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Review Article

Nano-Oncology: Clinical Application for Cancer Therapy and Future Perspectives

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Nano-oncology, the application of Nanomedicine to cancer diagnosis and treatment, has the potential to transform clinical oncology by enhancing the efficacy of cancer chemotherapy for a wide spectrum of invasive cancers. It achieves this by enabling novel drug delivery systems which target the tumour site with several functional molecules, including tumour-specific ligands, antibodies, cytotoxic agents, and imaging probes simultaneously thereby improving tumour response rates in addition to significant reduction of the systemic toxicity associated with current chemotherapy regimens. For this reason, nano-oncology is attracting considerable scientific interest and a growing investment by the global pharmaceutical industry. Several therapeutic nano-carriers have been approved for clinical use and others are undergoing phase II and III clinical trials. This paper describes the current approved formulations, such as liposomes and polymeric nanoparticles, and discusses the overall present status of nano-oncology as an emerging branch of nanomedicine and its future perspectives in cancer and therapy.

1. Introduction

Nanotechnology is defined as the development of small devices in a range of 1 to 100 nm. Such nano-structures/devices can offer to the clinical practice of medicine in general, and to oncology in particular, many potentially significant and desirable applications which address unmet clinical needs [1].

These nano-structures by virtue of the quantum effects acquire at the nanoscale unique physical and chemical properties not present at their macroscale. Additionally, by virtue of molecular scale, they are able to interact with biological systems at cellular level.

The current focus of new technologies is to design and develop novel pharmaceutical formulations or drug carriers, which are both size- and site-specific aimed at targeted delivery of the active drug to the tumour site whilst evading clearance by the reticuloendothelial system (RES).

The ideal *nano-carrier* for drug delivery and cancer chemotherapy should (i) stabilize without altering the pharmacological activity of the drug, (ii) prevent premature metabolic degradation of the drug in the systemic circulation such that it arrives in a pristine state at the intended target, (iii) release the drug at the intended site/tumour, and (iv) exhibit similar or lower toxicity than that of the free drug. Other ideal characteristics include ability for visualisation by MRI for guided chemotherapy and molecular imaging. In general, schedule-dependent regimes requiring steady state drug levels are ideal for these controlled drug delivery systems (CDDSs), for example, cytotoxic agents when a prolonged sustained drug concentration in the tumour is necessary to kill cancers cells as these enter and exit the sensitive phase of the cell cycle.

Such CDDSs overcome the problems encountered when cytotoxic agents are administrated systemically as these chemotherapeutic drugs lack specificity and thus cause significant damage to noncancerous tissues (systemic toxicity), including bone marrow suppression, hair loss (alopecia) and gut mucosal damage. Lack of specificity for cytotoxicity of these drugs is further compounded by escalating doses required in chemotherapy for solid cancers because of their rapid excretion and low therapeutic index [2]. The use of nano-carriers should improve the pharmacokinetics by prolonging the half-life of drugs in the systemic circulation. Moreover, nano-carriers can improve the aqueous solubility of poorly soluble drugs. In this respect, the majority of

Table 1: Goals of targeted nanoscale drug delivery systems.

Characteristic of an ideal carrier for cancer therapy:
Biocompatible and biodegradable
Facilitate cellular uptake and intracellular trafficking
Retain the drug at the target site for the desired period of time
Protect the drug from the degradation and from premature clereance
Ensure minimal drug leakage during transit to target
Decrease drug localisation in sensitive, non target tissue
Increase drug localisation in the tumour
(a) Passive targeting
(b) Active targeting

cytotoxic agents used in cancer chemotherapy are water insoluble and need to be dissolved in toxic organic solvents, such as Cremophor EL, for their intravenous systemic [3, 4]. The relevant advantages of nano-carriers as CDDS over free drugs are summarised in Table 1.

The following sections provide an overview of the arsenal of most promising nano-carriers, underlined by their current established clinical usage and evaluation in on-going clinical trials.

2. Nano-Carriers for Oncology

2.1. Liposomes. Liposomes have a long history as drug carrier systems because of their easy preparation, acceptable toxicity, and biodegradability profiles [5, 6]. Liposomes are self-assembling colloid structures composed of lipid bilayers surrounding an aqueous compartment(s) and can encapsulate a wide variety of (chemo)therapeutic drugs whether hydrophilic or hydrophobic in nature [7, 8]. Drug loading in liposomes can be achieved through (i) liposome formation in an aqueous solution saturated with soluble drug; (ii) the use of organic solvents and solvent exchange mechanisms; (iii) the use of lipophilic drugs; and (iv) pH gradient methods [9].

Because liposomes are of the order of 400 nm in size they are rapidly cleared by mononuclear phagocytic system (MPS) which requires preliminary opsonisation by the immune system. A useful method for evading opsonisation of carriers was developed at Rutgers University in the 1960s by a process called PEGylation: a biocompatible polymer, poly(ethylene glycol) (PEG; [CH2CH2O]n), is conjugated to the drug carrier [10]. The coating by PEG chains of the surface of the nanoparticles results in significantly increased blood circulation half-life. The opsonisation process is blocked or delayed by the hydrophilic protective layer around the nanoparticles which repels the absorption of opsonin proteins.

