Clinically Relevant Strategies of Actively Targeted Nanomedicine

Vehmeijer, L.J.C.

Abstract

Nanocarriers, particles in the size range of 1 to 1000 nanometer, are the application of nanotechnology to drug delivery. Delivery of therapeutic agents through these nanoparticles allows tailoring of physicochemical and biological qualities to provide control over the pharmacokinetic and pharmacodynamic properties of the therapeutics. Nanoparticle-based drug delivery is most often applied to solid cancers and classes of nanocarriers include polymer conjugates, liposomes, micelles, polymersomes and dendrimers. Drug delivery strategies can be broadly classified into triggered drug release, passive targeting and active targeting. Several studies have demonstrated that active targeting increases nanoparticle internalization into cells but not tissue localization, being largely reliant upon the Enhanced Permeability and Retention (EPR) effect. As such, few actively targeted nanoparticles have made it into clinical trials and none have made it into medical practice. Actively targeted nanocarriers that have been reported to have entered clinical trials include BIND-014, CALAA-01, MBP-426, PK2, MCC-465, Lipovaxin-MM, SGT-53 and MM-302, most of which are currently in phase I trials. Most of these nanoparticles feature well-established nanocarriers and targeting ligands, all of which are targeted towards receptors that internalize upon antigen binding. All of the actively targeted nanoparticles have been designed for the treatment of solid cancers and most target the cancer cells directly to deliver therapeutics that would otherwise exhibit a short half-life, low tumor accumulation and/or adverse side-effects. The only exception, Lipovaxin-MM, is designed for immunotherapy of cancer through delivery of antigens and interferon-gamma to dendritic cells. Actively targeted nanoparticles were demonstrated to have similar biodistribution, tumor localization and clearance to passively targeted nanoparticles. Nevertheless, in most cases active targeting showed improved cellular uptake, tumor retention and antitumor efficacy in *in vitro*, and in some cases *in vivo*, studies. While these actively targeted nanoparticles showed promising results, there has not yet been a definitive proof-of-principle of the clinical benefit of active targeting in humans and further studies are needed to fully assess the capabilities, benefits and complications of active targeting.

Introduction to Nanomedicine

Nanomedicine, as the name suggests, is the medical application of nanotechnology. Nanotechnology in its turn is defined as the manipulation of matter at the atomic and molecular scales, specifically at scales of 1 nanometer to 1 micrometer[1] (figure 1). While nanomedicine encompasses a broad variety of disciplinary fields, from neuro-electronic interfaces to nanosensors, one field of nanomedicine that is receiving a large amount of attention is that of nanoparticle-based drug delivery[2].

Nanoparticle-based drug delivery could be applied to many different clinical indications, including infections, central nervous system diseases as well as metabolic and autoimmune diseases[3]. Nevertheless, most scientific literature focuses on cancer, medically known as malignant neoplasm, and on solid tumors in particular. Cancer has been a rising problem in healthcare, having become one of the leading causes of death in the world[4]. The development of new imaging agents and molecularly targeted therapeutics, which are small molecules that specifically interfere with cellular processes common to cancer, also known as the 'hallmarks of cancer'[5], have opened up new avenues of cancer management. However, many of these therapeutics, such as hydrophobic molecules or nucleic acids, exhibit unfavorable pharmacokinetics, tumor site localization and cellular internalization[6].

Nanotechnological drug delivery systems are a possible solution to this problem. As particle size is a major determinant of transvascular transport in tumors[7] nanoparticles can exhibit improved tissue and vascular penetration. These characteristics, as well as others such as bioavailability[8], pharmacokinetics and renal clearance[9], can be optimized by adjusting the size and molecular architecture of the nanoparticles, providing control over biodistribution, blood half-life and tissue localization[10,11]. Particles can be modified to exhibit controlled drug release[12] or increased adhesion to tissues or cells to increase local drug retention, which can also contribute to controlled drug release[3]. Encapsulation of therapeutic agents in nanocarriers such as liposomes or micelles can bestow the benefits of nanoparticles onto traditional therapeutics as well as improve solubility of

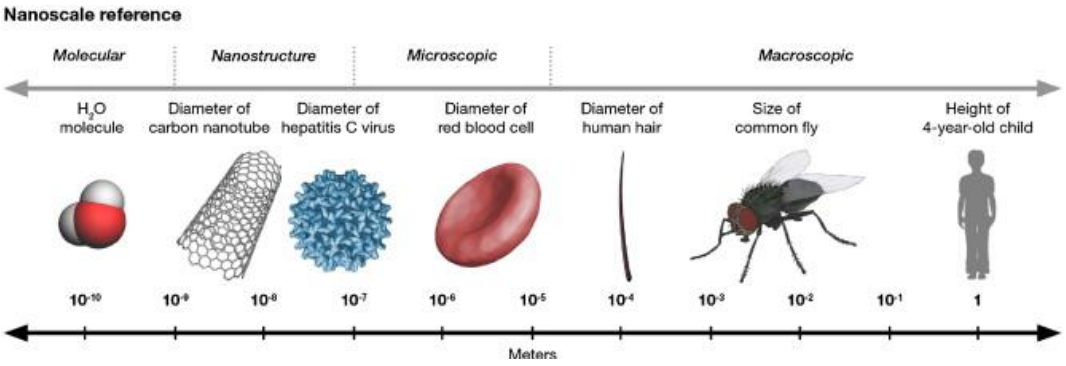

Figure 1: relative size of nanoparticles, reprinted from Kateb et al[1].

previously insoluble therapeutics[13], reduce toxicity and therapeutic side-effects[14] and/or decrease immunogenicity, which also increases blood half-life[15]. It also allows the simultaneous delivery of multiple drugs[16]. Importantly, nanoparticles can also be modified to exhibit targeted drug delivery, whether passively, through selective localization in tumor tissue due to the Enhanced Permeability and Retention (EPR) effect[17], or actively, through the use of targeting ligands[18].

Despite holding great theoretical and preclinical promise, relatively few examples of targeted nanotechnological drug delivery systems have made it through clinical trials and into medical practice[6]. This review will focus on actively targeted nanomedicine, specifically at examples of products that recently have been showing great promise during their development and (pre-)clinical testing, and attempt to distinguish what patterns emerge; what makes a successful actively targeted nanomedicine, how would it work and how could it be applied? First, the concept of actively targeted nanomedicine will be discussed, followed by an analysis of actively targeted nanotherapeutics that are currently being evaluated in clinical trials. Finally, there will be a critical evaluation of possibilities, limitations and potential patterns that emerge from this analysis.

Nanotechnological drug delivery systems

There are many different classes of nanocarriers that could be used for nanoparticle-based drug delivery, including polymers, liposomes, micelles, polymersomes, dendrimers (figure 2). The different classes of nanoparticles have different structural and chemical characteristics (table 1), which might be advantageous or disadvantageous depending on for example the therapeutic application, drug payload, target tissue and target cell. For example, intravenously administered hydrophobic therapeutics would benefit from encapsulation in micelles to increase blood half-life and bioavailability. Incorporation into for example polymer conjugates would not be advantageous, since the hydrophobic therapeutic would still be exposed to the hydrophilic environment.

Strategies of nanoparticle-based drug delivery can be broadly classified into three categories: triggered drug release, passive targeting and active targeting. Triggered drug release features the design of carrier systems that circulate the body, holding onto their payload until triggered by a stimulus to release the active components. This stimulus could be internal, due to the local conditions at the tumor site such as an acidic or reductive environment, or external, through remote induction such as acidity, ultrasound or heat[19–22]. Passive targeting constitutes the design of nanoparticles that cause drug

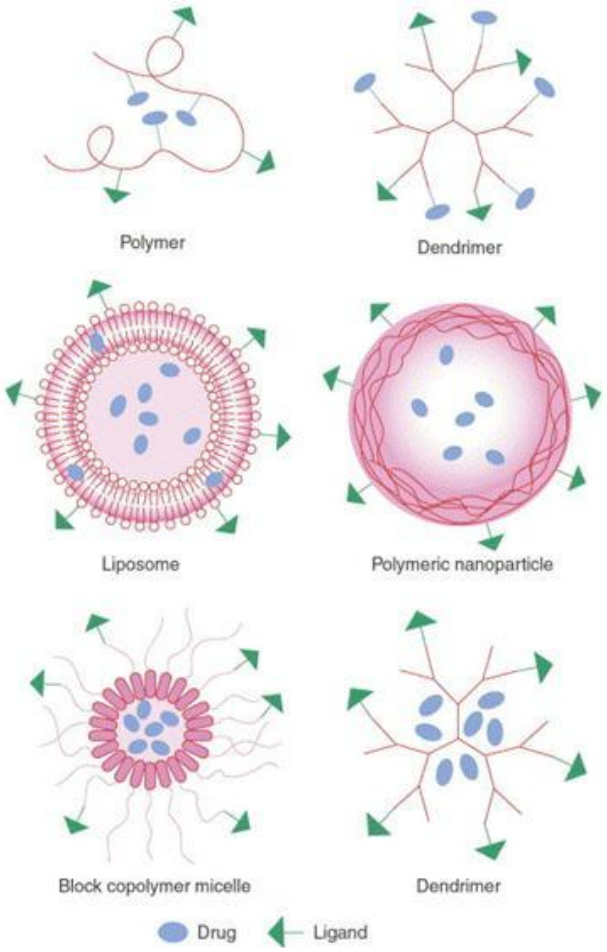

Figure 2: schematic representations of several different types of actively targeted nanocarriers, adapted from Marcucci&Lefoulon[236].

accumulation at the tumor site due to exploitation of the Enhanced Permeability and Retention (EPR) effect. This EPR effect is caused by the vascular and lymphatic irregularities of many solid tumors, which can cause increased extravasation and decreased elimination of particles, resulting in nanoparticle accumulation[17]. Active targeting constitutes the use of targeting ligands that bind to molecular markers, often receptor structures. These markers, whether exclusively expressed or overexpressed, are characteristic to the target, which could improve target specificity and reduce off-target effects, as well as increase cellular uptake. Examples of these cellular markers include the transferrin, folate and HER2 receptors[23–26].

Nanoparticle administration and localization

Nanoparticles, whether actively targeted or not, need to reach the tumor site before being able to exert their therapeutic effect. There are a number of methods of administration available, including oral administration, inhalation, intravenous injection or even rectally, epidermally or ocularly. Nevertheless, these administration routes generally all share the fact that they have to enter and traverse the circulatory system.

Nanoparticle carrier	Description	Characteristics	Size (nm)
Polymer conjugate[47]	Polymer directly conjugated to drug and targeting ligand	Water-soluble, low toxicity, biodegradable, small, low immunogenicity, chemically stable. Some can be polydisperse.	6-15
Liposome[16]	Hydrophobic membrane formed by phospholipid bilayer with hydrophilic core and hydrophilic exterior, drug encapsulated in core or lipid bilayer	Amphiphilic, biocompatible, self-assembling, large loading capacity, can be loaded with multiple payloads, can have multiple membrane layers, biologically inert, well-established. Rapid clearance unless modified., polydisperse, can have lower *in vivo* stability.	50-150
Micelle/Nanoemulsion[48,49]	Phospholipid or amphiphilic co-polymer monolayer with hydrophobic core and hydrophilic exterior, drug encapsulated in core	Amphiphilic, suitable for hydrophobic drugs, biocompatible, self-assembling, can be phospholipid or polymer-based. Polydisperse, can have lower *in vivo* stability.	10-100
Polymersome[50]	Amphiphilic co-polymer bilayer with aqueous core and hydrophilic shell, drug encapsulated in core	Amphiphilic, biocompatible, self-assembling, large loading capacity.	100
Dendrimer[51]	Branching polymer composed of cores and repeating units, drug encapsulated in cores	Adjustable size, shape and biodistribution and pharmacokinetics, high structural and chemical homogeneity, high ligand density, large loading capacity, controlled degradation, multifunctional, multivalent, highly soluble. Charge-dependent cytotoxicity and hemolysis, can be polydisperse.	Varies

Table 1: examples of nanoparticle carrier platforms used in actively targeted nanomedicine[11,52–54].

Upon entering the circulatory system, whether through intravenous administration or otherwise, the body treats nanoparticles the same as other foreign substances and attempts to remove them from circulation through the mononuclear phagocyte system (MPS), which is also known by its older term, the reticuloendothelial system (RES)[27]. After recognition and endocytosis of the particles, phagocytes will attempt to break down the foreign substance with enzymes and other oxidative-reactive chemical factors[28]. Non-biodegradable nanoparticles are not susceptible to this degradation process and are removed in other ways; smaller particles, generally under a molecular weight of 5 000, or up to 100 000 for dense particles (for example dendrimers), are removed through the renal system while larger particles are sequestered and stored in the MPS organs, mostly the liver and spleen, where accumulation of these particles can cause adverse effects in the long term[27]. The MPS can clear nanoparticles from circulation within seconds of injection, negating any potential therapeutic effect[29]. Removal by the MPS can be reduced, and the blood half-life of nanoparticles increased, in a number of ways, such as adjusting the size of the nanoparticles[30], increasing the hydrophilicity of the particles[31] or camouflaging the particle surface with a stealth coating such as poly(ethylene glycol) (PEG)[32]. Unfortunately, addition of stealth coatings may have unforeseen adverse effects, for example PEGylation has been demonstrated to lead to decreased internalization efficiency[33].

After entering and transversing systemic circulation, the nanoparticles must also be able to penetrate into the diseased tissue. Due to the fact that the smallest blood vessels in the human body, the capillaries, have a diameter of 5-10 μm[34], nanosized (up to 1 μm) particles should be able to reach any tissue connected to the circulatory system, provided they do not aggregate and remain in circulation for a sufficiently long period. As mentioned earlier, a major focus of nanomedical research is on solid cancers, medically known as malignant neoplasms. Neoplasms are abnormal masses of tissue resulting from aberrant cell proliferation and may be benign or malignant, which includes the solid tumors formed by most cancers. Neoplasms generally consist of vascular, interstitial and cellular compartments[35]. The vascular compartment is often highly irregular and damaged, featuring densely vascularized as well as necrotic and hemorrhaging regions, generally having highly 'leaky' blood vessels[17] and abnormal tumor blood flow[36]. While the pore size of the endothelium of most healthy blood vessels is approximately 2 nm, or 6 nm in the postcapillary venules, in tumor vasculature the size can vary from 100 to 780 nm[37]. The pore size is sufficient for the passage of most, if not all, nanocarriers, which cross the vessel wall through passive diffusion or convection[38]. Due to the tissue irregularities, this 'leakiness' is very heterogeneous throughout tumor vasculature[7,39]. The tumor interstitium features a collagen and elastic fiber network, which, in combination with the interstitial fluid and certain macromolecules, forms a hydrophilic gel[35]. Tumor interstitium features higher pressures than healthy tissues and often lacks functional lymphatics[35,40–43], potentially hindering the passage of nanocarriers[44,45]. The cellular compartment includes tumor cells as well as non-tumor cells, such as connective tissue cells[46], and often exhibits a high cell density. When the nanoparticles have penetrated into the cellular compartment of the tumor, they can finally deliver their payload and/or exert their therapeutic effect.

Passive targeting

As mentioned earlier, passive targeting is dependent on the EPR effect (figure 3). Despite a long history[55,56], the extent of the EPR effect seems to have been overestimated due to

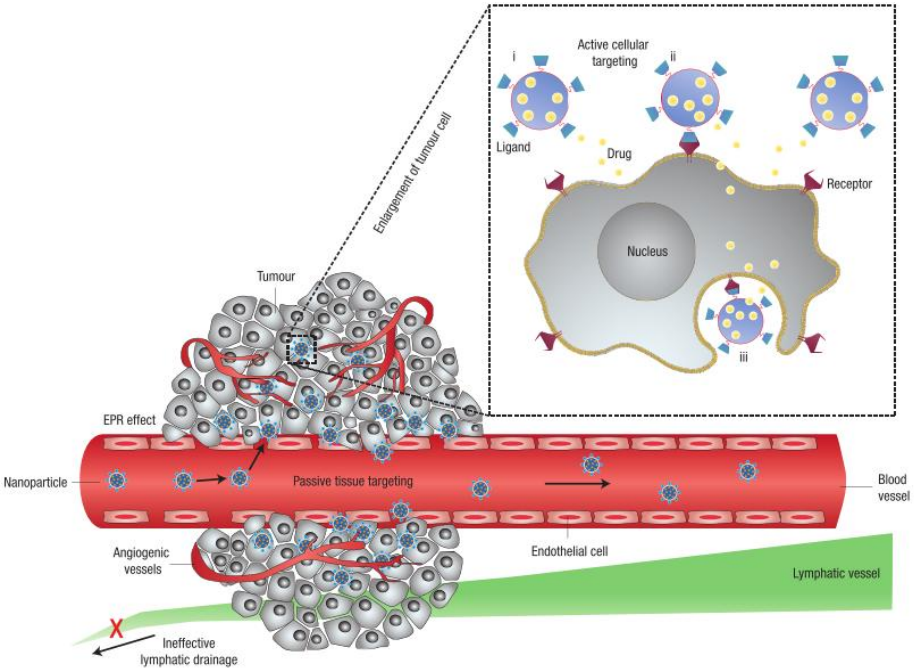

Figure 3: schematic depiction of passive and active targeting into tumors, reprinted from Peer et al[53]. Nanoparticles (blue circles) carrying a drug payload (yellow circles) passively target tumor tissue through the EPR effect caused by increased extravasation, due to increased permeability of tumor vasculature, and increased retention, due to ineffective lymphatic drainage. Active targeting of nanocarriers with targeting ligands causes increased cellular association. The nanocarriers can either (i) release the drug near the target cells, (ii) attach to the cell surface for extracellular sustained release of drugs or (iii) be internalized.

a number of reasons[6]. Firstly, in the early stages of drug development, research is primarily performed in animal models. Since tumors in animal models, especially in the case of rodent models, often grow much faster than in humans, with doubling times of days and months to years respectively[57]. The much faster growth of tumors in rodents may show 'exaggerated' responses to therapeutics, especially those drugs that depend on proliferation for their activity[57], and increased tissue irregularities, which may cause an increased EPR effect[6]. Secondly, it was demonstrated in animal models that the EPR effect may vary greatly depending on the implantation site of the tumor xenograft[58] and between xenograft cell lines, even when implanted at the same sites[59]. Similarly, it was demonstrated in humans that the accumulation of liposomes varied greatly between patients with head and neck, bronchus and breast tumors[60]. Thirdly, two major determinants of the flow of fluids across the walls of vessels are hydrostatic and oncotic pressure differences[35,61]. As mentioned earlier, hydrostatic pressures in tumor vessels and interstitium are elevated[41–43] as is the oncotic pressure in the tumor interstitium[62]. These features, combined with high cell densities and abnormal tumor blood flow[36], may reduce the delivery and thus efficacy of antitumor therapeutics[44,45]. Fourthly, the compartment commonly includes layers of non-tumor connective tissue cells, such as fibroblasts, pericytes and smooth muscle cells[46], and often exhibits a high cell density, which could hinder nanoparticle tissue penetration. Lastly, the irregularities that give rise to the 'leakiness' of tumor blood vessels, on which the EPR effect is dependent, are not spread homogeneously through the tumor vasculature, resulting in heterogeneous extravasation and delivery of therapeutic agents[7,39].

Actively targeted nanomedicine was envisioned as a method to further improve drug localization and targeting specificity of nanocarriers.

Active targeting

Actively targeted nanomedicine is defined as the branch of nanomedicine that uses targeting ligands that specifically bind to biological markers to increase local drug delivery (figure 3). This binding is the result of specific interactions between the targeting moiety and the target molecule, which in many cases is overexpressed or specifically expressed on the therapeutic target. In short, the targeting capabilities of actively targeted nanomedicine function much like that of the monoclonal antibodies in therapeutic use today[63]. Table 2 shows a number of examples of targeting ligands.

