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# Targeted Drug Delivery in Cancer Therapy

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**Jaspreet K. Vasir, M.S.[1]**
**Vinod Labhasetwar, Ph.D.[1,2,*]**

[1]Department of Pharmaceutical Sciences
College of Pharmacy
986025 Nebraska Medical Center
Omaha, NE 68198-6025, USA
[2]Department of Biochemistry and
Molecular Biology
Nebraska Medical Center
Omaha, NE 68198-6025, USA

Chemotherapy has been the main modality of treatment for cancer patients; however, its success rate remains low, primarily due to limited accessibility of drugs to the tumor tissue, their intolerable toxicity, development of multi-drug resistance, and the dynamic heterogeneous biology of the growing tumors. Better understanding of tumor biology in recent years and new targeted drug delivery approaches that are being explored using different nanosystems and bioconjugates provide optimism in developing successful cancer therapy. This article reviews the possibilities and challenges for targeted drug delivery in cancer therapy.

Keywords: Nanoparticles; Bioconjugates; Tumor cells; Vasculature; and Anticancer agents.

## *Introduction*

Cancer remains one of the leading causes of death in most parts of the world (1). Early prognosis of the disease due to regular screening and better understanding of the pathophysiology of tumor progression has opened many new vistas as therapy options. In most solid tumors, after its surgical removal, the remaining cancer cells are managed with a variety of treatment options including, radiotherapy, chemotherapy, immunotherapy, *et cetera* (2). However, once the cancer is metastasized, the treatment options are limited, and chemotherapy remains the choice of treatment. The main reason for failure of chemotherapy is the poor accessibility of antineoplastic agents to the tumor, requiring higher doses, and the nonselective nature of these agents causes severe toxicity (3). Thus, targeted drug delivery holds immense potential to improve the treatment of cancer by selectively providing therapeutically effective drug concentrations at the tumor site. This review is an attempt to present an overview of the problems related to targeted drug delivery in cancer, and to provide an insight into the issues related to the development of targeted drug delivery systems for cancer.

## *Need for Targeted Drug Delivery*

The quest for specificity of therapeutic agents is implicit in all treatment modalities. In cancer treatment, where chemotherapeutic and radiotherapeutic options are designed to kill cells, the specificity of drug action gains paramount importance. These strategies are based on the basic principle of preferentially killing cancer cells, without having any significant toxic effect on normal cells. It is necessary that all the cancer cells must be killed, either directly as a result of drug effect or indirectly due to bystander effect of the therapy in order to achieve a complete remission in patients presenting a disseminated disease (4). Chemotherapy regimens alone are not entirely satisfactory in aggressive carcinomas and often produce only transient responses. Combination therapy, which involves high dose of radiation (~60-70 Gy) with continuous infusion of

* Corresponding Author:
Vinod Labhasetwar, Ph.D.
Email: vlabhase@unmc.edu

chemotherapeutic agents (like paclitaxel) has been investigated for the management of unresectable locally advanced tumors (5). Paclitaxel radiosensitizes tumor cells, and hence the combination therapy is more effective than drug or radiation therapy alone. Also, achieving therapeutically relevant drug concentrations in the tumor mass, especially in case of solid tumors for a time sufficient to allow therapeutic activity of the drug is a major problem. Poor penetrability of these drugs into the biologically heterogeneous tumor mass leads to residual tumor cells even after prolonged treatment with these cytotoxic agents (3). High dose therapy required to maintain a state of complete remission causes intolerable systemic adverse effects, forcing the discontinuation of therapy in many patients. Most of these adverse effects impose significant compromises on the quality of life (QoL) of patients. Thus, the low therapeutic indices of these treatment options have resulted in a hunt for efficient delivery systems for the currently available drugs, which can enable maximizing the therapeutic efficacy of the drugs with minimal adverse effects. Targeting drugs with specially designed drug delivery systems offers a lucrative option to enhance the therapeutic efficacy and to reduce the event of systemic toxicity of anti-cancer agents. Thus, the need for developing specifically targeted drug delivery systems arises from not only the clinical perspective but can also help in eradicating cancer from the patient before it kills the patient.

