Lipid nanoparticles for cancer therapy: state of the art and future prospects

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Introduction: Cancer is a leading cause of death worldwide and it is estimated that deaths from this disease will rise to over 11 million in 2030. Most cases of cancer can be cured with surgery, radiotherapy or chemotherapy if they are detected at an early stage. However, current cancer therapies are commonly associated with undesirable side effects, as most chemotherapy treatments are cytotoxic and present poor tumor targeting.

Areas covered: Lipid nanoparticles (LN) are one of the most promising options in this field. LN are made up of biodegradable generally recognized as safe (GRAS) lipids, their formulation includes different techniques, and most are easily scalable to industrial manufacture. LN overcome the limitations imposed by the need for intravenous administration, as they are mainly absorbed via the lymphatic system when they are administered orally, which improves drug bioavailability. Furthermore, depending on their composition, LN present the ability to cross the blood–brain barrier, thus opening up the possibility of targeting brain tumors.

Expert opinion: The drawbacks of chemotherapeutic agents make it necessary to invest in research to find safer and more effective therapies. Nanotechnology has opened the door to new therapeutic options through the design of formulations that include a wide range of materials and formulations at the nanometer range, which improve drug efficacy through direct or indirect tumor targeting, increased bioavailability and diminished toxicity.

Keywords: anticancer therapy, lipid nanoparticles, nanomedicine, nanotechnology, tumor targeting

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1. Introduction

According to the World Health Organization [1], cancer is one of the leading causes of death worldwide, and cancer deaths are projected to continue rising to over 13.1 million in 2030. The main types of cancer are: lung, stomach, liver, colorectal, breast and cervical cancer; nevertheless, it can affect any part of the body and people of any age. Early detection of this disease through screening prevents the cancer from spreading to other parts of the body (metastasization) and thus improves survival rates. Cancer treatment frequently comprises a combination of surgery, radiotherapy and chemotherapy. Cure rates of surgically removable primary tumors that have not spread to other parts of the body are high (e.g., breast, colorectal). However, even when complete resection of the tumor is possible, chemotherapy is generally required.

Chemotherapy has been used for more than 70 years, since mustard gas was used for the first time in the treatment of lymphomas [2], but it still presents severe side effects and limited efficacy. Most chemotherapeutic drugs act through interaction with DNA

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Article highlights.

- Lipid nanoparticles (LN) have been widely studied since their discovery in the 80s. The broad spectrum of fabrication methods and targeting strategies have improved nanoparticles efficacy in tumor treatment.
- Their composition based on generally recognized as safe (GRAS) lipids for oral administration, guarantees less potential toxicity than other nanovehicles and their scaling-up is currently feasible.
- LN reduce drug toxicity and enhance antitumor activity mainly due to: i) passive and active targeting and ii) multi-drug resistance (MDR) overcoming (P-glycoprotein (P-gp) inhibition).
- Current investigations suggest two relevant advantages of LN in cancer treatment: i) the oral administration and further absorption through the lymphatic system and ii) BBB penetration due to certain formulation components.

This box summarizes key points contained in the article.

that causes irreparable damage or by impeding cell division, which finally leads to cell apoptosis. Chemotherapeutic drugs are generally classified as: alkylating agents (platinums, nitrogen mustard derivates, oxazophosphorines), antimetabolites (pyrimidine analogs, antifolates), mitotic inhibitors (vinka alkaloids, taxanes), topoisomerase inhibitors (topoisomerase-I inhibitors, topoisomerase-II inhibitors) and antitumor antibiotics (anthracyclines, bleomycin, mitoxantrone (MTO)). Even if these drugs present efficacy against the disease, multi-drug resistance (MDR) to chemotherapeutic agents complicates cancer treatment. This mechanism is mainly related to P-glycoprotein (P-gp), which extrudes the drug from the cell, decreasing the intracellular drug concentration and thus inhibiting its antitumor action. The group of alkyl lysophospholipids, which are non-DNA affecting molecules, comprises another class of antitumor agents. Edelfosine, the prototype of these new drugs discovered in the late 1980s, presents several advantages over conventional antitumor drugs. It is a drug that can be administered orally, it acts selectively in tumor cells sparing healthy tissues and its mechanism of action is not based on DNA targeting but membrane triggered apoptosis [3]. Chemotherapy is mainly administered intravenously, a route which is generally associated with poor patient well-being and compliance, and high clinical cost [4]. Moreover, it is also associated with a wide variety of severe side effects (mainly due to the poor targeting of cancer cells) such as myelosuppression, gastrointestinal toxicity, alopecia, neuropathy, infertility or cardiac ischemia, among others. Bearing in mind all the drawbacks of chemotherapy, researchers are still investigating into new drugs and new delivery systems to obtain safer and more effective therapies that allow oral administration.

Among drug delivery systems, lipid nanoparticles (LN) are promising drug carriers due to their effectiveness in targeting tumor tissue. They provide higher drug efficacy, as a result of an increased concentration of drug in tumor cells, and lower side effects [5]. LN can be divided into solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid–drug conjugates (LDC). In general, they can be defined as nanometer-sized solid particles made up of biodegradable generally recognized as safe (GRAS) lipids. Besides the above-mentioned advantages, LN can be administered orally, avoiding all the disadvantages of the intravenous route. Like other nanosystems, LN are passively targeted to the tumor tissue due to the well-known enhanced permeability and retention effect (EPR effect) [6,7]. Moreover, when given orally, they are absorbed via the lymphatic system avoiding first-pass hepatic metabolism and targeting lymph nodes [8,9]. Depending on their composition, they also have the ability to cross the blood–brain barrier (BBB), thus opening up the possibility of targeting brain tumors [10]. Furthermore, active targeting offers the possibility of directing the drug toward different tissues. This review focuses on the most recent advances in the use of LN in the treatment of cancer. Specifically, studies published in the last 5 years will be reviewed and discussed.

2. Lipid-based nanosystems

The LN concept begins with lipid nanosuspensions. Oil-in-water (O/W) emulsions were first used in clinic in the 1950s to administer parenteral nutrition. Afterward, Etomidat-Lipuro® and Diazepam-Lipuro® were successfully marketed [11]. At this time, the only purpose of these emulsions was to reduce the side effect of pain after diazepam injection. Despite the success of the O/W emulsions, the number of products on the market is low due to their physical instability and low drug solubility.

Lipid-based nanosystems were first launched on the market in 1986 by the Dior brand [12]. The Dior commercial formula was followed by the first pharmaceutical liposome formulations. Epi-Pevaryl® (antimycotic topical therapy) was introduced in the market in the 1980s, and Alveofact® (pulmonary instillation) and Ambisome® (intravenous injection) in the following decade. One of the major disadvantages of liposomes is their rapid plasma clearance. Consequently, pegylated liposomes (stealth liposomes) were developed by Allen in 1994 [13] as a solution to the short half-life of liposomes in plasma as a result of the reticuloendothelial system (RES) clearance. However, the number of commercialized liposomal formulations is low due to disadvantages such as physical instability, insufficient drug solubility and the need for expensive technology. Besides, regardless of the potentiality of these formulations in reducing drug side effects, their poor controlled release posed a challenging drawback.

In this sense, LN, invented in the 1980s, represent significant progress in the development of lipid-based nanosystems.

