Treating metastatic cancer with nanotechnology

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Abstract | Metastasis accounts for the vast majority of cancer deaths. The unique challenges for treating metastases include their small size, high multiplicity and dispersion to diverse organ environments. Nanoparticles have many potential benefits for diagnosing and treating metastatic cancer, including the ability to transport complex molecular cargoes to the major sites of metastasis, such as the lungs, liver and lymph nodes, as well as targeting to specific cell populations within these organs. This Review highlights the research, opportunities and challenges for integrating engineering sciences with cancer biology and medicine to develop nanotechnology-based tools for treating metastatic disease.

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doi:10.1038/nrc3180

Metastasis, the spread of cancer cells from a primary tumour to seed secondary tumours in distant sites, is one of the greatest challenges in cancer treatment today. For many patients, by the time cancer is detected, metastasis has already occurred. Over 80% of patients diagnosed with lung cancer, for example, present with metastatic disease[1]. Few patients with metastatic cancer are cured by surgical intervention, and other treatment modalities are limited[2]. Across all cancer types, only one in five patients diagnosed with metastatic cancer will survive more than 5 years[1].

Although cancer therapies are improving, many drugs are not reaching the sites of metastases, and doubt remains over the efficacy of those that do. Methods that are effective for treating large, well-vascularized tumours may be inadequate when dealing with small clusters of disseminated malignant cells. As the biological mechanisms of metastasis (FIG. 1) are being unravelled, it is becoming clear that new approaches to treat this condition may become available. We expect that the expanding capabilities of nanotechnology, especially in targeting, detection and particle trafficking, will enable novel approaches to treat cancers even after metastatic dissemination[3].

Nanotechnology has encountered several hurdles in its quest towards application, but it is not alien to the clinic; for example, liposome-encapsulated doxorubicin (Doxil; Johnson & Johnson) is widely used to treat ovarian cancer and Karposi's sarcoma (more than 300,000 patients are treated annually) while protecting patients from the cardiotoxicity of the unencapsulated drug[4]. Protein nanoparticles containing paclitaxel (Abraxane; Abraxis Bioscience), which are approved to treat metastatic breast cancer, have been shown to enhance tumour uptake of the drug[5]. Iron oxide nanoparticles (ferumoxytol), which are approved for the treatment of iron deficiency anaemia, have shown efficacy for the early staging of lymph node metastasis in patients with prostate and testicular cancers[6,7]. Although the first generation of more than 40 nanotherapeutics has reached the clinic[8], we expect that future systems will introduce new capabilities, including advances in targeting metastatic sites and their earlier detection through more sensitive imaging techniques.

The rationale of this Review is to outline the current state of nanotechnology with respect to its use in treating metastatic cancer. For the purpose of this Review, we define nanoparticles as synthetic constructs that are composed of organic or inorganic matter, the dimensions of at least two axes of which are between 1 and 1,000 nanometers.

There is a wide array of nanomaterial-based therapeutic approaches under development. For example, nanoparticles can be engineered to detect a stimulus, such as a molecular binding event or ionic concentration change, and respond by releasing cargo, degrading or even carrying out the chemical modification of drugs *in vivo*. Importantly, in addition to their potential to combine multiple therapeutic functions into a single platform, nanoparticles can be targeted to specific tissues, reach particular subcellular compartments or target malignant cells in circulation (FIGS 1,2). As we enter an era of personalized cancer medicine, nanomaterials may even provide platforms for the delivery of modular

At a glance

- In most patients, by the time cancer is detected, metastasis has already occurred. More than 80% of patients diagnosed with lung cancer, for example, present with metastatic disease.
- Nanotechnology is not alien to the clinic; more than 40 nanotherapeutics have reached patients, including anticancer drugs and imaging agents.
- Many current therapies are not reaching the sites of metastases. Nanomaterials have the potential to combine multiple therapeutic functions into a single platform, can be targeted to specific tissues and can reach particular subcellular compartments.
- Primary targeting is the act of steering nanoparticles to the specific organ or organs in which the metastases reside.
- Secondary targeting is the direction of these delivered materials to the cancer cells and potentially to a specific subcellular location within the cancer cell.
- Many solid tumours exhibit the enhanced permeation and retention (EPR) effect through which nanomaterials may accumulate and be retained in the tumour. However, this effect is limited to tumours larger than ~4.6 mm in diameter, hindering its use for targeting small, unvascularized metastases.
- To treat the complex problem of metastatic cancer, we must combine the expertise of engineers, biologists and clinicians.

personalized therapies. However, to realize all of these goals, we need to carefully consider the biology of the metastatic process and engineer nanomaterials accordingly.

Therapeutic mechanisms

Nanoparticle therapeutics vary from carriers of small-molecule drugs or biomacromolecules, such as proteins or small interfering RNA (siRNA)[9–18], to vehicles for imaging and thermal absorption (FIG. 2). The potential benefits of loading drugs into nanoparticles (or alternatively, conjugating drugs to their surface), include targeting drugs to the disease site; triggering drug release in specific locations in the body[19,20]; and changing the pharmacokinetic profile of a drug to increase its half-life at the disease site[21]. These capabilities can potentially reduce off-target effects and lower the amount of drug that must be administered[4,22]. Nanoparticles offer more complex delivery-related capabilities, such as concomitant delivery of a drug with a molecule that modulates the vasculature[23,24], administration of a prodrug with its activator enzyme[25,26], or the administration of immunotherapy with a targeting ligand[27–30]. The physiological microenvironment[31,32], or, alternatively, external stimuli, such as ultrasound[33–35], light[36–38] or radio-frequency electromagnetic fields[39,40], can be used to trigger local drug release.

The encapsulation and delivery of drugs is not the only therapeutic function of nanoparticles. Thermoablative therapy (the heating of tissues in order to kill tumour cells) can be enhanced by activating nanomaterials, which are localized in the diseased tissue, with magnetic fields, infrared light and radio-frequency[41–44]. Each of these external triggers has both advantages and limitations; for example, electromagnetic fields can penetrate deeply (>15 cm) into tissue with minor energetic losses, but they are difficult to focus[39,45]. High-intensity focused ultrasound (HIFU) can penetrate deeply into tissue and can be focused to a volume of several mm^3, but its capabilities are diminished when applied within bones or gas-filled organs[46]. Infrared light, of wavelengths spanning ~750–1,300 nm, penetrates tissue to depths of up to 1 cm, after which penetration decreases substantially[47]. Therefore, the use of infrared would mostly apply to lesions near the skin surface, during surgical procedures or with a minimally invasive laser catheter. Recently, radiofrequency-induced heating of gold nanoparticles has generated interest, and deep tissue-penetration is predicted to be possible[48].

