Nanomedicines for Breast Cancer Theranostics

Micro and Nano Technologies

Nanomedicines for Breast Cancer Theranostics

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Breast cancer nanomedicine market update and other industrial perspectives of nanomedicine

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Chapter outline

16.1 Introduction

Breast cancer (BC) is the second leading cause of mortality in females after lung malignant neoplasms. Dedicated, noninvasive diagnostic screening methods for early detection and surveillance (e.g., mammography/echography) have indeed increased the five-year relative survival rate of women with BC from 75% in the 1970s to 91% in the middle 2010s. Sadly, however, the same life expectation for BC patients diagnosed at late stages is still as low as 26% [1], the leading cause of related mortality being metastasis to lymph nodes, lung, liver, bone, and brain [2]. Therefore, more efficacious BC treatments beyond the current standards of chemotherapy, radiation, and surgery [3]—all of which may damage not only disease cells but also healthy tissues—are heavily needed.

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Nanomedicine (NM) is a multidisciplinary arena aiming at the design, synthesis, characterization and application of materials and devices with nanoscale dimensions (1−100 nm) [4]. As a rapidly developing field, NM offers concrete opportunities in human cancer theranostics since, contrarily to conventional drugs, nanobased platforms may be designed and tailored for, e.g., overcoming biological barriers, allowing for prolonged blood circulation time, simultaneously exploiting both active tumor targeting and enhanced permeability and retention (EPR) effects, efficient drug delivery, and reduced or eliminated side effects [5].

Approximately 50 cancer NMs approved by the Food and Drug Administration (FDA) are available in the clinics [6,7] and, according to the National Institute of Health (NIH), 243 clinical trials involving NMs in cancer theranostics are currently ongoing [8]. Of these NMs, this chapter will review those targeting BC, briefly discussing the advantages they offer and underlying the challenges this emerging trend in the medical field still must face in paving the road to fulfill the European Union "Beating cancer by 2030" [9] promise.

16.2 Approved nanomedicines for breast cancer therapy

The nanosystems approved for clinical BC treatments can be generally classified into lipid, polymer, inorganic, viral, and drug-conjugated nanoparticles (NPs). These include a variety of structures (Fig. 16.1) with different sizes, shapes, and charge, each having

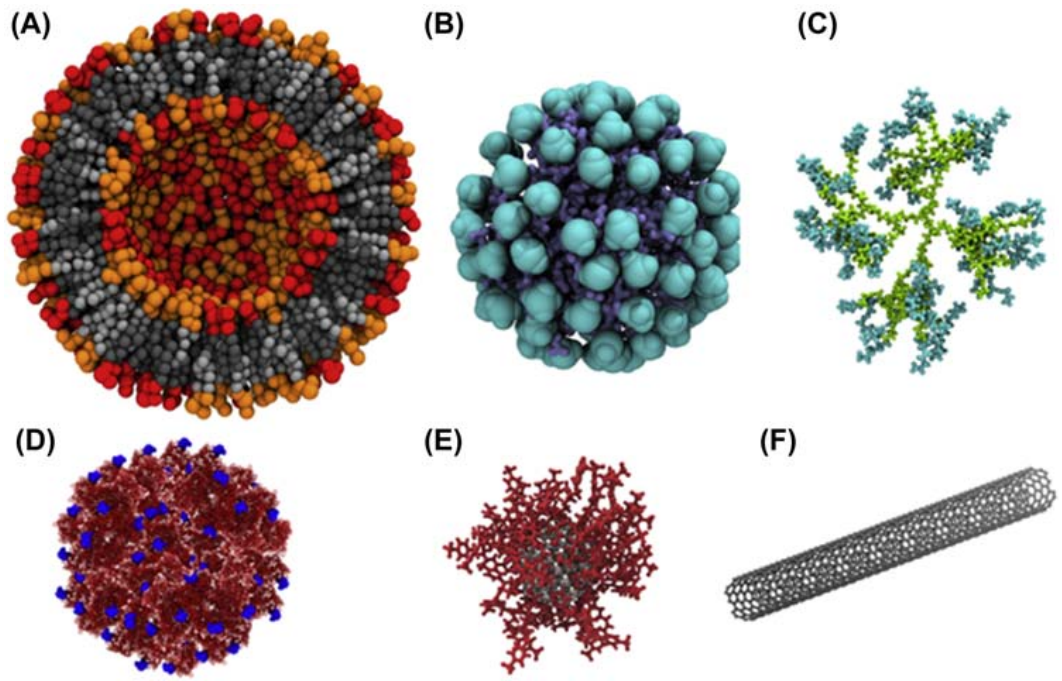

FIGURE 16.1 Representative examples of different nanoparticles (NPs) used as cancer nanomedicines: (A) liposomes; (B) micelles; (C) dendrimers; (D) protein NPs; (E) surface-modified metallic NPs; and (F) carbon nanotubes.

different properties such as, among others, drug loading capacity, release profile, cellular targeting, and stability [10].

Table 16.1 lists the nanomedicines actually in clinical use for breast cancer. As seen from this table, liposomal, protein, and polymeric nanoformulations are greatly represented among approved BC nanotherapeutics [11]. Yet, most of them mainly show reduced toxicity rather than effective improved efficacy compared to conventional free drugs formulations [12].

Table 16.1 Approved nanodrugs in clinical use for breast cancer treatment.

Trade name manufacturer	Nanoplatform/agent	Indication(s)	Status
Liposomal NPs			
Doxil (Janssen products)	PEGylated liposome/ doxorubicin HCl	Kaposi's sarcoma, ovarian cancer, multiple myeloma, metastatic breast cancer	Approved in 1995
Myocet (Sopherion Therapeutics)	Non-PEGylated liposomal doxorubicin	Metastatic breast cancer	Approved in EU and Canada in combination with cyclophosphamide in 2000
Lipodox (Sun Pharma Global FZE)	PEGylated liposome/ doxorubicin HCl	Ovarian cancer; breast cancer	Approved as a generic of Doxil since 2013; preclinical studies
DaunoXome (NeXstar Pharmaceuticals)	Heat-activated liposome/ daunorubicin	AIDS-related Kaposi's sarcoma	Approved in 1996
Lipusu (Sike Pharmaceutical Co. Ltd)	Liposome/paclitaxel	NSCLC and breast cancer	Approved in China 2006
Protein NPs			
Abraxane (Celgene)	Albumin-bound paclitaxel (nab-paclitaxel)	Breast cancer	Approved in 2005
Polymeric NPs			
Genexol-PM (Samyang Biopharm)	PEG-PLA micelles/ paclitaxel	Breast cancer, advanced lung cancer, ovarian cancer	Approved in South Korea in 2007
Nanoxel (Fresenius Kabi India Pvt Ltd)	mPEG-PDLLA/paclitaxel	Gastroesophageal cancers, breast cancer	Approved in India in 2006, Phase I
Inorganic NPs			
NanoTherm (MagForce AG)	SPIONs	Glioblastoma and other brain tumors, prostate cancer	Approved in 2010 in EU; late clinical trials in US
Others			
Kadcyla (Hoffmann-La Roche)	Ado-trastuzumab emtansine (MCC-DM1 complex)	HER2-positive metastatic breast cancer	Approved in Canada in 2018, and in USA in 2019

The main goal of liposomal drug encapsulation is to alter tissue distribution and pharmacokinetics (PK) of the active principle, ultimately improving its therapeutic index. Caelyx/Doxil, Myocet, lipodox all are marketed liposomal formulations of doxorubicin (lipo-dox) [13], one of the most effective small molecule anticancer drug currently used against both early- and late-stage BCs [14]. However, the potential therapeutic benefits of doxorubicin are severely limited by its dose-dependent toxicity (cardiotoxicity and myelosuppression in particular), the emergence of multidrug resistance (MDR), and its low specificity against cancer cells [15–17]. To overcome these hurdles, its first lipodox formulation was developed, approved by FDA in 1995, and introduced to the market as Doxil, with over 600 million USD in annual sales [18]. Doxil liposomes are composed of phospholipid hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and N-(carbonyl-methoxy polyethylene glycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (mPEG-DSPE) in a molar ratio 56:38:5. The mean size of Doxil particles is in the range of 80–90 nm, with up to 15,000 doxorubicin molecules encapsulated in its internal core [19]. The PEGylation technology provides stability to these self-assembled NPs and, most importantly, endows them with the so-called *stealth effect*, that is the ability of eluding their reticuloendothelial system (RES)-mediated identification and subsequent clearance [20] (Fig. 16.2). Accordingly, substantially extended circulation times (74 h) can be achieved with respect to free drug formulations (5 min) [21]. When administered at a dose of 50 mg/m^2 once per month, Doxil has the same efficacy as 60 mg/m^2 of conventional doxorubicin dispensed every 3 weeks. Moreover, the risk of developing cardiotoxicity associated with peak concentrations of free doxorubicin is significantly reduced (∼threefold) when a BC patient is

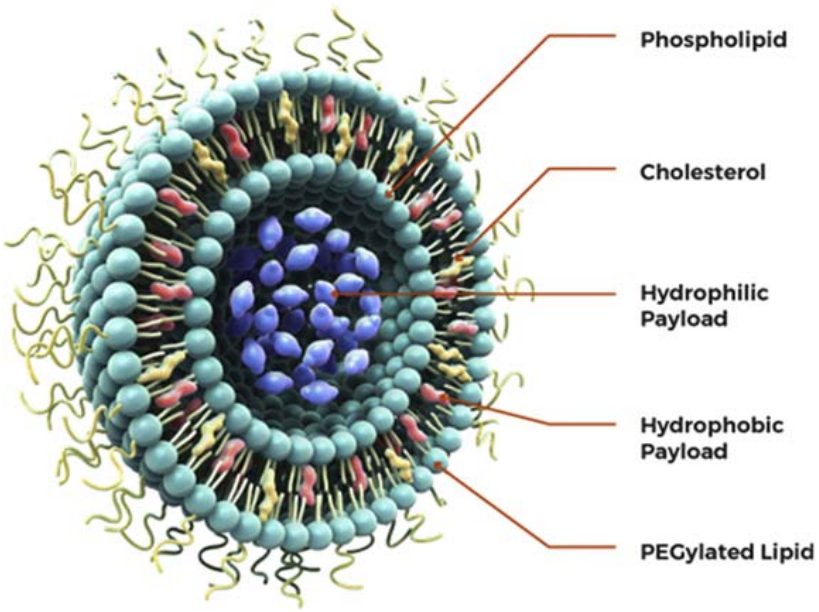

FIGURE 16.2 Cartoon showing the generic structure of a liposome-based cancer NM.

treated with the liposomal formulation of the same therapeutic [21]. Although Doxil treatments induce some new adverse effects such as skin toxicity (e.g., palmar-plantar erythrodysestesia, PPE) and mucositis, these are considerably less relevant than cardiotoxicity from the clinical standpoint [22].

Another liposomal formulation that encapsulates doxorubicin for BC therapy is Myocet, which received approval from the European Medicine Agency (EMA) and Health Canada in 2000. At variance with Doxil, Myocet phospholipidic composition includes cholesterol and acidic egg phosphatidylcholine (EPC) in a molar ratio of 45:55; moreover, the resultant NPs (150−250 nm in diameter [23]) lack PEG functionalization. As the circulation time of Myocet is shorter compared to Doxil (∼2.5 h), these NPs are not associated with PPE effects. In a Phase III clinical study in patients with metastatic breast cancer (MBC) this lipo-dox showed response rates and progression-free survival times comparable to free drug, with substantially lower cardiotoxic side effects [24]. In another multicentric clinical trial in patients with MBC, Myocet (60 mg/m^2) was administrated in combination with cyclophosphamide (600 mg/m^2) and showed equivalent efficacy with minimal toxicity compared to free doxorubicin/cyclophosphamide combination at the same dose [25].

Lipodox (doxorubicin hydrochloride liposome injection) is considered a generic of Doxil. In 2012−13, due to a Doxil shortage in the USA, FDA approved the temporary importation of Lipodox from Sun Pharma Global FZE (Mumbai, India) to quickly provide this critical agent to the American recurrent ovarian cancer patient population [26]. Notwithstanding direct comparative clinical or preclinical data were not available at that time, in February 2013 FDA finally approved generic liposomal doxorubicin, marketed and distributed under the brand name Lipodox, and rated it as AB (i.e., therapeutically equivalent to Doxil) [27].

DaunoXome, originally developed in 1996 by NeXstar Pharmaceuticals (USA) for the treatment of AIDS-related Kaposi's sarcoma [28], is the last example of market-available liposomal formulation for BC nanotherapeutics delivering daunorubicin citrate. DaunoXome liposomes (∼50 nm) consist of a bilayer membrane of distearoyl phosphatidylcholine and cholesterol at a 2:1 molar ratio, encapsulating the citrate salt of daunorubicin within the inner aqueous core at a lipid:drug weight ratio of 18.7:1. DaunoXome is able to avoid RES sequestration, has a circulation half-life of 2−4 h [29], is endowed with an improved PK profile compared to free daunorubicin [30,31], and its tumor site targeting relies on the so-called EPR effect [32]. A study evaluating DaunoXome in BC treatment involved 16 women with MBC treated with increasing doses of the nanoformulation (80−100, 120, and 150 mg/m^2) over 2 h in 21-day cycles [33]. The maximum tolerated dose was 120 mg/m^2, and the related toxicity observed was mild and manageable (asymptomatic cardiotoxicity, neutropenia or neutropenic pyrexia) [34,35]. Since a considerable antitumor activity of DaunoXome was shown in these trials, its approval in MBC therapy is expected to be delivered soon.

Together with anthracyclines, taxanes represent another important class of antitumor agents that play a substantial role in the treatment of early stage and advanced BCs [36].

