REVIEW

NEW PHARMACEUTICAL DOSAGE FORMS USED IN THE TREATMENT OF BREAST CANCER. POLYMERIC MICELLES

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Abstract

Among all types of cancer encountered in women, breast cancer is the most prevalent, with the highest mortality rate. An increased survival rate is closely related to early diagnosis, the use of high performing screening methods and of selective and adequate treatments.
By using the nanotechnologies, the therapeutic effectiveness of the drugs may be improved by a controlled release of the active substances to the tumoral site. The aim of this review is to present the current state of knowledge and to mention the new treatment trends in breast cancer, focusing on a pharmaceutical form that, thanks to its advantages, is already used in the therapy of this disease – the polymeric micelles.
Several examples of anticancer agents loaded polymeric micelles are mentioned, illustrating the preparation methods and the current state of clinical studies in which polymeric micelles are used.

Keywords: *breast cancer, pharmaceutical dosage form, nanomedicines, polymeric micelles*

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Introduction

Among all types of cancer, breast cancer is the most prevalent in women, representing the highest mortality rate(1).

Depending on the histological characteristics, breast cancer is classified in HR+ (hormone-receptor-positive), HER2+ (human epidermal growth factor receptor-2 overexpressing) and TNBC (triple-negative) breast cancer (2). For the metastatic breast cancer, endocrine therapy represents a mainstay treatment (3), that involve the use of SERMs (selective estrogen receptor modulators), SERDs (selective estrogen receptor downregulators) and AIs (aromatase inhibitors) (4).

Lung, breast and colorectal are the most frequent diagnosed cancers worldwide. There are 4.4 million survivors up to 5 years following breast cancer diagnosis (5, 6). Breast cancer's exact etiology is not known, but some associated risk factors were observed, such as: family history of breast cancer (7), aging (8), obesity (9), nutrition (10), low physical activity (11), gene alteration (12), use of oral hormone replacement therapy and contraceptives(13) and chest radiation (14). Genetic mutations involve: high/low

penetrance genes (BRCA1, BRCA2, p53, ATM, NBS1, PTEN, LKB1, respectively: CYP1A1, CYP2D6, CYP19), glutathione S-transferase genes (GSTM1, GSTP1), alcohol and 1C metabolism genes (ADH1C, MTHFR), DNA repair genes (XRCC1, XRCC3, ERCC4/XPF), cell signaling genes (PR, ER, TNF-alpha, HSP70) (15). Furthermore, HER-2/neu antigen was found to be overexpressed not only in breast cancer, but also in gastric, ovarian, oral and lung cancers (16).

There are several criteria that are used to categorize this disease. Considering the histogenetic characteristics and the DNA microarrays, the subtypes of breast cancer are presented in the figure 1.

