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The Potential Legacy of Cancer Nanotechnology: Cellular Selection

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Abstract

Overexpression of oncogenes or loss of tumour suppressors can transform a normal cell to a cancerous one, resulting in uncontrolled regulation of intracellular signaling pathways and immunity to stresses, which both pose therapeutic challenges. Conventional approaches to cancer therapy, although they are effective at killing cancer cells, may still fail due to inadequate biodistribution and unwanted side effects. Nanotechnology-based approaches provide a promising alternative, with the possibility of targeting cells at an early stage, during their transformation into cancer cells. This review considers techniques that specifically target those molecular changes, which begin in only a very small percentage of normal cells as they undergo transformation. These techniques are crucial for ‘early-stage’ diagnosis and therapy.

Introduction

The efficiency of chemotherapeutic protocols depends on the extent to which the drug can abate and eradicate cancerous cells without destroying normal healthy cells. Successful chemotherapy thus depends on the selective targeting of tumour cells [1, 2]. Indeed, a paradigm shift in chemotherapeutic research has occurred over the past decade, with the focus moving from the discovery of new cytotoxic drugs to the development of target-specific protocols, aided by nanotechnology, for the selective treatment of cancer [3]. Such specific targeting of cancer cells and the subsequent administration of a drug to a selected site can potentially enhance the chemotherapeutic prognosis and reduce unwanted side effects, as illustrated in the cartoon in Figure 1 (see <http://www.cancer.gov/> for a detailed description of chemotherapy side-effects). In turn, the extent of selectivity of a chemotherapeutic drug towards the diseased cell is directly correlated with improved quality of life and survival rate of patients.

In 1906, Paul Ehrlich coined the term “magic bullet” to describe the ideal therapeutic reagent, possessing excellent targeting ability with super specificity [4]. Despite the many thousands of research articles that have since been published on drug targeting, this vision has remained an elusive goal in clinical practice. Although the available chemotherapeutic library has achieved moderate size and includes drugs that can target the intra-cellular signalling process, the cytoskeleton, DNA, the cell cycle and more, most drugs cannot differentiate between normal and cancer cells and, as a result, millions of patients are dying even after medication [5]. Moreover, during chemotherapy, patients often suffer from an increasing range of side effects ranging from infertility and memory loss to heart attack [6].

Nanoscale research has taken up this challenge. By improving site specificity,

nanoscale technology offers the possibility of exceptional improvements in early-stage diagnosis and therapeutics, and also the potential for circumventing multi-drug resistance (MDR) [7,8].

Nanoscale materials are currently being investigated to improve their specificity towards cancer cells and towards sub-cellular compartments in order to reduce systemic toxicity. A substantial number of such materials have received clinical approval for reduction of drug toxicity, but have yet to be translated into therapies, due to concern about their eventual fate. Current efforts are therefore aimed at integrating specific targeting, real-time monitoring, biocompatibility, and excellent pharmacokinetic profiles [9, 10]. The short- and long-term effects of such nanoscale materials are still unknown, but nanoscale research is presenting some tantalising questions. Might it be possible in the future for the pathologist to trace and diagnose the very first transformed cell that might lead to cancer? Could a doctor track down individual cancer cells and remove them from the circulation? Would it be possible to specifically instruct a cancer cell to self-destruct? Can a nanoscale material be designed to sense when, where and to what extent a drug should be released to avoid side effects and MDR?

Each of these functionalities could bring further complexity to the design [11]. Here, we focus on the trade-off between achieving such multifunctional “magic bullets” and specificity at the nanoscale in the effort to improve early stage detection and reduce collateral damage as side effects, central issues in the field of nanotechnology in cancer treatment.

Nanoscale diagnosis: molecular and cellular selection

Cancer is a heterogeneous disease that rapidly progresses to an uncontrollable stage after onset [12, 13]. Thus, the survival and quality of life of patients depend

directly on the stage at which the cancer has been detected; the most important factor in cancer management therefore is the detection of cancer cells at an early stage. Conventional detection systems that are regularly used by pathologists include the vacuum-assisted Mammotome® Breast Biopsy System for breast cancer, Papanicolaou test to detect potentially pre-cancerous and cancerous processes for cervical cancer, Hemoccult Test for colorectal cancer, sputum cytology for lung cancer, digital rectal examination and prostate-specific antigen (PSA) level for prostate cancer, combined with imaging (endoscopy, X-ray, CT, MRI and ultrasound). However, none of these are very sensitive for an early-stage detection [12, 14]. Nanotechnology-based approaches offer the possibility of reliably detecting and amplifying weak signals arising from a very early-stage transforming cell.