2.1 Types of lipid-based nanosystems

2.1.1 Solid lipid nanoparticles

SLN were discovered by Speiser [14] and Eldem *et al.* [15]. in the 1980s when they formulated SLN by spray drying and

nanopellets for peroral administration for the first time. SLN are colloidal carriers composed of lipids that are solid at body temperature. The use of solid lipids prevents the drug from immediate release. The drug is included in a solid matrix that makes the diffusion of the drug to the surface difficult. In addition, the lipids used to form SLN provide low acute and chronic toxicity [5]. In the 1990s, SLN were further developed by Müller *et al*. using high-pressure homogenization (HPH) methods [16,17] and by Gasco, who used a warm microemulsion technique [18,19]. The most important advantages of SLN over liposomes are controlled drug release and the physical stability of the preparations. Nevertheless, they still present some limitations such as limited drug loading and drug expulsion during storage. Anticancer drugs have been encapsulated into SLN by many different authors [5]. Most of these studies have developed SLN to be administered intravenously, with successful results; nevertheless, SLN can also be a very promising oral drug delivery system. Several studies have demonstrated that these nanocarriers are absorbed via the lymphatic system, improving drug bioavailability [8,9,20]. Consequently, the oral administration of antitumor agents might have a large impact on clinical practice both in patient well-being and in treatment costs [4].

2.1.2 *Nanostructured lipid carriers*

NLC are the second generation of LN. They were developed by Müller *et al*. to solve the low drug loading capacity of SLN [21]. The difference between the two formulations is their lipid composition: in NLC the solid lipid is mixed with a liquid lipid in order to obtain a solid structure and to avoid crystallization after particle solidification. The applications of NLC are the same as Müller *et al*. described for SLN [17]. Several recent studies endorse the efficacy of NLC in cancer treatment [22-26].

2.1.3 *Lipid–drug conjugates*

Although SLN and NLC are able to incorporate hydrophilic drugs, their lipophilic nature makes them more suitable to incorporate lipophilic compounds. LDC were developed in the late 1990s in order to achieve better drug loading rates for hydrophilic drugs [27]. Their manufacture consists of binding the drug to the lipid prior to forming the O/W emulsion. The drug is first conjugated with the lipid by salt formation or by covalent linkage, and afterward, LDC are formed by homogenizing the drug–lipid complex with a surfactant aqueous solution by HPH.

2.2. Preparation methods

To date, different methods have been developed to produce LN. Most of them are based on traditional emulsion techniques. The two principal methods used are the HPH, patented by Müller and Lucks in 1993 [17], and microemulsion techniques patented by Gasco in 1993 [19]. However, several variations of these methods have been proposed in order to optimize the characteristics of LN formulations. **Table 1** brings together all these methods and variations along with the drugs used in cancer therapy that have been successfully loaded in these systems. Research efforts have been focused on the improvement of particle stability, surfactants at considerable concentration, particle size control according to the administration routes, functionalization of the particle surface for targeting a specific cell, drug controlled release, minimal mechanical and thermal energy input, risk of organic solvent residues, cost-effective process and industrial scalability, among others. The main advantages and drawbacks of all the production techniques are summarized in **Table 2**.

3. Scaling-up of LN

After the development and optimization of a formulation on a small scale, the next step is usually to find the way to produce it on a larger scale. However, in most cases, the scaling-up of a process implies an increase in problems [28]. In the case of LN production based on HPH, which is the most widely used method in the pharmaceutical industry, it has been observed that the use of large-scale machines leads to an even better quality of the product with regard to a smaller mean particle size and polydispersity index (PDI) [29].

The most typical devices for lab-scale production are the Avestin C5 (capacity: 5 l/h, batch: 7 ml to 1 l, Avestin Europe GmbH, Mannheim, Germany) and the Micron LAB 40 (batch: 20 – 40 ml, APV Deutschland GmbH, Unna, Germany). In the case of very high-cost drugs, or if there is a limited amount available (e.g., new chemical entities), it is positive to reduce the batch size. Avestin B3 (Avestin Europe GmbH) can be employed in order to reduce the batch size, achieving a final volume of 0.5 – 3.2 ml [30].

The next scaling-up step implies a minimum batch size of 2 kg and a maximum of 10 kg. This aim can be achieved using the Micron LAB 60 (APV Deutschland GmbH), which has a homogenization capacity of 60 l/h. The next step in scale-up is the use of a Gaulin 5.5 (APV Deutschland GmbH) with a homogenization capacity of 150 l/h (nearly 150 kg) [31]. In this case, the pre-emulsion is formed in larger containers. The product containers and homogenizer are manufactured from pharmaceutical grade materials. Another feature is that the product containers can be sterilized by autoclaving; formation of the pre-emulsion under protective gas is also feasible. It is noteworthy that a batch size of about 500 kg can be produced in approximately 3-h homogenization time using this machine.

For even larger scales, a Rannie 118 (APV Deutschland GmbH) or an Avestin EmulsiFlex C1000 (Avestin Europe GmbH) can be used [30,31]. Their capacity is much higher than that of the previous machines, ranging from 1000 to 2000 l/h at the low pressure required for the production of LN.

4. Physical–chemical characterization of LN

Physical–chemical characterization of the LN is essential due to the fact that these systems present colloidal-sized

Table 1. Examples of drugs incorporated in LN and preparation methods.

Preparation method	Drugs loaded	Ref.
Hot HPH	Palmityl prodrug analog of capecitabine, all-trans retinoic acid, chlorambucil, docetaxel, coenzyme Q10	[93,126-129]
Cold HPH	VB	[84]
Microemulsion technique	Trans-resveratrol, gold (III) porphyrin and camptothecin	[130,131]
Microemulsion precursor technique	Idarubicin and DOX, paclitaxel, gadolinium	[108,132,133]
Coacervation method	Cisplatin, curcumin, methotrexate	[134-136]
PIT method	Etoposide, tripentone 3-(3-hydroxy-4-methoxyphenyl)-8H-thieno[2,3-*b*]pyrrolizin-8-one, paclitaxel	[137-139]
Emulsion formation solvent-evaporation or -diffusion method	Methotrexate, edelfosine, DOX, paclitaxel and siRNA	[8,36,53,140]
w/o/w double emulsion method	Edelfosine, 5-FU	[36,87]
Emulsification dispersion followed by ultrasonication	MTO, cisplatin	[90,141]
Hot homogenization by high shear homogenization and/or ultrasonication	Edelfosine, simvastatin and tocotrienol, all-trans retinoic acid, paclitaxel, γ-tocotrienol	[10,142-145]
Solvent injection method	Paclitaxel, 5-FU	[146,147]

DOX: Doxorubicin; 5-FU: 5-fluorouracil; HPH: High-pressure homogenization; LN: Lipid nanoparticles; MTO: Mitoxantrone; PIT: Phase inversion temperature; VB: Vinorelbine bitartrate; siRNA: Small interfering RNA; w/o/w: Water-in-oil-in-water.

Table 2. Advantages and disadvantages of LN preparation methods.

