Lipid-based nanomaterials in cancer treatment and diagnosis

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Abstract

Cancer consists of a wide range of diseases that are mainly driven by continuous unregulated proliferation of cancer cells. Current treatment options include the use of chemotherapies, radiotherapy, and surgery. Recently, there were an increased interest in the application of nanoparticles (NPs) in the field of cancer diagnosis and treatment. NPs are materials in the size range 1 to 100 nm, and can be classified based on their properties, shape, or size. They have attracted wide attention because of their versatile physico-chemical properties, nanoscale sizes, high surface-to-volume ratios, favourable drug release profiles, and targeting modifications. Nanotechnology can be used to improve personalisation of cancer diagnosis and treatment by enhancing the detection of cancer-specific biomarkers, imaging of tumours and their metastases, specific drug delivery to target cells, and real-time observation of treatment progression. This chapter will highlight the main types of lipid NPs with their preparation methods. The clinical applications of these lipid NPs in the field of cancer diagnosis and treatment will be presented along with the currently approved drugs that are based on these NPs.

Key words:

Lipid nanoparticles, liposomes, micelles, cancer treatment, cancer diagnosis

1. Cancer Background

Cancer is considered one of the most fatal diseases that has reported high rates of incidence and mortality globally[1]. For instance, the Global Cancer Observatory (GLOBOCAN) 2020, an online database of global cancer statistics and estimates of occurrence and death rates in 185 countries for 36 types of cancer, has indicated that 19.3 million new cancer patients were diagnosed and around 10 million deaths caused by cancer in 2020 [1]. Therefore, interest in

studying cancer continues to progress at a high rate in order to investigate the underlying causes of cancer and progression.

On a biological level, cancer consists of a wide range of diseases that are mainly driven by continuous unregulated proliferation of cancer cells [2]. Over growth of cells may develop a mass of tissues called a tumour. However, tumours can be benign or malignant [3]. Benign tumours usually non-invasive and can be removed without the risk of reoccurrence. Also, cells of benign tumours do not circulate or spread to other parts of the body [4]. Whereas malignant tumours have the ability to invade nearby tissues, and to spread to other parts of the body in a process known as metastasis [4].

Cancer types are classified into five main categories according to their origin, for instance, carcinoma usually originating from epithelial cells, sarcoma arising from bone, cartilage, fat, muscle, blood vessels, leukemia from the bone marrow while lymphoma and myeloma are found to be derived from cells of the immune system, and central nervous system cancers from brain tissues and spinal cord [2, 4]. Therefore, different types of cancer exhibit a variety of behaviors and response to treatment [2].

At a cellular level, cancer initiation and progression are viewed as a multi-step process, where progressive genetic alterations take place to transform normal human cells into highly malignant ones. These genetic changes are found to affect three gene classes: proto-oncogenes, tumour suppressor genes, which are both involved in normal cell growth and division, and DNA repair genes that are responsible for fixing damaged DNA [2, 5]. For instance, any modifications, amplifications or deletions in these genes may cause a de-coupling of the biological mechanisms of normal cell growth and differentiation [5, 6]. Moreover, these changes in the genetic material of the cell may arise unexpectedly or be induced by a factor or carcinogen that causes cancer [2].

Carcinogens include radiation such as solar ultraviolet radiation, chemicals in tobacco smoke, and viruses. However, carcinogenesis, the process of cancer development, does not rely only on single causes in most cancers as it has been found that many factors contribute in both animal models and humans [2].

However, preventive measures can be taken against some carcinogens such as radiation and smoking in order to minimise the incidence rate of cancer as it has been found that more than half of all cancers are preventable [7]. However, the integration of effective therapeutic approaches and developing new ones are still limited [8].

In recent years, different forms of cancer have been effectively treated by immunotherapies such as antibodies [9], stem cell therapies [10], and chimeric antigen receptor (CAR)-T cell therapies [11, 12]. The success of these approaches is attributed to the high specificity and efficacy of these molecules in treating both primary and metastasised tumours. Despite the high promise of these approaches, few undesired side effects can also arise, like autoimmune disease [13]. Furthermore, lymphoma and other non-solid tumours have generally shown better responses to immunotherapies than solid tumours [14, 15], due to the expected difficulty in penetrating solid tumours [16]. The immune-suppressive tumour microenvironment can similarly contribute to this efficacy reduction against solid tumours [17]. These limitations can be surpassed through the utilisation of nanotechnology.