Currently, paclitaxel and docetaxel are included in the regimens for adjuvant chemotherapy of recurrent and metastatic BCs [37]. Unfortunately, paclitaxel causes various formulation problems due to its very low solubility in water (<0.01 mg/mL) and in most suitable pharmaceutical solvents. Taxol, the conventional clinical formulation of paclitaxel consisting of Cremophor EL and ethanol (50:50 mixture), is diluted prior to administration via slow infusion to avoid its precipitation in the blood. However, Cremophor EL, a lipid-based solvent used as vehicle, causes number of adverse reactions itself (e.g., acute hypersensitivity, aggregation of erythrocytes, and neuropathy) [38–40]. The formulation including docetaxel (Taxotere) is prepared using solvents like polysorbate 80 (Tween 80) and ethanol diluent in order to increase drug solubility. However, hypersensitivity reactions can also occur with Tween 80, although to a lesser extent with respect to Cremophor EL [37]. Additionally, both these two excipients (Cremophor EL and Tween 80) may have a negative impact on drug efficacy because they can limit tumor penetration [41]. Thus, during recent years special efforts in nanomedicine have been devoted to formulate alternative platforms for delivery of taxanes, as detailed below.

Nanoparticle albumin-bound (nab)-paclitaxel (Abraxane) has been approved by FDA in 2005 to treat MBCs. In this Cremophor EL-free nanoformulation, nanoparticles of approximately 130 nm are formed during physical complexation of unmodified paclitaxel with albumin under high pressure [42–44] (Fig. 16.3).

Abraxane allows safe administration of paclitaxel to a much higher dose and shorter injection times compared to Taxol (2–10 mg/mL vs. 0.3–1.2 mg/mL, respectively) [45]. In clinical trials involving patients with advanced breast cancer Abraxane showed remarkably increased efficacy of paclitaxel [46]. It has been proposed that this result is due to enhanced uptake of nab-paclitaxel from the intravascular space and augmented

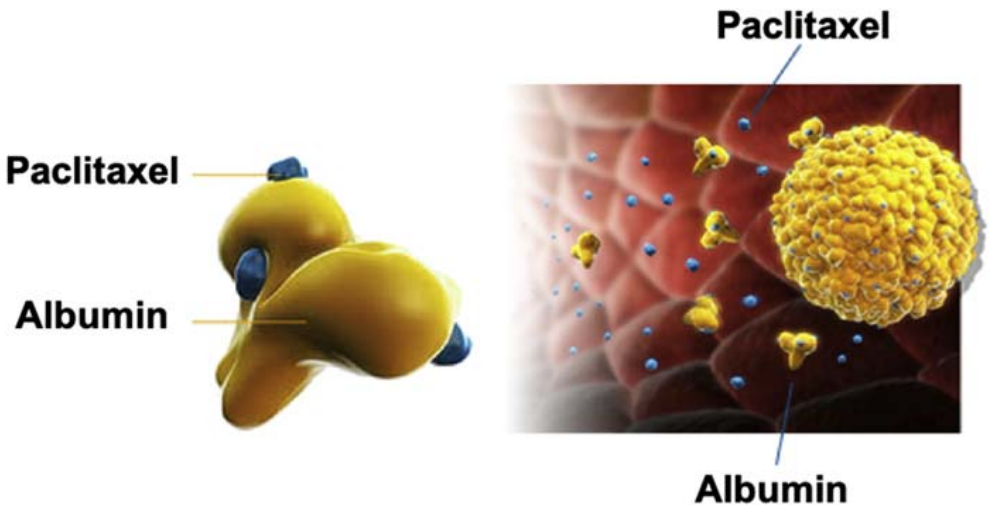

FIGURE 16.3 Cartoon showing (left) paclitaxel molecules (in blue) bound to albumin protein chains (in yellow) and its release from the Abraxane NPs (right).

transport into cancer cells. Once administered, Abraxane albumin binds to the endothelial glycoprotein 60 (gp60). This initiates a process of cell membrane invagination to form vesicles, which are then transported through the endothelial cell before fusing with the membrane of the other side of the cell, thereby releasing the vesicle contents to the interstitial space [47]. In an in vivo study Desai and coworkers further showed that, when administered at equivalent doses, Abraxane has a 33% higher tumor accumulation compared to Taxol [48]. Furthermore, in a Phase III trial for MBC (*nab*-)paclitaxel monotherapy demonstrated superior overall response rate (34%) with respect to Taxol (19%) [49]. Here, an injection dose of 260 mg/m^2 of Abraxane was administrated over 30 min every 3 weeks. Remarkably, this well-tolerated regimen is approximately 50% higher than the typical tolerated dose of Taxol (175 mg/m^2 over 3 h every 3 weeks). The drug clearance rate and volume of distribution were also higher for (*nab*-)paclitaxel (21.13 L/h/m^2, 663.8 L/m^2) than for Cremophor EL formulated paclitaxel (14.76 L/h/m^2, 433.4 L/m^2) [49]. Also, no hypersensitivity side effects were observed in the 229-patient cohort, due to the absence of Cremophor EL [45]. In summary, the solvent-free Abraxane represents a taxane nanoplatform that may provide clinical benefit combined with a reasonable toxicity profile. Moreover, the particularly favorable pharmacokinetics and pharmacodynamics of nab-paclitaxel likely contribute to its enhanced clinical safety and efficacy with respect to its Taxol alternative [50].

In 2006 China allowed Lipusu, another liposome injection formulation of paclitaxel produced by Sike Pharmaceuticals, to enter the clinical use in that country. Although the composition information for Lipusu has not been publicly released [51], this nanoformulation was shown to retain the antitumor activity of the free drug while the toxicity was reduced compared to Taxol(R) both in vitro and in vivo [52]. One study reported that, although Lipusu had beneficial therapeutic effect in BC, substantial premedication is recommended. This includes (1) methylprednisolone (40 mg) administered intravenously 30 min before Lipusu, and granisetron (antiemetic) 30 min before chemotherapy, (2) dexamethasone 2.25−3 mg taken orally 12 h and 2 h before Lipusu, and granisetron 30 min before chemotherapy [53].

Another nanoformulation of traditional paclitaxel that has been approved in South Korea in 2007 is Genexol-PM, which consist of paclitaxel-doped poly(D,L)-lactide (PDLLA) micelles. This nanoformulation is also able to deliver a dose of paclitaxel (300 mg/m^2) higher than conventional therapy (175 mg/m^2) without dose-limiting toxicity [54,55]. The half-life of Genexol-PM is 1.8-times longer compared to free paclitaxel (18.3 ± 3.1 h vs. 6.8 ± 1.4 h, respectively). In a USA-based Phase II clinical trial where Genexol-PM was used for the treatment of MBC, 41 patients were treated with 300 mg/m^2 of Genexol-PM over a period of 3 h every 3 weeks [56]. According to this clinical investigation, the overall response rate was in the range 43.5%−73.7%, and the median time to progression for all patients was 9 months. The lower systemic toxicity and fewer side effects (hypersensitivity reaction and neuropathy) were again ascribed to the absence of Cremophor EL [56]. One recent Phase III clinical trial that recruited 212 patients with recurrent or metastatic HER2-negative breast cancer documented an

improved overall response rate of Genexol-PM compared to standard paclitaxel treatment, with manageable toxicities [57]. Genexol-PM allowed administration of an increased dose of paclitaxel, offering improved anticancer efficacy. However, Genexol-PM toxicity remains an issue if compared to Abraxane; moreover, instability of Genexol-PM micelles was verified, resulting in precipitation of paclitaxel in the form of large needle-like crystals between 2 and 4 h at 40°C [58].

Nanoxel is another polymeric micelle-based NM that encapsulates paclitaxel. In this formulation, NPs are PEGylated (mean particle size = 80 nm), and their accumulation in the tumor cells relies on passive targeting via the EPR effect [59]. Nanoxel has been approved in India in 2006 for the treatment of progressive and metastatic BCs. More precisely, Nanoxel is indicated for the adjuvant treatment of node-positive breast cancer administered sequentially to standard doxorubicin-containing combination chemotherapy, or after failure of anthracycline therapy [60].

Since 2011 pilot studies are conducted on inorganic NPs for the treatment of BC. The most promising one, NanoTherm, has already received the approval from European Medicines Agency (EMA) in 2010 yet for the treatment of glioblastoma [61]. NanoTherm consists of superparamagnetic iron oxide nanoparticles (SPIONs, Fe_3O_4, iron concentration 112 mg/mL) with a mean diameter of 12 nm and an aminosilane-type shell for the easy formation of injectable colloidal solutions [62] (Fig. 16.4).

NanoTherm mechanism of action is based on the principles of induced magnetic hyperthermia and tumor thermal ablation. Accordingly, after delivering SPIONs to the

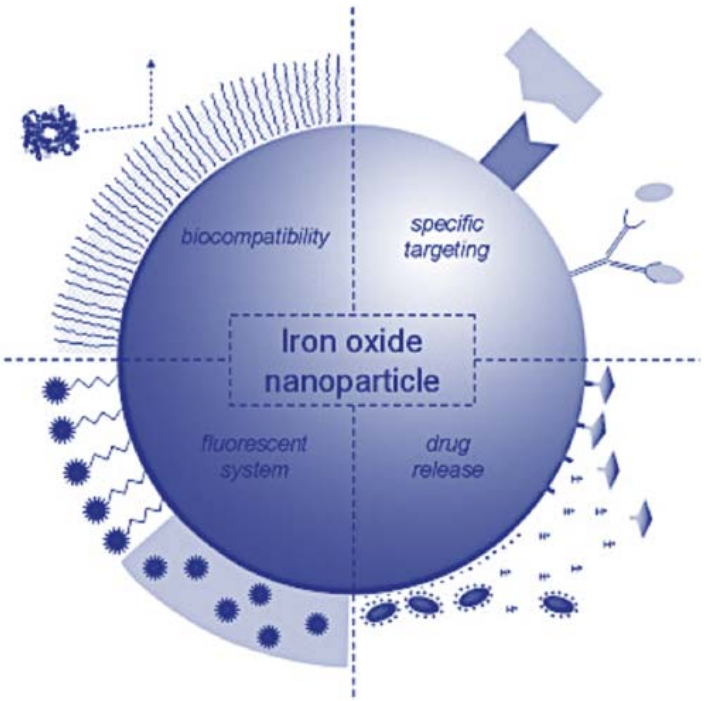

FIGURE 16.4 Schematic representation of a supermagnetic iron oxide nanoparticle (SPION) and its possible application in cancer NM.

cancer lesion, an alternating magnetic field is applied, which sets the NPs in rapid rotation. This, in turn, induces heat within tumor due to NP friction. The tumor temperature can be controlled by changing the duration of exposure to the oscillating magnetic field until the intratumoral temperature reaches the ablative region (>50°C), in which heat irreversibly destroys the cancer cells. Induced magnetic hyperthermia without the subsequent tumor ablation can also be used to weaken or make tumors more sensitive to concomitant radiotherapy or chemotherapy. Other studies are currently being conducted with SPIONs to ascertain their efficacy in other cancer types (pancreatic, prostate, and esophageal cancer inter alia) [63].

Very recently, FDA approved Kadcyla (trastuzumab emtansine), another nanoformulation from Roche as adjuvant treatment for patients with HER2-positive early breast cancer with residual invasive disease after neoadjuvant treatment. Kadcyla is an antibody-drug conjugate (ADC) that selectively delivers the drug to HER2-expressing BC cells (Fig. 16.5).

The antibody (Ab) component of Kadcyla is trastuzumab (Herceptin), a monoclonal Ab (class IgG1) approved for the clinical treatment of HER2+ BCs [64], while the chemotherapeutic agent—covalently bond to the Ab via a stable thioether linker—is a microtubule inhibitor consisting of a molecular complex of 4-[N-maleimidomethyl] cyclohexane-1-carboxylate and a derivative of maytansine (MCC:DM1), aka emtasine. Approximately 3.5 molecules of DM1 are conjugated to one molecule of trastuzumab. Once administrated, Kadcyla binds to HER2 and prompts the entry of this complex into the cell via receptor-mediated endocytosis [65,66]. Due to the high stability of the linker, the active chemotherapy agent (DM1) release occurs only in the lysosome as a result of

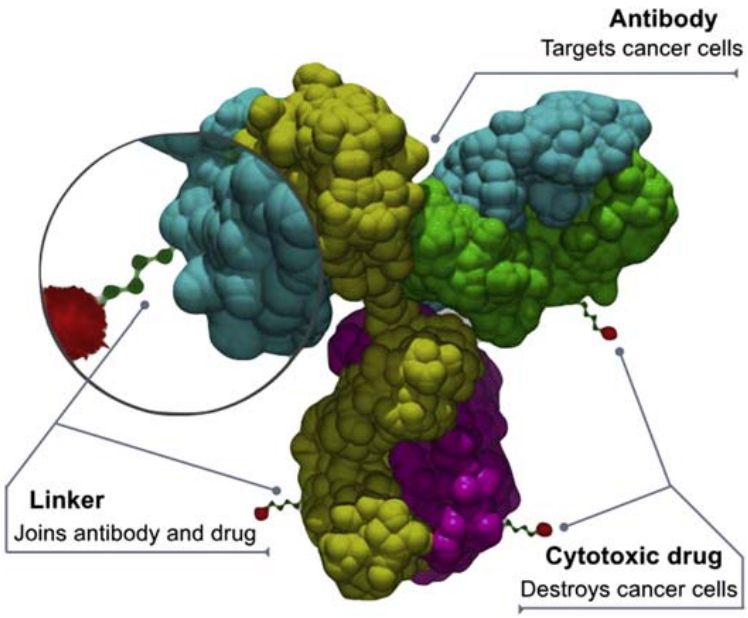

FIGURE 16.5 Schematic representation of an antibody-drug conjugate.

proteolytic degradation of the antibody part [67,68]. After the release, DM1-containing metabolites inhibit microtubule assembly, eventually causing cell death. This targeted-therapy increases the antitumor effect due to the combinational approach and potentially limits damage to healthy tissues [69,70].

16.3 Nanomedicines in clinical trials for breast cancer therapy

Most nanoformulations that gained regulatory approval in BC therapy are based on previously FDA-licensed drugs. However, a plethora of new nanodrugs enter preclinical and clinical investigations every year [12]. Table 16.2 lists the NMs that are currently undergoing clinical trials for BC treatment. As for the BC NMs currently on the market, some of the items in Table 16.2 already constitute clinical regimens in the treatment of tumors other than BC.