Liposomes can be classified as first generation or naked liposomes with an unmodified phospholipid surface, second generation or stealth liposomes with a layer of hydrophilic carbohydrates or polymers, usually PEG, onto the surface of the vesicles, and third generation liposomes that incorporate surface ligands to improve the therapeutic index of the drug by increasing the selectivity and the specificity of the complex. Figure 1 is a schematic representation of the three classes of the liposomes.

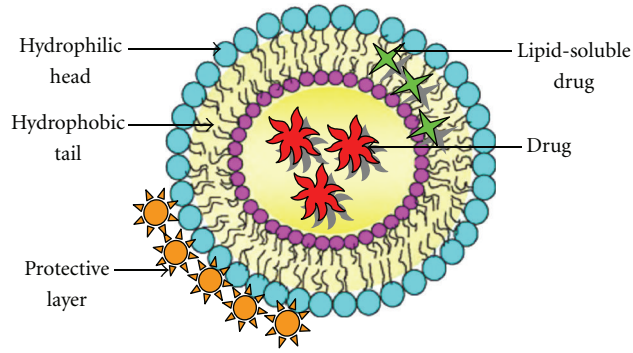

Figure 1: Diagram of bilayered membrane structure. The internal core entraps hydrophilic drug, while lipid soluble drugs are entrapped between the hydrophobic tails of the phospholipids. The outer surface can be functionalized with PEG and ligands for active targeting.

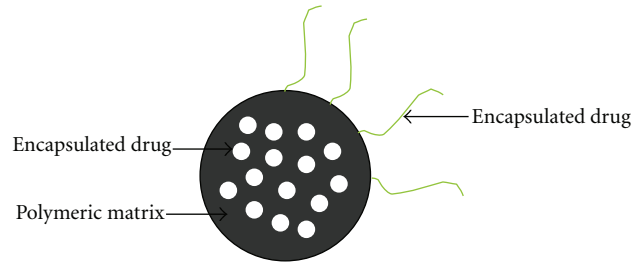

Figure 2: A schematic representation of polymeric particle as drug carrier: drug is entrapped in polymeric matrix and functional moieties lead to active targeting.

2.2. Polymeric Nanoparticles. Delivery devices made from biodegradable polymers are an attractive option as carriers of therapeutic drugs in cancer therapy. Polymeric nanoparticles (NPs) (Figure 3), which include nanospheres and nanocapsules, are solid carriers ranging from 10 to 1000 nm in diameter made of natural or artificial polymers which are generally biodegradable and in which therapeutic drugs can be adsorbed, dissolved, entrapped, encapsulated, or covalently linked to the polymer backbone by means of a simple ester or amide bond that can be hydrolyzed *in vivo* through a change of pH (Figure 2).

Synthetic polymers, which include poly(lactic acid) (PLA) [12], poly(glycolic acid) (PGA) [13], poly(ethylene glycol) (PEG) [14], and their copolymers, have been among the most extensively researched due to their biocompatibility, biodegradability, and regulatory approval. Also natural polymers such us chitosan, alginate, and gelatine have been extensively tested [15].

When systemically administered, nanoparticles are generally more stable than liposome but are limited by poor pharmacokinetic properties that is, uptake by the RES. As with liposomes, the surface of nanoparticles can be coated with molecules, or intercalated into their structure,

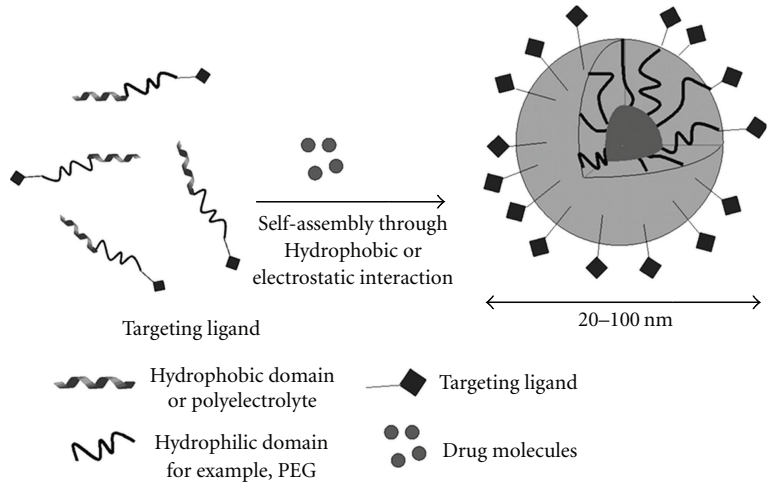

Figure 3: Polymeric micelle core-shell structure and drug encapsulation (reproduced from [11]).

to increase pharmacokinetics and even enable targeting for delivery and imaging purpose [16].