Targeting metastatic cancer

Targeting nanoparticles to sites of metastasis can be divided into two steps. Primary targeting is the act of steering nanoparticles to the specific organ or organs in which the metastases reside. Secondary targeting is the direction of these delivered materials to the cancer cells and even to a specific subcellular location within the cancer cell. The current progress in primary and secondary targeting is described below.

Primary targeting — getting to the organ. Particle size, surface charge, mechanical properties and chemistry, as well as the route of administration, all have major roles in determining the localization of nanoparticles to specific organs (TABLE 1). Several studies have provided informative trends regarding how these properties affect nanoparticle biodistribution and targeting. For example, only particles of a limited size will be able to exit or enter fenestrated vessels in the liver endothelium or in the tumour microenvironment[49]. Particle surface charge can cause arrest in certain tissues, and material composition can also change the fate of nanoparticles, such as lipid complexes, which accumulate in the liver. Ultimately, life-threatening metastases occur most often in the brain, lungs, liver, lymph and bone (FIG. 3); we will therefore focus on targeting these organs.

The brain is considered to be the most challenging organ to target with intravenously administered nanoparticles[50]. Protected by a lining of endothelial cells that are tightly bound to one another, the blood–brain barrier (BBB) permits only certain materials to cross from the circulation into the cerebrospinal fluid. In general, gases (such as CO_2 and O_2), metabolic products (such as glucose) and hormones, as well as small, electrically neutral lipid-soluble molecules are exchanged across the BBB[51]. However, diseases of the central nervous system, including brain tumours and metastases, can disrupt the integrity of the BBB[3], thereby altering the ability of therapeutics to access the brain[52–55].

Unlike other organs that permit the uptake of materials in the higher nanoscale range, it has been reported that transportation of particles across the BBB requires a particle size of under 15 nm[55,56]. Particles in the range of 15–100 nm may also penetrate the brain, but with uptake efficiency that decreases exponentially with size[56,57]. Modifying the particle surface with lipophilic moieties and reducing surface charge may contribute to BBB passage[57–60]; such nanoparticles bind apolipoprotein E (ApoE) following systemic administration[61,62]. These ApoE-decorated particles can mediate BBB delivery[63,64]. Interestingly, ApoE also facilitates the trafficking of

Shear rates
The velocity gradient that is the relative velocity at which one layer of the fluid flows over an adjacent layer of the fluid.

Kupffer cells
A type of macrophage that lines the sinusoid walls of the liver and that removes toxins present in blood coming from the digestive tract. Involved in the breakdown and recycling of red blood cells and haemoglobin.

siRNA-loaded lipid nanoplexes to liver hepatocytes[62,65,66]. Conjugating monoclonal antibodies that target BBB receptors to the surface of nanoparticles has also been reported to increase uptake into the brain parenchyma[67].

Nanoparticles have been shown to be trafficked to the brain after being taken up by cells in the circulation. For example, sugar-coated nanoparticles can be phagocytosed by leukocytes and macrophages[55,68]. Such cells accumulate at sites of BBB degradation that are associated with disease, and they have the capacity to infiltrate the brain[69–71]. By targeting these cells in the circulation, nanoparticles might be trafficked into the brain, where they can ultimately be released[68,72]. The ability of cells to infiltrate deep tissue, cross biological membranes and target disease sites has made them attractive carriers of nanoparticles[73,74].

Lymph nodes, which are linked by lymphatic vessels, are distributed throughout the body and have an integral role in the immune response. Dissemination of cancer cells through the lymph network is thought to be an important route for metastatic spread. Tumour proximal lymph nodes are often the first site of metastases, and the presence of lymph node metastases signifies further metastatic spread and poor patient survival[75]. As such, lymph nodes have been targeted using cell-based nanotechnologies.

Certain characteristics are associated with preferential (but not exclusive) nanoparticle trafficking to lymph nodes following intravenous administration[76–79]. Targeting is often an indirect process, as receptors on the surface of leukocytes bind nanoparticles and transfer them to lymph nodes as part of a normal immune response[76]. Several strategies have been used to enhance nanoparticle uptake by leukocytes in circulation. Coating iron-oxide nanoparticles with carbohydrates, such as dextran, results in the increased accumulation of these nanoparticles in lymph nodes[78–80]. Conjugating peptides and antibodies, such as immunoglobulin G (IgG), to the particle surface also increases their accumulation in the lymphatic network[81]. In general, negatively charged particles are taken up at faster rates than positively charged or uncharged particles[76,77]. Conversely, 'stealth' polymers, such as polyethylene glycol (PEG), on the surface of nanoparticles, can inhibit uptake by leukocytes[82–84], thereby reducing accumulation in the lymph nodes.

Lymph node targeting may be achieved by other routes of administration. Tsuda and co-workers[85] reported that non-cationic particles with a size range of 6–34 nm, when introduced to the lungs (intrapulmonary administration), are trafficked rapidly (<1 hour) to local lymph nodes. Administering particles <80 nm in size subcutaneously also results in trafficking to lymph nodes[86,87]. Interestingly, some studies have indicated that non-pegylated particles exhibit enhanced accumulation in the lymphatics and that pegylated particles tend to appear in the circulation several hours after administration[86].

The liver is a frequent site of metastasis. This well-vascularized tissue, with low shear rates and accessible capillaries, provides a 'friendly soil' for cancer cells. Many researchers think that targeting the liver is a simple task because various injected agents can accumulate in this organ. However, recent studies that aimed to deliver siRNA to liver hepatocytes showed that precise particle engineering is required to pass through the fenestrae that are present in the liver endothelium[88]. In general, intravenously administered nanoparticles accumulate in activated Kupffer cells that reside within and near the liver vasculature and so do not reach the hepatocytes[89]. Active targeting through endothelial fenestrations to hepatocytes has been facilitated via ApoE adherence to the particle surface while in the circulation, or by conjugating carbohydrates, such as *N*-acetylgalactosamine (GalNAc), to the surface of the particle[90]. This is an example of how organ and cell targeting are often interconnected. An ongoing clinical trial is using lipid nanoparticles as siRNA carriers for treating liver cancer and metastases[91].