There are currently two novel formulations of liposomal doxorubicin under clinical investigation. ThermoDox, developed by Celsion Corporation, is an original lipo-dox which is thermosensitive and designed to undergo bilayer disruption and subsequent drug release when exposed to heat. These liposomes are composed of DPPC, myristoylstearoyl phosphatidylcholine (MSPC), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG-2000). The phase transition temperature of DPPC is 41.5°C, a temperature which can be clinically reached by local hyperthermia. The addition of MSPC accelerates drug release by slightly reducing the transition temperature of DPPC, while DSPE-PEG-2000 enhances the circulation time of liposomes. The presence of the PEG lipid chains also helps in attaining lysolipid-induced permeability at a faster rate [71]. Interestingly, the coupling of this nanodrug with radiofrequency thermal ablation allows the drug to be released in a site-specific manner to the cancer lesion. ThermoDox is currently present is several clinical trials (from Phase I to Phase III) for hepatocellular carcinoma and recurrent chest wall breast cancer (RCWBC) in combination with mild hyperthermia. Indeed, in the DIGNITY Phase I/II study conducted by Celsion Corporation, which was designed to evaluate the safety and antitumor activity of ThermoDox in combination with mild hyperthermia for the treatment of RCWBC, a total of 28 patients were administered with NM doses of 40 or 50 mg/m^2. In addition to a local response rate of 61.9% among evaluable patients, a combined local response rate was observed in 46.4% of the cohort, demonstrating five durable local responses lasting more than 3 months, four complete responses and one partial response. Patients who received the lower NM dose (40 mg/m^2) displayed a comparable response rate and a more favorable safety profile to those receiving the higher nanodrug amount (50 mg/m^2). As a result, 40 mg/m^2 was recommended as the dose to be adopted in future ThermoDox clinical trials [72].

MM-302 (Merrimack Pharmaceuticals) is an Ab-drug conjugate liposomal nanoformulation. The drug is composed of a HER2-targeted antibody linked to doxorubicin.

Table 16.2 Nanodrugs as breast cancer therapeutics undergoing clinical investigation.

Trade name manufacturer	Nanoplatform/agent	Indication(s)	Status
Liposomal NPs			
ThermoDox (Celsion corporation)	Lysolipid thermally sensitive liposome/doxorubicin	Various solid tumors (hepatobiliary tumors, liver metastases and hepatocellular carcinoma) including BC	Phase III
MM-302 (Merrimack Pharmaceuticals)	HER2-targeting antibody liposomal-doxorubicin conjugate	HER2-positive, locally advanced/metastatic BC	Phase II
EndoTAG-1 (Medigene)	DOTAP/paclitaxel	BC, pancreatic cancer	Phase II
LEP-ETU (NeoPharm)	NeoLipid technology liposomes/Paclitaxel	Ovarian cancer	Approved orphan drug 2015
LEM-ETU (NeoPharm)	NeoLipid technology liposomes/Mitoxantrone	BC, leukemia, stomach, liver, ovarian cancer	Phase I
2B3-101	GSH PEG-liposome/ doxorubicin	Metastatic BC/brain cancer	Phase II
Onyvide (Merrimack pharmaceuticals, Inc.)	PEGylated liposome/ Irinotecan sucrosofate salt	Metastatic pancreatic adenocarcinoma, small cell lung cancer (SCLC) Metastatic BC, triple-negative BC	Approved in 2005; Phase II and III; Phase I
Lipolatin (regulon Inc.)	PEGylated liposome/ cisplatin	Pancreatic cancer, non-small cell lung cancer (N) SCLC, metastatic BC	Approved in 2015, metastatic pancreatic adenocarcinoma; Phase III for metastatic BC
Protein NPs			
ABI-008 (originator: Abraxis BioScience; developer: Celgene Corporation)	Albumin-bound docetaxel	Prostate cancer, NSCLC, metastatic BC	Phase I/II, preclinical studies
Polymeric NPs			
NK105 (Nippon Kayaku Co., Ltd.)	PEG polyaspartate micelle/ paclitaxel	Metastatic or recurrent BC	Phase III
Accurins (BIND-014, BIND Therapeutics, now Pfizer)	PEG-PLGA/docetaxel	Solid tumors (BC, prostate, endometrial cancer, head and neck cancer, melanoma)	Phase II
Inorganic NPs			
AuroLase (Nanospectra Biosciences Inc.)	Silica core coated with gold shell and PEG/PTA with gold NPs	Refractory and/or recurrent tumors of the head and neck, lung and prostate cancers	Phase I for prostate preclinical
Aurimune (CYT-6091, CytImmune Sciences)	PEGylated colloidal GNPs/ CYT-6091 THF-targeting ligand	Metastatic BC, adenocarcinoma, colorectal cancer	Phase I/II

Continued

Table 16.2 Nanodrugs as breast cancer therapeutics undergoing clinical investigation.—cont'd

Trade name manufacturer	Nanoplatform/agent	Indication(s)	Status
Others			
Rexin-G (Epeius Biotechnologies Corporation)	Tumor-targeted retroviral expression vector/micro-RNA-122	Recurrent or metastatic BC	Approved in Philippines in 2007, in Phase II in USA

DOTAP, 1,2-dioleoyl-sn-glycero-3-phosphocholine; *PTA*, photothermal ablation.

The Phase II HERMIONE trial was designed to evaluate MM-302 (30 mg/m^2 every 3 weeks) plus trastuzumab versus a chemotherapy of physician's choice (gemcitabine, capecitabine, or vinorelbine) plus trastuzumab. The 250 patients enrolled in HERMIONE were an anthracycline-naïve cohort with locally advanced/metastatic HER2-positive breast cancer following previous treatments with trastuzumab, pertuzumab, and ado-trastuzumab emtansine [73]. However, although no serious safety problems were concerned, unfortunately also no benefits were observed over the comparator group; accordingly, the trial for MM-302 was suspended in 2017 [74].

2B3-101 is another liposomal doxorubicin formulation (glutathione PEGylated) that has completed a Phase I/IIa clinical trial [75]. This NM has been developed for people suffering from multiple brain cancer indications, with an initial focus on patients with brain metastases originating from BC and patients with glioma. Liposome coating with PEG ensured the prolonged circulation time in plasma, while conjugation of glutathione (GSH) at the end of the PEG molecules targets the NPs to the active GSH transporters located on the blood brain barrier to enhance doxorubicin delivery to the brain [75].

EndoTAG-1 is an alternative liposomal formulation of paclitaxel. These liposomes are composed of cationic dioleoyloxypropyltrimethylammonium (DOTAP) and neutral (DOPC) lipids (DOTAP:DOPC:paclitaxel in 50:47:3 ratio). This product has been developed by Medigene, which has an agreement with SynCore Biotechnology Co. for the complete technology transfer of EndoTAG-1 [76,77]. These cationic NPs interact with newly developed, negatively charged endothelial cells thereby preventing tumor angiogenesis and, hence, tumor growth. The product is currently under Phase II clinical trial investigation for breast and pancreatic cancer, where it showed promising antitumor activity [78].

The Liposome Entrapped Paclitaxel-Easy-To-Use (LEP-ETU) liposomes developed by NeoPharm Labs and composed of a 90:5:5 molar ratio of DOPC, cholesterol and cardiolipin, contain encapsulated paclitaxel with at final total lipid to drug molar ratio of 33:1. As for Abraxane, this NM is Cremophor EL-free; therefore, adverse effects related to this nonionic solubilizer are excluded [79]. The clinical trial development of LEP-ETU formulation has been sponsored by Insys Therapeutics, Inc [79]. and in 2015 the FDA

has granted orphan drug status for the treatment of ovarian cancer. However, the use of these paclitaxel formulations declined after the success of albumin-bound and polymeric formulations of paclitaxel. The LEP-ETU story highlights an important underlying, general concept in cancer NM development: substantial clinical improvements are hardly achieved using already existing drugs, even if the latter are delivered exploiting new (nano)formulations. Thus, further preclinical studies are heavily needed in order to design new anti-tumor drugs and mark a breakthrough in cancer NM.

Liposome encapsulated mitoxantrone (LEM)-ETU is also developed by NeoPharm Labs by using its proprietary NeoLipid technology, as for LEP-ETU. However, while the liposome composition is the same as for LEP-ETU, in this case the encapsulated active agent is mitoxantrone, a DNA intercalating agent and inhibitor of topoisomerase II [80]. LEM-ETU is currently present in Phase I clinical trial for breast cancer and other malignancies (leukemia, stomach, liver, and ovarian cancer) [81].

Onivyde (Merrimack Pharmaceuticals, Inc.) is a PEGylated lipid bilayer liposomal injection of irinotecan, another topoisomerase I inhibitor. The vesicles (110 nm in diameter) are composed of DSPC, cholesterol and methoxy-terminated PEG (M_W 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) in the ratio 3:2:0.015 [82]. Liposomal irinotecan was approved in October 2015 by the FDA and is indicated, in combination with fluorouracil and leucovorin, for the treatment of patients with metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy. This new nanoformulation of irinotecan presents advantageous pharmacokinetic properties, endowing Onivyde with higher tumor accumulation of the prodrug and its active metabolite SN-38 compared with free irinotecan. This, in turn, results in improved antitumor activity with low systemic toxicity [82]. Irinotecan is not a drug commonly adopted in BC treatments; however, in a Phase I study on advanced refractory solid tumors that include breast cancer, the disease control rate achieved with Onivyde was 45.5% [83]. Moreover, a more recent preclinical investigation reported by Zheng et al. show its efficacy in the treatment of triple-negative BC [84].

Cisplatin is a milestone achievement in clinical oncology starting from its approval in 1978 [85]. It has been used for treatment of numerous human malignancies including breast, bladder, head and neck, lung, ovarian, and testicular cancers. Due to its square planar geometry, this molecular complex is able to crosslink with the purine bases of DNA. This induces a structural damage in the nucleic acid double helix that impedes its replication and, ultimately, leads to tumor cell apoptosis. The principal limitation for its use is set by its severe systemic toxicity (e.g., nephrotoxicity, neuropathy, ototoxicity, and hematological toxicities). To limit these adverse effects. Regulon Inc. developed Lipoplatin, a liposomal formulation of cisplatin [86] which presents the additional qualities of higher targeting properties and longer half-life [87–89]. The EMA granted orphan drug status to this product for pancreatic cancer treatment in 2015. Lipoplatin is composed of 91.1% of lipids (DPPG, soy PC (SPC3), MPEG-DSPE lipid conjugate, and cholesterol), and 8.9% of cisplatin. The average diameter of this nanoformulation is 110 nm [90] (Fig. 16.6).

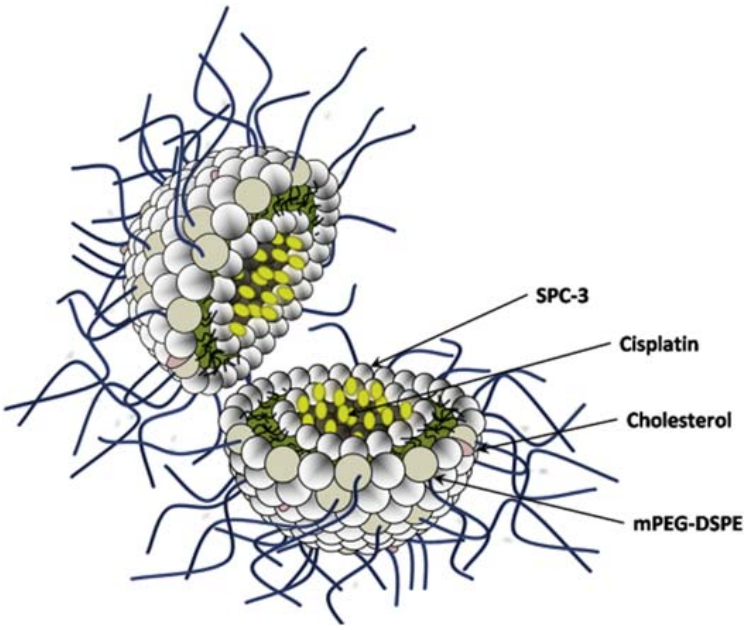

FIGURE 16.6 Schematic representation of a liposomal nanoformulation of cisplatin.

As mentioned above, encapsulation of cisplatin offers various benefits in terms of long-term circulation in vivo, high encapsulation efficiency that leads to ability to attain 10 to 200-fold higher concentration in tumors compared to free cisplatin, and ability to penetrate the cell membrane [86]. Given its high efficacy, Lipoplatin is present in numerous clinical trials: in Phase I trials for malignant pleural effusion [91], Phase II trials for BC and gastric cancer [92], Phase II/III trials for pancreatic cancer [93], and Phase III trials for nonsmall-cell lung cancer (NSCLC) [94,95]. The use of a combinatory approach, in which a Lipoplatin/vinorelbine regimen has been evaluated, showed to be clinically effective and good tolerability in the treatment of MBC. Indeed, the Phase II trial showed that Lipoplatin/Vinorelbine achieved comparable results in relation to overall response rate and better median survival when compared with classical cisplatin/vinorelbine treatment [96]. Moreover, the adopted regimen was well tolerated with mild grade 1/2 and no grade 3/4 nephrotoxicity or neuropathy. Nevertheless, as the majority of patients in this trial received previous chemotherapeutics, a survey of this combinatory regimen as de novo first-line treatment in patients with MBC would be advisable [92].

The generation of albumin-bound drug NPs, patented as *nab* Technology platform, has great potential for improving the delivery of an active drug, especially in the case of water-insoluble compounds. Examples exploiting drug *nab*-application are currently in preclinical and clinical studies. Rapamycin (an allosteric inhibitor of mTOR complex 1, mTORC1) has proven to be effective in various solid tumors including BCs. However, it has low oral bioavailability, poor solubility, and dose-limiting intestinal toxicity [97]. Therefore, Celgene Corporation developed ABI-009, i.e., *nab*-rapamycin. In their study

performed on MDA-MB-231 using human-tumor-xenografts Desai and coworkers showed that these NPs were endowed with dose-linear pharmacokinetics, very limited side effects up to 90 mg/kg, and effective antitumor activity at 40 mg/kg, with corresponding tumor growth inhibition of 71%–88% [98]. The same company also developed ABI-008 (*nab*-docetaxel), which is currently in Phase I/II trial for patients with MBC [99].

