

# Smart Targeting To Improve Cancer Therapeutics

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Moraima Morales-Cruz<sup>1,\*</sup>  
Yamixa Delgado<sup>2,\*</sup>  
Betzaida Castillo<sup>3</sup>  
Cindy M Figueroa<sup>4</sup>  
Anna M Molina<sup>1</sup>  
Anamaris Torres<sup>2</sup>  
Melissa Milián<sup>2</sup>  
Kai Griebenow<sup>1,\*</sup>

<sup>1</sup>Department of Chemistry, University of Puerto Rico, Río Piedras Campus, San Juan, PR, USA; <sup>2</sup>Department of Biochemistry & Pharmacology, San Juan Bautista School of Medicine, Caguas, PR, USA; <sup>3</sup>Department of Chemistry, University of Puerto Rico, Humacao Campus, Humacao, PR, USA; <sup>4</sup>Department of Math and Sciences, Polytechnic University of Puerto Rico, San Juan, PR, USA

\*These authors contributed equally to this work

**Abstract:** Cancer is the second largest cause of death worldwide with the number of new cancer cases predicted to grow significantly in the next decades. Biotechnology and medicine can and should work hand-in-hand to improve cancer diagnosis and treatment efficacy. However, success has been frequently limited, in particular when treating late-stage solid tumors. There still is the need to develop smart and synergistic therapeutic approaches to achieve the synthesis of strong and effective drugs and delivery systems. Much interest has been paid to the development of smart drug delivery systems (drug-loaded particles) that utilize passive targeting, active targeting, and/or stimulus responsiveness strategies. This review will summarize some main ideas about the effect of each strategy and how the combination of some or all of them has shown to be effective. After a brief introduction of current cancer therapies and their limitations, we describe the biological barriers that nanoparticles need to overcome, followed by presenting different types of drug delivery systems to improve drug accumulation in tumors. Then, we describe cancer cell membrane targets that increase cellular drug uptake through active targeting mechanisms. Stimulus-responsive targeting is also discussed by looking at the intra- and extracellular conditions for specific drug release. We include a significant amount of information summarized in tables and figures on nanoparticle-based therapeutics, PEGylated drugs, different ligands for the design of active-targeted systems, and targeting of different organs. We also discuss some still prevailing fundamental limitations of these approaches, eg, by occlusion of targeting ligands.

**Keywords:** active targeting, drug delivery systems, EPR effect, nanoparticles, passive targeting, stimulus-responsive targeting

## Introduction

The American Cancer Society estimates for 2018 more than 1.7 million of new cancer cases in the United States of America, and 1600 million cancer-related deaths with lung cancer being the primary cause of death (43%, [www.cancer.org](http://www.cancer.org)). These statistics are expected to increase in the coming decades “unless we make more progress today” (Joe Biden, Vice President at the American Association for Cancer Research Annual Meeting, 2016).

Currently, surgery, radiation therapy (RT), and chemotherapy are the principal treatment strategies against cancer. Surgery is usually recommended at an early stage of the disease and is most effective when all the cancer cells can be excised.<sup>1,2</sup> It is also used in later stages but mostly to debulk tumors and improve quality of life. Thus, chemotherapy and RT are the most widely used interventions for the treatment of cancer.<sup>1–3</sup> In contrast to surgery, chemotherapy and RT are mostly only capable of killing a fraction of tumor cells during each treatment regimen and typically never completely cure the disease.<sup>3</sup> Cytotoxic anticancer drugs are used in

Correspondence: Yamixa Delgado  
PO Box 4968 Caguas, PR 00726-4968, USA  
Tel +1 787 743 3038  
Fax +1 787 746 3093  
Email ydelgado@sanjuanbautista.edu

Kai Griebenow  
17 Ave Universidad STE 1701, San Juan, PR 00925-2537, USA  
Tel +1 787 764 0000  
Fax +1 787 746 3093  
Email kai.griebenow@upr.edu

chemotherapy to primarily kill metabolically active cells. Most normal cells do not divide as often as cancer cells and thus are proportionately less affected by these cytotoxic drugs. However, although chemotherapy and RT are employed to improve the patient's quality of life or to prolong it, they are frequently associated with severe side-effects related to systemic toxicity due to the lack of tumor specificity.<sup>3-7</sup> Similar to chemotherapy, RT also damages healthy cells, organs, and tissues. For example, the term "mucositis" describes one of the common adverse effects of RT and chemotherapy treatments. Mucositis may limit the patient's ability to tolerate chemotherapy or RT, and the nutritional status may become compromised.<sup>3,4</sup> In addition, one of the leading causes of treatment failure in cancer therapy is the phenomenon of multidrug resistance syndrome (MDRS), typically acquired during prolonged exposure to chemotherapy.<sup>8-12</sup> MDRS is characterized by the ability of cancer cells to efflux drugs by molecular pumps, which results in reducing the therapeutic effect.<sup>12</sup>

With this in mind, in the last decade, a diverse range of drug delivery systems (DDS) has been developed to improve cancer therapies. There are two main types: targeted and non-targeted drug delivery systems. Both types of DDS have been designed at the nanoscale (in this review loosely defined as 10–1000 nm) to enable efficient transport in blood vessels, to overcome biological barriers during the transport, and to reach pathological cells.<sup>13</sup>

In this review, we will focus on some recent developments of smart targeting in cancer treatment, particularly promising data and advanced preclinical and clinical studies. We will evaluate the use of different nanoparticles (NPs) to facilitate drug delivery by both, passive and active targeting mechanisms and highlight the potential advantages of utilizing nanotechnology within the field of cancer therapy.

## Passive Targeting For Enhanced Drug Tumor Accumulation By DDS

In cancer applications, passive targeting is defined as the preferential accumulation of the drug in the tumor. The accumulation and delivery of the drug are determined by the capability of the DDS to overcome biological barriers and the inherent characteristics of the DDS itself (eg, size, material, and charge).

## Overcoming Physiological Barriers

The delivery of drugs from nanosized DDS offers a multitude of advantages over their delivery from larger-sized

particles (eg, microparticles) or their delivery as single-molecule drugs (<10 nm). In general, nanosized drugs are better suited than larger delivery systems in the micrometer range for important delivery routes, eg, intravenous (i.v.) and oral delivery.<sup>13-16</sup> In the case of oral delivery, the size limit for the particles to be able to cross the intestinal mucosal barrier of the gastrointestinal tract is the main restriction. Particles in the intestinal lumen within a size range of 3–10  $\mu\text{m}$  cannot migrate through the lymphatic system whereas particles larger than 10  $\mu\text{m}$  are not taken up in the gastrointestinal tract.<sup>17-19</sup> NPs are also better suited for i.v. delivery in comparison with microparticles. Since the smallest capillaries in the body are 5–6  $\mu\text{m}$  in diameter, the size of particles being distributed into the bloodstream must be significantly smaller than 5  $\mu\text{m}$  and they must show no propensity for forming aggregates, to ensure that the particles do not cause an embolism.<sup>20,21</sup> NPs with a diameter of >100 nm are susceptible to phagocytic clearance by the reticuloendothelial system (RES), an essential component of the immune system located in different organs of the human body (eg, liver, spleen, and lymph nodes). In this context, the effective diameter must be >10 nm and <100 nm because particles with a diameter of <10 nm are secreted readily by the kidney.<sup>22-24</sup> In addition to the size restriction by drug administration routes and to avoid urinary excretion, the optimum particle size will depend on the specific target organ (also called primary targeting) and the purpose of the delivery (Table 1). At this point, it is important to elucidate that studies may show a discrepancy for what the optimal recommended size is for delivery to a specific organ. This discrepancy may be due to the use of different delivery materials and diverse methods to determine the particle size with potentially limited accuracy.<sup>25</sup> One of the most challenging organs to be targeted is the brain, due to the blood-brain barrier which consists of tightly bound endothelial cells forming a lining that blocks the entrance of a lot of molecules into the brain. However, in general, the optimum particle size to deliver a drug to specific organs (eg, brain, liver, and lymph nodes) is in the nanoscale range.<sup>26-28</sup>

## Exploiting The Enhanced Permeation And Retention Effect

Small molecule drugs can enter and exit tumors and normal tissues and typically do not accumulate in them. In contrast, nano-sized DDS have been proven to be able to cause drug accumulation in tumors through exploiting the

**Table I** The Nanoparticle Size Recommended For Intravenous Delivery To Specific Organs

Organ	Specifications	Recommended Size <sup>a,b</sup>	References
Brain	Particles should be able to across the blood–brain barrier (BBB) and exhibiting a molecular weight of <400 Da	<12 nm (<200 nm may also penetrate the brain but with less efficiency)	195,196
Liver	For rapid uptake 10–20 nm. However, particles ~150 can cross the fenestrae present in the liver endothelium	<150 nm	197
Lung	For long accumulation times, cationic nanoparticles are recommended	>300 nm <1000 nm	198,199
Colon	Carbon nanotube and polymeric NPs	~100 nm	200
Pancreas	L-fucose-receptor-mediated delivery of nanoparticles was recommended to target fucosyltransferases	20–100 nm	201
Breast	Albumin nanoparticles are recommended to diminish dose-limiting toxicities of extremely hydrophobic drug formulations	<150 nm	202

**Notes:** <sup>a</sup>In general, ~200 nm is the recommended size to avoid RES. <sup>b</sup>The recommended size can vary by the tumor size that also influences the irregular vasculature gaps.