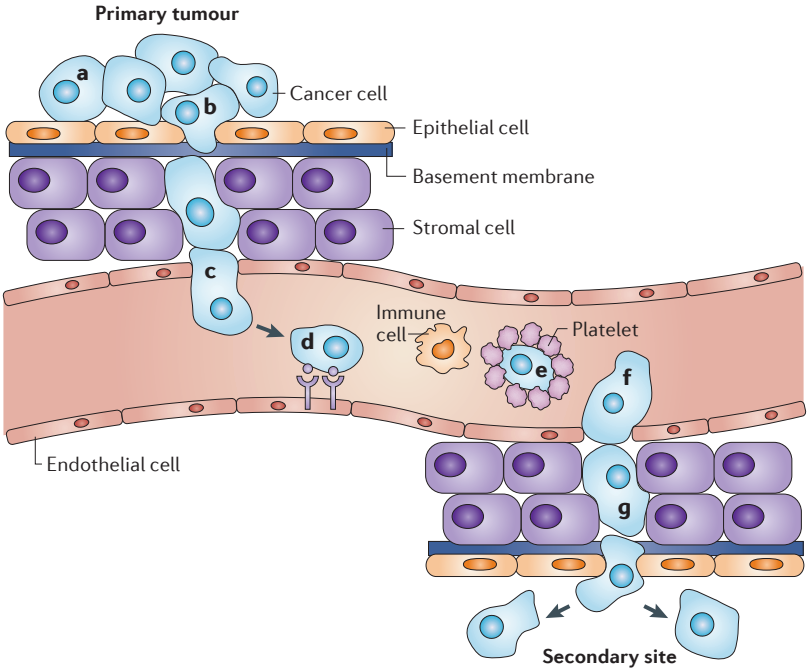

Figure 1 | **The steps of metastasis and opportunities for therapeutic intervention.** Metastasis requires several steps, each of which presents an opportunity for new therapies. First, metastatic cells must break free from the primary tumour. To accomplish this, cancer cells (**a**) reduce adhesion to neighbouring cells and (**b**) clear a path for migration into the vasculature-rich stroma[210–213]. Once at the vasculature, cells can freely enter the bloodstream if the vasculature is discontinuous, such as in certain regions of the liver, bone marrow and kidneys. Intravasation (**c**) is required if the vasculature is continuous; cells either cause endothelial cell retraction by releasing compounds such as vascular endothelial growth factor (VEGF) or endothelial cell death by releasing reactive oxygen species and factors including matrix metalloproteinases (MMPs)[214,215]. In the bloodstream, cancer cell distribution is determined by blood flow and interactions between cancer cells and the secondary organs that they colonize: cells can get trapped in narrow capillary beds, such as those of the lung and liver, and can also express receptors that bind to metastasis-supporting sites (**d**) or to platelets (**e**), which protect the cancer cells from the immune system[216–220]. Cancer cells can circulate for more than 2 hours, suggesting that they do not always become lodged in the first capillary beds that they reach[221]. After reaching the secondary site, cancer cells can exit the bloodstream (**f**) by inducing endothelial cell retraction or death[222,223]. To proliferate in the secondary site, cancer cells co-opt the local environment by releasing pro-inflammatory compounds and proteinases that induce their neighbours to release growth factors[224,225] (**g**).

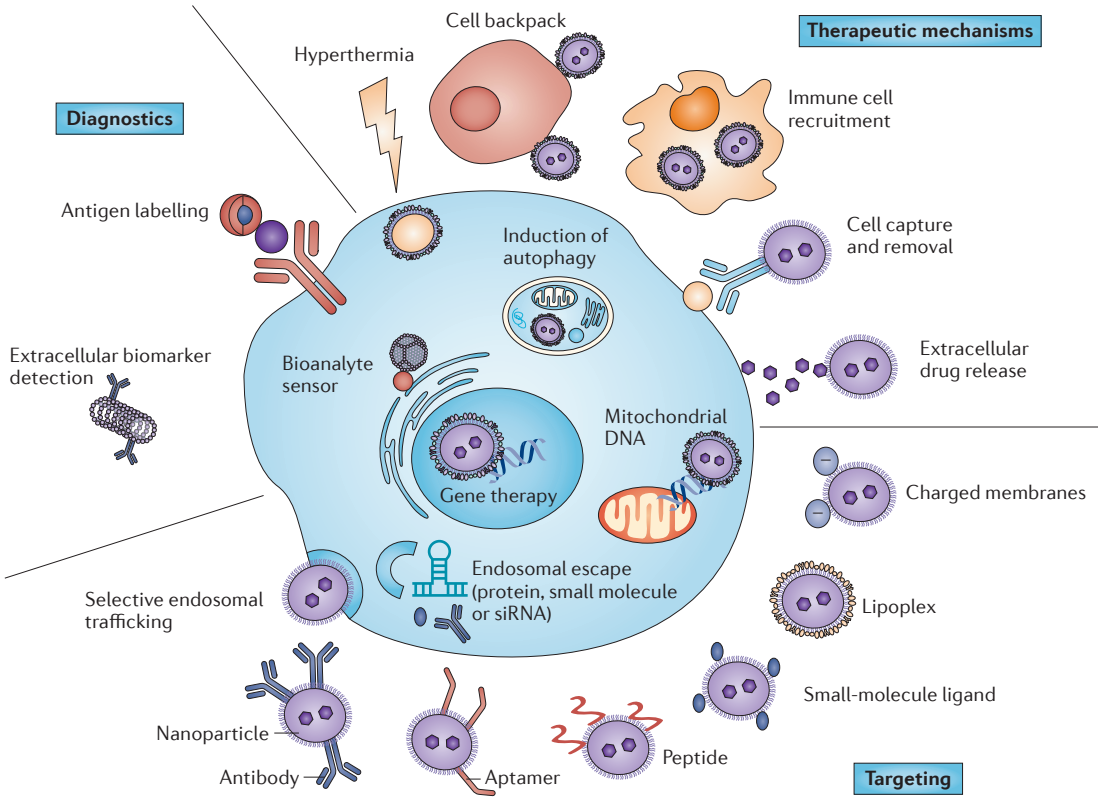

Figure 2 | **Nanomaterial strategies from the point-of-view of the cell.** The ability to target nanoparticles to cancer cells (secondary targeting) and to influence their uptake into specific cellular compartments (tertiary targeting) is now feasible. This figure summarizes unique targeting, diagnostic and therapeutic mechanisms as they relate to the cancer cell. siRNA, small interfering RNA.

