Bridging Bio–Nano Science and Cancer Nanomedicine

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Abstract

The interface of bio–nano science and cancer medicine is an area experiencing much progress, but also beset with controversy. Core concepts of the field—*e.g.*, the enhanced permeability and retention (EPR) effect, tumor targeting and accumulation, and even the purpose of "nano" in cancer medicine—are hotly debated. In parallel, considerable advances in neighboring fields are occurring rapidly, including the recent progress of "immuno-oncology" and the fundamental impact it is having on our understanding and the clinical treatment of the group of diseases collectively known as cancer. Herein, we: (i) revisit how cancer is commonly treated in the clinic and how this relates to nanomedicine; (ii) examine the ongoing debate on the relevance of the EPR effect and tumor targeting; (iii) highlight ways to improve the next-generation of nanomedicines; and (iv) discuss the emerging concept of working *with* (and not *against*) biology. While discussing these controversies, challenges, emerging concepts, and opportunities we explore new directions for the field of cancer nanomedicine.

Our continuously improving ability to engineer nanomaterials with tailored properties has provided a strong foundation for applications across a range of biomedical settings.[1,2] To date, a diverse set of engineered nanomaterials have been developed, including both inorganic (*e.g.*, gold,[3] iron oxide,[4] silver,[4] and silica or silicon[5]) and organic nanoparticles (*e.g.*, lipid-based,[6] templated,[7] cell-membrane derived,[8] and layer-by-layer assembled[9,10]). These developments have led to rapidly growing interest in the area of nanomedicine, which leverages the strengths of nanoscience and nanotechnology to achieve improved patient outcomes.[11–13] For the purposes of this article we focus on approaches using the type of engineered nanomaterials listed above and consider biologics (such as antibodies) a separate entity.

Cancer nanomedicine is the application of nanomedicine to the treatment of cancer, and while the field has seen enormous progress in recent years (especially academically) there is still much to achieve.[14–17] Key concepts in the field, including the enhanced permeability and retention (EPR) effect, tumor targeting and accumulation, as well as the role of "nano" in cancer medicine, are all subjects of ongoing debate;[18–20] a debate that is a cornerstone to the concepts discussed in this article.

Herein, we provide an overview of the field of cancer nanomedicine, and discuss controversies and challenges, and emerging concepts and opportunities (**Figure 1**). We recently discussed strategies for accelerating the field of bio–nano science,[13] and our focus in this article is on the ongoing debates and controversies associated with key concepts in cancer nanomedicine. Our intention is to provide an overview accessible to the wide range of researchers active in the area (*e.g.*, chemists, biologists, oncologists, engineers, material scientists), with a special focus on exploring opportunities and new directions for the field. We start by revisiting the standard treatment of cancer to provide background for the subsequent discussions on the concept of "tumor targeting", and emerging concepts in the development of the next-generation of cancer nanomedicines.

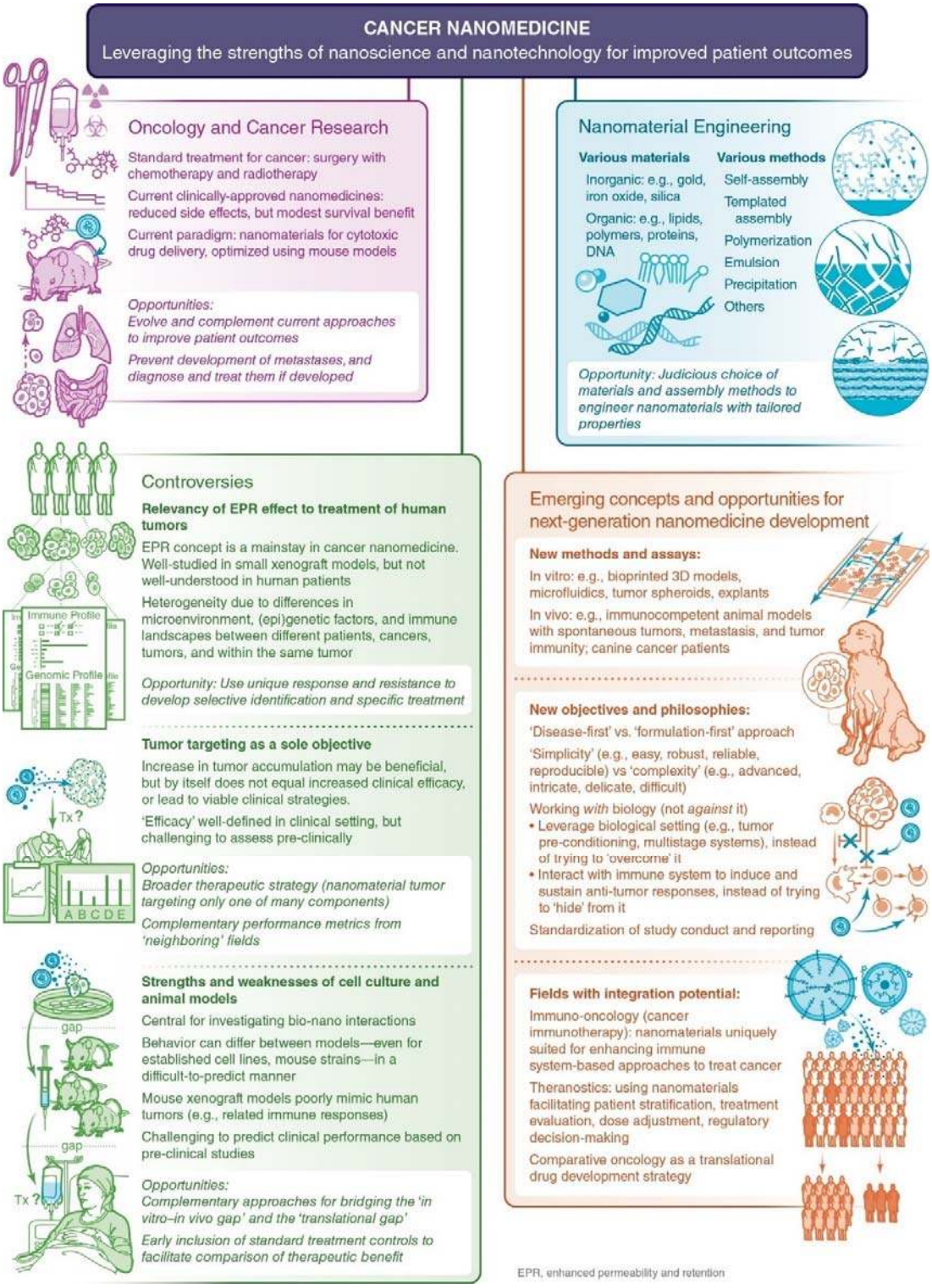

Figure 1. Overview of current challenges and opportunities in field of cancer nanomedicine, at the intersection of oncology, cancer research, chemistry, materials science and nanomaterial engineering.

Standard treatment in the clinic

Cancer is a highly heterogeneous and multifaceted disease. The treatment approach is divided into curative or non-curative therapy, subject to the extent of disease and the general clinical state of the patient.[21–24] Determination of the extent of disease ("staging") can involve both imaging as well as direct visualization strategies (*e.g.*, endoscopy or visualization at time of surgery). In the curative ("radical") setting the aim is to remove all of the tumor either by surgery or by surgery combined with radio- and chemotherapy (**Figure 2**). These well-established treatment options can cure many primary tumors (tumor at the site where cancer developed), but the vast majority of cancer-related deaths are instead due to metastatic tumors (tumors forming after cancer cells migrate from a primary tumor into other tissues and locations in the body).[25] Where a curative approach is not feasible, the focus is on improvements in overall survival and quality of life; for example through chemotherapy and targeted therapy (*e.g.*, with molecular targeted agents and antibodies). Metastatic disease is therefore an attractive target for the development of new treatments that aim to provide substantial patient survival benefit.[26]

