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Role of nanoparticles in targeted drug delivery system

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Introduction

Foundation of nanotechnology to deliver therapeutic and diagnostic agents with improved efficiency and effectivity was laid more than 40 years ago. Number of nano-therapeutics and nano-diagnostics that have reached the clinical stage and are being commercialized has increased ever since [1]. Traditionally, non-specific drug administration resulted in distribution of drug throughout the body, with very little drug reaching the desired physiological target tissue or cell type. This resulted in lower drug efficacy and unwanted side-effects on other parts of the body. Delivering drugs, such that its concentration is increased in the target tissue and reduced in healthy tissues, thus increasing efficacy and lowering side-effects, can be achieved through targeted drug delivery. Targeted drug delivery can be used in treating various physiological disorders like diabetes and cardiovascular diseases, but it finds its promising application in the area of cancer treatment [2].

Cancer is the uncontrolled growth of abnormal cells in the body. Surgery and radiotherapy are two most effective therapies for localized cancers or tumours, but where the cancer becomes metastatic, these therapies become ineffective and chemotherapy remains the most sought after and promising therapy as the anticancer drug could reach every organ via the blood circulation. The primary function of these drugs is to inhibit rapid proliferation of cancer cells, unfortunately they also inhibit rapidly growing cells of hair follicles, bone marrow and gastrointestinal tract leading to various severe and life threatening side-effects [3,4]. In spite of significant advancement in cancer treatment like adjuvant and combinatorial chemotherapies or the approval of important anticancer drugs like cisplatin, doxorubicin, paclitaxel etc., the haphazard killing of cells and toxic side-effects were the only possible approach for treatment of metastatic cancers, until late 1990s, when the discovery of cell signalling pathways for proliferation and differentiation opened new doors for therapies against specific pathways or proteins for cancer treatment [3].

In order to divide in an uncontrolled fashion, cancer cells over-express certain molecules (tumour specific and/or tumour associated antigens) that allow tremendous cell signalling for cell survival and division and inhibit cell death or apoptosis. Objective of targeted therapy includes blocking these signalling pathways or targeting those molecules which are over-expressed in cancer cells but are normally expressed or unexpressed in non cancerous cells, thereby inhibiting proliferation and leading to cancer cell apoptosis. The importance of these new and revolutionized anticancer drugs can be deduced by looking at the number of FDA approved anticancer drugs in the last two decades. FDA approved 19 drugs from 2000-2006, among which 14 were used in targeted therapy. Further, from 2007 to 2012, 40 anticancer drugs were approved for different cancer, among which 30 targeted specific cancer molecules and between 2012 to 2014, 18 anticancer drugs among the 19 approved by FDA, either inhibited or blocked biological signal transduction or blocked specific cancer molecules or proteins [3,5].

Nanoparticles as drug delivery systems

According to NNI (National Nanotechnology Initiative) definition, nanoparticles are structures of sizes ranging from 1 to 100 nm in at least one dimension. However, the prefix “nano” is commonly used for particles with several hundred nanometers in size. Nanosized materials that can carry a drug/multiple drugs and/or imaging agent is called a nanocarrier. Use of nanocarriers as drug delivery vehicles has various advantages over free drug administration. Cells take up the nanoparticles with optimized physicochemical and biological properties more easily than larger molecules, thus they can be used as drug delivery tools for bioactive compounds. Nanocarriers
have a high surface area to volume ratio which makes them suitable to carry a large number of ligand on its surface for targeting. Nanocarriers increase local drug concentration by encapsulating the drug and releasing it in a controlled manner to the target cells and tissues [6,7].

Advantages of nanocarrier over free drug:

1. Protection of drug from premature degradation
2. Increased blood circulation time
3. Increased shelf life
4. Enhanced absorption of drugs by target tissue
5. Controlled release of drug in target cells/tissues
6. Improved intracellular penetration

Liposomes, Solid lipid nanoparticles, Dendrimers, Polymers, Silicon or Carbon materials, Protein nanoparticles and Magnetic nanoparticles are various examples of nanocarriers that have been tested as a drug delivery system.

Figure 2.1 represents various nanomaterials with different shape, size and surface characteristics. Functionalization of nanoparticle surface allows efficient attachment of various targeting moieties like aptamers, antibodies, folic acid, peptides, transferrin etc. Drugs can also be attached to the nanoparticle surface via functional groups. Polyethylene glycol (PEG), cholesterol, etc. could be conjugated for increasing circulation time of nanoparticles in the bloodstream.

Effect of shape, size and surface properties of nanoparticles in drug delivery

The size, shape and surface property of nanoparticles could affect the penetration ability of nanotherapeutic platforms. Only extremely small particles (<20 nm diameter) can properly penetrate tumour tissue. But then, such small particles have the danger of being rapidly cleared off from the system through the kidneys without effectively being accumulated in the tumour. Thus, larger particles are necessary for increased circulation time, while smaller particles for better tumour penetration. Addressing the situation, Wong et al., [8] developed a 10 nm gelatin quantum dot nanoparticle (QD) that breaks down upon entering tumour environment by tumour associated proteases into 10nm size which then can effectively penetrate the tumour. The particle consists of gelatin core with amino-PEG quantum dots attached to the surface by 1-Ethyl-3-(3 dimethylaminopropyl) carbodiimide/ N-hydroxysulfosuccinimide (EDC/NHS) coupling chemistry. The nanoparticle is cleaved when encountered by matrix metalloproteases (MMPs), that are involved in tumourogenicity and metastasis and are present in abundance in tumour microenvironment [8,9].
FIGURE 2.1
Different types of nanomaterial with varying shape, surface functionalization and size [10].

Previous studies show that nanoparticles with high aspect ratio have better tumour penetration i.e., particles that are more cylindrical than spherical [9,11]. A negative surface charge on nanoparticles allows better penetration ability [12].

Pre treating tumours with enzymes like collagenase, hyaluronidase and gelatinase [12,13,14], priming tumours with drugs (low doses of paclitaxel and doxorubicin) [15] or inflammatory mediators or co-delivery of these molecules with nanoparticles could break-down dense ECM(extracellular matrix) barrier or increase the interstitial space, thus allowing deep penetration of nanoparticles ranging from 85-200 nm size.