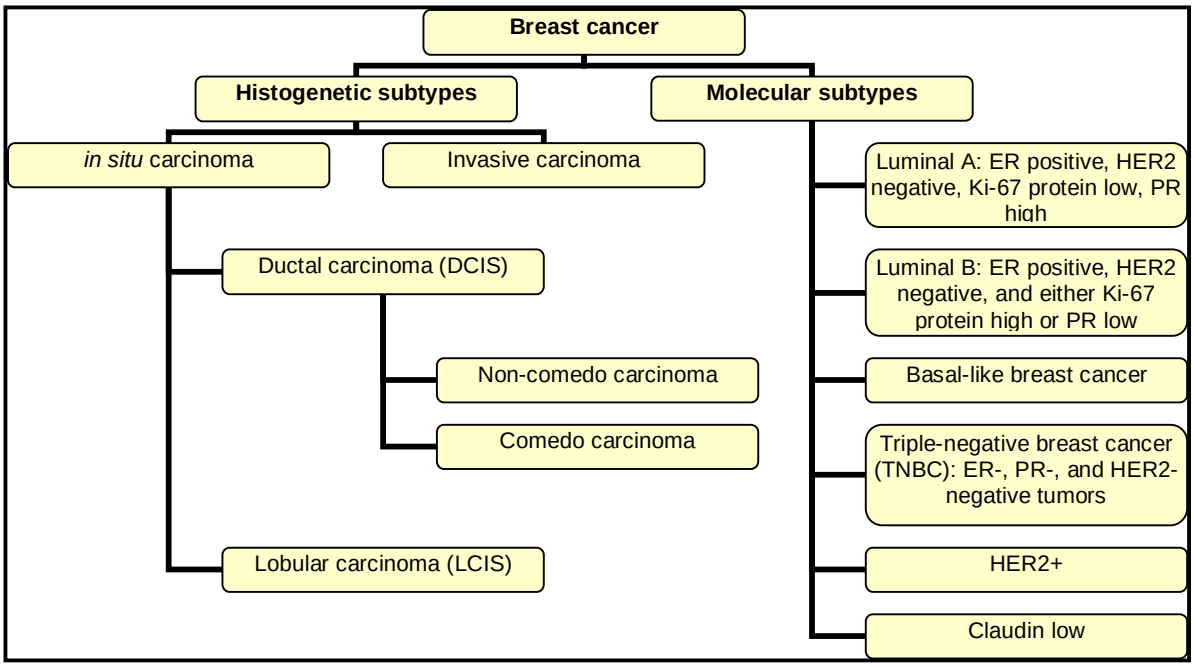

Figure 1 Breast cancer classes (17, 18)

In the past century, many diagnostic methods have been developed for cancer in general, and breast cancer in particular: mammography, magnetic resonance imaging (MRI), breast biopsy, HER-2/neu detection assay (immunohistochemistry, FISH test), Blood-based assay (CA 15-3, carcinoembryonic antigen CEA, CA 27-29, proteins, cancer cells, DNA/RNA, autoantibodies, genomics/proteomics) (19).

Although MRI has a low selectivity, breast cancer may be discovered in an early stage due to its high sensitivity. However, side effects and drawbacks may appear because of the use of contrast agents. Furthermore, new drug-delivery carriers such as nanosized formulations are recently used for the delivery of contrast agents to the tumor site (20).

Nanomedicine is in continuous development, with many applications both in the clinical and nonclinical field. Taking into account the significant side effects and the high toxicity of anti-tumor treatments, it

is easy to understand that the use of drugs that act directly on the target is a very important aspect. By using the nanotechnologies, the therapeutic effectiveness of the drugs may be improved by a controlled release of the active substances to the tumoral site (21).

Compared to conventional dosage forms, nanomedicines have particular characteristics that allow them to provide augmented safety, bioavailability and specificity. Their physicochemical properties may lead to changes in pharmacokinetics (absorption, distribution, elimination, metabolism) (22), with an increased potential to cross more easily biological barriers and a higer persistence in the body (23, 24). By the year 2013, from 11 commercially available nanomedications, EMA has approved 8, and 3 were withdrawn. Other nanomedicine products are in preclinical trials(25). By the year 2016, the FDA allready approved 51 nanomedicines, which can be classified in:

- polymer based nanomedicines (e.g. Paxone®, Ulasta®, and PLEGRIDY®),
- hydrophilic polymers (e.g. Venofer®, Ferrlecit®, Dexferrum®, Feraheme®),
- liposomes (e.g. Onivyde®, Doxil®, Visudyne®, Thermodox®),
- micelles(e.g. Estrasorb®, BIND-014, CALAA-01),
- nanocrystals (e.g. Rapamune®, Tricor®, Emend®, Megace ES®),
- antibody-drug conjugates (e.g. Brentuximab emtasine, Tratuzumab),
- proteic and inorganic nanoparticles (e.g. Abraxane®, Ontak®) (26).

Before FDA aproval, the properties of nano-formulation must be evaluated: physicochemical properties, formulations aspects, pharmacokinetics, distribution to blood and tissues, metabolism, elimination, accumulation in target tissues, elimination, toxicity (27).

Two major limitations that imped the entry of novel nanomedicines into the pharmaceutical market is the high cost and the fact that most of the reagents and inactive substances used in the formulation are not included in the FDA approved database of inactive ingredients (28).