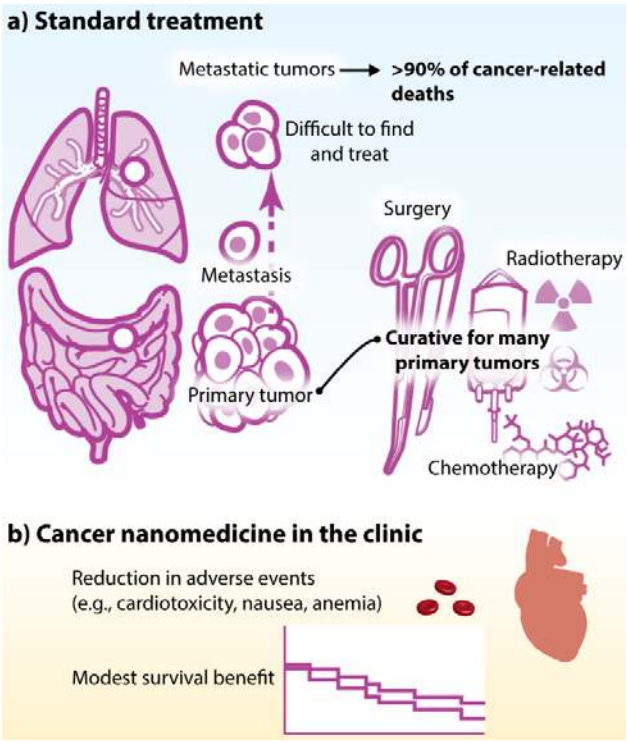

Figure 2. a) Current cancer treatment typically involves surgery combined with chemotherapy and radiotherapy. This is curative for many primary tumors. However, metastasis (cancer cells that migrate from the primary site and establish secondary tumors in other tissues) remains difficult to treat and causes most cancer-related deaths. **b**) Cancer nanomedicines that are being used in the clinic today are primarily used for their capacity to reduce side effects, as the survival benefit is often modest.[27]

To date, thousands of cancer nanomedicines have been developed, with around a dozen approved for clinical use.[14,15] For most of these, the main benefit is the reduction of adverse events: *e.g.*, decreased nausea/vomiting, hair loss, anemia and cardiotoxicity (**Figure 2b**).[28] While some recent clinical trials are showing promise,[18] the survival benefit afforded by using nanomedicines compared to standard treatment is typically low.[28] For example, in a recent meta-analysis comparison of liposomal *versus* conventional non-liposomal chemotherapy (14 clinical trials, 2589 patients in total) no increase in survival was found.[27] These results are in stark contrast to preclinical mouse studies where significantly increased survival was observed.[27] Reasons for this discrepancy include differences between human and mouse tumor microenvironments, dosing regimens, bioavailability, pharmacokinetics and pharmacodynamics, as well as a lack of standardization in the conduct and reporting of preclinical anticancer efficacy studies. Importantly, while many of the drug delivery systems included in these clinical trials were developed many years ago, the fundamental concepts and rationale underpinning much of the field of cancer nanomedicine have remained largely unchanged. For example, the approach of using nanomaterials to directly kill tumors through cytotoxic drug delivery optimized using mouse models. Moving forward, we should consider how we can evolve and complement current approaches to both increase our fundamental understanding of the behavior of nanomaterials in cancer nanomedicine, and to facilitate translation into improved patient outcomes.

EPR controversy

Human tumors are highly complex and heterogeneous, with differences observed from patient to patient, between multiple tumors in the same patient (*e.g.*, primary tumor and metastasis, and between metastases), and even within the same tumor microenvironment (**Figure 3**).[29–32] This has important implications for the response to therapies and for the development of resistance.[33] Nevertheless, there are several hallmarks that cancers have in common, including sustained proliferation and growth, changes in the behavior of the immune system, and the induction of angiogenesis (*i.e.*, formation of new blood vessels).[34,35]

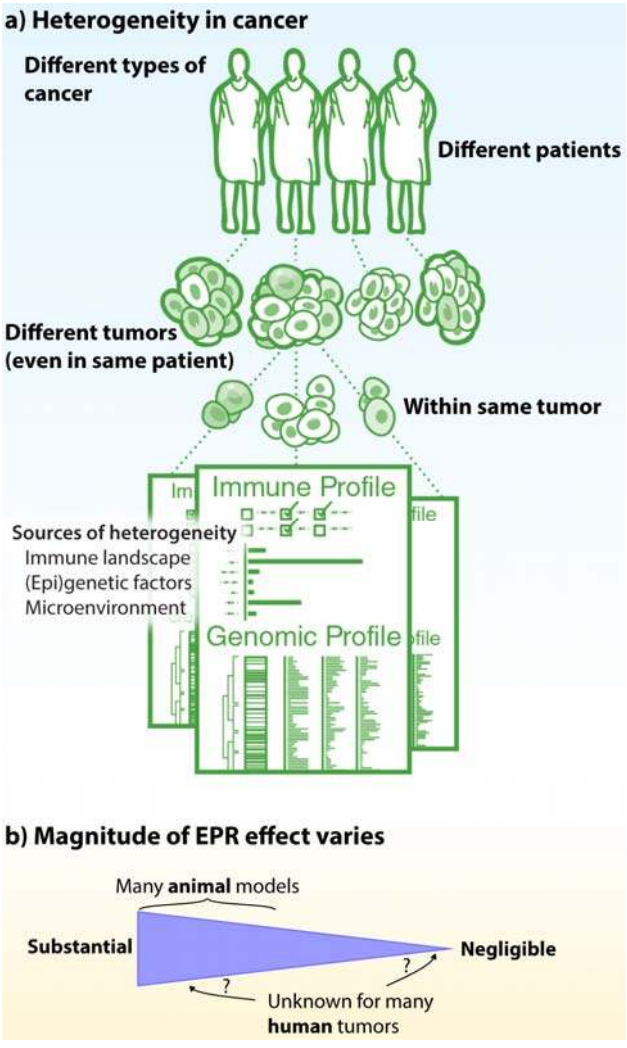

Figure 3. a) Tumors have distinct features that can change with the type of cancer, the patient, the type of tumor, and even within the tumor. **b**) The existence and magnitude of an EPR effect for a tumor is governed

by all of the factors listed in (a). Some of these factors are well-established for transplanted tumors in small animal models, but remain largely unknown for many human cancers.

The abnormal tissue environment associated with cancer raises both challenges and opportunities for the development of treatments. Challenges include vascular and interstitial barriers for the delivery of therapeutics to tumors,[36] which has important implications for tumor penetration of anticancer drugs,[37] antibodies,[38] and nanomedicines.[28,39] On the other hand, unique features associated with tumors poses opportunities, as they may enable selective identification and treatment.

It has been known since the late 1950s that dyes injected into the blood of mice and rats (bearing implanted tumors) or cats and dogs (with spontaneous tumors) can extravasate out of the blood stream and accumulate in tumors.[40–42] In 1986, two independent studies showed that: (i) tumor vessels are typically more permeable to large molecules than many normal vessels (studied using rabbits with transplanted tumors[43]), and (ii) that some tumors can retain and accumulate large molecules due to reduced clearance (studied using mice with transplanted tumors[44]). These studies formed the foundation to what is known as the "enhanced permeability and retention" (EPR) effect.

Since these original studies, the EPR effect has become a mainstay of much of cancer nanomedicine. While it has been well-studied in the case of small animal models with transplanted tumors, its relevancy to human tumors remains controversial.[45–49] Recent examples highlighting this controversy include statements such as the "EPR effect fails in the clinic" and "works in rodents but not in humans",[50] while others assert that the "EPR effect is the main mechanism of tumor penetration by nanocarriers and is a clinically relevant phenomenon".[51]

Much of the current knowledge on the EPR effect in patients and human tumors is based on early, relatively low-resolution imaging (both spatial and temporal) using radiolabeled lipid vesicles.[52–56] While

patient biopsies have further shown that nanomaterials can preferentially accumulate in human tumors,[57] the extent to which the EPR effect varies between different patients and tumor types remains to be explored, and therefore its relevance to the clinical use of cancer nanomedicines remains uncertain (**Figure 3b**). It is noteworthy that the first clinically approved nano-sized anticancer drug carrier—Doxil/Caelyx—was approved for treatment of Kaposi's sarcoma,[58] a cancer of the endothelial cells of blood and lymph vessels which makes endothelial barriers highly permeable, so that even red blood cells can leak out.[45,58] For this cancer, Doxil/Caelyx (pegylated-liposomal doxorubicin) was found to be clinically more effective and less toxic than the standard combination chemotherapy (doxorubicin, bleomycin, and vincristine).[59] For other, less leaky tumors, such as many breast cancers, Doxil was not as effective, but it did reduce side-effects (*e.g.*, cardiotoxicity) and is currently used for that reason.[60] (Additional examples and more extensive discussion on this topic are available elsewhere[45]). The varying efficacies reported for these examples across different cancers and patient groups further highlight the complexities and heterogeneities associated with clinically relevant tumors. A recent study[61] using pet dogs (companion animals with cancers that developed spontaneously), in what is known as "comparative oncology",[62,63] provides further evidence to the heterogeneity of the EPR effect. For the seven dogs that had carcinomas (a cancer of epithelial tissues), six (ca. 85%) displayed high uptake levels of liposomes, as determined using high resolution imaging. However, only one of the four dogs (25%) that had sarcomas (a cancer of soft tissues) displayed signs of liposome retention. Similar results have been observed in clinical studies.[64] Taken together, while the EPR effect can be strong in some tumors, it can also be negligible in others, and should therefore not be considered a general feature of all cancers and tumors. Emerging hybrid imaging techniques such as simultaneous positron emission tomography–magnetic resonance imaging (PET–MRI)[65–68] may help in expanding our understanding of the EPR effect in human cancers and patient tumors, and its relevance to nanomaterials.