2.3. Micelles. Polymeric micelles are biodegradable spherical nano-carriers with a usual size range of 10–200 nm. They are formed by self-assembly of block copolymers consisting of two or more polymer chains with different hydrophobicity. These copolymers spontaneously assemble to form a core-shell structure in an aqueous media to minimize the system's free energy (Figure 3). The hydrophobic segments form the hydrophobic inner core to minimize their exposure to environment, whereas the hydrophilic chains form the outer hydrophilic corona-like shell to stabilize the core through direct contact with water [11].

Micelles are considered ideal drug delivery vehicles because they provide a set of important advantages. The hydrophobic core can be used to carry pharmaceuticals, especially lipophilic drugs which are solubilized and physically entrapped in the inner region with high loading capacity. It must be remembered that hydrophobic drugs can only be administered intravenously after addition of solubilizing adjuvants like ethanol or Cremophor EL, which often induce toxic side effects. The incorporation of these drugs in micelles avoids the use of adjuvants. The hydrophilic shell not only provides a steric protection that increases micellar stability in blood, but also provides functional groups suitable for further micelle modification. Polymeric micelles can simultaneously codeliver two or more therapeutic agents and are capable of releasing drugs in a regulated manner. The encapsulated drugs can be released through erosion of the biodegradable polymers, diffusion of the drug through the polymer matrix, or polymer swelling followed by drug diffusion. External conditions such as change of pH and temperature can also induce drug release from micelles. Moreover, the surface modification of micelles with ligands such as antibodies, peptides, or other small molecules can be used for targeted delivery and uptake of these nano-carriers, thereby reducing their systemic toxicity and improving their specificity and efficacy [11].

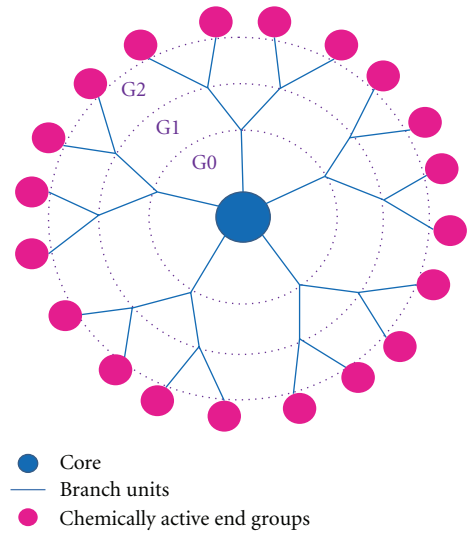

Figure 4: Structure of a dendrimer.

2.4. Dendrimers. Dendrimers are spherical, highly branched, and synthetic macromolecules with adjustable size and shape. They contain multiple layers with active end groups, also known as generations, that extend outwards from an initiator core called generation zero (Figure 4). The size of dendrimers is usually in the range of 1–15 nm. The branches of these polymers provide a large surface area to which chemotherapeutic drugs and targeting molecules can be attached through covalent conjugation or electrostatic adsorption. Alternatively, therapeutic agents can be loaded in the cavities of the core regions through hydrophobic interaction, hydrogen bonds, or chemical linkage [11].

The most commonly studied dendrimers belong to the family of PAMAM (polyamidoamine) dendrimers. These polymers have shown great potential for drug delivery because they are biodegradable and biocompatible and have high water solubility [17]. Recently, a G5-PAMAM dendrimer has been developed with a diameter of 5 nm and more than 100 functional amines on the surface. This nano-carrier

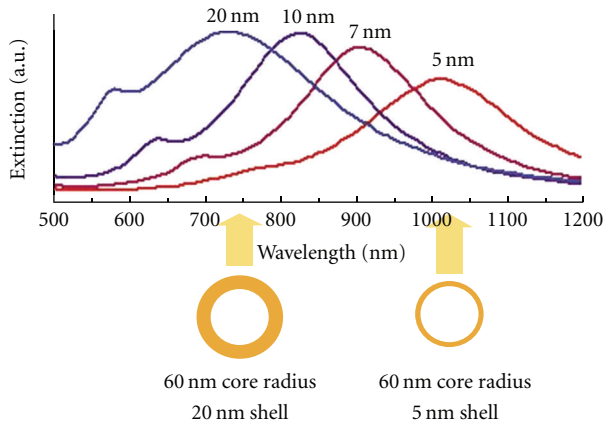

Figure 5: Extinction spectra of gold nano-shells depending on the core: shell diameter ratio (reproduced from [21]).

was used for the delivery of methotrexate in a preclinical study. The dendrimer surface charge was first reduced by modifying peripheral amines with acetyl groups. Then, the G5-PAMAM dendrimer was conjugated with methotrexate (as the cytotoxic agent) and with folate as the targeting molecule. A biodistribution study in mice with subcutaneous tumors showed internalization and intracellular accumulation of dendrimers in xenograft human KB tumors that overexpressed folate receptors. The *in vivo* delivery of the G5-PAMAM dendrimer conjugated with methotrexate induced a tenfold reduction in tumor size compared with that observed following systemic administration of free methotrexate at the same molar concentration [18]. This study provided the basis for further preclinical studies on dendrimers, which are now under investigation for cancer chemotherapy.

