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# Lipid-core nanoparticles: classification, preparation methods, routes of administration and recent advances in cancer treatment.

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Nanotechnological drug delivery platforms represent a new paradigm for cancer therapeutics as they improve the pharmacokinetic profile and distribution of chemotherapeutic agents over conventional formulations. Among nanoparticles, lipid-based nanoplatforms possessing a lipid core, that is, lipid-core nanoparticles (LCNPs), have gained increasing interest due to lipid properties such as high solubilizing potential, versatility, biocompatibility, and biodegradability. However, due to the wide spectrum of morphologies and types of LCNPs, there is a lack of consensus regarding their terminology and classification. According to the

current state-of-the-art in this critical review, LCNPs are defined and classified based on the state of their lipidic components in liquid lipid nanoparticles (LLNs). These include lipid nanoemulsions (LNEs) and lipid nanocapsules (LNCs), solid lipid nanoparticles (SLNs) and nanostructured lipid nanocarriers (NLCs). In addition, we present a comprehensive and comparative description of the methods employed for their preparation, routes of administration and the fundamental role of physicochemical properties of LCNPs for efficient antitumoral drug-delivery application. Market available LCNPs, clinical trials and preclinical *in vivo* studies of promising LCNPs as potential treatments for different cancer pathologies are summarized.

## 1. Introduction

Cancer encompasses a heterogeneous group of disorders defined by an abnormal and uncontrolled growth of the cells of a tissue or organ beyond their usual limits.[1] Worldwide, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020, being the first or second leading cause of death before the age of 70 years in 112 of 183 countries.[2] Cancer can develop anywhere in the body, with surgical resection, chemotherapy and radiotherapy being the three main treatment strategies.[3] Chemotherapy consists of the administration of an antitumoral drug such as tamoxifen (TMX), paclitaxel (PTX), docetaxel (DTX), doxorubicin (DOX) or methotrexate (MTX), that can kill cancer cells throughout the body, even those at the edges of tumors, which may not be removed by surgery. However, the poor aqueous solubility and permeability of these drugs have resulted in low bioavailability and decreased treatment efficiency.[4] Furthermore, this limitation along with the non-specificity of treatment, results in patients being treated at the maximum tolerated dose of these antitumor compounds, which entails several adverse and off-target effects. These obstacles decrease the therapeutic value of many anticancer drugs and their respective chemotherapy treatment.[5]

Nanotechnology stands out as an attractive strategy to avoid the aforementioned problems associated with conventional antitumor drug delivery. Nanosystems can overcome biological barriers and control the release of drugs to target sites, enabling the use of lower doses while decreasing the side effects and increasing the treatment efficiency.[6,7] Anticancer drug-loaded nanoparticles (NPs) possess several advantages compared to conventional chemotherapy due to their reduced toxicity, high-loading capacity, stability, specificity, tolerability and efficacy.[5] Nowadays, nanotechnology plays an important role in the targeted delivery of drugs for cancer treatments.

Among NPs, lipid nanoparticles (LNPs) have gained increasing interest due to their potential to load and release drugs from the biopharmaceutical classification system (BCS) of class II

(low solubility and high permeability) and class IV (low solubility and low permeability).[8] Due to lipid properties such as high solubilizing potential, flexibility and biodegradability, poorly water-soluble drugs can be loaded into LNPs, increasing their bioavailability. Furthermore, lipids as carriers have the potential to improve oral drug delivery due to their ability to enhance gastrointestinal solubilization and absorption, as well as topical application.[6,9]

In the last decade, LNPs have also been intensively studied for enabling gene therapies. LNPs technology is proposed to be a dominant non-viral technology in gene therapy, mainly due to their design flexibility.[10] In August 2018, the first ever approved LNP delivery of nucleic-acid based drugs was reported by the Food and Drug Administration (FDA) and by the European Commission (EC): Onpattro™ ((Patisiran (ALN-TTR02)), a lipid complex encapsulating a siRNA for the treatment of the hereditary transthyretin-mediated amyloidosis.[11] In this sense, the COVID-19 vaccines BNT162b2 (Comirnaty®; BioNTech and Pfizer) and Moderna COVID-19 (mRNA-1273) (ModernaTX, Inc; Cambridge, Massachusetts), which are based on LNPs encapsulating a modified mRNA, are outstanding examples.[12–14] LNPs are also being investigated as the formulation vehicle for mRNA vaccines for cancer immunotherapy.[15] Among LNPs for nucleic-acids delivery, liposomes are the most employed systems.[16]

Liposomes represent the first generation of LNPs [17] and, since their discovery in 1965 by Sir Alec Bangham,[18] several studies have been reported. In fact, the FDA has already approved liposomal formulations for cancer treatment such as Daunoxome®, Doxil® or Myocet®. Liposomes are spherical lipid vesicles formed mainly by natural or synthetic phospholipids organized in a bilayer structure.[19] In addition, other membrane bilayer constituents, such as cholesterol or hydrophilic polymers conjugated lipids, can be added to their formulation.[20]. Liposomes have an aqueous core in which water-soluble drugs can be dissolved and encapsulated. However, liposomes can also entrap lipophilic drugs into their lipidic bilayer membrane. This structure causes liposomes to have a reduced drug loading capacity for lipophilic drugs compared to LNPs with a lipidic core, *i.e.*, lipid core nanoparticles (LCNPs). Other significant drawbacks related to liposomes are production scalability, the need to use organic solvents during the production and the relatively low stability in biological fluids.[21]

LNPs composed of a lipid core *i.e.*, lipid core nanoparticles (LCNPs) (LCNPs) such as microemulsions (MEs) and nanoemulsions (NEs) are also widely employed. MEs were described almost simultaneously to liposomes in 1959 by Schulman.[22] The description and characterization of MEs and, subsequently, NEs, lead to extensive research into this type of

drug delivery systems.[23] Furthermore, these emulsion-type LCNPs are considered to be the starting point for the development of solid lipid nanoparticles (SLNs) and nanostructured lipid nanocarriers (NLCs) in the 90s. LCNPs represent an alternative to liposomes for the encapsulation and delivery of hydrophobic drugs. The different systems included in the category of LCNPs have different characteristics in terms of stability and drug release kinetics, which will be discussed in the subsequent sections.[24] Overall, LCNPs offer higher hydrophobic drug loading capacities and physical stability than liposomes.[25] Moreover, production methods for LCNPs include organic solvent-free methods, making easier to scale up their production (Section 3).

In this review, we focus on the description of LCNPs, along with their methods of synthesis, routes of administration and applications as antitumoral drug delivery systems. Since liposomal formulations are based on an aqueous core, they will not be included in this review, although they are extremely important in LNPs technology development.

## 2. Classification

LCNPs are differentiated according to the physical state of their lipid core component (Figure 1). Accordingly, they are classified in liquid lipid nanoparticles (LLNs), which includes lipid nanoemulsions (LNEs) (Figure 1A) and lipid nanocapsules (LNCs) (Figure 1B), SLNs (Figure 1C) and NLCs (Figure 1D).

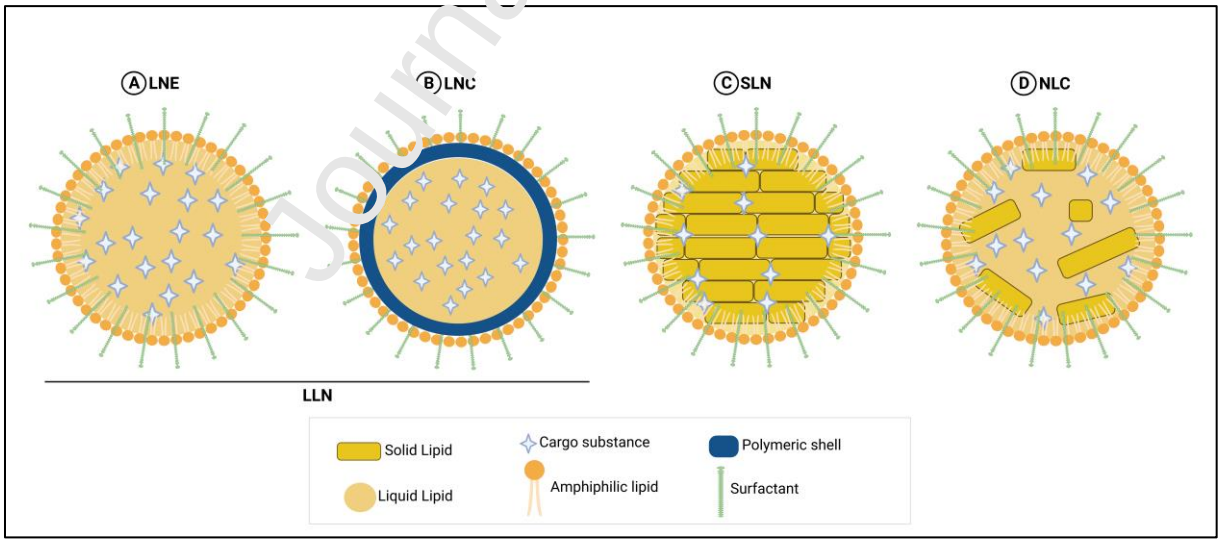


**Figure 1**. Schematic representation of LCNPs. They are classified according to the physical state of their lipidic component in LLNs, which include (A) LNEs and (B) LNCs; (C) SLNs and (D) NLCs. Due to the various morphologies and preparation methods existing for LCNPs, the representation presented here could vary. Created with BioRender.com.

2.1. Liquid Lipid Nanoparticles (LLNs)

LNPs presenting a liquid lipid core at body temperature are widely described in the literature. LNEs and LNCs are the main types of nanocarriers included in this category.

Nanocapsules (NCs) can be defined as vesicular nanosystems with an inner cavity (core) surrounded by a polymeric protective shell, *i.e.*, a core-shell structure.[26,27] The drug or cargo substance can be either confined in the core material or attached to the polymeric shell. Different types of NCs can be formulated according to the nature of their core, which can consist of a liquid phase, either an oily or an aqueous core, a solid phase of a polymeric matrix or a hollow internal structure.[28] In the case of NCs comprising a liquid oily core, generally formed by vegetable oils or fatty acids, their lipidic component plays an undeniably leading role. The oily core affords a safe and efficient dissolving media for hydrophobic drugs that, in the case of oils with therapeutic potentials, can also provide a synergistic and beneficial functionality. Furthermore, despite the development of NCs manufactured with an aqueous core, a hollow inner structure or, to a lesser extent, a solid core, oily liquid NCs surrounded by a polymeric layer are the most widely reported.[29] In fact, some reports directly define NCs as vesicular structures containing an oily core surrounded by a rigid shell, ignoring other feasible compositions.[30–33] Therefore, this review includes polymeric oily NCs as part of LCNPs and categorizes this type of nanosystem as LLNs because of their liquid lipidic inner core. Moreover, there is a need to use a term that includes nanosystems with a core-shell structure, *i.e.*, NCs, based on a liquid lipid core. In this regard, we propose coining a term already employed in the literature,[34] which accurately describes the structure of these nanocarriers: LNCs.

2.1.1. Lipid Nanoemulsions (LNEs)

NEs are defined as biphasic dispersions of two immiscible liquids, in which one liquid is dispersed within the other in the form of nanodroplets stabilized by an amphiphilic surfactant. While MEs and NEs can appear similar, their stability and structure differ. Contrary to MEs, which are equilibrium systems thermodynamically stable under certain conditions, NEs are non-equilibrium systems that tend to separate into the two phases but possess a high kinetic stability. Interestingly, MEs, as thermodynamically stable systems, are sensitive to conditions of temperature and composition, unlike NEs, which are relatively immune to physical and chemical changes. In the pharmacological and drug delivery field, as some administration routes involve physicochemical changes to the environment, NEs are more suitable for clinical

applications. For further insights, readers are referred to reports detailing the differences between NEs and MEs.[35,36]

NEs can be either oil in water (O/W) or water in oil (W/O).[37] However, in clinical applications the aqueous dispersion medium is adopted, LNEs, *i.e.*, nanodroplets of oil dispersed in water, are the most investigated and employed. LNEs can be directly employed for active delivery and targeting as drug delivery systems or can act as a template for the preparation of polymeric NPs and LNCs. [38–40]

2.1.2. Lipid Nanocapsules (LNCs)

The term nanocapsule was first employed in 1977 by Couvreur *et al.*[41,42] Since then, an exhaustive development of this type of nanoparticulated system has been achieved. However, the denomination of LNCs was not firmly employed until the 2000s, when it appeared in the patent No. WO02688000 by Herault *et al.*[34] These LNCs were composed of an oily core built up with capric and caprylic acid medium-chain triglycerides (Labrafac®) and a surfactant shell of a polyethylene glycol(PEG)ylated nonionic surfactant (Solutol®) and lecithin (Lipoid®).[43–46] The patented LNCs are described as nanoemulsion-template NCs formulated by a novel phase inversion temperature (PIT) methodology. This technique includes an additional stage of cycling temperatures, which provokes the over-concentration of the surfactant at the interface of the oil droplets. As a result, a thick surfactant layer is created around the nanodroplets, forming a core-shell structure. These authors state that the major difference between LNCs and LNEs comes from the type of energy provided to their formation, thus, LNCs are more rigid than LNEs.[47]

Some authors demonstrate the existence of two types of LNCs, based on the structure and composition of their shells: polymer-shelled LNCs and surfactant shelled NCs.[48,49] The former includes a polymer in their shell, whereas the latter are the described and patented LNCs produced by the novel PIT method. Surfactant-shelled LNCs give rise to an interesting debate on whether the use of a polymer is necessary for the preparation of oil-based NCs. The diversity of synthesis methods and the broad range of possible compositions generate a wide number of conformations and formulations of LNCs, which leads to a lack of consensus in the classification of these nanocarriers. From a structural point of view, we consider that any nanocarrier possessing a liquid lipid core and a core-shell structure, regardless of the composition and synthesis method employed, should be considered as LNCs.

Furthermore, since a large part of LNCs are prepared from LNEs, it is important to evaluate the transition from LNEs into LNCs, *i.e.*, the characteristics of the nanosystem that will define the

subtle line which differentiates them. In a practical sense, the main difference is the inclusion of a polymer during the nanocarrier synthesis. However, given that many surfactants are proper polymers[50,51] and surfactant-shelled LNCs exist, according to some authors, this phenomenon should be accurately evaluated. The most frequent technique employed for studying the structure of NCs and determining their core-shell structure is TEM performed after freeze-fracture. However, because of the nanometer scale of the samples, this method remains very difficult and, often subjective, in determining the wall thickness.[52] Preetz *et al.* applied atomic force microscopy to study the shell structure of three different preparations: LNCs prepared by the layer-by-layer deposition technique based on a LNE template, the LNE itself prepared by a high-energy method and PEG-PLA LNCs prepared by interfacial deposition of preformed polymer.[52] The stiffness of the shell of PEG-PLA LNCs and 5-layer LNCs were found to be 33.3 and 14.3 %, respectively, higher than the original LNE. This study proved atomic force microscopy as a suitable technique to distinguish NCs from NEs. Some reports compare the stability and physicochemical properties of LNEs and LNCs, which essentially differ in the addition (or not) of polymer during synthesis.[53–55] Clearly, the addition of polymer and the consequent formation of the polymeric shell improve the performance of the carrier. These studies indicate that there are significant differences between LNEs and LNCs. However, this is truly evident only when polymers are used. In the absence of polymers, as it is the case of LNCs prepared by the novel PIT technique, it is necessary to study the rigidity of the shells in order to distinguish between the two types of nanocarriers and, frequently, the limit is not well defined.

From our point of view, LNCs, defined as a core-shell structure where the core is composed of a liquid lipid phase, can be considered an evolution of LNEs. LNCs are able to maintain the advantages of LNEs, mainly the high loading capacity due to the liquid core, while integrating a stiffer protective barrier, which provides stability and less drug leakage during preparation compared to typically employed surfactants.[56] LNEs can be distinguished from LNCs based on the absence of the core-shell structure and a lower surface thickness and rigidity. However, this difference is not always so clear, as discussed in the previous paragraph. Moreover, it is important to note that LNEs and LNCs can be used for the same applications and some authors employ these terms indistinguishably. In this regard, we consider it appropriate to employ an inclusive designation that consolidates both concepts, as the term LLNs, encompassing both LNEs and LNCs, that accurately and faithfully describes the fundamental characteristics of these nanosystems.

2.2. Solid Lipid Nanoparticles (SLNs)

In their attempts to avoid burst release and produce systems for controlled drug release, Speiser and co-workers started to work with small-sized particles. They created micro and nano-sized solid lipid matrixes as drug carriers, which they named as micro and nanopellets.[57] These studies laid the foundation for the later development of SLNs in the same decade. In 1993, Gasco and co-workers patented the production of solid lipid micro-spheres, 50-800 nm in size, through a hot emulsification process.[58] However, Müler and co-workers were the first to name those colloidal systems as SLNs. They described a production method using high pressure homogenization of melted lipid in water at high temperature.[59]

SLNs are made of lipids which are solid at room and body temperature.[60] These form a crystalline and organized structure, which is stabilized by emulsifiers and where the drug is entrapped. They were developed to overcome fast degradation and toxicity problems associated with LNEs and liposomes, and with polymeric NPs, respectively. The slower diffusion of the drug through the solid matrix allows a sustained drug release from SLNs over a longer period of time.[61,62] On the other hand, lipids used in SLNs formulations are biocompatible, which reduces their toxicity.[62]

SLNs present other advantages, such as the possibility of encapsulating lipophilic or hydrophilic drugs with increased solubility, large-scale production and sterilization, and the existence of production methods where the use of solvents can be avoided.[62] However, the crystalline structure of the lipidic core may represent a disadvantage, since the highly organized lipid structure promotes the exclusion of the encapsulated drug. The crystallization of the lipid molecules during the synthesis process leads, in some cases, to low drug encapsulation efficiency and drug loading.[63,64] On the other hand, pure lipids, as found in SLNs, undergo polymorphic changes, *i.e.*, lipids which have recrystallized in a low melting and less stable morphology can change their configurations to a more stable one over time. These modifications involve changes in the crystalline structure which can cause drug expulsion from the lipid core, as well as lead to precipitation of large drug crystals in the aqueous phase.[63]

2.3. Nanostructured lipid carriers (NLCs)

SLNs were presented as an avant-garde alternative to LNEs, liposomes, and polymeric NPs. However, problems related to the crystalline lipid core, such as low drug loading, drug exclusion, and slow drug release, lead to the development, in 1999, of a new generation of LCNPs, the so-called NLCs.[64,65] In these particles, the matrix is composed not only of one solid lipid, but of a blend of solid and liquid lipids. The main advantage of NLCs, when

compared to SLNs, is an increased loading capacity of actives, which was first shown for retinol.[66] The solid and liquid lipid blend of NLC remains solid at body temperature. The addition of the oil compound distorts the formation of perfect lipid crystals, thus creating imperfections, which increase the uptake capacity for drug or active. This "structure" increases the drug loading capacity. The localization of the drug does not only depend on the structure of the lipid matrix but also on the lipophilicity and structure of the drug itself. An additional advantage of NLCs is a minimized risk of drug expulsion over time. The addition of oil to a solid lipid has recently proven to be able to prevent the re-crystallization of the lipid in a less stable morphology.[67,68] Hence, no changes in morphology occur, and thus, the drug expulsion from NLCs over time is reduced. Therefore, NLCs generally possess higher physical stability than SLNs.

## 3. Preparation methods

In this section, synthesis and preparation methods of LNPs are classified. We provide an overview of the current methodology in this area, highlighting procedural differences between techniques with a shared physical basis but which may need an adaptation depending on the nature of the lipid component of the carrier.

### 3.1. Methods for LNEs preparation

LNEs, being non-equilibrium systems, require an energy input to be formed. This energy can proceed from the potential energy stored in the system or from mechanical devices that create powerful disruptive forces. Consequently, two broad categories of techniques for the preparation of LNEs can be distinguished: high-energy methods and low-energy methods.

#### 3.1.1. High-energy methods

High-energy methods employ intense disruptive forces which break up the oil and water phases to form nanodroplets. Typically, a coarse emulsion is first produced by mixing both phases. The coarse emulsion is then homogenized, employing mechanical devices such as high-pressure homogenizers, high-shear homogenizers, microfluidizers or ultrasonicators (Figure 2). Those techniques use different mechanisms to produce cavitation and shear stress on the sample and break down the particles.[69] The shear stress is the force per area of lateral interaction between the fluid layers.[70] This force causes the deformation of the material by glissade along the plane parallel to the acting stress. Shear stresses are caused by friction between the fluid particles, and they are the consequence of different velocities within the fluid. In turbulent flow,

the bigger velocity differences within the particles leads to a greater shear stress in the fluid. Homogenization devices promote shear stress within the fluid to reduce particles size, which ultimately will depend on the type of instrument and their operating conditions, as energy, time, number of cycles and sample formulation and properties. These methods are industrially scalable, but they have some disadvantages such as cost and high temperatures needed for some of the processes, which can inactivate thermolabile drugs and macromolecules, including proteins, enzymes and nucleic acids.

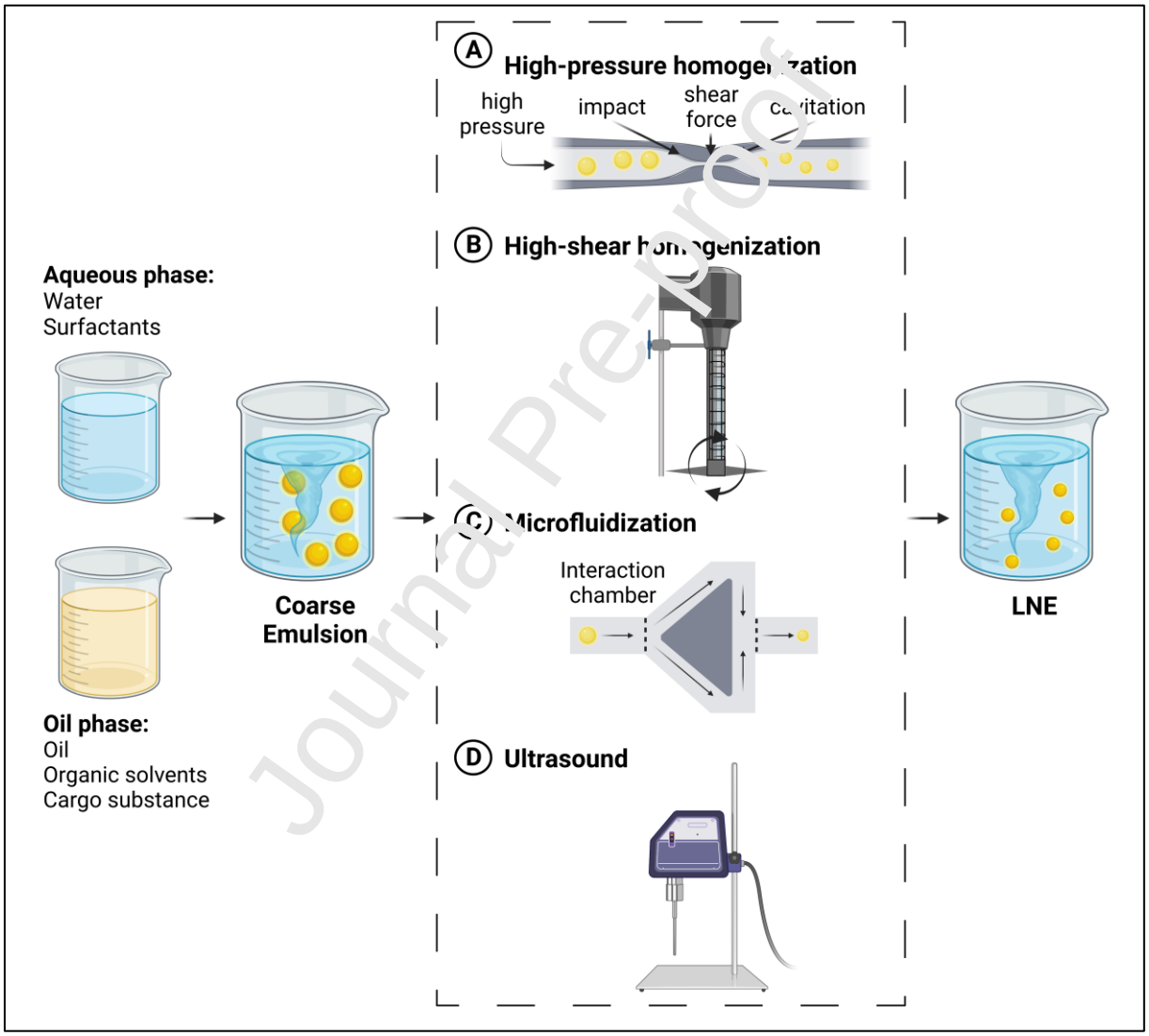


**Figure 2.** Representation of typical procedures for LNEs preparation through high-energy methods. First, a coarse emulsion is prepared by mixing both phases. Then, it is subjected to (A) high-pressure homogenization, (B) high-shear homogenization, (C) microfluidization, (D) Ultrasound, or a combination, to obtain a final LNE suspension. Created with BioRender.com.

A) High-pressure homogenization

This method involves the use of high-pressure homogenizers or piston gap homogenizers. These instruments consist of high-pressure pumps which impel the fluid towards a disruption

unit producing several forces, such as hydraulic shear, intense turbulence and cavitation, acting together to generate shear stresses and contributing to particle size reduction (Figure 2A).[71] The droplet size and polydispersity index depend on the pressure, which usually ranges from 50 to 200 MPa, the number of cycles and the temperature of the system, along with the emulsion composition itself. High-pressure homogenization can be processed in high temperature (hot homogenization) or in low temperature (cold homogenization). This method has various advantages such as an easy scale up, short process time and the avoidance of organic solvents.

B) High shear homogenization or high-speed stirring

The reduction of particle size in this technique is mainly driven by shear stress. The high shear mixers use a rotor/stator system to produce the shear stresses.[72] This system makes the fluid flow between a static platform and an inner-rotary one (Figure 2B). The rotation of the inner device acts as an impeller and produces a turbulent flow, which further enhances the shear stresses.[73]

C) Microfluidization

A microfluidizer is a patented mixing device that uses a high-pressure positive displacement pump (5 to 135 MPa) which repeatedly forces a coarse emulsion through an interaction chamber consisting of small channels, called microchannels, until the desired particle size is obtained (Figure 2C).[74] Turbulent flow along with cavitation causes droplet disruption and NE formation. The bulk emulsion is then filtered to remove large droplets, resulting in a uniform NE. This technique is suitable for its use at industrial scale.[75]

D) Ultrasound

Ultrasound waves produce changes in the pressure within the fluid over time. At some points in the fluid, pressure reduction is enough to allow fluid evaporation and the formation of bubbles. This phenomenon is known as cavitation. When bubbles collapse, they generate the projection of the liquid and high pressure which can disrupt the fluid droplets or erode the solid surfaces, leading to the formation of smaller particles.[76] The ultrasound waves also produce motion of the fluid and the particles, generating shear stress on the particles surface and contributing to their reduction in size. Ultrasound is a highly effective technique but is not suitable for industrial scale, unlike high-pressure homogenization and microfluidization. Its performance range is limited to laboratory scale and small batches (Figure 2D).

3.1.2. Low-energy methods

Nanoemulsification methods involving a low quantity of applied energy rely on the stored internal chemical energy of the system to form nanodroplets. These methods are very attractive because of their low equipment cost.[77] Low-energy methods can be classified according to whether a phase inversion of the surfactant is produced or not. If changes in the surfactant spontaneous curvature happen, they are designated as phase inversion methods. Phase inversion methods employ the chemical energy released by a phase transition produced during the emulsification process. This phase transition can be triggered either by changing the temperature (PIT) or the composition (PIC). If no phase inversion is involved, methods are termed as spontaneous emulsification.

A) PIT method

The PIT method (Figure 3A) was introduced by Shinoda and Saito.[78,79] This method is based on the ability of nonionic surfactants, such as polyethoxylated surfactants, to modify their affinities for water and oil as a function of the temperature. Polyethoxylated surfactants tend to become lipophilic upon heating as a consequence of the dehydration of polyoxyethylene groups.[80] In the PIT method, oil, water and nonionic surfactant are mixed together at its PIT or hydrophobic lipophilic balance (HLB) temperature, where the surfactant exhibits a similar affinity for the two immiscible phases, forming an unstable emulsion.[81] At a fixed composition, this method consists of a rapid change in temperature from the PIT through rapid heating or cooling to generate kinetically stable W/O or O/W NEs, respectively.[82] It is a simple, low-energy consuming and solvent-free method suitable for industrial scale-up. However, a limitation of this procedure is that it can only be applied to surfactants sensitive to changes in temperature.

B) PIC method

In the PIC method (Figure 3B), also known as emulsion inversion point (EIP) method, the transition in spontaneous curvature is achieved by changes in the composition during emulsification, at constant temperature.[83] The PIC method is a solvent-free technique that yields kinetically stable NEs at room temperatures. PIC is preferred from a scale-up point of view, since it is industrially easier to add one component to a large volume of emulsion rather than to generate a sudden change in temperature. Furthermore, the PIC method is more suitable for thermosensitive components and drugs. Components (water or oil) are added over a mixture of the other components (oil-surfactant or water-surfactant). Typically, water is added

progressively and dropwise into the oil phase comprising a W/O emulsion. As the fraction of water increases, surfactant hydrophilic-lipophilic properties begin to balance. When the transition composition is exceeded, phase inversion occurs and a O/W LNE is formed.[84]

C) Spontaneous emulsification

In this process, two contacting immiscible liquids that are not in equilibrium can form droplets without the need of an external energy input, taking advantage of the chemical potential gradients between both phases. Spontaneous emulsification is driven mainly by the rapid diffusion of a water-miscible solvent present in the organic phase through the aqueous phase, producing a local supersaturation near the interface that gives rise to the emulsification.[85] Other factors, such as interfacial turbulence and low interfacial tension values, play a secondary role in defining colloidal characteristics of the final resulting system.[86]

Spontaneous emulsification can be produced by the so-called Ouzo effect,[87] also called solvent-displacement method or nanoprecipitation (Figure 3C). In this method, the oily phase is dissolved in water miscible organic solvents comprising the organic phase. The aqueous phase consists of water and a hydrophilic surfactant. Both phases are mixed under magnetic stirring. Oil nanodroplets are instantaneously formed by rapid diffusion of the organic solvent in the aqueous phase and the consequent change in oil solubility.[88] Later, organic solvents are removed by suitable means, such as vacuum evaporation, and oil droplets remain dispersed in the aqueous phase. Oil viscosity, the HLB of the surfactant and the water solubility of the organic solvent are important parameters determining the size and quality of the oil nanodroplets prepared by this process. This methodology has also been reported for free-surfactant systems.[85] However, surfactants stabilize the interface of the formed nuclei and contribute to obtain smaller sizes and/or better polydispersity indexes, as well as provide a higher colloidal stability.[88] With this method, LNEs can be spontaneously fabricated at room temperature with simple equipment. The main drawback is the use of organic solvents, which need to be removed and generates several difficulties during scale-up. Other spontaneous emulsification methods are the dilution of microemulsions technique, also termed the microemulsion method, reported by Taylor and Ottewill,[89] and the dilution of surfactant aggregates.[90]

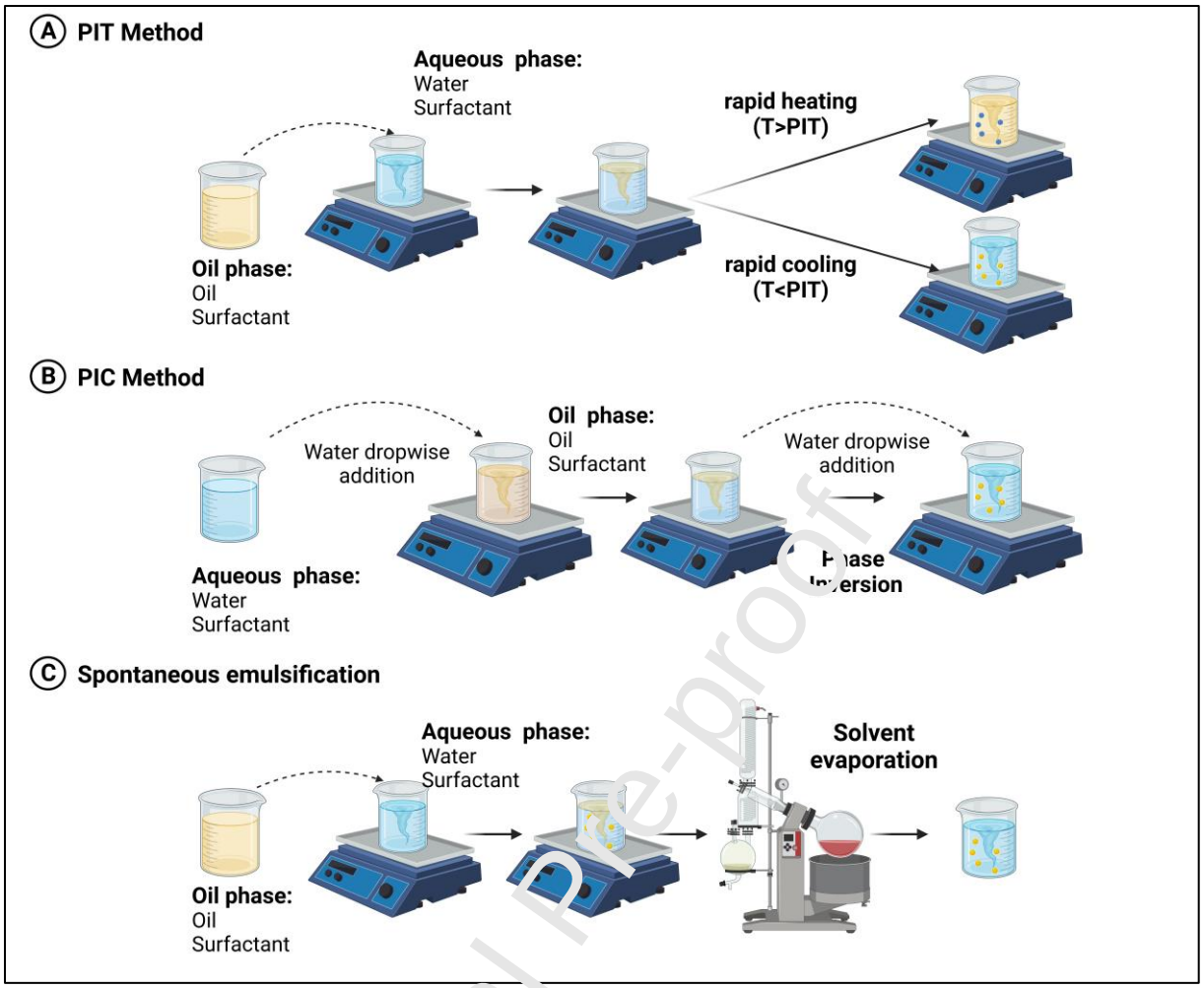


**Figure 3.** Low-energy methods for LNCs preparation. (A) PIT method and (B) PIC methods are based on a phase inversion phenomena, while (C) spontaneous emulsification is driven mainly by the rapid diffusion of a water-miscible organic solvent through the aqueous phase and the subsequent local supersaturation at the interface. Created with BioRender.com.

### 3.2. Methods for LNCs preparation

Techniques describing the manufacturing of LNCs involve the preparation of LNEs.[27] Nanodroplets of emulsions act as bioreactors where a protective layer is formed, generating the core-shell structures of LNCs. As mentioned in section 2.1.2, the core-shell structure of LNCs can be produced with or without the addition of a polymer during their preparation. Surfactant-shelled LCNs can be formed through an adapted PIT methodology. When LNCs preparation is based on the formation of a polymeric wall, methods can be classified depending on how the polymeric shell is obtained: by interfacial polymerization of monomers or employing preformed polymers during the preparation.