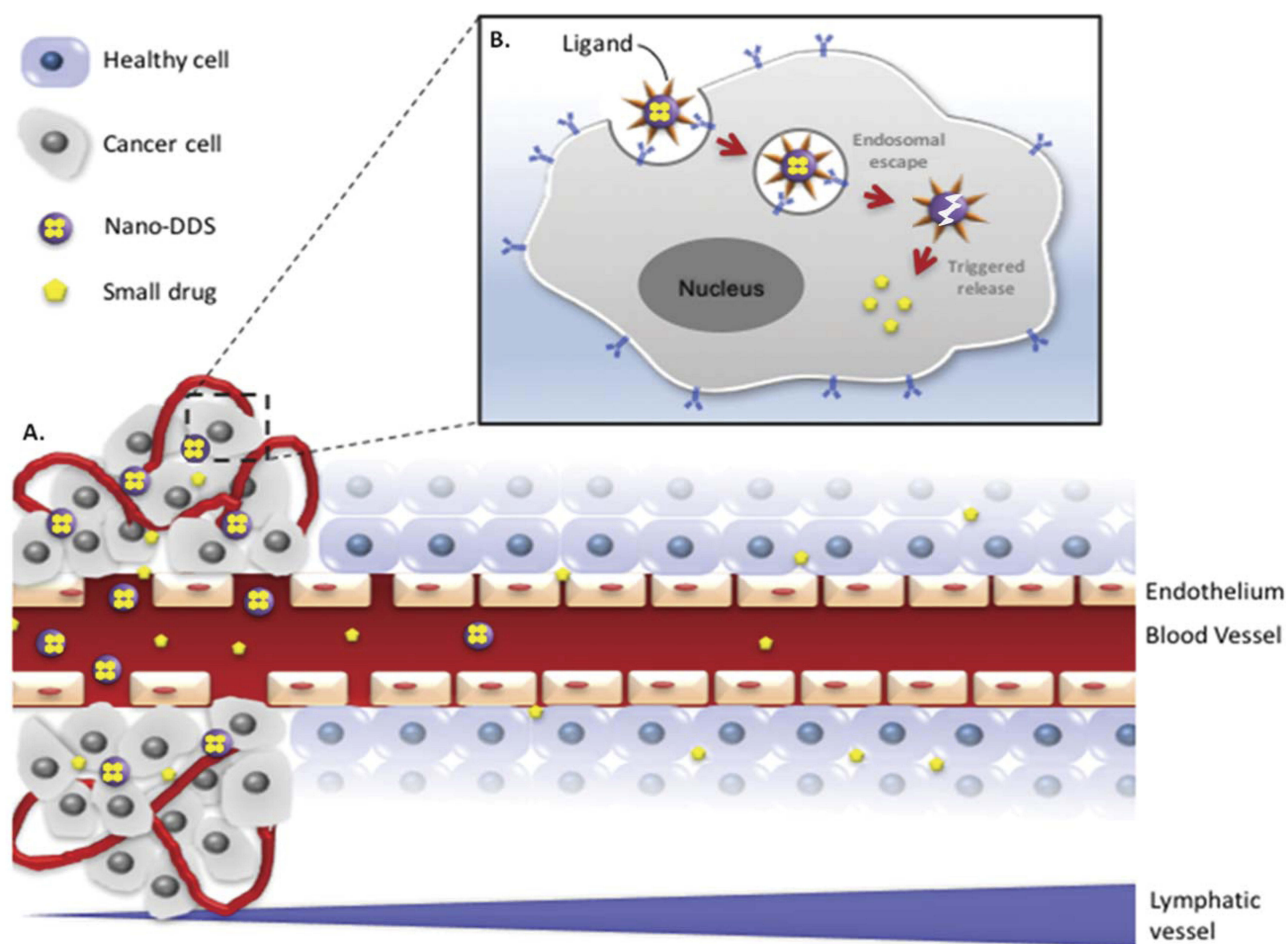
dimensions of fenestrae in tumor blood vessels and the lack of a proper lymphatic system.<sup>29,30</sup> This effect has been referred to as “enhanced permeation and retention effect” (EPR effect).<sup>31–33</sup> The EPR effect in tumor tissues is the consequence of rapid tumor growth. Angiogenesis, the process of growing new blood vessels, is necessary to satisfy the increased oxygen and nutrient demand of fast-growing cancers. Angiogenesis is strictly necessary once the tumor reaches a size of just over 1 mm in diameter to allow for further growth and produces blood vessels with large fenestrae, ie, pores between the endothelial cells.<sup>34</sup> The irregular gaps of the vasculature have been shown to be increased by vascular endothelial growth factor (VEGF) and nitric oxide in the endothelia.<sup>13,35,36</sup> In consequence, NPs can penetrate through the fenestrae into the tumor tissue, whereas tight junctions between healthy endothelial cells do not allow such penetration.

The EPR effect is also being caused by inefficient lymphatic drainage in tumors due to the absence or malfunction of lymphatic vessels.<sup>24,37</sup> This, in consequence, leads to a high interstitial pressure (10–50 mm Hg) and enhanced interstitial space retention of NPs in the tumor.<sup>38</sup> The EPR effect (Figure 1A) was first reported by Matsumura and Maeda in 1986<sup>39</sup> and was validated by Maeda and coworkers.<sup>32</sup> Matsumura and Maeda found that polymer-protein conjugates of approximately 80 kDa  $M_w$  accumulated preferentially in tumor tissues.<sup>39</sup> Therefore, due to their high molecular weight, macromolecules (>40 kDa) can be internalized and retained in the tumors by the EPR effect.<sup>40–42</sup> Many studies show an increment in drug efficacy when the system is designed to utilize the EPR effect.<sup>27,31</sup>

In order to optimize the accumulation of nano-sized drugs in tumor tissues via the EPR effect, one must consider

that the size of gap junctions between endothelial cells in tumor vasculature may vary from 100 to 800 nm<sup>25,29,40</sup> unlike the tight gap junctions between endothelial cells of normal vessels (5–10 nm).<sup>43</sup> The irregularity of “leaky” vasculature varies depending on cancer type, affected organ, and furthermore, from patient-to-patient.<sup>44</sup> Usually, the unique pathophysiological characteristics of the tumor vasculature enable NPs of less than 400 nm to accumulate in the interstitial space in tumor tissues.<sup>17,29,31</sup> Therefore, NPs can accumulate in tumor tissues and deposit anticancer agents passively in the interstitium or cytoplasm of tumor cells after cellular uptake.<sup>40,41,45</sup> In contrast, conventional small anticancer drugs (<10 nm) and microparticles have a limited plasma half-life and do not take advantage of the EPR effect. The DDS size plays a significant role in passive tumor targeting in order to minimize or even overcome the toxic side effects of most current chemotherapies using small cytotoxic drugs. The basic idea is that due to the accumulation of nano-sized DDS in the tumor tissue, the local drug concentration can be higher in the tumor than in other body parts.<sup>36,40,41,46</sup> Many scientists have designed particles in the range of 200 nm for optimum delivery to solid tumors.<sup>47–49</sup>

There are several factors which might limit the EPR effect in tumors. One of the most obvious limitations of passive targeting is that when small molecule drugs are released from DDS in cancer tissues, they can in principle diffuse out of the tumor area and interact with normal tissues. Therefore, it is important that DDS can address issues associated with the low target-site specificity. Ideally, cancer cells should do both: 1) take the DDS up preferentially and 2) the drug should only be released from the delivery device after cellular internalization. Strategies that accomplish this



**Figure 1** Scheme of (A) a free drug (eg, chemotherapy) versus encapsulated drug in a DDS for tumor delivery by passive targeting via the EPR effect and (B) active targeting using a ligand-mediated cellular internalization of the encapsulated drug via receptor-mediated endocytosis. The nanosize of well-designed DDS allows the drug to circulate for a longer period of time in the bloodstream to eventually extravasate and accumulate in the tumor tissue through “leaky” tumor vasculature. Decorating the nanocarriers with targeting ligands allows the specific binding to receptors overexpressed on tumor cells.

require much more intimate knowledge of the specific characteristics of the targeted cancer. In the next sections, we will discuss these more evolved strategies.

