

ADVANCED TARGETED THERAPIES IN CANCER: DRUG NANOCARRIERS, THE FUTURE OF CHEMOTHERAPY

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Abstract

Cancer is the second worldwide cause of death, exceeded only by cardiovascular diseases. It is characterized by uncontrolled cell proliferation and an absence of cell death that, except for hematological cancers, generates an abnormal cell mass or tumor. This primary tumor grows thanks to new vasculatization and, in time, acquires metastatic potential and spreads to other body sites, which causes metastasis and finally death. Cancer is caused by damage or mutations in the genetic material of the cells due to environmental or inherited factors. While surgery and radiotherapy are the primary treatment used for local and non-metastatic cancers, anti-cancer drugs (chemotherapy, hormone and biological therapies) are the choice currently used in metastatic cancers. Chemotherapy is based on the inhibition of the division of rapidly growing cells, which is a characteristic of the cancerous cells, but unfortunately, it also affects normal cells with fast proliferation rates, like the hair follicles, bone marrow and gastrointestinal tract cells, generating the characteristic side effects of chemotherapy. The indiscriminate destruction of normal cells, the toxicity of conventional chemotherapeutic

drugs, as well as the development of multidrug resistance, support the need to find new effective targeted treatments based on the changes in the molecular biology of the tumor cells. These novel targeted therapies, of increasing interest as evidenced by FDA-approved targeted cancer drugs in recent years, block biologic transduction pathways and/or specific cancer proteins to induce the death of cancer cells by means of apoptosis and stimulation of the immune system, or specifically deliver chemotherapeutic agents to cancer cells, minimizing the undesirable side effects.

Although targeted therapies can be achieved directly by altering specific cell signaling by means of monoclonal antibodies or small molecules inhibitors, this review focuses on indirect targeted approaches that mainly deliver chemotherapeutic agents to molecular targets overexpressed on the surface of tumor cells. In particular, we offer a detailed description of different cytotoxic drug carriers, like liposomes, carbon nanotubes, dendrimers, polymeric micelles, polymeric conjugates and polymeric nanoparticles, in passive and active targeted cancer therapy, by enhancing the permeability and retention or by the functionalization of the surface of the carriers, respectively, emphasizing those that have received FDA approval or are part of the most important clinical studies up to date. These drug carriers not only transport the chemotherapeutic agents to tumors, avoiding normal tissues and reducing toxicity in the rest of the body, but also protect cytotoxic drugs from degradation, increase the half-life, payload and solubility of cytotoxic agents and reduce renal clearance. Despite the many advantages of all the anticancer drug carriers analyzed, only a few of them have reached the FDA approval, in particular, two polymer-protein conjugates, five liposomal formulations and one polymeric nanoparticle are available in the market, in contrast to the sixteen FDA approval of monoclonal antibodies. However, there are numerous clinical trials in progress of polymer-protein and polymer-drug conjugates, liposomal formulations, including immunoliposomes, polymeric micelles and polymeric nanoparticles. Regarding carbon nanotubes or dendrimers, there are no FDA approvals or clinical trials in process up to date due to their unresolved

toxicity. Moreover, we analyze in detail the more promising and advanced preclinical studies of the particular case of polymeric nanoparticles as carriers of different cytotoxic agents to active and passive tumor targeting published in the last 5 years, since they have a huge potential in cancer therapy, being one of the most widely studied nano-platforms in this field in the last years. The interest that these formulations have recently achieved is stressed by the fact that 90% of the papers based on cancer therapeutics with polymeric nanoparticles have been published in the last 6 years (PubMed search).

Keywords

Cancer, chemotherapy, targeted therapy, nanocarriers, polymeric nanoparticles, passive targeting, active targeting, clinical status.

1. Cancer and chemotherapy.

Cancer is a group of diseases that involve uncontrolled cell division, replicative immortality and resistance to cell death. Cancer cells grow into an abnormal cell mass called tumor, except for hematologic cancers, where cancer cells grow and spread throughout the blood and lymph systems and the bone marrow [1, 2]. Cancer processes are mainly originated by damage or mutation of proto-oncogenes that code for proteins implicated in the induction of cell proliferation and differentiation, and tumor suppressor genes that code for proteins that produce inhibitory signals of cell growth and/or stimulate apoptosis. Alterations in both oncogenes and tumor suppressor genes are necessary for tumor development and are favored by mutations in the tumor susceptibility genes, which encode for a family of proteins implicated in the control of DNA damage. The mutations that initiate a tumor are clonally selected to favour aberrant and uncontrolled cell division, absence of inhibition of the excessive cell growth, avoidance of the immune system, blockage of cell death and transmission and accumulation of genetic material errors [3-9].

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Surgery and radiotherapy are the most effective and valuable treatments for local and non-metastatic cancers, but are inefficient when the cancer has spread throughout the body. The use of cancer drugs (chemotherapy, hormone and biological therapies) is the current choice for the treatment of metastatic cancers, since they are able to reach every organ in the body via the bloodstream [10]. Chemotherapeutic drugs are based on toxic compounds that primarily inhibit the fast proliferation of the cancer cells but, unfortunately, they also inhibit the rapid growth needed for the maintenance of hair follicles, bone marrow and gastrointestinal tract cells which leads to the undesirable side effects observed in cancer treatment [10]. Since the first drugs approved by the Food and Drug Administration (FDA) for the treatment of hematological cancers and solid tumors back in the forties and fifties (nitrogen mustards, antifolate drugs, and methotrexate, etc.), chemotherapy drugs have

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evolved towards increasingly effective treatments [10-12]. In spite of important progresses in the cancer treatment, like combinatory and adjuvant chemotherapies [13, 14], or the approval of important anticancer drugs, like cisplatin [15] and paclitaxel [16]; the indiscriminate destruction of cells, and the toxic side effects of the chemotherapeutic agents, was for many years the only possible approach for the treatment of metastatic disease. This unspecific and less than ideal strategy changed with the discovery of the cell signaling networks involved in the control of cell proliferation and differentiation, that allowed the design of drugs specifically affecting those networks, and opened the door to the use of the targeted therapy, in the late 1990s [10].

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Targeted treatments are aimed to block specific biologic transduction pathways or cancer proteins that are involved in tumor growth and progression, i.e. molecular targets (receptors, growth factors, kinase cascades or molecules related with apoptosis and angiogenesis) that are present in normal tissues, but are found overexpressed or mutated in cancer. The idea of these revolutionary therapies is either to block the signals that help malignant cells to grow and divide uncontrollably, produce the death of cancer cells by means of induction of apoptosis, stimulate the immune system, or target the delivery of chemotherapy agents specifically to cancer cells, minimizing the death of normal cells and avoiding the undesirable side effects [5, 10]. The importance of these new anticancer drugs can be deduced looking at the FDA-

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approved drugs in the oncology area in the last ~~decade~~ fourteen years. Among the 19 anticancer drugs approved in the 2000-2006 period, 14 were targeted therapies. This data increased between 2007 and 2012 when 40 drugs were approved for the treatment of different types of cancer, and 30 of them targeted specific cancer molecules. It should be noted that among 16-19 cancer drugs approved by the FDA ~~in-between~~ 2012-2014, 14-18 were targeted cancer drugs based on inhibiting or blocking biologic transduction pathways and/or specific cancer proteins [17-19].

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Targeted therapies can be achieved by direct approaches that alter specific cell signaling events by means of monoclonal antibodies or small molecules inhibitors [20] , or by indirect approaches using molecular targets, overexpressed or exclusively expressed on the surface of tumor cells, to send cytotoxic molecules, like chemotherapeutic agents, toxins, cytokines or radionuclides that can be conjugated to monoclonal antibodies or peptide ligands via a chemical linker or included in nanocarriers to avoid the lack of specificity of the conventional chemotherapy, this way achieving higher concentrations of cytotoxic molecules in tumors and decreasing the peripheral toxicity [21-24].

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Monoclonal antibodies [20, 25] can be designed to be attached to specific proteins in cancer cells, so that the immune system can recognize these cells and kill them [26] . They can also be selected for their ability to bind to the growth factor receptors overexpressed in certain cancer cells, blocking the docking sites of the growth factors and stopping the mitogenic signals [27].

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Rituximab, the first targeted therapy cancer drug approved by the FDA (1997), was also the first monoclonal antibody available for cancer treatment [28]. Small molecule inhibitors are selected for its ability to block signaling pathways involved in abnormal proliferation, anti-apoptotic and angiogenic events produced in cancer cells [20]. Many of the inhibitors lately approved for its clinical use have been designed to interfere with the kinase domain of tyrosine kinases [29]. Thus, BCR-ABL (the product of the Philadelphia chromosome and the cause of Chronic Myelogenous Leukemia), c-KIT, or the platelet derived growth factor receptor- β (PDGFR β) are tyrosine kinases that are inhibited by Imatinib, the first drug of this type to be approved by FDA for the treatment of cancer (chronic myeloid leukemia, gastrointestinal stromal tumors, and other rare malignancies) [30, 31]. Another example is the inhibition of the epidermal growth factor receptor (EGFR, HER1) by gefitinib and erlotinib, also approved by the FDA, for advanced non-small cell lung cancer [32]. Tumours need new blood vessels to feed the tumor mass. They are induced from already existent vessels in a process known as angiogenesis. Both, monoclonal antibodies and small molecules inhibitors have also

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been used as anti-angiogenic therapies to target the angiogenic proteins that produce the vascularization of tumors, for example, the anti-VEGF monoclonal antibody, bevacizumab (the first anti-angiogenic drug approved by FDA), or the small molecule SU-11246 that inhibits VEGF [33].

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Antibody drug conjugates combine the targeting properties of monoclonal antibodies with the cytotoxicity of chemotherapeutic drugs, leading to a selective accumulation of anticancer agents in the tumor cells [34, 35]. Gemtuzumab ozogamicin, a humanized IgG4 monoclonal antibody coupled with calicheamycin, was the first antibody drug conjugate approved by FDA in 2000 and has been used for the treatment of acute myelogenous leukemia [36], however, because of their significant side effects, this drug was withdrawn in 2010 [37-39]. Brentuximab vedotin, a chimeric monoclonal antibody anti CD-30 coupled with monomethyl auristatin E, approved by FDA in 2011 and currently in clinical use for Hodgkin's lymphoma and anaplastic large cell lymphoma, and ado-trastuzumab emtansine, a HER2-targeting monoclonal antibody (trastuzumab) conjugated to the cytotoxic compound DM1 (mertansine, a maytansine derivative), approved by FDA in 2013 for the treatment of HER2-positive metastatic breast cancer, are the only two antibody drug conjugates approved by FDA up to date, [40-43].

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Nowadays, there are numerous drugs of this type under clinical trials, and some of them probably will be approved in the next few years [44-48]. With the new advances in peptide research, cytotoxic peptide conjugates are being considered as good alternatives to antibody-drug conjugates, combining small peptides (up to 100 times smaller than antibodies) of low cytotoxicity with the ability to selectively bind to overexpressed receptors of some cancer cells, with the cytotoxicity of anticancer drugs. Several interesting cytotoxic peptide conjugates are under clinical trials, but none have been approved by FDA for the clinical use, because some issues, like their short half-lives, still have to be solved [49-54]. In both antibodies and peptides, the linker of the conjugates should be stable during blood transportation, maintaining the drug inactive (prodrug) to preserve normal tissues, but it has to be cleaved

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intra or extracellularly in the target cells by specific enzymatic or chemical degradation, releasing the active cytotoxic agents into the cancer cells [55, 56].

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2. Nanocarriers: promising anticancer drug carriers in indirect cancer targeted therapy.

Clinical status.

Nanocarriers are colloidal nano-scale systems capable of transporting anticancer agents, like small molecular weight drugs or macromolecules as genes or proteins, so that, as an indirect approach of targeted therapy, allow these anticancer agents to avoid normal tissues and be accumulated in tumors, achieving a cytotoxic concentration several-fold higher in this tumors with a reduced toxicity for the rest of the body compared with free drugs, in the same way that antibodies and peptide-drug conjugates do [24, 57, 58]. But in addition, nanocarriers protect the drug from degradation and, reduce the renal clearance and increase its half-life in the bloodstream, augment the payload of cytotoxic drugs, allow the control of the release kinetics of the anticancer drugs, and improve the solubility of those insoluble [24, 59, 60].

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Angiogenesis in cancer generate new blood vessels to the tumor, but these new vessels have got increased permeability (enhanced permeability and retention or EPR effect), that together with the poor lymphatic drainage of tumors allow the passive accumulation of the nanocarriers in tumoral tissues, releasing the chemotherapeutic agents in the vicinity of the tumor (Figure 1) [60-62]. To exploit the singularities of the tumor vasculature, nanocarriers must have enough circulation half-life to passively target the tumor environment, avoiding the action of the mononuclear phagocyte system (MPS) and the reticulo-endothelial system (RES) during their transportation throughout the bloodstream, and releasing the anticancer drugs in tumor [63-65]. For this purpose, nanocarrier size must not exceed 400 nm to escape from the MPS and achieve the extravasation into tumors by the EPR effect, which is much more effective with diameters below 200 nm [66-68]. In addition, the surface of these nano-scale carriers must be hydrophilic and neutral or slightly anionic to avoid the plasma proteins

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(opsonins) and to delay the macrophage attack [69]. This is achieved through the decoration of the surface of the carriers with hydrophilic polymers like poly(ethylene glycol) (PEG) [70], or amphiphilic polymers like synthetic copolymers of polyethylene oxide (hydrophilic block) and propylene oxide (hydrophobic block) [71]. Also, it should be noted that the surface of blood vessels and cells contain negatively charged constituents that might repel nanocarriers with negatively charged surfaces, being a good choice the use of slightly negative or positive surfaces [72].