### *Cellular Barriers*

The emergence of multi-drug resistance in tumor cells due to the expression of drug-efflux proteins on cell surface has raised concerns regarding long term treatment with the chemotherapeutic agents (6, 7). Delivering the cytotoxic drugs into the tumor cells, packaged in drug delivery systems can overcome the problems associated with multi-drug resistance (MDR) (8). Several mechanisms have been proposed for drug resistance. The membrane-bound p-glycoprotein (P-gp)-mediated efflux mechanism is known to reduce the intracellular accumulation of anticancer agents in most resistant cells (9). Furthermore, some of the anticancer drugs are sequestered into the cytoplasmic vesicles and extruded out, preventing their effective cytoplasmic delivery or localization into the nucleus, the site of action of certain anticancer agents (*e.g.*, doxorubicin, cisplatin, *et cetera*) (10). Some other anticancer agents (*e.g.*, doxorubicin) are also the substrates of the membrane-associated multi-drug resistance proteins (MRPs), which reduce their intracellular accumulation (11). Various drug delivery approaches such as polymer-drug conjugates (12), nano and microparticles (11), liposomes, and polymeric micellar systems (13) are being investigated to overcome the problem of drug resistance in cancer therapy (14, 15). Although the above systems could improve the intracellular delivery of chemotherapeutic agents as compared to that with drug in solution, they do not target drug directly to the nucleus. Drug transport to the nucleus with the above delivery systems is mostly dependent on the passive diffusion of free drug from the cytoplasm to the nucleus, which could be inefficient. This is because, besides the plasma membrane, P-gp has been shown to express on intracellular organelles such as the Golgi apparatus and the nuclear membrane envelop. Clacabrini *et al*. (16) have shown the presence of P-gp on the nuclear membrane of multidrug resistant variants (MCF-7/Adr) whereas another study has demonstrated the efflux of doxorubicin from the nucleus, thus reducing the available drug in the nucleus for DNA intercalation (17, 18). Thus, P-gp on the nuclear membrane envelope represents a further defense mechanism that is developed by resistant cells against antineoplastic agents. Therefore, simply delivering the drug into the cytoplasmic compartment may not overcome the problem of drug resistance unless there is greater drug localization in the nucleus. Further, the efficacy of some of the currently used drug delivery systems could be limited because they remain trapped in the endo-lysosomal vesicles upon intracellular internalization (19). Based on our recent studies with transferrin-conjugated nanoparticles, it appears that the duration of drug retention in cancer cells, especially in resistant cell line is critical to overcome the problem of drug resistance (20). Therefore, sustained release formulations may be more effective in anticancer therapy than other drug delivery mechanisms. Thus, understanding the mechanism of drug resistance is critically important in developing effective drug delivery strategy.

### *Targeting Drugs for Cancer Treatment*

The rapid increase in understanding of molecular pathogenesis of diseases and emergence of newer techniques in the field of molecular biology has produced an over-abundance of molecular information. Indeed, the pace of these developments has been such that a lot of molecular targets for drug action have been identified at a rate far exceeding our present abilities to utilize this molecular information. Currently numerous efforts are in progress to discover and develop drugs, which specifically interfere with various signal transduction pathways present exclusively in cancer cells and thus, offer opportunities to tailor individualized treatments based on the unique set of molecular targets produced by the patient's tumor.

Drugs can be delivered either by themselves or in a drug delivery system targeted to specific organs where tumor is residing or specifically to the cancer cell surface. The major components of such a targeted drug delivery include: the presence of specific targets, ligands for these targets, and ways of delivering the drug to its target using different delivery systems conjugated to the ligands. In addition, cancers can be hematologic or solid tumors and different strategies need to be evolved for each type of cancer. Solid tumors

present a heterogenous and dynamic biology, which keeps changing with time and thus offers further challenges for drug delivery (21). A thorough understanding of the tumor cell biology, microenvironment of tumor cells, and their growth patterns allow developing effectively targeted drug delivery options. Drug targeting can be achieved by taking advantage of the distinctive pathophysiological features of a tumor tissue or by actively targeting drug carrier making use of some target specific ligands (Fig. 1).

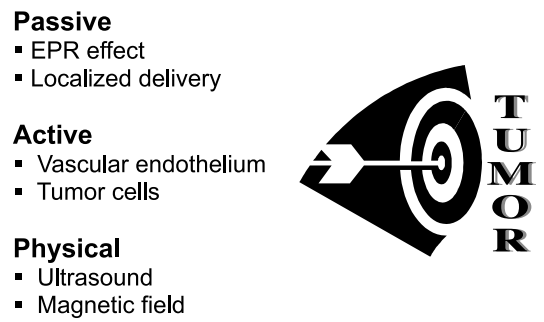


**Figure 1:** Schematic representing different drug targeting approaches to tumor.

***Passive Targeting***

Passive targeting approaches make use of the anatomical and functional differences between the normal and the tumor vasculature to allow a selective accumulation of drugs at the tumor site (22).

*EPR Effect*

Tumor vasculature is generally, more heterogeneous in distribution, larger in size, and more permeable than the vasculature present in normal tissues (23, 24). Unlike the tight endothelium of normal blood vessels, the vascular endothelium in tumor microvessels is discontinuous and leaky. It has been determined that the size of gaps between the endothelial cells ranges from 100-780 nm depending on the anatomic location of tumor (25, 26). Further, the elevated levels of growth factors like VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor) in tumor vasculature result in vasodilation and enhance the extravasation of drugs in tumors (27). This coupled with the impaired lymphatic drainage in solid tumors allows an enhanced accumulation and retention of high molecular weight drugs in solid tumors, known popularly as the enhanced permeability and retention (EPR) effect (28, 29).

The EPR effect has been predominantly used for passive targeting of drugs more than 40 kDa and for low molecular weight drugs presented in drug-carriers such as polymeric-drug conjugates, liposomes, polymeric nanoparticles, and micellar systems to solid tumors (28, 30). To cash in on this pathophysiological opportunity, the targeted drug/drug-carriers should have long circulation time and should not lose therapeutic activity while in circulation (31). Other factors which influence EPR include the size of tumors, degree of tumor vascularization, and angiogenesis (32). Thus the stage of the disease is critical for drug targeting using EPR effect. Two liposomal formulations, which target drugs to tumors by means of EPR effect, are currently available commercially. Daunosome™ liposome (NeXtar, Inc.) encapsulating daunorubicin and Doxil™ (Sequus Pharmaceuticals) based on doxorubicin, are sterically stabilized liposomal formulations with extended circulation times which efficiently accumulate in the tumor cells. This passive targeting of anthracycline anti-cancer agents results in reduced drug levels in plasma and thus, minimizes the frequently occurring cardiac adverse effects of these drugs (33).

