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NANOPARTICLES FOR BREAST CANCER TREATMENT

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Abstract

Early detection and treatment of breast cancer remains a challenge today. Although conventional therapy is currently the first-line treatment, it produces remarkable side effects by damaging healthy cells. As a novel therapy approach, nanoparticles are intended to increase the systemic circulation of anticancer drugs, enhancing their accumulation at the tumor site and consequently reducing their toxicity in healthy tissues. In order to achieve this goal, scientists have been studying how to shape the structural and physicochemical properties of nanoparticles, such as their nature, size and surface, in order to camouflage and evade the immune system, increase therapeutic efficacy and reduce toxicity of the anticancer drug. Nowadays, the FDA (Food and Drug Administration) has already approved more than eight types of nanomedicines applied to the routine treatment of breast cancer.

Keywords: nanoparticles, nanotechnology, breast cancer.

Resum

La detecció precoç i el tractament del càncer de mama segueixen suposant un repte en l'actualitat. Per molt que la teràpia convencional segueixi essent de primera elecció, provoca notables efectes secundaris i danya les cèl·lules sanes. Com a teràpia innovadora, les nanopartícules pretenen augmentar la circulació sistèmica dels fàrmacs anticancerosos, potenciant d'aquesta manera la seva acumulació al teixit tumoral i reduint en conseqüència la seva toxicitat als teixits sans. Per assolir aquest objectiu, la comunitat científica ha estudiat com modelar les propietats estructurals i fisicoquímiques de les nanopartícules, com ara la seva naturalesa, la seva mida i la seva superfície, amb la finalitat de reduir la toxicitat del fàrmac anticancerós. Actualment, la FDA (Food and Drug Administration) ha aprovat més de vuit tipus de nanomedicines amb la finalitat de tractar el càncer de mama.

Paraules clau: nanopartícules, nanotecnologia, càncer de mama.

Resumen

La detección precoz y el tratamiento del cáncer de mama siguen presentando muchos retos en la actualidad. Aunque la terapia convencional siga siendo el tratamiento de primera elección, produce notables efectos secundarios y daña las células sanas. Como enfoque terapéutico novedoso, las nanopartículas pretenden aumentar la circulación sistémica de los fármacos anticancerígenos, potenciando su acumulación en el tejido tumoral y reduciendo, en consecuencia, su toxicidad en los tejidos sanos. Para lograr este objetivo, la comunidad científica ha estudiado cómo moldear las propiedades estructurales y fisicoquímicas de las nanopartículas, tales como su naturaleza, tamaño y superficie, con el fin de camuflarse y evadir el sistema inmunitario, aumentar la eficacia terapéutica y reducir la

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toxicidad del fármaco anticanceroso. En la actualidad, la FDA (Food and Drug Administration) ya ha aprobado más de ocho tipos de nanomedicinas aplicadas a la rutina del cáncer de mama.

Palabras clave: nanopartículas, nanotecnología, cáncer de mama.

1. Introduction

Cancer is the second leading cause of death around the world, having killed 9.6 million people according to the ("Cancer," n.d.). Conventional treatment methods have shown some limitations, like multi drug resistance (MDR), unspecific cell death (chemotherapy), limited dosage, and low biocompatibility of hydrophobic anti-cancer drugs. In order to address these limitations, scientists have been trying to develop a new strategy for cancer treatment, focusing on nanotechnology (Khan *et al.*, 2019).

Nanotechnology for clinical use has been developed since 1956, according to the physician Richard P. Feynman, with the aim of taking advantage of the special physical and chemical features of atomic or molecular structures in nano-scale, 1-100nm, to treat several diseases (Khan *et al.*, 2019). The main purposes of nanotechnology-based formulations in cancer treatment are the improvement of pharmaco-kinetic traits, like bioavailability, and the selective targeting of tumor cells, overcoming problems of drug delivery to the tumor site. This would lead to increased circulation time of the anti-cancer drug and therefore of its effectiveness as well as reduced side effects like weakness, hair loss and organ disfunction (Khan *et al.*, 2019).

Despite all the advantages mentioned above, it is necessary to take into consideration the impact that nanoparticles (NPs) can have, after chronic exposition, on our health. In 11 scientific studies, possible respiratory and dermal nanotoxicity side effects have been exhibited (Gutiérrez González *et al.*, 2013).

Even though this field has shown some challenges and limitations, the FDA has already approved more than 50 drugs involving nanomaterials in their formulation, with more than a dozen having been authorized in the last decade (Lammers and Ferrari, 2020).

In order to fully understand the mechanism of nanotherapeutics, it is important to have knowledge of physiological changes caused in cancerous tissue and how they affect the features of NPs to specifically target the tumor site and liberate the anti-cancer drug.

2. Targets

- Description of physiopathological changes induced by a tumor and further study of the physicochemical features of NPs to be taken into account for the optimal design of nanomedicines, treatment and diagnosis of cancer.
- Review FDA approved nanoparticles and those still in clinical trials, for breast cancer treatment. Additionally, analyze the progress/usefulness of nanoparticles for the treatment of breast cancer.

3. Methodology

In order to write this paper, extensive bibliographic research was carried out. Nanomedicine is a very large field in which many articles have been published. Entering the term "nano-

medicine" in google academics showed more than 422,000 results. To shorten the list of articles I took into account their date of publication. Finally, after searching for "Recent advances in nanotechnology", I noticed that many nanoparticles under study are aimed at improving the treatment of breast cancer so I decided to focus my search on this field of study.

4. Results and discussion

4.1. Nanoparticles drug delivery systems

Tumor tissues are dynamic systems that differ from normal tissues, having a specific tumor microenvironment (TME) designed to promote the extension and progression of cancer cells. This dynamic system has a different blood flow, oxygenation, redox microenvironment, temperature and enzyme activity; traits that will be used to design tumor-specific NPs intended to perceive the TME-stimuli and release the drug specifically at the tumor site (Boix-Montesinos *et al.*, 2021; Raju *et al.*, 2019). Nanoparticles respond to two types of tumoral stimuli:

- Endogenous stimuli: pH, peptides, redox, enzymes.
- External stimuli: temperature, light, ultrasound magnetic fields, and electric fields.

4.1.1 Endogenous stimuli

4.1.1.1 pH-responsive drug delivery system

Tumors have an acidic microenvironment (pH≈6.5) compared to physiological pH (≈7.4) due to excessive production of lactate and hydrogen, whose function is to enhance the tumor growth and increase its energy (Raju *et al.*, 2019; Zhang *et al.*, 2010). Adhering ionizable groups with different pks and chemical structures to the NPs surface, nanomaterials will be able to alter their structure depending on the microenvironments pH, liberating the drug into the extracellular matrix of the TME (Lee *et al.*, 2008).

4.1.1.2 Peptide-based drug delivery system

Peptides are amino acids linked together that can be designed to specifically bind overexpressed receptors at tumoral cells ("Definition of Peptide," n.d.). Their small size makes it possible for them to penetrate into tissues and bind these receptors.

4.1.1.3 Redox-responsizve drug delivery systems

The TME oxidation and reduction reactions are regulated by NADPH/ $NADP^+$ and glutathione (GSH), where GSH is responsible for the regulation of metabolic processes, interacting with reactive oxygen species (ROS) and breaking disulfide bonds (Guo *et al.*, 2018). The concentration of GSH in a resistant tumoral cell is four times higher than in a normal matrix (Raju *et al.*, 2019). Redox-sensitive NPs are designed incorporating redox-sensitive bonds, which will release the cargo in the presence of GSH (Guo *et al.*, 2018).

4.1.1.4 Enzyme-responsive drug delivery

This type of NPs are designed to be recognized and cut by catalytic enzymes, containing enzyme-labile linkers on their main or side-chain groups, in order to bind the target. Furthermore, the construction of the enzyme-specific substrate has to be very precise, because of the existence of several enzyme subtypes with similar cleavage (Shahriari *et al.*, 2019). Enzymes used for cleavage of NPs are: lipases, metalloproteinases, proteases, cathepsins, glycosidases and oxidoreductases (Shahriari *et al.*, 2019).

4.1.2 Exogenous stimuli

4.1.2.1 Thermo-responsive drug delivery systems

Tumor microenvironment has a temperature of 40-42ºC, with the normal temperature of a living body being around 37ºC (Liu *et al.*, 2017). NPs can be specifically designed to deliver the anti-cancer drug under high-temperature conditions.

