Nanomaterial-based therapy: a new generation of cancer treatment

"A clear need for treatment functionalities that can be met via nanomaterial-based solutions has been identified, and their continued development and fruition will thus catalyze a transformative vision for cancer treatment."

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The eradication of cancer represents one of the most compelling medical challenges of our time, and optimized chemotherapy requires the innovation and adoption of modalities that can circumvent the barriers against its interventional and perpetual elimination. To meet these demands, global efforts in nanomaterial research have culminated in multifunctional technologies with integrated cancer-cell-targeting moieties and pH-dependent therapeutic unloading to enhance the specificity of drug release, high drug-carrying capacities that have reduced therapeutic dosages towards side-effect mitigation and fluorescent payloads/properties, such as quantum dots or innately fluorescent nanocarbons, which have enabled simultaneous imaging and tracking of nanomaterial distribution and clearance. To address cancer in its metastatic form, nanomaterial, or particle-based strategies that are capable of injection and effective targeting towards ultimate specificity of activity have been envisioned; for example, lipid, polymer, metal and carbon-based nanoparticles have been utilized to deliver a broad range of small-molecule, protein and RNA-based therapeutics with improved specificity [1–15]. With regards to operable tumors and the prevention of recurrence, devices that are complementary to the injected nanoparticles have been developed for implantation and localized drug release, which may reduce dosing/side effects from the repeated administration of systemic therapies [16–20]. A clear need for treatment functionalities that can be met via nanomaterial-based solutions has been identified, and their continued development and fruition will thus catalyze a transformative vision for cancer treatment. The roadmap for nanomaterial-driven cancer treatment can be viewed from two categories, which include particle-mediated delivery (systemic) and device-mediated delivery (localized). Both components serve as important directions that should be pursued, which can result in significantly decreased dose-dependent patient side effects and toxicity, improved targeting, as well as prolonged chronicity of efficacy. Given their innate versatility in their capacity as building blocks towards drug delivery system construction, nanomaterials carry innate properties that may add new dimensions to the way that interventional routes in oncology are practiced.

Requisite properties for nanomaterial-driven therapy

The integration of several properties into a single drug-delivery platform represents an important first step towards the realized clinical relevance of nano-enabled cancer therapy. These include the scalability of material production, platform performance (the ability to interface multiple classes of drugs with the nanomaterial), sustained and targeted release, as well as innate biocompatibility that can be both qualitatively and quantitatively verified. More specifically, on the topic of scalability, it is of paramount importance that roadmaps towards large-scale production with high yield/consistent performance metrics are developed that allow for widespread patient impact. A prevalent example of scalability includes the advent of nanodiamond particles that have been utilized for doxorubicin delivery, which exhibit very narrow particle diameter ranges of 4–6 nm, arrived at through a facile process of ball milling, ultrasonication and acid washing [15,16]. With regards to platform functionalization, a host of materials have been explored that have been linked with small-molecule drugs such as cyclophosphamide, doxorubicin and paclitaxel (Taxol®), among others, as well as RNAinterference molecules that have not only been delivered by nanoparticle agents, but have also been stabilized by these nanoparticles for prolonged activity [21–24]. Sustained and targeted release represents a cornerstone of the nano-enabled therapy community, as high surface area-to-volume ratios of a spectrum of nanoparticle agents, coupled with

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customizable activation with antibodies and other analogs towards the unique surface properties of cancerous cells, have improved the prospects for technology-enabled enhancements towards the pharmacological specificity of existing drugs and drugs in development. Finally, perhaps one of the most critical determining factors of the translational potential for nano-enabled cancer agents is the examination of the post-drug-release properties of the delivery agent. Quantitative insight from both short- and long-term nanomaterial exposure allows the investigator to accurately determine if, following therapeutic intervention, the body is able to interact favorably with the residual material, exhibited by attenuated inflammatory response and toxicity, followed by comprehensive clearance.

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Injectable nanomaterial platforms for systemic therapy

Specific benefits afforded by nanoparticle-based therapy include noninvasive delivery strategies such as injection, as well as the opportunity for prolonged drug activity from sustained drug release. A vast range of particle delivery methods have been studied with foundational materials based upon lipids, polymers and nanocarbons. Recently, lipid-based vehicles have been developed with integrated targeting, therapeutic and imaging components [25]. For example, immunoliposomes (ILs) were created via conjugation to anti-HER2 single-chain Fv fragment-targeting moieties against HER2-overexpressing breast cancer SK-BR-3 and MCF-7/HER2 cell lines, which resulted in increased cancer cell targeting and specificity *in vitro*. Quantum dots (QDs) were conjugated to the immuno-liposome exterior (QD–ILs) to enable simultaneous immunoliposome internalization and localization tracking. Efficient receptor-mediated QD-IL endocytosis with anti-HER2 single-chain Fv fragment-targeting elements was observed with HER2-overexpressing SK-BR-3 and MCF-7/HER2 cell lines, while control MCF-7 cells revealed minimal uptake, demonstrating the potential of QD-ILs as multifunctional nanotherapeutic agents.