Finally, Chauhan et al., [16] showed that on administration of angiotensin inhibitor, losartan, tumour stromal ECM and hyaluronan production reduced significantly, which in turn decreased profibrotic signalling expression and tumour vessel compression [16]. This resulted in an increased tumour oxygen level and delivery of drugs to tumour tissues, thus enhancing the efficacy of chemotherapeutic drugs and reducing hypoxia in breast and pancreatic cancer mouse models.

Drug delivery to tumour site can be achieved in two ways: Passive and Active targeting.

Passive Targeting

Certain macromolecules have the ability to preferentially accumulate in tumours. This kind of accumulation of therapeutic macromolecules in tumour was first reported for a polymer conjugate poly(Styrene-co-Maleic Acid)-NeoCarzinoStatin (SMANCS) 30 kDa, that binds to albumin in circulation and accumulate in tumour vicinity. Matsumura and Maeda [17] then further investigated this and found that proteins larger than 30 kDa could preferentially distribute to the tumour interstitium and remain in the site for longer period [1,17]. This is due to fenestrations
present in tumour blood vessels and poor lymphatic drainage. The combination of these two is called EPR effect or Enhanced Permeation and Retention effect.

![Diagram of EPR effect in malignant tissue](image)

**FIGURE 2.2**
EPR effect of macromolecules in malignant tissue [18]

The term EPR however, encompasses dozens of biological phenomenon like vascular permeability, heterogeneities in tumour genetic profile and tumour microenvironment, angiogenesis, hemodynamic regulation and lymphangiogenesis. When a tumour reaches a particular size, blood vessels present in its vicinity is not sufficient to provide the required oxygen supply, hence cells start dying and as they die, they secrete growth factors that results in the formation of new blood vessels from surrounding capillaries. The new vasculature lacks the basal membrane and presents discontinuous epithelium [119,20]. The fenestration in tumour capillaries can attain a size of 200 to 2000 nm depending on the type of tumour, its localization and its environment [1,21]. When blood components reach the abnormal vasculature, these fenestrations slightly resist extravasation to the tumour interstitium. This elucidates the enhanced permeation of the EPR effect.

Also in tumours, the lymphatic system is defective. As in normal tissue, where the interstitial fluid is constantly drained in the lymphatic vessels (mean flow velocity around 0.1–2 μm/s) [1,22] and renewed, thereby recycling extravasated colloids and solutes constantly, the defective lymphatic system of tumour could not completely uptake interstitial fluid. Only molecules smaller than 4 nm could return back to circulation, leaving macromolecules or nanoparticles with larger hydrodynamic radii retained in the tumour interstitium [1,22–25]. This explains the enhanced retention of the EPR effect. The lymphatic system is heterogeneous in tumour mass. Vessels that are in the bulk region experience more mechanical stress than those on the margin, thus they show a functional loss [1,26].

**Active Targeting**

Active tumour targeting involves the use of affinity ligand on the nanoparticle surface to bind
specific receptors or surface molecules overexpressed on cancer cells, tissues or organs. Ligand could be antibodies, peptides, proteins, nucleic acids, sugars and small molecules like vitamins. Target molecules could be proteins, sugar and lipids. The target-ligand interaction is enhanced by the multivalent nature of nanoparticles as more number of ligand in the nanoparticle increases its avidity for the target. Efficiency of the active targeting system depends on targeting specificity and delivering capacity. Specificity of ligand functionalised nanoparticles is decided by its distribution in different tissues and interaction with non-targeted cells, whereas their delivering capacity is related to nanoparticle material and structure. Often very less concentration of nanoparticle reaches the tumour site due to their systemic clearance in the blood stream and low tumour blood flow. The affinity of nanoparticle ligand to target molecules cannot always compensate for the clearance process. Thus, they need to be designed such that they spend longer time in circulation. Nanoparticles rely on EPR effect to reach the target, as target molecules are extravascular. These factors explain why the active targeting strategy alone cannot change the distribution profiles of nanoparticles and why methods for increased circulation of nanoparticles in the blood are required. The efficacy of targeted system is determined by the nanoparticle architecture, type of ligand attached and their conjugation chemistry. Other factors affecting efficacy are, the administration route, non-specific binding of ligand during its journey to the target cells in the bloodstream and physicochemical properties like the choice of ligand, ligand density and nanoparticle size [1].

Active targeting has been greatly exploited to increase internalization of nanoparticles in target cells and improve the efficacy of loaded drugs. Kirpotin et al., [28] showed that anti-HER2 targeting ligands attached to liposomal nanoparticle surfaces resulted in increased uptake by cancer cells as opposed to non targeted liposomes or targeted liposomes administered to mice bearing non HER2
expressing tumours, where the nanoparticles accumulated in perivascular and stromal spaces in high proportion. In this case liposomes was captured by macrophages and had reduced interactions with the cancer cells [28].

**Aptamers**

Aptamers are single stranded oligonucleotides (20-80 nucleotides) that could fold into unique tertiary structures and bind to specific proteins with dissociation constant of 10 pmol/l to 10 μmol/l (high affinity). They exhibit remarkable properties which make them an attractive molecule to be used as a ligand in drug delivery platform. They are stable over a wide range of pH (4-9), could tolerate moderate temperature change, non-immunogenic and greater ionic strength through intramolecular interactions. Also, on processing with organic solvents, they do not lose their activity [29]. Aptamers are chemically synthesized and could be modified as per our requirement, example, 30 or 50 amino or thiol groups could be attached to them to facilitate covalent conjugation to nanoparticles. These properties favour them to withstand production conditions of nanoparticle preparation. Since aptamers have small size, they could efficiently penetrate and accumulate within the tumour tissue. However, they could also get easily eliminated from the system by the kidneys due to their size. To delay their clearance and increase their circulation time in the system, PEG (Polyethylene glycol) or cholesterol can be attached to the aptamer nanoparticles [30,31].

