Advanced Drug Delivery Reviews 108 (2017) 25–38

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

# Challenges and strategies in anti-cancer nanomedicine development: An industry perspective☆

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ARTICLE INFO

*Article history:*
Received 24 February 2016
Received in revised form 20 April 2016
Accepted 21 April 2016
Available online 29 April 2016

*Keywords:*
Nanomedicine
EPR effect
Clinical translation
Pre-clinical models
Industry
Companion diagnostics
Patient pre-selection

ABSTRACT

Successfully translating anti-cancer nanomedicines from pre-clinical proof of concept to demonstration of therapeutic value in the clinic is challenging. Having made significant advances with drug delivery technologies, we must learn from other areas of oncology drug development, where patient stratification and target-driven design have improved patient outcomes. We should evolve our nanomedicine development strategies to build the patient and disease into the line of sight from the outset. The success of small molecule targeted therapies has been significantly improved by employing a specific decision-making framework, such as AstraZeneca's 5R principle: right target/efficacy, right tissue/exposure, right safety, right patient, and right commercial potential. With appropriate investment and collaboration to generate a platform of evidence supporting the end clinical application, a similar framework can be established for enhancing nanomedicine translation and performance. Building informative data packages to answer these questions requires the following: (I) an improved understanding of the heterogeneity of clinical cancers and of the biological factors influencing the behaviour of nanomedicines in patient tumours; (II) a transition from formulation-driven research to disease-driven development; (III) the implementation of more relevant animal models and testing protocols; and (IV) the pre-selection of the patients most likely to respond to nanomedicine therapies. These challenges must be overcome to improve (the cost-effectiveness of) nanomedicine development and translation, and they are key to establishing superior therapies for patients.



**Contents**



☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Editors' Collection 2016".

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http://dx.doi.org/10.1016/j.addr.2016.04.025

## 1. Introduction

Nanomedicines have been investigated for the targeted delivery of drugs to treat a large variety of diseases. This industry perspective focusses on oncology-based nanomedicinal therapeutics only, as they receive about two-thirds of the research attention [1]. The concept that nanomedicines aim to improve the therapeutic index of anti-cancer drugs by modifying their pharmacokinetics and tissue distribution to improve delivery to the site of action is well known and has also been demonstrated clinically. Designed to exploit the enhanced permeability and retention (EPR) effect [2–3], liposomal doxorubicin (Doxil™/Caelyx™) was the first anti-cancer nanomedicine approved by the FDA in 1995 [4–7]. Doxil™/Caelyx™ achieves a differential distribution of doxorubicin *versus* the free drug and is now approved for several indications based on improved safety with equivalent or superior efficacy *versus* standard therapies [8]. In patients, Doxil™ has achieved a nearly 300-fold increase in area under the curve, relative to free doxorubicin [4], although this includes free (bioavailable) and liposome-encapsulated (non-bioavailable) doxorubicin.

Other nanomedicines approved for clinical use for cancer treatment include: Myocet™, DaunoXome™, Depocyt™, Abraxane™, Genexol-PM™, and, mostly recently, Onivyde™ (see Table 1). The approval of new nanomedicines has been based primarily on improving therapeutic benefit by enhancing safety, with patient survival being equivalent to that resulting from the use of standard treatments [9]. The significant anti-cancer activity demonstrated pre-clinically by many novel nanomedicines has yet to be recapitulated clinically [10], and, as a result, the development of the marketed nanomedicines has often been slow. Although valuable, the lack of or limited gain in overall survival challenges the field to improve patient survival further with more effective nanomedicine-based therapies. Many of the key opinion leaders in the nanomedicine field have written excellent articles detailing the challenges facing the successful development of novel nanomedicinal therapeutics and suggestions to overcome these hurdles, including refs. [11–16], amongst others.

At AstraZeneca, improved success in translating new drug projects to the clinic has been achieved by evaluating a drug's 5Rs: 'right target/efficacy', 'right tissue/exposure', 'right patients', 'right safety', and 'right commercial potential' [17]. This means that the pre-clinical data, at a minimum, must be consistent with the agent being able to achieve target engagement or inhibition in man—through the appropriate level of drug exposure at the target tissue, as determined from *in vitro* and *in vivo* screening. Projects that are more likely to be progressed have a well-defined therapeutic margin and detailed understanding of the agent's adverse toxicity profile. Further, there must be a patient selection hypothesis and appropriate biomarkers in place. Finally, the project must target the correct, commercially attractive, patient population. The 5Rs are the pivotal technical determinants of project success.

Applying a 5Rs framework to nanomedicine development requires definition of the key relationships between biology and technology: the influence of tumour pathophysiology on nanomedicine accumulation, distribution, retention, and efficacy, and the correlation between delivery system properties and *in vivo* behaviour. Until now, drug delivery system engineering has been the priority in nanomedicine research [18]. However, there has been little focus on defining the design of the nanomedicine based on tumour biology, and optimising nanomedicine use has been largely empirical. Using more clinically relevant models to test nanomedicines will enable the biology of the target population to drive the fine-tuning of the system properties. By changing our approach to nanomedicine development, it will be possible to build data sets supporting translatable clinical development and patient pre-selection strategies that will help these effective therapies reach the right patients.

### 1.1. Anti-cancer nanomedicines in pre-clinical and clinical development

Anti-cancer nanomedicines in clinical development can be broadly divided into five main types: liposomes, polymeric conjugates, polymeric nanoparticles, polymeric micelles, and others, although there is some overlap between categories. Antibody-drug conjugates were considered outside of the scope of this article, as an important therapeutic class distinct from the particulate nanomedicine systems discussed here. Examples of marketed anti-cancer nanomedicines and those in clinical development are summarised in Table 1.

The majority of approved anti-cancer nanomedicines have been designed to exploit the concept of the EPR effect, with a small subset of nanomedicines seeking to alter nanomedicine behaviour further with ligand-mediated targeting (*e.g.*, BIND-014 (BIND Therapeutics; [19]) and MM-302 (Merrimack Pharmaceuticals; [20])) [21]. Generally, EPR-based therapeutics aim to improve efficacy and tolerability by changing the pharmacokinetics and biodistribution of the drug. They can minimise the peak free drug concentration (Cmax) while often increasing the area under the curve in plasma and tumour to provide prolonged exposure to therapeutic levels of drug at the target. By achieving the 'right target' and 'right exposure', several nanomedicines have conferred a significantly enhanced therapeutic index to an existing therapy or enabled new innovative treatment approaches (*e.g.*, CRLX101 (Cerulean; [22]) and AZD2811 nanoparticle (AstraZeneca; [23])). The AZD2811 nanoparticle employed a novel encapsulation of an Aurora-B kinase inhibitor to mitigate dose-limiting bone marrow toxicity in pre-clinical testing and is currently in early clinical trials.

An important benefit of some nanomedicines is the ability to formulate a drug without using dose-limiting toxic excipients present in current marketed formulations, often improving tolerability and enabling more drug to be administered to patients. For instance, higher doses of paclitaxel can be administered to patients using Abraxane™ (Celgene) or the polymeric micelle formulation Genexol-PM™ (Samyang Biopharmaceuticals) because these formulations avoid the use of Cremophor™ needed to formulate Taxol™. While not considered to be the major focus for many nanomedicine research projects, such solubilisation benefits can be considerably cost-effective. Moreover, by achieving the 'right safety' profile, this approach can make a significant difference to the patients and the clinical outcome, as the maximum tolerated dose of the active agent can be increased by avoiding the tolerability problems caused by the solubilising surfactants. However, without improved efficacy, the increased cost of nanomedicine systems can prevent them from being a mainstream treatment choice. For this next generation of therapeutics, it is important to engage with