Like the liver, the tumour vasculature can have fenestrae; however, these spaces are even larger — up to 600 nm in the tumour compared with <200 nm in the liver[92–95]. Furthermore, tumour-associated lymphatic drainage is often inefficient[93]. Consequently, some solid tumours will exhibit the enhanced permeation and retention (EPR) effect whereby nanomaterials may accumulate in the tumour and be retained[92,93,96] (FIG. 4). This effect has been harnessed to direct macromolecule-based therapies to a tumour site[92,96], but it has limitations: it is present in tumours of more than ~100 mm^3 in volume, hindering its use for targeting small or unvascularized metastases[97].

The lungs can be accessed directly through inhalation or indirectly following intravenous administration. Aerosolized, low-density (<0.4 g per cm^3) micron-sized particles (>5 μm) can be engineered so that they are retained in the lungs for prolonged periods of time[98]. Alternatively, targeting the lungs by intravenous administration has been reported to be more effective using particles that are larger than 300 nm in diameter[99]. Smaller particles tend to reside in the lungs during the first 1–2 hours post-administration and are thereafter detected in other organs[100]. This is explained by larger particles being physically trapped in the intricate capillary beds of the alveoli[101]. For both intravenous and intratracheal routes of administration, cationic nanoparticles tend to accumulate at higher levels and have longer residence times in the lungs than negatively charged or neutral nanoparticles[104]. It has been hypothesized that positively charged particles bind to erythrocytes and serum proteins, resulting in their inability to exit the lung owing to the large size of the complex[105,106]. Recently, Cohen and co-workers used intravenously administered 300 nm particles to target laminin receptors that are overexpressed in melanoma metastasis residing in the lungs of mice[107].

The sinusoidal vasculature of bone marrow facilitates the migration of haematopoietic progenitor cells and circulating cancer cells. The majority of clinically detectable prostate metastases and breast metastases are present in the bone, suggesting that the bone has an active role in cancer cell recruitment, survival and outgrowth[108]. Bone metastases, which often occur in high multiplicity, can result in bone degradation and complications such as fractures, hypercalcaemia and nerve compression[109,110]. Targeting nanoparticles to bone is still in its infancy[110], and current investigative agents include small molecules and proteins targeted to hydroxyapatite, the calcium-containing mineral which constitutes up to 50% of bone[111]. Compounds, such as bisphosphonate, which has been used to treat osteoporosis and bone metastases, have been shown to increase the accumulation of nanoparticles in the bone[112]. Nanoparticles targeting the bone marrow could also make use of the sinusoidal endothelium to increase the delivery of agents that directly target cancer cells[113].

Table 1 | **Primary targeting — general considerations for nanoparticle delivery to specific organs**

Target organ	Particle size	Surface property	Comments
Brain	5–100 nm: uptake efficiency decreases exponentially with size	Lipophilic moieties and neutral charge enhance brain uptake	Leukocytes can take up nanoparticles in circulation and then carry them to disease sites in the brain
Lung	>200 nm: particles are trapped in lung capillaries	Positive surface charge	Inhaled particles with low density (<0.4 g per cm^3) and of large size (>5 mm) are also retained in the lung
Liver	<100 nm, to cross liver fenestrae and target hepatocytes. >100 nm particles will be taken up by Kupffer cells	No specificity needed	Lipid and lipid-like materials tend to accumulate in the liver
Lymph nodes	6–34 nm: intra-tracheal administration. 80 nm: subcutaneous administration	Non-cationic, non-pegylated and sugar-based particles	200 nm particles in circulation can be taken up by leukocytes and trafficked to lymph nodes
Bone	Unknown	Compounds such as alendronate and aspartic acid adhere to bone and have been used for bone targeting	Despite great importance, bone targeting is under-researched

Once at the target organ, steering nanoparticles towards the malignant cells poses an additional challenge. This has been addressed using several approaches. One approach is to use external driving forces, such as magnetic fields to concentrate iron oxide nanoparticles[114,115], or acoustic waves to trigger micro-bubble localization[116]. Recently, an active nano-signalling system was developed, in which one targeted nanomaterial triggers a local biological cascade that, in turn, recruits other therapeutic nanoparticles to the disease site[117]. This ability to amplify a local signal may be especially important for locating and treating metastases. Another mechanism for steering nanomaterials to a disease site is by using self-propelled nanoparticles that can navigate autonomously[118,119].

Secondary targeting — the cancer cell. Targeting a metastatic cancer cell, either in transit from the primary tumour or buried within a population of non-cancerous cells, presents a unique challenge. In contrast to primary targeting, secondary targeting is the precise homing of a particle or a drug to a specific cell type (FIGS 2,3). It can require a chemical specificity that enables the nanoparticle to bind to unique moieties that are presented by the cancer cell. Cancer cells, whether metastatic or part of the primary tumour, can upregulate certain cell-surface molecules and secreted factors, and may even express proteins that are usually only expressed during embryonic development[120]. A metastatic cell will also express endogenous surface proteins from its site of origin, which will differ from its site of implantation. For example, a metastatic pancreatic cancer cell is distinct from cells within the liver strictly by virtue of its pancreatic origin. These characteristics provide investigators with handles to target cancer cells. They may also limit the potential side effects of targeting proteins that are expressed by a given cell type that are not exclusive to the cancer cell.