Conventional and novel therapies for breast cancer

Early stage of breast cancer is considered to be potentially curable. In the past years, breast cancer therapy has substantially progressed and new therapies are emerging (29). In order to establish proper therapy concepts, a multidisciplinary setting is needed, focusing on the molecular subtype and locoregional tumour load (30).

The main types of breast cancer treatment are: surgery, RT (radiation therapy), CT (chemotherapy), ET (endocrine therapy), and targeted therapy (31).

For the localized breast cancer, the first step is neoadjuvant therapy, in order to shrink tumor bulk, then the trending approach is surgery, usually followed by an adequate adjuvant therapy to minimize the apparition of metastases and to ensure full recovery.

To reduce the local recurrence of cancer, radiation therapy may be performed (31).

At the base of the selection of systemic adjuvant therapies is the surrogate intrinsic phenotype determined by HER-2, ER/PR and Ki-67 assessment (Figure 2):

- ET for endocrine-responsive histology (tubular, cribriform, and mucinous);
- CT for endocrine-nonresponsive (medullary, adenoid cystic, apocrine, and metaplastic)(32).

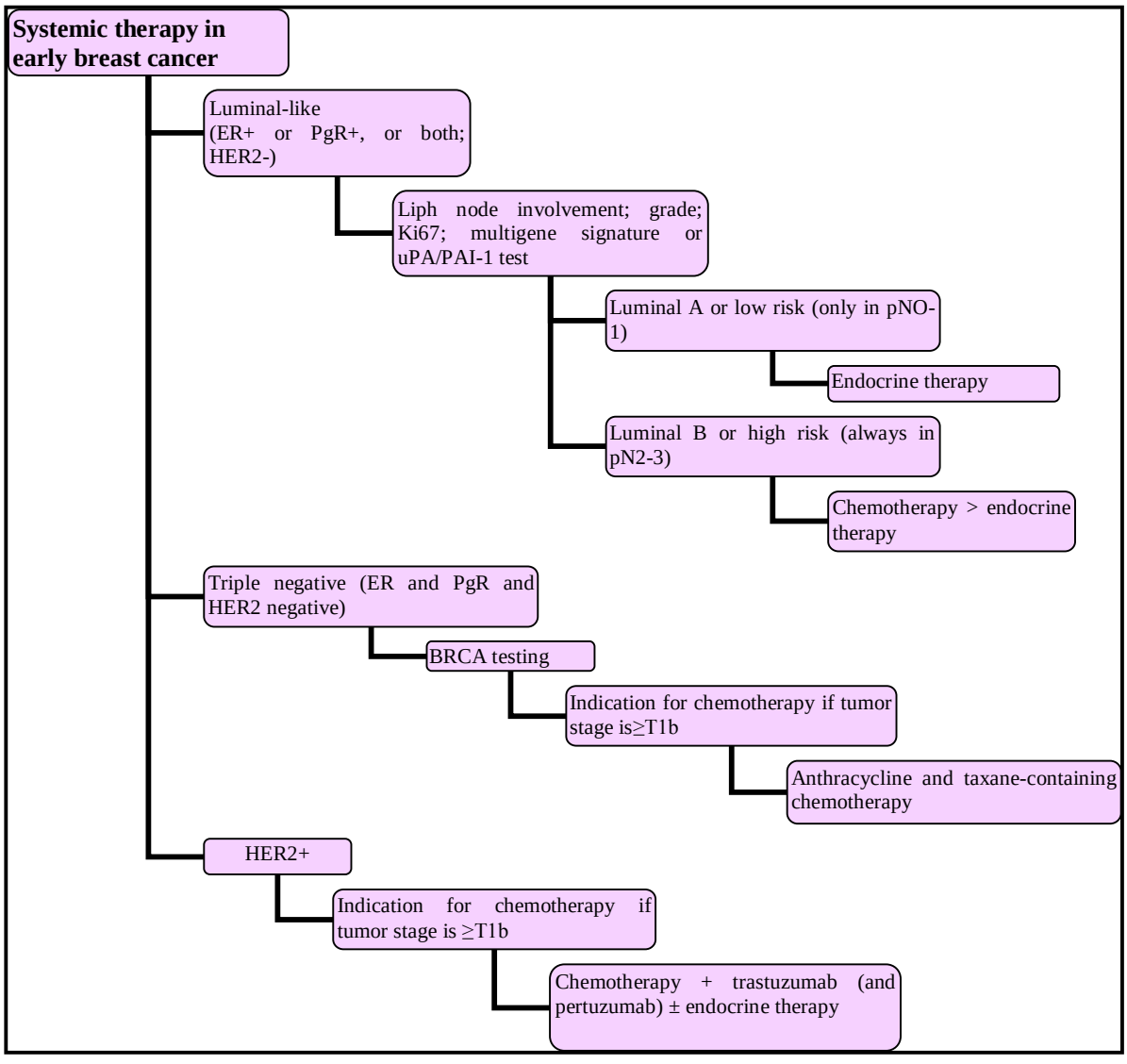

Figure 2 Principles of systemic therapy in early breast cancer (30)

LOCAL TREATMENTS	**Surgery**	
	Radiation	