FIGURE 2.4
Aptamer molecular recognition principle, nt=nucleotide; RNA=ribonucleic acid; ssDNA=single stranded deoxyribonucleic acid [32]

Dhar et al., [33] used A10 aptamer conjugated to the surface of PLGA-PEG nanoparticles (150 nm) by carbodiimide chemistry, in targeted delivery of cisplatin (5% w/w Pt(IV)) to prostate-specific membrane antigen (PSMA) of prostate cancer cells. As compared to the free drug, aptamer formulation of nanoparticles were 80 times more cytotoxic to the cancer cells, with enhanced pharmacokinetics in vivo, increasing its circulation time in blood and decreasing its accumulation in the kidneys [33]. The formulation improved drug efficacy, rising maximum tolerated drug dose and therapeutic index. In rat and mouse models, the formulation reduced the tumour size considerably at lower doses of anticancer drugs [34].
In another study, nucleolin (protein overexpressed in the plasma membrane of cancer cells) was employed as the target molecule for the delivery of PEGylated PLGA nanoparticle conjugated to DNA aptamer AS1411 for the delivery of paclitaxel to C6 glioma cells. The nanoformulation showed improved anti glioma properties, increased circulation time in the blood, increased cytotoxicity and ultra internalization of the nanoparticles due to specific binding of A1411 to nucleolin. In C6 glioma xenografts and intracranial rat C6 gliomas, the formulation showed enhanced inhibition of tumour growth and increased drug accumulation in tumour as compared to non targeted therapies and Taxol [35]. Recently they found through flow cytometry analysis, that TuTu22 aptamer with $K_d \pm 7.3$ nM could specifically recognize a variety of cancer cells expressing EGFR and bind with high affinity but did not bind to EGFR-negative cells [36].

**Epithelial Growth Factor Receptor (EGFR)**

EGFR is a 140KDa glycoprotein consisting of a single polypeptide chain that spans cell membrane, that comprises of an extracellular domain, transmembrane domain and an intracellular cytoplasmic domain with tyrosine kinase activity. It belongs to HER (Human Epidermal receptor) family of 4 structurally related receptor tyrosine kinases consisting of (HER1, erbB1), HER2 (neu, erbB2), HER3 (erbB3) and HER4 (erbB4). EGFR plays a critical role in many cell signalling pathways that influence cell division, apoptosis, motility and adhesion in response to binding of growth factor ligands. When a ligand binds to the EGFR, it forms a homo- or heterodimeric complexes (usually with HER2), activating receptor tyrosine kinase via autophosphorylation which in turn activates an intracellular signalling cascades (ras/MAP kinase, phosphatidylinositol-3-OH (PI3) kinase, and signal transducer and activator of transcription (STAT)-3 signal transduction pathways), culminating in the activation of nuclear gene. After binding and activation, the receptor/ligand complex is internalized for destruction or recycling, resulting in downregulation of surface EGFRs [37].

**TABLE 2.1**
Epidermal growth factor receptor (EGFR) expression in solid tumors

<table>
<thead>
<tr>
<th>Type of Tumor</th>
<th>Range of tumors expressing EGFR (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>40–80</td>
<td>[37,38,39]</td>
</tr>
<tr>
<td>Prostate</td>
<td>40–80</td>
<td>[37,40,41]</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>30–50</td>
<td>[37,42,43]</td>
</tr>
<tr>
<td>Bladder</td>
<td>53–72</td>
<td>[37,44]</td>
</tr>
<tr>
<td>Cervical</td>
<td>54–74</td>
<td>[37,45]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>35–70</td>
<td>[37,46,47]</td>
</tr>
<tr>
<td>Breast</td>
<td>14–91</td>
<td>[37,48,49]</td>
</tr>
<tr>
<td>Head and neck</td>
<td>80–100</td>
<td>[37,42]</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>40–50</td>
<td>[37,50]</td>
</tr>
<tr>
<td>Esophageal</td>
<td>71–88</td>
<td>[37,51]</td>
</tr>
<tr>
<td>Colorectal</td>
<td>25–77</td>
<td>[37,52]</td>
</tr>
<tr>
<td>Renal cell</td>
<td>50–90</td>
<td>[37,53]</td>
</tr>
</tbody>
</table>
Anticancer therapeutic agents can be successfully delivered to the intended cell/tissue/organ by active targeting of nanoparticle ligands to specifically bind with over expressed EGFR on the cell surfaces. EGFR targeting could be done by full antibodies, antibody fragments, epithelial growth factors, aptamers and peptides. Kim et al., [54] conjugated EGFR targeting antibodies with pH sensitive liposomes to study the antitumour activity of gemcitabine in a non-small-cell lung carcinoma in animal model and found that it stopped tumour growth. However, the tumor was not eradicated during the time frame studied [54]. Polymers like poly(lactic acid-co-lysine), poly(ethylene glycol-co-caprolactone) and poly(lactic acid-co-glycolic acid), have been studied vastly for EGFR targeted drug delivery as they could be easily bioconjugated with antibodies via maleimide and amide chemistries [55]. Gold nanoparticles are a very good choice in the area of cancer cell killing using photothermal and radiofrequency that causes tissue heating when excited by certain wavelengths of electromagnetic radiation but the method imposes the predicament of killing the neighbouring healthy tissues as well. Park et al., [56] used cyclodextrin covered gold nanoparticles for the delivery of drug β-lapachone to cancer cells targeted by anti-EGFR for glutathione-mediated drug release. The drug release could be tuned by the amount or concentration of glutathione present in the cells [56]. Many studies, including those of El-Sayed et al., [57] and Melancon et al., [58] have revealed the potential of antibody targeted gold nanoparticles to cause 100% cell death in vitro [57,58]. Strategies for targeting contrast agents for imaging purpose, to preferentially accumulate in tumours that over-express EGFR have also been accomplished by designing multifunctional nanoparticles using combinations like gold with iron oxide coatings, quantum dot/magnetite hybrids, and silica-coated polystyrenes loaded with ferric oxide and quantum dots. FDA approved of EGFR immunotherapy in 2004, and since then researchers have made tremendous efforts and succeeded in attaching cetuximab (a chimeric monoclonal antibody), which is an EGFR specific antibody to various nanoparticle surfaces like gold, dendrimers, polymers, liposomes, carbon nanovectors etc. for targeting EGFR over-expression on cancer cells, with drugs like gemcitabine and methotrexate, and achieved 80-100% cell death in vitro. [3,59-61]. Though the antibody mediated targeting have shown successful and promising results, there are certain disadvantages of the strategy like antibodies are expensive as they are first raised in animals and then humanized to render it safe for clinical use. Even after that it can pose immunogenic issues in some patients. Also, antibodies are of large size that limits the number of molecules to be decorated on nanoparticles and results in low affinity and suboptimal targeting. Thus, researchers have tried using antibody fragments as targeting moieties. Antibody molecule has certain regions that bind to its target more strongly than the other regions. Researchers have used single chains of the variable antibody fragment (ScFv) for conjugation to nanoparticles for target recognition in EGFR upregulated cancer cells [3,62,63]. While researchers have used ScFv to target EGFR overexpressing tumours, they got successful results upto 80% cell death in vitro and unaffected cell line that did not express EGFR, they could not ensure same results in vivo [62-64]. Another analogous EGFR targeting motif is single-domain antibody (denoted sdAb). It is a single monomeric variable antibody fragment called a Nanobody® and an order of magnitude smaller than full antibodies. It showed 100% cell death in vitro with excellent uptake and binding when cross linked with thermo-sensitive polymeric micelles [65-66]. Natural Epithelial growth factor (EGF) has also been used to target EGFR expressing cells, both for drug delivery and photothermal ablation and gave excellent results in vitro, although little work has been carried out with these systems [67-69]. Although EGFs are an attractive choice for drug
delivery, the commercially available EGFs are from murine sources that can generate antigenecity in the human system. EGF is also found in human platelets, macrophages and plasma, but its purification is time consuming, very expensive and could cause immunogenicity.