The be-all and end-all of targeting

One of the core principles of cancer nanomedicine is the concept of using nanoparticles to selectively or specifically accumulate at tumor sites. From a clinical perspective, this "targeting" is only of interest if it leads to increases in efficacy, *i.e.*, improved patient outcomes. But while efficacy is well-defined in the clinical setting (*e.g.*, reduction of adverse events, increase in response rate, and increase in progression-free and overall survival), it is a more fluid concept that can be challenging to assess pre-clinically. This has led to a large focus on "targeting" in and of itself, as it can be easier to assess and try to optimize, for example using rodent models (**Figure 4**).

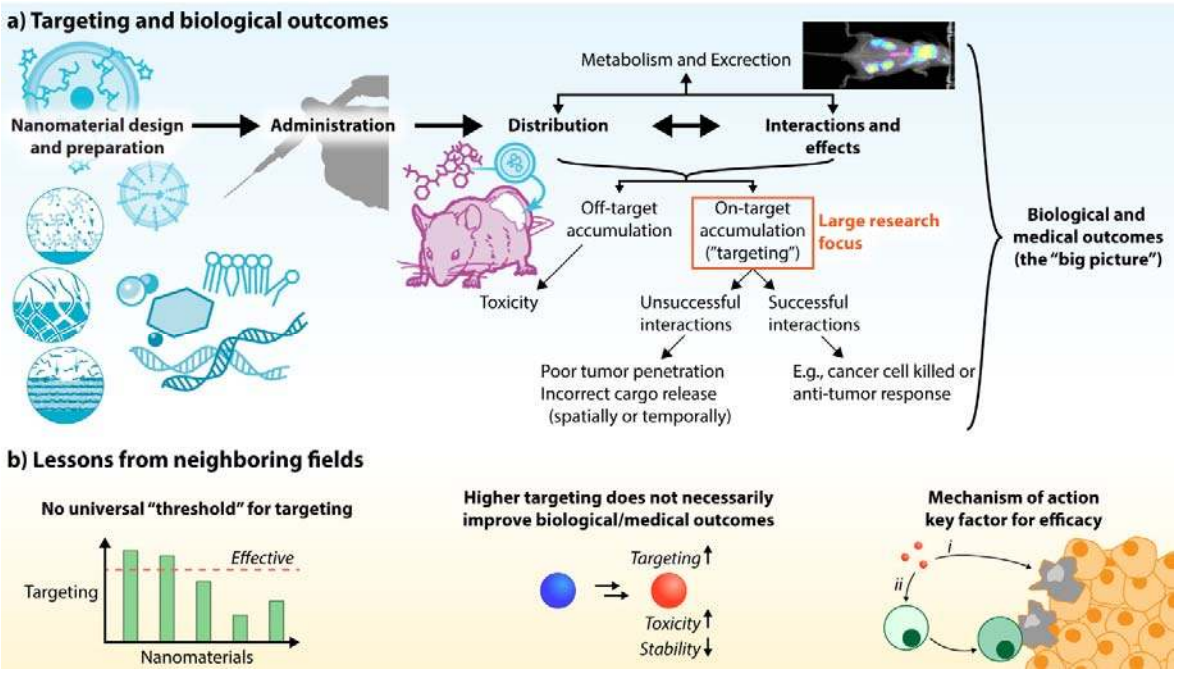

Figure 4. a) The efficacy of nanomaterials for cancer medicine depends on a range of factors, some of which are challenging to assess pre-clinically. Substantial efforts have focused on improving the "targeting" of nanomaterials (*e.g.*, increase in tumor accumulation) and it is important to remember that one factor in isolation only represents a small part of the bigger picture. **b**) Lessons from neighboring fields (*e.g.*, therapeutic affinity proteins) include that there is no universal "threshold" value for targeting, *i.e.*, at which an ineffective therapeutic becomes effective, and that an increase in targeting does not necessarily improve efficacy. The mechanism of action is also a key factor for therapeutic efficacy. For example, if the target

cells are cancer cells, or they are other, associated cells (*e.g.*, immune cells); or if the intended molecular target (*i.e.*, site of action) is accessible on the surface of the cell; or if internalization is required. The mouse imaging figure in (a) is adapted with permission.[69] Copyright 2015 American Chemical Society.

While the concept of tumor targeting of nanomaterials has been a controversial topic for many years,[70–75] a recent meta-analysis[76] surveyed the literature from the past ten years and found that the amount of injected dose (ID) of particles that accumulated at the tumor was typically less than 1% (**Table 1**). This seemingly low number re-energized the debate on whether tumor targeting is a viable concept.[77–80] While this type of debate can be healthy and constructive for a field as active as cancer nanomedicine, there are some important points to note. First, while an increase in tumor targeting may be of interest scientifically, if it does not lead to improved patient outcomes, then clinically it is not a viable therapeutic strategy. Therefore, care should be taken not to overemphasize the importance of numbers such as percentage of injected dose accumulated at a tumor (Figure 4). Second, similar to what we have discussed previously,[13] many of the challenges faced in the field of cancer nanomedicine are not unique to the field and there are lessons that can be learnt from neighboring areas of research. For example, much of the work, and terminology, of developing engineered nanomaterials for cancer therapies has roots in the field of targeted anti-cancer therapies using antibodies.

Table 1. Tumor accumulation of nanomaterials in rodent models. Based on data from online repository introduced by Wilhelm *et al.*[76] containing 238 data sets from 118 publications. "Active" and "passive" refers to nanomaterials functionalized and not functionalized with targeting ligands, respectively.

Targeting	**%ID at tumor, median (min–max)**	**%ID per gram of tumor tissue, median (min–max)**
Active	1.00 (0.001–18.9)	4.60 (0.02–45.8)
Passive	0.60 (0.0002–14)	2.60 (0.01–28.8)
Data combined	0.70 (0.0002–18.9)	3.17 (0.01–45.8)

Over the last few decades, therapeutic antibodies have revolutionized the treatment of cancer and today form part of the backbone in cancer therapy.[81–83] To address questions such as "how does the accumulation of nanomaterials compare to other targeted therapies?", it may be informative to compare the accumulation described above for nanomaterials with tumor accumulation values observed for antibodies. In mice, the accumulation of antibodies in "xenografts" (*e.g.*, human cancer cells implanted into mice) can vary greatly, typically between 0.5 to 50 %ID per gram of tumor tissue.[84] In contrast, accumulation of antibodies in human patient tumors is much lower, typically much less than 0.01 %ID per gram of tumor tissue (**Table 2**).

Table 2. Examples of tumor accumulation of antibodies in human cancer patients.