**Table 1**
Examples of anti-cancer nanomedicines in clinical trials or on the market.

| Nanomedicine type | Drug | Product name/company | Indication | Phase |
|---|---|---|---|---|
| **Liposomes** | Doxorubicin | Myocet™/Teva UK | Metastatic breast cancer | Approved |
| | | Doxil™/Janssen | Kaposi's sarcoma | Approved |
| | | | Ovarian cancer (post-first line failure) | |
| | | | Multiple myeloma | |
| | | ThermoDox™/Celsion | Primary hepatocellular carcinoma | Phase III |
| | | | Refractory chest wall breast cancer | |
| | | | Colorectal liver metastases | |
| | | | | Phase II |
| | | 2B3–101/2-BBB Medicines BV | Brain metastases Glioma | Phase II |
| | Vincristine | Marqibo™/Spectrum Pharmaceuticals | Acute lymphoblastic leukaemia | Approved |
| | Daunorubicin | DaunoXome™/Galen | HIV-related Kaposi's sarcoma | Approved |
| | Cytarabine | Depocyt™/Pacira Pharmaceuticals | Lymphomatous meningitis | Approved |
| | Irinotecan | Onivyde™/Merrimack Pharmaceuticals | Metastatic pancreatic cancer (2nd line) | Approved |
| | | | Gastric cancer | |
| | | | | Phase II |
| | Cytarabine: daunorubicin 5:1 fixed ratio | CPX-351/Celator | Acute myeloid leukaemia | Phase III |
| | Cisplatin | Lipoplatin/Regulon | Non-small cell lung cancer | Phase III |
| | | SPI-77/ALZA Pharmaceuticals | Ovarian cancer | Phase II |
| | | Aroplatin/Aronex Pharmaceuticals | Malignant mesothelioma | Phase II |
| | Oxaliplatin | MBP-426/Mebiopharm | Gastrointestinal adenocarcinoma | Phase II |
| | Paclitaxel | LEP—ETU/Insys | Breast cancer | Phase II |
| | | EndoTAG-1/MediGene | Breast cancer | Phase II |
| | | PNU-91934/MSKCC | Esophageal cancer | Phase II |
| | SN-38 | LE-SN38/Neopharm | Metastatic colorectal cancer | Phase II |
| | Irinotecan: Floxuridine 1:1 ratio | CPX-1/Celator | Colorectal cancer | Phase II |
| **Polymeric conjugates** | Camptothecin | CRLX101 (cyclodextrin adamantane)/Cerulean | Renal cancer | Phase II |
| | | | Small cell lung cancer | |
| | | | Ovarian cancer | |
| | Asparaginase | Oncaspar™ (PEG)/Baxalta | Acute lymphoblastic leukaemia | Approved |
| | Paclitaxel | Opaxio™ (Polyglycerol adipate)/CTI Biopharma | Ovarian cancer | Phase III maintenance |
| | | | | Phase II |
| | | | Non-small cell lung cancer (women) | |
| | Irinotecan | NKTR102 (PEG)/Nektar | Metastatic breast cancer | Phase III |
| | Camptothecin | CRLX101 (nanoparticle)/Cerulean | Renal cell carcinoma (3rd/4th line) | Phase II |
| | | | Ovarian cancer (2nd/3rd line) | |
| | | XMT1001 (Fleximer™)/Mersana | Gastric cancer (2nd line) | Phase II |
| | | | Non-small cell lung cancer (2nd/3rd line) | |
| | Diaminocyclohexane (DACH) Platinum | AP 5346 (Hydroxypropylmethacrylate)/ProLindac™ | Ovarian cancer | Phase II |
| | Docetaxel | DEP™ (G5 PEG-Polylysine)/StarPharma | Advanced cancers | Phase I |
| | | CriPec™ docetaxel (nanoparticle)/Cristal Therapeutics | Solid tumours | Phase I |
| **Polymeric nanoparticles** | Docetaxel + Prostate-Specific Membrane Antigen (PSMA) | BIND-014 (Accurin™)/BIND Therapeutics | Cholangiocarcinoma | Phase II |
| | | | Cervical cancer | |
| | | | Bladder cancer | |
| | | | Head and neck cancer | |
| | | | Non-small cell lung cancer subtypes | |
| | AZD2811 (AZD1152 hydroxyquinazoline pyrazol anilide; Aurora-B Kinase Inhibitor) | AZD2811 (Accurin™) nanoparticle/AstraZeneca | Advanced solid tumours | Phase I |
| **Polymeric micelles** | Paclitaxel | Genexol-PM™/Samyang Biopharmaceuticals | Breast cancer | Approved |
| | | | Non-small cell lung cancer | |
| | | | Ovarian cancer | |
| | | NK105/NanoCarrier™ | Stomach cancer | Phase III |
| | | | Breast cancer | |
| | | NC-4016/NanoCarrier™ | Solid tumours | Phase I |
| | | Nanoxel™/Samyang Biopharmaceuticals | Advanced breast cancer | Phase I |
| | DACH-platin | NC-6004 Nanoplatin™/NanoCarrier™ | Pancreatic cancer | Phase III |
| | | | Head and neck cancer | |
| | | | Non-small cell lung cancer | |
| | | | Bladder cancer | |
| **Other** | Irinotecan | HA-irinotecan HyACT™/Alchemia | Colorectal cancer | Phase II |
| | | | Lung cancer | Phase III |
| | Tumour Necrosis Factor (TNF) | CYT-6091/CytImmune | Non-small cell lung cancer | Phase II |
| | Paclitaxel | Abraxane ™/Celgene | Advanced breast cancer | Approved |
| | | | Advanced non-small cell lung cancer | |
| | | | Advanced pancreatic cancer | |

physicians and healthcare providers to demonstrate (what they regard as) meaningful clinical differentiation.

The nanomedicine field has devoted significant effort towards developing insight into the technological and biopharmaceutical advantages and disadvantages of different nanomedicine systems. Many different nanomedicines have been developed that improve the stability, solubility, pharmacokinetics/biodistribution, toxicity, and/or efficacy of cytotoxics and other classes of payloads [18,24]. Delivery system characteristics like size, charge, shape, type of surface modification, and biocompatibility have an important influence on the biodistribution and clearance of the nanomedicine [25–26].

As shown in pre-clinical models and patients, 'stealth' nanomedicines, with prolonged circulation times, are able to achieve considerable accumulation at sites of leaky vasculature [27–32]. In pre-clinical models, smaller (sub-100 nm) nanomedicine systems and lower molecular weight macromolecules have been shown to extravasate to a greater extent and/or penetrate farther from the vasculature than do larger systems [33–37]. This size effect has also been associated with improved efficacy [34,37]. However, it is too premature to generalise this size-dependency, which is likely tumour- and nanomedicine-dependent. The ability to control the release rate of a drug from a nanomedicine can significantly impact its safety and efficacy. Tuning drug release to exploit the therapeutic window can be achieved by modulating diffusion through a polymer matrix or by using chemical conjugation linkers with different degradation (*e.g.*, hydrolysis) rates *in vivo* [23,38–42]. Building clearer insight into the relationship between disease biology and nanomedicine behaviour will allow data-driven manipulation of the properties of the delivery system. Focussing nanomedicine development to align a delivery system, tumour, and drug with a specific clinical line of sight is discussed further in Section 3.1.

### *1.2. Perceived challenges for the nanomedicine field*

The attrition of anti-cancer agents in clinical trials is high; to improve success, it is important to learn why. For some nanomedicines, the root cause of the failure has been investigated. The paclitaxel-polyglutamic acid conjugate Opaxio™ was tested in phase III clinical trials for the treatment of non-small cell lung cancer. However, a survival benefit was only observed for females, but not males, treated with Opaxio™ [43]. Opaxio™ relies on cathepsin B-mediated activation, and since the clinical trial, a relationship between oestrogen levels and cathepsin B activity has been reported [44]. As a result, subsequent clinical studies with Opaxio™ have been restricted to women with oestrogen levels above a pre-defined threshold.

While Opaxio™ has developed a sound strategy to address the issue going forward, for most nanomedicines that fail in the clinic, the reason for the disappointing efficacy or increased toxicity is unknown. Moreover, the biological drivers behind the poor clinical translation are likely to be multi-factorial. Further, early stage clinical trials are typically completed in heavily pre-treated patients presenting with advanced, metastatic disease, and co-morbidities. Predicting many of these variables from pre-clinical testing alone is challenging; therefore, interfacing with the clinicians is important.

As was the case for early antibody therapeutics, the probability of success with nanomedicines is perceived to be low [9–10,45–47]. Progressing nanomedicines therapeutics to market is often slow. This may be because their clinical efficacy is not sufficient to warrant accelerated development, or that technical or cost challenges in scale-up and manufacturing can delay (or necessitate further) investment. However, the greatest drivers of failure may be our poor understanding of the disease heterogeneity in the patient population, inability to fine-tune the system based on the disease biology or stage of the target patients, and failure to build a platform of evidence supporting a specific end clinical application.