Active targeting could increase tumor localization and target specificity, potentially decreasing off-target effects and increasing treatment efficacy. Depending on the choice of cell surface marker and ligand, for example folate receptor and folic acid[64], binding of the ligand to the marker may induce internalization of the nanoparticle into the cell through for example the endocytic pathway[65]. Many therapeutics, such as siRNAs, are intracellularly active[66] and may thus benefit from the internalization induced by active targeting. Since many tumor cell markers are also expressed in other tissues, albeit at lower levels, there could hypothetically be a risk of off-target binding and internalization when particles are targeted with a single type of ligand; ligand-targeted nanoparticles have been demonstrated to be internalized in cells with relatively low expression of the target marker[67,68]. Nanoparticles could be engineered to use two or more ligands simultaneously for enhanced selectivity and thus decreased off-target effects[69,70]. The size of nanoparticles can also be adjusted to optimize characteristics such as binding and activation of membrane receptors[71]. Interestingly, nanoparticles could even be actively targeted to prevent endocytosis, as was demonstrated for insulin-targeted magnetic nanoparticles[72]. Inhibition of endocytosis might be useful in applications where

Ligand class	Example targeting ligand	Corresponding target	Size (nm)	MW (kDa)	Characteristics
Proteins	Monoclonal antibody (mAb)[85]	Cell-surface markers	15-20	150	Whole antibody. High affinity, versatile, immunogenic, rapid clearance. May be animal-based, humanized or even human antibodies.
	Fab'[86]	Cell-surface markers	5-10	50-55	Antibody fragment. Versatile, longer circulation than mAb.
	$F(ab')_2$[87]	Cell-surface markers	10-15	100	Antibody fragment. High affinity, versatile, longer circulation than mAb.
	scFv[88]	Cell-surface markers	3-5	25-30	Antibody fragment. Lower affinity, versatile, rapid clearance, less stable. Can be engineered to be multivalent and multispecific.
	Nanobody/V_HH[89]	Cell-surface markers	2-3	15	Antibody fragment. Versatile, simple, stable, modifiable, longer circulation than mAb. Can be engineered to be multivalent and multispecific.
	Transferrin[90,91]	Transferrin receptor	12	80	Iron-binding blood plasma glycoprotein. Naturally-occuring, non-immunogenic. Transferrin receptor often overexpressed on cancer cells.
Peptides	RGD[92]	avβ3 integrin receptor	*	0.35	Amino acid motif recognized by many integrins. Simple, modifiable.
Nucleic acids	Oligonucleotide aptamer[93–95]	Nucleic acids, proteins, peptides and small molecules	2-3	5-30	Short single-stranded nucleotide sequence. Stable, non-immunogenic, easy to synthesize and modify, high specificity and affinity. Binds through pockets formed by secondary and tertiary structure. Rapidly cleared from circulation unless modified.
Small molecules	Folic acid[64]	Folate receptor	*	0.44	Essential vitamin B_9. Simple, stable, not biologically active, naturally occurring, non-immunogenic. Folate receptor often overexpressed in cancer cells.

*Table 2: examples of targeting ligands used in actively targeted nanomedicine[11,96]. Ligands marked with * have insignificantly small sizes. Peptide aptamers, which are peptides designed to recognize a specific protein domain, analogous to oligonucleotide aptamers, were not included as a ligand for active targeting since they are predominantly used for applications such as drug discovery[97], protein detection[98] and intracellular inhibition[99].*

internalization could be detrimental to functionality, such in imaging.

Despite being envisioned to improve targeting, antibody-mediated targeting of liposomes was observed to increase cellular internalization but not tumor localization in *in vivo* mouse models[73]. These findings were confirmed when it was demonstrated that passively and actively targeted nanomedicine exhibited similar biodistribution and tumor localization, although targeted nanomedicine did result in significantly higher cell uptake[74]. Similarly, while the use of folic acid as a targeting ligand increased *in vitro* uptake of liposomes approximately 12-fold over passively targeted liposomes, it did not show increased tumor localization in *in vivo* xenograft mouse models[75]. Other groups have reported similar observations[76,77]. These results suggest that actively targeted nanomedicine is dependent on the EPR-mediated passive targeting to reach and accumulate at the tumor site. In fact, the term 'active targeting' might be a misnomer and inspire a mental image akin to a guided missile seeking its target. In fact, the ligand-receptor interaction upon which active targeting is based occurs only when ligand and the target are very close to one another (less than 0.5 nm)[78], functioning more like a 'key and its lock' than a 'missile and its target'.

Since active targeting is largely reliant on passive localization of the nanoparticles, it features the same limitations as passively targeted nanoparticles. These limitations include the reliance upon animal models in preclinical development[6,57], the variability of the EPR effect between tumor sites[58–60], elevated fluid pressures in tumor tissue[41–45,62], the high density of tumor and non-tumor cells[46] and the heterogeneous 'leakiness' of tumor vasculature[7,39]. Additionally, the availability and capacity of the tumor cell markers may be limited as receptor expression in tumor tissue is often heterogeneous[79,80] and saturation of binding sites or internalization of tumor markers upon binding may provide a bottleneck for the efficacy of ligand-mediated targeting[81]. A converse bottleneck may also present itself, known as the binding-site barrier, which constitutes the depletion of ligands before penetrating deeply into the diseased tissue, due to the fact that the targeting moieties may bind the first markers they encounter[82]. Addition of a targeting ligand to nanoparticles may also increase the immunogenicity of the particle, especially in the case of antibodies, reducing the blood half-life, as was shown for immunoliposomes[83,84].

Reflecting these issues, while the results of preclinical studies in animal models have been promising[100–102], few examples of actively targeted nanomedicine have made it into clinical trials[6]. In short, the tissue localization of active targeting may fall short of earlier expectations and not have the qualities of a 'guided missile' but rather those of a 'key to a lock'. As such, while active targeting contributes little to tissue localization, it may still yield other benefits to the delivery and efficacy of certain therapeutics, such as increased internalization into the cell. It would therefore still be worthwhile to further investigate the benefits and possibilities of active targeting and how they could be applied to nanotherapeutics.

Nanoparticle internalization into cells

Many nanoparticle-delivered drugs, such as siRNAs[103] or molecularly targeted therapeutics[6], are intracellularly active, requiring internalization into the cell upon reaching the tumor tissue. Actively targeted nanocarriers can deliver their payload in two ways after binding to a marker on the target cell: extracellular or intracellular release[53]. In extracellular release the nanocarrier acts like an extracellular drug reservoir, releasing the payload extracellularly after binding a target marker, followed by internalization of the drug while the nanocarrier remains attached to the exterior of the cell. Intracellular release constitutes the internalization of the nanocarrier as a whole, followed by release of the payload.

There are several possible internalization routes, including phagocytosis, macropinocytosis, caveolae and clathrin-mediated endocytosis[65]. The mechanism of internalization depends on a number of factors, such as structural or physiochemical factors and ligand-receptor binding[65]. The majority of scientific literature concerning ligand-targeted therapeutics features internalization through a form of receptor-mediated endocytosis.

Phagocytosis is an internalization mechanism that captures large solid objects through engulfing them with the cell membrane to form internal phagosomes. This mechanism is limited to certain cell types such as dendritic cells, neutrophils, monocytes and macrophages[65]. Active targeting of microparticles has already been shown to induce ligand-specific receptor-mediated phagocytosis but not non-specific phagocytosis[104,105]. This active targeting could also be applied to nanoparticles, as these can also be taken up through phagocytosis[106]. Macropinocytosis constitutes a non-selective internalization mechanism that is primarily involved in the uptake of large macromolecules, such as nucleic acids, cell-penetrating peptides and antigen-presenting objects, such as pathogens[107]. It functions through the formation of invaginations on the plasma membrane that non-selectively capture volumes of extracellular fluid. The captured fluid, as well as any suspended particles, is internalized, forming large vesicles called macropinosomes. In some cells the macropinosomes are routed back to the cell membrane but in certain cells, such as leukocytes or renal cells, they are directed into the endolysosomal system[65,108]. Interestingly, while considered a non-specific internalization pathway, macropinocytosis has been shown to be induced by receptor binding[109,110]. Since nanoparticles can internalize through this pathway[111,112], active targeting could be used to induce nanoparticle macropinocytosis. Caveolae-mediated endocytosis is a selective internalization mechanism for small volumes[113]. It functions through the use of special lipid rafts called caveolae, which are small invaginations that bud off the plasma membrane in response to receptor binding[113]. While still being disputed, it is thought that the budded caveolae fuse with the early endosomes to be transported to the Golgi complex, avoiding the lysosomal route[65]. Addition of the plant lectin WGA to nanoparticles has been shown to increase cellular uptake through a receptor-mediated, caveolae-dependent pathway 5-to-8-fold over untargeted nanoparticles[114]. Finally, clathrin-mediated endocytosis, one of the best studied forms of endocytosis, is a selective receptor-mediated internalization mechanisms for the uptake of small volumes. It functions through formation of plasma membrane invaginations and the subsequent budding of clathrin-coated vesicles. These vesicles then uncoat and fuse with the early endosomes and eventually lysosomes[65].

If internalized through endocytosis, the nanoparticles need to be able to escape the endolysosomal pathway. This can happen at the endosomal level, as some drugs are able to simply diffuse out of the endosomes. Other mechanisms, such as pH-sensitive polymers[115] or swellable dendritic polymers[116] respond to the physiochemical changes in the vesicle during endolysosomal transport to escape the pathway.

In conclusion, there are several endocytic routes available for nanoparticle internalization. The exact mechanism for internalization of nanocarriers or therapeutic payloads depends on a number of factors. These include ligand-binding, nanoparticle size and physicochemical factors as well as the type of cell that is targeted[65]. While most actively targeted nanocarriers reported in literature utilize receptor-mediated endocytosis, the nanocarrier, ligand and/or payload could hypothetically be optimized for one specific internalization route, depending on the nature of the therapeutic, treatment or target cell type. For example, vaccination through actively targeted nanoparticles, loaded with antigens, could benefit from phagocytic internalization into immune cells.

Product	Organization	Nanoparticle qualities	Size (nm)	Ligand	Target marker	Clinical indication	Payload	Clinical phase	Notes
BIND-014[117]	BIND Biosciences	Polymeric nanoparticle, PEG coating	~100	Peptide	Prostate specific membrane antigen	Solid tumors	Docetaxel	Phase I	Self-assembling components. Other applications of same platform (Accurins™) in lead discovery and preclinical phase. Nature of ligand unclear
CALAA-01[118]	Calando Pharmaceuticals	Polymeric nanoparticle, PEG-coating	~70	Transferrin	Transferrin receptor	Solid tumors	αRRM2 siRNA	Phase I	Self-assembling components. Platform (RONDEL™) is highly modular. Similar system (IT-101) licensed to Cerulean Pharma Inc.
MBP-426[119]	Mebiopharm	Liposome, NGPE (N-glutaryl-phosphatidylethanolamine) coating	50-200	Transferrin	Transferrin receptor	Gastric and esophageal tumors	Oxaliplatin	Phase II	Several other applications of same platform in preclinical phase. Limited specific information available.
PK2 or FCE28069[120,121]	Pharmacia (now Pfizer)	Galactosamine-drug-polymer conjugate		Galactosamine	Asialoglycoprotein receptor	Liver tumors	Doxorubicin	Phase II	Discontinued in 2008.
MCC-465[122]	National Cancer Center Japan and Mitsubishi Chemical Holding Corporation	Liposome, PEG coating	125-160	GAH $F(ab')_2$ fragment	Unknown antigen	Gastric and colorectal tumors	Doxorubicin	Phase I	Antigen of ligand unknown. Despite phase I completion, no news since 2004.
Lipovaxin-MM[123]	Lipotek	Liposome-like nanoparticle formulated from cancer cells		V_H domain antibody fragment	DC-SIGN	Dendritic cells (target cell), melanoma (target disease)	Melanoma antigens and cytokine interferon gamma	Phase I	Targeted vaccine. Antigen provided by cancer cells. Hypothetically allows personalization. Other applications of same platform (Lipovaxin) in preclinical phase.
SGT-53, SGT53-01 or Synerlip p53[124]	SynerGene Therapeutics	Liposome		scFv fragment	Transferrin receptor	Solid tumors	Plasmid DNA with p53 gene	Phase I	Little information available
MM-302[125]	Hermes Biosciences (now Merrimack)	Liposome, PEG coating		Antibody fragment	ErbB2 (HER2)	Breast cancer	Doxorubicin	Phase I	Little information available

Table 3: an overview of actively targeted nanocarriers currently in clinical trials.

BIND-014

Founded in 2007, BIND Biosciences is a company active in the development of actively targeted nanomedicine. BIND-014, the first product developed with their Accurins™ system, entered phase I clinical trials in early 2011 for evaluation in the treatment of advanced or metastatic cancers and solid tumors[126]. This product is based on the Accurins™ technology, which features particles composed of therapeutic payloads encapsulated in controlled release polymers and a stealth protective layer covered in targeting ligands[127]. In the case of BIND-014, the encapsulated drug is Docetaxel, the active ingredient in Taxotere®, a clinically well-established chemotherapeutic agent of which the patent expired in 2010. Docetaxel is a mitotic inhibitor that acts through the disruption of microtubule functionality and is effective against a wide range of cancer cells[128]. The basis of BIND-014 is a self-assembling biodegradable PLGA-PEG polymer that can be conjugated to targeting ligands, which in this case targets prostate specific membrane antigen (PSMA), a cell surface glycoprotein (over)expressed in prostate cancer[117,129]. PSMA has been demonstrated *in vitro* to undergo a three-fold increase in internalization through endocytosis when subjected to antibody binding, which could be advantageous in combination with intracellularly active therapeutics[130].

Accurins™, the technology on which BIND-014 is based, allows the formulation of nanoparticles through the use of macromolecular self-assembly. The backbone of these self-assembling nanoparticles is a biodegradable polymer composed of PLGA (poly(lactic-co-glycolic acid)) and PEG (polyethylene glycol)[129]. By conjugating a ligand, in this case a peptide targeting PSMA, to this PLGA-PEG polymer and nanoprecipitating it with 'normal' PLGA-PEG polymers and the drug, single-step macromolecular assembly is achieved. Additionally, the biophysicochemical properties can be adjusted by varying the ratios of the nanoparticles components (figure 4)[131]. Optimization of the nanoparticles qualities, such as the size, drug loading, drug release or differential targeting, allows for optimization for therapeutic applications[131,132]. The pipeline of the Accurins™ system features a wide range of different products. These include chemotherapeutics and molecularly targeted therapeutics for the treatment of cancer (in the preclinical and lead optimization phases respectively) as well as therapeutics for the treatment of inflammatory indications and cardiovascular indications (in the lead optimization and discovery phases respectively)[133].

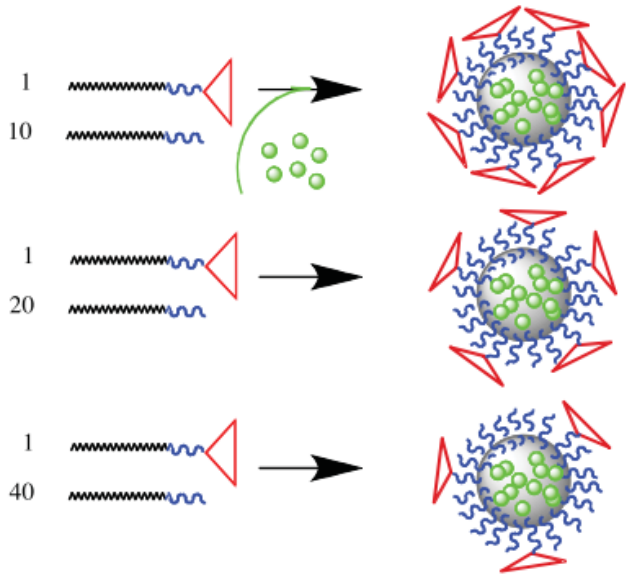

Figure 4: adjustment of the biophysicochemical properties of PLGA-PEG polymer nanoparticles by varying the composition of the nanoparticles. PLGA is black, PEG is blue, the targeting ligand is red and the drug is green. Reprinted from Gu et al[131].

Before discussing the research behind BIND-014, it is important to note that the authors focus on illustrating the concept through the use of A10, a known RNA aptamer targeting PSMA[134], as a targeting ligand, which may not be the actual targeting moiety used in BIND-014. *In vitro* studies revealed that the conjugation of the A10 anti-PSMA RNA aptamer to PEGylated nanoparticles increased binding to the PSMA-expressing prostate cell line LNCaP cell line 77-fold, when compared to PEGylated nanoparticles without the aptamer, while neither bound PC3, a prostate cell line that does not express PSMA[135]. Similar effects were observed *in vivo* in LNCaP xenograft mouse models, where the addition of the A10 aptamer to PLGA-PEG polymers resulted in a 3.77-fold increase in drug levels in the tumor after 24 hours, when compared to optimized PLGA-PEG polymers without the aptamer[132]. *In vitro* cytotoxicity assays performed on LNCaP cell lines with PLGA-PEG nanoparticles loaded with docetaxel (Dtxl) showed an approximately 1.5-fold increase in cytotoxicity when the A10 aptamer was added[136]. When this test was repeated in an *in vivo* 109-day study in a mouse LNCaP xenograft model, 100% of the mice treated with the Dtxl-loaded PLGA-PEG-A10 nanoparticles survived, in contrast with 57% of those treated with 'normal' Dtxl-loaded PLGA-PEG nanoparticles and 14% of those treated with non-encapsulated Dtxl. The addition of the targeting ligand also resulted in significantly smaller tumors (figure 5) and less body weight loss than in the untargeted nanoparticles and non-encapsulated Dtxl[136].

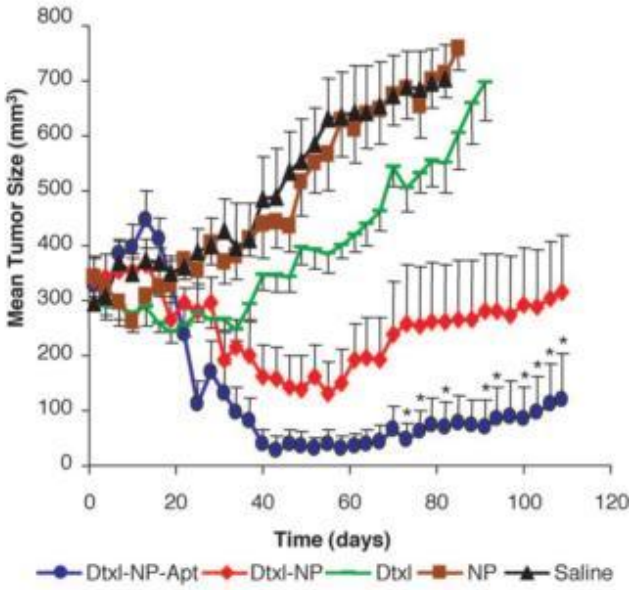

Figure 5: mean tumor size in mouse xenograft models. Administered formulations constitute A10 aptamer-targeted docetaxel-encapsulated nanoparticles (Dtxl-NP-Apt), docetaxel-encapsulated nanoparticles (Dtxl-NP), docetaxel emulsion (Dtxl), unloaded nanoparticles (NP) or saline. Reprinted from Farokhzad et al[136].

The *in vitro* and *in vivo* results of Dtxl-loaded PGLA-PEG-A10 nanoparticles demonstrate the efficacy of the system, despite the fact that the benefit of adding a targeting ligand at first resulted in a 77-fold difference in particle binding *in vitro* and later resulted in only a 1.5 difference in cytotoxicity *in vitro*. Nevertheless, the *in vivo* studies revealed that, while non-targeted nanoparticles are already an effective delivery system, the addition of a ligand does yield significant increases in drug localization and therapeutic efficacy.

It should be noted that, while most of the research published by the scientists involved in BIND Biosciences feature the use of RNA aptamers as targeting ligand for proof-of-principle, it was stated in one article[117] and in one patent[129] that the targeting ligand is a peptide. It is never explicitly stated what targeting ligand is used on the BIND-014 nanoparticles. While a peptide aptamer could be considered be comparable to the A10 RNA aptamer the molecular formula in the patent[129] does not seem to feature the protein scaffold typical of peptide aptamers[99]. There are several other known peptides that selectively bind PSMA[137,138], so it might be a different peptide ligand altogether. The discrepancy and lack of transparency make it therefore unclear how comparable the results of the A-10 targeted nanoparticles and BIND-014 are, potentially casting doubt on the claims of the authors.

CALAA-01

CALAA-01, a transferrin-targeted nanoparticle in development by Calando Pharmaceuticals, entered phase I clinical trials for the treatment of solid tumors in April 2008, which are expected to be completed in 2012[139]. This drug is the first example of Calando Pharmaceuticals' RONDEL™ (RNAi/Oligonucleotide Nanoparticle Delivery) platform, which uses self-assembling cyclodextrin-containing polymers to deliver anti-RRM2 siRNA to solid tumors. This nanoparticle delivery system consists of three components: a linear cyclodextrin-containing polymer (CDP) backbone, adamantane-conjugated polyethylene glycol (PEG-AD) and transferrin-conjugated AD-PEG (Tf-PEG-AD) as a targeting agent[118] (figure 6).