Cancer	**Number of patients**	**Days since antibody administered**	**Accumulation of antibody (% administered dose / g tumor)**	**Reference**
Lymphoma	10	2	0.0002–0.009	[85]
Leukemia	1	1	0.01	[86]

Leukemia	10	1	0.005–0.011	[87]
Neuroblastoma	6	1	0.08 [a]	[88]
Colorectal	27	6–7	0.0002–0.01	[89]
Colorectal	12	7	0.0021–0.011	[90]
Colorectal	32	3–17	0.001–0.009	[91]
Colorectal	4	>1	0.007	[92]
Ovarian	2	5	0.002–0.006	[93]
Ovarian	1	>3	0.009	[94]
Carcinoma	7	5–7	0.005–0.01	[95]
Melanoma	17	7–10	0.001–0.026	[96]
Melanoma	6	3–4	0.007–0.0003	[97]
Sarcoma	14	2–3	0.0003–0.006	[98]

[a] per mL tumor

Despite the very low accumulation of antibodies commonly observed in human tumors compared to mouse xenograft models, many antibodies display substantial clinical efficacy, both for the imaging and treatment of cancer.[81–84,99–101] Therefore, when comparing these metrics of accumulation (**Figure 5**) it is important to remember that they only capture one aspect of a bigger picture (Figure 4a). Inspired by the success of antibodies, other high affinity proteins for targeted therapies are also being developed. Examples include nanobodies,[102] antibody fragments,[103] repeat proteins,[104] bispecific affinity proteins,[105,106] and other non-immunoglobulin based protein scaffolds.[107] For some of these, first-in-human clinical trials have recently been published.[108–110] A key message from these and similar studies (and the nanomaterial–antibody comparison, Figure 5) is that a single parameter (*e.g.*, %ID accumulated at tumor) forms only a small part of evaluating the performance of targeted therapies, and care should be taken not to overemphasize this aspect when engineering nanomaterials for targeted therapies (Figure 4).

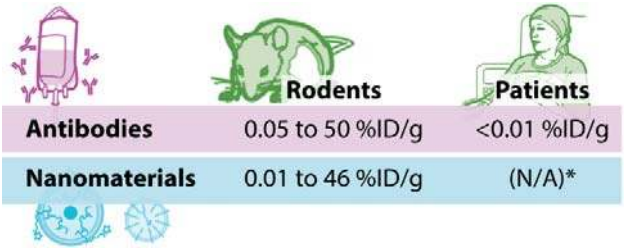

Figure 5. Summary of typical tumor accumulation metrics: 0.01 to 45.8 %ID/g for nanomaterials in rodents (Table 1), 0.5 to 50 %ID/g for antibodies in rodents,[84] and typically much less than 0.01 %ID/g for antibodies in patients (Table 2). *Larger scale, quantitative and systematic studies of tumor accumulation of different nanomaterials in various human tumors are yet to be conducted.

There is growing interest in using antibodies and antibody-like molecules for generating antibody–drug conjugates[111,112] and for functionalizing nanomaterials.[113–116] When the tumor accumulation (in animal models) for a wide range of nanomaterials with and without targeting moieties are compared, an increase is observed but a large overlap also exists between the two groups (Table 1, compare "active" and "passive"). Several studies on direct head-to-head comparisons have demonstrated that the functionalization of nanomaterials with targeting moieties does not always increase tumor accumulation, but it often improves cellular uptake.[117–122] That is, even if the total amount of nanomaterial deposited in a tumor does not increase with the use of a targeting moiety, for the fraction of nanomaterial deposited in the tumor, a targeting moiety can facilitate internalization into the tumor cells. However, antibody-functionalization and efficient accumulation does not always lead to efficient internalization, as this depends on the receptor targeted and potentially other, receptor-independent mechanisms involved.[123,124] Additionally, an increase in internalization efficiency does not necessarily translate into increased activity, due to processes such as endocytic recycling[125,126] and the mechanism of action of the therapeutic, which may require endosomal release/escape and further translocation (*e.g.*, into the nucleus).[1,127,128] All of these mechanisms are further complicated by the presence of dynamic biomolecular coronas on nanomaterials in

biological environments.[129] These endogenous molecules (*e.g.*, lipids and proteins) can interact with off-target receptors, possibly inducing difficult-to-predict off-target effects and toxicity.[130]

In addition to functionalizing nanomaterials with targeting ligands, it has also been shown that the tumor accumulation (and more generally, biodistribution and pharmacokinetics) in small animal xenograft models for both functionalized and non-functionalized nanomaterials strongly depends on time after administration of particles,[131,132] the dose administered,[133] the diameter of the particles,[134] and the amount of targeting molecules attached to each particle.[135,136] The strategy used to attach targeting ligands onto nanomaterials is also important, with recent data indicating that for some methods only ~4% of attached targeting ligands have a favorable orientation for recognition by their target receptor, which can lead to poor (and heterogeneous) outcomes.[137] The addition of targeting ligands may also impose a "binding site barrier" effect, which retards or even prevents tumor penetration of therapeutics into tumor tissues due to strong interactions of targeting ligands with cells in the periphery of tumors.[138–141] This "binding site barrier" is only one of many obstacles tumor-targeted nanomaterials encounter inside the body, and is an additional factor that must be considered when trying to elucidate mechanisms that drive the efficacy of nanomedicines.

Strengths and weaknesses of animal models

When nanomaterials are administered *in vivo*, they interact with the physiological environment (be it in a mouse or a human) at multiple levels: the sub-cellular and cellular levels, the tissue level, and the organ and organism levels.[142,143] Depending on the intended application, many of these interactions can be thought of as "barriers" that need to be overcome.[144,145] When studying these barriers for cancer nanomedicine using mouse xenograft models it is important to consider the substantial differences that exist between different xenograft models and mouse strains. For example, when comparing liposomal tumor accumulation and plasma clearance rates in multiple xenograft mouse models, order-of-magnitude differences have been observed.[146,147] The age and sex of mice may also affect results, which, if not accounted for, can make

comparisons difficult.[148,149] It has also been shown that there are substantial differences between intratumor distribution of liposomes in xenograft mouse models, with macro-accumulation not always reflecting micro-accumulation in specific regions inside tumors.[150] These results highlight the complexities that exist in xenograft mouse models (even when well-established cell lines and mouse strains are used), complexities that are even more intricate for human patients.

Cell culture studies and small animal models (*e.g.*, mice with xenografts) have been and continue to be essential for the field of nanomedicine, as they are central for the investigation of fundamental bio–nano interactions. However, while examples of successful correlations between preclinical animal studies and clinical human trials exist,[151,152] it remains challenging to use these type of models to predict clinical performance. An approach that is being investigated to accelerate discovery, development, and translation of antibodies and antibody–drug conjugates from animal models to the clinic is pharmacokinetic–pharmacodynamic (PK–PD) modelling.[153,154] These types of strategies integrate pre-clinical and clinical data, and therefore require judicious choice of the pre-clinical models employed. Thus, it is important to remember what these type of models can and cannot tell us.[155–158] A relevant quote from a recent commentary[155] reads: "the reality is that the value of a model depends on what the modeler is trying to accomplish. A good use of human tumor xenograft models would be to support an experimental hypothesis, a bad use would be to present animal data that add little to the value of *in vitro* data, and an ugly use of tumor xenografts would be to facilitate publication of a manuscript or give a false sense of safety or efficacy."

A key difference between human patient tumors and many of the studies that utilize tumors implanted in mice is their relative size. This introduces challenges in interpretation of data obtained using mouse models, as exemplified by this quote:[100] "The relative mass of a tumor (0.1–4.0 g) xenografted into and growing in a nude mouse (15–30 g) is in the range of 0.3–30%. By contrast, the relative mass of a tumor (2–10 g) in a patient (70 kg) is in the range of 0.003–0.01%." For the human patient, a tumor of

equivalent relative size (to the mouse model) would be the size of a basketball (**Figure 6**). While it is not impossible for tumors to reach this size, it does not represent the standard clinical situation. Larger, resectable (*i.e.*, can be operated on) tumors are surgically removed in patients so even if a tumor had grown to be large before diagnosis then it would typically be surgically removed or debulked (through surgery or other local therapies) as a first step in treatment.[21–24] By knowing these details, a simple mathematical model can be used to estimate the likelihood that a nanoparticle encounters the tumor during circulation.[142] In a xenograft mouse model, with a relatively large tumor (*e.g.*, 10% of body weight), the likelihood of nanoparticle–tumor encounter reaches 50% after only 6 seconds.[142] For a human tumor (*e.g.*, 0.005% of body weight), this takes over 10 days (Figure 6b).[142] These numbers do not account for factors such as more variable vascularity, higher interstitial pressure and the more pronounced hypoxia present in many human tumors, all factors that can further increase the differences observed between mouse models and patients. This further highlights some of the challenges of using mouse tumor models for predicting nanomaterial performance in the clinic.

