

## CANCER NANOTECHNOLOGY: OPPORTUNITIES AND CHALLENGES

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Abstract | Nanotechnology is a multidisciplinary field, which covers a vast and diverse array of devices derived from engineering, biology, physics and chemistry. These devices include nanovectors for the targeted delivery of anticancer drugs and imaging contrast agents. Nanowires and nanocantilever arrays are among the leading approaches under development for the early detection of precancerous and malignant lesions from biological fluids. These and other nanodevices can provide essential breakthroughs in the fight against cancer.

### NANOVECTOR

A hollow or solid structure, with diameter in the 1–1,000 nanometre range, which can be filled with anticancer drugs and detection agents. Targeting moieties can also be attached to the surface. Nanovectors can be used for targeted gene therapy.

### LIPOSOME

A type of nanovector made of lipids surrounding a water core.

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The past quarter century of outstanding progress in fundamental cancer biology has not translated into even distantly comparable advances in the clinic. Inadequacies in the ability to administer therapeutic moieties so that they will selectively reach the desired targets with marginal or no collateral damage has largely accounted for the discrepancy<sup>1,2</sup>. Most striking is the recognition that only between 1 and 10 parts per 100,000 of intravenously administered monoclonal antibodies reach their parenchymal targets *in vivo*<sup>3</sup>. Similar limitations apply to contrast agents for imaging applications.

There are two general, synergistic goals that should be striven for to increase the efficacy per dose of any therapeutic or imaging contrast formulation: to increase its targeting selectivity<sup>4</sup> and to endow the agent(s) comprising the therapeutic formulation with the means to overcome the biological barriers that prevent it from reaching its target<sup>5</sup>. An ideal therapeutic system would be selectively directed against cell clusters that are in the early stages of the transformation towards the malignant phenotype<sup>6</sup>.

The realization of such a system faces formidable challenges, including the identification of suitable early markers of neoplastic disease, and understanding their evolution over time; the deployment of these markers in screening and early detection protocols; and the development of technology for the biomarker-targeted delivery of multiple therapeutic agents, and for the simultaneous capability of avoiding biological and biophysical barriers. The hypothesis offered in this

article is that nanotechnology, if properly integrated with established cancer research, provides extraordinary opportunities to meet these challenges.

### What is cancer nanotechnology?

Formal definitions of nanotechnological devices typically feature the requirements that the device itself or its essential components be man-made, and in the 1–1,000 nm range in at least one dimension. Cancer-related examples of nano-technologies include injectable drug-delivery NANOVECTORS such as LIPOSOMES for the therapy of breast cancer<sup>7</sup>; biologically targeted, nanosized magnetic resonance imaging (MRI) contrast agents for intraoperative imaging in the context of neuro-oncological interventions<sup>8,9</sup>; and novel, nanoparticle-based methods for high-specificity detection of DNA and protein<sup>10</sup>. In his definition of nanotechnology, George Whitesides<sup>11</sup> places less stringent limitations on the exact dimensions, and defines the ‘right’ size in bionanotechnology in an operational fashion, with respect to addressable unmet needs in biology. Robert Langer and colleagues<sup>12</sup> argue similarly, in the context of drug-delivery applications. In harmony with these approaches, this review’s basic approach is that the defining features of cancer nanotechnology are embedded in their breakthrough potential for patient care. This article discusses prominent, largely unsolved, cross-cutting problems in cancer, and proposes nanotechnology-based approaches to solving them. Greater emphasis is placed on highlighting promising directions than on consensus taxonomies of scientific

**Summary**

- Nanotechnology concerns the study of devices that are themselves or have essential components in the 1–1,000 nm dimensional range (that is, from a few atoms to subcellular size).
- Two main subfields of nanotechnology are nanovectors — for the administration of targeted therapeutic and imaging moieties — and the precise patterning of surfaces.
- Nanotechnology is no stranger to oncology: liposomes are early examples of cancer nanotherapeutics, and nanoscale-targeted magnetic resonance imaging contrast agents illustrate the application of nanotechnology to diagnostics.
- Photolithography is a light-directed surface-patterning method, which is the technological foundation of microarrays and the surface-enhanced laser desorption/ionization time-of-flight approach to proteomics. Nanoscale resolution is now possible with photolithography, and will give rise to instruments that can pack a much greater density of information than current biochips.
- The ability of nanotechnology to yield advances in early detection, diagnostics, prognostics and the selection of therapeutic strategies is predicated based on its ability to ‘multiplex’ — that is, to detect a broad multiplicity of molecular signals and biomarkers in real time. Prime examples of multiplexing detection nanotechnologies are arrays of nanocantilevers, nanowires and nanotubes.
- Multifunctionality is the fundamental advantage of nanovectors for the cancer-specific delivery of therapeutic and imaging agents. Primary functionalities include the avoidance of biobarriers and biomarker-based targeting, and the reporting of therapeutic efficacy.
- Thousands of nanovectors are currently under study. By systematically combining them with preferred therapeutic and biological targeting moieties it might be possible to obtain a very large number of novel, personalized therapeutic agents.
- Novel mathematical models are needed, in order to secure the full import of nanotechnology into oncology.

disciplines. The development of novel mathematical models will be required to reap the full rewards of the deployment of nanotechnology.

**The nanotechnology toolbox**

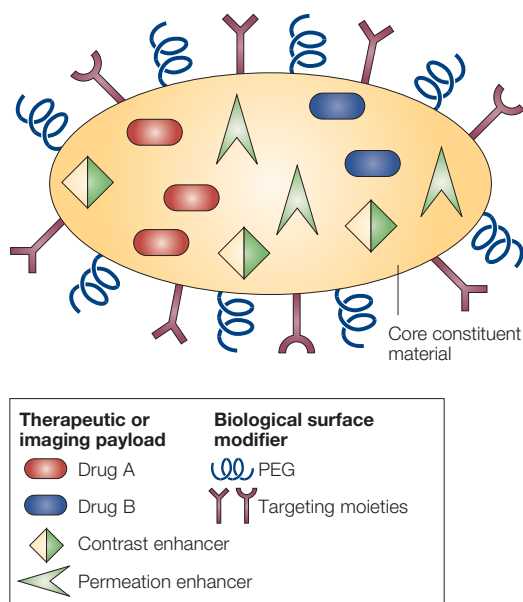
Before entering into the discussion of the challenges that define the potential breakthrough that nanotechnology might help attain, it is necessary to present an overview of current nanotechnologies. I will focus on nanovectors in various stages of development for targeted imaging and therapeutics, and on different emerging approaches to biomolecular identification from tissue and serum samples. Some nanotechnologies have been demonstrated for applications outside of cancer, and seem ready for transition into oncology — these are also reviewed here.

