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Mechanisms and Barriers in Cancer Nanomedicine: Addressing Challenges, Looking for Solutions

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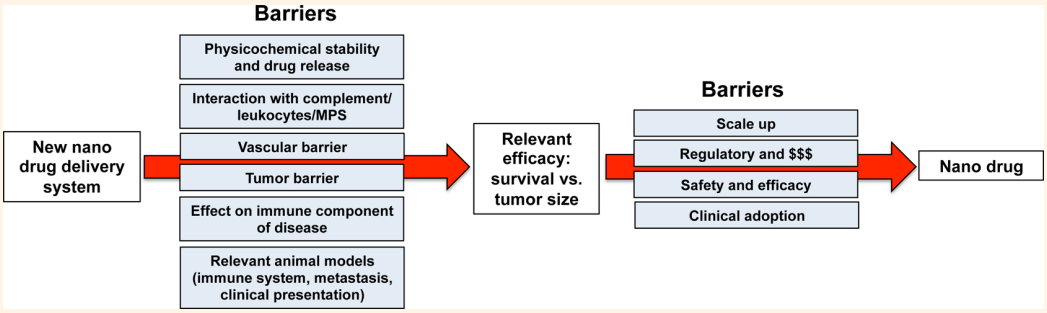

ABSTRACT: Remarkable progress has recently been made in the synthesis and characterization of engineered nanoparticles for imaging and treatment of cancers, resulting in several promising candidates in clinical trials. Despite these advances, clinical applications of nanoparticle-based therapeutic/imaging agents remain limited by biological, immunological, and translational barriers. In order to overcome the existing *status quo* in drug delivery, there is a need for open and frank discussion in the nanomedicine community on what is needed to make qualitative leaps toward translation. In this Nano Focus, we present the main discussion topics and conclusions from a recent workshop: "Mechanisms and Barriers in Nanomedicine". The focus of this informal meeting was on biological, toxicological, immunological, and translational aspects of nanomedicine and approaches to move the field forward productively. We believe that these topics reflect the most important issues in cancer nanomedicine.

In the last 25 years, there has been remarkable progress in the synthesis/fabrication and characterization of engineered nanoparticles for imaging and therapy of tumors. This activity resulted in several Food and Drug Administration (FDA)-approved nanodrug products being put on the market, mostly liposomes for intravenous administration,[1] and promising candidates in clinical trials. Despite the fact that FDA-approved nanodrugs succeeded in reducing life-threatening toxicities associated with the active pharmaceutical ingredients (APIs), the clinical use of nanodrugs has thus far resulted in limited improvement in the overall survival of patients.[2] Moreover, nanodrugs are subject to multiple interactions with the host immune system, resulting in premature clearance, immune system activation, and toxicity.[3] The clinical success of nanoparticles is limited due to (i) biobarriers en route to the affected loci, (ii) their fate at the disease site, and (iii) safety issues. In order to overcome the *status quo* in cancer drug delivery and to meet high expectations,[3−5] there is a need for open and frank discussion in the fundamental, translational, clinical, and regulatory aspects of nanomedicine.

In July 2016, a group of drug-delivery experts, clinicians, and industrial scientists convened in Breckenridge, Colorado, for a two-day "Mechanisms and Barriers in Nanomedicine" workshop. The main focus of this informal meeting was on biological, tox 117 icological, immunological, and translational barriers of nanomedicine (as shown in Figure 1). Instead of presenting only positive results and "progress reports", the speakers were encouraged to present a broad critical view and analyses of their perceived problems. Herein, we report the main points presented at the workshop and discuss approaches to move the field forward. Although it was impossible to cover all issues related to nanomedicine, the topics below are deemed critical for clinical translation at various stages of development.

IMMUNE SYSTEM: FRIEND OR FOE?

Interactions of nanocarriers with the immune system was a topic of intense discussions among the participants. Although some types of adaptive immunity may play a role in toxicities associated with nanomedicine administration (*e.g.*, anti-poly(ethylene glycol), PEG, antibody formation), nanoparticles mainly activate/affect innate immunity responses. Several factors involved in the nanoparticle−immune system relationship were discussed, including complement activation, association with circulating and organ-residing cells of mononuclear phagocyte system (MPS), and utilizing interactions with immune cells in the tumor microenvironment (see below).