The therapeutic payload of CALAA-01 is an anti-RRM2 siRNA[118]. Small interfering RNA (siRNA) is able to induce gene silencing in mammalian cells through RNA interference[140–142]. Unfortunately, it is burdened by a number of challenges, including off-target silencing of homologous genes[143–145], immunogenicity[146–148] and an extremely short half-life in human plasma[149,150]. More importantly, siRNAs are exclusively intracellularly active but, due to their large size, strong negative charge and hydrophilicity, are unable to cross the cellular membrane through diffusion[103]. CALAA-01 addresses these issues by encapsulating the siRNA into a protective polymeric nanoparticle that uses the transferrin receptor to trigger clathrin-mediated endocytosis. The target mRNA that is silenced by the CALAA-01-induced RNAi is the M2 subunit of ribonucleotide reductase (RRM2), an essential enzyme for cell replication and considered to be an important target in cancer therapeutics[151]. RNAi against RRM2 was shown to significantly reduce cell proliferation in several cancer types *in vitro* and *in vivo*[152].

Cyclodextrins (CDs), also called cycloamyloses, are cyclic oligosaccharides produced from starch that exhibit resistance to degradation by human enzymes, water-solubility, low toxicity and low immunogenicity[153]. Their three-dimensional structure features a cup-like torus with a hydrophilic outside and a hydrophobic interior cavity. This interior cavity forms a hydrophobic micro-environment which preferably encapsulates a-polar molecules[154]. In CALAA-01, these cyclodextrins, specifically β-cyclodextrins, are formed into short cationic, water-soluble polymers that are used as a scaffold for the attachment of structural and functional agents[155].

The targeting ligand, transferrin (Tf), is an iron-binding blood plasma glycoprotein that is recognized and bound by transferrin receptors on the cell membrane. Transferrin

receptor is often overexpressed on the surface of cancer cells and is internalized through the clathrin-dependent endocytic pathway upon binding transferrin[90]. In CALAA-01, the transferrin ligand is conjugated to a number of PEG-AD polymers on the surface of the nanoparticle, which are described by Bellocq et al[156].

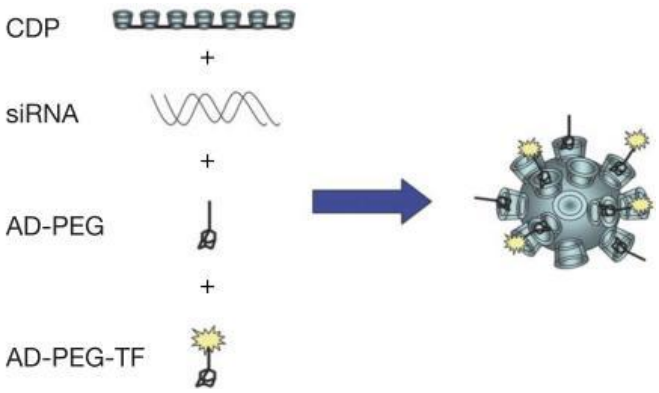

Figure 6: a schematic depiction of the self-assembly of CDP, siRNA and adamantane-conjugated polymers, with and without ligand, into inclusion complexes. Reprinted from Davis et al[157].

The components of the CALAA-01 nanoparticle self-assemble into inclusion complexes, formed by the interaction between the cationic CDP and the anionic siRNA molecules[118,157] (figure 6). The interaction between the hydrophobic adamantane molecule and the similarly hydrophobic interior cavity of the β-cyclodextrin cups on the CDP 'click' the AD group of the PEG-AD molecules into place on the CDP scaffold, while the hydrophilic PEG extends out from the nanoparticle. This modular construction allows modification of the particle through conjugation of various structural or functional agents to the PEG-AD molecules[155,158]. Additionally, the PEG-AD polymers impart salt stability by covering the particle with a hydrophilic layer[159]. The resulting nanoparticles are water-soluble, approximately 70 nanometers in size and can encapsulate approximately 2 000 siRNA molecules at a 20:1 ratio to the transferrin ligand[118,158].

In vitro the nanoparticles exhibited little aggregation and were tested for protection from nuclease degradation in serum and, while naked siRNA was rapidly degraded, the nanoparticle-encapsulated siRNA remained intact after 4 hours in 50% mouse serum at 37 °C and 5% CO_2[158]. Attachment of a transferrin ligand was *in vitro* demonstrated to result in a ligand-density-dependent increase in cell binding and an approximately 40% increase in siRNA uptake in HeLa cells, which was reduced to the level of untargeted nanoparticles through outcompetition by free transferrin[158]. In another *in vitro* experiment, the use of transferrin as a ligand was observed to result in a 4-fold increase in luciferase expression of K562 cells after transfection with plasmids carrying a luciferase gene, while there was no increase in transfection efficiency when outcompeted by free transferrin[156] (figure 7A). *In vivo* studies in mice implanted with luciferase-expressing tumors revealed little difference in tumor localization and biodistribution between non-targeted and transferrin-targeted nanoparticles, although luciferase signal was approximately 50% decreased in the targeted nanoparticles, indicating increased transfection efficiency[74] (figure 7B). There was rapid siRNA clearance from the blood through the liver and kidneys, possibly the result of release of the siRNA from the nanoparticles quickly after administration[74]. During *in vivo* pilot safety studies in non-human primates escalating doses of CALAA-01 were well-tolerated, although at higher doses there were indications of kidney damage as well as some liver damage[160]. Early results of CALAA-01 clinical trials in three patients with solid tumors showed dose-dependent intracellular localization in tumor cells but not in the adjacent epidermis as well as decreased expression of RRM2 in one patient (mRNA levels were also lowered in the other two patients but, due to the age of the pre-treatment samples, efficacy was not certain)[157].

In conclusion, active targeting through transferrin was demonstrated *in vitro* to increase cellular uptake[158] and transfection efficiency[156], which could be outcompeted by free transferrin, and *in vivo* to increase transfection efficiency but not tumor localization[74]. CALAA-01 was well tolerated in primates[160] and early results in humans seem encouraging[157]. Nevertheless, some critical notes should be placed. While the

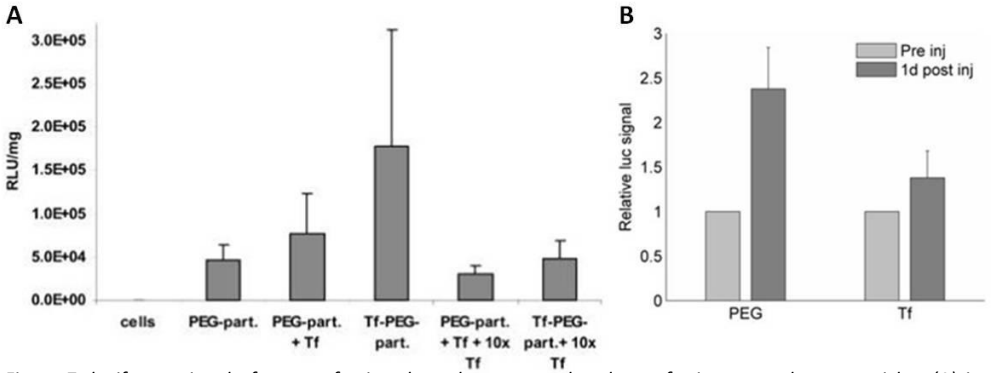

Figure 7: luciferase signal after transfection through untargeted and transferrin-targeted nanoparticles. (A) in vitro transfection of cells with plasmids carrying a luciferase gene. Formulations include nanoparticles containing PEG-AD (PEG-part.), PEG-AD mixed with holo-Tf (PEG-part. + Tf) or PEG-AD and Tf-PEG-AD (Tf-PEG-part.). Ligand competition was assessed through the addition of free transferrin (10x Tf). Reprinted from Bellocq et al[156]. (B) Luciferase-expressing xenograft tumors in mice were transfected in vivo with siRNA targeting luciferase mRNA. Formulations include nanoparticles containing PEG-AD (PEG) or PEG-AD and Tf-PEG-AD (Tf). Reprinted from Bartlett et al[74].

description of the nanoparticle remains consistent, it should be noted that the earlier preclinical studies do not mention the name "CALAA-01", presumably because it was named as such later in the development. It is therefore not completely certain to what extend the results of these studies represent CALAA-01 itself. CALAA-01 features a very high siRNA load per particle, approximately 2 000 individual molecules per nanoparticle[158]. While siRNA is already effective in specifically silencing gene expression at low concentrations, higher concentrations can result in increased off-target silencing[161], which might result in adverse side-effects. The core component of CALAA-01, β-cyclodextrin, has a high affinity for cholesterol and is able to extract it and other molecules from the cell membrane, even causing haemolysis of erythrocytes at high concentrations[153]. It was demonstrated to greatly inhibit clathrin-mediated endocytosis of transferrin receptor through cholesterol depletion[162]. Nevertheless, the results of CALAA-01 showed adequate cell uptake, presumably because the hydrophobic 'cups' on the CDP were occupied by the adamantane-ligated polymers, making them unable to extract the cholesterol. Additionally, β-cyclodextrin is poorly soluble without modification, can cause haemolysis at high concentrations and may cause kidney damage[153]. It can only be assumed that the modification of CDPs, including PEGylation, enabled CALAA-01 overcome these issues, although kidney damage was observed in primates at high concentrations[160]. While CALAA-01 seems to be a promising solution for the systemic administration of siRNA, its efficacy in humans still needs to be assessed.

MBP-426

MBP-426 is an actively targeted nanoparticle in development by Mebiopharm Co. It consists of an intravenously administered transferrin-conjugated liposome loaded with oxaliplatin (L-OHP). Phase I trials to study the safety for treatment of advanced or metastatic solid tumors started in July 2006 and ended in November 2008[163]. These were followed by phase II trials in August 2009, which were scheduled to end in March 2011, for treatment of second line gastric, gastroesophageal or esophageal adenocarcinomas in combination with leucovorin (folinic acid, FA) and fluorouracil (5-FU)[164]. There are several other formulations of the same platform in the preclinical stage of development: MBP-Y003, loaded with methotrexate for the treatment of cancer, MBP-Y003b, a version of MBP-Y003 for the treatment of chronic inflammatory diseases, MBP-Y004, loaded with docetaxel for the treatment of ovarian, breast and non-small cell lung cancer, and MBP-Y005, loaded with gemcitabine for the treatment of non-small cell lung, pancreatic and biliary cancer[165].

The nanoparticle itself is a liposome approximately 50-200 nm in size and has a lipid bilayer in which N-glutaryl-phosphatidylethanolamines (NGPEs) are located, which can be used as a linker for transferrin attachment[166–168] (figure 8).

The conjugated ligand, transferrin, targets transferrin receptor and was discussed earlier in this paper. Transferrin receptor is often overexpressed on cancer cells and, upon binding transferrin, the complex is internalized through the receptor-mediated endocytic pathway[91].

The encapsulated payload, trans-L-diaminocyclohexane oxalatoplatinum, or oxaliplatin (L-OHP), is a derivative of cisplatin. It is a platinum coordination complex that is intracellularly active and inhibits DNA synthesis and transcription[169]. It is typically used in FOLFOX, a combination chemotherapy regimen consisting of L-OHP, FA and 5-FU for the treatment of colorectal cancer[170]. Unfortunately, in blood and plasma, oxaliplatin quickly forms reactive platinum complexes that irreversibly bind to various molecules in the blood or on cells and are eventually eliminated, resulting in low tumor accumulation[171]. Encapsulation in a liposome might therefore increase oxaliplatin availability and treatment efficacy and the conjugation to transferrin could increase intracellular localization[172].

Addition of transferrin as a targeting ligand to PEG-liposomes was demonstrated *in vitro* to result in an approximately 10-fold increase in binding to Colon-26 cells at 4°C (temperature was lowered to prevent other cellular processes such as internalization), unless in the presence of free transferrin[173]. Additionally, cell association of transferrin-targeted PEG-liposomes increased approximately 4-fold when temperature was raised to 37°C and approximately 75-80% of the liposomes were found to be internalized, although these tests were not performed on untargeted liposomes or in the presence of free transferrin[173].

In vivo in Colon 26-tumor xenograft mice, transferrin-targeted and untargeted PEG-liposomes showed similar clearance, organ distribution and tumor localization, although the transferrin-targeted PEGylated liposomes were retained longer in tumor tissue; after 48 hours the level of untargeted PEG-liposomes began to decrease and after 120 hours transferrin-targeted PEG-liposomes showed approximately twice as much tumor-associated liposomes[173]. *In vitro* cytotoxicity tests on Colon-26 cells showed that, while L-OHP

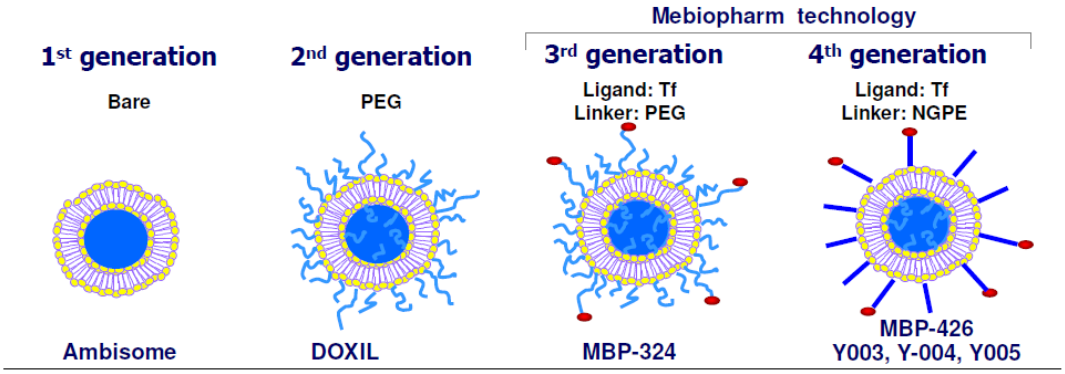

Figure 8: comparison of Mebiopharm technologies with other liposomal carriers. Reprinted from a presentation by Tadashi Fujisawa at the Japan Biotech Forum 2009[168].

in solution was the most effective, L-OHP delivery through transferrin-targeted PEG-liposomes resulted in higher cytotoxicity than delivery through untargeted PEG-liposomes and unPEGylated liposomes, when not outcompeted by free transferrin[174]. *In vivo* tests on Colon-26 tumor xenograft mice demonstrated similar clearance for targeted and untargeted PEG-liposomes, while bare liposomes and L-OHP in solution were rapidly cleared from the blood plasma[174]. L-OHP in solution was taken up by erythrocytes, while all liposomal formulations showed localization in the liver and spleen, although the concentration of unPEGylated liposomes was twice that of the PEGylated liposomes[174]. Transferrin-targeted PEG-liposomes showed significantly increased tumor localization over untargeted PEG-liposomes, although both were higher than bare liposomes and L-OHP in solution[174] (figure 9A). While all other formulations showed similar tumor growth ratios, transferrin-targeted PEG-liposomes showed approximately 2.5-3-fold increased tumor growth suppression after 30 days[174] (figure 9B).

Early results of clinical trials, presented at the AACR-NCI-EORTC International Conference 2009, showed hints of efficacy in treating solid tumors in patients with previous disease progression and platinum resistance[167]. In phase I studies MBP-426 was shown to have a favorable safety profile, the dose limiting toxicity was caused by thrombocytopenia and, despite the majority of the patients having had extensive prior treatment with oxaliplatin or cisplatin, approximately half of the subjects had stable disease over time and 2 of the 39 patients experienced tumor size reduction[119]. Results presented at the American Association for Cancer Research (AACR) Annual Meeting 2007 of *in vivo* research into human pancreas tumor xenograft models suggested that treatment with MBP-426 resulted in a dose-dependent upregulation of transferrin receptor in tumor tissue[166], which could hypothetically result in increased therapeutic efficacy over time.

The limited availability of scientific literature specifically concerning MBP-426 creates difficulties in assessing the composition and qualities of the nanoparticle. While multiple studies about transferrin-targeted liposome-encapsulated oxaliplatin have been published, it is rather unclear which studies apply to MBP-426, especially since it, as opposed to earlier generations of transferrin-targeted L-OHP-encapsulating liposomes, utilizes NGPE instead of PEG. Nevertheless, from what data is available, it seems a valid solution for the delivery of oxaliplatin, which has a low functional half-life upon systemic administration[171]. Encapsulation of L-OHP in PEG-liposomes showed increased blood half-life and tumor localization, the latter of which was

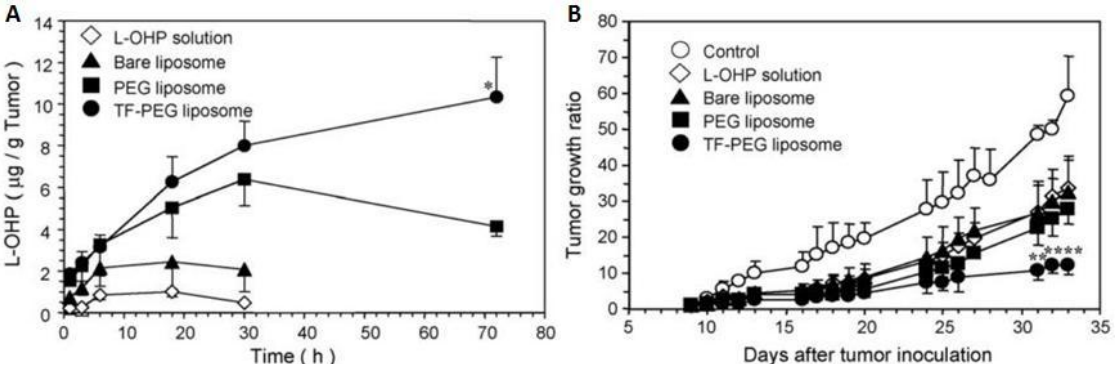

Figure 9: tumor accumulation and growth suppression in Colon-26 tumor xenograft mouse models. Formulations constitute L-OHP in solution and L-OHP encapsulated in bare (unPEGylated) liposomes, untargeted PEG-liposomes and transferrin-targeted PEG-liposomes. (A) Time course of L-OHP accumulation in tumor after intravenous injection. (B) Tumor growth ratio after tumor inoculation, formulations were injected at days 9 and 12. Reprinted from Suzuki et al[174].

improved by the addition of transferrin[174]. Interestingly, despite the fact that untargeted unPEGylated and PEGylated liposomes showed improved tumor localization over L-OHP in solution, this did not result in a significant increase in tumor growth suppression, while addition of transferrin as a targeting ligand did result in lower tumor growth[174], demonstrating the benefit of the ligand. It should be noted that, while free L-OHP was mostly internalized by erythrocytes, L-OHP in targeted and untargeted liposomal formulations showed much greater degree of localization in the liver and spleen[174], which, while lowering haemotoxicity, could increase toxicity to these organs.

PK2

PK2 also known as FCE28069, is a galactosamine-conjugated N-(2-hydroxypropyl)methacrylamide (HPMA)-polymer that is used to transport doxorubicin to liver cancer cells[120,121] (figure 10). It was originally developed by Pharmacia, a Swedish biotechnological and pharmaceutical company that was acquired by Pfizer in 2003[175]. While results of phase I clinical trials for the treatment of primary and metastatic liver cancer were reported in 2002[120] and phase II trials were initiated, it was discontinued in 2008 for unspecified reasons[176].

The backbone of PK2, a HPMA polymer, is part of a versatile and biocompatible class of molecules that can be conjugated to various other groups, such as therapeutic drugs, to act as nanocarriers. They have already been demonstrated in a number of occasions to be able to deliver payloads to cells or tissues in both *in vitro* and *in vivo* research[177,178]. An untargeted but otherwise identical therapeutic, called PK1 or FCE28068, which consists of a HPMA polymer conjugated to doxorubicin, has progressed through phase I[179] and phase II clinical trials[180].

The galactose-based ligand of PK2 was designed to mimic asialoglycoprotein, which is bound by hepatocyte asialoglycoprotein receptors (ASGPR) on liver cells. Upon binding to the receptor, the complex has been demonstrated to be internalized through receptor-mediated endocytosis and directed to the lysosomes[181].

