Published in final edited form as:

ACS Nano. 2013 April 23; 7(4): . doi:10.1021/nn4015399.

Design principles for clinical efficacy of cancer nanomedicine: a look into the basics

Shiladitya Sengupta* and **Ashish Kulkarni**

Laboratory of Nanomedicine, Division of Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard-MIT Division of Health Science Technology, Harvard Medical School; Dana Farber Cancer Center, Boston, MA, USA

Abstract

With the 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? This perspective builds on a fundamental study by Stefanick et al on the significance of the right design principles in the engineering of a nanomedicine, such as peptide-PEG-linker length and ligand density in cellular uptake of liposomal nanoparticles. The perspective addresses additional design parameters that can potentially facilitate clinical translation as well as how emerging insights into tumor biology will inspire the next generation cancer nanomedicines.

SUMMARY: As we rapidly race cancer nanomedicines towards the clinics, what are the fundamental design parameters that will influence outcome? What can we learn from antibody-drug conjugates that will facilitate nanomedicines passing the 'efficacy test'? This perspective addresses some of these questions.

Some commentaries in the recent times 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 technology³, and that the loss of enthusiasm for nanomedicine could in part be due to the growing understanding of the inherent constraints¹. If one compares with the evolution of antibody-drug conjugates (ADCs), an analogous field, the early work in the 1980's translated into the first approved drug in 2000⁴. It is important to keep in perspective that it has only been a decade since the nanomedicine revolution really took off, and a decade is a very 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 towards the clinics.

To enable success in the clinics, it is also important to understand the factors that pose challenges. Early nanomedicines such as Doxil that reached the clinics were designed to ameliorate the side effects of the payload, for example the cardiotoxicity of doxorubicin⁵. Similarly, Abraxane, the albumin-bound taxane, can overcome the challenges associated with administering hydrophobic taxol in vehicles that induced hypersensitivity reactions⁶. However, while 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 more developed than delivery of entrapped cytotoxic agents in a nanovector. This requires a

*Corresponding author: Dr. Shiladitya Sengupta, 65 Landsdowne street, Room 317, Cambridge, MA 02139, USA. shiladit@mit.edu.

fundamental re-look at the design of nanomedicines including challenging the dogmas. In this issue of ACS Nano, Stefanick et al go back to the basics to establish design principles for liposomal nanovectors⁷.

Nanomedicines, like ADCs, offer the exciting potential of homing to the tumor with limited exposure to normal tissues. However, unlike the ADCs, which home to specific epitopes on cancer cells, nanomedicines exhibit a more dynamic form of tumor targeting, where increased circulation time, the enhanced permeability of tumor vasculature as compared to normal tissues, and the impaired tumor drainage contribute to increased intratumoral concentration of the nanoparticles as compared with free drugs⁸. An established dogma is that coating of a nanoparticle with polyethylene glycol (PEG) prevents binding of plasma proteins (opsonization), which reduces clearance by the reticuloendothelial system resulting in increased circulation time. Interestingly, most studies 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 logic⁹. However, results reported by Stefanick et al indicate that this may not be true, and that shorter chain lengths could be just as good. Indeed this is consistent with previous reports that liposomes with shorter PEGs can exhibit similar circulation half-life. However, an issue remains that PEGylation can potentially hinder internalization into the cells, and this 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. If one applies the 'efficacy test' to this conflict, the question that comes up is, how much concentration is enough? Would an incremental increase in amount of drug into 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 (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 a topic for debate. This could 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 is being 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 non-pegylated and a PEG mushroom surface respectively (mushroom conformation is defined by low density, <4 mol% DSPE-PEG2000, while brush mode is defined by >8 mol% PEGylation)¹⁰. Interestingly, Stefanik et al demonstrate that the tethering of a HER2- or a VLA-4-targeting peptide to a PEG2000 did not increase cellular internalization as compared with a non-targeted 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 repeats of ethylene glycol) and EG18 enhanced internalization by almost 9 fold as compared with control, while linkers EG6 or greater than 24 had lower internalization (completely diminishes 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 (Fig.1).

While 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 as the drug failed to release efficiently¹¹. In contrast, low sustained concentration of taxanes, also called metronomic dosing, has been shown to improve antitumor efficacy¹²,

indicating low sustained intratumoral concentrations might be clinically effective. This indicates that understanding the drug mechanisms, whether efficacy is driven by C_{max} or AUC (and hence whether loading efficiency and release kinetics from a nanoparticle enable the desired therapeutic thresholds), will be a critical factor for 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, Ruoslahti group have 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 α_v integrins, and a proteolytic cleavage then exposes a binding motif for neuropilin-1, which mediates penetration into tissues and cells¹³. Similarly, another less explored but interesting observation comes from a pathophysiological observation where the 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 nanoparticles¹⁴. A futuristic strategy might encompass the integration of nanoscale motors that use distinct propulsion mechanisms such as catalytic, magnetic or ultrasound to drive tumor penetration¹⁵. Clearly any strategy that improves on the tumor penetration metric can potentially result in enhanced clinical efficacy of nanomedicines outcome over existing therapeutics.