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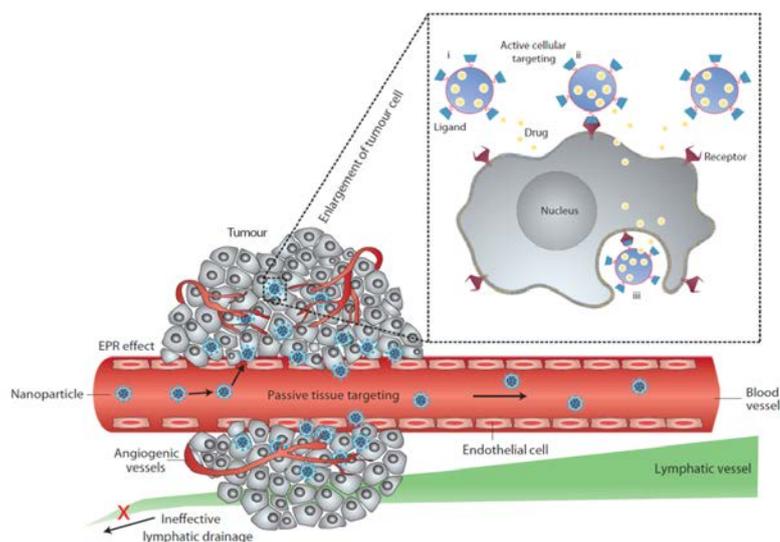


Figure 1. Nanocarriers as promising transporters of anticancer drugs to tumors by passive tissue targeting and active cellular targeting. **Passive tissue targeting** uses the increased permeability of tumor vasculature and the poor lymphatic drainage of tumors (EPR effect), allowing the release of chemotherapeutic agents in the vicinity of the tumor. **Active cellular targeting** is achieved by functionalization of the surface of nanocarriers, containing chemotherapy drugs, with targeting moieties that provide selective recognition of different receptors or antigens overexpressed in cancerous cells, increase their therapeutic efficacy, and overcomes the multiple-drug resistance. Nanocarriers, once in the vicinity of the tumor, can: (i) release their cytotoxic content next to the cancer cells; (ii) bind to the membrane of the cancer cells and release their content in a sustained way; (iii) be internalized into the cells.

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It is possible to achieve an active targeting by the conjugation of different moieties, like monoclonal antibodies, antibody fragments, peptides, growth factors, etc., to the surface of nanocarriers containing chemotherapy drugs (Figure 1). Indeed, nanocarriers allow the incorporation of multiple targeting ligands due to their high surface-area-to-volume ratio resulting in multiple binding possibilities [24, 73]. Active targeting does not enhance the overall tumor accumulation of cytotoxic drugs in the target site, not delivering a higher amount of cytotoxic drugs to the tumor than passively targeted systems since the initial accumulation of the nanocarriers in the tumor relies on EPR effect, before active targeting takes place [24, 74]. However, active cellular targeting improve therapeutic efficacy by decreasing unspecificity and increasing uptake [74, 75]. Moreover, decoration of nanocarriers with targeting moieties overcomes the multiple-drug resistance (MDR), using peptides, [76-80] and avoids the limitations of passive targeting, since the EPR effect is not produced in certain hypovascular tumors [81, 82] and the permeability of blood vessels can vary in a single tumor [83]. Active targeting nanocarriers are able to increase by many folds the antitumor efficacy compared to untargeted carriers [84].

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However, no nanocarriers with active targeting have achieved the FDA approval to date, and there are only few clinical trials in progress [85]. This clinical failure can be attributed to the fact that, after they penetrate into the tumor vasculature, there are different barriers that have to be crossed to reach and enter the cancer cells [85, 86]. Furthermore, in rapidly growing tumors, cancer cells are located adjacent to the endothelial barrier, and nanocarriers with targeting moieties will bind to the first receptors they find, not penetrating into the rest of the tumor. In the literature, different strategies have been described to solve these shortcomings, for example, an active targeting to angiogenic endothelial cells, so that nanocarriers do not have to permeate through the different layers that can be found between the endothelium and the tumor cells. This increases the antitumor potential and efficacy of nanocarriers, since they decrease the nutrients and oxygen delivery to the tumors, and release

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the low molecular anticancer drugs in the vicinity of the tumor vasculature [49, 85]. This promising strategy is under preclinical development, with a few clinical examples in phase I [87-89]. An additional drawback, that contributes to the current clinical failure of active targeting nanocarriers, comprises the increased immunogenicity and plasma protein adsorption when targeting moieties are included in the nanocarriers, decreasing their bloodstream circulation time and their ability to passively target the tumors [85]. Up to date, all these problems make these active targeted nanoparticles to behave in vivo with the same or less antitumor efficiency than untargeted particles [85, 90], being necessary more research to attain clinical success.

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Nanocarriers comprise mainly polymer therapeutics (polymer-protein and polymer-drug conjugates), where the drug is covalently bound or conjugated to a polymeric structure, and particulate drug nanocarriers, where the drug is physically entrapped within molecular assemblies with different structures made from different materials, such as polymers (polymeric micelles, dendrimers and polymeric nanoparticles), lipids (liposomes), or organometallic compounds (carbon nanotubes). The first generation of anticancer nanocarriers to be approved by FDA included both liposomal drugs and polymeric conjugates [91, 92].

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2.1. Polymer therapeutics

Polymer therapeutics that include polymer-protein conjugates and polymer-drug conjugates, among other structures, can be defined as nanoscale linear water-soluble polymeric macromolecular structures conjugated to anti-tumor proteins or small-molecule anticancer drugs, via cleavable linkers that are stable during the transportation of the cytotoxic

component, and release the anticancer drug in the tumor [93]. The covalent conjugation of anticancer proteins to polymers reduces its immunogenicity and increases its stability and circulation time in blood [94] , while, in the case of polymer-drug conjugates, polymers give cytotoxic drugs an increased circulation time in blood, an improved aqueous solubility, a passively targeted delivery to tumors, and a reduced toxicity, improving the therapeutic value of the anticancer drug [93, 95, 96]. In both cases, these structures can be considered as “new chemical entities” rather than drug carriers, having a reduced drug loading and a restricted capacity of active targeting because of the limited number of conjugation sites available in the polymer [94]. In addition, as the drug release is produced by breakage of the linker that bounds the drug to the polymer by enzymatic or chemical degradation, there is no control over the drug release.

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There are two polymer-protein conjugates approved by the FDA up to date: the anti-tumor protein neocarzinostatin conjugated to styrene maleic anhydride (SMANCS or Zinostatin Stimalamer) that it is administered locally [97, 98], and the PEG-protein conjugate, PEG-L-asparaginase (Pegaspargase or Oncaspar) that it is administered parenterally [99-101].

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Pegaspargase was the first polymer-protein conjugate to be approved by the FDA in 1994 for the treatment of acute lymphoblastic leukemia [102, 103]. From these approvals, twenty years ago, numerous clinical trials of polymer-proteins conjugates have been or are being performed. Those include enzymes (arginase [104, 105], arginine deiminase [104, 106, 107], glutaminase [108, 109]) and biological response modifiers (interleukin 2 [110-112], interferon-alpha [113-119], antibody fragment angiogenesis inhibitor [120, 121]) bound to PEG [93, 122-125], but to the best of our knowledge there are no new FDA approvals for cancer treatment [126-128].

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After 30 years of development, there are no polymer-drug conjugates in the market [126, 129]

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However, clinical trials have grown exponentially in the last years [130]. Unlike the polymer-

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protein conjugates, where the last FDA approval covered different diseases [126-128] such as hepatitis C [131], gout [132], acromegaly [133-135], neutropenia [136-138], Crohn's disease [139], renal anemia [140], and age-related macular degeneration [141-143], the research on polymer-drug conjugates has been focused on cancer treatment, with at least 20 conjugates currently in clinical trials (some of them in discontinued status) [127, 130, 144]. They are based on the traditional cytotoxic drugs (e.g. platinates [145, 146], doxorubicin [147, 148], camptothecin and analogues [149-159], paclitaxel/docetaxel [160-164], methotrexate [165], and irinotecan [166, 167]). Xyotax (CT-2103 or OPAXIO), a PGA-paclitaxel conjugate, and NKTR-102, a polymer conjugate of irinotecan, are both currently in phase III clinical trials, being the conjugates closest to approval and market availability [164, 166-168].

N-(2-hydroxypropyl)methacrylamide (HPMA)-doxorubicin conjugate was the first synthetic polymer-drug conjugate to enter clinical trials in 1994 [169-173]. Since then, other polymer-drug conjugates, based on synthetic polymers like HPMA, polyglutamic acid (PGA) or PEG, have been submitted to clinical trials [93, 125, 129, 172-175]. Also, many natural polymers can be found in literature as polymer-drug conjugates [96], but only few polysaccharides, hyaluronic acid [161], human serum albumin (HSA) [165], dextran [148, 151] and cyclodextrin [155, 176] have reached clinical trials [96]. The most remarkable advance towards the clinical use of polymer-drug conjugates is DHA (docosahexaenoic acid)-paclitaxel conjugate (Taxopresin), which recently entered a phase III clinical trial [18, 177-180].

Although, almost all the polymer-drug conjugates exploit the passive targeting, active approaches with targeting ligands like antibodies, peptides and folates have been developed in the last years [170, 181-185]. In addition, new promising lines of research are being followed to achieve active targeting by polymer-drug conjugates that inhibit specific kinases [186-189], activate apoptosis [190-192] or decrease angiogenesis [183-185, 193-196], being all these lines

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of research in preclinical status, except for a polyacetal-fumagillin (antiangiogenic) conjugate (XMT-1107) which is under a phase I trial [18, 197].

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2.2. Particulate drug nanocarriers

Particulate drug nanocarriers that include liposomes, carbon nanotubes and polymeric nanocarriers, among other structures, are nanosized molecular structures that provide an additional protection of the anticancer agent over the polymeric conjugates since the anticancer drugs are isolated from the environment and macrophages. In addition, they provide the possibility of a controlled drug release, which, as mentioned before, is not possible in the case of the polymer therapeutics [198].

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Liposomes

Liposomes are self-assembled colloidal vesicles with a characteristic lipid bilayered membrane composed of amphiphilic phospholipids that not only allow the encapsulation of numerous hydrophilic anticancer drugs and siRNAs in its aqueous core, but also can host hydrophobic cytotoxic agents in its hydrophobic membrane. However, the drug loading capacity of poorly soluble drugs is limited due to the small space available in the membrane and the destabilization effect of the drug on the outer space, being then considered mainly as carriers of water-soluble drugs, although also with low loading limits [199, 200]. In addition to the amphiphilic drug loading characteristics, these structures exhibit additional advantageous properties like biocompatibility and almost biologically inert profiles in most patients, not causing antigenic or toxic reactions in a high percentage of cases. However, note that intravenous injection of liposomal drugs can cause the so-called “complement activation-related pseudoallergy” (CARPA), a drug-induced acute immune toxicity manifested in

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hypersensitivity reactions [201-203]. Liposomes exhibit long time circulation in blood, that can be enhanced by conjugation of PEG to the liposome surface (stealth liposomes); and an easy tunable surface. Moreover, the physiochemical properties of liposomes can be modified by mixing different commercial lipid molecules, being able to control their size, surface charge, and utility in a simple way, without the additional chemical synthesis steps and complicated modifications that are used for the preparation of other carriers, i.e. polymer conjugates [204-207]. However, in addition to their low drug loading mentioned above, they have some other limitations, like problems with stability and industrial reproducibility, difficulties in sterilization, the oxidation of phospholipids, and the limited control of drug release by the conventional formulations, which have profiles of release in rapid bursts, being possible to improve this behavior by strategies including thermo-, pH-sensitive and ultrasound triggered drug release [208-210]. Liposomes can also be used as active targeting carriers by bounding monoclonal antibodies or antibody fragments to the surface of liposomes (immunoliposomes) [211-214], increasing the antitumor activity of the anticancer agent, free or encapsulated in simple liposomes, and reducing the systemic toxicity of the free drug [214, 215]. These immunonanocarriers, like a simple liposome, permit much higher amounts of cytotoxic drugs than antibody drug conjugates that only can be coupled with few molar equivalents of drugs to avoid interference with antigen binding [22, 211]. Additionally, immunoliposomes are able to incorporate multiple antibodies and other targeting moieties, which increase the targeting avidity of antibodies [22]. However, the size of these carriers and their limited circulation in blood (that can be enhanced by coating with PEG [216]) may restrict their penetration in solid tumors [217].

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Despite the above-mentioned advantages of liposomal nanocarriers, only 5 liposomes based anticancer drugs have been approved by FDA to date in contrast to the 16 FDA approval of monoclonal antibodies (including antibody drug conjugates) [17, 18, 25, 126, 144, 218]. Doxorubicin HCl stealth liposome injection (Doxil) was, in 1995, the first liposomal carrier

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approved by FDA [219], and vincristine sulfate liposome injection (Marqibo, ONCO TCS) have been the last liposomal cytotoxic drug to be approved by FDA in August 2012 [220]. However, many promising clinical studies are under development to reduce the toxicity, and increase the antitumoral activity of the cytotoxic drugs, by their association to liposomal formulations with increased circulation time in blood. The most remarkable examples of these liposomal formulations are included in the Table 1.