The payload of PK2 is doxorubicin, also known as Adriamycin or hydroxydaunorubicin. Doxorubicin is a anthracycline antibiotic used in chemotherapeutic treatment of various forms of cancer. It is able to interfere in DNA, RNA and protein synthesis and is highly cytotoxic[182]. Unfortunately, doxorubicin treatment can inflict serious, potentially life-threatening, adverse effects, including cardiomyopathy and

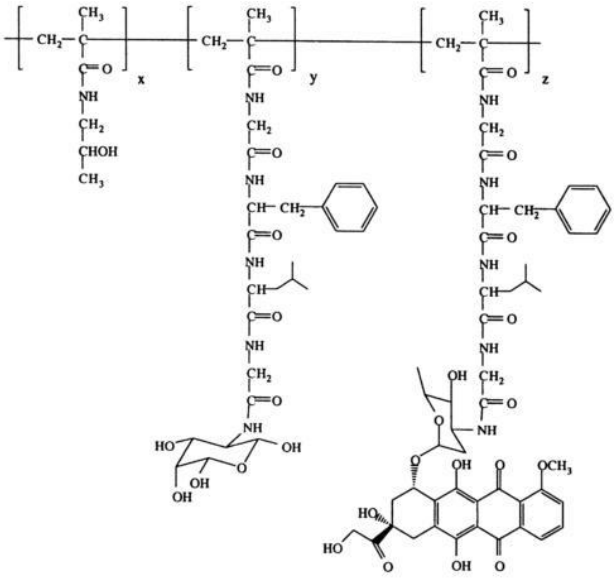

Figure 10: The chemical structure of the galactosamine- and doxorubicin-conjugated N-(2-hydroxypropyl)methacrylamide (HPMA)-polymer PK2. Reprinted from Hopewell et al[121].

congestive heart failure in the long term[183]. Nanoparticle-mediated delivery of doxorubicin in the treatment of liver cancer might alleviate these side effects, taking advantage of both active targeting and the fact that nanoparticles are often cleared from the bloodstream through MPS organs, which includes the liver.

Early *in vivo* studies with radiolabelled HPMA polymers demonstrated that rats injected with galactosamine-carrying polymers showed approximately 6-8-fold and 3-9-fold higher radioactivity in the liver after 1 and 5 hours respectively when compared to control polymers and polymers carrying other monosaccharide residues[184]. In a similar experiment, it was demonstrated that increasing the number of galactosamine residues per polymer led to increased liver localization in rats *in vivo*, where they were found to be internalized by hepatocytes, and increased affinity for hepatocyte plasma membranes *in vitro*[185]. *In vivo* studies in mice injected with HPMA polymers containing doxorubicin and galactosamine demonstrated relatively decreasing clearance and liver accumulation with escalating doses, indicating receptor saturation[186]. Additionally, injection with doxorubicin- and galactosamine-conjugated HPMA polymers showed a 100-fold decrease in doxorubicin concentration in the heart, when compared to injection of free doxorubicin[186]. Preclinical *in vivo* studies in rats demonstrated that PK2 had 2-3-fold less acute toxicity and approximately 5-fold less cardiotoxicity than free doxorubicin[121]. Phase I studies for the treatment of

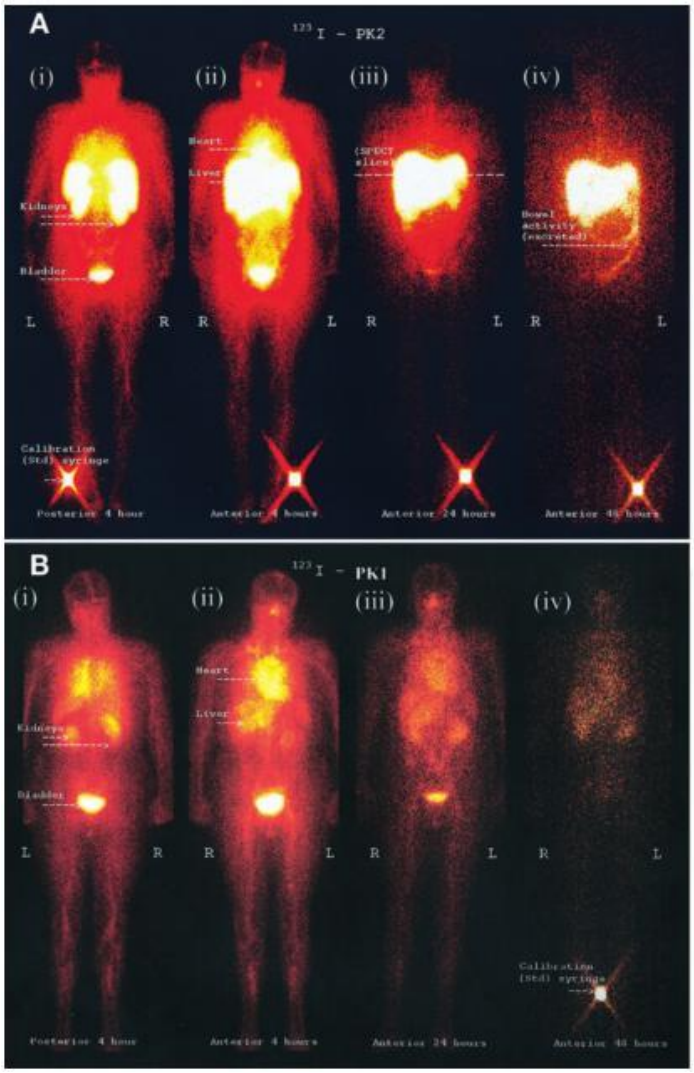

Figure 11: Planar gamma-camera images of patients at 4, 24 and 48 hours after therapeutic administration. From left to right: posterior after 4 hours and anterior after 4, 24 and 48 hours respectively. (A) Galactosamine-targeted doxorubicin-conjugated HPMA polymer PK2. (B) Untargeted doxorubicin-conjugated HPMA polymer PK1. Reprinted from Seymour et al[120].

solid primary and secondary hepatic tumors with a combination of conventional PK2 and radiolabelled analogs (for *in vivo* imaging) revealed high levels of radioactivity in the hepatic region (figure 11A), while a parallel study featuring the untargeted PK1 showed much lower levels of radioactivity in that region (figure 11B)[120]. The uptake of radiolabelled PK2 was 5-fold higher in normal liver tissue than in tumor tissue but there were indications of antitumor efficacy in several patients[120]. Although higher than that of free doxorubicin, the maximum tolerated dose and recommended dose of PK2 were significantly lower than that of PK1 and infusion of PK2 caused pain at conditions that were suitable for PK1[120]. Interestingly, there were no indications of receptor saturation[120], unlike in rodent models[186].

While the targeted HPMA nanoparticles did show increased localization and uptake over untargeted nanoparticles in rodents[184–186], receptor saturation quickly occurred at higher concentrations[186]. In humans, liver localization was improved by the addition of the targeting ligand, although more severe adverse effects resulted in lower tolerated doses[120]. Interestingly, later studies demonstrated that PK2 is less soluble in water and has a significantly altered structure, leading to a more "open" coil structure that could have increased exposure of doxorubicin relative to PK1[187]. This structural change, and the fact that galactosamine has been shown to be hepatotoxic[188], could be the underlying reason for the increased toxicity of PK2 and indicates that conjugation of an active targeting ligand may have unforeseen consequences. While phase II trials were started, little information has been made public since 2002 and PK2 was reported to be discontinued in 2008, alongside the untargeted HPMA-doxorubicin copolymer PK1[176], although both showed decreased toxicity to free doxorubicin. It is unknown why PK2 and PK1 were discontinued.

MCC-465

MCC-465 is a doxorubicin-loaded PEGylated immunoliposome, which is a liposome using an antibody-based targeting ligand, in this case a F(ab')$_2$ fragment of the human monoclonal antibody named "GAH" (the name has not been specified any further). While the drug has progressed through phase I clinical trials, for treatment of metastatic stomach cancer, and was recommended for phase II trials[122], no further information has been made available since 2004.

The targeting ligand, the F(ab')$_2$ fragment, is based on the human monoclonal IgG$_1$ antibody GAH, which was selected for cancer-specific reactivity by Hosokawa et al[189]. While the antigen of the GAH antibody is currently unknown, it was demonstrated to have over 90% reactivity to gastric cancer tissue cells and gastric cancer tissue sections[189,190]. F(ab')$_2$ fragments are obtained through pepsin digestion of the GAH antibody and the fragments are thiolated prior to linkage to the liposomal surface[191].

Multilamellar liposomes are created and sized through extrusion with a series of filters of decreasing pore size[191,192]. The nanoparticles are loaded with doxorubicin through the pH gradient method, which involves migration of the drug through the liposomal membrane in response to a transmembrane pH gradient, into the more acidic interior of the liposome[193,194]. Afterwards thiolated antibody fragments and subsequently PEG are conjugated to the liposome surface through thioesther linkage[191,192]. The resulting

immunoliposomes are approximately 125-160 nm in size, on average 143 nm[191,195].

During *in vitro* tests on a GAH-reactive human stomach tumor cell line, GAH-conjugated doxorubicin-loaded liposomes exhibited strong dose-dependent cytotoxicity, up to 90% at 10 μg/ml, while non-targeted doxorubicin liposomes showed no significant toxicity[191]. Additionally, intracellular localization was demonstrated *in vitro* with fluorescence-labelled immunoliposomes, which was inhibited by the addition of free GAH antibodies[191]. While *in vitro* experiments demonstrated that free doxorubicin exhibited strong cytotoxicity to both GAH-reactive and GAH-non-reactive cell lines, incorporation into immunoliposomes greatly decreased cytotoxicity to GAH-non-reactive cells but not to GAH-reactive cells[191]. In GAH-reactive human gastric and colorectal cancer mouse xenograft models, treatment with doxorubicin-loaded immunoliposomes were shown to result in approximately 2.5-fold lower tumor weight than treatment with free or liposome-encapsulated doxorubicin, even when the latter was administered in combination with free GAH antibodies[191]. Mouse xenograft models of 10 different human cancer cell lines were treated with immunoliposomes[191]. These cell lines varied in GAH-reactivity, which was determined through quantification of fluorescence-labeled GAH-binding to the cell surface, in and doxorubicin sensitivity[191]. The antitumor effect of the immunoliposomes but not of non-targeted liposomes was shown to correlate with the GAH-reactivity of the cell lines, even in cell lines that normally exhibited limited sensitivity to doxorubicin treatment[191].

In an *in vivo* study comparing MCC-465, free doxorubicin and PEGylated liposome-encapsulated doxorubicin in the treatment of three different cancer cell lines in mouse xenograft models, treatment with MCC-465 was demonstrated to result in tumor weights that were approximately 3-fold lower in the first and 7-fold lower in the second of the two GAH-reactive cell lines, when compared to free and liposomal doxorubicin, but showed little effect in the GAH-non-reactive cell line (figure 12)[195].

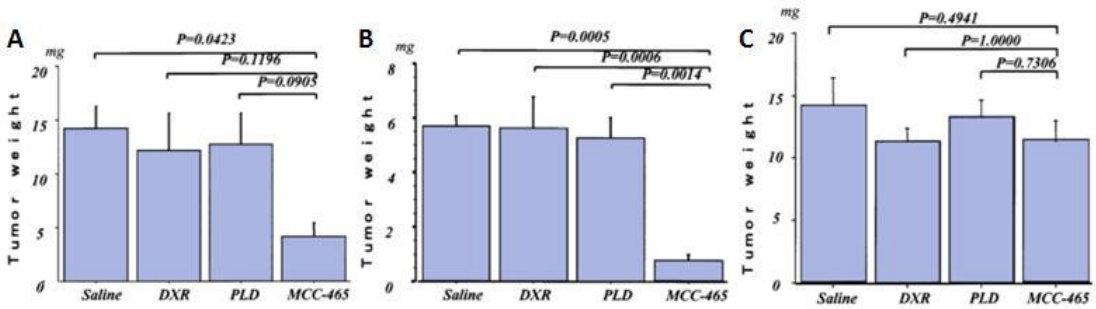

Figure 12: in vivo antitumor effect of MCC-465, PEGylated liposome-encapsulated doxorubicin (PLD) and free doxorubicin (DXR) in mouse xenograft models. Formulations were administered 1, 8 and 15 days after tumor inoculation and tumor weights were measured on day 22. (A) and (B) GAH-reactive tumor cell lines. (C) GAH-non-reactive tumor cell line. Adapted from Hamaguchi et al[195].

Phase I trials of MCC-465 for the treatment of metastatic stomach cancer revealed that the nanoparticles were stable in the blood circulation and had similar pharmacokinetics to Doxil, which is a non-targeted doxorubicin-containing PEGylated liposome[122,196]. Treatment was well-tolerated and, while there were no indications of antitumor effect, over half of the patients showed stable disease[122].

While the nature of the antibody fragment has not been clarified and the corresponding antigen was not identified as of 2004, active targeting was shown in several preclinical *in vitro* and *in vivo* studies to contribute to an increased antitumor effect of liposomal doxorubicin. It remains unclear what the mechanism behind this increased cytotoxicity is, especially since it is unknown whether GAH-binding results in cellular internalization of the liposome. Notably, in a more recent *in vivo* study in which mice were implanted with a GAH-reactive tumor cell-line and were treated with radiolabeled free or PEG-liposome-bound GAH $F(ab')_2$ fragments, both agents showed significantly more localization in several organs other than the tumor, even after several days[197], which might cast doubt on the efficacy of targeting through GAH. No new studies have been made public since 2004 and there is little information available regarding the current clinical status. Since one of the organizations involved in the development of MCC-465, Mitsubishi Chemical Corporation, has merged with Mitsubishi Pharma Corporation, resulting in Mitsubishi Chemicals Holdings Corporation in October 2005, the project was possibly abandoned in the restructuring of the corporations.

Lipovaxin-MM

Lipovaxin-MM, in development by the Australia-based company Lipotek, is a liposomal vaccine for the immunotherapy of malignant melanoma. It is based on the Lipovaxin platform, which features immune-activating liposomal nanovesicles that are actively targeted towards dendritic cells (DCs), and includes Lipovaxin-TB, a vaccine for tuberculosis, in the preclinical phase of the pipeline[123,198]. Lipovaxin-MM entered phase I

clinical trials for the treatment of malignant melanoma in September 2009, which were scheduled to be completed in November 2011[199].

The nanoparticle itself consists of a immunoliposome that derives part of its lipid membrane from the MM200 melanoma cancer cell line. Cancer cells are lysed and the membrane fraction is isolated to produce membrane vesicles, which bear a range of melanoma antigens. The membrane vesicles are then fused with synthetic liposomes, which carry the other components of Lipovaxin-MM, in the presence of interferon-gamma (IFN-γ), which serves as a 'danger signal' to activate DCs[200].

One component of the synthetic liposomes, and thus ultimately Lipovaxin-MM, is a chelating lipid, composed of 3-nitrilotriacetic acid (3NTA) and ditetradecylamine lipid (DTDA), that is used to bind and anchor histidine-tagged proteins, such as the targeting ligand, to the liposome[198,200].

The targeting ligand is a His-tagged single variable heavy chain (V_H) domain antibody fragment named DMS5000, which is linked to the liposome through the 3NTA-DTDA lipids and actively targets the DC-SIGN receptor[198,200]. DC-SIGN (Dendritic Cell-Specific Intracellular adhesion molecule-3-Grabbing Non-integrin), also known as CD209, is a receptor present on macrophages and dendritic cells. DC-SIGN mediates antigen internalization into dendritic cells and activation of cytotoxic T-cells[201]. DC-SIGN, as well as a number of other related receptors such as C-type lectins, have been proposed to be utilized in dendritic cell immunotherapy for the treatment of cancer through *in vivo* targeting of DCs with antigens, which could induce an immune response[202,203]. Targeting antigen to DCs via anti-DC-SIGN antibodies has been shown to induce both naïve as well as recall antigen-specific T-cell responses[204]. Lipovaxin-MM utilizes the antibody fragment DMS5000 to achieve the same effect[200].

An *in vivo* study was performed in mice, which were immunized with a Lipovaxin-MM-like compound prior to being injected with highly metastatic murine melanoma model B16-OVA. The compound was a murine-adapted Lipovaxin platform, produced from B16-OVA cells and incorporating antibody fragments against murine DC surface receptors, since DC-SIGN is not expressed in mice. After immunization, splenic T-cells were isolated and restimulated with B16-OVA cells *in vitro* to measure the cytotoxic T-cell (CTL) activity. While immunization with non-targeted antigen-carrying liposomes or DC-targeted antigen-carrying liposomes resulted in near-background levels of CTL activity, incorporation of both a targeting ligand and a 'danger signal', such as IFN-γ or a bacterial cell wall component, resulted in up to a 10-fold increase in specific lysis[205]. When the lungs were checked for metastases 16 days after injection with the B16-OVA cells, the mice immunized with the targeted antigen- and danger signal-carrying liposomes showed 100-200-fold less tumor foci than the non-immunized mice, while the liposomes that were non-targeted or did not carry a danger signal showed little-to-no tumor protection[205]. Additionally, when the targeted antigen- and danger signal-carrying liposomes were injected several days after B16-OVA injection, the mice did not show any signs of tumor development for up to 8 months, while the control mice had to be euthanized within 22 days due to massive tumor loads[205].

Although not published in scientific journals, the patent for Lipovaxin-MM describes *in vivo* studies in pigtailed macaques, which have a highly conserved DC-SIGN receptor[200]. Although *in vitro* studies demonstrated that DMS5000 had a much lower affinity for the macaque DC-SIGN, vaccine-specific antibodies and cytokine production were detected after immunization[200]. It should be noted that this study did not include negative controls; there were only two groups of macaques, one of which was immunized with a lower dose and the other with half the equivalent maximum dose. Nevertheless, the immunological response was most evident in the group with the higher dose[200].

Interestingly, Lipovaxin-MM is rather unique in this roster of actively targeted nanomedicines as it has an indirect method of action; it does not target the pathogens directly but delivers antigens to immune cells to induce an immunological response to melanoma cancer cells. Targeting antigen to DCs through anti-DC-SIGN antibodies was previously shown to induce immunological responses, indicating the efficacy of this kind of approach[204]. While studies in mice showed increased immunological anti-tumor activity, it should be noted that the platform was highly modified to correspond to the mice model; the liposomes themselves were produced from a mice melanoma cell-line, instead of the human MM200, and the ligand was changed, since DC-SIGN is not expressed in mice[205]. While this could be seen as a proof-of-principle of this immunizing immunoliposome platform, it is doubtful whether these results could be considered convincing evidence of Lipovaxin-MM itself. Studies in primates showed induction of antibody and cytokine production although it should be noted that no negative controls were included and that the antibody fragment had a low affinity for macaque DC-SIGN, despite a high degree of

conservation between humans and macaques[200]. Since there have been no publications of studies in humans as of yet, it remains to be seen whether delivery of antigens through immunoliposomes carries therapeutic benefits. However, if the functionality of this platform is demonstrated, it could hypothetically allow an even more personalized treatment regimen. Since the loading and production of the liposomal nanoparticles have been combined into one step, which involves fusion of the membrane fraction of lysed melanoma cells with targeting-ligand carrying synthetic liposomes, the cancer cells of individual patients could be extracted, cultured and lysed to produce personalized vaccines.

SGT-53

SGT-53, also known as SGT-53-01 or Synerlip p53, is drug being developed by SynerGene Therapeutics Inc. for the treatment of cancer. It consists of plasmid DNA encapsulated by a liposome that is targeted to the transferrin receptor through an antibody fragment[124]. It is currently in phase I clinical trials for the treatment of solid tumors, which were started in February 2008 and are scheduled to end in March 2012, to evaluate the safety of combinational therapy with docetaxel and to establish the recommended dose for further studies[124].

While SGT-53 is targeted towards the transferrin receptor like several other actively targeted nanomedicines, it utilizes an antibody fragment instead of transferrin. An advantage of targeting the transferrin receptor with an antibody fragment instead of transferrin itself is that the scFv has a much smaller size than transferrin[91,206]. Additionally, since the scFv is a recombinant protein and not a blood product like transferrin, it allows stricter quality control and larger scale production[206,207].

Like CALAA-01, SGT-53 is essentially an agent for gene therapy of cancer cells. Unlike CALAA-01 however, which transfects cancer cells with siRNA, SGT-53 is loaded with a DNA plasmid coding for wild-type p53. The p53 tumor suppressor protein is encoded in humans by the TP53 gene and is crucial for cell cycle regulation, as well as preventing genome mutation[208]. Aside from the native tumor-suppressor effects of wild-type p53, ligand-targeted liposome-mediated p53 transfection of cancer cells was demonstrated to sensitize them to chemo- and radiotherapy[209,210]. Expression of p53 was also demonstrated to correlate with sensitivity to antiangiogenic tumor therapy[211].