**Transferrin receptor**

Transferrin (Tf) is a glycoprotein (80kDa), synthesized by the liver and released into plasma where it binds to endogenous iron, forming an iron-transferrin chelate and acts as an important source of iron for cells. Proliferating cells require iron as a co-factor for DNA synthesis and also for the synthesis of haemoglobin, thus the chelate provides for the required iron to the cells via transferrin receptors [70-72]. Since cancer cells are highly proliferating cells, transferrin can act as a biomarker for tumour detection.

Transferrin receptor (TfR) is a dimeric transmembrane glycoprotein (180kDa) that binds to its natural ligand transferrin, with a dissociation constant of approximately 40nM. The receptor is also known as CD71. Transferrin receptor 1 (TfR1) is expressed ubiquitously in most of the normal human tissues at low levels, whereas Transferrin receptor 2 (TfR2), second member of the transferrin receptor family is restricted to hepatocytes. TfR1 is a type II receptor, that when receive its ligand converts into acidic endosomes inside the cell in clathrin/dynamin dependent manner [72,73]. Low pH environment triggers the iron out of the complex and Tf is then released out of the cell to be recycled.

Cancer cells need high levels of nutrient, in this case iron, to support its rapid proliferation, hence, they express high levels of these receptors on their surface. Many studies have shown that the expression of transferrin receptor is much higher on tumour cells (prostate cancer, breast cancer, squamous cell carcinoma, surface of cerebral endothelium and brain tumour cells), than on normal cells. This receptor could be exploited as an attractive molecule for target therapy, to kill and inhibit cancer cell proliferation.

There are 2 ways to target this receptor, (i) Delivering therapeutics inside the cell through the receptor or (ii) Blocking the natural function of the receptor, i.e., cutting off the transport of iron in cancer cells thereby resulting in cell death. Therapeutic agents that have been used for TfR-targeted cancer therapy includes anticancer drugs, plant and bacterial toxins, enzymes, siRNA, DNA and oligonucleotides. Various anticancer drugs have used ligands like the natural transferrin, anti-TfR antibodies and peptides that bind to TfR with or without nanoparticles and viruses.
FIGURE 2.5
Strategies for targeting therapeutics through transferrin receptor (TfR) in malignant cells. Therapeutic agents for cancer treatment could be delivered mediated by its natural ligand Tf, monoclonal antibodies, antibody fragments or specific peptides. Therapeutic cargo like drugs, proteins, aptamers, radionuclides etc. can be delivered via nanocarriers or viral carriers [74]

Transferrin has a number of properties that render it suitable to be used as a TfR targeting ligand, like stability over a large range of pH (3.5-11), unaffected in freeze-thaw cycles thus keeping it safe during coarse procedures of nanoparticle formation. As it is a human protein, it has low immunogenicity when it enters the system. It is also available in recombinant form (Optiferrin). Tf-TfR mediated targeted therapy increases the uptake of therapeutic nanoparticles by cancer cells as compared to ligand free nanoparticles. Li et al., [75] observed that gold nanoparticles conjugated with Tf for imaging and therapy of Hs578 breast cancer cell line showed sixfold cellular uptake of nanoparticles than the Tf free nanoparticles [75]. Anticancer drug paclitaxel(PTX) loaded in PLGA
polymer with Tf ligand conjugated via epoxy linkage showed increased cellular uptake, reduced exocytosis, greater antiproliferative activity and sustained effect as compared to ligand free nanoparticle and free drug [76]. In another study effect of PTX loaded PLGA nanoparticles with Tf ligand on PC3 cells (human prostate cancer) was observed and found that the formulation inhibited 70% of proliferation which was much more than ligand free nanoparticles (25%) or drug in solution (35%) [77].

Hong et al., [78] combined active and passive delivery strategies and developed transferrin modified PEG nanoparticles (TF-PEG-NP), encapsulating poly(ethylene) glycol-hydroxycamptothecin conjugate (PEG-HCPT). These particles showed a sustained release profile in vitro, and better behaviour in S180 solid tumours induced in mice, than the PEG–HCPT conjugates alone, with longer circulation times in blood, enhanced tumour accumulation, and increased antitumor activity [3].