SYSTEMIC TREATMENTS	**Chemotherapy**	• Anthracyclines: doxorubicin and epirubicin • Taxanes: paclitaxel and docetaxel • 5-fluorouracil • Cyclophosphamide • Carboplatin Advanced breast cancer: Taxanes and albumin-bound paclitaxel, Anthracyclines, Platinum agents, Vinorelbine, Capecitabine, Gemcitabine, Ixabepilone, Eribulin HER2+ cancers: drugs that target HER2 may be used with chemotherapy.
	Hormone Therapy	• Estrogen receptors blockers: Tamoxifen, Toremifene, Fulvestrant • Estrogen level modifiers: Aromatase inhibitors (Letrozole, Anastrozole, Exemestane), Ovarian suppression • Other hormone therapy: Megestrol acetate, Androgens, High doses of estrogen
	Targeted therapy	• HER2-positive breast cancer: Trastuzumab, Pertuzumab, Ado-trastuzumab emtansine, Lapatinib, Neratinib • Hormone receptor-positive breast cancer: CDK4/6 inhibitors (Palbociclib, Ribociclib, Abemaciclib), Everolimus • BRCA gene mutations: Olaparib, Talazoparib

Table 1 Current local and systemic treatments for breast cancer (33)

Because of the extended side effects and low bioavailability of the conventional antineoplastic drugs (Table 1), novel aproaches for breast cancer treatment were investigated in the past 15 years (Figure 3).

Nanotechnology comes with the advantage of a targeted delivery of the active substances (31). Furthermore, new active substances were discovered, due to the advances in molecular biology (34) and pharmacology(29, 35).

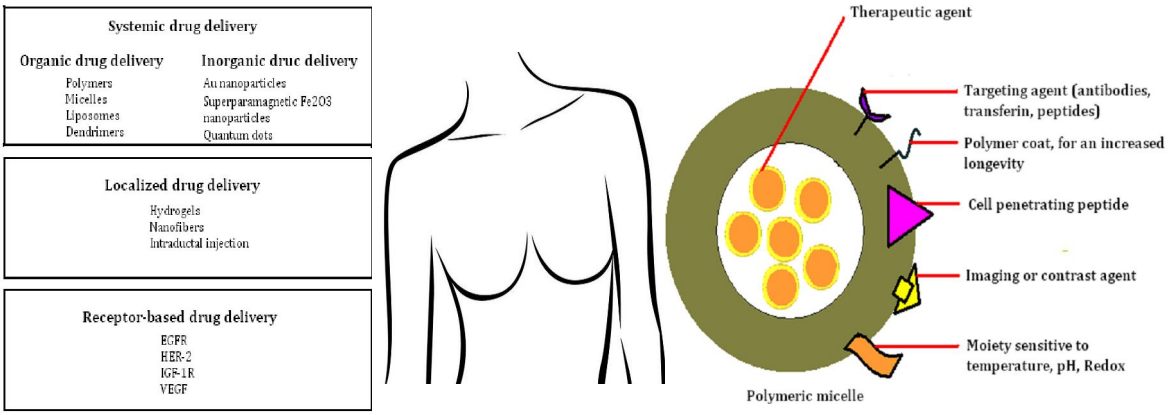

Figure 3 Drug delivery systems and different types of linkers used in breast cancer (44, 49)