3.2.1. PIT method for LNCs preparation.

The PIT method is normally employed for the preparation of LNEs. However, Herault et al. reported an adapted PIT method for the preparation of LNCs.[91] This novel methodology included an additional stage of cycling temperatures PIT (Figure 4). In this technique, an oily phase (Labafrac) and a water phase in the presence of tensioactives (Lipoïd and Solutol) are first mixed under magnetic stirring. The mixture is then heated from room temperature to 85 ºC and subsequently to 60 ºC at a rate of 4 ºC/min. Three temperature cycles (85-60-85-60-85 ºC) are then applied to achieve phase inversion. The formed emulsion is then rapidly cooled through dilution with cold water to produce LNCs. The temperature cycling produces surfactants to be trapped and concentrated in the interfacial zone, generating a thick tensioactive shell that acts as a barrier to the oil diffusion (i.e., a nanocapsular system).[92]

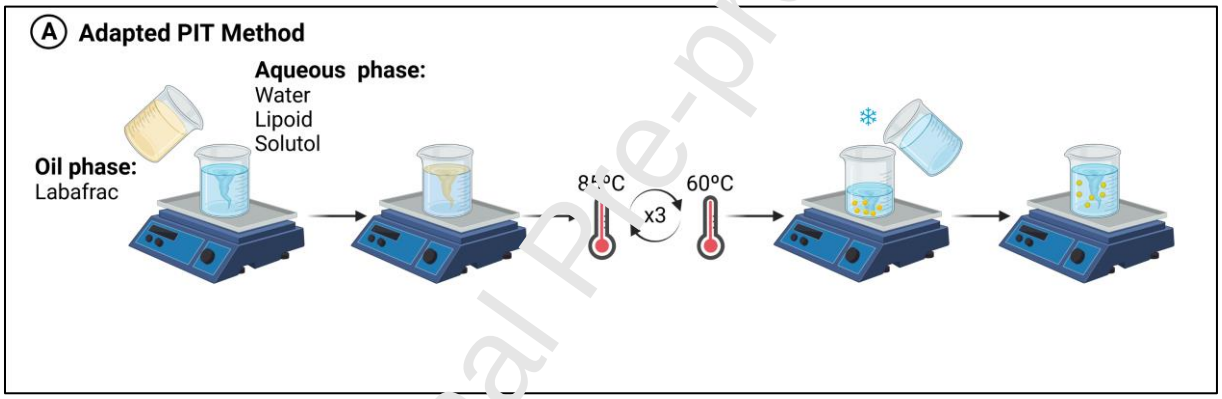


**Figure 4.** Adapted PIT method for the preparation of LNCs. Created with BioRender.com.

3.2.2. Interfacial polymerization

This technique is based on the fast polymerization of a monomer at the interface of an emulsion, and can be considered independent of the technique chosen to generate the LNE. The polymerization phenomena can occur either during the emulsion process: A) *in situ* polymerization (Figure 5A); or once LNEs are prepared: B) polymerization in emulsion (Figure 5B). *In situ* polymerization was the first reported method of an oil-in-water system where the isobutylcyanoacrylate monomer polymerized at the oil/water interface of the nanodroplets.[93,94] Alkylcyanoacrylates are the most commonly employed monomers for *in situ* polymerization. In a general procedure, oil, monomer and the active compound to be encapsulated are dissolved in a water-miscible solvent. This organic phase is injected, under magnetic stirring, into an aqueous phase containing a hydrophilic surfactant. Polymerization starts with the addition of the initiator/activator in the continuous phase or induced by UV, ultrasonication or enzymes.[38] This technique presents some major drawbacks: the lack of

control of the polydispersity and molecular weight of the polymer obtained, the presence of reactive monomers or oligomers, which can cause unwanted chemical reactions and drug inactivation, as well as the possibility of cross-reactions with the drug.[95,96] To avoid these issues during the polymerization process, methods based on preformed polymers are preferred.

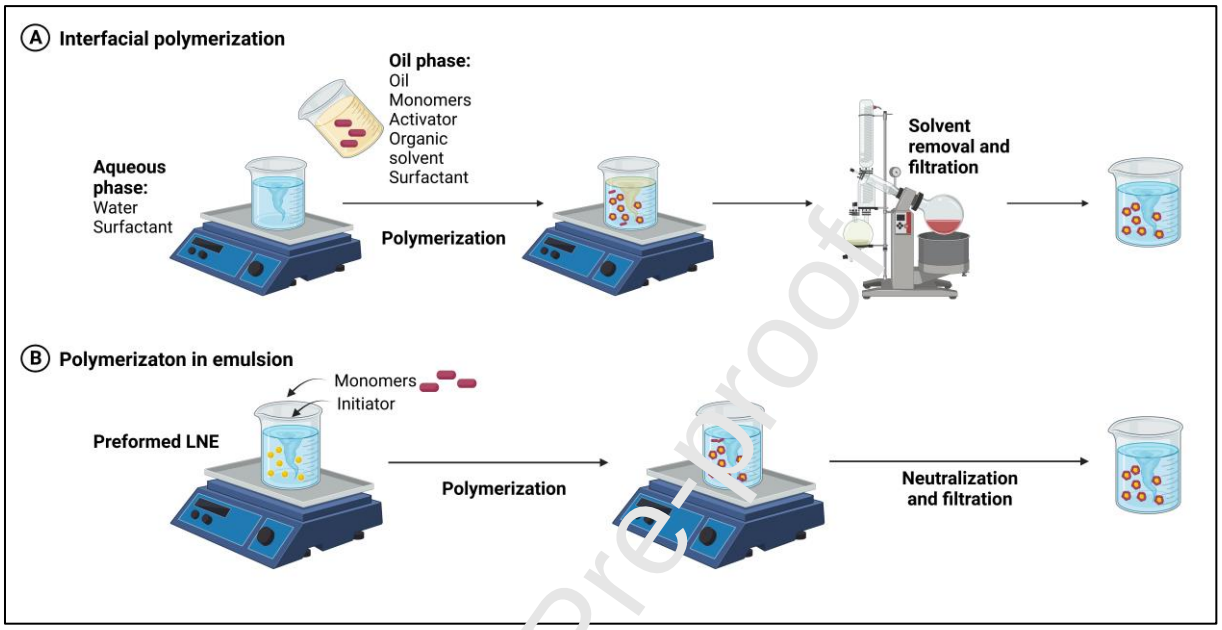


**Figure 5.** Methods for the preparation of LNCs through *in situ* polymerization of monomers. (A) interfacial polymerization method and (B) polymerization in emulsion technique. Created with BioRender.com.

3.2.3. LNCs preparation from preformed polymers

For these methods, polymers are dissolved or suspended into the continuous or dispersed phase during LNCs preparation, depending on their nature and solubility. Emulsion-diffusion/evaporation, emulsion-coacervation, solvent-displacement and layer-by-layer are the main methodologies employed to obtain LNCs from preformed polymers. Furthermore, double emulsions, either water in oil in water (W/O/W) or oil in water in oil (O/W/O), can be applied to obtain LNCs. The principle of these double emulsions is associated with emulsion-diffusion/evaporation and coacervation methods.[26]

A) Emulsion-diffusion/evaporation

The preparation of LNCs using the emulsion-diffusion/evaporation method is based on emulsion of the organic phase into the aqueous one and the subsequent elimination of the organic solvent. This is achieved either by the addition of water and dilution of the system,

which provokes the diffusion of the solvent (emulsion-diffusion) (Figure 6A), or through evaporation (emulsion-evaporation) (Figure 6B). LNCs are formed by a combination of polymer precipitation and interfacial deposition phenomena during the diffusion or evaporation event.[28]

B) Emulsion-coacervation

In the emulsion-coacervation method (Figure 6C), a LNE is employed as a template where the polymeric wall is formed on the surface of the nanodroplets through the formation of a coacervate, which causes polymer precipitation. The physical coacervation process can be provoked by: electrolytes, the addition of a water miscible non-solvent or a dehydrating agent, or temperature modification.[26]

C) Solvent-displacement method

This method, also called interfacial deposition or nanoprecipitation, was first described by Fessi *et al* [97] (Figure 6D). It is driven by the Ouzo effect, as in the case of the spontaneous emulsification process presented in the previous section. For instance, for LNCs preparation, the preparation procedure only differs in the employment of a polymer to produce the core-shell structure on the surface of LNEs. In this method, a solvent phase consisting of a solution of polymer, drug, oil and, if needed, a lipophilic tensioactive, is added with moderate stirring to a non-solvent phase, usually water, containing a hydrophilic surfactant.[88] Generally, the solvent and non-solvent phases are called organic and aqueous phases, respectively. Polymeric substances and surfactants can be added in the organic or water phase according to their properties. After LNCs formation, the organic solvent is removed. Polymer precipitation and solvent diffusion are the key factors that drive the process of LNCs formation. Polymeric NPs are also prepared with this technique by using an organic phase in the absence of a liquid lipid.[98]

D) Layer-by-Layer

The Layer-by-Layer method was developed by Sukhorukov *et al.* (1998).[99] In this method, polymer layers of polycations and polyanions are adsorbed by irreversible electrostatic attraction on the surface of a colloidal template (Figure 6E). The polymer layer can be adsorbed either by incubation in the polymer solution or by decreasing polymer solubility through the addition of a miscible solvent. The procedure is then repeated with multiple polymer layers that are deposited sequentially. Therefore, the main advantage of this technique is that it allows to

control the composition and thickness of polymeric shells. When LNEs are employed as template material, LNCs are obtained. Other colloidal templates, such as inorganic particles made of iron oxide, gold, calcium carbonate and silica, are widely employed.[100–102] These materials can be easily removed under mild conditions in order to obtain hollow NCs. The payload entrapment into these hollow NCs is achieved through diffusion (hydrophilic substances) and hydrophobic effect (hydrophobic substances). Polymeric NCs methods that do not lead to the obtention of LNCs, have also been extensively employed and reviewed. Readers are referred to polymeric NCs formulation techniques reported by Mora-Huertas *et al.*[26] , Kothamasu *et al.*,[103] Vauthier *et al.*[40] and by Deng *et al.*[28]

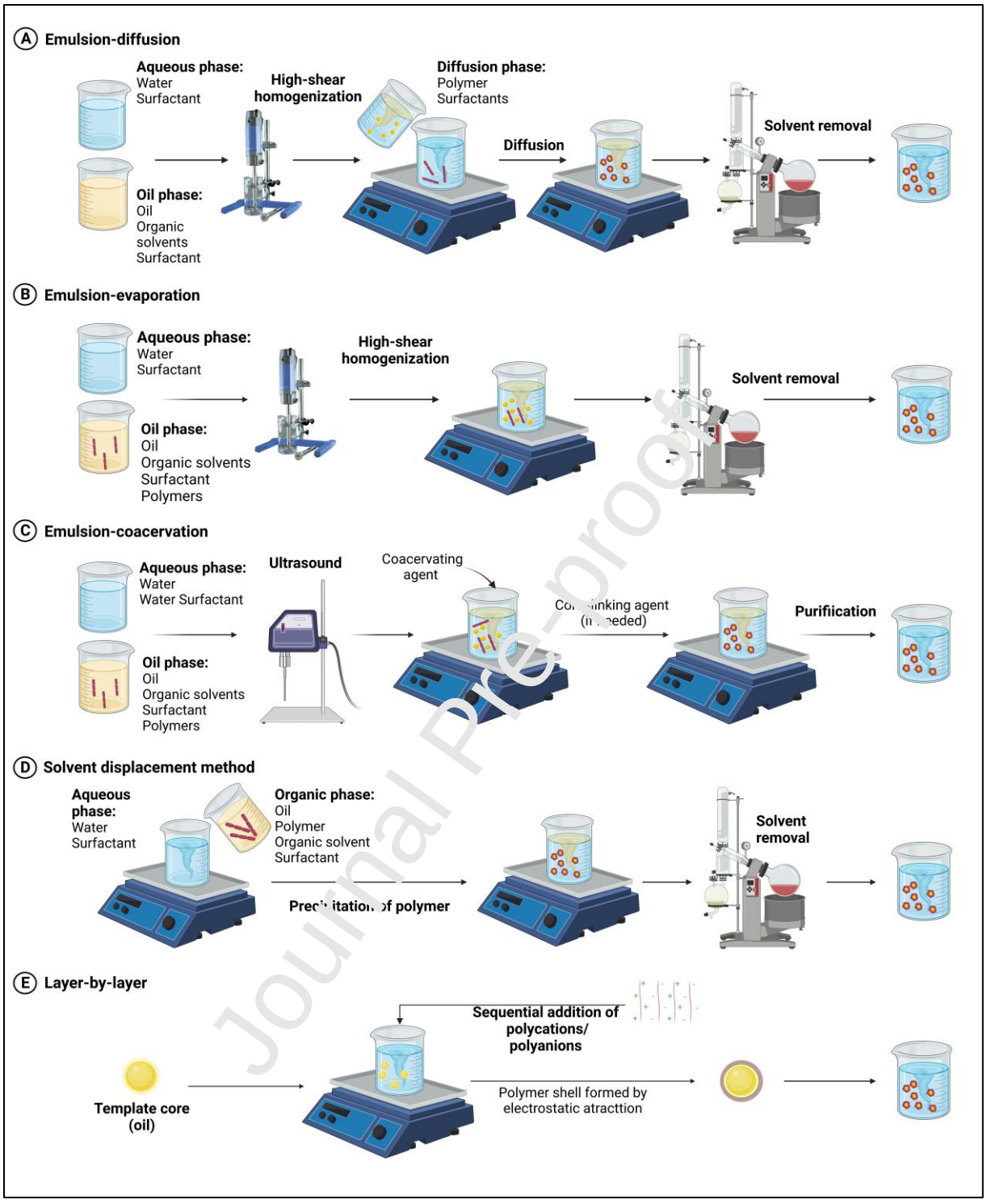


**Figure 6.** Methods for the preparation of LNCs by preformed polymers: (A) emulsion-diffusion, (B) emulsion-evaporation, (C) emulsion-coacervation, (D) solvent displacement and (E) layer-by-layer methods. When no oil is employed during the preparation procedure, the layer-by-layer and solvent displacement methods give rise to polymeric NPs and NCs. Created with BioRender.com.

### 3.3. Methods for SLNs and NLCs preparation

Synthesis of SLNs and NLCs usually employs similar methodologies as those described for LNEs. However, the different physical nature of the lipid component precises adaptations in

some cases. In this section, we focus on modifications of the previously described methodologies.

3.3.1. High-energy methods

The solid nature of the lipid component, where hydrophobic drugs are supposed to be included, usually requires the inclusion of a lipid melting step. The homogenization techniques employ different mechanisms to produce cavitation and shear stress on the sample and break down the particles.[63]

A) High-pressure homogenization

There are two different variants of the high-pressure homogenization method: hot homogenization and cold homogenization (Figure 7A). In the hot homogenization technique, the melted lipids are pre-emulsified with the drug and then introduced into the high-pressure homogenizer, which is kept at a temperature above the lipids melting point. The resulting final mixture is cooled to let the lipid solidify. During this process, the drug stability can be compromised due to the high temperatures maintained during the process.[63] *Schwarz* and coworkers used this method in 1994 to produce SLNs consisting of the triglyceride trilaurin stabilized with soy lecithin and Poloxamer 188. They compared the particle sizes obtained with those obtained using ultrasound, stirring, or the combination of both, and found that the most efficient technique, in terms of particle size and size distribution, was the high-pressure hot homogenization.[61] The cold homogenization process was later developed to avoid drug instability and degradation due to high temperatures. Sample preparation consists of fast mixing of the drug with melted lipids to achieve drug solubilization. This step is developed at high temperature. Subsequently, the mixture is quickly cooled down to produce a dispersion of the solid drug-lipid mixture. This mixture is then subjected to high-pressure homogenization to produce the small-sized particles.[104]

B) High shear homogenization or high-speed stirring

In this method, the drug is homogeneously dispersed in the molten lipids. A hot aqueous phase, containing the surfactants, is then added to the molten lipids, and the mixture is homogenized in a high-shear device (Figure 7B).[105] This technique is frequently combined with a final step of ultrasonication to further reduce particle size and narrow size distribution.

C) Ultrasound

The use of this technique with solid lipids yields large particle sizes and wide size distributions. This is the reason why this technique is usually employed in combination with others, as a further step to reduce the size of preformed NPs (Figure 7C).[105]

D) Membrane contractor method

In this method, pressure is applied to promote the passing of the melted lipids through a membrane (Figure 7D). The lipids form droplets, whose size depends on the pore size of the membrane. The aqueous phase, which contains the surfactants, flows tangentially to the membrane and removes the formed lipids droplets. The emulsion is then cooled down to allow lipids to solidify and form SLNs or NLCs.[106]

E) Film ultrasonic method

The lipidic component and stabilizers are added to the organic phase. This phase is heated to dissolve lipids. The organic solvent is then removed under vacuum, usually at a high temperature. The film formed on the evaporating recipient is then mixed with water and sonicated, to allow the film to redisperse in the aqueous solution (Figure 7E).[107,108]

F) Solvent emulsification and evaporation

In this technique, the hydrophobic ingredients are dissolved in a water-immiscible organic solvent, which is added to the aqueous phase containing the surfactant. After emulsification of the organic phase into the water phase, the solvent is removed from the mixture, usually through evaporation, which leads to the precipitation of the hydrophobic component into the water phase, resulting in SLNs/NLCs (Figure 7F). This method was first described by Sjöström and coworkers, and the emulsification of the organic phase was achieved through high shear homogenization followed by high-pressure homogenization.[109]

G) Supercritical fluids

In this method, a lipid emulsion is first prepared using an organic phase. Supercritical fluids are then employed to extract the organic solvents (Figure 7G). The rapid removal of the solvent leads to lipid precipitation and SLNs or NLCs formation with narrow size distribution.[110] This tecnique achieves higher solvent extraction efficiency than other methods such as evaporation or dilution. $CO_2$ is the most employed supercritical fluid for this method.

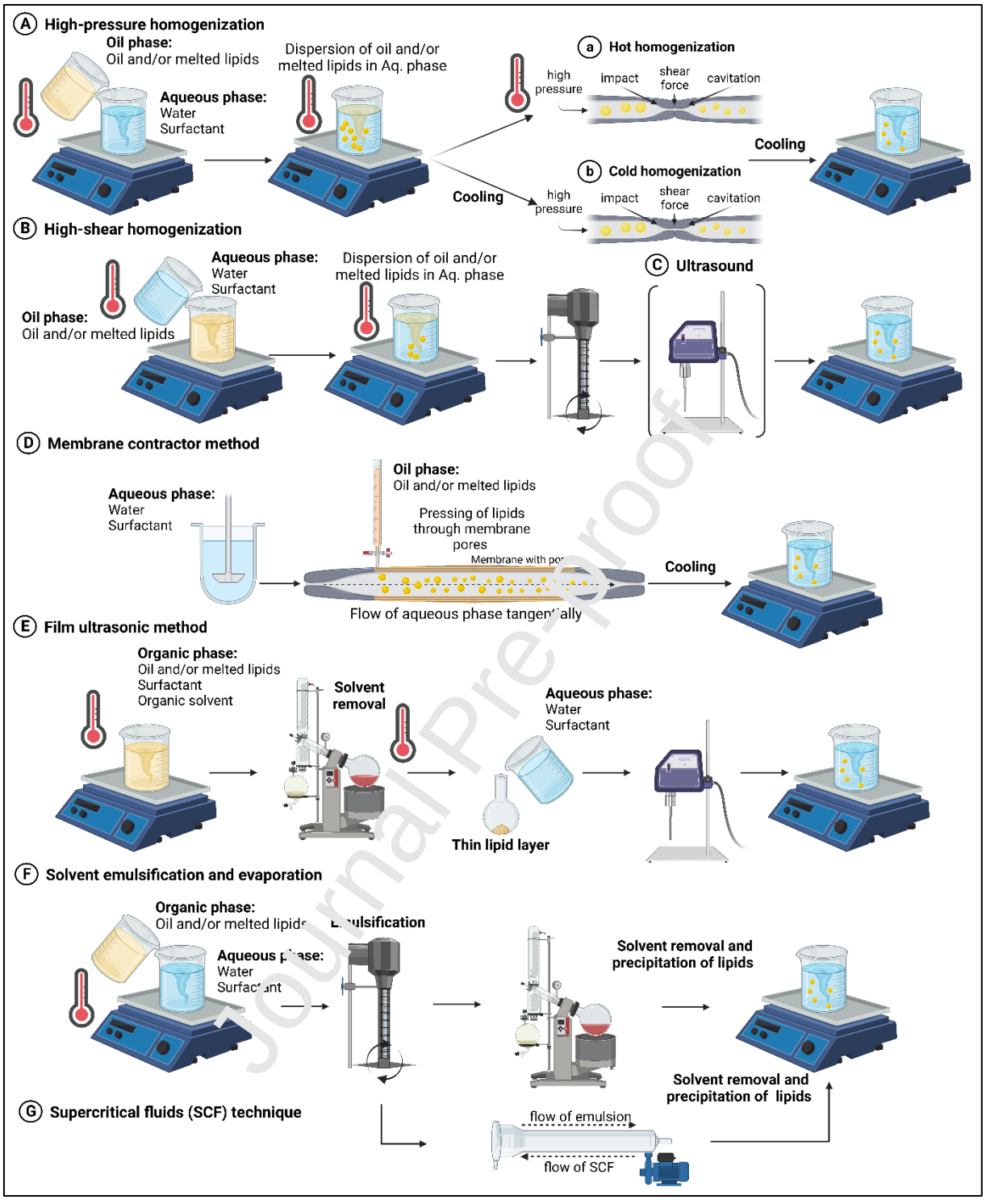


**Figure 7.** High-energy methods for the preparation of SLNs and NLCs: (A) high-pressure homogenization, (B) high-shear homogenization, (C) ultrasound method, (D) membrane contractor method (E) film ultrasonic method, (F) solvent emulsification and evaporation, and (G) supercritical fluids. Created with BioRender.com.

### 3.3.3. Low-energy methods

SLNs and NLCs can be also obtained using low-energy methods. Those preparation procedures are based on LNEs and LNCs techniques reviewed in the previous sections. The solvent-

displacement method, driven by the Ouzo effect,[111–113] the emulsification-diffusion method,[114,115] the PIT method,[116] and the double emulsion procedure [117,118] have all been employed, without adaptation, to prepare SLNs and NLCs. The coacervation method can be adapted to produce SLNs and NLCs. Furthermore, SLNs and NLCs can be prepared with a ME as the starting point.

A) Coacervation method

This technique does not require the use of solvents and uses pH to achieve the precipitation of fatty acids from their sodium salt micelles (Figure 8A). The method was first used by Battaglia *et al*. to prepare SLNs in 2010.[119] The fatty acids sodium salt solution is briefly dispersed with the polymeric stabilizer solution. The mixture is heated to achieve fatty acid solubilization. The pH of the solution is then acidified, leading to the fatty acid precipitation. Ultimately, the solution is cooled down to achieve lipid solidification. The starting point of this method is a soap, i.e., a fatty acid sodium salt. In an acidic medium, it exchanges the ionic sodium with a proton, which makes the molecule more soluble. This process, defined as coacervation, reduces the solubility of the molecule and drives precipitation of the fatty acids.[119] .

B) Microemulsion method

This method was developed and patented in the nineties by Gasco *et al.* to produce solid lipid microparticles (Figure 8B). Briefly, the lipids and the cargo are heated above the melting point of the lipids. The lipid solution is mixed with an aqueous phase, which is at a temperature equal to or above the lipids melting point and contains the surfactant and co-surfactant. This mixture is kept at a high temperature to obtain the ME. Finally, the hot ME solution is added, under stirring, over a cold-water solution (2 – 10 °C), resulting in lipid solidification and the formation of the microspheres.[120]. Another variant of this technique consists of the cooling of the hot ME without water addition. The cooling process of the solution under stirring drives the solidification of the lipids and the reduction of the particle sizes, yielding SLNs and NLCs with sizes below 300 nm.[121]

C) Cold-burst method

Recently, Cholakova et al. reported a novel cold-burst process for the preparation of SLNs. In this novel low-energy method, a preformed LNE is cooled so that the dispersed nanodrops freeze into solid lipid particles (Figure 8C). The dispersion is then heated to the lipid melting point provoking lipid particles to spontaneously disintegrates into SLNs. The low surfactant

content (<2%) and high drug loading (50%) of this method demonstrates a new strategy for scalable emulsification technology.[122]

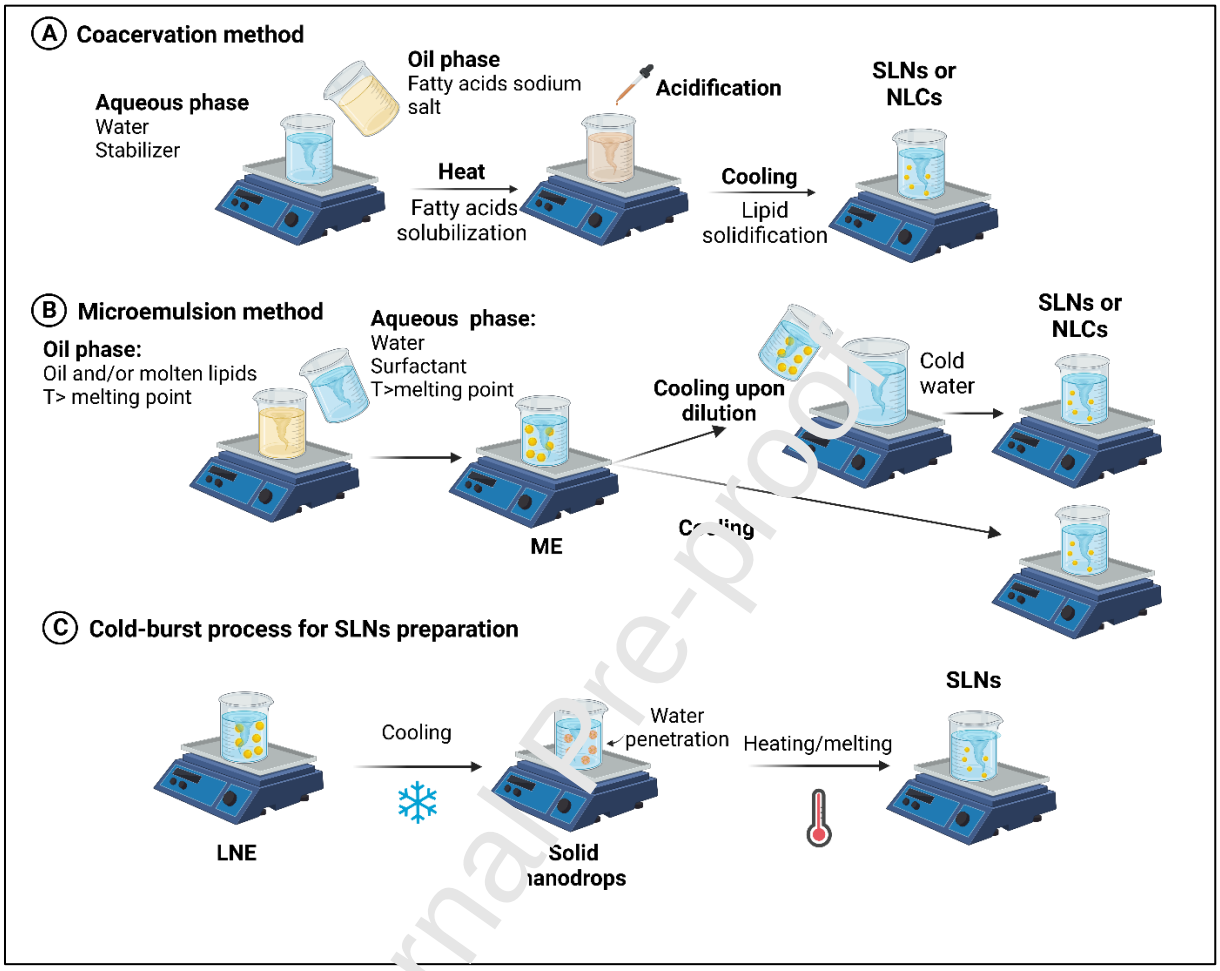


**Figure 8.** Specific low-energy methods for the preparation of SLNs and NLCs: (A) coacervation method, (B) microemulsion method, and (C) cold-burst method. Created with BioRender.com.

## 4. Drug administration routes and tumor targeting

In a successful treatment, therapeutic agents must pass a series of biological barriers depending on the administration route employed. Nanomaterials used for targeting tumor cells aim to increase the local concentration of drugs in and around tumor cells, thereby reducing potential toxicity toward healthy cells and decreasing the off-target effects of the treatment.[123] Nanocarriers can improve the biodistribution, bioactivity and bioavailability of the encapsulated therapeutic products. The fate of drugs in the organism is no longer determined only by their properties, but also by the type of drug-delivery nanosystem. Table 1 summarizes the main factors and barriers of each route of administration when working with colloidal systems.

Table 1. Summary of the main threatening factors and characteristics of each route of administration regarding colloidal drug-delivery systems.

| Administration route | Barriers and threats for the colloidal systems | | | | Distribution in the organism | Advantages | Disadvantages | References |
|---|---|---|---|---|---|---|---|---|
| | Physical | Chemical | Biological | Interfacial changes | | | | |
| Intravenous | | | | Protein-corona | Systemic circulation | Direct route to systemic circulation | Qualified person for drug administration | [124–127] |
| Oral | Peristalsis<br>Mucus layer<br>Gastrointestinal epithelium | pH changes<br>Surfactant (bile salts) | Immune system<br>Digestive enzymes<br>First pass metabolism effect (intestinal and hepatic)<br>Microbiota activity | Protein-corona<br>Enzymatic digestion | Systemic circulation<br>Lymphatic drainage | Self-administration<br>Bypass first pass hepatic effect<br>Great absorptive surface<br>High drainage | Digestive process | [128–137] |
| Rectal | Mucus layer<br>Epithelium | Enzymes | Immune system<br>Microbiota activity | Protein-corona | Systemic circulation (Hemorrhoidal vein)<br>Lymphatic drainage | Self-administration<br>Bypass first pass metabolism effect | Low patient compliance<br>Smaller absorption surface owed to the absence or villi and microvilli | [138–140] |
| Ocular | Blinking | Tears | Immune system<br>Enzymes | Protein-corona | Systemic circulation | Self-administration | Continuous and fast tears turnover<br>Ocular structural barriers | [139,141,142] |
| Intranasal | Mucus layer<br>Epithelium | Enzymes | Immune system | Protein-corona | Systemic circulation<br>Direct route to brain | Bypass blood-brain barrier (via olfactory bulb)<br>Self-administration | Control the aerosol characteristics to control the deposition area of the particles<br>Mucus turnover | [143–146] |
| Pulmonary | | Surfactant | Immune system | Protein-corona<br>Surfactant | Systemic circulation<br>Lymphatic drainage | Great absorptive surface<br>High drainage<br>Self-administration | | [143,144] |
| Vaginal | | Acidic pH | Immune system<br>Microbiota activity | Protein-corona | Lymphatic drainage | Self-administration<br>Bypass first pass metabolism effect<br>Prolonged and continuous release | Changes in the pH, cervicovaginal fluid efflux, thickness of the mucus layer depending on age, hormonal or physiological state | [141,147,148] |
| Transdermal | Epithelium: epidermis, dermis, hypodermis | | Immune system | Protein-corona | Systemic circulation<br>Lymphatic drainage | Self-administration<br>Bypass first pass metabolism effect<br>Prolonged and continuous release | Interaction with the extracellular matrix | [141,142] |
| Subcutaneous | Extracellular matrix<br>Lymphatic vessels epithelium | Enzymes | Immune system | Protein-corona | Lymphatic drainage | Self-administration<br>Bypass first pass metabolism effect | Small volume doses<br>Interaction with the extracellular matrix | [149,150] |
| Intramuscular | Extracellular matrix | Enzymes | Immune system | Protein-corona | Systemic circulation | Self-administration | Small volume doses | [151,152] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blood and lymphatic vessels epithelium | | | | Lymphatic drainage | Bypass first pass metabolism effect | | |

4.1. Intravenous route

The intravenous route (IV) is the most common route of administration of nanomaterial-based anticancer drugs, as it is the most direct one to the systemic circulation.[124]. Effectiveness of the treatment is achieved when the administered drug arrives with proper dosage and displays activity in cancer cells. However, this is not easy to achieve. Once NPs enter the bloodstream, they find a complex environment designed to recognize external elements. NPs must overcome different obstacles such as the interaction with plasma proteins and the formation of a protein corona (PC) or their clearance from the bloodstream by the mononuclear phagocyte system (MPS) and the complement system. In addition, the delivery of NPs to the target tissues can be classified as passive or active targeting. Furthermore, properties such as size and charge will determine their biodistribution and performance.

4.1.1. Protein corona formation

When nanosystems are in a physiological environment, they quickly adsorb biomolecules, such as proteins and lipids, on their surface. This PC can be divided into “hard” and “soft” corona, depending on the strength of the interaction. The PC may change its composition if NPs moves to another compartment or biological fluid.[153] This corona surrounding the particle changes its original surface charge, size, solubility, aggregation and, therefore, the interaction of NPs with cells, thus influencing traffic, biodistribution, and cellular absorption.[125] Furthermore, PC influences macrophage uptake. For instance, opsonins such as IgG, complement factors, and fibrinogen promotes phagocytosis, removal of NPs from the bloodstream and concentration in the liver and spleen, while dyopsonins, such as albumin and apolipoprotein, promote longer circulation times of NPs in the body.[126] While the clearance of many nanocarriers from the bloodstream is a question of minutes, interaction with distant cells may take hours or days. Therefore, the success of the nanocarrier highly depends on its blood circulation lifetime. Furthermore, PC can cover the surface of nanosystems and, therefore, strongly reduce the ability to target and recognize cellular receptors.[154] Hence, understanding the formation of the PC around the NPs is essential in predicting the system performance.