Low molecular weight cytotoxic drugs have also been conjugated to polymers to explore the possibility of passively targeting tumor tissues using EPR effect. Therapeutic agents are conjugated to polymers by means of a hydrolysable or a degradable peptide linker, which releases the active drug only after the degradation of linker inside the tumor cell. A variety of block co-polymers, dextran, inulin, polysaccharide B, polyglutamate, alginic acid have been used to prepare such polymer-drug conjugates (34-36). Maeda developed SMANCS, which is a conjugate of polystyrene-co-maleic acid half-n-butylate (SMA) with neocarzinostatin (NCS), a potent cytotoxic agent, used for treatment of primary hepatoma (12, 37). The typical outcomes of using such systems have been an initial tumor regression followed by remission of tumor from the residual cells in the non-accessed regions of the tumor (38, 39). This occurs as the extravasation and permeation of drugs is limited to the peripheral regions of the tumor. The interstitial fluid pressure is high in the center of tumor and relatively low in the periphery and the surrounding tissues. Thus, there is a substantial permeation of macromolecules in the peripheral regions of a tumor mass, in contrast to relatively less drug diffusion into the center of solid tumors. For these macromolecules to diffuse into the more necrotic interior regions of the tumor, they have to overcome the outward flow of interstitial fluid, which can carry these drugs by convection to normal tissues (2). Thus, the systems which make use of the EPR effect need to be optimized for deep tumor penetration or some adjunctive physiological modulators can be co-administered to increase the tumor blood flow. VEGF, bFGF, angiotensin converting enzyme inhibitors like enalapril can variably increase the tumor blood flow and thus the tumor penetration of drugs (28, 40). However, there are limitations to the use of the above mentioned growth factors due to their involvement in tumor growth and metastasis. Further, these theoretical options have not yet been confirmed in clinical settings.

*Localized Delivery*

It involves direct delivery of the drug to a localized tumor site, thus excluding the systemic side effects of the drugs, while concentrating drug levels at their site of action. However, not all tumor types are amenable to such an approach, for example, lung cancer. However, for prostate cancer treatment such an approach can be effective. Over the past decade, substantial improvements in diagnosis and staging of the disease have been made with the combined use of digital rectal examination, measurement of serum PSA (prostate specific antigen) levels, and transrectal ultrasound (41, 42). Almost 90% of the men diagnosed with prostate cancer in North America are present with localized disease (43). Therefore, an early intervention to treat the disease with a less invasive local treatment could be effective in such patients. We have recently shown that direct intratumoral delivery of paclitaxel in biodegradable nanoparticles, which were conjugated to transferrin (Tf) ligand, demonstrated complete tumor regression in subcutaneous mice model of prostate carcinoma (44). The mechanism of greater efficacy of Tf-conjugated nanoparticles was determined to be due to greater cellular uptake and sustained intracellular retention of the encapsulated drug than that with drug in solution or unconjugated nanoparticles (20).

***Physical Targeting***

It is a new targeting strategy which makes use of an external stimulus to target the release of drug at a specific site in the body.

*Ultrasound*

Focusing ultrasound waves at the tumor tissue can be used to trigger the release of anti-cancer agents from polymeric micelles, and thus allow an effective intracellular uptake of the encapsulated drug. In addition, polymeric micelles can sensitize multidrug resistant (MDR) cells to the action of drugs (45, 46). Precise mechanism of targeting is yet unresolved, though the possibilities include ultrasound promoted extravasation of micelles into tumor tissue and a triggered release of drug from the micelles only at the ultrasound irradiated tumor site (47). This technique of targeting has been studied *in vitro* for delivering anthracycline drugs to drug-sensitive as well as MDR ovarian A2780 carcinoma cells (46, 48). Ultrasound can either induce drug diffusion out of the micelles or promote micelle degradation. An important advantage of this technique is that it is non-invasive, penetrates deep into the body and can be precisely controlled and focused at specific target sites. However, concerns have been raised with respect to the effect of energy of ultrasound radiation on plasma membranes of cells (49). Low ultrasound energies required for this kind of targeting approach increase the intracellular uptake of drug, while energies greater than the cavitation threshold can severely damage the cell membranes. Animal studies are underway, and can provide some insights into the ultimate usefulness of this tumor targeting technology in humans (45).

*Magnetic Field*

Magnetic targeting approach involves intravenous injection of a therapeutic agent bound or encapsulated in a magnetic drug carrier, which can then be directed and preferentially localized in the tumor tissue, upon application of an external localized magnetic field. Magnetic responsive drug carriers usually incorporate materials such as magnetite, iron, nickel, cobalt, *et cetera*. Such drug carriers include the magnetic liposomes, microspheres, nanospheres, and colloidal iron-oxide solution (magnetic ferrofluids) (50). Magnetic ferrofluid (particle size 100 nm) coated with a special carbohydrate that can reversibly bind drugs was explored for targeting tumor tissues by means of properly arranged external magnets (51). They were designed to desorb the carried drug, triggered by certain physiological conditions (pH, osmolality). Magnetic targeting of the drug-epirubicin was attempted in first ever Phase 1 clinical trials using the abovementioned targeting system in patients with advanced sarcomas (52). Although this new approach of drug targeting was found to be clinically well tolerated and safe, more than 50% of the carriers ended up in liver due to low magnetic susceptibility. Thus, the trials concluded that the targeting system needs improvements to make it more effective and independent of patient or disease related problems. Magnetic drug carriers are under active preclinical investigation for various chemotherapeutic agents – mitoxantrone, etoposide, and paclitaxel (53). The use of magnetic carriers should address some other issues like the drug-carrying capacity, aqueous dispersion stability, and biocompatibility with the tissues. We have recently developed a novel water-dispersible oleic acid-