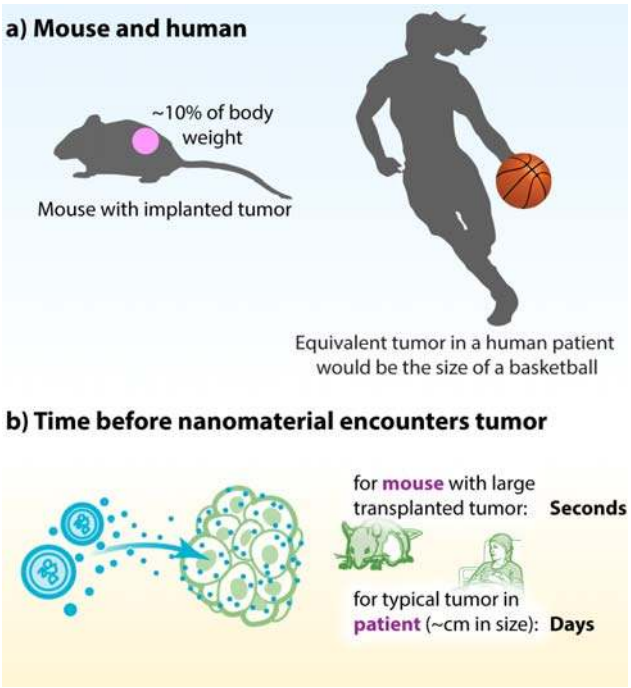

Figure 6. **a**) While rodent models are well-suited for investigating fundamental bio–nano interactions, it is important to remember that substantial differences exist between xenograft tumors and human tumors. In

addition to the difference in size, rodent models also typically have compromised immune systems. **b**) Based on these differences the typical time from administration to nanomaterial–tumor encounter can be estimated using a simple mathematical model.[142]

Despite the explosive growth of cancer nanomedicine, there are many publications today that follow a similar pattern: nanomaterial synthesis, *in vitro* cell culture, and *in vivo* mouse xenograft studies. This pattern is so common that it has been identified as an issue by researchers and journal editors,[159] as it can be difficult to appreciate exactly what the new knowledge and insights are for studies following this pattern. Suggestions to improve this situation include careful consideration of study design and models used. If comparative therapeutic benefit is being investigated, then "standard treatment" controls should be included and compared against (as is standard procedure in clinical studies). This can, for example, include comparing a new cancer nanomedicine against clinically-used liposomal formulations of cancer drugs. If fundamental bio–nano interactions are being investigated (*e.g.*, biodistribution, function under physiological conditions, interactions with tissues and organs), small animal models are often perfectly suitable. But when the main objective is development of new therapies to be translated into the clinic, then it is important to complement these studies with other approaches that can help bridge the "translational gap" (**Figure 7**). Examples for more mature nanomaterials include the emerging fields of "comparative oncology"[62,63] and "phase 0" trials.[160]

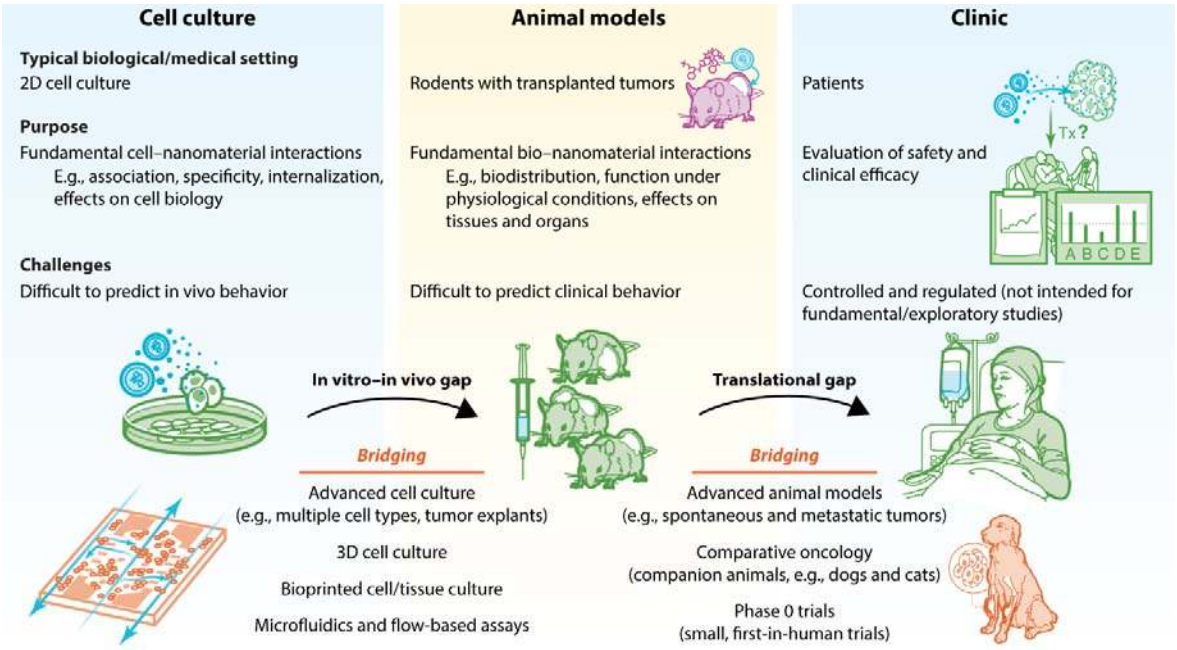

Figure 7. Overview of approaches for developing nanomaterials for cancer nanomedicine. The "*in vitro–in vivo* gap" is the fact that *in vitro* results can be difficult to translate to *in vivo* settings. The "translational gap" is the fact that strategies developed with the help of animal models can be difficult to translate to human patients. Example approaches that can facilitate the bridging of these gaps are listed. Specific examples of advanced animal models include syngeneic tumor models (*e.g.*, using immunocompetent mice bearing tumors derived from the same mouse strain), orthotopic tumor models (*e.g.*, colon cancer cells implanted into the colon— *i.e.*, the organ of origin—of mice, instead of subcutaneously), patient-derived tumor xenograft (PDX) models (*e.g.*, using cancerous tissue from patients, instead of cell lines, to establish tumors in mice), and transgenic models (*e.g.*, mice genetically engineered with cancer-causing 'oncogenes', that develop cancers spontaneously).

Developing the next-generation of cancer nanomedicines

There are several challenges and opportunities associated with the large-scale synthesis, characterization, evaluation, and commercialization of particles that are currently being explored.[161–163] An important part of these are recent advances in the development of new *in vitro* and *in vivo* assays (Figure 7). *In vitro*,

engineered 3D tumor models,[164,165] microfluidic-based assays,[166–168] the use of tumor spheroids,[169,170] and the culturing of tumor explants ex vivo are being pursued and have shown promising results.[171–174] Alternatives that are being explored *in vivo* include the use of animals and animal models in which tumors develop spontaneously; importantly, these animals typically have an intact immune system, in contrast to the immune-deficient mice commonly used in many xenograft models. Examples include mouse models of advanced spontaneous metastasis,[175,176] mouse models of tumor immunity,[177] and canine cancer patients.[62,63,178] This involves the emerging field of comparative oncology—the study of naturally occurring cancers in companion animals—which has shown promise as a translational development strategy.[62,63]

In parallel with these developments, opportunities exist for improving how these types of studies are conducted and reported. In a meta-analysis[179] of 74 quantitative, pre-clinical studies, 35 different cell types were used for the xenografts. How tumor accumulation was reported also varied widely, with many studies reporting only normalized accumulation (*e.g.*, %ID per cubic centimeter or %ID per gram of tumor tissue) but not the size or mass of the tissue, making comparisons difficult. An example highlighting this is a study using a mouse model where the accumulation of liposomes was found to depend on the size of the tumor.[180] For small tumors (≤0.1 g), the accumulation was observed to be around 15 %ID per gram of tumor tissue, whereas for larger tumors (≥1 g) the uptake was only 3 %ID per gram.[180] This study used the same type of tumor cell and the same type of liposome, but despite this, when comparing the normalized %ID per gram of tumor tissue, a 5-fold difference in accumulation was observed. For a study investigating the effect of tumor size on accumulation (as this was[180]), this is suitable. But consider if the study instead intended to compare different nanomaterials. If the comparisons are not performed appropriately (*e.g.*, by accounting for differences in tumor size) then the results would be dominated by external effects (in this case, the size of the tumor). Therefore, all of these parameters—both normalized value (*e.g.*, %ID per gram tumor tissue) and the measurement used to normalize (*e.g.*, tumor mass) need to be reported. To avoid these types of issues, standardization of reporting (and when possible, standardization of experimentation) is

vital.[13,181–184] To this end, guidelines and recommendations to facilitate comparison and benchmarking of preclinical studies of nanomedicines have recently been proposed.[185]