This situation should encourage us to optimise and refine how projects are designed and conducted. To exploit the significant advancements in nanomedicine engineering, focussing how nanomedicine therapeutics are tested clinically is important. Investing in the translational science will improve clinical outcomes, as it has for other classes of cancer drugs. Demonstrating improved clinical performance with nanomedicines will enable them to be broadly established as a credible and viable drug development option. As highlighted above, traditionally, nanomedicine research projects have been structured to adapt the physico-chemical parameters of a delivery system – loading, chemistry, size, charge, surface modification – to control its *in vivo* behaviour. What has been largely lacking is insight into the features of patient tumours that present unique challenges for nanomedicines to display optimal performance.

Considerably less research effort has been dedicated to the challenge of understanding the correlations between patient biology and nanomedicine behaviour. While for now the nanomedicine field is primarily embracing the attractive, but often evidence-lacking, assumption of a positive correlation between EPR and efficacy [15–16,46,48–50], the successful clinical translation of nanomedicine projects would be improved by greater focus in four key areas (Fig. 1):

1) Building the understanding of the interaction between tumour pathophysiology and nanomedicine behaviour in tumours, to enable the

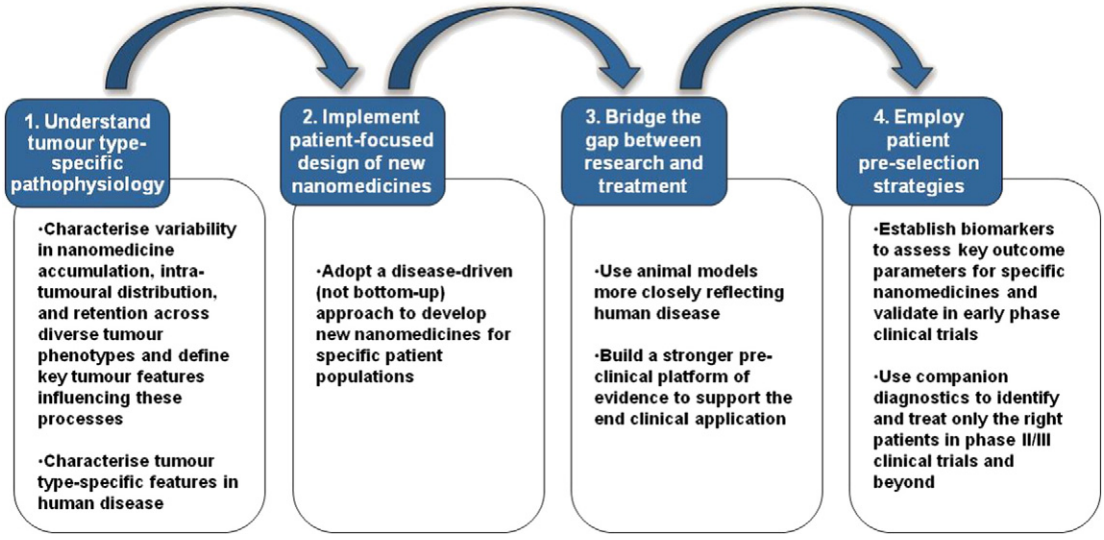


**Fig. 1.** Improving the successful clinical translation of nanomedicines.

optimisation of tumour accumulation, intra-tumoural distribution, and retention of distinct nanomedicines
2) Transitioning from formulation-driven research to disease-driven rational development
3) Developing and exploiting more clinically relevant animal models to optimise nanomedicine properties, dosing schedules, and treatment combinations with a clinical line of sight to the target disease as it develops in patients
4) Pre-selecting patients likely to respond to nanomedicine-based therapy

Together, drawing on the principles of the 5Rs framework and implementing these changes in nanomedicine science would underpin a more precise and an improved translatable approach to nanomedicine development that adopts a patient-focussed and disease-driven mindset from the outset.

These four areas are important for improving the successful clinical translation of nanomedicines; however, prioritising where to focus investment is also key. This will depend on the specific challenges in the development of each novel nanomedicine. Two common scenarios in nanomedicine development are novel delivery systems with established drugs and established delivery systems with novel payloads. Developing a new nanomedicine using a well-characterised delivery system will benefit from selecting the right patients. Here, the parameters driving the efficacy of the free drug (concentration *versus* exposure time) and the *in vivo* behaviour and critical quality attributes of the delivery system affecting *in vivo* performance should already be understood. Conversely, when testing a novel delivery system, investment should be focussed to gain insight into the behaviour of the delivery system across a range of tumour models to aid in defining the most suitable clinical populations. When beginning the development of a novel drug with a novel delivery system, implementing patient-driven design and building a strong pre-clinical platform of evidence will be the most advantageous areas in which to focus investment.

## 2. The EPR effect in nanomedicine development

The EPR phenomenon elegantly explains the enhanced accumulation and prolonged retention of macromolecules observed in solid tumours, relative to normal tissue [2–3,51]. The EPR effect has become a dogma in the literature to explain the targeting of nanomedicines to tumours after intravenous administration [33]. Despite the fact that EPR-mediated accumulation has only been reported for some tumour types [52–53], it is often claimed as a universal property of all solid tumours and is regularly cited as the driving 'passive targeting' principle underlying most nanomedicine research and development in cancer [48,54].

However, the EPR effect is unlikely to be present and equal in all tumours, nor likely to be the sole driver of nanomedicine efficacy. Nanomedicine activity is not only related to tumour accumulation/retention (EPR effect); it is also influenced by the intra-tumoural distribution of the delivery system, the extent and kinetics of drug release within the tumour, and the exposure to drug released in circulation. The contribution and importance of these elements will vary with the delivery system, drug, and properties of the tumour, and each must be considered when optimising nanomedicine systems.

### 2.1. Measuring nanomedicine biodistribution in patient tumours

The practicalities of evaluating the EPR effect in human tumours are relatively costly and time-consuming [15,50,55]. However, the potential rewards of a reliable evaluation method could make the costs acceptable. For long-term success, however, investing in more structured approaches to assess the EPR effect in pre-clinical and patient tumours would be invaluable. At present, it is challenging to complete an in-depth analysis because the majority of the currently available methodologies for tracing nanomedicines *in vivo* do not provide sufficient resolution [50]. While greater detail can be achieved using more sophisticated techniques (*e.g.*, imaging-based, such as gamma-scintigraphy or dynamic contrast-enhanced magnetic resonance) [50,55], they require expensive equipment that is often not accessible for many laboratories or hospitals. Moreover, few tracking techniques are useful across a range of delivery systems [50]. This capability becomes increasingly important when directly comparing different delivery systems for their suitability for a specific clinical application.

Nanomedicine biodistribution and accumulation in human tumours have only been documented in a small number of patients by gamma-scintigraphy/SPECT imaging of indium/technetium-labelled liposomes [27,53,56–58] and detection of drug fluorescence in patient biopsies [59]. Tumour accumulation of indium-labelled liposomes varied between tumour types, from 5 ± 3% of the injected dose/kg in breast cancers to 33 ± 16% of the injected dose/kg in head and neck cancers [56]. These data illustrate the concern that the access and/or accumulation of the nanomedicine may be disease-dependent and differ from tumour to tumour [55–56]. The implications of this concept should not be underappreciated. The inter-tumour variability in nanomedicine delivery is confirmed by a recent analysis of the EPR effect in spontaneous canine carcinomas and sarcomas that showed substantial heterogeneity in the level of liposome uptake, as measured by CT/PET scanning [60].

Therefore, if tumour EPR is a driving principle in the design characteristics of a nanomedicine, then it is essential to treat tumours that present an EPR effect, as obviously variability in the level of EPR effect, or even a complete lack of an EPR effect, would significantly impact the clinical outcome. Defining the biological conditions driving EPR variability is essential. However, there is currently no systematic investigation of the magnitude or diversity of the EPR effect in patient tumours, making patient or tumour type pre-selection challenging at present and requiring coordinated investment to make it a reality.

### 2.2. The tumour microenvironment and nanomedicine behaviour

In addition to circulation kinetics, drug release, and tumour clearance rates, the overall tumour exposure to a nanomedicine and its payload is governed by entry into the tumour, distribution across, and retention within the tumour. Human cancers have been shown to exhibit features (*e.g.*, fenestrated or permeable vasculature, dysfunctional or underdeveloped lymphatic system, and high interstitial fluid pressure [61–67]) that affect these processes. In human tumours, the dominant features determining tumour drug exposure are poorly understood. Moreover, such tumour features may either be polarised (biased to one or two dominant features), or manifested only subtly.