*Drug-delivery and imaging nanovectors.* Intravascularly injectable nanovectors are a major class of nanotechnological devices of interest for use in cancer. Their envisioned use is for the *in vivo*, non-invasive visualization of molecular markers of early stages of disease; the targeted delivery of therapeutic agents, with a concurrent, substantial reduction of deleterious side effects; and — by a combination of the first two — the interception and containment of lesions before they reach the lethal or even the malignant phenotype, with minimal or no concurrent loss of quality of life.

Liposomes are the archetypal, simplest form of a nanovector. They use the overexpression of fenestrations in cancer neovasculature to increase drug concentration at tumour sites. Liposome-encapsulated formulations of doxorubicin were approved 10 years ago for the treatment of **Kaposi’s sarcoma**, and are now used against breast cancer and refractory **ovarian cancer**. Liposomes continue to be refined and applied to more cancer indications<sup>4,7,13</sup>. They are only the first in an ever-growing number of nanovectors under development for novel, more efficacious drug-delivery modalities<sup>1,2,14</sup>.

Several types of nanoparticle for the enhancement of MRI contrast have been used clinically and in research protocols. These include gadolinium-based<sup>15</sup>, iron-oxide-based nanoparticles<sup>16–21</sup> and multiple-mode imaging contrast nano-agents that combine magnetic resonance with biological targeting<sup>22</sup> and optical detection<sup>9,22,23</sup>. Low-density lipid NANOPARTICLES have been used to enhance ultrasound imaging<sup>24,25</sup>. For each current clinical modality it is actually possible to develop nanoparticles that can provide signal enhancement, combined with biomolecular targeting capabilities<sup>26</sup>.

Nanovectors in general have at least a tripartite constitution, featuring a core constituent material, a therapeutic and/or imaging payload, and biological surface modifiers, which enhance the biodistribution and tumour targeting of the nanoparticle dispersion (FIG. 1). A major clinical advantage sought by the use of nanovectors over simple immunotargeted drugs is the specific delivery of large amounts of therapeutic or imaging agents per targeting biorecognition event. Targeting methods that have been investigated range from covalently linked antibodies<sup>2,27</sup> to mechanisms



**Figure 1 | Multifunctional nanoparticle.** The following are illustrated: the ability to carry one or more therapeutic agents; biomolecular targeting through one or more conjugated antibodies or other recognition agents; imaging signal amplification, by way of co-encapsulated contrast agents; and biobarrier avoidance, exemplified by an endothelial tight-junction opening permeation enhancer, and by polyethylene glycol (PEG) for the avoidance of macrophage uptake by macrophages.

**NANOPARTICLE**  
A solid nanovector, typically made of a single material.

based on the size and physical properties of the nanovector<sup>28</sup>. Nanovector formulations are designed to reduce the clearance time of small peptide drugs, provide protection of active agents from enzymatic or environmental degradation, and avoid obstacles to the targeting of the active moiety. Examples of such obstacles include the protective exclusion by the blood–brain barrier or the vascular endothelium; the augmented osmotic pressure states in cancer lesions, resulting in outward convection of the therapeutic moiety<sup>29</sup>; and nanoparticle sequestration by the RETICULO-ENDOTHELIAL SYSTEM (RES)<sup>7,30</sup>.

Nanovectors might act as carriers for the therapeutic and imaging payloads, or their constituent materials might also possess image-enhancement properties, such as in the case for iron oxide for MRI, and semiconductor nanocrystals or quantum dots for optical imaging<sup>31–34</sup>. Many polymer-based nanovectors have been investigated<sup>2,14,35</sup>, and seem most promising for clinical translation. For instance, dendrimers are self-assembling synthetic polymers with exquisitely tunable nanoscale dimensions<sup>36</sup>, which were recently used for the MRI of the lymphatic drainage in a mouse model of breast cancer<sup>37</sup>. This indicates that dendimer-based contrast agents might be used to non-invasively detect cancer cells in the lymph nodes in patients, to provide early signals of disease, or information about patterns of metastatic spread.

Silicon<sup>27,38,39</sup> and silica<sup>40,41</sup> are emerging as interesting candidate materials for injectable nanovectors. Porosified silicon is biodegradable<sup>42</sup>, with kinetics that are much more rapid (minutes to hours) than those of biodegradable polymers (weeks to months), and therefore release drugs with previously unattainable time profiles. Metal-based nanovectors include NANOSHHELLS<sup>43,44</sup>, which comprise a gold layer over a silica core. The thickness of the gold layer can be precisely tuned, so that the nanoshell can be selectively activated through tissue irradiation with near-infrared light to perform localized therapeutic thermal ablation. The approach was recently used to eradicate transmissible venereal tumours in mice<sup>44</sup>. Beyond its specific merits, this approach introduces the concept that nanovectors can be used as highly selective, externally activated therapeutic agents.

It is estimated that several thousand different nanovector types have been reported in the literature. Just a minute fraction of their potential uses against cancer have been explored, yet these offer technological foundations for meeting the fundamental cancer nanotechnology challenges discussed below.

**Nanocomponents of macroscopic devices.** Beyond nanovectors, a very diverse array of novel devices, concepts and fabrication methods are emerging for potential use against cancer, starting with the high-precision patterning of biological molecules on substrates. Microarrays, as a prime example, are used for molecular diagnostics, genotyping and biomarker-guided therapeutic targeting, and are fabricated by synthesizing single-stranded DNA probes one

oligonucleotide at a time<sup>45</sup>, in a spatially directed manner that is governed by the selective ultraviolet irradiation of a substrate through a patterned mask (FIG. 2). With the ability to control the molecular depositions now in the nanometre range, a million-fold increase in information density might be packed in ‘nanoarrays’, directed both at nucleic acids or at the detection of proteomic profiles<sup>46–49</sup>. Another example of nanoscale patterning for cancer applications is the substrate preparation for surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) proteomic analysis protocols, for non-invasive, early cancer diagnostic applications<sup>50–52</sup> (FIG. 2).

Biomolecular sensors with the ability to ‘multiplex’ massively — that is, to detect a large number of different molecular species at the same time — are being developed for serum and tissue proteomics-based cancer diagnostics, prognostics and therapeutic-efficacy monitoring. Promising emerging approaches to multi-molecular sensing include mechanical sensors such as microcantilever and NANOCANTILEVER arrays<sup>53–55</sup> (FIG. 3). These comprise a large number of beams that deflect when the biomolecules of interest bind. The deflections are either observed directly by laser light or generate detectable shifts in the physical properties of the beam, such as their resonant-vibration frequency. Microcantilever-based, multiplexed DNA assays to detect *BRCA1* mutations were recently introduced<sup>56</sup>.