The detection of molecular biomarkers expressed during cancer onset is now an intensely researched area of nanotechnology and Tables 1 and 2 illustrate some recent developments. One such advanced example are ‘Nanobiochips’ that have been developed as integrated biosensors using quantum dots (QDs) that can potentially be used as multiplexed tools for quantitation of cancer markers such as carcinoembryonic antigen (CEA), cancer antigen (CA) 125, and Her-2/*Neu* (C-erbB-2). They offer very high specificity in serum and saliva and may also be integrated with nanofluidic arrays to facilitate detection of sub cellular constituents [15, 16]

Early stage *in vivo* selection

In vivo selection: In the ideal case, the transforming event would be probed by non-invasive means for the early-stage detection of cancer onset. An example of this type of approach was reported by Harisinghani *et al.* [17]. The authors used Ferumoxtran-10 (Combidex; Advanced Magnetix Inc, Cambridge, MA, USA), an

ultrasmall, superparamagnetic, biodegradable iron oxide particle that is covered with a low-molecular-weight dextran as lymphotropic paramagnetic nanoparticles that facilitate detection of clinically occult lymph-node metastases by enhancing MRI contrast in patients with prostate cancer. Such metastases are undetectable by any other non-invasive approach. Probing the surrounding microenvironment by nanotechnology can provide further information on the onset and status of the early-stage disease [18]. Sustained angiogenesis is one of the important signatures of disease onset. The imaging of such events with very low amounts of epitope targeted by nanoparticles could be an important step for early-stage detection of cancer [19].

Platform selection:

Currently, there is no suitable platform to detect the presence of cancer biomarkers at ultralow concentrations, but promising results are emerging from nanotechnology-based approaches for the amplification and detection of such ultra-low signatures. Nanoscale surface patterning is one such example, where carbon nanotube patterning has been used for the detection of carcinoembryonic antigen (CEA), a glycosyl phosphatidyl inositol (GPI) cell surface-anchored glycoprotein involved during foetal development. The production of CEA stops before birth and its reappearance serves as an important cancer biomarker [20]. Surface-Enhanced Laser Desorption / Ionisation Time-of-Flight Mass Spectrometry (SELDI-TOF-MS), is a promising MS platform for the detection of trace amounts of peptides and small molecules at the specific tumour microenvironment, which is now under investigation in combination with nanomaterials for early-stage detection of cancer [21,22].

Nanotechnology-based multiplex platforms are also emerging as future diagnostic approaches for sensitive and reliable detection. One of the best examples is the 'bio-barcode' developed by Mirkin, which consists of two-component oligonucleotide-modified nanoparticle probes [23], which can detect zeptomolar concentrations. Such nanotechnology-based platforms thus show considerable promise for probing early cell transformations by both *in vivo* and *ex vivo* measurement [24].

Nanocarriers in cancer cell selection

Nanocarriers also offer a new mode of chemotherapeutic drug delivery. The unique physicochemical properties of nanomaterials can be exploited for developing such systems. Examples include: (i) tunable surface charge (+ve) to promote electrostatic interaction with the comparatively negatively charged cancer cell surface (compared to normal cells) in order to stimulate endocytosis; (ii) tunable hydrophilic surfaces with stealth properties to prevent clearance by the reticulo-endothelial systems (RES) to prolong circulation times, thus avoiding opsonisation, the process by which foreign particles are altered by opsonins so as to become more readily and efficiently engulfed by phagocytes; and (iii) modification on the surface of nanoparticles to target specifically cancer cell membranes, the tumour microenvironment, or the sub cellular compartment. As a result of these approaches, nanocarriers can deliver high concentrations of drug to a selected site with reduced systemic cytotoxicity. One such example is described by Han *et al.*, where they demonstrate that the antitumour efficacy of nanoparticles carrying the anticancer drug camptothecin, can be enhanced by incorporating on average a single antibody Herceptin [25]. Table 3 summarises some nanotechnology-based drug delivery

systems approved by the US FDA and European Medicines Agency (EMA) for clinical use (see <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/>):

Nanoscale cell selection

Passive selection:

The biology of tumour growth and progression is exploited for passive selection by nanoparticle systems. Cancer cells have a faster proliferation rate than the surrounding normal cells and thus monopolise nutrients while generating more waste products, hence exerting stress on healthy cells. Gradually, cancer cells replace healthy cells and form a small cluster of rapidly proliferating cells, until the tumour reaches the diffusion limit size of around 2mm^3 [26]. At this point the normal cells cannot compete for an adequate supply of amino acids, nutrients, oxygen, and glucose, and are unable to manage the excreted waste. However, cancer cells survive in this limiting environment by inducing angiogenesis, overcoming the stress by increasing the surrounding vasculature. In such cases, there are always abnormalities in the basement membrane of the vessels due to defective angiogenesis, resulting in leaky vessels (gap size ranges from the nanometre to micrometer scale). Also, the lymphatic drainage network is abnormal and the asymmetric interstitial pressure (interstitium is the collagen-rich matrix that surrounds cancer cells and separates them from the vasculature) in the tumour inner core prevents normal diffusion of molecules [27].