2.5. Gold Nano-Shells. Gold nano-shells are nanospheres composed of an ultrathin layer of gold around a dielectric core, typically silica. The size of these nanoparticles ranges from 50 to 500 nm in diameter but the outer shell can be only few nanometers thick. Gold nano-shells have peculiar optical properties due to their unique interaction with light and related to a nanotechnological phenomenon known as surface plasmon resonance (SPR). In this process, the conducting surface electrons of the metal nano-shell oscillate collectively in the presence of the oscillating magnetic field of light. After absorption of radiation, the surface plasmon decays radiatively resulting in light emission or nonradiatively as heat. The SPR effect depends on nanoparticle size and shape (Figure 5), so the absorption characteristics of gold nano-shells can be tuned by adjusting the core: shell diameter thickness ratio [19, 20].

Gold nano-shells can be used for medical applications because they are resistant to corrosion, are physiologically inert and thus quite safe [19]. The primary application of gold nano-shells in cancer treatment is the photothermal ablation based on their absorption/heating properties (temperatures $\geq 42^{\circ}$C) in tumors. With other forms of hyperthermic ablation, for example, radiofrequency probes, it is difficult to avoid thermal damage to the normal tissues surrounding the tumor. Gold nano-shells offer a solution to this problem. Indeed, gold nano-shells targeted to tumor site can be locally heated by radiation with a near-infrared laser (wavelength in the range of 650–950 nm), allowing selective tumor ablation without any collateral damage to normal tissues [19–22]. Recent studies have demonstrated the efficacy of gold nano-shells in the destruction of mouse carcinoma tumors and in the photo-thermal ablation of human breast carcinoma tumors implanted in mice [23, 24]. These experimental studies have led to the initiation of a clinical trial using gold nano-shells as hyperthermia agents for cancer therapy in patients with oropharyngeal malignancies. However, near-infrared thermal ablation with gold nano-shells can only be effective for the treatment of superficial tumors, because near-infrared radiation is significantly attenuated by biomolecules and cannot penetrate the deeper tissues [22].

The ability of gold nano-shells to scatter light can also be used for imaging, thus allowing the detection and diagnosis of cancer. In particular, gold nano-shells have been useful as *in vivo* contrast agents [25], and when conjugated to antibodies to epidermal growth factor receptor, they have been used for the imaging of early cervical cancers [26].

2.6. Superparamagnetic Iron Oxide Nanoparticles (SPIOs). SPIOs are nanoparticles usually composed of Fe_3O_4 (magnetite) with a size of less than 20 nm [21]. These materials show peculiar superparamagnetic properties related to nanoscale dimensions. Like ferromagnetic materials, SPIOs strongly magnetize under the influence of a magnetic field, but as with other paramagnetic materials, the removal of the field eliminates the phenomenon. The magnetic behavior of paramagnetic and superparamagnetic species is due to the presence of unpaired electrons, whose spins align with the applied magnetic field. Pools of adjacent electrons aligned in the same direction form the so-called magnetic domains. The difference between paramagnetic and superparamagnetic materials is that the latter are smaller and do not possess multiple domains, but only a single magnetic domain where all spins are mutually aligned. This produces a large magnetic moment, which can be exploited for medical applications. SPIOs have intrinsic toxicity, so they have to be suitably modified through surface coatings (e.g., dextran or PEG coatings) for biocompatibility before any medical use [19] SPIO surface can also be functionalized through the attachment of targeting ligands [21].

Like gold nano-shells, SPIOs are attracting particular interest as hyperthermia agents for cancer thermal ablation. It is known that oscillating magnetic fields (in the range of kHz–MHz) applied to SPIOs result in generation of heat because of the great relaxation loss of the single magnetic domain. Energy can be dissipated through Brownian relaxation (heat due to total particle oscillation) or Neél relaxation (heat due to the rotation of the magnetic moment in the oscillating magnetic field) [27]. After their delivery and accumulation at the tumor site, SPIOs can be remotely activated through oscillating magnetic fields for the ablation of malignant tumors situated in deep regions of the body. Magnetic fields are not absorbed by normal tissues, so that

Table 2: Examples of liposomes available on the market and in clinical use.