4.1.2.2 Photo-sensitive drug delivery

This DDS uses light (visible, near-infrared reflection, UV) for a controlled release of the drug. Near infrared light has most potential and activates NPs to convert light into heat through light thermal agents, which excites heat-susceptible materials embedded in NPs and disrupts their structure to release the anti-cancer drug (Wang *et al.*, 2016).

4.2. Tumor targeting

Inserting biomarkers, like fluorescent molecules, on the NPs makes it possible to diagnose tumors at an early stage and delimit their location in vivo (Khan *et al.*, 2019). With a view to understanding how NPs target the tumor and release the drug, first one needs to understand how tumors physiologically affect our bodies.

4.2.1. Tumor microenvironment

Tumors are complex dynamic systems consisting of irregular vasculature, fibroblast and immune cells in the extracellular matrix (ECM) (Annaratone *et al.*, 2020). Their TME has an essential role in the progression and metastasis of the tumor. When the ECM is altered, it suppresses the immune system, allowing cancer to spread unrestrained to different body parts (Hashemzadeh *et al.*, 2021). Last but not least, tumors have an acidic (pH=6.5) and hypoxic microenvironment, conditions that will provide the tumor with nutrients and energy (Annaratone *et al.*, 2020; Khan *et al.*, 2019).

4.2.2. Passive tumor targeting, enhanced permeability and retention effect

Passive tumor accumulation is based on the enhanced permeability and retention effect (EPR) of cancerous tissues (Perry *et al.*, 2017). As an instance, during the extension of the tumor mass, the existing vessels will expand rapidly to provide nutrients to the tumor tissue. In ad-

dition, new leaky vessels can be created in a process called angiogenesis (Folkman, 1985). The before-mentioned newly formed vessels contain huge pores in their walls which will be essential for passive tumor targeting as can be seen in figure 1. NPs with a specific size, will extravasate through the pores from the blood circulation into the tumor masses and accumulate. The more they accumulate at the tumor site, the more treatment efficiency and the less adverse effects (Khan *et al.*, 2019; Perry *et al.*, 2017).

Even though passive targeting is essential for anti-cancer treatment with nanoparticles, some factors should be taken into consideration. At first, for NPs to extravasate through blood vessels, they need to have a long circulation time and thus a low clearance. As for the NPs design, to counteract this limitation, scientists will have to choose a specific size and coating of NPs, so extravasation can take place. Likewise, the metastatic area will be oxygenated with NO-releasing factors and angiotensin II infusions for the purpose of EPR effect to take place (Fang *et al.*, 2010; Khan *et al.*, 2019).

4.2.3. Active tumor targeting

Active targeting is based on receptor-mediated endocytosis of the NPs into the cancer cell. Depending on their size and shape different kinds of endocytosis will take place delivering the NP to the desired compartment or to the nucleus (Harisa and Faris, 2019). To perform such internalization, NPs surface will be modified incorporating ligands, such as carbohydrates, anti-bodies, peptides or aptamers that specifically bind cell surface receptors of tumor cells (Choi *et al.*, 2010; Khan *et al.*, 2019). Nevertheless, to reach the subcellular compartment, NPs first will have to escape degradation by endosomes.

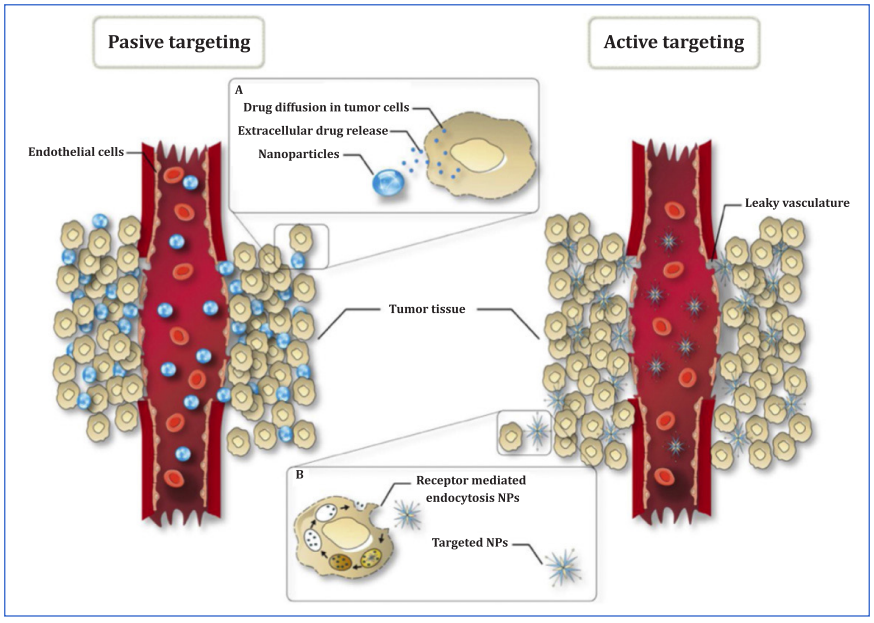

Figure 1. Active (B) vs passive (A) targeting approaches for anti-cancer drug delivery systems (Khan *et al.*, 2019).

4.3. Types of nanoparticles for cancer treatment

NPs are classified in two different groups: inorganic and organic, according to their structure/components. The former are currently used for cancer diagnosis (imaging and hyper-

thermia treatment), and the latter for drug release and gene-therapy (Fukumori and Ichikawa, 2006). The design of NPs is essential for achieving the aimed accumulation on the tumor site and therefore a located drug release. There are some factors that need to be taken into account, including: stimuli-responsive drug release, tumor targeting capability, cellular internalization, medicine loading levels and circulation time.

4.3.1. Liposomes and polymeric micelles

PEG-coated liposomes have the ability to avoid their uptake by the RES, resulting in a longer circulation time, slower clearance and a small volume of distribution (Gabizon *et al.*, 1997). Their good physicochemical properties allow these NPs to reach a higher drug concentration at the tumor site of liposomal formulations, compared to the free drug. Liposomes are considered biodegradable, non-toxic and non-antigenic (Fukumori and Ichikawa, 2006). Furthermore, these kind of formulations are created by self-assembly in aqueous solutions, resulting in hydrophilic heads of phospholipids headed to the water, allowing the encapsulation of hydrophilic drugs inside the aqueous core (figure 2). On the other hand, polymeric micelles (PMs), contain a hydrophobic core, allowing hydrophobic drugs to be carried to the targeted tissue (figure 3) (Zhou *et al.*, 2018). Comparing to liposomes, they can accumulate more easily on the tumor site and seem to be more flexible when molding their size, surface and shape (Fukumori and Ichikawa, 2006).

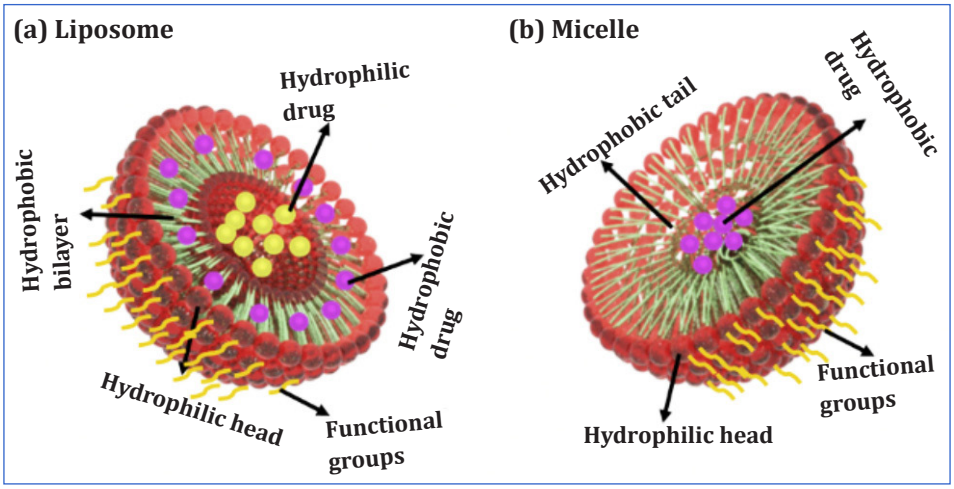

Figure 2. Illustration of liposomes (a) and polymeric micelles (b) (Raju *et al.*, 2019).

