

Opinion

Cancer Nanomedicine:
Lessons for Immuno-
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Cancer nanotechnology and cancer immunotherapy are two parallel themes that have emerged over the last few decades in the search for a cure for cancer. Exciting applications can emerge at the intersection of these two fields. However, it is important to learn from the past successes and failures of cancer nanomedicines for its future applications in cancer immunotherapy. This review discusses the two key parameters that defined clinical success in the case of cancer nanomedicines: (i) physicochemical design principles, and (ii) clinical trial design, which are frequently overlooked in most analyses of the state of the field. Learning from the design principles that defined success for the clinically-used cancer nanomedicines can enable the design of next-generation nanomedicines that can address some of the emerging challenges in cancer immunotherapy, for example (i) enabling combinations of molecularly targeted therapies with immunotherapies that are pharmacologically incompatible; (ii) early monitoring of efficacy of immunotherapies; and (iii) personalizing an immune response to a patient's tumor. Currently, only a subset of patients treated with immunotherapy exhibit durable response; the integration of nanomedicine and immunotherapy to address the above challenges can lead to new paradigms in the treatment of cancer.

Possibilities and Challenges with Cancer Nanomedicines

Cancer nanomedicine is one of the promising approaches that have emerged in the continuing search for a cure for cancer [1]. The possibility of engineering miniature robots that circulate in the body and selectively detect and kill cancer cells drives the excitement around cancer nanomedicine. This has led to the development of a diverse array of nanoparticles in terms of composition, for example, organic, inorganic, and organometallic [2–4]; physicochemical properties, such as size, charge, shape, or surface functionalization [3,5]; and functionality like imaging, diagnostic, therapeutic, or theranostics [6–8]. However, results from some recent clinical trials have been disappointing [9]. This has led to calls for introspection on the way forward for cancer nanomedicines [10]. This article examines whether the field of cancer nanomedicines has failed to deliver on its promise, and explores the lessons from successes and failures of the last decade that can impact the paradigm shifts that we are seeing in the treatment of cancer.

Postulated Barriers to Clinical Success

After a nanomedicine is injected into the body, it is subjected to various barriers that can limit the total quantity of nanomedicine that reaches the tumor, which in turn can impact the outcome. For example, macrophages present in different organs, such as liver, spleen, lungs, lymph nodes, and skin, can phagocytose and degrade the nanoparticles [10]. Phagocytosis is

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Cancer nanomedicines are currently facing challenges as a result of some recent clinical failures. However, a deeper analysis of the field reveals several nanomedicines that were approved in the past few years. There are several commonalities between the nanomedicines that succeeded in the clinics, including the physicochemical design of the nanomedicines as well as the design of the clinical trials.

Cancer nanomedicines preferentially home into tumors, and can result in more than threefold higher drug concentration in the tumor than can be achieved using conventional formulations. There is a trend towards optimizing the design such that more nanomedicines reach the tumor while minimizing systemic loss. A key area of research is to minimize phagocytic uptake by the reticuloendothelial system.

With the evolving immunotherapy-based approach to treat cancers, there is an early move in the nanomedicine field to apply the existing nanomedicine knowledge to immunotherapy. It is important to learn from failures in the past as nanotechnology is applied to immunotherapy to prevent repeating the same mistakes. Any studies applying nanotechnology in immuno-oncology need to be mechanistically based and not just observational.

Fundamental challenges facing immunotherapy, including early monitoring of efficacy, enabling combinations of immunotherapy and molecularly targeted therapeutics, or converting cold tumors to hot tumors are open topics that can be explored using nanotechnology. The integration of

enhanced if the nanoparticles are opsonized with serum proteins, undergo aggregation, are large sized, or possess a cationic surface charge [10,11]. To address the above challenges and escape macrophage uptake, nanoparticles (ideally less than 100 nm in diameter) are classically coated with polyethylene glycol (PEG), which reduces opsonization and acts as a 'stealth' cloak around the nanoparticles [12]. The PEG coating has been shown to confer an increased circulation lifetime to the nanoparticles, which correlates with enhanced accumulation in the tumor [13]. Optimization of PEG configuration can further increase tumor targeting and internalization of the nanoparticles into cancer cells [14]. The long-circulating nanoparticles extravasate into the tumor microenvironment through the highly abnormal tumor vasculature, known as the enhanced permeability and retention (EPR) effect [15]. Indeed, as compared with normal physiological vessels that prevent the extravasation of nanoparticles, tumor vasculature is characterized by a thin endothelial barrier that is devoid of pericyte support, disrupted basement membrane, and interendothelial gaps that are as large as 500 nm [10]. Theoretically, this distinction between normal and tumor vasculature confers the safety advantage with nanoparticles compared with Angstrom-sized small molecules that can leak out of physiological vasculature into normal tissue (Figure 1A–1 B).