The EPR effect, and more generally the tumour exposure to a nanomedicine, is dependent on many more factors than 'just' local permeability of endothelia lining tumour blood vessels and the prolonged circulation characteristic of a nanomedicine. The importance of and interaction between such factors is increasingly being recognised, including the following:

- tumour—type, size, proliferation rate, necrosis, intra-tumoural volume, and anatomical location
- vasculature—density, volume, permeability, distribution relative to stromal and tumour cells, and blood flow
- stroma—architecture, density, composition, and matrix rigidity
- macrophages—number and function
- lymphatics—density, function, and location within and around the tumour
- interstitial fluid pressure—local effects and cross-tumour pressure gradients

To gain insight into aspects of the complex tumour biology, we analysed over 200 different patient tumours from eight different cancer types. This revealed marked variability in the vasculature, specifically its

intra-tumoural location and density, stromal morphology and density, as well as in macrophage number and distribution across human tumours. This variation was observed between tumour types, between tumours of the same type, and even within individual tumours (Fig. 2). Nanomedicine scientists often fail to appreciate the significance and implications of this heterogeneity when designing and testing new formulations. While some consistent trends in tumour features can be observed between cancers (see Fig. 2), the high degree of variability even within specific tumour types makes it critical to evaluate tumour features together in individual tumours, and to pre-select the 'right patients' with a greater likelihood of benefiting from nanomedicine-based therapy.

Tumour histology is not sufficient to predict tumour EPR. More bespoke analyses of tumour features using high-resolution imaging can be employed pre-clinically to gain functional insight into the relationship between the microenvironment and nanomedicine behaviour in different pre-clinical models that recapitulate the clinical setting.

Several imaging modalities, including CT, multi-spectral optoacoustic tomography, MRI, and functional ultrasound, have been used to investigate the influence of tumour vasculature, permeability, perfusion, and/or interstitial fluid pressure on the magnitude and heterogeneity of nanomedicine accumulation and intra-tumoural distribution in pre-clinical models [68–73]. These important studies primarily used liposomes, and as a major finding, a strong correlation between vascularisation and intra-tumoural accumulation/distribution was noted. This suggests that tumour vascular density and perfusion may greatly influence the therapeutic outcome of passively targeted nanomedicine therapy. Moreover, the variation in local/regional vessel permeability, blood volume, and interstitial pressure throughout a tumour will also dictate the carrier deposition and should be included in expanded analyses using more clinically relevant models [15–16,74–76]. These same tumour features are expected to influence nanomedicine behaviour in humans. Therefore, continued investment is required to develop additional clinically relevant imaging techniques that can validate the significance of these parameters in patients.

### 2.3. Why invest in patient pre-selection?

Within large pharma, very early on in drug development projects, there is a strong focus on the definition of the 'right patients' to treat. Molecularly targeted therapies have benefited significantly from rational patient selection strategies and personalised treatment approaches. Clinical trials with more focus on clearly defined outcome criteria, biomarker profiles, and treatment schedules have improved results in patients. A similar strategy in nanomedicine projects could improve clinical performance.

Identifying a suitable companion diagnostic or clinically useful biomarker that is predictive of therapeutic outcome presents a significant challenge, but also a significant reward. Selecting the 'right patients' is complex for nanomedicines and other classes of anti-cancer agents. For instance, remarkably, a therapeutic response to trastuzumab or cetuximab treatment is not always observed in patients with high tumour expression of HER2 and EGFR, as measured by *ex vivo* diagnostic staining. Clearly though, such diagnostic approaches provide important information but do not guarantee the correct identification of the 'right patients' to treat. However, patient pre-selection efforts enable clear decision-making and serve as a starting point for stratification of treatment groups.

Patient stratification strategies are attractive, but more demanding, for nanomedicines, as patient pre-selection for both delivery system and payload must align. Moreover, as tumour pathophysiology varies with growth and disease stage, selecting the 'right patients' for nanomedicinal therapies requires data-based knowledge of nanomedicine suitability for each delivery system in individual tumours [55].

#### 2.3.1. Using companion diagnostics to predict treatment outcome

The development of companion diagnostics to screen a patient's 'nanomedicine suitability' has received only limited attention so far [77–78]. A drug-free version of a nanomedicine system labelled with an imaging agent can be used to identify patients likely to benefit from the therapeutic version [79]. This requires an understanding of

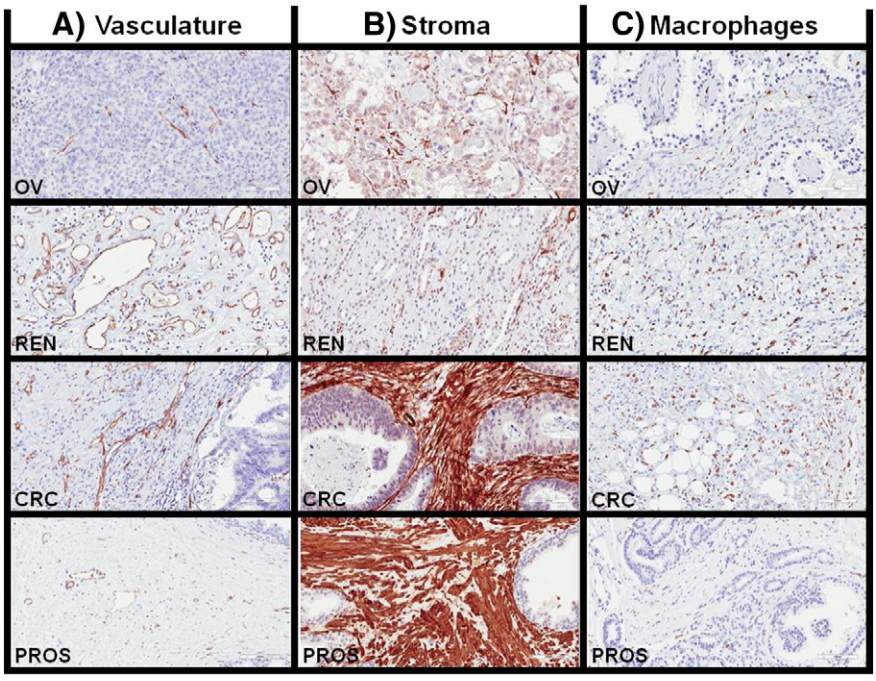


**Fig. 2.** Heterogeneity in tumour features across human clinical cancers. Representative immunohistochemical staining of human prostate, ovarian, colorectal, and renal tumour material for *vasculature* (CD31; A), *stroma* (alpha-smooth muscle actin; B), and *macrophages* (CD68; C). Positive staining represented by brown colour. Greater vascular density was observed in colorectal and renal cancers than in ovarian and prostate cancers. Colorectal and prostate cancer vasculature was situated in the stromal compartment; ovarian and renal cancer vasculature was located amidst the tumour cells. A significantly higher stromal content was observed in prostate and colorectal cancers, compared to ovarian and renal tumours. The lowest macrophage content was observed in prostate tumours. However, it must be noted that substantial variability was seen within each tumour and between patients in each disease type.

the relationship between the delivery system and the trafficking of the drug throughout the body, as discussed later. Significant cost reductions and improvements in the speed of generating key data informing project progression will be aided by state-of-the-art imaging techniques, integration with *in silico* modelling, and advances in bioanalytical methods for drug and nanomedicine visualisation and quantification.

Following a labelled delivery system is most useful when it can be related to the fate of the drug, based on robust understanding of the kinetics of drug release in plasma, and ultimately, in the future, in the tumour and tissues. Further, it is also necessary to define the parameters driving anti-cancer efficacy in order to evaluate therapeutic potential, choose the appropriate patient pre-selection criteria, and define potential biomarkers. For instance, if a prolonged duration of exposure is key to a drug's activity, evaluating the duration of delivery system retention in the tumour may prove to correlate with treatment outcome. However, if a high Cmax drives drug efficacy, it may be more appropriate to evaluate the peak accumulation of the delivery system, provided that its release kinetics achieve therapeutic levels of bioavailable drug in tumour.

Moreover, it may be possible in some circumstances to avoid using a companion diagnostic nanomedicine, and instead use the accumulation of small molecules to predict the accumulation of the therapeutic nanomedicines. Published pre-clinical findings have shown strong agreement between the magnitude and intra-tumoural distribution of the CT enhancement patterns for a CT contrast agent and a liposomal carrier [70]. Employing clinically relevant imaging techniques to translate this research into a strategy to visualise nanomedicine therapeutics in patients could transform decision-making early in development.

