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REVIEW ARTICLE

Current status of nanomedicine in the chemotherapy of breast cancer

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Abstract
Despite the efforts that have been made in the field of breast cancer therapy, it is a leading cause of cancer death in women and a major health problem. The current treatments combine several strategies (surgery, radiotherapy, immunotherapy, hormone therapy, and chemotherapy) depending on cancer subtype and tumour stage. The use of chemotherapy is required in certain circumstances, like before or after surgery or in advanced stages of the disease. Chemotherapeutic regimens that include anthracyclines (e.g. doxorubicin), taxanes (e.g. paclitaxel), 5-fluorouracil and/or cyclophosphamide show, in general, a high toxicity that limit their clinical use. The use of targeted chemotherapy allows to get a selective location of the drug at tumour mass, decreasing the toxicity of these treatments. An increase of the antitumour efficacy can also be achieved. The use of nanocarriers containing anticancer drugs can be a good strategy to get targeted chemotherapy. In fact, several nanoformulations containing paclitaxel and doxorubicin have been approved or are under clinical trial for breast cancer therapy. The main advantage of these nanomedicines is their lower toxicity compared to conventional formulations, which can be attributed to the elimination of the solvents of the formulation (e.g. Cremophor-EL in paclitaxel conventional formulations) and the more selective location of the drug at tumour site (e.g. cardiotoxicity related to free doxorubicin). However, some adverse events (e.g. hand foot syndrome or infusion reactions) have been related to the administration of some nanomedicines, which have to be considered.

Keywords Breast cancer · Chemotherapy · Doxorubicin · Micelles · Liposomes · Paclitaxel

Introduction

Breast cancer is the most common neoplasm in women with 2.09 million cases and 627,000 of deaths worldwide in 2018 [1]. Although in the last decades the implementation of early detection techniques and novel therapies have significantly improved breast cancer survival; this disease is still a leading cause of death in women between 35 and 54 years of age and a major health problem.

Breast cancer is a heterogeneous disease that is divided in several subtypes according to: (1) the aggressiveness grade: in situ and infiltrating (invasive) carcinomas; (2) the histopathology, e.g. tubular, medullary and ductal; (3) the molecular profile considering the expression of oestrogen receptor (ER), progesterone receptor (PR), and the overexpression of human epidermal growth factor receptor 2 (HER-2) and (4) the disease progress: in stage 1 (primary tumour within the breast), stage 2–3 (spreading to tissues and lymph nodes nearby) and stage 4 (spreading to distant organs like lung, bone and brain). Tumours with no expression of ER, PR and HER-2, called triple negative breast cancers (TNBC), are more aggressive and tend to metastasise, hampering the treatment. In fact, compared to hormone receptor or HER-2 positive breast tumours, TNBC shows the poorest prognosis and the most treatment challenge. Nowadays, an intensive research to identify novel therapeutic targets and new treatment agents is being undertaken, including inhibitors of phosphoinositide 3-kinase pathway, poly-ADP-ribosyl polymerase (PARP), cyclin-dependent kinase and immune checkpoint. Two PARP inhibitors (olaparib and talazoparib) have been approved by FDA for the treatment of metastatic gBRCA1/2-associated TNBC, revolutionizing the therapy scenario of this neoplasm [2].

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Depending on cancer subtype, there are available several treatment strategies: (1) surgery (mastectomy or lumpectomy), (2) hormone therapy, (3) radiotherapy, (4) antibody-based therapy and (5) drug therapy (chemotherapy) [3]. Surgery and radiotherapy are usually used for treating primary breast tumours and loco-regional lesions, and drug therapy to reduce tumour burden and to control and eradicate metastasis [4]. Although new antitumour agents have been investigated in the last decades and introduced in therapeutics with good results, chemotherapy (necessary treatment in high aggressive tumours) still shows a limited efficacy due to the difficulty to reach cancer cells. Although not all breast carcinomas required the use of chemotherapy, this is necessary in certain circumstances like before (neoadjuvant chemotherapy) or after surgery (adjuvant chemotherapy), or in advance tumours where chemotherapy could be the main cancer treatment strategy, like in TNBC where represents the mainstay of systemic therapy. The used drugs depend on cancer stage and cancer subtype. However, most of the chemotherapeutic regimens include anthracyclines like doxorubicin (Dox), taxanes like paclitaxel (PTX), 5-fluorouracil and/or cyclophosphamide.

The deployment of targeted chemotherapy may overcome the handicaps of the chemotherapeutic treatments, increasing their efficacy and decreasing their toxicity. In this sense, the development of nanotechnology-based anticancer drugs can be a good approach to get their selective location at tumour mass level (Fig. 1) [5].

Nowadays, there are several nanomedicines approved for the treatment of cancer, including several formulations for the treatment of breast tumours (Table 1). This review is focused on the formulations based on nanocarriers for breast cancer therapy that have already been approved or are in clinical trials. Antibody–drug conjugates (ADC), like trastuzumab-emtansine that is marketed for the treatment of breast cancer, have not been included.

Targeted chemotherapy strategies

There are several available strategies to get this targeted therapy: (1) passive targeting, (2) active targeting and (3) stimuli responsive drug release.

Passive targeting in cancer chemotherapy is mainly based on enhanced permeability and retention effect (EPR) characteristic of tumours. EPR effect is attributed to: (1) the presence of hyperpermeable tumour vasculature, with a fenestrated endothelium with higher gaps (100–780 nm) compared to healthy tissues and (2) the impaired lymphatic drainage that limits the clearance of substances in this way. Due to EPR, intravenously administered nanomedicines trend to accumulate at tumour site. As a consequence, the drug biodistribution to healthy tissues is restricted and its toxicity decreased. However, tumours show high heterogenicity, thus EPR effect depends on several tumour characteristics like the degree of tumour vasculature development and of pericyte coverage, the degree of lymphatic development, the density of tumour stroma, the size and location of the tumour and the intratumoral pressure [6]. For example, the central area of metastatic tumour does not exhibit an EPR effect, showing a lower accumulation of nanomedicines. Therefore, some tumours show a marked EPR effect while in others it is not so marked, resulting in an inefficient accumulation. For example, hepatocellular and renal carcinomas show a high vascular density and usually an increased EPR effect, or Kaposi sarcoma, which also shows a high vascular permeability. On the contrary, pancreatic and prostate tumours show a low vascular density and low EPR effect [7].

The active targeting strategy involves the binding of high-affinity moieties to the nanocarrier surface to get its "more selective" location at tumour site. These ligands have to be specifically recognised by cancer cells or tumour microenvironment (blood vessels or extracellular matrix). A high number of ligands have been investigated, including proteins, antibodies, peptides, aptamers, carbohydrates and small molecules like folic acid. The election

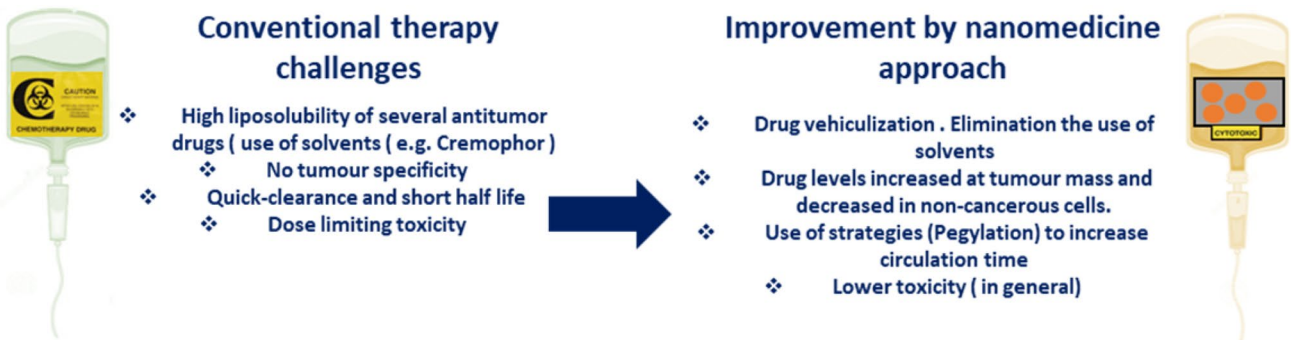

Fig. 1 Improvement of conventional chemotherapy challenges by nanomedicine approach