4.1.2. How to Avoid Immune System Clearance

NPs can be designed to prevent immune system elimination and increase their circulating half-life in the blood, allowing for a continuous and controlled drug release in the vascular

compartment. A relatively successful approach to prolong blood circulation time of NPs is to create a steric/hydrophilic surface barrier of sufficient density. Hydrophilic polymers, such as PEG, or surfactants, such as poloxamers and poloxamines, have been investigated to reduce the adsorption of blood proteins and opsonins and, therefore, increase the half-life of nanosystems. The addition of PEG has been widely used with this purpose and is the most employed method for "masking" NPs. This process is also known as PEGylation.[155]. Several studies have been conducted to determine how a change in the thickness and density of a PEG coating affects opsonization and biodistribution, showing that the degree of protein adsorption depends on the size of the PEG and graft density.[156,157] Poloxamers, also known as Pluronic® and poloxamines or Tetronic®, are non-ionic block copolymers of hydrophobic propylene oxide (PPO) and hydrophilic ethylene oxide (PEO). Poloxamers consist of a central PPO moiety, flanked on both sides by two PEO chains while poloxamines are tetrafunctional block copolymers with four coupled PEO-PPO blocks linked by a central bridge of ethylenediamine.[158] The adsorption of these molecules on the surface of NPs through their hydrophobic PPO fragments provides stability to the suspension by a repulsion effect through a steric stabilization mechanism. NPs designed with poloxamers and poloxamines exhibit reduced adsorption of blood proteins and opsonins and, as a result, resist ingestion by phagocytic cells and remain in the systemic circulation for a prolonged period. [159] However, the foreign nature of synthetic polymers should be considered. For instance, an acquired immune-response to PEG moiety that compromises PEG-NPS performance has been reported. [160] To solve these limitations, biomacromolecules such as polysaccharides and proteins have been also employed as coating material for colloids due to their biocompatibility and biodegradability. [161] In addition, an emerging approach in the masking of NPs is cell membrane nanotechnology.[162] This technique, first reported in 2011, [163] consists of the deposition of a bioactive layer of a cell membrane directly onto the surface of NPs. The consequent transference of its lipids, protein and carbohydrates, enables the resultant membrane coated-nanoparticle to take on characteristics of the source cell, such as their biocompatibility and immune-evasion properties, along with tropic and targeting effects.[162,164]

4.1.3. Passive targeting

At tumor sites, the vascular barrier is disrupted due to failed rapid growth of blood vessels in angiogenesis, thus enabling nanocarriers to cross and accumulate in the tumor tissue.[165] The gaps between endothelial cells in the tumor vasculature can range up to 2000 nm depending on the tumor type, localization and environment.[165] Moreover, due to poor lymphatic function,

NPs are not rapidly cleared and are accumulated in the tumor interstitium.[166] This is known as the enhanced permeability and retention (EPR) effect, which is the basis of passive targeting.[167] This accumulation of the drug at tumor sites is a passive process, and requires prolonged circulation of the drug for appropriate delivery. The accumulation of nanocarriers is essentially dependent on their physicochemical properties such as size, morphology, surface charge and chemistry as will be explained in section 4.1.5 mentioned.[168] Furthermore, biodistribution of the drug is also influenced by blood perfusion, passive interactions with biomolecules along the route and immunological clearance processes such as phagocytosis or renal clearance.[169,170]

4.1.4. Active targeting

Active targeting, also known as the ligand-mediated targeted approach, involves affinity-based recognition, retention and facilitated uptake by the target cells.[171] Biomolecules such as antibodies, proteins, nucleic acids, peptides, carbohydrates and vitamins are employed as ligands.[172,173] The target substrates can be: surface molecules expressed in target cells, proteins, sugars, lipids or molecules present in the organs or in the microenvironment of cells.[174] Intelligent and targeted systems based on nanomaterials exploit the multivalent nature of ligand interactions with the target antigens. When multiple ligand molecules accumulate in nanosystems, there is a general increase in the avidity of NPs to their related objective.[175] In addition, the binding of a ligand molecule generally facilitates the binding of consequent molecules through cooperative effects, collectively improving binding efficiency and subsequent actions.[176] However, there are other aspect related to the concentration of ligand in the NPs that should be considered. For instance, the concentration and nature of the ligand in the NPs surface will determine the orientation of such ligands at NPs' surface, and therefore their targeting capabilities.[123] Generally, covalent conjugation methods are employed, but systems with physical adsorption using affinity complexes are also used effectively.[177] The critical aspect of this conjugation is to maintain the stability of the conjugated ligands during the adverse physiological environment.[178] The main angiogenic targets explored by NP systems for therapeutic benefit include vascular endothelial growth factor receptors (VEGFRs), αvβ3 integrins, matrix metalloproteinase receptors (MMPs), and vascular cell adhesion molecule-1 (VCAM-1).[179]

Monoclonal antibodies (mAb) were the first and are still the preferred class of targeting molecules since conjugated antibodies enhance uptake and cytotoxic potential of NPs in tumor cells. The first mAb to gain FDA approval for the treatment of cancer was Rituximab in 1997,

a chimeric mAb used for the treatment of B-cell non-Hodgkin's lymphoma. Trastuzumab, in 1998, a humanized mAb used for the treatment of HER2 expressing breast cancer, quickly followed. Cetuximab, which binds to epidermal growth factor receptors (EGFR), was approved for treating colorectal cancer in 2004 and head/neck cancer in 2006. Bevacizumab, a tumor angiogenesis inhibitor that binds to VEGF, was approved for treating colorectal cancer in 2004. Recent studies have tried to encapsulate chemotherapeutic drugs into NPs and then functionalize the particle surface with mAbs to maintain targeting efficacy.[180–182] Some peptide sequences have been also employed due to the high affinity for tumor-associated receptors and, in this context, peptide-based targeting of tumor-associated receptors has emerged as a potential tumor-specific chemotherapeutic agent. Cell permeating and fusogenic peptides from pathogens or toxins and peptides randomly derived from technologies such as phage display, are commonly used for targeting purposes.[183] Among single nuclear localization peptides, the trans-activating transcriptional activator peptide has been shown to be an efficient molecule for translocating NPs into cell nuclei *via* the binding import receptors importin α and β. In 2012, a peptide was used to conjugate onto mesoporous silica NPs for nuclear-targeted drug delivery of DOX for the first time.[184]

Ligand conjugation on the NP surface changes the properties of the targeting molecules along with the nanocarrier.[185,186] The ligand-NP conjugation provides a greater targeting capacity for the resulting nanocarrier, although with a detriment of the rotational and translational freedom of the conjugated ligand.[187] On the other hand, the size, geometry, surface properties (charge and hydrophobicity) and the composition of the NPs can also be altered. In some cases, NPs have demonstrated benefits that go beyond simple drug release, such as greater resistance to degradation by nucleases of nucleic acid chains immobilized on the surface of nanomaterials.[188]

4.1.5. Properties affecting NPs performance

A) Size and morphology

The size and shape of the nanomaterial should be considered when designing NPs, as it affects the way in which the organism "sees" them and, therefore, determines their distribution and pharmacokinetic profile.[189] For spherical particles, smaller sizes represent higher curvatures, which may be problematic for ligand functionalization after synthesis, along with increased toxicity.[190] The kidneys effectively remove, through blood filtration, NPs with diameters smaller than 10-20 nm. Filtration through inter-endothelial slits in the walls of the splenic sinus removes particles of more than 200 nm. These filters suggest that the size of NPs should be

greater than 20 nm but not more than 200 nm if prolonged circulation within the body is desired. In addition to the effect on circulation properties and accumulation in tumors,[191] the shape of NPs seems to influence the kinetics of cell internalization by modulating the interactions between the nanomaterial and the cell surface.[192]

B) Surface and ligand charge

Surface characteristics of NPs, such as charge, chemical moieties, and nature of the materials, can define the NPs toxicity. Most of the currently available studies point out to the surface charge as the main surface-related parameter affecting the toxicity of the system. [193,194] Positively charged surfaces seem to be more toxic than negatively charged and neutral NPs.[195,196] Moreover, the surface charge can favor or hinder the approach between NPs and cells. Thus, surface charge defines the interacting relations of the NPs with the different cells in the organism, affecting their uptake and distribution. In addition, surface properties determine the stability of the system in the biological medium. They influence the formation of the protein-corona and the aggregation or not of the system.[197] The surface is the site of ligand functionalization. From a synthetic perspective, the charge of both the non-functionalized NPs and the ligand, can affect conjugation performance and spatial configuration of the ligand on the surface, due to repulsive or attractive forces.[198,199] A chemical spacer of reasonable length, such as those based on PEG units, can help reduce this effect, but can simultaneously complicate synthesis and increase final particle size.[200] In addition, since most ligands are charged molecules, the final surface charge of NPs is determined by the combination of ligand densities, materials, and formulation strategies.

C) Hydrophobic/Hydrophilic surface

From a colloidal point of view, hydrophilic nanoparticles are more stable than their hydrophobic counterparts. Solvation, *i.e.* the adhesion of water molecules onto hydrophilic surfaces, makes difficult for hydrophilic particles to touch because of the present “hydration pressure”, whereas “hydrophobic effects” may cause the aggregation of the system.[201] In terms of cellular uptake, hydrophobic NPs are more rapidly internalized. However, they also tend to agglomerate and are earlier removed by the MPS.[201] Hydrophobicity can also affect the presentation of the ligand.[202] This is especially evident for NPs possesing a hydrophobic nucleus since the ligand could be trapped within the core and, thus, not properly exposed on the surface. [203]

4.2. Oral route

The oral route is the oldest route used for drug administration due to ease of use, less expensive manufacturing, and high patient compliance. However, in cancer therapies, the oral route is not the most employed due to the physiological conditions and barriers at the gastrointestinal tract (GIT). Poor solubility of some of the chemotherapeutic drugs and their degradation along the GIT lead to low compound bioaccessibility. Moreover, low permeability at the intestinal level further reduces the bioavailability of chemotherapy drugs. On the other hand, this administration route can be used for local drug administration along the GIT or for systemic drug delivery.

With regard to oral administration, the bioavailability of a compound depends on its bioaccessibility and bioactivity. In this case, bioactivity includes absorption at the intestinal level and pre-systemic metabolism, which includes intestinal and hepatic first pass metabolism, its ability to enter the systemic circulation and maintain its functionality regardless of the interaction with other biological entities, and finally, the ability to reach the target entity.[128] The first problem that NPs help to solve is the solubilization of hydrophobic drugs. Some of the antitumor drugs currently used in clinics, as well as many bioactive compounds which are under investigation as antitumor drugs or as co-adjuvant in cancer therapies, are insoluble in aqueous media. The lipidic core of LCNPs is able to solubilize hydrophobic compounds in a highly efficient way. Thus, LCNPs contribute to increased bioaccessibility of those compounds by increasing their solubility.

4.2.1. GIT absorption

The role of the GIT is to break down food into absorbable components and allow the absorption of such nutrients. At the same time, the GIT provides a physical barrier which hinders the entrance of microorganism and toxic compounds into systemic circulation.[204] From the mouth to the anus, the GIT is divided into compartments, each with different functions and physicochemical conditions, posing a threat to orally administered drugs and limiting bioaccessibility and bioavailability. Those adverse conditions comprise ionic strength and physiological medium pH, which are important factors to consider when working with colloidal systems,[129] as well as enzymatic digestion and peristaltic movement, which can further contribute to nanocarrier aggregation. Moreover, bile salts released in the intestine are also concerning when working with NPs due to their ability to displace surfactants from NPs shells.[130–132] Finally, microbiota from the large intestine metabolizes components which cannot be digested by human enzymes, such as complex carbohydrates and proteins.[128] This

fact can be used to achieve colon-targeted drug delivery by providing the LCNP with a chitosan, pectin or alginate shell.[205] Shell composition of the nanocarriers can determine the behavior of the system along with the changing conditions on the GIT. For instance, the inclusion of hyaluronic acid (HA) on the shell of albumin-coated LLNs improves the retention of curcumin (CUR) under gastric *in vitro* simulated digestion conditions.[206] Moreover, we can take advantage of different conditions along the GIT to achieve a controlled release of a loaded compound or to achieve the absorption of NPs in a specified portion of the GIT.[207]

If NPs or their loaded compounds achieve to survive the digestion process, then, they have to diffuse through the mucus layer, a viscoelastic gel which acts as a filter and allows some particles to arrive at the brush border surface, *i.e.*, the absorptive intestinal surface, while hindering pathogens or toxins diffusion.[133] A too weak interaction with the mucus layer will lead to the direct transit and excretion of the NPs from the GIT. The inclusion of mucoadhesive polymers on the shell is a commonly used strategy to prolong the residence time of the nanocarrier in the GIT and to enhance the absorption of carried drugs at intestinal level. However, a too strong interaction with the mucus may result in the retention of the nanocarrier and its excretion, together with the mucus, owing to the continuous turnover of this layer.[208] On the other hand, mucus penetrating NPs easily diffuse through the mucus layer. These nanocarriers can modify the mucus structure and open 'gaps' in the mucus mesh, which allow them to diffuse. These nanocarriers have coatings which weakly interact with the mucus barrier.[209,210]

4.2.2. First pass metabolism

Once drugs arrive at the epithelial surface, they must deal with the so-called first pass metabolism, first carried out by the intestinal cells and, later, by the hepatic cells. First pass intestinal metabolism comprises the action of brush border enzymes and intracellular metabolism in the gut cells.[134] Both, enteric and hepatic intracellular metabolisms, follow the same metabolic procedure and are divided into three different phases. Enzymes involved in phases I and II carry out the chemical modification of xenobiotics to make them more chemically-reactive and more soluble.[211] Phase III includes the traffic of molecules into and out of the cells. The main efflux transporter limiting drug absorption both in enterocytes and hepatocytes, is P-glycoprotein (P-gp).[212] During this phase, some of the compounds will be excreted from enteric cells back to the lumen or from hepatocytes to the bile canaliculus. In the case of first pass enteric metabolism, 'surviving' compounds will enter portal vein circulation thanks to the mentioned transporter and will be further exposed to suffer first pass hepatic

metabolism. The compound which ultimately surpasses hepatic metabolism will enter central vein and systemic circulation.[135]

However, for lipids, there is an alternative pathway to enter systemic circulation. This way implies passing across the cells, in the form of chylomicrons, and arriving at the lamina propria. Here, lipids can enter the lymphatic circulation, helping carried-drugs bypass first pass hepatic metabolism and reaching systemic circulation.[134] Once there, the absorbed system will face the same challenges as those of intravenously administered systems (section 4.1).

Structural differences between blood vessels and lymphatic vessels are responsible for the differentiated absorption.[136] The more compact structure of blood vessel endothelium leads to the absorption of molecules mainly through transcellular transport (across the cell), while the less compact structure of lymphatic endothelium makes them more permeable and allows for absorption *via* paracellular transport (through intercellular spaces). Thus, high molecular weight molecules and bigger particles, such as colloidal structures (where chylomicrons are included) have preferential access to lymphatic vessels.[137] The lymphatic system plays an important role in metastasis.[136,213] Hence, NPs and drug circulation through the lymphatic system can promote immune response and become a pathway to reduce metastasis by attacking circulating cancer cells and acting on lymphoid metastasis. A schematic and comparative representation of both intravenous and oral administrations and their main challenges are presented in Figure 9.

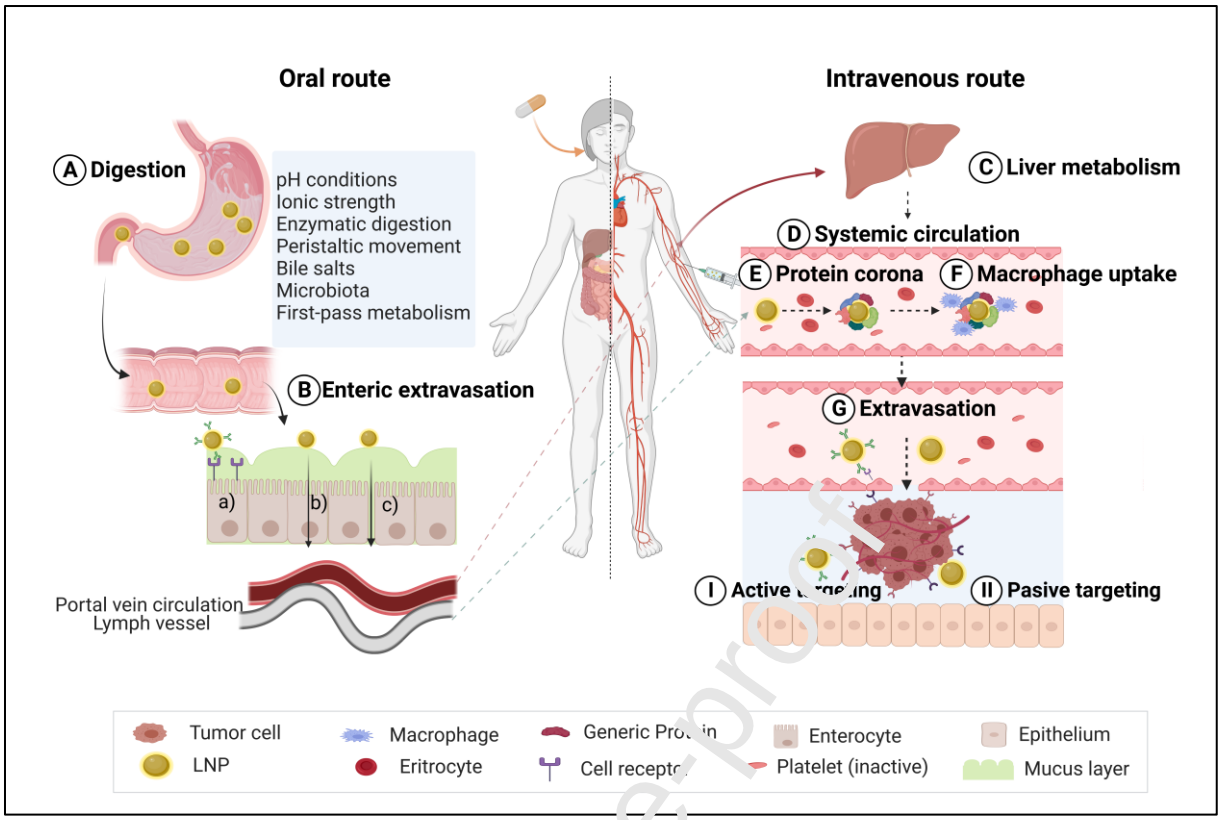


**Figure 9**. Schematic and comparative representation of the oral and intravenous routes of administration for a colloidal drug-delivery systems. When the colloidal nanosystem is administered orally, it is absorbed by the GIT and needs to surpass the (A) digestion process and (B) the enteric epithelial barrier along with the first-pass metabolism. Compounds absorbed into portal vein circulation will suffer (C) hepatic metabolism, and finally, surviving compounds and those absorbed into the lymphatic system will enter (D) systemic circulation. Once in the systemic circulation, the colloidal nanosystem will encompass the (E) protein corona and the (F) macrophage uptake before it can (G) extravasate and arrive at the site of action, where it performs its therapeutic effect. The protein corona and the macrophage uptake are also active biological barriers in the lymphatic system. Created with BioRender.com.

### 4.3. Mucosal absorption for systemic drug delivery

Mucosal administration does not require special training, making patient self-administration possible, which is advantageous when compared to the intravenous route. Mucosal surfaces include all the biological surfaces producing mucus, a viscoelastic gel secretion, to protect a part of the organism in contact with the external medium. Therefore, the digestive system is included in this category. However, considering the relevance and the complexity of the digestive process, we have dedicated a separate section to the oral route of administration (section 4.2). Barriers that NPs encounter during mucosal absorption are similar: mucus protective layer and its clearance rate, epithelial barrier which determines transport (transcellular or paracellular) and, finally, absorption by blood or lymphatic vessels. Nevertheless, the previously described first pass metabolism, a limiting factor in the oral administration route, can be avoided by using other transmucosal administration routes, such

as rectal, nasal, pulmonal or vaginal. Each mucosal surface possess different characteristics, such as enzymatic activity, mucus layer thickness, pH and hydration, presence of specific microorganisms or immune system entities (cells, like macrophages, or molecules) and different absorptive surface or different drainage (portal drainage to the liver or systemic vein or lymphatic drainage, which bypasses liver first pass effect).[138,139,214]

Drug administration via the skin, vagina, eye and nose are considered topical administration forms when the objective is localized treatment. Topical application of drugs is frequently used to act directly on the deposition area, since it avoids the drawbacks involved in systemic circulation.[141] However, those administration routes also offer a pathway for the systemic administration of drugs. In this section we will focus on the use of mucosal surfaces for systemic drug administration, emphasizing the unique characteristics and challenges of each route.

4.3.1. Digestive system-related administration routes. Rectal, sublingual, and buccal administration

Rectal, sublingual, and buccal drug administration are alternative routes involving the digestive system. Rectal administration is a promising route, however not as widely used as the oral route due to less patient compliance. Physiological characteristics of the rectum include lower water content and the absence of villi or microvilli, which leads to a smaller absorption surface compared to the small intestine. The rectum is surrounded by rectal (hemorrhoidal) veins and lymphatic vessels. Moreover, in this part of the large intestine, enzymatic threatening activity is very low. It is also interesting to take into account that drainage of the upper part of the rectum occurs in the portal vein, following first pass liver metabolism, while the lower part of the rectum empties into the vena cava, bypassing first pass metabolism, similar to lymphatic drainage.[138,139]

4.3.2. Intranasal administration

The respiratory system also provides a window for the entrance of drugs. This administration route involves the inhalation of aerosols. Controlling the characteristics of the aerosolized particles is crucial to ensure drug deposition on the targeted area.[143] Nasal and pulmonary delivery are the most frequently used routes in the respiratory system. In these areas, the mucus layer and its clearance rate are limiting factors for drug absorption, with 20 min being the renewal rate for the nasal mucus layer and 10-20 min for the respiratory tract. [143] On the protective mucus layer, NPs are also exposed to macrophage attack and to surfactants such as phospholipids,[144] which may compromise NPs stability. Pulmonary administration can be

driven to local treatment, but since the alveolar region provides a great absorption surface, thanks to ciliated cells, and high drainage to the lymphatic and circulatory systems, drugs may enter systemic circulation quickly, bypassing hepatic metabolism. This provides the possibility of efficient systemic drug administration through the pulmonary system. On the other hand, the nasal route is especially interesting for brain drug administration, since delivery of drugs through the olfactory pathway allows to bypass the blood-brain barrier (BBB).[145] Moreover, the olfactory bulb provides a direct neural route to the brain, avoiding systemic circulation and consequent metabolism.[146]

4.3.3. Vaginal administration

With regard to vaginal administration of drugs, it is necessary to consider the special characteristics of this area, such as the acidic pH because of the resident microbiota, the thickness of the mucus secretion layer, which can vary depending on age, hormonal and physiological state, and the continuous efflux of cervicovaginal fluid, which can reduce the retention time of the drug, limiting its absorption [141] This administration route is mainly used for topical treatment. However, systemic administration of drugs though this route has been proved to be effective and, even preferable, in some cases. An example of vaginal administration of compounds at a systemic level is the widely used vaginal ring for hormone sustained delivery over long periods of time.[147] This route allows prolonged and continuous release, maintaining more constant levels of the drug in the systemic circulation, and requires smaller amounts, when compared with the oral route.[148] On the other hand, the extensive vascularization of the vagina, as well as the lymphatic drainage in the area, allow for the absorption of compounds into the systemic circulation, avoiding the first pass hepatic metabolism. [147]

4.3.4. Ocular administration

The administration of drugs to the eye is normally used for topical local treatment because the physiological and anatomical barriers to overcome hinder the task. Ocular surface is continuously cleaned by tears, which dilute and reduce the residence time of the administered drugs.[141,142] Besides tear turnover and drainage, ocular administration presents other barriers. The cornea provides a tightly packed cell layer that hinders the passage of hydrophilic and ionic compounds. After crossing the cornea epithelium, lipophilic compounds find the stroma of the cornea, which is a hydrophilic space acting as a barrier and retaining hydrophobic compounds.[139] This is a disadvantage because drug accessibility to the systemic circulation

is reduced. However, the retention of hydrophobic compounds provides a drug reservoir and allows sustained drug delivery.[139]

4.4. Other administration routes

4.4.1. Topic application

The skin barrier is about 3 mm thick and is divided in three layers: the epidermis, which is the avascularized outer layer, the dermis, widely vascularized and located immediately under the epidermis and, finally, the innermost hypodermis layer. The epidermis is the biological barrier that protects the body from microorganism invasion and preserves body homeostasis. The dermis acts as thermal protection and the hypodermis provides a protective mechanical barrier. Despite skin drug administration being mostly used for topical application, some drugs are permeable through the skin barrier and can achieve systemic circulation. This transdermal route bypasses first pass metabolism, allowing the plasma drug level of certain drugs to be maintained.[141,142]

4.4.2. Intramuscular application

Intramuscular administration of drugs allows for fast systemic absorption, avoiding first pass hepatic metabolism. However, this absorption will depend on the vascularization and blood flow of the chosen muscle. Thus, high blood flow will promote faster drug absorption in the systemic circulation.[151] Moreover, muscle choice for drug administration will depend not only on previously mentioned blood flow, but also on the volume dose to be administered, which is frequently low (2–5 mL in humans).[152]

4.4.3. Subcutaneous application

Subcutaneous administration implies the deposition of the administered formula in the hypodermis.[149] The extracellular matrix of this interstitial area is mainly composed of collagen and hyaluronan. This last molecule forms a gel, which limits the diffusion of the component injected in the area.[150]

The subcutaneously administered formula is drained from the extracellular matrix to the circulatory system or the lymphatic system, depending on the size and the physicochemical characteristics of the molecules or the colloidal system. The higher flow rate of the vascular system would provide more efficient drainage from the interstitial area. However, the permeability of vascular endothelium limits absorption, mainly absorbing only small molecules. An alternative drainage route is through the lymphatic system.[150] Lymphatic

absorption of the drug, as previously discussed, will allow the drug to enter the systemic circulation and avoid the first pass metabolism effect. However, interactions with the extracellular matrix should be carefully evaluated for the specific system, since high interaction between both would hinder the diffusion and absorption of the NPs. [149] Other factors that must be taken into account are the possible enzymatic degradation or cellular immune system attack.

## 5. LCNPs in cancer therapy

Cancer is considered a heterogeneous disease which includes a variety of subtypes with unique morphologies and clinical behaviors. The first line chemotherapy treatment for cancer is the use of broad spectrum anticancer drugs such as DOX, DTX, and PTX.[215] These antineoplastic compounds present major drawbacks, such as the lack of specificity and their rapid clearance from the body. This limitation causes patients to be treated at the maximum-tolerated dose of these antitumor compounds, thus suffering several adverse and off-target effects. Another major cause of treatment failure is multi-drug resistance (MDR). The most studied mechanism of MDR is the overexpression of drug efflux pumps, belonging to the adenosine triphosphate binding cassette (ABC) transporters family, which pump drugs from inside the cell to the outside. The main ABC transporters clinically associated with MDR are P-glycoprotein (Pgp/ABCB1) and MDR related proteins (MRPs/ABCCs).[216] Since anticancer drugs are used at maximum-tolerated doses, a small increase in drug resistance is enough to make chemotherapy ineffective and making it impossible to overcome drug resistance by increasing the dose.[217]

At present, nanotechnological solutions are employed to surpass those limiting aspects. However, clinical translation of NPs remains a challenge, as it requires a detailed understanding of the physicochemical properties of nanosystems, internal and external structure, chemical reactivity and stability, biodistribution, toxicity and biocompatibility, among other factors, especially for biomedical applications and cancer therapy. In this regard, LCNPs offer significant advantages compared to other nanoparticulate drug-delivery systems.

LCNPs can solubilize promising hydrophobic compounds with antitumoral effects with limited application through conventional methodologies, thus allowing their application in cancer nanotherapy. For instance, aromatase inhibitors (AIs), employed in the treatment of estrogen-receptor (ER) positive breast cancers, have poor aqueous solubility. Since about 75% of breast cancers are ER-positive, the development of nanocarriers that can effectively encapsulate and deliver AIs arises as a promising approach to breast cancer treatment. [218] Similarly,

LCNPs allow the application of other interesting hydrophobic antitumoral agents such as Camptothecin (CPT), fisetin, melphalan, β-carotene, or Citral.[219–223] Furthermore, lipids can serve as a skeleton for the preparation of interesting lipid-drug conjugates, such as the lipidated C16-DOX prodrug, which can then be included within LCNPs.[224]

In addition, lipids which possess bioactive activities can be employed as the core of LCNPs, thus generating LCNPs with interesting inherent properties that can be further loaded with other chemotherapeutics. That is the case of $\omega$-3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA). Several reports indicate that DHA is an effective adjuvant of conventional anticancer drugs that improves the antitumoral efficacy while reducing the side effects of the therapy.[225] Selol, an oily mixture of triglycerides with reported cytostatic effect, has also been employed to prepare LNCs co-encapsulating vincristine and DOX.[226] Similarly, we reported the preparation of maslinic acid (MA) SLNs, a plant-derived low water-soluble triterpene with antitumor properties, which can be employed as nanocarriers of hydrophobic compounds.[227] Interestingly, El-Gogary et al. reported the preparation of LNCs and polymeric NPs of ferulic acid, a polyphenolic compound with anticancer properties but with low solubility and bioavailability in aqueous media. [228] They reported that LNCs were superior to polymeric NPs both on the physicochemical and cellular level.

LCNPs are widely employed for combinatorial therapy of different chemotherapeutic agents, as they allow the co-encapsulation and combination of different drugs due to their higher drug loading capacity compared to other types of nanocarriers. Combination chemotherapy is an attractive strategy in cancer treatment because reduces side effects, since a lower concentration of each drug is needed to get the desired antitumoral effect.[229,230] The combination of chemotherapeutics with agents that can inhibit the MDR effect are very promising. Furthermore, it is reported that LCNPs help in overcoming the MDR phenomenon as they can carry the encapsulated compounds into cells by endocytosis, thus bypassing the P-gp drug efflux mechanism.[231,232] In addition, LCNPs allow for the administration of therapeutic biomacromolecules such as as peptides or RNA/DNA-based agents, which are usually co-administrated with traditional antitumoral drugs.

Furthermore, due to their biocompatibility, stability, and versatility, LCNPs can be administered not only through parenteral administration, but also through other not so widely employed routes for NPs administration, such as oral, intranasal, or topical pathways. For instance, they offer significant advantages when using these pathways as they improve oral drug solubility and intestinal permeability, can cross the BBB barrier, and enhance skin

penetration. [142,145,227] As mentioned in section 2, they differ in important features such as the release kinetics of their cargo substance or the physicochemical and colloidal properties of the system, mainly due to the different physical state of their lipidic components. In this sense, there are preferred LCNPs for each target tissue and delivery route. From a broader point of view, SLNs and NLCs, more resistant to mechanical forces, are preferred for intranasal and topical administration, whereas LLNs, due to their higher drug-loading capacity, are widely employed for intravenous and oral administration.

In this section, we examine and summarize LCNPs, in both the clinical stage or market state of production, as well as those in preclinical state, for the treatment of the most frequent types of cancer.

5.1. Market-available LCNPs and clinical trials

A literature review was performed on different databases (Medline *via* PubMed, Cochrane Library, Web of Science, and ClinicalTrials) with the aim of reviewing clinical trials of LCNPs as cancer treatments. Reports are listed in Table 2.

Table 2. Clinical trials regarding the use of LCNPs as cancer treatment.

| Cancer | LCNP | Administration Route | Cargo substance | Status | Identifier | Ref |
|---|---|---|---|---|---|---|
| Actinic keratoses | LNE | Topical | 5-ALA | Commercial and Phase IV | NCT02799069 | [233–241] |
| Actinic keratoses (face and scalp) | | | | Phase I | NCT05060237 | [242] |
| Superficial basal cell carcinoma | | | | Phase II and Phase III, active | NCT02367547 NCT03573401 | [243–245] |
| Ovarian cancer | | IV | PTX | Phase II, terminated | NCT02195973 | [246,247] |
| Breast cancer | | | | Pilot clinical study | - | [248] |
| Canine lymphoma | | | Carmustine | Pilot clinical study | - | [249] |
| Solid tumors | | | 17-AAG | Phase I, terminated | NCT00319930 | [250,251] |
| | Non-specified | | Akt-1 Antisense Oligonucleotide | Phase 1 | NCT05267899 | [252] |
| | | | mRNA-2416 | Phase 2, terminated | NCT03323398 | [253] |
| | | | mRNA-2752 | Phase 1 | NCT03739931 | [254] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Oligonucleotide Targeting MYC | Phase I, terminated | NCT02110563 | [255] |

It should be noted that several clinical trials do not describe the type of lipid nanocarrier employed. These studies are listed as "non-specified" in Table 2. No reports including LNCs, SLNs or NLCs as antitumor drug delivery-systems were found. During this literature search, we only found one market available LCNP formulation: Ameluz® (Biofrontera Pharmaceuticals, Wakefield, MA).

Ameluz® was developed in 1998 by Hürlimann *et al.* as a novel LNE-based gel formulation, containing 10% of 5-aminolaevulinic acid (ALA), for topical treatment of Actinic keratoses.[233] These disorders are *in situ* squamous cell carcinomas which need to be treated to prevent their potential progression. Photodynamic therapy with ALA is an accepted treatment option for this disease. However, the main problem with ALA formulations is the instability of this active compound in aqueous media. The nanoemulsion formulation, termed as BF-200 and patented by Biofrontera Pharmaceuticals (Wakefield, MA),[256] confers improved ALA stability and skin penetration.[257,258] Several successful clinical studies reported the effectiveness and security of this platform as a treatment for actinic keratoses.[234–241] The BF-200 formulation was approved by the FDA in 2016 under the commercial name of Ameluz®. Currently, BF-200 is under clinical trial to evaluate its safety and tolerability as treatment of actinic keratosis on face and scalp.[242] This LNE-based formulation is also proposed as a photodynamic treatment for nonaggressive basal cell carcinoma (BCC). BCC is the most common non-melanoma skin cancer, with superficial BCC (sBCC) being the second most frequent non-aggressive form. Along with excision and surgical procedures, photodynamic therapy has been demonstrated to be an effective therapy alternative for SBCC. Currently, different clinical reports, including a non-controlled single-center clinical study,[243] a Phase II clinical trial [259] and a randomized, intraindividual, non-inferiority, Phase III clinical trial,[245] are assessing the effectiveness and security of this promising formulation.

Another interesting LNE platform is a cholesterol-rich nanoemulsion that binds to low-density lipoprotein (LDL) receptors, termed as LDE. LDE is recognized by LDL receptors and can be used to target antineoplastic drugs against cancer cells that overexpress LDL, such as ovarian and breast carcinomas. Dai *et al.* conducted a pilot study on eight patients with gynecologic carcinoma.[246] These authors studied the pharmacokinetics of LDE associated with PTX oleate, a derivatized form of PTX, and the ability of this nanosystem to concentrate the drug in the tumor sites. Results showed that PTX oleate associated to LDE is stable in the bloodstream,

has longer half-life and greater absolute bioavailability, that is**,** AUC, when compared to the commercial formulation. Furthermore, a Phase II study supports the use of PTX-LDE as third-line chemotherapy for ovarian cancer. The results also suggest that PTX-LDE can be eligible for clinical trials at first or second line setting in combined chemotherapy.[247] A pilot clinical study of LDE-PTX was also carried out to evaluate the tumoral uptake, pharmacokinetics and toxicity in breast cancer patients.[248] Results showed that LDE-PTX preparation can be advantageous for use in breast cancer treatment as the pharmacokinetic profile is improved, the drug is concentrated in the neoplastic tissue and the toxicity of PTX is reduced. Interestingly, these authors also performed a pilot clinical study of LDE as a carmustine carrier combined with vincristine (VCR) and prednisone for the treatment of canine lymphoma.[249] LDE-carmustine was shown to be safe and effective in a drug combination protocol, which encourages larger studies to investigate the use of this novel formulation. Although not in the cancer treatment field, an active Phase III trial is assessing the potential of LDE-PTX as a non-invasive treatment to reduce lesion size and inflammation in patients with aortic and coronary atherosclerotic disease.[260] Similarly, a prospective, randomized, double-blind, placebo-controlled, Phase III trial is studying LDE associated with MTX as an atherosclerotic disease treatment.[261]

17-(Allylamino)-17-demethoxygeldanamycin (17-AAG) is a benzoquinone ansamycin that inhibits the Hsp90 family of molecular chaperones, which leads to the proteasomal degradation of client proteins critical in malignant cell proliferation and survival. Therefore, it is a promising antitumor compound. Saif *et al.* undertook an open-label, dose-escalation, safety, pharmacokinetic and pharmacodynamic Phase I trial of CNF1010, a LNE loaded with17-AAG,[250] in patients with solid tumors. Unfortunately, the maximum tolerated dose was not formally established and the CNF1010 clinical program is no longer being pursued due to the drug toxicity profile and the development of fully synthetic second and third generation Hsp90 molecules.[251] Several studies under clinical trials are currently evaluating the feasibility of the delivery of genetic materials within LNPs as another interesting approach to treat solid tumors.[252–255]

5.2. Preclinical *in vivo* LCNPs studies

In this section, we present significant reports from 2015 to date regarding the *in vivo* use of LCNPs in the treatment of the most frequent types of cancer. Medline *via* PubMed, Scopus and Web of Science were employed as databases. Reports were excluded if they did not include *in*

*vivo* studies, LCNPs composition was not properly defined or the therapeutic application was not antitumoral treatment. Results are summarized in Table 3.

