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Multifunctional Nanoparticles: Cost versus benefit of adding targeting and imaging capabilities

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Abstract

Nanoparticle-based drug delivery systems have been developed to improve the efficacy and reduce the systemic toxicity of a wide range of drugs. While clinically-approved nanoparticles have consistently shown value in reducing drug toxicity, their use has not always translated into improved clinical outcomes. This has led to the development of “multifunctional” nanoparticles, where additional capabilities like targeting and image contrast enhancement are added to the nanoparticles. However, additional functionality means additional synthetic steps and costs, more convoluted behavior and effects *in vivo*, and also greater regulatory hurdles. The trade-off between additional functionality and complexity is the subject of ongoing debate and the focus of this review.

Introduction

The continual identification and development of new drugs has led to an appreciable reduction in both mortality and morbidity for most life-threatening diseases, including cardiovascular diseases and cancer, the two leading causes of death in the world. However, due to poor pharmacokinetic profiles and broad mechanisms of action, most small-molecule drugs still carry a substantial risk of systemic toxicity (1). Moreover, only a relatively small fraction of the administered drugs are delivered to and act at the disease site. Nanoparticles offer an opportunity to alter the pharmacokinetic profile of drugs, reduce off-target toxicity and improve the therapeutic index. An example of such a formulation is Doxil®, a PEGylated liposome carrier loaded with the cytotoxic anticancer drug doxorubicin. While doxorubicin indiscriminately diffuses across blood vessels into normal tissues and tumors alike, Doxil is generally retained within the blood pool except at sites of increased vascular permeability, such as in the liver, spleen, and within tumors. As a result, Doxil exhibits a circulation half-life that is 100-times longer than free doxorubicin and carries a risk of cardiotoxicity that is seven-fold lower than free drug (2). Yet, the use of Doxil, does not always afford a significant improvement in survival compared with doxorubicin, e.g. when used as first-line therapy in breast cancer patients (2).

The design and development of “multifunctional” nanoparticles seeks to expand upon the benefits realized by Doxil and other first generation, clinically-tested nanoparticles (Table 1), by adding functionalities intended to improve delivery, therapeutic efficacy, and

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ultimately patient outcome. Multifunctional nanoparticles have been devised with stealth-like features to evade the immune system and prevent opsonization, protective layers to prevent the degradation of biologic cargo (e.g. proteins, DNA), targeting moieties to improve specificity and tumor accumulation, membrane-permeation moieties to improve cell uptake, imaging agents to assess delivery and dosing, endosome escape mechanisms, target-dependent assembly or disassembly to control drug release, microenvironment sensors (pH, proteases, phospholipases) to trigger drug release and cell uptake, and intracellular targeting moieties to direct drugs to specific intracellular compartments. Some of these features are considered necessary for the delivery of therapeutic biologics, such as peptides, proteins, siRNA, and genes; however, the cost/benefit ratio of these modifications in improving the delivery of many small-molecule drugs is less certain. Each new functionality elevates complexity (e.g. multi-step syntheses, purification and characterization) and cost (e.g. lower yields, more costly materials), and regulatory barriers arise (e.g. owing to multi-component, heterogeneous formulations). This review focuses on the benefits and caveats of adding targeting and imaging features to nanoparticles as a means to improve the efficacy of small-molecule anti-cancer drugs (Figure 1), since these modifications are expected to see the most immediate translation into the clinic. In fact, several targeted nanoparticles have already shown sufficient promise for entry into clinical trials (Table 2).

Targeting: the Good, the Bad, and Cell Uptake

The accumulation of non-targeted nanoparticles, which includes all current Food and Drug Administration (FDA)-approved nanoparticles, within malignant lesions is generally attributed to passive (pathophysiological) targeting. Passive targeting is a consequence of enhanced permeability and retention (EPR), whereby the leakiness of the tumor vasculature combined with poor lymphatic drainage, enable nanoparticles to accumulate within the tumor matrix. Animal studies suggest that EPR can lead to more than a 50-fold increase in nanoparticle accumulation within tumors, compared with healthy tissues (3). In general, the longer the nanoparticle circulation time, the greater the EPR-induced accumulation (4).

While there is no single optimal particle size or surface charge for maximizing EPR, due to the high degree of tumor-to-tumor variability, nanoparticles between 10nm to 100nm typically demonstrate the most effective tumor penetration (5), but particles up to ~ 400nm in size have been shown to extravasate into tumors in animal models (6). The EPR effect, however, is not commonly observed in some types of cancers, including gastric and pancreatic cancer (7). In other cases, the tumor core may not be well-perfused (8).