2. Nanoparticle applications

Nanoparticles (NPs) are materials in the size range 1 to 100 nm, and can be classified based on their properties, shape, or size [18]. They have attracted wide attention because of their versatile physico-chemical properties, nanoscale sizes high surface-to-volume ratios, favourable drug release profiles, and targeting modifications [19, 20]. Nanotechnology can be used to improve personalisation of cancer diagnosis and treatment by enhancing the detection of cancer-specific

biomarkers, imaging of tumours and their metastases, specific drug delivery to target cells, and real-time observation of treatment progression [21, 22].

The crucial challenge in treating cancer resides in the ability to engineer an effective treatment capable of specifically targeting cancer cells without affecting surrounding healthy cells [23]. The NPs must pass through several physiological and biological barriers to be effective. So their use as delivery systems inflicts necessities to optimise their size, surface chemistry, and biocompatibility to avoid non-specific interactions, and to enable specific binding to their targets. Attia *et al.* [24], specified different criteria that should be maintained by all therapeutic NPs, including the ability to remain stable in the blood and tumour microenvironment (TME); to evade reticuloendothelial system (RES) clearance, and prevent being seized by the mononuclear phagocyte system (MPS); to accumulate in tumour tissues through irregular tumour vasculature; and to infiltrate into tumour interstitial fluid with high pressure.

Various articles have comprehensively reviewed the use of different nanomaterials as carriers of therapeutic molecules for the treatment of cancer [23, 25-27]. The implemented NPs can be commonly classified into organic (liposomes, micelles, lipids, poly-lactic acid, exosomes, and poly(lactic-co-glycolic acid) [28, 29], inorganic (gold, silver, iron, carbon quantum dots, silica, and graphene), and composites (metal organic frameworks and transition metals dichalcogenide) [30]. These systems can be additionally extended by introducing external effects of laser light, magnetic waves, or heat to improve the delivered drug effect at tumour sites [31]. Therefore, combining these external effects with NPs can create a foundation for more innovative approaches exemplified by photodynamic [32], photothermal [33, 34], magnetic [35], and neutron-capturing systems [36]. These multi-disciplinary approaches undoubtedly provide additional armoury to enhance the effect and selectivity of cancer treatment.

NPs are commonly directed to tumour sites through active or passive targeting. Passive targeting represents the ability of NPs to accumulate at cancer sites due to tumour-associated conditions like inflammation and hypoxia [37]. Matsumura and Maeda [38] described the phenomenon of the Enhanced Permeation and Retention (EPR) effect during cancer, which contributes to the accumulation of NPs in cancer tissues. Whereas active targeting relies on ligand-receptor binding that improves selective accumulation at the targeted sites, and thus distinguishes between cancerous and healthy tissues [39]. Active targeting involves the implementation of one or more targeting moieties conjugated to the NP surface, which interact precisely with antigens or receptors that are either uniquely expressed or overexpressed on tumour cells in comparison to normal tissues [40]. This concept was initially tested with antibodies grafted on the surface of lipid NPs known as liposomes [41].

Several passively targeted NPs have been used over the past two decades; nevertheless, none of their actively targeted counterparts have progressed through clinical trials [42]. Additionally, over 40,000 published articles in the last decade have focused on active targeting strategies, and yet we still need to address several technical challenges [42]. These include low drug-loading efficiency and solubility, poor ability to cross *in vivo* barriers, low tumour targeting and penetration, and *in vivo* unpredictability; whilst additional problems arise from nonspecific interactions of hydrophobic compounds, unfavourable bio-distribution, and adverse drug release profiles [43]. Consequently, the NP type should be carefully selected to suit each specific cancer type. Likewise, the conjugated molecules should be attached to NPs using a suitable linker to ensure high stability of the conjugates at the site of action.

The Food and Drug Administration (FDA) has approved a selection of polymers for the synthesis of NPs for cancer immunotherapy, such as polyethylene glycol (PEG), poly (lactide-o-glycolic) acid, and chitosan, attributed to their biocompatibility, non-toxicity, and

biodegradability [44]. For instance, PEGylating NP surfaces can improve their hydrophilic properties, and promote characteristics to avoid their recognition by immune system, and to increase the chance of targeting the desired cells [45]. Polymer-based nanoparticles are the most popular systems in cancer immunotherapy [46]. Liposomes were discovered in 1965, and were the first approved class of therapeutic NPs for cancer treatment [47]. The lipid features, pharmacological properties of liposomal formulations and the clinical studies of their application have been extensively reviewed in other articles [48-51].

3. Types of lipid based nanomaterials

The main lipid-based nanomaterials include: liposomes, niosomes, micelles, solid lipid nanoparticles and nanostructured lipid carriers.