Over-expression of transferrin receptor on the surface of the endothelial cells of the blood–brain barrier (BBB) is often exploited to deliver anticancer drugs to the brain [3,79]. Gan and Feng [80] conjugated transferrin as ligand to develop biodegradable poly(lactide)-D-alpha-tocopheryl polyethylene glycol succinate diblock copolymer nanoparticles (PLA–TPGS NPs) for delivery of docetaxel across the blood brain barrier. As compared to non-targeted delivery of docetaxel in PLGA and PLA–TPGS nanoparticles, the free drug docetaxel (Trade name: Taxotere), the formulation displayed more cellular uptake and cytotoxicity [3,80]. Similarly Jain et al., [81] showed an increased in vitro cytotoxicity of temozolomide as compared to the free drug when transferrin was conjugated to temozolomide encapsulated PLGA–PEG nanoparticles [3,81]. In an in-vivo study, when fluorescence imaging was done with a confocal laser scanning microscope, enhanced cellular uptake of these particles and their localization in the brain tissue of rats was observed. This affirms that anticancer drug delivery to the brain can be achieved by conjugating transferrin as a moiety on therapeutic nanoparticles.

Folate receptor

Folate or folic acid (FA) (441Da) belongs to a vitamin B complex group and is essentially needed by the cells for biosynthesis of purines and pyrimidines, in epigenetic processes, DNA and RNA synthesis and cell proliferation and survival. It is non-toxic, non-immunogenic, stable and inexpensive, features that are needed for in a nanoparticle-drug-ligand conjugate preparation and application. They can deliver variety of therapeutic agents in the cytoplasm of tumour cells via FA receptor mediated endocytosis. Drug-carrier-FA conjugate binds to folate receptor on the cell surface with high affinity [72].

Three folate receptor (FR) isoforms have been identified. They are FRα, FRβ and FRY. FRα expression in normal tissue is insignificant, FRβ is expressed at low levels in the liver and FRY is expressed only in hematopoietic cells. FRα and FRβ are exceedingly overexpressed in tumours of the uterus, colon, lung, prostate, ovaries, mammary glands, nose, throat and brain [72,82-84]. However, immunohistochemistry studies have shown FR overexpression in kidneys and placenta. At the tumour site, FR has a very high affinity for folic acid and they are rapidly internalized into tumour cells (3 * 10^5 folic acid molecules/hr) [72,85].

Zhang et al., [86] developed a novel folate conjugated poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) nanoparticles (240 nm) for targeted delivery of Doxorubicin. The formulated nanoparticle showed efficient uptake and enhanced cytotoxicity in HeLa cells as compared to non folate mediated nanoparticles. In vivo, it showed better therapeutic efficacy and the final tumour volume was significantly smaller than the control group [3,86].
Beyond Tumour Targeting

While tremendous research efforts have been undertaken for many years in developing nanotherapeutic platform based on active and passive targeting, in recent years studies have begun to address and overcome current limitations on using nanoparticles as drug carriers. There are many barriers encountered by nanoparticles upon extravasation from tumour vessels. One strategy to solve the problem is to target nanoparticles to the tumour vasculature [9,87]. Tumour blood vessels express or over-express many cell surface receptors or extracellular proteins that are either absent or present in very low levels in normal blood vessels which makes them as potential targets. Since circulating compounds have complete access to the luminal surface of tumour vessels, nanoparticles that target tumour endothelium could bind to the target molecules of tumour vessels, without having to penetrate into the tumour to deliver its cargo. Thus, eliminating the need of a nanoparticle delivery system to penetrate the high density of cells between the endothelium and tumour cells, with obstruction from extracellular matrix and interstitial pressure common within tumours. Targeting of tumour vessels has been done by adding ligands like antibody fragments and peptides that bind to tumour vessel associated extracellular matrix proteins like the EDB domain of fibronectin and the fibrin-fibronectin complex, and peptides that could bind to specific receptors and molecules that are highly expressed on tumour endothelial cells e.g, nucleolin, certain integrin receptors and aminopeptidase-N (CD13) and nucleolin [9,12,87].

While having some obvious benefits of this strategy, it is not suitable for avascular or poorly perfused tumours. And also, anticancer drugs delivered in tumour vessels could still suffer from poor distribution in the tumour tissue.

It has been shown that chemotherapeutics could only penetrate three to five cell diameters from the blood vessels with little or no drug reaching the distant tumour cells and this could result in drug resistance [87,88]. The ability of nanoparticle to penetrate deep within the tumour tissue, enhances the efficacy of anticancer drugs. Certain tumour vessel targeting peptides has shown to possess the ability of deep tumour penetration e.g., addition of cyclic iRGD peptide sequence to nanoparticle surface to target first, the integrin receptors of tumour vasculature. Upon binding, the peptide gets cleaved (proteolysis), thus exposing a new binding motif that then targets nucleophilin-1 in tumour tissues [87,88,89]. Further studies have shown that combining CendR (R/KXXR/K peptide motif) tumour penetrating property of iRGD with tumour homing peptide (NGR) to create iNGR that has superior tumour penetrating and homing properties [90]. These peptides have been identified by phage display, emphasizing the need for a cross disciplinary approach to develop an advanced nanodelivery system.

Integrin

Integrins are heterodimeric protein on the cell surface and comprises of α and β subunits like αvβ5 and αvβ3. These receptors are absent from normal blood vessels, but vastly expressed in tumour associated endothelial cells. αvβ3 integrin is associated with a calcium dependent signalling pathway, causing endothelial cell migration. Endothelial cells that undergo angiogenesis, experience increase in proliferation, locomotion and interaction with the ECM. These phenomena are directly related to αvβ3 integrin adhesion processes [91]. Thus integrins serve as likely targets for antiangiogenic therapy. Several antibodies and peptides have been used for functional blocking of αvβ5 and αvβ3 resulting in inhibition of neovascularization in tumour bearing mice. Integrin αvβ3 is associated with VEGFR2 signalling Three amino acid sequence RGD plays an important role in
targeting this integrin. Components that harbour RGD sequence when binds to integrin $\alpha_v\beta_3$, VEGF signalling in cell cultures get upregulated. When its binding is blocked, VEGF signalling is reduced, confirming the use of blocking agents for antiangiogenesis. Xiong et al., [92] developed RGD-mimetic-modified SSL (sterically stabilized liposomes) (RGDm-SSL) for delivery of anticancer drug Doxorubicin and observed through flow cytometry and confocal microscopy that the formulation facilitated DOX uptake into the melanoma cells via integrin-mediated endocytosis and displayed higher cytotoxicity. RGDm-SSL-DOX exhibited similar DOX accumulation in tumour tissues as SSL-DOX but showed significantly lower levels of the drug in blood and exceptionally high levels in the spleen. RGDm-SSL-DOX administration at 5 mg DOX/kg dose eventuated in efficient tumour growth check and prolonged survival times [92].