Table 1 Approved nanomedicines for cancer therapy

Brand name	Drug	Nanocarrier	Cancer type	Country
Abraxane®	Paclitaxel	Albumin nanoparticles	Metastatic breast cancer	EU, USA, Argentina, Australia, Japan, New Zealand
Genexol®	Paclitaxel	Polymeric micelles	Breast, lung and pancreatic carcinomas	Australia, New Zealand, China
Paclical®	Paclitaxel	Polymeric micelles	Ovarian cancer	USA, EU, Russia
Myocet®	Doxorubicin	Liposomes	Breast cancer	EU, Canada
Doxil/Caelyx®	Doxorubicin	Pegylated-liposome	Metastatic breast cancer, ovarian cancer and Kaposi sarcoma	USA[a], EU, Australia, New Zealand[b]
Daunoxome®	Daunorubicin	Liposome	Kaposi sarcoma	USA, France Ireland
Onco-TCS®	Vincristine	Liposome	Non-Hodgkin lymphoma	USA
Marquibo®	Vincristine	Liposome	Leukaemia	USA
Mepact®	Mifamurtide	Liposome	Osteosarcoma	EU
Onyvide®	Irinotecan	Liposome	Metastatic pancreas cancer	EU, USA
Nanoxel®	Paclitaxel	polymeric micelles	Metastatic breast cancer, non-small-cell lung carcinoma and Kaposi's sarcoma	India
Lipusu®	Paclitaxel	Liposome	Non-small lung cancer, breast cancer	China
PICN®	Paclitaxel	Liposome	Metastatic breast cancer	USA, India[c]
Transdrug®	Doxorubicin	Nanoparticle	Hepatocarcinoma	EU, USA
Oncaspar®	Asparraginase	Polymeric conjugate	Acute lymphoblastic leukaemia	EU, USA, New Zealand
Depocyt®	Cytarabine	Liposome	Neoplastic meningitis	EU

EU European Union

[a]Under the trade name of Doxil®

[b]Under the trade name of Caelyx®

[c]Under the trade name of Bevetex®

of the ligand depends on the targets overexpressed in each cancer subtype [8]. For example, folate receptor (FR) is overexpressed in 60–85% of breast cancers and is a promising target. In HER-2 positive tumours, the use of monoclonal antibodies against this receptor can be a good active targeting strategy. In fact, the first FDA-approved active targeting therapy (not involving nanomedicines) was trastuzumab (the anti-HER-2 antibody), which revolutionized the treatment of HER-2 positive tumours and became a standard treatment in this type of breast tumours [9]. The conjugation of this antibody with drugs or with nanocarriers, would let a selective location of the chemotherapeutics. In fact, ado-trastuzumab emtansine (an antibody–drug conjugate) is marketed for the treatment of this kind of breast cancer. Several aptamers have also be designed to target to HER-2 receptors and coupled to drugs or nanoparticles to get a selective location of chemotherapeutics [10]. In TNBC, epidermal-growth-factor receptor (EGFR) could be a therapeutic target, because it is overexpressed in more than 50% of the cases. Therefore, the use of anti-EGFR antibody coated nanocarriers can be a promising therapeutic tool. The transmembrane glycoprotein NMB (gpNMB) could also be a good therapeutic target. Glembatumumab vedotin is other antibody–drug conjugate in a phase II clinical trial for the treatment of Metastatic gpNMB Over-expressing Triple Negative Breast Cancer (NCT01997333) [11].

Stimuli-responsive release strategy is based on the induction of drug release from the nanocarriers at cancer tissue, due to internal or external stimuli. The selective release of the drug at tumour site increases its efficacy and decreases its toxicity. Internal or external stimuli can trigger changes in the phase, structure or conformation of nanocarrier and, as consequence drug release. Regarding internal stimuli, differences between tumour and healthy tissue have been exploited, including changes in pH, redox, enzyme expression or temperature. For example, tumour have a more acidic pH than normal tissue (~6.7 vs 7.4 respectively) [12]. Therefore, the use of nanocarriers that release the drug in this acidic microenvironment may get a selective location of the drug at tumour mass. This is the mechanism that follow some micelle systems. The higher tumour temperature (~40 °C) could also be exploited. The application of external stimuli, including temperature, light, ultrasound, magnetic force and electric field; has also been investigated. It could be applied locally at tumour site, without damaging normal tissue.

Approved nanoformulations

Nowadays, the approved nanoformulations in breast cancer are based on the passive targeting strategy, to try to get a certain selective location of the drug at tumour mass, decreasing the toxicity of these treatments.

Paclitaxel nanoformulations

Paclitaxel (PTX), an antimicrotubular agent belonging to taxane group, is largely used for the treatment of solid tumours, including breast cancer. In fact, a combination of taxanes and anthracyclines remains the standard neoadjuvant chemotherapy in breast cancer.

PTX is a poorly water-soluble drug. The available conventional formulation, named Taxol®, uses polyoxyethylated castor oil, known as Cremophor EL (Cre-EL) as solubilizing agent. Cre-EL is responsible for several adverse effects (including hypersensitivity reactions, neurotoxicity and nephrotoxicity). Therefore, it significantly contributes to the dose-limiting toxicity related to PTX conventional formulation. Patients need to be pre-treated with antihistamines and corticoids to prevent hypersensitivity reactions [13]. Although Cre-El is also used to solubilise other hydrophobic drugs such as cyclosporine A, Taxol® contains much more quantity (around 26 ml per administration) [14]. The use of nanocarriers for PTX administration, eliminates the use of Cre-EL as solubilising agent and as a consequence, the toxicity related to this agent. Moreover, it has been reported that Cre-EL inhibit the PTX binding to endothelial cells and to plasmatic albumin, limiting the uptake of PTX by tumour cells [15]. In this context, nanoformulations may also increase PTX efficacy [16, 17]. Nowadays, there are approved four nanoformulations containing PTX as anticancer for breast cancer therapy: (1) Abraxane®, (2) Genexol®, (3) Nanoxel® and (4) Lipusu® (Fig. 2).

Abraxane®

Abraxane (nab-PTX) is a Cremophor-EL free nanoformulation that uses albumin nanoparticles (particle size around 130 nm) as PTX carrier. It was approved by FDA in 2005 for the treatment of metastatic breast cancer. Nab-PTX has a lower toxicity than conventional PTX formulations, showing a minimal risk of hypersensitivity reactions and allowing the elimination of corticoid premedication. While conventional PTX has a maximum tolerated dose (MTD) of 175 mg/m^2, nab-PTX reported a higher value of 260 mg/m^2 (Table 2). It also showed a higher antitumour efficacy, due

Table 2 Maximum tolerated doses of PTX formulations

	Maximum tolerated dose (mg/m^2)
Taxol®	175
Abraxane®	260–300
Genexol®	300–390
Nanoxel®	220–300
LEP-ETU	325
NK-105	180

