# Design Principles for Clinical Efficacy of Cancer Nanomedicine: A Look into the Basics

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**ABSTRACT** With recent advances in cancer nanomedicine, there is an increasing expectation for clinical translation. However, what are the parameters of a nanomedicine that will define clinical success, which will be measured by increased efficacy and not just ease of delivery or reduction in toxicity? In this Perspective, we build on a fundamental study by Stefanick *et al.* on the significance of the design principles in the engineering of a nanomedicine, such as peptide-PEG-linker length and ligand density in cellular uptake of liposomal nanoparticles. We address additional design parameters that can potentially facilitate clinical translation as well as how emerging insights into tumor biology will inspire next-generation cancer nanomedicines.


Recent commentaries have posed critical questions regarding the future of nanomedicine, including "Is the wave cresting?"[1] or "Why so many papers and so few drugs?"[2] These commentaries have cautioned against "overselling" the benefits of this technology,[3] and that the loss of enthusiasm for nanomedicine could in part be due to the growing understanding of the inherent constraints.[1] If one looks at the evolution of antibody–drug conjugates (ADCs), a field that is analogous to nanomedicine, early work in the 1980s translated into the first approved drug in 2000.[4] It is important to keep in perspective that it has only been a decade since the nanomedicine revolution began, and a decade is a short period of time for translation to the clinics. It is therefore especially critical now to keep the momentum up as we enable the evolution of cancer nanomedicines toward the clinics.

**In this issue of *ACS Nano*, Stefanick *et al.* go back to the basics to establish design principles for liposomal nanovectors.**

To enable success in the clinic, it is also important to understand the factors that pose challenges. Early nanomedicines that reached the clinic, such as Doxil, were designed to ameliorate the side effects of the payload, for example, the cardiotoxicity of doxorubicin.[5] Similarly, Abraxane, the albumin-bound taxane, can overcome the challenges associated with administering hydrophobic taxol in vehicles that induced hypersensitivity reactions.[6] However, although reduced adverse effects can improve quality of life, this alone might not be enough of a driver today for clinical translation, especially in the context of increasingly stringent economic criteria being applied for healthcare reimbursements. It is clear that the benchmark for clinical translation will be increased efficacy, which will require approaches that are more developed than the delivery of entrapped cytotoxic agents in a nanovector. This requires a central re-examination of the design of nanomedicines, including challenging current dogmas. In this issue of *ACS Nano*, Stefanick *et al.* go back to the basics to establish design principles for liposomal nanovectors.[7]

Nanomedicines, like ADCs, offer the exciting potential of homing to the tumor with limited exposure to normal tissues. However, unlike ADCs, which home to specific epitopes on cancer cells, nanomedicines

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exhibit a more dynamic form of tumor targeting, where increased circulation time, the enhanced permeability of tumor vasculature as compared to normal tissues, and impaired tumor drainage contribute to increased intratumoral concentration of nanoparticles as compared to free drugs.[8] An established dogma is that coating a nanoparticle with polyethylene glycol (PEG) prevents binding of plasma proteins (opsonization), which reduces clearance by the reticuloendothelial system and results in increased circulation time. Interestingly, most studies have focused on longer chain PEGs, with the incorporation of a 5 mol % of DSPE-PEG2000 (average ~45 repeating units of ethylene glycol) emerging as the clinical and research gold standard more as a general consensus rather than through scientific logic.[9] However, results reported by Stefanick *et al.* indicate that this may not be true, and that shorter chain lengths could be just as effective. Indeed, this is consistent with previous reports that liposomes with shorter PEGs can exhibit similar circulation half-lives. However, the issue remains that PEGylation can potentially hinder internalization into cells, and this consideration has inspired elegant designs of smart nanoparticles that address this challenge. Interestingly, an equally large volume of reports show cellular internalization of PEGylated nanoparticles resulting in efficacy (Figure 1). If one applies the "efficacy test" to this conflict, the question that comes up is, what concentration is enough? Would an incremental increase in the amount of drug entering the cell confer a significant difference in clinical outcome?