Silicon NANOWIRES<sup>57,58</sup> also yield highly multiplexed, real-time detectors of simultaneous molecular binding events. They operate as nanoscale field-effect biotransistors; that is, by reporting changes in their conductance that are generated by molecular binding events on their surface (FIG. 3).

Following the Nobel-prize-winning discovery of FULLERENES by Richard Smalley and the identification of nanotubes<sup>59</sup>, carbon nanotechnology has been intensely studied as a platform for high-specificity sensing in several biomedical applications<sup>60,61</sup>. For instance, NANOTUBES have been reported as high-specificity sensors of antibody signatures of autoimmune disease<sup>62</sup> and of single-nucleotide polymorphisms (SNPs)<sup>63</sup>.

Instrumentation for the exquisitely precise movement and analysis of picolitre-to-microlitre amounts of fluid has been developed and refined over the past decade<sup>64,65</sup>. Descending into the nanoscale domain, channels and pores of exquisitely controlled dimensions in the 5–100 nanometre range have been fabricated on silicon chips<sup>66–69</sup>. Their applications have been reported in molecular separation, controlled-release drug delivery<sup>70</sup>, the immunoisolation of CELL XENOGRAFTS<sup>71</sup> and DNA transport and characterization<sup>69,72</sup>.

### Cancer nanotechnology: the challenges

In an ideal scenario, the onset of the transformational processes leading towards malignancy would be detected early, as a matter of routine screening, by non-invasive means such as proteomic pattern analysis from blood samples, or the *in vivo* imaging of molecular profiles and evolving lesion contours. The biology of the host and the disease would be accurately determined, and dictate

#### RETICULO-ENDOTHELIAL SYSTEM

A system composed of monocytes and macrophages that is located in reticular connective tissue (for example, in the spleen). These cells are responsible for phagocytosing and removing cellular debris, pathogens and foreign substances from the bloodstream.

#### NANOSHHELLS

A nanoparticle composed of a gold shell surrounding a semiconductor. When nanoshells reach their target they can be irradiated to make the nanoshell hot — the heat kills the cancer cell.

#### NANOCANTILEVERS

Flexible beams, resembling a row of diving boards, that can be coated with molecules capable of binding to cancer biomarkers.

#### NANOWIRES

Nanoscale sensing wires that can be coated with molecules such as antibodies to bind to proteins of interest and transmit their information through electrodes to computers.

#### FULLERENE

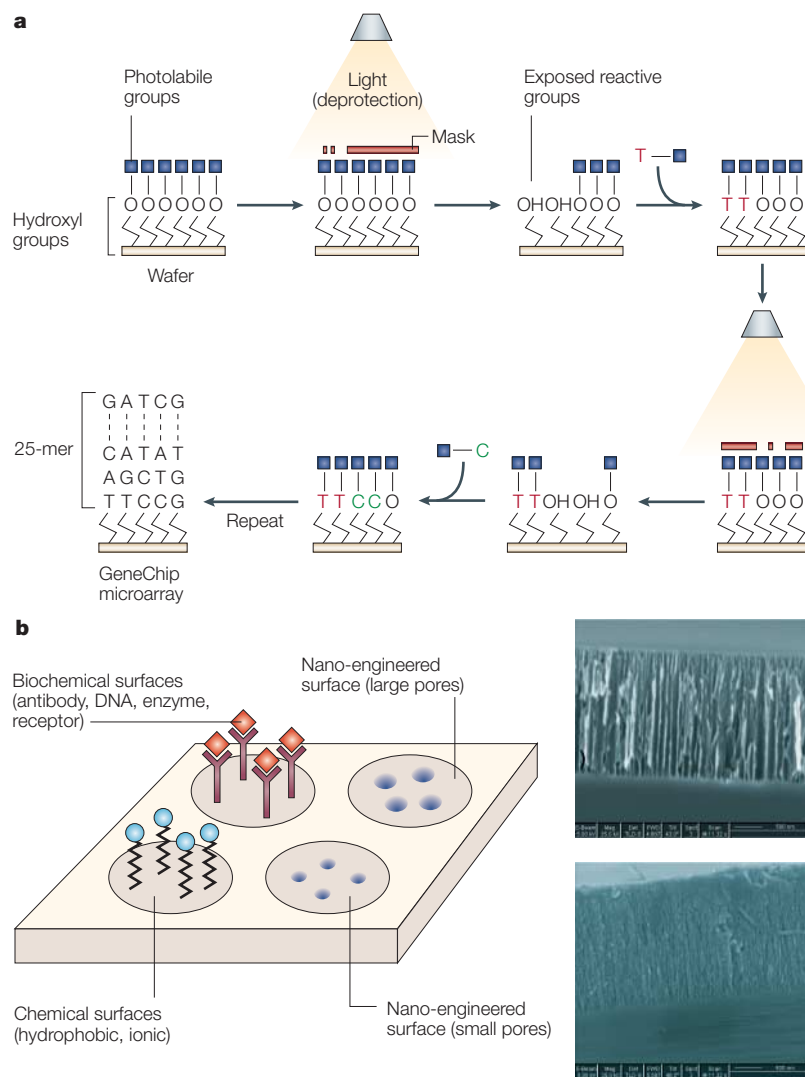
A nanoscale structure, composed of carbon atoms arranged in a specific soccer-ball-like architecture. Fullerenes are a form of carbon (C-60), which also forms nanotubes.

#### NANOTUBES

Cylinder-like assemblies of carbon atoms, with cross-sectional dimensions in the nanometre range, and lengths that can extend over a thousand times their diameters.

#### CELL XENOGRAFTS

Cross-species, therapeutic cell transplants.



**Figure 2 | Nanotechnologies for molecular detection, identification and diagnostics.**

**a** | Microarrays exemplify the patterning of biological molecules on surfaces, with exquisite control over their spatial placement, for instance to obtain DNA sequencing by hybridization on a chip<sup>45</sup>. In the figure, blue squares represent photolabile groups, which are selectively illuminated through a mask (a process known as photolithography) and removed to expose reactive groups. Sequential application of the procedure yields single-stranded hybridization probes of preselected vertical sequences at predetermined locations on the microarray. The technique of photolithography was adapted from the microelectronic industry. The ability to control the lateral dimensions of each square in the checkerboard of a microarray was originally of the order of 100 microns (or 100,000 nanometres). Now, the linear spatial resolution of lithography is 1,000 times better, indicating that up to a one-million-fold increase in information density could be packed in 'nanoarrays'.