Over the years there have been a number of adjunct strategies that have been shown to increase vascular permeability, which may be used enhance nanoparticle accumulation at the tumor site. One such approach involves the administration of vasoactive agents such as vascular endothelial growth factor, thrombin, bradykinin, substance P, and lipopolysaccharide endotoxin. These agents initiate a cascade of cellular events that ultimately result in disruption of endothelial cellular junctions and increase vascular permeability. An alternative method for increasing vascular permeability includes transiently raising systemic pressure, by infusing vasoconstrictors (e.g. angiotensin II) to overcome the high interstitial pressure of tumors (9). One general concern with all of these methods is that altering blood vessel permeability may affect both healthy blood vessels and diseased blood vessels and therefore may increase the occurrence of off-target effects. More targeted approaches involve using external stimuli such as ionizing radiation to alter vascular permeability (10) or photodynamic therapy to disrupt vascular integrity (11). It has been suggested that systemic injection of the tumor-penetrating peptide iRGD may also

increase vascular and tissue permeability in a tumor-specific manner, through interactions with integrin and neuropilin-1 (12).

Presumed benefits of developing receptor-specific targeted nanoparticles

Active targeting mediated by affinity ligands may complement EPR or provide an alternative delivery mechanism. The value of targeting therapeutic agents can be seen from early clinical trials with antibody-conjugated chemotherapeutics. T-DM1, which consists of the antibody trastuzumab (Herceptin®) linked to the cytotoxin mertansine, was shown to reduce the death rate of patients with HER2/neu-positive metastatic breast cancer by 37% and side effects by nearly 50% in phase III clinical trials (13). The selective toxicity of this and similar agents is attributed to an increase in the target/non-target distribution ratio. Similar results have also been reported in many animal studies with targeted nanoparticles. For example, in a murine tumor model, folate-targeted paclitaxel-loaded micelles exhibited a tumor accumulation of 10 ± 2 percent injected dose per gram of tissue (% ID/g) compared to only $1 \pm 0.3\%$ ID/g for non-targeted micelles, and a markedly reduced systemic toxicity (14). Nanoparticles offer an opportunity to carry a larger and more diversified drug payload than direct antibody conjugates, including poorly water soluble drugs, which can account for ~40% of the active substances identified through combinatorial screening (15).

A selective increase in the delivery of small-molecule drugs to cancer cells requires the functionalization of nanoparticles with targeting ligands that bind to cognate counterparts preferentially expressed on the target cells (Table 3). Targeting can be particularly valuable in treating small metastases ($<100 \text{ mm}^3$), since these sites are poorly vascularized and do not evoke EPR (39). Accordingly, in a murine orthotopic model for pancreatic carcinoma, $\alpha_v\beta_3$ -targeted doxorubicin-loaded nanoparticles led to an 82% reduction in the growth of metastatic lesions compared with untreated control animals. In contrast, non-targeted nanoparticles did not show any significant effect on metastatic lesions (40). Furthermore, it has been shown that targeted nanoparticles are able to overcome multiple drug resistance as glycoprotein efflux pumps are unable to remove drug-nanoparticle complexes that have entered via receptor-mediated events (41).

Drug-loaded nanoparticles may also exhibit a synergistic tumoricidal effect when actively targeted with therapeutic antibodies, i.e. combining chemotherapy and immunotherapy. For example, when doxorubicin-loaded nanoparticles were functionalized with the therapeutic antibody trastuzumab they exhibited more effective inhibition of tumor growth compared to the additive effect of therapeutic antibodies and non-targeted doxorubicin-loaded nanoparticles, when each was administered individually (42).

As an alternative to targeting tumor cells, other options include targeting the endothelium, to choke the blood supply and starve the cancer cells of nutrients and oxygen, and targeting tumor-supporting cells such as tumor-associated macrophages. When DNA vaccines were targeted to legumain, a stress protein that is overexpressed on tumor-associated macrophages, mouse models of metastatic breast, colon and non small cell lung cancer displayed prolonged survival in 75% of mice and 62% were completely free of metastasis (43).

Challenges facing the development of receptor-specific targeted nanoparticles

While it has been more than 30 years since the concept of targeted nanoparticles was introduced (44), few formulations have reached clinical trials and to date none have been clinically approved (Table 2). Although targeted therapeutics hold much promise, there are also potential risks and challenges, ranging from synthesis and purification, choosing the right ligand-receptor pair, to altering nanoparticle properties. Even the conjugation

technique can affect binding features of a ligand due to conformational changes, insufficient steric freedom or inadequate orientation. Promising novel bioconjugation methods including click chemistry are being introduced to overcome some of these limitations, but their adaptability, biological inertness, and clinical potential remain to be thoroughly appraised (45).

In addition to practical constraints, some have questioned whether targeting actually improves nanoparticle accumulation in tumors. A fundamental paradox here is that the addition of targeting moieties compromises the stealth feature of nanoparticles and can accelerate their clearance by the host. For example, it has been shown that non-targeted liposomes can exert comparable tumor accumulation as folic acid-functionalized liposomes since they benefit from longer circulation times and higher EPR (46). The density of the target receptor on tumor cells may also be a limiting factor. In a metastatic breast cancer model, it was found that a receptor density of 10^5 HER2/neu receptors per cell was required to achieve an improved therapeutic effect with anti-Her2/neu-targeted liposomal doxorubicin over non-targeted liposomal doxorubicin (47).