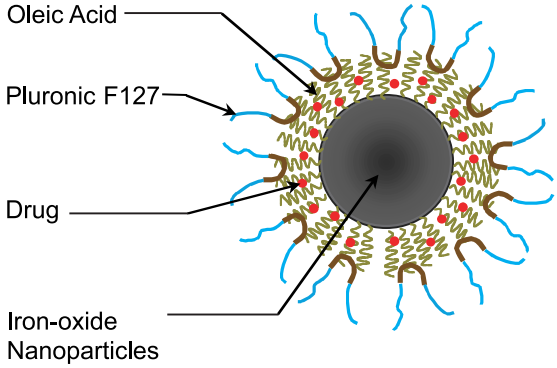


**Figure 2:** Schematic representing water-dispersible oleic acid-Pluronic®-coated iron-oxide magnetic nanoparticle loaded with doxorubicin. Doxorubicin salt was converted to base before incorporation into formulation. Drug loading in formulation was 8.2 ± 0.5 wt %. [Figure reproduced with permission from Ref. (54)].

Pluronic coated iron-oxide magnetic nanoparticle formulation that can be efficiently loaded with high doses of water-insoluble anticancer agents like paclitaxel (54) (Fig. 2). This nanoparticle formulation sustained the release of the incorporated drug for over two weeks under *in vitro* conditions. Most importantly, the formulation parameters had no effect on the magnetic properties of the core iron-oxide nanoparticles.

Radioisotopes encapsulated in such magnetic drug carriers can be used to deliver a targeted high dose of radiation to the tumor cells, without adversely affecting the surrounding normal cells (55). Radioisotopes are not released; rather the entire magnetic carrier is delivered to and held in close proximity to the area where irradiation is required. This is a promising approach of targeting drugs to the tumor site, and further improvements in the system would require collaborative efforts between biologists and physicists. An effective targeting *via* systemic administration would require a large targeting magnetic force that can overcome the force due to linear blood flow rates in arteries and blood capillaries (50). Thus, efforts are concentrated to prepare targeting carriers with high magnetic moment or developing magnets which can provide higher magnetic field gradients to externally direct these magnetic drug carriers to the tumor site.

### *Active Targeting*

#### *Tumor Vascular Endothelium*

Targeting tumors by means of their vascular endothelium is a promising strategy which utilizes targets that are easily accessible and endothelial cells that are genetically stable and do not develop resistance against therapeutic agents (56, 57). The vascular endothelium in solid tumors differs from that of normal tissues with respect to their anatomy and the expression of functional receptors on the cell surface. The existing vasculature in all solid tumors (more than 1-2 mm in diameter) gives rise to new blood vessels in order for providing the increasing demands for nutrients and oxygen by the rapidly proliferating tumor cells. This process is known as angiogenesis, and is marked by activation of existing endothelial cells, which show an elevated expression of cell adhesion molecules and proteolytic enzymes (58).

Thus, the vascular endothelium provides many targets for cancer therapy, including the endothelial cells, and specific stromal components which are highly accessible to any system present in circulation and thus, can be used to target drugs/drug-carriers (59). Endoglin (CD105) which is the receptor for tumor growth factor (TGF-$\alpha$) is the most favored target for tumor imaging and therapy (60, 61). Proliferation of tumor neovasculature is maintained due to the presence of many growth factors including vascular endothelial growth factor (VEGF). Anti-VEGF antibodies were attempted for inhibiting the growth of human tumor xenografts, however, these MAbs could not eradicate tumors, and tumor growth resumed after cessation of therapy (62). Thus, anti-VEGF antibodies are now being explored in combination with chemotherapeutic agents to control tumor growth (63). Other potential targets in vascular endothelium include the targets present in the sub-endothelial matrix. These include the targets involved in angiogenesis: matrix metalloproteinases (MMPs), angiopoeitins and their receptors (tie1 and tie2), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), endothelial growth factor (EGF), and their receptors (56). Vascular endothelial cadherin (VE-cad) is a specific endothelial cell adhesion molecule critical for vascular integrity and angiogenesis during tumor growth. MAbs against VE-cad suppressed the tumor growth and its metastasis to distant organs (64). Integrins (especially $\alpha_v \beta_5$ integrin) are very interesting molecular targets since they are exclusively expressed during angiogenesis and are not found on normal mature blood vessels (65, 66) (Fig. 3). MAbs and ligands especially, peptides rich in Arg-Gly-Asp (RGD) have been found to bind to integrins and thus exhibit the opportunity to target the tumor endothelium (67, 68).