To date, hundreds of nano-sized DDS are being researched and tested (in vitro and in vivo) to improve therapeutic and diagnostic outcomes in different types of cancers. At least seven nanoparticle-based cancer therapeutic drugs are approved by the US FDA, four additional nano-sized drugs were approved in Europe and Asia, and there are other formulations in various stages in current clinical studies (Table 2).

Drug delivery by exploiting the EPR effect is certainly an important starting point in improving drug efficiency and thus cancer treatment, but limitations remain with the approach. Foremost, the particle size is by far not the sole determinant of tumor specificity or lack thereof.<sup>33</sup> There are literature data that suggest that surface charge is also

an important factor to consider for DDS tumor accumulation and physiological interactions.<sup>50</sup> Cell membranes have large negatively charged domains, which could explain the commonly reduced cellular adsorption by negatively charged nanoparticles and the effective cellular uptake of positively charged ones. Although a positively charged DDS should increase the therapeutic efficacy in vitro, molecules with a positive charge rapidly bind to vascular endothelial cells, which reduces the in vivo tumor drug accumulation.<sup>31</sup> Likewise, highly negatively charged NPs have been shown to be taken up rapidly by the RES in the liver and spleen.<sup>42</sup> Due to this, it is recommended to create neutral or slightly negatively charged NPs. In addition, the uptake by the target cell can be improved incorporating an active targeting modality (eg, antibodies, ligands, etc.) into the DDS; this approach will be discussed later in this review.

**Table 2** Nanoparticle-Based Cancer Therapeutic Drugs For Clinical Use

Commercial Name	Composition (Drug/ Nanocarrier)	Company (Manufactured/ Distributed)	Type Of Cancer	Year Or Stage Of Approval
<b>US FDA approved</b>				
Doxil (Caelyx)	Doxorubicin HCl/ Pegylated Liposome	Johnson & Johnson	Ovarian cancer, Kaposi sarcoma, multiple myeloma	1995
Gliadel	Carmustine/ Polymeric Wafer	Guilford Pharmaceuticals	Brain tumors	1996
DaunoXome	Daunorubicin Citrate/Liposome	Galen Pharmaceuticals	Kaposi sarcoma	1996
DepoCyt	Cytarabine/Liposome	Pacira Pharmaceuticals	Lymphomatous meningitis	1999
Tocosol	Paclitaxel/Tocopheryl-based Emulsion	Sonus Pharmaceuticals	Urothelial cancer, bladder cancer	2005
Abraxane	Paclitaxel/Albumin-bound Nanoparticles	Celgene Corporation	Metastatic breast cancer, non-small cell lung cancer, adenocarcinoma of the pancreas	2005
Marqibo	Vincristine Sulfate/ Liposome	Talon Therapeutics	Philadelphia chromosome-negative acute lymphoblastic leukemia	2012
Lipoplatin	Cisplatin/Liposome	Regulon	Pancreatic, head and neck, and breast cancer	2012
Onivyde	Irinotecan Liposome	Merrimack Pharmaceutical	Pancreatic cancer	2015
<b>Approved by other countries</b>				
Myocet	Doxorubicin/ Liposome	Elan Pharmaceuticals	Breast cancer	2000-Europe and Canada
Nanoxel	Paclitaxel/Polymeric Micelle	Dabur Pharma	Breast cancer, non-small-cell lung cancer, and ovarian cancer	2006- India
Genexol-PM	Paclitaxel/Polymeric Micelle	Samyang Pharmaceuticals	Breast and small cell lung cancer	2007- South Korea and Europe
Rexin-G	Cytocidal Cyclin G1/ Retroviral Vector	Epeius Biotechnologies Corporation	Sarcoma, osteosarcoma, pancreatic cancer, and other solid tumors	2007-Philippines
<b>Ongoing clinical trials<sup>a</sup></b>				
ThermoDox	PEG-liposome/ Doxorubicin	Celsion	Hepatocellular carcinoma	Phase III/Phase I
Paclical	Paclitaxel Micelles	Oasmia Pharmaceutical	Ovarian cancer	Phase III
Nektar –102	PEGylated Irinotecan Liposome	Nektar Therapeutics	Breast/colorectal cancer	Phase III
Autoimmune Auroshell	TNF- $\alpha$ – Gold NPs Gold Nanoshells	Cytimmune Sciences Nanospectra Biosciences	Head and neck cancer Cancer aurolace therapy	Phase II Phase I
NKTR-105	PEG-Docetaxel	Nektar Therapeutics	Solid tumors	Phase I

**Notes:** <sup>a</sup>This is just a summary of the most important formulations for different types of cancers. There are more drugs in clinical trials.

## PEGylation As The Main Strategy For Increasing DDS Circulation Half-Life

To protect NPs from the RES and increase their circulation half-life, the most commonly used strategy is to conjugate poly-ethylene glycol (PEG) onto the nanoparticle surface to cover any undesired charge or surface properties.<sup>36</sup>

PEGylation is considered one of the best methods to stealth DDS of immunological responses.<sup>51–53</sup> In addition, it has been shown that PEGs increase the solubility, size, molecular mass, and pharmacokinetic and pharmacodynamic properties of drugs.<sup>54–56</sup> For example, Zhang et al encapsulated paclitaxel (PTX) into PEGylated polyphosphoester-based

nanocarriers, increasing the aqueous drug solubility from <2.0 µg/mL to 4.8 mg/mL.<sup>57</sup> In addition, Ma et al showed an increase in the therapeutic efficacy of recombinant human interleukin-11 mutein (mIL-11) when modified with PEG.<sup>55</sup> A single-dose administration of PEGylated mIL-11 was comparable to the effect obtained with 7 to 10 consecutive daily administrations of native mIL-11.

PEG has been utilized to improve DDS based on NPs. Jain et al developed a targeted PEG-siRNA/peptide NP which demonstrated improved biophysical properties, including high serum stability and high cellular uptake.<sup>58</sup> Zhao et al developed a biocompatible VEGF-targeted poly [bis(ε-Lys-PEI)Glut-PEG] (PLEGP) NP for triple-negative breast cancer. The authors determined that PLEGP-1800 was the most effective among others in the DDS construction leading to high cellular uptake, high tumor penetration, and downregulation of VEGF expression.<sup>59</sup> This

study showed how modulating the PEG and polymer content can influence the delivery outcome.

Due to the promising features displayed by PEGylation, some PEGylated protein drugs, summarized in Table 3, have received US FDA approval, including PEG-L-asparaginase (Oncaspar<sup>®</sup>), PEG-interferon (PEGIntron<sup>®</sup>), and PEG-granulocyte colony-stimulating factor (Neulasta<sup>®</sup>).

However, PEGylation can also impart certain disadvantages. In contrast to the general thought that PEGylation inhibits the opsonization process, some published studies have claimed that in their DDS, PEGylation actually accelerates the clearance.<sup>60,61</sup> In another study, Mohamed et al recently reviewed different studies demonstrating an unanticipated immune response to PEGylated NPs after repeated administration and called this “the accelerated blood clearance (ABC) phenomenon”.<sup>62</sup> In a very recent review, Shiraishi and Yokoyama analyzed different results from

**Table 3** FDA-Approved PEGylated Drugs<sup>#</sup>

Product	Trade Name	PEGylated Molecule	PEG Size (kDa)	Disease	Approval Year	Company
PEGadamasase	Adagen <sup>®</sup>	Adenosine deaminase	5	Immunodeficiency	1990	Enzon
PEGaspargase	Oncaspar <sup>®</sup>	L-asparaginase	5	Leukemia	1994	Enzon
PEGinterferon-α2b	PEG-Intron <sup>®</sup>	Interferon-α2b	12	Hepatitis C	2000	Schering-Plough/ Enzon
PEGinterferon-α2a	Pegasys <sup>®</sup>	Interferon-α2a	20	Hepatitis C	2001	Hoffmann-La Roche
PEGfilgrastim	Neulasta <sup>®</sup>	filgrastim	20	Neutropenia	2002	Amgen
PEGvisomant	Somavert <sup>®</sup>	Growth hormone antagonist	5	Acromegaly	2003	Pfizer
PEGaptanib	Macugen <sup>®</sup>	Anti-EVGF aptamer	20	Macular degeneration	2004	Pfizer
Doxorubicin HCl liposome	Doxil/Caely <sup>®</sup>	Liposome of doxorubicin	2	Cancer	2005	Ortho Biotech/ Schering-Plough
Epoetin beta-methoxy PEG	Mircera <sup>®</sup>	Erythropoietin-beta	30	Anemia	2007	Roche
PEG-Certolizumab pegol	Cimzia <sup>®</sup>	Tumor necrosis factor inhibitor	20	Rheumatoid arthritis and Crohn's	2008	Nektar/UCB Pharma
PEGloticase	Krystexxa <sup>®</sup>	Uricase	10	Gout	2010	Savient Pharma
PEGinesatide	Omontys <sup>®</sup>	dimeric peptide	40	Anemia	2012	Affymax/Takeda Pharmaceuticals
PEGinterferon β1a	Plegridy <sup>®</sup>	interferon β1a	12	Multiple sclerosis	2014	Biogen
Naloxegol	Movantik <sup>®</sup>	opioid	0.34	Opioid-induced constipation	2014	AstraZeneca
PEG-growth hormone	Jintrolong <sup>®</sup>	growth hormone	40	Growth deficiency	2014	GeneScience
PEG-antihemophilic Factor VIII	Adynovate <sup>®</sup>	Coagulation factor (VIII)	~2	hemophilia A	2015	Baxalta
Nonacog β pegol	Rebiny <sup>®</sup>	Coagulation factor (IX)	40	hemophilia B	2017	Novo Nordisk
Pegvaliase	Biomarin <sup>®</sup>	phenylalanine NH <sub>3</sub> -lyase	20	Phenylketonuria	2018	BioMarin Pharmaceutica