Not all cancer cell types overexpress the same unique receptors and often, overexpressed receptors are also present on normal tissue. A recent study of gastric cancer found that significant intratumoral heterogeneity also exists with 79.3% of HER2-positive tumors having regions with different immunohistochemical staining scores (48). Some cancer cells, e.g. cancer stem cells, may be void of any known upregulated receptors (49). The dynamic nature of tumor markers is also a challenge. In an animal model it was found that Sigma receptor density decreased by 30% after a single dose of doxorubicin (50) and Her2/neu receptor density decreased by 40% after treatment with trastuzumab-conjugated nanoparticles (51). In this context, nanoparticles with multiple unique targeting ligands have generally been seen as an advantage that may help to defuse this problem. Testing of this “multispecificity” approach is ongoing (52).

Nanoparticle multivalency and multi-specificity can also help to overcome the weak affinity of some ligand-antigen pairs and promote endocytic uptake. Hong et al. showed that a 5-fold increase in the number of folic acid on dendrimer nanoparticles gives a 68-fold improvement in binding avidity (53). However, while multivalency can increase avidity, it can also cause unexpected adverse cellular responses. Wang et al. showed that transferrin and transferrin receptor antibodies, neither of which are toxic individually, can be toxic to selected cells when multivalently conjugated to nanoparticles (54). Although multivalency induced toxicity can be leveraged against cancer cells, further investigation into the optimal level of multivalency and related adverse effects for each nanoparticle formulation is nonetheless warranted (55). Further, an increase in valency does not necessarily translate into more effective targeting. In some cases, nanoparticles with an intermediate ligand density display higher binding than nanoparticles with higher ligand densities, likely due to steric limitations imposed on congruency inter-molecular ligand-receptor interactions by too closely adjacent ligands (56).

While higher nanoparticle avidity is generally seen as an advantage, the use of targeted nanoparticles with high avidity may elicit a “binding site barrier” wherein binding to target cells paradoxically reduces penetration in deep layers of the tumors (57). This was originally observed with antibodies and may be particularly problematic for nanoparticles due to higher diffusion limitations. Since the binding site barrier is more prominent at low doses, this may negate the perceived advantage of administering lower dosages with targeted nanoparticles. Finally, high avidity may compromise selectivity, as nanoparticles may be depleted upon binding to non-tumor cells expressing low levels of “tumor-specific determinants” (58). An absolute specificity of a target molecule for cancer cells (or any

other targets) is unlikely to exist. In fact, many “tumor specific” target molecules including receptors for folate, integrins and transferrin exist in non-tumor cells and pose the danger of off-target (yet specific) binding and effects (Table 3).

These considerations help to explain a co-existence of reports of increased tumor accumulation of targeted nanoparticles with those showing increased cellular internalization but not tumor accumulation. The view that targeting does not necessarily increase localization is supported by theoretical works, which suggest that nanoparticles reach cancer cells by enhanced permeability (59). Ligands then bind target receptors, which helps to retain the nanoparticles within the tumor. However, nanoparticles must still overcome high intratumoral fluid pressure and penetrate the dense tumor extracellular matrix (60, 61). Adding a targeting moiety may hamper this process by increasing the nanoparticle size (which further impedes diffusion) (62) and creating the affinity barrier problem discussed above. These sobering considerations on the real value of targeting pertain mostly to the targeting of cancer cells in primary solid tumors. Tumor penetration is much less relevant in situations where the tumor endothelium or other readily accessible targets are pursued. Recent findings suggest that coordinated targeting approaches may provide a solution to poor tumor penetration. It has been shown that iRGD can specifically home to tumors and promote tissue extravasation through coordinated interactions with both integrin and neuropilin-1 (63).

Cellular internalization

If targeting does not help nanoparticles actively reach the tumor, then the true value of targeted nanoparticles may lie in their ability to be internalized upon receptor-mediated endocytic processes. Cell internalization is considered to be important since many of the FDA-approved nanoparticle-based medications, such as Abraxane (paclitaxel) and Doxil (doxorubicin), contain drugs that act on intracellular targets. Therefore, internalization is expected to result in higher intracellular drug concentrations and facilitate drug-target interactions. Commonly utilized targeting ligands, such as transferrin, epidermal growth factor and many antibodies, already benefit from receptor-mediated endocytic processes. Even ligands that do not naturally internalize often undergo internalization when displayed multivalently on the nanoparticle surface (64).