4.3.2. Dendrimers

Dendrimers are uniformly dispersed synthetic polymers of an amphiphilic nature, having a hydrophobic core and hydrophilic surface (Chowdhury *et al.*, 2021; Raju *et al.*, 2019). They are formed of a core with branched polymers resulting in a large surface, able to target a specific area. As can be seen in figure 3, the amount of polymeric branches will determine the number of generations of the formed nanoparticle. In addition, dendrimers can carry hydrophobic drugs, by forming complexes with the highly branched polymers. However, they must be combined with other nanoparticles in order to overcome their limitations, such as low tumor penetration and rapid in vivo elimination (Sunoqrot *et al.*, 2013).

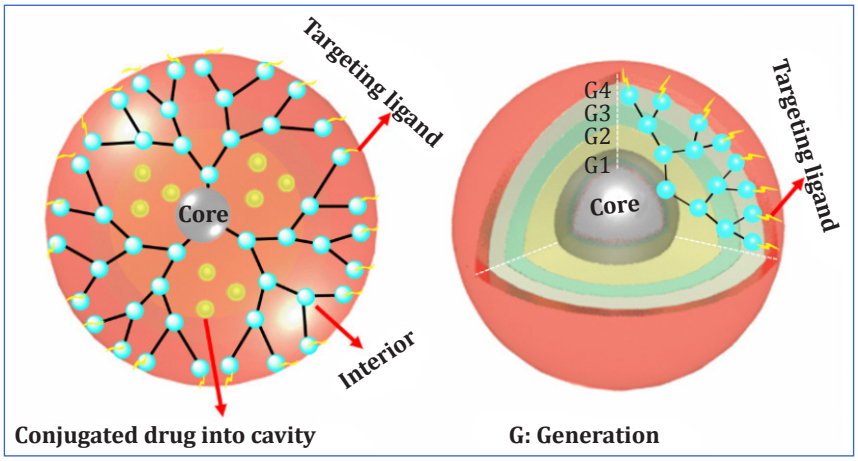

Figure 3. Illustrated dendrimer with its different generations (Raju *et al.*, 2019).

4.3.3. Mesoporous silica nanoparticles

Mesoporous silica NPs (MSNPs) are biodegradable drug delivery vehicles, with reduced side effects, a high loading capacity and an approximate size of 2-10 nm (Raju *et al.*, 2019; Slowing *et al.*, 2008). Furthermore, they have the ability to be internalized by endocytosis, delivering the drug inside the targeted cancer cell, despite their relatively long size, to perform extravasation through leaky vessels (EPR-effect).

4.3.4. Carbon nanotubes

Carbon nanotubes (CNT) are insoluble cylindrical structures of benzene rings with low bioavailability, which can be functionalized to be water-soluble with coatings like PEG (Chowdhury *et al.*, 2021). They have a thin needle structure and are normally used as biosensors. CNT can detect ion movement and therefore biological activity in different cells (Zhang *et al.*, 2014).

4.3.5. Polymeric-glycol nanoparticles

TABLE 1. GLYCOL-NANOPARTICLES USED IN CANCER THERAPY (KHAN ET AL., 2019)

Cancer	Nanoparticle	Drug	Model	Type of cell line
Human tumors	β-Cyclodextrin-Bearing Gold Glyco-nanoparticles	Methotrexate	*In vitro*	–
Melanoma cancer	Gold glycol-nanoparticles		*In vitro* *In vitro*	B16OVA, A37, Mel JuSo, B16.F10, MeWo, SKMel24, CHO
Prostate cancer	Gold nanoparticles + NaBH4 + Either thio-glucose or sodium citrate		*In vitro*	DU-145
adenocarcinoma	Gold glycionanoparticles + glucose + biotin + siRNA		*In vitro*	CMT/167

Cancer	Nanoparticle	Drug	Model	Type of cell line
Leukemia cancer	Gold nanoparticles + thio-PEG + thio-glucose + Glycopolymer-Stabilized Gold Nanoparticles		*In vitro*	THP-1, MCF-7
Breast cancer	Metformin Loaded gold glycol-nano-particles	Metformin	*In vitro*	MCF-7
Neuroblastoma	Glycol-polymer-coated gold nanoparticles	DOX	*In vitro*	SH-SY5Y
Prostate cancer	Galacto-glycogen nanoparticles	–	*In vitro*	PC3
Hepatocellular carcinoma	Magnetic iron oxide nanoparticles + polydopamine + Hypericin + Lac	Hypericin	*In vitro*	HepG2, MCF-7
Breast cancer	Iron oxide nanoparticles + polydo-pamine + glucose oxidase	–	*In vitro* *In vitro*	MDA-MB-231, MCF-10A, 4T1
Multiple cancers	Magnetic glyco-nanoparticle	–	*In vitro*	TA3-ST TA3-HA, MCF-7, B16-F1, B16-F10, SKOV-3, HT29, A549, A498, 184B5
Lung cancer	Fluorescein isothiocyanate- (FITC)-doped mesoporous silica Nanoparticles	DOX	*In vitro*	A549 PCC
Hepatocarcinoma	Galactose-based glycopolymer -drug conjugates nanoparticle	DOX	*In vitro*	HepG2, COS7
Breast cancer	Glucose-conjugated chitosan Nanoparticles	DOX	*In vitro*	4T1

Polymeric-glycol NPs are carbohydrate functionalized nanomaterials that were created to perform as carriers of a multi-valent binding, higher biocompatibility, controlled release and better uptake to the intracellular compartment (Khan *et al.*, 2019). In table 1 different kinds of glycol NPs in investigation for cancer treatment are listed (Khan *et al.*, 2019).

4.3.5.1 Poly-(lactic-co-glycolic acid) nanoparticles (PLGA)

PLGA are the biodegradable polymers with highest success, because of their incorporation of glycolic and lactic acid, endogenous monomers that can be easily metabolized via Krebs-cycle (Mir *et al.*, 2017). In addition, PLGAs are internalized via pinocytosis or clathrin-mediated endocytosis, escaping degradation with endo-lysosomes (figure 4) (Mir *et al.*, 2017; Akash *et al.*, 2016; Li *et al.*, 2001). These NPs possess high stability in biological fluids and can avoid the enzymatic metabolism better than liposomes. Furthermore, PLGA-NPs show a greater toxicity than free drugs in targeted cancerous cells (Khan *et al.*, 2019). Nowadays, PLGA nanoparticles have been implemented in several cancer therapies in combination with anti-cancer drugs like docetaxel (Chen *et al.*, 2011) or paclitaxel (Win and Feng, 2006) for colon or breast cancer.

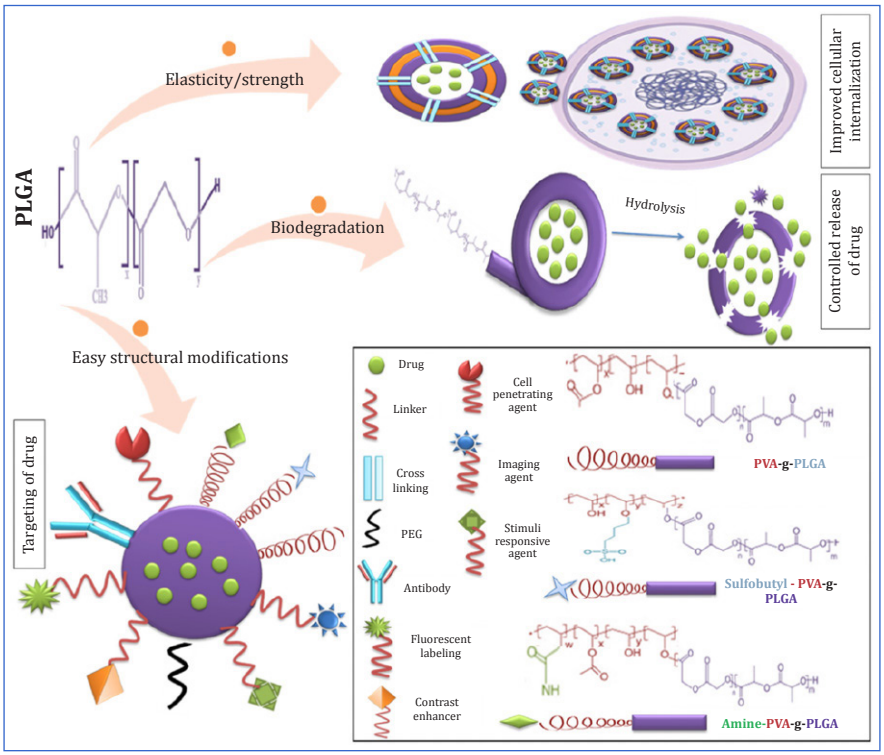

Figure 4. Properties of PLGA to improve drug delivery (Mir *et al.*, 2017).