Table 3. Preclinical studies of LCNPs as anticancer treatment.

| Organ/Cancer | LCNP | Administration Route | Cargo substance | Core | Shell | Ref |
|---|---|---|---|---|---|---|
| Colon | LNE | Oral | CSB-INH | Triacetin | Tween-80 and Transcutol-HP | [262] |
| | | | OXA and 5-FU | Capryol 90 | Labrasol, Cremophor EL and Transcutol HP | [263] |
| | | IV | CS-5-FU | Cholesteryloleate, PC, triolein and cholesterol | Tween-80 and Labrasol | [264] |
| | LNC | Oral | CPT | Miglyol 812 | PCL, CD and Chitosan | [265] |
| | | | Ferulic acid | Ferulic acid | Solutol HS 15 and Epikuron 200 | [228] |
| | | IV | CUR | Castor oil, Soybean oil, Miglyol 812 | PEGylated PLGA | [266] |
| | | | 5-FU,DOX,OXA, SN38 and IRI | Labrafac™ lipophile WL 1349 and Labrafil 1944 Cs | Solutol, Lipoid and Transcutol® HP | [267] |
| | SLN | Oral | CPT | GMS and CPT-PA conjugate | Poloxamer 188 | [268] |
| | | | 5-FU | Glyceryl monooleate | Poloxamer 407 and Chitosan-TPP | [269] |
| | | IV | CPT | Trilaurin and egg yolk PC | Poloxamer 188 and PEG-PE | [270] |
| | | | SN38 | Compritol 888 ATO and Precirol 5 ATO | Hydrogenated soy PC, Poloxamer 188, and PEG-PE | [271] |
| | | | miRNA | PC, cholesterol, DOTAP and PEG-PE conjugate | Tween 80 | [272] |
| | NLC | IV | 5-FU | Compritol® ATO 888 and oleic acid | Tween 80 and Eudragit S100 | [273] |
| Breast | LNE | Oral and IP | ClFH, CHA | Labrafac™ lipophile WL 1349 | Span 80 and Tween 80 | [274] |
| | | Intraductal | C6 ceramide | Monoolein, tributyrin and tricaprylin | Tween 80, poloxamer 407 and chitosan | [275] |
| | | IP | Prodrug C16-DOX | Castor oil | PEG-35 | [224] |
| | | IV and IP | α-TOS | Ethylis oleas | Cremophor EL and PEG 400 | [276] |
| | | IV | CS-5-FU | Phospholipid mixture containing cholesteryloleate, PC, triolein and cholesterol | Tween-80 and Labrasol | [264] |
| | | | PTX | MCT, LCT and oleic acid | Glycerol and PL–100 M | [277] |
| | | | DOX and α-linolenic acid | α-linolenic acid and cholesterol | Lecithin, Tween 80 and FA | [278] |
| | | | DAC and PAN | Cod liver oil | LPC, PA and carboxylated PEG | [279] |
| | | | DOX and W198 | Oleic acid and soybean oil | soy lecithin | [280] |
| | | | CPT | Captex 300 | Chitosan Tween 80 and TPGS | [281] |
| | | | PTX and vitamin E | MCT, LCT and cholesterol | soy lecithin, poloxamer 188 and glycerol | [282] |
| | | | CPT | Capmul MCM, Captex 300 and Captex 810D | Simulsol P 23, Poloxamer 407 and Solutol HS 15 | [219] |
| | LNC | Oral | EXM and RES | Capryol 90 | Lipoid-S75 and Zein protein | [218]. |
| | | IV | MTX | Maisine 35 –1 | Stearic acid-valine conjugate | [283] |
| | | | PTX and CUR | Oleic oil | PEG-PE and Poloxamer 407 | [284] |
| | | | DOX and Selol | Selol | PVM/MA-DOX | [285] |
| | | | DTX and THQ | Caprylic (C8) and Capric (C10) triglycerides | TPGS | [286] |
| | | | Honokiol | Almond oil, Castor oil, and Isopropyl myristate | PEG-PLGA | [287] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Fisetin | PC, cholesteryl oleate and cholesterol | HA and chondroitin sulfate | [220] |
| | SLN | Oral | CUR | GMS | Soya lecithin, Poloxamer 188 and Chitosan | [288] |
| | | | B-carotene | GMS, gelucire50/13, and Phospholipid S-100 | Tween-80 and Pluronic F68 | [222] |
| | | | Raloxifene | GMS and Compritol® 888 ATO | Phospholipid S-100 and TPGS-1000 | [289] |
| | | Intratumoral | PTX | stearic acid | Lecithin, Poloxamer 188 and CD | [290] |
| | | IP | DTX | Compritol | Span 80, and Pluronic ® F127 | [291] |
| | | IV | PTX and pEGFP | GMS and stearic acid | PC, DDAB and PE-HA | [292] |
| | | | DTX | Trimyristin, Ceramide and TMP-I | PC, Pluronic ® P85 and Emulsiflex EF-B3 | [293] |
| | | | Termoporfin | 1-tetradecanol | PEO-PC | [294] |
| | | | MTX | Gelucire and stearyl amine | phospholipid-90 NG, Tween 80 and fucose | [295] |
| | | | DTX and CUR | Compritol and GMS | Poloxamer 188 | [296] |
| | | | DOX | stearic acid | Soy lecithin and PEG-PE | [297] |
| | | | Melphalan | Tristearin | Soya lecithin, Polaxamer 188 and PEO-PPO | [221] |
| | | | DOX | RGD-PEG-GMS | RGD and Myrj52 | [298] |
| | | | CUR | Trilaurin and Cholesterol-Chitosan | Chitosan, Epikuron®200, NaTC, Cremophor®RH60 and Pluronic®F68 | [299] |
| | | | Radiolabeled trastuzumab | Stearic acid | Lecithin | [300] |
| | NLC | Oral | Citral | Hydrogenated palm oil and olive oil | Lipoid S-100, thimerosal, D-Sorbitol and Tween 80 | [223] |
| | | | EXE | Precirol® ATO 5 and flaxseed oil | Poloxamer 188, Tween 80, and Tween 20 | [301] |
| | | IP | Calycosin | Miglyol and steric acid | Tween 80, Span 60, PEG 400 and sucrose stearate | [302] |
| | | IV | DOX, DHA and α-TOS | OmeRx™ DHA 500 TG and Compritol | Tween 80 | [303] |
| | | | PTX | Precirol ATO5 and Maisine 35-1 | Cremophor RH40 and PEG | [304] |
| | | | Cembegic acid | Compritol 888 ATO and MCT 812 | Lecithin, Myrj 52 and RGD | [305] |
| | | | DOX and CDDP | Stearic acid and Precirol® ATO | Soy PC | [306] |
| | | | DOX and β-lapachone | Compritol® 888 ATO, oleic acid and GMS | Soy PC and PEG-Succinic Acid | [307] |
| | | | RES | Stearic acid and oleic acid | Phospholipon® 90 G and Poloxamer 188 | [308] |
| | | | DOX-TS | Compritol, DHA and TEA | Tween 80 and glycerol | [309] |
| | | | DOX and Sclareol | Compritol® 888 ATO, peanut oil and oleic acid | Tween 80 | [310] |
| Lung | LNE | Oral | CUR | Ethyl oleate, cremorphor EL 35and GMS | Lipoid S 75, PEG and Tween 80 | [311] |
| | | IV | Lycobetaine | Oleic acid and soybean oil | PEGylated lecithin and Lipoid E80 | [312] |
| | | | PTX | DL-α-tocopheryl acetate and soybean oil | Tween 80 and HA | [313] |
| | LNC | IV | Selol | Selol | PMV/MA | [314] |
| | | | Erlotinib | Lecithin and Transcutol | DDAB and PEG-Aspartic Acid | [315] |
| | SLN | Oral | PTX | GMS, lyceryl tripalmitate, glyceryl trimyristate, glyceryl tristearate and stearic acid | Soy lecithin, Tween 80, poloxamer 188, and poly(vinyl) alcohol | [316] |
| | | | Erlotinib | Glyceryl behenate and stearic acid | TPGS and soy lecithin | [317] |
| | | | PTX and artemether | GMS and stearyl amine | Span® 80 and $MPEG_{2000}$-DSPE | [318] |
| | | IV | Transferrin etoposide | GMS and stearic acid | Soy lecithin and Tween 80 | [319] |
| | NLC | Intranasal | 9-bromo-noscapine | Stearic acid | PC and sodium glycocholate | [320] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | IV | PTX and salinomycin | ATO-5, MCT, Solutol HS15 and Kolliphor EL | PEO (40) stearate and PEG-PE | [321] |
| | | | PTX and DOX | Oleic acid and Compritol® 888 ATO | Soybean PE | [322] |
| | | | DOX and β-elemene | Compritol® 888 ATO and Miglyol® 812 | PEG-PE, lecithin, and Tween 80 | [323] |
| | | | pEGFP | precirol ATO-5 and olive oil | Lipoid S100, soybean lecithin, tween 80 and Transferrin | [324] |
| | | | DCT and CUR | Dynasan 114, Precirol ATO5 and Labrafac lipophileWL 1349, | Phospholipon 90 G, PEG and FA | [325] |
| | | | PTX and 5-Demethylnobiletin | Oleic acid and Compritol® 888 ATO | Soylecithin, soy PC and Cetuximab | [326] |
| | | | PTX and DNA | GMS and oleic acid | Soy lecithin, Tween-80 and Transferrin | [327] |
| Melanoma | LNE | Topical | Zinc phthalocyanine | MCT, Lipoid E80 and DOTAP | Tween 80 and Poloxamer 188 | [328] |
| | | Oral and IV | Piplartine | Capmul PG-8 | Tween 80 and PE-PEG | [329] |
| | | IP | PTX | Cholesteryl oleate, cholesterol and Miglyol® 812 N | Egg PE andTween 80 | [330] |
| | | IV and IP | 7-Ketocholesterol | 7-Ketocholesterol, cholesteryl oleate, triolein and cholesterol | Egg PC | [331] |
| | | IV | DTX | olive oil, cholesterol, α-TOS and oleic acid, | Egg lecithin, stearyl amine, albumin and glycerol | [332] |
| | LNC | IP | Eugenol and Ace (eugenol acetylated) | Caprylic (C8) and Capric (C10) triglycerides and sorbitan monoesterate | PCL | [333] |
| | | IV and IP | Ferrociphenol and Ansa-Ferrociphenol and Bcl-2 siRNA | Labrafac® | Kolliphor® HS15 | [334] |
| | SLN | Oral | DHA-dFdC | GMS | Tween 20, soy lecithin and TPGS | [335] |
| | | IV and Intratumoral | PTX | Stearic acid | Lecithin, Poloxamer 188, Tyr-3-octreotide-PEG-PE | [336] |
| | | IV | PTX | GMS, Cholesterol and DDAB | soy PC and HA | [337] |
| | | | DTX | GMS | Octadecylamine, Soy PC, HA and tetraiodothyroacetic acid | [338] |
| | | | PTX and ascorbyl palmitate | GMS and DDAB | Pluronic F68 | [339] |
| | NLC | Topical | Silymarin | Lipid Sefsol R 218 and Geleol | Cremophor R RH40 and bile salt | [340] |
| | | Subcutaneous | Bupivacaine | Lavender and melaleuca oils | Pluronic F68 | [341] |
| | | Topical and IV | PTX and lidocaine | Myristyl myristate and Miglyol 812® | Pluronic F68 | [342] |
| Brain | LNE | Intranasal | Kaempferol | MCT | Egg lecithin and Tween 80 | [343] |
| | | | CD73siRNA | MCT | Lecithin and DOTAP | [344] |
| | LNC | Intracraneal | peptide NFL-TBS.40-63 | Labrafac® | Solutol HS15 and Lipoïd® | [345] |
| | | | Anti-Galectin-1 and anti-EGFR siRNA | Labrafac® | Lipoïd® S75-3, Solutol® H15 and Chitosan | [346] |
| | | | RES | Caprylic (C8) and Capric (C10) triglycerides and sorbitan monoesterate | PCL | [347] |
| | | | PTX and CpG DNA | Captex1 8000 | Lipoid1S75-3, Solutol® H15 and chitosan | [348] |
| | | Oral | Diphenyl diselenide | MCT | PCL, Span 80 and Tween 80 | [349] |
| | | Oral and IV | MTX | Caprylic (C8) and Capric (C10) triglycerides and sorbitan monoesterate | PCL | [350] |
| | | | PTX and CUR | Labrafac® | Lipoïd® S75-3, Solutol® H15, PEG and chitosan | [351] |
| | | IV | Nonpsychotropic cannabinoids | Labrafac® | Lipoïd® S75-3 and Solutol® H15 | [352] |
| | | | CUR | Labrafac® | Lipoïd® S75-3 and Solutol® H15 | [353] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | MTX | Caprylic (C8) and Capric (C10) triglycerides and sorbitan monoesterate | PCL | [354] |
| | SLN | Oral | PTX and naringenin | Percirol ATO5 and Dynasan 114 | DSPE-mPEG-2000, Lutrol F188 and RGD peptide | [355] |
| | | IV | DTX | GMS and Stearic acid | Soya lecithin, Tween 80 and Angiopep-2 (conjugated via EDC/NHS) | [356] |
| | | | PTX | Stearic acid | Lecithin, Poloxamer 188 and Tyr-3-octreotide | [357] |
| | | | VCR and TMZ | 888 ATO | PC, Cremophor ELP, soy lecithin and DDAB | [358] |
| | | | IR-780 iodide | PA | PEG-PE, P407-Tween 80 andpeptide (cyclo (Arg-Gly-Asp-d-Tyr-Lys) | [359] |
| | | | Diosgenin | Stearic acid | Lecithin and polysorbate 80 | [360] |
| | NLC | Intanasal | CUR | Precirol and capmul MCM | soy lecithin | [361] |
| | | IP | CUR | tripalmitin acid and oleic acid | Tween 80 | [362] |
| | | | Atorvastatin and CUR | Precifac® ATO 5 and Labrasol® | Tween 80, Lipoid S75, Transcutol® HP, HA, FA and cRGDfK and H7K(R2)2 peptides | [363] |
| | | IV | VCR and TMZ | Stearic acid and COMPRITOL® 888 ATO | PC, soy lecithin and Lactoferrin and RGD | [364] |
| | | | TMZ | COMPRITOL® 888 ATO | , Cremophor ELP, PC, Soya lecithin and RGD | [365] |
| | | | TMZ and CUR | GMS and MCT | Poloxamer 188 | [366] |
| | | | TMZ and VCR | COMPRITOL® 888 ATO | PC, soy lecithin and Cremophor ELP | [358] |
| | | | DTX | Caprylic/capric triglyceride, Polyoxyethylene stearate and PEG-hydrogenated castor oil | DSPE-PEG2000-Maleimide and bevacizumab | [367] |
| Gastric cancer | LNE | IV | Ginsenoside Rg3 | Labrafac and Suppocire NC | Lipoid s75, Myrj s40 and VEGFR-3 antibody | [368] |
| | SLN | IV | PTX and tanespimycin | Stearic acid | Myrj 52 and lecithin | [369] |
| | NLC | IV | PTX | MCT | Solutol HS, Myrj 52 and Peptide GX1 | [370] |
| | | | 5-FU and CDDP | GMS and soybean oil | Soylecithin, Tween 80 and HA | [371] |
| | | | Etoposide | GMS and oleic acid | DOTAP, Soya lecithin and Labrafac PG | [372] |
| | | | Chlorin e6 | Oleic acid | Folic acid-PEG-PE | [373] |
| Pancreatic cancer | LNE | IV | Dodecafluoropentane | Dodecafluoropentane | Emulsiflex C-5 | [374] |
| | LNC | IV | GlaB | Castor oil | PLGA–PEG–DTPA | [375] |
| | | | PTX | Olive oil | DCA, Epikuron 145, Pluronic F68 and αCD44 | [376] |
| | SLN | Oral | Aspirine and CUR | Stearic acid | poloxamer | [377] |
| | | IV | CUR | Trilaurin | Epikuron® 200, Tween (20, 40, 80), Cremophor and Pluronic® F68 | [378] |
| | LNC | IV | Gemcitabine and Baicalein | Stearic acid | PC, Tween 80 and HA | [379] |
| Prostate | LNE | IV | DHA-SBT-1214 | Fish oil | Lipoid E80®, Tween 80® and PEG-PE | [380] |
| | SLN | IV | DTX and Adenosie | Stearic acid and GMS | Soya lecithin, Tween 80 and Adenosine | [381] |
| | | | DOX and magnetite | Trialurin | TPGs and histamine dodecyl carbamate | [382] |
| | NLC | Intragastrical and IP | Tripterine | Precirol ATO-5 and Labrafil M 1944CS | Soy lecithin and d-α-TOS-PEG | [383] |

OXA:Oxaliplatin; 5-Fu:5-fluorouracil; CPT:Camptothecin; PCL:Poly-e-caprolactona; CD:2-hydroxypropyl-β-cyclodextrin; CUR:Curcumin; PLGA:poly(lactic-co-glycolic acid);

DOX:Doxorubicin; SN38:7-ethyl-10-hydroxycamptothecin; IRI:irinotecan; PA:Palmitic acid; GMS:Glycerin monostearate; PC:Phosphatidylcholine; PEG:Poly(ethylene glycol); PE:Phosphatidyl-ethanolamine; DOTAP:Dioleoyl-3-trimethylammonium propane; PUFA:Polyunsaturated fatty acids; ClFPh-CHA-16:(4-chloro-3-trifluorophenyl)carbamoylamino]hexadecenoic acid; α-TOS;α-tocopherol succinate; PTX:Paclitaxel; MCT:Medium chain triglycerides; LCT:Long chain triglycerides; DAC:Decitabine; PAN:Panobinostat; W198:Bromotetrandrine; TPGS:D-α-tocopheryl polyethylene glycol succinate; EXM:Exemestane; RES:Resveratrol; MTX:Methotrexate; PVM/MA:Poly(methyl vinyl ether-co-maleic anhydride); DTX:Docetaxel; THQ:Thymoquinone; pEGFP:Plasmid encoding enhanced green fluorescent protein; DDAB:Dimethyldidodecylammonium bromide; HA:Hyaluronic acid; PEO:Poly(ethylene oxide); PPO:Poly(propylene oxide); RGD:Arginine-glycine-aspartic tripeptide; HZ:Adipic acid dihydrazide; DHA:docosahexaenoic acid; CDDP:Cisplatin; DHA-dFdC: 4-(N)-docosahexaenoyl-2,2-difluorodeoxycytidine; VCR:Vincristine; TMZ:Temozolomide; DTPA:Diethylene triamine pentaacetic acid; CSB-INH-¡:Carvone Schiff base of isoniazid; CS-5-FU:cholesteryl-succinyl-5-fluorouracil; FA:Folate.

5.2.1. Colon cancer

Colorectal cancer is currently the third most common type of cancer and the second most cancer-related cause of death worldwide.[2] In recent years, several LCNPs have been described to obtain more effective therapies against colorectal cancer. Among these studies, a frequently studied parameter is drug loading. In this sense, Tsakiris *et al.* encapsulated six different drugs, three hydrophobic and three hydrophilic, into LCNCs. Apart from using the same synthesis process for the three hydrophobic and the three hydrophilic drugs, drug loading changed depending on the drug. In fact, the formulation with smaller drug loading was chosen for the subsequent *in vitro* and *in vivo* assays, since the bioactivity of this encapsulated drug was higher than that of the others.[267] Bhat *et al.* developed a carvone Schiff base of isoniazid (CSB-INH) loaded LNEs as an orally administered treatment for colorectal cancer. They evaluated the drug release under gastric simulated conditions. Under this acidic condition, they found a higher release of the compound from LNEs than from the insoluble drug suspension. In this case, a higher release means an improvement in terms of bioavailability and, therefore, a higher amount of compound available to be absorbed at the intestinal level. These authors also compared the plasma concentration profile of the compound in rats, after oral administration of drug-loaded LNEs or free drug suspension, and found a higher drug

concentration in the plasma of LNEs-fed rats.[262] On the other hand, Yawei *et al.* evaluated the stability of a camptothecin and palmitic acid conjugate (CPT-PA) in its free form and encapsulated inside SLNs. CPT is very unstable under physiological conditions, especially under reductive ones, which hinder the drug from achieving its target and lead to severe side effects. Moreover, it is a poor water-soluble component and is non-soluble in lipids. The strategy used to protect the compound from premature physiological degradation and increase its solubility was the encapsulation in SLNs by synthetizing a CPT-PA conjugate to improve drug solubility in lipids and drug loading. Unlike the previous study, conjugate release from SLNs was smaller than the release from the suspension under simulated digestion conditions. In this case, the slower release provided an advantage, since the instability of the free compound would reduce its bioavailability.[268] A fast release of CPT from SLNs under reductive conditions was also reported, demonstrating that CPT could be effectively released from the conjugate. This fast release under reductive conditions provides a further advantage to the system, since the tumor environment is also reductive, owing to the overexpression of glutathione.[268] They reported that the encapsulated conjugate could effectively cross an *in vitro* Caco-2 simulated intestinal epithelium. Khat *et al*. and Yawei *et al*. equate plasma drug concentrations and bioavailability.[262,268]. However, according to the bioavailability definition, evaluation of this parameter requires the analysis of the effect on the tumor, because a higher plasma concentration of the drug without a visible effect on the tumor target would not be relevant in practical terms. In this sense, *in vivo* assays performed with tumor bearing mice to study tumor growth inhibition or tumor drug accumulation provide a better characterization of the nanocarrier.[228,266,267,269–272] For instance, the study of CUR-loaded PEGylated PLGA LNCs in mice showed a prolonged blood circulation time of these LNCs thanks to the PEG coating. Moreover, radio-labelled LNCs were confirmed to accumulate on the tumor site and furthermore, a significant reduction of tumor volumes was observed in CUR-loaded PEGylated PLGA-LNCs treated mice when compared with empty LNCs-treated mice.[266] Similary, the accumulation of LNCs into reticuloendothelial system rich-organs for their subsequent elimination is also common and is one of the factors limiting bioavailability of encapsulated drugs.[266,270] The strategy followed by Jang *et al.* to reduce LCNPs elimination was to pre-inject tumor bearing mice with empty SLNs before treating animals with CPT-SLNs. This way, they achieved the saturation of the reticuloendothelial system rich-tissues and improved targeting and accumulation of drug-loaded SLNs in tumors.[270] On the other hand, these authors also reported the protective role of SLNs on encapsulated CPT, an *in vitro* long term sustained release, an improvement in the *in vitro* cytotoxic effect of CPT-SLNs compared

to free CPT, as well as *in vivo* prolonged blood circulation compared with free CPT and significant tumor growth inhibition.[270]

Another interesting strategy to improve drug release at the tumor site is the use of stimuli-responsive NPs. Along these lines, pH-responsive PEG-lipid-derivate SLNs and liposomes are found.[272] These SLNs and liposomes take advantage of the acidic pH of the tumor microenvironment to achieve specific release of the encapsulated microRNA and irinotecan (IRI), respectively, at the tumor site. Moreover, microRNA loaded PEG-coated SLNs and Iri-loaded PEG-coated liposomes were further functionalized with tumor targeting peptides, which resulted in improved inhibition of tumor growth and reduction of the side effects and systemic toxicity on tumor bearing mice. In addition, microRNA-SLNs and IRI-liposomes were administered in a combined treatment, which showed an improvement in *in vitro* cytotoxicity outcomes in HCT116 cells and higher significant reduction of tumor size and side effects in mice.[272] Recently, Borderwala *et al.* prepared NLCs containing 5-fluorouracil (5-FU) as the chemotherapeutic agent and coated with Eudragit S-100, a pH-sensitive polymer found to provide release in the colonic region.[273] *In vitro* and *in vivo* experiments demonstrated the capacity of the prepared NLCs to retain the integrity and to pass through the stomach and intestine without releasing the drug until reaching the colon, where the coating is dissolved.[273]

The combination of drugs to enhance anticancer activity is an approach currently applied in clinics. We can find studies where the combined drugs are included in separate nanocarriers, which improve drug solubility and/or stability, its cytotoxic effect, drug targeting, or reduce side effects.[267,272] LCPNs offer the possibility to co-encapsulate several drugs in the same nanocarrier, such as the CPT derivative/IRI loaded NLCs coated with HA to target colon adenocarcinoma.[384] However, this study does not include *in vivo* characterization of the nanocarrier.

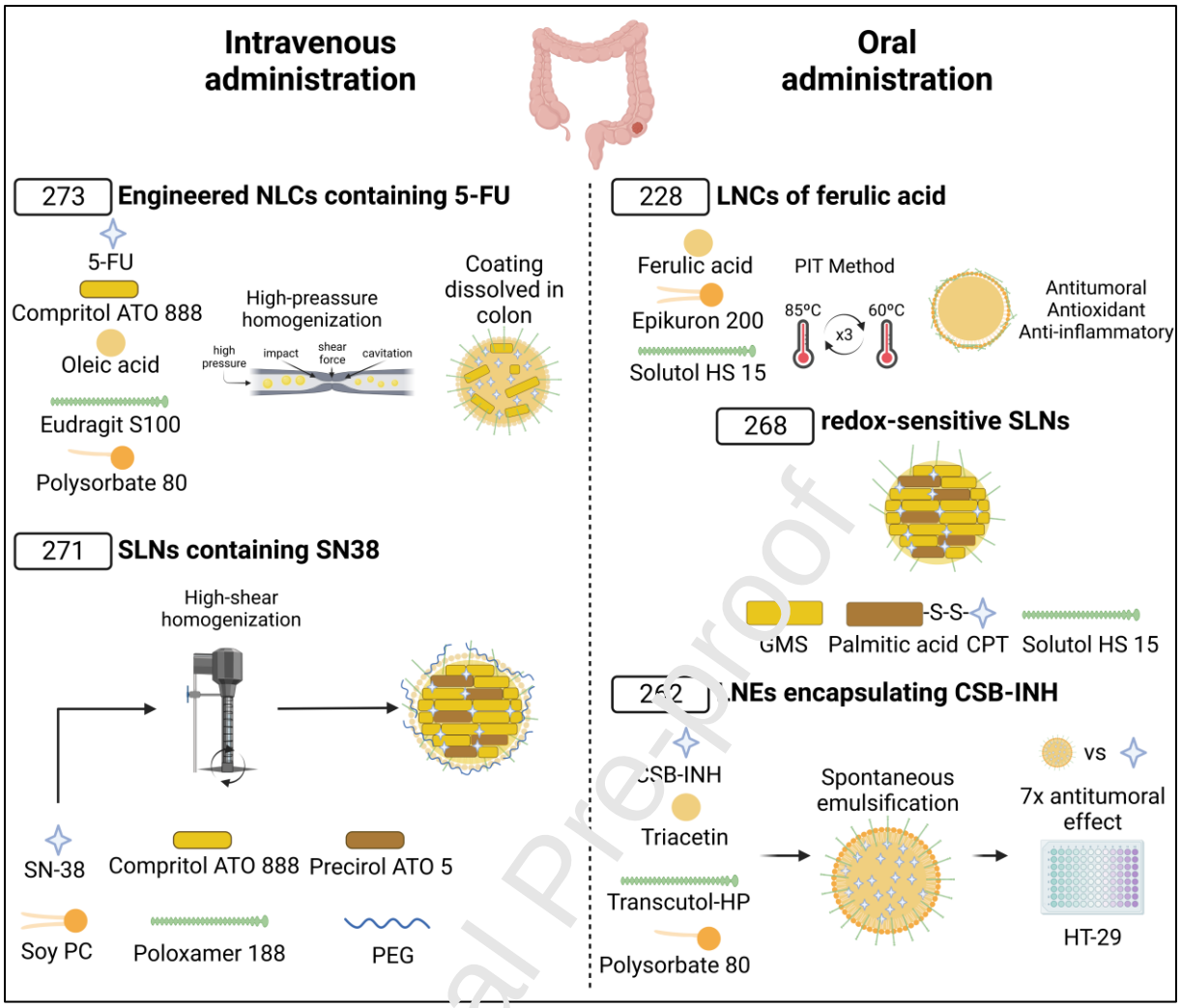


Figure 10. Representative preclinical studies of LCNPs to target colon cancer.[228,262,271,273]

5.2.2. Breast cancer

Breast cancer is the most commonly occurring cancer in women.[2] DOX, DTX and PTX are broad spectrum antineoplastic compounds frequently employed in breast cancer chemotherapy.[215] To enhance the chemotherapeutic behavior of these compounds and reduce off-target effects, LCNPs have been applied to encapsulate DOX [224,385], DTX [291], and PTX.[277] Interestingly, Dos Santos Câmara *et al.* encapsulated the prodrug C16-DOX, a lipidated, inactive and pH-sensitive form of DOX, in castor oil LNEs prepared using a spontaneous emulsification procedure. Once the nanocarrier reached the tumor tissue, its acidic environment cleaved the hydrazone bond of the prodrug, resulting in a localized DOX release. *In vivo* studies on 4T1 murine cancer model revealed that this nanoformulation allowed for the use of a higher dose of DOX and improved the chemotherapeutic index and tumor control efficacy.[224] Similarly, Burgarelli-Lages *et al.* entrapped the pH-sensitive doxorubicin-tocopherol succinate (DOX-TS) prodrug in DHA-based NLCs. The *in vitro* and *in vivo*

experiments demonstrated better DOX-TS-NLCs pharmacokinetics compared to free DOX and DOX-NLCs, as well as the prevention of short-term cardiotoxic effects of DOX after intravenous injection in 4T1 tumor-bearing mice model.[309]

Combination chemotherapy is an attractive strategy for addressing multifaceted challenges associated with cancer.[229] An interesting approach is combinatorial therapy employing established chemotherapeutic agents with compounds that can inhibit the MDR effect. As an example, Cao *et al.* prepared LNEs of oleic acid containing DOX and bromotetrandrine (W198), a potent P-gp inhibitor that can prevent them from pumping out drugs. *In vitro* cytotoxicity assays revealed that at the same concentration level, DOX+W198 and DOX/W198-LNEs exhibited much greater inhibitory effects than DOX solution and DOX-LNEs in MCF-7/ADR resistant breast cancer cells. After intravenous injection in MCF-7/ADR-bearing xenograft mice, DOX/W198-LNEs demonstrated enhanced tumor uptake and higher plasma concentrations along with reduced cardiac toxicity of both drugs.[280] Different LCNPs have also been developed to co-deliver DOX in combination with other drugs to treat breast cancer. CUR, which possesses P-gp inhibitor properties along with antitumor activity, was included in SLNs;[299] β-lapachone, a novel therapeutic agent that dramatically influences various P-gp-related pathways, co-delivered with DOX in NLCs;[307] thymoquinone and Tocopheryl polyethylene glycol 1000 succinate, a soluble natural derivative of Vitamin E, co-delivered with DTX in LNCs;[286] as well as sello and sclareol.[285,310] Similarly, LCNPs are also employed for the combinatorial therapy of PTX and DTX.[282,284,386]

Decreasing estrogen levels through the inhibition of aromatase, the enzyme that turns androgens into estrogens, is a selective and effective therapy for hormone-dependent breast cancer patients. Aromatase inhibitors (AIs) are successfully used in the treatment of estrogen receptor (ER) positive breast cancer but have poor aqueous solubility.[387]. Elzoghby *et al.* prepared protamine-coated LNCs containing the AI Letrozole and the COX-2 inhibitor Celecoxib. COX-2 inhibitors can reduce the expression of Prostaglandin E2, which promotes aromatase gene expression, consequently reducing estrogen production in breast cancer cells. The developed LNCs demonstrated antitumor effects *in vivo* as evidenced by the reduction of tumor volume and aromatase level.[218] This group also reported the development of LNCs coated with a crosslinked shell of zein, a natural hydrophobic protein, for oral codelivery of Exemestane (EXE), a third-generation AI clinically approved, and Resveratrol (RES), a polyphenolic phytoestrogen.[218] Similarly, Singh *et al.* prepared NLCs of Precirol® ATO 5 and flaxseed oil as the solid and liquid lipid, respectively, encapsulating EXE. An *in vivo* pharmacokinetic study on female Wistar rats found an increase of 3.9 fold in oral bioavailability of EXE through

NLCs compared with EXE suspension.[301] Recently, Jain *et al.* developed SLNs containing Reloxifene, a second-generation selective estrogen receptor modulator, showing promising results both *in vitro* and *in vivo*.[289]

LCNPs can be used to solubilize and deliver other promising chemotherapeutic compounds in breast cancer treatment, which have limited clinical application. This is the case of α-tocopherol succinate (α-TOS), a derivative of Vitamin E. Gao *et al.* designed LNEs mainly composed of ethyl oleate encapsulating α-TOS using an emulsification-evaporation procedure. α-TOS-LNEs showed stronger inhibitory effects on MCF-7 cells compared to free α-TOS solution and, *in vivo* experiments showed significant improvement on the metabolism time of α-TOS in rats, both by intravenous and intraperitoneal injection.[276] α-TOS is also applied in combinatorial therapy nanosystems as the NLCs co-encapsulating DOX, DHA and α-TOS proposed by Lages *et al.*[303] *In vitro* cell studies indicated that DOX, DHA, and α-TOS have synergistic effects against 4T1 tumor cells. The *in vivo* study showed that DHA-DOX-α-TOS-NLCs exhibited the greatest antitumor efficacy by reducing tumor growth in 4T1 tumor-bearing mice and reduced mice mortality, prevented lung metastasis, and decreased DOX-induced toxicity to the heart and liver. Recently, Arshad *et al.* designed NLCs containing Calycosin, a novel anti-cancer drug under clinical trials, showing significant recovery in mammary glands weight loss, which occurred due to cancer, to their normal level.[302] Similarly, Talaat *et al*. formulated LNCs encapsulating fisetin through the layer-by-layer method. Fisetin is a promising flavonol that has proved to inhibit cancer growth without causing toxicity to healthy cells.[220] Compared to the free drug, the nanoformulation showed a 4-times decrease of the IC50 in the i*n vitro* cytotoxicity and a superior therapeutic effect in the *in* vivo model.

Another interesting approach is the codelivery of a chemotherapeutic agent and DNA, which can overcome drug resistance, decrease side effects, and achieve enhanced antitumor efficiency.[292] Yu *et al.* prepared SLNs of glycerol monostearate coated with HA and coencapsulating PTX and pDNA. *In vitro* experiments on MCF-7 cells and *in vivo* in breast cancer xenograft BALB/c nude studies, confirmed that the developed SLNs could inhibit the tumor and, at the same time, deliver and transfect genes into cancer cells.[292]

Several ligands and targeting moieties can be attached onto the surface of LNPs in order to achieve active targeting in breast cancer treatment. In this sense, Folate (FA) is one of the most employed and studied. FA receptors are upregulated in different types of cancers such as breast, lung and colon. Therefore, FA decorated nanosystems can act as a selective drug delivery system to positive FA receptors cancer cells/tissues.[388] Furthermore, compared to antibody ligands, FA is advantageous due to its smaller size, non-immunogenicity, non-toxicity, ease of

handling, stability and low cost.[389] Tripathi *et al.* prepared FA decorated LNEs of α-linolenic acid encapsulating DOX. *In vivo* studies in 7,12-dimethylbenz[a] anthracene(DMBA)-induced breast cancer tumor Albino Wister rats revealed that, after tail vein injection, decorated LNEs enhanced antitumor targeting potential and therapeutic safety compared to other non-decorated LNEs and free DOX, thus corroborating the effectiveness of active targeting.[278] Similarly, Poonia *et al.* reported the synthesis of RES NLCs decorated with FA as the targeting moiety. Cell cytotoxicity experiments revealed high cytotoxic effects of FA-NLCs compared to unmodified NLCs on MCF-7 cells along with enhanced bioavailability and pharmacokinetic behavior *in vivo*. This study suggested the high potential of targeted NLCs in enhancing the therapeutic concentration of RES to breast cancer cells.[308] FA is also employed for the active targeting of different LCNPs.[296,304] Bombesin [297], Arginine-glycine-aspartic (RGD) peptides,[298,305] 2-Hydroxypropyl-b-cyclodextrin,[290] adenosine,[381] and lysophosphatidic acid [279] are also employed as target moieties to decorate and functionalize LCNPs.

In relation to administration routes of LCNPs in breast cancer treatment, most of the developed nanosystems are engineered to reach the tumor tissue by parenteral administration. However, LCNPs offer important advantages and can be conveniently used for oral delivery, as, for example, the aforementioned LNCs prepared by Elzoghby *et al.*[218] or the NLCs developed by *Singh et al.*[301] and Nordin *et al.*[223], encapsulating AIs and Citral, respectively. Baek *et al.* included CUR in chitosan coated SLNs prepared by hot homogenization.[288] Coated SLNs exhibited suppressed burst release in simulated gastric fluid, prevented by the polymer coat, while sustained release was observed in simulated intestinal fluid. Furthermore, the prepared SLNs exhibited increased cytotoxicity and cellular uptake on MCF-7 cells. The lymphatic uptake and oral bioavailability evaluated using male Sprague Dawley rats were found to be 6.3 fold and 9.5 fold higher than that of CUR solution, respectively.[288] Similarly, Garrastazu-Pereira *et al.* developed LNEs prepared by the PIT process for the encapsulation of a synthetic derivative of ω-3 polyunsaturated fatty acid, namely CIFPh-CHA.[274] Oral administration in xenografted mice of the drug-loaded LNE was able to significantly reduce tumor mass to ~50% of untreated control at doses of 10 and 40 $mg \cdot kg^{-1}$.[274]

Due to breast cancer usually beginning in the lining of the ducts, intraductal administration arises as a promising administration route to combine efficacy and reduce systemic adverse effects. Migotto *et al.* developed chitosan-coated positive bioadhesive LNEs encapsulating C6 ceramide as the chemotherapeutic agent. LNEs decreased the $IC_{50}$ of C6 ceramide in MCF-7 cells by 4.5 fold when compared to the free solution.[275] *In vivo* experiments of C6-containing

LNEs conducted in Female Wistar rats revealed that drug localization, after intraductal administration, remained for more than 120 h in the mammary tissue compared to its solution.