Interaction of Nanocarriers with the Complement System. The complement system is a group of over 30 soluble

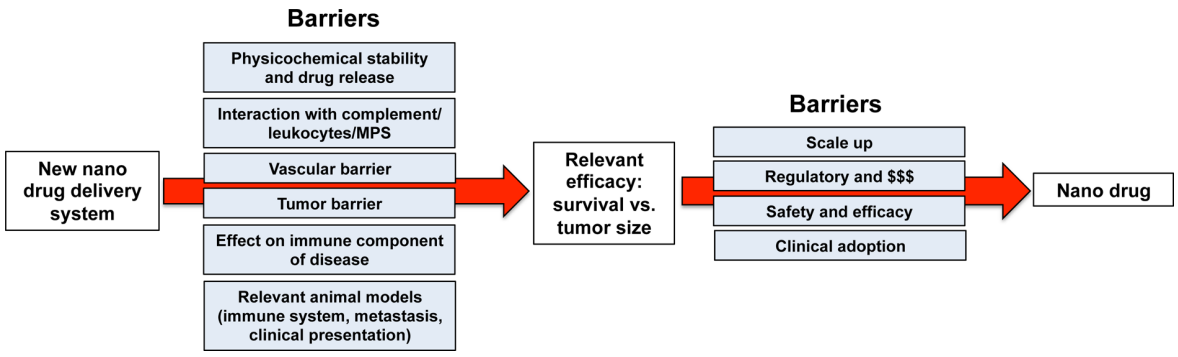

Figure 1. Perceived biological and translational barriers to nano-drug development.

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and membrane-bound proteins that function to neutralize invading pathogens, and it represents an important arm of innate immunity. The effect of complement activation by nanosurfaces is dual: uncontrolled liberation of highly pro-inflammatory mediators such as C3a, C4a, and C5a, also known as anaphylatoxins, may induce adverse reactions in sensitive individuals, whereas opsonization of nanoparticles with C3b/iC3b may lead to their uptake by phagocytic cells.[6] Although a complement activation-related pseudoallergy (CARPA) induced by nanocarriers is a topic of concern,[7] the opinions at the meeting were split on the need to ensure avoidance of complement activation by nanomedicines. While PEGylated liposomal doxorubicin (Doxil), the first approved anticancer nanodrug, induces significant complement activation *in vitro*, the occurrence of acute infusion reactions in patients in typically less than 10% and can be mitigated with premedication and by slowing the rate of infusion.[8] This difference points to the involvement of other integrative immune pathways regulating adverse responses, where complement activation may play a secondary role. However, other attendees stressed that products of the complement activation also induce strong pro-inflammatory responses that could nullify the therapeutic efficacy of the payload. Indeed, there is evidence that some nanocarriers actually promote tumor growth,[9] likely due to liberation of C5a and subsequent recruitment of pro-inflammatory macrophages and regulatory T-cells.[10] While some of these effects may be due to the presence of anti-PEG antibodies, which elicit complement activation in a proportion of patients, it is likely that there are other mechanisms that remain to be elucidated. Collectively, these data suggest that the tumor-promoting potential of the carrier may mitigate the benefits of carrier-mediated drug delivery and could partially explain why there has been insufficient improvement in the clinical efficacy of liposomal drugs over free drugs.[11] Arguably, more preclinical and clinical research is needed to understand the implications of complement activation on nanocarrier performance.

Clearance by Leukocytes and Mononuclear Phagocyte System. Peripheral blood leukocytes and tissue-resident macrophages efficiently take up foreign materials entering the body, with the aim of eliminating putative dangers as well as presenting the signal to mount the adaptive immune response. Some of this uptake is a consequence of complement opsonization in blood and tissues, but other mechanisms can play a role, as well. It is desirable to avoid premature clearance of nanocarriers/nanodrugs by the MPS, particularly in the liver and spleen, in order to enable the nanocarriers to reach other target tissues (*e.g.*, tumor), while also ensuring that nanocarriers are effectively eliminated in order to avoid safety and regulatory problems. Nanoparticle uptake by leukocytes could be beneficial since the leukocytes (*e.g.*, monocytes) could act as transporters that subsequently take nanoparticles into the tumor. Also, the interaction of nanocarriers with leukocytes can potentially trigger inflammatory reactions or alter the normal course of an immune response, events that may have an impact on tumor progression. As an example, the macrophage infiltrate in solid tumors is primed by the tumor microenvironment toward a "healing" phenotype (promoting angiogenesis and cell proliferation), whereas nanoparticles triggering an inflammatory reaction may divert the functional phenotype of macrophages toward an inflammatory "cytocidal" direction, thereby favoring tumor destruction. On the other hand, inflammation may contribute to tumor development in different circumstances, and in such cases, the effect of nanoparticles would be tumor promotion. There was agreement that the effects of nanomedicine on the function of immune cells in the context of the disease needs to be understood more fully.