Despite the above design parameter optimizations, a recent analysis of data from 117 reports over the last 10 years revealed that only 0.7% (median) of the injected nanomedicine dose reaches the tumor [10]. It is possible that a fraction of this dose is available to the cancer cells, with the rest being trapped in the stromal compartment. This provocative analysis attributed the limited success in the clinical translation of cancer nanomedicine to this small fraction of the administered drug reaching the desired site of action. Attempts have been made to enhance tumor targeting by integrating homing mechanisms, such as tumor-binding antibodies, aptamers, and peptides on the surface of the nanomedicines [15–17]. However, such approaches were shown to increase the intratumoral delivery to 0.9% of injected dose. Based on these observations, the authors proposed a 30-year strategy to achieve 10% of the injected dose reaching the tumor, starting with understanding the interactions of nanoparticles with tissue and organs at a fundamental level, exploring mechanisms of transport, and determining the relationships between biological systems and the physicochemical properties of the nanoparticles [10]. Naturally, this analysis of the state of cancer nanomedicine has seen a robust rebuttal from the nanomedicine community [18].

It is a fact that ~1% of the nanoparticles injected into the body reach the tumor, and there is indeed a need for a systematic approach towards addressing the barriers that limit the delivery of the nanoparticles into the tumor. Indeed, overcoming phagocytosis by macrophages can increase intratumoral delivery [19]. However, is it true that nanomedicine is all 'hype' that can cure cancer only in mice? Are Doxil and Abraxane the only two nanomedicines that have been successfully translated despite \$1 billion in investments in North America in the last 10 years [10]? In the following section, I shall examine whether there are any lessons from historical research into nanomedicines, especially from those that were successfully translated into approved products.

Lessons from Successfully Translated Cancer Nanomedicines

Doxil and Abraxane are two examples of drug delivery nanoparticles, a form of cancer nanomedicines; Doxil is a liposomal formulation of doxorubicin, and Abraxane is an albumin-bound nanoparticle of paclitaxel [3,20]. Beyond these, semiconductor quantum dots (QDs) are commercially available and are routinely used for imaging in cancer biology [21]. Nanoparticles have also been approved for human use in magnetic resonance imaging (MRI) applications as contrast enhancers [22]. Physically, nanoparticles are structures bound by the dimension range of 1–999 nanometers (although significant arbitrariness has been used by the community in defining nanomedicine in terms of dimension). Therefore, antibody drug conjugates (ADCs),

immunotherapy and cancer nanomedicine can emerge as the new paradigm in the treatment of cancer.