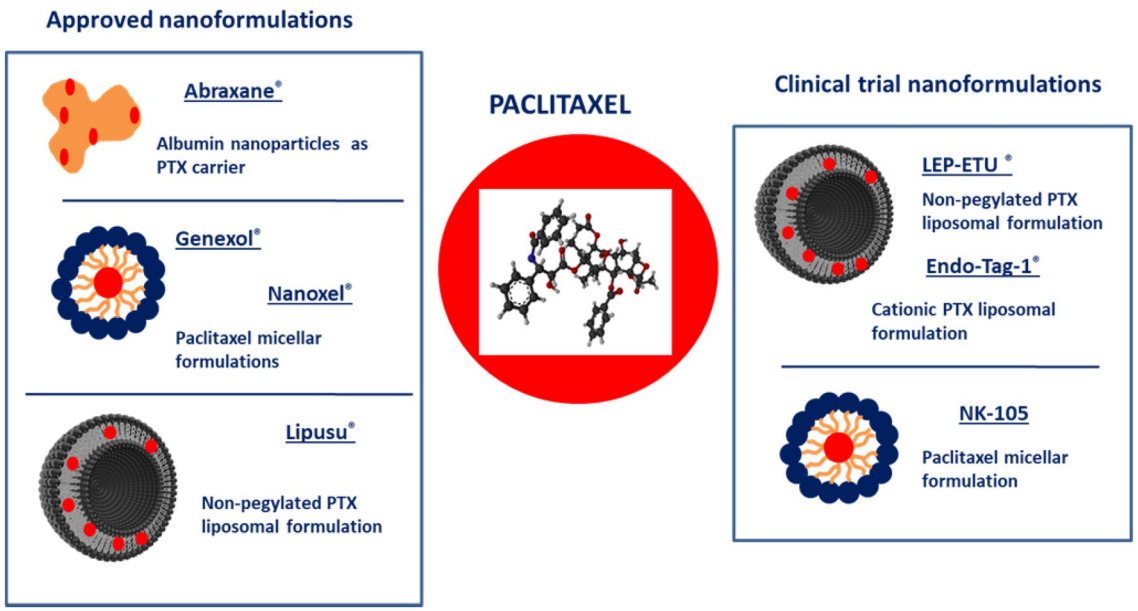

Fig. 2 Paclitaxel nanoformulations that are approved or in clinical trials for the treatment of breast cancer

to an increased intratumoral concentration of PTX (around 33% of increase) [18].

Several mechanisms are involved in "albumin nanoparticle-based targeting". On the one hand, drug delivery involves glyprotein-60 (gp-60) receptors of vascular endothelium and that are also known as albondin receptors. Albumin shows high affinity for gp-60 receptors and their binding induces the activation of caveolin-1 pathway (an intracellular protein), triggering the invagination of cell membrane, the formation of vesicular structures called caveolae and consequently the internalization of PTX-albumin bounded to tumour interstice. On the other hand, albumin tends to accumulate at tumour site due to SPARC proteins; which are located in tumour interstice and on the surface of cancer cells and have been found overexpressed in the 50–60% of breast carcinomas and have been associated with a poor prognosis [19]. Albumin nanoparticles bind to these proteins and are accumulated at tumour site [20].

Nowadays, nab-PTX is approved for the treatment of breast cancer (second line treatment when combination chemotherapy fails or cancer relapses within 6 months of adjuvant chemotherapy), metastatic pancreatic tumours (first line treatment in combination with gemcitabine) and in metastatic or locally advanced non-small cell lung cancer (first line treatment in combination with carboplatin when the use of curative surgery or radiotherapy is not possible). Several clinical trials are also ongoing for the treatment of: (1) breast cancer in combination with epirubicin (NCT03647280), (2) early breast cancer in combination with capecitabine (NCT03647514), (3) HER-2 negative breast cancer in combination with PU-H17 (NCT03166085), (4) metastatic or advanced breast cancer in combination with phenelzinesulfate (NCT03505528) and (5) in TNBC in combination with pembrolizumab, epirubicin and cyclophosphamide (NCT03289819).

Genexol®

Micelles have gained a deal of interest as chemotherapeutic agent nanocarriers. Genexol® is a PTX polymeric micellar formulation (with a particle size around 20–50 nm), formed by monomethoxy-poly(ethylene–glycol)-block-poly(D,L-lactide) di-block copolymer and that devoid of Cre-EL as solubilising PTX [21].

Several clinical studies have evaluated the efficacy and toxicity of this formulation in patients with metastatic breast cancer. For example, Genexol® was effective as chemotherapeutic agent, with an acceptable toxicity, in patients with metastatic breast cancer who failed to respond to standard chemotherapy. 37.5% of patients showed a partial response to this formulation [22]. In another clinical trial, a higher response rate (58.5% of overall response rate) was detected, with complete response rate of 12% [23]. The combination of this formulation with epirubicin was also effective. An open-label phase I study reported a good antitumour efficacy in patients with locally breast cancer receiving Genexol® (175 mg/m^2) and epirubicin (60 mg/m^2) for four cycles as neoadyuvant chemotherapy, showing a cumulative 5-year disease-free survival rate of 70.0% in patients with complete or partial remission [24]. Genexol® did not show a lower efficacy than conventional PTX formulation (containing Cre EL), even it was higher (with values of objective response rate of 39.1% and 24.3% in Genexol® and conventional PTX respectively), showing an acceptable safety profile. With respect to its safety profile, neutropenia rate was more common in micellar formulation (66.8%) than in conventional PTX (40.2%) [25]. Nevertheless, in general the safety profile of this micellar PTX formulation is better than conventional PTX, with a higher MTD (around 300–390 mg/m^2) (Table 2).

Nowadays, Genexol® is approved for the treatment of metastatic breast cancer (first line treatment) and metastatic or locally advanced non-small cell lung cancer in combination with cisplatin. Several clinical trials are also ongoing for the treatment of advanced breast cancer in combination with doxorubicin (NCT01784120), for gynaecological cancer (NCT02739529), for recurrent pancreatic cancer in combination with gemcitabine (NCT02739633) and for hepatocellular carcinoma after failure of sorafenib (NCT03008512).

Nanoxel®

Nanoxel® is another PTX micellar formulation marketed in India since 2006. Nowadays, it is approved for the treatment of metastatic breast cancer, non-small cell lung carcinoma and Kaposi's sarcoma [26]. This formulation consists of polymeric micelles, with a particle size around 80–100 nm [27], formed by a pH sensitive co-polymer of *N*-isopropyl acrylamide and vinylpyrrolidone monomers. At physiological pH, the micelles are stable and PTX is entrapped in the formulation, but at acid pH values the polymer is degraded and PTX is released. Thus, the acidic microenvironment of tumours triggers PTX release [28].

It is also a cremophor-EL free formulation, avoiding the adverse reactions related to this solvent. Although this micellar formulation reported similar adverse effects compared to conventional PTX formulations (including anorexia, nausea, vomiting, diarrhoea, stomatitis, alopecia, and myelosupression), the incidence and severity were lower, showing in general a better safety profile (MTD = 220–300 mg/m^2) [26, 29]. A study undertaken in India in patients with several types of carcinomas (mainly breast, oesophagus and ovary tumours), most of them in advanced stages of the disease (stages 3 and 4), reported similar results in terms of safety, with no infusion reaction related to nanomicellar formulation. In terms of efficacy, the group treated with Nanoxel®

showed higher antitumour activity compared to conventional PTX, with an overall survival of 22 and 12 months, respectively [30].

Apart from breast cancer, several phase II clinical trials are being undertaken to evaluate the efficacy of this nanoformulation at doses of 75 mg/m^2 as monotherapy in patients with head and neck carcinoma (NCT02639858) or in combination with trastuzumab as first-line therapy in the treatment of metastatic salivary duct carcinoma (NCT03614364) and with oxaliplatin in patients with metastatic oesophagus squamous cell carcinoma (NCT03585673). Finally, a phase III clinical trial is ongoing to evaluate the efficacy of Nanoxel administered intravesically in patients with bacillius Calmette-Guerin refractory non-muscle invasive bladder cancer (NCT02982395).