Composition	Trade name	Company	Indication	Administration
Liposomal daunorubicin	DaunoXome	Gilead Sciences	Kaposi's sarcoma	intravenous
Stealth liposomal doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	Kaposi's Sarcoma; refractory ovarian cancer; refractory breast cancer	intramuscular
Liposomal doxorubicin	Myocet	Zeneus	Metastatic breast cancer in combination with cylophosphamide	intravenous
Liposomal muramyl Tripeptide phosphatidyl Ethanolamine	MEPACT	Taked	Osteosarcoma	intravenous
Cytarabine	Depocyt	SkyePharma PLC	Lymphomatous meningitis	intrathecal
Liposomal vincristine	Onco-TCS	Inex Enzon	Non-hodgking lymphoma	intravenous

magnetic thermal ablation of tumors does not damage normal tissues [21]. Recent clinical trials have demonstrated the feasibility and good tolerability of magnetic thermotherapy for the treatment of human prostate cancer after the transperitoneal injection of iron oxide nanoparticles and the application of an alternating magnetic field [1]. However, the efficacy of magnetic thermo-ablation of tumors as monotherapy is limited and requires improvement. Thus, it seems likely that SPIOs thermotherapy will probably be used as part of a combination regimen.

The peculiar magnetic properties of SPIOs can also be useful for cancer diagnosis and detection by magnetic resonance imaging (MRI), an important medical imaging technique depending on signals from water protons of the body. By virtue of their large magnetic moment, SPIOs can enhance image contrast in MRI, thus producing distinct images and allowing the discrimination between neoplastic and healthy tissues. Several SPIOs have been in long established clinical use as contrast agents for MRI [19].

3. Nano-Carriers in Clinical Usage

3.1. Liposomes: Clinical Use. Many liposomal formulations of anticancer drugs have been approved for human use and are already available on market. The list of the cytotoxic agents marketed for clinical use by various pharmaceutical companies are shown in Table 2.

The first liposome formulations approved by the regulatory authorities were Doxil and Myocet. Both products contain the cytotoxic drug doxorubicin, a chemotherapeutic agent used widely in the treatment of breast, ovarian, bladder, and lung cancers. The two liposomal formulations, Myocet and Doxil, differ in PEG coating: Doxil is a PEG-liposome formulation designed to prolong blood circulation time. Free doxorubicin has an elimination half-life time of 0.2 h. This value is prolonged to 2.5 h and 55 h for Myocet and Doxil, respectively (Table 3) [28].

Liposomal encapsulation and consequently polymer coating can substantially affect a drug's functional properties relative to those of the unencapsulated drug. Harris et al., have reported on the advantages of Myocet over free doxorubicin in terms of cardiotoxicity by evaluating two parameters: the incidence of cardiac events and congestive heart failure with significant decrease of cardiac events and congestive cardiac failure of 16% and 6%, respectively [29] (Table 4).

Table 3: Values for half-life and AUC for two liposomal formulations and free doxorubicin.

	Half-life time	AUC (Area Under Curve)
Free doxorubicin	0.2 h	4 μgh/mL
Myocet	2.5 h	45 μgh/mL
Caelyx/Doxil	55 h	900 μgh/mL

Table 4: Effect of Myocet on cardiotoxicity than free doxorubicin.

	Incidence of cardiac events	Congestive Heart failure
Free doxorubicin	29%	8%
Myocet	13%	2%

Myocet is currently used in the chemotherapy of breast cancer in combination with other chemotherapeutic agent (cyclophosphamide). Doxil is used to treat women with metastatic breast cancer who have an increased risk of heart damage, in patients with advanced ovarian cancer and in AIDS-related Kaposi's sarcoma.

Other liposomal systems have been approved and are currently on the market such as MEPACT, DepoCyt and Onco-TCS. MEPACT is a liposomal formulation of mifamurtide, an immune modulator proposed for clinical use in adjuvant chemotherapy of children and young adults with high grade resectable non-metastatic osteosarcoma.

DepoCyt, used in the treatment of lymphomatous meningitis, is a sustained-release liposomal formulation of the active ingredient cytarabine designed for direct administration into the cerebrospinal fluid (CSF). Onco-TCS is a non-PEGylated liposomal formulation (about 50 nm in diameter) of daunorubicin and vincristine. DaunoXome is a liposomal preparation of daunorubicin, formulated to maximize the selectivity of daunorubicin in AIDS related Kaposi's sarcoma. As with Myocet, both the pharmacokinetic parameters and incidence of side effects are decreased by DaunoXome (Tables 5 and 6).

Table 5: Pharmacokinetic parameters of DaunoXome compared to free daunorubicin.

	Half-life time (h)	Plasma Clereance (mL/min)
Conventional daunorubicin	0.77 + 0.3	236 + 181
DaunoXome	4.41 + 2.33	17.3 + 6.1

Table 6: Comparative toxicity (neuropathy and alopecia) of DaunoXomeand free daunorubicine.

	Neuropathy (%)	Alopecia (%)
Conventional daunorubicin	41	36
DaunoXome	13	8

4. Nano-Carriers in Clinical Trial

4.1. Liposomes. Drug-encapsulated liposomes dominate clinical trials designed to study the effects of these CDDS in overcoming rapid clearance from the blood by phagocytic cell of the RES and thus improving the therapeutic index.

The main liposome formulations currently in clinical trials are listed in Table 7.

Aroplatin is a novel liposomal third generation formulation of cisplatin (platinum). Its antitumour activity has been demonstrated in the treatment of colorectal cancer. SPI-77, a pegylated liposomal formulation of cisplatin developed specifically to reduce systemic toxicity and improve cisplatin delivery, is currently undergoing a phase III clinical trial [30].