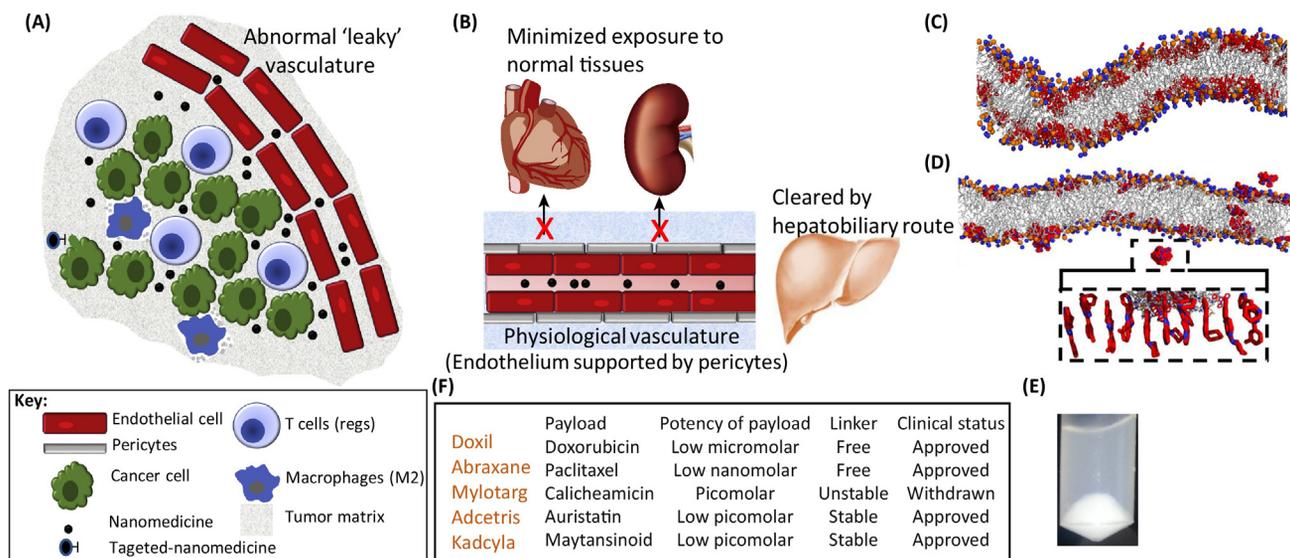
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Figure 1. Parameters That Can Impact Success of Cancer Nanomedicines. (A) Schematic shows that nanoparticles can passively home into tumors via the leaky tumor vasculature. Typically, 0.7% of the administered dose reaches the tumor. This can be increased to 0.9% by actively targeting the nanoparticles to epitopes expressed by tumor cells. (B) The vasculature supplying normal tissue prevents the leakage of nanoparticles. These blood vessels are composed of endothelial cells that are supported by pericytes. This difference in pathophysiology means that the therapeutic index of a drug can be increased using nanoparticles. However, nanoparticles are still phagocytosed by macrophages in the reticuloendothelial system, such as in the liver. Strategies to evade the macrophages can increase tumor delivery. Despite these barriers, actual drug delivered to the tumor using nanoparticles is twofold to fourfold greater than when delivered using classical formulations. A major cause of limited clinical translation is the inability to form stable nanoparticles at high mol% of drug. (C) An all-atomistic simulation of paclitaxel (red) in a lipid bilayer shows that it sits at the lipid–water interface, causing ripples and rifts in the bilayer that enable water to enter the bilayer, and conferring instability to the nanostructure. (D) A PI3K inhibitor (red) precipitates out of a lipid bilayer by forming π – π stacking (adapted from [35]). (E) Image shows a taxane precipitating out of a lipid-based nanoparticle with time. (F) Table shows unique properties of nanomedicines that were successfully translated, suggesting that these are critical parameters that can define success even if the total delivery to tumor is low.

which are ~ 10 nm in size [23], fall well within the definition of a nanomedicine. ADCs are structures, where a highly toxic payload is conjugated to an antibody that can home into specific antigens expressed by the cancer cells [24]. Indeed, ADCs such as T-DM1 (Kadcyla) and brentuximab vedotin (Adcetris) have been approved for clinical use, while the FDA approval of a third, gemtuzumab ozogamicin (Mylotarg) was withdrawn (although it was the first ADC to be launched) [24]. Our calculations based on the analysis of some of the published literature [25–27], which revealed that 2.5–5.5% of the total injected dose of the ADC reaches the tumor, although the median is likely to be 1–2% [28].

The barriers described earlier should have applied to these nanomedicines too. However, these nanomedicines were successfully translated. Interestingly, a key point that was lost in the debate over the $<1\%$ number, and the emphasis on increasing the delivery to the tumor, is that despite the low fraction of administered nanomedicines reaching the tumor, the concentration of drug achieved in the tumor with the nanomedicines is often more than twofold to fourfold the concentration that is achieved with traditional formulations of the same drug [4,29]. Clearly, there is more to successful translation than increasing the delivery to the tumor. An analysis of the nanomedicines that were successfully developed highlights a few cross-cutting themes: (i) Design principles: each building block needs to be fully characterized and easily scalable, should form stable nanoparticles, pharmacologically make sense, and pathophysiologically be suitable for use in humans. For example, it is unlikely that a graphene or a carbon nanotube (CNT) will ever translate clinically. In a study, we observed that they can accumulate in lungs, which means that long-term side effects remain a major concern [30]. However, we still see reports where CNTs are being studied as a drug delivery platform [31]. (ii) A ‘smart’ clinical trial

design. A nanomedicine is unlikely to be approved (nor would funding be available for development) solely based on the premise that it can reduce the side-effects of a currently used drug. Therefore, clinical trials need to be designed to showcase a clear efficacy advantage. Next, I shall elaborate on each of these points.