Passive targeting - The enhanced targeting may be passive or active. The cancer tissue's physical and chemical properties may represent good tools in the passive targeting (increased angiogenesis, slightly acidic pH) (36).

Active targeting - A potential target is represented by an overexpression of antigens or receptors in cancers. For example, in 2017, alumina NP–autophagosome conjugates were used to manage breast cancer (37).

New active pharmaceutical entities and targeting moieties

Thank to the newest discoveries in molecular biology, genetics, pharmacology, new generations of active and tailored active molecules are emerging for breast cancer management:

- human prolactin antagonist-interleukin 2 [hPRLA-IL-2]), a novel fusion protein acts as positive immunomodulator (38).
- tetrahydrocannabinol and phytocannabinoid cannabidiol have antiproliferative effect and inhibit id-1 gene expression in some breast cancers (39).
- gamma secretase inhibitor 1 (GSI1) inhibits the production of the substrate binding component (nicastrin, Nct) (40).
- retinamides inhibited the growth of established breast tumor xenografts blocking retinoic the acid metabolism via apoptosis (41).
- expression of human genes A7322 and F3374 (SEQ ID no: 79) is elevated in breast cancer (42).

Nanoparticles

Nanoparticles are new pharmaceutical forms used in the treatment of cancer in general and breast cancer in particular thanks to their capacity to encapsulate both hydrophilic and hydrophobic active substances and to their ability to be conjugated to various systems in order to provide a targeted therapy (43).

Systemic drug delivery approaches - polymers, micelles, liposomes, dendrimers and nanogels (44).

Localized drug delivery approaches - PEG, PLGA, chitosan, PLA, PVA, PCL or polyethylene oxide based nanofibers, chitosan hydrogels based on temperature-responsive hydroxyl butyl, poly (vinyl alcohol), thermo sensitive poly (ethylene lycol)-grafted, chitosan chloride/glycerophosphate and chitosan/bifunctional aldehyde and intraductal injection (44).

PEG may be used to conjugate nanoparticles for a longer circulation time(45) Some polymers like polylactic-co-glycolic acid (PLGA), that are used in NPs formulations, have high cell adhesion property (46). Beside all the advantages, nanoparticles may have many side effects such as: altered cellular redox balance and tissue inflammation, causing abnormalities

in cell function, leading even to cell death (47).

Receptor based drug delivery - HER-2, epidermal growth factor receptor [EGFR], vascular endothelial growth factor receptor [VEGFR], insulin-like growth factor I receptor [IGF-IR])-based targeting (44).

Depending on the materials they are synthesized of, NPs can be classified into: polymers (polimeric nanoparticles, micelles, dendrimers), lipids (liposomes), viruses (viral nanoparticles), organometallic compounds (nanotubes), metals colloids (Ag, Au), metal oxides (TiO2, SiO2), inorganic materials (carbon nanotubes, quantum dots, nanocrystals, nanoshells, nanowires, nanorods, nanopores, nanospheres, nanobelts, nanorongs, nanocaps, fullerenes) (48).

Polymeric micelles

Polymeric micelles are colloidal particles formed from amphiphilic block copolymers with a core-shell nanostructure. In aqueous media, the hydrophilic heads are arranged to outside and the hydrophobic tails to inside to stabilize the structure, structure that makes them suitable to deliver water-insoluble chemotherapeutic drugs (50).