While it is not certain that it is the same particle as SGT-53, in a paper by the same authors behind SynerGene Therapeutics, cationic lipoplexes of approximately 60-70 nm, targeted through anti-transferrin scFvs and loaded with the p53 gene, were demonstrated *in vitro* and *in vivo* to have enhanced tumor cell binding, gene delivery and transfection when compared to untargeted liposomes and liposomes targeted through transferrin[209]. *In vitro*, in human breast cancer cells, both the transferrin- and the scFv-targeted liposomes showed a 2-fold increase in transfection efficiency with the E. coli LacZ gene compared to the untargeted liposomes[209]. *In vitro* in human prostate cancer cells the scFv-targeted liposomes were twice as effective as transferrin-targeted liposomes and 4 times as effective as untargeted liposomes[209]. In a similar *in vitro* study by the same authors, which were supported by a grant from SynerGene Therapeutics Inc., anti-transferrin scFv-targeted liposomes were 4-10 times as effective in transfecting human breast and prostate cancer cells, when compared to untargeted liposomes and twice as effective when compared to transferrin-targeted liposomes[206]. Similar results were found *in vitro* in four of five other cell lines; transfection activity was limited only in normal human fibroblast cells[206]. In an *in vivo* experiment with mice xenografted with prostate tumors, treatment with anti-transferrin scFv-targeted p53-loaded liposomes was shown to induce high levels of p53 expression in the tumors but not in the liver[206].

The lack of scientific literature concerning SGT-53 creates difficulties in assessing this actively targeted nanoparticle. In related articles by the same authors, the *in vitro* transfection of cancer cells through anti-transferrin scFv-targeted liposomes was demonstrated to be more effective than untargeted and transferrin-targeted liposomes. While these results were promising, the limited amount of *in vivo* research and the lack of transparency concerning the nanoparticle, SGT-53 could be considered to be unproven as of yet.

MM-302

MM-302 is a cancer therapeutic, in development by Merrimack Pharmaceuticals, that is currently in phase I clinical trials for the treatment of advanced breast cancer. This study started in March 2011 and is planned to be completed in March 2012[125] The nanoparticle consists of a PEGylated liposome encapsulating doxorubicin that is targeted towards HER2 through antibodies attached to the liposome[212]. The original developer, Hermes Biosciences, was

acquired by Merrimack Pharmaceuticals in 2009[213] and little information concerning MM-302 has been made public since.

MM-302 consists of doxorubicin encapsulated in an immunoliposome, which is a liposome that is actively targeted through an antibody-based targeting ligand. As such, it is similar to MCC-465 and, to a lesser extent, SGT-53, which were discussed earlier in this article. The ligand is an anti-HER2 antibody fragment[214], although its exact nature is unclear, especially since the scientists behind Hermes Biosciences have published several studies featuring doxorubicin-encapsulating liposomes targeted through different anti-HER2 antibody fragments, including Fab' and scFv fragments[215,216]. The target of the ligand, HER2, also known as ErbB2, Neu, p185 or CD340, is an epidermal growth factor receptor encoded by a proto-oncogene that is often involved in breast and other forms of cancer[217] and is associated with poor prognosis[218]. HER2 is already used as a target for actively targeted therapeutics[219], although HER2-targeted therapy has been associated with cardiotoxicity[220–222]. HER2 has been demonstrated to be internalized upon antibody binding[223].

Preclinical studies to assess the cardiotoxicity of MM-302, presented at the AACR-NCI-EORTC International Conference 2010, demonstrated *in vitro* nuclear accumulation, leading to cell death, in human stem-cell derived cardiomyocytes when these were treated with free doxorubicin but not for MM-302 or untargeted liposomes[212]. Results of *in vitro* studies, presented at the CTRC-AACR San Antonio Breast Cancer Symposium 2010, demonstrated increased levels of doxorubicin in HER2 over-expressing cancer cell lines for MM-302, when compared to free or untargeted liposome-encapsulated doxorubicin[214]. *In vivo* studies in mouse tumor xenograft models, presented at the same conference, demonstrated similar organ distribution, half-life and clearance for MM-302 and untargeted liposomes[214]. Nevertheless, MM-302 was indicated to have a stronger anti-tumor effect than both free and untargeted liposome-encapsulated doxorubicin[214].

Similar to SGT-53, MM-302 is characterized by a lack of transparent scientific literature, creating difficulties in assessing the platform. While HER2 is a proven therapeutic target[219], actively targeted delivery of doxorubicin could prove problematic. Notably, the two of the major components of MM-302 are cardiotoxic; both the ligand, as it is based on an anti-HER2 antibody[220–222] and the payload, doxorubicin[183] could present side-effects upon utilization in human treatment.

Discussion

As reported earlier, nanomedical drug delivery can utilize several types of nanocarriers, including polymers, liposomes, micelles, polymersomes and dendrimers. Nanocarrier-mediated drug delivery can utilize triggered drug release, passive targeting or active targeting. This study focused on the latter technique, active targeting. Active targeting has been demonstrated to increase internalization into the cell but not tissue localization, when compared to passively targeted nanoparticles[73–77]. These findings indicate that, like passive targeting, active targeting mostly relies upon the EPR effect for tissue localization. The clinical relevance of the EPR effect in tumor targeting is compromised by a number of factors, including abnormal blood flow[36], high hydrostatic[41–43] and oncotic[62] pressures in tumor tissue and interstitium, heterogeneous extravasation from tumor vasculature[7,39] and overestimation of the EPR effect due to the reliance upon animal xenograft models for initial therapeutic development[57–59]. As a result of these issues, few actively targeted nanoparticles have made it into clinical trials[6], despite promising preclinical research results[100–102].

In this study it was attempted to create an overview of actively targeted nanoparticles that have progressed into the clinical phase of development to determine what the capabilities and limitations of active targeting are. What patterns emerge? What are the hallmarks of a successful actively targeted nanomedicine? What avenues of exploration are still open or what new possibilities have arisen?

This study reported eight different actively targeted nanoparticles that had progressed through preclinical research and into clinical trials: BIND-014, CALAA-01, MBP-426, PK2, MCC-465, Lipovaxin-MM, SGT-53 and MM-302. The majority of these drugs have currently not progressed beyond phase I. Only MBP-426[164] and PK2[176] have entered phase II, although there is little information available for the former and the development of the latter was reported to be discontinued in 2008[176].

There are many different nanoparticle carrier platforms available, many of which could benefit from active targeting (table 1). Nevertheless, five of the eight nanocarriers that progressed into clinical trials were reported to be liposomal; only two are polymeric nanocarriers (BIND-014 and CALAA-01) and one is a polymer conjugate (PK2). Five of the eight nanocarriers feature a stealth coating, mostly PEG. There is insufficient information on SGT-53 to assess whether or not it

has a stealth coating. Lipovaxin-MM has not been reported to have a stealth coating, presumably because a stealth coating could hinder its function as an immunization agent. In most cases, the nanocarriers are actively targeted reflections of well-established passively targeted nanoparticle platforms that have shown promise; stealth liposomal nanocarriers[224] or, in the case of PK2, an HPMA polymer[178,225]. Notably, both MCC-465 and MM-302 seem to be close adaptations of the passively targeted Doxil. Doxil is a PEGylated liposomal nanocarrier loaded with doxorubicin[226], designed to reduce the cardiotoxic side effects of doxorubicin, although dosage is limited by localization in the skin causing 'hand-foot' syndrome[227]. Active targeting of Doxil-like nanocarriers could serve as a relatively simple and clinically relevant proof-of-principle of actively targeted nanocarriers. In short, while there are several different nanocarriers available (table 1), most of the actively targeted nanocarriers are very similar to well-established passively targeted platforms. Active targeting is presumably applied to existing platforms, opposed to developing nanocarriers de novo, to avoid a lengthy development process while still providing clinical proof-of-principle of actively targeted nanomedicine.

Half of the nanocarriers feature antibody fragments as targeting ligands, including $F(ab')_2$, scFv and V_H domain antibody fragments. Two of the nanoparticles utilize transferrin and one galactosamine, which are molecules that naturally occur in the human body. Although the nature of the targeting ligand on BIND-014 is unclear, it seems that the majority of the nanocarriers feature ligands that are well-studied[228,229] and/or (semi-)naturally occurring.

Three of the eight nanoparticles are targeted to the transferrin receptor, whether through transferrin or through an antibody fragment. The exact nature of the antigen of the ligand is unknown in the case of MCC-465. Lipovaxin-MM, as a vaccination agent, features active targeting as an indirect method of action, since the ligand targets DC-SIGN on DCs to activate them to combat melanoma cells. MM-302 targets HER2, which is a clinically well-established target for monoclonal antibody therapy[219,230]. It is notable that all of the known targets of the nanocarriers are receptors that internalize upon antigen binding[90,130,181,201,223], which could facilitate drug efficacy.

Despite the fact that active targeting could be applied to a vast variety of clinical indications, all of the nanocarriers were designed for the therapeutic treatment of solid cancers. Lipovaxin-MM does so through an indirect method-of-action, as mentioned previously. There could be many reasons for selecting solid cancer as a therapeutic target, including the fact that cancer has been a rising health care problem[4] or that is relatively easy to research. Most *in vitro* cell lines are cancerous, solid cancer xenograft models permit simple and manageable *in vivo* research, efficacy is easy to measure through measurement of tumor size and weight. Solid cancers also feature a relatively long and gradual disease progression, which facilitates observation of efficacy over time and increases the demand for long-circulating nanoparticles. As cancer cells are more similar to healthy cells than most other pathogens, there might be a greater need for specifically targeted drugs. Lastly, all of the nanoparticles were designed for intravenous administration, limiting the clinical application to diseases featuring intravenously accessible diseased tissues, which is often the case in advanced solid cancers.

Three of the eight nanocarriers feature doxorubicin as a payload, two of those three are essentially actively targeted Doxil, as mentioned earlier. Doxorubicin, as discussed earlier in this study, is a chemotherapeutic agent that is burdened by potentially lethal side-effects[183]. Delivery of doxorubicin through passively targeted liposomes was demonstrated to improve pharmacokinetics[196] and reduce cardiotoxicity[231], although it was burdened by development of hand-foot syndrome[227] caused by accumulation in the skin[232]. Active targeting might alleviate these adverse effects. Four of the other nanocarriers feature Oxaliplatin, Docetaxel or oligonucleotides, which have a relatively short *in vivo* half-life and low tumor accumulation. These therapeutic agents are delivered through nanocarriers to decrease toxicity and/or improve half-life. Active targeting could improve increase retention in tumor tissue and improve efficacy through increased cellular uptake, potentially mitigating the decreased internalization efficiency caused by PEGylation[33]. Lipovaxin-MM, being unique among these nanocarriers in that it does not deliver a compound to cancer cells but to DCs, carries both melanoma antigens, derived from a melanoma cell line, and IFN-γ. Lipovaxin-MM is actively targeted to DC-SIGN to improve T-cell response[200,204]

In most cases, the studies into the actively targeted nanoparticles demonstrated similar biodistribution, localization and clearance to passively targeted nanoparticles. Nevertheless, in several cases cellular uptake and tumor retention were improved, which resulted in increased *in vitro* and *in vivo* efficacy. *In vivo* treatment of rodent tumor xenograft models with actively targeted nanoparticles showed improved antitumor efficacy[136,191,195,205,214] and increased survival[136,205], when compared to passively targeted

nanoparticles. Several of the nanocarriers showed decreased toxicity to off-target cells, although PK2 demonstrated increased *in vivo* side-effects over untargeted nanoparticles[120]. In conclusion, active targeting has been demonstrated to have significant benefits, generally resulting in higher concentrations of drugs in target cells and lower concentrations in off-target cells.

It should be noted that there might be a selection bias in the information presented here as this study specifically focuses on actively targeted nanocarriers that were approved for at least phase I clinical trials, which necessitate promising preclinical research results. This limits the selection to those actively targeted nanoparticles that have already shown positive results and excludes nanoparticles that have been terminated prior to clinical trials or are currently still in preclinical development. Additionally, since all of the nanocarriers are in development by commercial organizations, there might be strong incentives to only report studies that shed a favorable light on the nanocarriers.

In light of the results of this study, a number of qualities seem to characterize successful actively targeted nanocarriers. The target tissue should logically be accessible to the nanoparticles, which usually traverse the circulatory system. The nanoparticles should have a favorable *in vivo* half-life and clearance profile, especially since active targeting does not seem to increase localization but does increase retention. Additionally, since active targeting does not seem to increase drug localization, it should mainly be used in clinical indications that feature an EPR effect, such as solid tumors or inflammation, since active targeting contributes little to drug localization. As increased internalization efficiency is one of the main benefits of active targeting, the delivery system should capitalize upon this. The antigen of the targeting ligand should preferably internalize upon antigen binding, which should increase uptake of the nanoparticles. Lastly, care should be taken in assessing unforeseen side-effects of active targeting. For example, while treatment with both anti-HER2 antibodies and free doxorubicin resulted in increased cytotoxicity[233], both individual agents have been demonstrated to be cardiotoxic[183,220–222], potentially creating serious adverse effects. In another example, the structure of PK2 was changed by the addition of the targeting ligand[187], which might have been the reason for the increased adverse effects in humans[120]. This effect might have been avoided through the use of a separate nanocarrier, onto which the ligand is attached and into which the therapeutic is loaded, as opposed to polymer conjugates such as PK2; for example, the structure of a liposome is virtually independent from the ligand and payload.

While the actively targeted nanoparticles reported in this study have demonstrated that active targeting can yield benefits to nanocarrier-mediated delivery of therapeutic agents to solid cancer, there are still areas that should be explored further. Firstly, studies in animals and phase I studies were promising but there still has been no definite proof-of-principle of an increased antitumor effect in humans. Since active targeting is still a relatively young field-of-expertise and the clinical development process can be lengthy, it may simply be a matter of time. Nevertheless, this can discourage studies and investment in this field. Additionally, while the current proof-of-principle of active targeting relies on the use of animal xenograft models, it is still unclear to what extent these models are comparable to humans. The effect of factors such as the location of the xenograft implant, xenograft cell line and metabolism of the animal model should be investigated further to provide more insight into this matter. For example, MCC-465, an actively targeted nanocarrier for the treatment of colorectal cancer, was studied in mice by implanting colorectal cell lines in the kidneys[195]. Hypothetically, the difference in location could alter localization efficacy, local environmental conditions could alter cellular activity and receptor expression of the xenograft tumor and the use of animal models with a higher metabolism, such as rodents, could result in an exaggerated image of therapeutic efficacy. Similarly, there is still relatively little known about the full extent, efficacy and clinical relevance of the EPR effect in human therapy, partially due to the reliance upon animal models. Since the tissue localization of active targeting is largely dependent on the EPR effect, this is crucial information for the design of nanoparticles and choice of clinical target. For example, the *in vivo* effect of tumor location on the EPR effect could be studied through site-specific tumor induction by irradiating specific tissues in the body. By inducing 'natural' tumors in animal models, as opposed to xenograft implants, this would provide a more clear image of the EPR effect in humans. Lastly, it would be interesting to provide a large-scale overview of the extent of the EPR effect in different human cancers and other diseases. For example, this could be explored through a large-scale study in which human patients would be injected with nanocarriers loaded with an imaging agent, allowing measurement of the EPR effect across a wide variety of afflictions. Further development of passively and actively targeted nanocarriers should focus upon those diseases that showed the strongest EPR effect. Characterization of the

cellular activity and expression patterns of these diseases could also allow for more rational drug design by optimizing the ligand and therapeutic payload.

Due to the focus on solid cancer in research into active targeting, there are still several possible avenues of exploration that have as of yet received relatively little attention. Actively targeted drug delivery to the endothelial cells of tumor vasculature has been demonstrated[101] and might not be burdened by some of the disadvantages of active targeting, such as high fluid pressures[44,45]. Targeting of metastatic or non-solid cancers such as lymphomas, could also be interesting for similar reasons. Especially metastatic cancers could be an important target for active targeting, since solid tumors are often treated through surgery or irradiation and metastatic tumors are difficult to localize and treat. Inflammation of other tissues is rather similar to the EPR effect of solid tumors, causing increased extravasation of macromolecules[234] and might be interesting for actively targeted treatment of inflammatory disorders such as atherosclerosis. Clinical indications caused by other pathogens, such as bacteria, viruses and fungi, provide a greater specificity of target markers but also less need for active targeting, due to the biological differences between the pathogens and the host body. Active targeting could also be applied to gene therapy to allow *in vivo* transfection of certain cells. For example, transfection of stem cells with the CCR5Δ32 gene could be used in the treatment of HIV[235]. While Lipovaxin-MM is a vaccination agent for solid cancers, similar actively targeted platforms could be used to vaccinate against a wide variety of diseases. Drug delivery platforms could also be combined for increased specificity and efficacy, such as a combination of actively targeted nanocarriers and triggered drug release, which could greatly enhance tissue-specific drug release. Lipovaxin-MM is also exceptional in the fact that the nanocarrier features multiple payloads; antigens as well as an adjuvant, in this case IFN-γ[200]. Similar strategies could also be explored for other therapeutics, for example encapsulating multiple therapeutics for combination chemotherapy regimens or including agents that alter the tumor vasculature for optimal drug delivery[45]. All nanoparticles reported in this study focus on intravenous administration, neglecting other routes of administration. For example, treatment of respiratory diseases could benefit from administration through inhalation or treatment gastrointestinal diseases could benefit from oral or rectal administration. Lastly, since monoclonal antibody therapy has been shown to be effective in the treatment of certain diseases, it could hypothetically be possible to design actively targeted nanocarriers that feature a synergy between a therapeutically active targeting ligand and the therapeutic payload.

In conclusion, while the actively targeted nanoparticles that are currently in clinical development have reported promising findings in preclinical studies and, in some cases, early clinical trials, therapeutic efficacy in humans has not been convincingly demonstrated. Further studies are needed to fully assess the clinical relevance of actively targeted nanomedicine.