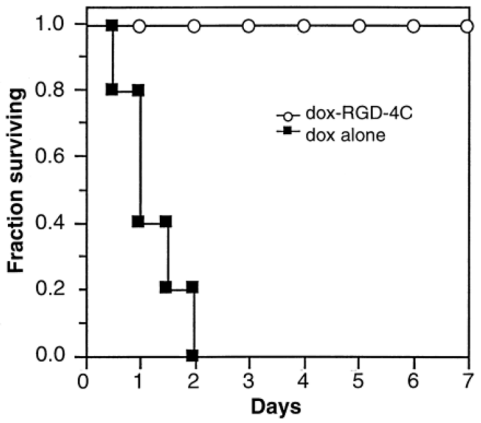


**Figure 3:** Treatment of mice bearing large (~5 cm$^3$) MDA-MB-435 breast carcinomas (four animals per group) were randomized to receive a single dose of free doxorubicin or doxorubicin-RGD-4C conjugate at 200 □g of doxorubicin-equivalent per mouse. A Kaplan-Meier survival curve is shown. [Figure reproduced with permission from Ref. (66)].

#### *Tumor Cells*

Cancer cells express some new proteins and over express many known proteins in comparison to normal cells due to their transformed nature. These proteins can serve as significant biomarkers for the progression of disease and also as surrogate markers providing an indirect measure of the effectiveness of drug therapy in patients (69). Such specific proteins/biomarkers which are preferentially expressed only in or on the cancer cells, or are at least highly over expressed on these cells have been named as "tumor associated antigens" (TAA) (70). Antibodies or ligands, specific

to these TAA can be used to target drugs to tumor cells. Further a variety of cell surface receptors for peptides, hormones and essential nutrients like iron and folic acid are over expressed in many cancer cells, thus providing opportunity for targeting of drugs to tumor cells.

The first TAA to be identified and cloned was melanoma antigen E (MAGE)-1, which was found over expressed on tumor surfaces (71). Advances in the fields of proteomics and bioinformatics, have triggered off a race for identification of novel TAA (72). An ideal tumor associated antigen should be expressed preferentially on the surface of tumor cells. An antigen which serves a critical function for the cancer cells would be of high importance as antibodies raised against this antigen would selectively kill cancer cells, without the need for any cytotoxic agent.

Herceptin® (Trastuzumab, Genentech) which is the antibody against Her-2, a tyrosine kinase found in breast cancer cells induces apoptosis of tumor cells (69). Such tumor specific functional targets are most desirable, however, only a few of these have been identified till now. Another possibility of tumor cell targeting is conjugating therapeutic moieties to the antibodies or ligands against the non-functional TAA and utilizing the specificity of these ligands to deliver toxins to the tumor cells in a highly specific manner. Some of the notable classes of tumor specific targets that have been used in immunotherapy include the transferrin receptor, selectins, and integrins.

*Folate Receptor*

The folate receptor (FR) is a highly specific tumor marker frequently over expressed in more than 90% of ovarian carcinoma patients and in many other cancer types (choriocarcinoams, uterine sarcomas, osteocarcinomas). It has acquired considerable attention for drug targeting purposes since it is absent in most normal tissues with the exceptions of placenta, choroid plexus, and low levels in lungs and kidney (73). In addition, the possibility of targeting the folate receptor either using the small molecule ligand-folic acid or antibody against the receptor further increases the opportunities to target drugs/drug-carriers to tumor cells *via* FR.

*LDL Receptor*

Low density lipoprotein (LDL) receptors are endocytic receptors which transport cholesterol rich lipoproteins (LDL) into the cells *via* a receptor mediated endocytosis process (74). Liposomes can be designed to mimic the LDL and thereby interact with the LDL receptors on the cancer cells, allowing an increased uptake of drug loaded liposomes in the cancer cells *via* LDL receptor mediated endocytosis (75).

*Hormone Receptors*

Hormone receptors, another class of tumor cell specific targets, are found on the cell surface of many hormone dependent tumors (76). Gonadotropin/leutanising hormone releasing hormone (GnRH/LHRH) receptors have been reported in some solid tumors, and also in cell-lines of many hormone dependent tumors such as breast, prostate, endometrial, and ovarian carcinoma. Anti-tumor therapies can be targeted to tumor cells using specific ligands-LHRH or its synthetic peptide analog or using antibody against this receptor (77).

Identification and validation of tumor associated antigens/targets has given rise to an explosion in the field of new tumor associated targets. Meanwhile, for successful utilization of these potential targets for drug targeting, it is imperative to understand the presence of these targets in light of the tumor pathophysiology at a molecular and cellular level. Tumor tissues are mostly heterogeneous in nature, and this heterogeneity further depends not only on the stage of disease and its aggressiveness, but also varies from patient to patient (69). Thus, it is not necessary that the molecules found over expressed in a particular cancer type would be present in all patients. This further necessitates defining subsets of patient populations, (using gene-micro array analysis) who over express specific proteins on the tumor cells (78, 79). The expression of these cell surface molecules may not be homogeneously uniform throughout the tumor mass. This may partly result from genetic instability induced by hypoxia in the interior regions of tumor, or from differences in the patterns of post-translational modifications of the protein within the tumor mass. Further differences in the degree of glycosylation of a tumor specific antigen can lead to diminished or non-existent antibody reactivity in certain regions of the tumor. Heterogeneity in the expression of surface proteins may not be a major limiting factor, if the cytotoxic drug is stable and delivered in amounts large enough to provide a 'bystander effect' to the antigen negative cells (due to the drug released into the interstitium by the antigen-positive cells). A very high antigen expression is also problematic for a tightly binding antibody, since it reduces the tumor penetration of the antibody or antibody-drug conjugate. Furthermore, most cancer patients are treated on a multi-drug therapy, so it becomes important to verify that the expression of the target on the cancer cells is present even after following these treatments. In advanced stages of cancer, when the tumor cells start metastasizing to distant regions, many of the cells release the surface antigen molecules into the systemic circulation. Such antigens circulating in the blood stream are more accessible to a targeted drug delivery system and thus, compete with the bound antigen on tumor cells, decreasing the effectiveness of therapy.