Another NM that encapsulates docetaxel yet in polymeric NPs is BIND-014 (BIND Therapeutics). It targets tumor prostate-specific membrane antigen (PSMA), that is a protein expressed on the surface of prostate cancer cells and on the neovasculature of many solid tumors [100]. BIND-014 is the first targeted polymeric nanoformulation (approximately 100 nm in. diameter) composed of a hydrophobic polylactic acid (PLA) core and a hydrophilic PEG corona decorated with small PSMA-targeting ligands. BIND-014 is designed to specifically accumulate in cancerous tissues (as healthy vasculature does not present PSMA), and release docetaxel in a controlled manner. PEG is incorporated into the outer shell of the NPs to exploit again the stealth property for immune evasion (Fig. 16.7). Currently, BIND-014 is in Phase II clinical development in patients with metastatic castration-resistant prostate cancer (mCRPC) [101]. Interestingly, initial clinical data for BIND-014 in patients with advanced solid tumors indicated that this NM displays a pharmacological profile differentiated from the free drug, including pharmacokinetics characteristics consistent with preclinical data and cases of tumor shrinkage at doses below docetaxel dose typically used in the clinic [102]. However, whether the development of docetaxel polymeric NPs will continue after BIND Therapeutics' bankruptcy and subsequent acquisition by Pfizer in July 2016 [103] remains to be ascertained.

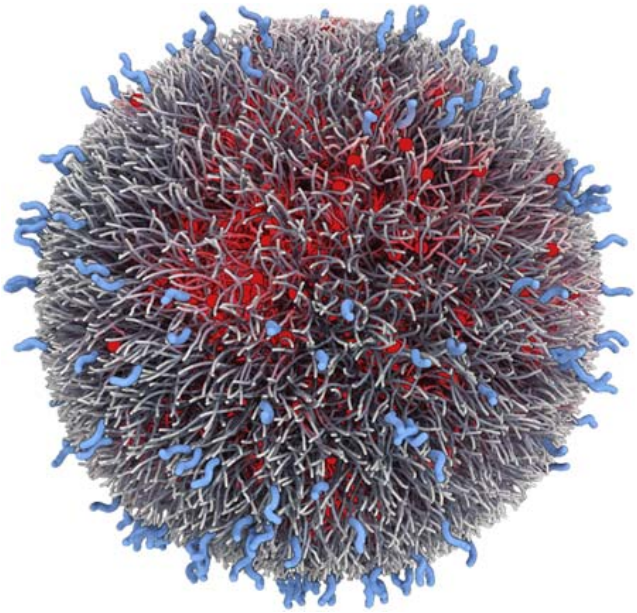

FIGURE 16.7 Cartoon showing the structure of the BIND-014 NM. The hydrophobic PLA core is in red, the hydrophilic PEG corona is in gray, and the PSMA-targeting ligand is in light blue.

NK105 is a novel NP drug delivery formulation that encapsulates paclitaxel in polymeric micelles [104]. NK105 polymers are constructed using the so-called PEG polyaspartate, where PEG is used as the hydrophilic segment and a 4-phenyl-1-butanol-modified polyaspartate as the hydrophobic segment. Paclitaxel is incorporated into the inner core of the micelle via hydrophobic interactions with the aspartate chains. The average dimension of NK105 micelles is 85 nm, with a remarkable polydispersity (20−430 nm). In one nonclinical study it was shown that NK105 is endowed with superior efficacy with respect to that of paclitaxel, and that this property was ascribed to its notable EPR effect [105]. A recent Phase III clinical study in patients with breast cancer, sponsored by Nippon Kayaku Co., Ltd. showed that NK105 was well tolerated, and its peripheral sensory neuropathy profile was particularly favorable in comparison with that of free paclitaxel. As an additional advantage, in contrast to paclitaxel-Cremophor EL formulation no premedication was needed with NK105, the administrated dose was lower (65 vs. 80 mg/m^2), and the infusion time was shorter (30 vs. 60 min) [106].

Currently, few inorganic NPs are present in clinical trials for cancer therapy, while only preclinical investigations are conducted for breast cancer treatment. Nanospectra Bioscience Inc. developed silica NPs (120 nm) coated with a thin layer of gold (12−15 nm) and PEG, called AuroLase. These particles absorb light and convert it to heat for solid tumor thermal ablation. This site-selective approach has the potential to significantly reduce adverse effects, pointing to excellent tolerability in humans [107]. To date, AuroLase is present in clinical trials for the treatment of subjects with refractory and/or recurrent tumors of the head and neck [108], primary and/or metastatic tumors of the lung [109], and prostate cancer [110]. Preclinical investigations showed antitumor activity of these NPs when incubated with SK-BR-3 human breast carcinoma cells for 1 h and then exposed to laser light (820 nm, 35 W/cm^2) for 7 min [111,112].

Preclinical study conducted on multidrug resistant MCF-7/ADR breast cancer cell line showed enhanced toxicity in culture when the cells were exposed to DOX-Hyd@AuNP, that is gold nanoparticles (GNPs) bound to doxorubicin via an acid labile linker [113]. In addition, an in vivo study demonstrated the ability of DOX-Hyd@AuNP to efficiently transport and release doxorubicin to cancer cells, causing inhibition of tumor growth in murine models [114]. Also, SPIONs can be coated with gold and loaded with doxorubicin (SPIONs@Au). This combined complex efficiently releases drugs at acidic conditions and efficiently reduces the cell viability and proliferation in MCF-7 cells, as reported by Mohammad and coworkers [115].

GNPs with the toxic agent TNF-α linked to their surface is another approach to treat cancer. TNF-α is a potent antitumor agent; however, its extreme systemic toxicity (profound cardiovascular side effects) limits its use in clinic. Therefore, CytImmune Sciences developed its PEGylated gold nanoformulation (Aurimune) [116] that in a Phase I study in patients with advanced cancer was well tolerated [117]. One preclinical study suggests the use of combinatory therapy of Aurimune with radiation, since a synergistic enhancement of tumor growth inhibition in breast and head and neck cancer models was observed [118].

The first targeted injectable molecular genetic medicine, Rexin-G, received its approval in Philippines in 2007 [119,120], and is currently in Phase I/II trials in USA for advanced pancreatic cancer [121]. Rexin-G is a mixed system that is based on the murine leukemia virus. These retrovirus-derived NPs (∼100 nm) are potent inhibitors of the human cyclin G1 pathway (CCNG1 proto-oncogene) that is overexpressed in over 50% of various malignancies (including breast, pancreas, prostate, ovarian, and colon cancer). Rexin-G specifically targets collagen, which constitutes the scaffold of tumor microenvironment and affects tumor microenvironment (TME) such that it regulates the extracellular matrix remodeling by collagen degradation and redeposition, and promotes tumor infiltration, angiogenesis, invasion and migration [122]. The vector exposes the collagen-binding peptide from human von Willebrand factor (vWF) in the modified viral envelope and permits NPs accumulation. Indeed, after administration, Rexin-G accumulates in the TME, and subsequently enters into the rapidly proliferating cells [120]. The genetic payload produces a cytocidal dominant-negative mutant of human cyclin G1 (dnG1) that effectively blocks the cell division cycle; this, in turn, results in cancer cell apoptosis and elimination of proliferative tumor vasculature and associated malignant fibroblasts [123,124]. In 2008, Rexin-G was granted orphan drug status for soft tissue sarcoma and osteosarcoma by FDA and 2 years later Phase I and II clinical trials were successfully completed using Rexin-G for the treatment of these two tumors [125]. Already in 2006 Gordon et al. reported the utility of Rexin-G formulation in the treatment of BC patients with an overall survival from diagnosis of approximately 5 years [126]. Since then, however, no further studies on the use of Rexin-G as BC therapeutics were reported.

16.4 Limitations and strategies employed in current breast cancer therapies with NMs

16.4.1 Multidrug resistance

Among the numerous factors hampering the successful performance of NMs, multidrug resistance (MDR) has been estimated to contribute to over 90% of patient treatment failures, ultimately leading to tumor recurrence and progression [127]. The mechanisms of MDR are complex, and they include: (1) overexpression of ATP-binding cassette pumps (e.g., P-glycoprotein, P-gp); (2) defective apoptotic mechanisms; (3) structural alterations of the drug targets; and (4) existence of cancer stem cells (CSCs). Therefore, there is an urgent need to develop NP-based delivery systems able to bypass the cancer cell efficient MDR machinery. While mechanisms (2) and (3) are very variegated and need to be addressed on a case-by-case basis, concerning mechanism (1) of MDR one in vitro study performed on BC cellular models (MCF-7 and resistant MCF-7/ADR cells) showed that the treatment with Pluronic polymer micelles containing doxorubicin (SP1049C) were able to sensitize the BC resistant cancer cells, resulting in cytotoxic activity two to three orders of magnitude higher than the free drug. The reasons for these

results were attributed to the different mechanisms presiding Pluronic NPs and free drug cellular internalization: while the former enter cells via endocytosis - a P-gp-independent pathway - the latter is transported across the membrane through diffusion, a pathway affected by P-gp expression in MDR cells [128,129]. Similar evidence was also reported in other of cancers in vivo, and SP1049C is in Phase II clinical study in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction [130].

The existence of breast cancer stem cells (BCSCs) (mechanism (4)) is a particularly important issue of MDR in this cancer type. Generally speaking, CSCs are a unique subpopulation of tumor cells that possess tumor initiation and self-renewal capacity with high resistance to current cancer treatments (including chemotherapy and radiation therapy). In BC, these cells can be identified by the presence of the cell surface antigens $CD44^{high}/CD24^{low}$, and CD133 and by an increased enzymatic activity of aldehyde dehydrogenase (ALDH1) [131]. Accordingly, these biomarkers can be used as targets for new therapeutic strategies based on NMs. An example of the approach targeting BCSC biomarkers on a breast cancer murine model was recently reported by Al Farah and collaborators. The authors used the combination of paclitaxel- and salinomycin-CD44 Ab conjugates linked via an hydrazone spacer to single wall carbon nanotubes (SWCNTs) as carrier. The results obtained from the in vivo study with this complex NM confirmed its enhanced therapeutic effect on BCSCs population [132]. Additional strategies that use lipid NPs loaded with paclitaxel and coated with hyaluronic acid (HA) [133] or complexed with micro-RNA-200 [134] showed enhanced cytotoxicity of the payload drugs against BCSCs. Other valuable strategies target membrane proteins on BCSCs. Int his respect, Swaminathan et al. developed polymeric NPs loaded with paclitaxel and conjugated to an anti-CD133 monoclonal Ab [135]. Interestingly, this in vivo study performed in MDA-MB-231 xenograft mice showed that while free paclitaxel initially effectively inhibited tumor growth, disease recurrence started very quickly as soon as the treatment was stopped. On the contrary, tumor regrowth was significantly lower when paclitaxel was delivered through CD133NPs (e.g., tumor volume was 518.6 ± 228 vs. $1370.9 \pm 295\ mm^3$ for free paclitaxel after 63days) [135]. However, BCSCs likely remain one of the biggest hurdles on the road to BC NMs development, as in all cancer types CSCs are always correlated with poor clinical outcome due to their notorious contribution to chemotherapy resistance and metastasis [136].

16.4.2 Interstitial fluid pressure

Another important factor that limits the efficacy of chemotherapeutic agents is interstitial fluid pressure (IFP) [137]. In solid tumors like BC, the angiogenic factors are dysregulated and this leads to the development of a disorganized network of vasculature. These abnormal features together with poorly formed fenestrations lead to leaky tumor vessels and irregular blood-flow [138]. Because of this leaky vasculature, fluids and proteins are released into the interstitial space. The absence of drainage by the lymphatic

system results in accumulation of these factors; as a consequence, an increase in IFP is observed (up to 100 mmHg as compared to normal interstitial pressure, which is equal to the atmospheric pressure) [139]. In addition, tumor cells also compress blood vessels, which exacerbates the problem. Therefore, in BC, IFP may operate as a barrier to interstitial transport of the drug, ultimately resulting in its poor distribution and penetration in tumor cells. As a consequence, tumor cells exposed to suboptimal drug concentration could develop acquired resistance. Under this perspective, several strategies have been developed in order to decrease IFP; i.e., normalization of tumor vasculature by inhibiting angiogenic growth factors, appliance of hyaluronan-degrading enzymes in order to reduce the swelling pressure of the extracellular matrix (ECM), or usage of nicotinamide to lower the vascular resistance [140].

16.4.3 Triple-negative breast cancer

Another population of breast cancer subtypes that is difficult to target is constituted by triple-negative breast cancers (TNBCs), which neither express hormone receptors, nor overexpress HER2. They are associated with poor prognosis, as defined by low 5 year survival and high recurrence rates after adjuvant therapy [141]. TNBC (15%−20% of BCs) is the most aggressive form of breast cancer, mostly because, when diagnosed, the disease is already in an advanced stage [142]. Several studies have been performed in order to discover molecular markers of TNBC; however, only few of them might have clinical utility, namely epidermal growth factor (EGFR or HER1), folate receptor, and chemokine (C-X-C motif) receptor 4 (CXCR4) [143]. Shu et al. developed siRNA loaded NPs that were linked to an anti−EGFR targeting peptide (GE11), and their studies revealed that GE11-conjugated NPs are suitable nanocarriers for siRNA delivery specifically to MDA-MB-468 cells (TNBC cell model) [144]. More recently, a similar strategy was used to target TNBC cells using anti-EGFR decorated NPs that were loaded with paclitaxel [145]. These in vivo study on mice xenografts showed that anchoring of anti−EGFR targeting protein to the NPs surface increase the antitumor effect of the payload by specifically binding with the EGFR protein on TNBC cell membrane, finally resulting in a significant reduction of tumor mass over time [145].

Based on many reports, folate receptor is overexpressed in TNBC patients [146] and is associated with poorer disease-free survival [147]. Folate receptor-targeted NPs loaded with orlistat, an antiobesity drag that demonstrated its antitumor effect through fatty acid synthase pathway that is upregulated in TNBC, induced almost 90% apoptosis in MDA-MB-231 cells [148]. In another NM formulation, doxorubicin was also encapsulated in protein NPs that was conjugated with folic acid, and showed significant inhibition of cell proliferation in MDA-MB-231 cells at much lower doses compared to free drug [149].