A significant learning experience in the design of clinically effective nanomedicines can come from studying the evolution of ADCs. Both platforms can meet the requirement of tumor homing and internalization. However, while nanomedicine has focused on the delivery of existing therapeutics, ADCs were built around highly potent cytotoxic agents. For example, calicheamycin, maytansinoids and auristatins exhibit IC₅₀ values in the range of 10⁻⁹M to 10⁻¹¹M¹⁶. In comparison, doxorubicin, which has been widely explored for fabrication of nanoparticles, typically exhibits an IC₅₀ in the high nanomolar range. The advantage of using such potent cytotoxics together with the knowledge of permissive sites that allow conjugation to linkers is that the cleaved metabolites can retain activity that translates into significant efficacy¹⁶. Indeed, the linker chemistry is crucial, with properties that confer stability in plasma (to avoid premature release of the drug) but is labile once internalized to release the drug in an active form. From a technical viewpoint, this knowledge could be transferred to the design of nanomedicines (Fig.2). 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, as the former is a significantly more potent therapeutic agent than the latter¹⁷ (Fig.3). 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, the understanding of the structure activity relationships of the cytotoxics will need to come in to the design of nanomedicines as in 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 analogs that were significantly more potent than the parent molecule and furthermore facilitated the supramolecular assembly into nanostructures that could bypass renal clearance and hence exhibit reduced nephrotoxicity¹⁸ (Fig.3). Interestingly, Stefanick et al use 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 allowed 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.

In conclusion, it is increasingly becoming evident that increased efficacy will be the benchmark with quality of life as only a secondary end point. Hence for a 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 right design principles that confer significant increase in potency or activity. Indeed, rather than reinventing the wheel, some of this knowledge can be drawn from analogy with the more advanced ADC platform. However, will such 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 increasingly becoming clear that cancer is a complex disease, and understanding this complexity will be critical to developing the next generation nanotherapeutics that are significantly more effective. For example, here is increasing realization of the heterogeneity that exists within a single tumor¹⁹. Indeed, subsets of cells can exhibit non-mutational 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. Additionally, it is also becoming evident that the right temporal sequencing of combination drugs will be critical for optimal efficacy²⁰. It is likely that a nanomedicine that integrates the right design principles, is mechanistically inspired, and enables a combinatorial approach that addresses heterogeneity will possess the maximal scope to enable a cure for cancer.

Acknowledgments

S.S is supported by a DoD BCRP Era of Hope Scholar Award (W81XWH-07-1-0482), DoD BCRP Collaborative Innovator Grant (W81XWH-09-1-0700), a NIH RO1 (1R01CA135242-01A2), and a Joint Center Grant from IUSSTF. S.S. is a cofounder at Cerulean Pharmaceuticals and Invictus Oncology, holds equity and serves on the board of these companies.

REFERENCES

1. Juliano R. Nanomedicine: is the wave cresting? *Nat Rev Drug Discov.* 2013; 12:171–172. [PubMed: 23449291]
2. Venditto VJ, Szoka FC Jr. Cancer nanomedicines: so many papers and so few drugs! *Adv Drug Deliv Rev.* 2013 Jan; 65(1):80–88. [PubMed: 23036224]
3. Duncan R, Gaspar R. Nanomedicine(s) under the microscope. *Mol Pharm.* 2011 Dec 5; 8(6):2101–2141. [PubMed: 21974749]
4. Teicher BA, Chari RV. Antibody conjugate therapeutics: challenges and potential. *Clin Cancer Res.* 2011; 17:6389–6397. [PubMed: 22003066]
5. Lasic DD, Papahadjopoulos D. Liposomes revisited. *Science.* 1995; 267:1275–1276. [PubMed: 7871422]
6. Hawkins MJ, Soon-Shiong P, Desai N. Protein nanoparticles as drug carriers in clinical medicine. *Adv Drug Deliv Rev.* 2008; 60:876–885. [PubMed: 18423779]
7. Stefanick JF, Ashley JD, Kiziltepe T, Bilgicer B. A systematic analysis of peptide linker length and liposomal polyethylene glycol coating on cellular uptake of peptide-targeted liposomes. *ACS Nano.* 2013 XXX.
8. Prabhakar U, Blakey DC, Maeda H, Jain RK, Sevick-Muraca EM, Zamboni W, Farokhzad OC, Barry ST, Gabizon A, Grodzinski P. Challenges and key considerations of the enhanced permeability and retention effect (EPR) for nanomedicine drug delivery in oncology. *Cancer Res.* 2013 [Epub ahead of print].
9. Gref R, Lück M, Quéllec P, Marchand M, Dellacherie E, Harnisch S, Blunk T, Müller RH. 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids Surf B Biointerfaces.* 2000; 18:301–313. [PubMed: 10915952]
10. Perry JL, Reuter KG, Kai MP, Herlihy KP, Jones SW, Luft JC, Napier M, Bear JE, DeSimone JM. PEGylated PRINT nanoparticles: the impact of PEG density on protein binding, macrophage association, biodistribution, and pharmacokinetics. *Nano Lett.* 2012; 12:5304–5310. [PubMed: 22920324]