TUMOR BARRIERS

It is generally agreed that the major obstacle in increasing the efficacy of anticancer treatment lies in poor tumor penetration of therapeutic nanoparticles as well as suboptimal drug release rates (discussed below). The physiological barriers include extracellular barriers, such as hemodynamics and margination in the capillaries, endothelial permeability, and intratumoral diffusion. Active targeting of nanoparticles to specific epitopes in tumors can enhance intracellular uptake; however, it does not dramatically increase the net amount of nanoparticles in the tumor and frequently limits penetration deep within the tumor.[4,12] Vascular targeting (angiogenesis receptors, trans-endothelial transport pathways) appears to be a more efficient process that can significantly enhance the dose of nanodrug that reaches the tumor.

Overcoming the Tumor Endothelium Barrier. Presenters strongly felt that the enhanced permeability and retention (EPR) effect[13] is the main mechanism of tumor penetration by nanocarriers and is a clinically relevant phenomenon. The data on vascular bursts in tumors[14] contribute to our understanding of the tumor vascular permeability to nanoparticles but, at the same time, highlights the heterogeneity of the EPR effect even within the same tumor. Other strategies to improve tumor vasculature penetration of compounds have been discussed. One proposed solution is to design supramolecular carriers that adhere to the tumor endothelium and release nanomedicine over time. At the same time, numerous studies with various nanodrugs demonstrate that even though passive targeting improves accumulation in tumors, this is not sufficient to provide a "quantum leap" in improvement of therapeutic efficacy. Among the main reasons are the effect of the dense extracellular matrix in suppressing convection flow and the high tumor interstitial fluid pressure. These effects reduce intratumoral distribution of the nanoparticles and associated APIs.

Enhancing Transport in the Tumor Stroma. Despite numerous attempts to engineer synthetic nanocarriers for tumor-specific drug delivery, systemically administered therapeutic compounds or nanoparticles remain predominantly in the perivascular region, reducing the overall therapeutic effect.

Several strategies to improve tumor penetration of compounds have been discussed. An approach to boost tumor permeability by tumor-penetrating peptides was presented. These peptides bind to a primary receptor specific for tumor blood vessels, tumor cells, and stroma, then undergo proteolytic cleavage and shift their affinity from the primary receptor to neuropilin-1. Binding to neuropilin-1 subsequently activates an endocytic/exocytic trans-tissue transport pathway, leading to a transient increase in tumor penetration of nanoparticles and small-molecule drugs.[15] This mechanism works especially well in starved tumors due to dependence of the transport process on metabolism.

It was pointed out that, unlike intravascular targets, extravascular/tumor receptors are not easily accessible to nanomedicines, and the concentration of these target receptors is typically in the picomolar range. Thus, strategies should be developed to open the tumor endothelial barrier. Several other novel approaches were presented that could enhance tumor penetration. Changing the tumor microenvironment by radio frequency (RF) or by high-intensity focused ultrasound (HIFU) can effectively remodel the tumor microenvironment while concomitantly enhancing nanodrug accumulation, thereby

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leading to significantly improved therapeutic efficacy.[16] However, these methods are obviously insufficient when treatment of disseminated disease is contemplated. Extracellular vesicles, a natural transport system, can be hijacked to mediate intercellular migration of exogenous hydrophobic compounds through multiple cell layers, both *in vitro* and *in vivo*. This approach would significantly improve the therapeutic efficacy of hydrophobic compounds in poorly vascularized tumors.[17] Another strategy presented was to use the natural tropism of cell-derived vesicles to tumors. Thus, ghosts of stem cells (nanoghosts) associate with tumor cells through cellular uptake (endocytosis pathways) and *via* cellular binding (adsorption, lipid exchange, fusion). These interactions enable targeting of anticancer therapeutics to different compartments of the cancer cell, significantly enhancing delivery of payloads to tumors.[18]

RELEVANCE OF TUMOR TARGETS USED IN NANOMEDICINE

Besides tumor vasculature and tumor cells, the relevant nanomedicine targets include tumor stroma, immune cells, and disseminated metastatic cells.