Karathanasis *et al.* used a rat model of breast cancer to demonstrate the feasibility of using mammography to quantify the tumour extravasation of a nanoscale probe in individual tumours to predict therapeutic benefit of liposomal doxorubicin treatment [76]. A strong correlation between probe accumulation/extravasation and positive treatment outcome was observed [76], highlighting the potential utility and importance of patient pre-selection approaches. Moreover, it suggests that vascular permeability may be a useful selection parameter to evaluate in some instances.

Using high-resolution fluorescent imaging of xenograft tumours, Miller *et al.* have shown, with 85% accuracy, the co-localisation of two different nanomedicine delivery systems—a magnetic nanoparticle suitable for magnetic resonance-based diagnostics and a polymeric system designed to deliver therapeutics [80]. Further exploration of the concept of nanomedicine-based companion diagnostics with other delivery systems could identify additional "imaging-friendly" nanomedicine systems useful for predicting the accumulation and localisation of different therapeutic nanomedicines. However, validation on a case-by-case basis would be necessary.

#### *2.3.2. Early efforts to apply patient pre-selection and companion diagnostics in the clinic*

Merrimack Pharmaceuticals is investing in the use of a companion diagnostic to identify the patients most likely to benefit from treatment with their novel liposomal irinotecan formulation. Preliminary experiments utilised magnetic resonance to image the tumour accumulation of a 30 nm iron oxide nanoparticle (Feraheme™, AMAG Pharmaceuticals) with subsequent assessment of the therapeutic response of the same patients receiving liposomal irinotecan therapy [81]. Feraheme™ is approved by the FDA for the treatment of iron deficiency anaemia. When employed off-label to evaluate the EPR characteristics of patient tumours, a strong correlation between Feraheme™ tumour uptake and shrinkage of tumour lesions following liposomal irinotecan treatment was observed [81].

Despite the difference in size and composition between the two nanosystems, the data suggest that imaging tumour accumulation of the iron oxide nanoparticles is a highly useful pre-selection tool for liposomal irinotecan treatment [81]. This approach might be able to be generalised to other passively targeted nanomedicines as well, if enhanced accumulation underpins their therapeutic activity. The potential translational benefits of such a clinically validated pre-selection tool to determine broad applicability across tumour types and nanomedicine systems warrants substantial continued effort.

Beyond patient pre-selection, other nanomedicine-based approaches to companion diagnostics are being utilised in the clinic as part of comprehensive cancer treatment strategies. For example, Lymphoseek™ is a mannose-derived dextran conjugate that has been FDA-approved as a radioactive diagnostic for locating tumour-draining lymph nodes [82–83]. As well, LUM015, a nanoparticle in phase I trials, can be used to identify, in real time, cancer cells in the tumour margin during surgery [84].

## 3. Developing nanomedicines using 'industry-style' thinking to enhance clinical translation

Whilst pre-clinical experimentation has been used effectively to generate proof-of-principle and drive optimisation of new nanomedicine technologies, it is important to identify weaknesses and remain objective about their relevance for later development. The primary aim of early pre-clinical testing should be to identify both the therapeutic potential and any clinical risks, to select formulations that will be safe and efficacious and possess the required pharmacokinetic and biodistribution properties. In the past, anti-cancer nanomedicine research has used the standard formulation-driven approach: novel nanomedicines are developed and then evaluated using *in vitro* cytotoxicity assays, *in vivo* pharmacokinetics/biodistribution studies, and anti-tumour experiments in xenograft models sensitive to the payload. This paradigm has not generated the data that yield insight into the key issues that enable the successful translation of nanomedicines to the clinic. Instead, using a decision-making framework like the 5Rs should enable scientists to make 'go/no-go' investment decisions earlier in the development process, before making significant financial investment in clinical trials. Realising the need to change current nanomedicine development strategies, this section focusses on the goal of disease-driven design and generating pre-clinical project data that more reliably inform clinically relevant therapeutic end points, to be implemented in treating the 'right patients'.

### *3.1. Adopting a structured approach to nanomedicine projects*

No single nanomedicine will achieve the 'right exposure' and the 'right efficacy' in all tumour types. For large pharma, the range of nanomedicine systems available at present makes it possible and favourable to adopt a disease-driven development strategy and transition away from formulation-driven (bottom-up) approaches. From the start of the project, it is important to build a clinical line of sight and to understand the specific challenges with the standard-of-care, such as excessive normal organ toxicity or unsuitable pharmacokinetic profile. Designing a nanomedicine to overcome a well-defined challenge in a particular cancer has a greater chance of success than developing a delivery system and then attempting to align it with an existing clinical challenge. It is essential to consider the relationship between the heterogeneous disease and patient pathophysiology and the physicochemical properties of different nanomedicines to enable the data-driven selection of the nanomedicine systems that are most appropriate for specific disease types. Further, this requires the generation of more informative data in the clinic which can be bridged back to improve development strategies.

Thus, rational selection criteria are critical in the development of clinically successful and translatable nanomedicines. A disease-driven approach to development focusses on aligning a drug, delivery system, and target patient population to balance many different variables to maximise therapeutic activity (Fig. 3). For example, human cancers are sensitive to specific drugs. The physico-chemical properties of different nanomedicine systems dictate their suitability for delivering certain drugs, and any off-target effects that may result from the "dose" of the

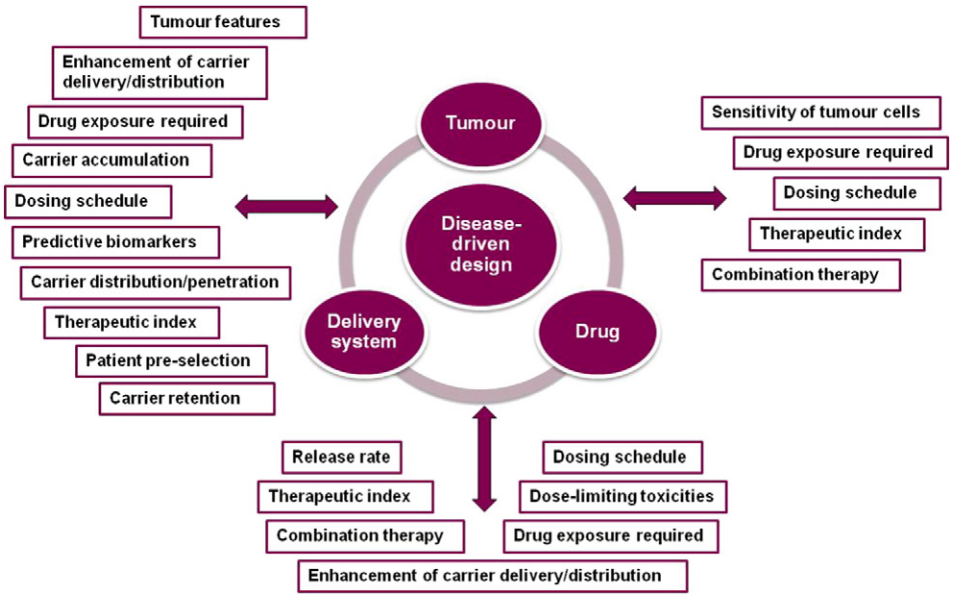


**Fig. 3.** Considerations when selecting the delivery system, drug, and target patient population for disease-driven design and development of new anti-cancer nanomedicines.

delivery system required to achieve therapeutically active drug concentrations in patients. Further, the tumour features of the target patient population will influence the levels of accumulation and retention of the carrier that can be expected, which will determine whether the system can achieve the drug release rate required to deliver drug to the tumour at therapeutic levels/exposures. The off-target accumulation of the carrier will determine whether it achieves an appropriate safety profile for the drug, particularly when administered in combination with the typical standard-of-care regimens for the target cancer. Although complex, considering these patient- and disease-focussed parameters from the design phase should produce more translatable nanomedicines.

It is not practical, however, to develop nanomedicines for individual patients. Using a structured framework, the aim is to focus the development of a nanomedicine to a specific patient population. In-depth knowledge of tumour genetics has generated a translatable patient focus for therapies targeting specific genetic drivers. To progress towards patient-driven nanomedicine design, initial investment is required to build a comprehensive understanding of the complex criteria highlighted in Fig. 3. Such insight will underpin the ability to achieve focussed, accelerated, and translational development with a clinical line of sight.