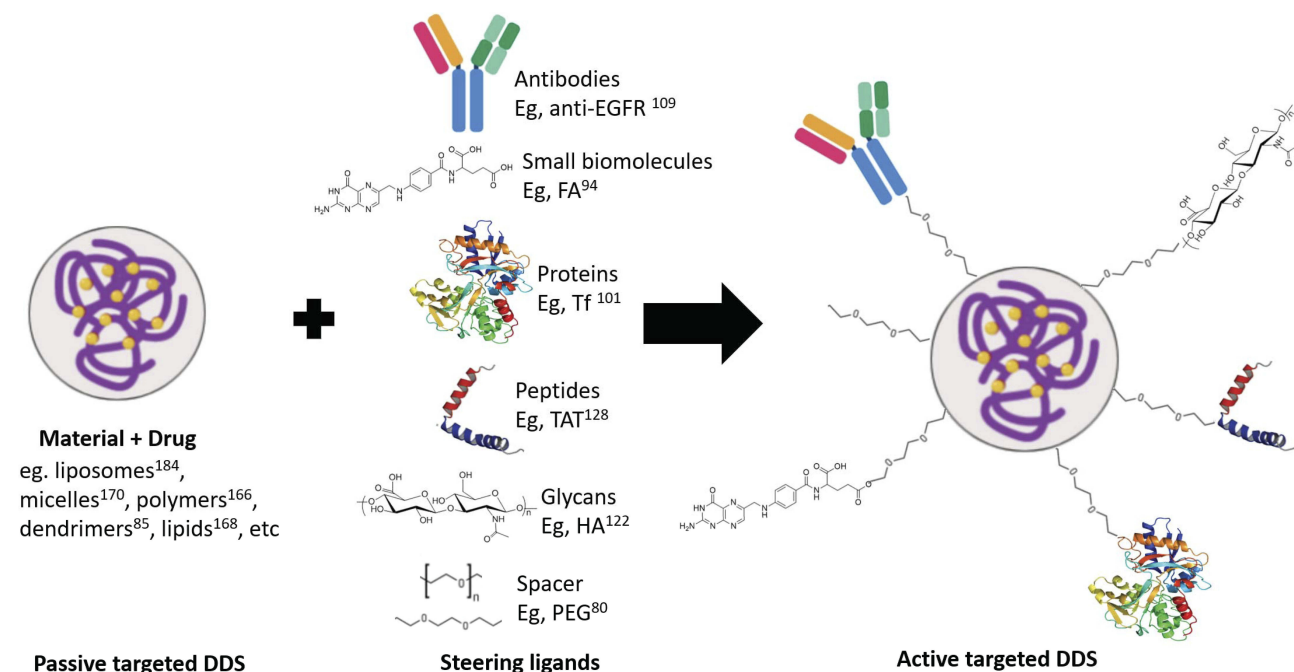
**Note:** <sup>#</sup>All data from publicly available sources.

them and other researchers about the use of PEG and this ABC phenomenon. They concluded that PEG-liposomes and PEG-coupled to nonhuman enzymes easily exhibit the ABC phenomenon, whereas other PEG-NPs do not.<sup>63</sup> These immunogenic responses could be deeply related to inherent characteristics of the polymer that encapsulate the drug in these PEG-conjugates than of the PEG itself. In addition, PEG-modified DDS sometimes display slower uptake into tumor cells which have coined the term “PEG dilemma”.<sup>64</sup> To overcome this, Hatakeyama et al designed a gene delivery system modified with an enzymatically cleavable PEG-lipid which confers higher stability in systemic circulation and higher uptake in tumor cells.<sup>65</sup> Thus, despite of some of the adverse effects PEGylation might cause, the PEG dilemma can be reduced by the using cleavable PEGs and by efficiently conjugating targeting moieties (eg, antibodies, ligands, etc.) to the PEGylated NPs,<sup>66,67</sup> an approach referred to as active targeting discussed later in this review.

The reported disadvantages caused by protein or DDS PEGylation demonstrate the importance to construct and evaluate each system in the desired therapeutic application and optimize it. An improvement in the DDS efficacy in one specific application could cause a drawback in others depending on the core material used, the route of administration, its target, among others.

## Active Targeting For Enhanced Tumor Cell Uptake By Steering Moieties

Tumor cells undergo rapid proliferation and uncontrolled cell growth due to their self-sufficiency in growth signals, insensitivity to anti-growth signals, apoptosis evasion, and sustained angiogenesis.<sup>68</sup> All of these tumor hallmarks have been explored to target cancer cells. One example is the targeting of receptors overexpressed on tumor cells that respond to growth signals or facilitate nutrient uptake for DNA and protein synthesis. Such overexpressed receptors constitute excellent targets for ligands to afford docking and uptake of the delivery device by the targeted cells and thereby promoting intracellular delivery of anti-tumor agents.<sup>29,31,69</sup> DDS are conjugated to targeting moieties to take advantage of ligand–receptor, antigen–antibody, or any other form of molecular recognition.<sup>70–72</sup> The use of this strategy is called “active targeting”. Active tumor targeting (Figure 1B) results in more specific secondary targeting after primary targeting based on the EPR effect takes place (Figure 1A). It has been suggested that for active targeting to be effective, passive targeting must be attained first.<sup>73</sup> While the EPR effect is considered a common phenomenon in many tumor tissues, the nature of the specific receptor overexpressed on a tumor cell is an event depending on the



**Figure 2** From passive to active targeting by the attachment of steering molecules to the surface of the NPs for molecular recognition by cancer cell membrane.  
**Abbreviations:** FA, folic acid; HA, hyaluronic acid; Tf, transferrin; EGF, epidermal growth factor.

specific type of tumor.<sup>74–76</sup> The moiety ligand selected (Figure 2) for the active targeting must be based on the specific cell surface properties, ie, membrane overexpressed targets of the tumor cell. To obtain an optimum and specific DDS involving active targeting, the selected targeting moiety (eg, ligand) must preferentially bind to the target receptor overexpressed by tumor cells and the target receptor must be homogeneously expressed on all target cells. Also, this targeted molecule on the membrane, ie, receptor, must be promptly recycled back on the cell membrane after uptake and mostly not released into circulation.

It is relatively straightforward to investigate which receptors are overexpressed in a certain tumor by employing ligand-decorated chromophores for imaging.<sup>77</sup> The same ligand can then be used to facilitate drug targeting to the tumor. Table 4 shows some examples for tumor-receptor specific delivery via small molecule ligands, such as folic acid (FA), sugar residues (eg, hyaluronic acid (HA)), peptides (eg, RGD), and proteins (eg, cytokines, lectins, and transferrin (Tf)). Other strategies include the release of a small therapeutic cargo in the tumor area or the reduction of the nanoparticle size mediated by some tumor microenvironment stimulus after nanoparticle accumulation near the blood vessels.<sup>78</sup>

Because active targeting can promote receptor-mediated endocytosis for increased cellular uptake, it could help overcome the reduced or slower uptake caused by PEGylation. PEGylation of DDS NPs employing carefully controlled

conditions (eg, PEG chain length, shape, density, molecular weight) and incorporation of different targeting ligands are emerging as promising in DDS development for anticancer therapy.<sup>53,67,76,79,80</sup> Bao et al designed a novel DDS consisting of the chemotherapeutic agent daunorubicin (DNR) loaded into poly(lactic-co-glycolic acid) (PLGA)-poly-L-lysine (PLL)-PEG-Tf NPs.<sup>81</sup> Results showed that DNR-loaded NPs increased the intracellular concentration of DNR in K562 cells in vitro and induced a remarkable improvement in anticancer activity in vivo. Cruz et al covalently linked PEG molecules of various chain lengths (Mw 2,000 to 20,000 g/mol) to PLGA NP vaccines coated with various antibodies recognizing the dendritic cell-specific receptor DC-SIGN.<sup>82</sup> These DDS were designed to study the effects of shielding on antibody–receptor interactions. Results demonstrate that binding and uptake of NPs by human DCs were affected by PEG chain length, showing that NPs coated with PEG-3,000 had the optimal chain length for antibody–receptor interactions and induction of antigen-specific T-cell responses. In another study, Su et al generated bispecific PEG-binding antibodies (PEG engagers) for targeted delivery of PEGylated nanomedicines to tumors.<sup>83</sup> Their experiments demonstrate that pre-targeting of PEG engagers allowed subsequent accumulation and endocytosis of PEGylated nanocarriers in tumors, leading to enhanced antitumor efficacy of PEG-modified therapeutic agents in vitro and in vivo.