The strategies needed for targeting specific cells (secondary targeting) may differ from those used for targeting the organ (primary targeting). The researcher must take into account the binding affinity of the nanoparticle to the molecules of interest, as well as binding specificity and immunological effects. Antibody conjugates — drug, polymer or radioisotope-labelled antibodies — are currently in the clinic for targeting cancer. For instance, ^{131}I-tositumomab (Bexxar; GlaxoSmithKline) is a combination therapy that involves a radiolabelled CD20-specific antibody for targeting follicular B cell lymphoma[121]. Antibody-based targeting ligands have been used on various nanodelivery systems[122–126]. Likewise, short peptides, including those with integrin-binding domains RGD and IKVAV, can be appended to nanoparticles and can increase their binding to specific cell types within a tissue[107,127–129].

High-throughput methods, such as phage display, ribosome display, *in vitro* evolution and *in vitro* selection are being used to discover new targeting ligands[130], such as antibodies, peptides and nucleic acid-based ligands (aptamers)[131,132]. Pegaptanib, an anti-angiogenic aptamer-based agent, is being used clinically for the treatment of macular degeneration[133]; however, aptamers have not yet been approved for cancer treatment. Peptide nucleic acids (PNAs) can bind with a high affinity to complimentary DNA strands, and the peptide backbone allows covalent modification with targeting ligands and fluorophores[134]. PNAs have been used to target pro-metastatic genes and to inhibit their expression[135].

Small-molecule-binding domains, such as the folate receptor which is overexpressed in human oral carcinoma, metastatic breast, colorectal and other cancers, are also under investigation and demonstrate affinity to nanoparticles coated with folic acid[136–139]. Certain cells, such as macrophages, which routinely phagocytose particles, can be targeted with materials, such as dextran[78], which resemble lipopolysaccharides that are expressed on the surface of bacteria. Phagosomes tend to fuse with lysosomes, however, resulting in the degradation of the contents; thus, strategies must be used to control the route of uptake[140].

The route through which nanoparticles enter a cell can also be engineered, potentially affecting the cellular compartment into which a drug is released. This is an emerging area and will probably evolve into a separate field of tertiary targeting[141], because the intracellular fate of the particle can determine the resulting efficacy of the encapsulated drug[142]. Clathrin-dependent endocytosis, one of the most well-characterized pathways of cell uptake, primarily results in entrance to the lysosomal pathway[143]. This type of endocytosis can be triggered by

Phage display
A selection technique in which a library of peptide or protein variants is expressed on the outer membrane of virus-infected bacteria (phage virion) and then screened for binding affinity using a process called panning.

Ribosome display
A selection technique in which diverse gene sequences encoding functional proteins are produced by ribosomes and then screened for their affinity to bioactive targets using a process called panning.

Aptamers
Oligonucleotides with high binding affinity to proteins or other molecules.

Peptide nucleic acids
Artificial polymers that mimic the DNA or RNA base structure, but that replace the negatively charged deoxyribose and ribose sugar backbone with *N*-(2-aminoethyl)-glycine units linked by peptide bonds.