An additional nanoparticle property of emerging importance under development involves their capacity as simultaneous polar/nonpolar molecular delivery platforms, a highly sought-after characteristic given the widely varied properties of therapeutic compounds which, in several cases, are not readily soluble in water and thus require alternate routes towards delivery. To address this issue, researchers have developed nanoemulsions with water-in-oil-in-water architectures [26]. Further stabilized via amphiphilic Kx(*rac*-L)y surfactant diblock racemic copolypeptides, robust water-in-oil-in-water double nanoemulsion stability and resultant compartmentalization of polar and nonpolar therapeutic analogs was enabled for several months. The feasibility of scalable emulsion processing and size determination was demonstrated via initial ultrasonic homogenization-induced formation of a polydisperse nanoemulsion population with a minimum diameter of 400 nm. Subsequent centrifugation reduced the droplet range to 10–100 nm, which resulted in the formulation of noninvasive multiagent transporters, and also confirmed the amenability of the stabilized double emulsion droplets towards large-scale processing techniques. The multifaceted encapsulation capabilities of the copolypeptide-stabilized nanoemulsions were confirmed via simultaneous carrying of polar, nonpolar and peptide imaging agents, which included water-soluble InGaP/ZnS quantum dots, hydrophobic pyrene-containing silicone oil and fluorescein-labeled K40(*rac*-L)10. Fluorescence imagery revealed the robust compartmentalization of all three imaging agents for at least 3 months. This work thus illuminated the translational potential of nanoemulsions as robust vehicles for multiagent delivery [26].

Nanocarbons, including nanotubes, nanohorns and nanodiamonds, have also served as therapeutic delivery agents, or therapeutic agents themselves given their unique properties including infrared-driven heating, which has been previously investigated for targeted cancer cell death [27]. Their biostability has also been utilized for the solubilization of water-insoluble compounds and other small-molecule drugs [28]. Recent studies of nanotube distribution and clearance *in vivo* have generated evidence that they can be removed from the body as evidenced by the monitoring and confirmation of nanotube clearance in mice, providing important insight and support towards medical utility.

Localized therapy

In addition to the conventionally-viewed applications of nanomaterials in particle format for therapy, nanostructured devices have also been developed that allow for small footprint,

localized drug release, which can circumvent the need for continual systemic injection [18,19]. For example, tunable and localized drug release has been developed, which allows for on-demand therapeutic delivery, and therefore plays an important role in maintaining dosing control and drug reserves [4,5,18]. In addition, gene delivery has been achieved using tissue-engineering scaffolds on the months-scale, reducing possible side effects associated with potential nonspecific nanoparticle internalization [19]. Nanodiamond-embedded microfilm devices have also previously been explored, which have enabled the continuous release of chemotherapeutic compounds for a minimum of 1 month [17]. In addition to long-term release, implantable devices also possess a unique opportunity for localized sequential and combinatorial release. For example, existing patient treatment regimens for breast cancer include the initial release of doxorubicin/adriamycin, followed by the administration of a dose of cyclophosphamide that may also be accompanied by an additional dose of doxorubicin. This dual-staged drug release may then be followed up by an additional three-drug course of cyclophosphamide, methotrexate and fluorouracil to constitute 'CMF' therapy, which has resulted in improved treatment outcomes [29]. Nanomaterial-based platforms, such as small footprint scaffolds, or nanocarbon-embedded microfilms, possess the unique advantage of enabling high drug-loading capacities with minimal impact upon the architecture of the device. As such, several of these technologies are carrying very large drug payloads, but possess minimally-invasive dimensions, further improving their amenability with their surrounding biological environment. Furthermore, film/scaffold-based platforms may also be particularly applicable towards localized 'zero-order' release kinetics, eliminating the 'burst' release that is often observed when an initial spike of drug is released instantaneously, which can induce major side effects due to excess initial dosing while also significantly depleting the drug supply [30–34]. Thus, it can be envisioned that, following a tumor resection, surgeons may be able to implant these localized drug administration devices over areas where residual cancer cells might remain, resulting in a potent and sustained dosing of the overlying cancer cells, and reducing the need for systemically administered drugs. Furthermore, the aforementioned combinatorial and sequential release strategy, which has been shown to improve tumor reduction efficacy, may then potentially be performed using this device. As therapeutics such as cyclophosphamides and fluorouracil are particularly cytotoxic, the need for localized release and its benefits such as reduced toxicity, brought upon by reduced dosing, is clearly evident and potentially addressed via nanomaterial-based devices.

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Requisite properties for translation: post-release amenability

To complement the confirmation of nanomaterial-mediated therapeutic efficacy via drug loading and subsequent release/functionality assays, quantitative assessment coupled with tissue/animal model assessment of nanomaterial compatibility is a vital determinant of the translational relevance of these platforms for cancer treatment [35–38]. Elevated levels of inflammatory cytokines such as IL-6, for example, have resulted in decreased chemotherapeutic efficacy and the promotion of tumor spreading, among other complications [35]. In addition to the delivery-associated advantages that can be offered by nanomaterial platforms, a deep understanding of post-release interaction and nanomaterial fate, including the mechanisms of clearance, is required to understand the short- and long-term effects of material exposure. Qualitatively speaking, cells that are not adversely affected by their surroundings can resemble cells that are stressed and overexpressing the genes for inflammatory cytokine production, which can then be followed by increased endogenous levels of the cytokines. As such, conventional cell viability assays have provided important quantitative insight into the amenability of a broad array of nanomaterials towards basic cell function, including proliferation and metabolism. Further studies including microarray analysis of a broad spectrum of gene-expression profiles, large-scale ELISA assays for protein release, as well as quantitative real-time PCR, all serve as evaluative strategies to provide further information into the innate effects that can be elicited by prolonged nanomaterial exposure following drug delivery. Towards clinically-relevant nanomaterial administration scenarios, biodistribution trials, liver toxicity, Kaplan–Meier survival curves and additional *in vivo* methodologies will definitively forge a path for the adoption of new avenues for cancer therapy.

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