Doxil is a sterically stabilized 100 nm liposome formed using cholesterol, soy phosphatidylcholine and mPEG-diastearoylethanolamine with doxorubicin encapsulated in the aqueous compartment at a 12% drug to lipid ratio (wt/wt), while Abraxane comprises of paclitaxel complexed with albumin in the ratio of 1:9 wt:wt^{i,ii}. An earlier variant of Doxil, where the drug was entrapped in the lipid bilayer, failed in the clinic, primarily due to the drug being released rapidly in circulation [29]. Earlier generations of ADCs, where doxorubicin was conjugated as the cytotoxic agent to an antibody, also failed [32]. In contrast, successful ADCs are synthesized using highly toxic molecules with potency in the subnanomolar range such that even after they are conjugated to the antibody the construct is still highly effective. In the case of the ADCs too, the stability in circulation is paramount; the clinical failure of Mylotarg was attributed to an unstable linker that allowed premature release of the cytotoxic drug [24]. Taken together, these observations suggest that for clinical translation of nanomedicines, a low potency drug (nano- to micro-molar efficacy range) should ideally be entrapped and not conjugated. Furthermore, an ideal composition should have at least 10% wt/wt of drug, and need to be stable in circulation. Interestingly, in our studies, we were amazed by how unstable most nanoconstructs become beyond 5 mol% drug loading [33]. Using quantum mechanical-all atomistic simulations, we observed this can arise from completely different behaviors of drug molecules (Figure 1C–1 E) [33,34]. Unfortunately, few preclinical studies address this aspect; most studies exclusively focus on encapsulation efficiency, which can be misleading. For example, one can get high loading efficiency by starting with a low quantity of drug compared to excipients, but such a nanomedicine will never achieve therapeutic dosing. Similarly, it makes no sense to use nanoparticles that are stable only when freshly made but the drug rapidly precipitates over time. The challenges of low mol% loading vs stability can be addressed by anchoring the drug to the building blocks of the nanoparticle. However, in this case, the drug should ideally be highly potent (subnanomolar), as in the case of ADCs. Here, the choice of the linker chemistry also becomes important – not only for optimal release of the drug intratumorally but also to facilitate the nanoparticle assembly. Interestingly, it is now possible to use computational algorithms to optimize these parameters, and increase the mol% of the drug molecules in the nanoparticle [34].