**Table 4** Examples Of Ligands For Active Tumor Targeting

Ligand	Targeted Membrane Molecule	Cancer With Frequent Expression	References
FA	FAR <sup>a</sup>	High expression: ovarian, uterus, testicular, lung, brain, and pituitary cancers. Variable expression levels: breast, colon, and renal tumors.	89–97
HA	HA-binding receptors (eg, CD44 and RHAMM)	Epithelial, ovarian, colon, stomach, and acute leukemia tumor.	116–125
Exposed RGD tripeptide proteins	$\alpha\text{v}\beta\text{3}$ integrin receptor <sup>b</sup>	Melanoma, glioma, pancreatic, prostate, ovarian, cervical, and breast cancer.	71
Cytokines	Cytokine-binding receptors (eg, IL-2, IL-4, and IL-13 receptors)	Cutaneous T-cell lymphoma, renal cell carcinoma, glioblastoma, Kaposi's sarcoma, ovarian, head and neck, and prostate cancer.	203,204
Lectin	Lectin-binding glycoproteins (eg, P-glycoprotein)	Pancreas, kidney, ovarian, and breast cancer.	112,115,205
Tf	TfR	Ovarian, lung, colon, and brain cancer.	99–104,206
EGF, EGF-like ligands, TGF- $\alpha$ , and HRGs.	EGFR	Colon, lung, head, neck, ovarian, kidney, pancreatic, and prostate cancer, and especially in breast cancer.	106–111,207
VEGF	VEGFR-1 and VEGFR-2 <sup>c</sup>	Breast, colon, lung, gastric, renal, and oropharyngeal cancers.	207,208

**Notes:** <sup>a</sup>Present in normal lung, brain, and kidney cells are inaccessible to the NPs via i.v. route. <sup>b</sup>Upregulated in both tumor cells and angiogenic endothelial cells. <sup>c</sup>Upregulated in tumoral endothelium.

While ligand-selection requires specific knowledge of the type of receptor overexpressed, another type of macromolecules, aptamers, does not require such knowledge. Aptamers are nucleotides that bind to cell surface groups and can be selected from vast libraries and also allow for recursive optimization by mutational approaches.<sup>84</sup> Similar to antibodies, they can be directed towards a wide range of targets and be useful for personalized medicine.<sup>85</sup> While this potentially offers huge advantages, it is a tricky type of targeting because, in principle, for each patient, one or more aptamers have to be selected and it has to be assured that no unwanted cells are targeted. In how far this can be made clinically relevant remains to be seen.

It is important to mention that the aim to incorporate different targeting ligands on DDS is to increase the targetability to cancer cells, not cytotoxicity. Nevertheless, when the targetability is enhanced, this results in an increase of the intracellular drug concentration thus simultaneously enhancing therapeutic efficacy.<sup>86</sup>

## Membrane Overexpressed Targets

### Folic Acid Receptor (FAR)

Folic acid (FA) is one of the most frequently used tumor-targeting ligands in DDS because the FAR is a well-known tumor marker and the conjugation chemistry to therapeutic and imaging agents is well characterized.<sup>37,87–93</sup> FA is important in the formation of new cells because it is required in one carbon metabolic reactions and consequently is essential for the synthesis of nucleotide bases.<sup>94</sup> In fact, FARs are a family of 35–40 kDa glycoproteins which can be divided into four different isoforms (FAR $\alpha$ , FAR $\beta$ , FAR $\gamma$ , and FAR $\delta$ ). Very few places in the body express the FAR and these are typically not targeted with nanosized DDS.<sup>95</sup> In contrast, FAR- $\alpha$  and FAR $\beta$  have been overexpressed in lots of cancer cells, ie, ovarian, breast, lung, bladder, pancreas, prostate, colon, and kidney.<sup>96</sup> Overall, 40% of the tumors showed FAR $\alpha$  and 25% showed FAR $\beta$  expression. In addition, FAR $\beta$  is also highly expressed in activated myeloid cells of the immune system.<sup>97</sup> This makes FA an attractive molecule to target cancer and inflammatory/autoimmune diseases. Due to the FAR overexpression in different cancer types, Morales-Cruz et al designed a smart nanosized DDS of cytochrome c modified with PEG-FA. They confirmed the FAR targeting, selective internalization, and cytotoxicity of the system on FAR-positive HeLa cells compared to FAR deficient cell lines. This DDS also showed great potential on an in vivo tumor model.<sup>98</sup>

### Transferrin (Tf) Receptor

The transferrin receptor (TfR) is a cell-membrane-associated glycoprotein involved in the cellular uptake of holo-Tf (iron-bound to Tf) and the regulation of cell growth.<sup>99,100</sup> Transferrin is a plasma protein responsible to transport iron to proliferating cells. Therefore, various studies have shown elevated levels of TfR particularly in lung, ovarian, colon, and brain cancer.<sup>101</sup> Due to this, Tf is an important potential ligand in the active targeting of tumors. Thus, there are some promising results of TfR-targeted drugs against different types of tumors showing strong cytotoxic effects.<sup>37,102–104</sup> For example, Saxena et al developed a DDS of a Tf-modified cytochrome c to target TfR on A549 lung cancer cells. The results suggest that this conjugate design would be effective in cancers with enhanced expression of TfR due to its nontoxic pattern to lung normal cells.<sup>105</sup> These outcomes make TfR an important molecular target in the study of more effective cancer therapies.

### Epidermal Growth Factor Receptor (EGFR)

The EGFR is a member of the family of tyrosine kinase receptors (also known as the HER family).<sup>106,107</sup> In humans, more than 30 ligands have been identified that bind to EGFR, including EGF and EGF-like ligands, transforming growth factor (TGF)- $\alpha$ , and heregulins (HRGs, also known as neuregulins).<sup>74</sup> EGFR is activated upon ligand-binding stimulating key processes involved in tumor growth and progression, including proliferation, differentiation, angiogenesis, cell motility, metastatic spread, and survival.<sup>74,107</sup> EGFR is frequently overexpressed in many different cancers, including colon, lung, head, neck, ovarian, kidney, pancreatic, prostate, and especially breast cancer.<sup>108</sup> Based on this, there are two classes of anti-EGFR agents that are currently approved for the treatment of cancer: monoclonal antibodies directed at the extracellular domain of the receptor and competitive inhibitors of its tyrosine kinase. Anti-EGFR and anti-VEGFR monoclonal antibodies are mostly employed to play the role of both, targeting ligand and drug (eg, Herceptin<sup>®</sup>).<sup>109</sup> Recently, several drugs binding to EGFR have received FDA approval (ie, Kisqali<sup>®</sup> and Nerlynx<sup>®</sup>) (Table 5). Taking advantage of active targeting based on antigen-antibody recognition to promote the intracellular delivery of drugs, BESPONSA<sup>®</sup> has been designed and approved by the FDA in 2017 (Table 5). BESPONSA<sup>®</sup> (inotuzumab ozogamicin) is composed of a monoclonal antibody linked to the cytotoxic agent calicheamicin. Its mechanism of action is based on targeting of the CD22 antigen, a cell

**Table 5** Active-Targeted Drugs To Treat Different Cancer Types Approved By FDA In 2017 And 2018 (cancer.org)<sup>a</sup>