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Tabla 1: Main examples of liposomal formulations in clinical trials.

Product name	Composition	Status	Therapeutic indication	References
Lipoplatin	Pegylated liposomes with cisplatin	Phase III	Non-small lung cancer and pancreatic, gastric, breast, head and neck cancers	[221-226]
Mepact (mifamurtide, L-MTP-PE)	Liposomal muramyl tripeptide phosphatidyl ethanolamine	Phase III (approved in Europe)	Nonmetastatic resectable osteosarcoma	[227-229]
9NC-LP	9-nitrocamptothecin liposomes	Phase II/III	Hepatocellular carcinoma	[230, 231]
L-BLP25 (Stimuvax)	BLP25 liposome vaccine that induce an immune response to cancer cells that express MUC1	Phase II/III	Colorectal cancer, prostate cancer, non-small cell lung cancer, multiple myeloma, and endocrine-sensitive advanced breast cancer (in combination with hormonal treatment)	[18, 218]
SPI-077	Pegylated liposomal cisplatin	Phase I/II/III	Non-small cell lung cancer, ovarian cancer, head and neck cancer	[18, 224, 232-234]
Lipoxal	Liposomal formulation of oxaliplatin	Phase II	Advanced cancer	[222-224, 235]

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Product name	Composition	Status	Therapeutic indication	References
INGN 401	Liposomal formulation of the tumor suppressor gene FUS1	Phase II	Metastatic lung cancer	[236-238]
EndoTAG-1	Paclitaxel-loaded cationic liposomes	Phase II	Anti-angiogenesis, breast, liver and pancreatic cancers	[18, 218, 239]
Atragen	Liposomal retinoic acid (tretinoin)	Phase II	Recurrent or refractory Hodgkin's disease ¹ and metastatic kidney cancer (in combination with Interferon alfa)	[18, 126]
SLIT² cisplatin	Nebulized liposomal formulation of cisplatin	Phase I/II	Osteosarcoma metastatic to the lung	[18, 240, 241]
OSI-211	Liposomal formulation of lurtotecan (analogue of camptothecin)	Phase I/II	Phase II: recurrent small cell lung cancer, advanced ovarian cancer, metastatic or locally recurrent head and neck cancer Phase I: advanced or metastatic solid tumors	[18, 242-245]
OSI-7904L	Liposomal thymidylate synthase inhibitor	Phase I/II	Phase II: gastric or gastroesophageal cancer, biliary tract cancer, and head and neck cancer Phase I: refractory or recurrent advanced colorectal cancer (in combination with oxaliplatin), and solid tumors (in combination with cisplatin)	[18, 246-248]
L-Annamycin	Liposomal annamycin	Phase I/II	Breast cancer and acute lymphocytic leukemia	[18, 238, 249-251]

¹ Study withdrawn

² SLIT: sustained release lipid inhalation targeting

Product name	Composition	Status	Therapeutic indication	References
LE-DT	Liposome encapsulated docetaxel	Phase I/II	Phase II: advanced or metastatic pancreatic cancer, metastatic castrate resistant prostate cancer Phase I: solid tumors	[18, 252]
LEP-ETU	Liposome-based paclitaxel formulation	Phase I/II	Phase II: metastatic breast cancer Phase I: advanced cancer	[18, 239, 253]
LE-SN-38	Liposomes with SN-38 (the active metabolite of irinotecan)	Phase I/II	Phase II: colorectal cancer and small cell lung cancer Phase I: advanced cancer	[18, 144, 254, 255]
2B3-101	Pegylated liposomal doxorubicin	Phase I/II	Solid tumors, brain metastases of breast cancer, recurrent malignant glioma, and meningeal carcinomatosis	[18, 256]
Aroplatin, DACH platinum LNDDP	Liposomal cisplatinum derivative agent	Phase I/II	Advanced pancreatic and colorectal cancer, malignant pleural mesothelioma, advanced solid malignancies, B-cell lymphoma	[18, 224, 257, 258]
PLD-EIA	Cationic liposomal EIA pDNA	Phase I/II	Breast and ovarian cancers	[259]
S-CKD602	Pegylated liposomes with CKD-602 (camptothecin derivative)	Phase I	Advanced malignancies	[18, 260]
Alocrest	Vinorelbine liposomes injection	Phase I	Breast and lung cancer	[197]
ATI-1123	Liposomal formulation of docetaxel	Phase I	Solid tumors	[18, 261, 262]
IHL-305	Pegylated liposomal irinotecan	Phase I	Advanced solid tumors	[18, 207, 263]
LE-rafAON	Liposomal formulation containing c-Raf antisense oligodeoxynucleotides	Phase I	Advanced malignancies	[264, 265]
Oncolipin	Liposomal interleukin 2	Phase I	Non-Hodgkin lymphoma	[259, 266]

Product name	Composition	Status	Therapeutic indication	References
C-VISA BikDD	Cholesterol liposome formulation of C-VISA BikDD (plasmid)	Phase I	Advanced pancreatic cancer	[18, 197]
LiPlaCis	Liposomal formulation of cisplatin	Phase I	Advanced or refractory tumors	[18, 224]
TL1	Topotecan liposomes injection	Phase I	Small lung cancer, ovarian cancer, and other advanced solid tumors	[18]
NL CPT-11	Pegylated liposomes with irinotecan	Phase I	Recurrent high-grade gliomas	[18, 197]

Recently, new strategies in liposomal research have gained interest, and some clinical trials based on the combination of different chemotherapeutic agents, and stimuli-responsive release approaches, have been started [267, 268] This is the case of the phase I/II/III trials with liposomes containing cytarabine and daunorubicin (CPX-351) in the treatment of acute myeloid leukemia [18, 269-271], the phase II trials of liposomes containing irinotecan·HCl and floxuridine (CPX-1) as a therapy for advanced colorectal cancer [18, 218, 272], and the thermo-sensitive liposomes containing doxorubicin (ThermoDox) in phase III for the treatment of hepatocellular carcinoma [18], and in phase I/II to recurrent chest wall breast cancer, locally recurrent breast cancer, primary and metastatic tumors of the liver, and metastatic colorectal cancer [18, 273, 274].

With respect to immunoliposomes, although many preclinical studies can be found in the literature [24, 215, 275, 276] and a few clinical trials are in progress [18, 75, 277-279], no immunoliposome has reached the FDA approval. Moreover, there are other active targeting liposomes in clinical trials, like the phase I/II trials of MBP-426, a transferrin-targeted liposomal oxaliplatin formulation to treat advanced or metastatic solid tumors, second line gastric, gastroesophageal, or esophageal adenocarcinoma [18, 279-281].

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Furthermore, active targeting liposome formulations are also being used in gene cancer therapy as delivery vehicles of either synthetic short interfering siRNA that silence certain genes involved in cancer promotion or plasmid encoding cDNAs for the recovery of tumor suppressor expression. Liposomes provide the nucleic acids with protection and reduced macrophage clearance [282-284]. There are several siRNA-loaded liposomal formulations under phase I clinical trials, like ALN-VSP that includes siRNA targeting VEGF and kinesin spindle protein for the treatment of solid tumors with liver involvement [18, 285], Atu07 with an siRNA that targets PKN3 [18, 286], or TKM-PLK1 that includes siRNA against PLK1 for the treatment of neuroendocrine tumors and adrenocortical carcinomas (phase I/II clinical trials) [283, 287, 288]. The phase I liposomal formulation SGT-53 includes plasmidic DNA that expresses the tumor suppressor gene p53 to treat solid tumors [280, 289].

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Carbon nanotubes

Carbon nanotubes are tubular hydrophobic networks of carbon atoms with a diameter of approximately 1-4 nanometers (depending on the number of graphene layers), 1-100 micrometers in length and unique structural, electronic, optical and mechanical properties [290, 291]. Although carbon nanotubes are completely insoluble in all solvents, creating toxicity problems, limiting their length and diameter diminish this toxicity, and chemical modifications to their structure transform them in water-soluble carriers, increasing their biocompatibility and decreasing their toxicity [292-294]. Different anticancer drugs can be included in their inner cavity [295, 296] or in their surface [297] with a high payload due to their ultrahigh surface area. These novel carriers have the ability of cross the plasma membrane and enter into the cancerous cells (by endocytosis or penetration like a needle), having no influence the type of functionalization of the nanotubes or the type of cancer cells [298, 299]. In addition, the functionalized surface of the nanotubes can be bound to different

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targeting moieties, leading to an enhanced tumor cell specificity of the cytotoxic agents, compared with the non-targeted carbon nanocarriers, and overcoming the multidrug resistance [300-306].

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There are no FDA approvals or clinical trials in process up to date using carbon nanotubes, although the encouraging preclinical results *in vitro* and *in vivo* in cancer treatment via passive targeting show that this structures are promising nanocarriers for cancer treatment [18, 126, 296, 297, 300, 307-316]. Active tumor targeting has been rarely reported *in vivo* [300, 317, 318], usually without drug loading [301, 303, 319]. However, it should be noted the promising studies reported by Bhirde et al. [320, 321]. These authors, using a first-line cytotoxic agent (cisplatin), and a targeting moiety (epidermal growth factor, EGF) conjugated to the sidewall of single-walled nanotubes (SWNTs), have shown an enhanced efficacy to target squamous cancer cells that overexpress the epidermal growth factor receptor (EGFR), increased accumulation and uptake of the nanotubes by the tumor tissue, and an augmented efficacy to reduce the size of a head and neck tumor, compared with untargeted nanotubes without EGF molecules [320, 321]. This work is an example of multiple functionalization of the sidewall of carbon nanotubes that permit to carry several types of molecules with different functions in the same carrier, being very useful in cancer treatment [322-324]. More recently, Wang et al. [325] reported water-soluble single-walled carbon nanotubes conjugated with docetaxel and NGR (Asn-Gly-Arg) peptide to target tumor angiogenesis. These authors showed enhanced *in vitro* and *in vivo* efficacy in the PC3 cell line and in a murine S180 cancer model, compared to free docetaxel [325]. In the last two years, the number of *in vivo* studies with targeted carbon nanotubes has slightly increased. Ji et al. [326] reported a new chitosan modified single walled carbon nanotubes that selectively deliver doxorubicin to SMMC-7721 liver cancer cells due to the folic acid conjugated to the chitosan outer layer. These modified carbon nanotubes are able to kill the HCC SMMC-7721 hepatocellular carcinoma cell line and to minimize the growth of tumors on nude mice with liver cancer, showing enhanced pharmaceutical efficiency and

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decreased *in vivo* toxicity compared to free doxorubicin [326]. Datir et al. [327] described a multiwalled doxorubicin-loaded carbon nanotube-hyaluronic acid conjugate that target and is internalize by A549 human lung adenocarcinoma cells via hyaluronan receptors. This targeted system showed higher cytotoxicity and apoptotic activity than free doxorubicin. *In vivo*, the doxorubicin-loaded carbon nanotube-hyaluronic acid showed enhanced accumulation in Ehrlich ascites tumor bearing mice model, compared with free doxorubicin and non-targeted carbon nanotubes, and higher inhibition of tumor growth in breast cancer-induced rats, compared with free doxorubicin, not detecting any toxicity in mice and rats [327]. Ren and collaborators [328] developed doxorubicin-loaded PEGylated oxidized multi-walled carbon nanotubes with angiopep-2 as targeting ligand to the treatment of brain glioma. The superior anti-glioma effect of this system, compared to free doxorubicin, was demonstrated *in vitro* by its cytotoxicity on C6 glioma cells and *in vivo* by an increase in the median survival time of mice with glioma [328]. Singh et al. [329] functionalized multi-walled carbon nanotubes with folic acid to specifically deliver gemcitabine to breast cancer cells. These smart carbon nanotubes are able to generate a sustained release of gemcitabine at the lysosomal pH at the tumor site, generating an enhanced cytotoxic response on MCF-7 human breast cancer cell line, compared to free gemcitabine and non-targeted carbon nanotubes. *In vivo* studies revealed that folic acid-conjugated multi-walled carbon nanotubes released appreciable gemcitabine in the systemic circulation, with lesser accumulation in liver, kidney, spleen and lungs, compared to the free drug and the non-targeted carbon nanotubes. The *in vivo* pharmacokinetic parameters showed an enhanced residence time and half-life with respect to the free drug and the non-targeted system [329].

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Polymeric nanocarriers

Polymeric nanocarriers are polymer-based drug nanocarriers with different possible structures: amphiphilic core/shell (polymeric micelles), hyperbranched macromolecules (dendrimers), or capsules/particles (polymeric nanoparticles). Despite the numerous advantages and the clinical validation of liposomes, they show problems of stability and a limited control of drug release. Polymeric nanocarriers are able to overcome these liposomal limitations, since they are more stable in vivo, show higher drug circulation times, higher loading capacities, and the ability to produce more controlled and targeted drug release profiles, both during prolonged periods of time and at different predetermined rates [330, 331].