Targeting Tumor-Associated Macrophages. Macrophages represent a prominent cell population in the microenvironment of many tumors because they are recruited to the tumor as an immune reaction to a damage event. Targeting tumor-associated macrophages (TAMs) cells could be a major advantage, as macrophage infiltrate is abundant and is a hallmark of aggressive cancers. For instance, although blood flow is significantly impaired in some tumor lesions in the liver, the number of macrophages in the tumor proximity is frequently increased. This disparity can be used as a strategy to localize therapeutics and imaging agents within the tumor. Consequently, using solid particles, transport of albumin-bound nanotherapeutics can be significantly shifted toward the tumor-associated macrophages, increasing the therapeutic efficacy and enabling survival benefits.[19]

Data were presented showing that the inclusion of a macrophage-depleting drug (alendronate) in doxorubicin liposomes greatly enhanced therapeutic effects in immunocompetent mice by targeting TAMs.[20] In terms of imaging markers, using clinically available superparamagnetic iron oxide nanoparticles (SPIONs) ferumoxytol, recent studies have demonstrated that iron uptake is proportional to the numbers of TAMs. Tumor-associated macrophage uptake of ferumoxytol was noninvasively assessed by T_2-weighted magnetic resonance imaging (T2w-MRI) and was used as a surrogate measurement to predict penetration efficacy of delivery systems[21] and to provide additional diagnostic parameters for clinical decision-making as well as for selecting patients for nanoparticle-based therapy. Interestingly, recent research demonstrated that accumulation of SPIO in TAMs promotes a phenotypic switch from the M2 (anti-inflammatory) to the M1 (inflammatory) functional phenotype and promotes an antitumor effect.[14] However, due to the complex relationship between the tumor and the immune system,[22] not every phagocytic cell is a relevant target. For example, dendritic cells in the tumor may promote antitumor immunity by delivering tumor antigens to the draining lymph nodes.[23] As nanomedicine is relatively nonselective toward immune cells, it was suggested that more research should be dedicated to the effect of nanomedicine on immune response and immunotherapy in relevant mouse models.

Targeting Metastases. Metastatic tumor cells present a great challenge because they are sequestered next to the blood vessels in the bone marrow and other organs[24] and often become refractory and resistant to therapy. The ability of nanomedicine to cross that barrier and either to enhance the dormancy or to destroy the dormant cells is key to achieving an antimetastatic effect. There was an opinion that unleashing the immune system may be an optimal way to target tumor metastases, and the delivery of specific immunostimulatory signals to the immune cells may be a promising approach. In that regard, the strategy for targeted delivery of payload to a variety of circulating lymphocytes (*e.g.*, cytotoxic and regulatory T-lymphocytes, chimeric antigen receptor T-lymphocytes, natural killer cells, neutrophils, and monocytes) in order to boost antimetastatic response/immunity, possibly as an adjuvant to immunotherapy, could be an exciting perspective for nanotherapy. At the same time, the science of delivery into the heterogeneous population of blood cells remains a challenge. Seemingly, systemic drug delivery to leukocytes should be less challenging in comparison to solid tumors, as there are fewer barriers to cross. However, due to the fact that leukocytes are notoriously hard to transfect and are spread not only in peripheral blood but also in the bone marrow, lymph nodes, lymphatic organs, and other organs, there is an unmet need for developing designated delivery strategies to leukocytes.[25]

NANOCARRIER ASPECTS OF TUMOR TARGETING

Cancer nanomedicine dogma states that nanoparticles that exhibit sufficiently long circulation times are able to accumulate passively within malignant tissues *via* the EPR effect. However, a recent literature survey conducted on a large body of work published over the past 10 years shows that, in many cases, less than 1% of the administered nanoparticle dose reaches the malignant tissue.[26] The opinions were split as to whether 1% is enough to cause a therapeutic effect. In small tumors, 1% of the total injected dose may achieve high enough concentration. Moreover, many felt that the pertinent issue is not only how much of the dose reaches the tumor but also how much active drug gets into the tumor cells. Nevertheless, for effective treatment of tumors *via* nanoparticles, accumulation at the targeted tissue and the extent of release of the API need to be improved.