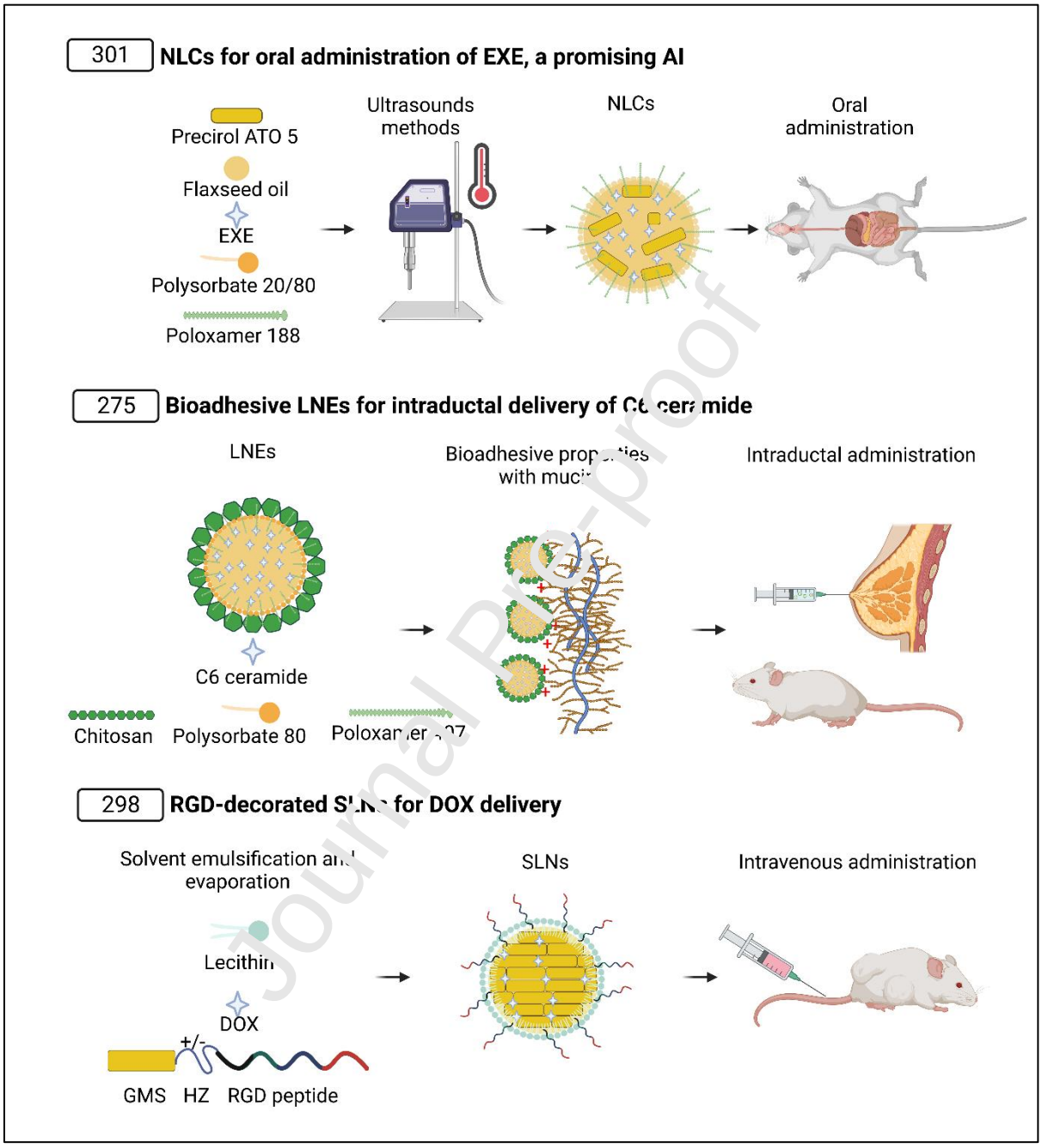


Figure 11. Representation of interesting preclinical studies of LCNPs for breast cancer treatment.[275,298,301]

5.2.3. Lung cancer

Lung cancer is currently the second most common cancer type and the first cancer-related cause of death worldwide.[2] Despite advances in chemotherapeutics to improve survival, median survival remains limited to less than 12 months. Escalation in global lung carcinoma mortality

presents a grave concern. Chemotherapy of non-small cell lung cancer, the most common form of lung carcinoma, employs DTX, PTX, DOX and cetuximab, among others, as promising molecules. However, an amalgamation of the issues pertaining to poor safety and toxicity profile, pharmacologic resistance and poor tumor bioavailability of these second generation taxanes calls for strategies that may promote its clinical worth.[390] Combinatorial chemotherapy is also employed in lung cancer treatment. Rawal *et al.* employed co-delivery of DTX and CUR through the development of FA–appended NLCs (FA-DTX/CUR-NLCs) with promising results, such as significantly better *in vivo* relative bioavailability of DTX (24.85 fold) with FA-DTX/CUR-NLCs compared with Taxotere®.[325] Immunostaining of the tumor sections with tumor differentiation biomarkers suggested considerably higher apoptotic, anti-proliferative, anti-angiogenic and anti-metastatic potential of FA-DTX/CUR-NLCs compared with Taxotere®. *In vivo* toxicity assessment of the NLCs demonstrated a noteworthy reduction in DTX associated side effects.[325] Recently, Khatri *et al.* prepared FA appended PEGylated SLNs for the encapsulation of PTX and Artemether. *In vitro* and *in vivo* experiments concluded that the anticancer potential of PTX was improved without any renal or hepatic toxicity, which indicated that the developed formulation is able to reduce dose related toxicity of PTX.[318]

β-elemene (ELE) is an antitumor agent extracted from the chinese medicinal plant Radix Curcumae. Previous studies have shown that ELE exhibited anti-cancer effects in many cancer cells, especially lung cancer cells, by inducing apoptosis.[323] Cao *et al.* developed DOX and ELE co-loaded, pH sensitive NLCs (DOX/ELE-NLCs) with greater lung tumor inhibition ability.[323] Guo *et al.* developed cetuximab functionalized, PTX and 5-Demethylnobiletin co-loaded NLCs, exhibiting a remarkable *in vivo* tumor inhibition efficiency, high tumor accumulation amount and low toxicity.[326] Moreover, Zhou *et al.* demonstrated that PTX and salinomycin active-targeting NLCs killed cancer cells and cancer stem cells (CSCs) at the same time.[321]

Additionally, there are other recent studies where LNEs are used as drug nanocarriers against lung cancer. Typically, these LNEs are functionalized with active targeting RGD peptides.[312] LNEs can be used as an imaging agent, targeting different receptors like PARP1,[391] as well as for the treatment of lung cancer, carrying different drugs like CUR or PTX.[311,313] LNCs are also useful in the treatment of lung cancer. Erlotinib (ERL), an EGFR tyrosine kinase inhibitor, is currently available on the market as tablets for oral administration. However, poor oral bioavailability associated with poor solubility and permeability results in limited therapeutic efficacy. In addition, traditional oral delivery of ERL is accompanied by severe side effects including rash, diarrhea, gastrointestinal perforations, ocular lesions, and hematological

disorders.[315] Kim *et al.* developed PEGylated polypeptide LNCs to enhance the anticancer efficacy of ERL in non-small cell lung cancer with high drug entrapment efficiency (~95%), effective controlled release, efficient internalization *via* receptor-mediated endocytosis, and improved antitumor efficacy upon intravenous administration.[315] Recently, Rampaka *et al.* prepared ERL-loaded SLN for oral administration. *In vivo* pharmacokinetic studies revealed an improvement in bioavailability of ERL around 2.12 fold and a significant reduction in fed to fasted variability.[317]

5.2.4. Melanoma

Melanoma is the most aggressive skin cancer because of its high metastatic rate.[392,393] Current available therapies are ineffective, and dacarbazine, the main chemotherapist used against metastatic melanoma, induces a low response rate.[334] Some studies explore the use of new compounds such as those reported by Fofaria *et al.*, who developed LNEs to encapsulate piplartine (PL), a hydrophobic anticancer active compound found in black pepper.[329]. The PL-loaded LNEs achieved solubilization of the compound, provided increased bioavailability compared with the free compound when orally administered and reduced the weight of the tumor in tumor bearing mice melanoma model.[329] On the other hand, the encapsulation of diphenyl diselenide [(PhSe)2], a synthetic organoselenium compound that has multiple pharmacological properties,[394] in LNCs, improved the stability of the compound upon exposure to UV radiation compared to the free form. Moreover, encapsulation significantly reduced its cytotoxic effects on healthy cells, proving the safety and highlighting the added value of the nanocarrier.[393]

Another approach is the use of LCNPs as the platform for codelivery of synergistic drugs. For instance, Zhou *et al.* developed palmitate and PTX-loaded SLNs as a dual drug delivery system, which improved the cytotoxic effect *in vitro*, inducing cell apoptosis, and reduced tumor sizes and density of tumor cells *in vivo*, compared to the individual drugs.[339] Following this lead, Resnier *et al.* chose a dual therapy, combining gene therapy with ferrocifen.[334] They managed to encapsulate Bcl-2 siRNA and Ansa-ferrociphenol on the same LNC. The siRNA was able to down-regulate Bcl-2 expression. This protein blocks the oligomerization of Bax and Bak proapoptotic proteins, avoiding the activation of the apoptosis pathway. Therefore, the downregulation of this overexpressed protein in melanoma cells would facilitate apoptosis activation and would reduce chemoresistance in tumor cells. The siRNA-LNCs achieved Bcl-2 gene silencing and the Ansa-ferrociphenol-LNCs showed the same cytotoxicity as the free compound. The combination of siRNA-LNCs with the free compound demonstrated the

synergistic effect of dual therapy and yielded better results than the combination of siRNA-LNCs with dacarbazine. In addition, the co-encapsulation of both siRNA and Ansa-ferrociphenol, improved siRNA encapsulation efficiency. The *in vivo* outcomes of intravenously administered siRNA/Ansa-ferrociphenol-LNCs also reported a higher tumor size reduction with respect to mice treated with one-drug LNCs.[334]

A commonly used strategy to improve chemotherapy effectiveness is the design of targeted nanocarriers to achieve higher drug concentration into the tumor and reduce side effects. In this regard, albumin-decorated DTX-loaded LNEs and HA-coated SLNs have been developed.[107,332] HA is one of the biomolecules frequently used to decorate nanocarriers. This molecule binds to the CD44 receptor, overexpressed in cancer cells from different cancer types.[337,338] Shi *et al.* used synergistic dual targeted SLNs based on HA to target CD44 receptor and tetraiodothyroacetic acid (tetrac) to target integrin $\alpha_v\beta_3$, which is overexpressed in tumor endothelial cells.[338] The DTX-loaded HA/tetrac SLNs improved the cytotoxic effect *in vitro* and the cellular uptake of $CD44^+/\ \alpha_v\beta_3^+$ cells but not of $CD44^-/\ \alpha_v\beta_3^-$ cells. *In vivo* assays reported the effective synergistic dual targeting of HA/tetrac SLNs to the tumor environment and cells in xenograft and lung metastasis models. Moreover, HA /tetrac SLNs not only provided higher tumor growth inhibition when compared to the non-targeted system, but also when compared to the HA-coated SLNs and tetrac-coated SLNs.[338] The LDL receptor is also overexpressed in cancer cells. Kretzer *et al.* synthesized PTX-loaded LNEs, which specifically bind to the LDL receptor [330] as the 7-ketocholesterol LNEs developed by Favero *et al.*[331]

When the skin tumor is superficial, a topical route for drug administration is possible. Drug encapsulation into LCNPs can enhance drug permeability through the skin. An example of this is the one developed by Iqbal *et al.* using silymarin-loaded SLNs.[331] De Moura *et al.* prepared DTX and lidocaine co-loaded NLCs embedded into a xanthan-chitosan hydrogel for topical administration. *In vivo* assays indicated that the hybrid hydrogel was able to inhibit tumor growth in an equivalent manner to the conventional (free DTX) treatment and showed no adverse effects, as revealed by physical, biochemical, and histopathological parameters.[342] Recently, Geronimo *et al.* prepared NLCs of lavender and melaleuca oils for the encapsulation of Bupivacaine, the most widely used local anesthetic agent in surgery, which presents anticancer properties. *In vitro* cytotoxicity tests revealed that the optimized NLC increased melanoma cell death and greater *in vivo* anesthetic activity after subcutaneous application.[341] Furthermore, topical photodynamic therapy is a possible alternative treatment, which requires a photosensitizing agent on the tumor site that will then be excited by a specific wavelength to produce reactive oxygen species in the tumor environment.[328] Dalmolin *et al.* encapsulated

the lipophilic photosensitizing agent zinc phthalocyanine into LNEs and used iontophoresis, *i.e.*, the application of a constant and weak electric field on the skin to generate a voltage gradient, to facilitate the penetration of Zinc Phthalocyanine LNEs through the skin's inner layers to the tumor site. The iontophoresis process enhanced skin permeability of the emulsions, demonstrating improved permeability when particle size was smaller.[328]

A less explored possibility is the use of the intrinsic toxicity of the nanocarriers themselves against tumor cells. Drewes *et al.* reported the inhibition of melanoma development in mice orally treated with LNCs, both loaded with eugenol or not. This antitumor effect was not associated with systemic toxicity or side effects. In view of these findings, they argue that the antitumor efficiency of the system is due to the very structure of the carrier and not to its role as drug delivery system.[333]

5.2.5. Brain cancer

Brain cancer is currently the 19th most common cancer type and the 12th cancer-related cause of death worldwide.[2] Glioblastoma multiforme (GBM) is the most common and malignant type of brain tumor in adults. After initial diagnosis, the median survival of GBM patients is about 12–15 months, even with aggressive treatment.[395,396] Unlike other tumors, GBM treatment represents a major challenge, mainly due to its location in the brain, which hinders complete surgical resection, and the presence of the BBB, which limits drug entry into the central nervous system. Despite all the recent biomedical advances, current treatment is still confined to surgical resection, radiotherapy, and chemotherapy epitomized by temozolomide (TMZ). This standard treatment is applied to newly diagnosed GBM patients, but median survival remains unsatisfactory.[397] The development of new drugs has not been sufficient in improving GBM treatment. Most drugs have poor solubility in water, cannot cross the BBB, and require high doses to achieve the effective concentration, leading to toxicity consequences and adverse effects. To overcome these limitations, attempts have been made to develop LCNPs as promising drug-delivery nanocarriers to treat GMB.[398] LCNPs can cross the BBB and are suitable carriers for a wide spectrum of GBM treatments such as large molecules, genes, oligonucleotides, siRNA, and enzymes, with SLNs and NLCs being the most employed.[399]

SLNs have proved to cross the BBB carrying DTX and functionalized with peptides such as angiopep-2,[356] or carrying PTX and Tyr-3-octreotide[357] or diosgenin,[360] with promising antitumor effects in orthotopic *in vivo* models. Interestingly, Wang *et al.* developed surface-modified with RGD peptides SLNs containing PTX and naringerin peptide for a combinatorial therapy against GMB upon oral administration.[355] The dual drug-loaded SLNs

showed significant improvement in drug pharmacokinetics and higher cytotoxicity and chemoprotective effect versus the free drug suspension.[355]

Regarding NLCs, different studies have demonstrated their capabilities of crossing the BBB, active targeting, and antitumor *in vivo* effects with different drugs such as TMZ and CUR, and even using synergic therapy with both drugs.[362,365,366] Di Filippo et al. prepared bevacizumab-coated NLCs encapsulating DTX to target GBM.[367] *In vitro* anti-tumor assays showed that BVZ-NLC-DTX selectively increased the cytotoxic of DTX in cells overexpressing VEGF (U87MG and A172) though not in healthy cells (PBMCs). An *in vivo* orthotopic rat model demonstrated that free-DTX was not capable of reducing tumor growth whereas BVZ-NLC-DTX reduced up to 70% tumor volume after 15-days of treatment. Another example of NLCs as nanocarriers for synergic cancer therapy in GBM is the combination of TMZ and VCR-coloaded NLCs functionalized with RGD peptides, which exhibited sustained-release behavior, high cellular uptake, high cytotoxicity and synergy effects, increased drug accumulation in the tumor tissue, and notorious tumor inhibition efficiency with low systemic toxicity.[364] In fact, Wu *et al.* carried out a study in which they compared SLNs and NLCs for the dual drug delivery of VCR and TMZ. They state that NLCs can deliver VCR and TMZ into U87MG cells in an orthotopic brain tumor implant more efficiently, and inhibition efficacy is higher than SLNs. The inhibition efficacies of dual drugs-loaded NLCs *in vitro* and *in vivo* are also higher than single drug-loaded vectors.[358] Finally, Qu *et al.* conducted a study with the aim of comparing which type of drug delivery nanosystem (SLNs, NLCs, and polymeric NPs) was better for GBM chemotherapy using TMZ.[400] They concluded that NLCs exhibited significantly better targeting ability as well as tumor growth inhibition rate.[400]

LNCs have been used to treat brain tumors using different strategies. Lollo *et al.* prepared LNCS loaded with PTX and the immuno-stimulant single-stranded DNA molecule containing methylated cytosine-guanine dinucleotides (CpG), which increased the survival rate of GL261 glioma-bearing mice.[348] Using PTX, Groo *et al.* developed different LNCs in order to compare their pharmacokinetics and efficacy in a subcutaneous isograft model in rats.[351]

MTX is an antifolate drug that has been used for more than a half century in cancer research and clinical treatment. MTX has been studied in a wide range of cancer types, including solid brain tumors.[401] Figueiro *et al.* observed a decrease in tumor size and an increase in apoptosis in the tumor microenvironment using MTX-LNCs. This treatment decreased the leukocyte number but did not alter toxicological tissue marker expression or metabolic parameters.[354] Furthermore, Pereira *et al.* employed MTX-LNCs for oral administration.[350] They showed a higher therapeutic efficacy of MTX-LNCs in relation to MTX in GBM treatment, suggesting

that low oral doses of MTX-LNCs are a safer and effective alternative to the current expensive and invasive intravenous high-dose MTX regimens. The beneficial effect of MTX-LNCs could be due to the improved ability of LNC-loaded drugs to cross the BBB and the efficient MTX-LNCs uptake by cancer and immune cells in the brain.[350] Despite the pharmacological properties of (PhSe)2, some toxic issues limit its therapeutic use, such as the inhibition of enzymes and oxidation of biomolecules.[402,403] In addition, (PhSe)2 is a poorly water-soluble compound,[404] which leads to low oral bioavailability [405] and hinders its administration by other routes, such as the parenteral one. Ferreira *et al.* demonstrated a decrease in C6 glioma cell viability without causing any adverse effect in astrocyte cells (healthy control) by employing (PhSe)2-LNCs.[349] Importantly, the (PhSe)2-LNCs had a superior cytotoxic effect compared to its free form, as well as increased nitrite content. Intragastric treatment reduced brain tumor size and did not cause alteration in the plasma renal and hepatic markers of function or in the parameters of oxidative balance in the brain, liver, or kidneys.[349]

Mucoadhesive-LNEs are also employed intranasally as a brain-tumor drug-delivery system. Colombo *et al.* demonstrated that LNEs carrying Kaempferol (KPF), an anti-oxidant, anti-inflammatory, neuroprotective and anti-tumor agent, showed no toxicity towards nasal mucosa.[343] *Ex vivo* permeation studies and *in vivo* biodistribution studies confirmed the superiority of the developed chitosan-coated LNEs for brain targeting after intranasal administration compared to KPF-LNEs and free KPF. The mucoadhesive LNEs decreased the viability of glioma cells by enhancing apoptosis.[343] Similarly, Azambuja *et al.* prepared cationic LNEs delivering siRNA for CD73, an enzyme responsible for adenosine production involved in a variety of tumor-progression actions, as a gene therapy for GBM treatment.[344] Upon nasal delivery, LNE-siRNA CD73R reduced tumor growth by 60% in glioma-bearing Wistar rats and achieved a 95% decrease in adenosine levels in tumor expression, confirming CD73 silencing.[344]

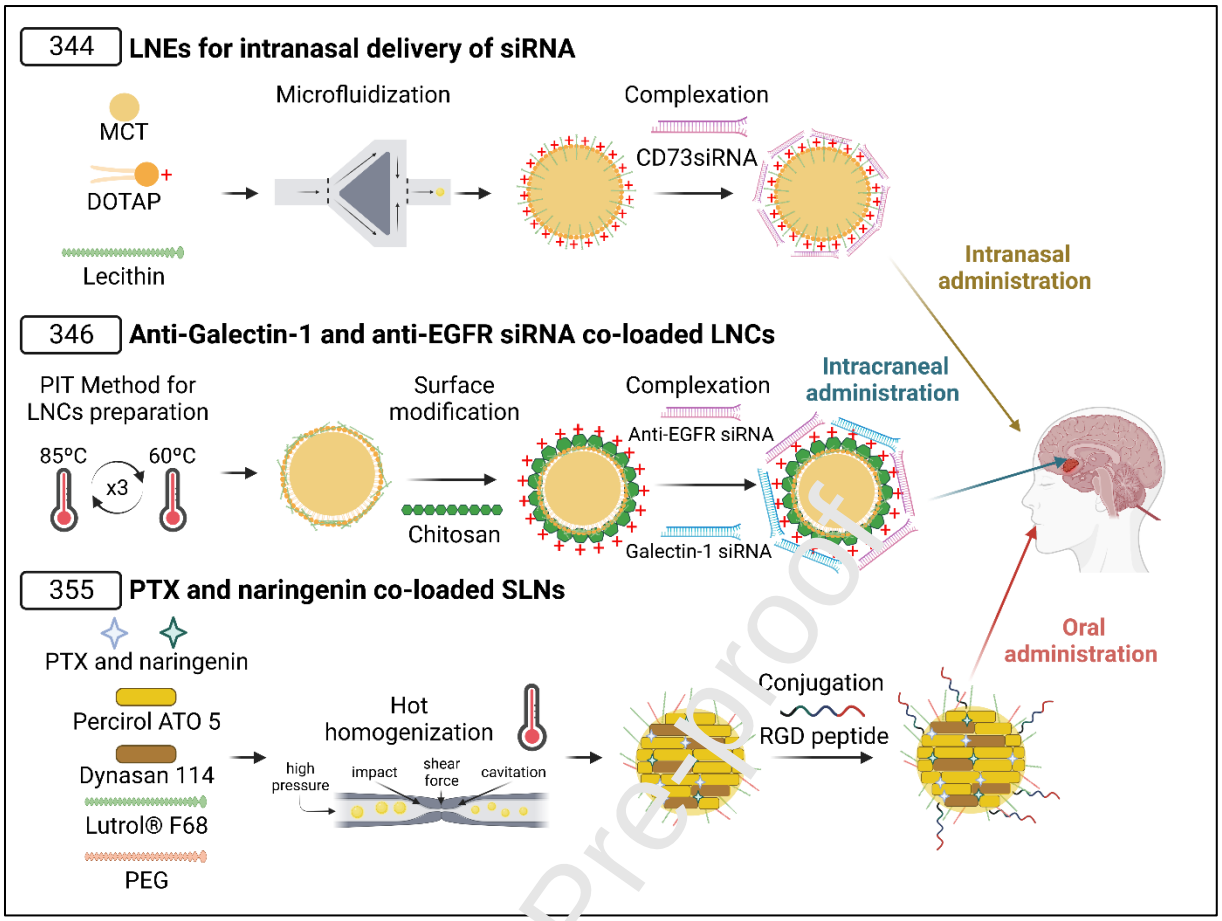


Figure 12. Representative studies of LCNPs for brain cancer treatment.[344,346,355]

5.2.6. Gastric cancer

Gastric cancer is the fifth most common cancer around the world and the fourth cancer-related cause of death.[2] Among the most recent reviewed literature, we find studies that improve the *in vitro* and *in vivo* performance of already used drugs, such as etoposide.[372] This drug inhibits DNA synthesis, but its poor water-solubility limits its bioavailability, and cancer cells show resistance to this compound. The encapsulation in NLCs improved both the solubility and the stability of the drug, contributing to higher *in vitro* cytotoxicity and better *in vivo* tumor growth inhibition.[372]

A targeting strategy also provides interesting results. Dai *et al.* functionalized ginsenoside Rg3 LNEs with a vascular endothelial growth factor receptor (VEGFR-3) antibody. The VEGFR-3, together with VEGF-C and VEGF-D, is involved in the metastatic spread of tumor cells throughout lymphatic vessels.[368] VEGFR-3 coated LNEs down-regulated the expression of these three factors and reduced gastric tumor growth in mice when compared with the control group. However, the most remarkable result is that LNEs reduced lymph-node metastasis in mice when compared with the control and 5-FU treated mice. [368] Jian *et al.* used an

oligopeptide, which bound to the blood vessels irrigating the gastric adenocarcinoma, GX1 peptide, to coat PTX-loaded NLCs. The encapsulation of the drug slowed down its release. They also studied the effect of GX1-PTX-NLCs, PTX-NLCs, and PTX on HUVEC cells, as vascular epithelial cells model, and on MKN45 cells, as gastric cancer cells. GX1-PTX-NLCs showed the strongest cytotoxic effect on HUVEC cells, while PTX-NLCs were more cytotoxic for MKN45 cells than the GX1-coated NLCs. GX1-coating also improved the NLCs uptake by HUVEC cells and reported a better inhibition on tumor growth *in vivo*, compared to PTX-NLCs and free PTX.[370]

5-FU and cisplatin (CDDP) combination is the first line chemotherapy treatment for gastric cancer. Qu and coworkers developed HA-coated NLCs for the co-delivery of these two drugs. They combined both drugs at different proportions and studied the synergistic effects on BGC823 human gastric cancer cell line. The *in vivo* results in mice reported higher tumor growth inhibition on 5-FU and CDDP NLCs. Moreover, the HA-coated 5-FU and CDDP LNCs reported the best results regarding tumor growth inhibition, as well as reduced side effects of treatment.[371] Combinatorial nanotherapy of PTX and tanespimycin co-loaded into SLNs was proposed by Ma *et al*. The encapsulated SLNs reduced cell viability and colony formation in gastric cell lines and could also induce apoptosis in MKN45 cells and inhibit growth of xenografts.[369]

Immunotherapy has become a promising strategy in cancer research. Along these lines, FA-coated chlorin e6-loaded-NLCs is an *in-situ* vaccine that stimulates dendritic cells and the subsequent immunologic response in gastric cancer.[373] Since chlorin e6 is used as a photodynamic therapy agent, this compound is expected to produce cell damage after being excited by the appropriate light wavelength. Tumor cell damage will facilitate the exposure of tumor associated antigens to dendritic cells, which will contribute to immunological activation and cancer vaccination. Their results confirmed the activation of dendritic cells after photodynamic therapy and proved to not only reduce the sizes of the primary tumors, but also of the distant tumor *in vivo* when mice were treated with FA-coated chlorin e6 -loaded- NLCs. On the other hand, the coating with FA improved the NLCs targeting at the tumor site.[373]

5.2.7. Pancreatic cancer

The high mortality rate associated with pancreatic cancer, with a five year survival rate below 7%, makes this cancer the seventh cancer related cause of death.[2] The aggressivity of this illness is linked to the existence of a subpopulation of CSCs within the tumor, which is responsible for metastatic tumor initiation, growth, recurrence and chemotherapy

resistance.[406] In light of the relevance of CSCs in pancreatic cancer prognosis, Ingallina and coworkers focused their work on targeting the Hedgehog signaling pathway, which regulates normal cell growth and differentiation.[375] The dysregulation of this pathway leads to tumorigenesis and to more aggressive phenotypes associated with CSCs. Ingallina *et al.* loaded Glabrescione B (GlaB), a natural compound that binds to the nuclear Hedgehog modulator GLI1, in GlaB-LNCs. [375,407] GlaB-loaded LNCs were more cytotoxic for CSCs compared with the Hedgehog-down regulated-non-stem population. However, the *in vivo* assays showed accumulation of LNCs on first-pass organs and the concentrations achieved at the tumor site were below the therapeutic threshold. In another study, Navarro-Marchal *et al.* encapsulated PTX into olive oil LNCs functionalized with anti-CD44 antibody in an attempt to selectively target pancreatic CSCs.[376] *In vitro* efficient targeted delivery to PCSCs and the *in vivo* noninvasive imaging cell tracking suggest their promising potential in targeted tumor theragnostic.[376]

To improve the treatment response of pancreatic tumors, Johnson *et al.* developed dodecafluoropentane-loaded LNEs to enhance tumor oxygenation. Their final objective was to raise the effect of tumor radiation therapy, since hypoxia in tumors has proved to reduce radiation effectiveness.[374] Dodecafluoropentane binds to $O_2$ and acts as an oxygen carrier. Dodecafluoropentane-LNEs achieved oxygenation of the tumor for a short period of time, which was enough to attain tumor sensibilization to the radiotherapy. Moreover, the combined treatment of carbogen breathing therapy, radiotherapy, and LNEs provided higher reductions of tumor sizes compared with the combined treatment of carbogen breathing therapy and radiotherapy (2 fold reduction) and compared with the non-treated group (25 fold reduction).[374]

In terms of chemoprevention, oral administration of aspirin and CUR-loaded SLNs combined with oral administration of free sulforaphane have been proved to suppress the progression of pancreatic intraepithelial neoplasms, the precursor of pancreatic ductal adenocarcinoma.[377] The toxicity of the combined therapy was studied for different time periods, and its lack of toxic effect was proved up to 90 days of administration.[377]

In addition to the lack of efficient treatments for pancreatic cancer, little is found in the literature about this subject. This seems to reflect the difficulty involved in the development of effective therapies against this tumor type, partially due to the special characteristics of the cellular population within the tumor.

5.2.8. Prostate cancer

Prostate cancer is the fourth most common cancer and the second cancer related cause of death in men.[2] In the most recent literature, the search for effective therapies has led researchers to explore the use of new drugs, such as the new generation toxoid SBT-1214. This drug is active against CSCs, which usually show resistance to other drugs used in clinics.[380] A conjugated omega-3 fatty acid/SBT-1214 prodrug has been effectively encapsulated into LNEs.[380] The LNE showed an efficient uptake by PPT2 cells in both the total population and the enriched-CSCs population. Moreover, the encapsulation of the drug increased its cytotoxic effect. The *in vivo* assays showed an improvement in tumor growth inhibition in LNEs-treated mice, even at low drug concentrations. In addition, the LNEs treatment inhibited tumor cell proliferation abilities in *in vitro* CSCs selective culture conditions.[380]

An innovative approach has been recently reported by Liu *et al.* who developed pH-sensitive DOX and superparamagnetic iron oxide nanoparticles (SPIONs)-loaded SLNs.[382] The magnetic component provided the possibility of guiding the SLNs toward the desired area due to an external electrostatic field and thermomagnetic therapy. The pH-sensitive coating allowed for the release at the acidic pH found in the tumor. They confirmed the enhanced cytotoxicity of the pH sensitive DOX/SPIONs-SLNs when the hyperthermia treatment was applied *in vitro*. Moreover, they achieved an *in vivo* increased accumulation of SLNs in the tumor due to the electrostatic field guidance and the pH-sensitive coating. Tumor growth was effectively inhibited, and the application of the hyperthermia treatment further improved tumor growth inhibition.[382]

## 6. Conclusions and outlook

At present, nanotechnology plays an important role in the targeted delivery of drugs for cancer treatments. LNPs constitute a diverse and extensive group of lipid-based nanotechnological platforms that have been widely studied and employed in the treatment of numerous pathologies due to their versatility and biocompatibility, among other interesting properties. In cancer therapy research, LNPs demonstrate promising potential, as confirmed by the existence of market-available nanopharmaceuticals and numerous clinical trials, with liposomes being the most exploited formulation. In this critical review, LCNPs, defined as LNPs with a lipid core, are classified into LLNs (including LNEs and LNCs), SLNs and NLCs. The great variety of LCNPs types is derived from the large and increasing number of preparation methods. Their production is feasible and effective in traditional laboratories and includes the possibility of large-scale production. Furthermore, due to the fact that their components are usually of GRAS status or accepted by the FDA, they possess a high safety threshold. The selection of ingredients

affects the performance of LCNPs, such as their uptake, drug release, or the solubility of drugs as well as their physicochemical properties. Moreover, although LCNPS are well established as safe drug-delivery systems, it is necessary to assess their possible toxicological concerns, especially for new nanoformulations and preparations techniques.

As reviewed, LCNPs not only allow for the inclusion of traditional drugs such as hydrophobic chemotherapeutics, but are very promising as carrier systems for larger molecules such as peptides or nucleic acids. At present, the delivery of genetic material in LCNPs is very promising for different biomedical applications. For cancer therapy, biomolecules are mainly included to achieve a combinatorial therapy with chemotherapeutics that develop resistances or are not effective. Further understanding is required to determine how LCNPs accommodate biomacromolecules, as it remains unclear. Techniques such as freeze-fracture electron microscopy (FFEM), NMR, or X-ray scattering are needed to fully understand the structure of the formulated complexes.

Due to their versatility and adaptability, LCNPs may be introduced into pharmaceutical formulations administered via different pathways. As for most types of colloidal nanocarriers, intravenous injection is the preferred route of administration for cancer treatment. LCNPs are very promising as oral drug delivery systems as they improve oral drug solubility and intestinal permeability. However, the gastrointestinal tract is complex and not easy to replicate in models. It remains difficult to predict the interactions between NPs and the gut. More sophisticated models are required to study the performance of orally administered nanocarriers. In this regard, microfluidics devices could allow the replication of the gastrointestinal environment, including peristaltic movements. Similarly, LCNPs, especially SLNs and NLCs, administered intranasally, establish a promising approach for targeting drugs to the brain. Nevertheless, it is essential to identify factors influencing nasal absorption of the drug such, as mucociliary clearance or enzymatic degradation. LCNPs offer advantages for topical applications such as the use of lipids that enhance skin penetration and modulate drug release. Furthermore, they can be applied directly onto damaged skin due to the safety of their components.

In the future, LCNPs development will lead to the adaptation of the formulation methods to encapsulate more complex drugs, especially therapeutic biomacromolecules, and to functionalize particles to achieve target-specific therapy. In this sense, drug-delivery systems to target the brain through intranasal administration is one of the most promising fields in cancer nanotherapy. However, further work is required to study the interactions of LCNPs and biological environments to fully understand and predict their *in vivo* performance. Despite the presented challenges, LCNPs are a versatile group of nanoparticles that can adapt different

formulations to overcome a great number of difficulties related to drug delivery, with increasing interest in the treatment of different types of tumors.

Declaration of Competing Interest

All authors declare that there is no conflict of interest.

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References

[1] Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17–48. https://doi.org/10.3322/caac.21763.

[2] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209–49. https://doi.org/10.3322/caac.21660.

[3] Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, et al. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med 2021;9:20503121211034366. https://doi.org/10.1177/20503121211034366.

[4] Tran P, Lee SE, Kim DH, Pyo YC, Park JS. Recent advances of nanotechnology for the delivery of anticancer drugs for breast cancer treatment. J Pharm Investig 2020;50:261–70. https://doi.org/10.1007/s40005-019-00459-7.

[5] Din F ud, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S, et al. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. Int J Nanomedicine 2017;Volume 12:7291–309. https://doi.org/10.2147/IJN.S146315.

[6] Borgheti-Cardoso LN, Viegas JSR, Silvestrini AVP, Caron AL, Praça FG, Kravicz M, et al. Nanotechnology approaches in the current therapy of skin cancer. Adv Drug Deliv Rev 2020;153:109–36. https://doi.org/10.1016/j.addr.2020.02.005.

[7] Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol 2015. https://doi.org/10.1038/nbt.3330.

[8] Teixeira MC, Carbone C, Souto EB. Beyond liposomes: Recent advances on lipid based nanostructures for poorly soluble/poorly permeable drug delivery. Prog Lipid Res 2017;68:1–11. https://doi.org/10.1016/j.plipres.2017.07.001.