Much of the history of nanomaterials for biomedicine, and many of its researchers, have a background in the chemical sciences, and much of the emphasis has therefore been focused on the development of new and exciting nanomaterials (and not on the pathology and biology of the disease). There have been recent calls[186] for the field to adopt "industry-style frameworks" where strategies for the development of nanomedicines would be focused around the disease and the patient from the outset, instead of on the chemistry and material science: a "disease-first approach" instead of a "formulation-first approach".[159] While these frameworks are of interest for projects aiming to accelerate clinical translation of nanomedicine, there is also a broader ongoing discussion on the objective and purpose of cancer nanomedicine.

As the field of cancer nanomedicine matures—in parallel with advancements within oncology and cancer biology—the full complexity of the challenge before us has started to emerge. The response of the field towards this complexity can broadly be put into two categories. On the one hand, the seemingly ever-increasing complexity of cancer can be met with ever-increasing complexity in material design. An example of this is the recent proposal of a framework based around "nanoproperty integration and synchronization",[187] which is based around a so-called C-A-P-I-R cascade with 2-R-2-S-P requirements and 3-S transitions, many of which contain difficult balances and trade-offs between conflicting properties such as retention/release, stability/degradation and "stealthy-ness/stickiness". While it is an interesting attempt to combine many of the seemingly conflicting results of the field, it also represents a multi-dimensional optimization problem for which the solution (when it exists) is most likely different for different cancers, patient groups, and patients, and perhaps even for different tumors in the same patient (*e.g.*, primary or metastasis).[29–33] On the other hand, this increasing complexity has been met by calls towards simplicity and robustness,[188–190] for example by focusing translational nanomedicine development

around combining robust approaches in a stepwise manner. The concept of "minimal design" can be helpful in this pursuit.[191] Of course, there is rarely a single answer to multifaceted questions, so future research can benefit from pursuing several paths, while keeping the advantages and disadvantages of each in mind.

Using the next-generation of cancer nanomedicines

The best treatment option for most cancers is based around a combination of several interventions, including well-established options such as surgery, radiation therapy and chemotherapy,[21,22] often combined with biological therapy: which can include antibody therapy,[81–83] immunomodulation and T cell engineering.[192,193] Nanomedicine can facilitate these approaches, for example by: (i) guiding surgical removal of tumors,[194] (ii) enhancing radiotherapy,[195,196] (iii) co-delivering therapeutics to reduce the likelihood of multi-drug resistant cancers developing,[197–199] and (iv) by stimulating the immune system to induce or sustain anti-tumor responses.[200]

Complementing the approaches outlined above is the possibility of using cancer nanomedicine to stratify patients based on imaging and response.[201–203] Early work on the imaging of antibodies facilitated their clinical translation and regulatory approval[84,100] (as knowing where the material is and its ultimate fate facilitates development and regulatory decision making[204]) and molecular imaging is today an important tool in drug development and trial design.[205,206] Imaging techniques are also playing an increasingly important role for nanomedicine.[207,208] While it is important to remember the costs associated with adding imaging capabilities,[209] there are ways to reduce these costs. An interesting recent example is the use of "companion particles" with well-established imaging capabilities co-administered with the therapeutic particles.[210] Another example is using nanomaterials or drugs that are both inherently fluorescent and therapeutic (*e.g.*, cytotoxic), for example quantum dots[211] or doxorubicin.[212,213] Imaging in nanomedicine can help provide feedback on the treatment ("is it working?") as well as help with dose adjustments. This forms the rationale of the field of "theranostics".[214–216] There have also been recent advances in preparing nanoparticles that can be cleared rapidly and safely (*e.g.*, very small (<10 nm) nanoparticles), intended for

tumor therapy and imaging.[217,218] An example of this is a first-in-human clinical trial of inorganic nanoparticles for the imaging of cancer in patients with metastatic melanoma.[219] These types of rapidly clearing nanomaterials may facilitate imaging (similar to how small affinity proteins can facilitate imaging compared to larger antibodies[108,109]) and may also provide new avenues for drug delivery using carriers that are stable enough that the excess is excreted (*e.g.*, through kidneys and urine) before release of the drug, thus potentially minimizing off-target toxicity.

Working with—and not against—biology

We have recently proposed a framework centered around "convergent science" towards facilitating the development and translation of materials for biomedical applications that aims to integrate many of the ideas discussed herein.[13] Recently, an industry/pharma-focused perspective was also published centered around many of these translational challenges.[186] While there are differences between research focused on translational work and "blue-sky" exploratory work, there is also substantial overlap and lessons that can be learnt.[13,186] An overarching theme that is emerging in cancer nanomedicine is a shift away from working *against* biology (overcoming obstacles and barriers), towards working *with* biology (leveraging and taking advantage of physiology and disease pathologies).

"Working with biology" involves adjustments in both the methodology and objectives of cancer nanomedicine. For example, it has been shown that many drugs have difficulty penetrating more than a few cell diameters away from blood vessels and into extravascular tumor tissue.[220] Similar problems have been observed with both inorganic and organic particles using mouse models and flow-based *in vitro* assays.[221–225] One way to address this challenge is the use of "tumor-penetrating peptides" (**Figure 8a**). These peptides activate an endocytic transport pathway (the "CendR pathway") related to, but distinct from, macropinocytosis.[226] This pathway can transport compounds and nanoparticles both directly from cell to cell and "through" cells (*i.e.*, be taken up on one side and released on the other) in a process that is faster

than diffusion.[226] Tumor-penetrating peptides can either be conjugated to or co-administered with the nanomaterial.[227–229]

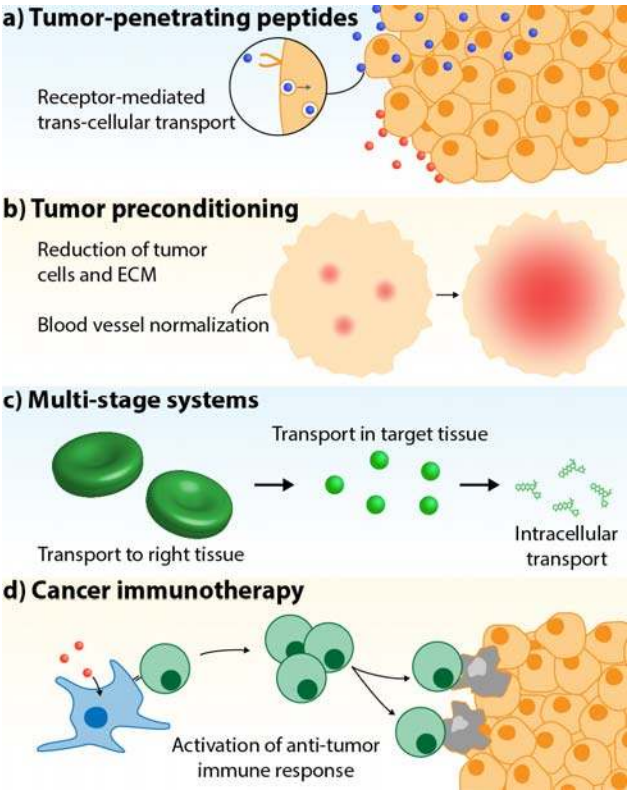

Figure 8. Examples of the concept of "working with biology". **a**) Using peptides (blue particles) to hitchhike on trans-cellular transport pathways. Red particles illustrate control particles without peptide that get stuck at the periphery. **b**) Adjusting the biological environment (*e.g.*, through blood vessel normalization) to facilitate anti-tumor drugs and immune cells to reach and distribute within the tumor. **c**) Multi-stage nanomaterial systems that change depending on the biological setting. For example, blood cell-mimicking microparticles with favorable circulation characteristics (transport to tissue) that break into nanoparticles with favorable tissue transport characteristics (transport within tissue) and release a drug that is easily taken up by cells (intracellular transport). **d**) Using nanomaterials to activate or sustain anti-tumor immune responses.