4.3.6. Inorganic

Inorganic NPs have been developed as intravascular probes for in vivo and ex vivo imaging, and also therapeutics. Their success depends on their ability to escape the RES and target a specific tissue or cell type. Inorganic NPs are normally more difficult to eliminate and can produce toxicity.

4.3.6.1 Quantum dots

Quantum dots (QDs) are 2-10 nm fluorescent nanocrystals that have a semi-conducting capacity (Lim *et al.*, 2014). They are normally used for imaging/diagnosis, enabling the detection of biomarkers, like specific proteins or metabolites, produced by cancer cells. The QDs fluorescent light intensity changes after being internalized into tumor cells, indicating changes on the TME. This kind of NPs are often used in photodynamic therapy and as real-time imaging agents to visualize metastatic cancer (Lim *et al.*, 2014). Furthermore, QDs are able to destroy tumor cells, producing singlet oxygen (Raju *et al.*, 2019). Finally, it is important to mention that QDs proved to have a greater photostability than other NPs (Huang *et al.*, 2011).

4.3.6.2 Magnetic NPs

Magnetic NPs are used directly or dispersed as cores in the polymeric matrix and their ther-

apeutic application for cancer is hyperthermia treatment and magnetic field targeting of NPs (Fukumori and Ichikawa, 2006; Mahmoudi *et al.*, 2011). For diagnosis, magnetic NPs are used for magnetic resonance imaging (MRI) as contrast agents, targeted molecular imaging, hyper-fusion region visualization, cell labeling in T-cell based therapy and detection of angiogenesis, apoptosis and gene expression (Fukumori and Ichikawa, 2006).

4.3.6.3 Superparamagnetic iron oxide NPs

Superparamagnetic iron oxide NPs (SPIONs) are spherical magnetite nanoparticles with diameters less than 20 nm which acquire paramagnetic abilities in presence of a magnetic field (Revia and Zhang, 2016). These features allow SPIONs to be used as drug delivery systems and also as contrast agents for MRI. SPIONs are iron oxide cores that can be targeted to the required area via external magnets. For imaging, SPIONS have a high signal range (Huang *et al.*, 2011). It is for that reason that small quantities of NPs are needed for imaging, reducing their toxicity. In addition, this type of NPs can transform energy to heat and end up killing the targeted cancer cells. Nevertheless, due to the iron core, their elimination, retention and biodegradability should be taken into special consideration.

4.3.6.4 Gold NPs

Gold NPs (AuNPs) have special optical and photothermal properties, because of the collective oscillation of free electrons in their conduction bands, high absorption and scattering intensity (Huang *et al.*, 2011). They are good contrast agents for MRI and radiosensitizers. Furthermore, gold-NPs are able to absorb near infrared light (650-900 nm) and generate heat, feature that can be used in thermal ablation procedures in combination with chemotherapy. Nevertheless, AuNPs can be unspecific and toxic for healthy cells (Huang *et al.*, 2011).

4.3.6.5 Silver NPs

Silver NPs (AgNPs) have the ability to interact at a specific wavelength showing optical features and can enhance the anti-cancer drug's effectiveness. AgNPs are used as drug delivery systems, diagnosis, nanocomposites and antimicrobial agents (Burduşel *et al.*, 2018).

4.4. Nanoparticles physical-chemical features

The biodistribution, circulation time and therefore efficiency of the therapy with NPs is determined generally by their size, administration route, composition and surface charge. Nanotechnology works by modulating these parameters in order to achieve better physicochemical features.

4.4.1. Size

Size is an essential parameter for reaching a sufficient biodistribution and accumulation, on

the tumor site, of nanomedicines. The longer the NPs remain at systemic circulation, the more likely they are to overflow, through a leaky blood vessel, to the tumor (Khan *et al.*, 2019). For efficient delivery of NPs, the main criteria are: penetration through leaky blood vessels, lymph node draining and internalization by APC (Raju *et al.*, 2019).

First of all, it is important to mention that normal tissues' pores, in vessel walls, have a 9-50 nm diameter. Otherwise, tumor tissues with discontinue capillary walls, allow particles less than a 100 nm to penetrate easily. This means that particles from 50-100 nm will be able to extravasate specifically into the tumor mass, due to the EPR effect (Fukumori and Ichikawa, 2006) (figure 5).

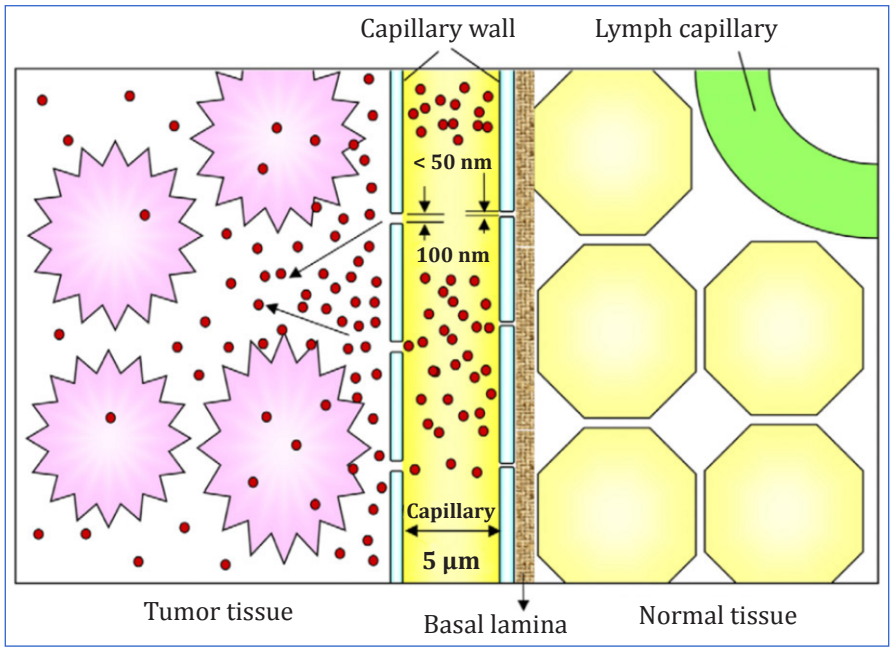

Figure 5. Particle extravasation to tumor and normal tissue through leaky vessels depending on NPs and pore size (Fukumori and Ichikawa, 2006).

Generally, NPs size is around 10-100 nm. In case of being too small (<10 nm), NPs will end up being filtered through the kidneys and if they exceed the established dimensions (> 100 nm), they will be easily cleared by the reticuloendothelial organs, spleen and liver (Fukumori and Ichikawa, 2006; Huang *et al.*, 2012; Zhou *et al.*, 2018).

Moreover, the drug release is affected by the NPs size, more specifically by their area. Small NPs have a bigger area, implying that more drug is bound to the NPs surface and therefore a faster release (Kumar *et al.*, 2017). Besides, it has been shown that both uptake- and ligand-binding forces increase with the size of the NP, while small NPs have the risk to aggregate during dispersion in biological fluids diminishing their uptake (Behzadi *et al.*, 2017).

4.4.2. Surface charge (ζ Potential) and chemistry

The zeta-potential is a parameter used to determine the charge of NPs, an essential data to get information about repulsion or attraction among them and thus predict their stability. In order to have a great stability in suspensions, preventing NPs from aggregation, their charge should be around ± 30 mV (Kumar *et al.*, 2017). This parameter also determines the opsoni-

zation of NPs and its interactions with membrane receptors to be internalized (Behzadi *et al.*, 2017). Furthermore, hydrophobic coatings are normally avoided due to their poor stability and enhanced formation of protein corona (Li and Lane, 2019).

In reference to cellular trafficking, cationic nano-shuttles are internalized through clathrin-mediated endocytosis (CME) and anionic nano-shuttles via caveolae-mediated endocytosis (CvME) (figure 6) (Akash *et al.*, 2016; Harisa and Faris, 2019). Nano-shuttles are gold, iron oxide and polylysine drug delivery systems that cross the cell membrane and are transported via motor proteins to the desired organelles (Harisa and Faris, 2019).