1. Kateb B, Chiu K, Black KL, Yamamoto V, Khalsa B, Ljubimova JY, et al. Nanoplatforms for constructing new approaches to cancer treatment, imaging, and drug delivery: what should be the policy? NeuroImage [Internet]. 2011 Jan [cited 2011 Jul 18];54 Suppl 1:S106–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20149882

2. Moghimi SM, Hunter a C, Murray JC. Nanomedicine: current status and future prospects. The FASEB journal [Internet]. 2005 Mar [cited 2011 Jul 7];19(3):311–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15746175

3. Emerich DF, Thanos CG. Targeted nanoparticle-based drug delivery and diagnosis. Journal of drug targeting [Internet]. 2007 Apr [cited 2011 Aug 3];15(3):163–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17454354

4. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer [Internet]. 2010 Dec 15 [cited 2011 Jul 15];127(12):2893–917. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21351269

5. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell [Internet]. 2000 [cited 2011 Nov 10];100(1):57–70. Available from: http://www.citeulike.org/group/1390/article/710800

6. Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. Journal of controlled release [Internet]. 2011 Sep [cited 2011 Sep 23];Available from: http://linkinghub.elsevier.com/retrieve/pii/S0168365911008480

7. Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, et al. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer research [Internet]. 1995 [cited 2012 Jan 2];55:3752–6. Available from: http://cancerres.aacrjournals.org/content/55/17/3752.short

8. Shaikh J, Ankola DD, Beniwal V, Singh D, Kumar MNVR. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. European journal of pharmaceutical sciences [Internet]. 2009 Jun 28 [cited 2011 Sep 15];37(3-4):223–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19491009

9. Fox ME, Szoka FC, Fréchet JMJ. Soluble polymer carriers for the treatment of cancer: the importance of molecular architecture. Accounts of chemical research [Internet]. 2009 Aug 18 [cited 2011

Dec 12];42(8):1141–51. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2759385&tool=pmcentrez&rendertype=abstract

10. Galindo-Rodriguez SA, Allemann E, Fessi H, Doelker E. Polymeric nanoparticles for oral delivery of drugs and vaccines: a critical evaluation of in vivo studies. Critical reviews in therapeutic drug carrier systems [Internet]. 2005 [cited 2011 Dec 31];22(5):419–63. Available from: http://cat.inist.fr/?aModele=afficheN&cpsidt=17358199

11. Scheinberg D, Villa CH, Escorcia FE, McDevitt MR. Conscripts of the infinite armada: systemic cancer therapy using nanomaterials. Nature reviews clinical oncology [Internet]. 2010 May [cited 2011 Jun 14];7(5):266–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20351700

12. Verdun C, Couvreur P, Vranckx H, Lenaerts V, Roland M. Development of a nanoparticle controlled-release formulation for human use. Journal of controlled release [Internet]. 1986 [cited 2012 Jan 3];3(1-4):206–10. Available from: http://www.sciencedirect.com/science/article/pii/0168365986900817

13. Ulrich AS. Biophysical aspects of using liposomes as delivery vehicles. Bioscience reports [Internet]. 2002 Apr [cited 2012 Mar 1];22(2):129–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12428898

14. Herman E, Rahman A, Ferrans V, Vick J. Prevention of chronic doxorubicin cardiotoxicity in beagles by liposomal encapsulation. Cancer research [Internet]. 1983 [cited 2012 Jan 3];43:5427–32. Available from: http://cancerres.aacrjournals.org/content/43/11/5427.short

15. Baran ET, Ozer N, Hasirci V. In vivo half life of nanoencapsulated L-asparaginase. Journal of materials science materials in medicine [Internet]. 2002 Dec [cited 2011 Oct 11];13(12):1113–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15348652

16. Cosco D, Paolino D, Maiuolo J, Russo D, Fresta M. Liposomes as multicompartmental carriers for multidrug delivery in anticancer chemotherapy. Drug delivery and translational research [Internet]. 2010 Dec 29 [cited 2011 Nov 1];1(1):66–75. Available from: http://www.springerlink.com/index/10.1007/s13346-010-0007-x

17. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer research [Internet]. 1986 [cited 2011 Nov 11];46:6387–92. Available from: http://cancerres.aacrjournals.org/content/46/12_Part_1/6387.short

18. Sapra P, Allen TM. Ligand-targeted liposomal anticancer drugs. Progress in lipid research [Internet]. 2003 Sep [cited 2011 Aug 27];42(5):439–62. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0163782703000328

19. Meng F, Hennink WE, Zhong Z. Reduction-sensitive polymers and bioconjugates for biomedical applications. Biomaterials [Internet]. 2009 Apr [cited 2011 Jul 12];30(12):2180–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19200596

20. Engin K, Leeper D, Cater J, Thistlethwaite A, Tupchong L, McFarlane J. Extracellular pH distribution in human tumours. International journal of hyperthermia [Internet]. 1995 [cited 2011 Nov 11];11(2):211–6. Available from: http://informahealthcare.com/doi/abs/10.3109/02656739509022457

21. Klibanov AL, Shevchenko TI, Raju BI, Seip R, Chin CT. Ultrasound-triggered release of materials entrapped in microbubble-liposome constructs: a tool for targeted drug delivery. Journal of controlled release [Internet]. 2010 Nov 20 [cited 2011 Jul 6];148(1):13–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3085724&tool=pmcentrez&rendertype=abstract

22. Landon CD, Park J-Y, Needham D, Dewhirst MW. Nanoscale drug delivery and hyperthermia: the materials design and preclinical and clinical testing of low temperature-sensitive liposomes used in combination with mild hyperthermia in the treatment of local cancer. The open nanomedicine journal [Internet]. 2011 [cited 2011 Nov 10];3:38–64. Available from: http://www.celsioninvestor.com/Nano Drug Delivery 2011.pdf

23. Daniels TR, Delgado T, Rodriguez J, Helguera G, Penichet ML. The transferrin receptor part I: biology and targeting with cytotoxic antibodies for the treatment of cancer. Clinical immunology [Internet]. 2006 Nov [cited 2011 Jun 13];121(2):144–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16904380

24. Daniels TR, Delgado T, Helguera G, Penichet ML. The transferrin receptor part II: targeted delivery of therapeutic agents into cancer cells. Clinical immunology [Internet]. 2006 Nov [cited 2011 Jul 21];121(2):159–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16920030

25. Wang X, Li J, Wang Y, Cho KJ, Kim G, Gjyrezi A, et al. HFT-T, a targeting nanoparticle, enhances specific delivery of paclitaxel to folate receptor-positive tumors. ACS nano [Internet]. 2009 Oct 27 [cited 2011];3(10):3165–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19761191

26. Cirstoiu-Hapca A, Bossy-Nobs L, Buchegger F, Gurny R, Delie F. Differential tumor cell targeting of anti-HER2 (Herceptin) and anti-CD20 (Mabthera) coupled nanoparticles. International journal of pharmaceutics [Internet]. 2007 Mar 1 [cited 2011 Oct 3];331(2):190–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17196347

27. Owens D, Peppas N. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. International journal of pharmaceutics [Internet]. 2006 [cited 2012 Jan 15];307(1):93–102. Available from: http://www.sciencedirect.com.proxy.library.uu.nl/science/article/pii/S037851730500668X

28. Mitchell R. Innate and adaptive immunity: the immune response to foreign materials. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, editors. Biomaterials science: an introduction to materials in medicine. Amsterdam, NL: Elsevier Academic Press; 2004. p. 304–18.

29. Gref R, Minamitake Y, Peracchia M, Trubetskoy V, Torchilin V, Langer R. Biodegradable long-circulating polymeric nanospheres. Science [Internet]. 1994 [cited 2012 Mar 1];263(5153):1600–3. Available from: http://www.sciencemag.org.proxy.library.uu.nl/content/263/5153/1600.short

30. Moghimi SM, Hedeman H, Muir IS, Illum L, Davis SS. An investigation of the filtration capacity and the fate of large filtered sterically-stabilized microspheres in rat spleen. Biochimica et biophysica acta [Internet]. 1993 Jul 11 [cited 2012 May 1];1157(2):233–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8323953

31. Hans M, Lowman A. Biodegradable nanoparticles for drug delivery and targeting. Current opinion in solid state and materials science [Internet]. 2002 [cited 2012 Jan 15];6(4):319–27. Available from: http://www.sciencedirect.com.proxy.library.uu.nl/science/article/pii/S1359028602001171

32. Kaul G, Amiji M. Long-circulating poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. Pharmaceutical research [Internet]. 2002 Jul [cited 2011];19(7):1061–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12180540

33. Song LY, Ahkong QF, Rong Q, Wang Z, Ansell S, Hope MJ, et al. Characterization of the inhibitory effect of PEG-lipid conjugates on the intracellular delivery of plasmid and antisense DNA mediated by cationic lipid liposomes. Biochimica et biophysica acta [Internet]. 2002 Jan 2 [cited 2012 Jul 1];1558(1):1–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11750259

34. Anthea M, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, et al. Human biology and health. 1st ed. Englewood Cliffs, NJ: Pearson Prentice Hall; 1993.

35. Jain RK. Transport of molecules in the tumor interstitium: a review. Cancer research [Internet]. 1987 [cited 2012 Jan 1];47:3039–51. Available from: http://cancerres.aacrjournals.org/content/47/12/3039.short

36. Jain RK. Determinants of tumor blood flow: a review. Cancer research [Internet]. 1988 [cited 2012 Jan 2];48(10):2641–58. Available from: http://cancerres.aacrjournals.org/content/48/10/2641.short

37. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacological reviews [Internet]. 1999 Dec [cited 2012 Jan 10];51(4):691–743. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10581328

38. Yuan F. Transvascular drug delivery in solid tumors. Seminars in radiation oncology [Internet]. 1998 Jul [cited 2012 May 1];8(3):164–75. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1053429698800428

39. Yuan F, Leunig M, Huang SK, Berk DA, Papahadjopoulos D, Jain RK. Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. Cancer research [Internet]. 1994 [cited 2012 Jan 2];54(13):3352–6. Available from: http://cancerres.aacrjournals.org/content/54/13/3352.short

40. Jain RK. Delivery of molecular medicine to solid tumors: lessons from in vivo imaging of gene expression and function. Journal of controlled release [Internet]. 2001 Jul 6 [cited 2011];74(1-3):7–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11489479

41. Nathanson SD, Nelson L. Interstitial fluid pressure in breast cancer, benign breast conditions, and breast parenchyma. Annals of surgical oncology [Internet]. 1994 Jul [cited 2012];1(4):333–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7850532

42. Boucher Y, Kirkwood JM, Opacic D, Desantis M, Jain RK. Interstitial hypertension in superficial metastatic melanomas in humans. Cancer research [Internet]. 1991 [cited 2012 Jan 1];51(24):6691–4. Available from: http://cancerres.aacrjournals.org/content/51/24/6691.short

43. Gutmann R, Leunig M, Feyh J, Goetz AE, Messmer K, Kastenbauer E, et al. Interstitial hypertension in head and neck tumors in patients: correlation with tumor size. Cancer research [Internet]. 1992 [cited 2012 Jan 1];52(7):1993–5. Available from: http://cancerres.aacrjournals.org/content/52/7/1993.short

44. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nature reviews clinical oncology [Internet]. 2010 Nov [cited 2011 Jul 19];7(11):653–64. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3065247&tool=pmcentrez&rendertype=abstract

45. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science [Internet]. 2005 Jan 7 [cited 2012 Jan 1];307(5706):58–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15637262

46. Sivridis E, Giatromanolaki A, Koukourakis MI. "Stromatogenesis" and tumor progression. International Journal of Surgical Pathology [Internet]. 2004 Jan 1 [cited 2012 Mar 27];12(1):1–9. Available from: http://ijs.sagepub.com/cgi/doi/10.1177/106689690401200101

47. Duncan R. Polymer conjugates as anticancer nanomedicines. Nature reviews cancer [Internet]. 2006 Sep [cited 2011 Jul 18];6(9):688–701. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16900224

48. Mikhail AS, Allen C. Block copolymer micelles for delivery of cancer therapy: transport at the whole body, tissue and cellular levels. Journal of controlled release [Internet]. 2009 Sep 15 [cited 2011 Aug 30];138(3):214–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19376167

49. Sutton D, Nasongkla N, Blanco E, Gao J. Functionalized micellar systems for cancer targeted drug delivery. Pharmaceutical research [Internet]. 2007 Jun [cited 2011 Sep 2];24(6):1029–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17385025

50. Discher BM, Won Y-Y, Ege DS, Lee JC-M, Bates FS, Discher DE, et al. Polymersomes: tough vesicles made from diblock copolymers. Science [Internet]. 1999 May 14 [cited 2011 Jun 13];284(5417):1143–6. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.284.5417.1143

51. Baker Jr JR. Dendrimer-based nanoparticles for cancer therapy. Nanotechnology for hematology [Internet]. 2009 [cited 2011 Nov 8];2009(1):708–19. Available from: http://171.66.125.162/cgi/content/abstract/2009/1/708

52. Chacko A-M, Hood ED, Zern BJ, Muzykantov VR. Targeted nanocarriers for imaging and therapy of vascular inflammation. Current opinion in colloid & interface science [Internet]. 2011 Jun [cited 2011 Aug 19];16(3):215–27. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3121175&tool=pmcentrez&rendertype=abstract

53. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nature

nanotechnology [Internet]. 2007 Dec [cited 2011 Nov 12];2(12):751–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18654426

54. Cho K, Wang X, Nie S, Chen ZG, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clinical cancer research [Internet]. 2008 Mar 1 [cited 2011 Jul 5];14(5):1310–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18316549

55. Maeda H. Tumor-selective delivery of macromolecular drugs via the EPR effect: background and future prospects. Bioconjugate chemistry [Internet]. 2010 [cited 2012 Feb 6];21(47):797–802. Available from: http://pubs.acs.org/doi/abs/10.1021/bc100070g

56. Maeda H, Greish K, Fang J. The EPR effect and polymeric drugs: a paradigm shift for cancer chemotherapy in the 21st century. Advances in polymer science [Internet]. 2006 [cited 2012 Feb 6];193:103–21. Available from: http://www.springerlink.com/index/F3876600J4541856.pdf

57. Kerbel RS. What is the optimal rodent model for anti-tumor drug testing? Cancer metastasis reviews [Internet]. 1999 [cited 2012 Feb 10];17(3):301–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10352884

58. Jain RK, Safabakhsh N, Sckell A, Chen Y, Jiang P, Benjamin L, et al. Endothelial cell death, angiogenesis, and microvascular function after castration in an androgen-dependent tumor: role of vascular endothelial growth factor. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 1998 Sep 1;95(18):10820–5. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=27979&tool=pmcentrez&rendertype=abstract

59. Yuan F, Salehi HA, Boucher Y, Vasthare US, Tuma RF, Jain RK. Vascular permeability and microcirculation of gliomas and mammary carcinomas transplanted in rat and mouse cranial windows. Cancer research [Internet]. 1994 [cited 2012 Feb 4];54(17):4564–8. Available from: http://cancerres.aacrjournals.org/content/54/17/4564.short

60. Harrington KJ, Mohammadtaghi S, Uster PS, Glass D, Peters AM, Vile RG, et al. Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled pegylated liposomes. Clinical cancer research [Internet]. 2001 [cited 2012 Feb 4];7(2):243–54. Available from: http://clincancerres.aacrjournals.org/content/7/2/243.short

61. Jain RK. Transport of molecules across tumor vasculature. Cancer metastasis reviews [Internet]. 1987 Jan [cited 2012 Apr 2];6(4):559–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3327633

62. Stohrer M, Boucher Y, Stangassinger M, Jain RK. Oncotic pressure in solid tumors is elevated. Cancer research [Internet]. 2000 [cited 2012 Jan 1];60:4251–5. Available from: http://cancerres.aacrjournals.org/content/60/15/4251.short

63. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature [Internet]. 1975 [cited 2012 Feb 1];256:495–7. Available from: http://www.nature.com.proxy.library.uu.nl/nature/journal/v256/n5517/abs/256495a0.html

64. Zhao X, Li H, Lee RJ. Targeted drug delivery via folate receptors. Expert opinion on drug delivery [Internet]. 2008 Mar [cited 2012 Mar 1];5(3):309–19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18318652

65. Zaki NMNM, Tirelli N. Gateways for the intracellular access of nanocarriers: a review of receptor-mediated endocytosis mechanisms and of strategies in receptor targeting. Expert opinion on drug delivery [Internet]. 2010 Aug [cited 2011 Nov 21];7(8):895–913. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20629604

66. Sapra P, Allen TM. Internalizing antibodies are necessary for improved therapeutic efficacy of antibody-targeted liposomal drugs. Cancer research [Internet]. 2002 [cited 2011];62:7190–4. Available from: http://cancerres.aacrjournals.org.proxy.library.uu.nl/content/62/24/7190.short

67. Ghaghada KB, Saul J, Natarajan JV, Bellamkonda RV, Annapragada AV. Folate targeting of drug carriers: a mathematical model. Journal of controlled release [Internet]. 2005 May 5 [cited 2012 Jan 6];104(1):113–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15866339

68. Saul JM, Annapragada A, Natarajan JV, Bellamkonda RV. Controlled targeting of liposomal doxorubicin via the folate receptor in vitro. Journal of controlled release [Internet]. 2003 Sep 19 [cited 2012 May 1];92(1-2):49–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14499185

69. Kibria G, Hatakeyama H, Ohga N, Hida K, Harashima H. Dual-ligand modification of PEGylated liposomes shows better cell selectivity and efficient gene delivery. Journal of controlled release [Internet]. 2011 Jul 30 [cited 2011 Oct 25];153(2):141–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21447361

70. Saul JM, Annapragada AV, Bellamkonda RV. A dual-ligand approach for enhancing targeting selectivity of therapeutic nanocarriers. Journal of controlled release [Internet]. 2006 Sep 12 [cited 2011 Sep 14];114(3):277–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16904220

71. Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. Nature nanotechnology [Internet]. 2008 Mar [cited 2011 Jul 4];3(3):145–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18654486

72. Gupta AK, Berry C, Gupta M, Curtis A. Receptor-mediated targeting of magnetic nanoparticles using insulin as a surface ligand to prevent endocytosis. IEEE transactions on nanobioscience [Internet]. 2003 Dec [cited 2012 Feb 5];2(4):255–61. Available from: http://ieeexplore.ieee.org/xpl/freeabs_all.jsp?arnumber=1254529&abstractAccess=no&userType=inst

73. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, et al. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer research [Internet]. 2006 Jul 1 [cited 2011 Jul 9];66(13):6732–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16818648

74. Bartlett D, Su H, Hildebrandt I, Weber W, Davis M. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2007 Sep 25 [cited 2011 Dec

1];104(39):15549–54. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1978218&tool=pmcentrez&rendertype=abstract

75. Leamon CP, Cooper SR, Hardee GE. Folate-liposome-mediated antisense oligodeoxynucleotide targeting to cancer cells: evaluation in vitro and in vivo. Bioconjugate chemistry [Internet]. 2003 [cited 2012];14(4):738–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12862426

76. Choi CHJ, Alabi C, Webster P, Davis ME. Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2010 Jan 19 [cited 2011 Aug 8];107(3):1235–40. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2824286&tool=pmcentrez&rendertype=abstract

77. Mamot C, Drummond DC, Noble CO, Kallab V, Guo Z, Hong K, et al. Epidermal growth factor receptor-targeted immunoliposomes significantly enhance the efficacy of multiple anticancer drugs in vivo. Cancer research [Internet]. 2005 Dec 15 [cited 2011 Aug 20];65(24):11631–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16357174

78. Bae YH, Park K. Targeted drug delivery to tumors: myths, reality and possibility. Journal of controlled release [Internet]. 2011 Jun 6 [cited 2011 Jul 29];153(3):198–205. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21663778

79. Miller DW, Graulich W, Karges B, Stahl S, Ernst M, Ramaswamy A, et al. Elevated expression of endoglin, a component of the TGF-beta-receptor complex, correlates with proliferation of tumor endothelial cells. International journal of cancer. [Internet]. 1999 May 17 [cited 2012 Apr 2];81(4):568–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10225446

80. Yasui W, Sumiyoshi H, Hata J, Kameda T, Ochiai A, Ito H, et al. Expression of epidermal growth factor receptor in human gastric and colonic carcinomas. Cancer research [Internet]. 1988 [cited 2012 Feb 4];48:137–41. Available from: http://cancerres.aacrjournals.org/content/48/1/137.short

81. Klotz IM. Ligand-receptor energetics: a guide for the perplexed. [Internet]. 1st ed. New York, NY: John Wiley & Sons; 1997 [cited 2012 Mar 1]. Available from: http://books.google.nl/books?hl=en&lr=&id=yM8RLqr7h-kC&oi=fnd&pg=PR11&ots=nQudsZkOxx&sig=JwOpB8QaBtdmQCPll4WgROZKoDM&redir_esc=y#v=onepage&q&f=false

82. Juweid M, Neumann R, Paik C, Perez-Bacete MJ, Sato J, van Osdol W, et al. Micropharmacology of monoclonal antibodies in solid tumors: direct experimental evidence for a binding site barrier. Cancer research [Internet]. 1992 [cited 2012 Jan 3];52(19):5144–53. Available from: http://cancerres.aacrjournals.org/content/52/19/5144.short

83. Aragnol D, Leserman LD. Immune clearance of liposomes inhibited by an anti-Fc receptor antibody in vivo. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 1986 Apr [cited 2012 Mar 1];83(8):2699–703. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=323367&tool=pmcentrez&rendertype=abstract

84. Derksen J, Morselt H, Scherphof G. Uptake and processing of immunoglobulin-coated liposomes by subpopulations of rat liver macrophages. Biochimica et biophysica acta. 1988;971(2):127–36.