A different approach, focused instead on adapting the biological environment, is "tumor preconditioning" (**Figure 8b**). For example, alleviation of interstitial fluid pressure can be accomplished by reducing the number of tumor cells and the tumor-associated extracellular matrix, as well as through blood vessel normalization; these strategies have all shown promise for increasing nanomaterial penetration and retention (reviewed elsewhere[230]). These types of approaches are especially important for difficult-to-treat cancers, such as pancreatic cancer.[231] A related concept is to instead leverage features of the cancer-related microenvironment,[232] for example by targeting tumor-associated macrophages,[233–236] using neutrophils for cell-mediated delivery of liposomal anticancer drugs,[237] or using particles to assemble drug-depots in the tumor microenvironment.[238] In a recent study,[239] it was demonstrated that local tumor irradiation of tumor-bearing mice increased the accumulation of tumor-associated macrophages and enhanced "vascular bursting", which in some cases lead to a sixfold increase in nanoparticle accumulation in the tumor *via* a cascade of changes to the tumor vasculature and microenvironment.

Multi-stage systems form the basis for a strategy in which multiple components are combined and where each component is intended for different parts of the process, from administration to tumor treatment.[240] One way to achieve this is the "pre-targeting approach".[241] In tumor pre-targeting, the targeting component and the effector component are administered sequentially. For example, long-circulating tumor-targeting antibodies are first administered and sufficient time is allowed for unbound excess of antibodies to clear. Subsequently, a rapidly clearing effector component (*e.g.*, nanoparticle with an imaging component or drug) with specific, complementary functionality to the antibody can then be administered.[242] Rapid clearance (*e.g.*, through kidneys into urine) can decrease non-specific tissue accumulation and thus improve imaging and reduce toxicity.[241]

A different approach to multi-stage systems involve "particle generators" and "particle clusters".[243–245] In these types of systems, smaller components with favorable tumor penetration and distribution characteristics (*e.g.*, nanoparticles or molecules that easily diffuse), can be combined into larger structures

and assemblies with favorable vascular transport characteristics (*e.g.*, microparticles mimicking blood-circulating platelets) (**Figure 8c**).[243–245] These types of structures may also provide new ways to address the challenge of low drug-loading of nanomaterials, a common challenge for the applications of nanomaterials in chemotherapy.[246] Most of these approaches discussed above (Figure 8a-c) represent subtle shifts in concepts and reasoning where aspects of the biological setting are being leveraged to facilitate improved cancer nanomedicines, but the core principle of drug delivery of cytotoxic drugs to tumors remains largely the same. But there are also new concepts emerging that are fundamentally different to this principle, involving approaches for which particle-based systems may be uniquely suited, such as cancer immunotherapy (**Figure 8d**).

Cancer immunotherapy and nanomedicine

In the rapidly developing field of immuno-oncology, strategies for activating and stimulating the immune system to treat cancers are being explored. The core idea is to interact with the immune system in constructive ways to induce and sustain anti-tumor responses.

Cancer cells are different to normal cells in several fundamental ways.[34,35] These changes give rise to "tumor-specific antigens" that the immune system can use to distinguish cancer cells from non-cancer cells.[247] However, it has been shown that in some patients with progressive disease tumors and tumor-specific immune cells coexist.[248] This demonstrates that the induction of an immune response is insufficient to fully prevent disease progression; there are additional ways the tumor prevents effective attacks by the immune system.[248–250]

"Immune checkpoints" are inhibitory pathways that are a natural part of the immune system and whose purpose is to help distinguish between "self" and "foreign", to modulate the duration and amplitude of an ongoing immune response, and to ensure elimination of any threat with minimal damage to healthy cells and tissues.[251] In the last few years, there have been substantial clinical advances in the field of cancer

treatment using “immunotherapies” that target immune checkpoints and similar pathways, providing new opportunities for nanomedicine. For example, several high-profile clinical studies have recently been published demonstrating the strong clinical potential of strategies utilizing “immune checkpoint blockades” (reviewed elsewhere[252]) that use antibodies to block parts of these inhibitory pathways, thus enabling the immune system to attack previously resistant tumors. Although immuno-oncology (also known as cancer immunotherapy) approaches are being used clinically, and have led to improved patient outcomes, a proportion of patients respond poorly.[253–256] Therefore, it remains a challenge to constructively interact with and activate the immune system for a broader range of patients and types of cancers. This is a challenge that nanomedicines may be uniquely suited for, as many types of nanomaterials are inherently capable of interacting with (and enhancing the function of) key immune cells and organs.[257,258]

Nanomedicine systems are in the size range of viruses and bacteria (dimensions of nanometers to micrometers), and the human body has evolved intricate mechanisms to identify, inactivate, and remove or destroy foreign objects of this size.[259] Consequently, much of the work in cancer nanomedicine has been focused on “hiding” from the immune system (*e.g.*, to limit the induction of immune responses and to reduce the sequestration of nanomaterials) which, due to the effectiveness and robustness of the vast array of defense mechanisms that exists in the human body, has proven to be an uphill battle. Therefore, a shift away from “hiding from the immune system” towards instead enabling constructive interactions with it, represents a fundamental change in the rationale and design of nanomedicine systems (**Figure 9**).

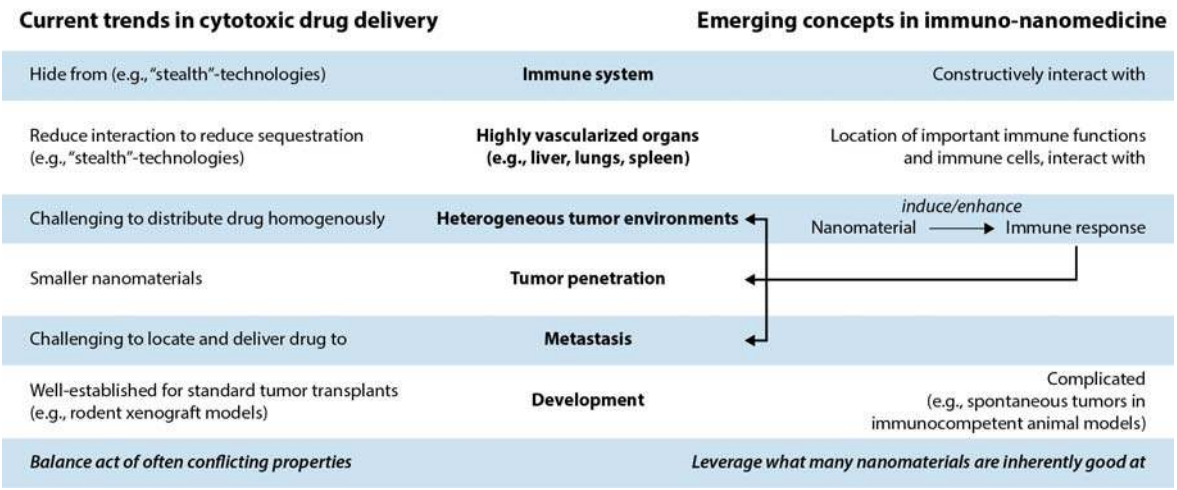

Figure 9. Comparison of the current paradigm of cytotoxic drug delivery to tumors with emerging trends in using nanomaterials for cancer immunotherapy. A key distinction is that the target is no longer killing tumors directly, but instead to interact constructively with the immune system.

We recently published a review article outlining immunological principles for the rational design of particles,[260] and review articles from the last few years outlining how particle systems can be used to combine nanomedicine with immuno-oncology also exist.[261–266] In the following we will highlight recent developments and insights in the emerging field of "immune-nanomedicine" for the treatment of cancer.