Nanotechnology-based approaches exploit these opportunities to target tumour cells. Nanomaterials can escape through the leaky vasculature and can become trapped within the tumour due to the dysfunctional lymphatic drainage system. This phenomenon in fact generates an enhanced permeation and retention (EPR) effect for the nanomaterials in the tumor compared to conventional drug molecules, while

avoiding normal healthy tissue, which has non-leaky vessels and a proper lymphatic drainage system [18]. Another mode of passive selection depends on the tunable surface charge of nanoparticles. The surface of tumour endothelial cells contains more negative charges due to the presence of sialic acids and overexpressed phospholipids, and these can be selected by positively charged nanoparticles due to electrostatic interactions [28].

Active selection:

Active selection of cancer cells is based on conjugation of biomolecules (“active biomolecules”) to the surface of the nanomaterial system. In an ideal case, the targets are specific cancer surface molecules that are not expressed in other cell types and that bind to the active biomolecules. The primary objectives in such cases include: (i) specific recognition of cells via interaction of active biomolecules with respective ligands on the cell surface; (ii) avoidance of non-specific deposition of active biomolecules conjugated nanoparticles on the surface of non-target cells; (iii) increase in the local concentration of nanoparticles on target cells, to achieve the functional dose, before the drug becomes diluted through normal circulation and renal clearance; (iv) increase of cellular uptake of the drug in cancer cells through cell signalling and endocytosis; and (v) trafficking of nanomaterials to the target site through EPR. Surface-active biomolecules can bind either non-internalising target molecules or endocytosis-active molecules. In the former case, the nanoparticle is not engulfed, but resides on the surface of vascular endothelial cells; the drug-loaded nanoparticles can then release the drug with higher local concentration near the target site. In the latter case, a broad range of chemotherapeutic agents can be delivered including siRNAs and DNA binding drugs etc. Table 4 lists some nanoparticle systems under trial for active and passive selection of different cancer types. One such example is EndoTAG,

formulated with neutral and positive lipids, and paclitaxel as cytostatic drug. The idea is to target passively target newly developing, negatively charged endothelial cells, which are especially needed for the growth of tumour blood vessels by the EndoTAG with positively charged lipids on the surface. The EndoTAG loaded with paclitaxel works on the activated dividing endothelial cells. As a result, EndoTAG is targeting the blood supply system to tumours without affecting the supply to non-activated endothelial cells of the surrounding healthy tissue. EndoTAG is thus expected to prevent new blood vessel formation to inhibit tumour growth. Figure 2 illustrates active versus passive modes of selection by nanomaterials.

In summary, the passive selection of cancer cells is guided by the enhanced permeability and retention effect, electrostatic interactions (in case of positively charged nanomaterial systems) and the high metabolic rate of the cancer cells that indirectly leads to engulfment of nanomaterials - already present in the vicinity of cancer cells - due to EPR and phagocytosis-like activities. In active selection the nanoparticles are attached to either an antibody, or carrier protein or a ligand, which will allow it to selectively find and dock on the target cell having the receptor for the attachment.

Systemic selection

Systemic selection targets uncontrolled replication of cancer cells, angiogenesis during tumour growth, and metastasis. These events are not independent and may occur simultaneously, depending on the stage of the cancer. This heterogeneity of cancer is also being tackled by the design of multifunctional nanoscale systems for anticancer therapy.

Selection through uncontrolled proliferation

Cell cycle regulation is highly conserved to ensure normal growth and the proper architecture of tissues and organs. Damage to this regulation can induce an uncontrolled cell growth [29], which leads to the emergence of a more efficient proliferating machinery that replicates the cell without control. Such cells over express altered surface molecules, which can become targets for nanoparticle-based systems equipped with antibodies (against surface peptides or glycoproteins), aptamers (against surface antigens), or specific ligands (for receptors) [30]. Cancer cell surface host molecules that are regularly selected for study are: (i) folate receptor; (ii) transferrin receptors; and (iii) endothelial receptors.