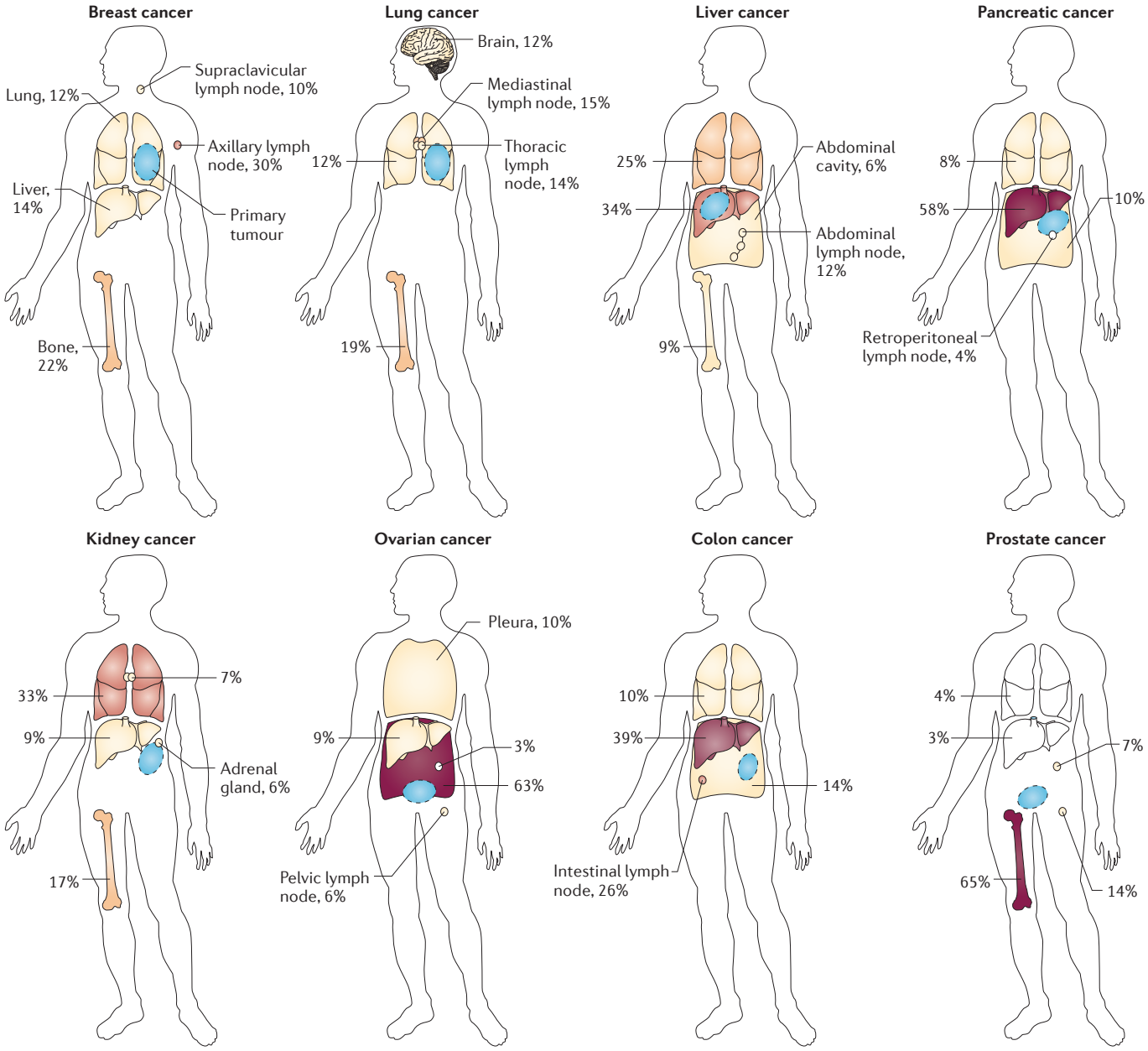

Figure 3 | **Metastatic spread to different organs.** Blood flow patterns can predict the specific regions of metastases in approximately two-thirds of cancers[108]. For example, blood from the gastrointestinal tract flows through the hepatic-portal vein to the liver, where metabolic and detoxification processes are carried out. Following this pattern, the vast majority of metastatic colorectal tumours and the majority of metastatic pancreatic tumours spread to the liver[108]. In such a manner, magnetic nanoparticles, which are bound to an affinity ligand, can be used to remove circulating cancer cells[151,152]. Polymeric nanomaterials can scavenge cancer cell debris from circulation[22], and certain non-spherical, worm-like polymeric micelles (known as filomicelles), which have been reported to have long circulation times in the blood, may also be used for such applications[155,226]. Percentages refer to the relative incidence of metastatic spread to a specific organ for a specified cancer type. Adapted from REF. 108.

the protein transferrin or ligands for glycosylated receptors[144]. Lysosomes sequester their contents from the cytosol and are rich in enzymes that degrade their contents. Therefore, lysosomal components may also degrade nanoparticles and their cargo or may otherwise inhibit drug function by preventing access to the cytoplasm and nucleus. To bypass the lysosome, a nanoparticle can be engineered to break out of endosomes or enter the cell through non-lysosomal pathways. One proposed mechanism with which to disrupt lysosomes is the proton sponge effect, whereby nanoparticles with cationic surface groups induce osmotic lysis upon endosome acidification[145,146]. Uptake through caveolin-dependent endocytosis, which can bypass the lysosome, can be mediated by nanoparticles that are coated with folic acid, cholesterol or albumin[144]. Micropinocytosis, a less well-understood

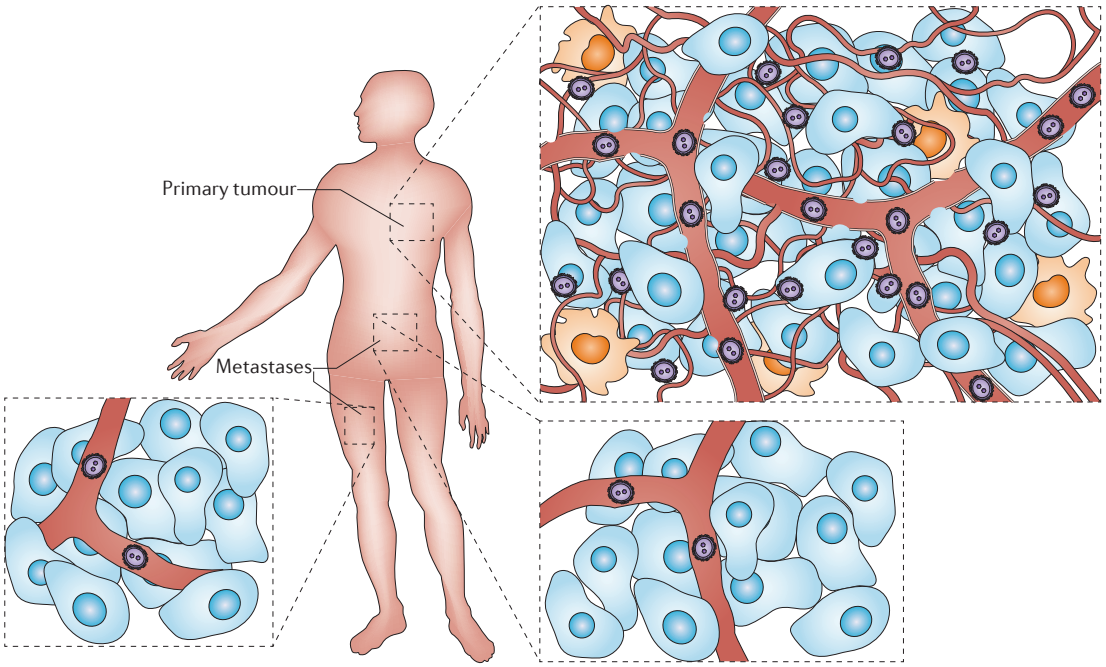

Figure 4 | **The enhanced permeation and retention (EPR) effect.** The EPR effect enables nanomaterials to accumulate and be retained by a tumour. A large primary tumour and its secondary metastasis are shown. Nanoparticles circulating in the blood can accumulate in a large and well-vascularized tumour by extravasating through leaky blood vessels at the tumour site. The particles are retained at the tumour site owing to poorly functioning lymphatic drainage. Small metastases (<100 mm^3 in size) are poorly vascularized and are not well accessed by nanoparticles via the EPR effect; therefore, alternative targeting methods are necessary.

process that forms vacuoles that are larger and distinct from clathrin and caveolin-coated vesicles[143], can be initiated by certain cell-penetrating peptides and lipid-like materials[147,148].

Therapeutic nanocarriers

Nanoscale vehicles have been derived from biological, organic and inorganic origins to address a wide variety of biological mechanisms and targets (TABLE 2). Lipid-based and polymeric materials constitute the majority of nano-vehicles that have been used to date because of their properties that enable the addition of targeting moieties, such as antibodies, their ability to degrade under specific conditions, and their capacity to carry a large amount of drug. A variety of new materials with potential as delivery agents include DNA origami cages[149], macrophage-specific nanoparticles[150], targeted magnetic nanoparticles[151,152], gold nanomaterials[153], functionalized carbon nanotubes[154], worm-like filomicelles[155], silica particles[156,157], modified plant viruses[158,159], nanodiamonds[160] and others. This abundance of options, in fact, creates a new challenge for engineers who need to identify the appropriate combination of materials that will produce the most effective therapies. From liposomes and polymeric formulations to iron oxide particles and modified plant viruses, various materials and methods have been finding their niche in targeting metastasis.