Engineering Nanoparticles with Improved Vascular Transport and Tumor Deposition. The size, shape, surface properties, and mechanical stiffness (abbreviated as "4S parameters") of nanoparticles are critical for vascular transport, MPS elimination, blood longevity, and tumor deposition. Specifically, nonspherical nanoparticles are known to resist MPS sequestration more efficiently and to exhibit a tropism for the tortuous and moderately perfused malignant microvasculature.[27] Modulating the mechanical stiffness of nanoparticles is also gaining increasing interest. Importantly, soft nanoconstructs manifest longer circulation half-lives and lower accumulation in the liver, lungs, and spleen as compared to their rigid counterparts.[28,29]

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This growing evidence strongly suggests that researchers should challenge the conventional dogma of cancer nanomedicine by engineering nanoparticles with 4S-parameter combinations

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specifically tailored to the disease type, stage, and biological signature.

Payload Design. Most nanodrugs are supramolecular assemblies, and their physicochemical features largely determine pharmacokinetics/pharmacodynamics and interaction with the biological milieu. Therefore, in-depth physicochemical characterization is a prerequisite for their successful application. This characterization should include an accurate way to determine size distribution, morphology, high-resolution structure, thermotropic behavior of the carrier, and its encapsulated/associated active API. However, there should be a balance between drug retention *versus* drug release at the target site. For any API, loading efficiency and release should be measured rigorously. The ability of the drug to remain associated with the delivery vehicle *in vivo* is an underappreciated problem that contributes to the limited gain in therapeutic performance and inability to improve the therapeutic index (which is particularly important for inherently toxic anticancer agents) that is frequently seen and reported for clinically tested liposomes. Thus, stealth liposomal cisplatin failed in human clinical trials in spite of good tumor accumulation because of lack of release of drug in tumors and other tissues.[30] In the case of remote-loaded doxorubicin liposomes, the encapsulated doxorubicin may be released from the liposomes by the high concentration of ammonia due to tumor-specific glutaminolysis.[31] For other liposomal nanodrugs, application of physical stimuli such as HIFU or RF can be used to assist in drug release from the carrier (especially in the case of thermosensitive liposomes).[32] However, there is still insufficient understanding about the optimal rate of drug release in the tumor and how this rate is affected by tumor type, anatomy, intratumoral pressure, and microenvironment.

Conversely, a growing number of studies point to rapid loss of the drug cargo with different types of nanocarrier-based formulations, often immediately after their systemic administration.[32] Re-engineering drug molecules into reversibly hydrophobized constructs is a promising strategy to improve nanocarrier-drug association. An example was provided whereby a prodrug conjugate of a vitamin E derivative and a camptothecin analogue SN-38 was stably retained in sub-100 nm biodegradable polymeric nanoparticles for sustained local presence of the pharmacologically active drug, resulting in extended survival in animal neuroblastoma models.[33] Another example is a lipophilic prodrug of mitomycin C delivered by liposomes that is currently in clinical testing.[34]

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TRANSLATIONAL CHALLENGES

Efficacy and safety are two important criteria of every new drug. A considerable amount of discussion was dedicated to delineating the preclinical findings that are considered necessary and sufficient for a nanodrug in order to embark on more costly and time-consuming clinical development. It is without doubt that the efficacy and safety of nanodrugs need to be compared to standard-of-care and to demonstrate superiority in animal models. Moreover, several aspects of preclinical models can seriously affect the conclusions about the efficacy and safety profiles of nanodrugs.

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Preclinical Efficacy. The issue of testing drug-delivery systems in artificial xenograft models with leaky vasculature *versus* more realistic orthotopic or genetic mouse models, which may more closely resemble the tumor biology in patients, has been discussed previously.[5] One important limitation of mouse models, as presented at the meeting, is that while primary tumors are enriched with blood neovasculature, metastatic lesions have insufficient blood flow or nonfunctional blood vessels.[35] These transport variables affect the efficacy of therapeutics, which critically depend on perfusion for transport to the target site. As metastatic disease is the primary cause of death in cancer patients, preclinical testing should include metastatic models with measurement of survival (or quantifiable tumor burden) as the primary outcome. In addition, genetically engineered and allograft models might be a better option than immunodeficient animal models to account for the complex interplay between tumor immunologic milieu and nanomedicine efficacy.