Targeting to specific epitopes may also help to control the nanoparticle's intracellular trafficking and destination. The transferrin receptor, which recycles to the plasmalemma avoiding lysosomal degradation (54) and glycoproteins, localizes in caveoli, favoring transcellular transport (65). Dovetailing on such natural transport mechanisms may assist in sub-cellular delivery of vulnerable biotherapeutics. However, the uptake and trafficking of ligand-guided nanoparticles may drastically differ from those of ligands in their natural form. Bhattacharyya et al. showed that using nanoparticles conjugated with the anti-EGFR antibody cetuximab, compared to free cetuximab alone, uniformly enhanced EGFR endocytosis, changed the compartmentalization pattern and implicated the role of a distinct pathway involving dynamin-2 in the endocytic processes (66).

The use of high-affinity ligands may also impede physiological mechanisms including nanoparticle detachment from the receptor and intracellular transport. For example, low affinity antibody conjugates targeted to transferrin receptor provide more effective transport across the vascular barrier, by virtue of more dynamic interaction with the receptor than high-affinity counterparts (and likely due to an elevation in the ratio of free/bound conjugate in the vasculature) (41). Parameters of nanoparticle design, such as size, shape, valency and plasticity greatly modulate sub-cellular trafficking (67) and need to be more fully understood in order to capitalize on the potential advantages provided by targeting.

New targeting strategies that exploit the tumor microenvironment or external stimuli

Targeting cell surface biomarkers is complicated and mostly empiric. New targeting strategies that exploit the altered tumor microenvironment may avoid many of the complications and pitfalls associated with receptor-specific targeting and achieve broader disease coverage (Figure 2). For example, targeting agents have recently been designed to employ local factors of the tumor microenvironment to trigger cell uptake. In one approach, nanoparticles were linked to neutralized cell penetrating peptides that become activated upon cleavage by the metalloproteinases present in the tumor parenchyma, enabling local intracellular delivery of the cargo (68).

A plethora of pH-sensitive drug release, cell-penetrating and disassembly mechanisms have also been devised to assist tumor targeting, by taking advantage of the lower pH observed in most tumors. Metabolic differences that exist between tumors and normal tissues have been known for a long time - increased glucose uptake and glycolysis lead to tumor acidification, a phenomenon known as the Warburg effect. Recent examples of nanoparticles that target the acidic tumor microenvironment include nanoparticles functionalized with glycol chitosan, a water-soluble polymer with a pH-titrable charge (69). These metabolic sensors undergo a switch from a near neutral charge at pH 7.4 to a positive charge at lower pH, enabling them to remain in the acidic tumor microenvironment due to electrostatic interactions with surrounding tissue. In another approach, pH low insertion peptides have been developed that are soluble in physiologic pH, but form a rigid transmembrane α -helix that spontaneously inserts across lipid bilayers at low pH (<7.0) (70). It has been shown that these insertion peptides can be used to translocate nanoparticles across lipid membranes (71). Preliminary studies have suggested that these peptides can insert into primary tumors, metastatic lesions, and lipid bodies in necrotic tissues (72). Environment-responsive liposomes have also been prepared by anchoring phospholipids with cell-penetrating peptides and a pH-sensitive PEG shield. At low pH, the liposomes lose their PEG coating and are internalized into cells via the now-exposed cell penetrating peptide moieties (73). In a similar fashion, nanoparticles have also been designed to shed their PEG coating in response to other stimuli, including reducing agents and proteases, exposing targeting ligands or positive charges (74). Although PEGylation is required to impart stealth-like properties on nanoparticles, it also limits drug release, therefore, shedding of the PEG coating can facilitate drug delivery. Overall, it is expected that these microenvironment-sensitive delivery approaches may provide a new mechanism for the treatment of tumors. On a cautionary note, tissue acidosis is not a tumor-specific phenomenon; areas of pathological (ischemia) and physiological (e.g., skeletal muscle in exertion) acidification may become victims of off-target localization of pH-regulated delivery systems.

As an alternative to relying on environmental stimuli to drive nanoparticle localization, some nanoparticle formulations can be externally manipulated. It has been shown that iron oxide nanoparticles with bound anti-cancer agents can selectively accumulate in cancer tissues under the guidance of a magnetic field gradient. Researchers found that by using magnetic drug targeting of ferrofluids, only 20% of the systemic dose was necessary for complete remission of tumors and no side effects were observed (75). This approach mirrors other targeted approaches where external stimuli are applied to limit treatment to the tumor site, such as photodynamic therapy (76), nanoparticle-mediated hyperthermal therapy (77), and photoactivatable nanoparticles that release their cargo in response to light (78).

Combining Drugs with Imaging Agents: Synergistic or Counter Productive?