Polymeric materials make up the largest category of vehicles for carrying drug payloads. Several subtypes include core-shell particles, which often involve a material that surrounds a drug payload using non-covalent forces[161,162]. A noteworthy example is poly(lactic-*co*-glycolic acid) (PLGA)-based biodegradable nanoparticles, which are made from US Food and Drug Administration (FDA)-approved materials that incorporate hydrophobic drugs[163]. Polymeric micelles are non-crosslinked particles involving block co-polymers, a single polymer chain which incorporates more than one block of identical molecules. A simple polymeric micelle will contain many amphipathic polymers, which mimic the tail and head portions of the micelle. These spontaneously form micelles around hydrophobic drugs. Polymeric nanoparticles with controlled sizes and shapes have been generated to permit cell attachment but to prevent internalization, allowing a cell to carry a drug payload to a second site for delivery, in effect creating a 'cell backpack' (REF. 73). Polymers with long circulating times may be used for targeting circulating tumour cells (FIGS 1,2). Hydrogel nanoparticles, also known as nanogels, are crosslinked, hydrophilic polymer networks that swell when in contact with water in aqueous environments[164]. Nanogels can be engineered to covalently or non-covalently bind drugs or targeting ligands. They can also swell or shrink in response to factors such as pH or temperature.

Lipids are amphiphilic small molecules that can self-organize into vesicles (lipid bilayers and liposomes), micelles or lipoplexes (amorphous particles)[165]. These vehicles can be modified for the targeted delivery of both water-soluble and insoluble therapeutics. Properties such as size, carrying capacity and targeting capabilities can also be modified. Coupled with appropriate targeting ligands, such as integrin-binding peptides, liposomes can

DNA origami cages
The specificity between complementary DNA base pairs enables constructing nanoscale architectures using a combination of predesigned long and short DNA strands.

Filomicelles
Worm-like micelles that are composed mainly of biodegradable materials that can reach up to several microns in length and that can remain in circulation for long periods of time after intravenous administration.

Table 2 | **Nanoparticulate building blocks and their uses**

Building block	Vehicle	Uses
Polymers	Core-shell nanoparticles, nanogels and polymer micelles	Well-characterized, biocompatible and modular delivery vehicles
Lipids	Liposomes, lipoplexes, micelles and filomicelles	Delivers water-soluble and -insoluble drugs effectively
Metals	Gold nanorods, gold nanoparticles, iron oxide nanoparticles and quantum dots	Imaging agents for diagnosis. Thermoablative therapies
Carbon	Carbon nanotubes, nanodiamonds and graphene	Near-infrared emissions allow for tissue-transparent imaging for diagnosis and tracking. Therapies to potentially sidestep MDR in some leukaemias
Biologicals	Viruses, nucleic acid nanoparticles, DNA origami and protein nanoparticles	Viruses deliver a non-covalently bound payload without loss from passive diffusion

MDR, multi-drug resistance.

accumulate in the tumour vasculature during angiogenesis[166] and can deliver a therapeutic payload. pH-sensitive and temperature-sensitive formulations have been developed to control the release of the payload[167,168]. Synthetic, lipid-like materials that form lipoplexes have been produced by combinatorial techniques for applications such as siRNA delivery[88,148].

Gold nanoparticles have been used for thermoablative therapies[41]. Gold shells, spheres and rods respond to near-infrared light by releasing energy in the form of heat that induces the coagulation of the tumour vasculature and that can cooperatively increase the therapeutic effect of other targeted therapies[169]. Gold nanoparticles can also be used as scaffolds to which multiple ligands are attached[170]. Other nanomaterial classes, such as iron nanoparticles and carbon nanotubes or spheres (buckyballs), have been used to deliver therapies, often by binding the drug to the outer surface or by filling the interior, where applicable[171].

Biological response to nanomaterials

Different types of materials exhibit varying bio-distribution, compatibility, degradation and circulation properties. No single parameter can be denoted as the most important prerequisite for effective cancer therapy. Recent studies have identified cytokines that are upregulated after the administration of positively charged nanoparticles[104,172]. Compliment activation has been associated with nanoparticle administration. Particles with positive surface charge activate the classical compliment pathway, and negatively charged particles activate the alternative (lectin) pathway[173,174]. Interestingly, it has been shown that different degrees of PEG on the surface of nanoparticles affect the complement activation pathways; lower levels of PEG are associated with the classical pathway, while higher degrees of PEG are associated with moderate activation of the lectin pathway[175]. Particle size also has a role in this process; the larger the nanoparticle, the higher the extent of opsonization[176]. In many cases, adverse biological responses to nanoparticle administration, such as inflammation or compliment activation, can be treated with pre-therapy or post-therapy medication[177].

Tunable imaging agents
The emission wavelength of quantum dots can be modulated by changing their size.

In an attempt to improve the biocompatibility of nanoparticles *in vivo*, a hybrid biomimetic approach has been undertaken. Nanoparticles were disguised by coating them with a naturally derived erythrocyte membrane (also known as 'red-blood-cell ghosts')[178–182] or by physically loading the particles into stem cells, thereby evading reticuloendothelial system (RES) clearance and using natural pathways to target cancer[180,183,184]. A different approach uses cell membranes as scaffolds for constructing nanoparticles, using targeting moieties that are naturally present on the cell surface and of the biocompatibility of biologically derived materials[185,186]. Taking advantage of the body's natural trafficking modalities (that is, cells and complex proteins) is a new and promising approach for delivering nanoparticles to specific tissue compartments.

The toxicity of nanomaterials is under investigation; a meta-analysis illustrates that their effect on tissues depends on the physicochemical properties of the materials used, including size, charge and coating ligands[187]. For example, the semiconductor cores of quantum dots can be cytotoxic, but certain polymer coatings have reduced toxic effects *in vivo*[187]. Nanoscale gold particles exhibit minimal toxicity on mammalian tissues, but they do not naturally degrade *in vivo* and can accumulate in organs unless their surface is decorated with stealth materials such as PEG[188]. Careful engineering of drug carriers can potentially reduce the amount of foreign material, both drug and nanoparticle, that is administered to the patient[8,148].