85. Pastan I, Hassan R, FitzGerald DJ, Kreitman RJ. Immunotoxin treatment of cancer. Annual review of medicine [Internet]. 2007 Jan [cited 2012 Oct 1];58:221–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17059365

86. Park J, Hong K, Carter P, Asgari H, Guo L, Keller G, et al. Development of anti-p185HER2 immunoliposomes for cancer therapy. Proceedings of the National Academy of Sciences [Internet]. 1995 [cited 2012 Jan 10];92(5):1327–31. Available from: http://www.pnas.org/content/92/5/1327.short

87. Heath TD, Fraley RT, Papahdjopoulos D. Antibody targeting of liposomes: cell specificity obtained by conjugation of F(ab')2 to vesicle surface. Science [Internet]. 1980 Oct 31 [cited 2012 Apr 1];210(4469):539–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7423203

88. Chen Y, Zhu X, Zhang X, Liu B, Huang L. Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. Molecular therapy [Internet]. 2010 Sep [cited 2011 Jul 19];18(9):1650–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2956922&tool=pmcentrez&rendertype=abstract

89. Oliveira S, Schiffelers RM, van der Veeken J, van der Meel R, Vongpromek R, van Bergen en Henegouwen PMP, et al. Downregulation of EGFR by a novel multivalent nanobody-liposome platform. Journal of controlled release [Internet]. 2010 Jul 14 [cited 2011 Aug 30];145(2):165–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20362020

90. Qian ZM, Li H, Sun H, Ho K. Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway. Pharmacological reviews [Internet]. 2002 Dec [cited 2011];54(4):561–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21862329

91. Daniels TR, Bernabeu E, Rodríguez J, Patel S, Kozman M, Chiappetta D, et al. The transferrin receptor and the targeted delivery of therapeutic agents against cancer. Biochimica et biophysica acta [Internet]. 2011 Aug [cited 2011 Aug 10];Available from: http://linkinghub.elsevier.com/retrieve/pii/S0304416511001826

92. Gehlsen KR, Argraves WS, Pierschbacher MD, Ruoslahti E. Inhibition of in vitro tumor cell invasion by Arg-Gly-Asp-containing synthetic peptides. The journal of cell biology [Internet]. 1988 May [cited 2012 Sep 1];106:925–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21769437

93. Wilson DS, Szostak JW. In vitro selection of functional nucleic acids. Annual review of biochemistry [Internet]. 1999 Jan [cited 2011];68:611–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10872462

94. Farokhzad OC, Karp JM, Langer R. Nanoparticle-aptamer bioconjugates for cancer targeting. Expert opinion on drug delivery [Internet]. 2006 May [cited 2011 Dec 30];3(3):311–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16640493

95. Zhou J, Rossi JJ. Cell-specific aptamer-mediated targeted drug delivery. Oligonucleotides [Internet]. 2011 Feb [cited 2011 Aug 3];21(1):1–10. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3043981&tool=pmcentrez&rendertype=abstract

96. Yu MK, Park J, Jon S. Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. Theranostics [Internet]. 2011 [cited 2011 Nov 10];1:371–412. Available from: http://thno.org/v01p0371.pdf

97. Baines IC, Colas P. Peptide aptamers as guides for small-molecule drug discovery. Drug discovery today [Internet]. 2006 Apr [cited 2011 Nov 24];11(7-8):334–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16580975

98. Johnson S, Evans D, Laurenson S, Paul D, Davies AG, Ko Ferrigno P, et al. Surface-immobilized peptide aptamers as probe molecules for protein detection. Analytical chemistry [Internet]. 2008 Feb 15 [cited 2011];80(4):978–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18186614

99. Borghouts C, Kunz C, Groner B. Peptide aptamers: recent developments for cancer therapy. Expert opinion on biological therapy [Internet]. 2005 Jun [cited 2011 Jan 12];5(6):783–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15952909

100. Hood JD, Bednarski M, Frausto R, Guccione S, Reisfeld R, Xiang R, et al. Tumor regression by targeted gene delivery to the neovasculature. Science [Internet]. 2002 Jun 28 [cited 2011 Aug 8];296(5577):2404–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12089446

101. Murphy E a, Majeti BK, Barnes L a, Makale M, Weis SM, Lutu-Fuga K, et al. Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2008 Jul 8 [cited 2012 Apr 1];105(27):9343–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2453735&tool=pmcentrez&rendertype=abstract

102. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, et al. Tissue-penetrating delivery of compounds and nanoparticles into tumors. Cancer cell [Internet]. 2009 Dec 8 [cited 2011 Jul 6];16(6):510–20. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2791543&tool=pmcentrez&rendertype=abstract

103. Meade BR, Dowdy SF. Enhancing the cellular uptake of siRNA duplexes following noncovalent packaging with protein transduction domain peptides. Advanced drug delivery reviews [Internet]. 2008 Mar 1 [cited 2011 Oct 26];60:530–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2293332&tool=pmcentrez&rendertype=abstract

104. Faraasen S, Vörös J, Csúcs G, Textor M, Merkle HP, Walter E. Ligand-specific targeting of microspheres to phagocytes by surface modification with poly(L-lysine)-grafted poly(ethylene glycol) conjugate. Pharmaceutical research [Internet]. 2003 Feb [cited 2012 Apr 1];20(2):237–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12636162

105. Brandhonneur N, Chevanne F, Vié V, Frisch B, Primault R, Le Potier M-F, et al. Specific and non-specific phagocytosis of ligand-grafted PLGA microspheres by macrophages. European journal of pharmaceutical sciences [Internet]. 2009 Mar 2 [cited 2011 Sep 23];36(4-5):474–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19110055

106. Schäfer V, von Briesen H, Andreesen R, Steffan AM, Royer C, Tröster S, et al. Phagocytosis of nanoparticles by Human Immunodeficiency Virus (HIV)-infected macrophages: a possibility for antiviral drug targeting. Pharmaceutical research [Internet]. 1992 [cited 2012 Feb 5];9(4):541–6. Available from: http://www.springerlink.com/index/X131944320544137.pdf

107. Swanson J, Watts C. Macropinocytosis. Trends in cell biology [Internet]. 1995 [cited 2012 Mar 9];5(11):424–8. Available from: http://www.sciencedirect.com/science/article/pii/S0962892400891011

108. Falcone S, Cocucci E, Podini P, Kirchhausen T, Clementi E, Meldolesi J. Macropinocytosis: regulated coordination of endocytic and exocytic membrane traffic events. Journal of cell science [Internet]. 2006 Nov 15 [cited 2011 Jul 30];119(Pt 22):4758–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17077125

109. Ogden CA, DeCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok V a, et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. The journal of experimental medicine [Internet]. 2001 Sep 17 [cited 2012 Apr 1];194(6):781–95. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2195958&tool=pmcentrez&rendertype=abstract

110. Hoffmann PR, DeCathelineau a M, Ogden CA, Leverrier Y, Bratton DL, Daleke DL, et al. Phosphatidylserine (PS) induces PS receptor-mediated macropinocytosis and promotes clearance of apoptotic cells. The journal of cell biology [Internet]. 2001 Nov 12 [cited 2012 Apr 1];155(4):649–59. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2198875&tool=pmcentrez&rendertype=abstract

111. Dausend J, Musyanovych A, Dass M, Walther P, Schrezenmeier H, Landfester K, et al. Uptake mechanism of oppositely charged fluorescent nanoparticles in HeLa cells. Macromolecular bioscience [Internet]. 2008 Dec 8 [cited 2011 Aug 1];8(12):1135–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18698581

112. Harush-Frenkel O, Debotton N, Benita S, Altschuler Y. Targeting of nanoparticles to the clathrin-mediated endocytic pathway. Biochemical and biophysical research communications [Internet]. 2007 Feb 2 [cited 2011 Aug 16];353(1):26–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17184736

113. Nabi IR, Le PU. Caveolae/raft-dependent endocytosis. The journal of cell biology [Internet]. 2003 May 26 [cited 2012 Mar 6];161(4):673–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2199359&tool=pmcentrez&rendertype=abstract

114. Mo Y, Lim L-Y. Mechanistic study of the uptake of wheat germ agglutinin-conjugated PLGA nanoparticles by A549 cells. The journal of pharmaceutical sciences [Internet]. 2004 Jan [cited 2012 Aug 1];93(1):20–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14648632

115. Lackey CA, Press OW, Hoffman AS, Stayton PS. A biomimetic pH-responsive polymer directs endosomal release and intracellular delivery of an endocytosed antibody complex. Bioconjugate chemistry [Internet]. 2002 [cited 2011];13(5):996–1001. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12236781

116. Padilla De Jesús OL, Ihre HR, Gagne L, Fréchet JMJ, Szoka FC. Polyester dendritic systems for drug delivery applications: in vitro and in vivo evaluation. Bioconjugate chemistry [Internet]. 2002 [cited 2011 May 12];13(3):453–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12009933

117. Shi J, Xiao Z, Kamaly N, Farokhzad OC. Self-assembled targeted nanoparticles: evolution of technologies and bench to bedside translation. Accounts of chemical research [Internet]. 2011 Oct 18 [cited 2011];44(10):1123–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21692448

118. Davis ME. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. Molecular pharmaceutics [Internet]. 2009 [cited 2011 Nov 29];6(3):659–68. Available from: http://pubs.acs.org/doi/abs/10.1021/mp900015y

119. Sankhala K, Mita A, Adinin R, Wood L, Beeram M, Bullock S, et al. A phase I pharmacokinetic (PK) study of MBP-426, a novel liposome encapsulated oxaliplatin. Journal of clinical oncology [Internet]. 2009 [cited 2011 Dec 11];27(15):2535. Available from: http://www.mebiopharm.com/topics/MBP-426_ASC2009.PDF

120. Seymour LW, Ferry DR, Anderson D, Hesslewood S, Julyan PJ, Poyner R, et al. Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin. Journal of clinical oncology [Internet]. 2002 Mar 15;20(6):1668–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11896118

121. Hopewell JW, Duncan R, Wilding D, Chakrabarti K. Preclinical evaluation of the cardiotoxicity of PK2: a novel HPMA copolymer–doxorubicin–galactosamine conjugate antitumour agent. Human & experimental toxicology [Internet]. 2001 Sep 1 [cited 2011 Dec 13];20(9):461–70. Available from: http://het.sagepub.com/cgi/doi/10.1191/096032701682693017

122. Matsumura Y, Gotoh M, Muro K, Yamada Y, Shirao K, Shimada Y, et al. Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer. Annals of oncology [Internet]. 2004 Mar 1 [cited 2011 Sep 7];15(3):517–25. Available from: http://annonc.oupjournals.org/cgi/doi/10.1093/annonc/mdh092

123. Lipotek Pty Ltd. Lipovaxin - our technology [Internet]. Lipotek.com.au. 2011 [cited 2011 Dec 12];Available from: http://www.lipotek.com.au/technology/lipovaxin.htm

124. U.S. National Institutes of Health. Safety study of infusion of SGT-53 to treat solid tumors [Internet]. clinicaltrials.gov. 2011 [cited 2011 Dec 18];Available from: http://clinicaltrials.gov/show/NCT00470613

125. U.S. National Institutes of Health. Safety and pharmacokinetic study of MM-302 in patients with advanced breast cancer [Internet]. Clinicaltrials.gov. 2011 [cited 2011 Dec 27];Available from: http://clinicaltrials.gov/ct2/show/NCT01304797?term=mm-302&rank=1

126. U.S. National Institutes of Health. A study of BIND-014 given to patients with advanced or metastatic cancer [Internet]. Clinicaltrials.gov. 2011 [cited 2011 Sep 11];Available from: http://clinicaltrials.gov/ct2/show/NCT01300533

127. BIND Biosciences. Accurins^{TM} [Internet]. Bindbio.com. 2011 [cited 2012 Jan 27];:Technology. Available from: http://www.bindbio.com/content/pages/technology/index.jsp

128. Lyseng-Williamson K. Docetaxel: a review of its use in metastatic breast cancer. Drugs [Internet]. 2005 [cited 2011 Nov 8];65(17):2513–31. Available from: http://www.ingentaconnect.com/content/adis/dgs/2005/00000065/00000017/art00007

129. Zale SE, Troiano G, Ali MM, Hrkach J, Wright J. Drug loaded polymeric nanoparticles and methods of making and using same [Internet]. 2009 Jun 16 [cited 2012 Feb 13];Available from: http://www.google.com/patents?hl=en&lr=&vid=USPATAPP12485399&id=QM_NAAAAEBAJ&oi=fnd&dq=Drug+loaded+polymeric+nanoparticles+and+methods+of+making+and+using+same&printsec=abstract

130. Liu H, Rajasekaran AK, Moy P, Xia Y, Kim S, Navarro V, et al. Constitutive and antibody-induced internalization of prostate-specific membrane antigen. Cancer research [Internet]. 1998 Sep 15 [cited 2011];58(18):4055–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9751609

131. Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic-Moreno AF, et al. Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2008 Feb 19 [cited 2011];105(7):2586–91. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2268180&tool=pmcentrez&rendertype=abstract

132. Cheng J, Teply B a, Sherifi I, Sung J, Luther G, Gu FX, et al. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. Biomaterials [Internet]. 2007 Feb [cited 2011 Jun 13];28(5):869–76. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2925222&tool=pmcentrez&rendertype=abstract

133. BIND Biosciences. Pipeline [Internet]. Bindbio.com. 2011 [cited 2012 Feb 6];Available from: http://www.bindbio.com/content/pages/rd/index.jsp

134. Lupold SE, Hicke BJ, Lin Y, Coffey DS. Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. Cancer research [Internet]. 2002 [cited 2012 Feb 6];62(14):4029–33. Available from: http://cancerres.aacrjournals.org/content/62/14/4029.short

135. Farokhzad OC, Jon S, Khademhosseini A, Tran T-NT, Lavan DA, Langer R. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer research [Internet]. 2004 Nov 1 [cited 2011 Sep 16];64(21):7668–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15520166

136. Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, et al. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2006 Apr 18 [cited 2011 Feb 12];103(16):6315–20. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1458875&tool=pmcentrez&rendertype=abstract

137. Aggarwal S, Singh P, Topaloglu O, Isaacs JT, Denmeade SR. A dimeric peptide that binds selectively to prostate-specific membrane antigen and inhibits its enzymatic activity. Cancer research [Internet]. 2006 Sep 15 [cited 2012 Feb 6];66(18):9171–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16982760

138. Lupold SE, Rodriguez R. Disulfide-constrained peptides that bind to the extracellular portion of the prostate-specific membrane antigen. Cancer research [Internet]. 2004 [cited 2011 Dec 12];3:597–603. Available from: http://mct.aacrjournals.org.proxy.library.uu.nl/content/3/5/597.short

139. U.S. National Institutes of Health. Safety study of CALAA-01 to treat solid tumor cancers [Internet]. Clinicaltrials.gov. 2011 [cited 2011];Available from: http://clinicaltrials.gov/ct2/show/study/NCT00689065?term=calaa-01&rank=1

140. Hamilton AJ, Baulcombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science. 1999;286(5441):950–2.

141. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature [Internet]. 2001 May 24 [cited 2011];411(6836):494–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11373684

142. de Fougerolles A, Vornlocher H-P, Maraganore J, Lieberman J. Interfering with disease: a progress report on siRNA-based therapeutics. Nature reviews drug discovery [Internet]. 2007 Jun [cited 2011];6(6):443–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17541417

143. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, et al. Expression profiling reveals off-target gene regulation by RNAi. Nature biotechnology [Internet]. 2003 [cited 2011 Nov 29];21(6):635–7. Available from: http://www.blatny.com/Epigenetika2007/2007-12-04/papers/Jackson 2003.pdf

144. Lin X, Ruan X, Anderson MG, McDowell JA, Kroeger PE, Fesik SW, et al. siRNA-mediated off-target gene silencing triggered by a 7 nt complementation. Nucleic acids research [Internet]. 2005 Jan [cited 2011 Jul 29];33(14):4527–35. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1184219&tool=pmcentrez&rendertype=abstract

145. Qiu S, Adema CM, Lane T. A computational study of off-target effects of RNA interference. Nucleic acids research [Internet]. 2005 Jan [cited 2011 Jul 27];33(6):1834–47. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1072799&tool=pmcentrez&rendertype=abstract

146. Reynolds A, Anderson EM, Vermeulen A, Fedorov Y, Robinson K, Leake D, et al. Induction of the interferon response by siRNA is cell type- and duplex length-dependent. RNA [Internet]. 2006 Apr 17 [cited 2011 Sep 25];12:988–93. Available from: http://www.rnajournal.org/cgi/doi/10.1261/rna.2340906

147. Hornung V, Guenthner-Biller M, Bourquin C, Ablasser A, Schlee M, Uematsu S, et al. Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nature medicine [Internet]. 2005 Mar [cited 2011 Jul 21];11(3):263–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15723075

148. Manche L, Green SR, Schmedt C, Mathews MB. Interactions between double-stranded RNA regulators and the protein kinase DAI. Molecular and cellular biology [Internet]. 1992 [cited 2011 Nov 29];12(11):5238–48. Available from: http://mcb.asm.org/cgi/content/abstract/12/11/5238

149. Layzer JM, McCaffrey AP, Tanner AK, Huang Z, Kay MA, Sullenger BA. In vivo activity of nuclease-resistant siRNAs. RNA [Internet]. 2004 May 1 [cited 2011 Aug 29];10:766–71. Available from: http://www.rnajournal.org/cgi/doi/10.1261/rna.5239604

150. Choung S, Kim YJ, Kim S, Park H-O, Choi Y-C. Chemical modification of siRNAs to improve serum stability without loss of efficacy. Biochemical and biophysical research communications [Internet]. 2006 Apr [cited 2011 Nov 8];342:919–27. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0006291X06003366

151. Cerqueira NMFSA, Pereira S, Fernandes PA, Ramos MJ. Overview of ribonucleotide reductase inhibitors: an appealing target in anti-tumour therapy. Current medicinal chemistry [Internet]. 2005 [cited 2011];12(11):1283–94. Available from: http://www.ingentaconnect.com.proxy.library.uu.nl/content/ben/cmc/2005/00000012/00000011/art00002

152. Heidel JD, Liu JY-C, Yen Y, Zhou B, Heale BSE, Rossi JJ, et al. Potent siRNA inhibitors of ribonucleotide reductase subunit RRM2 reduce cell proliferation in vitro and in vivo. Clinical cancer research [Internet]. 2007 Apr 1 [cited 2011 Sep 14];13:2207–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17404105

153. Davis ME, Brewster ME. Cyclodextrin-based pharmaceutics: past, present and future. Nature reviews drug discovery [Internet]. 2004 Dec [cited 2011 Aug 14];3(12):1023–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15573101

154. Szejtli J. Introduction and general overview of cyclodextrin chemistry. Chemical reviews [Internet]. 1998 Jul 30 [cited 2011];98(5):1743–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11848947

155. Davis ME, Bellocq NC. Cyclodextrin-containing polymers for gene delivery. Journal of inclusion phenomena and macrocyclic chemistry [Internet]. 2002 [cited 2011 Nov 29];44(1):17–22. Available from: http://www.springerlink.com/index/P16K463027T71377.pdf

156. Bellocq NC, Pun SH, Jensen GS, Davis ME. Transferrin-containing, cyclodextrin polymer-based particles for tumor-targeted gene delivery. Bioconjugate chemistry [Internet]. 2003 [cited 2011 Dec 1];14:1122–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14624625

157. Davis ME, Zuckerman JE, Choi CHJ, Seligson D, Tolcher A, Alabi CA, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature [Internet]. 2010 Apr 15 [cited 2011 Jul 17];464(7291):1067–70. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2855406&tool=pmcentrez&rendertype=abstract

158. Bartlett DW, Davis ME. Physicochemical and biological characterization of targeted, nucleic acid-containing nanoparticles. Bioconjugate chemistry [Internet]. 2007 [cited 2011];18(2):456–68. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2517011&tool=pmcentrez&rendertype=abstract

159. Pun SH, Davis ME. Development of a nonviral gene delivery vehicle for systemic application. Bioconjugate chemistry [Internet]. 2002 [cited 2011 Dec 1];13(3):630–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12009955

160. Heidel J, Yu Z, Liu J, Rele S, Liang Y, Zeidan R, et al. Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2007 Apr 3 [cited 2011 Dec 1];104(14):5715–21. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1829492&tool=pmcentrez&rendertype=abstract

161. Persengiev S, Zhu X, Green M. Nonspecific, concentration-dependent stimulation and repression of mammalian gene expression by small interfering RNAs (siRNAs). RNA [Internet]. 2004 Jan 1 [cited 2011 Aug 7];10:12–8. Available from: http://www.rnajournal.org/cgi/doi/10.1261/rna5160904

162. Subtil A, Gaidarov I, Kobylarz K, Lampson MA, Keen JH, McGraw TE. Acute cholesterol depletion inhibits clathrin-coated pit budding. Proceedings of the National Academy of Sciences [Internet]. 1999 Jun 8 [cited 2011 Dec 1];96:6775. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20499258

163. U.S. National Institutes of Health. Safety study of MBP-426 (liposomal oxaliplatin suspension for injection) to treat advanced or metastatic solid tumors [Internet]. Clinicaltrials.gov. 2009 [cited 2011 Dec 10];Available from: http://clinicaltrials.gov/ct2/show/NCT00355888?term=mbp-426&rank=2

164. U.S. National Institutes of Health. Study of MBP-426 in patients with second line gastric, gastroesophageal, or esophageal adenocarcinoma [Internet]. Clinicaltrials.gov. 2011 [cited 2011 Dec 10];Available from: http://clinicaltrials.gov/ct2/show/NCT00964080?term=mbp-426&rank=1

165. Mebiopharm Co. L. Mebiopharm - products/pipeline [Internet]. Mebiopharm.com. 2011 [cited 2011 Dec 10];Available from: http://www.mebiopharm.com/english/pro.html

166. Izbicka E, Diaz A, Campos D, Wick M, Takimoto C, Nagasawa M, et al. Evaluation of antitumor activity and biomarkers for MBP-426 in human pancreatic cancer xenograft models in vivo [Internet]. In: AACR Annual Meeting. Los Angeles, CA, USA: 2007 [cited 2011 Dec 10]. Available from: http://www.mebiopharm.com/english/aacr5586.html