Lipusu®

Lipusu® is a non-pegylated liposomal formulation approved in China since 2006 for the treatment of ovarian cancer as first line chemotherapy, breast cancer as follow-up treatment in patients receiving radiotherapy or doxorubicin-based chemotherapy, lung cancer in combination with cisplatin and gastric cancers (Table 1). It is constituted by vesicles with a bilayer of phospholipids (mainly phosphatidylcholine) and cholesterol, in which PTX is entrapped, and with a particle size around 400 nm [26]. This liposomal formulation resolves PTX solubility challenges, eliminating the need of Cre-EL as PTX solvent and decreasing overall side effects [31]. In fact, similar anticancer efficacy [31, 32] and a lower toxicity has been reported compared to conventional PTX formulation. For example while anaphylaxis and peripheral nerve toxicity are common adverse effects in patients treated with Taxol®, the liposomal formulation considerably decreases hypersensitivity risk and peripheral nerve toxicity. In this way, the maximum tolerated dose of liposomal PTX is higher (200 mg/kg) than conventional PTX solution (30 mg/kg), letting the increase of the dose and consequently the antitumour effects [31, 33] (Table 2).

Nowadays, this liposomal formulation has only been approved in China. A clinical study to evaluate the efficacy and safety of Lipusu® plus carboplatin compared to gemcitabine plus carboplatin as first-line treatment is being undertaken in patients with advanced squamous cell carcinoma of lung (NCT02996214).

Doxorubicin nanoformulations

Doxorubicin (or adryamicin) is the most used anthracycline in chemotherapeutic regimens of a vast number of cancer types due to its high antitumour efficacy. In the case of breast carcinomas, it is one of the most efficacious drugs in both early- and late- stage tumours. Although dox interacts and interferes in several cell processes, the main accepted mechanism responsible for dox cytotoxicity is the inhibition of DNA topoisomerase-II; with the inhibition of the progression of the S-cell cycle phase. Dox also interacts with mitochondria inducing the overproduction of reactive oxygen species (ROS) [34].

Despite dox anticancer efficacy, its clinical use is limited by the risk of both acute and chronic cardiotoxicity. Acute cardiotoxicity, with an incidence around 11%, is often reversible and occurs during and 2–3 days after dox therapy. On the contrary, chronic cardiotoxicity, although is less prevalent, with an incidence of 1.7%, is currently irreversible and shows a bad prognosis (1 year mortality rate around 50%) [35]. Cardiotoxicity has been attributed to ROS production and to an increase in oxidative stress. In fact, cardiomiocytes are much more sensitive to dox oxidative stress than tumour cells, and the generation of ROS is enhanced by the intracellular non-chelated iron. Dox interaction with cardiomyocyte topoisomerase II-β is also considered a central mechanism responsible for cardiomyocyte damage; thus mice lacking this enzyme are resistant to Dox damage [34].

The use of nanocarriers for the administration of doxorubicin may let a more selective location of the drug at tumour mass, overcoming drug-associated cardiotoxicity. In this way, several nanoformulations containing this anthracycline are currently approved. In the case of breast carcinoma, there are two dox liposomal formulations in the market: (1) non-pegylated liposomal doxorubicin (LDox) (known as Myocet®) and (2) pegylated liposomal doxorubicin (PLDox) (known as Doxil®, Caelyx® or its FDA-approved generic formulation Lipodox®) (Fig. 3).

Doxil®/Caelyx®

Pegylated liposomal doxorubicin (Doxil® in EEUU and Caelyx® in EU) was the first FDA-approved nanoformulation for cancer treatment in 1995. Nowadays, it is indicated for the treatment of Kaposi's sarcoma, advanced ovarian cancer, metastatic breast cancer, and multiple myeloma.

This formulation consists of lamellar vesicles with a particle size around 80–90 nm. The external lipid bilayer is formed by fully hydrogenated soy phosphatidylcholine, cholesterol and PEG-modified phosphatidyl-ethanolamine, at a molar ratio of 55:40:5. The doxorubicin is encapsulated into the aqueous core [36]. Pegylation provides a hydrophilic cover to liposomes (known as stealth liposomes), increasing their stability in bloodstream and diminishing the uptake by macrophages. This is why the ratio of free dox circulating in plasma is reduced [37]. This liposomal formulation is prepared by an active loading method using an ammonium sulphate salt gradient. In this technique liposomes are elaborated in an aqueous solution of ammonium sulphate. Then, a pH gradient between the internal aqueous phase of the

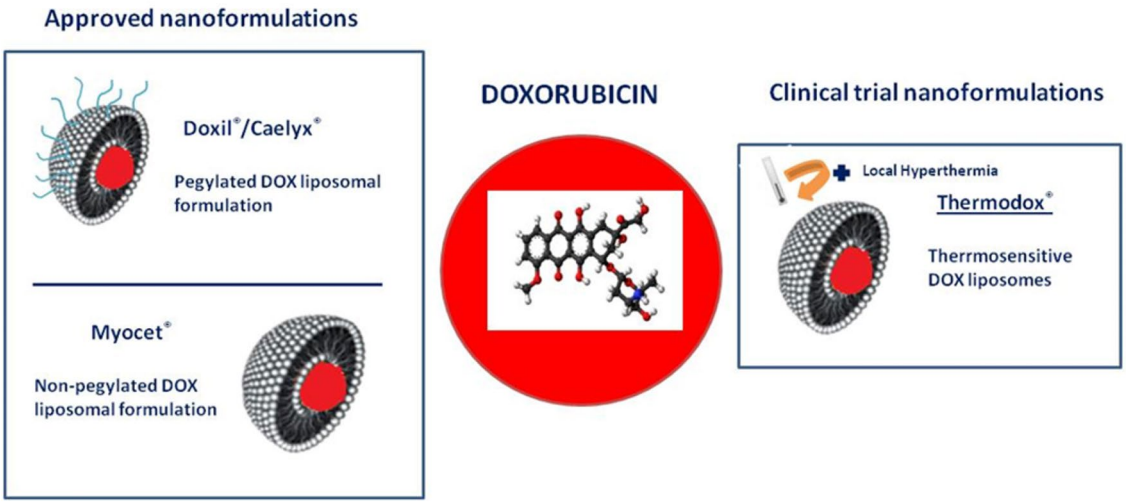

Fig. 3 Doxorubicin nanoformulations that are approved or in clinical trials for the treatment of breast cancer

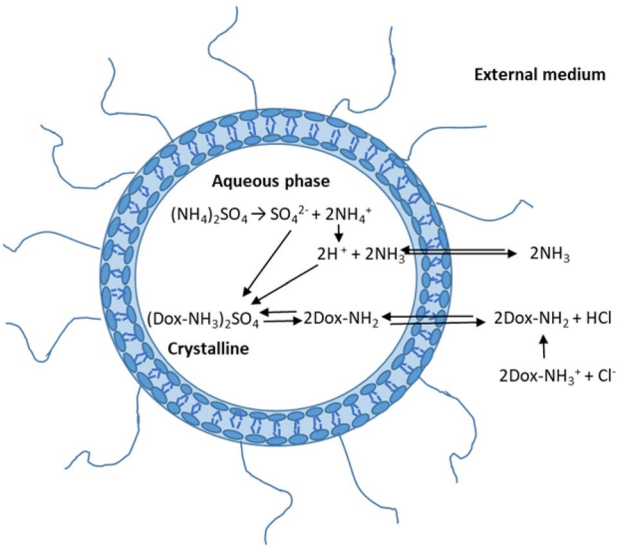

Fig. 4 Scheme of the Doxil® encapsulation method

liposomes and the extra-liposomal medium (pH 7.4 buffer) is generated. As a consequence, NH_3 molecules cross liposomal bilayer, leaving behind one proton for each molecule, and Doxorubicin, that is in the external medium as hydrochloride salt, penetrates into liposome (Fig. 4) [38].