Product	Trade Name	Mechanism Of Action	Type Of Cancer	Company
Ribociclibactive	Kisqali <sup>®</sup>	Cyclin-dependent kinase inhibitor	Breast cancer	Novartis
Neratinib	Nerlynx <sup>®</sup>	Kinase inhibitor	Breast cancer	Puma Biotechnology
Midostaurin	Rydapt <sup>®</sup>	Kinase inhibitor	Acute myeloid leukemia	Novartis Pharmaceuticals/ Invivoscribe Technologies
Inotuzumab ozogamicin	Besponsa <sup>®</sup>	Antibody-drug conjugate	Acute lymphoblastic leukemia	Pfizer
Dabrafenib/Trametinib combination	Tafinlar <sup>®</sup> / Mekinist <sup>®</sup>	Kinase inhibitor	Anaplastic thyroid cancer	Novartis
Acalabrutinib	Calquence <sup>®</sup>	Bruton tyrosine kinase inhibitor	Mantle cell lymphoma	AstraZeneca
Niraparib	Zejula <sup>®</sup>	Poly ADP ribose polymerase inhibitor	Ovarian cancer	Tesaro
Apalutamide	Erleada <sup>®</sup>	Androgens hormone inhibitor	Prostate cancer	Janssen
Lutetium Lu 177- dotatate	Lutathera <sup>®</sup>	Peptide receptor radionuclide therapy	Gastroentero-pancreatic neuroendocrine tumors	Advanced Accelerator Applications
Paclitaxel poliglumex	Xyotax <sup>®</sup>	Protease-reactive lysosomal degradation	Lung, breast, and ovarian cancer with high levels of estrogen	Cell Therapeutics

**Notes:** <sup>a</sup>These drugs are not nanosized or encapsulated in a drug delivery system. Except Xyotax<sup>®</sup>, a macromolecular taxane.

surface antigen expressed on cancer cells in almost all B-cell precursor acute lymphoblastic leukemia patients. Once attached to the CD22 antigen, the system is internalized by the cell and the cytotoxic agent released causing cell death. Additional examples in clinical trials of humanized anti-EGFR are necitumumab, zalutumumab, and nimotuzumab.<sup>37,110</sup> In a preclinical in vivo study, Hartimath et al developed a PEGylated nimotuzumab DDS using maytansine as the drug. Their results showed higher cellular internalization due to the PEGylation versus the non-PEGylated system. However, the PEGylation has to be maintained at a low ratio (ie, ~1:3 nimotuzumab-to-PEG molar ratio) to avoid the disruption to the EGFR affinity.<sup>111</sup>

### Lectins

Lectins are proteins of non-immunological origin which preferentially recognize and bind certain carbohydrates attached to glycoproteins.<sup>112</sup> Carbohydrate-lectin interaction-based systems have been developed to target cancer cells.<sup>37,40</sup> The interaction of lectins with particular carbohydrates can be as specific as the interaction between those of antigen and antibody or substrate and enzyme. Therefore, lectins are used for the detection of glycoproteins on cell surfaces. Lectins mediate biological processes, such as tumor cell survival, adhesion to endothelium, or extracellular matrix, as well as tumor vascularization and

other processes that are crucial for metastatic spread and growth.<sup>112</sup> Cancer cells often express glycoproteins that are different to those of normal cells. For example, P-glycoprotein is widely expressed in many human cancers including those of the liver, pancreas, kidney, ovary, and breast.<sup>113</sup> P-glycoprotein is one of the molecules responsible for the MDR syndrome in many types of cancer.<sup>113,114</sup> However, if these glycoproteins could be specifically targeted, treatment would consequently be more effective. Shimomura et al conducted in vivo experiments and demonstrated how lectin could be used for active targeting and as a drug carrier in the treatment of pancreatic ductal adenocarcinoma.<sup>115</sup>

### Hyaluronic Acid (HA) Receptor

HA is a linear, negatively charged polysaccharide, containing two alternating units of D-glucuronic acid and N-acetyl-D-glucosamine with a molecular weight range from 105 kDa up to 1 MDa. HA is distributed widely throughout connective, epithelial, and neural tissues. As one of the principal components of the extracellular matrix, HA is responsible for various functions within it, such as cell growth, differentiation, and migration.<sup>116</sup> HA is the main ligand for the CD44 receptor, a multifunctional cell surface glycoprotein.<sup>117</sup> It is well known that various tumors, such as epithelial, ovarian, colon, stomach, and acute leukemia, overexpress this receptor.<sup>118,119</sup>

Furthermore, CD44 expression is upregulated in important subpopulations of cancer cells and is recognized as a molecular marker for cancer stem cells.<sup>120,121</sup> This makes HA a key molecule in the development of novel cancer therapies, due to there are no drugs able to kill cancer stem cells. Consequently, various anticancer therapeutics have been linked or modified with HA to increase their cellular uptake and therefore their pharmacokinetic effect on cancer cells.<sup>122,123</sup> For example, Figueroa et al showed how pro-apoptotic protein-HA bioconjugates can reduce at least 3-fold the cell viability of CD44 overexpressing cancer cells versus normal cells.<sup>124</sup> The ONCOFID™-S bioconjugate, which links the anticancer drug irinotecan and HA, showed a significant increment in drug efficacy in vitro and improved mice survival in ovarian cancer.<sup>125</sup>

### Cell-Penetrating Peptides (CPP)

CPPs (also known as tumor-penetrating peptides) are promising to target the nanocarriers to specific cells and penetrate cells. CPPs can be attached to the nanocarrier surface conferring the potential to translocate across the membrane via micropinocytosis.<sup>126,127</sup> CPPs are normally short peptides of 1 to 5 kDa of less than 20 basic amino acids and can be attached to >30 kDa cargoes (eg, TAT).<sup>128</sup> CPP-based drug strategies are considered a form of subcellular targeting due to preferential accumulation in the cell nuclei and sub-nuclear regions.<sup>129</sup> It has been shown that CPPs are effectively internalized in lung, breast, and heart cancer cells.<sup>126–130</sup> Hsieh et al synthesized a TAT-derived peptide that specifically targets the  $\beta$ -catenin signaling in breast cancer, resulting in cell growth inhibition.<sup>130</sup> El-Sayed et al reported the conjugation of Paclitaxel and Camptothecin to a cyclic CPP containing tryptophan and arginine residues.<sup>131</sup> Results revealed that the antiproliferative activities of the CPP-drug conjugates were less than that of the free hydrophobic drugs in the breast cancer cell line MCF-7 after 72-hr incubation. Lastly, the modification of DDS with CPP (eg, RGD peptide) made NPs capable of penetrating deeper into tumor tissue, resulting in several-fold higher therapeutic efficacy indices.<sup>71,72</sup>

Considering the targeting of CD44 receptor again, several peptides sharing sequence homology with the HA-binding domain of CD44 have been discovered for treatment and diagnosis of cancers.<sup>132–134</sup> For example, it was found that the RP-1 peptide showed affinity and specificity to the HA-binding domain of CD44. The results demonstrate the potential of RP-1 peptide to detect gastric

cancer.<sup>132,133</sup> Furthermore, studies have shown that binding of A6-peptide to the HA-binding domain of CD44 results in the inhibition of migration, invasion, and metastasis of tumor cells, and the modulation of CD44-mediated cell signaling.<sup>134</sup>

## Protein And Peptide-Based Therapeutics For Anticancer Targeting

In general, it has been shown that when DDS are modified with any of these ligands (eg, FA, HA, Tf, CPPs, and anti-EGFR) (Figure 2), therapeutic performances are improved in cellular and animal models when compared to their non-targeted counterpart. Furthermore, the use of proteins as drug carriers (as mentioned in the section “Passive targeting for enhanced drug tumor accumulation”), as targeted ligands (as discussed in the section “Membrane overexpressed targets”), or as therapeutic agents has had great impact in the development of nanomedicines for cancer therapies. The high chemical specificity, biodegradability, low toxicity, non-antigenicity, and non-mutagenic character of proteins and peptides make them attractive substitutes of small cytotoxic drugs.<sup>135,136</sup> Highlighting this is the fact that since the early 1980s, a total of 239 therapeutic proteins and peptides have been approved for clinical use by US FDA.<sup>136,137</sup>

However, the potential of proteins has not been translated into successful clinical trials most of the time. At this point, Abraxane® is the only nanosized protein-based formulation currently approved by the FDA taking advantage of both, passive and active targeting mechanisms to treat cancer. Its mechanism of action is based on the high affinity of SA to the Gp60 receptor in endothelial cell walls.<sup>85</sup> The use of human SA as drug carrier is based on the fact that SA is an important carrier of endogenous and exogenous hydrophobic molecules in the human circulation.<sup>138</sup> Studies have demonstrated that the SA-bound paclitaxel formulation differs favorably from unbound paclitaxel in terms of tumor accumulation of SA, patient toleration, response rate, and extends the time to tumor progression.<sup>139</sup> Besides Abraxane, an active-targeted NP, Rexin-G, is commercially available outside of the USA (Table 1) and there are other drug nanocarriers that incorporate an active targeting ligand in clinical trials (eg, HA-Irinotecan in phase III and RGD-PET-CT in phase II).<sup>71,140–142</sup> Indeed, a small ligand-targeted therapeutic agent avoids deficiencies in tumor penetration sometimes shown by bigger nanoparticles (>100 nm) to cells deep within a tumor mass which can be

dense and not well supported by blood vessels.<sup>79,143</sup> Also, the anti-metabolite methotrexate covalently bound to human SA has been evaluated, demonstrating its great potential as carrier for targeted delivery of antitumor drugs.<sup>144</sup>