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Polymeric micelles: They are promising vehicles for the delivery of poorly soluble cytotoxic drugs that allow a controlled drug release. Polymeric micelles are composed of amphiphilic block copolymers that form nanosized spheroidal micellar structures with a hydrophobic core which can contain poorly-water soluble anticancer drugs and a hydrophilic shell that allows the inclusion of hydrophilic drugs and provides stability to the micelle. This results in long circulation times of the drug in blood and makes this formulation an appropriate carrier for intravenous administration [332-334]. In addition to solve the solubility problems of numerous anticancer drugs by including them into polymeric micelles, the reduced size of the micelle (20-80 nm), which is sufficient to accommodate a high amount of anticancer drugs, and their uniformity, increase even more the circulation time of the drug in the bloodstream and provide better permeability to the anticancer drugs, improving their delivery from the blood vessels deep into the tumors, and generating a uniform distribution of the cytotoxic drug throughout the anomalous tissue [335, 336]. Polymeric micelles will get more clinical significance when some drawbacks of the conventional formulations, like their insufficient stability in systemic circulation and the premature drug leakage, that may cause side effects

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and a decrease of effectiveness, are overcome [332, 337]. Some research is being done in this direction [338, 339] either by stimuli-responsive approaches [340-344], core crosslinking, drug entrapment in the core by covalent binding with linkers [345, 346], functionalization strategies with targeting moieties [347-350], or combinations [351, 352].

In spite of the promising characteristics of the polymeric micelles, there are only eight polymeric micelle-based formulations that include anticancer agents currently in clinical trials [338, 353, 354]. Genexol-PM (paclitaxel encapsulated in monomethoxy-poly(ethylene glycol)-block-poly(D,L-lactide)), that is currently involved in 13 clinical trials, is the polymeric micelle with a more advanced state of development. In fact, it is under phase II, III and IV clinical trials for the treatment of advanced, recurrent or metastatic breast cancer; is under phase II for the treatment of advanced urothelial cancer, advanced head and neck cancer, and advanced non-small-cell lung cancer; is under phase I/II clinical trials for the treatment of ovarian cancer and advanced or metastatic pancreatic cancer; and has been approved by the Korean drug administration for the treatment of breast and lung cancer [18, 85, 354-356]. The other clinical trials currently under development with these structures are: the phase I trial with polymeric micelles composed of methoxy-poly(ethylene glycol)-block-poly(D,L-lactide) loaded with docetaxel (Nanoxel-PM) [357], the phase I trial of NC 4016, a PEG-poly(glutamic acid) polymeric micelle of oxaliplatin [18, 197], the polymeric micelles composed of PEG and PAA that incorporate doxorubicin (NK911) (phase I/II trials) [358] or paclitaxel (NK105) (phase II/III trials) [18, 359, 360], the phase I/II trials of the pegylated polymeric micelles NC-6004 (Nanoplain: PEG + poly-glutamic acid) [18, 361] and NK012 (PEG + poly-glycolic acid) [18, 362], containing cisplatin or SN38, respectively, and the phase II/III trials of the P-glycoprotein-targeting pluronic micelle of doxorubicin (SP1049C) that was labeled as orphan drug by the FDA in 2008 [347, 354].

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Dendrimers: These are highly branched three-dimensional synthetic polymeric macromolecules (10-100 nm). Since dendrimers are synthesized from a central core by consecutive and controlled polymeric reactions that allow a high level of control over their architecture, it is possible to adjust the biocompatibility and the pharmacokinetics of the carrier by tuning the chemical synthesis of the dendrimer. This novel and emerging carrier family bears promising features for its use in the oncology field, like uniform properties (monodisperse size and well-defined shape), biodegradability and biocompatibility, good water solubility, high drug loading capacity, and multiple functional groups in its surface that influence its toxicity and permit the conjugation of multiple molecules at the same time, like anticancer drugs, targeting moieties, and PEG to increase the water solubility and the circulation time of the drugs in blood [363-366]. The unique uniformity of dendrimers gives them the ability to cross the membrane of cancer cells and decreases the carrier clearance by macrophages. Despite these promising characteristics, dendrimers share a limiting feature with polymer therapeutics: the multistep synthesis that increases production costs. The anticancer drug can be either non-covalently encapsulated in the core of the dendrimer or covalently conjugated to its surface, being possible to customize the drug release profiles by controlled depolymerization processes [334, 365, 367, 368]. The encapsulation of anticancer drugs in amphiphilic dendrimers with a hydrophobic core surrounded by hydrophilic branches permits only the utilization of these dendrimers in local treatments (intratumoral injections), because, although it solubilizes the hydrophobic drugs and leaves the drug unaltered, produces toxicity and generates an uncontrolled drug release [365, 369-373]. The covalent attachment of anticancer prodrugs to the surface groups of the dendrimer by chemical conjugation offers, in addition to the enhanced solubilization of the drugs, some advantages compared to the non-covalent encapsulation of the drugs into the dendrimer, namely the multiple attachment of different hydrophobic or hydrophilic anticancer drugs and the controlled release of the drugs depending on the linkers used [374-376].

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Dendrimers have been used as passive anticancer nanocarriers [365, 369, 370, 377-379]. An early example of *in vivo* passive targeting is the conjugation of a sodium carboxyl-terminated G-3.5 polyamidoamine (PAMAM) dendrimer with cisplatin for the treatment of B16F10 induced melanomas, achieving an enhanced antitumor efficacy compared with the free drug [372]. Also, there are preclinical promising *in vitro* and *in vivo* results with active targeting dendrimers [73, 365], for example antibody-dendrimer conjugates showed better efficacy than free antibodies [380-383], peptide (RGD) dendrimer conjugates showed enhanced tumor targeting [384-386], and folic acid functionalized dendrimers generated better tumor accumulations than untargeted controls or free drug, producing a stronger reduction of the tumor size [73, 387-390]. The slow translation of these preclinical studies to clinical trials may be due to the current toxicity of dendrimers [391, 392], with the aim of the current research in the development of new biocompatible and less toxic alternatives [367, 393-399].

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Polymeric nanoparticles: This group may be the most effective carriers for controlled and prolonged anticancer targeted drug delivery. They can be defined as biodegradable colloidal systems that include spherical nano-sized polymeric particles, where cytotoxic drugs can be encapsulated or physically entrapped within a polymeric matrix (nanospheres) or entrapped into a cavity surrounded by a polymeric membrane (nanocapsules), being also possible the conjugation of the anticancer drug to the surface or the core of the particles [198, 400]. In the case of insoluble drugs, it is possible to produce a hydrophobic interaction between the drug and the core of the particle, increasing its solubility [401]. When a drug is conjugated to the particle, the properties of the linkers play a crucial role in the pharmacological properties of the complex, for example, they can make them stable in the bloodstream at pH=7 and be decomposed at pH=5.5, the typical pH in tumors, or be stable in blood, but be cleaved by lysosomal enzymes in the tumors [402]. In addition to their protective properties, these

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carriers also offer: multiple delivery of synergic drugs, reduced toxicity with a limited interaction with healthy cells, long circulation times (stealth nanoparticles), and enhanced uptake by cancer cells, characteristics they share with other nanocarriers; but in addition they show: better stability, more homogeneous size distribution, better controllable physicochemical properties, higher drug payload, and more controlled drug release via diffusion through the polymer matrix or by erosion and degradation of the particles, compared with other colloidal systems (e.g. liposomes, polymeric micelles) [403]. The generation of nanoparticles includes biodegradable and biocompatible natural or synthetic polymers that already have FDA approval. Synthetic polymers, like polyglutamic acid and polyglycolic acid (PGA), polyethylene glycol (PEG), polycaprolactone (PCL), polylactic acid (PLA), polyaspartate (PAA), poly(D, L-lactide-co-glycolic) acid (PLGA), and N-(2-hydroxypropyl)-methacrylamide copolymer (HPMA), are frequently used, since they are easily manufactured and degraded after use, and produce a sustained release of the active compounds over the time [280]. However, natural polymers, like chitosan, alginate, dextran, heparin, albumin, gelatin or collagen, are less used since, in spite of being non-toxic, abundant in nature, inexpensive, and easily biodegraded, they present relatively fast release profiles, and they are not naturally pure and homogeneous, requiring a purification step before their use [250, 404]. Nevertheless, natural polymers are recently gaining interest as usable options, since the generation of nanocarriers with them is performed by mild methods (ionic gelation, coacervation, or complex complexation) [405].

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Polymeric nanoparticles are currently in preclinical and clinical development, but in spite of their promising characteristics described above, in general, they haven't reached FDA approval. In fact, currently there is only one polymeric nanoparticle available in the market, Abraxane (ABI-007), an albumin-bound paclitaxel nanoparticle that was approved by the FDA in 2005 for the treatment of metastatic breast cancer. Later, it was approved for the first-line treatment of advanced non-small cell lung cancer (October, 2012) and for the metastatic

pancreatic cancer (September, 2013). Also, it is in phase III trials for the treatment of malignant melanoma [18, 406-412]. Moreover, there are another 10 formulations under clinical trials that are included in the Table 2 [126].

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Table 1. Main examples of polymeric nanoparticles in clinical trials.

Product name	Composition	Status	Therapeutic indication	References
BA-003³ (Doxorubicin Transdrug, Livitag)	Nanoparticulate formulation of polyisohexylcyano-acrylate containing doxorubicin	Phase III	Advanced hepatocellular carcinoma	[18, 197, 413, 414]
DHAD-PBCA-NPs	Mitoxantrone-loaded polybutylcyanoacrylate nanoparticle formulation	Phase II	Hepatocellular carcinoma	[415]
IT-101 (CRLX101)	Camptothecin coupled to cyclodextrin-PEG copolymer nanoparticles	Phase I/II	Phase II: ovarian cancer and recurrent small cell lung cancer Phase I/II: advanced solid tumors Pilot trial: advanced or metastatic stomach, gastroesophageal, or esophageal cancer	[18, 155, 176, 416]
BIND-014 (DTXL-TNP)	(PLA-PEG or PLGA-PEG) PSMA ⁴ -targeted nanoparticle formulation with docetaxel ⁵	Phase I/II	Phase II: metastatic castration-resistant prostate cancer and non-small cell lung cancer Phase I: advanced or metastatic cancer	[18, 417-419]
ABI-008	Nanoparticle albumin-bound formulation of docetaxel (nab-docetaxel)	Phase I/II	Metastatic breast cancer and hormone-refractory prostate cancer	[18, 197, 420, 421]

³ First nanoparticle to enter in clinical trials.

⁴ PSMA: prostate-specific membrane antigen.

⁵ The nanoparticles were targeted to PSMA using ACUPA (S,S-2-[3-[5-amino-1-carboxypentyl]-ureido]-pentanedioic acid)

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Product name	Composition	Status	Therapeutic indication	References
ABI-009	Nanoparticle albumin-bound formulation of rapamycin (nab-rapamycin)	Phase I/II	Advanced non-hematologic malignancies and nonmuscle invasive bladder cancer.	[18, 197, 420-422]
ABI-010	Nanoparticle albumin-bound formulation of 17-AAG (nab-17-AAG)	Phase I	Solid tumors	[18, 197, 420, 421]
ABI-011	Nanoparticle albumin-bound formulation of thiocolchine dimer	Phase I	Advanced solid tumors or lymphomas	[18, 197, 420, 421]
CALAA-01	Cyclodextrin-PEG-transferrin receptor-targeted nanoparticles containing anti-RRM2 siRNA	Phase I	Solid tumors	[18, 423-426]
Docetaxel-PNP	Polymeric nanoparticle formulation of docetaxel	Phase I	Advanced solid malignancies	[18, 85, 427]

In the next section we will discuss in detail some of the more advanced preclinical studies with polymeric nanoparticles for cancer treatment.

3. Preclinical studies of polymeric nanoparticles for targeted cancer therapy.

As mentioned before, polymeric nanoparticles are promising carriers in cancer therapy because they have enabled the efficient co-delivery of multiple cytotoxic compounds and other therapeutic agents with synergic properties to tumors, allowing a longer bloodstream half-life, showing reduced toxicity and improving pharmacokinetics. In addition to these characteristics that they share with other carriers in the nano-sized scale, polymeric nanoparticles offer enhanced features, like higher stability in different biological fluids, tunable

surface chemistry, size distributions near to monodispersion, controllable physicochemical properties, higher drug loading, and controlled drug release [403, 428]. For the liberation of the active compounds from the polymeric nanoparticles, the use of biodegradable and biocompatible synthetic and natural polymers produces controlled release profiles by diffusion or erosion and degradation mechanisms [330, 331]. The long-term sustained drug release supplied by polymeric nanoparticles is more effective and produces fewer side effects, than the pulse supply of chemotherapeutic agents at high concentrations (traditional chemotherapy) [429]. The properties of nanoparticles, like their size, surface charge, structure, stability, and porosity, which are determined by the properties of the components of the particles, and the process used to generate them, will determine their *in vitro* and *in vivo* behavior.