11. Seetharamu N, Kim E, Hochster H, Martin F, Muggia F. Phase II study of liposomal cisplatin (SPI-77) in platinum-sensitive recurrences of ovarian cancer. *Anticancer Res.* 2010; 30:541–555. [PubMed: 20332467]
12. Ng SS, Sparreboom A, Shaked Y, Lee C, Man S, Desai N, Soon-Shiong P, Figg WD, Kerbel RS. Influence of formulation vehicle on metronomic taxane chemotherapy: albumin-bound versus cremophor EL-based paclitaxel. *Clin Cancer Res.* 2006; 12:4331–4338. [PubMed: 16857808]
13. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, Hanahan D, Mattrey RF, Ruoslahti E. Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Cancer Cell.* 2009; 16:510–520. [PubMed: 19962669]
14. Demers M, Wagner DD. Targeting platelet function to improve drug delivery. *Oncoimmunology.* 2012; 1:100–102. [PubMed: 22720224]
15. Wang J, Gao W. Nano/Microscale motors: biomedical opportunities and challenges. *ACS Nano.* 2012; 6:5745–5751. [PubMed: 22770233]
16. Beck A, Haeuw JF, Wurch T, Goetsch L, Bailly C, Corvaia N. The next generation of antibodydrug conjugates comes of age. *Discov Med.* 2010; 10:329–339. [PubMed: 21034674]
17. Cheng J, Khin KT, Davis ME. Antitumor activity of beta-cyclodextrin polymer-camptothecin conjugates. *Mol Pharm.* 2004; 1:183–193. [PubMed: 15981921]
18. Sengupta P, Basu S, Soni S, Pandey A, Roy B, Oh MS, Chin KT, Paraskar AS, Sarangi S, Connor Y, Sabbisetti VS, Koppam J, Kulkarni A, Muto K, Amarasiriwardena C, Jayawardene I, Lupoli N, Dinulescu DM, Bonventre JV, Mashelkar RA, Sengupta S. Cholesterol-tethered platinum II-based supramolecular nanoparticle increases antitumor efficacy and reduces nephrotoxicity. *Proc Natl Acad Sci U S A.* 2012; 109:11294–11299. [PubMed: 22733767]
19. Marusyk A, Polyak K. Cancer. Cancer cell phenotypes, in fifty shades of grey. *Science.* 2013; 339:528–529. [PubMed: 23372002]
20. Lee MJ, Ye AS, Gardino AK, Heijink AM, Sorger PK, MacBeath G, Yaffe MB. Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. *Cell.* 2012; 149:780–794. [PubMed: 22579283]

Fig. 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 sub-optimal binding and internalization. Interestingly, (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 length this internalization is reduced, suggesting hindrance in interacting with the cell or the epitope. (Adapted from Stefanick et al, ACS Nano 2013).

Fig. 2. Design parameters highlight analogy between ADCs and cancer nanomedicines that can facilitate clinical translation

The cartoons show a representative ADC shown is T-DM1, the conjugate of trastuzumab with mertansine and a typical nanoparticle. The table shows overlapping similarities between ADCs and nanomedicines.

Fig. 3. Selecting the right drug is critical for efficacy as also selecting the right comparator arms (a-b) A cyclodextrin-camptothecin conjugate nanoparticle that results in greater antitumor efficacy than irinotecan, a clinically-approved drug in the same topoisomerase I inhibitor class (Adapted from Ref. 17). (c-d) 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 undergo aquation more efficiently than the dicarboxalo-linkage in carboplatin. The analog facilitates supramolecular nanoassembly (SACNs) as seen using cryo-TEM, and results in greater antitumor efficacy in an RAS/Pten ovarian cancer model (adapted from Sengupta P, et al, Proc Natl Acad Sci U S A. 2012, 109, 11294-9).

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 IC50) 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