Benefits of developing theranostic nanoparticles

There has been a trend toward combining therapeutic and diagnostic functions within a single formulation at the nanoscale, i.e. “theranostic” agents. Compared to delivering drugs

or imaging agents alone, theranostic agents can simultaneously deliver imaging and therapeutic agents to specific sites or organs, enabling detection and treatment of disease in a single procedure. When drugs and imaging agents are combined into a single formulation, they are usually assumed to have similar biodistribution and tumor localization in living subjects. As a result, theranostics agents are expected to inform us about the localization of the drug and pathological process longitudinally. Direct visualization of theranostic pharmacokinetics can provide important insight into heterogeneities between tumors and patients. This will help physicians make informed decisions about timing, dosage, drug choice, and treatment strategies. Such “personalized medicine” can lead to improved efficacy, lower off-target toxicity and an overall increase in quality of life and patient outcome.

In an example illustrating these aspirations (79), a theranostic agent was prepared by the encapsulation of iron oxide nanoparticles and the photosensitizer Photofrin within polyacrylamide nanoparticles. Dynamic scanning magnetic resonance imaging (MRI) revealed that tumor-targeting resulted in an increase in tumor half-life from 39 to 123 minutes, within a rat brain tumor model. The therapeutic efficacy of nanoparticles was subsequently evaluated in comparison to the free photosensitizer, and was monitored by changes in tumoral diffusion, as measured by MRI. Treatment of glioma-bearing rats with targeted Photofrin-encapsulated nanoparticles resulted in a higher mean tumor apparent diffusion coefficient, compared with non-targeted control nanoparticles and free Photofrin.

Challenges facing the employment of theranostic nanoparticles

In many cases the addition of a contrast agent to a drug carrier might enable some imaging capabilities, but will still be below the threshold for obtaining images with sufficient quality or resolution. For example, micelles and liposomes that have their outer surfaces labeled with chelated gadolinium (Gd) will typically only possess several hundred to several thousand Gd per nanoparticle (80). In contrast, state-of-the-art Gd-based MR contrast agents can have tens to hundreds of thousand Gd per particle (81–83). Similarly, loading nanoparticle-based imaging agents with anti-cancer drugs is not necessarily a winning proposition. For example, labeling the surface of superparamagnetic iron oxide nanoparticles with small molecule drugs such as doxorubicin would only allow for the incorporation of several hundred to several thousand doxorubicin molecules per particle (< 4% w/w) (84), which is far below some of the state-of-the-art drug delivery vehicles, which can have drug loading efficiencies of 30–70% w/w (85–89).

The design of theranostic agents can require a compromise between optimal features of sole modalities. Therefore, the question is, how much imaging and therapeutic efficacy is one willing to sacrifice in order to achieve the benefits of having dual functionality? This will likely depend on how much value the imaging agent can provide and whether it will affect patient management. In general, the combination of imaging and therapeutic agents is not a natural fit. For example, while long circulation times are generally preferred for therapeutic nanoparticles, to maximize total tumor accumulation via EPR, this is not ideal for imaging. Imaging can only be conducted once sufficient contrast agent has been cleared from circulation, otherwise the background signal may be too high to accurately quantify tissue biodistribution. Moreover, nanoparticles can often have long residence time at the tumor site, particular those possessing inorganic materials. This may limit the ability of nanoparticle-associated imaging agents to provide temporal information on changes in biomarker expression, tumor response, and vascular reorganization. Consequently, adding imaging agents to therapeutic nanoparticles may simply serve as a way to track nanoparticle biodistribution.

The addition of imaging capabilities to therapeutic nanoparticles increases the cost and the complexity of synthesis and purification and will lead to more heterogeneous formulations. Therefore, it must be carefully considered whether or not individualized information on nanoparticle distribution warrants the additional expense. With a dedicated imaging agent, the contrast enhancement would likely be superior to theranostic agents, images can be acquired at more convenient times and intervals, and targets can be chosen that are independent of the therapeutic target - to better reflect tumor response, with less concern about altered expression owing to drug resistance. Perhaps, an optimal resolution is to adopt two mutually exclusive imaging agents (e.g. different energies or modalities) that will allow for the parallel tracking of nanoparticle biodistribution and monitoring of tumor response. Some imaging agents may also be able to be temporally resolved, owing to distinct radionuclide decay and/or clearance profiles.

The immediate future of theranostic nanoparticles

Theranostic agents are being devised with diverse contrast media including those for optical imaging. Arguably, isotope probes enabling single-photon emission computed tomography (SPECT) and positron- emission tomography (PET) offer preferable features for clinical translation: high sensitivity, accurate quantification, deep tissue penetration and real-time non-invasive longitudinal imaging. Labeling nanoparticles with isotopes enables the accurate assessment of biodistribution, circulation half-life, and pharmacokinetics of nanoparticles. Due to the high sensitivity of nuclear imaging and well controlled labeling, patients can be treated with carriers loaded with high therapeutic doses, yet labeled with rather trace amounts of isotopes that do not compromise drug loading and safety. Nonetheless, when combining radioisotopes with therapeutic nanoparticles, there are several issues that must be taken into consideration.