Preparation method	Advantages	Disadvantages
HPH (hot and cold)	Good reproducibility, well-established homogenization technology on large scale, organic solvent-free method	High temperature process, high energy input, complex equipment required, possible degradation of the components caused by HPH
Microemulsion technique	Reduces mean particle size and narrow size distribution, organic solvent-free method, non-energy-consuming method, easy to scale–up	High concentration of surfactants and co-surfactants, concentration of final formulation is required
Microemulsion precursor technique	Rapid, reproducible and cost-effective method, dilution of the final formulation is not needed, organic solvent-free method, non-energy-consuming method	High concentration of surfactants and co-surfactants
Coacervation method	Allows incorporation of thermosensitive drugs, inexpensive for laboratory and industrial application, possibility to control shape and size of SLN by reaction conditions	Possible degradation of the components under acidic conditions
PIT method	Organic solvent-free method, non-energy-consuming method, easy to scale-up	Not suitable for thermosensitive molecules like peptides or proteins
Emulsion formation solvent-evaporation or -diffusion method	Allows incorporation of thermosensitive drugs, reduces mean particle size and narrow size distribution, good reproducibility	Concentration of final formulation is required, possible organic solvent residues in the final formulation
w/o/w double emulsion method	Allows incorporation of hydrophilic drugs	Concentration of final formulation is required, large particle size of the final formulation
Emulsification dispersion followed by ultrasonication	Allows incorporation of thermosensitive drugs	Possible metal contamination
Hot homogenization by high shear homogenization and/or ultrasonication	Easy to handle, no complex equipment is required, high concentration of surfactants and co-surfactants are not required, organic solvent-free method	High energy input, polydisperse distributions, possible metal contamination, concentration of final formulation is required
Solvent injection method	Easy to handle and fast production process	Possible organic solvent residues in the final formulation

HPH: High-pressure homogenization; LN: Lipid nanoparticles; PIT: Phase inversion temperature; w/o/w: Water-in-oil-in-water.

particles [32]. Nevertheless, proper characterization of the formulations is necessary to control the product quality, stability and release kinetics. The most important parameters of LN to be characterized include particle size and shape, the surface charge, the degree of crystallization and the kind of lipid modification. All these properties must be well characterized because any contact of the LN dispersion with new surfaces might be able to induce changes in their structure, causing, for example, an alteration in the lipid crystallization or modification leading to the formation of a gel, or to the drug expulsion. Among all the parameters that should be considered for characterization of LN, size is crucial and critical for determining the interactions of nanoparticles with living systems. For instance, particle sizes below 300 nm are suitable for intestinal transport to the thoracic duct [33], while sizes no larger than 5 μm are required in order not to cause embolisms after parenteral administration of LN due to the blocking of the thin capillaries [34]. Besides, particle size also plays a very important role in the clearance of the LN by the RES. A great number of methods are available for determining the size of nanoparticles [35]; however, dynamic light scattering (DLS) is generally used to determine the size distribution profile of LN. Alternatively, electron microscopy and/or atomic force microscopy (AFM) are often used to corroborate the results.

The zeta potential is the overall charge a particle gains in a specific medium, and its value indicates the degree of repulsion between close and similarly charged particles in dispersion. Most authors calculate this value by laser-Doppler anemometry [36-40]. Colloids with high zeta potential (negative or positive) are electrically stabilized, while colloids with low zeta potentials tend to coagulate or flocculate. In general, absolute values greater than 30 mV have been found to be enough for good stabilization, and hence indicate good physical stability [41]. In terms of stability, any contact of the LN dispersion with new surfaces might be able to induce changes in their structure, causing, for example, an alteration in the lipid crystallization or modification leading to the formation of a gel or to the drug expulsion [42]. Therefore, the crystallinity and polymorphic behavior of the components of the LN should be studied, as these both influence drug incorporation and release rates to a high degree. Differential scanning calorimetry (DSC) and X-ray diffractometry (XRD) are two of the main tools employed. Bunjes *et al.* [43-46]. reported on crystalline properties of lipids and their recrystallization patterns during nanoparticle preparation and the influence of nanoparticle size on recrystallization pattern in a very extensive way.

It is imperative to obtain a dry product to ensure their stability, thus allowing their long-term storage. Lyophilization is one of the most widely used techniques for obtaining dry powders from nanoparticulate suspensions [47-51] and provides an increase in chemical and physical stability over extended periods of time [34]. In general, cryoprotectant agents are used so as not to achieve a final LN aggregated product, which will commonly acquire a rubbery appearance. Saccharides are the most widely employed cryoprotectant agents in the formulation of LN, namely trehalose, sucrose, sorbitol, maltose, glucose and mannose [52-54].

5. Drug release from LN

The solubility of the drug in both the aqueous release medium and the lipid component of the formulation, and the partitioning between them, are considered very important factors in predicting the *in vitro* drug release behavior. It is known that increasing the production temperature and surfactant concentration leads to increased drug solubility in the water phase [55]. Cooling the LN suspension again will decrease the water solubility and the repartition to the lipid, forming drug core-enriched or drug shell-enriched LN, depending on the lipid recrystallization temperature [56]. These two drug distribution models lead to too slow and too fast release rates of the drugs, respectively.

In order to study the drug release kinetic profile of drugs from LN, various assays can be performed. The most widely employed assays are based on the use of dialysis membranes, Franz-type diffusion cells and rotating vials.

5.1 Dialysis membranes

Among all assays, dialysis tubes are the most widely used to study the drug release kinetics from LN formulations [53,57-59]. Briefly, a definite amount of prepared LN, free from any unentrapped drug, is separately placed in the dialysis tube of different molecular weight cut-off (MWCO) (usually between 12 and 14 kDa), tied at both ends and suspended in different beakers (receptor compartment) each containing the appropriate medium to study the release (namely phosphate buffered saline (PBS), gastric or intestinal media). The medium is stirred continuously to favor the crossing of the membrane, and the whole system is usually assembled at physiologic temperature throughout the experiment (**Figure 1**). Samples are withdrawn periodically and after each withdrawal of sample the same volume of appropriate medium is added in the receptor compartment so as to maintain a constant volume throughout the study.

5.2 Franz-type diffusion cell

This assay is relatively similar to the method based on dialysis membranes, with the difference of the use of a specific system [52,60]. A Franz diffusion cell system is composed of a receptor and a donor cell (**Figure 2**). This cell has a static receptor solution reservoir with a side-arm sampling port. The membrane (usually of a MWCO of 12 kDa) is mounted between the cell compartments. The receptor compartment is filled with the appropriate medium to study the release (namely, PBS, gastric or intestinal media). It is kept at physiological temperature by circulating water through an external water jacket. After a certain time of equilibration of the membrane with the receptor solution, a definite amount of

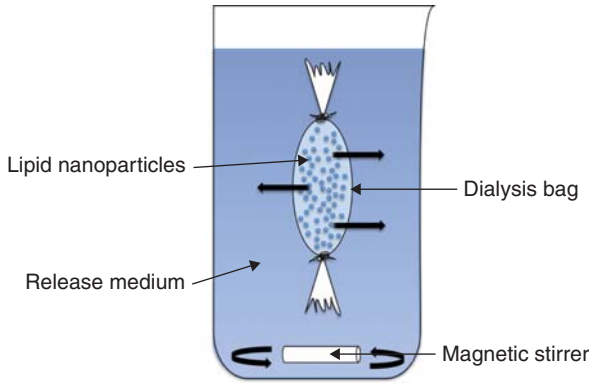

Figure 1. Representative scheme of the determination of drug release by dialysis membrane bag.