3.1. Liposomes

Liposomes were first described by Bangham in 1965, where they were found to display several dimensional, structural and functional properties similar to that of biological membranes [52]. Since then, liposomes have been established as vehicles for drug delivery [53] and have been extensively studied. Liposomes are the most studied lipid-based system investigated and have been the first to achieve clinical approval [47, 54, 55].

Liposomes are enclosed vesicular structures which form spontaneously, by self-assembly of lipids upon hydration, into colloidal particles consisting of a lipid bilayer surrounding an aqueous core [56]. The lipid bilayer is arranged so that the hydrophobic tail moieties are shielded from the aqueous environment within the bilayer space by the hydrophilic head groups facing the aqueous phase on both sides [57]. As a result, liposomes serve as versatile carrier

vehicles where drugs of varying physicochemical properties can be entrapped in the aqueous core, contained within the bilayer space or interact with the bilayer surface [58].

Phospholipids, either glycerophospholipids or sphingomyelin, are the main lipid constituent of liposomes. Phosphatidylcholine (PC), also known as lecithin, is the most popular glycerophospholipid used in liposome formation along with others such as phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE) [59]. The inclusion of cholesterol into the liposomal structure helps to improve bilayer fluidity, reduce permeability and reduce liposome identification by plasma proteins and subsequent uptake [60].

Different criteria have been used in the classification of liposomes; the most predominant is based on size and lamellarity. Barba *et al.* (2019) classified them into unilamellar vesicles (UVs), further subclassified into small (SUVs, 20-200 nm), large (LUVs, 0.2-1 μm) and giant (GUVs, >1 μm), and vesicle with multiple bilayers including multilamellar vesicles (MLVs, 0.5-5 μm) in which the bilayers are arranged in a concentric manner and multivesicular vesicles (MVVs) consisting of a non-concentric arrangement of close-packed vesicles [59].

The most basic, conventional liposomes can be anionic, cationic or neutral depending on the charge of the phospholipid used [61]. However, once administered into the blood circulation, these liposomes are subjected to rapid clearance through opsonisation by plasma proteins and subsequent recognition and uptake by the MPS. This can be limiting if a drug is required to be delivered to sites beyond the MPS, such as for instance solid tumours. Functionalising conventional liposomes by attaching a hydrophilic carbohydrate or polymer to their surfaces, most likely a lipid derivative of polyethylene glycol (PEG), prolongs their circulation time [62, 63] and have been termed as long-circulating, sterically stabilised, PEGylated or stealth liposomes. Both of these liposomes deliver their payload through passive targeting which will

be discussed in later sections. Other types of functionalised liposomes include ligand-targeted liposomes such as immunoliposomes [61] and stimuli-responsive liposomes such as pH-sensitive liposomes, thermal-sensitive liposomes and magnetic liposomes [64].

3.2. Niosomes

Niosomes, also known as non-ionic surfactant vesicles, are colloidal particles structurally and functionally similar to liposomes formed by the self-assembly of non-ionic amphiphiles upon lipid hydration into vesicular structures [65]. They were first described and used by researchers in the cosmetic industry [66] but with the discovered potential of liposomes in drug delivery, the motivation to use niosomes as drug carriers as well was prompted.

As with liposomes, niosomes too are composed of a lipid bilayer surrounding an aqueous core and can therefore be loaded with drugs of different physicochemical properties. They are also classified in the same way as liposomes based on size and lamelllarity into SUVs, LUVs and MLVs [67]. They can also be conventional or functionalised to be sterically stabilised, ligand-targeted or stimuli-responsive [68].

However, niosomes are considered cheaper alternatives to liposomes as they have less stringent storage and handling requirements, thereby offering greater stability and comparable toxicity [65, 69, 70]. The various types of non-ionic surfactants used in niosome formation include alkyl ethers, alkyl esters, and alkyl amides fatty acid [71]. Similarly to liposomes, cholesterol is included in niosomal composition providing the same benefits by reducing bilayer fluidity, improving entrapment efficiency and reducing membrane permeability [72, 73]. Charged lipids can also be used to impart electrostatic retention between vesicle thereby preventing aggregations [73].

3.3. Micelles

Micelles are small colloidal structures, formed from the self-assembly of amphiphilic molecules, ranging in size between 5-100 nm [74]. In aqueous media, micelles are formed spontaneously into monolayer structures where the hydrophilic heads are arranged so that they face the aqueous phase and protect the hydrophobic tails which form the inner core. Depending on drug solubility they can be entrapped in the hydrophobic core, adsorbed onto the micelle surface or dispersed between the surfactant molecules [75]. Depending on the type of amphiphilic molecules, the micelles may be divided into lipid micelles, polymeric micelles or lipid-polymeric hybrid structures [54]. Micelles can be modified to be PEGylated, ligand-targeted or stimuli-responsive [76].