Continuing with charge-dependent cellular internalization, neutral nano-shuttles generally have less protein adsorption than charged NPs but interact in an unspecific way, limiting their cellular uptake (Harisa and Faris, 2019; Li and Lane, 2019) Moreover, anionic NPs enter cells via endocytosis while positively charged NPs have stronger electrostatic interactions with the phospholipidic bilayer, inducing a disruption on the membrane (i.e., creating pores), resulting in a higher uptake of positively charged NPs (Behzadi *et al.*, 2017; Li and Malmstadt, 2013). Additionally, it has been proven that, the higher the charge density, the more penetration and disruption (Behzadi *et al.*, 2017).

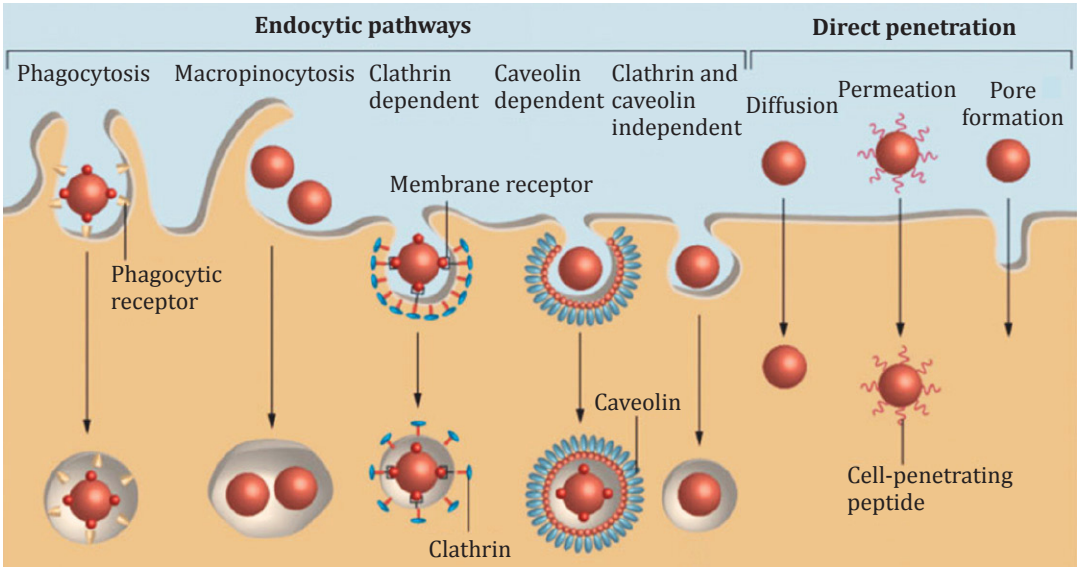

Figure 6. Direct penetration and endocytic pathways to internalize nanoparticles (Akash *et al.*, 2016).

4.4.3. Protein corona

The protein adsorption layer, formed on the surface of colloidal NPs when administered intravenously to our bloodstream, is called protein corona. Protein corona is formed during diffusion of the NP as a result of electrostatic and hydrophobic interactions between NP and proteins, usually generating a monolayer (Lee *et al.*, 2015). The protein corona has the function of removing foreign agents from the bloodstream in a process called opsonization. It will target the nanoparticle to enhance its uptake by phagocytic cells to the RES organs and enhance its elimination (Li and Lane, 2019).

4.4.4. Shape

How the shape of the NPs influences their protein adsorption is something that has not yet been studied as thoroughly as the other physical-chemical parameters. It is known that NPs shape has a great impact in its intracellular uptake and can trigger or avoid the immune response (Behzadi *et al.*, 2017). As an example, due to its large surface and lower curvature, nanorods exhibited 10 times greater protein adsorption than the spheres (Li and Lane, 2019). Furthermore, Chithrani and Chan (2007) studied the internalization differences between positively charged gold nanorods and anionic nanospheres with the same volume. As a result, they saw that the nanorods had a decreased internalization even though generally cationic NPs favor their uptake. They thought it was because of the larger surface/volume ratio of nanorods, that it is more difficult for the cell to surround and internalize the NP, which implies an energetic expense and usually ends up in a frustrated endocytosis (Chithrani and Chan, 2007; Li and Lane, 2019).

Moreover, Black *et al.* (Black *et al*., 2014) studied tumor accumulation of 50 nm PEGylated gold NPs with different shapes. They determined that spheres accumulated 3 times more than nanorods, 6 times more than nano-discs and 10 times more than cages.

In conclusion, as mentioned above and according to the studies cited, the shape of NPs can influence their protein adsorption, accumulation, internalization and finally their elimination.

4.5. Biomimetic nanomedicine (coating)

In order to address one of the biggest limitations of NPs, their circulation time, and avoid the uptake by the reticuloendothelial system, different surface modifications have to be made. When administered intravenously, NPs are detected as foreign by the immune system, they are targeted with opsonins, which will be detected by macrophages to enhance phagocytosis and degradation (Kumar *et al.*, 2017). It has been proven that covering liposomes with natural polymers or synthetic biodegradable copolymers with hydrophilic sections, like polyethylene glycol (PEG) or lactic-co-glycolic acid (PLGA), prolongs the NPs half-time (Li *et al.*, 2001; Mirza and Karim, 2021).

As an innovative approach scientists have been studying how to create biomimetic NPs (figure 7); camouflaged NPs, covered with a cell membrane to avoid immune elimination, prolonged circulation rime an target a specific area (Wang *et al.*, 2020).

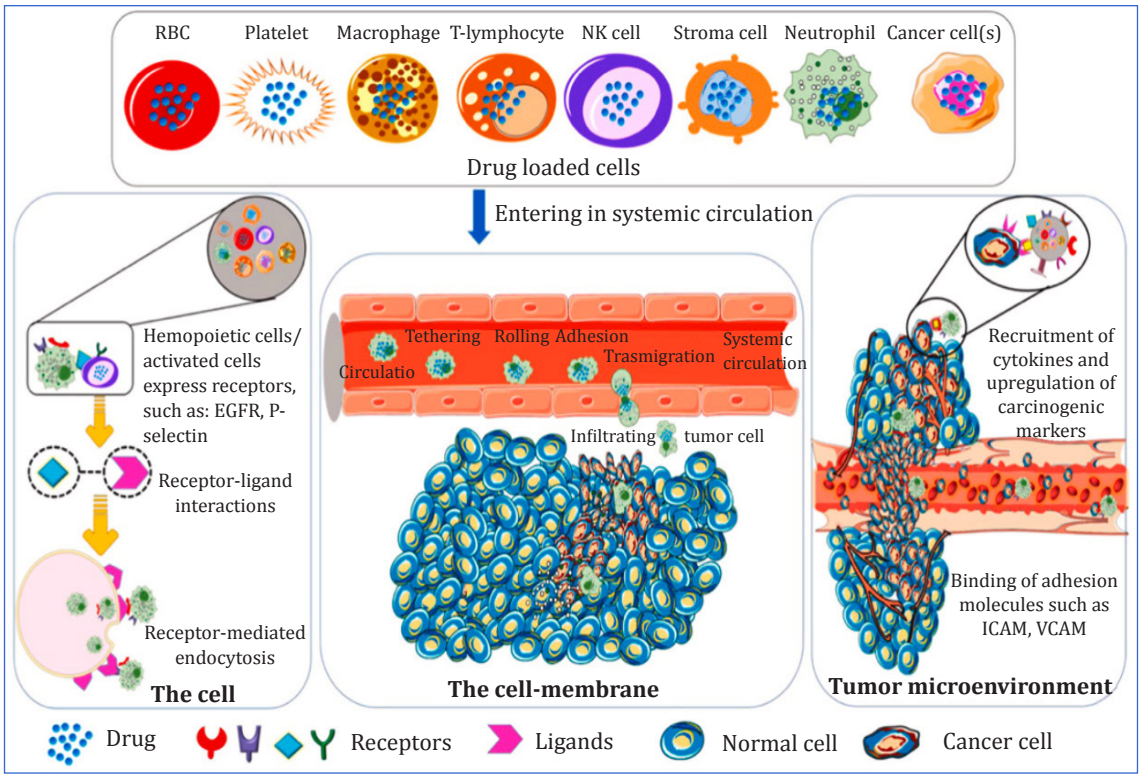

Figure 7. Delivery options of the anti-cancer drug via extravasation and accumulation of the nanoparticles cellular vehicle to the tumor site (Chowdhury *et al.*, 2021).