Also, overexpression of the chemokine receptor CXCR4 upholds tumor propagation and chemotaxis and predicts a poor outcome in patients with TMBC [150]. Accordingly, Misra et al. developed a pH-sensitive immunoliposome conjugated with an anti-CXCR4

antagonist that not only targeted CXCR4 but also inhibited CXCL12-induced signaling in an in vitro model of CXCR4-expressing breast cancer cells [151]. Specifically, these NPs are comprised of a novel poly(lactide-co-glycolide) derivative that allows for straightforward immobilization of 1,1'-[1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (Plerixafor), a small molecule with affinity for CXCR4. The work of Misra and coworkers [151] showed that these targeted nanocarriers are selectively taken up by CXCR4-expressing cells and effectively block CXCR4 signaling, suggesting that CXCR4 may be an effective target for nanocarrier-based therapies.

16.5 Challenges in the clinical translation of BC NMs

While a number of NM products are already on the market and a plethora of new compounds are currently entering clinical development, the existing weak points and regulatory gaps hampering the way to their effective clinical translation must be analyzed. In BC therapy, the most frequently observed clinical benefit NM is limited to reduced toxicity, with little evidence of enhanced efficacy. However, a deeper understanding of the BC molecular biology and an exhaustive preclinical investigation of the possible NM strategies discussed above is undoubtedly expected to lead to products with higher efficacy that will ultimately obtain regulatory approval. Major concerns in the application of different strategies in new NM design are the precise characterization of the final NM structure, and the physicochemical complexity of the formulations themselves. Indeed, this latter aspect not only increases the risk of toxicity and immunogenicity of the relevant NM but also implies high production costs and problems related to large-scale manufacturing. Issues in large-scale good manufacturing production (cGMP)—mandatorily required for NM clinical translation—render quality assurance (QA) and quality control (QC) evaluations of such nanoformulations quite laborious. Indeed, multiple features of the manufactured NMs have to be defined in detail, e.g., chemistry, manufacturing, and controls (CMC) information is required for investigational new drugs (IND) at each phase of investigation to ensure proper identity, strength or potency, quality, and purity of the drug substance and final product [152]. Importantly, the reproducibility of manufactured NMs can be particularly challenging taking into consideration the number of parameters that have to be validated (e.g., size distribution, morphology, charge, purity, drug encapsulation efficiency, coating efficiency, and density of conjugated ligand/s) [153]. Indeed, batch-to-batch variation of NMs can cause substantial alterations to their physicochemical properties (e.g., polarity and size), PK parameters (i.e., absorption, distribution, metabolism and excretion), and/or pharmacodynamics (PD) interactions (e.g., cellular interaction and activity). Thus, the reproducibility has to be guaranteed according to GMP standards during large-scale production.

Features like PK and PD are critical also during the preclinical investigation and they have to be guaranteed in in vivo studies; thus, appropriate animal models must be

selected for their strict in vivo evaluation. Prior to in vivo studies in animal models, comprehensive in vitro and/or ex vivo assays for tracking NMs safety profiles have to be performed. The preclinical phase of testing usually involves animal studies to demonstrate efficacy, safety, toxicity profile, and to identify appropriate dose ranges. To avoid the development of unpredictable adverse effects a complete toxicity profile of NMs should be analyzed before entering the market. Nevertheless, there are no standardized assays and protocols for early detection of toxicity. Although large-scale research attempts have been pursued, specific toxicity protocols to characterize NMs are still limited and insufficient [154]. The Nanotechnology Characterization Laboratory (NCL; established by the National Cancer Institute) has published documents about innovative platforms for the development of NMs for cancer treatment and others are under development [11].

Moving forward, the clinical evaluation of NMs is characterized by the lack of clear regulatory and safety guidelines as well. Currently, the FDA approval process for NMs is essentially the same as that for any other drug, biologic or device [7,11]. More precisely, the FDA classifies nanotechnology-based products as combination products, assigning a primary regulatory route and supplementing with ad hoc requirements as necessary to assure safety and efficacy [155]. Due to the complex structure of new NMs, their unclear interactions with cells and tissues in the human body, these regulations are no longer appropriate to confirm their quality, safety, and efficacy for clinical use [11]. Therefore, new regulatory standards and protocols should be developed considering the increasing structural complexity of NMs, but also route of administration, PK, PD, and safety profile. They should also ensure an optimal clinical trial design providing information on the most appropriate patient selection [156]. Moreover, not only safety and quality profile of NMs but also documentation on possible prevention of environmental issues should be ensured as well [157].

The lack of specific regulatory requirements translates into uncertainty for investors and reduced public acceptance. Indeed, from the pharma perspective considering the risks that may hinder clinical success and, most vitally, whether the agent might succeed with a minimal initial investment is of outmost importance. The cost−benefit analysis is the key aspect that has to be taken in consideration prior to and all along an NM therapy development pipeline. Generally, the costs associated with the development of a nanodrug are much higher compared to standard therapy, as a consequence of the lack of in vitro and preclinical standardized tests that can predict adequately a given NM performance in the human body. As discussed in the previous Sections, the majority of approved nanodrugs in cancer therapy are conventional, already approved, chemotherapeutics which formulation has been modified. Therefore, so far, the decision to develop these nanoformulations implicated relatively reduced financial risk since the efficacy and safety of the active agent was already investigated and proven [6]. On the other hand, investments are decidedly heavier when a novel chemical entity has to be developed. However, pharmacoeconomic studies need to be performed to determine the economic and social value of NMs compared to traditional therapy [11]. Concerns such

as improvement in quality and life expectancy of patients and reduction of personnel costs and hospitalization days as well as the savings in the number of medical procedures have to be carefully taken into account. These cost savings will be decisive for the overall cost-effectiveness of NM products [158]. In addition, public awareness of NMs is still low. Therefore, citizens and physicians should be properly informed about the benefits and risks of NM [159].

Considering all these shortcomings and benefits, collaborations between pharmaceutical industry and scientists across academia, medical doctors and most importantly, regulatory agencies will help shaping the new era of clinical NMs in cancer therapy. Yet the biggest challenge is to overcome the lack of precisely defined regulatory requirements and to harmonize globally the existing regulations in different countries. Some improvements in this respect have already been achieved starting from 2009 when EMA's Committee for Medicinal Products for Human Use (CHMP) established an ad hoc expert-group on NMs. CHMP chairs schedule regular meetings with licensing authorities from USA, Japan, Canada and Australia. This group of selected experts review the guidelines on NM development and tries to direct the progress toward appropriate and effective clinical translation. Although this might seem like a herculean effort, with the strengthening of cross collaborations and the adoption of multidisciplinary approaches the safe and efficient clinical translation of NMs will shortly no longer be a "mission impossible."

16.6 Future perspectives in breast cancer nanomedicine

One of the main, further promises brought along by cancer NMs is the possibility to merge therapeutic and diagnostic features in one single nanoformulation (aka theranostics). Actually, theranostic NMs are NPs that combine imaging and therapeutic agents and emerge as an alternative to the separate administration of diagnostic probes and pharmacologically active molecules. Although there is an increasing number of in vitro studies in this field, progress in the use of theranostic agents is somewhat slower compared to therapeutic NPs. This is mainly due to the fact that these nanosystems have difficulties in achieving acceptable PK properties and standardization in particle features, as well as concerns about toxicity, biodegradation, and elimination. Indeed, imaging has a crucial role in BC detection; e.g., mammographic screening drastically reduced breast cancer mortality. Therefore, the combination therapeutic and diagnostic features in one single nanoentity is crucial since it would enable simultaneous diagnostic imaging and therapy efficiency monitoring. SPIONs are one of the most feasible NPs that could be used as contrast agents for noninvasive MRI and targeted drug delivery, since their surface can be coated with targeting macromolecules, therapeutics payloads, or additional imaging tags [160]. In a study reported by Yang et al. active targeting of tumor marker on breast cancer cells (HER2) was used as a strategy, and showed a combination of doxorubicin and iron oxide NPs, which surface was functionalized with the anti-HER

antibody [161]. After administration of these NPs the authors observed an increase of 50.5% in MR image contrast, compared to animals that were treated with NPs functionalized with an irrelevant antibody. Furthermore, injection of these NPs did not only show preferential tumor accumulation of the NPs, but also a significant therapeutic activity compared to controls [161]. Iron oxide was used also in complex with quantum dots (QDs) and doxorubicin in order to perform QD/MRI-based imaging and therapy in a breast cancer mouse model [162]. In addition, QDs were used in complex with the monoclonal anti-HER2 Ab trastuzumab both for target imaging and therapeutic effect on BC [163]. Also, thermosensitive liposomes that coencapsulate Gd^{3+} and doxorubicin (HaT-DOX-Gd) were tested in vivo in combination with hyperthermia, on a mouse model of breast cancer (EMT-6), and showed promising theranostic effect [164].

Other combinational therapeutic modalities such as chemotherapy and hyperthermia can be co-administered to take advantage of synergistic effects that can improve efficacy and decrease side effects. Indeed, the photothermal ablation (PTA) was shown to have tremendous therapeutic potential. The PTA focuses a light laser source on the tumor and the absorbed light energy is transformed into heat that is lethal to cancer cells. This effect can be enhanced with the addition of plasmonic photothermal sensitizers, such as GNPs, which have very high optical cross-sections at illumination wavelengths. Therefore, when GNPs are activated by the lasers, there is a rapid increase in temperatures. A good example of such an agent is the AuroLase nanosystems discussed previously. Once injected, laser irradiation causes a temperature increase in the AuroLase NPs which eventually leads to tumor ablation. Efficacy of PTA has been shown in an array of cancer cells including breast [165,166], liver [167], prostate [168], brain [169], lung [170], and pancreatic cancers [171].

Combinational therapies are advantageous in breast cancer treatment as well. First of all because the dose of each agent can be reduced, thus the compliance of the patient is improved; moreover, the combination of two agents can lead to higher target selectivity (depending on the mechanism) or even better, to synergic effect and thereby to higher therapeutic efficacy [172]. Indeed, drug delivery systems derived from liposomes, polymeric and inorganic NPs are currently under preclinical and clinical development as innovative NMs that can deliver a combination of multiple drugs to various cancers. Deng et al. designed liposomal NP coated with PLA and HA (active targeting for the CD44 biomarker on breast cancer cells) that simultaneously delivered doxorubicin and siRNA, with high in vivo stability and low toxicity, that reduced the tumor growth, and in some cases, led to a complete tumor shrinkage compared to treatment with doxorubicin alone [173]. Using similar approach with micelles composed of PLGA and poly(ethylene imine) (PEI), coated with HA and loaded with doxorubicin and micro-RNA (miR-542-3p), Wang et al. [174] showed effective codelivery of the agents and synergic effects on apoptosis of breast cancer cells. Alternatively, an elegant study has shown that the sequential codelivery of two anticancer agents is even more efficacious [175]. Indeed, to obtain such sequential effect GNPs that are responsive to near-infra-red (NIR) light were used. In the latter study, the GNPs were attached to miR-21i and doxorubicin, once administrated the

miR-21i enters the cells by endocytosis and gets released in cytoplasm, escaping endosomes. After that, NIR was applied for 4 h which caused a collapse of GNPs realizing a burst release of doxorubicin. This kind of sequence events produced a synergistic effect to exhibit a superior anticancer efficacy.

Photodynamic therapy (PDT) is another emerging technique for the treatment of many cancers which uses a photosensitizer-based drug, oxygen and light of specific wavelength to generate reactive oxygen species (ROS), which can promote cell death (apoptosis or necrosis depending on the intensity of delivered light) [176]. Since it is possible to focalize the radiation beam to a precise area of interest, there is a significant reduction of adverse effects on nearby healthy tissues. Portilho et al. [177] showed in their in vivo study that PDT mediated by a new formulation based on albumin nanospheres containing zinc-phthalocyanine tetrasulfonate (ZnPcS4-AN) inhibited tumor growth causing no adverse effects. This constitutes a proof-of-concept for PDT as an emergent promising nanotechnology-based approach for the treatment of BC. One clinical trial using Visudyne, a liposomal formulation of verteporfin (photosensitizer) in combination with PDT, demonstrated that the cytotoxic effect of the compound is obtained only after the light activation and in the presence of oxygen [178]. The resulting ROS cause damage to biological structures, leading to local vascular occlusion, cell damage and ultimately cell death [178]. Other inorganic and protein NPs were also successfully used for delivery of photosensitizers; however, they are still at preliminary stages of research [179,180]. Not only combination of therapeutic agents but also a combination of two techniques (e.g., simultaneous radiotherapy and superficial hyperthermia) have shown good therapeutic outcomes in the treatment of high-risk breast carcinoma [181].

In summary, the current efforts and progresses made in understanding NMs effects both in vitro and in vivo breast cancer models are undoubtedly paving the way of these nanoformulations to the clinical practice, notwithstanding stringent requirements for more cost-effective nanobased systems which allow BC diagnosis and treatment at an early stage with a high level of specificity still posing important obstacle. The future is bright, however, and full of opportunities as well as tremendous near-term rewards for BC patients.

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References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, Cancer J. Clin. 68 (1) (2018) 7−30.

[2] J.L. Caswell-Jin, S.K. Plevritis, L. Tian, C.J. Cadham, C. Xu, N.K. Stout, G.W. Sledge, J.S. Mandelblatt, A.W. Kurian, Change in survival in metastatic breast cancer with treatment advances: meta-analysis and systematic review, JNCI Cancer Spectr. 2 (4) (2018) pky062.

[3] A.G. Waks, E.P. Winer, Breast cancer treatment: a review, J. Am. Med. Assoc. 321 (3) (2019) 288−300.

[4] S. Soares, J. Sousa, A. Pais, C. Vitorino, Nanomedicine: principles, properties, and regulatory issues, Front Chem. 6 (2018) 360.

[5] C.M. Hartshorn, L.M. Russell, P. Grodzinski, National Cancer Institute Alliance for nanotechnology in cancer-catalyzing research and translation toward novel cancer diagnostics and therapeutics, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 11 (6) (2019) e1570.