The short half-life of most clinically-suitable radionuclides has generally led to their placement on the surface of therapeutic nanoparticles, to facilitate expedited labeling and purification. When utilizing nanoparticles with surface-bound radionuclides, it is important to ensure stability of the radiolabel. For example, if a radiometal is lost from its chelate or if the radiochelate is removed prematurely from the nanoparticle, acquired images would not accurately reflect actual nanoparticle biodistribution and could lead to misleading findings. Studies suggest that the *in vivo* behavior of metal chelates exposed to a complex protein environment cannot be predicted by classical equilibrium constants, so *in vivo* evaluation is often warranted (90). Approaches for stable radiolabeling of nanoparticles are needed; however, recent animal studies employing nanoparticles stably labeled with PET isotopes show promising results (91).

To close the gap between the desired long circulation time for therapeutic nanoparticles and the short timeframe desired for nuclear imaging, devise of targeted theranostics seems an interesting avenue. Although targeting may not always lead to elevation of tumor accumulation, it may accelerate this process, which is advantageous for imaging. For example, whole body gamma scintigraphic imaging of mice revealed noticeable tumor contrast 6 h post-injection when administering targeted ¹¹¹In-radiolabeled liposomes, compared to 24 h for non-targeted nanoparticles (92).

As an alternative to radiolabeled nanoparticles, nanomaterials exhibiting an intrinsic utility for both imaging and therapy seem ideal for theranostic agents. For example, iron oxide nanoparticles can be used for both MR imaging and thermal ablation therapy (93), gold nanoparticles for computed tomography (CT) imaging and radiation therapy (94), gold nanoparticles for CT imaging and thermal ablation therapy (94), and porphyrins for photoacoustic imaging and photodynamic therapy (95). When irradiated with the appropriate form of energy, such as near infrared light, radiowaves, or an alternating magnetic field,

porphyrins, iron oxide and gold nanoparticles absorb the incident energy and convert it to heat, which kills the cancerous cells. In the case of radiation therapy, the presence of gold results in radiation dose enhancement primarily owing to a photoelectric effect.

While still in a relatively early stage of technological development, these theranostic agents show promise for cancer therapy due to their simplicity, cost-effectiveness, and their ability to treat chemotherapy-resistant cancers. However, it should be noted that the delivery of high doses of these agents to tumors are often needed and in many cases a direct intratumoral injection is required.

Perspective

Multifunctional nanoparticles featuring targeting ligands and other auxiliary moieties remain to be translated into the clinical domain and the discussion of their utility and cost/benefit ratio seems timely. At the moment the conventional point of view is that adding targeting ligands to therapeutic nanoparticles is worth the additional complexity of synthesis, cost, and regulatory hurdles. The targeted nanoparticles that minimize these issues are likely to see the most immediate clinical translation. Nanoparticles that do not require post-synthesis modification and purification may provide one such solution. This can be accomplished by coupling low-molecular weight targeting ligands (e.g. small molecules like folate, peptides, and aptamers) or bio-responsive materials (e.g. pH-responsive polymers) directly onto nanoparticle starting materials prior to nanoparticle formation. This allows tighter control over synthesis, uniformity, and target ligand density. Although natural biological proteins, e.g. transferrin, require post-synthesis conjugation and purification, their abundance and relatively low cost also make them a favorable option. Nearly all of the targeted nanoetherapeutics that are in early clinical trials use transferrin as a targeting ligand (Table 2). Of course, the choice of natural ligands is very limited. Antibodies present the most flexible and perhaps most desirable option, due to their high specificity and broad selection of targets. However, antibody production, conjugation, and subsequent nanoparticle purification can be extremely costly and time consuming. Immunotherapies such as trastuzumab can cost ~\$70,000 for a full course of treatment (96). Trastuzumab-labeled nanoparticles can easily cost many times this amount due to low nanoparticle conjugation efficiencies alone, which can be <10% (97). Then there is the cost of nanoparticle production, purification, and sterilization to consider. These limitations highlight the need for the continual development of more efficient nanoparticle-antibody coupling strategies. The possibility of lower administered doses and a higher therapeutic index that manifests from the addition of targeting capabilities can also help to offset these costs. Ultimately, however, the motivation to invest in antibody-targeted nanoparticles will likely be driven by the clinical benefits observed with first generation targeted nanoparticles.

The costs and regulatory hurdles associated with adding imaging capabilities to nanoparticles is likely to be significantly lower than that of targeting. However, while theranostic agents can provide insight into tumor accessibility and heterogeneity, this is unlikely to be consistently predictive of tumor response due to a tumors ability to acquire drug resistance. Therefore, imaging agents that are introduced onto therapeutic nanoparticles are likely to play a supporting role to dedicated imaging agents. In cases where the nanomaterial itself possesses both imaging and therapeutic capabilities, the benefits are likely to be more profound. For example, the ability to identify the distribution of gold nanoparticles within a tumor via CT can help demarcate tumor boundaries and identify poorly perfused necrotic or hypoxic regions. This insight can guide radiation treatment planning and highlights the potential impact that some theranostic agents can have on patient management.