A new targeting strategy consisting in "activable" nanocarrier is also being evaluated in a clinical trial. The liposomal formulation developed by Needham and Dewhirst's groups at Duke (USA) underwent further pharmaceutical development by the biopharmaceutical company Celsion, which has now reached the stage of phase III clinical trial and is marketed as Thermodox. Two main studies are currently on-going combining Thermodox with hyperthermia in patients with loco-regional breast carcinoma of the chest wall and Thermodox with radiofrequency ablation in patients with primary or metastatic liver cancer. Results of these trials have not yet been published. However, the reported results to date have indicated the need for confirmatory clinical phase III trials in patients with liver cancer patients (http://www.ClinicalTrials.gov/). These promising developments indicate the high potential of combining hyperthermia with thermosensitive liposomes for delivery of chemotherapy to solid cancers. Nano-carriers maintain the stealth function during circulation; upon arrival at the tumour site the drug release is triggered by application of external stimuli allowing a controlled and selective targeting of the cells now referred to as environmentally responsive DDS.

Another strategy adopted to increase the accumulation of liposomes in the desired tumour tissue is by attaching targeting ligands such as antibodies, peptides, and small molecules (i.e., folate, transferrin) to the liposome surface. The targeted liposome formulations involved in clinical trials are summarized in Table 8.

Two examples of liposomal formulations for targeted drug/gene delivery are MBP-426 and SGT-53, which are currently undergoing phase I and phase II of clinical trials. They utilize transferrin and an antitransferrin receptor single-chain antibody fragment as targeting moieties, respectively.

MBP-426, transferrin-conjugated liposomal oxaliplatin formulation, was developed to improve the safety and efficacy of oxaliplatin through the prolongation of drug plasma circulation time and thus bioavailability and by targeting transferrin receptor on tumour cell.

Many human tumours possess loss or mutation of wild-type p53 (wtp53). In addition to playing a crucial role in cell cycle control, the p53 gene is a critical component in two of the pathways involved in regulating tumor cell growth: cell death (apoptosis) and the regulation of angiogenesis. The loss of such critical tumour suppressor activity is believed to be responsible for p53's involvement in such a broad array of human tumors and resistance to chemo/radiotherapy. SGT-53 is a complex composed of a wild-type p53 gene (plasmid DNA) encapsulated in a liposome that is targeted to tumor cells by means of an antitransferrin receptor single-chain antibody fragment (TfRscFv) attached to the outside of the liposome.

4.2. Polymeric Nanoparticles. The majority of polymeric nanoparticles are still in preclinical phase of development but have potential for targeted drug delivery of anticancer drugs owing to the ease with which ligands can be attached (Table 9).

Albumin-bound nanoparticles of paclitaxel (Abraxane) have been successfully used to deliver paclitaxel for the treatment of metastatic breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. The advantages with the use of Abraxane include (i) albumin is nontoxic and well tolerated by immune system because it is a plasma protein (molecular weight of 66 kDa); (ii) its use eliminates the need for toxic solvent (Cremophor EL polyoxyethylated castor oil) which has been shown to limit the dose of Taxol that can be administered [31]. Abraxane has also intriguing properties due to the pharmacokinetics of the albumin, especially its long half-life which is particularly attractive to the design of drug carriers for passive targeting. It has been proposed that Abraxane targets cancer tissues because of the high metabolic demand and active transport of plasma proteins for anabolic processes [31]. Albumin seems to help endothelial transcytosis of protein bound and unbound plasma constituents via the binding to a cell surface receptor (gp60). Gp60 binds to caveolin-1 with subsequent formation of transcytolic vesicles. Abraxane could be transported into tumour by secreted protein acidic rich in cysteine (SPARC) or osteonectin, which binds albumin because of a sequence homology with gp60. SPARC as caveolin-1 is often expressed in some cancers (e.g., breast, lung and prostate), which could explain why albumin is known to accumulate in some tumors and thus facilitates intratumoral accumulation of albumin-bound drugs. In a Phase III study, Abraxane resulted in higher tumour response rates, a better safety

Table 7: Liposomes in clinical trials.

Compound	Name	Status	Indication
Liposomal cisplatin	SPI-77	Phase III	Non-small cell lung cancer
Liposomal interleukin 2	Oncolipin	Phase I	Non-hodgking lymphoma
Liposomal annamycin	L-Annamycin	Phase I	Acute lymphocytic leukemia
Liposomal oxaliplatin	Aroplatin	Phase II	Advanced colorectal cancer
Liposomal lurtotecan	OSI-211	Phase II	Ovarian cancer
Cationic liposomal c-Raf AON	LErafAON	Phase I/II	Various
Cationic liposomal EI A pDNA	PLD-EIA	Phase I/II	Breast, Ovarian
Thermosensitive liposomal doxorubicin	Thermodox	Phase III	Breast, liver

Table 8: Examples of targeted liposome in clinical trials.