[6] H.A. Havel, Where are the nanodrugs? An industry perspective on development of drug products containing nanomaterials, AAPS J. 18 (6) (2016) 1351−1353.

[7] H. Havel, G. Finch, P. Strode, M. Wolfgang, S. Zale, I. Bobe, et al., Nanomedicines: from bench to bedside and beyond, AAPS J. 18 (6) (2016) 1373−1378.

[8] https://clinicaltrials.gov/ct2/home.

[9] J.E. Celis, D. Pavalkis, A mission-oriented approach to cancer in Europe: a joint mission/vision 2030, Mol. Oncol. 11 (12) (2017) 1661−1672.

[10] T. Lammers, S. Aime, W.E. Hennink, G. Storm, F. Kiessling, Theranostic nanomedicine, Acc. Chem. Res. 44 (10) (2011) 1029−1038.

[11] V. Sainz, J. Conniot, A.I. Matos, C. Peres, E. Zupancic, L. Moura, et al., Regulatory aspects on nanomedicines, Biochem. Biophys. Res. Commun. 468 (3) (2015) 504−510.

[12] J.M. Caster, A.N. Patel, T. Zhang, A. Wang, Investigational nanomedicines in 2016: a review of nanotherapeutics currently undergoing clinical trials, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 9 (1) (2017).

[13] S.T. Duggan, G.M. Keating, Pegylated liposomal doxorubicin: a review of its use in metastatic breast cancer, ovarian cancer, multiple myeloma and AIDS-related Kaposi's sarcoma, Drugs 71 (18) (2011) 2531−2558.

[14] R. Abe, J.F. Forbes, N. Wilcken, M. Ploner, et al., Early breast cancer trialists' collaborative group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomized trials, Lancet 365 (9472) (2005) 1687−1717.

[15] K. Shan, A.M. Lincoff, J.B. Young, Anthracycline-induced cardiotoxicity, Ann. Intern. Med. 125 (1) (1996) 47−58.

[16] S.M. Swain, F.S. Whaley, M.S. Ewer, Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials, Cancer 97 (11) (2003) 2869−2879.

[17] E. Bria, F. Cuppone, M. Fornier, C. Nisticò, P. Carlini, M. Milella, et al., Cardiotoxicity and incidence of brain metastases after adjuvant trastuzumab for early breast cancer: the dark side of the moon? A meta-analysis of the randomized trials, Breast Cancer Res. Treat. 109 (2) (2008) 231−239.

[18] Y.C. Barenholz, Doxil-the first FDA-approved nano-drug: lessons learned, J. Control. Release 160 (2) (2012) 117−134.

[19] A.A. Gabizon, Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy, Cancer Invest. 19 (4) (2001) 424−436.

[20] N.J. Butcher, G.M. Mortimer, R.F. Minchin, Drug delivery: unravelling the stealth effect, Nat. Nanotechnol. 11 (4) (2016) 310−311.

[21] M.E. O'Brien, N. Wigler, M. Inbar, R. Rosso, E. Grischke, A. Santoro, et al., Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYXTM/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer, Ann. Oncol. 15 (3) (2004) 440–449.

[22] L. Ansari, F. Shiehzadeh, Z. Taherzadeh, S. Nikoofal-Sahlabadi, A.A. Momtazi-Borojeni, A. Sahebkar, et al., The most prevalent side effects of pegylated liposomal doxorubicin monotherapy in women with metastatic breast cancer: a systematic review of clinical trials, Cancer Gene Ther. 24 (5) (2017) 189–193.

[23] J. Balazsovits, L. Mayer, M. Bally, P. Cullis, M. McDonell, R. Ginsberg, et al., Analysis of the effect of liposome encapsulation on the vesicant properties, acute and cardiac toxicities, and antitumor efficacy of doxorubicin, Cancer Chemother. Pharmacol. 23 (1989) 81–86.

[24] L. Harris, G. Batist, R. Belt, D. Rovira, R. Navari, N. Azarnia, et al., Liposome encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma, Cancer 94 (2002) 25–36.

[25] G. Batist, G. Ramakrishnan, C.S. Rao, A. Chandrasekharan, J. Gutheil, T. Guthrie, et al., Reduced cardiotoxicity and preserved antitumor efficacy of liposome encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer, J. Clin. Oncol. 19 (5) (2001) 1444–1454.

[26] FDA approval of generic version of cancer drug Doxil is expected to help resolve shortage, Oncology 35 (6) (2013) 25. http://journals.lww.com/oncology-times/Citation/2013/03250/FDA_Approves_Generic_Version_of_Doxil__Expected_to.16.aspx.

[27] J.A. Smith, L. Mathew, M. Burney, P. Nyshadham, R.L. Coleman, et al., Equivalency challenge: evaluation of Lipodox® as the generic equivalent for Doxil® in a human ovarian cancer orthotropic mouse model, Gynecol. Oncol. 141 (2) (2016) 357–363.

[28] C.E. Petre, D.P. Dittmer, Liposomal daunorubicin as treatment for Kaposi's sarcoma, Int. J. Nanomed. 2 (2007) 277.

[29] T.M. Allen, F.J. Martin, Advantages of liposomal delivery systems for anthracyclines, Semin. Oncol. 31 (6 Suppl. 13) (2004) 5–15.

[30] L. Fumagalli, M. Zucchetti, I. Parisi, M.G. Viganò, B. Zecca, A. Careddu, et al., The pharmacokinetics of liposomal encapsulated daunorubicin are not modified by HAART in patients with HIV-associated Kaposi's sarcoma, Cancer Chemother. Pharmacol. 45 (6) (2000) 495–501.

[31] D.S. Alberts, N.R. Bachur, J.L. Holtzman, The pharmacokinetics of daunomycin in man, Clin. Pharmacol. Ther. 12 (1) (1971) 96–104.

[32] S.K. Golombek, J.N. May, B. Theek, L. Appold, N. Drude, F. Kiessling, et al., Tumor targeting via EPR: strategies to enhance patient responses, Adv. Drug Deliv. Rev. 130 (2018) 17–38.

[33] K.J. O'Byrne, A.L. Thomas, R.A. Sharma, M. DeCatris, F. Shields, S. Beare, et al., A phase I dose-escalating study of DaunoXome, liposomal daunorubicin, in metastatic breast cancer, Br. J. Cancer 87 (1) (2002) 15–20.

[34] https://www.cancernetwork.com/breast-cancer/daunoxome-shows-promise-breast-cancer-treatment.

[35] National Cancer Institute (NCI), Clinicaltrials.gov Identifier: NCT00004207, 2004. Retrieved from, https://clinicaltrials.gov/ct2/show/NCT00004207.

[36] J.M. Nabholtz, J. Gligorov, The role of taxanes in the treatment of breast cancer, Expert Opin. Pharmacother. 6 (7) (2005) 1073–1094.

[37] J. Crown, M. O'Leary, W.S. Ooi, Docetaxel and paclitaxel in the treatment of breast cancer: a review of clinical experience, Oncologist 9 (Suppl. 2) (2004) 24–32.

[38] E.K. Rowinsky, R.C. Donehower, Paclitaxel (Taxol), N. Engl. J. Med. 332 (1995) 1004–1014.

[39] R.B. Weiss, R.C. Donehower, P.H. Wiernik, T. Ohnuma, R.J. Gralla, D.L. Trump, et al., Hypersensitivity reactions from Taxol, J. Clin. Oncol. 8 (7) (1990) 1263−1268.

[40] H. Gelderblom, J. Verweij, K. Nooter, E.L. Sparreboom Cremophor, The drawbacks and advantages of vehicle selection for drug formulation, Eur. J. Cancer 37 (2001) 1590−1598.

[41] A.H. Kyle, L.A. Huxham, D.M. Yeoman, A.I. Minchinton, Limited tissue penetration of taxanes: a mechanism for resistance in solid tumors, Clin. Cancer Res. 13 (9) (2007) 2804−2810.

[42] N.K. Ibrahim, N. Desai, S. Legha, P. Soon-Shiong, R.L. Theriault, E. Rivera, B. Esmaeli, et al., Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel, Clin. Cancer Res. 8 (5) (2002) 1038−1044.

[43] E. Miele, G.P. Spinelli, E. Miele, F. Tomao, S. Tomao, Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer, Int. J. Nanomed. 4 (2009) 99−105.

[44] K. Paal, J. Muller, L. Hegedus, High affinity binding of paclitaxel to human serum albumin, Eur. J. Biochem. 268 (7) (2001) 2187−2191.

[45] A. Sparreboom, C.D. Scripture, V. Trieu, P.J. Williams, T. De, A. Yang, et al., Comparative preclinical and clinical pharmacokinetics of a cremophor free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol), Clin. Cancer Res. 11 (11) (2005) 4136−4143.

[46] V. Guarneri, M.V. Dieci, P. Conte, Enhancing intracellular taxane delivery: current role and perspectives of nanoparticle albumin-bound paclitaxel in the treatment of advanced breast cancer, Expert Opin. Pharmacother. 13 (3) (2012) 395−406.

[47] M.J. Hawkins, P. Soon-Shiong, N. Desai, Protein nanoparticles as drug carriers in clinical medicine, Adv. Drug Deliv. Rev. 60 (8) (2008) 876−885.

[48] N. Desai, V. Trieu, Z. Yao, L. Louie, S. Ci, A. Yang, et al., Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel, Clin. Cancer Res. 12 (4) (2006) 1317−1324.

[49] W.J. Gradishar, S. Tjulandin, N. Davidson, H. Shaw, N. Desai, P. Bhar, et al., Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer, J. Clin. Oncol. 23 (2005) 7794−7803.

[50] N. Chen, Y. Li, Y. Ye, M. Palmisano, R. Chopra, S. Zhou, Pharmacokinetics and pharmacodynamics of nab-paclitaxel in patients with solid tumors: disposition kinetics and pharmacology distinct from solvent-based paclitaxel, J. Clin. Pharmacol. 54 (10) (2014) 1097−1107.

[51] V.M. Steffes, M.M. Murali, Y. Park, B.J. Fletcher, K.K. Ewert, C.R. Safinya, Distinct solubility and cytotoxicity regimes of paclitaxel-loaded cationic liposomes at low and high drug content revealed by kinetic phase behavior and cancer cell viability studies, Biomaterials 145 (2017) 242−255.

[52] A. Yang, J. Li, H. Xu, H. Chen, A study on antitumor effect of liposome encapsulated paclitaxel in vivo and in vitro, Bull. Chin. Cancer 15 (2006) 862−864.

[53] Q. Zhang, X.E. Huang, L.L. Gao, A clinical study on the premedication of paclitaxel liposome in the treatment of solid tumors, Biomed. Pharmacother. 63 (8) (2009) 603−607.

[54] T.Y. Kim, D.W. Kim, J.Y. Chung, S.G. Shin, S.C. Kim, D.S. Heo, et al., Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clin. Cancer Res. 10 (11) (2004) 3708−3716.

[55] E. Chu, V.T. Devita, Physicians' Cancer Chemotherapy Drug Manual 2013, Jones & Bartlett Learning LLC, MA, USA, 2012.

[56] K.S. Lee, H.C. Chung, S.A. Im, Y.H. Park, C.S. Kim, S.B. Kim, et al., Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer, Breast Cancer Res. Treat. 108 (2) (2008) 241−250.

[57] I.H. Park, J.H. Sohn, S.B. Kim, K.S. Lee, J.S. Chung, S.H. Lee, et al., An open-label, randomized, parallel, phase III trial evaluating the efficacy and safety of polymeric micelle-formulated paclitaxel compared to conventional cremophor EL-based paclitaxel for recurrent or metastatic HER2-negative breast cancer, Cancer Res. Treat. 49 (3) (2017) 569–577.

[58] N. Ron, J. Cordia, A. Yang, S. Ci, P. Nguyen, M. Hughs, et al., Comparison of physicochemical characteristics and stability of three novel formulations of paclitaxel: Abraxane, Nanoxel, and Genexol-PM, Cancer Res. 68 (9 Suppl.) (2008) 5622. AACR Annual Meeting–Apr 12–16, 2008; San Diego, California, USA.

[59] Y. Matsumura, Polymeric micellar delivery systems in oncology, Jpn. J. Clin. Oncol. 38 (12) (2008) 793–802.

[60] Fresenius Kabi Oncology Ltd, ClinicalTrial.org Identifier: NCT00915369, 2009. Retrieved from: http://clinicaltrials.gov/ct2/show/NCT00915369.

[61] Clinical treatment with Nano Therm™ therapy. www.magforce.de/en/studien.html.

[62] K. Li, H. Nejadnik, H.E. Daldrup-Link, Next-generation superparamagnetic iron oxide nanoparticles for cancer theranostics, Drug Discov. Today 22 (9) (2017) 1421–1429.

[63] S. Palanisamy, Y.M. Wang, Superparamagnetic iron oxide nanoparticulate system: synthesis, targeting, drug delivery and therapy in cancer, Dalton Trans. 48 (26) (2019) 9490–9515.

[64] M. Mazzotta, E. Krasniqi, G. Barchiesi, L. Pizzuti, F. Tomao, M. Barba, P. Vici, Long-term safety and real-world effectiveness of trastuzumab in breast cancer, J. Clin. Med. 8 (2) (2019) E254.

[65] Y.V. Kovtun, V.S. Goldmacher, Cell killing by antibody-drug conjugates, Cancer Lett. 255 (2007) 232–240.

[66] M. Ritchie, L. Tchistiakova, N. Scott, Implications of receptor-mediated endocytosis and intracellular trafficking dynamics in the development of antibody drug conjugates, mAbs 5 (2013) 13–21.

[67] G.D. Lewis Phillips, G. Li, D.L. Dugger, L.M. Crocker, K.L. Parsons, E. Mai, et al., Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate, Cancer Res. 68 (2008) 9280–9290.

[68] H.K. Erickson, P.U. Park, W.C. Widdison, Y.V. Kovtun, L.M. Garrett, K. Hoffman, et al., Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing, Cancer Res. 66 (2006) 4426–4433.