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The use of polymeric systems in cancer therapy started upon the description in 1976 by Langer and Folkman of the first controlled release system of macromolecules with polymers [430]. In 1979, Couvreur and colleagues described *in vitro* and *in vivo* studies using polymeric nanoparticles composed of polyalkylcyanoacrylate for the release of doxorubicin [431]. The early formulations of polymeric nanoparticles applied to cancer therapy in the 1980s were used for the treatment of hepatocarcinoma, bronchopulmonary tumors and myelomas (macrophage-infiltrated tumours) since they were delivered directly to the mononuclear phagocytic system (MPS), exploiting the opsonisation and clarification of the particles by the macrophages once in the bloodstream [432, 433]. The next step was the targeting of the rest of tumours by “stealth” polymeric nanoparticles with long circulation times in the bloodstream and reduced opsonisation by macrophages [434-436]. In fact, in 1994, Langer and colleagues described poly(lactic acid)/poly(lactic-co-glycolic acid) (PLA/PLGA) and PEG block copolymer nanoparticles as long-circulating stealth polymeric nanoparticles with therapeutic applications [437]. Later, biodegradable polymeric nanoparticles have been developed as promising and

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effective carriers for an advanced and personalized chemotherapy due to the progresses in material science and engineering [432, 438-441].

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In spite of the low quantity of clinical trials operative, and the fact that only one formulation has gotten FDA approval, polymeric nanoparticles have a huge potential in cancer therapy, being one of the most widely studied nano-platforms in this field [442], being only second to studies with conventional platforms like “liposomes” [443].

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90% of the papers based on cancer therapeutics with polymeric nanoparticles have been published in the last 6 years, which shows the interest that these formulations have recently achieved [443]. The most promising and advanced preclinical studies of polymeric nanoparticles applied to active and passive cancer treatment in these years will be analyzed in detail in this section.

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A) Passive targeting.

Nanoparticles accumulate in tumors mostly because of the enhanced permeability and retention effect (EPR effect), which is due to angiogenic processes that produce highly permeable blood vessels in tumors and their characteristic abnormal lymphatic drainage that leads to the accumulation of the nano-scale particles in them, allowing the release of the cytotoxic drugs close to the tumor cells [444]. Next, the most promising and advanced polymeric nanoparticulate formulations using passive targeting to deliver different cytotoxic agents will be presented.

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Formulations loaded with cisplatin.

Kim et al. [445] encapsulated **cisplatin** in the hydrophobic cores of nano-sized-aggregates formed in an aqueous medium with hydrophobically modified glycol chitosans, achieving a

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drug loading efficiency of about 80 %. *In vitro*, these nanoparticles of about 300-500 nm released the cytotoxic agent in a sustained manner, showing less drug cytotoxicity than the free drug, probably due to this sustained drug release. *In vivo*, the nanoparticles applied to tumour-bearing C3H/HeN male mice, showed prolonged circulation in blood, better targeting ability, successful accumulation in tumours, enhanced antitumor activity, and less toxicity compared to free drug.

Mattheolabakis and co-workers [446] developed long-circulating **cisplatin**-loaded PLGA-monomethoxy-PEG nanoparticles to passively target tumors. This nanoparticulate formulation allowed a prolonged residence time of cisplatin in systemic circulation after its intravenous administration in mice [447], while maintaining an anticancer activity in prostate cancer cells comparable with free cisplatin [448]. In addition, high doses of cisplatin included in the PLGA-mPEG nanoparticles were well tolerated in BALB/c mice [446]. The authors demonstrated the antitumor activity of the system by a reduction in tumor growth in SCID (severe combined immunodeficiency) mice with HT29 colon adenocarcinoma tumors, showing higher survival rates compared to the treatment with free cisplatin [446]. In another work with a similar loading of **cisplatin** to PLGA nanoparticles to avoid its side effects without decreasing the drug efficacy, Moreno et al. [449] used the nanoparticulate formulation in tumor-bearing mice, decreasing the tumor volume and activating apoptosis, leading to a significant antitumor activity with lower side effects than conventional cisplatin.

Cisplatin loaded on to gelatin-poly(acrylic acid) nanoparticles (100 nm), which allow a high drug load and stability, produced a sustained drug release and showed enhanced *in vivo* antitumor activity and high accumulation of the particles in the vicinity of the tumor in a mice liver cancer model [450].

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Nanoparticles loaded with doxorubicin.

Park and co-workers [451] reported that pegylated PLGA nanoparticles enhanced the anti-tumoral activity of **doxorubicin** and reduced the cardiomyopathies produced by the commercially available liposomal formulation of doxorubicin, Doxil. This formulation had enhanced cytotoxicity compared to free doxorubicin, *in vitro* on A20 murine B-cell lymphoma cells, and *in vivo* in subcutaneously implanted solid tumors in mice due to the increased uptake of the nanoparticles by the malignant cells.

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An upgrade of these particles was achieved by Gelperina and collaborators, which found a way to cross the blood-brain barrier by **doxorubicin**-loaded PLGA nanoparticles coated with poloxamer 188 or polysorbate 80 that generated enhanced anti-tumor activity against intracranial glioblastoma in rats [452]. Shortly after, this formulation was slightly modified by Wohlfart et al. and tested by histological and immunohistochemical methods to ensure their anti-tumor efficacy in a glioblastoma rat model [453]. The modifications proposed by these authors were the stabilization by polyvinylalcohol (PVA) and human serum albumin (HAS), and the inclusion of lecithin in the core of the PLGA/HSA formulations that stabilize the coating of poloxamer 188 due to the affinity between both components. Studies on a rat glioblastoma model demonstrated the enhanced anti-tumor effect of the doxorubicin-loaded lecithin-containing PLGA/HSA formulation coated with poloxamer 188, the key ingredient for the delivery of the drug to the brain [453].

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Paclitaxel encapsulation.

Paclitaxel has been encapsulated into PEGylated PCL-PLGA nanoparticles, enhancing the *in vitro* and *in vivo* cytotoxic effect of the commercial formulation of paclitaxel, Taxol [454]. *In vitro* studies in Human Cervix Carcinoma Cells (HeLa) revealed higher anti-tumoral activity and

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cytotoxicity than Taxol. The paclitaxel-loaded nanoparticles also showed *in vivo* greater inhibition of the tumor growth on TLT-tumor-bearing mice than the Taxol formulation.

Paclitaxel encapsulation into PLGA nanoparticles has been also used as a promising formulation to eradicate hypoxic tumor cells. Jin and co-workers [455] demonstrated an enhanced *in vitro* cytotoxicity of the paclitaxel in breast carcinoma (MCF-7) and cervix carcinoma (HeLa) cell lines, compared to the free drug, observing cellular uptake by fluorescence microscopy. In addition, using mice they observed the biodistribution of the paclitaxel-loaded particles to the liver and the spleen. In order to achieve long-circulating **paclitaxel**-loaded PLGA nanoparticles, Parveen and Sahoo [456] coated PLGA nanoparticles with a blend of chitosan and polyethylene glycol. This system was able to encapsulate hydrophobic drugs due to the PLGA core, and to bypass the phagocytic attack (increasing their circulation time) due to their hydrophilic coating with a blend of chitosan and polyethylene glycol. *In vitro* studies in retinoblastoma, breast, and pancreatic cancer cell lines revealed an increased antiproliferative activity over PLGA nanoparticles and the free drug due to an enhanced cellular uptake and cytotoxicity of the PLGA-CS-PEG nanoparticles.

In an approach for improving the traditional **paclitaxel** formulation in Cremophor EL, Oh and collaborators, included paclitaxel in nanoparticles generated by a temperature-induced phase transition, with a mixture of Pluronic F-68 and liquid PEG, being this process solvent free [457]. This particulate system was applied *in vivo* to male mice with tumors induced by the subcutaneous injection of SCC-7 (squamous cell carcinoma) cells, showing increased circulation time in bloodstream, enhanced targeting efficiency, and higher antitumor efficacy, compared to the conventional drug.

Paclitaxel has also been loaded in poly(N-vinylpyrrolidone)-b-poly(epsilon-caprolactone) (PVP-b-PCL) nanoparticles (100 nm) generated by a modified nano-precipitation method, achieving a good drug loading (>25 %), and high encapsulation efficiency (>85 %) [458]. The antitumor

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efficacy was studied *in vitro* on different cancer cell lines, showing a sustained drug release profile, similar drug cytotoxicity (once released) compared to Taxol, and no cytotoxicity if they were empty (no drug). They also analyzed the efficacy of this formulation *in vivo* using a mice model of hepatic tumors, finding a significantly higher antitumor activity compared to the conventional Taxol formulation, increased half-life in the bloodstream, and enhanced targeting efficiency.

Nanoparticles which incorporate curcumin.

Anand et al. [459] prepared **curcumin**-loaded biodegradable nanoparticles (80.9 nm) based on PLGA and PEG-5000 as stabilizer. The encapsulated curcumin had enhanced cellular uptake and bioactivity *in vitro* (antitumor, anti-invasive and antiangiogenic effects) compared to free curcumin, inducing apoptosis in leukemic cells and suppressing proliferation in different tumor cell lines. *In vivo* studies in mice revealed also superior bioavailability and half-life than free curcumin. This drug has been also encapsulated in a nano-scale particulate system using glycerol monooleate and pluronic F-127, which is able to solubilize the hydrophobic **curcumin** and protect this drug from hydrolysis and biotransformation, and to deliver the anti-cancer agent in a sustained way, producing longer antiproliferative effects [460]. This nanoparticulate system showed *in vitro* an enhanced uptake and accumulation in different cancer cell lines compared to free curcumin, inducing apoptosis. *In vivo*, the encapsulated curcumin displayed an increased half-life and higher bioavailability in mice, compared to native curcumin [460].

Also working with **curcumin** Duan and co-workers [461] synthesized novel positive-charged curcumin-loaded poly(butyl) cyanoacrylate nanoparticles coated with chitosan (200 nm) with high encapsulation efficiencies (90 %), increasing the solubility of curcumin and avoiding the drug clearance by the RES system. The curcumin particles showed comparable *in vitro* efficacy to the free drug in hepatocarcinoma cells, and inhibited the growth of the tumors *in vivo* in

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murine xenograft models of hepatic carcinoma, having an important antiangiogenic effect [461].

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Other drugs used for incorporation in nanoparticles.

Bernardi and collaborators [462] demonstrated *in vivo* the reduction of the tumor size in a xenograft rat model of glioma treated with **indomethacin**-loaded biocompatible polymeric nanocapsules that achieved an increase in the intracerebral drug concentration, with half of the animals carrying just residual cancerous cells after the treatment. The gliomas treated with the indomethacin-loaded particles showed less invasive characteristics according the pathological analyses. The survival of the rats was much longer with the nanocapsules that contained indomethacin than within the control groups (animals treated with unloaded nanocapsules or with the free drug).

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Zhang et al. [463] prepared safe chitosan-polyaspartic acid nanoparticles by ionic gelification technique to deliver **5-fluorouracil** in a sustainable way in mice. In a human gastric carcinoma model was observed enhanced anti-tumor effects with reduced bone marrow suppression compared to the free drug.

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Other promising formulations in a very early stage of development can be found in the literature of this increasingly important field. For example, ImaRx Therapeutics is developing a branching block copolymer self-assembled nanoparticulate formulation of irinotecan metabolite (MRX-952) [197, 464].

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Polymeric nanoparticles that incorporate macromolecules.

In addition to small molecular weight anticancer drugs, polymeric nanoparticles are able to incorporate, macromolecules as **genes or proteins**. Kim et al. [465] encapsulated efficiently the

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RGD peptide that targets the integrin α V- β 3 which is the most important integrin during the angiogenic processes that feed the tumors, and is expressed and exposed on the surface of activated endothelial cells but not in other endothelial cells or normal tissue. Encapsulation enhances the antiangiogenic and anti-tumoral efficacy of this peptide. The authors used self-assembled glycol chitosan nanoparticles (230 nm) to increase RGD *in vivo* half-life, obtaining a stronger anti-angiogenic effect. The intratumoral administration of this formulation enhanced the tumor growth inhibition, decreasing the microvessel density compared to the free peptide formulation. Hu and Zhang [466] loaded the recombinant human endostatin-endostar in PEG-PLGA nanoparticles to increase *in vivo* their anticancer effect in mice and rabbits. *In vitro*, the encapsulated endostar showed longer circulation times in blood. *In vivo*, the PLGA nanoparticles were able to distribute, in a sustainable way, a larger amount of endostatin to solid tumors, and enhanced significantly the inhibition of the tumor growth, augmenting the antitumor effect of endostar.

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Oral anticancer passive nanoparticulate formulations.