4.5.1. RBC-membrane

Red blood cells (RBC) have a lifespan of 115 days and can last up to 40h in in vivo circulation not inducing an immunological response (Chowdhury *et al.*, 2021; Harisa and Faris, 2019). Furthermore, they can be targeted to cancer cells and have also shown an increased loading capacity and enhanced efficiency (Chowdhury *et al.*, 2021).

Targeting ligands

- CD47: binds macrophages surface to evade phagocytosis (Wang *et al.*, 2020).

4.5.2. Platelet membrane

Platelets have a half-time of 7 to 10 days and accumulate in injured tissues (Chowdhury *et al.*, 2021; Wang *et al.*, 2020). Moreover, thrombocytes are associated to tumor growth and metastasis and can be recruited by vascular damaged components of the subendothelial matrix. Nevertheless, NPs with platelet membranes cannot be internalized (Wang *et al.*, 2020).

Targeting ligands:

- CD47, CD55 and CD59: to suppress the immunological complement system and avoid macrophages (Wang *et al.*, 2020).
- CD40 + p-selectin: binds CD44 overexpressed in tumor cells and promotes NPs aggregation (Wang *et al.*, 2020).

4.5.3. Immune-cell membrane

NPs covered with an immune-cell membrane can evade the immune system and cross biological barriers. They present reduced opsonization and have a self-recognition mechanism that delays phagocytosis (Wang *et al.*, 2020).

Targeting ligands:

- Leukocyte membrane
  Targeted to deliver nanoparticles to diseased tissues (Chowdhury *et al.*, 2021).
  - LFA-1: binds ICAM-1 on inflamed endothelium(Wang *et al*., 2020).

- Macrophage and monocyte membrane
  Monocytes differentiate into tumor-associated macrophages (TAMS) after crossing the endothelial barrier. TAMs are able to deliver the anti-cancer drug in reaching the hypoxic areas of the TME. There are two types of TAMs being the variant M1 the one who kills tumor cells and M2 the responsible for tumor progression and growth (Chowdhury *et al.*, 2021).
  - Cytokines (CCR2): can be recruited by CCL2-ligans on breast cancer cells(Wang *et al*., 2020).
  - Alfa-4-integrin: can bind VCAM1, a receptor expressed in cancer cell (Wang *et al*., 2020).
- Neutrophile membrane
  They are first-line defenders reaching the inflamed site through neutrophil transmigration across endothelial layer (Chowdhury *et al.*, 2021). Tumor associated neutrophiles (TANs) are recruited to the TME because of its surface composition:
  - CD44 + p-selectin: bind circulating tumor cells (CTCs).
  - LFA-1.
  - Beta-1-integrin: binds VCAM1 on tumor cells (Wang et al., 2020).
- Cytotoxic t lymphocytes membrane (CTLs)
  - CD8+: can be recruited to the tumor site.
  - High level of adhesion molecules (Wang *et al*., 2020).
- Natural killer membrane (NKsome)
  - DNAM-1 and NKG2D: receptors that target tumor cells (Wang *et al*., 2020).

4.5.4. Cancer-cell membrane

NPs coated with a cancer-cell membrane inherit its functionality of homologous targeting and antigen pool from their source cell. CTCs are resistant to the immune system and can target homotypic tumors. They have an improved uptake by homologous cancer cells. Nevertheless, this type of coating wouldn't work in heterogenous cancer types which are the most common ones.

Targeting ligands:

- CD47: to avoid phagocytosis by macrophages (Wang *et al.*, 2020).

4.6. Toxicity of nanoparticles

Even though nanotechnology offers our society a new way of developing therapeutics and diagnosis, it is important to take into account which harmful effects nanomaterials can cause on our health, after exposure. Because of their small size, NPs are easily absorbed through the respiratory tract and through the skin barrier arriving to the bloodstream and in the same way to the organs. Parameters like its size, shape, charge, composition and hydrophobicity, can influence the NPs toxicity. One of the most decisive features is its solubility in biological fluids, where depending on its composition, NPs can cause systemic toxicity and local adverse effects (Boix-Montesinos *et al.*, 2021). In addition, physical and chemical properties of NPs can activate stress-related genes, membrane disruption and release pro-inflammatory cytokines activating an inflammatory response as mentioned in figure 8 (Pandey and Prajapati, 2018).

Nevertheless, scientists have been trying to develop biodegradable NPs with decreased its in vivo toxicity (Pandey and Prajapati, 2018).

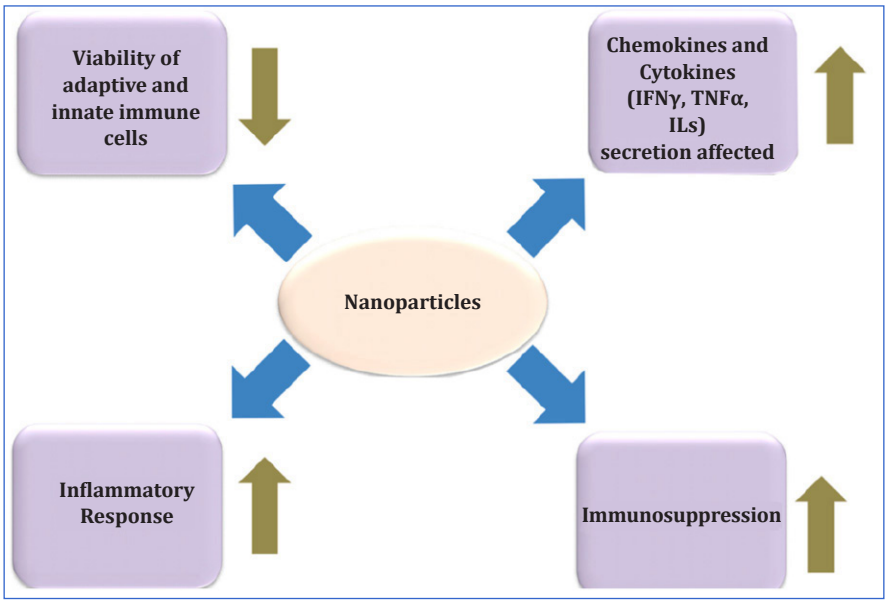

Figure 8. Toxicological effects of nanoparticles in our organism (Pandey and Prajapati, 2018).

4.7. Nanoparticles for breast cancer treatment

4.7.1. Breast cancer

Breast cancer (BC) is the most common cancer among women worldwide, with 2.3 million diagnosed cases and 685,000 deaths in 2020 (Boix-Montesinos *et al.*, 2021).

In order to understand the treatment of breast cancer using NPs, it is firstly necessary to point out the mechanism of this tumor type. There are different ways how normal breast cells can develop to be tumorigenic. On the one hand, it can be triggered by a mutation in oncogenes like PI-3-KCA (phosphatidylinositol-3-kinase) and HER-2 (epidermal growth factor 2). On the other hand, a loss of function of tumor suppression genes occasioned by mutation can occur (Boix-Montesinos *et al.*, 2021).

In order to detect breast cancer, there are several biomarkers we have to take into consideration (Boix-Montesinos *et al.*, 2021).

- Estrogen receptor (ER): expressed in 70% of invasive breast cancers.
- Progesterone receptor (PR): involved in ER signaling.
- Epidemic growth factor 2 (HER-2): which appears in 20% of BC cases and is related to poor prognosis.

In addition, HER-2 and Ki67 (proliferation marker) divide BC into 5 clinical subtypes:

TABLE 2. CLINICAL SUBTYPES OF BREAST CANCER, BIOMARKERS AND PROGNOSIS (BOIX-MONTESINOS *ET AL.*, 2021; CHOWDHURY *ET AL.*, 2021)

Clinical subtypes	Biomarkers	Prognosis
LUMINAL A	Increased ER and PR, but low HER2 and Ki67	Slow growth and good prognosis. High response to therapy.
LUMINAL B	High ER and PR. Ki67 positive and HER2 can be positive or negative.	Accelerated growth. Less favorable than luminal A.
HER2-ENRICHED	High HER2 and ER and PR absence.	More rapid and aggressive development and poor prognosis.
TNBC	Mutation in the tumor suppressor gene BRCA1. Lack of ER, PR and HER2 but expression of KI67.	Least favorable prognosis.