**b** | Photolithography can be used to pattern different chemistries, biological moieties and physical textures on substrates, for the purpose of prefractionation of protein mixtures before investigation by time-of-flight spectrometry. Different proteomic patterns are produced by different substrate treatments, on contact with the same biological sample. The panels to the right illustrate different nanochanneled surfaces, which selectively retain proteins and proteolytic fragments. This has the effect of 'focusing' the resulting protein profiles in different molecular-weight ranges<sup>51</sup>.

**SENTINEL LYMPH-NODE BIOPSY**

A surgical approach for the assessment of the metastatic involvement of lymph nodes. It is based on the hypothesis that if the node that is nearest to a tumour is negative, the others along the same pattern of spread will also be negative.

choices for targeting and barrier-avoiding strategies for an intervention plan. Transforming cellular populations would be eradicated or contained, without collateral effects on healthy tissues, in a routine that could be repeated many times. Treatment efficacy would be monitored in real time. Therapeutics would be supplanted by personalized prevention.

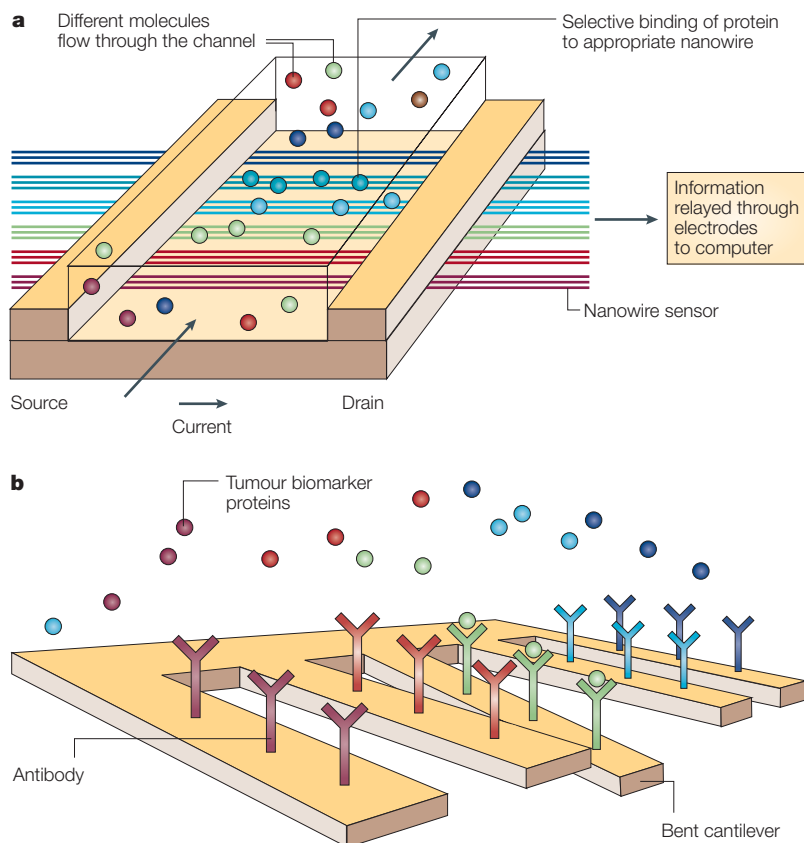
If fully integrated with the established cancer-research enterprise, nanotechnology might help this vision become reality. Some of the principal challenges along this path are discussed below.

*Developing approaches for the in vivo detection and monitoring of cancer markers.* The effective early detection of precancerous and neoplastic lesions remains an elusive goal. Clinical cancer imaging technologies do not possess sufficient spatial resolution for early detection based on lesion anatomy. To identify malignancies based on their molecular expression profiles, all imaging technologies require contrast agents, comprising a signal-amplifying material conjugated to a molecular recognition and targeting agent such as an antibody. Nanoparticle technologies are under development and testing as candidate multifunctional, molecularly or physically targeted contrast agents for all clinical imaging modalities, with the objectives of detecting smaller and earlier-stage cancer tumours, identifying molecular expressions of neoplasms and their microenvironment, and providing improved anatomical definition for lesions<sup>26</sup>.

For instance, Weissleder and colleagues<sup>17</sup> recently demonstrated that lymphotropic paramagnetic nanoparticles allow the MRI imaging of clinically occult lymph-node metastases in patients with prostate cancer, which are not detectable by any other non-invasive approach. Polymeric dendrimers were used as gadolinium nanocarriers to image the lymphatic drainage of breast cancer in mice<sup>37</sup>, indicating that this procedure could be used clinically instead of SENTINEL LYMPH-NODE BIOPSY. Dextran-coated, ultra-small paramagnetic iron-oxide nanoparticles were shown to outperform conventional gadolinium MRI contrast in terms of intraoperative permanence of imaging enhancement, inflammatory targeting, and detectability at low magnet strength in the surgical treatment of brain tumours<sup>9</sup>. Bimodal nanoparticles, carrying a near-infrared optically detectable fluorochrome conjugated to an MRI contrast agent —crosslinked iron oxide— were used for the preoperative, contour-defining imaging of a brain tumour, and the intraoperative visualization of the lesion<sup>8</sup>.

Nanoparticle probes with molecularly targeted recognition agents might provide information on the presence, relative abundance and distribution of cancer signatures and markers associated with the tumour microenvironment<sup>3,26</sup>. Crosslinked iron oxide nanoparticles were conjugated to annexin-V, which recognizes the phosphatidylserine that is present on apoptotic cells, and were used for MRI identification of camptothecin-induced apoptosis of Jurkat T cells *in vitro*<sup>16</sup>. Telomerase activity, a marker of limitless replicative potential<sup>73</sup>, was detected by MRI in cell assays, by the use of biologically 'smart' nanoparticles that switch their magnetic state on by annealing with telomerase-synthesized TTAGGG sequences<sup>74</sup>.

Sustained angiogenesis is an important marker for use in the early detection of cancer, as it is found in pre-malignant lesions of the cervix, breast and skin<sup>75</sup>, and might be expected to be an early-to-midstage



**Figure 3 | Nanowires and nanocantilevers. a** | Nanowires deployed within a microfluidic system. Different colours indicate that different molecules (coloured circles) adsorb or affinity-bind to different nanowire sensors. The binding causes a change in conductance of the wires, which can be electronically and quantitatively detected in real time. The working principle is that of a (biologically gated) transistor and is illustrated in the insert. The charges of the binding protein disrupt electrical conduction in the underlying nanowire. The 'nano' size of the wire is required to attain high signal-to-noise ratios. **b** | Nanocantilever array. The biomarker proteins are affinity-bound to the cantilevers and cause them to deflect. The deflections can be directly observed with lasers. Alternatively, the shift in resonant frequencies caused by the binding can be electronically detected. As for nanowire sensors, the breakthrough potential in nanocantilever technology is the ability to sense a large number of different proteins at the same time, in real time.

event in human cancers<sup>76</sup>. Several groups have successfully imaged angiogenesis with MRI in animal models by various formulations of derivatized nanoparticles, targeted by  $\alpha_v\beta_3$ -integrin<sup>18,77–79</sup>. MRI was recently shown to detect signals from very low picomolar concentrations of epitopes targeted by suitable nanoparticles<sup>80</sup>, and this shows promise for future clinical applications.