Diagnosis and detection

The treatment of metastatic disease increasingly depends on imaging and diagnostics (FIG. 2; TABLE 2). Some tools, such as directed radiotherapy, require precise tumour localization, and treatment decisions are based on understanding the extent of disease spread. Diagnostics, such as contrast agents for radioimaging, visualization aids for surgeons and molecularly activated sensors, comprise an active area of investigation for materials engineers working at the nanoscale. Much of the excitement in this area stems from the unique material properties that appear at this scale. For example, the fluorescent properties of highly photostable tunable imaging agents, such as quantum dots, only appear when semiconductor crystals are synthesized with nanometer dimensions. For patients with metastatic cancer, the work in this field has the potential to reduce toxicity while increasing the specificity and signal strength of imaging agents; enable the visualization of metastases during surgery; and provide molecular sensors to aid in many areas, from the dosing of chemotherapy to defining the onset of malignancy.

For magnetic resonance imaging (MRI), superparamagnetic nanoparticles consisting of iron oxide (SPIONs) can yield higher contrast at lower concentrations than gadolinium, a common MRI contrast agent. Such particles that are decorated with dextran, which localize within lymph nodes, have been studied for nodal tumour detection in patients with prostate cancer[80]. Targeted SPIONs, coated with RGD peptide, have been investigated *in vivo* to image integrin $\alpha_V\beta_3$-positive tumour neovasculature[189]. Nanoparticles have been explored for

targeting gadolinium-based contrast agents. For example, gadolinium-encapsulated carbon fullerenes and gadolinium-DOTA-decorated liposomes can change the pharmacokinetics and localization of gadolinium[190,191].

Recently, silica nanoparticles have entered clinical trials for detecting lymph node metastases in patients with melanoma using positron emission tomography (PET)[192]. Dual-modality nanoparticles, which combine two imaging methods into a single entity, can provide the advantages of two different techniques, such as the important anatomical information gained from the soft-tissue contrast of MRI with the high sensitivity and/or functional information of PET[193]. Examples include radiolabelled iron oxide nanoparticles for both PET and MRI imaging[194,195].

Computed tomography contrast agents often involve small molecules with short half-lives in the body[196]. Encapsulating the agents in nanoparticles can prolong the residence time, thereby reducing the required dose and allowing more logistical flexibility in the clinical setting[196]. Low-sensitivity techniques such as single photon emission computed tomography (SPECT) can be improved by nanoparticle administration of higher contrast-agent doses, such as with ^{110}In-labelled perfluorocarbon nanoparticles[197]. Nanoparticles are also being used to image tissue microstructure and to delineate tumour margins by techniques such as optical coherence tomography (OCT)[198,199].

In addition to their use in diagnosis, diverse classes of nanomaterials have been used to aid surgical resection, to identify cancer cells in the blood and to detect unique tumour subregions[200]. Optical nanomaterials that have been synthesized to emit visible to near-infrared light and conjugated to targeting ligands have been developed for *in vivo* diagnostic applications. Quantum dots have been used efficaciously to track metastatic cells[201] and to differentiate between cells in heterogeneous tumour subpopulations *in vivo*[202].

Nanoscale sensors promise to aid the early detection of cancer and metastasis to improve patient prognosis by lowering the detection limit and the specificity of biomarker recognition. Sensitivity down to the single molecule has been reached using nanomaterials with unique electronic and optical properties. For example, single-walled carbon nanotubes have been used to measure single molecules of specific reactive oxygen species (ROS) and chemotherapeutic drug concentrations in real-time[203]. Localized surface plasmon resonance (LSPR) nanoparticles[204] and nanowires[205] detect cancer markers and other proteins with extremely high sensitivity through the modulation of surface electrons. Schemes have been developed using nanoparticles to quench a fluorophore, such as a fluorescent polymer, until a specific protein binds[206]. Biologists are currently discovering important disease biomarkers in several molecular classes; for example, microRNA-141 and carcinoembryonic antigen were discovered to be prognostic markers for metastatic colorectal cancer[207], and the small-molecule metabolite sarcosine indicates the presence of metastatic prostate cancer[208]. When coupled with a microfluidic device (devices that allow multiplexed biomarker assays using nanoliter volumes of whole blood[209]) nanoengineered materials might allow advances in minimally invasive methods for detecting cancer at an early stage.

Anti-metastatic nanotechnology: the future

New strategies are needed to treat the complex problem of metastatic cancer, which is currently considered to be largely incurable. Nanomaterials represent tools with many potential benefits that are only now starting to be realized in the clinic. To date, most nanotechnology cancer therapies have focused on the treatment of primary tumours, but it is important to leverage the potential of nanotechnology to combat cancer spread at each stage of the metastatic process.

The biological mechanisms that specifically drive each step of metastasis (angiogenesis, intravasation, tumour cell circulation, extravasation and growth in secondary sites) may be addressable using nanoparticle therapies. The characteristics that make an environment susceptible to metastasis may also make specific and targeted therapeutic intervention possible. Despite these advances, additional research is needed to develop robust methods for targeting nanoparticles to metastatic sites, in particular to the bone, brain and tumour microenvironment.

As our knowledge of cancer biology becomes more complete, it is increasingly important for clinicians, biologists and engineers to discuss ideas for diagnostics and treatments of metastatic cancer[3]. Developing nanoparticle therapies that are aimed in the right directions with the right therapies will improve the outcome for patients with metastatic cancer.

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Acknowledgments
The authors thank R. Weinberg, R. Weissleder, J. Kolodny, C. A. Alabi, B. Chertok, V. Frenkel and D. Siegwart for helpful discussions. A.S. thanks the Misrock Foundation and D.A.H. thanks the Damon Runyon Cancer Research Foundation for postdoctoral support. M.M.W. thanks the US National Institutes of Health (NIH; grant K99-CA151968). J.D. thanks the National Defense Science and Engineering Graduate fellowship, National Science Foundation and MIT Presidential Fellowships for support as well as A. Bell and J. Haught for motivation. The authors thank the MIT-Harvard Center of Cancer Nanotechnology Excellence (CCNE) for NIH grants U54CA151884 and EB000244.

Competing interests statement
The authors declare no competing financial interests.