4.7.2. Breast cancer therapeutics

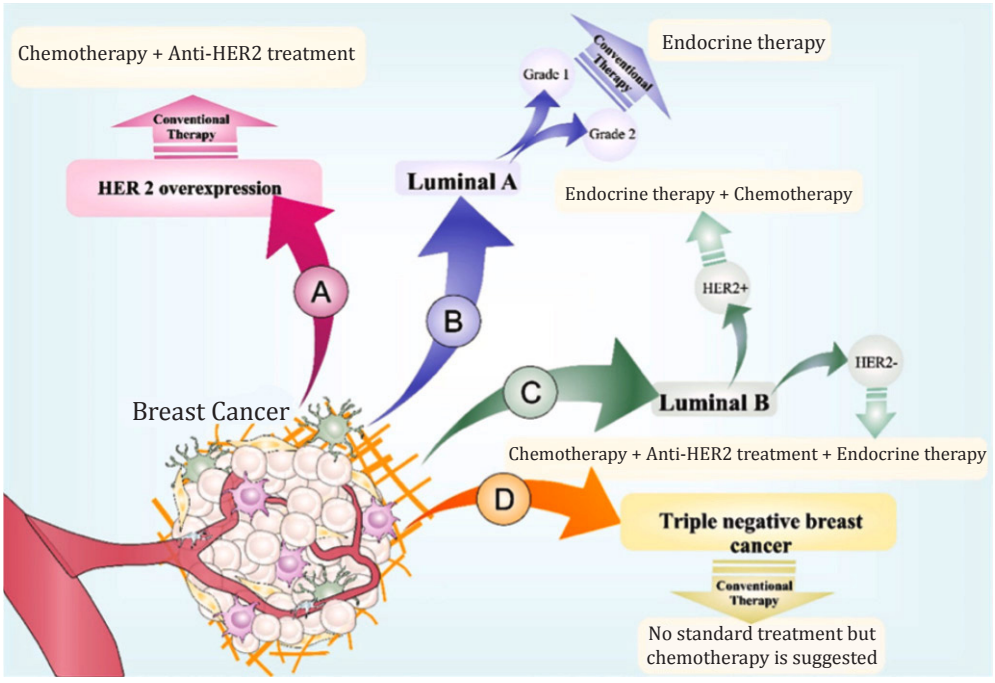

Figure 9. BC subtypes, biomarkers and traditional treatments (Hashemzadeh *et al.*, 2021).

In order to tackle breast cancer, we firstly have to identify the subtype which will determine the treatment. Breast cancers that express hormone receptors (ER,PR) will be treated with endocrine therapy (antiestrogens + aromatase inhibitors), while TNBC (HER2+) tumors will be treated with HER-2 targeted therapies or monoclonal antibodies, being some-

times concluded with chemotherapy (figure 9) (Boix-Montesinos *et al.*, 2021; Hashemzadeh *et al.*, 2021). Although these treatments have been successful in curing many people, they have certain limitations and side effects (Boix-Montesinos *et al.*, 2021). For this reason, scientists have been trying to develop a new treatment based on encapsulation of the anti-cancer drugs in NPs, for more localized treatment with less toxicity for healthy cells, greater circulation time and efficiency of the loaded medicine.

4.7.3. Nanoparticles for breast cancer therapeutics

4.7.3.1. FDA approved

DOXIL®

Doxil® was the first FDA-approved anticancer nanomedicine in 1995 for treatment of Kaposi Sarcoma and second-line treatment in metastatic breast cancer, multiple myeloma and ovarian cancer cells (Barenholz, 2012). It is a PEGylated liposomal bilayer formulation with a 80-90 nm diameter, which has 15,000 molecules of doxorubicin (anthracycline) loaded in its core. Because of its PEG coating, it has an extended circulation time of 72h and a melting point of Tm=53ºC, due to phosphatidylcholine and cholesterol molecules (Barenholz, 2012). Based on the aforesaid composition, the liposome can free the anti-cancer drug in 5 minutes, enhancing the efficiency of the treatment (Boix-Montesinos *et al.*, 2021). Furthermore, the formulation can prevent from being cleared by the RES and shows a reduced cardiotoxicity of DOX.

ABRAXANE®

Abraxane® (ABI-007) is an albumin-bound NP loaded with paclitaxel approved by the FDA in 2005 for metastatic and recurrent breast cancer (Boix-Montesinos *et al.*, 2021). It has six or seven non-covalently bonded PTX to form aggregates that result in a NP of 130nm of diameter (Chowdhury *et al.*, 2021). Due to its albumin composition it is tolerated by the immune system, reducing its toxicity, unlike conventionally in toxic cremophor solved hydrophobic paclitaxel. Furthermore, Abraxane® targets tumors due to its higher metabolic demand or active transports of the plasma proteins (albumin) for anabolic procedures (Miele *et al.*, 2009). The TME synthetizes cysteine (SPARC) or osteonectin in the acidic pH of the TME which can bind albumin. SPARC is normally overexpressed in various tumor types allowing the passive accumulation of the NP at the tumor site (Khan *et al.*, 2019). Last but not least, Abraxane® has shown in phase III clinical trials that this formulation can lead to a greater tumor accumulation (33%), superior response rate (34%) in comparison with common paclitaxel (19%), and an inhibited elimination of 4-fold times decrease (Boix-Montesinos *et al.*, 2021; "Neoadjuvant Chemotherapy With Nab-paclitaxel in Women With HER2-negative High-risk Breast Cancer - Full Text View - ClinicalTrials.gov," n.d.).

To conclude, Abraxane® has shown a high anti-tumoral response rate of cell death in combination with other treatments like radiotherapy, without increasing side effects in normal cells (Miele *et al.*, 2009).

MYOCET®

Myocet® is a 150nm liposomal doxorubicin citrate NP approved by the EMA in the year 2000. This NP is composed by phosphatidylcholine/cholesterol (55:45) and isn't PEGylated, leading to a shorter circulation time (2,5h) and fewer side effects (Boix-Montesinos *et al.*,

2021). Doxorubicin administration is limited by its cardiotoxicity at high dosage. Even though Myocet® does show a similar antitumoral activity than free doxorubicin, it triggers less side effects like cardiotoxicity and can therefore load more doxorubicin in its core (Swenson *et al.*, 2001). As shown in figure 10 this formulation achieves greater concentration of the anti-cancer drug in systemic circulation and will therefore result in an enhanced accumulation at the tumor site.

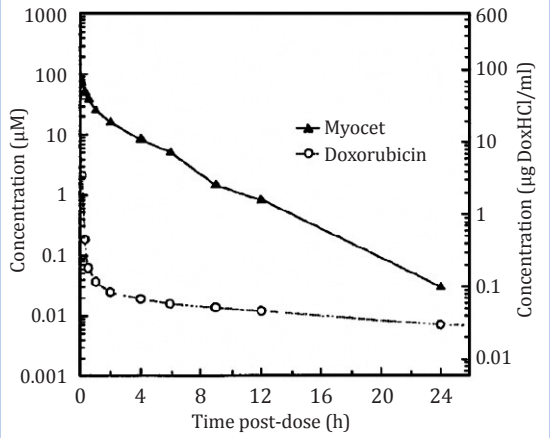

Figure 10. Concentration of doxorubicin in systemic circulation after an intravenous slow bolus injection of Myocet® and conventional doxorubicin (Swenson *et al.*, 2001).

KADCYLA®

Kadcyla® is a FDA nanomedicine approved in 2013 focused on the treatment of HER2 positive metastatic breast cancer previously treated with trastuzumab and taxanes (Boix-Montesinos *et al.*, 2021). It encapsulates the chemotherapeutic agent DM1 (emtansine) covalently bound to trastuzumab monoclonal Ab with a stable thioether linker. This combination is well tolerated and significantly reduces the risk of invasive breast cancer relapse compared to trastuzumab. It has recently been approved by the EMA as an adjuvant treatment for HER2 breast cancer. However, it has 0.5% of probability to develop lung toxicity (Glassman *et al.*, n.d.).