Compound	Therapeutic agent	Status	Targeting agent
MCC-465	Doxorubicin	Phase I	F(ab')2 fragment of human antibody GAH
MBP-426	Oxaliplatin	Phase II	Transferrin
SGT-53	Plasmid DNA with p53 gene	Phase I	Transferrin receptor antibody fragment
CALAA-01	Small interfering RNA	Phase I	Transferrin

profile and improved survival compared with conventional paclitaxel, in patients receiving second-line chemotherapy [32].

4.3. Micelles. In cancer chemotherapy, a multitude of preclinical studies on polymeric micelles has been published, which have shown that micelle-based drug delivery is advantageous over free drug delivery in laboratory animals, resulting in less adverse effects and toxicity to nontargeted areas. To-date, five products for anticancer therapy has been investigated in clinical trials, of which Genexol-PM has FDA approval for use in patients with breast cancer (Table 10).

Genexol-PM is a novel Cremophor EL-free polymeric micelle formulation of paclitaxel (Taxol) consisting of two block copolymers: poly-(ethylene glycol), which is useful as a non-immunogenic carriers, and the core-forming poly-(D,L-lactide) that allows the solubilization of the hydrophobic drug. Preclinical *in vivo* studies have shown that compared with free paclitaxel, the bio-distribution of paclitaxel administered as Genexol-PM was 2-3 times higher in various tissues, including liver, spleen, kidneys, lungs, heart, and tumor. Moreover, Genexol-PM demonstrated a 3-fold increase in the maximum tolerated dose (MTD) and a significantly increased antitumor efficacy compared to free paclitaxel [33]. In phase I studies, no acute hypersensitivity reactions occurred in patients at the MTD 390 mg/m^2 administered every 3 weeks or 120 mg/m^2 weekly [34, 35]. Phase II studies have demonstrated the safety and efficacy of Genexol-PM with high response rates in patients with metastatic breast cancer and advanced pancreatic cancer. In patients with metastatic breast cancer, however, hypersensitivity reactions were seen in the 19.5% of patients [36, 37]. Moreover, Genexol-PM plus cisplatin combination chemotherapy has shown significant antitumor activity and allowed the administration of higher doses of paclitaxel compared to the Cremophor EL-based formulation in patients with advanced non-small-cell lung cancer. Furthermore, no additional toxicity was reported, although hypersensitivity reactions were observed [38]. Clinical studies are now being conducted on Genexol-PM for the treatment of several malignancies, including a phase III and IV study in patients with recurrent breast cancer.

5. Drawbacks and Future Challenges

In this paper, we have focused on the main achievements obtained with organic and inorganic nanoparticles in cancer therapy, but we must also consider their drawbacks, current limitations, and the important challenges for the future development of nano-oncology. Additionally, aspects of higher performance, nanosafety, and regulatory issues need to be addresses in the near future.

The first requirement relates to improvement of the targeting efficacy of nano-vectors to specific cancers and their immediate microenvironment in order to concentrate delivery of the cytotoxic agents to the tumor site. Targeting methods involve the conjugation of specific recognition molecules to the surface of nano-vectors. Another requirement is the development of effective triggers for release of the cytotoxic drugs, for example, nano-carriers which release their payload of active drugs at the intended site by external energy (e.g., light and electromagnetic fields) or environmentally responsive by conditions preferentially expressed at tumor site (e.g., low pH) [38–40]. Equally important is progress in the ability of nano-carriers to escape or overcome physiological barriers such as cellular multidrug resistance, clearance by the RES, blood-brain barrier, hypersensitivity

Table 9: Examples of polymeric nanoparticles in clinical trials.

Compound	Name	Status	Indication
Albumin-paclitaxel	Abraxane	Approved	Metastatic breast cancer
Doxorubicin	Transdrug	Approved	Hepatocarcinoma
Paclitaxel	Nanoxel	Phase I	Advanced breast cancer
Paclitaxel	Paclimer	Phase I	Various

Table 10: Polymeric micelles in clinical trials.

Polymeric micelle	Block copolymer	Drug	Diameter (nm)	Indication	Clinical phase
NK012	PEG-PGlu(SN-38)	SN-38	20	Breast cancer	II
NK105	PEG-P(aspartate)	Paclitaxel	85	Advanced stomach cancer	II
SP1049C	Pluronic L61 and F127	Doxorubicin	22–27	Adenocarcinoma of esophagus, gastro esophageal junction and stomach	III
NC-6004	PEG-PGlu(cisplatin)	Cisplatin	30	Solid tumors	I/II
				Breast cancer	I
				Pancreatic cancer	V
Genexol-PM	PEG-P(D,L-lactide)	Paclitaxel	20–50	Non-small-cell lung cancer in combination with carboplatin	II
				Pancreatic cancer in combination with gemcitabine Ovarian cancer in combination with carboplatin	I/II

reactions induced by carrier, increased osmotic pressure within cancer lesions [27].