The main advantage of PLDox compared to free doxorubicin formulation is its lower cardiotoxicity risk (around 3 fold reduction) [39]. In metastatic breast cancer patients PLDox showed a comparable efficacy to the free doxorubicin formulation. However, the cardiotoxicity was significantly lower in PLDox-treated patients. The incidence of myelosupression, vomiting and alopecia was also decreased [40]. PLDox also demonstrated a lower cardiotoxicity and a similar efficacy than epirubicin (another anthracycline) in patients with invasive breast cancer who received this drug as neoadjuvant chemotherapy [41]. Despite pegylated liposomal formulation decreases doxorubicin overall toxicity, two adverse events that are not typical for free doxorubicin, were observed in PLDox-treated patients: Palmar–Plantar Erythrodysthesia (PPE), also known as "hand–foot syndrome", and a complement activation-related pseudoallergy (CARPA). Hand-foot syndrome is characterised by tenderness, redness and peeling of the skin, and constitutes the major adverse effect of this formulation. There is no a complete solution for this effect, but the increasing of the treatment intervals decreases its incidence and severity [42]. CARPA is an acute hypersensitive reaction that usually appears with the first use of this formulation and includes facial and neck flushing, dyspnoea, and, less commonly, back pain, cyanosis, and hypotension. It is not a classical hypersensitivity reaction, and it is attributed to complement activation via the alternate pathway. This complement protein activation triggers the stimulation of inflammatory mediators [43]. It is also known as "infusion reaction", because it seems to be related to fast infusion rates of PLDox. In fact, it can be reduced using slow infusion rates and the administration of pre-medication [48]. Although, overall, PLDox toxicity is well tolerated, these adverse events limit the clinical use of this formulation. PLDox is only approved as monotherapy. However, a marginal progression-free survival improvement was detected in patients with platinum-sensitive ovarian carcinomas that received carboplatin plus PLD compared to those not administered liposomal doxorubicin as second-line therapy [44].

Due to the lower cardiotoxicity of PLDox compared to free doxorubicin, this liposomal formulation could be administered in combination with trastuzumab or lapatinib (anti-HER-2 antibodies) [39]. In fact, a clinical study

in patients with HER-2 positive breast cancer reported that the concomitant administration of PLDox, cyclophosphamide and trastuzumab had a significantly lower cardiotoxicity compared to the administration of free doxorubicin and cyclophosphamide [45]. The co-administration of PLDox and lapatinib was also effective in this kind of carcinoma, reporting a tolerated safety profile [46]. In women with locally advanced breast cancer, especially in patients with inflammatory breast carcinoma, the combination of PLDox, cisplatin and 5-fluoracil was also useful [47].

PLDox could also be useful in elderly patients, where anthracyclines are not commonly used due to their cardiotoxicity. In a study undertaken in patients (aged > 65) with stage II–IIb breast carcinomas, the treatment with the combination of PLDox and paclitaxel was feasible and reported a well-tolerated profile [48]. Nevertheless, in patients with operable and locally advanced breast cancer receiving low doses of PLDox (biweekly) and paclitaxel (weekly), the hand–foot syndrome was a major side effect, and even in some patients (4 of 35) the last dose of PLDox was suspended due to severity of this syndrome [49]. In another study, a "feasible tolerated" safety profile was detected in patients with locally advanced breast cancer receiving low doses of PLDox plus cyclophosphamide, but a limited activity was observed [50]. On the contrary, in patients with metastatic breast cancer, the combinations of PLDox and gemcitabine, or PLDox and cyclophosphamide were useful, showing an acceptable safety profile. Hand–foot syndrome was detected in the 14% of PLDox-gemcitabine treated patients [51].

In summary, it could be concluded that although the hand–foot syndrome could limit the clinical use of PLDox, the significantly lower cardiotoxicity compared to free doxorubicin is a great advantage and makes liposomal formulation useful for patients for whom doxorubicin could be a good option, but not used due to the cardiac risk (e.g. elderly people), even in combination with other anticancer drugs. Nowadays, several clinical studies are still evaluating the safety and efficacy of PLDox in breast cancer patients as monotherapy or in combination with cisplatin, docetaxel, cyclophosphamide, atezolizumab, everolimus and/or bevazizumab (NCT03712956, NCT03123770, NCT03164993, NCT02456857).

Myocet®

This formulation consists of lamellar vesicles in which lipid bilayer is formed by phosphatidylcholine and cholesterol. Compared to stealth liposomes of Doxil®, Myocet® has a larger particle size (around 190 vs 80–90 nm) and a much faster dox release rate. Consequently, the half-life is shorter (6.7 vs 45 h) and doxorubicin distribution volume is higher (18.8 vs 4.1 L). This liposomal formulation is also loaded by an active method approach based on a pH gradient, but in this case liposomes are elaborated in citrate buffer (pH ≈ 4) and the pH gradient is generated when external solution is changed to an HEPES buffer (pH ≈ 7.4) [38].

Probably, the major advantage of this formulation is the reduction of doxorubicin cardiotoxicity without presenting the side effects related to PLDox, specifically the hand–foot syndrome which limits the clinical use of pegylated nanoformulation [52]. The absence of this adverse effect in non-pegylated formulation could be attributed to PEG absence, in such a way that PEG could be responsible for the lower particle size of PLDox and consequently for its higher accumulation at skin level, triggering hand–foot syndrome.

Nowadays, the non-pegylated liposomal formulation of Dox is approved for the treatment of metastatic breast cancer as monotherapy in Europe and Canada. Moreover, several clinical studies have evaluated its therapeutic potential in combination with other drugs. In this sense, in combination with cyclophosphamide this liposomal formulation has been reported to have a similar antitumour efficacy and a lower cardiotoxicity than free doxorubicin in patients with metastatic breast cancer, becoming a promising chemotherapeutic regimen [53]. Similar results were obtained in elderly patients with breast cancer, where the combination of non-pegylated liposomal formulation and cyclophosphamide resulted in a low rate of cardiotoxicity [54]. Other clinical trial undertaken in patients with metastatic breast cancer not previously treated with chemotherapy reported that the combination of LDox and cyclophosphamide was more efficient than the combination of LDox and vinorelbine. No clinical evidence of cardiotoxicity was observed in both LDox plus cyclophosphamide and LDox plus vinorelbine treated patients [55].

Several clinical studies also showed that the co-administration of LDox and docetaxel, with or without trastuzumab, was beneficial and well tolerated in metastatic carcinomas, showing an acceptable cardiac toxicity [56–58]. On the contrary, other study reported that the combination of LDox and docetaxel in patients with metastatic breast cancer, although efficient, produced an unexpected cardiac dysfunction in 15% of treated patients [59]. Although a deeper investigation should be undertaken, these data suggest than LDox could be used instead of doxorubicin injectable solution in chemotherapeutic standard regimens, in combination with docetaxel and trastuzumab, with an acceptable cardiac toxicity. This combination seems to be useful in patients with metastatic breast cancer not previously treated with chemotherapy and could be particularly interesting in people with cardiac risk (e.g. elderly people).

Nanoformulations in clinical trial

Numerous clinical trials are ongoing to evaluate the safety and efficacy of nanoformulations of antitumour drugs in breast cancer therapy. Most of these formulations are also based on passive targeting strategy. However, some of them go further and rely on a drug release stimulation at tumour mass due to internal or external stimuli (Figs. 2 and 3) or the incorporation of ligands recognised specifically by cancer cells.

Paclitaxel nanoformulations

LEP-ETU®

LEP-ETU® (liposome-entrapped paclitaxel easy to use), with a particle size around 150 nm, is composed of egg phosphatidylcholine, cardiolipin, cholesterol and D-α-tocopheryl acidsuccinate, with a molar lipid ratio (1,2-Dioleoyl-sn-glycero-3-phosphocholine: cardiolipin:cholesterol) of 90:5:5 and loaded with paclitaxel at a lipid:drug ratio of 33:1 [60].