The core of combining nanomedicines with immuno-oncology is to leverage what many nanomaterials are intrinsically good at (*e.g.*, interacting with phagocytic cells and the reticuloendothelial or mononuclear phagocyte system[267]), instead of trying to engineer nanomaterials that can hide from the immune system. For example, both soft and hard nanomaterials have been shown to be capable of accumulating in lymph nodes and lymphoid tissues, and to strongly interact with different types of immune cells (*e.g.*, dendritic cells, B cells, and T cells) to induce controlled immune responses.[268–271]

Circulating nanomaterials tend to strongly interact with the liver and the spleen (*e.g.*, Kupffer cells, hepatic B cells, and splenic macrophages).[272,273] While much work has focused on reducing this very efficient sequestration of circulating nanomaterials, there is also potential to leverage this capability for inducing and modulating immune responses. For example, Kupffer cells form an important part of the innate immune response and have been shown to have important roles in the prevention of the formation of liver metastases, and could facilitate and enhance the sequestration of circulating tumor cells.[274,275]

With these notable features in mind, nanomaterial-based approaches have the potential to be transformative for activating and enhancing anti-tumor immune responses. Examples include the activation of pre-existing tumor-specific immune cells, or through the delivery of tumor-associated antigens to antigen-presenting cells to mount new immune responses.[276–279] One example of this is using antigen-capturing particles to improve the effect of radiotherapy administered with immunotherapy, through the "abscopal effect".[280] Another example is the recent translation into clinical trials of a lipid nanoparticle that targets dendritic cells to induce anti-tumor immune responses.[281] A related recent example is the use of nanoparticles for reprogramming circulating immune cells (*i.e.*, *in situ*) into having tumor-recognizing capabilities.[282]

More fundamentally, the immune system is in constant balance between immunosuppressive and immunostimulatory compounds and interactions, and nanomedicine-based approaches can help shift this balance towards enhanced anti-tumor responses while maintaining tolerance towards healthy tissues (**Figure 10**). A strategy for achieving this include the spatiotemporally controlled delivery of immunomodulatory compounds to lymphoid tissues and/or the tumor site.[283–288] Particles can also be attached to cells ex vivo and then administered as particle-cell constructs, which can improve the efficacy of vaccination,[289] and enhance the activity of administered T cells in "adoptive cell therapy" approaches.[290] Particles have also been functionalized with multiple antibodies for combinatorial immunotherapy, which showed increased effectiveness compared to administration of soluble antibodies by themselves.[291]

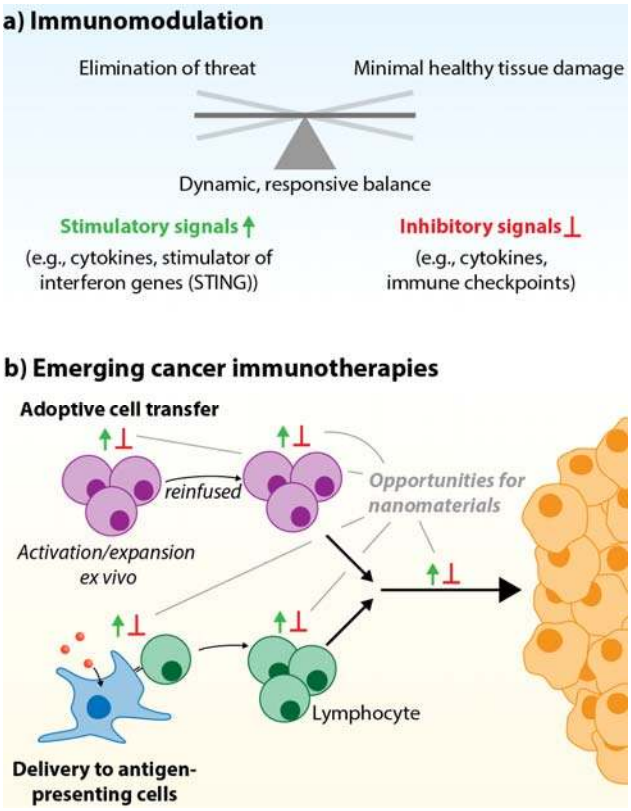

Figure 10. a) The immune system is in a constant balance between stimulatory and inhibitory signals that for healthy individuals assures that any threats (*e.g.*, infections) are eradicated with minimal side effects. In cancer, this balance is upset (*e.g.*, too strong inhibitory signals). **b**) Cancer immunotherapy is based around adjusting this balance to induce and sustain anti-tumor immune responses. Virtually all steps in immunological cascades have stimulatory and inhibitory signals acting on them, and drug delivery to specific immune cells and tissues to help control this balance pose opportunities for nanomaterials.

Despite these advances, the eradication of well-established tumors through the induction of immune responses remains difficult. A main reason for this is the complex and robust network of immunosuppressive pathways present in established tumors. The most promising results when treating complicated and advanced tumors have been achieved through the use of combination therapies that engage both innate and adaptive immune responses,[292] locally and systemically.[293] It is in this broader biological context that we believe cancer nanomedicine can have the greatest impact.

Outlook

The field of nanomedicine is currently undergoing substantial changes, reinventing itself as old ideas become obsolete and are replaced by new directions and emerging concepts.[294,295] At such a stage in the evolution of a field, it may be informative to look at neighboring fields for inspiration. A related area that has been part of cancer research for a longer time, so much so that it is today considered an integral part, is molecular biology. In a recent essay,[296] Weinberg (one of the authors of the highly influential "Hallmarks of Cancer" paper[34]) outlines lessons from more than half-a-century of molecular biology in relation to cancer, and the wild fluctuations (both up and down) in confidence and enthusiasm that the field has experienced as it has matured. Some of these descriptions are similar to the experience and ongoing debate within cancer nanomedicine. Weinberg describes the early excitement among molecular biologists entering into cancer research as an atmosphere of feeling like "knights on white horses" about to "save the day" for the oncologists and clinicians who had toiled for so many years with only limited success. Armed with the new tools and the emerging power of molecular biology, the complexity of cancer could (the idea was) be reduced to easy, universally applicable rules that could then lead to cures. But despite many years of explosive growth in molecular biology, this did not happen. Since the initial enthusiasm and flurry of activity decades ago, Weinberg argues that molecular biology has gone (and is still going) through cycles of viewing cancer from perspectives ranging from simplistic reductionism to intractable complexity, as the field has matured to the point where it is today, where it is contributing both impactful scientific and clinical advances.

As the field of cancer nanomedicine is maturing, it is important to keep these lessons in mind: while it is not helpful to consider cancer a problem of intractable complexity, it is also not helpful to go too far down the path of reductionism, to reduce the complexity of the broad collection of diseases collectively known as cancer (and the associated human biology and disease pathology), to a set of "barriers" to be "overcome". Thinking of nanomedicine as a set of tools for "conquering biology" is engaging in a battle

there is little chance of winning. Instead, we should consider the inherent properties of nanomedicine systems and work towards leveraging these. For example, if the vast majority of one type of intravenously injected nanoparticle gets sequestered in large, highly vascularized organs such as the liver and the lungs, can we use that to help arrest and sequester circulating (blood-borne) tumor cells or to prevent the formation of liver and lung metastasis, either directly or by inducing local mechanisms? And if another type of nanomaterial is instead subcutaneously injected and transported to lymph nodes where it strongly interacts with immune cells, can this be used for inducing anti-tumor immune responses? The key questions are: what are cancer nanomedicine systems good at, and how do we exploit these properties? How can we work with biology, and not against it?

Nanomaterials are having a growing impact on the on-going development of next generation therapeutics, and are indeed helping to redefine what we consider a medicine for cancer. By combining the concepts described herein with emerging broader themes such as the pursuit towards increasing robustness and convergence in science,[13,297] along with the many innovative approaches being explored in oncology, nanomedicine has the potential to be transformative for the treatment of cancer.

ASSOCIATED CONTENT

The authors declare no competing financial interest.

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VOCABULARY

Nanomedicine, leveraging the strengths of nanoscience and nanotechnology to achieve improved patient outcomes; **Comparative oncology**, the study of cancers that occurs naturally in animals (*e.g.*, companion animals such as pet dogs); **Syngeneic mouse tumor models**, using immunocompetent mice-bearing tumors derived from the same mouse strain; **Orthotopic mouse tumor models**, tumors established by implanting cancer cells into the organ of origin (*e.g.*, colon cancer cells implanted into the colon of mice instead of subcutaneously); **Patient-derived tumor xenograft (PDX) mouse models**, using cancerous tissue from patients (*e.g.*, instead of cell lines) to establish tumors in mice; **Transgenic tumor mouse models**, mice genetically engineered with cancer-causing "oncogenes" that develop cancers spontaneously.

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