The development of **oral anticancer polymeric nano-scale formulations** is very interesting due to their easy uptake and stress-less characteristics. Thus, Jain and colleagues have shown that **oxaliplatin**-loaded hyaluronic acid-chitosan nanoparticles encapsulated in Eudragit S100-coated pellets efficiently targeted colorectal tumors in mice upon oral delivery, and a prolonged accumulation once in the tumor, providing an enhanced antitumor activity with reduced systemic toxicity [467]. Moreover, Shaikh and collaborators [468] improved up to 9-fold the *in vivo* oral bioavailability of **curcumin** by long-term PLGA nanoparticles using cetyl trimethylammonium bromide (CTAB), polyvinyl alcohol (PVA), Pluronic F-68, or vitamin E TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate) as stabilizers, being PVA the most promising. It is also of interest the preclinical oral formulation of **irinotecan** BA-018 (Irinotecan Transdrug) for the treatment of colorectal cancer that continues the Transdrug nanoparticles

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technology with one formulation on phase III trial, the BA-003 or Doxorubicin Transdrug (already mentioned above) [197]. In addition, Kalaria et al. [469] improved the *in vivo* oral bioavailability and decreased the cardiotoxicity of **doxorubicin** in female Sprague-Dawley rats with 185 nm PLGA nanoparticles using PVA as stabilizer. *In vitro* studies revealed a sustained release of the drug during 24 days and the ability of the nanoparticles to decrease the drug induced toxicity. **Paclitaxel, docetaxel and tamoxifen** have been other cytotoxic agents to be formulated for oral administration in association with polymeric nanoparticles. Bhardwaj et al. [470] formulated PLGA nanoparticles using a quaternary ammonium salt didodecyl dimethylammonium bromide (DMAB) as stabilizer to deliver orally **paclitaxel** to treat chemical-induced breast cancer in rats, being safe and non-toxic to cells, and reducing the tumor burden in a Sprague Dawley rats model. Zhao and Feng [471] developed **paclitaxel**-loaded nanoparticles composed of PLGA and vitamin E TPGS that increased 10 times the *in vivo* oral bioavailability of Taxol. *In vitro* experiments in MCF-7 human breast cancer cell line showed higher effectiveness of this formulation over Taxol. Agüeros et al. [472] significantly increased the *in vivo* oral bioavailability of **paclitaxel** in rats by its encapsulation into nanoparticles based on cyclodextrins (beta-cyclodextrin and 2-hydroxypropyl-beta-cyclodextrin) that inhibit the detoxification activity of P-glycoprotein and P450 cytochrome and solubilize the lipophilic paclitaxel, and poly(anhydride) with bioadhesive properties. Zabaleta et al. [473] reported the use of PEGylated poly(anhydride) nanoparticles for the oral delivery of **paclitaxel**. PEGylation enhanced the intestinal permeation of paclitaxel compared to free Taxol, obtaining the best results with low molecular weight PEGs (2000 and 6000) that give enhanced bioadhesive properties to the nanoparticles and inhibit the activity of P-glycoprotein and P450 cytochrome, increasing the drug half-life. *In vivo*, pharmacokinetic studies of PEGylated poly(anhydride) nanoparticles in rats revealed high and prolonged plasma levels of paclitaxel, being also dependent on the molecular weight of PEG. The oral bioavailability of paclitaxel-loaded nanoparticles also increased significantly when the molecular weight of PEG decreased, being

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always higher than that in the case of non-PEGylated nanoparticles. Feng et al. [474] developed four nanoparticle formulations based on PLGA, PLA, vitamin E TPGS and montmorillonite (MMT) for the oral delivery of **docetaxel**. *In vitro*, cell viability studies showed that PLA-TPGS/MMT and PLA-TPGS nanoparticles were more effective than the commercial formulation of docetaxel, Taxotere. *In vivo*, pharmacokinetic studies in Sprague-Dawley rats demonstrated that the PLA-TPGS/MMT and PLA-TPGS nanoparticle formulations showed up to 26.4- and 20.6-fold longer half-life, respectively, compared to the intravenous injection of Taxotere at the same dose. In addition, both nanoparticle formulations gave better oral bioavailability of docetaxel than Taxotere. Jain et al. [475] reported **tamoxifen**-loaded PLGA nanoparticles that increased 11 times the *in vivo* oral bioavailability of the free drug. *In vitro* experiments with the mouse breast cancer cells C1271, showed an increased cytotoxicity of the nanoparticulate formulation over the free drug since, as shown by imaging techniques, the particles were localized in the nuclear region of the cells. As shown in a model of chemically induced breast tumors, the accumulation in the tumors was higher than commercial tamoxifen, with a better anti-tumoral activity and stronger reduction of the tumor size [475].

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The **combination of chemotherapy-loaded nanoparticles with radiotherapy** has shown to have enhanced tumor accumulation and a better intratumoral distribution of the anticancer drugs, improving the native antitumor activity of the cytotoxic agents. Radiotherapy produces the overexpression of VEGF and FGF and the induction of endothelial cell apoptosis that increases the tumor blood vessels permeability, producing a reduction of the interstitial fluid pressure and the tumor cell density that increases the particle retention and distribution inside the tumors [476, 477]. In addition, the **combination of chemotherapy-loaded nanoparticles and photo-hyperthermia therapy** allows to deliver anticancer drugs and heat to the tumorigenic regions, demonstrating a clear synergistic effect compared with chemotherapy or

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hyperthermia alone. Thus, Park et al. [478] developed doxorubicin-loaded PLGA-gold half-shell nanoparticles by coating biodegradable doxorubicin-loaded PLGA nanoparticles with a film of gold. The multifunctional nanoparticles were endocytosed and accumulated within human cervical cancer (HeLa) cells, where particles were degraded, releasing the doxorubicin, and the heat was generated with near-infrared irradiation (NIR) over the gold layer that is NIR resonant. NIR irradiation, in addition to produce the hyperthermic treatment, generated a photothermal controlled delivery of doxorubicin that increased the release rate of the cytotoxic drug. The combined chemo- and photothermal treatment obtained with these nanoparticles generated enhanced cytotoxic effects, decreasing treatment times.

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B) Active targeting.

As mentioned above, active targeting is achieved by attaching specific ligands to the nanoparticle structure, allowing a selective recognition of different receptors or antigens overexpressed in the tumor cell surfaces, increasing the cytotoxicity of the anticancer agents in tumors and avoiding most of their side effects, since the exposure of healthy cells to the drug is minimized [479]. The functionalization of the surface of the polymer nanoparticles, not only provides active targeting characteristics to the particles, but also improves therapeutic efficacy of cytotoxic drugs and overcomes the multidrug resistance (MDR) [480, 481].

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In this section we summarize some of the more promising and advanced polymeric nanoparticulate formulations developed to date.

Albumin-based targeting:

Albumin-bound nanoparticles not only accumulate in the tumors by the EPR effect, but also by binding to glycoprotein 60 receptor that facilitates the endothelial transcytosis. Once in the

tumor interstitium, tumor cells overexpress the albumin binding protein BM-40 (SPARC, osteonectin) that binds to the albumin-nanoparticles, leading to the uptake of the carrier into the tumor cells by endocytosis [482, 483].

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After the already mentioned success of the albumin-bound platform (nab), with one formulation on market (Abraxane), and four in clinical trials (ABI-008 to 011), where the anticancer drug is mixed with human serum albumin (3-4 %) in an aqueous solvent and pass under a high pressure jet to form the 100-200 nm nanoparticles, there is currently a preclinical study of a novel taxane nab of 70 nm including docetaxel (ABI-013) [484]. This nanoparticle formulation showed *in vivo* superior activity, improved stability, and enhanced antitumor activity over the solvent-based docetaxel, when tested on mice models of human breast, colon and lung cancer [484]. In addition, studies in monkeys and rats demonstrated that ABI-013 is a

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Moreover, an albumin-based Apo2L nanoparticle system was designed by Kim et al. to improve the solubility and the pharmacokinetic properties of the apoptotic agent Apo2 ligand (tumor necrosis factor-related apoptosis-induced ligand) that selectively binds to tripartite death receptors, which are overexpressed on tumor cells [486].

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Hyaluronic acid-based targeting:

Hyaluronic acid (HA), in addition to its biodegradability and biocompatibility, is able to bind specifically to some cancer cells that overexpress the glycoprotein CD44 receptor [487]. Choi et al. [488] designed self-assembled amphiphilic hyaluronic acid nanoparticles to actively target the CD44 receptor overexpressed by SCC7 cancer cells. The authors demonstrated the *in vitro* efficient intracellular uptake of the nanoparticles by fluorescence labeling, and the accumulation *in vivo* of the particles into the tumor in tumor-bearing mice after two days in the bloodstream by a fluorescence imaging system [488]. The PEGylation of these self-

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assembled hyaluronic acid nanoparticles can improve active and passive tumor targeting in tumor-bearing mice, reducing the liver uptake, increasing the circulation time and the accumulation of the particles [489, 490]. Cho et al. [491] reported non-toxic self-assembled nanoparticles composed of a hyaluronic acid-ceramide conjugate and Pluronic 85 for intravenous delivery of docetaxel. The small size of the particles (< 150 nm) and the outside location of the hyaluronic acid allowed the delivery of docetaxel by passive and active targeting. The incorporation of Pluronic 85 enhances the drug solubility, stabilizes the nano-scale formulation, and helps to overcome the multidrug resistance. *In vitro* studies showed an enhanced cellular uptake in CD44-overexpressing MCF-7 cells by receptor-mediated endocytosis, and multidrug resistance avoidance in MCF-7 and ADR cell lines. *In vivo* studies by near-infrared fluorescence imaging demonstrated the tumor targeting of this nanoparticulate formulation in mice bearing tumors overexpressing the CD44 receptor.

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Biotin-based targeting:

Vitamin H or biotin can be used as a targeting ligand against tumors. Surface receptors for vitamin H have usually a higher concentration in tumors than in normal tissues, since cancer cells require an extra amount of this vitamin due to their fast proliferation [492]. Taheri et al. [493, 494] developed human serum albumin (HSA) nanoparticles for the targeted delivery of methotrexate, using biotin as targeting ligand. These biotin-conjugated methotrexate-loaded human serum albumin nanoparticles showed higher cytotoxicity *in vitro* than non-functionalized particles and free methotrexate. Moreover, *in vivo* studies in mice with 4T1 breast carcinoma that overexpresses the biotin receptor revealed that this biotin functionalized HSA nanoparticles enhanced the methotrexate antitumor efficacy and reduced its toxic effects compared to non-targeted nanoparticles or the free drug, decreasing the tumor volume and increasing the survival of the animals. Patil et al. [80] used PLGA nanoparticles to achieve the simultaneous targeted delivery of an anticancer drug, paclitaxel,

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and tariquidar, an inhibitor of P-glycoprotein, to drug-resistant tumors, using also biotin as targeting ligand. These dual agent nanoparticles showed an enhanced cytotoxicity *in vitro*, compared to the same formulation loaded with paclitaxel alone, since they produced an increased accumulation of the drug in the drug-resistant cells due to the inhibition of P-glycoprotein. Enhanced tumor growth inhibition was also observed in a mouse drug-resistant model, at a dose of paclitaxel that was ineffective without the presence of tariquidar.

Folate-based targeting:

Folate or folic acid is used as a targeting ligand because it selectively binds to cell surface folate receptors that are overexpressed mainly on epithelial cancers of the mammary gland, ovary, lung, nose, throat, colon, prostate, and brain, as well as in hematologic cancers, like the chronic and acute myelogenous leukemias, or the non-Hodgkin's lymphomas, and in sarcomas, such as uterine sarcoma and osteosarcoma [495, 496]. Folate conjugated nanoparticles bind to folate receptors and are internalized by the cancerous cells, being able to release the cytotoxic drug contained in the particle to the cytoplasm of the tumor cell [497].

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Patil et al. functionalized the surface of paclitaxel-loaded PLA-PEG nanoparticles with biotin and folic acid in a single step, achieving an *in vivo* enhanced tumor accumulation and efficacy in a mouse MCF7 tumor xenograft model [498].

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Seemingly Parveen and co-workers conjugated folic acid to the surface of chitosan nanoparticles for active and sustainable targeted delivery of doxorubicin to retinoblastoma [499]. The high affinity of folic acid to the folate receptors generated an enhanced intracellular uptake of the particles by the retinoblastoma cells, increasing the cytotoxic effects of the free doxorubicin or the doxorubicin-loaded particles without the targeting moiety. In another approach for increasing the efficiency of doxorubicin, Zhang and colleagues [500] designed a novel biocompatible poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) nanocarrier (240 nm)

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with folic acid as targeting ligand for selective delivery of the drug. *In vitro* studies showed an enhanced cellular uptake and cytotoxicity in folate receptor-overexpressed cancer cells compared to non-targeted nanoparticles of the same formulation. *In vivo*, this new formulation revealed a stronger anti-tumor activity than control nanoparticles.

Nukolova et al. [501] reported folate-targeting nanogels using diblock copolymer poly(ethylene oxide)-b-poly(methacrylic acid) (PEO-b-PMA). *In vitro* studies with human ovarian carcinomas cell lines that overexpress folate receptors, demonstrated enhanced cellular uptake compared to non-targeted nanogels. *In vivo* studies showed that this formulation enhanced the anti-tumor activity and reduced the renal toxicity of cisplatin in a mice model of ovarian cancer.

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Wang et al. [481] studied the potential of paclitaxel-loaded folate-targeted heparin nanoparticles to minimize the P-glycoprotein mediated multidrug resistance. Studies with the epidermal carcinoma KB-8-5 cancer cells, that overexpress the P-glycoprotein, showed the superior activity of these folate-targeted nanoparticles in inhibiting proliferation, compared to the non-targeted particles or the free drug. This effect was produced by the enhanced cellular uptake and intracellular retention of these paclitaxel-loaded targeted particles, and *in vivo*, using a mice model, they produced a delay in the growth of drug-resistant tumors, being demonstrated that folate-targeted nanoparticles help to overcome drug resistance in this model.

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Liang et al. [502] demonstrated that paclitaxel-loaded PLGA-PEG nanoparticles decorated with folate (220 nm) showed enhanced cytotoxicity against endometrial carcinoma, observing cellular uptake, particle accumulation by both passive and active targeting, and anti-tumoral activity in endometrial carcinoma HEC-1A cells and tumor-bearing mice.