[69] S.A. Hurvitz, L. Dirix, J. Kocsis, G.V. Bianchi, J. Lu, J. Vinholes, et al., Phase II randomized study of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with human epidermal growth factor receptor 2–positive metastatic breast cancer, J. Clin. Oncol. 31 (9) (2013) 1157–1163.

[70] S. Verma, D. Miles, L. Gianni, I.E. Krop, M. Welslau, J. Baselga, et al., Trastuzumab emtansine for HER2-positive advanced breast cancer, N. Engl. J. Med. 367 (19) (2012) 1783–1791.

[71] J. Chen, C.Q. He, A.H. Lin, W. Gu, Z.P. Chen, W. Li, et al., Thermosensitive liposomes with higher phase transition temperature for targeted drug delivery to tumor, Int. J. Pharm. 475 (1–2) (2014) 408–415.

[72] Celsion, ClinicalTrial.org Identifier: NCT00826085, 2009. Retrieved from, https://clinicaltrials.gov/ct2/show/results/NCT00826085.

[73] K. Miller, J. Cortes, S.A. Hurvitz, I.E. Krop, D. Tripathy, S. Verma, et al., HERMIONE: a randomized phase 2 trial of MM-302 plus trastuzumab versus chemotherapy of physician's choice plus trastuzumab in patients with previously treated, anthracycline-naïve, HER2-positive, locally advanced/metastatic breast cancer, BMC Cancer 16 (2016) 352.

[74] Merrimack Pharmaceuticals, Clinicaltrials.gov Identifier: NCT01304797, 2011. Retrieved from: https://clinicaltrials.gov/ct2/show/NCT01304797.

[75] Otilia Dalesio, The Netherlands Cancer Institute Clinicaltrials.gov Identifier: NCT01304797. Retrieved from: https://clinicaltrials.gov/ct2/show/NCT01818713.

[76] M.E. Eichhorn, I. Ischenko, S. Luedemann, S. Strieth, A. Papyan, A. Werner, et al., Vascular targeting by EndoTAG™-1 enhances therapeutic efficacy of conventional chemotherapy in lung and pancreatic cancer, Int. J. Cancer 126 (5) (2010) 1235–1245.

[77] G. Schuch, EndoTAG-1. MediGene, Curr. Opin. Investig. Drugs 6 (12) (2005) 1259.

[78] SynCore Biotechnology Co, Ltd, ClinicalTrials.gov Identifier: NCT03002103, 2016. Retrieved from: https://clinicaltrials.gov/ct2/show/NCT03002103.

[79] INSYS Therapeutics Inc, ClinicalTrials.gov Identifier: NCT03002103, 2004. Retrieved from: https://clinicaltrials.gov/ct2/show/NCT00080418.

[80] S. Ugwu, A. Zhang, M. Parmar, B. Miller, T. Sardone, V. Peikov, et al., Preparation, characterization, and stability of liposome-based formulations of mitoxantrone, Drug Dev. Ind. Pharm. 31 (2005) 223–229.

[81] H.I. Chang, M.K. Yeh, Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy, Int. J. Nanomed. 7 (2012) 49–60.

[82] H. Zhang, Onivyde for the therapy of multiple solid tumors, OncoTargets Ther. 9 (2016) 3001–3007.

[83] T.C. Chang, H.S. Shiah, C.H. Yang, K.H. Yeh, A.L. Cheng, B.N. Shen, et al., Phase I study of nanoliposomal irinotecan (PEP02) in advanced solid tumor patients, Cancer Chemother. Pharmacol. 75 (3) (2015) 579–586.

[84] N. Bernards, M. Ventura, I.B. Fricke, B.S. Hendriks, J. Fitzgerald, H. Lee, et al., Liposomal irinotecan achieves significant survival and tumor burden control in a triple negative breast cancer model of spontaneous metastasis, Mol. Pharm. 15 (9) (2018) 4132–4138.

[85] L. Kelland, The resurgence of platinum-based cancer chemotherapy, Nat. Rev. Cancer 7 (8) (2007) 573–584.

[86] G.P. Stathopoulos, T. Boulikas, M. Vougiouka, G. Deliconstantinos, S. Rigatos, E. Darli, et al., Pharmacokinetics and adverse reactions of a new liposomal cisplatin (Lipoplatin): phase I study, Oncol. Rep. 13 (4) (2005) 589–595.

[87] T. Boulikas, G.P. Stathopoulos, N. Volakakis, M. Vougiouka, Systemic Lipoplatin infusion results in preferential tumor uptake in human studies, Anticancer Res. 25 (2005) 3031–3039.

[88] T. Boulikas, Low toxicity and anticancer activity of a novel liposomal cisplatin (Lipoplatin) in mouse xenografts, Oncol. Rep. 12 (2004) 3–12.

[89] P. Devarajan, R. Tarabishi, J. Mishra, Q. Ma, A. Kourvetaris, M. Vougiouka, et al., Low renal toxicity of lipoplatin compared to cisplatin in animals, Anticancer Res. 24 (2004) 2193–2200.

[90] G.P. Stathopoulos, Liposomal cisplatin: a new cisplatin formulation, Anti Cancer Drugs 21 (2010) 732–736.

[91] S. Peters, Centre Hospitalier Universitaire Vaudois, Clinicaltrials.gov Identifier: NCT03002103. Retrieved from: https://clinicaltrials.gov/ct2/show/NCT02702700.

[92] F.S. Farhat, S. Temraz, J. Kattan, K. Ibrahim, N. Bitar, N. Haddad, et al., A phase II study of lipoplatin (liposomal cisplatin)/vinorelbine combination in HER-2/neu–negative metastatic breast cancer, Clin. Breast Cancer 11 (6) (2011) 384–389.

[93] J. Chiramel, A.C. Backen, R. Pihlak, A. Lamarca, M. Frizziero, N.U. Tariq, et al., Targeting the epidermal growth factor receptor in addition to chemotherapy in patients with advanced pancreatic cancer: a systematic review and meta-analysis, Int. J. Mol. Sci. 18 (5) (2017) 909.

[94] C. Kosmas, J. Angel, A. Athanasiou, A. Rapti, C. Karanikas, S. Lambaki, N. Politis, et al., Phase III study of lipoplatin plus gemcitabine versus cisplatin plus gemcitabine in advanced NSCLC; interim analysis, Eur. J. Cancer Suppl. 7 (2009) 531.

[95] G.P. Stathopoulos, D. Antoniou, J. Dimitroulis, P. Michalopoulou, A. Bastas, K. Marosis, et al., Liposomal cisplatin combined with paclitaxel versus cisplatin and paclitaxel in non-small-cell lung cancer: a randomized phase III multicenter trial, Ann. Oncol. 21 (11) (2010) 2227−2232.

[96] M. Fantini, L. Gianni, C. Santelmo, F. Drudi, C. Castellani, A. Affatato, M. Nicolini, A. Ravaioli, Lipoplatin treatment in lung and breast cancer, Chemother. Res. Pract. 2011 (2011) 125192.

[97] R. Yuan, A. Kay, W.J. Berg, D. Lebwohl, Targeting tumorigenesis: development and use of mTOR inhibitors in cancer therapy, J. Hematol. Oncol. 2 (2009) 45.

[98] N. Desai, O. D'Cruz, V. Trieu, Combination regimens of nab-rapamycin (ABI-009) effective against MDA-MB-231 breast-tumor xenografts, Cancer Res. 69 (2009) 6106.

[99] Celgene Corporation, Clinicaltrials.gov Identifier: NCT03002103. Retrieved from https://clinicaltrials.gov/ct2/show/NCT00531271.

[100] S.S. Chang, D.S. O'Keefe, D.J. Bacich, V.E. Reuter, W.D. Heston, P.B. Gaudin, Prostate-specific membrane antigen is produced in tumor-associated neovasculature, Clin. Cancer Res. 5 (1999) 2674−2681.

[101] BIND Therapeutics, Clinicaltrials.gov Identifier: NCT03002103. Retrieved from https://clinicaltrials.gov/ct2/show/NCT01812746.

[102] J. Hrkach, D. Von Hoff, M.M. Ali, E. Andrianova, J. Auer, T. Campbell, et al., Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile, Sci. Transl. Med. 4 (2012) 128ra39.

[103] https://www.biospace.com/article/pfizer-wins-bid-buys-bind-therapeutics-bankruptcy-assets-for-40-million-/.

[104] T. Saeki, H. Mukai, J. Ro, Y.-C. Lin, Y. Fujiwara, S. Nagai, et al., 250P A global phase III clinical study comparing NK105 and paclitaxel in metastatic or recurrent breast cancer patients, Ann. Oncol. 28 (Suppl. _5) (2017).

[105] T. Hamaguchi, Y. Matsumura, M. Suzuki, K. Shimizu, R. Goda, I. Nakamura, et al., NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumor activity and reduce the neurotoxicity of paclitaxel, Br. J. Cancer 92 (7) (2005) 1240−1246.

[106] Nippon Kayaku Co, Ltd, Clinicaltrials.gov Identifier: NCT03002103. Retrieved from https://clinicaltrials.gov/ct2/show/NCT01644890.

[107] J.M. Stern, V.V.K. Solomonov, E. Sazykina, J.A. Schwartz, S.C. Gad, G.P. Goodrich, Initial evaluation of the safety of nanoshell-directed photothermal therapy in the treatment of prostate disease, Int. J. Toxicol. 35 (1) (2016) 38−46.

[108] Nanospectra Biosciences, Inc, Clinicaltrials.gov Identifier: NCT03002103. Retrieved from https://clinicaltrials.gov/ct2/show/NCT00848042.

[109] Nanospectra Biosciences, Inc, Clinicaltrials.gov Identifier: NCT03002103. Retrieved from https://clinicaltrials.gov/ct2/show/NCT01679470.

[110] Nanospectra Biosciences, Inc, Clinicaltrials.gov Identifier: NCT03002103. Retrieved from https://clinicaltrials.gov/ct2/show/NCT02680535.

[111] L.R. Hirsch, R.J. Stafford, J.A. Bankson, S.R. Sershen, B. Rivera, R.E. Price, et al., Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance, Proc. Natl. Acad. Sci. USA 100 (23) (2003) 13549−13554.

[112] J. Lee, D.K. Chatterjee, M.H. Lee, S. Krishnan, Gold nanoparticles in breast cancer treatment: promise and potential pitfalls, Cancer Lett. 347 (1) (2014) 46−53.

[113] F. Wang, Y.C. Wang, S. Dou, M.H. Xiong, T.M. Sun, J. Wang, Doxorubicin-tethered responsive gold nanoparticles facilitate intracellular drug delivery for overcoming multidrug resistance in cancer cells, ACS Nano 5 (5) (2011) 3679−3692.

[114] T.M. Sun, Y.C. Wang, F. Wang, J.Z. Du, C.Q. Mao, C.Y. Sun, et al., Cancer stem cell therapy using doxorubicin conjugated to gold nanoparticles via hydrazone bonds, Biomaterials 35 (2014) 836–845.

[115] F. Mohammad, N.A. Yusof, Doxorubicin-loaded magnetic gold nanoshells for a combination therapy of hyperthermia and drug delivery, J. Colloid Interface Sci. 434 (2014) 89–97.

[116] Attacking Metastatic Breast Cancer July 6, 2017. https://cytimmune.blog/2017/07/06/attacking-metastatic-breast-cancer/.

[117] S.K. Libutti, G.F. Paciotti, A.A. Byrnes, H.R. Alexander Jr., W.E. Gannon, M. Walker, et al., Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine, Clin. Cancer Res. 16 (2010) 6139–6149.

[118] N.A. Koonce, C.M. Quick, M.E. Hardee, A. Jamshidi-Parsian, J.A. Dent, G.F. Paciotti, et al., Combination of gold nanoparticle-conjugated tumor necrosis factor-α and radiation therapy results in a synergistic antitumor response in murine carcinoma models, Int. J. Radiat. Oncol. Biol. Phys. 93 (3) (2015) 588–596.

[119] E.M. Gordon, F.L. Hall, Critical stages in the development of the first targeted, injectable molecular-genetic medicine for cancer, in: C. Kang (Ed.), Gene Therapy Applications, InTech, 2011, pp. 461–492.

[120] E.M. Gordon, F.L. Hall, Rexin-G, a targeted genetic medicine for cancer, Expert Opin. Biol. Ther. 10 (5) (2010) 819–832.

[121] S.P. Chawla, H. Bruckner, M.A. Morse, N. Assudani, F.L. Hall, E.M. Gordon, A phase I-II study using Rexin-G tumor-targeted retrovector encoding a dominant-negative cyclin G1 inhibitor for advanced pancreatic cancer, Mol. Ther. Oncolytics 12 (2018) 56–67.

[122] F.L. Hall, L. Liu, N.L. Zhu, M. Stapfer, W.F. Anderson, R.W. Beart, et al., Molecular engineering of matrix-targeted retroviral vectors incorporating a surveillance function inherent in von Willebrand factor, Hum. Gene Ther. 11 (2000) 983–993.

[123] E.M. Gordon, P.X. Liu, Z.H. Chen, L. Liu, M.D. Whitley, C. Gee, et al., Inhibition of metastatic tumor growth in nude mice by portal vein infusions of matrix-targeted retroviral vectors bearing a cytocidal cyclin G1 construct, Cancer Res. 60 (2000) 3343–3347.

[124] E.M. Gordon, Z.H. Chen, L. Liu, M. Whitley, L. Liu, D. Wei, et al., Systemic administration of a matrix-targeted retroviral vector is efficacious for cancer gene therapy in mice, Hum. Gene Ther. 12 (2001) 193–204.

[125] J. Torres-Mora, S. Dry, X. Li, S. Binder, M. Amin, A.L. Folpe, Malignant melanotic schwannian tumor: a clinicopathologic, immunohistochemical, and gene expression profiling study of 40 cases, with a proposal for the reclassification of 'melanotic schwannoma', Am. J. Surg. Pathol. 38 (2014) 94–105.

[126] E.M. Gordon, F.F. Lopez, G.H. Cornelio, C.C. Lorenzo, J.P. Levy, R.A. Reed, et al., Pathotropic nanoparticles for cancer gene therapy Rexin-G™ IV: three-year clinical experience, Int. J. Oncol. 29 (2006) 1053–1064.