A different approach to molecular detection *in vivo* involves the use of implantable sensors, equipped with technology to relay sensed information extracorporeally. Despite many years of research towards this vision, the unsolved challenge for the clinical deployment of implantable molecular sensors remains the unwanted, non-specific adsorption of serum proteins on the sensing surfaces<sup>81</sup>. This phenomenon is known as biofouling, and results in the rapid loss of the ability of the sensor to detect the protein of interest over the background signal. A challenge for nanotechnology researchers is to develop surface nanostructures that will prevent non-specific

adsorption. More realistically, however, nanotechnology might be expected to yield novel, biofouling-indifferent sensing strategies, based for instance on the measurement of physical properties, from which the contributions of the fouling molecules might be systematically decoupled by appropriate mathematical algorithms.

**Refining technology platforms for early detection of cancer biomarkers *ex vivo*.** Serum markers for the early detection of most cancers are not available. The markers that are in clinical use, such as prostate-specific antigen (PSA) and carcinoembryonic antigen (CEA), are non-specific and have widely different baseline expressions in the population, so are of limited effectiveness for early detection. The goal of developing reliable early detection approaches from serum, other biological fluids, or any sample obtained through minimally or non-invasive procedures remains of paramount importance<sup>6</sup>.

Several nanotechnologies are realistic candidates for early detection platforms, starting with surface patterning approaches including firmly established technologies such as DNA microarrays<sup>45</sup>, and SELDI-TOF mass spectroscopy for proteomics<sup>52</sup>. For these, the transition from the micron- to the nanoscale dimensional control on surface features translates into increases in information quality, quantity and density.

Ushering in entirely new approaches to molecular recognition, James Gimzewski and colleagues pioneered the concept that biomolecular binding events yield forces and deformations that might be detected and recognized by appropriately selective sensing nanostructures<sup>82</sup>. Primary examples of such devices are micro- or nanocantilevers, which deflect and change resonant frequencies as a result of affinity binding and as a result of nucleic-acid hybridization events occurring on their free surfaces (FIG. 3). Arun Majumdar and colleagues used microcantilevers to detect SNPs in a 10-mer DNA target oligonucleotide without the use of extrinsic fluorescent or radioactive labelling<sup>53,83</sup>. They also demonstrated the applicability of microcantilevers for the quantitation of PSA at clinically significant concentrations<sup>54</sup>. The specificities and sensitivities of these assays do not yet offer substantial advantages over conventional detection methods, although the use of nanoparticle probes might allow for individual single-pair mismatch discrimination<sup>53</sup>. Rather, the breakthrough potential afforded by nanocantilevers resides in their extraordinary multiplexing capability<sup>84</sup>. It is realistic to envision arrays of thousands of cantilevers constructed on individual centimetre-sized chips, allowing the simultaneous reading of proteomic profiles or, ultimately, the entire proteome. Nanowire<sup>57</sup> and nanotube<sup>60,63,85</sup> arrays might contain several thousand sensors on a single chip, and therefore offer even greater multiplexing advantages<sup>58</sup>. For both nanowires and microcantilevers, it is the nanofabrication protocols that afford very large numbers of identical structures per unit area, and therefore the massive multiplexing capabilities. The many similarities that these protocols share with the fabrication of microelectronic components indicate that they will be comparably suitable for production scale-up at low cost and with high reliability.

Nanocantilever, nanowire and nanotube arrays might be the approaches that enable the transition from single-biomarker to multiple-biomarker cancer diagnostic, prognostics and treatment selection. However, areas of concern and current limitations of these approaches include the need for covalent binding of different antibodies or other biological recognition molecules to the devices; and the deconvolution of noise from the signal, especially in regard to biofouling. For the analysis of proteomic signatures, a major challenge will be the identification of signatures from low-concentration molecular species, in the presence of extremely high concentrations of non-specific serum proteins. Issues that pertain specifically to the cantilever arrays include the need to develop further mathematical models for the determination of stresses and biological identification signatures from the beam curvatures<sup>83,86</sup>.

Nanoparticles are also showing promise for the *ex vivo* detection of biomarkers. For instance, fluorophore-laden silica beads have been used for the optical identification of leukaemia cells in blood samples<sup>87</sup>; gold-nanoshell-based immunoassays have been developed<sup>43</sup>; fluorescent nanoparticles have been used for an ultrasensitive DNA-detection system<sup>88</sup>; and QUANTUM DOT bioconjugates with targeting antibodies have been used to recognize molecular signatures including ERBB2 (REFS 89,90). Furthermore, as a quantitative measure of the response of cells to the compound *m*-dinitrobenzene, fluorescent nanoparticles have been used to detect intracellular calcium, a precursor of cell death, in human SY5Y neuroblastoma and C6 glioma cells<sup>91,92</sup>.

Nanoparticles have the advantages of stability and 'tunability' over conventional staining methods. For instance, quantum dots do not lose their signal intensity over time; that is, they do not 'photobleach'. Furthermore, populations of nanoparticles, each with one of many different colours might be conjugated with antibodies to different molecular targets. When irradiated with a light beam of single wavelength<sup>31</sup>, a precise map of the distribution of many molecular markers in a single cell, cell population or tissue is generated. This offers the potential advantages of readily identifying the conjugate markers, yielding specific information on their tissue distribution, introducing new protocols that include cell surface, endocellular and microenvironmental antigens in the same test.

The use of nanoparticles as selective, enriching harvesting agents for serum proteomics has been proposed<sup>93</sup>. The emphasis for this approach is on low-molecular-weight proteolytic fragments, which are found in trace quantities in ovarian and other cancers<sup>51</sup>. The use of nanoparticles for this approach has two objectives: the maintenance of fragments in the circulation that otherwise would be rapidly cleared; and the selectivity of the uptake of the desired molecular signals over the 'noise' of the most abundant serum proteins. This approach raises the possibility, used in SELDI-TOF proteomics, that appropriate surface treatment can significantly increase protein uptake per unit area, and help pre-fractionate the sample to focus on the spectral domains of interest.