Due to the absence of Cre-El, this nanoformulation shows, in general, a lower toxicity than conventional PTX. A phase I study undertaken in patients with several types of carcinoma (breast and ovarian tumours were the most common) who had received prior chemotherapy in the 99% of the cases reported that liposomal formulation could be administered safely at higher doses compared to Taxol®. While the maximum tolerated dose of conventional PTX was 175 mg/m^2, liposomal formulation reported almost a two-fold increase tolerated dose (325 mg/m^2) and a dose-limiting toxicity of 375 mg/m^2 (Table 2), being febrile neutropenia and neuropathy the associated adverse events. 23% of the liposome-treated patients suffered infusion-related reactions. Their severity was similar to conventional paclitaxel [61]. Other study undertaken in patients with advanced cancer (including breast tumours) showed similar results, reporting that liposomal formulation was well tolerated and also bioequivalent with Taxol® [60]. These data suggest that LEP-ETU could be an alternative to conventional PTX.

Nowadays, this nanoformulation is under a phase II clinical trial to evaluate its efficacy and safety in patients with metastatic breast cancer who have received prior chemotherapy (NCT01190982).

Endo-Tag-1®

This is another liposome-entrapped paclitaxel formulation, also known as MBT-0206 or Lipopac®. It is a cationic liposomal formulation composed of 1,2-Dioleoyl-3-Trimethylammonium Propane (DOTAP), dioleolylphosphatidyl choline and paclitaxel at a molar ratio of 50:45:5, showing a particle size of 180–200 nm and a positive zeta potential [62]. Due to this positive surface charge, this formulation could target at tumour mass blood vessels. The endothelial cell lining tumour vascularization tends to have a negative charge, and positive charge complexes tend to be takenup by these cells, triggering microvascular damage at tumour mass and an increase in tumour permeability [63]. Thus, the use of cationic liposomes as PTX carriers could improve antitumour efficacy.

A phase II clinical trial undertaken in patients with advanced triple negative breast cancer reported that the administration of Endo-Tag-1® (44 mg/m^2), Endo-Tag-1® (22 mg/m^2) plus Taxol® (70 mg/m^2) or Taxol® (90 mg/m^2) shows similar efficacy. The median overall survival did not differ significantly for all treatments (13.0, 11.9 and 13.1, respectively). Although infusion-related reaction was more common in patients receiving liposomal formulation, their severity was classified as mild to moderate. Finally, in combination-treated patients higher rates of 3/4 neutropenia reactions were detected [64]. Similar results were found in patients with HER-2 negative breast cancer who received Endo-Tag-1® (22 mg/m^2) plus Taxol® (70 mg/m^2) for 12 cycles, followed by 3 cycles of a combination of fluorouracil, cyclophosphamide and doxorubicin as neoadjuvant chemotherapy. In terms of efficacy, the combination of cationic liposomal and conventional paclitaxel formulations was useful, showing an 81% reduction in tumour mass. In terms of safety, infusion-related hypersensitive reactions to Endo-Tag-1® (grade 3) were observed, and 4 of 15 patients required permanent discontinuation of the Endo-Tag-1® due to these adverse events. The study was finally suspended due to these hypersensitivity reactions [65].

Nowadays, a clinical trial in patients with Visceral Metastatic Triple Negative Breast cancer is evaluating the safety and efficacy of Endo-Tag-1® in combination with paclitaxel and gemcitabine versus paclitaxel in combination with gemcitabine (NCT03002103).

NK-105

This is a micellar formulation of PTX with a particle size of 85 nm. It is composed of polyethyleneglycol and modified polyaspartate as hydrophobic block and contains 23% (w/w) of PTX [66].

A phase II clinical trial undertaken in patients with several kind of carcinomas (not breast cancer patients were included) reported that NK-105 micelles were in general well tolerated, showing a maximum tolerated dose of 180 mg/m^2 that is similar to Taxol® (175 mg/m^2) [67]. In another phase II study it was reduced up to 150 mg/m^2 with a faster infusion rate (30 min) [68]. The main advantage

of this formulation compared to conventional PTX is the elimination of Cre-El.

Compared to Genexol®, another PTX micellar formulation, NK-105, shows higher AUC and Cmax values, probably due to a superior plasma micellar stability [69], and a lower MTD (Genexol® shows a MTD around 390 mg/m^2) (Table 2) [70]. In another clinical trial (phase I study) undertaken in patients with several carcinomas (including breast cancer), weekly doses of 80 mg/m^2 were recommended (doses from 50 to 100 mg/m^2 were evaluated with infusion rates of 30 min). An objective response rate of 60% in patients treated with this recommended dose was also reported, leading to the conclusion that NK-105 showed desirable antitumour effects. In terms of safety, this micellar formulation was in general well tolerated, haematological toxicity manifestations (neutropenia and leucopoenia) being the most common adverse events [71].

Finally, a phase III clinical trial in patients with metastatic or recurrent breast cancer receiving NK-105 (65 mg/m^2, lower dose than recommended previously) or PTX (80 mg/m^2) reported a similar antitumour efficacy in terms of progression-free survival (8.4 and 8.5 months, respectively), and also similar safety profile. However, some major adverse events like peripheral sensory neuropathy showed a lower incidence in patients treated with micellar formulation (1.4 vs 7.5% ≥ grade 3) [72].

All these studies support the idea that NK-105 nanoformulation shows a similar antitumour efficacy compared to Taxol®, but eliminates the need of Cre-El and allows the use of faster infusion rates (30 min).

Doxorubicin nanoformulation

Thermodox®

Thermodox® is the first thermosensitive formulation to reach clinic. It consists of liposomal formulation containing doxorubicin whose lipid bilayer is formed by of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1-stearoyl-2-hydroxy-sn-glycero-3-phosphatidylcholine (a lysolipid component) and N (carbonyl-methoxypolyethyleneglycol2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (mPEG2000-DSPE), at a molar ratio of 86:10:4. Due to the presence of this lysolipid, above its transition temperature the release of the drug is really fast. In this way, at 42 °C almost 80% of doxorubicin is released in 20 s. To get this temperature and trigger drug release at tumour mass, a heat system like microwave hyperthermia or High Intensity Focused Ultrasound (HIFU) is "locally" applied prior or immediately after formulation administration [54].

In a phase I study undertaken in patients with inoperable hepatocarcinoma, the combination of Thermodox® and radiofrequency ablation reported a significant dose– response effect. In this way, patients receiving the maximum tolerated dose (50 mg/m^2) showed a median time to treatment failure of 374 days, while in patients that received lower doses it was 80 days [73]. However, in a phase III study in patients with intermediate liver tumours (3–7 cm), this combination did not enhance the antitumour activity of radiofrequency ablation [74].

In breast cancer therapy, there are two clinical trials in patients with loco regional breast cancer: (1) a phase I/II completed study to evaluate the maximum tolerated dose, bioequivalence, pharmacokinetics and efficacy of Thermodox® in combination with microwave hyperthermia (NCT00826085) and (2) a phase I study to evaluate the efficacy and safety of Thermodox® in combination with local hyperthermia (induced by Magnetic Resonance guided High Intensity Focused Ultrasound) and cyclophosphamide (NCT03749850). Apart from breast cancer, several clinical trials are ongoing with this formulation: (1) a phase I study to evaluate the efficacy of this thermosensitive formulation in combination with magnetic resonance high intensity focused ultrasound in paediatric refractory solid tumours (NCT02536183), (2) a phase II trial to evaluate its efficacy in combination with radiofrequency ablation (RFA) in the treatment of colorectal metastasis in liver (NCT01464593) and (3) phase II and III studies to determine the efficacy of Thermodox® in combination with RFA in primary and metastatic liver tumours (NCT00441376, NCT02112656).