Folic acid: Most cancer cell surfaces (e.g., breast, ovarian, prostate, lung, liver, head and neck, and brain cancer) express elevated levels of folate receptors compared to normal cells. Differences in folic acid transport between cancer and normal cells make the folate receptor an appropriate target. Although normal cells allow only transport the unconjugated reduced form of folic acid across their membrane, cancer cells bind the nanoconjugated form of folic acid with high affinity. In addition, folate receptors are expressed asymmetrically on the luminal surface of epithelia and are thus inaccessible to the circulation. However, the precise expression pattern of folate receptors is lost in cancer cells and the polarised distribution of folate receptors due to overexpression helps selection by folic-acid-conjugated nanomaterials, because it no longer is hidden from circulation [31].

Transferrin: The uncontrolled rapid proliferation rate of cancer cells compared to normal cells requires a large amount of iron for cell division. For this reason, cancer cells express extremely high levels of transferrin; a membrane receptor that binds and transports iron into cells via endocytosis. Transferrin is thus another potential target molecule for nanoconjugated systems for the selection of cancer cells

[26]. In addition, during metastasis and drug resistance (the two most difficult situations in cancer management), the expression of transferrin receptors is upregulated compared to normal healthy cells. Clinical trials are ongoing to validate transferrin targeting for cancer cell selection [32].

Epidermal growth factor receptors (EGFRs): Intracellular growth signalling is essential for the rapid growth and proliferation of cancer cells, leading to upregulation of growth factor receptor (EGFR) and human epidermal receptor (HER) on the cancer cell surface. These receptors transduce intracellular proliferation signals upon binding growth factor ligands. These receptors are favoured targets for cancer cell selection [33]. Herceptin, comprising a monoclonal antibody that targets HER, provides one example. It has been used in nanoconjugated systems for the selection of cancer cells expressing HER [34].

Nanomaterials: intrinsic cell selection

Recent reports describe nanomaterials without any targeting molecules on the surface, which can nevertheless be selectively taken up and produce a cell-specific response. The first report in this direction was from Patra *et al.*, who showed that citrate-capped gold nanoparticles can selectively target human lung epithelial cells (A549), without interacting with BHK21 kidney cells and HepG2 liver cells, as illustrated in Figure 3A [35]. This report first coined the term ‘cell selective’ nanoparticles and is now supported by a large number of new reports along the same lines [36], although the exact molecular mechanism is still unknown. The asymmetric distribution of the nanoparticles just outside the nucleus suggests the possibility of sub-cellular selection, as illustrated in Figure 3B and C. Later, Fraga *et al.* extensively investigated HepG2 cells [37] and also found that citrate and 1-mercaptoundecanoic acid (11-MUA)

coated gold nanoparticles are taken up by the human liver cancer cells with indicative DNA damage.

The intrinsic selection by nanoparticles is likely dependent not only on the composition of the material itself, but also its size, shape, charge and surface conjugations. Thus, different cell types may react to different aspects of the particle. For example, Sohaebuddin *et al.*, using a set of multi-wall carbon nanotubes, silicon oxide and titanium oxide nanoparticles, showed that the mechanism of response to the particles, even using the same nanomaterial, varied from one cell type to the next [38]. In addition, they have found that the response is variable between cell types and they explained such selection due to the physiological function of the cell types. Patra *et al.* undertook extensive investigations to find the optimum hydrodynamic parameters of the nanoparticles for such selections and found neither size nor surface zeta potential (the indirect measurement of the surface charge of the particles in colloidal system) alone determined the cellular interactions of gold nanoparticles. A complex interplay among hydrodynamic diameter, surface charge, surface functionalisation, and cell cycle stage determined the outcome [28, 39-41].

The geometry of a nanoparticle may also play a role. Jin *et al.* proposed a quantitative model for endocytosis rate based on nanoparticle geometry. They have shown size- and geometry-dependent cellular uptake and expulsion of single-walled carbon nanotubes and gold nanoparticles by single particle tracking [42]. Several investigations are ongoing to correlate the intrinsic effect of such nanomaterial properties with cellular function, uptake, transportation and toxicity *in vitro* and *in vivo* [43, 44]. Below, we highlight the rationale for these studies and review the unusual interactions of nanomaterials with tissue types to provide a perspective on the long-term implications of these findings.