Methods of preparation of drug-loaded micelles

Usually, 3 approaches may be used to prepare drug-loaded micelles:

- DIRECT DISSOLUTION METHOD - At a concentration ≥ CMC (critical micelle concentration), the amphiphilic copolymer and the therapeutic agent self assemble to form the drug-loaded micelles. To increase the drug loading, high temperature or a thin drug film may be used.
- SOLVENT EVAPORATION METHOD (SOLUTION CASTING METHOD) – The copolymer and the drug are dissolved in a volatile organic solvent. Then, the solvent is evaporated and removed, leading to a thin film of drug and copolymer. The micelles are formed when water is added over the film (51).
- DIALYSIS METHOD is very suitable for long and very hydrophobic copolymers. The organic solution of the polymer and drug is put in a dialysis bag, then the bag is immersed into water, exchanging the organic solvent with this second solvent, leading to micelle assembly. To shorten this method, a mixture of water/tert-butanol is used as solvent, afterwhich, this solution is lyophilized. The micelles are obtained after redispersion in a suitable vehicle (52) (Figure 4). Different polimers may be used as seen in Table 2. Di-blocks (A-B), triblocks (A-B-A) and graft co-polymers may be used to form micelles.

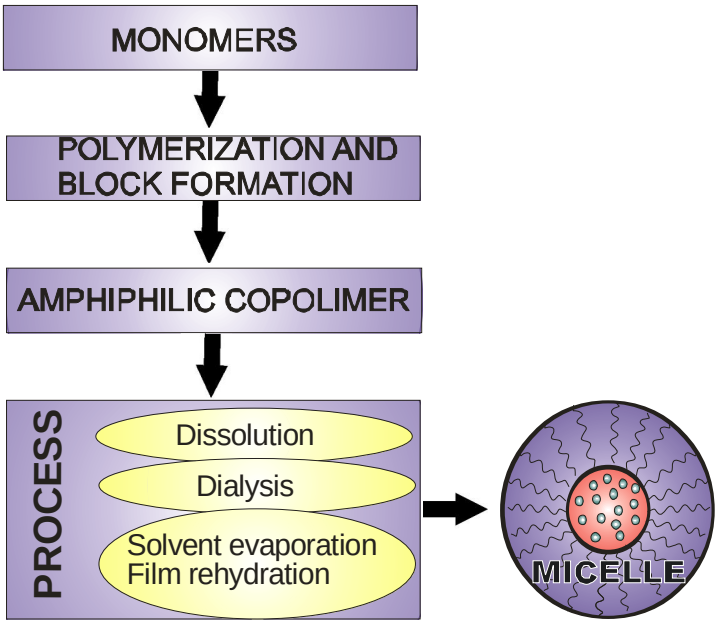

Figure 4 Micelle preparation steps (53)

POLYMER CLASS	EXAMPLES	FEATURES
Chitosan derivatives	N-Phthaloylcarboxymethylchitosan Oleoyl-carboxymethyl chitosan N-palmitoyl chitosan	• Chitosan has also bacteriostatic and fungistatic action.
Pyrolidone derivatives	Poly(N-vinyl pyrrolidone) (PVP)	• High water solubility, and flexibility.
Polyamides	Poly(Nisopropylacrylamide) (pNIPAAm)	• It may be used to prepare thermo-sensitive polymeric micelles.
Polyethers	Poly(ethylene oxide) (PEO) or poly(ethylene glycol) (PEG)	• The most utilized hydrophilic block to coat nanoparticles; • Non-toxic, flexible, hydrophilic, and electrically neutral; • PEG-coating prolongs the circulation time.
	Poly(propylene oxide) (PPO)	• They are very dynamic when assembled.
Polyesters	Poly(L-lactide) (PLA), poly-ε -caprolactone (PCL), poly(lactide-co-glycolic acid) (PLGA), poly(β-aminoesters)	• The block co-polymers hydrolyse in the biological system and are degraded to non-toxic monomers.
Polyamino acids	Poly(L-histidine) (pHis), poly(L-aspartic acid) (pAsp)	• They can transport drugs by chemical modifications (unlike other classes of core-forming polymers).
Lipids	Distearoyl(phosphatidylethanolamine) (DSPE), Dioleoyl(phosphatidylethanolamine) (DOPE)	• Excellent biocompatibility and a amphiphilicity.