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Figure 1.

Schematic of a multifunctional nanoparticle. Multifunctional nanoparticles can be prepared with a wide range of therapeutic, imaging, and targeting agents. Representative examples are shown, with relative expenses indicated by dollar symbols. The addition of each new functionality is expected to have a beneficial impact on patient survival; however, this will come at the cost of additional regulatory, production, and financial hurdles. The trade-off between these “costs” and clinical benefit will be highly dependent on the choices made when designing multifunctional nanoparticles. The following agents are shown: Targeting (left: folic acid, center: antibody, right: aptamer); Imaging (top: chelated Technetium-99m, center: chelated gadolinium, bottom: near infrared fluorescent dye-isocyanine green); Therapy (left: doxorubicin, center: paclitaxel, right: camptothecin).

Figure 2.

Schematic diagrams of targeted nanoparticles that utilize local factors from the tumor microenvironment to improve drug delivery to tumors. (A) Carriers containing MMP-sensitive cell-penetrating peptides. A polycationic cell penetrating peptide is connected to a neutralizing polyanion via a matrix metalloproteinase (MMP)-cleavable linker. Dissociation of the polyanion, following MMP-mediated cleavage enables the activated cell penetrating peptide to associate with and enter cells. (B) pH-responsive glycol chitosan (GC)-nanoparticles. At physiologic pH, the surface amines on the GC-nanoparticles are deprotonated and carry a net neutral charge. Within the acidic tumor microenvironment, the GC-nanoparticles acquire a net positive surface charge and exhibit electrostatic interactions with negatively charged cell membranes, leading to enhanced tumor retention. (C) Action of pH low insertion peptide (pHLIP). pHLIP is a water-soluble membrane peptide that shows little to no interaction with cell membranes at physiologic pH, but at slightly acidic pH forms a stable transmembrane alpha-helix and inserts into the cell membrane. (D) pH-responsive nanoparticles. Nanoparticles shed their protective coating within an acidic tumor microenvironment, exposing an underlying positive surface charge that promotes electrostatic interactions with surrounding cells. This ‘unmasking’ process may also be triggered by other stimuli including reducing agents and proteases.

Table 1

Non-targeted nanoparticles that have been approved for clinical use or undergoing clinical trials

Brand Name	Composition	Indication	Status
Liposome-Based Nanoparticle			
Doxil/Caelyx	PEGylated liposomal doxorubicin	Ovarian cancer, Kaposi's sarcoma	Approved
DaunoXome	Liposomal daunorubicin	Kaposi's sarcoma	Approved
Myocet	Non-PEGylated liposomal doxorubicin	Breast cancer	Approved
Micelle-Based Nanoparticle			
Genexol-PM	Paclitaxel-loaded PEG-PLA Micelle	Breast cancer, lung cancer	Approved
NK911	Doxorubicin-loaded PEG-pAsp Micelle	Various cancers	Phase 2
NK012	SN-38-loaded PEG-PGlu(SN-38) micelle	Breast cancer	Phase 2
NC-6004	Cisplatin-loaded PEG-PGlu Micelle	Various cancers	Phase 1
SP1049C	Doxorubicin-loaded Pluronic Micelle	Gastric cancer	Phase 3
NK105	Paclitaxel-loaded PEG-PAA Micelle	Breast Cancer	Phase 3
Polymer-drug conjugates-Based Nanoparticle			
Xyotax	Paclitaxel combined with a poliglumex	Metastatic breast cancer	Phase 2
IT-101	Camptothecin conjugated to cyclodextrin-based polymer	Various cancers	Phase 1/2
HPMA-DOX (PK1)	Doxorubicin bound to HPMA	Lung cancer, breast cancer	Phase 2
HPMA-DOX-galactosamine (PK2)	Doxorubicin linked to HPMA bearing galactosamine	Hepatocellular carcinoma	Phase 1/2
CT-2106	Camptothecin poly-L-glutamate conjugate	Various cancers	Phase 1/2
Albumin-Based Nanoparticle			
Abraxane	Albumin-bound paclitaxel nanoparticles	Metastatic breast cancer	Approved

PEG, polyethylene glycol; PLA, poly(L-lactide); pAsp, poly(L-aspartic acid); PGlu, polyglutamate; PAA, poly(L-aspartate); HPMA, N-(2-hydroxypropyl)-methacrylamide copolymer.