After establishing a clinical line of sight, the genetic profile and intrinsic sensitivity of the target patient population influence many decisions. These include choosing the drug itself, selecting the relevant models for testing, defining the optimal drug release rate (to achieve the desired high maximum concentration, increased area under the curve, or improved therapeutic index, *etc.*), optimising the dosing schedule, and building combination therapy knowledge. Building this clinically translatable data set requires a series of focussed experiments to refine the nanomedicine and sequentially answer clinically relevant questions about the lead candidate. *In vivo* studies in realistic models are even more expensive; therefore, it is important to think carefully about the value of the data sets generated. Testing models and nanomedicines representing the extremes of different parameters (*e.g.*, tumour phenotype or drug release rate) can give broader insight and enable complementary *in silico* modelling approaches to be applied to reduce overall cost.

### *3.1.1. Using disease-driven design to achieve the 'right efficacy'*

Disease-driven design is rooted in understanding the implications of biology for nanomedicine behaviour in order to select a carrier able to exploit the pathophysiology. Heterogeneity in the tumour microenvironment between different tumours and cancers presents unique barriers to nanomedicine-based treatment, which can be overcome by some delivery systems but may be problematic for others. As a result, disease-driven design considers the target patient population from the outset and works to design the right nanomedicine to exploit the pathophysiology. The opportunity to use academic-industry collaborations to generate these data is discussed in Section 4.2.

For instance, tumour types like pancreatic cancers with complex, dense stroma, where penetration is severely limited, may be most susceptible to a small delivery system with a high drug:carrier ratio [34] or a depot of a sustained-release drug delivery system for an agent that does not require a high Cmax. These approaches are designed to overcome the limitations of the tumour biology by increasing penetration and delivering more drug away from the vasculature, or exploiting the prolonged retention that is experienced in highly stromal tumours to achieve greater drug exposure within the tumour. Moreover, in highly stromal phenotypes, combining nanomedicines with therapies designed to manipulate the tumour microenvironment may have the potential to overcome the physiological barriers that limit the therapeutic effect of some nanomedicines [85–88]. Nanomedicinal therapeutics may benefit from combination approaches with hyaluronidase [89–90], from degradation of collagen [91] or inhibition of its synthesis [92] or cross-linking [93], and from vascular normalisation [94].

Renal cancers, and some other highly vascularised tumours, present with the tumour cells in close proximity to the blood vessels; these tumours may exhibit a more classical enhanced accumulation effect, but the retention of the carrier may be reduced or limited to the periphery. Here, to achieve the 'right exposure', it may be necessary to employ a nanomedicine with a sufficiently rapid drug release rate that the payload becomes bioavailable before the delivery system is cleared from the tumour. Thus, slow release nanomedicines may not be optimal for tumour types where prolonged delivery system retention is unlikely. Alternatively, attaching a targeting ligand to the carrier may be able to improve its retention [95] to overcome this issue. Finally, in tumours with a high macrophage content, exploiting the mononuclear phagocytic system becomes a possibility, as does exploring immune-oncology therapies or combinations. These examples highlight the potential advantages of aligning an appropriate nanomedicine to the biology of a specific target population.

### *3.1.2. Using patient-focussed design to achieve the 'right safety'*

Understanding the off-target effects of nanomedicines is as important as evaluating efficacy. Nanomedicines will distribute into all tissues exhibiting the features, such as fenestrated vasculature, which permit

the accumulation of the delivery system. Thus, defining the features controlling the tissue localisation, particularly within the reticuloendothelial system, of different delivery systems is of equal importance to the assessment of the tumoural drug accumulation to increase efficacy. A recent paper from Kirtane *et al.* illustrates how a predictive model of size-related carrier accumulation can help us gain broader insight into nanomedicine behaviour and generate experimental hypotheses to test [96]. The model in this paper highlights that the EPR effect is not necessarily the key driver of benefit for every nanomedicine and proposes that the size of the delivery system is dictated by the features of the tumour, in particular the pore size [96].

Further, the levels of drug present in different target and off-target organs are controlled by the release rate of the drug from the delivery system, and the relative clearance from tumour *versus* normal tissues [96]. The importance of understanding therapeutic index is exemplified by the development of AstraZeneca's AZD2811, an Aurora-B kinase inhibitor delivered using a BIND Therapeutics Accurin™ polymeric nanoparticle. During pre-clinical development, the drug release rate from the nanoparticle was exploited to minimise bone marrow toxicity with therapeutic drug exposure. Nanoparticles with significantly different release rates showed equivalent efficacy in a rat xenograft model, but substantially different levels of bone marrow toxicity [97–98]. The importance of release rate has also been demonstrated in different scenarios, including by Hu *et al.* [42].

Knowledge of the activity and toxicities of the free drug, the behaviour of different delivery systems, and an understanding of the influence of drug release rate on target and off-target concentrations of bioavailable drug enable project teams to select an appropriate range of nanomedicines to test. A small amount of improvement in translational success through rational nanomedicine design would justify the initial investment required to build these essential baseline data sets. However, a key element of this change is further investment in broader strategic collaborations [16].

Refining basic models of nanomedicine behaviour by incorporating individual tumour types and normal tissues would significantly improve decision making when progressing nanomedicinal therapeutics. Defining these parameters across the nanomedicine toolbox provides an opportunity to match a delivery system with a suitable distribution profile to a specific drug's toxicity profile in an appropriate disease setting. This is particularly applicable when designing a nanomedicine to overcome a known problem—for instance, doxorubicin-induced cardiotoxicity limits the cumulative dose a patient can receive. By administering doxorubicin in a liposomal formulation, the cardiac exposure is eliminated or dramatically reduced, allowing patients to be treated with higher life-time doses. Moreover, well-tolerated nanomedicines like Doxil™ can be highly beneficial in combination regimens, to improve tolerability or enable the combination partners to be delivered at higher doses.

The off-target toxicities of nanomedicines may be different than the parent drug, as a result of the change in pharmacokinetics and biodistribution. An example of this is the risk of palmar-plantar erythrodysesthesia during Doxil™ therapy [99–100], in comparison to the cardiotoxicity of the free drug. Understanding the nanomedicine properties, dose, and scheduling parameters that deliver efficacy, as well as toxicity, enables more informed design of the clinical plan, drug combination opportunities, and population selection.

### 3.2. Building a stronger platform of evidence to justify project progression

EPR effect-based tumour accumulation is typically the primary focus during nanomedicine development. However, in some tumour types, nanomedicine accumulation is only minimal or highly variable, while altering the peripheral plasma pharmacokinetics of the drug can also yield therapeutic benefit. Thus, to evaluate nanomedicine efficacy, pre-clinical research needs to generate data sets that describe four properties of nanomedicine behaviour: the tumoural accumulation, intratumoural distribution, and tumoural retention of the system, as well as the additional contribution of the peripheral pharmacokinetics (or circulation) of the nanomedicine. It is likely that for any tumour, each of these features may independently contribute to potential efficacy; however, the dominant feature can influence the choice of delivery system and release kinetics desired. Further, as discussed above, understanding the off-target effects is as important as evaluating efficacy when taking a nanomedicine into development.

Translatable pre-clinical testing should strive to provide detailed insight into the key parameters that influence nanomedicine efficacy. Informative and translatable data sets should consider the following:

- Characterise the intra-tumoural carrier retention, drug release rates, and drug metabolism over time
- Differentiate between bioavailable/released drug and total concentrations of drug in the tumour, plasma, and other key organs (*e.g.*, liver, bone marrow, *etc.*)
- Define the intra-tumoural distribution of therapeutically active concentrations of bioavailable drug and drug metabolites
- Understand how the plasma, off-target tissue, and tumour pharmacokinetics of the nanomedicine are affected by repeat dosing
- Separate the evaluation of pharmacokinetics/biodistribution from efficacy/mechanism of action
- Evaluate treatment efficacy in tumours having reached less EPR-rich sizes
- Understand the degree of therapeutic benefit from extravasation *versus* simple accumulation/residence in the tumour vasculature *versus* alteration of the pharmacokinetics of the drug
- Evaluate the inter-tumour variation within a group
- Maintain clear focus on the end clinical application (such as combination with standard-of-care) of the nanomedicine;
- Evaluate its efficacy in that context to define an appropriate dose and schedule

While it is easy to list a set of activities, it has to be recognised that it is currently technically difficult to generate insight into these questions, particularly across delivery systems. Many imaging approaches label and follow the distribution of the delivery system (agnostic of the fate of the associated drug). To improve our understanding, we need to consider the release, trafficking, and target engagement of the payload which ultimately exerts the therapeutic effect. Further, it is important to invest in developing and refining the necessary analytical approaches because the ability to determine the concentration of bioavailable *versus* bound/encapsulated drug throughout the body is fundamental to many of the considerations listed above. It may only be possible to achieve these complex data sets through more strategic collaborations. Once in place, the data packages that can be produced during nanomedicine development will be substantially more informative and discriminatory when selecting lead candidates and when progressing to the clinic. Maintaining a clear focus on the end clinical application and building a thorough understanding of the therapeutic margin of novel nanomedicines are likely to have a significant impact on improving translation.