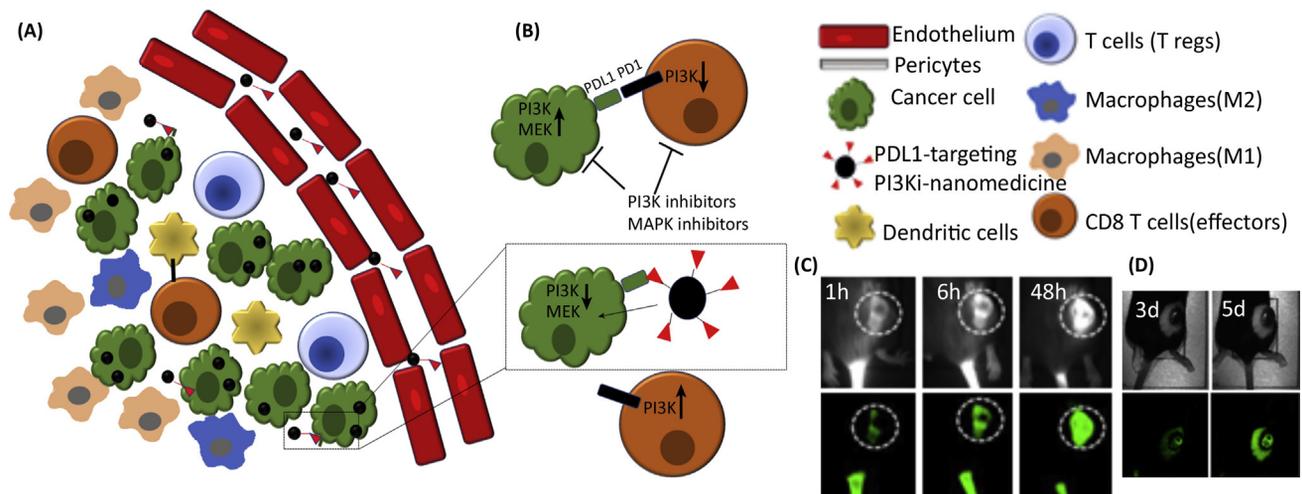
An analysis of pivotal clinical trials that led to the approvals for the above nanomedicines also throws up some common themes. For example, the first study with Doxil was an open label, single arm study with 77 patients of AIDS-related Kaposi sarcoma, using two endpoints for analysis of tumor response, (i) changes in lesion, and (ii) changes in up to five prospectively-identified representative indicator lesions. Its recent approval in multiple myeloma was based on an open-label study with 646 patients, previously not treated with bortezomib. The patients were randomized into two treatment arms: bortezomib alone, and a combination of Doxil and bortezomib. It is well known that doxorubicin is pharmacologically active in multiple myeloma [35]. Hence the combination of Doxil and bortezomib versus bortezomib alone in bortezomib-naïve patients, with time to progression as the primary endpoint for analysis, was a smart clinical trial design based on pharmacological evidence. Similarly, Abraxane was first approved in metastatic breast cancer, based on data from 106 patients accrued in two single arms, and a randomized comparative pivotal study of 460 patients. The single-arm, open-label studies tested Abraxane at 175 mg/m² and 300 mg/m² of paclitaxel equivalent, resulting in response rates of 39.5% and 47.5% respectively. The randomized study compared Abraxane (260 mg/m² paclitaxel equivalent) and a classical formulation of paclitaxel (175 mg/m²), with primary end point being a reconciled target lesion response rate. The single arm study had already established a dose-dependent efficacy for paclitaxel, and hence the design of comparing

Abraxane at a higher dose (260 mg/m² paclitaxel equivalent) with a classical formulation at a lower dose of paclitaxel (175 mg/m²) was smart. Interestingly, in many of these studies, no statistically significant difference in overall survival was evident at the end of the studies. Adcetris was approved for classical Hodgkin lymphoma, based on an open-label, single-arm trial of 102 patients with overall response rate and duration of response as a measure of efficacy evaluationⁱⁱⁱ. Taken together, these indicate that nanomedicines that succeeded in clinical translation took a de-risked approach of first being tested in niche indications, typically single arms, at higher doses than the parent drug, or in combinations that were rationally supported by pharmacology (based on evidence that the parent drug works in that indication), and used clinical end points such as target lesion response rate, objective response rates, duration of response and only rarely benchmarks such as progression free survival (PFS) or overall survival (OS). In contrast, CLRX101, a cyclodextrin-based camptothecin nanoparticle, was tested in a randomized study compared with the standard of care, with PFS and OS as the clinical end-points. Furthermore, BIND-014, a docetaxel-containing nanoparticle, was tested in the clinics at a dose that was surprisingly lower than the dose of docetaxel that is traditionally used in the clinics, although a clear dose-dependency was evident in preclinical studies [36]. Both nanomedicines had disappointing outcomes. Therefore, an optimally designed nanomedicine and a smart clinical trial design are critical features for successful clinical translation of nanomedicines (Figure 1F).

Current Nanomedicine-Based Approaches in Immuno-Oncology

Cancer immunotherapy is the emerging paradigm in the search for a cure for cancer [37,38]. Cancer cells have developed mechanisms by which they co-opt both innate and adaptive immune cells for tumor progression. For example, the tumor can polarize tumor-associated macrophages (TAMs) towards a protumorigenic M2 lineage and decrease the levels of inhibitory classical M1 macrophages. Similarly, cancer cells can activate immune checkpoints resulting in the anergy of T cells (Figure 2A). The emerging understanding of various mechanisms that underlie immunosuppression means that we can pharmacologically activate the immune response against cancer. For example, ipilimumab blocks the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) checkpoint in T cells leading to activation of T cells. Similarly, pembrolizumab inhibits PD-1 in T cells, blocking the inhibitory signaling induced by ligation with PD-L1, which is overexpressed by cancer cells. Multiple colony stimulating factor receptor 1 inhibitors are in currently clinical trials for their ability to polarize TAMs to an M1 phenotype.