Tissue specific cell selection

Recent statistics show that one in four deaths are due to cancer. In males, estimated new cases in 2013 for prostate cancer are estimated at 28% and at 14% for lung cancer. Similarly, in females, new cases are estimated at 29% for breast cancer and 14% for lung cancer [5]. Thus, it is reasonable to focus on these cancer types in the search for cellular target selection by nanomaterial systems.

Prostate cancer selection: The prostate cancer cell surface contains prostate specific membrane antigen (PSMA). This antigen (a trans-membrane glutamate carboxypeptidase II glycoprotein) is widely used for selecting prostate cancer cells [45]. Antibodies and aptamers specific for PSMA are used for diagnostic and therapeutic purposes [46]. *In vivo* studies show that targeted nanoparticle systems can effectively select prostate cancer cells (LNCaP prostate cancer cells) and inhibit tumour growth compared to non-targeted and bare drug [47]. An alternative involves folate, as discussed in the section above. Similarly, σ factors expressed by the prostate cancer cell surface can be used for selection, but this is not restricted to prostate cancer only [48].

Breast cancer selection: As discussed previously, one of the most important surface markers overexpressed in breast cancer is HER-2, and Herceptin is commercially used for the successful treatment of metastatic breast cancer [49]. Nanoparticle systems conjugated with HER-2 antibody fragment are being investigated for selective imaging, drug delivery and gene silencing through siRNA [50, 51]. As in the case of prostate cancer, folate-receptor-mediated nanoparticle systems are also being developed. For localised breast cancer selection, another important molecular marker is luteinising hormone-releasing hormone (LHRH) receptor on breast cancer cells. A superparamagnetic iron oxide nanoparticle (SPIONs) based system has been

developed for detection of breast cancer metastasis (LHRH-conjugated magnetic iron oxide nanoparticles) [52]. Importantly, the *in vivo* study showed that the LHRH-SPIONs nanoparticle system did not accumulate in the livers of normal mice, suggesting that off-target effects were limited. By contrast, the non-targeted controls accumulated in the liver, making this study one of the best examples of successful selection of specific cancer cells by nanoparticles [52].

Lung cancer selection: Although lung cancer is one of the leading causes of death worldwide, there is no established specific selection system developed for its diagnosis and therapy [5]. As a result, conventional chemotherapy and radiation protocols used along with surgical procedures, resulting in extensive side effects and quality poor life [53]. Thus there is a great need to design better therapies. The landmark work of Chen *et al.* [54] resulted in an aptamer that can specifically select small-cell lung cancer. The *in vitro* study showed specific cellular selection over different kinds of cancer cells including leukaemia, and liver and even lung adenocarcinoma. The aptamer was conjugated with magnetic nanoparticles for the selective targeting and isolation of small-cell lung cancer cells and later on, detection through fluorescent nanoparticles. This is the first successful example of small-cell lung cancer cell selection, isolation and detection by a nanoparticle system [26, 54].

Specific selection using nanomaterials

Species-specific selection: The first reported species specificity showed that gold nanoparticles can interact with the human lung epithelial cancer cell line (A549), but not with baby hamster kidney cell line (BHK21) [35]. Such evidence of selectivity was again reported recently using different sizes of copper nanoparticles. The differential behaviour has been observed for mammalian (H4IIE and HepG2) and piscine cell lines (PLHC-1 and RTH-149) [55]. These results may aid in the design of

therapies against cancers induced by other species such as oncoviruses. In such cases the nano-aided system might be helpful in differentiating the transformed cells from the host.

Sex-specific selection: Recent *in vivo* reports show gold nanoparticles can induce different interactions in males and females and indicate a sex bias in nanoparticle activity. One study showed that gold nanoparticles can induce significant kidney damages in female mice, while inducing liver damage in males [56]. The mechanism is yet to be established, but this effect should be considered when metallic nanoparticles are applied for chemotherapeutic purposes.

Multidrug resistance: cell selection

Development of tumour drug resistance is now considered a major hurdle restricting chemotherapeutic success. In multidrug resistance (MDR), a cancer cell can escape the effects of a wide range of chemotherapeutic drugs. In such cases, exposure of a cancer cell to a single drug gradually stimulates the cell to develop direct resistance to that group of drugs (structurally similar) or even cross-resistance with other structurally non-similar drugs. The molecular basis of this effect is the presence of ATP-binding cassette (ABC) transporters that pump out the chemotherapeutic toxic drugs from cancer cells. P-glycoprotein (P-gp) is one of the well-established ABC transporters present on the cell surface of multidrug resistant tumour cells. For a summary of the current status and the strategies to overcome MDR, see Saraswathy *et al.* [57]. MDR and possibilities for nano-based approaches to conquer MDR, are illustrated in Figure 4 and discussed below. Table 5 lists nanotechnology-based approaches for overcoming MDR that are currently undergoing clinical trials.