GENEXOL-PM®

Genexol®, developed by Samyang Biopharmaceuticals Corporation, is a 20-50 nm NP approved in 2007 in South Korea and Europe for breast, lung and ovarian cancer (Boix-Montesinos *et al.*, 2021). This NP consists of a PEG-b-poly(D,L-lactide) deblock polymer that encapsulates paclitaxel (PTX) in polymeric micelles (Chowdhury *et al.*, 2021). It has a high loading capacity of 16.7% (Chowdhury *et al.*, 2021), prolonged circulation time (1.8 fold) and an improved response rate with few secondary effects (Boix-Montesinos *et al.*, 2021). Its amphiphilic PEG reduces the activation of the RES, while PLGA reduces the multi-drug resistance.

4.7.3.2. Emerging nanoparticles

TABLE 3. EMERGING NANOPARTICLES FOR BREAST CANCER TREATMENT (BOIX-MONTESINOS *ET AL.*, 2021 AND CHOWDHURY *ET AL.*, 2021)

Product	Nanoplatform/ agent	Features	Status	Reference
EndoTAG-1 **(MediGene/ SynCore Biotechnology)**	Paclitaxel integrated in cationic liposomal membranes with a positive zeta potential (25-100mV) in physiological ph.	For TNBC HER2 (-) breast cancer. Benefit of 53% in combined therapy, compared to 31% and 36% on EndoTAG-1 and paclitaxel monotherapy. This NPs are around 200 nm and has a higher uptake to tumor cells because of its cationic membrane. It also shows increased side (3/4) effects (neutropenia) in combination.	Phase II	(Ignatiadis *et al.*, 2016a, 2016b; Mirza and Karim, 2021)
Lipoplatin ® (Regulon)	Liposomal cisplatin	For HER-2(-)metastatic BC. Similar efficacy to cisplatin with significant toxicity reduction (nephrotoxicity).	Phase III	("Photo-induction as a Means to Improve Cisplatin Delivery ClinicalTrials.gov," n.d.; Stathopoulos and Boulikas, 2012)
NKTR-102 **(Nektar Therapeutics)**	PEGylated liposome loaded with irinotecan.	For advanced breast cancer and solid tumors. Increased penetration to the TME and active targeting. Side effects can be severe diarrhea and suppression of the immune system.	Phase III	(Pillai, 2014; "Breast Cancer Outcomes With NKTR-102, ClinicalTrials.gov," n.d.)
LEP-ETU® **(INSYS Therapeutics, Neopharma)**	Liposomal Paclitaxel	A 150 nm NP for treatment of advanced breast cancer. Highly stable: less than 6% of paclitaxel was released after 120 h at physiologic temperature. It has a higher drug loading efficiency and reduced toxicity compared to Taxol® (PHASE I trial).	Phase II	("Efficacy and Safety Study of LEP-ETU, ClinicalTrials.gov," n.d.; Zhang *et al.*, 2005)
NK-012 **(Nippon Kayaku)**	PEG-polyglutamic acid/SN-38	For treatment of triple negative metastatic BC. No results published yet.	Phase II	("A Study of NK012, ClinicalTrials.gov," n.d.)
Xyotax® (CT-2103) (Dana-Farber Cancer Institute)	Paclitaxel poliglumex	In metastatic breast cancer. Increases therapeutic index of paclitaxel by passive accumulation via EPR effect. Enhanced safety and efficacy relative to paclitaxel.	Phase II	("Study of Xyotax (CT-2103), ClinicalTrials.gov," n.d.; Singer, 2005)

ThermoDox® **(Celsion)**	Heat-activated liposomal doxorubicin	Used in combination with hyperthermia or radiofrequency thermal ablation (RFA). Provides the release of 25 times more doxorubicin to the tumor compared to doxorubicin on its own.	Phase I / II	(Dou *et al.*, 2017; "Phase 1/2 Study of ThermoDox, ClinicalTrials.gov," n.d.)
Liposomal annamycin **(New York University, School of Medicine)**	Liposome semi-synthetic doxorubicin, analogue to annamycin	In locally advanced or metastatic breast cancers not responsive to chemotherapy. In Phase II studies of annamycin did not detect therapeutic effects in breast cancer, but showed antileukemic activity.	Phase I/ II	(Booser *et al.*, 2002; "Chemotherapy in Treating Patients With Breast Cancer ClinicalTrials.gov"; Wetzler *et al.*, 2013)
Rexin-G	Rexin-G is a replication-incompetent, pathotropic (disease-seeking),tumor matrix (collagen)-targeted retrovector encoding an N-terminal deletion mutant of the cyclin G1, (Erlinda M. Gordon quote NCI Thesaurus, C49082).	For recurrent and metastatic breast cancer. Rexin-G is able to bind the tumors extracellular matrix, specifically binding collagen. This nanoparticle carries a cytocidal cyclin G1 genetic payload inside the vector which is able to retard or eliminate tumor cell growth (63).	Phase I/ II	(Gordon and Hall, 2010; "Safety and Efficacy Study Using Rexin-G, ClinicalTrials.gov")
SPI-077 **(LiPlasome Pharma)**	Liposomal cisplatin	SPI-077 improves the therapeutic index and selectiveness of cisplatin in metastatic breast cancers.	Phase I/ II	("Safety and Tolerability of LiPlaCis, ClinicalTrials.gov," n.d.)
S-CKD602 **(ALZA)**	PEGylated liposomal/ CKD602, semisynthetic camptothecin analogue	In patients with refractory solid tumors. This NP has a prolongued plasma exposure and superior tumor delivery (68).	Phase I	("S-CKD602, ClinicalTrials.gov," n.d.; Zamboni *et al.*, 2009)
Nanoxel® **(Fresenius Kabi Oncology)**	PEG-poly(D,L-lactide)/doxataxel	Managed for metastatic breast cancer, triple negative breast cancer, in India. It is a pH-sensitive micelle which releases the anticancer drug at the TME.	Phase I	("Effects of Nanoparticle Based Paclitaxel Drug,ClinicalTrials.gov," n.d.)

5. Sex and gender

To introduce this section, it is firstly important to mention that sex and gender are not equivalent and do not represent the same concepts. Searching for scientific articles about gender I noticed that most of them referred to this term as feminine or masculine sex and not as what "gender" really stands for. "Sex" is "associated with physical and physiological features like chromosomes, gene expression, hormone levels and function anatomy", resulting in categorization as female or male, while "gender" is not binary (men/women) and englobes how people perceive themselves and each other in our society (Canadian Insti-

tute of Health Research [CIHR], 2020). Furthermore, "gender" takes into consideration socially constructed roles, behaviors among others and can change with time (MedicalNewsToday, 2021).

In the field of nanotechnology for cancer treatment it is sex that influences some biological parameters such as distribution, toxicology and internalization and therefore needs to be studied, not gender.

6. Final remarks and conclusions

Even though NPs for the treatment of cancer have been under investigation for more than fifty years making promising progress in preclinical phases, their clinical translation remains slow. Meta-analytic studies showed that only a 0.7% of the anti-cancer drug administered intravenously, accumulates at the tumor site (Lammers and Ferrari, 2020; Wilhelm *et al.*, 2016. This is partially due to the poor reproducibility of their manufacture, the insufficient availability of characterization methods, the instability of in vivo models and the lack of understanding of the biophysical and chemical interactions of NPs with the heterogeneous tumor microenvironment (Agrahari, 2018).

Understanding how NPs act according to their physicochemical characteristics at the tumor level and how their pharmacokinetics vary according to these characteristics, helps to predict their behavior and therapeutic efficacy. In addition, detailed knowledge of the tumor microenvironments features, along with the EPR effect, the immunological status of the patient and the site of tumor implantation, is essential for the effective design of nanomedicines (Boix-Montesinos *et al.*, 2021).

Currently, several treatments based on NPs, such as Kadcyla®, Genexol-PM®, Doxil®, Lipodox®, Myocet®, Lipusu®, Abraxane® and Nanoxel® have been approved by the FDA for the treatment of breast cancer, showing reduced toxicity and increased efficacy. Furthermore, in the last decade more than a dozen nanomedicines have been approved and marketed which is a great success in this relatively young field (Lammers and Ferrari, 2020). Likewise, many others are advancing in preclinical phases reaching NPs.

The optical, electrical and mechanical properties along with the ability to mold the shape, surface and size of NPs represent a promising hope in the field of science for the selective treatment of breast cancer among other severe diseases (Lammers and Ferrari, 2020).

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