There have been studies with other ligands for integrin targeted drug delivery as well. Hamano et al., [93] developed C16Y peptide modified liposomes (C16Y-L) to enhance intracellular uptake of drugs and genes specifically into tumour tissues. C16Y peptide is a modified C16 synthetic peptide (DFKLFAVYIKYRGGC) and is derived from the laminin $\gamma_1$ chain. It binds to integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ on endothelial cells. Cellular uptake of C16Y-L was higher than un-labelled and scramble peptide-modified liposomes. To evaluate whether the uptake depended on an integrin-ligand interaction, they examined the inhibition of C16Y-L uptake using recombinant integrin $\alpha_v\beta_3$ and found that the cellular uptake of C16Y-L treated with $\alpha_v\beta_3$ integrin decreased. This implied that C16Y-L can selectively target cells that vastly express integrin $\alpha_v\beta_3$. Thus, the refitting of the C16Y peptide in Drug Delivery System may be an advantageous approach for drug or gene delivery into tumours [93].

**From cells to clinics**

Various cytotoxic drug nanocarriers like liposomes, carbon nanotubes, dendrimers, polymeric conjugates, micelles and polymeric nanoparticles can be used for active and passive targeted therapies by functionalizing carrier surface and exploiting the permeability and retention properties of tumour vasculature. Despite many advantages of these carriers, only a few have been approved by FDA. However, various clinical trials are being carried out with polymer–protein and polymer–drug conjugates, liposomal formulations, including immunoliposomes, polymeric micelles and polymeric nanoparticles. There are five liposomal, two polymer–protein conjugates and one polymeric nanocarrier for anticancer drugs available in the market till date. There are no FDA approvals or clinical trials for dendrimers and carbon nanotubes up to date due to their unresolved toxicity [3]. However, numerous clinical studies are in progress with regard to reduced toxicity and enhanced antitumour activities of cytotoxic drugs. Tables 2.3 and 2.4 represent ongoing clinical trials based on liposome and polymeric nanocarriers.
TABLE 2.2
Few commercially available anticancer nanotherapeutics and their targets

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Company</th>
<th>Nanoconjugate</th>
<th>Type of Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxil/Caelyx</td>
<td>Ortho Biotech Schering-Plough</td>
<td>PEGylated liposome with Doxorubicin</td>
<td>Kaposi’s Sarcoma, breast cancer, ovarian cancer, multiple myeloma (in combination with bortezomib)</td>
</tr>
<tr>
<td>DaunoXome</td>
<td>Gilead Sciences</td>
<td>Liposomal formulation of Doxorubicin</td>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>DepoCyt</td>
<td>Skye Pharma</td>
<td>Liposomal formulation of Cytarabine</td>
<td>Malignant lymphomatous meningitis</td>
</tr>
<tr>
<td>Marqibo</td>
<td>Spectrum Pharmaceuticals</td>
<td>Liposomal formulation of Vincristine</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Myocet</td>
<td>Zeneus</td>
<td>Liposomal formulation of Doxorubicin</td>
<td>Breast cancer (in combination with cyclophosphamide)</td>
</tr>
<tr>
<td>Abraxane (ABI-007)</td>
<td>Abraxis Bioscience, Astrazeneca</td>
<td>Albumin bound paclitaxel</td>
<td>Advanced breast cancer and advanced non small lung cancer in combination with carboplatin</td>
</tr>
<tr>
<td>Zinoctratin Stimalmer</td>
<td>Yamanouchi Pharmaceuticals Co., Ltd.</td>
<td>Nanoconjugate of protein neocarzinostatin and poly(styrene-comaleic acid)</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Oncaspar</td>
<td>Enzon Pharmaceuticals Ltd.</td>
<td>PEG loaded with L-Aaparginase</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
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</table>
### Table 2.3: Liposome based nanomedicines in clinical trials

<table>
<thead>
<tr>
<th>Product name</th>
<th>Description</th>
<th>Current Status</th>
<th>Avg. Size</th>
<th>Type of cancers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSI-7904L</td>
<td>Liposomal formulation of potent non-competitive thymidylate synthase inhibitor (TSI). OSI-7904 is a benzoquinazoline analog with antineoplastic activity</td>
<td>Phase I/II</td>
<td>20-80 nm</td>
<td>Phase I: advanced colorectal cancer (in combination with oxaliplatin), and solid tumors (in combination with cisplatin)</td>
<td>[3,94]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phase II: gastric or gastroesophageal cancer, biliary tract cancer, and head and neck cancer.</td>
<td></td>
</tr>
<tr>
<td>L-Annamycin</td>
<td>Liposomal annamycin to inhibit topoisomerase II in cancer cells</td>
<td>Phase I/II</td>
<td>1.88 +/- 0.89 microns</td>
<td>Breast cancer and acute lymphocytic leukemia</td>
<td>[3,95]</td>
</tr>
<tr>
<td>2B3-101</td>
<td>Glutathione PEGylated liposomal doxorubicin</td>
<td>Phase I/II</td>
<td>95 nm</td>
<td>Solid tumors, brain metastases of breast cancer, recurrent malignant glioma, and meningeal carcinomatosis</td>
<td>[3,96]</td>
</tr>
<tr>
<td>Lipoxal</td>
<td>Liposomal formulation of oxaliplatin to reduce its adverse reaction while keeping the effectiveness intact</td>
<td>Phase II</td>
<td>100 nm</td>
<td>Advanced cancer</td>
<td>[3,97]</td>
</tr>
<tr>
<td>Product name</td>
<td>Description</td>
<td>Current status</td>
<td>Avg. Size</td>
<td>Types of cancer</td>
<td>References</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>Mepact&lt;sup&gt;®&lt;/sup&gt; (mifamurtide)</td>
<td>Liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE) to activates macrophages and monocytes to a tumoricidal state</td>
<td>Phase III (Approved in Europe)</td>
<td>1-5 μm</td>
<td>Metastatic Osteosarcoma</td>
<td>[3,98,99]</td>
</tr>
<tr>
<td>INGN 401</td>
<td>Liposomal formulation of FUS1 (tumor suppressor gene)</td>
<td>Phase II</td>
<td>NAD</td>
<td>Metastatic lung cancer</td>
<td>[3]</td>
</tr>
<tr>
<td>EndoTAG-1</td>
<td>Paclitaxel-loaded cationic liposomes that attack activated endothelial cells in tumor vasculature</td>
<td>Phase II</td>
<td>200 nm</td>
<td>Anti-angiogenesis, breast, liver and pancreatic cancers</td>
<td>[3,100]</td>
</tr>
<tr>
<td>SPI-077</td>
<td>cisplatin-loaded PEGylated liposome</td>
<td>Phase I/II/III</td>
<td>110 nm</td>
<td>Non-small cell lung cancer, ovarian cancer, head and neck cancer</td>
<td>[3,101]</td>
</tr>
<tr>
<td>Lipoplatin</td>
<td>cisplatin-loaded PEGylated liposome</td>
<td>Phase III</td>
<td>110 nm</td>
<td>Non-small lung cancer and pancreatic, gastric, breast, head and neck cancers</td>
<td>[3,102]</td>
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</table>