Anti-EGFR immunoliposomes loaded with doxorubicin

This is the first active targeted nanoformulation that has reached the clinic. It consists of pegylated liposomes containing doxorubicin as chemotherapeutic and coated with an antigen-binding fragment of the monoclonal antibody cetuximab that targets to EGFR. Nowadays, this nanoformulation is under a phase II clinical trial to evaluate its efficacy as first-line therapy in patients with advanced TNBC; thus approximately 66% of this tumour type overexpresses this receptor (NCT02833766). In this trial, anti-EGFR immunoliposomes are administered at doses of 50 mg/m^2 on day 1 of each cycle (a cycle lasts 28 days); thus this is the maximum tolerated dose established in a previous phase I study undertaken in patients with other solid tumours, including gastrointestinal neoplasms (colon and pancreas), head and neck tumours and urothelial cancer. This study reported that at this dose this nanoformulation was well tolerated, without detecting cardiotoxicity, palmar–plantar erythrodysaesthesia or cumulative toxicity in treated patients (26 patients were included in this study). Regarding efficacy, two patients exhibited complete or partial response and 10 showed a stable disease for 2–12 months [75].

Other anticancer drugs

Apart from paclitaxel and doxorubicin, several nanoformulations containing other anticancer agents are under clinical trials for the treatment of breast cancer or breast cancer metastases (Table 3).

CRL-X101

This nanoformulation consists of cyclodextrin polymeric nanoparticles containing camptothecin, a potent topoisomerase I inhibitor that shows a high anticancer activity in a broad range of tumours. However, this drug is highly toxic (producing haemorrhagic cystitis and myelotoxicity) and unstable at physiological pH, resulting its withdrawal in clinical trials [76]. In this new formulation, camptothecin is covalently bounded to a linear co-polymer of cyclodextrin and polyethylene glycol, which self-assembles forming nanoparticles. This nanoformulation has reported a good tolerability and antitumour efficacy in preclinical models of a broad range of tumours, including triple negative breast cancer [77]. It was also effective in combination with bevacizumab [78].

A phase I/II clinical study undertaken in patients with solid advanced tumours (including breast carcinomas) reported a maximum tolerated dose of 15 mg/m^2 bi-weekly. The most common adverse effects related to the treatment were fatigue, anaemia and neutropenia. In general, at 15 mg/m^2 biweekly, this formulation reported a promising safety profile. In terms of efficacy, encouraging activity was also detected showing a median progression-free survival of 3.7 months. This was higher (4.4 months) in patients with non-small lung cell carcinoma [79]. Despite these promising results, these are preliminary data and further studies are necessary.

NK-012

Irinotecan is a camptothecin derivate that shows high anticancer efficacy in a broad range of tumours, especially in colorectal cancer. Its active metabolite, called SN-38 is almost 100- to 1000-fold more potent than irinotecan. However, it shows a low aqueous solubility that makes difficult its administration. To overcome this, polymer–drug conjugates of SN-38 has been developed [80].

NK-102 is a nanoformulation formed by a copolymer of polyethylene glycol and poly-glutamic acid conjugated to SN-38. Due to the EPR effect, this nanocomplex trends to accumulate at tumour site, releasing the drug gradually. A phase I study undertaken in patients with several solid tumours, including breast, ovarian and colon carcinomas among others, reported a maximum tolerated dose of 28 mg/m^2 every 21 or 28 days at an infusion rate of 30 min, myelosuppression being the dose-limiting toxicity adverse effect. Patients with triple negative breast cancer reported a partial antitumour response, even at doses of 10.5 mg/m^2 for 8 cycles [81]. Due to these promising results, a phase II trial of NK012 efficacy was conducted in women with metastatic triple negative breast cancer who had been treated previously with one or two regimens that included a taxane (NCT00951054). Moreover, a phase I study is evaluating its efficacy when combined with carboplatin in TNBC patients (NCT01238952). Apart from breast cancer, there are ongoing other two clinical trials with this nanoformulation: (1) a phase II study to evaluate its efficacy in patients with small-cell lung cancer (NCT00951613) and a phase I study in

Table 3 Nanoformulations containing other anticancer agents (apart from paclitaxel and doxorubicin) that are under clinical trial

	Drug	Nanoformulation	Cancer type	Treatments	Clinical trial phase
CRL-X101	Camptothecin	Cyclodextrin polymeric nanoparticles	TNBC	Monotherapy	Phase I/II
NK-012	Irinotecan	Polymer micelle	Breast, ovarian and colon carcinomas	Monotherapy	Phase I
			Metastatic TNBC		Phase II
				In combination with carboplatin	Phase I
LE-DT®	Docetaxel	Liposome	Gastric carcinomas, breast, ovarian, pancreatic and non-small-cell lung tumours	Monotherapy	Phase I
LiPlaCis	Cisplatin	Liposome (stimuli responsive formulation)	Advanced or refractory solid tumours	Monotherapy	Phase I
			Metastatic breast cancer, prostate cancer and skin cancer		Phase II
Depocyt®	Cytarabine	Liposome	Central nervous system metastases from breast cancer	In combination with methotrexate	Phase II
			Leptomeningeal metastasis of breast cancer	Monotherapy	Phase III

combination with 5-fluorouracil in patients with metastatic colorectal cancer (NCT01238939).

LE-DT®

LE-DT® is a liposomal nanoformulation containing docetaxel, a taxane group anticancer drug used in a wide variety of solid tumours, including breast, prostate, gastric, non-small-cell-lung, neck and head carcinomas. As in the case of PTX, docetaxel shows a low aqueous solubility and needs the use of certain solvents (like ethanol) for its administration, which triggers several adverse events that limit the used dose [82, 83]. For example, a dose of 100 mg/m^2 produces severe neutropenia (grade 4) in the vast majority of the patients (75–86%). The encapsulation of docetaxel could overcome solubility challenge, with no need to use organic solvents and improving the safety profile. The clinical efficacy could also be increased. Docetaxel liposome's composition is similar to LEP-ETU® nanoformulation: Dioleoyl-sn-glycero-3-phosphocholine cholesterol, cardiolipin and α-tocopheryl acid succinate [84].

A phase I study undertaken in patients with solid tumours (gastric carcinomas were the most common malignance, but the study also included patients with breast and non-small-cell lung carcinomas among others) reported that LE-DT® was well tolerated at doses in the range of 50–110 mg/m^2. No dose-limiting experiences were appreciated even at the highest dose (110 mg/m^2), which is higher than that of free docetaxel. Regarding therapeutic efficacy; a clinical benefit, in terms of partial response (41%) and stable disease, was observed, recommending the administration of 85 mg/m^2 or 110 mg/m^2 in combination with a granulocyte colony-stimulating factor to solve neutropenia related to this dose [84]. Another phase I clinical trial undertaken in patients with solid breast, ovarian, pancreatic and non-small-cell lung cancers among others, evaluated the administration of liposomal docetaxel (15–110 mg/m^2) as neoadjuvant chemotherapy. At doses of 110 mg/m^2 two of six patients experienced severe stomatitis and neutropenia. For this reason, maximum doses of 85 mg/m^2 were also recommended. In terms of efficacy, a stable disease was observed in the 75% of treated patients.

LiPlaCis

This is a stimuli-responsive liposomal cisplatin formulation in which drug release is triggered at tumour site. Liposomes are formed by lipids that are specifically sensitive to degradation produced by secretory phospholipase A2 (sPLA 2), an enzyme present in tumours. Due to passive targeting strategy, liposomes trend to accumulate at tumour site, where due to the activation of this enzyme are degraded and, as a consequence, cisplatin is released. Nowadays, there is ongoing a phase I clinical trial to evaluate the safety and efficacy of this formulation in patients with advanced or refractory solid tumours and a phase II study in patients with metastatic breast cancer (NCT01861496). The preliminary results of this study showed that LiPlaCis is, in general, well tolerated. However, grade 3 and 4 adverse events were detected in 4 and 2 patients respectively (the study included 12 patients in total) after 43 cycles of treatment [85].