167. Senzer NN, Matsuno K, Yamagata N, Fujisawa T, Wasserman E, Sutherland W, et al. MBP-426, a novel liposome-encapsulated oxaliplatin, in combination with 5-FU/leucovorin (LV) – Phase I results of a Phase I/II study in gastro-esophageal adenocarcinoma, with pharmacokinetics [Internet]. In: AACR-NCI-EORTC international conference. Boston, MA, USA: 2009 [cited 2011 Dec 10]. Available from: http://www.mebiopharm.com/english/topics/MBP426 poster, AACR-NCI-EORTC 2009.pdf

168. Fujisawa T. Mebiopharm Co., Ltd. [Internet]. In: Japan biotech forum. London: 2009 [cited 2012 May 1]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21919604

169. Pendyala L, Kidani Y, Perez R, Wilkes J, Bernacki R, Creaven P. Cytotoxicity, cellular accumulation and DNA binding of oxaliplatin isomers. Cancer letters [Internet]. 1995 [cited 2011 Dec 11];97(2):177–84. Available from: http://www.sciencedirect.com/science/article/pii/0304383595039742

170. Wheate NJ, Walker S, Craig GE, Oun R. The status of platinum anticancer drugs in the clinic and in clinical trials. Dalton transactions [Internet]. 2010 Sep 21 [cited 2011 Dec 11];39(35):8113–27. Available from: http://pubs.rsc.org/en/content/articlehtml/2010/dt/c0dt00292e

171. Graham MA, Lockwood GF, Greenslade D, Brienza S, Bayssas M, Gamelin E. Clinical pharmacokinetics of oxaliplatin: a critical review. Clinical cancer research [Internet]. 2000 [cited 2011 Dec 11];6:1205–18. Available from: http://clincancerres.aacrjournals.org/content/6/4/1205.short

172. Maruyama K. Intracellular targeting delivery of liposomal drugs to solid tumors based on EPR effects. Advanced drug delivery reviews [Internet]. 2011 Mar 18 [cited 2011 Jul 26];63:161–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20869415

173. Ishida O, Maruyama K, Tanahashi H, Iwatsuru M, Sasaki K, Eriguchi M, et al. Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo. Pharmaceutical research [Internet]. 2001 [cited 2011 Dec 11];18(7):1042–8. Available from: http://www.springerlink.com/index/V443091166000680.pdf

174. Suzuki R, Takizawa T, Kuwata Y, Mutoh M, Ishiguro N, Utoguchi N, et al. Effective anti-tumor activity of oxaliplatin encapsulated in transferrin-PEG-liposome. International journal of pharmaceutics [Internet]. 2008 Jan 4 [cited 2011 Dec 11];346(1-2):143–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17640835

175. Pfizer. 2003: Pfizer and Pharmacia merger. [Internet]. Phizer.com. 2010 [cited 2012 Feb 10];Available from: http://www.pfizer.com/about/history/pfizer_pharmacia.jsp

176. Williams R. Discontinued drugs in 2008: oncology drugs. Expert opinion on investigational drugs [Internet]. 2009 Nov [cited 2012 Aug 1];18(11):1581–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19663760

177. Duncan R. Development of HPMA copolymer-anticancer conjugates: clinical experience and lessons learnt. Advanced drug delivery reviews [Internet]. 2009 Nov 12 [cited 2011 Jul 10];61(13):1131–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19699249

178. Kopecek J, Kopecková P. HPMA copolymers: origins, early developments, present, and future. Advanced drug delivery reviews [Internet]. 2010 Feb 17 [cited 2011 Jul 10];62(2):122–49. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2836498&tool=pmcentrez&rendertype=abstract

179. Vasey PA, Kaye SB, Morrison R, Twelves C, Wilson P, Duncan R, et al. Phase I clinical and pharmacokinetic study of PK1 [N-(2-Hydroxypropyl)methacrylamide copolymer doxorubicin]: first

member of a new class of chemotherapeutic agents—drug-polymer conjugates. Clinical cancer research [Internet]. 1999 [cited 2012 Feb 10];5(1):83–94. Available from: http://clincancerres.aacrjournals.org/content/5/1/83.short

180. Seymour LW, Ferry DR, Kerr DJ, Rea D, Whitlock M, Poyner R, et al. Phase II studies of polymer-doxorubicin (PK1, FCE28068) in the treatment of breast, lung and colorectal cancer. International journal of oncology [Internet]. 2009 [cited 2011];34(6):1629–36. Available from: http://www.ncbi.nlm.nih.gov.proxy.library.uu.nl/pubmed/19424581

181. Schwartz AL. The hepatic asialoglycoprotein receptor. CRC critical reviews in biochemistry [Internet]. 1984 Jan [cited 2011 Dec 13];16(3):207–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6383714

182. Momparler RL, Karon M, Siegel SE, Avila F. Effect of adriamycin on DNA, RNA, and protein synthesis in cell-free systems and intact cells. Cancer research [Internet]. 1976 [cited 2011 Dec 13];36(8):2891–5. Available from: http://cancerres.aacrjournals.org/content/36/8/2891.short

183. Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. New England journal of medicine [Internet]. 1998 [cited 2011 Dec 13];339(13):900–5. Available from: http://www.nejm.org/doi/full/10.1056/nejm199809243391307

184. Duncan R, Kopecek J, Rejmanova P, Lloyd J. Targeting of N-(2-hydroxypropyl) methacrylamide copolymers to liver by incorporation of galactose residues. Biochimica et biophysica acta [Internet]. 1983 [cited 2012 Feb 10];755(3):518–21. Available from: http://www.sciencedirect.com/science/article/pii/0304416583902581

185. Duncan R, Seymour LCW, Scarlett L, Lloyd JB, Rejmanova P, Kopeček J. Fate of N-(2-hydroxypropyl) methacrylamide copolymers with pendent galactosamine residues after intravenous administration to rats. Biochimica et biophysica acta [Internet]. 1986 [cited 2012 Feb 10];880(1):62–71. Available from: http://www.sciencedirect.com/science/article/pii/0304416586901200

186. Seymour LW, Ulbrich K, Wedge SR, Hume IC, Strohalm J, Duncan R. N-(2-hydroxypropyl)methacrylamide copolymers targeted to the hepatocyte galactose-receptor: pharmacokinetics in DBA2 mice. British journal of cancer [Internet]. 1991 Jun [cited 2012 Oct 2];63(6):859–66. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1972557&tool=pmcentrez&rendertype=abstract

187. Paul A, Vicent MJ, Duncan R. Using small-angle neutron scattering to study the solution conformation of N-(2-hydroxypropyl)methacrylamide copolymer-doxorubicin conjugates. Biomacromolecules [Internet]. 2007 May [cited 2012 Oct 2];8(5):1573–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17419585

188. Abdul-Hussain S, Mehendale H. Ongoing hepatocellular regeneration and resiliency toward galactosamine hepatotoxicity. Archives of toxicology [Internet]. 1992 [cited 2012 Feb 10];66(10):729–42. Available from: http://www.springerlink.com/index/x72l256521247p01.pdf

189. Hosokawa S, Hirakawa Y, Niki H, Tagawa T, Nagaike K. Establishment of human monoclonal antibody (hu-McAb) GAH, exhibiting a specific reactivity to viable cancer cells from fresh tissues and evaluation of the use of antibody GAH in targeting cancer therapy. In: Proceedings of the 92nd annual meeting of the American Association for Cancer Research. New Orleans: 2001. p. 737.

190. Hirakawa Y, Niki H, Oike S, Tagawa T, Hosokawa S, Yoshiyama Y. Antibody recognizing antigen [Internet]. 2010 [cited 2011 Dec 12];Available from: http://www.freepatentsonline.com/y2010/0226974.html

191. Hosokawa S, Tagawa T, Niki H, Hirakawa Y, Nohga K, Nagaike K. Efficacy of immunoliposomes on cancer models in a cell-surface-antigen-density-dependent manner. British journal of cancer [Internet]. 2003 Oct 20 [cited 2011 Sep 22];89(8):1545–51. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2394358&tool=pmcentrez&rendertype=abstract

192. Suzuki S, Watanabe S, Masuko T, Hashimoto Y. Preparation of long-circulating immunoliposomes containing adriamycin by a novel method to coat immunoliposomes with poly(ethylene glycol). Biochimica et biophysica acta [Internet]. 1995 [cited 2011 Dec 19];1245(1):9–16. Available from: http://www.sciencedirect.com/science/article/pii/030441659500066K

193. Mayer LD, Bally MB, Hope MJ, Cullis PR. Uptake of antineoplastic agents into large unilamellar vesicles in response to a membrane potential. Biochimica et biophysica acta [Internet]. 1985 [cited 2012 Feb 10];816(2):294–302. Available from: http://www.sciencedirect.com/science/article/pii/0005273685904973

194. Mayer LD, Tai LC, Bally MB, Mitilenes GN, Ginsberg RS, Cullis PR. Characterization of liposomal systems containing doxorubicin entrapped in response to pH gradients. Biochimica et biophysica acta [Internet]. 1990 Jun 27 [cited 2011];1025(2):143–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2364073

195. Hamaguchi T, Matsumura Y, Nakanishi Y, Muro K, Yamada Y, Shimada Y, et al. Antitumor effect of MCC-465, pegylated liposomal doxorubicin tagged with newly developed monoclonal antibody GAH, in colorectal cancer xenografts. Cancer science [Internet]. 2004 Jul [cited 2011];95(7):608–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15245599

196. Gabizon A, Catane R, Uziely B, Kaufman B, Safra T, Cohen R, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer research [Internet]. 1994 Feb 15 [cited 2011];54(4):987–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8313389

197. Kitamura N, Shigematsu N, Nakahara T, Kanoh M, Hashimoto J, Kunieda E, et al. Biodistribution of immunoliposome labeled with Tc-99m in tumor xenografted mice. Annals of nuclear medicine [Internet]. 2009 Feb [cited 2011 Dec 19];23(2):149–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19225938

198. Lipotek Pty Ltd. Lipotek Pty Ltd: a clinical-phase biopharmaceutical company with multiple platform technologies to address unmet medical needs in infection and cancer [Internet]. Lipotek.com.au. 2011 [cited 2011];46. Available from: http://www.lipotek.com.au/technology/Lipotek_technologies.pdf

199. U.S. National Institutes of Health. Safety study of a liposomal vaccine to treat malignant melanoma [Internet]. Clinicaltrials.gov. 2011 [cited 2011 Dec 12];Available from: http://clinicaltrials.gov/ct2/show/record/NCT01052142?term=lipovaxin&rank=1

200. Altin J, Atmosukarto I, De Wildt R, Parish C, Price J. Composition for targeting dendritic cells [Internet]. WO Patent WO/2010/. 2011 Apr 29 [cited 2011 Dec 23];Available from: http://www.wipo.int/patentscope/search/en/WO2010046338

201. Geijtenbeek TBH, van Vliet SJ, Engering A, 't Hart BA, van Kooyk Y. Self- and nonself-recognition by C-type lectins on dendritic cells. Annual review of immunology [Internet]. 2004 Jan [cited 2011 Jul 24];22:33–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15032573

202. Figdor CG, de Vries IJM, Lesterhuis WJ, Melief CJM. Dendritic cell immunotherapy: mapping the way. Nature medicine [Internet]. 2004 May [cited 2011 Jun 12];10(5):475–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15122249

203. Aarnoudse CA, Garcia Vallejo JJ, Saeland E, van Kooyk Y. Recognition of tumor glycans by antigen-presenting cells. Current opinion in immunology [Internet]. 2006 Feb [cited 2011 Dec 19];18(1):105–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16303292

204. Tacken PJ, de Vries IJM, Gijzen K, Joosten B, Wu D, Rother RP, et al. Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti-DC-SIGN antibody. Blood [Internet]. 2005 Aug 15 [cited 2011 Jul 30];106(4):1278–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15878980

205. van Broekhoven CL, Parish CR, Demangel C, Britton WJ, Altin JG. Targeting dendritic cells with antigen-containing liposomes: a highly effective procedure for induction of antitumor immunity and for tumor immunotherapy. Cancer research [Internet]. 2004 Jun 15 [cited 2011 Nov 3];64(12):4357–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15205352

206. Xu L, Huang CC, Huang W, Tang WH, Rait A, Yin YZ, et al. Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes. Molecular cancer therapeutics [Internet]. 2002 [cited 2012 Jan 4];1:337–46. Available from: http://mct.aacrjournals.org/content/1/5/337.short

207. Dass CR, Choong PFM. Selective gene delivery for cancer therapy using cationic liposomes: in vivo proof of applicability. Journal of controlled release [Internet]. 2006 Jun 28 [cited 2011 Dec 21];113:155–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16764960

208. Strachan T, Read AP. Chapter 18: Cancer Genetics [Internet]. In: Human molecular genetics. New York, NY: Wiley-Liss; 1999 [cited 2011]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK7580/

209. Xu L, Pirollo KF, Chang EH. Tumor-targeted p53-gene therapy enhances the efficacy of conventional chemo/radiotherapy. Journal of controlled release [Internet]. 2001 Jul 6 [cited 2011];74(1-3):115–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11489488

210. Xu L, Pirollo KF, Chang EH. Transferrin-liposome-mediated p53 sensitization of squamous cell carcinoma of the head and neck to radiation in vitro. Human gene therapy [Internet]. 1997 [cited 2012 Jan 4];8:467–75. Available from: http://www.liebertonline.com/doi/abs/10.1089/hum.1997.8.4-467

211. Yu JL, Rak JW, Coomber BL, Hicklin DJ, Kerbel RS. Effect of p53 status on tumor response to antiangiogenic therapy. Science [Internet]. 2002 Feb 22 [cited 2011 Jul 11];295:1526–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11859195

212. Geretti E, Reynolds J, Hendricks B, Eckelhofer I, Espelin C, Gaddy D, et al. Abstract C90: HER2-targeted liposomal doxorubicin MM-302 has a favorable cardiosafety profile in preclinical models. Molecular cancer therapeutics [Internet]. 2011 [cited 2011 Dec 28];10(11):C90. Available from: http://mct.aacrjournals.org/cgi/content/short/10/11_MeetingAbstracts/C90?rss=1

213. Ahmed T. Merrimack and Cubist on a biotech acquisition spree. PharmaDeals review [Internet]. 2009 Dec 1 [cited 2012 Jan 1];(12):130. Available from: http://www.pharmadeals.net/review/details/1291

214. Wickham T, Reynolds J, Drummond D, Kirpotin D, Lahdenranta J, Leonard S, et al. [P3-14-09] Preclinical safety and activity of MM-302, a HER2-targeted liposomal doxorubicin designed to have an improved safety and efficacy profile over approved anthracyclines. [Internet]. In: CTRC-AACR San Antonio breast cancer symposium. San Antonio: 2010 [cited 2012 Feb 13]. Available from: http://www.abstracts2view.com/sabcs10/view.php?nu=SABCS10L_961

215. Park JW, Hong K, Kirpotin DB, Colbern G, Shalaby R, Baselga J, et al. Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. Clinical Cancer Research [Internet]. 2002 [cited 2012 Feb 13];8:1172–81. Available from: http://clincancerres.aacrjournals.org.proxy.library.uu.nl/content/8/4/1172.short

216. Park JW, Kirpotin DB, Hong K, Shalaby R, Shao Y, Nielsen UB, et al. Tumor targeting using anti-her2 immunoliposomes. Journal of controlled release [Internet]. 2001 Jul 6 [cited 2012 Feb 13];74(1-3):95–113. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11489487

217. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. The EMBO journal [Internet]. 2000 [cited 2012 Feb 13];19(13):3159–67. Available from: http://stke.sciencemag.org.proxy.library.uu.nl/cgi/content/full/emboj;19/13/3159

218. Tan M, Yu D. Molecular mechanisms of ErbB2-mediated breast cancer chemoresistance. Breast cancer chemosensitivity [Internet]. 2007 [cited 2012 Feb 13];608:119–29. Available from: http://www.springerlink.com/index/U6V2216454762K34.pdf

219. Tagliabue E, Balsari A, Campiglio M, Pupa SM. HER2 as a target for breast cancer therapy. Expert opinion on biological therapy [Internet]. 2010 May [cited 2012 Feb 13];10(5):711–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20214497

220. Suter TM, Cook-Bruns N, Barton C. Cardiotoxicity associated with trastuzumab (Herceptin) therapy in the treatment of metastatic breast cancer. The breast [Internet]. 2004 Jun [cited 2012 Feb

13];13(3):173–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15177418

221. Feldman AM, Lorell BH, Reis SE. Trastuzumab in the treatment of metastatic breast cancer: anticancer therapy versus cardiotoxicity. Circulation [Internet]. 2000 [cited 2012 Feb 13];102(3):272–4. Available from: http://circ.ahajournals.org/content/102/3/272.short

222. Lenihan D, Suter T, Brammer M, Neate C, Ross G, Baselga J. Pooled analysis of cardiac safety in patients with cancer treated with pertuzumab. Annals of oncology [Internet]. 2011 Jun 10 [cited 2012 Feb 13];Available from: http://www.ncbi.nlm.nih.gov/pubmed/21665955

223. Tagliabue E, Centis F, Campiglio M, Mastroianni A, Martignone S, Pellegrini R, et al. Selection of monoclonal antibodies which induce internalization and phosphorylation of P185HER2 and growth inhibition of cells with HER2/neu gene amplification. International journal of cancer [Internet]. 1991 [cited 2012 Feb 13];47(6):933–7. Available from: http://onlinelibrary.wiley.com.proxy.library.uu.nl/doi/10.1002/ijc.2910470625/abstract

224. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends in pharmacological sciences [Internet]. 2009 Nov [cited 2011 Jul 16];30(11):592–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19837467

225. Kopecek J, Kopecková P, Minko T, Lu Z. HPMA copolymer-anticancer drug conjugates: design, activity, and mechanism of action. European journal of pharmaceutics and biopharmaceutics [Internet]. 2000 Jul [cited 2011];50(1):61–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10840193

226. Gabizon AA. Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. Cancer investigation [Internet]. 2001 Jan [cited 2012 Feb 26];19(4):424–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11405181

227. Lorusso D, Di Stefano A, Carone V, Fagotti A, Pisconti S, Scambia G. Pegylated liposomal doxorubicin-related palmar-plantar erythrodysesthesia (’hand-foot' syndrome). Annals of oncology [Internet]. 2007 Jul [cited 2011 Dec 6];18(7):1159–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17229768

228. Wagner E, Curiel D, Cotten M. Delivery of drugs, proteins and genes into cells using transferrin as a ligand for receptor-mediated endocytosis. Advanced drug delivery reviews [Internet]. 1994 [cited 2012 Feb 2];14:113–35. Available from: http://www.sciencedirect.com.proxy.library.uu.nl/science/article/pii/0169409X94900086

229. Nelson AL, Reichert JM. Development trends for therapeutic antibody fragments. Nature biotechnology [Internet]. 2009 Apr [cited 2012 Feb 2];27(4):331–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19352366

230. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nature biotechnology [Internet]. 2005 Sep [cited 2011 Jul 19];23(9):1147–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16151408

231. O’Brien MER, Wigler N, Inbar M, Rosso R, Grischke E, Santoro A, et al. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYXTM/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. Annals of oncology [Internet]. 2004 Mar 1 [cited 2011 Jul 21];15(3):440–9. Available from: http://annonc.oupjournals.org/cgi/doi/10.1093/annonc/mdh097

232. Lotem M, Hubert A, Lyass O, Goldenhersh MA, Ingber A, Peretz T, et al. Skin toxic effects of polyethylene glycol-coated liposomal doxorubicin. Archives of dermatology [Internet]. 2000 Dec [cited 2012 Aug 2];136(12):1475–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11115157

233. Baselga J, Norton L, Albanell J, Kim Y, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin^{TM}) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer research [Internet]. 1998 [cited 2012 Feb 27];58:2825–31. Available from: http://cancerres.aacrjournals.org/content/58/13/2825.short

234. Takakura Y, Mahato R, Hashida M. Extravasation of macromolecules. Advanced drug delivery reviews [Internet]. 1998 Oct 5 [cited 2012 Aug 2];34(1):93–108. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10837672

235. Hütter G, Nowak D, Mossner M, Ganepola S, Müßig A, Allers K, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. New England journal of medicine [Internet]. 2009 [cited 2012 Feb 27];360(7):692–8. Available from: http://www.nejm.org/doi/full/10.1056/NEJMoa0802905

236. Marcucci F, Lefoulon F. Active targeting with particulate drug carriers in tumor therapy: fundamentals and recent progress. Drug discovery today [Internet]. 2004 Mar 1 [cited 2012];9(5):219–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14980540