How does one meet the efficacy test? A potential strategy could be by harnessing active targeting, where the nanoparticles are coated with a "homing" beacon (*e.g.*, peptides, antibodies, aptamers) that can facilitate enhanced delivery to the tumor. However, the results have been inconsistent, and the advantage over passive targeting is still debated. This could

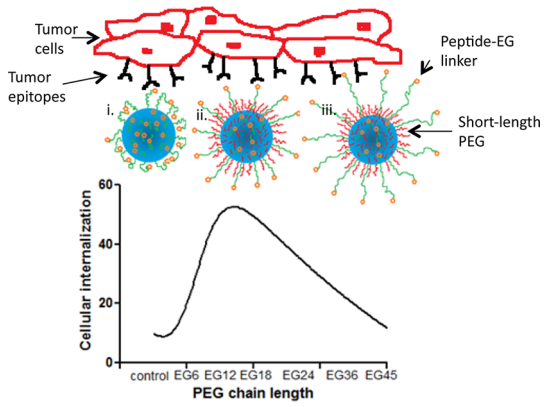


**Figure 1. Cartoon and graph show the effect of peptide-linker length on cellular internalization. The current dogma prefers the use of (i) PEG2000, which can assume a mushroom configuration and lower densities, and the targeting moiety can be buried in the PEG coat, resulting in suboptimal binding and internalization. However, (ii) PEGs (12 or 18 repeats) resulted in maximal internalization when supramolecularly assembled with lower chain PEGs, indicating optimal display of the ligand. (iii) At higher PEG-linker lengths, this internalization is reduced, suggesting hindrance in interacting with the cell or the epitope. Adapted from ref 7. Copyright 2013 American Chemical Society.**

have risen from early *ad hoc* approaches in the design of such nanostructures, where the homing molecule was conjugated to the PEG chains of nanoparticles that were optimized for passive targeting. It is only recently that the need for design principles has been recognized. For example, in a recent study, Perry *et al.* used PRINT nanoparticles to demonstrate that a PEG brush surface resulted in a 200-fold and 1.5-fold decrease in clearance *versus* a nonPEGylated and a PEG mushroom surface, respectively (mushroom conformation is defined by low density, <4 mol % of DSPE-PEG2000, while brush mode is defined by >8 mol % of PEGylation).[10] Interestingly, Stefanik *et al.* demonstrate that the tethering of a HER2- or a VLA-4-targeting peptide to PEG2000 did not increase cellular internalization as compared to a nontargeted nanoparticle, possibly because the mushroom conformation could bury a large fraction of the targeting ligand into the PEG coating and sterically hinder binding to the epitope. Indeed, there seems to be a biphasic response, where a peptide-PEG550 (or EG12, *i.e.*, 12 repeating units of ethylene glycol) and EG18 enhanced internalization by almost 9-fold as compared to the control, while linkers EG6, or EG greater than 24, had lower internalization (completely diminished by EG45 and EG72). Furthermore, they demonstrate that the peptide density also influences internalization, plateauing out at 2%, which could be a reflection of peptide valency.

> **Any strategy that improves tumor penetration can potentially result in enhanced clinical efficacy of nanomedicines over existing therapeutics.**

Although the above strategy can significantly increase the quantum of intratumoral drug concentration, it may not necessarily translate into enhanced efficacy. For example, increased delivery of cisplatin using a liposomal vehicle did not result in increased clinical efficacy because the drug failed to release efficiently.[11] In contrast, low sustained concentration of taxanes, also called metronomic dosing, has been shown to improve antitumor efficacy,[12] indicating that low sustained intratumoral concentrations might be clinically effective. This indicates that understanding the

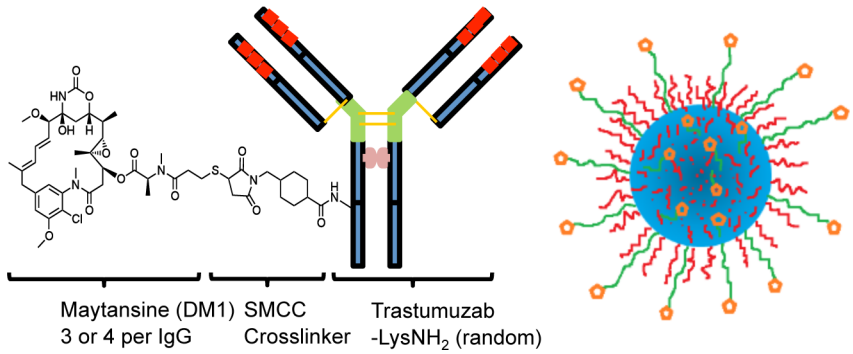


**Figure 2. Design parameter analogy between ADCs and cancer nanomedicines that can facilitate clinical translation. The ADC shown is T-DM1, the conjugate of trastuzumab with mertansine and a typical nanoparticle. The table shows overlapping similarities between ADCs and nanomedicines.**