Redressing chemotherapy regimens

Nanotechnology enables the simultaneous presentation of multiple drugs around a central nanoparticle scaffold [58], allowing clinicians to administer the maximum number of chemotherapeutic drugs at the highest possible dose in the hope that the cancer cell will not be resistant to all the drugs. It is expected that threshold drug concentrations can be reached for effective chemotherapy even after the drug has been pumped out by the multidrug-resistant cancer cell.

Targeting MDR key player P-gp

P-gp is one of the key players in MDR. Direct targeting of P-gp and its depletion using nano-conjugated antibody may improve chemotherapy protocols in two ways. Firstly, P-gp proteins are only overexpressed on multidrug-resistant cell surfaces, and thus conjugating nanoparticles with P-gp antibodies endows selectivity for multidrug-resistant cells. Secondly, by inhibiting the activity of the P-gp, the drugs allow conventional chemotherapeutic agents to exert their normal effects. Another option is use of an anti-sense RNA for knocking out the expression of P-gp at the translational level. Patil *et al.* reported one such example *in vitro* and *in vivo*, where coencapsulation of paclitaxel (a chemotherapeutic agent) and siRNA for P-gp in a single poly(D,L-lactide-co-glycolide) nanoparticle overcame tumour drug resistance [59].

Nanoscale cocktail

A combination of different modules in a single nanoparticle system to bypass tumor drug resistance is in a clinical trial. In this example, TOCOSOL, a paclitaxel and vitamin E nanoscale emulsion, is equipped with a P-gp inhibitor TGPS [60].

Next-generation nanodrugs: cell selection

Design of the first generation of nanodrugs sought mainly to increase the bioavailability of drugs at the tumour site and, to some extent, to reduce their side effects. The first nanodrug approved by the FDA was the paclitaxel protein-bound particle Abraxane that was indicated for the first-line treatment of patients with metastatic adenocarcinoma in 2005, and since then the list has expanded as shown in Table 3. In 2012 Abraxane was approved for injectable suspension in non-small cell lung cancer (NSCLC). The degrees of freedom allowed for next-generation nanodrugs is likely to expand compared to earlier liposome systems with next-generation nanotherapeutics for cancer management being designed for target selection along with increased efficiency, as illustrated in Figure 5. One such example is BIND-014 that targets the prostate-specific membrane antigen (PSMA). This compound has already completed a phase 1 clinical trial, which resulted in promising for anti-tumour activity in patients with advanced and metastatic solid-tumour cancers.

The next generation chemotherapy is expected to be based on nanotheranostics, the combination of both diagnostic and therapeutic elements at the nanoscale. Such systems, comprising drug and molecular targeting agents, deliver diagnostic capabilities along with therapy, and are emerging as the next generation of multifunctional remedies. Targeted delivery systems with nanoscale dimensions, such as liposomes, micelles, dendrimers, and different nanostructures have already been reported. However, real time tracking of the above-mentioned systems is still under investigation. Future delivery systems should release the drug from the carrier to its target in a well-controlled manner through site-specific stimuli such as inflammation, pH, and ionic strength. Again, the heterogeneity and super adaptive nature of cancer require novel therapeutic protocols that must be smart and custom-fitted to the cancer

type and individual patient. One such prototype system has been developed by Patra *et al* recently [61], where they have synthesised core-shell nanocomposite micelles using polystyrene block poly acrylic acid (PS-*b*-PAA) and SPIONs as a stimulus - responsive targeted (by folic acid) nanotheranostic module for the controlled release of drug into a tumour site with real-time monitoring possibilities through MRI. The findings suggest the possibility of next-generation smart theranostic micellar nanostructures as biocompatible, cancer cell target specific, tumour-microenvironment sensitive, pH-responsive systems with future provision for early-stage tumour sensing, tracking, and therapy, which is selective for cancer cells.