Table 2 Example of polymers used for micelle preparation (50, 53-57)

Micelles have special characteristics:

- The of size and morphology of amphiphilic block copolymer are easily controlled by an adequate selection of: molecular weight, aggregation number, proportion of hydrophilic/hydrophobic chains, volume of solvent inside the micellar core, preparation process.
- They may be sterilized by filtrative sterilization, due to their colloidal dimensions.
- The micellar core can entrap poorly water-soluble substances, overcoming solubility, incompatibility, instability problems (enhanced bioavailability)
- Compared to surfactant micelles, polymeric micelles are more stable when diluted, exhibiting minimal cytotoxicity.
- The polymeric micelles prevent mechanical clearance by renal filtration and reticuloendothelial system, prolonging the blood circulation of drug, with high rate of accumulation in low vascularized tumors (58). Increased intracellular drug concentration is achived by overcoming the P-gp efflux and by acting through receptor-mediated endocytosis (59, 60).
- The risk of embolism is minimized, due to nanoscopic size.
- Receptor-mediated targeted drug and gene delivery may benefit from polymeric micelles, which can be end-functionalizated with sugars and peptides.
- High binding specificity and targetability may be achieved by covalent bound of monoclonal antibody molecules (61).
- They may be stimuli-sensitive (pH, temperature) (53, 62) .

Recent implications of polymeric micelles in breast cancer

As promising drug-delivery nanocarrier, polymeric micelles attracted increasing attention in treating cancer in the last years (63) (Figure 5).

- The polymer of NIPAM-VP [poly(N-isopropylacrylamide)] was used to obtain micelles of **Paclitaxel (*Nanoxel*)** and several studies were conducted. In 2006, the Indian Drug Regulatory Authority approved its use in the indian pharmaceutical market as an alternative to Cremophor EL- paclitaxel (64).
- In 2008, **Paclitaxel** was entraped in mPEG-PDLLA: monomethoxy-poly (ethylene glycol)-block-poly(D,L-lactide) micelles ***(Genexol-PM)*** and after rigorous studies, it has been granted for FDA aproval for metastatic breast cancer (65, 66).
- In the same year, methoxy-capped poly(ethylene glycol)-block-poly(ε-caprolactone) (mPEG-b-PCL) micelles loaded with 17-allylamino-17-demethoxy **Geldanamycin** were formulated. That study concluded that, even in the absence of ligands, the nanoscale dimensions may offer specificity of the drug, because of the EPR effect (67).