The combined use of multiple-platform diagnostic nanotechnologies is beginning to emerge. A two-particle DNA-detection technology was developed by Chad Mirkin and colleagues<sup>10</sup>. Dubbed 'bio-barcode', it involves oligonucleotide-modified gold nanoparticles and magnetic particles that carry a predetermined nucleotide sequence acting as an identification label. This system has demonstrated 500 zeptomolar (zepto =  $10^{-21}$ ) sensitivity, and is therefore competitive with PCR. However, it has substantial advantages over PCR because it does not require enzymatic amplification and is applicable to proteins, as well as DNA. As a further example, gold-nanoparticle-modified probes have been used in conjunction with microcantilevers to develop a DNA assay with single mismatch discrimination<sup>55</sup> and to transduce molecular binding into readily detectable micrometre-scale deflections<sup>94</sup>.

#### *Improving the targeting efficacy of therapeutic or imaging agents to cancer lesions and their microenvironment.*

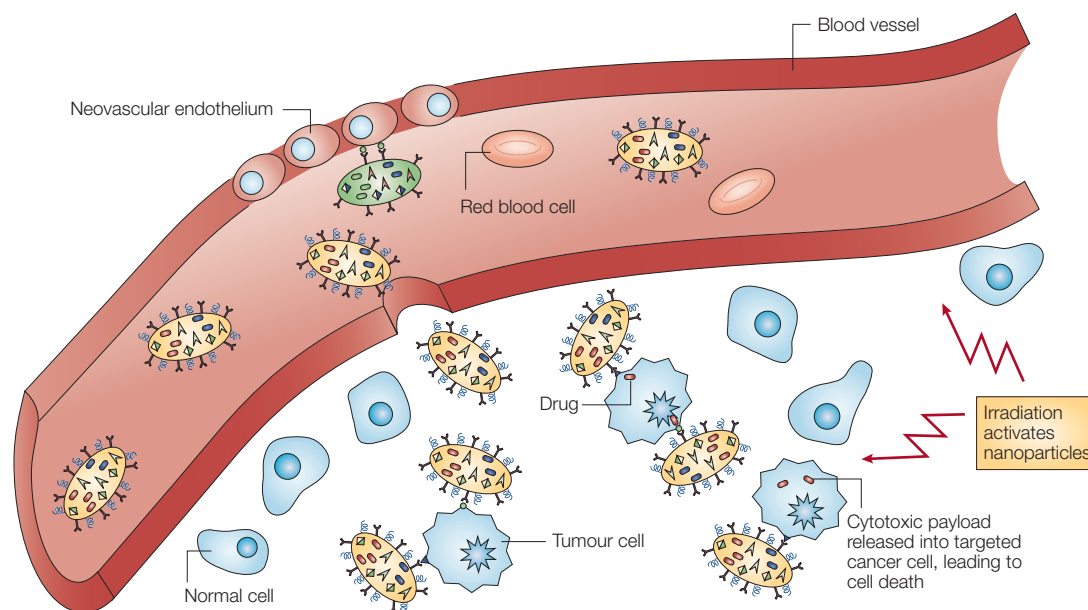
Multiple targeting strategies might be used to preferentially concentrate injected agents at tumour sites. For instance, the vasculature supplying cancer lesions might have increased endothelial fenestrations and architectural anarchy, resulting in the preferential extravasation and protracted lodging of injected particulates. This is a tumour-targeting mechanism known as enhanced permeation and retention (EPR), which was developed by Maeda and colleagues<sup>95</sup>. EPR is a selectivity strategy that is used in the clinic for particle-mediated delivery by liposomes, and is fundamental for novel emerging nanovector formulations<sup>2,95,96,97</sup>.

The molecular targeting of nanovectors containing active agents might be attained by the conjugation of active recognition moieties to the surface of a nanovector. Specificity is then increased, at the expense of added complexity in the nanoparticle preparation, increased particle size and the risk of biological adverse reactions to the targeting agent. The use of molecularly targeted nanovectors affords at least four potential advantages over conventional antibody-guided therapy: the delivery of much greater therapeutic payloads per target biorecognition event; the ability to carry multiple, potentially different targeting agents, providing selectivity enhancement<sup>98</sup>; the ability to integrate means to bypass biological barriers; and the colocalized delivery of multiple agents, resulting in targeted combination therapy.

Intracellular targeting of nanoparticles by folate has been demonstrated in the context of neutron-capture therapy of tumours with athymic mice bearing human nasopharyngeal carcinomas<sup>15</sup>. Dendritic polymers were demonstrated as multifunctional nanodevices with the ability to target folate in KB cells in culture, selectively deliver the cancer drug methotrexate intracellularly, and provide optical-imaging signals through the attachment of fluorescein to the nanovector<sup>99</sup>. A triplex-forming growth-inhibitory oligonucleotide was effectively delivered by dendrimers to breast, ovarian and prostate cell lines<sup>100</sup>. Several antigens have been used to preferentially direct nanoparticles to angiogenic

#### QUANTUM DOTS

Semiconductor particles with an inert polymer coating. The material used for the core can be chosen depending on the emission wavelength range being targeted. Targeting molecules can be attached to the coating.



**Figure 4 | Multicomponent targeting strategies.** Nanoparticles extravasate into the tumour stroma through the fenestrations of the angiogenic vasculature, demonstrating targeting by enhanced permeation and retention. The particles carry multiple antibodies, which further target them to epitopes on cancer cells, and direct antitumour action. Nanoparticles are activated and release their cytotoxic action when irradiated by external energy. Not shown: nanoparticles might preferentially adhere to cancer neovasculature and cause it to collapse, providing anti-angiogenic therapy. The red blood cells are not shown to scale; the volume occupied by a red blood cell would suffice to host 1–10 million nanoparticles of 10 nm diameter.

endothelium. For example, targeting  $\alpha_v\beta_3$ -integrin, which is found on endothelial cells, was used with per-fluorocarbon-based nano-emulsions for the MRI imaging of neovasculature<sup>18,79</sup> and anti-angiogenesis therapy in murine models of melanoma and colon adenocarcinoma<sup>3,101</sup>. Epidermal growth factor (EGF) receptor was proposed to target EGF-derivatized silicon particulates carrying the pore-forming protein melittin to provide selective action to lyse the membranes of cells in angiogenic endothelium<sup>39,102</sup>. The peptide-mediated nuclear targeting of gold nanoparticles was reported<sup>103</sup>. Phage-display methods might provide a broad range of organ- and lesion-specific nanoparticle targeting options<sup>104</sup>.