[9] Wang G, Wang J, Wu W, Tony To SS, Zhao H, Wang J. Advances in lipid-based drug delivery: Enhancing efficiency for hydrophobic drugs. Expert Opin Drug Deliv 2015;12:1475–99. https://doi.org/10.1517/17425247.2015.1021681.
[10] Cullis PR, Hope MJ. Lipid Nanoparticle Systems for Enabling Gene Therapies. Molecular Therapy 2017;25:1467–75. https://doi.org/10.1016/j.ymthe.2017.03.013.
[11] Yang J. Patisiran for the treatment of hereditary transthyretin-mediated amyloidosis. Expert Rev Clin Pharmacol 2019;12:95–9. https://doi.org/10.1080/17512433.2019.1567326.
[12] Lamb YN. BNT162b2 mRNA COVID-19 Vaccine: First Approval. Drugs 2021;81:495–501. https://doi.org/10.1007/s40265-021-01480-7.
[13] Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. Journal of Controlled Release 2015;217:345–51. https://doi.org/10.1016/j.jconrel.2015.08.007.
[14] Oliver SE, Gargano JW, Marin M, Wallace M, Curran KG, Chamberland M, et al. The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Moderna COVID-19 Vaccine — United States, December 2020. MMWR Morb Mortal Wkly Rep 2021;69:1653–6. https://doi.org/10.15585/mmwr.mm695152e1.
[15] Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. Mol Cancer 2021;20:1–23. https://doi.org/10.1186/s12943-021-01335-5.
[16] Barba AA, Bochicchio S, Dalmoro A, Lamberti G. Lipid Delivery Systems for Nucleic-Acid-Based-Drugs: From Production to Clinical Applications. Pharmaceutics 2019;11:360. https://doi.org/10.3390/pharmaceutics11080360.
[17] Yingchoncharoen P, Kalinowski DS, Richardson DR. Lipid-based drug delivery systems in cancer therapy: What is available and what is yet to come. Pharmacol Rev 2016;68:701–87. https://doi.org/10.1124/pr.115.012070.
[18] Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965;13:238–52. https://doi.org/10.1016/S0022-2836(65)80093-6.
[19] Wacker M. Nanocarriers for intravenous injection - The long hard road to the market. Int J Pharm 2013;457:50–62. https://doi.org/10.1016/j.ijpharm.2013.08.079.
[20] Sharma A, Sharma US. Liposomes in drug delivery: Progress and limitations. Int J Pharm 1997;154:123–40. https://doi.org/10.1016/S0378-5173(97)00135-X.
[21] Severino P, Andreani T, Macedo AS, Fangueiro JF, Santana MHA, Silva AM, et al. Current State-of-Art and New Trends on Lipid Nanoparticles (SLN and NLC) for Oral Drug Delivery. J Drug Deliv 2012;2012:1–10. https://doi.org/10.1155/2012/750891.
[22] Schulman JH, Stoeckenius W, Prince LM. Mechanism of formation and structure of micro emulsions by electron microscopy. Journal of Physical Chemistry 1959;63:1677–80. https://doi.org/10.1021/j150580a027.
[23] Sjöblom J, Lindberg R, Friberg SE. Microemulsions - Phase equilibria characterization, structures, applications and chemical reactions. Adv Colloid Interface Sci 1996;65:125–287. https://doi.org/10.1016/0001-8686(96)00293-X.
[24] Montenegro L, Lai F, Offerta A, Sarpietro MG, Micicchè L, Maccioni AM, et al. From nanoemulsions to nanostructured lipid carriers: A relevant development in dermal delivery of drugs and cosmetics. J Drug Deliv Sci Technol 2016;32:100–12. https://doi.org/10.1016/j.jddst.2015.10.003.
[25] Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid Nanoparticles from Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. ACS Nano 2021;15:16982–7015. https://doi.org/10.1021/ACSNANO.1C04996/ASSET/IMAGES/MEDIUM/NN1C04996_0026.GIF.

[26] Mora-Huertas CE, Fessi H, Elaissari A. Polymer-based nanocapsules for drug delivery. Int J Pharm 2010;385:113–42. https://doi.org/10.1016/j.ijpharm.2009.10.018.
[27] Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: A review. Crit Rev Ther Drug Carrier Syst 2002;19:99–134. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v19.i2.10.
[28] Deng S, Gigliobianco MR, Censi R, di Martino P. Polymeric Nanocapsules as Nanotechnological Alternative for Drug Delivery System: Current Status, Challenges and Opportunities. Nanomaterials 2020;10:847. https://doi.org/10.3390/nano10050847.
[29] Letchford K, Burt H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. European Journal of Pharmaceutics and Biopharmaceutics 2007;65:259–69. https://doi.org/10.1016/j.ejpb.2006.11.009.
[30] Kothamasu P, Kanumur H, Ravur N, Maddu C, Parasuramrajam R, Thangavel S. Nanocapsules: The weapons for novel drug delivery systems. BioImpacts 2012;2:71–81. https://doi.org/10.5681/bi.2012.011.
[31] Ferreira LM, Cervi VF, Sari MHM, Barbieri AV, Ramos AP, Copetti PM, et al. Diphenyl diselenide loaded poly(ε-caprolactone) nanocapsules with selective antimelanoma activity: Development and cytotoxic evaluation. Materials Science and Engineering C 2018;91:1–9. https://doi.org/10.1016/j.msec.2018.05.014.
[32] Figueiró F, Bernardi A, Frozza RL, Terroso T, Zanotto-Filho A, Jandrey EHF, et al. Resveratrol-loaded lipid-core nanocapsules treatment reduces in vitro and in vivo glioma growth. J Biomed Nanotechnol 2013;9:516–26. https://doi.org/10.1166/jbn.2013.1547.
[33] Lollo G, Gonzalez-Paredes A, Garcia-Fuentes M, Calvo P, Torres D, Alonso MJ. Polyarginine Nanocapsules as a Potential Oral Peptide Delivery Carrier. J Pharm Sci 2017;106:611–8. https://doi.org/10.1016/j.xphs.2016.09.029.
[34] Herault B, Saulnier P, Pech B, Proust J-E, Richard J, Benoit JP. WO02688000, 2000.
[35] Gutiérrez JM, González C, Maestro A, Solè I, Pey CM, Nolla J. Nano-emulsions: New applications and optimization of their preparation. Curr Opin Colloid Interface Sci 2008;13:245–51. https://doi.org/10.1016/j.cocis.2008.01.005.
[36] McClements DJ. Nanoemulsions versus microemulsions: Terminology, differences, and similarities. Soft Matter 2012;8:1719–29. https://doi.org/10.1039/c2sm06903b.
[37] Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, et al. Nanoemulsion: Concepts, development and applications in drug delivery. Journal of Controlled Release 2017;252:28–49. https://doi.org/10.1016/j.jconrel.2017.03.008.
[38] Anton N, Benoit JP, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates-A review. Journal of Controlled Release 2008;128:185–99. https://doi.org/10.1016/j.jconrel.2008.02.007.
[39] Abbas S, Karangwa E, Bashari M, Hayat K, Hong X, Sharif HR, et al. Fabrication of polymeric nanocapsules from curcumin-loaded nanoemulsion templates by self-assembly. Ultrason Sonochem 2015;23:81–92. https://doi.org/10.1016/j.ultsonch.2014.10.006.
[40] Vauthier C, Bouchemal K. Methods for the Preparation and Manufacture of Polymeric Nanoparticles. Pharm Res 2009;26:1025–58. https://doi.org/10.1007/s11095-008-9800-3.
[41] Couvreur P, Tulkenst P, Roland M, Trouet A, Speiser P. Nanocapsules: A new type of lysosomotropic carrier. FEBS Lett 1977;84:323–6. https://doi.org/10.1016/0014-5793(77)80717-5.
[42] Couvreur P, Kante B, Roland M, Guiot P, BAuduin P, Speiser P. Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and

sorptive properties. Journal of Pharmacy and Pharmacology 1979;31:331–2. https://doi.org/10.1111/j.2042-7158.1979.tb13510.x.
[43] Heurtault B, Saulnier P, Pech B, Proust J-E, Benoit J-P. A Novel Phase Inversion-Based Process for the Preparation of Lipid Nanocarriers n.d.;19:875–80. https://doi.org/10.1023/A:1016121319668.
[44] Groo A, Bossiere M, Trichard L, Legras P, Benoit J, Lagarce F. In vivo evaluation of paclitaxel-loaded lipid nanocapsules after intravenous and oral administration on resistant tumor n.d.;10:589–601. https://doi.org/10.2217/nnm.14.124.
[45] Lollo G, Matha K, Bocchiardo M, Bejaud J, Marigo I, Virgone-Carlotta A, et al. Drug delivery to tumours using a novel 5-{FU} derivative encapsulated into lipid nanocapsules n.d.;27:634–45. https://doi.org/10.1080/1061186X.2018.1547733.
[46] Lollo G, Ullio-Gamboa G, Fuentes E, Matha K, Lautram N, Benoit J-P. In vitro anti-cancer activity and pharmacokinetic evaluation of curcumin-loaded lipid nanocapsules n.d.;91:859–67. https://doi.org/10.1016/j.msec.2018.06.014.
[47] Hirsjärvi S, Dufort S, Gravier J, Texier I, Yan Q, Bibette J, et al. Influence of size, surface coating and fine chemical composition on the in vitro reactivity and in vivo biodistribution of lipid nanocapsules versus lipid nanoemulsions in cancer models n.d.;9:375–87. https://doi.org/10.1016/j.nano.2012.08.005.
[48] Nasr M, Abdel-Hamid S. Lipid based Nanocapsules: A Multitude of Biomedical Applications. Curr Pharm Biotechnol 2015;16:822–32. https://doi.org/10.2174/1389201016041502181053 55.
[49] Feng L, Mumper RJ. A critical review of lipid-based nanoparticles for taxane delivery. Cancer Lett 2013;334:157–75. https://doi.org/10.1016/j.canlet.2012.07.006.
[50] Wulff-Pérez M, Martín-Rodríguez A, Gálvez-Ruiz MJ, de Vicente J. The effect of polymeric surfactants on the rheological properties of nanoemulsions. Colloid Polym Sci 2013;291:709–16. https://doi.org/10.1007/s00396-012-2780-1.
[51] Wulff-Pérez M, Torcello-Gómez A, Martín-Rodríguez A, Gálvez-Ruiz MJ, de Vicente J. Bulk and interfacial viscoelasticity in concentrated emulsions: The role of the surfactant. Food Hydrocoll 2011;25:677–86. https://doi.org/10.1016/j.foodhyd.2010.08.012.
[52] Preetz C, Hauser A, Hause G, Kramer A, Mäder K. Application of atomic force microscopy and ultrasonic resonator technology on nanoscale: Distinction of nanoemulsions from nanocapsules n.d.;39:141–51. https://doi.org/10.1016/j.ejps.2009.11.009.
[53] Calvo P, Vila-Jato JL, Alonso MJ. Comparative in vitro evaluation of several colloidal systems, nanoparticles, nanocapsules, and nanoemulsions, as ocular drug carriers. J Pharm Sci 1996;85:530–6. https://doi.org/10.1021/js950474+.
[54] Maestrelli F, Mura P, Alonso MJ. Formulation and characterization of triclosan sub-micron emulsions and nanocapsules. J Microencapsul 2004;21:857–64. https://doi.org/10.1080/02652040400015411.
[55] Santos-Magalhães NS, Pontes A, Pereira VMW, Caetano MNP. Colloidal carriers for benzathine penicillin G: Nanoemulsions and nanocapsules. Int J Pharm 2000;208:71–80. https://doi.org/10.1016/S0378-5173(00)00546-9.
[56] Szafraniec-Szczęsny J, Janik-Hazuka M, Odrobińska J, Zapotoczny S. Polymer Capsules with Hydrophobic Liquid Cores as Functional Nanocarriers. Polymers (Basel) 2020;12:1999. https://doi.org/10.3390/polym12091999.
[57] Eldem T, Speiser P, Hincal A. Optimization of Spray-Dried and -Congealed Lipid Micropellets and Characterization of Their Surface Morphology by Scanning Electron Microscopy. Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists 1991;8:47–54. https://doi.org/10.1023/A:1015874121860.

[58] Maria R. Gasco. US5250236A 1993.
[59] Schwarz C, Mehnert W, Lucks JS, Müller RH. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. Journal of Controlled Release 1994;30:83–96. https://doi.org/10.1016/0168-3659(94)90047-7.
[60] Nasirizadeh S, Malaekeh-Nikouei B. Solid lipid nanoparticles and nanostructured lipid carriers in oral cancer drug delivery. J Drug Deliv Sci Technol 2020;55:101458. https://doi.org/10.1016/j.jddst.2019.101458.
[61] zur Mühlen A, Schwarz C, Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery - Drug release and release mechanism. European Journal of Pharmaceutics and Biopharmaceutics 1998;45:149–55. https://doi.org/10.1016/S0939-6411(97)00150-1.
[62] Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2001;47:165–96. https://doi.org/10.1016/S0169-409X(01)00105-3.
[63] Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2012;64:83–101. https://doi.org/10.1016/j.addr.2012.09.021.
[64] Müller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm 2002;242:121–8. https://doi.org/10.1016/S0378-5173(02)00180-1.
[65] Mueller R, Maeder K, Lippacher A, Jenning V. DE19945203A1, 1999.
[66] Jenning V, Gohla SH. Encapsulation of retinoids in solid lipid nanoparticles (SLN®). J Microencapsul 2001;18:149–58. https://doi.org/10.1080/02652040010000361.
[67] Keck CM, Specht D, Brüßler J. Influence of lipid matrix composition on biopharmaceutical properties of lipid nanoparticles. Journal of Controlled Release 2021;338:149–63. https://doi.org/10.1016/j.jconrel.2021.08.016.
[68] Kovačević AB, Müller RH, Keck CM. Formulation development of lipid nanoparticles: Improved lipid screening and development of tacrolimus loaded nanostructured lipid carriers (NLC). Int J Pharm 2020;576:118918. https://doi.org/10.1016/j.ijpharm.2019.118918.
[69] Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2001;47:165–96. https://doi.org/10.1016/S0169-409X(01)00105-3.
[70] Doran MP. Engineering Principles Second Edition. Academic Press; 2013.
[71] Gall V, Runde M, Schuchmann HP. Extending applications of high-pressure homogenization by using simultaneous emulsification and mixing (SEM) - An overview. Processes 2016;4:46. https://doi.org/10.3390/pr4040046.
[72] Zhang J, Xu S, Li W. High shear mixers: A review of typical applications and studies on power draw, flow pattern, energy dissipation and transfer properties. Chemical Engineering and Processing: Process Intensification 2012;57–58:25–41. https://doi.org/10.1016/j.cep.2012.04.004.
[73] Bosch ET, Langhorn KD, Cohen DM. High shear rotors and stators for mixers and emulsifiers. US 6241472 B1., 2001.
[74] Shepherd SJ, Issadore D, Mitchell MJ. Microfluidic formulation of nanoparticles for biomedical applications. Biomaterials 2021;274:120826. https://doi.org/10.1016/j.biomaterials.2021.120826.
[75] Uluata S, Decker EA, McClements DJ. Optimization of Nanoemulsion Fabrication Using Microfluidization: Role of Surfactant Concentration on Formation and Stability. Food Biophys, vol. 11, 2016, p. 52–9. https://doi.org/10.1007/s11483-015-9416-1.
[76] Wood RJK. Tribocorrosion. Shreir's Corrosion, Elsevier; 2010, p. 1005–50. https://doi.org/10.1016/B978-044452787-5.00041-X.

[77] Lovelyn C, Attama AA. Current State of Nanoemulsions in Drug Delivery. J Biomater Nanobiotechnol 2011;02:626–39. https://doi.org/10.4236/jbnb.2011.225075.
[78] Shinoda K, Saito H. The effect of temperature on the phase equilibria and the types of dispersions of the ternary system composed of water, cyclohexane, and nonionic surfactant. J Colloid Interface Sci 1968;26:70–4. https://doi.org/10.1016/0021-9797(68)90273-7.
[79] Shinoda K, Saito H. The Stability of O/W type emulsions as functions of temperature and the HLB of emulsifiers: The emulsification by PIT-method. J Colloid Interface Sci 1969;30:258–63. https://doi.org/10.1016/S0021-9797(69)80012-3.
[80] Ren G, Sun Z, Wang Z, Zheng X, Xu Z, Sun D. Nanoemulsion formation by the phase inversion temperature method using polyoxypropylene surfactants. J Colloid Interface Sci 2019;540:177–84. https://doi.org/10.1016/j.jcis.2019.01.018.
[81] Anton N, Gayet P, Benoit JP, Saulnier P. Nano-emulsions and nanocapsules by the PIT method: An investigation on the role of the temperature cycling on the emulsion phase inversion. Int J Pharm 2007;344:44–52. https://doi.org/10.1016/j.ijpharm.2007.04.027.
[82] Jintapattanakit A. Preparation of nanoemulsions by phase inversion temperature (PIT) method. Pharmaceutical Sciences Asia 2018;45:1–12. https://doi.org/10.29090/psa.2018.01.001.
[83] Heunemann P, Prévost S, Grillo I, Marino CM, Meyer J, Gradzielski M. Formation and structure of slightly anionically charged nanoemulsions obtained by the phase inversion concentration (PIC) method. Soft Matter 2011;7:3697–710. https://doi.org/10.1039/c0sm01556c.
[84] Solè I, Pey CM, Maestro A, González C, Porras M, Solans C, et al. Nano-emulsions prepared by the phase inversion composition method: Preparation variables and scale up. J Colloid Interface Sci 2010;344:417–23. https://doi.org/10.1016/j.jcis.2009.11.046.
[85] Solans C, Morales D, Homs M. Spontaneous emulsification. Curr Opin Colloid Interface Sci 2016;22:88–93. https://doi.org/10.1016/j.cocis.2016.03.002.
[86] Bouchemal K, Briançon S, Perrier E, Fessi H. Nano-emulsion formulation using spontaneous emulsification: Solvent, oil and surfactant optimisation. Int J Pharm 2004;280:241–51. https://doi.org/10.1016/j.ijpharm.2004.05.016.
[87] Botet R. The "ouzo effect", recent developments and application to therapeutic drug carrying. J Phys Conf Ser, vol. 352, Institute of Physics Publishing; 2012, p. 012047. https://doi.org/10.1088/1742-6596/352/1/012047.
[88] Lepeltier E, Bourgaux C, Couvreur P. Nanoprecipitation and the "Ouzo effect": Application to drug delivery devices. Adv Drug Deliv Rev 2014;71:86–97. https://doi.org/10.1016/j.addr.2013.12.009.
[89] Taylor P, Ottewill RH. The formation and ageing rates of oil-in-water miniemulsions. Colloids Surf A Physicochem Eng Asp 1994;88:303–16. https://doi.org/10.1016/0927-7757(94)02853-2.
[90] Solans C, Solé I. Nano-emulsions: Formation by low-energy methods. Curr Opin Colloid Interface Sci 2012;17:246–54. https://doi.org/10.1016/j.cocis.2012.07.003.
[91] Heurtault B, Saulnier P, Pech B, Proust JE, Benoit JP. A novel phase inversion-based process for the preparation of lipid nanocarriers. Pharm Res 2002;19:875–80. https://doi.org/10.1023/A:1016121319668/METRICS.
[92] Anton N, Gayet P, Benoit JP, Saulnier P. Nano-emulsions and nanocapsules by the PIT method: An investigation on the role of the temperature cycling on the emulsion phase inversion. Int J Pharm 2007;344:44–52. https://doi.org/10.1016/J.IJPHARM.2007.04.027.
[93] al Khouri Fallouh N, Roblot-Treupel L, Fessi H, Devissaguet JP, Puisieux F. Development of a new process for the manufacture of polyisobutylcyanoacrylate

nanocapsules. Int J Pharm 1986;28:125–32. https://doi.org/10.1016/0378-5173(86)90236-X.
[94] Tiarks F, Landfester K, Antonietti M. Preparation of polymeric nanocapsules by miniemulsion polymerization. Langmuir 2001;17:908–18. https://doi.org/10.1021/la001276n.
[95] Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: A review. Crit Rev Ther Drug Carrier Syst 2002;19:99–134. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v19.i2.10.
[96] Quintanar-Guerrero D, Allémann E, Doelker E, Fessi H. Preparation and characterization of nanocapsnles from preformed polymers by a new process based on emulsification-diffusion technique. Pharm Res 1998;15:1056–62. https://doi.org/10.1023/A:1011934328471.
[97] Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 1989;55:R1–4. https://doi.org/10.1016/0378-5173(89)90281-0.
[98] Rață DM, Cadinoiu AN, Atanase LI, Bacaita SE, Mihalache C, Daraba OM, et al. "In vitro" behaviour of aptamer-functionalized polymeric nanocapsules loaded with 5-fluorouracil for targeted therapy. Materials Science and Engineering C 2019;103:109828. https://doi.org/10.1016/j.msec.2019.109828.
[99] Sukhorukov GB, Donath E, Lichtenfeld H, Knippel E, Knippel M, Budde A, et al. Layer-by-layer self assembly of polyelectrolytes on colloidal particles. Colloids Surf A Physicochem Eng Asp 1998;137:253–66. https://doi.org/10.1016/S0927-7757(98)00213-1.
[100] Yunessnia lehi A, Shagholani H, Ghoreishi M, Nikpay A, Soleimani lashkenari M, Soltani M. Chitosan nanocapsule-mounted cellulose nanofibrils as nanoships for smart drug delivery systems and treatment of avian trichomoniasis. J Taiwan Inst Chem Eng 2019;95:290–9. https://doi.org/10.1016/j.jtice.2018.07.014.
[101] Belbekhouche S, Oniszczuk J, Pawlak A, el Joukhar I, Goffin A, Varrault G, et al. Cationic poly(cyclodextrin)/alginate nanocapsules: From design to application as efficient delivery vehicle of 4-hydroxy tamoxifen to podocyte in vitro. Colloids Surf B Biointerfaces 2019;179:128–35. https://doi.org/10.1016/j.colsurfb.2019.03.060.
[102] Elbaz NM, Owen A, Rannard S, McDonald TO. Controlled synthesis of calcium carbonate nanoparticles and stimuli-responsive multi-layered nanocapsules for oral drug delivery. Int J Pharm 2020;574:118866. https://doi.org/10.1016/j.ijpharm.2019.118866.
[103] Kothamasu P, Kanumur H, Ravur N, Maddu C, Parasuramrajam R, Thangavel S. Nanocapsules: The weapons for novel drug delivery systems. BioImpacts 2012;2:71–81. https://doi.org/10.5681/bi.2012.011.
[104] Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2012;64:83–101. https://doi.org/10.1016/j.addr.2012.09.021.
[105] Duong V-A, Nguyen T-T-L, Maeng H-J. Preparation of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers for Drug Delivery and the Effects of Preparation Parameters of Solvent Injection Method. Molecules 2020;25:4781. https://doi.org/10.3390/molecules25204781.
[106] Charcosset C, El-Harati A, Fessi H. Preparation of solid lipid nanoparticles using a membrane contactor. Journal of Controlled Release 2005;108:112–20. https://doi.org/10.1016/j.jconrel.2005.07.023.
[107] Shen H, Shi S, Zhang Z, Gong T, Sun X. Coating solid lipid nanoparticles with hyaluronic acid enhances antitumor activity against melanoma stem-like cells. Theranostics 2015;5:755–71. https://doi.org/10.7150/thno.10804.

[108] Shi S, Zhou M, Li X, Hu M, Li C, Li M, et al. Synergistic active targeting of dually integrin αvβ3/CD44-targeted nanoparticles to B16F10 tumors located at different sites of mouse bodies. Journal of Controlled Release 2016;235:1–13. https://doi.org/10.1016/j.jconrel.2016.05.050.
[109] Sjöström B, Bergenståhl B. Preparation of submicron drug particles in lecithin-stabilized o/w emulsions I. Model studies of the precipitation of cholesteryl acetate. Int J Pharm 1992;88:53–62. https://doi.org/10.1016/0378-5173(92)90303-J.
[110] Trucillo P, Campardelli R. Production of solid lipid nanoparticles with a supercritical fluid assisted process. J Supercrit Fluids 2019;143:16–23. https://doi.org/10.1016/J.SUPFLU.2018.08.001.
[111] Schubert MA, Müller-Goymann CC. Solvent injection as a new approach for manufacturing lipid nanoparticles - Evaluation of the method and process parameters. European Journal of Pharmaceutics and Biopharmaceutics 2003;55:125–31. https://doi.org/10.1016/S0939-6411(02)00130-3.
[112] Jiang H, Pei L, Liu N, Li J, Li Z, Zhang S. Etoposide-loaded nanostructured lipid carriers for gastric cancer therapy. Drug Deliv 2016;23:1370–82. https://doi.org/10.3109/10717544.2015.1048491.
[113] Ma L, Yang D, Li Z, Zhang X, Pu L. Co-delivery of paclitaxel and tanespimycin in lipid nanoparticles enhanced anti-gastric-tumor effect *in vitro* and *in vivo*. Artif Cells Nanomed Biotechnol 2018;46:904–11. https://doi.org/10.1080/21691401.2018.1472101.
[114] Trotta M, Cavalli R, Carlotti ME, Battaglia L, Debernardi F. Solid lipid micro-particles carrying insulin formed by solvent-in-water emulsion-diffusion technique. Int J Pharm 2005;288:281–8. https://doi.org/10.1016/j.ijpharm.2004.10.014.
[115] Quintanar-Guerrero D. Preparation and characterization of solid lipid nanoparticles containing cyclosporine by the emulsification-diffusion method. Int J Nanomedicine 2010;5:611. https://doi.org/10.2147/IJN.S12125.
[116] Carbone C, Tomasello B, Ruozi B, Renis M, Puglisi G. Preparation and optimization of PIT solid lipid nanoparticles via statistical factorial design. Eur J Med Chem 2012;49:110–7. https://doi.org/10.1016/j.ejmech.2012.01.001.
[117] Nabi-Meibodi M, Vatanara A, Najafabadi AR, Rouini MR, Ramezani V, Gilani K, et al. The effective encapsulation of a hydrophobic lipid-insoluble drug in solid lipid nanoparticles using a modified double emulsion solvent evaporation method. Colloids Surf B Biointerfaces 2013;112:408–14. https://doi.org/10.1016/j.colsurfb.2013.06.013.
[118] Becker Peres L, Becker Peres L, de Araújo PHH, Sayer C. Solid lipid nanoparticles for encapsulation of hydrophilic drugs by an organic solvent free double emulsion technique. Colloids Surf B Biointerfaces 2016;140:317–23. https://doi.org/10.1016/j.colsurfb.2015.12.033.
[119] Battaglia L, Gallarate M, Cavalli R, Trotta M. Solid lipid nanoparticles produced through a coacervation method. J Microencapsul 2010;27:78–85. https://doi.org/10.3109/02652040903031279.
[120] M.R. Gasco. Method for producing solid lipid microspheres having a narrow size distribution. US Patent 5250236, 1993.
[121] Mumper RJ, Jay M. Microemulsions as precursors to solid nanoparticles. US 7153535, 2006.
[122] Cholakova D, Glushkova D, Tcholakova S, Denkov N. Nanopore and Nanoparticle Formation with Lipids Undergoing Polymorphic Phase Transitions. ACS Nano 2020;14:8594–604. https://doi.org/10.1021/ACSNANO.0C02946/SUPPL_FILE/NN0C02946_SI_008.AVI.

[123] Navya PN, Kaphle A, Srinivas SP, Bhargava SK, Rotello VM, Daima HK. Current trends and challenges in cancer management and therapy using designer nanomaterials. Nano Converg 2019;6:1–30. https://doi.org/10.1186/s40580-019-0193-2.
[124] Zhao J, Castranova V. Toxicology of nanomaterials used in nanomedicine. J Toxicol Environ Health B Crit Rev 2011;14:593–632. https://doi.org/10.1080/10937404.2011.615113.
[125] Lynch I, Salvati A, Dawson KA. Protein-nanoparticle interactions: What does the cell see? Nat Nanotechnol 2009;4:546–7. https://doi.org/10.1038/nnano.2009.248.
[126] Joshi MD, Müller RH. Lipid nanoparticles for parenteral delivery of actives. European Journal of Pharmaceutics and Biopharmaceutics 2009;71:161–72. https://doi.org/10.1016/j.ejpb.2008.09.003.
[127] Thiele L, Diederichs JE, Reszka R, Merkle HP, Walter E. Competitive adsorption of serum proteins at microparticles affects phagocytosis by dendritic cells. Biomaterials 2003;24:1409–18. https://doi.org/10.1016/S0142-9612(02)00525-2.
[128] Verhoeckx K, Cotter P, López-Expósito I, Kleiveland C, Lea T, Mackie A, et al. The impact of food bioactives on health: In vitro and Ex Vivo models. Springer International Publishing; 2015. https://doi.org/10.1007/978-3-319-16104-4.
[129] Verwey EJW. Theory of the stability of lyophobic colloids. Journal of Physical and Colloid Chemistry 1947;51:631–6. https://doi.org/10.1021/j150453a001.
[130] Maldonado-Valderrama J, Muros-Cobos JL, Holgado-Terriza JA, Cabrerizo-Vílchez MA. Bile salts at the air-water interface: Adsorption and desorption. Colloids Surf B Biointerfaces 2014;120:176–83. https://doi.org/10.1016/j.colsurfb.2014.05.014.
[131] Pilosof AMR. Potential impact of interfacial composition of proteins and polysaccharides stabilized emulsions on the modulation of lipolysis. The role of bile salts. Food Hydrocoll 2017;68:179–85. https://doi.org/10.1016/j.foodhyd.2016.08.030.
[132] Maldonado-Valderrama J, Woodward NC, Patrick Gunning A, Ridout MJ, Husband FA, Mackie AR, et al. Interfacial characterization of β-lactoglobulin networks: Displacement by bile salts. Langmuir 2008;24:6759–67. https://doi.org/10.1021/la800551n.
[133] Petrou G, Crouzier T. Mucins as multifunctional building blocks of biomaterials. Biomater Sci 2018;6:2282–97. https://doi.org/10.1039/c8bm00471d.
[134] Thanki K, Gangwal RP, Sangamwar AT, Jain S. Oral delivery of anticancer drugs: Challenges and opportunities. Journal of Controlled Release 2013;170:15–40. https://doi.org/10.1016/j.jconrel.2013.04.020.
[135] Almazroo OA, Miah MK, Venkataramanan R. Drug Metabolism in the Liver. Clin Liver Dis 2017;21:1–20. https://doi.org/10.1016/j.cld.2016.08.001.
[136] Trevaskis NL, Charman WN, Porter CJH. Lipid-based delivery systems and intestinal lymphatic drug transport: A mechanistic update. Adv Drug Deliv Rev 2008;60:702–16. https://doi.org/10.1016/j.addr.2007.09.007.
[137] Porter CJH, Charman WN. Intestinal lymphatic drug transport: An update. Adv Drug Deliv Rev 2001;50:61–80. https://doi.org/10.1016/S0169-409X(01)00151-X.
[138] Hua S. Physiological and pharmaceutical considerations for rectal drug formulations. Front Pharmacol 2019;10:1196. https://doi.org/10.3389/fphar.2019.01196.
[139] Sofi HS, Abdal-hay A, Ivanovski S, Zhang YS, Sheikh FA. Electrospun nanofibers for the delivery of active drugs through nasal, oral and vaginal mucosa: Current status and future perspectives. Materials Science and Engineering C 2020;111:110756. https://doi.org/10.1016/j.msec.2020.110756.
[140] Ghadiri M, Young P, Traini D. Strategies to Enhance Drug Absorption via Nasal and Pulmonary Routes. Pharmaceutics 2019;11:113. https://doi.org/10.3390/pharmaceutics11030113.

[141] Singh Malik D, Mital N, Kaur G. Topical drug delivery systems: A patent review. Expert Opin Ther Pat 2016;26:213–28. https://doi.org/10.1517/13543776.2016.1131267.
[142] Guilherme VA, Ribeiro LNM, Tofoli GR, Franz-Montan M, de Paula E, de Jesus MB. Current Challenges and Future of Lipid Nanoparticles Formulations for Topical Drug Application to Oral Mucosa, Skin, and Eye. Curr Pharm Des 2017;23:6659–75. https://doi.org/10.2174/1381612823666171122103849.
[143] Ngan CL, Asmawi AA. Lipid-based pulmonary delivery system: a review and future considerations of formulation strategies and limitations. Drug Deliv Transl Res 2018;8:1527–44. https://doi.org/10.1007/s13346-018-0550-4.
[144] Weber S, Zimmer A, Pardeike J. Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) for pulmonary application: A review of the state of the art. European Journal of Pharmaceutics and Biopharmaceutics 2014;86:7–22. https://doi.org/10.1016/j.ejpb.2013.08.013.
[145] Costa CP, Moreira JN, Sousa Lobo JM, Silva AC. Intranasal delivery of nanostructured lipid carriers, solid lipid nanoparticles and nanoemulsions: A current overview of in vivo studies. Acta Pharm Sin B 2021;11:925–40. https://doi.org/10.1016/j.apsb.2021.02.012.
[146] Khan AR, Liu M, Khan MW, Zhai G. Progress in brain targeting drug delivery system by nasal route. Journal of Controlled Release 2017;268:364–89. https://doi.org/10.1016/j.jconrel.2017.09.001.
[147] Alexander NJ, Baker E, Kaptein M, Karck U, Miller L, Zampaglione E. Why consider vaginal drug administration? Fertil Steril 2004;82:1–12. https://doi.org/10.1016/j.fertnstert.2004.01.025.
[148] Dezarnaulds G, Fraser IS. Vaginal ring delivery of hormone replacement therapy - A review. Expert Opin Pharmacother 2003;4:201–12. https://doi.org/10.1517/14656566.4.2.201.
[149] McLennan DN, Porter CJH, Charman SA. Subcutaneous drug delivery and the role of the lymphatics. Drug Discov Today Technol 2005;2:89–96. https://doi.org/10.1016/j.ddtec.2005.05.006.
[150] Bittner B, Richter W, Schmidt J. Subcutaneous Administration of Biotherapeutics: An Overview of Current Challenges and Opportunities. BioDrugs 2018;32:425–40. https://doi.org/10.1007/s40259-018-0295-0.
[151] Dowd FJ. Pharmacokinetics: The Absorption, Distribution, and Fate of Drugs. Pharmacology and Therapeutics for Dentistry: Seventh Edition, Elsevier; 2017, p. 15–43. https://doi.org/10.1016/B978-0-323-39307-2.00002-3.
[152] Bolger GT. Routes of Drug Administration☆. Reference Module in Biomedical Sciences, Elsevier; 2018. https://doi.org/https://doi.org/10.1016/B978-0-12-801238-3.11099-2.
[153] Stoddart A. Corona creation. Nat Mater 2013;12:946–946. https://doi.org/10.1038/nmat3799.
[154] Salvati A, Pitek AS, Monopoli MP, Prapainop K, Bombelli FB, Hristov DR, et al. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. Nat Nanotechnol 2013;8:137–43. https://doi.org/10.1038/nnano.2012.237.
[155] Abuchowski A, van Es T, Palczuk NC, Davis FF. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. Journal of Biological Chemistry 1977;252:3578–81. https://doi.org/10.1016/s0021-9258(17)40291-2.
[156] Perry JL, Reuter KG, Kai MP, Herlihy KP, Jones SW, Luft JC, et al. PEGylated PRINT nanoparticles: The impact of PEG density on protein binding, macrophage

association, biodistribution, and pharmacokinetics. Nano Lett 2012;12:5304–10. https://doi.org/10.1021/nl302638g.
[157] Lee H. Molecular dynamics studies of pegylated single-walled carbon nanotubes: The effect of PEG size and grafting density. Journal of Physical Chemistry C 2013;117:26334–41. https://doi.org/10.1021/jp4093749.
[158] Moghimi SM, Hunter AC. Poloxamers and poloxamines in nanoparticle engineering and experimental medicine. Trends Biotechnol 2000;18:412–20. https://doi.org/10.1016/S0167-7799(00)01485-2.
[159] Moghimi SM, Muir IS, Illum L, Davis SS, Kolb-Bachofen V. Coating particles with a block co-polymer (poloxamine-908) suppresses opsonization but permits the activity of dysopsonins in the serum. BBA - Molecular Cell Research 1993;1179:157–65. https://doi.org/10.1016/0167-4889(93)90137-E.
[160] Yang Q, Lai SK. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2015;7:655–77. https://doi.org/10.1002/WNAN.1339.
[161] Schubert J, Chanana M. Coating Matters: Review on Colloidal Stability of Nanoparticles with Biocompatible Coatings in Biological Media, Living Cells and Organisms. Curr Med Chem 2018;25:4553–86. https://doi.org/10.2174/0929867325666180601101559.
[162] Fang RH, Kroll A v, Gao W, Zhang L. Cell Membrane Coating Nanotechnology. Advanced Materials 2018;30:1–34. https://doi.org/10.1002/adma.201706759.
[163] Hu CMJ, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. Proc Natl Acad Sci U S A 2011;108:10980–5. https://doi.org/10.1073/pnas.1106634108.
[164] Li R, He Y, Zhang S, Qin J, Wang J. Cell membrane-based nanoparticles: a new biomimetic platform for tumor diagnosis and treatment. Acta Pharm Sin B 2018;8:14–22. https://doi.org/10.1016/j.apsb.2017.11.009.
[165] Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Adv Drug Deliv Rev 2013;65:71–9. https://doi.org/10.1016/j.addr.2012.10.002.
[166] Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Rev Clin Oncol 2010;7:653–64. https://doi.org/10.1038/nrclinonc.2010.139.
[167] Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, et al. Regulation of transport pathways in tumor vessels: Role of tumor type and microenvironment. Proc Natl Acad Sci U S A 1998;95:4607–12. https://doi.org/10.1073/pnas.95.8.4607.
[168] Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev 2014;66:2–25. https://doi.org/10.1016/j.addr.2013.11.009.
[169] Navya PN, Kaphle A, Srinivas SP, Bhargava SK, Rotello VM, Daima HK. Current trends and challenges in cancer management and therapy using designer nanomaterials. Nano Converg 2019;6:1–30. https://doi.org/10.1186/s40580-019-0193-2.
[170] Rosenblum D, Joshi N, Tao W, Karp JM, Peer D. Progress and challenges towards targeted delivery of cancer therapeutics. Nat Commun 2018;9:1–12. https://doi.org/10.1038/s41467-018-03705-y.
[171] Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev 2014;66:2–25. https://doi.org/10.1016/j.addr.2013.11.009.