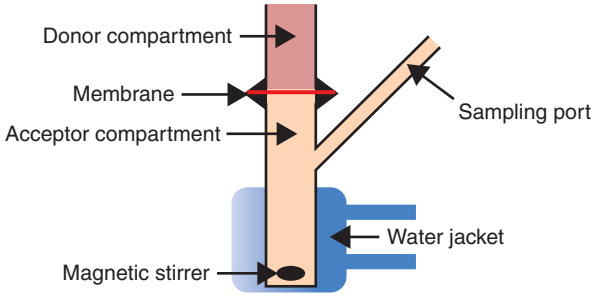

Figure 2. Schematic representation of a Franz diffusion cell system.

the LN formulation is applied in the donor compartment. The donor compartment can then be covered to prevent evaporation of the solvent. The receptor solution is continuously stirred by means of a spinning bar magnet. Receptor solution samples are withdrawn through the sampling port of the receptor compartment at various time intervals and the cells are refilled with receptor solution to keep the volume of receptor solution constant during the experiment.

5.3 Rotating vials

In this method, an amount of LN is placed in small vials containing the appropriate medium to study the release (PBS, gastric or intestinal media). The vials undergo continuous rotational mixing using a rotating device kept at physiologic temperature throughout the experiment (**Figure 3**). At the time of sample withdrawal, vials are centrifuged and the supernatant is recovered for analysis [36].

6. Application in cancer therapy

6.1 Surface-modified lipid-based nanosystems

Antitumor drugs imply many remarkable side effects as a result of their impaired toxicity. The poor selectivity of these compounds makes them accumulate in healthy tissues causing severe damage. This unspecific drug accumulation also decreases their effectiveness [2]. Nanotechnology has overcome this problem thanks to passive and active targeting of the tumor. Lipid-based nanocarriers are not only able to accumulate in tumor tissues passively, but these systems can also be actively targeted at tumors by attaching different molecules to their surface (**Figure 4**).

6.1.1 *Passive targeting*

The EPR effect is the principal mechanism of tumor accumulation of nanocarriers [7]. Tumor tissues grow very quickly, promoting special tissue architecture and the development of blood vessels with wide fenestrations between endothelial cells. These particular vessels permit an easier exchange of nutrients and oxygen to support the high demand of this abnormal growth. These wider spaces facilitate the extravasation and accumulation of nanoparticulated systems from the blood vessels into the tumor tissues. Therefore, lipid-based nanosystems are targeted at tumor tissues in a passive way, which is based on the shape and size and is independent of the surface nature.

In contrast to other nanocarriers, LN offer another possibility in passive targeting when they are administered orally. After oral administration, LN are absorbed via the lymphatic system and the drug is passively targeted at the lymph nodes [20,61]. This might represent a promising strategy in the treatment of general cancer metastases and in lymph-generated tumors (lymphomas) [9].

Another passive targeting approach is the use of certain tensoactive excipients that enable the lipid nanocarriers to penetrate into the central nervous system (CNS). Several studies suggest that LN including tensoactive excipients such as polysorbate 80 (Tween 80) or polyoxyethylene 20-stearyl ether (Brij 78) may overcome the BBB, allowing the drug to penetrate into the CNS [10,62]. Taking into consideration the difficulties of anticancer drugs in crossing the BBB, LN present high potential as therapeutic tools against brain tumors.

6.1.2 *Active targeting*

Passive targeting is mainly used in nanotechnology to target nanocarriers at the tumor; nevertheless, many authors have developed active targeted LN. In this section, the authors will discuss the main strategies developed to target LN to cancer cells. Efficacy studies will be described and documented in Section 6.2. Active targeting consists of attaching molecules to the surface of the nanoparticle. The main strategy in active targeting consists of using ligands that specifically bind to molecules that are selective or overexpressed in tumor cells. However, other approaches like hepatic cell targeting [63] and magnetic targeting [64] are also common.

Among all the molecules used for specific cancer cell targeting, transferrin (Tf) attachment is a widely used strategy [65-67]. Tf receptor is the ubiquitous cell surface glycoprotein related to cell proliferation and is overexpressed in malignant tissues because of the higher iron demand of malignant

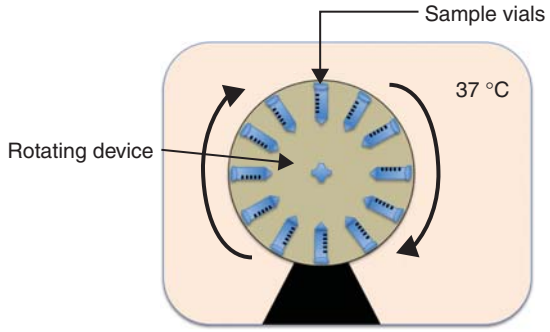

Figure 3. Diagram of a rotating device that can be used to determine drug release from LN formulations by rotating vials.

cells for fast growth and division [65]. Tf binds to its specific receptor on the cell surface and is internalized into the cell by endocytosis.

The attachment of ferritin to the nanoparticle surface is another approach related to the increased iron requirements of cancer cells. Ferritin is an intracellular protein complex, which is intended to store iron in the cell in a non-toxic form. Jain *et al.* [68] developed ferritin-mediated LN-containing 5-fluorouracil (5-FU) to assess their targetability to breast cancer cells.

Mannose has been also used as a ligand in active targeting of lipid nanocarriers [69]. Cancer cells tend to overexpress lectin-like receptors with high affinity for polysaccharide molecules on their surface. This occurs as a result of the increased requirement for carbohydrate molecules by tumor tissues. Mannosylated LN-containing doxorubicin (DOX) showed enhanced *in vitro* and *in vivo* efficacy compared with non-targeted LN or free drug [58].

Taking advantage of the cancer cell augmented metabolism, another targeted strategy developed is the use of hyaluronan (HA) of different molecular weights [70,71]. HA is a linear glycosaminoglycan with many biological functions that make it essential in tumor development. HA can be covalently attached to the surface of LN to target epithelial cancer cells and leukocytes overexpressing HA receptors (CD44 and CD168). Mizrahy *et al.* [70]. demonstrated that low molecular weight HA (LMW-HA) might be used as a secure substitute for polyethylene glycol (PEG), if macrophage or complement activation must be avoided. Previous studies demonstrated that LMW-HA but not high molecular weight HA (HMW-HA) induced inflammatory response [72]; however, Mizrahy *et al.* showed that macrophage activation avoidance was HA molecular weight independent. This could be explained by the low quantity of HA attached to the LN surface compared with preceding studies. Besides, HMW-HA may be used to efficiently target CD44 overexpressing tumors due to the strong binding of HMW-HA to the receptor.

Folate-mediated LN has also been developed to achieve active targeting [73,74]. Folate receptor has been identified as a useful tumor marker because it is overexpressed in cancer cells. Folate is essential in eukaryotic cells for the biosynthesis of nucleotide bases and, as in the previous cases, its requirement is increased in cancer cells by reason of its accelerated metabolism.

The $\alpha_v\beta_3$ integrins are another target in nanocarrier design. These receptors are overexpressed in angiogenic vessels and in some cancer cells. In a study carried out by Goutayer *et al.* [75]., NLC containing a fluorochrome were functionalized with cyclic triad peptide sequence RGD (Arg-Gly-Asp) in order to target $\alpha_v\beta_3$ integrins. Functionalized LN were shown to have a long half-life in plasma and were distributed widely except for the CNS. Fluorochrome signal was higher in tumor tissues overexpressing target receptors, indicating a targeted distribution of LN.