[127] N.R. Patel, B.S. Pattni, A.H. Abouzeid, V.P. Torchilin, Nanopreparations to overcome multidrug resistance in cancer, Adv. Drug Deliv. Rev. 65 (2013) 1748–1762.

[128] V. Alakhov, E. Klinski, S. Li, G. Pietrzynski, A. Venne, e Batrakova, et al., Block copolymer-based formulation of doxorubicin. From cell screen to clinical trials, Colloids Surf. B Biointerfaces 16 (1999) 113–134.

[129] V. Omelyanenko, P. Kopeckova, C. Gentry, J. Kopecek, Targetable HPMA copolymer–adriamycin conjugates. Recognition, internalization, and subcellular fate, J. Control. Release 53 (1998) 25–37.

[130] J.W. Valle, A. Armstrong, C. Newman, V. Alakhov, G. Pietrzynski, J. Brewer, et al., A phase 2 study of SP1049C, doxorubicin in P-glycoprotein-targeting pluronic, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction, Investig. New Drugs 29 (5) (2011) 1029–1037.

[131] E. Charafe-Jauffret, C. Ginestier, F. Iovino, J. Wicinski, N. Cervera, et al., Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature, Cancer Res. 69 (2009) 1302−1313.

[132] A. Al Faraj, A.S. Shaik, E. Ratemi, R. Halwani, Combination of drug-conjugated SWCNT nanocarriers for efficient therapy of cancer stem cells in a breast cancer animal model, J. Control. Release 225 (2016) 240−251.

[133] F. Wang, L. Li, Z. Chen, C. Li, Hyaluronic acid decorated pluronic P85 solid lipid nanoparticle as a carrier to overcome multidrug resistance in cervical and breast cancer, Biomed. Pharmacother. 86 (2017) 595−604.

[134] J. Liu, T. Meng, M. Yuan, L. Wen, B. Cheng, N. Liu, et al., MicroRNA-200c delivered by solid lipid nanoparticles enhances the effect of paclitaxel on breast cancer stem cell, Int. J. Nanomed. 11 (2016) 6713−6725.

[135] S.K. Swaminathan, E. Roger, U. Toti, L. Niu, J.R. Ohlfest, J. Panyam, CD133-targeted paclitaxel delivery inhibits local tumor recurrence in a mouse model of breast cancer, J. Control. Release 171 (3) (2013) 280−287.

[136] A. Desai, Y. Yan, S.L. Gerson, Concise reviews: cancer stem cell targeted therapies: toward clinical success, Stem Cells Transl. Med. 8 (1) (2019) 75−81.

[137] C.-H. Heldin, K. Rubin, K. Pietras, A. Östman, High interstitial fluid pressure—an obstacle in cancer therapy, Nat. Rew. Cancer 4 (10) (2004) 806−813.

[138] O. Trédan, C.M. Galmarini, K. Patel, I.F. Tannock, Drug resistance and the solid tumor microenvironment, J. Natl. Cancer Inst. 99 (19) (2007) 1441−1454.

[139] J.R. Less, M.C. Posner, Y. Boucher, D. Borochovitz, N. Wolmark, R.K. Jain, Interstitial hypertension in human breast and colorectal tumors, Cancer Res. 52 (22) (1992) 6371−6374.

[140] R.K. Jain, R.T. Tong, L.L. Munn, Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: insights from a mathematical model, Cancer Res. 67 (2007) 2729−2735.

[141] S. Sharma, M. Barry, D.J. Gallagher, M. Kell, V. Sacchini, An overview of triple negative breast cancer for surgical oncologists, Surg. Oncol. 24 (3) (2015) 276−283.

[142] A.A. Jitariu, A.M. Cîmpean, D. Ribatti, M. Raica, Triple negative breast cancer: the kiss of death, Oncotarget 8 (28) (2017) 46652−46662.

[143] T.C. de Ruijter, J. Veeck, J.P. de Hoon, M. van Engeland, V.C. Tjan-Heijnen, Characteristics of triple-negative breast cancer, J. Cancer Res. Clin. Oncol. 137 (2) (2011) 183−192.

[144] D. Shu, H. Li, Y. Shu, G. Xiong, W.E. Carson 3rd, F. Haque, et al., Systemic delivery of anti-miRNA for suppression of triple negative breast cancer utilizing RNA nanotechnology, ACS Nano 9 (10) (2015) 9731−9740.

[145] V. Venugopal, S. Krishnan, V.R. Palanimuthu, S. Sankarankutty, J.K. Kalaimani, S. Karupiah, et al., Anti-EGFR anchored paclitaxel loaded PLGA nanoparticles for the treatment of triple negative breast cancer. In-vitro and in-vivo anticancer activities, PLoS One 13 (11) (2018) e0206109.

[146] S. Ginter, P.J. McIntire, X. Cui, L. Irshaid, Y. Liu, Z. Chen, et al., Folate receptor alpha expression is associated with increased risk of recurrence in triple-negative breast cancer, Clin. Breast Cancer 17 (7) (2017) 544−549.

[147] L.C. Hartmann, G.L. Keeney, W.L. Lingle, T.J. Christianson, B. Varghese, D. Hillman, et al., Folate receptor overexpression is associated with poor outcome in breast cancer, Int. J. Cancer 121 (2007) 938−942.

[148] R. Paulmurugan, R. Bhethanabotla, K. Mishra, R. Devulapally, K. Foygel, T.V. Sekar, et al., Folate receptor−targeted polymeric micellar nanocarriers for delivery of orlistat as a repurposed drug against triple-negative breast cancer, Mol. Cancer Ther. 15 (2) (2016) 221−231.

[149] Z. Kayania, A.-K. Bordbarb, O. Firuzic, Novel folic acid-conjugated doxorubicin loaded β-lactoglobulin nanoparticles induce apoptosis in breast cancer cells, Biomed. Pharmacother. 107 (2018) 945−956.

[150] Q.D. Chu, L. Panu, N.T. Holm, B.D.L. Li, L.W. Johnson, S. Zhang, High chemokine receptor CXCR4 level in triple negative breast cancer specimens predicts poor clinical outcome, J. Surg. Res. 159 (2) (2010) 689−695.

[151] A.C. Misra, K.E. Luker, H. Durmaz, G.D. Luker, J. Lahann, CXCR4-targeted nanocarriers for triple negative breast cancers, Biomacromolecules 16 (8) (2015) 2412−2417.

[152] FDA, Guidance for Industry − INDs for Phase 2 and Phase 3 Studies: Chemistry, Manufacturing, and Control (CMC) Information, 2003. http://www.fda.gov/cder/guidance./index.htm.

[153] M.K. Teli, S. Mutalik, G.K. Rajanikant, Nanotechnology and nanomedicine: going small means aiming big, Curr. Pharm. Des. 16 (16) (2010) 1882−1892.

[154] A. Elsaesser, C.V. Howard, Toxicology of nanoparticles, Adv. Drug Deliv. Rev. 64 (2) (2012) 129−137.

[155] J.J.D. Paradise, Regulating nanomedicine at the food and drug administration, AMA J. Ethics 21 (4) (2019) E347−E355.

[156] S. Tinkle, S.E. McNeil, S. Muhlebach, R. Bawa, G. Borchard, Y.C. Barenholz, et al., Nanomedicines: addressing the scientific and regulatory gap, Ann. N. Y. Acad. Sci. 1313 (2014) 35−56.

[157] R. Gaspar, Regulatory issues surrounding nanomedicines: setting the scene for the next generation of nanopharmaceuticals, Nanomedicine 2 (2007) 143−147.

[158] R. Bosetti, Cost−effectiveness of nanomedicine: the path to a future successful and dominant market? Nanomedicine 10 (12) (2015) 1851−1853.

[159] JCR European Commission, Joint Research Center, Nanomedicine: drivers for development and possible impacts. http://publications.jrc.ec.europa.eu/repository/handle/JRC46744.Google Scholar.

[160] M.K. Yu, Y.Y. Jeong, J. Park, S. Park, J.W. Kim, J.J. Min, et al., Drug-loaded superparamagnetic iron oxide nanoparticles for combined cancer imaging and therapy in vivo, Angew Chem. Int. Ed. Engl. 47 (29) (2008) 5362−5365.

[161] J. Yang, C.H. Lee, H.J. Ko, J.S. Suh, H.G. Yoon, K. Lee, et al., Multifunctional magneto-polymeric nanohybrids for targeted detection and synergistic therapeutic effects on breast cancer, Angew Chem. Int. Ed. Engl. 46 (46) (2007) 8836−8839.

[162] J.H. Park, G. von Maltzahn, E. Ruoslahti, S.N. Bhatia, M.J. Sailor, Micellar hybrid nanoparticles for simultaneous magneto-fluorescent imaging and drug delivery, Angew Chem. Int. Ed. Engl. 47 (2008) 7284−7288.

[163] M. Takeda, H. Tada, H. Higuchi, Y. Kobayashi, M. Kobayashi, Y. Sakurai, et al., In vivo single molecular imaging and sentinel node navigation by nanotechnology for molecular targeting drug-delivery systems and tailor-made medicine, Breast Cancer 15 (2) (2008) 145−152.

[164] T. Tagami, W.D. Foltz, M.J. Ernsting, C.M. Lee, I.F. Tannock, J.P. May, et al., MRI monitoring of intratumoral drug delivery and prediction of the therapeutic effect with a multifunctional thermosensitive liposome, Biomaterials 32 (27) (2011) 6570−6578.

[165] Z. Yang, T. Liu, Y. Xie, Z. Sun, H. Liu, J. Lin, et al., Chitosan layered gold nanorods as synergistic therapeutics for photothermal ablation and gene silencing in triple-negative breast cancer, Acta Biomater. 25 (2015) 194−204.

[166] Y. Liu, M. Xu, Q. Chen, G. Guan, W. Hu, X. Zhao, et al., Gold nanorods/mesoporous silica-based nanocomposite as theranostic agents for targeting near-infrared imaging and photothermal therapy induced with laser, Int. J. Nanomed. 10 (2015) 4747−4761.

[167] L. Mocan, C. Matea, F.A. Tabaran, O. Mosteanu, T. Pop, T. Mocan, et al., Photothermal treatment of liver cancer with albumin-conjugated gold nanoparticles initiates Golgi apparatus-ER

dysfunction and caspase-3 apoptotic pathway activation by selective targeting of Gp60 receptor, Int. J. Nanomed. 10 (2015) 5435–5445.

[168] W. Zhang, X. Zheng, S. Shen, X. Wang, Doxorubicin-loaded magnetic nanoparticle clusters for chemo-photothermal treatment of the prostate cancer cell line PC3, Biochem. Biophys. Res. Commun. 466 (2) (2015) 278–282.

[169] R.J. Bernardi, A.R. Lowery, P.A. Thompson, S.M. Blaney, J.L. West, Immunonanoshells for targeted photothermal ablation in medulloblastoma and glioma: an in vitro evaluation using human cell lines, J. Neuro Oncol. 86 (2) (2008) 165–172.

[170] P. Sharma, S.C. Brown, A. Singh, N. Iwakuma, G. Pyrgiotakis, V. Krishna, et al., Near-infra-red absorbing and luminescent gold speckled silica nanoparticles for photothermal therapy, J. Mater. Chem. 20 (2010) 5182–5185.

[171] Y.1 Guo, Z. Zhang, D.H. Kim, W. Li, J. Nicolai, D. Procissi, et al., Photothermal ablation of pancreatic cancer cells with hybrid iron-ox-ide core gold-shell nanoparticles, Int. J. Nanomed. 8 (2013) 3437–3446.

[172] J.H. Lee, A. Nan, Combination drug delivery approaches in metastatic breast cancer, J. Drug Deliv. 2012 (2012), 915375–915317.

[173] Z.J. Deng, S.W. Morton, E. Ben-Akiva, E.C. Dreaden, K.E. Shopsowitz, P.T. Hammond, Layer-by-layer nanoparticles for systemic codelivery of an anticancer drug and siRNA for potential triple-negative breast cancer treatment, ACS Nano 7 (2013) 9571–9584.

[174] S. Wang, J. Zhang, Y. Wang, M. Chen, Hyaluronic acid-coated PEI-PLGA nanoparticles mediated co-delivery of doxorubicin and miR-542-3p for triple negative breast cancer therapy, Nanomed. Nanotechnol. Biol. Med. 12 (2015) 411–420.

[175] Y. Ren, R. Wang, L. Gao, K. Li, X. Zhou, H. Guo, et al., Sequential co-delivery of miR-21 inhibitor followed by burst release doxorubicin using NIR responsive hollow gold nanoparticle to enhance anticancer efficacy, J. Control. Release 228 (2016) 74–86.

[176] T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, et al., Photodynamic therapy, J. Natl. Cancer Inst. 90 (12) (1998) 889–905.

[177] F.A. Portilho, C.E. Cavalcanti, A.L. Miranda-Vilela, L.L. Estevanato, J.P. Longo, F. Almeida Santos Mde, et al., Antitumor activity of photodynamic therapy performed with nanospheres containing zinc-phthalocyanine, J. Nanobiotechnol. 11 (2013) 41.

[178] University College, Clinicaltrials.gov Identifier: NCT02872064, 2006. Retrieved from, https://clinicaltrials.gov/ct2/show/NCT02872064.

[179] E. Secret, M. Maynadier, A. Gallud, M. Gary-Bobo, A. Chaix, E. Belamie, et al., Anionic porphyrin-grafted porous silicon nanoparticles for photodynamic therapy, Chem. Commun. 49 (2013) 4202–4204.

[180] C.S. Shemesh, C.W. Hardy, D.S. Yu, B. Fernandez, H. Zhang, Indocyanine green loaded liposome nanocarriers for photodynamic therapy using human triple negative breast cancer cells, Photodiagn. Photodyn. Ther. 11 (2) (2014) 193e203.

[181] S. Varma, R. Myerson, E. Moros, M. Taylor, W. Straube, I. Zoberi, Simultaneous radiotherapy and superficial hyperthermia for high-risk breast carcinoma: a randomised comparison of treatment sequelae in heated versus non-heated sectors of the chest wall hyperthermia, Int. J. Hyperth. 28 (7) (2012) 583–590.