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In order to solve the low solubility of SN-38, the active metabolite of irinotecan, Ebrahimnejad and colleagues developed SN-38-loaded PLGA nanoparticles with efficient drug loading and gradual release profiles. The particles were surface modified with polyethylene glycol-folate,

showing higher plasma concentration in Wistar rats than free SN-38, and superior body distribution [503].

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Transferrin-based targeting:

Transferrin is a membrane glycoprotein that supports the transport of iron to rapidly growing cells [504]. The elevated demand of iron in tumorous tissues leads to an overexpression of transferrin receptors (TfRs) in the surface of the cancerous cells that can be used for the delivery of chemotherapeutic agents linked to transferrin that binds to the TfRs [504-506]. Like folate, transferrin-conjugated nanoparticles bind to these receptors in the tumoral cells, inducing its endocytosis and release the anticancer drug there [480].

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Hong and colleagues [507] developed stealth transferrin receptor-targeting nanoparticles (Tf-PEG-NP) containing a poly(ethylene) glycol-hydroxycamptothecin conjugate (PEG-HCPT conjugate). These particles showed a sustained release profile *in vitro*, and better behavior in S180 solid tumors induced in mice, than the PEG-HCPT conjugates, with longer circulation times in blood, enhanced tumor accumulation, and increased antitumor activity.

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Transferrin receptor can be used for the delivery of anticancer drugs to the brain due to the overexpression of this receptor on the surface of the endothelial cells of the blood-brain barrier (BBB) [508]. Gan and Feng [509] developed biodegradable poly(lactide)-D-alfatocopheryl polyethylene glycol succinate diblock copolymer nanoparticles (PLA-TPGS NPs) for targeted delivery of docetaxel across the blood-brain barrier, using transferrin as targeting ligand. This nanoparticle formulation showed more cellular uptake and cytotoxicity over PLGA and PLA-TPGS non-targeting nanoparticles containing docetaxel and the free commercial docetaxel under the trade name Taxotere. Moreover, Jain et al. [510] reported transferrin-conjugated PLGA-PEG nanoparticles to deliver temozolomide to the brain, showing an *in vitro* increased cytotoxicity compared to the free drug. Fluorescence imaging *in vivo* studies with a

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confocal laser scanning microscopy demonstrated an enhanced cellular uptake of these particles, and their localization in the brain tissues in rats, confirming the role of transferrin in the anticancer drug delivery to the brain.

Aptamer-based targeting:

Aptamers (short nucleic-acid-based ligands), like folate and transferrin, are effective ligands for the specific targeting of tumor cells [511].

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Dhar et al. delivered cisplatin to prostate cancer cells by nanoparticles conjugated to the A10 aptamer that targeted the prostate-specific membrane antigen (PSMA) overexpressed in prostate tumors. For this purpose they used non-toxic poly(D,L-lactic-co-glycolic acid)-b-poly(ethylene glycol) nanoparticles (150 nm) incorporating inactive platinum prodrugs (5% w/w Pt(IV)) with the A10 PSMA aptamer conjugated to their surface by the carbodiimide chemistry [49, 512, 513]. Comparing with the free drug, the PSMA aptamer-containing nanoparticles were 80 times more cytotoxic to prostate cancer cells [512]. The particles not only enhanced the drug pharmacokinetics *in vivo*, increasing its circulation times in blood and decreasing its accumulation in the kidneys, but also improved the drug antitumor efficacy compared to its conventional formulation, rising the drug maximum tolerated dose and the therapeutic index of the platinum drug [49]. The new formulation showed amplified antitumor efficacies in rat and mouse models, reducing the size of prostate tumors at considerably lower dosage of anticancer drugs [49].

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Nucleolin, a protein overexpressed in the plasma membrane of cancer cells was employed by Guo et al. [514] to design PEG-PLGA nanoparticles linked to AS1411, a DNA aptamer that specifically binds nucleolin, and used this combination to deliver paclitaxel to C6 glioma cells, improving the anti-glioma efficacy of the commercial formulation of the drug. The authors observed that longer circulation times and increased cytotoxicity were achieved due to the

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specific binding of the A1411 aptamer to nucleolin and ulterior internalization of the particles. *In vivo*, this formulation produced an enhanced inhibition of tumor growth and an increased drug accumulation at the tumor on mice bearing C6 glioma xenografts and intracranial rat C6 gliomas, compared to non-targeted nanoparticles and Taxol.

Monoclonal antibodies-based targeting:

Monoclonal antibodies (mAb), like aptamers, can be conjugated to the surface of nanoparticles (immuno-nanoparticles) being able to specifically target antigens or receptors overexpressed in tumors.

One of the mAb targets with a higher clinical relevance is the epidermal growth factor receptor (EGFR), which is found overexpressed in a wide variety of tumors, including: breast, ovarian, prostate, bladder, non-small cell lung, head and neck cancers and glioblastoma. Conjugation by carbodiimide chemistry of an EGFR-antibody to the surface of polymeric poly(lactide-co-glycolide) nanoparticles loaded with rapamycin has been used to selectively target the extracellular ligand binding domain of EGFR [515]. This approach showed an enhanced antiproliferative activity over the same nanoparticles without the targeting moiety and over free rapamycin, being observed an increased uptake on malignant MCF-7 breast cancer cells by confocal microscopy and fluorescence spectroscopy. Moreover, flow cytometry studies showed that the active targeted particles induced cell cycle arrest and apoptosis.

HER2 is also a member of the EGFR family that is overexpressed in breast and ovarian cancers and is associated with angiogenic processes [516]. The conjugation of anti-HER2 mAbs to the surface of polymeric nanoparticles promotes their intracellular accumulation due to the internalization of the antigens via receptor-mediated endocytosis [70, 84, 517]. Cirstoiu-Hapca and co-workers [518] developed paclitaxel-loaded poly (DL-lactic acid) immuno-nanoparticles with anti-HER2 mAbs (trastuzumab, Herceptin) covalently conjugated to their surface to

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actively target ovarian tumor cells that overexpress HER2 receptors. *In vitro* studies showed the enhanced cytotoxic effects of these immuno-nanoparticles at low drug concentrations compared to other paclitaxel formulations (either the same nanoparticles without the targeting moiety, with irrelevant mAbs or the free drug) [518]. The better therapeutic efficacy of this formulation was demonstrated by bioluminescence imaging in a disseminated xenograft ovarian cancer mouse model, showing, the polymeric immuno-nanoparticles, higher anti-tumor activity than free paclitaxel. These results were confirmed by the longer survival rate of mice treated with the immuno-nanoparticles, compared to mice treated with free paclitaxel, paclitaxel-loaded nanoparticles with a irrelevant quantity of rituximab, or trastuzumab alone [519]. HER2 antibodies (trastuzumab) have been also linked to non-toxic human serum albumin (HSA) nanoparticles for the specific delivery of methotrexate to tumors [520]. These trastuzumab-conjugated methotrexate-loaded human serum albumin nanoparticles presented higher cytotoxicity than non-functionalized particles or free methotrexate, showing an increased uptake by HER2 positive cells compared to non-targeted nanoparticles [520].

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In addition to their use as tools for the delivery of small molecular weight drugs, polymeric immunonanoparticles are able to deliver macromolecules as genes or proteins. Chen et al. [521] developed PLGA immuno-nanoparticles containing the proteic toxin PE38KDEL, by coupling Fab' (fragments antigen-binding) of a humanized anti-HER2 monoclonal antibody to the nanoparticles by the carbodiimide chemistry. The presence of the anti-HER2 Fab' enhanced the *in vitro* cytotoxicity of the PE38KDEL-loaded nanoparticles against breast cancer cell lines that overexpress HER2 receptors. *In vivo*, the immuno-nanoparticles showed an increased anti-tumor activity in tumor-bearing mice over the control immunotoxin (anti-HER2 Fab' conjugated to the toxin PE38KDEL), and a higher inhibition of the tumor growth, in a tumor xenograft model overexpressing the HER2 receptor, over the control immunotoxin. The toxicity of PE38KDEL on normal tissues was decreased dramatically by the inclusion of the toxin into the PLGA nanoparticles. Kos and colleagues [522] used poly(D,L-lactide-co-glycolide)

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(PLGA) nanoparticles with a cytokeratin specific monoclonal antibody (anti-cytokeratin mAb) conjugated to their surface for specific targeting of MCF-10A neoT invasive breast epithelial cells. The particles were loaded with cystatin, a potent protease inhibitor. The polymeric nanocarrier was able to specifically identify the cancerous cells that overexpress cytokeratin and released the inhibitor in the endosomes/lysosomes, inactivating the lysosomal cysteine protease cathepsin B that induces the tumor progression.

Peptidic targeting:

Peptides, like antibodies, can be used as ligands to specifically discern tumor cells, with the advantage of being less expensive and complex than antibodies.

One important aim of peptidic targeting is the integrin $\alpha V\beta 3$ that is overexpressed on tumor cells and is involved in angiogenesis. This integrin is recognized by the peptide sequence arginine-glycine-aspartic acid (RGD) [523, 524]. Wang et al. [525] coupled PEG and RGD peptide to PLGA nanoparticles to deliver doxorubicin to various malignant integrin-overexpressing cancer cell lines, inducing apoptosis specifically to malignant cells. Danhier and coworkers [526] developed RGD-conjugated PLGA-based nanoparticles to specifically deliver paclitaxel to the tumor endothelium, enhancing the anti-tumor efficacy of paclitaxel *in vitro* and *in vivo*. *In vitro*, the targeted particles showed an increased cellular uptake mediated by their binding affinity with $\alpha V\beta 3$ integrin, compared with the non-targeted particles of the same formulation. The authors also demonstrated the targeting characteristics of the particles and an enhanced survival of mice bearing syngenic transplantable liver tumors (TLT).

Another peptidic sequence with targeting characteristics is the Angiopep-2 that binds to the low-density lipoprotein receptor-related protein (LRP), which is overexpressed in the blood-brain barrier, in the malignant glioblastoma multiforme, and in benign pituitary gland tumors. Angiopep-2 has been coupled to the surface of paclitaxel-loaded poly(ethylene glycol)-co-

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poly(epsilon-caprolactone) nanoparticles to overcome the brain-blood barrier and target brain glioma [527]. This study, using U87 MG glioma cells, demonstrated enhanced apoptosis and antiproliferative activity, being possible to check the LRP-mediated endocytosis of the nanoparticles by the glioma cells using rhodamine-isothiocyanate labeling. Imaging analysis of mice bearing U87 MG gliomas, showed the accumulation of the Angiopep-2-conjugated nanoparticles in the tumors, showing their efficiency as carriers and the ability of Angiopep-2 to facilitate the crossing of the blood-brain barrier [527].

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Cyclo-(1-12)-PenITDGEATDSGC (cLABL) or lymphocyte function associated antigen-1 (LFA-1)-derived cyclic peptide is another peptidic formulation that can be used as ligand for targeting the ICAM-1 receptors, which are overexpressed in lymphomas, melanomas, renal, pancreatic, bladder and lung carcinomas [528]. Chuda et al. [529] developed PLGA nanoparticles coupled with cLABL as targeting ligand to specifically deliver doxorubicin to A549 lung epithelial cells that overexpress ICAM-1 receptors. This peptidic ligand, coupled to the particles, increased their cellular uptake being rapidly internalized into these cancer cells.

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In addition, certain peptide sequences, known as nuclear localization signals (NLS) can specifically induce cytoplasmic factors to enter and target the cell nucleus [530]. Thus, Misra and collaborators [531] coupled a NLS to the surface of poly(D,L-lactide-co-glycolide) nanoparticles loaded with doxorubicin allowing directly deliver of the drug to the nucleus of breast cancer cells. Studies in the MCF-7 breast cancer cell line revealed the sustained release of the cytotoxic agent, and the better antiproliferative activity of the NLS-conjugated nanoparticles, compared to the non-targeted particles and the free drug, due to an enhanced uptake and accumulation of the drug in the nucleus of the cells.

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Functionalization of nanoparticles with peptides that bind to specific receptors in the angiogenic endothelial cells results in an enhanced targeted drug delivery directly to the tumors, inhibiting the angiogenesis processes at the same time. RGD, that binds to integrins,

overexpressed on tumor vessels, and asparagine-glycine-arginine (NGR) peptides, that bind to CD13 isoforms, also overexpressed in tumor vasculature, have shown high affinity for angiogenic vessels [532, 533]. The use of a tumor-homing peptide, iRGD, an RGD peptide that contains a tumor penetrating peptide (CendR or C-end rule), results in an optimized delivery of anticancer drugs deeply to the tumors. These novel peptides are able, not only to bind to the surface of integrins-expressing vascular cells, but also to penetrate into the tumors once proteolytically cleaved into the CendR motif fragment that binds to the neuropilin-1 receptor responsible for tumor penetration [534, 535]. Conjugating the iRGD peptide and other tumor-homing peptides to the surface of abraxane (paclitaxel-loaded albumin nanoparticles) enhances significantly the anti-tumor activity of untargeted abraxane in mice, since the tumor-homing peptides facilitate the accumulation and penetration of the nanoparticles in tumors [534, 536]. Similarly, the F3 peptide binds to nucleolin, a protein that is overexpressed on the surface of tumor endothelial cells [537]. Winer et al. [538] used non-toxic F3-conjugated polyacrylamide nanoparticles to deliver cisplatin to tumor vessels in murine and human ovarian cancer models. *In vitro*, the nanoparticles showed to be highly specificity for both, tumor and tumor endothelial cells, being cytotoxic only against the tumor endothelial cells. *In vivo*, the nanoparticles bind mainly to tumor vessels, generating a rapid tumor regression due to their antivasular effect [538].