The excitement around cancer immunotherapy is driving an explosion of research into the application of nanomedicine in immuno-oncology [39,40]. For example, polymeric (PLGA) nanoparticles or liposomes are being studied for delivery of adjuvants, siRNAs, antigens, drugs that activate the immune system, or a combination of such payloads [40,41]. Unfortunately, the trend has been towards design of highly complex nanostructures, for example, carbon nanotubes conjugated to antigens as well as polymeric nanoparticles that contain both magnetite as well as interleukin 2 are being studied to amplify cytotoxic T cells [42]. Newer immunogenic properties of nanomaterials are being discovered, for example, the polarization of TAMs to a M1 phenotype by iron oxide nanoparticles. However, these studies lack any mechanistic or pharmacological underpinnings [43]. Similarly, single-walled carbon nanotubes (SWCNT) were reported to be exclusively taken up by a single immune cell subset, Ly-6C^{hi} monocytes, and this remarkable selectivity of this tumor-targeting mechanism was proposed as an advanced immune-based delivery strategy for enhancing specific tumor delivery with substantial penetration [44]. Not factoring in the lessons learnt from previous failures could mean that we may be repeating the same mistakes that limited the translation of cancer nanomedicines in the first place, but now in the context of immuno-oncology. Nanotechnology can indeed impact immuno-oncology, but we need to first overcome the challenges of high drug loading as well as stability in circulation. We also need to be cognizant of materials that will realistically translate



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Figure 2. Application of Cancer Nanomedicines in Immunotherapy. (A) Schematic shows the tumor immune contexture. The cancer cells express ligands for immune checkpoints or secrete factors that lead to immunosuppression. For example, a predominance of regulatory T cells, or expression of PD-L1 by cancer cells can lead to effector T cell suppression or anergy. Similarly, cancer cells can secrete CSF1 that binds to CSF1R on monocytes and skew them towards an M2 lineage that supports tumor progression. Nanomedicines can be designed to home specifically to specific cells using these very same signatures. For example, a nanoparticle can be designed to home specifically to PD-L1 expressed on cancer cells and deploy a PI3K inhibitor in the cancer cells as well as inhibiting PD-L1-PD1 immune checkpoint. This can spare the PI3K activation in the T cells. (B) Nanomedicines can be used to enable combinations of immunotherapy and molecularly-targeted therapy. For example, cancer cells are often driven by oncogenes such as PI3K and MAPK, which makes them attractive target for PI3K or MEK inhibitors. However, these pathways are also required for activation of T cells. A small molecule inhibitor of PI3K could potentially inhibit both the cancer cell as well as the T cells even in the presence of a T cell activating immunotherapy. A combinatorial nanomedicine can be designed to home specifically to PD-L1 on cancer cells and deploy a PI3K inhibitor in the cancer cells as well as inhibiting PD-L1-PD1 immune checkpoint. This can spare the PI3K activation in the T cells. (C) Images show that high-efficiency supramolecular MEK-inhibiting nanoparticle can preferentially home into tumors (adapted from [35]). Such nanostructures are engineered from MEK-inhibiting molecular building blocks that are designed to self-assemble. The nanostructures were tagged with a near-infrared (NIR) dye that allowed real-time visualization in a tumor (adapted from [35]). (D) Nanoparticles can also be used for imaging immunotherapy efficacy. Images show a PD-L1-inhibiting activatable 'reporter' nanoparticle that lights up as the cancer cells undergo apoptosis in response to an immune response post PD-L1 inhibition (adapted from [8]).

into humans, and whether the design is conducive to being scaled. If a liposome-based siRNA delivery system did not work earlier, it is unlikely that it will work in the case of immuno-oncology in the clinics. Similarly, observations need to be underpinned by a pharmacological and mechanistic understanding. There is also a need to tone down on the hyperbole. For example, it is not clear why the observation that a SWCNT is carried by a specific subset of monocytes will improve upon efficacy of SWCNT given that this mechanism was already functional in previous studies with SWCNTs that never translated. Finally, we need to consider how a clinical trial will look as we design these structures to activate the immune system against the tumor.

The Future of Nanomedicine in a World of Cancer Immunotherapy

There is pressure on the field of cancer nanomedicine to deliver on clinical translation. However, the field also needs some grand challenges that can be addressed in the near term for its redemption. The following grand challenges exist in immuno-oncology.