NAD: Non available data
### Types of Cancer

<table>
<thead>
<tr>
<th>Product name</th>
<th>Description</th>
<th>Current status</th>
<th>Avg. Size</th>
<th>Types of Cancer</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>OSI-211</td>
<td>Liposomal formulation of lurtotecan (analog of camptothecin) that inhibits Topoisomerase I</td>
<td>Phase I/II</td>
<td>150 nm</td>
<td>Phase I: advanced or metastatic solid tumors</td>
<td>[3,103]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NAD</td>
<td>Phase II: recurrent small cell lung cancer, advanced ovarian cancer, metastatic or locally recurrent head and neck cancer</td>
<td>[3,103]</td>
</tr>
<tr>
<td>SLIT (sustained release lipid inhalation targeting)</td>
<td>Formulation of cisplatin loaded nebulized liposome</td>
<td>Phase I/II</td>
<td>NAD</td>
<td>Osteosarcoma metastatic to the lung</td>
<td>[3]</td>
</tr>
<tr>
<td>C-VISA BikDD</td>
<td>cholesterol liposome nanoparticles complexed with the plasmid C-VISA BikDD, with potential antineoplastic activity</td>
<td>Phase I</td>
<td>NAD</td>
<td>Advanced pancreatic cancer</td>
<td>[3]</td>
</tr>
<tr>
<td>9NC-LP</td>
<td>9-Nitrocamptothecin liposomes as a potent Topoisomerase I inhibitor</td>
<td>Phase II/III</td>
<td>190 nm</td>
<td>Hepatocellular carcinoma</td>
<td>[3,104]</td>
</tr>
</tbody>
</table>

NAD: Non available data
<table>
<thead>
<tr>
<th>Product name</th>
<th>Description</th>
<th>Current status</th>
<th>Avg. Size</th>
<th>Types of cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE-DT</td>
<td>Liposomal formulation of docetaxel</td>
<td>Phase I/II</td>
<td>100 +/- 20 nm</td>
<td>Phase I: solid tumors</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phase II: advanced or metastatic pancreatic cancer, metastatic castrate resistant prostate cancer</td>
<td>[3]</td>
</tr>
<tr>
<td>LE-SN-38</td>
<td>Liposome formulated SN-38 (the active metabolite of irinotecan) to increase the solubility and stability</td>
<td>Phase I/II</td>
<td>&lt;200 nm</td>
<td>Phase I: advanced cancer</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phase II: colorectal cancer and small cell lung cancer</td>
<td>[3]</td>
</tr>
<tr>
<td>LEP-ETU</td>
<td>Liposomal formulation of paclitaxel</td>
<td>Phase I/II</td>
<td>150 nm</td>
<td>Phase I: advanced cancer</td>
<td>[3,103]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phase II: metastatic breast cancer</td>
<td>[3,103]</td>
</tr>
<tr>
<td>PLD-EIA</td>
<td>Cationic liposomal EIA pDNA</td>
<td>Phase I/II</td>
<td>NAD</td>
<td>Breast and ovarian cancers</td>
<td>[3]</td>
</tr>
<tr>
<td>TL1</td>
<td>Injection of Topotecan liposomes</td>
<td>Phase I</td>
<td>NAD</td>
<td>Small lung cancer, ovarian cancer, and other advanced solid tumors</td>
<td>[3]</td>
</tr>
<tr>
<td>Product name</td>
<td>Description</td>
<td>Current status</td>
<td>Avg. Size</td>
<td>Types of cancer</td>
<td>References</td>
</tr>
<tr>
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</tr>
<tr>
<td>IHL-305</td>
<td>PEGylated liposomal formulation of irinotecan with excellent anti-tumor activity</td>
<td>Phase I</td>
<td>70-120 nm</td>
<td>Advanced solid tumors</td>
<td>[3,105]</td>
</tr>
<tr>
<td>S-CKD602</td>
<td>PEGylated liposome formulation of CKD-602 (camptothecin analogue)</td>
<td>Phase I</td>
<td>100 nm</td>
<td>Advanced malignancies</td>
<td>[3,106]</td>
</tr>
<tr>
<td>LE-rafAON</td>
<td>Cationic liposomal formulation containing c-Raf antisense oligodeoxynucleotides that enhances cytotoxic effects of radiation and drugs</td>
<td>Phase I</td>
<td>467.2 ± 72.0 nm</td>
<td>Advanced malignancies</td>
<td>[3,107]</td>
</tr>
</tbody>
</table>
### Table 2.4: Polymeric nanoparticles in clinical trials