Apart from this selective targeting of cancer cells, there is another strategy that consists of targeting a specific tissue such as liver or brain. In this sense, selective targeting to hepatic cells is another common approach in nanomedicine. Asialoglycoprotein (ASGP) receptor is commonly used as a therapeutic target in hepatic disease [63]. In contrast to the previous approaches, attaching a hepatic ligand implies targeting of all hepatic cells including healthy tissue. Nevertheless, the EPR effect may help to overcome this drawback, by promoting uptake of nanocarriers by tumor.

CNS has also been targeted through the binding of ligands to the LN surface. Cationic bovine serum albumin (CBSA) promotes transport across the brain capillary endothelial cells [76,77]. CBSA has recently been used to target LN of DOX to the CNS [78]. In this case, ligand attachment delays *in vitro* drug release from the nanoparticle. Moreover, CBSA-mediated LN were uptaken by cells in a higher rate.

LN can be also targeted through physical approaches using magnetic fields [64]. Besides, drug release from magnetic LN can be controlled when nanoparticles are exposed to an alternating magnetic field [79].

Summarizing, all these possibilities of targeting lead to the conclusion that, although passive targeting has clearly increased antitumor drug efficacy, active targeting clearly improves drug efficacy and security. In fact, active targeting of lipid nanocarriers might be considered an added improvement of passive targeting. LN accumulate in tumor tissue not only due to their physical characteristics but also because of specific binding to cancer cells.

6.2 Cancer therapy using LN

This review is intended to discuss the treatment of cancer with lipid nanocarriers focusing on the past 5 years. Tumor extirpation combined with radiotherapy, chemotherapy and monoclonal antibodies are conventional treatments in early stages of the disease. However, these therapies are not always effective and entail severe side effects. For this reason, new therapeutic strategies are being investigated. Among all these possibilities, LN are promising drug delivery systems due to

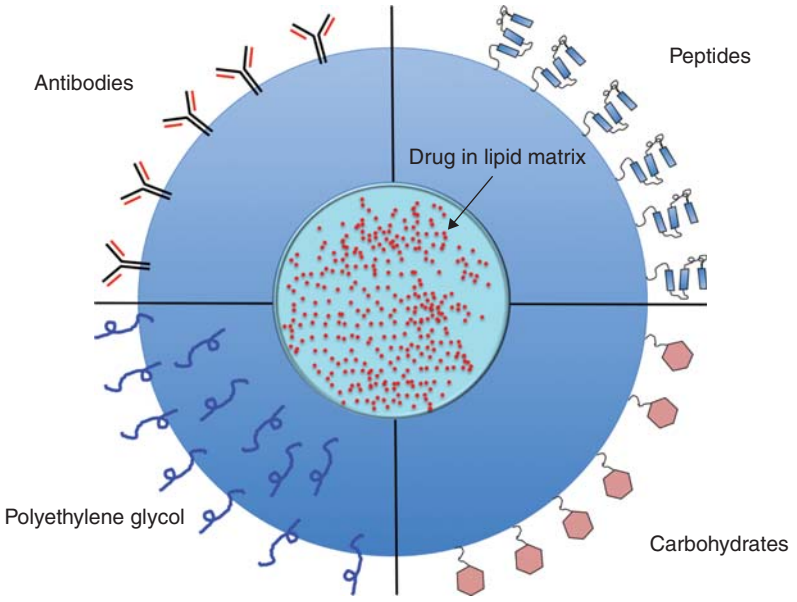

Figure 4. Representation of LN and different surface modifications for active and passive targeting.

the possibility of selectively targeting the nanoparticles at tumor tissues, providing effective and secure therapies.

6.2.1 *Lung cancer*

Lung cancer is the leading cause of cancer death in the world. This high mortality rates are mainly caused by a late diagnosis of the disease that is associated with non-operable stages. Non-small cell lung cancer (NSCLC) is the most common lung cancer type [80]. NSCLC is composed of heterogeneous aggregates of histologies that include epidermoid or squamous cell carcinoma, adenocarcinoma and large cell carcinoma. Despite improvements in NSCLC therapy, the overall survival at 5 years depends on the cancer stage at diagnosis varying from 49 or 16% to 2% for patients with local, regional and distant stage, respectively. NSCLC responds badly to radiotherapy and chemotherapy, so patients are frequently included in clinical trials [81]. With this basis in mind, novel formulations are being developed in order to obtain more secure therapies.

In the last 5 years, different authors have incorporated anti-tumor drugs into lipid nanocarriers to treat lung cancer [52,82,83]. These studies show that lipid vehicles protect labile drugs from degradation, increase drug bioavailability, enhance drug tumor uptake and decrease toxicity. Wan *et al.* [83] studied the *in vitro* efficacy of pegylated-LN-containing vinorelbine bitartrate (VB) in A-549 cancer cells. VB is a semi-synthetic vinca alkaloid currently registered for the treatment of NSCLC in many countries. It is a very labile and hydrophilic drug that possesses rapid clearance [84]. Pegylation, coupling of PEG to the surface of the nanocarriers, is a common strategy to avoid macrophage uptake and subsequent LN clearance by the RES [85]. Pegylated-LN-containing VB were able to reduce macrophage cell uptake by RAW264.7 cells because of PEG coupling on their surface; furthermore, they were internalized in a higher rate than the free drug in A-549 lung tumor cells. These results might increase *in vivo* efficacy of VB. Another study carried out by Jain *et al.* [58]. was based on the use of LN to encapsulate DOX, which is a cytostatic antibiotic with a narrow therapeutic index and severe cardiac toxicity. These authors developed a mannosylated LN formulation of DOX, which was tested *in vitro* in A-549 cells. The hemolytic effect of DOX was avoided when it was encapsulated into LN. *In vivo* studies in male BALB/c mice showed that intravenously administered LN increased bioavailability of DOX, which is cleared from plasma very quickly when it is administered in its free form. Moreover, vehiculized DOX accumulated in tumor tissue (xenograft A-549) at a higher rate than the free drug, avoiding toxicity in healthy cells.

Gene therapy has also been combined with lipid nanosystems in the treatment of lung cancer. Shi *et al.* [82]. investigated the effect of encapsulating anti-microRNA oligonucleotides (AMOs) for suppression of microRNA-21 (miR-21) functions in human lung cancer cells. A-549 human cancer cell line presents overexpression of miR-21, which causes cell proliferation and inhibits apoptosis. These AMOs cannot be administered in their free form due to their labile nature, and therefore LN constitute a promising drug delivery system in gene therapy. The *in vitro* results of the study clearly indicate that AMOs transfection efficacy is enhanced when it is encapsulated into LDC. Besides, this is the first time that AMOs is encapsulated instead of complexed. The high rate of transfection in A-549 cells inhibited cell proliferation and promoted apoptosis; moreover, cell motility was also inhibited.

6.2.2 Colon cancer

Colon cancer is one of the most common cancers worldwide. The prognosis of the disease is directly related to the penetration of the cancer through the bowel wall. Bowel localized cancer is removed by surgery and is curable in only 50% of the cases because recurrence is very frequent. Moreover, as in most cancer types, tumors are detected at an advanced stage and so radiotherapy and chemotherapy are the only feasible treatments [86].