Therapeutic peptides offer several advantages over protein-based therapeutics, such as less immunogenicity, lower manufacturing costs, and easier synthesis due to their simpler linear structures.<sup>145</sup> A total of 60 peptide-based drugs are already on the market and several other therapeutic peptides are currently being evaluated in different phases of clinical trials.<sup>137</sup>

Furthermore, it has also been shown that peptides can be an alternative as drug delivery vehicles. Peptide–drug conjugates (PDCs), which are usually composed of the peptide, the small molecule drug, and a cleavable or non-cleavable linker, are an emerging technology that has resulted in many PDCs in the developmental phase of preclinical and clinical studies.<sup>146</sup> PDCs have yet to get marketing approval from regulatory agencies.<sup>146</sup> Zoptarelin doxorubicin, consisting of doxorubicin linked to a small peptide agonist to the luteinizing hormone-releasing hormone (LHRH) receptor, has been evaluated in phase 3 clinical trials in a number of human cancers.<sup>147</sup> GRN1005 is a PDC composed of paclitaxel covalently linked to a peptide, angiopep-2, that targets the low-density lipoprotein receptor-related protein 1. This conjugate is in clinical trials for the treatment of advanced solid tumors in patients with brain metastases.<sup>148</sup>

A number of cancer-targeting PDCs, such as camptothecin–somatostatin conjugate,<sup>149</sup> camptothecin–bombesin conjugate,<sup>150,151</sup> H2009.1 peptide–polyglutamic acid–doxorubicin conjugate,<sup>152</sup> cyclic RGD peptide–paclitaxel conjugate,<sup>153</sup> RGD-4C peptide–doxorubicin conjugate,<sup>154,155</sup> aptide–SN38 conjugate,<sup>156</sup> and aptide–docetaxel conjugate,<sup>157</sup> have been developed and are being explored in targeted cancer therapy. Tai et al prepared a PDC consisting of HER2-specific peptide conjugated to TGX-221, a potent inhibitor of PI3K $\beta$ , a lipid enzyme that has been found to be over-activated in human cancers, such as prostate cancer.<sup>158</sup> This PDC exhibited a much higher cellular uptake in prostate cancer cells in comparison to the parent drug, indicating its tremendous potential as a targeted therapy for prostate cancer patients.

Although PEGylation has been used to obtain a PDC with an extended half-life, their efficient tumor penetration and cost-effectiveness were adversely affected.<sup>159</sup> Due to the need to develop a new approach that can significantly

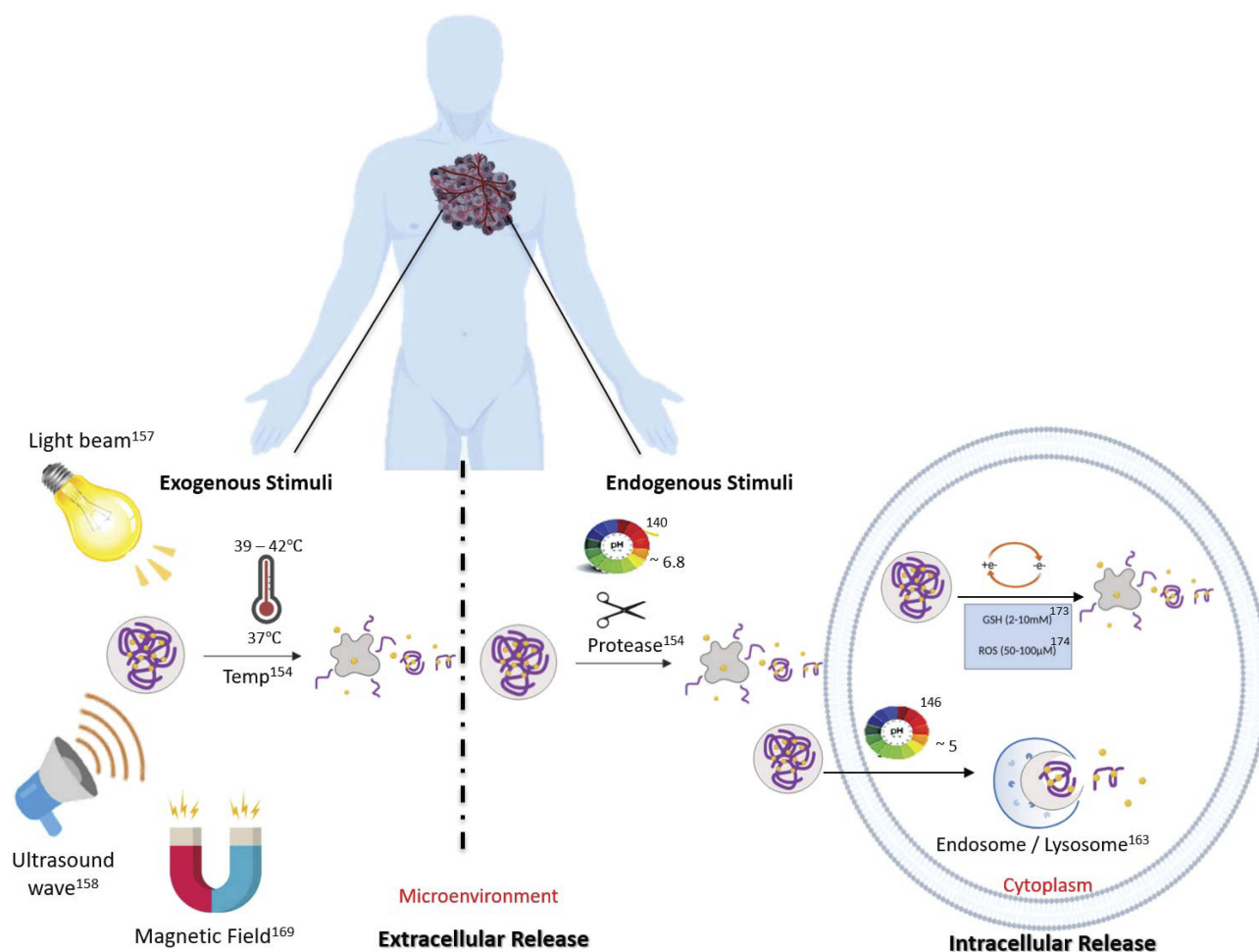
extend the half-life of PDCs while retaining the expected tumor-penetrating ability associated with the small size of PDCs, Kim et al studied the delivery of a PDC assisted by an antibody.<sup>160</sup> They showed that the conjugation of a cotinine-labeled PDC with an anticotinine antibody resulted in a therapeutic formulation with a significantly extended circulation half-life in blood, greater penetration and accumulation within the tumor, and, ultimately, inhibition of tumor growth. It has been demonstrated that due to the low manufacturing costs, excellent cell permeability, and a high drug-loading capability exhibited by PDCs, their development is promising for the targeted delivery of chemotherapy drugs.

## Stimulus-Responsive Targeting For Specific Release

An additional smart targeting strategy that takes advantage of specific characteristics of the chemical environments of the tumor interstitium or the cell interior consists in development of stimulus-responsive DDS. The DDS is designed to release or activate the drug only when triggered by specific internal or external stimuli<sup>161</sup> (this is also known as tertiary targeting). Such stimuli include pH, proteases, redox potential, heat and light, among others (Figure 3).

### Internal Stimuli

Since many cancer cells generate energy exclusively by glycolysis and are deprived of oxygen, the extracellular matrix of tumor sites frequently has a lower pH compared to normal tissues. The extracellular matrices of tumor sites frequently have an approximate pH range from 6.5 to 7.0, whereas in normal tissues and blood the typical pH is around 7.4.<sup>162,163</sup> These circumstances of cell metabolism abnormalities and resulting low pH values are known as the Warburg effect.<sup>163,164</sup> Exploiting the pH gradient between normal tissues and the tumor microenvironment to afford external drug release, and the low pH in cellular endosomes (~pH 5) to afford intracellular drug release, pH-sensitive NP systems have been designed to facilitate the release of anticancer drugs in a pH-controlled manner.<sup>165–168</sup> One release mechanism is a change in the ionization of acidic or basic groups in the NP upon exposure to acidic environments. The resulting electrostatic repulsion causes the particle to swell, releasing the encapsulated drug. Studies published by Zhao et al showed that their designed PEGylated pH-sensitive NPs were long circulated in blood and highly phagocytosed by tumor cells.<sup>169</sup> This delivery



**Figure 3** Scheme of the types of stimuli-responsive strategies utilized in the development of DDS. Drug release can in principle occur in the extracellular microenvironment or directly in the cell cytoplasm.

strategy resulted in several advantages including prolonged circulation, accumulation in tumors, highly efficient cellular internalization, and rapid intracellular drug release.<sup>170</sup>

The extracellular release of small drugs from DDS avoids some tumor penetration problems of nanosized DDS when tumors are very dense.<sup>24,170</sup> However, the main problem with using extracellular stimuli is that non-specific drug leakage is likely to occur (ie, after being released small-molecule drugs can exit the tumor since they are not trapped in the interstitium). Intracellular drug release overcomes this drawback, but it is required that the DDS is taken up by the cancer cells, which typically is accomplished by decoration with receptor-targeting ligands as discussed earlier. FAR-targeted-doxorubicin-loaded polymeric micelles are one example of using intracellular stimuli once the DDS are endocytosed by the cells.<sup>167</sup> The resulting endosome acidifies as it moves towards the lysosomes where the pH can be as low as 4.