### 3.3. Enhancing nanomedicine translation by using more clinically relevant models

Many of the first pre-clinical nanomedicine development projects were driven by data generated in sensitive subcutaneously implanted cell line-derived xenograft models [24]. The translatability of these results to the clinic has limitations, as the majority of these tumour lines present pathologies that bear little resemblance to the complexity and heterogeneity of the clinical tumours they are presumed to model [101–105]. This research has supported the conclusion that EPR-based efficacy should occur across all human tumours, and the drug delivery field has been founded on this belief. Further, relative to most clinical solid tumours, nanomedicine accumulation and intra-tumoural

distribution are likely to be exaggerated in typical xenografts. These models are highly vascularised, rapidly proliferating, have a high level of macrophage infiltrate, and possess a simple stromal architecture and low stromal density. Optimising nanomedicines to achieve efficacy in these types of models may not produce therapies that are effective in treating human solid tumours.

The *in vivo* models we rely on for developing new nanomedicines may only reflect a narrow spectrum of the human pathophysiology. Pre-clinical testing with liposomal doxorubicin shows that animal efficacy can be predictive of suitability for treating a specific clinical tumour phenotype closely reflecting the pathophysiology or histology of the models. For instance, early research demonstrated the efficacy of liposomal doxorubicin in tumours with a high tumour cell density, low stromal content, and dense and highly permeable vascularisation. These observations have successfully translated to the clinic, where Doxil™ has shown its greatest therapeutic efficacy in the treatment of multiple myeloma and AIDS-related Kaposi's sarcoma [24].

Past oncology research has relied on generating data in accessible pre-clinical models that often do not recapitulate the patient tumour features [106]. This is particularly problematic for therapies whose intra-tumoural behaviour and therapeutic efficacy are influenced by tumour heterogeneity and morphology, such as biologics and nanomedicines. The extent of pre-clinical testing completed in poorly representative models is a significant and often ignored obstacle for translating nanomedicine research. It is important to recognise that these pre-clinical data drive important clinical decisions, such as dose, schedule, and the expectation of efficacy. Striving to represent the target patient tumour population better will allow informed investment decisions. It is now possible to determine how well our *in vivo* models recapitulate human cancers by accessing tumour biobanks in collaboration with major oncology centres to characterise the genetics, pathophysiology, and heterogeneity of patient tumours.

Routine generation of data sets in a diverse panel of models that represent aspects of the target clinical tumour population is essential. Aligning the activity of a drug and a delivery system in models representative of the genetic profile of the target patient population, as well as the suitability of the delivery system in a specific tumour pathophysiology, often requires multiple pre-clinical models. This ensures that the profile of the system selected for further development is more broadly applicable across the tumour type, rather than optimised for one individual animal model.

More importantly, highlighting potential limitations early may inform on a 'stop decision' for a project before significant investment is required, or constrain the type of patients treated in early trials. Once the desired physico-chemical and biological characteristics of a nanomedicine are defined, it is then possible to use pre-clinical models to assess progress. For example, if the aim is to generate a formulation for a drug already known to be clinically active, then failure to show activity in multiple models of that disease type drives a stop decision. Conversely, toxicity concerns revealed by pre-clinical testing in relevant models may also drive stop decisions. For instance, beyond a certain threshold, some delivery systems can lead to additional adverse effects by virtue of their clearance properties, such as deposition in the liver and spleen with long-term effects on these organs upon repeat dosing. Finally, establishing the therapeutic index with agents the nanomedicine is likely to be combined with in the clinic can inform on the likelihood of success in early clinical trials. These objectives can be defined at the start of a programme, and have proven critical in improving success for classical therapeutics. The ability to make these decisions early in the pre-clinical testing phase is an essential part of cost-effective development of nanomedicines, and all other anti-cancer therapeutics.

### 3.3.1. The benefits of using more clinically relevant models

Patient-derived tumour explant (PDX) models and genetically engineered mouse models (GEMMs) more faithfully reflect the morphology, complexity, and heterogeneity of clinical tumours [102–103, 107]. Although PDX models are difficult to establish and resource-intensive to maintain, due to the typical slow growth rate and requirement for live passaging to help to maintain the clinical pathology, there are now thousands of models available through both contract research organisations and academic institutions. Likewise, GEMMs have similar drawbacks, but offer the potential to assess how nanomedicines achieve efficacy in tumours residing in specific organs (*e.g.*, liver *versus* lung). While the models may not be directly predictive of the clinic, they all have features that enable the assessment of the performance of a given nanomedicine, and generate insight into risks. A number of studies have now been published using these more relevant models to develop new perspectives on nanomedicines.

As mentioned, further insight can be developed by using GEMMs, where the tumour develops *in situ*. The Zamboni group recently published a paper that used GEMMs as relevant models of different breast cancer subtypes and showed that the components of the tumour microenvironment contributed to the heterogeneity of liposome, but not small molecule, accumulation in the tumour [108]. As more clinically relevant and diverse models become increasingly accessible to nanomedicine researchers, the ability to perform more realistic assessments of nanomedicine accumulation, intra-tumoural distribution, and retention will be highly valuable in generating a platform of evidence for the activity of different types of nanomedicines across tumour (pheno)types.

The vasculature in clinical tumours and many PDX models is more mature and less permeable than in xenografts, which develop over days, rather than weeks or months, and present properties that are less influenced by the overall rate of tumour cell proliferation [48, 102]. Further, in the majority of human tumour types, vessels are found either within the stromal compartment or in close proximity to tumour cells [109]. This differential vessel distribution is more faithfully recapitulated in PDX models [110] and is expected to influence nanomedicine behaviour in solid tumours. Assessing a number of different models with varied vessel distributions, as well as assessing tumour-to-tumour variation, is required to build a more realistic understanding of the likely outcome in different human disease segments.

### 3.3.2. Greater variety is possible with more clinically relevant models

Pre-clinical tumours are often considered to be uniform. However, sufficiently complex pre-clinical models can capture the influence of tumour heterogeneity and morphology on nanomedicine efficacy. Using a single PDX model that presented with a spectrum of stromal morphologies, detailed analysis showed that tumour architecture significantly impacts nanomedicine treatment outcome, in a cohort of tumours possessing the same intrinsic sensitivity [111]. Rather than reporting group means, capturing the variability in pharmacodynamic effect between individual tumours provides insight into the diversity in response possible within human disease segments. Particularly as tumours change with disease stage and in response to therapy, this proof-of-principle study highlights the importance of striving to develop biomarkers and imaging tools that enable patient pre-selection strategies.

A study of the distribution and activity of liposomal irinotecan (prodrug of SN-38) demonstrated that therapeutic efficacy was influenced by the accumulation of active drug in the tumour through enhanced vascular permeability and the presence of the enzyme that generates the active drug SN-38 [107]. The authors also showed that retention of active SN-38 was higher in tumour cell-derived xenografts than tumour cell explants [107]. This may imply that liposomes accumulate better in xenograft models derived from cells lines, and when models with a complex microenvironment are used, a different conclusion may be drawn. This study analysed the formulation across a panel of 13 models [107] and highlights that exploring more diverse models will yield valuable insight. Determining how tumour features are associated with reduced efficacy or drug penetration may inform a clinical plan, particularly when considering the potential influence of tumour

morphology on treatment outcome [111]. The breadth of models used in the study by Kalra *et al.* [107] gives broader insight into the potential of liposomal irinotecan and hence a data platform that helps focus the development and target population strategy.

## 4. Pharma perspective

A number of challenging questions are often asked of oncology projects: Why is the pre-clinical testing of anti-cancer agents so poor at predicting their clinical potential? Why is there a disconnect between animal models of cancer and human patients? What factors must be considered pre-clinically to improve success clinically? Project teams need to define the tumour type and stage, dose and dosing schedule, and potential currently used drugs with which the novel agent may be combined. As the project matures, they are also asked to articulate clearly the risks that may hinder clinical success. Although hard to address, some answers to these questions are required to improve the translation of oncology therapeutics and secure larger investment.