Antitumor drugs against colon cancer have been encapsulated into LN by several authors lately [87,88]. 5-FU is an antimetabolite widely used in colorectal cancer treatment but it presents large individual variability in pharmacokinetics and its toxicity is closely related to this variability. Yassin *et al.* [87]. incorporated this drug into LN successfully; however, they did not test the efficacy of the formulation either *in vitro* or *in vivo*.

6.2.3 Breast cancer

Breast cancer is the second leading cause of cancer death in women after lung cancer. It causes death in about 3% of the cases. The decline in death rates since 1990 is mainly a result of early detection programs. These preventive measures have allowed the complete elimination of most tumors by surgical resection. This measure is commonly associated with local radiotherapy, systemic chemotherapy, hormone therapy or targeted therapy [89].

Many drugs have been vehiculized through lipid nanocarriers to achieve better drug efficacy and decrease toxicity in breast cancer treatment [58,65,68,75,83,90-94].

Capecitabine is a prodrug of 5-FU that must be converted by enzymes that are mainly restricted to the liver and tumor site. In this sense, capecitabine and its analogs have fewer side effects than 5-FU; nevertheless, its rapid plasma clearance requires frequent dose regimens. LN are a promising tool due to their ability to provide controlled drug release and, subsequently, improved dose regimens. Capecitabine analog (5-FCPal) was encapsulated by Gong *et al.* in LN [93]. The *in vitro* results in MCF-7 breast cancer cells showed that encapsulated 5-FCPal was as effective as capecitabine and less toxic than 5-FU. *In vivo* study on a mouse breast cancer model in female BALB/c mice did not show any significant differences between free capecitabine and encapsulated 5-FCPal analog administered via orogastric gavage; however, a tendency to higher efficacy was observed in the LN group. The authors also postulate that LN-containing 5-FCPal might be administered on an intermittent basis obtaining similar efficacy due to controlled drug release. More studies are required in order to demonstrate that LN administered intermittently could provide similar efficacy to the free drug administered daily.

Lu *et al.* [90]. encapsulated the antitumor drug MTO in LN. Heart toxicity, myelosuppression and local toxicity at injection site are reported frequently when using this drug against breast cancer. Authors efficiently overcame these drug drawbacks by using LN to vehiculize the drug. A breast cancer model in BALB/c-nu nude mice was established and MTO-LN were subcutaneously injected. Not only were LN-containing MTO more effective in restricting the action of the drug to the tumor site, but additionally, they were also able to avoid macrophage uptake by using the PEG-derivated surfactant S-40. Local injection of MTO-LN avoided hepatonecrosis and interstitial pneumonia that is caused by the free drug. The breast tumor model was not satisfactory in all animals and, therefore statistically significant results were not obtained. Preliminary histopathological results showed more necrotic areas and thinner overgrown tumor layer when mice were treated with the encapsulated drug.

Wan *et al.* [83]. evaluated the *in vitro* efficacy of including the antitumor drug VB in LN. As in the preceding study, these authors also aimed to protect LN from macrophage uptake, and so they decided to cover the LN surface with PEG. The increment in PEG percentage on the surface of the LN increases its hydrophilicity, thus avoiding macrophage uptake. *In vitro* efficacy in MCF-7 cells of nanoencapsulated VB was enhanced about 6.5-fold compared with the free drug.

Another approach in breast cancer therapy is the use of hormonal therapy. Most breast cancers need estrogen to grow, and estrogen-receptor antagonists are therefore used to block the receptor and hamper cancer development. Tamoxifen citrate (TC) is a non-steroidal estrogen antagonist commonly used after mastectomy or in early breast cancer stages. One study from Reddy *et al.* [91]. incorporated TC in LN to evaluate the *in vivo* pharmacokinetics of the encapsulated drug in rats. They showed that nanoencapsulation of the drug produced higher plasma concentrations of TC and slower clearance, thus demonstrating again the potential use of LN as a secure and efficient drug delivery system in breast cancer.

Tumor targeting improvement through functionalized LN has been demonstrated in a wide variety of studies. In the last few years, many studies have focused on the treatment of breast cancer with lipid nanocarriers possessing active targeting. Goutayer *et al.* [75]. investigated the *in vivo* distribution of LN and the effect of functionalizing them in their biodistribution. They targeted the nanoparticles to $\alpha_v\beta_3$ integrins, overexpressed on angiogenic vessels and tumor tissues, achieving longer nanoparticle plasma circulation time. Nanoparticles were accumulated mainly in tumor tissue followed by uterus, ovarian and adrenal glands. Tumor targeting was achieved in the case of a cell line overexpressing the target: in this case, functionalized nanoparticles accumulate in tumor tissue in a higher rate than non-targeted LN. Another study conducted by Jain *et al.* [58]., in which LN were labeled with mannose, affirmed that functionalized lipid nanocarriers are more effective than free drug in inhibiting proliferation in breast cancer cells. Besides, *in vivo* bioavailability and tumor accumulation were enhanced when using LN, especially when they were coupled with mannose. Tf-mediated LN has also been utilized to target antitumor

drugs at breast cancer cells [65]. In this study, curcumin efficacy in MCF-7 cells was enhanced due to the use of functionalized nanoparticles. Curcumin is a physically labile antitumor drug that presents a low bioavailability profile. LN were effective in protecting the drug from degradation. Non-targeted LN were also effective but at a lower rate than Tf-mediated LN.

5-FU is one of the most commonly used drugs in the treatment of breast cancer due to its effectiveness against several solid tumors; however, it presents serious drawbacks due to a lack of specificity for tumor cells [95]. Jain *et al.* [68]. studied the possible advantages of using LN to target the drug toward the tumor tissue while avoiding its toxic effects. This also included the targeting of the LN with ferritin. *In vitro* results demonstrated that ferritin-mediated LN-containing 5-FU were internalized at a significant rate by breast cancer cells (MDA-MB-468) through a saturable mechanism. Furthermore, drug half-life in plasma was significantly enhanced when the drug was encapsulated in nanoparticles. 5-FU accumulates in the tumor 7.7 times more than drug included in non-targeted LN or free drug.

6.2.4 Brain cancer

Nowadays, brain diseases remain one of the most challenging pathologies to treat. Many circumstances make treatment of cerebral tumors particularly complicated. They are in many cases inoperable due to their location, and the BBB prevents drugs from crossing into the brain. BBB consists of physical (tight junctions) and metabolic (enzymes) barriers, which hamper the passing of drugs and toxins from circulation blood to the extracellular fluid of the brain. Lastly, the broad heterogeneity of brain malignancies makes the individual response to the treatment very unpredictable. Brain tumors are associated with high mortality rates despite their low incidence compared with other tumors. The pharmacology of brain cancer is always difficult but LN have provided a new insight in its treatment alternatives [10,59,78,96-98].

Several active and passive strategies have been used to enhance targeting of LN at the CNS. The inclusion of certain tensioactive agents has been demonstrated to be an effectively passive targeting strategy to bypass the BBB. Tensioactives such as Tween 80 enhance the binding of plasma proteins, with specificity for the BBB, to the LN surface [99]. Moreover, Tween 80 temporarily inhibits the MDR effect mediated by P-gp protein avoiding drug efflux [10]. Estella-Hermoso de Mendoza *et al.* investigated the *in vitro* efficacy and *in vivo* biodistribution of edelfosine-loaded LN. Edelfosine is an antitumor drug with *in vitro* activity against several cancer cells [5,10,100,101]. This study demonstrated that LN are able to inhibit P-gp *in vitro* and that they can thus revert the C6 cell line resistance to the free drug. Moreover, biodistribution studies showed drug accumulation in brain tissue after oral administration of the nanoencapsulated drug.