<table>
<thead>
<tr>
<th>Product name</th>
<th>Description</th>
<th>Current status</th>
<th>Avg. Size</th>
<th>Type of cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA-003</td>
<td>polyisoheylcryano-acrylate encapsulating doxorubicin</td>
<td>Phase III</td>
<td>NAD</td>
<td>Advanced hepatocellular carcinoma</td>
<td>[3]</td>
</tr>
<tr>
<td>BIND-014 (DTXL-TNP)</td>
<td>(PLA–PEG or PLGA–PEG) nanoparticle formulation with docetaxel targeted to PSMA(Prostate specific membrane antigen)</td>
<td>Phase I/II</td>
<td>100 nm</td>
<td>Phase I: advanced or metastatic cancer</td>
<td>[3,102]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phase II: metastatic castration-resistant prostate cancer and non-small cell lung cancer</td>
<td></td>
</tr>
<tr>
<td>DHAD-PBCA-NPs</td>
<td>polybutylcyanacrylate loaded with Mitoxantrone</td>
<td>Phase II</td>
<td>NAD</td>
<td>Hepatocellular carcinoma</td>
<td>[3]</td>
</tr>
<tr>
<td>IT-101 (CRLX101)</td>
<td>Formulation of cyclodextrin–PEG copolymer with Camptothecin</td>
<td>Phase I/II</td>
<td>NAD</td>
<td>Phase I/II: advanced solid tumors</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phase II: ovarian cancer and recurrent small cell lung cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pilot trial: advanced or metastatic stomach, gastroesophageal, or esophageal cancer</td>
<td></td>
</tr>
<tr>
<td>Product name</td>
<td>Description</td>
<td>Current status</td>
<td>Avg. Size</td>
<td>Types of cancer</td>
<td>References</td>
</tr>
<tr>
<td>--------------</td>
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<td>-----------------</td>
<td>------------</td>
</tr>
<tr>
<td>ABI-008</td>
<td>Formulation of albumin nanoparticles with docetaxel (nab-docetaxel)</td>
<td>Phase I/II</td>
<td>130 nm</td>
<td>Metastatic breast cancer and hormone-refractory prostate cancer</td>
<td>[3,108]</td>
</tr>
<tr>
<td>ABI-009</td>
<td>Formulation of albumin nanoparticles with rapamycin (nab-rapamycin)</td>
<td>Phase I/II</td>
<td>90 nm</td>
<td>Advanced non-hematologic malignancies and nonmuscle invasive bladder cancer</td>
<td>[3,108]</td>
</tr>
<tr>
<td>ABI-010</td>
<td>Chapter Two: Nanoparticle albumin-bound formulation of 17-AAG (17-(Allylamino)-17 demethoxygeldanamycin (nab-17-AAG)</td>
<td>Phase I</td>
<td>110 nm</td>
<td>Solid tumors</td>
<td>[3,108]</td>
</tr>
<tr>
<td>ABI-011</td>
<td>Formulation of polymer albumin with thiocolchicine dimer for dual inhibition of tubulin polymerization and topoisomerase I activity</td>
<td>Phase I</td>
<td>90 nm</td>
<td>Advanced solid tumors or lymphomas</td>
<td>[3,1083]</td>
</tr>
<tr>
<td>Docetaxel-PNP</td>
<td>PLGA nanoparticle formulation of docetaxel</td>
<td>Phase I</td>
<td>165±2.2 nm</td>
<td>Advanced solid malignancies</td>
<td>[3,109]</td>
</tr>
<tr>
<td>CALAA-01</td>
<td>Cyclodextrin–PEG-transferrin receptor-targeted nanoparticles containing anti-RRM2 siRNA</td>
<td>Phase I</td>
<td>70 nm</td>
<td>Solid tumors</td>
<td>[3,103]</td>
</tr>
</tbody>
</table>
A final area that has been researched for increased nanotherapeutic delivery and efficacy in tumours is by developing systems that can be triggered to release their contents upon application of external stimuli such as heat, light, magnetic fields, or ultrasound [110,111,112]. Drug release can be restricted to a specific region by confining the external stimulus within that region. Furthermore, these external stimuli have been shown to improve the effective distribution of larger nanoparticles throughout the tumour [111,113]. Example, Thermodox, a temperature sensitive PEGylated liposomal formulation for doxorubicin [12]. While this is a very promising strategy, poor stability of such systems and difficulties related to effectively and specifically applying loco-regional stimuli have often prevented them from clinical success.

Conclusion

Development of targeted drug delivery systems that modify the distribution, uptake and pharmacokinetics of the therapeutic agents is of great relevance in the field of biomedical research. The desirable properties of nanomedicines include their ability for controlling drug release, specific targeting of cancer tissues and their biocompatibility. Unique tumour attributes support the extravasation of nanomedicines through large pores on endothelial layer and via disarranged neoplastic tissue architecture. Thus, nanoconjugates could passively target tumours via EPR effect. Active targeting strategies can improve the efficacy of cancer therapy and reduce side-effects yoked with anticancer drugs, since not all nanoparticles can pass through the cell membrane barrier without targeting moiety. Therefore, active targeting, along with other targeting-based approaches, is conceived to provide an efficient strategy. Several ligand-targeted nanotherapeutics are either approved or under clinical evaluation, leading to second-generation nanomedicines.

The first anticancer drug delivery nanovehicles approved by the FDA were liposomal and polymeric. However, many clinical trials are currently in progress, which makes all new nano-platform promising carriers to passively or actively deliver numerous anticancer drugs, improving their efficacy and reducing their toxicity. Among the various carriers developed for the delivery of cytotoxic drugs, polymeric nanoparticles seem to the most promising carriers in cancer targeted therapy, as they exhibit enhanced stability in biological fluids, tunable surface conjugation chemistry, greater monodisperse size distributions, more controllable physicochemical properties, higher drug loading, more controlled drug releasing rates, long circulation in the blood, reduced toxicity, improved pharmacokinetics, and efficient co-delivery of multiple cytotoxic compounds to tumours.

Many tumours become resistant to drugs, thus novel strategies are required to deliver high concentrations of combinatorial therapeutics to the selected target. For this to happen, it is vital that these nanoconjugates are able to combat the body’s clearance and reaction to non-self particulates. The use of multiple nanoparticles that can be employed together may overcome current limitations of each individual nanoformulation alone. For example, AuNPs have demonstrated to be distinguished vectorisation systems for gene delivery and can be used to target molecular pathways, including those involved in drug resistance and in the survival of cancer cells. These NPs could be used in combination with other polymeric and/or metallic nanoparticles in cancer treatment that includes drug and thermal ablation.
References