[172] Mout R, Moyano DF, Rana S, Rotello VM. Surface functionalization of nanoparticles for nanomedicine. Chem Soc Rev 2012;41:2539–44. https://doi.org/10.1039/c2cs15294k.
[173] Weissleder R, Kelly K, Sun EY, Shtatland T, Josephson L. Cell-specific targeting of nanoparticles by multivalent attachment of small molecules. Nat Biotechnol 2005;23:1418–23. https://doi.org/10.1038/nbt1159.
[174] Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2007;2:751–60. https://doi.org/10.1038/nnano.2007.387.
[175] Weissleder R, Kelly K, Sun EY, Shtatland T, Josephson L. Cell-specific targeting of nanoparticles by multivalent attachment of small molecules. Nat Biotechnol 2005;23:1418–23. https://doi.org/10.1038/nbt1159.
[176] Navya PN, Kaphle A, Srinivas SP, Bhargava SK, Rotello VM, Daima HK. Current trends and challenges in cancer management and therapy using designer nanomaterials. Nano Converg 2019;6:1–30. https://doi.org/10.1186/s40580-019-0193-2.
[177] Gao J, Feng SS, Guo Y. Antibody engineering promotes nanomedicine for cancer treatment. Nanomedicine 2010;5:1141–5. https://doi.org/10.2217/nnm.10.94.
[178] Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev 2014;66:2–25. https://doi.org/10.1016/j.addr.2013.11.009.
[179] Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv Drug Deliv Rev 2008;60:1615–26. https://doi.org/10.1016/j.addr.2008.08.005.
[180] Taheri A, Dinarvand R, Atyabi F, Ghahremani MH, Ostad SN. Trastuzumab decorated methotrexate-human serum albumin conjugated nanoparticles for targeted delivery to HER2 positive tumor cells. European Journal of Pharmaceutical Sciences 2012;47:331–40. https://doi.org/10.1016/j.ejps.2012.06.016.
[181] Wang L, Su W, Liu Z, Zhou M, Chen S, Chen Y, et al. CD44 antibody-targeted liposomal nanoparticles for molecular imaging and therapy of hepatocellular carcinoma. Biomaterials 2012;33:5107–14. https://doi.org/10.1016/j.biomaterials.2012.03.067.
[182] Abdelghany SM, Schmid D, Deacon J, Jaworski J, Fay F, McLaughlin KM, et al. Enhanced antitumor activity of the photosensitizer meso -tetra(N -methyl-4-pyridyl) porphine tetra tosylate through encapsulation in antibody-targeted chitosan/alginate nanoparticles. Biomacromolecules 2013;14:302–10. https://doi.org/10.1021/bm301858a.
[183] Molek P, Strukelj B, Bratkovic T. Peptide Phage Display as a Tool for Drug Discovery: Targeting Membrane Receptors. Molecules 2011;16:857–87. https://doi.org/10.3390/molecules16010857.
[184] Pan L, He Q, Liu J, Chen Y, Ma M, Zhang L, et al. Nuclear-targeted drug delivery of tat peptide-conjugated monodisperse mesoporous silica nanoparticles. J Am Chem Soc 2012;134:5722–5. https://doi.org/10.1021/ja211035w.
[185] Shi J, Xiao Z, Kamaly N, Farokhzad OC. Self-assembled targeted nanoparticles: Evolution of technologies and bench to bedside translation. Acc Chem Res 2011;44:1123–34. https://doi.org/10.1021/ar200054n.
[186] Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC. Targeted polymeric therapeutic nanoparticles: Design, development and clinical translation. Chem Soc Rev 2012;41:2971–3010. https://doi.org/10.1039/c2cs15344k.
[187] Wang J, Tian S, Petros RA, Napier ME, Desimone JM. The complex role of multivalency in nanoparticles targeting the transferrin receptor for cancer therapies. J Am Chem Soc 2010;132:11306–13. https://doi.org/10.1021/ja1043177.

[188] Wu Y, Phillips JA, Liu H, Yang R, Tan W. Carbon nanotubes protect DNA strands during cellular delivery. ACS Nano 2008;2:2023–8. https://doi.org/10.1021/nn800325a.
[189] Rizvi SAA, Saleh AM. Applications of nanoparticle systems in drug delivery technology. Saudi Pharmaceutical Journal 2018;26:64–70. https://doi.org/10.1016/j.jsps.2017.10.012.
[190] Moradi E, Vllasaliu D, Garnett M, Falcone F, Stolnik S. Ligand density and clustering effects on endocytosis of folate modified nanoparticles. RSC Adv 2012;2:3025–33. https://doi.org/10.1039/c2ra01168a.
[191] Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, et al. Shape effects of filaments versus spherical particles in flow and drug delivery. Nat Nanotechnol 2007;2:249–55. https://doi.org/10.1038/nnano.2007.70.
[192] Petros RA, Desimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nat Rev Drug Discov 2010;9:615–27. https://doi.org/10.1038/nrd2591.
[193] Mukherjee B, Maji R, Roychowdhury S, Ghosh S. Toxicological Concerns of Engineered Nanosize Drug Delivery Systems. Am J Ther 2016;23:e139–50. https://doi.org/10.1097/01.MJT.0000433947.16654.75.
[194] Kang H, Mintri S, Menon AV, Lee HY, Choi HS, Kim J. Pharmacokinetics, pharmacodynamics and toxicology of theranostic nanoparticles. Nanoscale 2015;7:18848–62. https://doi.org/10.1039/C5NR05264E.
[195] Hossen S, Hossain MK, Basher MK, Mia MNH, Rahman MT, Uddin MJ. Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review. J Adv Res 2018;15:1–18. https://doi.org/10.1016/J.JARE.2018.06.005.
[196] Zhang X, Chen G, Zhang T, Ma Z, Wu B. Effects of PEGylated lipid nanoparticles on the oral absorption of one BCS II drug: a mechanistic investigation. Int J Nanomedicine 2014;9:5503. https://doi.org/10.2147/IJN.S73340.
[197] Soenen SJ, Rivera-Gil P, Montenegro JM, Parak WJ, de Smedt SC, Braeckmans K. Cellular toxicity of inorganic nanoparticles: Common aspects and guidelines for improved nanotoxicity evaluation. Nano Today 2011;6:446–65. https://doi.org/10.1016/J.NANTOD.2011.08.001.
[198] Vincent A, Babu S, Heckert E, Dowding J, Hirst SM, Inerbaev TM, et al. Protonated nanoparticle surface governing ligand tethering and cellular targeting. ACS Nano 2009;3:1203–11. https://doi.org/10.1021/nn9000148.
[199] Patil YB, Toti US, Khdair A, Ma L, Panyam J. Single-step surface functionalization of polymeric nanoparticles for targeted drug delivery. Biomaterials 2009;30:859–66. https://doi.org/10.1016/j.biomaterials.2008.09.056.
[200] Stefanick JF, Ashley JD, Kiziltepe T, Bilgicer B. A systematic analysis of peptide linker length and liposomal polyethylene glycol coating on cellular uptake of peptide-targeted liposomes. ACS Nano 2013;7:2935–47. https://doi.org/10.1021/nn305663e.
[201] Nel AE, Mädler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano–bio interface. Nature Materials 2009 8:7 2009;8:543–57. https://doi.org/10.1038/nmat2442.
[202] Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic-Moreno AF, et al. Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. Proc Natl Acad Sci U S A 2008;105:2586–91. https://doi.org/10.1073/pnas.0711714105.
[203] Wu J, Chu CC. Block copolymer of poly(ester amide) and polyesters: Synthesis, characterization, and in vitro cellular response. Acta Biomater 2012;8:4314–23. https://doi.org/10.1016/j.actbio.2012.07.027.
[204] Bellmann S, Carlander D, Fasano A, Momcilovic D, Scimeca JA, Waldman WJ, et al. Mammalian gastrointestinal tract parameters modulating the integrity, surface

properties, and absorption of food-relevant nanomaterials. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2015;7:609–22. https://doi.org/10.1002/wnan.1333.
[205] de Anda-Flores Y, Carvajal-Millan E, Campa-Mada A, Lizardi-Mendoza J, Rascon-Chu A, Tanori-Cordova J, et al. Polysaccharide-Based Nanoparticles for Colon-Targeted Drug Delivery Systems. Polysaccharides 2021;2:626–47. https://doi.org/10.3390/polysaccharides2030038.
[206] Aguilera-Garrido A, del Castillo-Santaella T, Galisteo-González F, José Gálvez-Ruiz M, Maldonado-Valderrama J. Investigating the role of hyaluronic acid in improving curcumin bioaccessibility from nanoemulsions. Food Chem 2021;351:129301. https://doi.org/10.1016/j.foodchem.2021.129301.
[207] Mackie AR, Goycoolea FM, Menchicchi B, Caramella CM, Saporito F, Lee S, et al. Innovative Methods and Applications in Mucoadhesion Research. Macromol Biosci 2017;17:1600534. https://doi.org/10.1002/mabi.201600534.
[208] Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. Adv Drug Deliv Rev 2012;64:557–70. https://doi.org/10.1016/j.addr.2011.12.009.
[209] Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Deliv Rev 2009;61:158–71. https://doi.org/10.1016/j.addr.2008.11.002.
[210] Ensign LM, Schneider C, Suk JS, Cone R, Hanes J. Mucus Penetrating Nanoparticles: Biophysical Tool and Method of Drug and Gene Delivery. Advanced Materials 2012;24:3887–94. https://doi.org/10.1002/adma.201201800.
[211] C.Y. Chow E, Sandy Pang K. Why We Need Proper PBPK Models to Examine Intestine and Liver Oral Drug Absorption. Curr Drug Metab 2012;14:57–79. https://doi.org/10.2174/138920021130010057.
[212] Wacher VJ, Salphati L, Benet LZ. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv Drug Deliv Rev 2001;46:89–102. https://doi.org/10.1016/S0169-409X(00)00126-5.
[213] Follain G, Herrmann D, Harlepp S, Hyenne V, Osmani N, Warren SC, et al. Fluids and their mechanics in tumour transit: shaping metastasis. Nat Rev Cancer 2020;20:107–24. https://doi.org/10.1038/s41568-019-0221-x.
[214] Ghadiri M, Young P, Traini D. Strategies to Enhance Drug Absorption via Nasal and Pulmonary Routes. Pharmaceutics 2019;11:113. https://doi.org/10.3390/pharmaceutics11030113.
[215] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11–30. https://doi.org/10.3322/caac.21166.
[216] Shapira A, Livney YD, Broxterman HJ, Assaraf YG. Nanomedicine for targeted cancer therapy: Towards the overcoming of drug resistance. Drug Resistance Updates 2011;14:150–63. https://doi.org/10.1016/j.drup.2011.01.003.
[217] Nurgali K, Jagoe RT, Abalo R. Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae? Front Pharmacol 2018;9:245. https://doi.org/10.3389/fphar.2018.00245.
[218] Elzoghby AO, El-Lakany SA, Helmy MW, Abu-Serie MM, Elgindy NA. Shell-crosslinked zein nanocapsules for oral codelivery of exemestane and resveratrol in breast cancer therapy. Nanomedicine 2017;12:2785–805. https://doi.org/10.2217/nnm-2017-0247.
[219] Sugumaran A, Ponnusamy C, Kandasamy P, Krishnaswami V, Palanichamy R, Kandasamy R, et al. Development and evaluation of camptothecin loaded polymer stabilized nanoemulsion: Targeting potential in 4T1-breast tumour xenograft model. European Journal of Pharmaceutical Sciences 2018;116:15–25. https://doi.org/10.1016/j.ejps.2017.10.005.

[220] Talaat SM, Elnaggar YSR, El-Ganainy SO, Gowayed MA, Abdel-Bary A, Abdallah OY. Novel bio-inspired lipid nanoparticles for improving the anti-tumoral efficacy of fisetin against breast cancer. Int J Pharm 2022;628:122184. https://doi.org/10.1016/J.IJPHARM.2022.122184.
[221] Rudhrabatla VSAP, Sudhakar B, Reddy KVNS. In Vitro and In Vivo Assessment of Designed Melphalan Loaded Stealth Solid Lipid Nanoparticles for Parenteral Delivery. Bionanoscience 2020;10:168–90. https://doi.org/10.1007/s12668-019-00680-6.
[222] Jain A, Sharma G, Thakur K, Raza K, Shivhare US, Ghoshal G, et al. Beta-carotene-Encapsulated Solid Lipid Nanoparticles (BC-SLNs) as Promising Vehicle for Cancer: an Investigative Assessment. AAPS PharmSciTech 2019;20:1–7. https://doi.org/10.1208/s12249-019-1301-7.
[223] Nordin N, Yeap SK, Rahman HS, Zamberi NR, Mohamad NE, Abu N, et al. Antitumor and Anti-Metastatic Effects of Citral-Loaded Nanostructured Lipid Carrier in 4T1-Induced Breast Cancer Mouse Model. Molecules 2020;25:2670. https://doi.org/10.3390/molecules25112670.
[224] dos Santos Câmara AL, Nagel G, Tschiche HR, Cardador CM, Muehlmann LA, de Oliveira DM, et al. Acid-sensitive lipidated doxorubicin prodrug entrapped in nanoemulsion impairs lung tumor metastasis in a breast cancer model. Nanomedicine 2017;12:1751–65. https://doi.org/10.2217/nnm-2017-0091.
[225] Merendino N, Costantini L, Manzi L, Molinari R, D'Eliseo D, Velotti F. Dietary ω-3 polyunsaturated fatty acid DHA: A potential adjuvant in the treatment of cancer. Biomed Res Int 2013;2013. https://doi.org/10.1155/2013/310186.
[226] de Souza LR, Muehlmann LA, Matos LC, Simón-Vázquez R, Lacava ZGM, De-Paula AMB, et al. Antitumor activity and systemic effects of PVM/MA-shelled selol nanocapsules in lung adenocarcinoma bearing mice. Nanotechnology 2015;26:505101. https://doi.org/10.1088/0957-4484/26/50/505101.
[227] Aguilera-Garrido A, Arranz E, Gálvez-Ruiz MJ, Marchal JA, Galisteo-González F, Giblin L. Solid lipid nanoparticles to improve bioaccessibility and permeability of orally administered maslinic acid. Drug Deliv 2022;29:1971–82. https://doi.org/10.1080/10717544.2022.2086937/SUPPL_FILE/IDRD_A_2086937_SM7558.DOCX.
[228] El-Gogary RI, Nasr M, Rahsed LA, Hamzawy MA. Ferulic acid nanocapsules as a promising treatment modality for colorectal cancer: Preparation and in vitro/in vivo appraisal. Life Sci 2022;298:120500. https://doi.org/10.1016/J.LFS.2022.120500.
[229] Rawal S, Patel MM. Threatening cancer with nanoparticle aided combination oncotherapy. Journal of Controlled Release 2019;301:76–109. https://doi.org/10.1016/j.jconrel.2019.03.015.
[230] Chou TC. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev 2006;58:621–81. https://doi.org/10.1124/pr.58.3.10.
[231] Dong X, Mattingly CA, Tseng MT, Cho MJ, Liu Y, Adams VR, et al. Doxorubicin and paclitaxel-loaded lipid-based nanoparticles overcome multidrug resistance by inhibiting P-glycoprotein and depleting ATP. Cancer Res 2009;69:3918–26. https://doi.org/10.1158/0008-5472.CAN-08-2747/654704/P/DOXORUBICIN-AND-PACLITAXEL-LOADED-LIPID-BASED.
[232] Garcion E, Lamprecht A, Heurtault B, Paillard A, Aubert-Pouessel A, Denizot B, et al. A new generation of anticancer, drug-loaded, colloidal vectors reverses multidrug resistance in glioma and reduces tumor progression in rats. Mol Cancer Ther 2006;5:1710–22. https://doi.org/10.1158/1535-7163.MCT-06-0289.

[233] Hürlimann AF, Hänggi G, Panizzon RG. Photodynamic therapy of superficial basal cell carcinomas using topical 5-aminolevulinic acid in a nanocolloid lotion. Dermatology 1998;197:248–54. https://doi.org/10.1159/000018006.
[234] Szeimies R-M, Radny P, Sebastian M, Borrosch F, Dirschka T, Krähn-Senftleben G, et al. Photodynamic therapy with BF-200 ALA for the treatment of actinic keratosis: results of a prospective, randomized, double-blind, placebo-controlled phase III study. Br J Dermatol 2010;163:386–94. https://doi.org/10.1111/j.1365-2133.2010.09873.x.
[235] Neittaanmäki-Perttu N, Karppinen TT, Grönroos M, Tani TT, Snellman E. Daylight photodynamic therapy for actinic keratoses: a randomized double-blinded nonsponsored prospective study comparing 5-aminolaevulinic acid nanoemulsion (BF-200) with methyl-5-aminolaevulinate. Br J Dermatol 2014;171:1172–80. https://doi.org/10.1111/bjd.13326.
[236] Reinhold U, Dirschka T, Ostendorf R, Aschoff R, Berking C, Philipp-Dormston WG, et al. A randomized, double-blind, phase III, multicentre study to evaluate the safety and efficacy of BF-200 ALA (Ameluz(®) ) vs. placebo in the field-directed treatment of mild-to-moderate actinic keratosis with photodynamic therapy (PDT) when using the BF-Rh. Br J Dermatol 2016;175:696–705. https://doi.org/10.1111/bjd.14498.
[237] Neittaanmäki-Perttu N, Grönroos M, Tani T, Snellman E. Long-term Outcome of Daylight Photodynamic Therapy with Amino-5-laevulinate Nanoemulsion vs. Methyl-5-aminolaevulinate for Actinic Keratoses. Acta Derm Venereol 2016;96:712–3. https://doi.org/10.2340/00015555-2345.
[238] NCT02799069. Evaluation of Safety and Efficacy of BF-200 ALA for the Treatment of Actinic Keratosis With Photodynamic Therapy. 2016.
[239] Serra-Guillén C, Nagore E, Bancalari E, Kindem S, Sanmartín O, Llombart B, et al. A randomized intraindividual comparative study of methyl-5-aminolaevulinate vs. 5-aminolaevulinic acid nanoemulsion (BF-200 ALA) in photodynamic therapy for actinic keratosis of the face and scalp. Br J Dermatol 2018;179:1410–1. https://doi.org/10.1111/bjd.17004.
[240] Dirschka T, Ekanayake-Bohlig S, Dominicus R, Aschoff R, Herrera-Ceballos E, Botella-Estrada R, et al. A randomized, intraindividual, non-inferiority, Phase III study comparing daylight photodynamic therapy with BF-200 ALA gel and MAL cream for the treatment of actinic keratosis. J Eur Acad Dermatol Venereol 2019;33:288–97. https://doi.org/10.1111/jdv.15185.
[241] Räsänen JE, Neittaanmäki N, Ylitalo L, Hagman J, Rissanen P, Ylianttila L, et al. 5-aminolaevulinic acid nanoemulsion is more effective than methyl-5-aminolaevulinate in daylight photodynamic therapy for actinic keratosis: a nonsponsored randomized double-blind multicentre trial. Br J Dermatol 2019;181:265–74. https://doi.org/10.1111/bjd.17311.
[242] Study to Evaluate Safety and Tolerability of BF-200 ALA (Ameluz®) for Photodynamic Therapy in the Treatment of the Expanded Field of Actinic Keratosis on Face and Scalp - Full Text View - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/NCT05060237?term=BF%E2%80%90200&draw=2&rank=2 (accessed January 30, 2023).
[243] Navarro-Triviño FJ, Ayén-Rodríguez Á, Llamas-Molina JM, Saenz-Guirado S, Ruiz-Villaverde R. Treatment of superficial basal cell carcinoma with 7.8% 5-aminolaevulinic acid nanoemulsion-based gel ( <scp>BF</scp> -200 <scp>ALA</scp> ) and photodynamic therapy: Results in clinical practice in a tertiary hospital. Dermatol Ther 2021;34:e14558. https://doi.org/10.1111/dth.14558.
[244] Salmivuori M, Grönroos M, Tani T, Pölönen I, Räsänen J, Annala L, et al. Hexyl aminolevulinate, 5-aminolevulinic acid nanoemulsion and methyl aminolevulinate in photodynamic therapy of non-aggressive basal cell carcinomas: A non-sponsored,

randomized, prospective and double-blinded trial. Journal of the European Academy of Dermatology and Venereology 2020;34:2781–8. https://doi.org/10.1111/jdv.16357.
[245] Morton CA, Dominicus R, Radny P, Dirschka T, Hauschild A, Reinhold U, et al. A randomized, multinational, noninferiority, phase III trial to evaluate the safety and efficacy of BF-200 aminolaevulinic acid gel vs. methyl aminolaevulinate cream in the treatment of nonaggressive basal cell carcinoma with photodynamic therapy. Br J Dermatol 2018;179:309–19. https://doi.org/10.1111/bjd.16441.
[246] Dias MLN, Carvalho JP, Rodrigues DG, Graziani SR, Maranhão RC. Pharmacokinetics and tumor uptake of a derivatized form of paclitaxel associated to a cholesterol-rich nanoemulsion (LDE) in patients with gynecologic cancers. Cancer Chemother Pharmacol 2007;59:105–11. https://doi.org/10.1007/s00280-006-0252-3.
[247] Graziani SR, Vital CG, Morikawa AT, van Eyll BM, Fernandes Junior HJ, Kalil Filho R, et al. Phase II study of paclitaxel associated with lipid core nanoparticles (LDE) as third-line treatment of patients with epithelial ovarian carcinoma. Medical Oncology 2017;34:1–7. https://doi.org/10.1007/s12032-017-1009-z.
[248] Pires LA, Hegg R, Valduga CJ, Graziani SR, Rodrigues DC, Maranhão RC. Use of cholesterol-rich nanoparticles that bind to lipoprotein receptors as a vehicle to paclitaxel in the treatment of breast cancer: pharmacokinetics, tumor uptake and a pilot clinical study. Cancer Chemother Pharmacol 2009;63:281–7. https://doi.org/10.1007/s00280-008-0738-2.
[249] Lucas SRR, Maranhão RC, Guerra JL, Coelho BMP, Barboza R, Pozzi DHB. Pilot clinical study of carmustine associated with a lipid nanoemulsion in combination with vincristine and prednisone for the treatment of canine lymphoma. Vet Comp Oncol 2015;13:184–93. https://doi.org/10.1111/vco.12033.
[250] Saif MW, Erlichman C, Dragovich T, Mendelson D, Toft D, Burrows F, et al. Open-label, dose-escalation, safety, pharmacokinetic, and pharmacodynamic study of intravenously administered CNF1010 (17-(allylamino)-17-demethoxygeldanamycin [17-AAG]) in patients with solid tumors. Cancer Chemother Pharmacol 2013;71:1345–55. https://doi.org/10.1007/s00280-013-2134-9.
[251] Phase 1, Dose-Escalation, Pharmacodynamic Study of IV CNF1010 in ZAP-70 Positive CLL. NCT00319930. 2005.
[252] A Phase I First in Human Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of WGI-0301 in Patients With Advanced Solid Tumors - Full Text View - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/NCT05267899 (accessed February 1, 2023).
[253] Dose Escalation and Efficacy Study of mRNA-2416 for Intratumoral Injection Alone and in Combination With Durvalumab for Participants With Advanced Malignancies - Full Text View - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/NCT03323398?term=lipid+nanoparticle*&cond=Neoplasms&draw=2&rank=2 (accessed February 1, 2023).
[254] Dose Escalation Study of mRNA-2752 for Intratumoral Injection to Participants in Advanced Malignancies - Full Text View - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/NCT03739931?term=lipid+nanoparticle*&cond=Neoplasms&draw=2&rank=3 (accessed February 1, 2023).
[255] Phase I, Multicenter, Dose Escalation Study of DCR-MYC in Patients With Solid Tumors, Multiple Myeloma, or Lymphoma - Full Text View - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/NCT02110563?term=lipid+nanoparticle*&cond=Neoplasms&draw=2&rank=6 (accessed February 1, 2023).
[256] Montserrat Foguet Roca. US 2009/0324727 A1, 2009.
[257] Maisch T, Santarelli F, Schreml S, Babilas P, Szeimies R-M. Fluorescence induction of protoporphyrin IX by a new 5-aminolevulinic acid nanoemulsion used for

photodynamic therapy in a full-thickness ex vivo skin model. Exp Dermatol 2009;19:e302–5. https://doi.org/10.1111/j.1600-0625.2009.01001.x.
[258] Schmitz L, Novak B, Hoeh AK, Luebbert H, Dirschka T. Epidermal penetration and protoporphyrin IX formation of two different 5-aminolevulinic acid formulations in ex vivo human skin. Photodiagnosis Photodyn Ther 2016;14:40–6. https://doi.org/10.1016/j.pdpdt.2015.11.004.
[259] Salmivuori M, Grönroos M, Tani T, Pölönen I, Räsänen J, Annala L, et al. Hexyl aminolevulinate, 5-aminolevulinic acid nanoemulsion and methyl aminolevulinate in photodynamic therapy of non-aggressive basal cell carcinomas: A non-sponsored, randomized, prospective and double-blinded trial. J Eur Acad Dermatol Venereol 2020;34:2781–8. https://doi.org/10.1111/jdv.16357.
[260] U.S National Library of Medicine. Treatment of Patients With Atherosclerotic Disease With Paclitaxel-associated to LDL Like Nanoparticles. NCT04148833. 2019.
[261] Treatment of Patients With Atherosclerotic Disease With Paclitaxel-associated to LDL Like Nanoparticles. NCT04616872. 2019. https://doi.org/10.21525/ct1-nct04148833.
[262] Bhat MA, Iqbal M, Al-Dhfyan A, Shakeel F. Carvone Schiff base of isoniazid as a novel antitumor agent: Nanoemulsion development and pharmacokinetic evaluation. J Mol Liq 2015;203:111–9. https://doi.org/10.1016/j.molliq.2014.12.037.
[263] Pangeni R, Choi SW, Jeon OC, Byun Y, Park JW. Multiple nanoemulsion system for an oral combinational delivery of oxaliplatin and 5-fluorouracil: Preparation and in vivo evaluation. Int J Nanomedicine 2016;11:6375–99. https://doi.org/10.2147/IJN.S121114.
[264] Shakeel F, Alanazi FK, Raish M, Haq N, Radwan AA, Alsarra IA. Pharmacokinetic and in vitro cytotoxic evaluation of cholesterol-rich nanoemulsion of cholesteryl-succinyl-5-fluorouracil. J Mol Liq 2015;211:164–8. https://doi.org/10.1016/j.molliq.2015.06.069.
[265] Ünal H, d'Angelo I, Pagano E, Borrelli F, Izzo A, Ungaro F, et al. Core–shell hybrid nanocapsules for oral delivery of camptothecin: formulation development, in vitro and in vivo evaluation. Journal of Nanoparticle Research 2015;17:42. https://doi.org/10.1007/s11051-014-2838-8.
[266] Klippstein R, Wang JTW, El-Gogary RI, Bai J, Mustafa F, Rubio N, et al. Passively Targeted Curcumin-Loaded PEGylated PLGA Nanocapsules for Colon Cancer Therapy in Vivo. Small 2015;11:4704–22. https://doi.org/10.1002/smll.201403799.
[267] Tsakiris N, Papavasileiou M, Bozzato E, Lopes A, Vigneron AM, Préat V. Combinational drug-loaded lipid nanocapsules for the treatment of cancer. Int J Pharm 2019;569:118588. https://doi.org/10.1016/j.ijpharm.2019.118588.
[268] Du Y, Ling L, Ismail M, He W, Xia Q, Zhou W, et al. Redox sensitive lipid-camptothecin conjugate encapsulated solid lipid nanoparticles for oral delivery. Int J Pharm 2018;549:352–62. https://doi.org/10.1016/j.ijpharm.2018.08.010.
[269] Patil P, Killedar S, More H, Vambhurkar G. Development and Characterization of 5-Fluorouracil Solid Lipid Nanoparticles for Treatment of Colorectal Cancer. J Pharm Innov 2022;17:1268–81. https://doi.org/10.1007/S12247-021-09605-X/TABLES/5.
[270] Jang DJ, Moon C, Oh E. Improved tumor targeting and antitumor activity of camptothecin loaded solid lipid nanoparticles by preinjection of blank solid lipid nanoparticles. Biomedicine and Pharmacotherapy 2016;80:162–72. https://doi.org/10.1016/j.biopha.2016.03.018.
[271] Mosallaei N, Mahmoudi A, Ghandehari H, Yellepeddi VK, Jaafari MR, Malaekeh-Nikouei B. Solid lipid nanoparticles containing 7-ethyl-10-hydroxycamptothecin (SN38): Preparation, characterization, in vitro, and in vivo evaluations. European Journal of Pharmaceutics and Biopharmaceutics 2016;104:42–50. https://doi.org/10.1016/j.ejpb.2016.04.016.

[272] Juang V, Chang CH, Wang CS, Wang HE, Lo YL. pH-Responsive PEG-Shedding and Targeting Peptide-Modified Nanoparticles for Dual-Delivery of Irinotecan and microRNA to Enhance Tumor-Specific Therapy. Small 2019;15:1903296. https://doi.org/10.1002/smll.201903296.
[273] Borderwala K, Rathod S, Yadav S, Vyas B, Shah P. Eudragit S-100 Surface Engineered Nanostructured Lipid Carriers for Colon Targeting of 5-Fluorouracil: Optimization and In Vitro and In Vivo Characterization. AAPS PharmSciTech 2021;22:1–15. https://doi.org/10.1208/s12249-021-02099-3.
[274] Pereira GG, Rawling T, Pozzoli M, Pazderka C, Chen Y, Dunstan CR, et al. Nanoemulsion-enabled oral delivery of novel anticancer ω-3 fatty acid derivatives. Nanomaterials 2018;8:825. https://doi.org/10.3390/nano8100825.
[275] Migotto A, Carvalho VFM, Salata GC, da Silva FWM, Yan CYI, Ishida K, et al. Multifunctional nanoemulsions for intraductal delivery as a new platform for local treatment of breast cancer. Drug Deliv 2018;25:654–67. https://doi.org/10.1080/10717544.2018.1440665.
[276] Gao Y, Qi X, Zheng Y, Ji H, Wu L, Zheng N, et al. Nanoemulsion enhances α-tocopherol succinate bioavailability in rats. Int J Pharm 2016;515:506–14. https://doi.org/10.1016/j.ijpharm.2016.10.026.
[277] Chen L, Chen B, Deng L, Gao B, Zhang Y, Wu C, et al. An optimized two-vial formulation lipid nanoemulsion of paclitaxel for targeted delivery to tumor. Int J Pharm 2017;534:308–15. https://doi.org/10.1016/j.ijpharm.2017.10.005.
[278] Tripathi CB, Parashar P, Arya M, Singh M, Kanoujia J, Kaithwas G, et al. QbD-based development of α-linolenic acid potentiated nanoemulsion for targeted delivery of doxorubicin in DMBA-induced mammary gland carcinoma: in vitro and in vivo evaluation. Drug Deliv Transl Res 2018;8:1313–34. https://doi.org/10.1007/s13346-018-0525-5.
[279] Kim B, Pena CD, Auguste DT. Targeted Lipid Nanoemulsions Encapsulating Epigenetic Drugs Exhibit Selective Cytotoxicity on CDH1-/FOXM1+ Triple Negative Breast Cancer Cells. Mol Pharm 2019;16:1813–26. https://doi.org/10.1021/acs.molpharmaceut.8b01065.
[280] Cao X, Luo J, Gong T, Zhang ZR, Sun X, Fu Y. Coencapsulated doxorubicin and bromotetrandrine lipid nanoemulsions in reversing multidrug resistance in breast cancer in vitro and in vivo. Mol Pharm 2015;12:274–86. https://doi.org/10.1021/mp500637b.
[281] Natesan S, Sugumaran A, Ponnusamy C, Thiagarajan V, Palanichamy R, Kandasamy R. Chitosan stabilized camptothecin nanoemulsions: Development, evaluation and biodistribution in preclinical breast cancer animal mode. Int J Biol Macromol 2017;104:1846–52. https://doi.org/10.1016/j.ijbiomac.2017.05.127.
[282] Ye J, Dong W, Yang Y, Hao H, Liao H, Wang B, et al. Vitamin E-rich Nanoemulsion Enhances the Antitumor Efficacy of Low-Dose Paclitaxel by Driving Th1 Immune Response. Pharm Res 2017;34:1244–54. https://doi.org/10.1007/s11095-017-2141-3.
[283] Katiyar SS, Kushwah V, Dora CP, Jain S. Novel biosurfactant and lipid core-shell type nanocapsular sustained release system for intravenous application of methotrexate. Int J Pharm 2019;557:86–96. https://doi.org/10.1016/j.ijpharm.2018.12.043.
[284] Anwar M, Akhter S, Mallick N, Mohapatra S, Zafar S, Rizvi MMA, et al. Enhanced anti-tumor efficacy of paclitaxel with PEGylated lipidic nanocapsules in presence of curcumin and poloxamer: In vitro and in vivo studies. Pharmacol Res 2016;113:146–65. https://doi.org/10.1016/j.phrs.2016.08.025.
[285] Ganassin R, Horst FH, Camargo NS, Chaves SB, Morais PC, Mosiniewicz-Szablewska E, et al. Selol nanocapsules with a poly(methyl vinyl ether-co-maleic anhydride) shell conjugated to doxorubicin for combinatorial chemotherapy against murine breast

adenocarcinoma *in vivo*. Artif Cells Nanomed Biotechnol 2018;46:1046–52. https://doi.org/10.1080/21691401.2018.1478423.

[286] Zafar S, Akhter S, Garg N, Selvapandiyan A, Kumar Jain G, Ahmad FJ. Co-encapsulation of docetaxel and thymoquinone in mPEG-DSPE-vitamin E TPGS-lipid nanocapsules for breast cancer therapy: Formulation optimization and implications on cellular and in vivo toxicity. European Journal of Pharmaceutics and Biopharmaceutics 2020;148:10–26. https://doi.org/10.1016/j.ejpb.2019.12.016.

[287] Haggag YA, Ibrahim RR, Hafiz AA. Design, Formulation and in vivo Evaluation of Novel Honokiol-Loaded PEGylated PLGA Nanocapsules for Treatment of Breast Cancer . Int J Nanomedicine 2020;Volume 15:1625–42. https://doi.org/10.2147/IJN.S241428.

[288] Baek JS, Cho CW. Surface modification of solid lipid nanoparticles for oral delivery of curcumin: Improvement of bioavailability through enhanced cellular uptake, and lymphatic uptake. European Journal of Pharmaceutics and Biopharmaceutics 2017;117:132–40. https://doi.org/10.1016/j.ejpb.2017.04.013.

[289] Jain A, Sharma T, Kumar R, Katare OP, Singh B. Raloxifene-loaded SLNs with enhanced biopharmaceutical potential: QbD-steered development, in vitro evaluation, in vivo pharmacokinetics, and IVIVC. Drug Deliv Transl Res 2021:1–25. https://doi.org/10.1007/s13346-021-00990-x.

[290] Cho C-W, Baek J-S, Kim J-H, Park J-S. Modification of paclitaxel-loaded solid lipid nanoparticles with 2-hydroxypropyl-β-cyclodextrin enhances absorption and reduces nephrotoxicity associated with intravenous injection. Int J Nanomedicine 2015;10:5397. https://doi.org/10.2147/IJN.S80474.

[291] da Rocha MCO, da Silva PB, Radicchi MA, Andrade BYG, de Oliveira JV, Venus T, et al. Docetaxel-loaded solid lipid nanoparticles prevent tumor growth and lung metastasis of 4T1 murine mammary carcinoma cells. J Nanobiotechnology 2020;18:43. https://doi.org/10.1186/s12951-020-00604-7.

[292] Yu DM, Li W, Zhang Y, Zhang P. Anti-tumor efficiency of paclitaxel and DNA when co-delivered by pH responsive ligand modified nanocarriers for breast cancer treatment. Biomedicine and Pharmacotherapy 2016;83:1428–35. https://doi.org/10.1016/j.biopha.2016.08.061.

[293] Balakrishnan P, Song CK, Jahn A, Cho HJ. Ceramide and N,N,N-Trimethylphytosphingosine-Iodide (TMP-I)-Based Lipid Nanoparticles for Cancer Therapy. Pharm Res 2016;33:206–16. https://doi.org/10.1007/s11095-015-1780-5.