Combination Therapy

Despite the promise of immunotherapy, only a fraction of the patients exhibit a long-term response to the currently approved immunotherapy agents. The emerging consensus is the use of combinations of immunotherapy with molecularly-targeted therapeutics for improving outcomes. Such combinations need to be rational, based on the compatibility of mechanisms for synergy [45]. For example, ample rationale exists to combine a BRAF inhibitor, vemurafenib and ipilimumab, both of which are approved for the treatment of advanced melanoma [46]. They have distinct mechanisms of action, and BRAF inhibitors can enhance immune-cell

function and antigen presentation [47]. Unfortunately, the clinical combination of vemurafenib and ipilimumab was associated with pronounced hepatotoxicity and hypersensitivity reactions, which put an early end to the trials [48]. Similarly, mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/mechanistic target of rapamycin (mTOR) signaling pathways are two of the most commonly mutated driver pathways in cancer, and thus targeted therapeutics that inhibit these pathways should form natural combinations with immune checkpoint inhibitors. The challenge is that the same pathways are critical for activation, proliferation, and cytotoxic activity of T cells [49]. For example, trametinib, a MEK inhibitor, reduced the proliferative T cell responses against cognate tumor antigens in tumor beds. MEK inhibition was also found to impair cross-presentation by dendritic cells, resulting in ineffective priming of CD8T cells [50,51]. Bupastisib, a PI3K inhibitor, was shown to inhibit tumor-specific T cells *in vivo* [52]. Therefore, while an immune checkpoint inhibitor can activate the T cells, the molecularly targeted inhibitors can negate that effect. However, there is a need to combine the two in tumors that are driven by these pathways (Figure 2B). The ability to modulate the pharmacological target at the desired site, for example only within the tumor, can be transformative for combination immunotherapy.

The above challenge can potentially be addressed by next-generation cancer nanomedicines that can shut down these molecular targets in the cancer cells while sparing the systemic immune system or systemic organs (Figure 2C). For example, cancer cells overexpress PD-L1, which can be targeted using antibodies. These antibodies can be used to deliver the molecularly targeted therapeutics, such as MEK or PI3K inhibitors, specifically to the cancer cells. However, given that most targeted therapeutics exhibit efficacy at a nanomolar range, based on previous lessons that we need the payload to exhibit efficacy at a subnanomolar concentration, it is unlikely that an ADC based on conjugation of a MEK or PI3K inhibitor to a PDL1-inhibiting antibody will be effective. Additionally, we have observed that a combination of nanomedicines can stochastically distribute to cancer cells, with some cells receiving only one of the nanomedicines [53]. Therefore, to truly enable a combination therapy, we need to design nanomedicines that combine a PD-L1 immune checkpoint inhibitor and a molecularly-targeted therapeutic within a single structure (Figure 2B). Such a nanomedicine needs to be stable, and the drugs should be freely available to therapeutically act on the target without any loss of potency. Additionally, both agents need to be present at >10 mol%, and the antibody needs to be displayed on the surface to be able to home onto PD-L1 proteins expressed on the cancer cell surface. Furthermore, the clinical study design needs creative thinking, which should also be captured in the preclinical studies. For example, an ideal design to test these next generation nanomedicines would be in cancers that overexpress PD-L1, known to be at least partially susceptible to an immune checkpoint inhibition, and are addicted to the molecular pathway. Some of these molecular targets can also exert an indirect activation of the immune system. For example, MEK inhibition reduces the mobilization of myeloid-derived suppressor cells (MDSCs) in KRAS-driven tumors. MDSCs suppress effector T cells, and thus MEK inhibitors can paradoxically, indirectly, activate T cells [49]. Hence the actual outcome is governed by the relative ratio of different immune players in the tumor immune contexture. Recruiting the right patients by matching the immune contexture may be more important than the presence of a single biomarker such as PD-L1 in designing the clinical trials. Therefore, enabling such combinations offer a ripe area for nanomedicines to evolve, leveraging existing knowledge, in the emerging world of cancer immunotherapy.