Table 2

Targeted NPs in clinical development for use in cancer therapy

Name	Composition	Target	Comments	Status
MCC-465	PEGylated liposomal doxorubicin containing antibody GAH targeting agent	Tumor antigen	Metastatic stomach cancer	Phase 1
MBP-426	Liposomal oxaliplatin containing human transferrin protein targeting agent	Transferrin receptor	Advanced or metastatic solid tumors	Phase 1/2
SGT-53	Liposomal p53 cDNA containing antibody fragment targeting agent	Transferrin receptor	Solid tumors	Phase 1
CALAA-01	siRNA-loaded polymeric nanoparticles containing human transferring protein targeting agent	Transferrin receptor	Solid tumors	Phase 1
BIND-014	Docetaxel-loaded polymeric nanoparticles containing peptide targeting agent	Prostate specific antigen	Advanced or Metastatic Cancer	Phase 1

Table 3

Common tumor biomarkers that can potentially be used as nanoparticle targets

Tumor Biomarker	Expression in cancer tissues (% of tumors that expression biomaker)	Expression in normal tissues/cells	Ref
Solid Tumors			
Folate receptor	Ovarian cancer (90%), renal cancer (86%), lung cancer (72%), breast cancer (43%), brain cancer (25%), pancreatic cancer (50%)	Kidney, colon, lungs, placenta, bladder	(16)
EGFR	Non-small cell lung cancer (40% – 80%), colorectal cancer (50% – 80%), ovarian cancer (35% – 70%), gastric cancer (41% – 83%), pancreatic cancer (30% – 50%), breast cancer (14% – 91%), bladder cancer (31% – 72%), head and neck (80% – 100%), glioma (40% – 63%)	Cells that originate from all three germ cell layers, particularly those of epithelial origin (e.g., the skin, liver, and gastrointestinal tract)	(17, 18)
HER2	Non-small cell lung cancer (18%–37%), colorectal (26% – 90%), ovarian cancer (10% – 15%), gastric (38% – 45%), breast cancer (25% – 30%), bladder cancer (9% – 36), glioma (20%–54%)	Skin, breast, placenta, epithelial cells on gastrointestinal, respiratory, reproductive and urinary tract	(17, 19)
PSMA	Prostate cancer (56.7% – 100%), high-grade prostatic intraepithelial neoplasia (48.6% – 100%)	Prostate, kidney, small bowel, colon	(20, 21)
PCLA	Prostate primary tumor (96.6%), metastatic prostate carcinoma (85.3%)	Brain vasculature, benign prostate tissue	(22)
Transferrin receptor	Colon cancer (48%), breast cancer, kidney cancer, lung cancer, stomach cancer, ovarian cancer *	Skin, pancreas, liver, brain (anterior pituitary), testis	(23, 24)
MUC1	Breast cancer (90%), lung cancer, prostate cancer and colorectal cancer *	Mammary gland, respiratory, urinary and reproductive tracts	(25, 26)
Tumor vasculature			
$\alpha_v\beta_3$ integrins	Melanoma, breast cancer, prostate cancer, pancreatic cancer, ovarian cancer, cervical cancer, glioblastoma, and tumor endothelial vessels *	Platelets, very low levels in resting endothelial cells and normal organs	(27)
VCAM-1	Leukemia, lung and breast cancer, melanoma, renal cell carcinoma, gastric cancer	Upregulated on endothelial cells in response to inflammation	(28)
VEGFR	Highly expressed on neovascular endothelial cells **, 73% – 100% in some non-small cell lung cancers	Monocytes, macrophages	(29)
Tem1	Highly expressed on neovascular endothelial cells ** including colon, brain and lung cancers	Expressed on normal endothelial cells	(30, 31)
APA	Upregulated on perivascular cells of tumor blood vessels, Stromal cells surrounding prostatic carcinoma cells (73%), non-keratinizing type cervical squamous cell cancer (90%) renal cancer (clear cell cancer) *	Expressed in the proximal tubules and glomerulus of nephron (kidney), upregulated in inflamed synovia, granulation tissue, low expression in capillaries and venules of pancreas, lymphoid tissue and intestinal mucosa	(32–35)
Supporting cells			
TAMs	Breast, prostate, ovary, cervix, stomach, lung, glioma and bladder cancers *	**	(36)
TAFs–FAP	Overexpressed in 90% of stromal fibroblasts in colon, lung and breast carcinoma	Fibroblasts in healing and inflammation	(37)
TEMs	*	Endothelial cells, hematopoietic cells	(38)

* Percentages of tumors expressing these receptors is not available;

** Not applicable

EGFR, Epidermal growth factor receptor; HER2, Human epidermal growth factor receptor 2; PSMA, Prostate specific membrane antigen; PCLA, Prostate cancer lipid antigen; MUC1, Mucin-1; VCAM-1, Vascular cell adhesion molecule 1; VEGFR, Vascular endothelial growth factor

receptor; Tem1, Tumor endothelial marker 1; TAMs, Tumor associated macrophages; TAFs-FAP, Tumor associated fibroblasts–fibroblast activation protein; TEMs, Tie-2 expressing monocytes; APA, Aminopeptidase A.