*Ligands*

A compound which can bind specifically to the cell surface proteins with high affinity can serve as a targeting moiety to target drugs/drug-carriers to the tumor site. An ideal targeting agent would be the one having high affinity and specificity of binding to the cell surface receptors, should be compatible for chemical modification for conjugating, and can be produced in sufficient quantities (80). Since the introduction of the concept of magic bullets, antibodies against specific cell surface targets have been used extensively for drug targeting. Introduction of hybridoma techniques to prepare monoclonal antibodies (MAbs) in large amounts, and the techniques to prepare smaller antibody fragments, or bispecific antibodies have further attracted considerable attention towards antibodies as targeting agents. In addition, new strategies have been evolved to prepare humanized monoclonal antibodies as opposed to the mouse monoclonal antibodies, so that immune response against murine antibody can be prevented (81). Such approaches include fusion of mouse variable regions to human constant regions (chimeric antibodies), removal of T-cell epitopes (de-immunization) and grafting mouse antigen binding regions onto human acceptor antibody frameworks (humanization of antibodies).

MAbs have been successfully used for the treatment of hematologic tumors like lymphomas and leukemias. On the contrary, the efficiency of antibodies as targeting agents is limited for solid tumors due to the poor penetration of these large molecules into the tumor mass. Thus, it was speculated that the efficiency of targeting of antibodies can be increased by using fragments of antibodies as small as possible so that these smaller molecules can penetrate tumors more uniformly (82). Such new antibody formats include the monovalent $F_{ab}$ fragments, single chain $F_v$ fragments (scFv) and the bispecific antibodies. As opposed to uniform penetrability of smaller fragments they are cleared relatively rapidly from the blood stream. Further, researcher's speculated that molecules smaller than antibodies (like cytokines, peptides, hormones, specific ligands) which have high affinity and specificity of binding to tumor associated proteins can also be explored for targeting purposes (83).

To summarize, the selection of a particular targeting agent is an important parameter that can affect the ultimate success of the drug targeting approach. It is important to keep this into consideration that all tumor-cell associated surface proteins are not receptors or binding proteins with specific ligands, and thus can be targeted using antibodies. Also, the specificity of antibodies can be exquisitely tailored to a specific sequence or some distinct fold in the tertiary structure of the cell surface target, while the promiscuity of ligands to bind to many different receptors with very similar affinities, further limits their use. The non-specific binding of a targeting ligand to non-target sites (acting as sink) would lead to reduced therapeutic availability of the targeted system at the targeted tumor site. The *in vivo* half-lives of the targeting agents also is a critical factor in selection of a targeting agent, which further depends on the stability of targeting agent in blood stream (31). Proteolysis, denaturation or binding to non-target sites results in short half-lives in the serum limiting the access of targeted drug delivery system to the target (tumor tissue). Extensive information about the structure and related activity of the targeting agent should be available as it would help in deciding the chemistry for conjugating the same to a drug or drug-carrier by making some modification to the structure of the targeting antibody or ligand. Therefore, antibodies may constitute a targeting agent of choice when the surface target is not a binding protein or receptor, and when specific small molecular ligands are unavailable. While, small molecular ligands like folic acid, peptides and cytokines gain advantage over antibodies due to their better penetration into the solid tumors, convenient availability and simple conjugation chemistry, with a presumed lack of immunogenicity.

***Drug Carrier Systems***

*Naked Antibodies*

Antibodies raised against the tumor associated antigens, which serve a critical function for cell growth can by themselves function as a therapeutic option for tumor treatment. Examples include Herceptin® (Trastuzumab, Genentech) which is the antibody against Her-2, a tyrosine kinase found in breast cancer cells. Herceptin® is an unconjugated humanized monoclonal antibody against Her-2 and induces apoptosis in tumor cells, thus clinically useful against metastatic breast cancers over expressing Her-2. Avastin® (bevacizumab) is another monoclonal antibody targeted against vascular endothelial growth factor (VEGF) that is involved in angiogenesis in tumors. Avastin improves the overall survival in patients of colorectal cancer, when given in combination with standard chemotherapy (63).

*Immunotoxins*

Immunotoxins are the products of conjugation of whole monoclonal antibodies to bacterial or plant toxins, for example, pseudomonas exotoxin and diphtheria toxin. Non-specific toxicity due to these natural toxins can be eliminated by mutating or deleting the ability of the toxin to bind to its own receptor (84, 85). Still these targeted toxins carry a high risk of non-specific toxicity to normal cells at higher doses.