Monitoring Immunotherapy Response

Another challenge facing the field of immuno-oncology is the limitation of current imaging techniques to monitor the efficacy of immunotherapy agents. Immunotherapy treatments are associated with stromal alterations resulting in discordant metabolic flux and anatomical changes due to which current imaging techniques such as FDG/PET and MRI lack the

sensitivity or specificity to enable an early-response assessment. For example, a productive immune response (T-cell infiltration) and the unimpeded growth of the tumor will both be manifested as ‘pseudoprogression’ on the conventional RECIST criteria but the biological underpinnings are 180 degrees apart. Indeed, in preliminary studies, we have observed that FDG-PET or CT are of limited use in this setting [8]. In some of the tumors we noted a transient increase in FDG uptake, consistent with the ‘flare response’ resulting from an increased glucose uptake by inflammatory cells and/or energy demand of the apoptosis process. There is an unmet need to design novel imaging tools to visualize the actual response to immunotherapy. Activatable nanomaterials could be used to image immunotherapy activity in real time (Figure 2D) [8]. Learning from currently approved imaging platforms, and adapting a mechanistic understanding of the immunological effects to design activatable nanosystems, can emerge as a paradigm shift in cancer immunotherapy.

Personalizing Immunotherapy

The concept of vaccination and long-term memory in cancer immunotherapy is very attractive. As a result, a large body of work has emerged on the use of nanostructures to present tumor antigens to the adaptive immune system [54,55]. These have focused on both indirect T-cell activation, that is, via activation of antigen presenting cells, and on direct T cell activation using ‘artificial antigen presenting cells (APCs)’. For example, microspheres displaying both tumor-associated antigens-relevant MHC complexes and anti-CD28 antibody to ligate and activate the T cell costimulator CD28 resulted in enhanced antitumor efficacy when cotransfused with T cells specific for the antigen [43]. However, not all cancer cells may present the antigen. Furthermore, while nanoscale platforms can increase the avidity of the tumor-associated antigen-specific T-cell responses, generation of memory T-cell responses against multiple tumor-associated antigens (TAAs) may be required for long-term prevention of relapse. An exciting but simple approach could be to engineer nanoparticles that result in amplification of TAAs *in situ*, that is, enhance the probability of an immune response that is personalized to the tumor. However, such an approach is unlikely to be effective unless efficiently presented to cytotoxic T cells. Such nanomedicines therefore need to be used in combination with activators of APCs as well as immune checkpoint inhibitors.

Concluding Remarks

While we have discussed some specific examples in this article, the observations can be generalized to multiple drugs and oncogenic mechanisms, and can be harnessed to rejuvenate the nanomedicine pipeline. The explosion of immunological and molecular targets, the need to modulate the immune response specifically within the tumor while sparing the systemic immune functions, and the pharmacological and mechanistic necessity to target some of these in combination and the inherent conflicts pose exciting grand challenges for cancer therapy. Such grand challenges can be addressed by next-generation cancer nanomedicines. However, these next-generation nanomedicines need to be designed based on lessons from past successes and failures (see Outstanding Questions). The integration of cancer nanomedicines and cancer immunotherapy can emerge as the next paradigm in the search for a cure for cancer.

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Resources

ⁱhttps://www.accessdata.fda.gov/drugsatfda_docs/label/2007/050718s029lbl.pdf

ⁱⁱhttps://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021660s037lbl.pdf

ⁱⁱⁱhttps://www.accessdata.fda.gov/drugsatfda_docs/label/2014/125388_S056S078lbl.pdf

Outstanding Questions

What are the key design parameters for successful translation of cancer nanomedicines into the clinics? Are choosing the right active agent, selecting the right loading approach based on potency of the drug, and stability of the nanostructure in circulation enough to define success? While the field is moving towards more complex nanostructures, can they translate to clinics? Do we need to think of elegant and simple designs instead? What is an ideal clinical trial design for testing cancer nanomedicines?

Does cancer nanotechnology have a role to play in the rapidly evolving cancer therapy scenario? Is it possible to harness nanotechnology to specifically focus an immune response within the tumor?

Can bifunctional nanomedicines be designed to inhibit both oncogenic drivers as well as immune checkpoint ligands in cancer cells? Would such bifunctionality result in synergistic efficacy? What are the best targets for combination therapy using such bifunctional nanomedicines?

Can the ability of nanomedicines to exert a spatiotemporal effect on the target pathways result in unmasking of unique biological effects? Can nanomedicines be used to probe the tumor immune contexture beyond T cells?

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