These DDS are optimized to release their drug only at a low pH. PH-sensitive nanocarriers can be constructed to be able to sense small changes in the microenvironment pH as well as the lower pH in endosomes or lysosomes.<sup>170</sup> Zhou et al reported a pH-sensitive PEG-doxorubicin stable at pH 7.4 and activated at an endosomal acidic pH with increased circulation time and drug accumulation compared with free doxorubicin.<sup>171</sup>

Differences in the redox potential between extra- and intracellular spaces provide another opportunity for the development of a stimulus-responsive DDS. The cell cytoplasm is significantly more reducing than the outside of the cell, with a concentration of reducing species, mainly glutathione (GHS), as much as 1000 times higher.<sup>172,173</sup> DDS that are redox-activated will exhibit intracellular drug release or activation.<sup>174</sup> Therefore, they are only cytotoxic to the cells that internalize them. Redox-responsive DDS can be generated by using thiol-cleavable crosslinkers,

which will be cleaved upon reduction by GHS. Such systems have been used to trigger the activation of pro-apoptotic proteins (eg, cytochrome c and caspase-3).<sup>98,175,176</sup> Recently, a system composed of a Cyt c NPs stabilized by an amphiphilic copolymer through a redox-sensitive bond (FA-PEG-PLGA-S-S-Cyt c NPs) exhibited excellent stability under extracellular physiological conditions (1  $\mu$ M GHS), whereas once in the intracellular reducing environment (10 mM GHS) Cyt c was released from the DDS.<sup>177</sup> Also, smart release nanosized (passive targeting) DDS combining HA-based active and redox-responsive targeting can release up to 92% of its payload once experiencing the characteristic reductive conditions of the intracellular space. Once the NPs are internalized, they can reduce the cell viability by up to 80%.<sup>178</sup>

Another characteristic of the interstitium of tumors is that it is frequently rich in proteases (eg, membrane-type matrix metalloproteinase, MMP) and thus protease-sensitive DDS (eg, peptide-based DDS) have been developed as well. Proteases play a key role in many processes in the human body, such as cell cycle, tissue remodeling, and homeostasis. However, upregulated proteolysis is a typical hallmark of the tumor microenvironment.<sup>179</sup> Graeser et al target the prostate-specific antigen, which is a protease overexpressed in prostate cancer, with a SA-bound doxorubicin DDS. Then, they showed that the formulation releases the doxorubicin drug effectively due to the cleavage of the SA by the protease. Interestingly, they demonstrated that even when the SA-bound doxorubicin DDS was ~100-fold less active than the free doxorubicin in *in vitro* experiments, in an *in vivo* model of human prostate tumor, the DDS showed greater efficacy compared to the free doxorubicin.<sup>180</sup> In the clinical context, Xyotax<sup>®</sup> is a pioneer protease-reactive drug in which its activity is dependent on the degradation of the poly-(L)-glutamic acid to release the paclitaxel drug.<sup>181</sup>

## External Stimuli

External stimuli, such as heat, ultrasound, and light have also been exploited to afford the specific release or activation of drugs at a specific time and location. ThermoDox<sup>®</sup>, a heat-activated doxorubicin liposome, is an example of a stimulus-responsive nanomedicine in Phase III of clinical trials.<sup>182,183</sup> In a recent study, the trial demonstrated that the ThermoDox<sup>®</sup>, designed to release targeted levels of doxorubicin into and around tumors with heat and ultrasound activation, increased doxorubicin delivery to tumors

in the majority of liver cancer patients in a 10-patient trial.<sup>184</sup>

In addition, photodynamic therapy (PDT) is a novel noninvasive medical application that utilizes a photosensitizing agent (PS) to kill pathological cells. The PS is a chromophore normally derived from porphyrins which absorbs light and then produces reactive oxygen species (ROS) that kill targeted cells.<sup>183</sup> After 1–2 days of PS administration, the tumor area is exposed to light to kill affected cells.<sup>185</sup> This photosensitizing agent could take up to 48 to 96 hrs to successfully reach cancer cells and its body elimination up to 50 days.<sup>186</sup> For example, patients treated with Photofrin<sup>®</sup> should avoid light for at least 1 month after administration and home equipment that emits heat (eg, hairdryer). Another limitation of this treatment is the unspecific accumulation of PS in healthy tissues near to pathological areas thus inducing adverse side effects, such as burns, swelling, and pain during treatment.<sup>186–188</sup> To overcome this, the PS is conjugated to a DDS to increase the selectivity for tumor tissues over healthy tissues.<sup>189–191</sup> Positive effects include accumulation of the PS in the tumor by the EPR effect and reduction of unwanted radical formation by the PS due to self-quenching prior to DDS disintegration.<sup>192,193</sup> Internal and external stimuli can be combined within a single system to reduce self-quenching of the drug and produce the therapeutic effect preferentially at the tumor site.<sup>194</sup>

## Conclusion

The present review discusses recent advances in smart-targeted delivery systems, including the use of NPs. Although stimulus-responsive strategies add tumor specificity to DDS, it is important to note that only a nanosized carrier increases the chances of the drug reaching the tumor tissue per se (See Figure 1). Both, active targeting and stimulus-responsive strategies are more efficient if the drug accumulates in the tumor tissue and is not distributed evenly throughout the body and/or eliminated from the bloodstream via renal or hepatic pathways. However, drug accumulation in the tumor tissue per se is not enough if the drug is released before reaching the target site or cannot enter the tumor cells. In summary, combining the use of passive targeting with additional targeting strategies such as active targeting and employing stimuli-responsive chemistry can potentially enhance the DDS selectivity towards cancer tissues and improve the overall therapeutic index.

Even though nanomedicine is a young science, it has demonstrated the potential to increase the therapeutic

efficacy in cancer therapy when compared with the conventional chemo- and radiation-therapy by the exploitation of smart targeting. Passive targeting can significantly contribute to diminish toxic side effects by accumulation of the NPs in the tumor area taking advantage of the EPR effect. Active targeting takes advantage of different molecules overexpressed in the tumor cell to design selective NPs-based DDS that recognize the specific target. The use of stimulus-responsive DDS can prevent the premature systemic release of the drug by the design of a biologically stable NP until exposure to a specific stimulus in the tumor or cellular site. These relatively new potential nanomedicines expose the possible multifunctionality of DDS designed using different smart targeting strategies. In the future, multidrug-loaded delivery systems incorporating passive, active, and stimulus-responsive targeting, alone or in combination with chemotherapy will become a powerful tool. Carefully designed and optimized DDS will enhance the therapeutic index, reduce side effects, avoid drug resistance, and increase patient compliance and survival.

## Abbreviations

ABC phenomenon, accelerated blood clearance; BBB, blood–brain barrier; CPP, cell-penetrating peptide; Cyt C, cytochrome c; MDRS, multidrug resistance syndrome; DDS, drug delivery system; DNR, daunorubicin; EGF, epidermal growth factor; EPR, enhanced permeation and retention effect; FA, folic acid; FAR, folic acid receptor; GHS, glutathione; HA, hyaluronic acid; HRG, heregulins; IL, interleukin; LHRH, luteinizing hormone-releasing hormone; MMP, membrane-type matrix metalloproteinase; NP, nanoparticle; PDC, peptide–drug conjugate; PDT, photodynamic therapy; PEG, poly(ethylene glycol); PLGA, poly(lactic-co-glycolic) acid; PLL, poly-L-lysine; PS, photosensitizing agent; PTX, paclitaxel; RES, reticuloendothelial system; RGD peptide, Arg-Gly-Asp peptide; RT, radiation therapy; ROS, reactive oxygen species; TGF, transforming growth factor; Tf, transferrin; TfR, transferrin receptor; SA, serum albumin; VEGF, vascular endothelial growth factor.

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## Author Contributions

MMC and YD contributed equally in the design and selection of the topics for this article. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. KG and YD coordinated and conceived the review article.

## Disclosure

The authors report no conflict of interest in this work.

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