The ideal system would be attained by the design and development of "smart" multifunctional nanoparticles concurrently able to image, target, and treat tumours imaging (Figure 6). These nanoparticles would be able to carry: one or more drugs, a specific targeting moiety, an imaging agent, a cell-penetrating agent, a stimulus-sensitive element for controlled release of drugs, and a stabilizing polymer for biocompatibility [1].

Before this can be materialized, however, there is an urgent need to resolve the outstanding issues relating to safety of nanoparticles and material, which have to be engineered for biocompatibility, biodegradability, and nontoxicity to enable safe use in patients. Unfortunately, little is known about the fate of nanoparticles in human body. As the size and surface properties of nano-vectors allow them to reach locations denied to larger particles, their biodistribution may be different from the expected and may result in accumulation in nontarget organs (such as liver, spleen and bone marrow), with possible undesired toxic effects [39].

Additionally, as with many applications in the nanofield, currently there is no internationally agreed regulation and legislation related to the development and subsequent clinical introduction of nanobased drug delivery systems [40]. Legislators have addressed the problem in the short term, by applying existing regulatory measures to nanomedical products. This is unsatisfactory and the only interim solution for nanomedicine consists of a governance-based pro-active regulatory system which, whilst not hindering research and development, governs what can and cannot be translated into clinical practice based on the best available information on the nanosafety of the product. There is a real need for more information on nanosafety as currently the published data are insufficient and at times conflicting especially on nanoparticle characterization, their detection and measurement, and persistence in humans and in the environment.

Research is essential to the future progress of nanomedicine and the realization of its potential in the treatment of various life-threatening disorders and others which severely impair the quality of life, and this research should encompass all aspects of nanosafety rather than the more limited field of nanotoxicity. The European Union has recognized the importance of this by establishing the "Nanosafety Network", by commissioning various reports and inclusion of Health Technology Assessment (HTA) calls within its more recent invitation for research projects within Framework 7 Programme. The two key issues are thus (i) improved knowledge on nanosafety and improved methods of HTA which, in addition to the conventional HTA measures, include additional nano-technology-related outcome measures. In turn these measures should provide the basis for effective regulation of newer nanomedicine products for healthcare.

Aside from regulatory and safety issues the translation of nanomedical products into clinical practice will remain restricted until the current limitations such as their selectivity, efficacy in protected drug carriage and release at the intended are resolved or improved by basic biological and

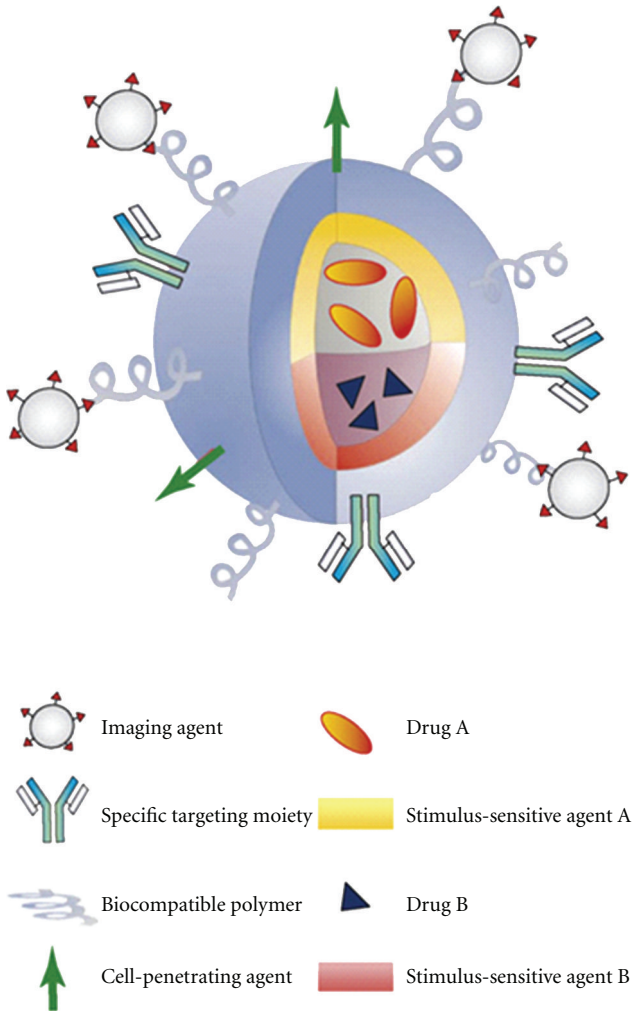

Figure 6: Structure of a smart multifunctional nanoparticle (reproduced from [1]).

in-vivo animal studies. Their full potential will then be realized as standard drug delivery systems for routine cancer chemotherapy. With the progress made in this field to-date, it is likely that in the not-distant future, nanoparticles-based approaches will usher a new era of personalized oncology, tailored to the phenotypical characteristics of the individual patient and his or her cancer—this is the ultimate objective for curative cancer chemotherapy and nano-oncology may be the means to provide this.

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