[294] Brezaniova I, Hruby M, Kralova J, Kral V, Cernochova Z, Cernoch P, et al. Temoporfin-loaded 1-tetradecanol-based thermoresponsive solid lipid nanoparticles for photodynamic therapy. Journal of Controlled Release 2016;241:34–44. https://doi.org/10.1016/j.jconrel.2016.09.009.

[295] Garg NK, Singh B, Jain A, Nirbhavane P, Sharma R, Tyagi RK, et al. Fucose decorated solid-lipid nanocarriers mediate efficient delivery of methotrexate in breast cancer therapeutics. Colloids Surf B Biointerfaces 2016;146:114–26. https://doi.org/10.1016/j.colsurfb.2016.05.051.

[296] Pawar H, Surapaneni SK, Tikoo K, Singh C, Burman R, Gill MS, et al. Folic acid functionalized long-circulating co-encapsulated docetaxel and curcumin solid lipid nanoparticles: In vitro evaluation, pharmacokinetic and biodistribution in rats. Drug Deliv 2016;23:1453–68. https://doi.org/10.3109/10717544.2016.1138339.

[297] Wang C, Sun X, Wang K, Wang Y, Yang F, Wang H. Breast cancer targeted chemotherapy based on doxorubicin-loaded bombesin peptide modified nanocarriers. Drug Deliv 2016;23:2697–702. https://doi.org/10.3109/10717544.2015.1049721.

[298] Zheng G, Zheng M, Yang B, Fu H, Li Y. Improving breast cancer therapy using doxorubicin loaded solid lipid nanoparticles: Synthesis of a novel arginine-glycine-

aspartic tripeptide conjugated, pH sensitive lipid and evaluation of the nanomedicine in vitro and in vivo. Biomedicine and Pharmacotherapy 2019;116:109006. https://doi.org/10.1016/j.biopha.2019.109006.

[299] Fathy Abd-Ellatef G-E, Gazzano E, Chirio D, Ragab Hamed A, Belisario DC, Zuddas C, et al. Curcumin-Loaded Solid Lipid Nanoparticles Bypass P-Glycoprotein Mediated Doxorubicin Resistance in Triple Negative Breast Cancer Cells. Pharmaceutics 2020;12:96. https://doi.org/10.3390/pharmaceutics12020096.

[300] Ozgenc E, Karpuz M, Arzuk E, Gonzalez-Alvarez M, Sanz MB, Gundogdu E, et al. Radiolabeled Trastuzumab Solid Lipid Nanoparticles for Breast Cancer Cell: In Vitro and in Vivo Studies. ACS Omega 2022;7:30015–27. https://doi.org/10.1021/ACSOMEGA.2C03023/ASSET/IMAGES/LARGE/AO2C03023_0008.JPEG.

[301] Singh A, Neupane YR, Mangla B, Kohli K. Nanostructured Lipid Carriers for Oral Bioavailability Enhancement of Exemestane: Formulation Design, In Vitro, Ex Vivo, and In Vivo Studies. J Pharm Sci 2019;108:3382–95. https://doi.org/10.1016/j.xphs.2019.06.003.

[302] Arshad S, Masood-Ur-Rehman, Asim MH, Mahmood A, Ijaz M, Alamgeer, et al. Calycosin-loaded nanostructured lipid carriers: In-vitro and in-vivo evaluation for enhanced anti-cancer potential. J Drug Deliv Sci Technol 2021:102957. https://doi.org/10.1016/j.jddst.2021.102957.

[303] Lages EB, Fernandes RS, Silva J de O, de Souza ÂM, Cassali GD, de Barros ALB, et al. Co-delivery of doxorubicin, docosahexaenoic acid, and α-tocopherol succinate by nanostructured lipid carriers has a synergistic effect to enhance antitumor activity and reduce toxicity. Biomedicine and Pharmacotherapy 2020;132:110876. https://doi.org/10.1016/j.biopha.2020.110876.

[304] Zhang Q, Zhao J, Hu H, Yan Y, Hu X, Zhou K, et al. Construction and in vitro and in vivo evaluation of folic acid-modified nanostructured lipid carriers loaded with paclitaxel and chlorin e6. Int J Pharm 2019;569:118595. https://doi.org/10.1016/j.ijpharm.2019.118595.

[305] Kebebe D, Wu Y, Zhang F, Yang J, Liu Y, Li X, et al. Dimeric c(RGD) peptide conjugated nanostructured lipid carriers for efficient delivery of Gambogic acid to breast cancer . Int J Nanomedicine 2019;Volume 14:6179–95. https://doi.org/10.2147/IJN.S202424.

[306] Di H, Wu H, Gao Y, Li W, Zou D, Dong C. Doxorubicin- and cisplatin-loaded nanostructured lipid carriers for breast cancer combination chemotherapy. Drug Dev Ind Pharm 2016;42:2038–43. https://doi.org/10.1080/03639045.2016.1190743.

[307] Li X, Jia X, Niu H. Nanostructured lipid carriers co-delivering lapachone and doxorubicin for overcoming multidrug resistance in breast cancer therapy. Int J Nanomedicine 2018;Volume 13:4107–19. https://doi.org/10.2147/IJN.S163929.

[308] Poonia N, Kaur Narang J, Lather V, Beg S, Sharma T, Singh B, et al. Resveratrol loaded functionalized nanostructured lipid carriers for breast cancer targeting: Systematic development, characterization and pharmacokinetic evaluation. Colloids Surf B Biointerfaces 2019;181:756–66. https://doi.org/10.1016/j.colsurfb.2019.06.004.

[309] Lages EB, Fernandes RS, Andrade MMS, Paiyabhroma N, de Oliveira RB, Fernandes C, et al. pH-sensitive doxorubicin-tocopherol succinate prodrug encapsulated in docosahexaenoic acid-based nanostructured lipid carriers: An effective strategy to improve pharmacokinetics and reduce toxic effects. Biomedicine and Pharmacotherapy 2021;144:112373. https://doi.org/10.1016/j.biopha.2021.112373.

[310] Borges GSM, Silva J de O, Fernandes RS, de Souza ÂM, Cassali GD, Yoshida MI, et al. Sclareol is a potent enhancer of doxorubicin: Evaluation of the free combination and

co-loaded nanostructured lipid carriers against breast cancer. Life Sci 2019;232:116678. https://doi.org/10.1016/j.lfs.2019.116678.
[311] Wan K, Sun L, Hu X, Yan Z, Zhang Y, Zhang X, et al. Novel nanoemulsion based lipid nanosystems for favorable in vitro and in vivo characteristics of curcumin. Int J Pharm 2016;504:80–8. https://doi.org/10.1016/j.ijpharm.2016.03.055.
[312] Chen T, Gong T, Zhao T, Fu Y, Zhang Z, Gong T. A comparison study between lycobetaine-loaded nanoemulsion and liposome using nRGD as therapeutic adjuvant for lung cancer therapy. European Journal of Pharmaceutical Sciences 2018;111:293–302. https://doi.org/10.1016/j.ejps.2017.09.041.
[313] Kim JE, Park YJ. Improved antitumor efficacy of hyaluronic acid-complexed paclitaxel nanoemulsions in treating non-small cell lung cancer. Biomol Ther (Seoul) 2017;25:411–6. https://doi.org/10.4062/biomolther.2016.261.
[314] de Souza LR, Muehlmann LA, Matos LC, Simón-Vázquez R, Lacava ZGM, De-Paula AMB, et al. Antitumor activity and systemic effects of PVM/MA-shelled selol nanocapsules in lung adenocarcinoma-bearing mice. Nanotechnology 2015;26:505101. https://doi.org/10.1088/0957-4484/26/50/505101.
[315] Kim J, Ramasamy T, Choi JY, Kim ST, Youn YS, Choi HG, et al. PEGylated polypeptide lipid nanocapsules to enhance the anticancer efficacy of erlotinib in non-small cell lung cancer. Colloids Surf B Biointerfaces 2017;150:393–401. https://doi.org/10.1016/j.colsurfb.2016.11.002.
[316] Pooja D, Kulhari H, Kuncha M, Rachamalla SS, Adams DJ, Bansal V, et al. Improving efficacy, oral bioavailability, and delivery of paclitaxel using protein-grafted solid lipid nanoparticles. Mol Pharm 2016;13:3903–10. https://doi.org/10.1021/acs.molpharmaceut.6b00691.
[317] Rampaka R, Ommi K, Chella N. Role of solid lipid nanoparticles as drug delivery vehicles on the pharmacokinetic variability of Erlotinib HCl. J Drug Deliv Sci Technol 2021;66:102886. https://doi.org/10.1016/j.jddst.2021.102886.
[318] Khatri H, Chokshi N, Rawal S, Patel BM, Badanthadka M, Patel MM. Fabrication and in vivo evaluation of ligand appended paclitaxel and artemether loaded lipid nanoparticulate systems for the treatment of NSCLC: A nanoparticle assisted combination oncotherapy. Int J Pharm 2020;583:119386. https://doi.org/10.1016/j.ijpharm.2020.119386.
[319] Pooja D, Kulhari H, Tunki L, Chinde S, Kuncha M, Grover P, et al. Nanomedicines for targeted delivery of etoposide to non-small cell lung cancer using transferrin functionalized nanoparticles. RSC Adv 2015;5:49122–31. https://doi.org/10.1039/c5ra03316k.
[320] Jyoti K, Kaur K, Pandey RS, Jain UK, Chandra R, Madan J. Inhalable nanostructured lipid particles of 9-bromo-noscapine, a tubulin-binding cytotoxic agent: In vitro and in vivo studies. J Colloid Interface Sci 2015;445:219–30. https://doi.org/10.1016/j.jcis.2014.12.092.
[321] Zhou J, Sun M, Jin S, Fan L, Zhu W, Sui X, et al. Combined using of paclitaxel and salinomycin active targeting nanostructured lipid carriers against non-small cell lung cancer and cancer stem cells. Drug Deliv 2019;26:281–9. https://doi.org/10.1080/10717544.2019.1580799.
[322] Wang Y, Zhang H, Hao J, Li B, Li M, Xiuwen W. Lung cancer combination therapy: co-delivery of paclitaxel and doxorubicin by nanostructured lipid carriers for synergistic effect. Drug Deliv 2016;23:1398–403. https://doi.org/10.3109/10717544.2015.1055619.
[323] Cao C, Wang Q, Liu Y. Lung cancer combination therapy: doxorubicin and β-elemene co-loaded, pH-sensitive nanostructured lipid carriers . Drug Des Devel Ther 2019;Volume 13:1087–98. https://doi.org/10.2147/DDDT.S198003.

[324] Han Y, Li Y, Zhang P, Sun J, Li X, Sun X, et al. Nanostructured lipid carriers as novel drug delivery system for lung cancer gene therapy. Pharm Dev Technol 2016;21:277–81. https://doi.org/10.3109/10837450.2014.996900.
[325] Rawal S, Bora V, Patel B, Patel M. Surface-engineered nanostructured lipid carrier systems for synergistic combination oncotherapy of non-small cell lung cancer. Drug Deliv Transl Res 2021;11:2030–51. https://doi.org/10.1007/s13346-020-00866-6.
[326] Guo S, Zhang Y, Wu Z, Zhang L, He D, Li X, et al. Synergistic combination therapy of lung cancer: Cetuximab functionalized nanostructured lipid carriers for the co-delivery of paclitaxel and 5-Demethylnobiletin. Biomedicine and Pharmacotherapy 2019;118:109225. https://doi.org/10.1016/j.biopha.2019.109225.
[327] Shao Z, Shao J, Tan B, Guan S, Liu Z, Zhao Z, et al. Targeted lung cancer therapy: preparation and optimization of transferrin-decorated nanostructured lipid carriers as novel nanomedicine for co-delivery of anticancer drugs and DNA. Int J Nanomedicine 2015;10:1223. https://doi.org/10.2147/IJN.S77837.
[328] Dalmolin L, Lopez R. Nanoemulsion as a Platform for Iontophoretic Delivery of Lipophilic Drugs in Skin Tumors. Pharmaceutics 2018;10:214. https://doi.org/10.3390/pharmaceutics10040214.
[329] Fofaria NM, Qhattal HSS, Liu X, Srivastava SK. Nanoemulsion formulations for anti-cancer agent piplartine - Characterization, toxicological, pharmacokinetics and efficacy studies. Int J Pharm 2016;498:12–22. https://doi.org/10.1016/j.ijpharm.2015.11.045.
[330] Maranhao R, Kretzer I, Maria D, Guido MC, Contente T. Simvastatin increases the antineoplastic actions of paclitaxel carried in lipid nanoemulsions in melanoma-bearing mice. Int J Nanomedicine 2016;11:885. https://doi.org/10.2147/ijn.s88546.
[331] Favero GM, Paz JL, Otake AH, Maria DA, Caldini EG, de Medeiros RSS, et al. Cell internalization of 7-ketocholesterol-containing nanoemulsion through LDL receptor reduces melanoma growth in vitro and in vivo: A preliminary report. Oncotarget 2018;9:14160–74. https://doi.org/10.18632/oncotarget.24389.
[332] Muzammil Afzal S, Naidu VGM, Harishankar N, Kishan V. Albumin anchored docetaxel lipid nanoemulsion for improved targeting efficiency – preparation, characterization, cytotoxic, antitumor and *in vivo* imaging studies. Drug Deliv 2016;23:1355–63. https://doi.org/10.3109/10717544.2015.1030715.
[333] Farsky S, Drewes C, Fiel L, Bexiga C, Asbahr AC, Uchiyama M, et al. Novel therapeutic mechanisms determine the effectiveness of lipid-core nanocapsules on melanoma models. Int J Nanomedicine 2016;11:1261. https://doi.org/10.2147/IJN.S101543.
[334] Resnier P, Galopin N, Sibiril Y, Clavreul A, Cayon J, Briganti A, et al. Efficient ferrocifen anticancer drug and Bcl-2 gene therapy using lipid nanocapsules on human melanoma xenograft in mouse. Pharmacol Res 2017;126:54–65. https://doi.org/10.1016/j.phrs.2017.01.031.
[335] Valdes SA, Alzhrani RF, Lansakara-P DSP, Cui Z. Effect of a Solid Lipid Nanoparticle Formulation on the Bioavailability of 4-(N)-Docosahexaenoyl 2′, 2′-Difluorodeoxycytidine After Oral Administration. AAPS PharmSciTech 2020;21:1–8. https://doi.org/10.1208/s12249-020-1617-3.
[336] Banerjee I, De M, Dey G, Bharti R, Chattopadhyay S, Ali N, et al. A peptide-modified solid lipid nanoparticle formulation of paclitaxel modulates immunity and outperforms dacarbazine in a murine melanoma model. Biomater Sci 2019;7:1161–78. https://doi.org/10.1039/c8bm01403e.
[337] Shen H, Shi S, Zhang Z, Gong T, Sun X. Coating solid lipid nanoparticles with hyaluronic acid enhances antitumor activity against melanoma stem-like cells. Theranostics 2015;5:755–71. https://doi.org/10.7150/thno.10804.

[338] Shi S, Zhou M, Li X, Hu M, Li C, Li M, et al. Synergistic active targeting of dually integrin αvβ3/CD44-targeted nanoparticles to B16F10 tumors located at different sites of mouse bodies. Journal of Controlled Release 2016;235:1–13. https://doi.org/10.1016/j.jconrel.2016.05.050.
[339] Zhou M, Li X, Li Y, Yao Q, Ming Y, Li Z, et al. Ascorbyl palmitate-incorporated paclitaxel-loaded composite nanoparticles for synergistic anti-tumoral therapy. Drug Deliv 2017;24:1230–42. https://doi.org/10.1080/10717544.2017.1370619.
[340] Iqbal B, Ali J, Ganguli M, Mishra S, Baboota S. Silymarin-loaded nanostructured lipid carrier gel for the treatment of skin cancer. Nanomedicine 2019;14:1077–93. https://doi.org/10.2217/nnm-2018-0235.
[341] Geronimo G, Rodrigues da Silva GH, de Moura LD, Ribeiro LN, Guilherme VA, Mendonça TC, et al. Development of S75:R25 bupivacaine-loaded lipid nanoparticles functionalized with essential oils for treating melanoma. Journal of Chemical Technology and Biotechnology 2021;96:2197–207. https://doi.org/10.1002/jctb.6715.
[342] de Moura LD, Ribeiro LNM, de Carvalho F v., Rodrigues da Silva GH, Lima Fernandes PC, Brunetto SQ, et al. Docetaxel and lidocaine co-loaded (Nlc-in-hydrogel) hybrid system designed for the treatment of melanoma. Pharmaceutics 2021;13:1552. https://doi.org/10.3390/pharmaceutics13101552.
[343] Colombo M, Figueiró F, de Fraga Dias A, Teixeira HF, Battastini AMO, Koester LS. Kaempferol-loaded mucoadhesive nanoemulsion for intranasal administration reduces glioma growth in vitro. Int J Pharm 2018;543:214–23. https://doi.org/10.1016/j.ijpharm.2018.03.033.
[344] Azambuja JH, Schuh RS, Michels LR, Gelsleichter NE, Beckenkamp LR, Iser IC, et al. Nasal Administration of Cationic Nanoemulsions as CD73-siRNA Delivery System for Glioblastoma Treatment: a New Therapeutical Approach. Mol Neurobiol 2020;57:635–49. https://doi.org/10.1007/s12035-019-01730-6.
[345] Carradori D, Saulnier P, Préat V, des Rieux A, Eyer J. NFL-lipid nanocapsules for brain neural stem cell targeting in vitro and in vivo. Journal of Controlled Release 2016;238:253–62. https://doi.org/10.1016/j.jconrel.2016.08.006.
[346] Danhier F, Messaoudi K, Lemaire L, Benoit JP, Lagarce F. Combined anti-Galectin-1 and anti-EGFR siRNA-loaded chitosan-lipid nanocapsules decrease temozolomide resistance in glioblastoma: in vivo evaluation. Int J Pharm 2015;481:154–61. https://doi.org/10.1016/j.ijpharm.2015.01.051.
[347] Figueiró F, Bernardi A, Frozza RL, Terroso T, Zanotto-Filho A, Jandrey EHF, et al. Resveratrol-loaded lipid-core nanocapsules treatment reduces in vitro and in vivo glioma growth. J Biomed Nanotechnol 2013;9:516–26. https://doi.org/10.1166/jbn.2013.1547.
[348] Lollo G, Vincent M, Ullio-Gamboa G, Lemaire L, Franconi F, Couez D, et al. Development of multifunctional lipid nanocapsules for the co-delivery of paclitaxel and CpG-ODN in the treatment of glioblastoma. Int J Pharm 2015;495:972–80. https://doi.org/10.1016/j.ijpharm.2015.09.062.
[349] Ferreira LM, Azambuja JH, da Silveira EF, Marcondes Sari MH, da Cruz Weber Fulco B, Costa Prado V, et al. Antitumor action of diphenyl diselenide nanocapsules: In vitro assessments and preclinical evidence in an animal model of glioblastoma multiforme. Journal of Trace Elements in Medicine and Biology 2019;55:180–9. https://doi.org/10.1016/j.jtemb.2019.06.010.
[350] Pereira NR, Loiola R, Rodrigues S, de Oliveira C, Büttenbender S, Guterres S, et al. Mechanisms of the effectiveness of poly(ε-caprolactone) lipid-core nanocapsules loaded with methotrexate on glioblastoma multiforme treatment. Int J Nanomedicine 2018;Volume 13:4563–73. https://doi.org/10.2147/IJN.S168400.

[351] Groo AC, Bossiere M, Trichard L, Legras P, Benoit JP, Lagarce F. In vivo evaluation of paclitaxel-loaded lipid nanocapsules after intravenous and oral administration on resistant tumor. Nanomedicine 2015;10:589–601. https://doi.org/10.2217/nnm.14.124.
[352] Aparicio-Blanco J, Romero IA, Male DK, Slowing K, García-García L, Torres-Suárez AI. Cannabidiol Enhances the Passage of Lipid Nanocapsules across the Blood-Brain Barrier Both in Vitro and in Vivo. Mol Pharm 2019;16:1999–2010. https://doi.org/10.1021/acs.molpharmaceut.8b01344.
[353] Lollo G, Ullio-Gamboa G, Fuentes E, Matha K, Lautram N, Benoit JP. In vitro anti-cancer activity and pharmacokinetic evaluation of curcumin-loaded lipid nanocapsules. Materials Science and Engineering C 2018;91:859–67. https://doi.org/10.1016/j.msec.2018.06.014.
[354] Figueiro F, de Oliveira CP, Rockenbach L, Mendes FB, Bergamin LS, Jandrey EHF, et al. Pharmacological improvement and preclinical evaluation of methotrexate-loaded lipid-core nanocapsules in a glioblastoma model. J Biomed Nanotechnol 2015;11:1808–18. https://doi.org/10.1166/jbn.2015.2125.
[355] Wang L, Wang X, Shen L, Alrobaian M, Panda SK, Almasmoum HA, et al. Paclitaxel and naringenin-loaded solid lipid nanoparticles surface modified with cyclic peptides with improved tumor targeting ability in glioblastoma multiforme. Biomedicine and Pharmacotherapy 2021;138:111461. https://doi.org/10.1016/j.biopha.2021.111461.
[356] Kadari A, Pooja D, Gora RH, Gudem S, Kolapalli VRM, Kulhari H, et al. Design of multifunctional peptide collaborated and docetaxel loaded lipid nanoparticles for antiglioma therapy. European Journal of Pharmaceutics and Biopharmaceutics 2018;132:168–79. https://doi.org/10.1016/j.ejpb.2018.09.012.
[357] Banerjee I, De K, Mukherjee D, Dey G, Chattopadhyay S, Mukherjee M, et al. Paclitaxel-loaded solid lipid nanoparticles modified with Tyr-3-octreotide for enhanced anti-angiogenic and anti-glioma therapy. Acta Biomater 2016;38:69–81. https://doi.org/10.1016/j.actbio.2016.04.026.
[358] Wu M, Fan Y, Lv S, Xiao B, Ye M, Zhu X. Vincristine and temozolomide combined chemotherapy for the treatment of glioma: a comparison of solid lipid nanoparticles and nanostructured lipid carriers for dual drugs delivery. Drug Deliv 2016;23:2720–5. https://doi.org/10.3109/10717544.2015.1058434.
[359] Kuang Y, Zhang K, Cao Y, Chen X, Wang K, Liu M, et al. Hydrophobic IR-780 Dye Encapsulated in cRGD Conjugated Solid Lipid Nanoparticles for NIR Imaging-Guided Photothermal Therapy. ACS Appl Mater Interfaces 2017;9:12217–26. https://doi.org/10.1021/acsami.6b16705.
[360] Khan H, Nazir S, Farooq RK, Khan IN, Javed A. Fabrication and Assessment of Diosgenin Encapsulated Stearic Acid Solid Lipid Nanoparticles for Its Anticancer and Antidepressant Effects Using in vitro and in vivo Models. Front Neurosci 2022;15:1914. https://doi.org/10.3389/FNINS.2021.806713/BIBTEX.
[361] Madane RG, Mahajan HS. Curcumin-loaded nanostructured lipid carriers (NLCs) for nasal administration: design, characterization, and *in vivo* study. Drug Deliv 2016;23:1326–34. https://doi.org/10.3109/10717544.2014.975382.
[362] Chen Y, Pan L, Jiang M, Li D, Jin L. Nanostructured lipid carriers enhance the bioavailability and brain cancer inhibitory efficacy of curcumin both *in vitro* and *in vivo*. Drug Deliv 2016;23:1383–92. https://doi.org/10.3109/10717544.2015.1049719.
[363] Basso J, Mendes M, Silva J, Sereno J, Cova T, Oliveira R, et al. Peptide-lipid nanoconstructs act site-specifically towards glioblastoma growth impairment. European Journal of Pharmaceutics and Biopharmaceutics 2020;155:177–89. https://doi.org/10.1016/j.ejpb.2020.08.015.
[364] Zhang J, Xiao X, Zhu J, Gao Z, Lai X, Zhu X, et al. Lactoferrin- and RGD-comodified, temozolomide and vincristine-coloaded nanostructured lipid carriers for gliomatosis

cerebri combination therapy. Int J Nanomedicine 2018;Volume 13:3039–51. https://doi.org/10.2147/IJN.S161163.
[365] Song S, Mao G, Du J, Zhu X. Novel RGD containing, temozolomide-loading nanostructured lipid carriers for glioblastoma multiforme chemotherapy. Drug Deliv 2016;23:1404–8. https://doi.org/10.3109/10717544.2015.1064186.
[366] Xu M, Li G, Zhang H, Chen X, Li Y, Yao Q, et al. Sequential delivery of dual drugs with nanostructured lipid carriers for improving synergistic tumor treatment effect. Drug Deliv 2020;27:983–95. https://doi.org/10.1080/10717544.2020.1785581.
[367] di Filippo LD, Lobato Duarte J, Hofstätter Azambuja J, Isler Mancuso R, Tavares Luiz M, Hugo Sousa Araújo V, et al. Glioblastoma multiforme targeted delivery of docetaxel using bevacizumab-modified nanostructured lipid carriers impair in vitro cell growth and in vivo tumor progression. Int J Pharm 2022;618:121682. https://doi.org/10.1016/J.IJPHARM.2022.121682.
[368] Dai X, Liu D, Liu M, Zhang X, Wang W, Jin F, et al. Anti-metastatic efficacy of traditional Chinese medicine (tcm) ginsenoside conjugated to a vefgr-3 antibody on human gastric cancer in an orthotopic mouse model. Anticancer Res 2017;37:979–86. https://doi.org/10.21873/anticanres.11407.
[369] Ma L, Yang D, Li Z, Zhang X, Pu L. Co-delivery of paclitaxel and tanespimycin in lipid nanoparticles enhanced anti-gastric-tumor effect *in vitro* and *in vivo*. Artif Cells Nanomed Biotechnol 2018;46:904–11. https://doi.org/10.1080/21691401.2018.1472101.
[370] Jian Y, Zhao M, Cao J, Fan T, Bu W, Yang Y, et al. A Gastric Cancer Peptide GX1-Modified Nano-Lipid Carriers Encapsulating Paclitaxel: Design and Evaluation of Anti-Tumor Activity . Drug Des Devel Ther 2020;Volume 14:2355–70. https://doi.org/10.2147/DDDT.S253023.
[371] Lei-Ming X, Qu C-Y, Zhou M, Chen Y, Chen M, Feng Shen F. Engineering of lipid prodrug-based, hyaluronic acid-decorated nanostructured lipid carriers platform for 5-fluorouracil and cisplatin combination gastric cancer therapy. Int J Nanomedicine 2015;10:3911. https://doi.org/10.2147/IJN.S83211.
[372] Jiang H, Pei L, Liu N, Li J, Li Z, Zhang S. Etoposide-loaded nanostructured lipid carriers for gastric cancer therapy. Drug Deliv 2016;23:1379–82. https://doi.org/10.3109/10717544.2015.1048491.
[373] Mao M, Liu S, Zhou Y, Wang G, Deng J, Tian L. Nanostructured lipid carrier delivering chlorin e6 as in situ dendritic cell vaccine for immunotherapy of gastric cancer. J Mater Res 2020;35:3257–64. https://doi.org/10.1557/jmr.2020.227.
[374] Johnson JLH, Leos RA, Baker AF, Unger EC. Radiosensitization of Hs-766T pancreatic tumor xenografts in mice dosed with dodecafluoropentane nano-emulsion-preliminary findings. J Biomed Nanotechnol 2015;11:274–81. https://doi.org/10.1166/jbn.2015.1903.
[375] Ingallina C, Costa PM, Ghirga F, Klippstein R, Wang JT, Berardozzi S, et al. Polymeric glabrescione B nanocapsules for passive targeting of Hedgehog-dependent tumor therapy *in vitro*. Nanomedicine 2017;12:711–28. https://doi.org/10.2217/nnm-2016-0388.
[376] Navarro-Marchal SA, GrinÌ án-Lisan C, Entrena JM, Ruiz-Alcalá G, Tristán-Manzano M, Martin F, et al. Anti-CD44-Conjugated Olive Oil Liquid Nanocapsules for Targeting Pancreatic Cancer Stem Cells. Biomacromolecules 2021;22:1374–88. https://doi.org/10.1021/acs.biomac.0c01546.
[377] Thakkar A, Chenreddy S, Thio A, Khamas W, Wang J, Prabhu S. Preclinical systemic toxicity evaluation of chitosan-solid lipid nanoparticle-encapsulated aspirin and curcumin in combination with free sulforaphane in BALalB/c mice. Int J Nanomedicine 2016;11:3265–76. https://doi.org/10.2147/IJN.S106736.

[378] Chirio D, Peira E, Dianzani C, Muntoni E, Gigliotti C, Ferrara B, et al. Development of Solid Lipid Nanoparticles by Cold Dilution of Microemulsions: Curcumin Loading, Preliminary In Vitro Studies, and Biodistribution. Nanomaterials 2019;9:230. https://doi.org/10.3390/nano9020230.
[379] Lu Z, Su J, Li Z, Zhan Y, Ye D. Hyaluronic acid-coated, prodrug-based nanostructured lipid carriers for enhanced pancreatic cancer therapy. Drug Dev Ind Pharm 2017;43:160–70. https://doi.org/10.1080/03639045.2016.1226337.
[380] Ahmad G, el Sadda R, Botchkina G, Ojima I, Egan J, Amiji M. Nanoemulsion formulation of a novel taxoid DHA-SBT-1214 inhibits prostate cancer stem cell-induced tumor growth. Cancer Lett 2017;406:71–80. https://doi.org/10.1016/j.canlet.2017.08.004.
[381] Swami R, Singh I, Jeengar MK, Naidu VGM, Khan W, Sistla R. Adenosine conjugated lipidic nanoparticles for enhanced tumor targeting. Int J Pharm 2015;486:287–96. https://doi.org/10.1016/j.ijpharm.2015.03.065.
[382] Liu TI, Lu TY, Chang SH, Shen MY, Chiu HC. Dual stimuli guided lipid-based delivery system of cancer combination therapy. Journal of Controlled Release 2020;318:16–24. https://doi.org/10.1016/j.jconrel.2019.12.002.
[383] Chen Y, Yuan L, Congyan L, Zhang Z, Zhou L, Qu D. Antitumor activity of tripterine via cell-penetrating peptide-coated nanostructured lipid carriers in a prostate cancer model. Int J Nanomedicine 2013;8:4339. https://doi.org/10.2147/IJN.S51621.
[384] Negi LM, Jaggi M, Joshi V, Ronodip K, Talegaonkar S. Hyaluronic acid decorated lipid nanocarrier for MDR modulation and CD-44 targeting in colon adenocarcinoma. Int J Biol Macromol 2015;72:569–74. https://doi.org/10.1016/j.ijbiomac.2014.09.005.
[385] Antonow MB, Asbahr ACC, Raddatz P, Beckenkamp A, Buffon A, Guterres SS, et al. Liquid formulation containing doxorubicin-loaded lipid-core nanocapsules: Cytotoxicity in human breast cancer cell line and in vitro uptake mechanism. Materials Science and Engineering C 2017;76:374–82. https://doi.org/10.1016/j.msec.2017.03.099.
[386] Wang F, Li L, Liu B, Chen Z, Li C. Hyaluronic acid decorated pluronic P85 solid lipid nanoparticles as a potential carrier to overcome multidrug resistance in cervical and breast cancer. Biomedicine and Pharmacotherapy 2017;86:595–604. https://doi.org/10.1016/j.biopha.2016.12.041.
[387] Fabian CJ. The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. Int J Clin Pract 2007;61:2051–63. https://doi.org/10.1111/j.1742-1241.2007.01587.x.
[388] Salazar MDA, Ratnam M. The folate receptor: What does it promise in tissue-targeted therapeutics? Cancer and Metastasis Reviews 2007;26:141–52. https://doi.org/10.1007/s10555-007-9048-0.
[389] Li W, Szoka FC. Lipid-based nanoparticles for nucleic acid delivery. Pharm Res 2007;24:438–49. https://doi.org/10.1007/s11095-006-9180-5.
[390] Gligorov J, Lotz JP. Preclinical Pharmacology of the Taxanes: Implications of the Differences. Oncologist 2004;9:3–8. https://doi.org/10.1634/theoncologist.9-suppl_2-3.
[391] Gonzales J, Kossatz S, Roberts S, Pirovano G, Brand C, Pérez-Medina C, et al. Nanoemulsion-Based Delivery of Fluorescent PARP Inhibitors in Mouse Models of Small Cell Lung Cancer. Bioconjug Chem 2018;29:3776–82. https://doi.org/10.1021/acs.bioconjchem.8b00640.
[392] Banerjee I, De M, Dey G, Bharti R, Chattopadhyay S, Ali N, et al. A peptide-modified solid lipid nanoparticle formulation of paclitaxel modulates immunity and outperforms dacarbazine in a murine melanoma model. Biomater Sci 2019;7:1161–78. https://doi.org/10.1039/c8bm01403e.

[393] Ferreira LM, Cervi VF, Sari MHM, Barbieri AV, Ramos AP, Copetti PM, et al. Diphenyl diselenide loaded poly(ε-caprolactone) nanocapsules with selective antimelanoma activity: Development and cytotoxic evaluation. Materials Science and Engineering C 2018;91:1–9. https://doi.org/10.1016/j.msec.2018.05.014.
[394] Nogueira CW, Rocha JBT. Toxicology and pharmacology of selenium: Emphasis on synthetic organoselenium compounds. Arch Toxicol 2011;85:1313–59. https://doi.org/10.1007/s00204-011-0720-3.
[395] Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, et al. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. Neuro Oncol 2013;15:ii1. https://doi.org/10.1093/neuonc/not151.
[396] Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114:97–109. https://doi.org/10.1007/s00401-007-0243-4.
[397] Koshy M, Villano JL, Dolecek TA, Howard A, Mahmood U, Chmura SJ, et al. Improved survival time trends for glioblastoma using the SEER 17 population-based registries. J Neurooncol 2012;107:207–12. https://doi.org/10.1007/s11060-011-0738-7.
[398] Upadhyay RK. Drug delivery systems, CNS protection, and the blood brain barrier. Biomed Res Int 2014;2014. https://doi.org/10.1155/2014/869269.
[399] Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev 2014;66:2–25. https://doi.org/10.1016/j.addr.2013.11.009.
[400] Qu J, Zhang L, Chen Z, Mao G, Gao Z, Lan X, et al. Nanostructured lipid carriers, solid lipid nanoparticles, and polymeric nanoparticles: which kind of drug delivery system is better for glioblastoma chemotherapy? Drug Deliv 2016;23:3408–16. https://doi.org/10.1080/10717544.2016.1189465.
[401] Wolff JE, Kortmann RD, Wolff B, Pietsch T, Peters O, Schmid HJ, et al. High dose methotrexate for pediatric high grade glioma - Results of the HIT-GBM-D Pilot study. J Neurooncol 2011;102:433–42. https://doi.org/10.1007/s11060-010-0334-2.
[402] Barbosa N. Effect of Organic Forms of Selenium on δ-Aminolevulinate Dehydratase from Liver, Kidney, and Brain of Adult Rats. Toxicol Appl Pharmacol 1998;149:243–53.
[403] Rosa RM, Roesler R, Braga AL, Saffi J, Henriques JAP. Pharmacology and toxicology of diphenyl diselenide in several biological models. Brazilian Journal of Medical and Biological Research 2007;40:1287–304. https://doi.org/10.1590/S0100-879X2006005000171.
[404] Prigol M, Nogueira CW, Zeni G, Bronze MR, Constantino L. Physicochemical and biochemical profiling of diphenyl diselenide. Appl Biochem Biotechnol 2013;169:885–93. https://doi.org/10.1007/s12010-012-0042-9.
[405] Prigol M, Brüning CA, Martini F, Nogueira CW. Comparative excretion and tissue distribution of selenium in mice and rats following treatment with diphenyl diselenide. Biol Trace Elem Res 2012;150:272–7. https://doi.org/10.1007/s12011-012-9464-z.
[406] Hermann PC, Sainz B. Pancreatic cancer stem cells: A state or an entity? Semin Cancer Biol 2018;53:223–31. https://doi.org/10.1016/j.semcancer.2018.08.007.
[407] Carpenter RL, Lo HW. Hedgehog pathway and GLI1 isoforms in human cancer. Discov Med 2012;13:105–13.

**Declaration of interests**

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

- LCNPs are a versatile group of drug-delivery systems for cancer treatment.
- LCNPs are classified depending on the physical state of their lipidic core.
- The preparation methods of LCNPs can be adapted for large-scale production.
- LCNPs formulations are effectively administered through different pathways.
- LCNPs administered intranasally are promising to target brain tumors.