To make a project attractive for pharma to take into development, there must be an opportunity to test a focussed hypothesis, and the ability to make a decision on whether the agent is likely to succeed with a minimal initial investment. As discussed above, projects are progressed when there is confidence in the 5Rs: 'right target/efficacy', 'right tissue/exposure', 'right safety', 'right patients', and 'right commercial potential' [17]. This general approach has been shown to improve success for projects to develop classical small molecules [17].

### *4.1. The cost of success … and failure*

The probability of success of a small molecule drug progressing from pre-clinical proof-of-principle to commercial launch is in the region of 6% [17]. There is a continuing demand for innovative, patient-personalised therapeutics to improve clinical outcomes. Nanomedicines have a tremendous potential to achieve this goal. However, industry, correctly or incorrectly, sees additional challenges when considering investment in nanomedicines, and other classes of therapeutics like antibodies, peptides, and DNA-/RNA-based agents. For these agents, the formulations are often more complex; there is poor understanding of the correlation between critical quality attributes and efficacy; the regulatory perceptions and environment are constantly evolving; manufacturing and scale-up are technically challenging; important analytical methods require further maturation; and the cost of goods is high (up to 15% higher than for tablets and standard parenterals). A key consideration when adopting nanomedicinal therapies is the cost–benefit provided by the nanomedicinal drug *versus* the cheaper (often generic) therapy. However, if such approaches can be delivered cost-effectively with the 'right commercial potential', the advantages should not be undervalued.

As a result, the development costs for novel classes of therapeutics can be disproportionately high. Moreover, with a lack of *in vitro* and pre-clinical tests to predict performance in man adequately, it requires a substantial investment to frontload formulation and process optimisation to avoid repeating long complex clinical studies. Looking to the future, rather than using nanomedicines to develop a solution to formulate the drug, it may be more rational to develop nanomedicine-friendly active pharmaceutical ingredients. For instance, it is possible for synthetic chemists to engineer specific features into the design of novel small molecules to develop drugs that are more compatible for conjugation, active-loading, encapsulation, or complexation with nanomedicine technologies.

For any company, but in particular smaller biotech companies or academic labs, the expense of taking a prototype nanomedicine into the clinic and beyond can be prohibitive. For large pharma, this cost can be a major blocker for investment, due to a lower perceived probability of success with added technical complexity potentially compounding the risk already associated with a novel drug. Therefore, the pre-clinical data sets supporting a nanomedicine therapeutic need to be stronger compared to those associated with classic drug therapeutics, which have more conventional formulations, defined patient populations, and fewer treatment obstacles.

### *4.2. Collaborations are essential to the future success of nanomedicines*

The development of nanomedicinal therapeutics is a multidisciplinary endeavour. Like many areas of drug development, it requires the successful integration of biology, chemistry, nanotechnology, imaging, and medicine. However, the diversity and complexity in nanomedicine systems necessitates highly varied expertise to develop translatable therapeutics. As such, it will become important to foster partnerships between large pharma, smaller companies, and academia early in pre-clinical development to capitalise on the unique strengths of each partner.

Effective collaborations between academia, industry, consortia, and cancer research hospitals will be essential to build the data sets and insight that link the physico-chemical properties of nanomedicine systems with biological implications. The Nanotechnology Characterisation Laboratory (NCL; www.ncl.cancer.gov) is a multidisciplinary laboratory set up in 2004 by the National Cancer Institute, National Institute of Standards and Technology, and FDA. The NCL's remit is to support the pre-clinical characterisation of nanomaterial-based drug delivery products. This includes establishing and standardising an analytical cascade for nanomaterial characterisation and facilitating the clinical development and regulatory review of nanomaterials for cancer clinical trials. The aim to provide robust nanomedicine charactrisation extends to the identification and characterisation of the critical parameters related to a nanomaterial's absorption, distribution, metabolism, and excretion (collectively termed ADME) and toxicity. As part of its effort, the NCL engages with and facilitates academic and industry-based knowledge sharing and interfaces with regulatory authorities. A European Nanomedicine Characterisation Laboratory (EU-NCL; www.euncl.eu) was set up 2015 with similar aims to the NCL.

Other consortia have also been established in Europe. These include the European Technology Platform for Nanomedicine (ETPN; www.etp-nanomedicine.eu), created in 2006, and more recently a Translational Advisory Board, which is part of the EU ENATRANS project (Enabling NAnomedicine TRANSlation; www.enatrans.eu). They aim to provide free of charge, non-binding, and strategic advice to promote and guide projects in the nanomedicine area.

The additional advantages of collaborative working are that new methods can be developed for improved characterisation, and testing in more than one lab or set of models improves the robustness, speed of data generation, and diversity of the platform of evidence. We are seeing the start of collaborative working with the National Cancer Institute's *Alliance for Nanotechnology in Cancer*, which has formed an expert public-private partnership between academia, government, and industry to accelerate nanotechnology development and translation. In 2012, the Translation of Nanotechnology in Cancer (TONIC) consortium met to discuss the importance of improving the "understanding of the EPR characteristics impacting the utility of nanoparticles in the clinic" [16].

Working consortia and expert collaborations like TONIC and EU ENATRANS have the potential to exploit the diverse expertise of their members to achieve significant progress in answering the major questions presently facing nanomedicine scientists. Broader collaboration has additional benefits. Valuable insight could be derived from comparing the efficacy or accumulation/intra-tumoural distribution of new nanomedicines against "benchmark" formulations (such as Doxil™), similar formulations, and different delivery systems with the same payload. This type of collaboratively generated information becomes highly informative to companies seeking to make investment decisions between different delivery systems and exploit disease-focussed design.

## 5. Future opportunities and concluding remarks

Many of the nanomedicine formulations in development and clinical trials are designed for cytotoxics, where broadening the therapeutic window can address issues with tolerability or sub-optimal target exposure that may limit the ability to develop the drug into a viable and effective product [40,100,112]. Future opportunities for nanomedicines are looking towards delivering the next generation of drugs: molecularly targeted agents, toxin-like agents that induce cell death, DNA-/RNA-based therapeutics, peptides, drug combinations, *etc.* The major delivery challenges for these agents include significant off-target accumulation, crossing the cell membrane, achieving synergistic drug ratios at the target, and a narrow therapeutic window. Turning these "undeliverable" next generation therapies into viable anti-cancer treatments has emerged as one of the main future directions for nanomedicines. The cost-effective delivery of nucleic acid-based therapeutics requires unique nanomedicine technology and knowledge, and some companies are making a focussed investment in this area, including Alnylam Pharmaceuticals, Calando Pharmaceuticals, Avidity NanoMedicines, Merck, and Arrowhead Research Corporation.

The standard practise of formulation-driven development has not achieved the expected patient benefit; however, nanomedicines still have the potential to enter the mainstream of cancer therapeutics, both for traditional and next generation agents. While scientists have attempted to overcome the challenges in achieving efficacy in patients by developing and investigating an overwhelming number of new nanomedicines, relatively few of the many promising pre-clinical nanomedicines have reached the market. There are important gaps in the translation of nanomedicines, and it is necessary to shift some of the long-established paradigms to overcome these hurdles.

Although poor clinical translation is also a concern with other classes of anti-cancer agents, at present, the costs of taking a novel nanomedicine into the clinic can be a significant obstacle. The best way to make nanomedicines cost-effective is to increase the probability that the patients who are treated will respond to the therapy, using focussed design and a decision-making strategy like the 5Rs framework. Moreover, without developing, validating, and implementing patient pre-selection tools, it will remain challenging to achieve the right cost-efficiency for nanomedicine therapeutics. Although not without its own challenges, a concerted effort across the nanomedicine field to adopt new and more clinically focussed ways of working will help the next wave of nanomedicines to address the obstacles, perceived or real, encountered in the current clinical trials. Investing in the science underpinning the fundamental principles in nanomedicine science could have a significant impact on bringing efficacious nanomedicine therapies to patients.

## Acknowledgements

This work was financially supported by AstraZeneca. The authors kindly acknowledge Dr. Colin Howes for helpful comments and discussions. The European Research Council is gratefully acknowledged for financial support (ERC Starting Grant 309495 (NeoNaNo) and proof-of-concept grant 680882 (CONQUEST); both to TL).

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