3.4. Solid lipid nanoparticles

A new generation of lipid-based nanomaterials have been presented as better alternatives that combine the advantages and overcome the major limitations of traditional colloidal systems such as liposomes and polymeric NPs [77]. These nanomaterials are based on solid lipids and include solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and lipid-drug conjugates (LDCs) [78].

SLNs, introduced in 1991, are colloidal particles (10-1000 nm) consisting of a lipid matrix which are solid at physiological temperature and can entrap both lipophilic and hydrophilic drugs either in dissolved or dispersed form [79, 80]. The solid matrix is composed of biodegradable and biocompatible lipids of natural or synthetic origin including mono-, di- or triglycerides, complex glyceride mixtures, fatty acids, waxes and steroids [81]. The lipid matrix is stabilised in the aqueous environment by a coating of surfactants or polymer and in some cases a co-surfactant can be added as well [82]. Drugs are incorporated into SLNs based on one of three models: they can be homogeneously distributed throughout the matrix (solid

solution), concentrated in the outermost portion of the lipid matrix forming a drug-free lipid core (drug-enriched shell), or concentrated in the core of the matrix (drug-enriched core) [83].

However, because the lipids are compactly packed in SLNs in an ideal crystalline structure, drug loading capacity is poor as a result of reduced encapsulation space. Furthermore, polymeric transition during storage can lead to drug expulsion from the carrier system [83]. As a result, NLCs were developed from SLNs by adding liquid lipids to the solid lipids in order to improve drug loading capacity, and prevent lipid crystallisation and subsequent drug expulsion during storage [80]. Liquid lipids used in NLCs include glyceryl tricaprylate, ethyl oleate, isopropyl myristate and glyceryl dioleate [81].

4. Methods of preparation of lipid based nanomaterials

Several approaches have been developed for the synthesis of lipid-based nanomaterials, which are used for the diagnosis, detection, or treatment of cancer. The selection of the appropriate method depends on the physicochemical properties of compounds within the nanoparticles. This section will describe the methods and their most significant characteristics according to their energy requirements.

4.1. High-energy methodologies

4.1.1. High-Pressure Homogenization (HPH) method

Firstly, the drug is dissolved or dispersed in the melted lipid to obtain a liquid formulation. The high operative pressure pushes the liquid formulation through a micrometric cavity, forming a suspension of homogenised lipid particles with reduced final size [77]. It can be performed either at elevated temperature (hot homogenisation) or below room temperature (cold homogenisation) [84] to produce excellent product characteristics. The principal limitation of this technique has been the high consumption of energy and the polydispersity of the final formulations. Over time, the HPH approach has shown some modifications/ improvements to

overcome its original limitations; in this way, narrowest droplets distributions can be produced keeping stable the pressure by alternative concepts such as microfluidics.

Microfluidics is a form of high-pressure homogenizer. The main difference with the conventional method is the shear rate applied at constant pressure; this is possible using a device or disruption unit with fixed geometry microchannels rather than valves [85].

The key component in this technology is the device used to make the particles. Microfluidic devices are small-scale circuits that consist of several channels (with dimensions of tens to hundreds of micrometres) with adjustable mixings of small volumes of several fluids [86, 87]. Fluid flow is established by external sources such as manual/digital peristaltic pumps or syringe pumps. There are two parameters to control the composition and size of the final formulation: Flow Rate Ratio (FRR) and the time settings for both inlet ports. The materials selected to make the NP, which encapsulates the drug, can be from any long-chain fatty acids to phospholipids or mixtures of glycerides. Some studies have shown reliable outcomes and synergistic effects of the combination lipids-drug, and an improvement in the loading capacity and stability of the combinations [70, 88].

4.1.2. High-speed homogenisation

The emulsion is created by external mechanical energy supplied by rotor mixers or high-speed stirrers. The speed used is between 6000 – 24,000 rpm [89]. The melted lipid and the surfactants in the water phase are mixed at the same temperature under constant stirring. In this way, an oil bath could be used to maintain the temperature until the particles are formed.

Higher stirring rates reduce the polydispersity, but do not show significant reproducibility when the process is scaled-up [90].

4.1.3. Ultrasonication

The most important parameters for the ultrasonication method are frequency, ultrasonication time, and power of irradiation. These parameters are related to the diameter of the probe tip, and they are tabulated according to the recommendations of the manufacturer of the ultrasonicator. The gradual temperature reduction of the final emulsion below the crystallisation temperature of the lipid yields a lipid NP dispersion [91].