Depocyt®

This is a liposomal formulation containing cytarabine/Ara C in which lipid bilayer is formed by dioleoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, cholesterol and triolerin, designed for intrathecal administration and approved for the treatment of neoplastic meningitis [52]. Nowadays, a phase 2 study is ongoing to evaluate the efficacy of this nanoformulation in combintion with methotrexate for the treatment of breast cancer central nervous system metastases (NCT00992602). A phase 3 study is also being undertaken to evaluate its efficacy as monotherapy in the treatment of breast cancer leptomeningeal metastases (NCT01645839).

Nanoformulations in research

As we mentioned previously, the approved or in clinical trials nanoformulations are mainly based on the concept of passive targeting to get a more selective location of the drug compared to conventional formulations. In research, the trend is to develop "more selective nanoformulations", using the active targeting and/or stimuli response approaches.

Active targeted nanoformulations

For the treatment of breast cancer, as aforementioned, trastuzumab was the first approved active-targeting system and is currently used in combination with paclitaxel in HER-2 positive metastatic breast cancer patients [86]. This antibody has been used as a ligand to decorate several chemotherapeutic nanoformulations in order to get a selective location of the drug at tumour cells and, as a consequence, increase their anticancer efficacy. For example, polymer nanoparticles loaded with PTX and coated with trastuzumab have reported a higher in vitro anticancer efficacy in HER-2 positive breast cancer cells than non-coated formulations [87]. Immunoliposomes containing PTX and coated with this antibody have also been developed, showing promising results in HER-2 receptor positive tumours [88]. Liposomes decorated with trastuzumab and loaded with other several anticancer drugs including, doxorubicin and docetaxel have also been designed with excellent antitumor results [89–92]. Interestingly, Eloy et al. demonstrated that liposomes coated with trastuzumab and loaded with rapamicyn and paclitaxel were

even better able to control HER-2 positives tumour growth in mice [93]. In fact, one of the advantages of nanoformulations is the possibility to incorporate more than one drug. The actives loaded into the same nanocarrier get tumour cells at the same time and a synergism could be achieved. All these data suggest that the use of anti-HER-2 immunoliposomes could be a great strategy to improve the treatment of HER-2 receptor positive tumours.

Other ligands like folic acid or transferrin are also being evaluated. For example, lipid-protein-nanocomplexes coated with folic acid as PTX carriers have showed an increased antitumor activity in mice models of triple negative breast cancers [94]. Transferrin-conjugated polymeric nanoparticles enhanced the anticancer activity of doxorubicin, being effective even in dox-resistant cells [95]. This supports the idea of using nanocarriers as a tool to overcome drug resistances.

Stimuli-responsive nanocarriers

Numerous authors are also evaluating the development of stimuli-responsive nanoformulations. As aforementioned, some strategies are based on the use of internal stimulus, like the acidic microenvironment of the tumours and the aberrant metabolism of cancer cells, showing higher glutathione concentrations compared to normal cells. For example, Chida et al. have developed pH sensitive polymeric micelles containing epirubicin. At the acidic pH values of the tumour microenvironment, micelles were destabilized and drug was released, improving the therapeutic efficacy of this anthracycline [96]. Yang and collaborators have developed pH- and redox-sensitive nanoparticles loaded with doxorubicin. This nanoformulation showed a preferential drug release at tumour cells and a higher in vivo antitumor efficacy than free doxorubicin [97].

Other strategy is focused on the application of external stimulus, such as temperature, light or magnetic field. This is the case of thermoliposomes, like Thermodox (discussed previously), in which drug release is triggered by the increase of temperature externally produced at tumour site. Thermoliposomes loaded with docetaxel have also been prepared, showing a high antitumor efficacy [98]. Shemesh et al. developed multi-stimuli-responsive systems, preparing thermoliposomes loaded with indocyanine, a light activable agent [99].

Finally, the current tendency is to use several targeted strategies in the same formulation. This could be really interesting, specially to overcome drug resistances. For example, Shin et al. have developed thermoliposomes coated with trastuzumab and loaded with gemcitabine, improving the selective delivery of the drug in HER-2 receptor positive breast cancer cells [91]. Kumar and collaborators followed a similar strategy with the development of redox responsive polymeric nanoparticles loaded with doxorubicin and coated with trastuzumab and folic acid, demonstrating higher anticancer activity compared to free doxorubicin in both in vitro and in mice tumour models [100].

Concluding remarks

The nanoformulations on antitumour drugs that are currently approved or under clinical trials are based, with few exceptions, on passive targeting strategies. Their main advantage is their lower toxicity; showing, in general, a better safety profile compared to conventional formulations. The lower toxicity of nanoformulations could be attributed, at least in part, to the elimination of the organic solvents that are necessary in conventional formulations to solubilize some anticancer agents. This is the case of PTX whose standard formulation (Taxol®) contains Cre-El, which produces several adverse effects (hypersensitivity reactions, neurotoxicity and nephrotoxicity among others), limiting the clinical doses of the drug product. The lower toxicity could also be related to a "particular biodistribution profile" of nanoformulations, which tend to accumulate at tumour site decreasing the systemic exposure of free drug. In fact, the maximum tolerated doses of nanosystems are, in general, higher than those of conventional formulations, allowing the increase of clinical doses and as a consequence, the antitumour efficacy, the use in combination with other anticancer drugs, or the use in certain groups of population in which standard formulations are not indicated. This is the case of doxorubicin. On the one hand, due to the higher cardiotoxicity of free doxorubicin, it cannot be concomitantly administrated with certain drugs (e.g. trastuzumab). However, this combination is possible with dox nanomedicines. On the other hand, these nanomedicines can be used in elderly people, where the administration of free dox is not recommended due to their higher cardiotoxicity risk. It has to be taken into account that several adverse reactions could also be attributed to these nanodevices, like in pegylated liposomes containing doxorubicin, where their different biodistribution triggers an undesirable and limiting reaction (the Palmar–Plantar Erythrodysesthesia). The elimination of polyethylene glycol coating resolved it. Hypersensitive reactions related to the administration by infusion have also be associated with certain nanocarriers (especially liposomes). Interestingly, this adverse effect improves with the use of lower infusion rates. Despite the more selective location of the currently approved nanoformulations, and as a consequence of the anticancer drug, at tumour mass level, an increase of antitumour efficacy (at the same drug concentrations than conventional formulations) is, in the most cases, not detected. Probably, the use of active targeting nanocarriers could lead to higher accumulation of the drug at tumour cell level, thus

increasing the antitumour effect. This improvement has been detected in research in vitro and in animal models. However, further studies are necessary to get this new nanomedicines to clinic. Finally, the use of "multi-targeted" nanoformulations is garnering a great deal of interest in research, especially to overcome tumour resistances.

Despite the potential use of nanomedicines in cancer disease, the transfer of research laboratory manufacturing methods to the industry is, in most cases, difficult due to the lack of appropriate methods to produce large quantities of nanoformulations in a controlled and reproducible manner. In the case of liposomes, the most approved type of nanosystems, numerous elaboration techniques are available. Among them, the homogenization technique based on extrusion through a French press cell "microfluidizer" method is scalable. The ethanol injection technique is also suitable for large-scale production. In addition, due to the absence of mechanical forces, the stability of the liposomes prepared by this method is higher compared to mechanical techniques. In fact, several companies have developed liposome production methods based on this technique.

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Compliance with Ethical Statement

Conflict of interest The authors declare that there are no conflict of interests.

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