*Chimeric Proteins*

These new and interesting targeted molecules recognize and specifically kill the tumor cells, which over express specif-

ic receptors. Chimeric proteins are chemical conjugates of some small cytokines, hormones, or growth factor based ligands with the natural toxins, such as pseudomonas exotoxin (PE) and diphtheria toxin (77). Chimeric proteins constructed using a GnRH analog fused to PE, inhibited tumor formation by 80% in a nude-mouse colon adenocarcinoma xenograft model (86). GnRH acts as the targeting moiety for adenocarcinoma cells, while PE kills the tumor cells by inhibiting protein synthesis (Fig. 4). Non-specific toxicity to hepatocytes and generation of human immune response to bacterial toxins limits the use of such chimeric proteins. A new generation of chimeric proteins based on the delivery of human pro-apoptotic proteins of low immunogenicity has been introduced (87). The concept of apoptotic protein delivery, in the form of targeting chimeric proteins was proved and the approach found to be successful at inhibiting tumor growth in a colon adenocarcinoma mice xenograft model. The new system is advantageous over the prior ones as it uses proteins of human origin and thus carries reduced risk of immunogenicity when given to humans. Also, the apoptosis inducing proteins have intracellular targets and therefore less chances of non-specific toxicity are expected due to the absence of surface receptors for the toxic proteins on normal cells.

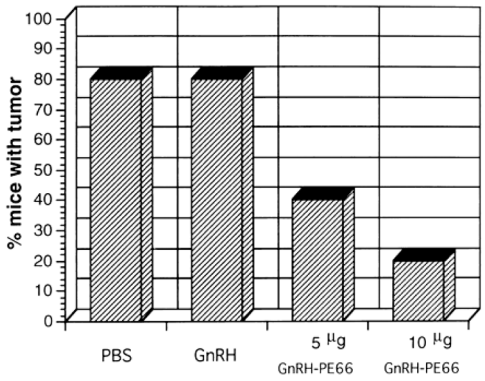


**Figure 4:** The effect of purified GnRH-$PE_{66}$ on xenograft formation in nude mice. Caco-2 colon carcinoma cells ($2.2 \times 10^6$) were injected subcutaneously into nude mice. After 36 h groups of mice ($n = 10$) were injected intraperitoneally every 12 h for 10 days with the following doses: 5 and 10 μg/day/mouse of purified GnRH-$PE_{66}$, an equivalent molar amount (0.176 μg/day/mouse) of the GnRH hormone, and an equal volume of PBS. On day 13, mice bearing tumors were sacrificed and the tumors were collected and examined. [Figure reproduced with permission from Ref. (86)].

*Nanosystems/Drug Carriers*

Anti-cancer drugs can be associated with the colloidal drug carrier systems such as polymeric micelles, nanoparticles, and liposomes, which can then be actively targeted to specific tumor cells by means of ligands or antibodies against tumor associated cell surface receptors. This strategy of targeted drug delivery can overcome the cellular based mechanisms of multi-drug resistance, and improves the selectivity of drug delivery to the cancer cells (80). Anticancer agents encapsulated in nanoparticles cannot be recognized by the cellular efflux mechanisms and thus circumvent the development of multi-drug resistance. Such nano-sized drug carriers are capable of passively accumulating in the tumor tissue using the EPR effect, when prepared in appropriate sizes and with long circulating properties in blood stream. Also, surface modifications of the nanoparticles can be achieved which would allow specific biochemical interactions with the proteins/receptors expressed on tumor cells. We have demonstrated increased efficacy of paclitaxel-loaded nanoparticles on conjugation with transferrin, in a murine model of prostate cancer (44). Transferrin receptors are overexpressed by 2-10 folds in tumor cells than in normal cells and thus transferrin and/or transferrin antibodies have been used for targeting drugs to tumor cells. Single-dose intratumoral injection of transferrin conjugated paclitaxel nanoparticles produced a complete regression and a significantly higher survival rate than the unconjugated nanoparticles or drug dissolved in Cremophor EL in a murine model of prostate cancer (Fig. 5). Greater cellular uptake of drug using transferrin conjugated nanoparticles was responsible for the greater efficacy of transferrin conjugated nanoparticles.

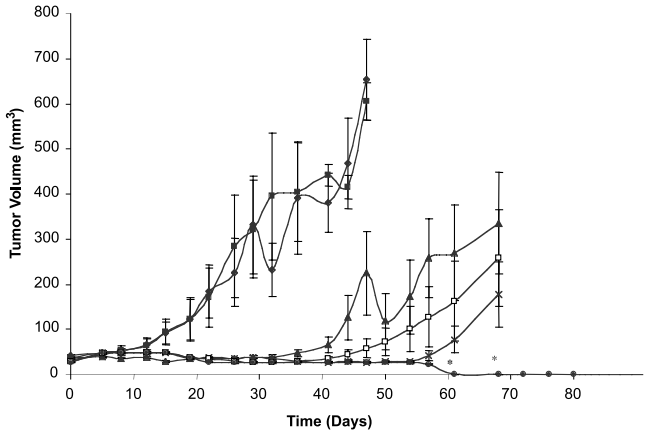


**Figure 5:** Antitumor activity of paclitaxel (Tx)-loaded nanoparticles (NPs) conjugated to transferrin (Tf) in prostate tumor model. PC3 cells ($2 \times 10^6$ cells) were implanted s.c. in athymic nude mice. Tumor nodules were allowed to grow to diameter of about 5 to 6 nm prior to receiving different formulations with a single intratumoral injection. Tx-NPs-Tf (●, 24 mg/kg; □, 12 mg/kg), Tx-NPs (✱, 24 mg/kg), Tx-Cremophor® EL (Δ, 24 mg/kg), (◆) control NPs and (■) Cremophor® EL. Data are means ± s.e.m. *$p$ <0.005 Tf-NPs-Tx groups versus Tx-NPs and Tx-Cremophor® EL. [Figure reproduced with permission from Ref. (44)].