Active targeting to the brain was also carried out by Agarwal *et al.* [78].. In this study, they conjugated DOX LN with CBSA. They based their strategy on previous studies that demonstrated that CBSA promotes transport of nanoparticles across the BBB [76,77]. The results of the study showed that CBSA-conjugated LN provided slower drug release rates than empty LN; this effect is commonly seen in lipid nanocarriers with attached ligands on their surface, which might happen because these added molecules act as extra barriers. Drug targeting was successfully achieved *in vitro* and *in vivo* through intravenous administration. CBSA-conjugated LN were able to target DOX to the CNS improving its brain concentration and avoiding side effects in healthy tissues. Kuo and Liang [59,97] also applied active targeting attaching anti-EGFR to the nanoparticle surface, and since EGFR is normally expressed in glioma, the attachment of an antibody against this receptor can certainly improve drug efficacy. These authors have published two studies in which they encapsulate DOX and carmustine into EGFR-targeted LN. Both studies evaluated the *in vitro* efficacy on U87MG cells, and showed that the efficacy of chemotherapy was enhanced as a result of the EGFR targeting.

6.2.5 Ovarian cancer

According to the American Cancer Society [102], ovarian cancer accounts for about 3% of all cancers in women and causes more deaths than any other cancer of the female reproductive system. It mainly affects older women, half of the diagnosed women being older than 60 years. As in other tumors, surgical removal of the tumor is the first option. Nevertheless, chemotherapy and radiotherapy must be administered in many cases if the main tumor cannot be removed or it has metastasized to other parts of the body. Chemotherapy in ovarian cancer is usually administered in combination therapy using a platinum compound, such as cisplatin or carboplatin, and a taxane, such as paclitaxel (Taxol®) or docetaxel (Taxotere®). Encapsulating, for example, docetaxel, into LN increased their efficacy compared with the commercial formulation (Taxotere) [92].

Many researchers are investigating new drug delivery systems that may overcome MDR to common chemotherapy drugs. Among these new strategies, LN have been successfully evaluated in ovarian cancer [62,70,92,103-105].

As it has been seen before, MDR can be overcome by using LN that include specific surfactants in their formulation. On this basis, one study developed by Dong *et al.* [62]. confirmed that Brij 78 can also inhibit P-gp efflux pump and, consequently, increase not only drug internalization but also drug retention inside the cells. The study, which consists of LN-containing DOX and paclitaxel, showed that both, blank LN and LN containing the antitumor drugs were able to inhibit the P-gp mechanism in P-gp overexpressing human ovarian carcinoma cell line NCI/ADR-RES. This inhibition is followed by a transitory adenosine triphosphate (ATP) depletion, which induces mitochondria stress and swelling as a desperate mechanism to obtain energy and supply ATP depletion. This study proves that LN containing certain

tensioactive agents have an effect on the MDR mechanism that helps to achieve higher intracellular drug accumulation. In fact, the addition of free DOX after treating the cells with non-loaded LN produced an *in vitro* cytotoxic effect similar to the drug-loaded nanocarriers, probably due to the transitory P-gp inhibition.

6.2.6 Hematological cancer

Blood cancer includes leukemia, lymphoma and myeloma. Leukemia develops in the bone marrow and affects white blood cells; it has different subtypes depending on its speed of development and the subtype of white cells involved. Childhood leukemia is the most common cancer in children. Lymphoma is a blood cancer that appears as a solid tumor and is commonly located in the lymph nodes. It causes the production of abnormal lymphocytes. There are two types of lymphoma: non-Hodgkin (more common) and Hodgkin. Non-Hodgkin lymphoma is the most common blood cancer in teenagers and young people. Myeloma affects the plasma cells on the blood unbalancing the immune system. Myeloma mainly occurs in people over the age of 40.

As has been mentioned before, lymphomas develop in lymph nodes, and so LN might be an appropriate tool to fight this cancer. Several studies support the theory that LN are absorbed by a lymphatic route after oral administration [8,20,61,106]. LN can passively target lymph nodes by concentrating unmetabolized drug at the cancer origin. So far, the only study of orally administered LN to treat hematological cancers was performed by Estella-Hermoso de Mendoza *et al.* [9]. In this work, very promising results were obtained after the oral administration of edelfosine-loaded LN to mantle cell lymphoma-bearing mice. These authors proved that the administration of drug-loaded LN every 4 days was as effective as the daily free drug in decreasing tumor growth. Moreover, while the daily administration of the free drug was able to reduce the metastases by a half, the administration of drug-loaded LN orally every 4 days completely eradicated the metastatization process. This study offers new hopes in orally administered chemotherapy to treat this kind of cancer.

In another study, Reddy *et al.* [107]. demonstrated that LN-containing etoposide were more effective than the free drug after intraperitoneal administration in Dalton's lymphoma ascites-bearing mice. Controlled release of etoposide in this kind of intraperitoneal tumors is essential due to the necessity of prolonged exposure to the drug to obtain a cytotoxic effect. LN remains in the peritoneal cavity after intraperitoneal administration, providing sustained release of the drug and thus increasing its antitumor efficacy. Antitumor drug encapsulation into LN has been carried out by several authors for treating hematological tumors [9,66,108,109]. Idarubicin and DOX were encapsulated into LN by Ma *et al.* [108]. in order to avoid P-gp-mediated MDR in leukemia patients and subsequent disease relapses. The results of the study showed that idarubicin inclusion into LN did not increase its efficacy. This could be explained because idarubicin uptake rate is much higher than its P-gp-mediated efflux because of its lipophilic properties. DOX-LN were, by contrast, more effective than the free drug, probably due to the P-gp inhibition mechanism mediated by the surfactants (Brij 78 and Vitamin E TPGS) included in the formulation.

Gene therapy has also been combined with LN in the treatment of leukemia leading to protection from serum nucleases, longer blood circulation and increased tumor concentration of oligodeoxyribonucleotides [109]. In addition, the coupling of these LN with Tf improves its targeting to leukemia cells overexpressing Tf-receptor. Moreover, targeting can be enhanced with a pretreatment with defeoxamine, a clinically used iron chelator which is known to up-regulate Tf-receptor expression in cells.

6.2.7 Other cancer types

Many other studies have been performed in relation to other cancer types such as prostate, tongue, hepatocellular cancer, melanoma and sarcoma [57,62,63,74,92,110-113]. In these studies, antitumor drugs and genetic material are encapsulated into LN.

As in other approaches, genetic material is protected from plasma nuclease degradation and LN show higher *in vitro* transfection efficiency than commercially available gene carriers [110,111]. Besides, an *in vivo* study carried out by Bauman *et al.* [110]. with oligonucleotides that down-regulate Bcl-x (an anti-apoptotic member of the Bcl-2 family) demonstrated that they are able to induce splicing modification in tumor cells.