Another class of targeting methods use external energy as a trigger for the localized activation of cytotoxic action, and have been demonstrated in animal models. Examples are the use of focused ultrasound to burst lipid-encapsulated ‘microbubbles’<sup>24</sup>; photodynamic therapy on silica-based carriers<sup>41,105</sup>; and the localized thermal ablation of cancer lesions by the combined use of gold nanoshells and optical activation in the near-infrared region, by which deep tissue penetration can be achieved<sup>43,44,106</sup>. Non-specific physicochemical interactions might also aid the localization of nanocarriers<sup>28,107</sup>. For instance, the size of the particle is largely responsible for its margination dynamics<sup>28</sup>. As a result of the balance of the acting forces, including hydrodynamic drag, van der Waals and steric interactions, particulates with size of about 100 nm display the greatest tendency to remain distal to the endothelium, and are therefore most suitable for proteomic enrichment and harvesting applications<sup>93</sup>.

Sizes smaller or larger than this crucial radius tend to marginate, and therefore are more likely to deliver therapeutic action to endothelial or parenchymal regions<sup>28</sup>. The *in vitro* use of pH sensitivity to trigger the release of the anticancer drug paclitaxel by biodegradable polymer nanocarriers<sup>108</sup> illustrates the activation of therapeutic action in response to conditions expressed preferentially at tumour sites; this is in itself a targeting strategy.

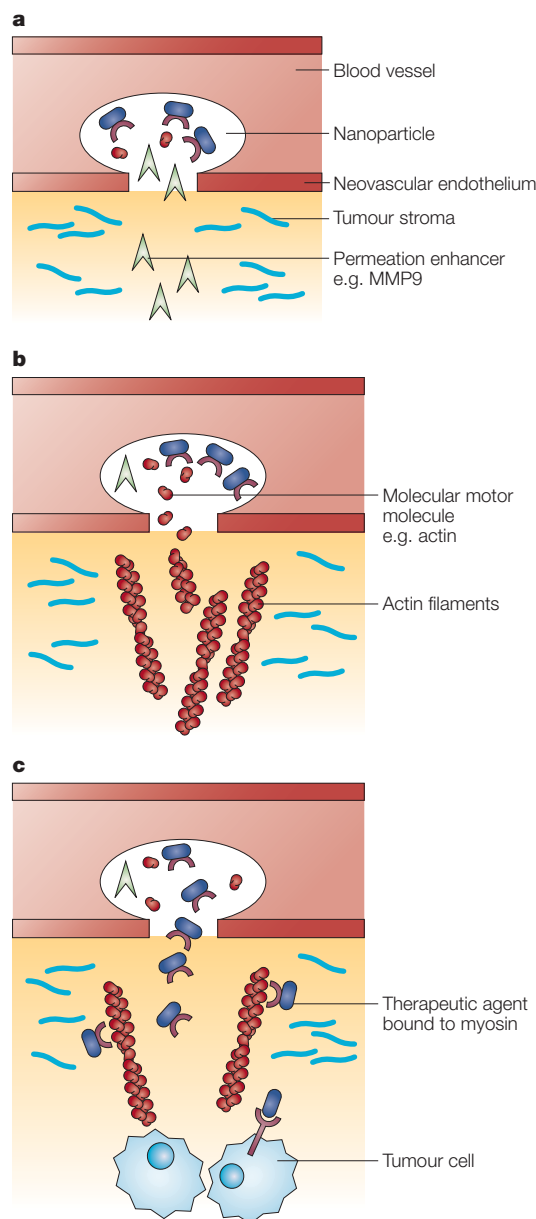
Effective as all of these targeting strategies might be by themselves, it is expected that the greatest gains in therapeutic selectivity will be achieved by synergistic combinations of these strategies (FIG. 4). An example is provided by the combined use of EPR and external activation<sup>43,44</sup>. Furthermore, multimechanism selectivity-enhancement approaches might involve EPR and physical targeting. For instance molecular charge influences the targeting efficiency of EPR<sup>109,110</sup>, and mathematical formulations have recently become available<sup>28</sup> that can guide future design of nanovectors so that margination properties and EPR are optimized.

One problem of delivering cytotoxic moieties in a targeted fashion to tumours has been highlighted by the modelling investigations of Vittorio Cristini and colleagues<sup>111</sup>. They have shown that the delivery of cytotoxic action to tumours, in particular of anti-angiogenic therapy, might be highly counterproductive, by fractionating the lesion into multiple satellite neoplasms. Termed ‘diffusional instability’, as it is driven by the therapy-generated rearrangement of the sources of oxygen and nutrient supply, this phenomenon illustrates the need to attain accurate spatial distribution — yet another challenge for directed nanovectors.

The achievement and maintenance of a desired biodistribution of therapeutic agents over time requires the tailoring of dosing and administration schedules. Drug-delivery systems might be implanted to attain desired time profiles of the plasma concentration of therapeutic agents, both nano-encapsulated or free, without the inconvenience of multiple injections or hospital stays. Future systems might be pre-programmable to have a time-variable rate of

delivery<sup>1,12</sup>, or to be self-regulating in response to sensor-detected environmental stimuli at the site of implantation. For the nearer-term future, however, a nanotechnology-enhanced objective is to realize delivery implants for the constant-rate release of a broad spectrum of agents. The constant-rate delivery of the hormonal agent leuprolide from an osmotic-pump-powered implant is already in clinical use for the treatment of prostate cancer, and exemplifies the potential benefits associated with controlled-release modalities: therapeutic advantage, reduction of side effects, regularity of dosing, localization of therapeutic action, and patient compliance. However, not many drugs can easily be delivered through osmotic pumps, and the maximum benefits of agents might be realized by time-variable delivery from implants<sup>112</sup>.

To address these issues, different nanotechnologies are under development. Silicon membranes with nanofabricated channels of exquisitely controlled dimensions in the 5–100 nm range were developed in our group<sup>71</sup> and shown to provide desired release rates for essentially any drug<sup>70</sup>, including interferon for the treatment of non-resectable melanoma<sup>113</sup>. Based on the nanochannel technology<sup>68</sup>, novel, actively controllable systems are being developed for the realization of pre-programmable, remotely controlled and self-regulating implants. Nanochannels were also shown to provide immunoprotection for cell xenografts for the treatment of diabetes<sup>67,114</sup>. This approach offers opportunities in cancer therapeutics, such as the grafting of cell clusters that secrete lipid-lowering drugs — statins — for the control of angiogenesis<sup>115</sup>.



**Figure 5 | A vision for a future multistage nanodevice with multiple-barrier-avoidance capability.** A

nanovector selectively binds to the cancer neovascular endothelium, releases a penetration enhancer, generates a fenestration, and deploys through it a track of molecular motor molecules such as actin. Therapeutic agents bound to a conjugate molecule such as myosin are then released by the nanovector, and travel along the 'molecular track' to reach deeply into the cancer lesion, despite the opposing oncotic osmotic pressure.