*Bioconjugation Chemistry/Linkers*

The cytotoxic drug or a drug carrier can be linked to the targeting agent by means of carefully designed chemical conjugation procedures. The targeting moiety may be directly conjugated to the drug or drug-carrier or a spacer/linker may be employed. The chemistry is chosen such that it has no adverse effect on the specificity or activity of the targeting

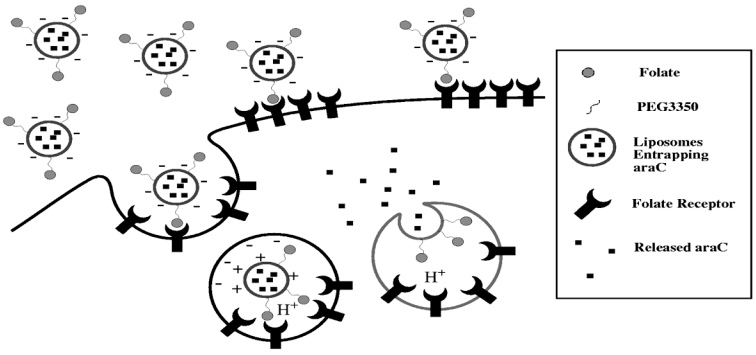


**Figure 6:** Possible mechanism of intracellular araC delivery by FR-targeted cationic lipid-based pH-sensitive liposomes. At first, the folate-derivatized liposomes are taken into the cell *via* binding to the FRs on the plasma membrane and FR-mediated endocytosis. This is followed by acidification of the endosome, which results in protonation of the anionic lipid component and generation of a net positive surface charge on the liposomes. Finally, the electrostatic interactions between the liposomal and endosomal membranes result in bilayer fusion and the cytosolic delivery of the encapsulated araC. [Figure reproduced with permission from Ref.(89)].

agent and the drug, respectively (88). The use of a linker between the therapeutic agent and the targeting moiety helps reduce steric hindrance and increases the mobility of the ligands, thus enhancing the efficiency of binding with the biological receptors. The linkers can be designed so as to confer additional control over the release of drug from its carrier when taken into the cell by means of endocytosis. This helps in integrating targeting and effective intracellular internalization of drug-carriers (Fig. 6) (89). Conjugation of a targeting agent to a drug or its carrier is carried out by chemical reactions using specific functional groups or chemical moieties in drug and targeting agent, which are not essential for maintaining the desired biological function of the compound.

### *Challenges*

Targeting drugs to solid tumors which are localized in a particular tissue involves numerous complications, however, the issues of targeting get more and more complicated when the tumor cells start disseminating to other tissues. Understanding the molecular factors affecting metastasis is critical to identifying appropriate future targets which can be utilized for targeting drugs to the tumor cells. The molecular targets would further depend on the site of metastasis of the cancer cells. Skeletal metastasis is a frequent complication of solid tumors, including breast and prostate cancer, and is usually incurable (90). Currently available treatment modalities are primarily palliative and have only a transient beneficial effect (91). Though many new therapeutic targets have been proposed, the lack of appropriate drug delivery systems has hampered any advances in clinical options available to patients. Thus, there is a crucial need for a drug delivery approach which can overcome the anatomical and physiological barriers and can deliver drugs specifically at the bone metastatic site (92). This would not only prevent non-specific systemic adverse effects of the drugs but also allow reaching therapeutic drug concentrations at the disease site.

### *Concluding Remarks*

Targeted drug delivery to tumors can increase the selectivity for killing cancer cells, decrease the peripheral/systemic toxicity and can permit a dose escalation. Advances in identification of tumor specific targets and development of different drug delivery approaches for tumor targeting have raised hopes for the development of a successful targeted drug delivery modality for cancer therapy. Though the ultimate aim is to eradicate cancer from the patient, more practical goals aiming at improving the quality of life of patients are close to fruition. The next few years will witness particular emphasis on the development of systems which can not only recognize specific targets on cancer cells but also are capable of efficiently internalizing into the cells. Combination of targeting approaches may provide solutions to some of these problems. Further, utilizing specific molecular addresses on the vascular endothelium, targeting using magnetic fields, and ultrasound are some of the emerging concepts which hold immense promise for drug targeting in cancer therapy. All these would require better understanding of the disease, identification of tumor specific markers, and simultaneous development of new drugs which are more potent and less toxic. For new drugs to make their way into the clinic, the drug discovery project should run in conjunction with new drug delivery system development, so that these drugs do not fall prey to unfavorable pharmacokinetics and are discarded in the development pipeline itself. Targeting strategies, involving nanotechnology and bioconjugation chemistry, which can alter a drug's biodistribution to avoid toxicity and maximize its efficacy, can enhance the prospects of new anticancer drugs reaching the patients.

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