**TABLE 1. Analogy between ADC Platform and Cancer Nanomedicine**

| parameters | ADC | nanomedicines |
|---|---|---|
| tumor targeting | active (Ab should bind to epitope after conjugation with drug)(differential/increased expression of epitope in tumor *vs* normal tissue a must) | passive (*via* EPR effect) (tumor vasculature is distinct from normal tissue); active (targeting moiety can be displayed on surface similar to ADC; multivalency can allow the use of lower affinity binders) (linker length and density needs to be optimized) |
| drug | highly potent (sub nM $IC_{50}$); validated mechanisms | adaptable to highly potent agents; also possible to use SAR in design; validated mechanisms |
| linker | stable in plasma, labile in tumor | stable in plasma, labile in tumor; entrapment only if therapeutic dose levels can be reached |

drug mechanisms, whether efficacy is driven by maximum concentration ($C_{max}$) or area under curve (AUC) of the drug concentration time profile (and hence whether loading efficiency and release kinetics from a nanoparticle enable the desired therapeutic thresholds), will be a critical factor for the design of nanoparticles to facilitate clinical translation. However, a key barrier that exists for successful chemotherapeutic outcome is the limited intratumoral penetration of active agents. Indeed, in a recent study, the Ruoslahti group demonstrated that iRGD peptides can facilitate delivery of compounds and nanoparticles deep into tumor parenchyma. These peptides home to tumors in a three-step process: the RGD motif binds to $\alpha_v$ integrins, and a proteolytic cleavage then exposes a binding motif for neuropilin-1, which mediates penetration into tissues and cells.[13] Similarly, another less explored but interesting observation comes from a pathophysiological observation where platelets were found to be implicated in the maintenance of tumor vessel integrity, and the injection of a platelet-depleting antibody with paclitaxel exhibited greater antitumor outcome, resulting from increased intratumoral drug access, a strategy that needs further exploration in the case of nanoparticles.[14] A futuristic strategy might encompass the integration of nanoscale motors that use distinct propulsion mechanisms such as catalytic, magnetic, or ultrasound to drive tumor penetration.[15] Any strategy that improves tumor penetration can potentially result in enhanced clinical efficacy of nanomedicines over existing therapeutics.

A significant learning experience in the design of clinically effective nanomedicines can come from studying the evolution of ADCs (Figure 2 and Table 1. Both platforms meet the requirement of tumor homing and internalization. However, whereas nanomedicine has focused on the delivery of existing therapeutics, ADCs were built around highly potent cytotoxic agents. For example, calicheamycin, maytansinoids, and auristatins exhibit $IC_{50}$ values in the range of $10^{-9}$ to $10^{-11}$ M.[16] In comparison, doxorubicin, which has been widely explored for fabrication of nanoparticles, typically exhibits an $IC_{50}$ in the high nanomolar range. The advantage of using such potent cytotoxins together with the knowledge of permissive sites that allow conjugation to linkers is that the cleaved metabolites can retain activity that translates into significant efficacy.[16] Indeed, the linker chemistry is critical, with properties that confer stability in plasma (to avoid premature release of the drug) but lability once internalized to release the drug in an active form. From a technical viewpoint, this knowledge could be transferred to the design of nanomedicines. For example, the selection of camptothecin in the design of a cyclodextrin–camptothecin nanoparticle resulted in enhanced therapeutic efficacy as compared with the current gold standard drug with the same mechanism of action, the topoisomerase I inhibitor irinotecan, because the former is a significantly more potent therapeutic agent than the latter.[17] Interestingly, the potential use of multivalent backbones in the design of nanoparticles can confer additional advantages over ADCs, and significantly higher number of cytotoxic molecules can be loaded. Indeed, understanding the structure–activity