Concluding remarks and future prospective

Lack of selectivity is still the major challenge in cancer management in terms of both diagnosis and therapy. Most current methods for cancer detection still lack sensitivity and have relatively high detection limits and are thus unable to track cancer at an early stage. Nanoparticle systems provide a new method for recognition, delivery and extension of effective drug lifetimes. Although still in its infancy, research on nanoparticles has resulted in systems that are able to target specifically cells, subcellular components, and the key molecules involved in tumour growth and metastasis. Recent reports include metallic, inorganic, and polymeric nanomaterials capable of changing the biodistribution of drug molecules at the cellular and sub-cellular level, but challenges still remain in terms of selectivity. In addition to improving this parameter, future work will seek to control when and how a drug is released at the target site to tackle the heterogeneity of cancer, where combinations of different drugs may need to be administered in different time frames. Nanoparticle systems offer the opportunity to integrate both the drug and the detection molecule to

create a multifunctional platform that can simultaneously target the correct site, and allow monitoring and release of the drug, thus fulfilling a prime requirement for personalised medicine. Such a platform would have the potential to control remotely the drug release profile and hence could also mitigate problems associated with MDR. However, most nanomaterials have yet to reach clinical trials. More work is thus needed before we can resolve whether nanoparticles are indeed the long-anticipated “magic bullets” for treating cancer.

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Figures

Figure 1: State-of-the-art conventional chemotherapy and its associated problems

One of the key problems associated with conventional chemotherapy is its unintentional toxicity for normal cells. Apart from the beneficial effects of killing cancer cells, anticancer drugs simultaneously destroy healthy tissue, resulting in systemic toxicity. The first course of chemotherapy may show highly beneficial effects. However a few cancer cells manage to find the escape route and often survive and proliferate against the drug. With the subsequent courses of high-dose chemotherapy, the drug-defiant cells thrive, showing increasing resistance to the administered drug and finally displaying virtual invulnerability to the conventional chemotherapy.

Figure 2: The mode of action for nanomaterials in cancer cell selection

Most of the conventional chemotherapeutic drugs have poor pharmacokinetic profiles and are unable to differentiate normal (Off-target) from the diseased cells (On-target), and are thus distributed non-specifically within the whole body, leading to systemic toxicity resulted with deleterious side effects. Due to the minute distinct characteristics of tumour microenvironment and tumour angiogenesis, it is possible to design nanotechnology-based drug delivery systems that can selectively target anti-cancer drugs to tumours. The mode of selection may be active (left half) or passive (right half). Nanomedicine can reach the tumour site passively through the leaky vasculature surrounding the tumours by the enhanced permeability and retention (EPR) effect (upper half). The newly developing endothelial cells with negative surface charge can be passively targeted due to electrostatic attraction to positively charged nanocarriers (upper-right). On the other hand active ligands grafted on the nano-surface allow active targeting by binding to the respective receptors present or overexpressed by cancer or angiogenic endothelial cells (upper left). As a result of the comparatively low EPR and lack of leaky vasculature, the healthy normal cells are unaffected by the nanotechnology-based approaches (lower half).

Figure 3: Cell selection by gold nanoparticles (GNPs):

(A) Phase contrast morphology of GNP-treated cancer cells. Only A549 cells are affected and change their morphology whereas another two cell lines (HepG2 and BHK21) are unaltered. Inset is the magnified view of the single A549 cell describing the round shaped morphology. (B) Confocal microscopic images of the selected A549 cells with Z-sectioning (left to right). (C) Asymmetric accumulation of GNP outside the nucleus (DAPI, 4',6-diamidino-2-phenylindole is a fluorescent stain that binds strongly to A-T rich regions in DNA, blue). Reprinted, with permission, from [35].

Figure 4: Multidrug resistance (MDR) mechanism and selection via nanotechnology:

Drug efflux by P-glycoprotein (P-gp) [1], an MDR1 gene product [2], or the MDR protein (MRP) can reduce the effective drug dose to sub-threshold levels. MRP co-transporters glutathione, which can detoxify a variety of alkylating agents through neutralisation [3]. Nanotechnology offers several different approaches to overcome these challenges. The nanotechnology-based approach can selectively target P-gp to stop drug clearance [I and ii]; the local concentration of the drug can be increased due to the positive enhanced permeability and retention (EPR) effect and the amount of drug delivered by the nanocarrier [iii]; a combinatorial drug delivery system can be used for delivery of multiple drugs simultaneously to avoid clearance of all drug molecules [iv]. In MDR, p53 alters apoptotic signalling molecule Bcl-2 and cell cycle regulatory molecule microtubule-associated protein (MAP)4 [4]. The release of transcription factor E2F regulates drug sensitivity (e.g., methotrexate, and fluorouracil etc) by producing dihydrofolate reductase (DHFR) and thymidylate synthetase (TS). Mutations in topoisomerases (topo) generate resistance to anthracyclines and etoposide, which can be countered by combinatorial therapy with multiple drugs in a single nanoparticle system [iv].