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Finally, it is important to highlight the use of active targeting nanoparticles for the treatment of **multi-drug resistant (MDR) cancers** that commonly overexpress the epidermal growth factor receptor (EGFR). For example, to overcome the MDR Milane and co-workers [539] modified nanoparticles with an EGFR-specific peptide (GE11) and loaded them with a drug combination of paclitaxel, that prevents cell division, and Iodinamine, that induces apoptosis and decreases MDR. The authors, using a nude mice bearing MDR human breast cancer, proved the safety and efficacy of this nanoparticulate formulate that uses a combination of chemotherapy and active targeting to overcome the MDR. This nanocarrier system showed a

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superior efficacy compared to a treatment with a single anticancer agent either in the form of nanoparticles or in solution, and produced an alteration of the MDR phenotype of the tumor, decreasing the expression of MDR associated proteins. The authors demonstrated that this combination of EGFR-targeted paclitaxel/iodinamine-loaded nanoparticles was the only treatment that decreased tumor volume during 28 days, being less toxic than the anticancer drugs in solution.

C) Polymeric nanoparticles in cancer gene therapy.

Small interfering RNA (siRNA) has recently emerged as a promising therapeutic approach for the treatment of different diseases, like neurodegenerative disorders, infectious diseases and cancer, due to their ability to silence certain genes involved in these threatening diseases [540, 541].

As we already mentioned above, liposomes have been used in gene cancer therapy, as delivery vehicles that provide protection from renal clearance and enzymatic digestion of siRNAs in plasma, allowing enhanced penetration through the capillary endothelium, and efficient cellular uptake [282, 284]. However, liposomes have not been the only platform used for delivering siRNA. Polymeric micelles [542-546], polymer conjugates [547-550], and polymeric nanoparticles [551-557] have also shown promising efficacy as non-viral carriers for the delivery of siRNAs in the treatment of different diseases.

Polymeric nanoparticles have interesting advantages with respect to other non-viral carriers for siRNA delivery: they are easy to scale-up, have improved stability and better safety regarding both to the materials used and to the manufacturing processes [552]. In fact, in addition to the above mentioned phase I trial of CALAA-01 (cyclodextrin-PEG-transferrin receptor-targeting nanoparticles with anti-RRM2 siRNA), there are promising preclinical works

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in cancer therapy using polymeric nanoparticles as non-viral carriers for siRNA delivery. Some of them will be described below.

Yang et al. [558] described the systemic delivery of specific siRNA by cationic lipid assisted polymeric nanoparticles (170-200 nm) with encapsulation efficiencies above the 90 %. The particles were generated by a double emulsion-solvent evaporation technique using poly(ethylene glycol)-b-poly(d,l-lactide), a cationic lipid, and siRNA. This novel formulation showed an efficient *in vitro* cellular uptake and apoptosis, escaping from endosomes. Using a mice model of liver cancer the authors showed that their formulation produced *in vivo* a down-regulation of gene expression. In a MDA-MB-435s murine xenograft model, using nanoparticles carrying siRNA against Plk1, a mitotic promoter highly expressed in tumors, a complete ablation of the tumor growth was observed.

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Other studies introduced a plasmid that contains an siRNA sequence, targeting the Methyl-CpG binding domain protein 1 (MBD1), into non-cytotoxic PLGA-Poloxamer nanoparticles [559]. MBD1 is a transcriptional repressor that mediates chromatin replication and has been found overexpressed in human pancreatic carcinomas and colorectal cancer [560]. These MBD1-siRNA-nanoparticles were uptake by the tumor cells and induced apoptosis, inhibiting the growth of cultured human pancreatic cancer cells [559].

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Andersen et al. [561] developed non-cytotoxic nanoparticles with a polymeric matrix of PLGA and a coating of polyethyleneimine (PEI) and cetylated PEI, and used it for *in vitro* siRNA delivery into human osteosarcoma (U2OS) and macrophage (J774.1) cell lines, targeting the antiapoptotic oncogene BCL-w and the inflammatory cytokine TNFalfa respectively. With this formulation they achieved a high cellular uptake and obtained a significant reduction in the expression of, both BCLw and TNFalfa, without a significant toxicity.

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Patil et al. [562] achieved *in vivo* the **active targeting** of tumors coupling biotin (as a ligand for breast cancer cells) to siRNA-loaded PLGA-based nanoparticles with a PEI coating. This system

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was efficient in silencing the overexpression of the drug efflux transporter P-glycoprotein (P-gp), and allowed to overcome tumor drug resistance. In addition, the authors loaded the particles with the anticancer drug, paclitaxel. A synergy between paclitaxel and the P-gp targeted siRNA was observed, since the particles loaded with both elements showed higher cytotoxicity *in vitro* than nanoparticles loaded with paclitaxel alone. Moreover, the inclusion of the ligand on the surface of the nanoparticles increased the drug accumulation in the tumor cells. Studies with a mouse model of a drug resistant breast cancer, showed enhanced inhibition of tumor growth using the nanoparticles loaded with both paclitaxel and P-gp targeted siRNA, being the dose of paclitaxel used ineffective without the siRNA. Similarly, Han et al. [563] coupled RGD peptide to chitosan nanoparticles for active targeting of siRNA to the integrin $\alpha v \beta 3$, which is overexpressed on tumor cells and the tumor vasculature, enhancing the antitumor activity compared to non-targeted siRNA-loaded nanoparticles. The authors demonstrated *in vivo* selective intratumoral and tumor vasculature delivery of the siRNA in mice models of ovarian cancer, generating targeted silencing of different genes that promote tumor growth.

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Moreover, there are other promising polymeric nanoparticulate formulations for siRNA delivery in very early stage of development, for example, a pharmaceutical company, with the support of a US NIH (National Institute of Health) grant, is developing STP503, a polymeric nanoparticulate formulation (Trisilensa) for the treatment of breast cancer by siRNA targeting EGFR, Raf-1 and mTOR transcripts [564]. The same company with the support of the NIH has other similar promising formulations in discovery stage, like STP801 that uses siRNA cocktail therapeutics to treat non-small lung cancer (NSCLC) by targeting EGFR, VEGF and Cox-2 gene expression, and STP523 that uses siRNA targeting EGFR, VEGF and AGT to treat glioblastoma multiforme [564].

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4. Conclusions and future perspectives.

Although conventional chemotherapy has been the cornerstone in the fight against cancer, is far from being totally satisfactory due to problems related with their formulation and pharmacokinetics, the acquired resistance to some cytotoxic agents, and, overall, their toxicity and indiscriminate action, that make necessary a more selective therapy. This selectivity is being achieved with the development of targeted chemotherapeutics that specifically target the biologic transduction pathways involved in tumor growth and differentiation. Many examples have arisen in the last years that show the efficacy of this approach. Thus, most of the anticancer drugs approved for clinical use in the last decade have a known signaling target. These new biological weapons include: a) monoclonal antibodies that are designed to bind to specific proteins in cancer cells so that the immune system can recognize and attack them, or specifically stick to and block the signals from growth factor receptors overexpressed in tumors; b) small molecules inhibitors, like the tyrosine kinase inhibitors, that are used to block signaling pathways involved in abnormal growth; c) anti-apoptotic molecules; or d) blockers of tumoral neo-angiogenesis.

Other approaches allow the delivery of cytotoxic drugs to molecular targets overexpressed on tumor cells by conjugating these drugs to monoclonal antibodies or peptide ligands via a chemical linker, or by the incorporation of cytotoxic drug into a nanocarrier that either passively targets the tumors by the enhanced permeability and retention effect, or actively targets the tumors by conjugating targeting moieties on the surface of the nanocarrier, helping also to overcome the multidrug resistance. Currently, in more than 50 % of the cancers treated a cure can not be achieved and drug nanocarriers might help to decrease this percentage.

Liposomes and polymer conjugates were the first nanocarriers to be approved by FDA, however only 5 liposomal drugs and 2 polymer-protein conjugates are in the market up to date. There are no polymeric micelles, polymer-drug conjugates, dendrimers, carbon

nanotubes or polymeric nanoparticles available for clinical use, except for Abraxane, an albumin-bound paclitaxel nanoparticle approved by the FDA in 2005 for the treatment of metastatic breast cancer, and that recently has got the approval for the first-line treatment of advanced non-small cell lung cancer (October, 2012) and for the metastatic pancreatic cancer (September, 2013). In spite of these data, numerous clinical trials are currently in development, which makes all these new nano-platforms promising carriers to passively or actively deliver numerous cytotoxic anticancer drugs, improving their clinical efficacy and reducing their toxicity.

Among the different carriers developed for the selective delivery of cytotoxic drugs, polymeric nanoparticles seem to be one of the more promising carriers in cancer targeted therapy, since they provide enhanced stability in biological fluids, tuneable surface conjugation chemistry, more monodisperse size distributions, more controllable physicochemical properties, higher drug loading, and more controlled drug releasing rates, in addition to their long circulation in blood, reduced toxicity, improved pharmacokinetics, and efficient co-delivery of multiple cytotoxic compounds to tumors, characteristics that they share with the others nanocarriers.

Looking into the future, the use of cancer theragnostics, combining anticancer targeted therapy and diagnosis by multifunctional nanocarriers that contain therapeutic and imaging agents, might become promising cancer treatments because they allow to detect selectively cancerous cells, kill them with minimal side effects, visualize them through real time in vivo imaging techniques, and monitor the effects generated by the treatment in real time [565].

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Magnetic nanoparticles are clear examples of theragnostics nanosystems since they are anticancer drug carriers with an inorganic core of iron oxide, manganese oxide, or other magnetic materials that act as contrast agent materials in magnetic resonance imaging (MRI) having higher magnetic susceptibility than traditional contrast agents [566]. By surface modification: targeting moieties, cytotoxic drugs or fluorescence dyes can be included in these

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magnetic particles to provide multifunctional properties [567]. In addition to active targeting, magnetic nanoparticles can be driven to tumors by magnetic fields (magnetic drug targeting). After anticancer drug delivery and accumulation in tumors (chemotherapy) is achieved, oscillating magnetic fields over magnetic nanoparticles can generate heat that will be used for the thermal ablation of tumors grown in deep/poorly accessible areas (hyperthermia). In addition, as the magnetic field is not absorbed by normal tissues, they are not damaged [259]. Unlike gold and carbon based inorganic nanoparticles, magnetic nanoparticles are able to degrade to Fe ions in acidic conditions, for example in the lysosomes of cells, decreasing the potential long-term toxicity of nanoparticles [281]. Quantum dots are another example of the emerging theragnostic nanosystems. In this case these semiconductor-based nanoparticles offer tumor imaging properties by acting as fluorescent probes that are able to emit intense signals at any wavelength between blue and infrared depending on their chemical composition and size [568]. These minuscule particles (2-8 nm) are usually covered by amphiphilic polymers that not only provide them biocompatibility and solubility properties, but also facilitate their functionalization by conjugation of targeting moieties and small molecule anticancer drugs to their surface, providing additional properties to this imaging agent, like active tumor targeting and anticancer drug therapy, without any loss of fluorescence intensity [568]. Moreover, quantum dots, in addition to be able to kill cancerous cells by chemotherapy, can act as photosensitizers that, in conjunction with light of specific wavelength and oxygen, produce reactive oxygen species that selectively induce apoptosis of cancer cells by oxidizing critical elements in them (photodynamic therapy) [568]. Inorganic nanocarriers, including mainly gold nanoparticles, magnetic nanoparticles and quantum dots, are the best examples of multifunctional theragnostic nanosystems that not only act as therapeutic agents, but also act as contrast agent materials in imaging and diagnosis applications. [However, t](#)This review focus on drug nanocarriers as cytotoxic drug carriers, and theragnostic materials, and magnetic hyperthermia in particular, could be the aim of a different extensive review.

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Although polymeric nanoparticles have a huge potential in the future cancer therapy, being one of the most widely studied nano-platforms in this field and offering a more effective and less toxic option to patients, a low quantity of clinical trials is operative, and only one formulation has gotten FDA approval. This clinical failure is due to the numerous challenges that need to be solved in the near future, such as the accumulation of the carriers in liver and spleen and, the low therapeutic efficiency inside the tumors, and the different barriers that have to be crossed to reach and enter the cancer cells, even after this carriers penetrate into the tumor vasculature. Another great challenge is the research on the discovery of new ligands or targeting moieties needed to drive the carrier to specific organs or tumors, so we in order to achieve the site-specific delivery of the chemotherapy-containing nanocarriers to cancerous tissue. Another innovation needed for polymeric nanocarrier improvement is to achieve the release of the anticancer agents in a controlled way by the development of stimuli sensitive carriers.

5. Acknowledgments.

Authors acknowledge financial support from University of Salamanca, Spain. AFM work is supported by grants FIS PI13/02846 and RTICC RD12/0036/0001 from Instituto de Salud Carlos III (ISCIII), and grant SA181U13 from JCyL, Spain. EPH work is supported by grant FS/25-2014 from Fundación en Memoria de D. Samuel Solórzano Barruso.

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