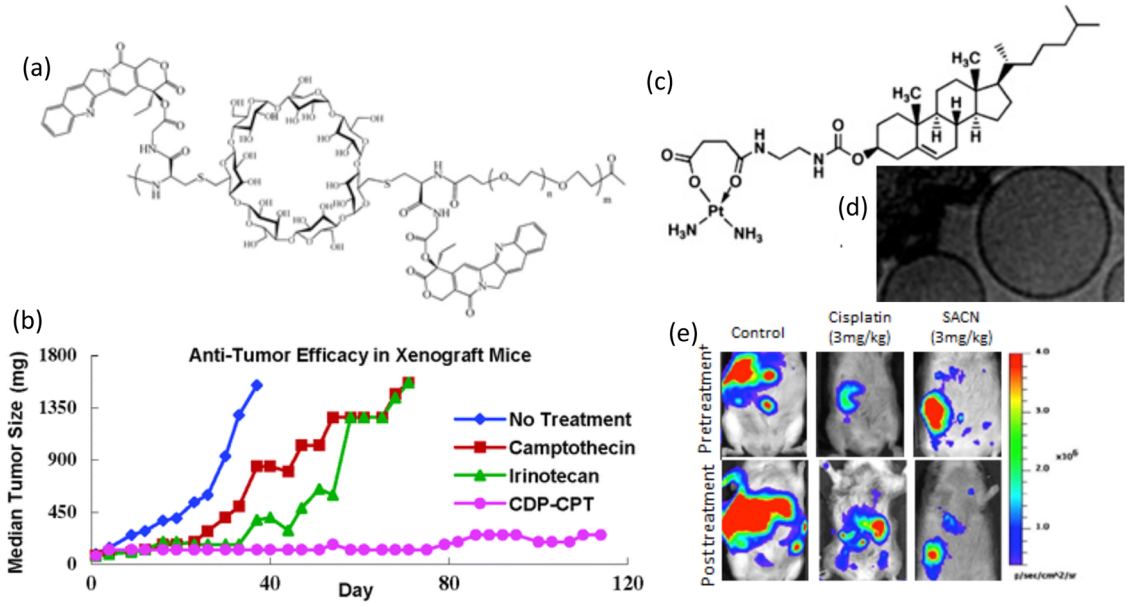


**Figure 3. Selecting the right drug is critical for efficacy. (a) Cyclodextrin−camptothecin conjugate nanoparticle that results in (b) greater antitumor efficacy than irinotecan, a clinically approved drug in the same topoisomerase I inhibitor class. Adapted with permission from ref 17. Copyright 2004 American Society for Pharmacology and Experimental Therapeutic. (c) Structure−activity studies can also be harnessed to increase efficacy, as in the case of a cisplatin/carboplatin analogue, where the leaving group is designed to aquate more efficiently than the dicarboxylato linkage in carboplatin. (d) Analogue facilitates supramolecular nanoassembly (SACNs) as seen using cryo-TEM, and (e) results in greater antitumor efficacy in an RAS/Pten ovarian cancer model. Adapted with permission from ref 18. Copyright 2012 National Academy of Sciences.**

relationships of the cytotoxins will be needed for the design of effective nanomedicines as is the case of ADCs. For example, in a recent study, the modification of the leaving group chemistry of Pt(II) enabled the design of carboplatin analogues that were significantly more potent than the parent molecule and furthermore facilitated supramolecular assembly into nanostructures that could bypass renal clearance and hence exhibit reduced nephrotoxicity (Figure 3).[18] Stefanick *et al.* used a similar strategy in the design of their nanoparticles, where they employed a multifaceted synthetic strategy of synthesizing the lipid-targeting ligand conjugate that was then mixed at the desired ratio during liposome synthesis. This enabled the precisely controlled stoichiometric loading of the targeting ligands as compared with the currently used post-insertion methods and also minimized batch-to-batch variability.

## CONCLUSIONS AND OUTLOOK

In conclusion, it is becoming increasingly evident that increased efficacy will be the benchmark, with quality of life as only a secondary end point. Hence, for successful clinical translation, nanomedicines will need to move beyond drug delivery of difficult-to-administer drugs or addressing side effects of existing drugs to the incorporation of design principles to confer significant increases in potency or activity. Rather than reinventing the wheel, some of this knowledge can be drawn from analogy with the more advanced ADC platform. However, will an approach that leads to more effective therapeutics lead to a cure? It is unlikely that such a milestone will ever be reached with a nanomedicine monotherapy. It is becoming increasingly clear that cancer is a complex disease, and understanding this complexity will be critical to developing next-generation nanotherapeutics that will be significantly more effective. For example, there is increasing realization of the heterogeneity that exists within a single tumor.[19] Indeed, subsets of cells can exhibit nonmutational tolerance to cytotoxic chemotherapy resulting in relapse. Increasing understanding of mechanisms underlying such "adaptive resistance" indicates that the future of cancer management will rely on precisely tailored combination therapy. In addition, it is also becoming evident that the right temporal sequencing of combination drugs will be critical for optimal efficacy.[20] It is likely that a nanomedicine that integrates design principles, is mechanistically inspired, and enables a combinatorial therapeutic approach that addresses heterogeneity will enable cures for cancers.

*Conflict of Interest:* The authors declare the following competing financial interest(s): S.S. is a cofounder at Cerulean Pharmaceuticals and Invictus Oncology, holds equity and serves on the board of these companies.

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