Figure 5: Nano bullet

Multiplex nanoparticles with all the required functionalities for cancer management may be the way to realise the ‘magic bullet’ proposed 100 years ago by Paul Ehrlich to treat cancer [4].

Cancer	Target	Probe	Methodology	Detection	Ref
Prostate	PSA	i) GNP	Fluorescence	0.032 pg/mL	62
		ii) Silicon	Label free	5 fg/mL	63
		Nanowire	Resonance	25 pg/mL	64
		iii) Dip-Pen Nanolithography	Raman		
Breast	PSMA	GNR	Optoacoustic	3D cell model	65
		PEDOT	Electrochemical	100pM	66
	Her-2	magneto-nanoclusters	Magnetic Imaging	-	67
		Nano array	Electrophoretic particle entrapment system	10 attomolar (aM)	68
Lung	CEA	Gold nanoshell	Surface Enhanced Raman Scattering	pg/mL in complex mixture	69
colon	KRAS	GNP	Colorimetric	Naked eye	70

GNP, Gold nanoparticles; GNR, Gold nanorods; PEDOT, Poly(3,4-ethylenedioxythiophene)

Table1: Molecular selection

Cell	Probe	Detection	Ref
Breast	GNP	SERS	71
	Quantum dots	PL	72
	Oval shaped GNP	Orthogonal	73
	Quantum dots	Amplification	74
	CdS	Two photon	75
	SWCNT	scattering	76
		Electrochemical Raman	
Circulating tumour cells Human epithelial cancer Human Lung Cancer cells Ovarian cancer cells	Nanobeads	Lateral Magnetophoresis	77
	MnFe ₂ O ₄ magnetic nanocrystals	Fluorescence/ MRI	78
	silica-coated superparamagnetic nanoparticles	Fluorescence/ MRI	79
	GNR	Photoacoustic Imaging	80

PL, Photoluminescence; SERS, Surface Enhanced Raman Scattering; SWCNT, Single wall carbon nanotubes

Table2: Cellular selection

Name	Target	Active component	Nano System
Myocet™ Abraxane	Breast Cancer	Doxorubicin	Liposomal
	Metastatic Breast Cancer	Paclitaxel	Albumin-bound
Doxil Eligard	Ovarian Cancer	Doxorubicin	PEGylated liposomal
	Prostate Cancer	Leuprolide acetate	Biodegradable polymer
Caelyx DaunoXome Genexol-PM	Kaposi's Sarcoma	Doxorubicin	PEGylated liposomal
	Kaposi's Sarcoma	Daunorubicin	PEGylated liposomal
	Breast and Lung Cancer	Paclitaxel	Polymeric micelle
Oncaspar	Lymphoblastic leukaemia	L-asparaginase	Polymeric micelle

Table3: Nanoscale delivery systems on the market

Passive				Active			
Nanosystem	Drug	Cancer	Clinical trial	Nanosystem	Cancer	Targeting agent	Clinical trial
SPI-77	Cisplatin	Epithelial ovarian cancer	Phase II	MCC-DM1	Metastatic Breast	Trastuzumab	Approved

					Cancer		
Marqibo®	Vincristine sulfate	Non-Hodgkin's Lymphoma and ALL	Phase II	BIND-014	Metastatic cancer	Prostate-specific membrane antigen (PSMA)	Phase I
DepoCyt®	Cytarabine	Meningeal Neoplasms	Phase IV	MBP-426	Metastatic solid tumour	Transferin	Phase I
EndoTAG®	Paclitaxel	Breast cancer	Phase II	CALAA-01	Solid Tumor Cancers	Human transferrin protein (Tf)	Phase I
CRLX101	Cyclodextrin	Adenocarcinoma	Phase II	ALN-VSP02	Advanced Solid Tumors and Liver cancer	VEGF	Phase I

VEGF, vascular endothelial growth factor

Table 4: Nanoparticle systems with active and passive selection undergoing clinical trials (see: <http://www.fda.gov/Drugs/default.htm> and <http://clinicaltrials.gov/ct2/home>)

Nanoscale formulation	Mode of actions	Current status	Ref
Pluronic®	Inhibition of transporters, ATP depletion	Clinical trial, Phase-II	[81,82]
Lipidic NP	P-gp inhibition, endocytosis, ATP depletion	<i>In vitro</i> & <i>In vivo</i>	[83-85]
Hybrid NP (Polymer and Lipid)	Phagocytosis	<i>In vitro</i>	[86,87]
Liposome	Reverse MDR, bind to P-gp	<i>In vitro</i>	[88,89]

NP: Nanoparticles;

Table 5: Nanoparticle systems that target MDR