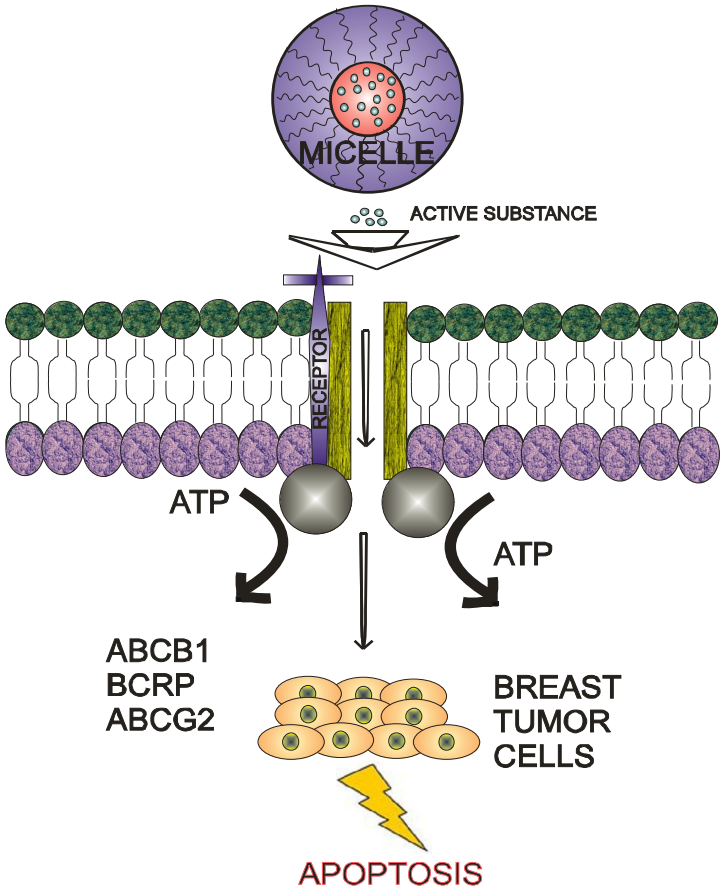

Figure 5 Polymeric micelles-based drug delivery mechanism in drug-resistant breast cancer cells (44)

- In november 2018, a multicenter phase IV study for breast bancer treatment started to evaluate **Docetaxel** polymeric micelles for safety, quality of life (QoL) and toxicity. The formulation was prepared using poly(N-vinylpyrrolidone)-block-poly(D,L-lactide) (PVP-b-PDLLA). The docetaxel loaded polymeric micelles are expected to have improved solubility, reduced toxicity and hypersensitivity compared to the drug alone (68).
- Nippon Kayaku Co.,Ltd formulated **Paclitaxel** loaded PEG-PAA plymeric micelles ***(NK105)*** which are now in Phase 3 of clinical study for NOS metastatic recurrent breast cancer (69).
- Several studies used antibody-bounded micelles to treat breast adenocarcinomas, by triggering immune response:

1. In 2009, Lee et al. used **Paclitaxel** loaded polymeric micelles conjugated with anti-HER-2 antibody. The complex shown high efficacy in HER-2-positive and HER-2-negative cells (70).
2. Anti-HER-2 monoclonal antibody (mAb) was conjugated with lysosomal P (LA-co-TMCC)-g-PEG-furan micelles for the treatment of HER-2-positive breast cancer (71).
3. Anti-HER2 antibody was conjugated with **Docetaxel** and polo-like

kinase 1 siRNA (siPlk1) loaded micelle nanoparticles composed of vitamin E d-α-tocopheryl polyethylene glycol succinate (TPGS) and TPGS-siRNA conjugates. The presence of receptor targeting antibody demonstrated higher toxicity compared to the nano-carriers that did not have an antibody against breast cancer cell (72).

4. For maximum therapeutic efficacy, intracellular localization must be reached. In 2012, Hoang et al. labeled the antibody, the peptide and the conjugated radio-sensitizer **(Methotrexate)** loaded block co-polymeric micelles with Indium-111. The indium-111 emitted radionuclides in the peri-nuclear as well as nuclear areas in breast cancer cells (73).

5. NK012 is a polymeric micelle of block copolymer PEG-Polyglutamate conjugated with 7-ethyl-10-hydroxy-**Campothecin** (SN-38) (74). It has completed phase II clinical trials for triple-negative breast cancer and relapsed small cell lung cancer (75). In 2016, Nippon Kayaku received orphan drug designation for NK012 from the US FDA (76).

Conclusions

In order to achieve better bioavailability and minimized negative effects, new pharmaceutical forms with controlled or targeted release have been developed. Nanoparticles represent a large part of these pharmaceutical formulations, that are currently involved in breast cancer treatment.

Nanoparticle systems such as liposomes, dendrimers, polymeric micelles and inorganic nanoparticles are under both preclinical and clinical trials for breast cancer.

In this review article, several examples of anticancer agents loaded polymeric micelles were mentioned, illustrating the preparation methods and the current state of clinical studies in which polymeric micelles are used. The micellar formulations must comply with the current Pharmacopoeias and the Good Manufacturing Process conditions. Clinical studies require team work between researchers and clinicians, for a multidisciplinary expertise.

Until 2018, sevaral targeted micelle formulations have been investigated in breast cancer clinical trials: Genexol-PM, Nanoxel, NK012 and NK105.(56)

Conflict of interest

The authors declare no conflict of interest.

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