Preclinical Safety/Toxicity. The main biological impurity affecting over 30% of preclinical grade nanomaterials is bacterial endotoxin.[36,37] According to the experience of the National Cancer Institute's Nanotechnology Characterization Laboratory, this number has not changed over the past 10 years. Endotoxin is a potent immunostimulant that elicits a cytokine storm that can confound the results of toxicity and efficacy studies. The selection of animal models for use in preclinical tests can have a great impact on the preclinical toxicology of nanoformulations. It is important to consider the differences in sensitivity and specificity of the given animal model to the given nanoformulation. Some conventional preclinical models (rats and nonhuman primates) may be relatively insensitive to endotoxin and to developing a cytokine storm.[38] In such cases, supplementing *in vivo* studies with *in vitro* assays utilizing human blood should be considered. Another consideration for selecting an animal model is related to the variable sensitivity of animal strains to a particular type of immunotoxicity. For example, rabbits are more sensitive to cytokine and complement-mediated toxicities than other rodents commonly used in preclinical studies. Among rodents, strains may differ in their selectivity to nanoparticle clearance. For example, BALB/c and C57BL/6 mice commonly used in preclinical studies demonstrate a different pattern of nanoparticle uptake due to their Th2 and Th1 bias, respectively.[39]

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Transitioning to Clinical Trials. Virtually all nanomaterials with the potential for healthcare use have been developed in research laboratory settings in which the batch size is relatively small. Successful commercialization requires significant scale-up of production and, ultimately, a good manufacturing practice (GMP) process. There appears to be consensus among researchers that transitioning transformative approaches from the lab to the clinic requires improved infrastructure for the clinical development of nanomaterials, such as access to GMP

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facilities. Nanotherapeutics face additional challenges and, arguably, a higher bar than more conventional devices, small molecules, or biologic therapies. In large part, these differences are due to the uncertain and evolving regulatory environment surrounding nanotherapeutics and the lack of established standards for many aspects of their manufacture and regulatory path. An example was presented of the regulatory challenge facing the commercialization and development of gold-nanoparticle-induced hyperthermia for the treatment of melanoma[40] and whether it should be evaluated as a medical device or a drug–device combination. Regardless, a full preclinical safety toxicology and absorption/distribution/metabolism/excretion (ADME) package had to be obtained in both a rodent species and a larger animal model, identified in collaboration with the FDA. There was an interesting discussion as to whether phase 0 (zero) clinical trials can speed up development of nanomedicines. Phase 0 trials are trials where an agent is administered in micro doses to test whether it behaves similarly to that observed in preclinical models in terms of pharmacokinetics and, if feasible, biodistribution. Such trials can help obtain valuable initial information before spending effort and money on costly phase I trials. Some industry scientists argued that phase 0 trials do not provide conclusive data on toxicology and efficacy, and many companies are likely to proceed directly to phase I. At the same time, phase 0 may be appropriate to university investigator-initiated trials.

A significant commercial challenge involves the current "standard-of-care" and physician behavior around the standard-of-care. New therapies positioned to replace or to augment a standard-of-care must demonstrate safety and efficacy, patient acceptance, and a workable reimbursement scenario, but—critically—new therapies must also work for providers and physicians in terms of their acceptance/compliance.

Future Perspective. The EPR effect and improved safety profiles *via* altered pharmacokinetics are the cornerstones of clinically approved nanomedicines. Three basic science aspects of nanomedicines need to be understood better: (1) interaction of nanoparticles with the immune system and how to manipulate that interaction for the benefit of the host; (2) relevance of the EPR effect in human cancer, particularly in metastatic lesions, and the role it plays in the performance of nanopharmaceuticals; (3) how much of the drug is released in the tumor and reaches tumor cells in its active form. The future of nanomedicine is likely to include a combined approach using imaging to assess tumor barriers and immunological makeup together with nanomedicines that are able to deploy spatiotemporal targeting strategies and to overcome biological barriers of the tumor microenvironment. More advanced "theranostic" nanoformulations could perform both imaging and therapeutic functions at the same time. However, the design of next-generation "smart" and dynamic materials must balance complexity with added therapeutic value and safety in order to enable reliable production and clinical adoption, as opposed to forcing applications of emerging nanomaterials regardless of the above considerations. We strongly believe that openly discussing these issues instead of refusing to acknowledge the associated difficulties is one of the characteristics of self-correcting science that propels the field forward.

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Notes

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