Radiotherapy has also been combined with nanotechnology in the treatment of head and neck cancers. Some studies show that β-emitting radionuclides that are included in LN better accumulate and localize radiation in the tumor, sparing healthy tissues after intratumor administration [112].

LN are also a good strategy for topical oral delivery of poorly water-soluble drugs used in oral cancer chemoprevention strategy [113]. Moreover, LN can reach connective tissue and, therefore, they could be used for systemic therapeutics through the oral mucosa. It is remarkable that this study also showed that LN must be in a high concentration in the treatment site to avoid MDR efflux; at a low concentration they conjugate with glutathione and are effluxed by cell proteins.

The taxanes, including paclitaxel and docetaxel, have broad activity and are extensively used in clinical practice in the treatment of cancer. As explained before, several authors have vehiculized them into LN to treat ovarian and colorectal cancer; however, prostate, hepatocellular or sarcoma, among others, have also been investigated [57,62,63,92]. As major antitumor drugs, taxanes comprise severe side effects because of their poor targeting and high toxicity; moreover, they exhibit poor water solubility. The studies mentioned above demonstrated that, in all cases, encapsulated drug effects

were more potent and toxic effects were avoided due to a lower accumulation in healthy tissues.

7. Biodegradation, safety and toxicity aspects

Over the past years, the development of lipid drug delivery systems has entailed a wide range of tasks such as the development of nanosystems that are suitable to specific applications, the type of release kinetics (pulsatile, fast, slow) and proof of efficacy. Furthermore, it is very important to prove the systems' safety, which implies at least two major entities: the biocompatibility of the delivery system and the safety of the systemically distributed drug [114]. The control of the systemic drug distribution can be a relatively simple matter of engineering release kinetics so that blood levels are lower. Being in the solid state, the lipid components of LN will be degraded more slowly providing a long-lasting exposure to the immune system. Degradation can be slowed down even more when using sterically stabilizing surfactants that hinder the anchoring of enzyme complexes. Reducing biocompatibility problems can be much harder, involving drug–tissue interactions and material properties that are still not well understood. However, LN are biocompatible and biodegradable and have been used for controlled drug delivery and specific targeting. Furthermore, in terms of safety issues, one clear advantage of the use of LN as drug carrier systems is the fact that the matrix is composed of physiological components, that is, excipients with GRAS status for oral, topical and intravenous administration [5,29,101,115,116], which decreases the possible cytotoxicity. LN have been already tested as site-specific carriers mainly for drugs that present a relatively fast metabolism and are quickly cleared from the blood, that is, peptides and proteins [117-119]. LN are generally well tolerated, and as stated above they are mainly formulated using biocompatible or physiological compounds that can be included in different metabolic pathways after degradation [120,121].

The biodegradation velocity of nanoparticles affects their toxicological acceptance (e.g., concentration of degradation products). As a result, many studies have been focused on the toxicology of LN, including genotoxicity and cytotoxicity studies [122]. It was observed that these effects usually occur at rather high concentrations, but the effects that happen at lower concentrations, without necessarily causing cell death, also should be taken into consideration.

8. Concluding remarks

LN have been shown to be effective carriers in cancer. The inclusion of anticancer drugs in LN improves drug efficacy and decreases side effects. Among all the advantages that these carriers offer, it is noteworthy that they protect labile drugs from degradation or rapid RES clearance. This is particularly relevant in the case of gene therapy or in antitumor drugs that have short plasma half-lives. Besides, they not only decrease toxicity but they also generally provide longer circulation times and higher concentration of the drug in tumor tissue. This proved efficacy is mainly based on passive and active targeting. Apart from these general considerations, LN present some particularly relevant advantages. First of all, they can be administered orally avoiding the tedious intravenous route in chemotherapy. When administered by this route, they are mainly absorbed via the lymphatic system, thus opening a new window in treatment of cancer metastases and lymphomas. Second, they can be targeted to the brain due to its capacity to cross the BBB when specific tensioactive compounds (Tween 80, Brij 78) are used in the formulation. *In vitro* studies have demonstrated that these molecules inhibit MDR by inhibiting P-gp efflux pump. LN can easily be scaled up, even obtaining improved results over those produced in the laboratory.

9. Expert opinion

Nowadays, LN are widely being investigated in the field of pharmaceutical technology. LN formulations are based on traditional emulsion techniques and a broad spectrum of manufacturing methods are currently available. Production methods for LN have been widely modified since their invention by Speiser [14] in the 1980s. Most of these methods are based on the HPH and warm microemulsion technique developed respectively by Müller and Lucks [17] and Gasco [19] in the 1990s. The investigations carried out in this field have led to improved nanoparticles due to the avoidance of degradation of thermolabile compounds, non-energy consuming methods, reproducibility and low surfactant concentrations, among other factors. Most of them can be easily scaled up, HPH-based procedures being the most suitable for this purpose, as homogenizers have been used for a long time in the pharmaceutical industry. Indeed, LN produced on a large scale have been seen to present better size quality [29]. Regarding safety issues, LN present the advantage of being composed by GRAS lipids for oral, topical and intravenous administration. Therefore, LN matrix composition would not be potentially toxic unless large non-ionic surfactant or organic solvent quantities are used in the formulation. Several *in vivo* studies demonstrate that the intravenous administration of LN lower than 5 μm does not produce macroscopic toxicity [57,58,75,110]. Besides, *in vitro* toxicity experiments have shown that LN do not affect Caco-2 cell viability [123], which makes this system suitable for oral administration. However, regardless of the potential safety of these nanosystems, further research is necessary in order to elucidate nanoparticle behavior after *in vivo* administration, emphasizing the study of LN barrier crossing (e.g., intestinal barrier or BBB) and cell interaction. The knowledge of this basis would enable us to anticipate possible toxic effects.

The antitumor activity of these nanosystems loaded with antitumor drugs has been widely demonstrated since their discovery. Studies carried out in the last 5 years show that

antitumor drug toxicity is dramatically reduced when the drug is encapsulated into LN [63,92,124]. Besides, LN provide higher bioavailability rates and prolonged plasma circulation times, thus improving drug efficacy [10,58,78,125]. The advantages of LN over the administration of free drugs can be mainly explained by the passive and active targeting of the tumor tissue, mediated by the lipid vehicle. Another important improvement in these systems is that when some tensioactive molecules are used in the formulation, LN are able to overcome MDR [10,62]. This benefit is due to the ability of LN to inhibit P-gp protein, which mediates the efflux of antitumor drugs from the cell and thus enhances intracellular drug concentration. Targeting anticancer drugs at the tumor avoids severe chemotherapy side effects. Although most studies in cancer treatment with LN are based on intravenous route, some authors have considered the oral route [9,10], which is better tolerated in terms of patient welfare. These studies suggest that LN are absorbed via the lymphatic system after oral administration, achieving high drug concentration in lymph nodes. This fact might be very relevant in the avoidance of metastases and in lymphoma treatment. Bearing in mind the benefits of orally administrated LN, current research efforts should be focused on this route. Further studies are required in order to fully characterize their lymphatic absorption. Besides, intracellular uptake and interactions between cells and LN must be evaluated with the aim of clarifying the biodegradation, safety and toxicity aspects of these vehicles. Despite the need for further research concerning these aspects of the field, and considering all the reviewed studies, in authors' opinion LN should provide more secure and effective antitumor treatments in the near future.

Declaration of interest

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