**Engineering nanoparticles to avoid biological and biophysical barriers.** The trek of a therapeutic or imaging agent from the point of administration to the intended target is full of perils, for both nanovectored and 'conventional' formulations. Biological barriers might arise in the form of tight junctions between epithelial cells, as is the case for the blood–brain barrier (BBB), which impedes the extravasation of vascularly injected agents. Nanotechnology-based systems have shown efficacy in crossing the BBB by virtue of the properties of their constituent core materials<sup>9,116–119</sup>. Endothelial vascular permeability might be increased by the co-administration of a bradykinin antagonist<sup>120</sup>. This indicates a strategy for the enhancement of EPR targeting of nanovectors.

The colocalized delivery of permeation enhancers such as zonula-occludens toxin, which reversibly opens tight junctions, affords the penetration of orally administered biomolecular agents through the intestinal epithelium, which is a very effective barrier, into the vascular compartment<sup>121,122</sup>. An illustration of the multifunctionality afforded by nanotechnology is given by synthetic particles that were designed to simultaneously carry biological therapeutic agents, permeation enhancers and intestinal-wall-targeting moieties<sup>102,122,123</sup>, while also providing protection from enzymatic degradation of the drug and the time delay of its release. Similarly complex, but smaller-scale, particulates might

be designed for intravascular injection (FIG. 5), to increase drug extravasation across the endothelium of cancer vasculature to enhance the effects of spontaneous EPR targeting or to facilitate its permeation through the BBB.

Cells of the RES act as immunological barriers to the effective targeting of nanoparticle-encapsulated drugs, as they sequester injected nanoparticles. Ten years of experience with liposomes have demonstrated that uptake by the RES is effectively avoided by using surface modification with polyethylene glycol<sup>17,30</sup> to increase circulatory half-life from minutes to many hours or days, therefore allowing for enhanced targeting of the liposomes within the tumour.

Nanovectors might also trigger sensitization reactions. For instance, antibodies to fullerenes have been described<sup>62</sup> and shown to also recognize carbon nanotubes. Early-generation dendrimers were shown to raise weak antibody response, but protein–dendrimer conjugates were strongly immunogenic in these studies<sup>124,125</sup>. These experiences indicate that sensitization to any nanoparticle-enhanced therapy is not unlikely, and appropriately engineered countermeasures will be required.

Biophysical barriers to the delivery of therapy include the increased osmotic pressure within cancer lesions, especially at later stages of their development<sup>29,126</sup>. By the resulting adverse force balance, the extravasation and diffusion of therapeutic agents into the tumour are countered, and agents directly injected into the lesions are readily ejected from it. Creative future solutions to this most daunting problem might involve multiple-stage, multiple-payload delivery systems (FIG. 5), which at present exist as theoretical constructs only.

Although relatively new, the field of barrier-avoiding multifunctional nanovectors might yield valuable advances in the development of anticancer therapeutic strategies with high efficacy and few side effects. Approved by the FDA in January 2005 for the treatment of metastatic breast cancer, **Abraxane** represents a promising advance in this direction. The drug consists of paclitaxel nanoparticles that are conjugated to albumin molecules. The nanoparticulate formulation renders unnecessary any pretreatment with steroidal anti-inflammatory drugs, which are required in conventional taxane therapy. Albumin enhances the transport of the nanoparticles across the vascular endothelium. The combination results in 50% greater clinical dosages of paclitaxel.

**Regulatory issues and opportunities for nanotechnologies.** However promising nanovector delivery systems might be, the enthusiasm for them must be placed against the backdrop of the proper considerations of safety for the patients and the health-care workers, and in the context of stringent regulatory approval perspectives. The relevant issues go well beyond considerations of biocompatibility of the carriers<sup>33</sup>, their biodistribution<sup>127</sup> and the reliability of their production protocols<sup>128</sup>, which of course remain central concerns. By their very tripartite nature, nanoparticles arguably fall under the purview of the three branches of regulatory agencies such as the

Food and Drug Administration (FDA): drugs, medical devices and biological agents. Therefore, they might have to be examined from these three perspectives accordingly<sup>129</sup>. The main advantage of nanoparticle resides in their multifunctionality — they can incorporate multiple therapeutic, diagnostic and barrier-avoiding agents. By current regulations, it could be expected that regulatory approval will have to be issued for each agent, and then for their combination. The time required for ascertaining their suitability for clinical use might therefore be quite substantial, and perhaps unnecessarily so.

The establishment of faster, safe regulatory approval protocols would ameliorate concerns about the length of time it takes for agents to be assessed by the FDA. This is especially true for multifunctional nanovectors, but applies to ‘conventional’ drugs, imaging agents and biological agents too. Nanotechnology might significantly contribute to realizing this goal. The development of approaches for the real-time assessment of the efficacy of therapeutic regimens would substitute for the direct observation of tumour size, molecular expression and efficacy in targeting the desired signalling pathways over, or in parallel with, conventional end point analysis, such as length of remission and extension of life. Research in this direction is steadily progressing, using the technology for molecular assessment both *in vivo* and *ex vivo*, as described earlier. The development of agents for *in vivo* molecular imaging<sup>26,34</sup>, the establishment of dual therapeutic/imaging nanovector technologies<sup>23</sup>, and the promise of *in vivo* microscopy<sup>130,131</sup> (with fluorescent multiphoton imaging reaching single-cell resolution<sup>132,133</sup>) all have the potential to transform regulatory processes. Therefore, nanotechnology might be expected to accelerate and render more accurate the regulatory approval process for all drugs, both nano-encapsulated and conventional, and assist in the determination of preferred therapeutic options.

The tripartite nature of nanoparticles might pose regulatory concerns, but also presents exciting opportunities for the development of a large number of novel therapeutic formulations: by combining 100 drugs of choice into the 100 most promising nanovectors, and directing them with 100 preferred biorecognition moieties, one would obtain 1,000,000 new targeted agents. Even allowing for an error of three orders of magnitude on this admittedly simplistic calculation, the number of resulting potential products with high efficacy and few side effects would compare very favourably with established drug-discovery routes.

#### **A look into the (nano)crystal ball**

Nanotechnology will have an important role in realizing the goal of detecting transforming cell populations early by *in vivo* imaging or *ex vivo* analysis. It will also allow the appropriate combination of agents to be chosen (based on accurate biological information on the tumour), targeting of these agents (while avoiding biological barriers) to the early cancer lesions to eliminate or contain them without collateral effects on healthy tissue, and monitoring the treatment effect in real time.

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The author declares **competing financial interests**: see web version for details.

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