Emulsion preparations by ultrasonic irradiations are very reproducible, but heat generation and potential metal contamination due to ultrasonication cause a significant problem with this method, so it is not easily scalable for the industry [92].

4.2. Low-energy methodologies

4.2.1. Spontaneous emulsification method

This method is based on the study of phase equilibria between the water, oil (lipids) and surfactant [93]. An important tool used to study and understand these emulsions is the ternary-phase diagram [94] where the compositions of the three components expressed in terms of the percentages are plotted; The greatest challenge for this method is achieving homogeneous and reproducible spontaneous emulsions [95].

4.2.2. Membrane emulsification

This method employs a cylindrical membrane module: internal water flow containing the surfactant allows the formation of small droplets when the melted lipid is pressed through the pores of the membrane. These droplets are detached from the membrane pores by tangential water flow [96]. The method is scalable, and the particle size can be tuned using membranes with different pore sizes [95]. However, clogging of the membrane reduces its industrial use.

4.2.3. Phase inversion temperature (PIT)

This method exploits the ability of specific temperature-sensitive non-ionic polyethoxylated surfactants to change their affinities for water and oil as a function of temperature [97]. Oil in water (o/w) emulsions can be obtained due to changes in the surfactant's spontaneous curvature at the oil-water interface and at some degrees below the phase inversion temperature PIT [98]. This technique is specific to thermal-sensitive surfactants such as polyethylene oxide (PEO) derivatives, thereby strongly restricting the choice in surfactants [99].

4.2.4. Coacervation

This is a simple method where the precipitation of lipid NPs is produced by acidification (or coacervation) of a micellar solution of fatty acid alkaline salts in the presence of an appropriate amphiphilic polymeric stabilising agent [100]. Using this technique, lipophilic, hydrophobic ion pairs of hydrophilic drugs and even thermosensitive drugs can be incorporated without using solvents. It is therefore a suitable method to scale up if the encapsulated drug is not pH-sensitive [101].

4.2.5. Double emulsion method

This technique has been more popular in the encapsulation of water-soluble anticancer drugs including peptides [95]. Double emulsions have been prepared using a two-step emulsification, where the initial step of making the primary emulsion involves the use of a low hydrophilic-lipophilic balance (HLB) surfactant in case of w/o/w emulsion and a high HLB surfactant in case of o/w/o emulsion. In the second step, a primary w/o emulsion is dispersed in an aqueous continuous phase containing high HLB or hydrophilic surfactant to form w/o/w double emulsion [102].

4.2.6. Methods based on the use of organic solvents to stabilise pH- or thermosensitive drugs and increase their bioavailability.

These can be divided into solvent injection [103], solvent evaporation from emulsions [104] and solvent diffusion from emulsions [105]. These methods require an additional step to remove the solvent from the final preparation, but this step does not ensure that the total solvent is removed; indeed residual organic solvent may remain in the final preparation. Moreover, for toxicological reasons, pharmaceutical manufacturers aspire to minimise the number and amount of solvents applied in drug production. Thus, these methods are the least attractive for the pharmaceutical industry [106].

4.2.7. Methods based on supercritical fluid

These methods solubilise a drug easily by a relatively small change in pressure. The most popular fluids used are carbon dioxide or nitrogen due to their relative low cost and non-toxicity [107]. There are four methods in this category: Rapid Expansion of Supercritical Solution (RESS) [108], Gas Anti-Solvent (GAS) [109] Process, particles from Gas-Saturated Solution/suspensions (PGSS) [110], and Supercritical Fluid Extraction of Emulsions (SFEE) [111]. These supercritical fluid technologies have great advantages for the environment as they pose no air or water pollution risks [112], although it is still an expensive method for mass production. Additional technological development would be needed for industrial scale-up.

4.3. Drying methodologies

After the emulsion/dispersion or suspension is formed by any of the methods previously described, one of the following drying technologies can be applied to get powdered lipid-based particles with better storage stability.

4.3.1. Nano-spray-drying

This is a vibration mesh technique with the ability to spray very small quantities of liquids (emulsion, suspension or dispersion) and drying them with a laminar stream of hot drying gas. This method provides NPs in powder form in a short processing time. The encapsulation efficiency and the size of the particles are significantly affected by the spraying parameters, the design of the nozzle, drying temperature and the physicochemical properties of the liquid feed [113]. The main constraints of this technique is that it cannot be scaled up and the yield of the process is low [114].

4.3.2. Freeze-drying

Freeze-drying or lyophilisation is a water removal process developed mostly for encapsulation of heat-sensitive compounds with no presence of solvents. In this process, the reduction of the pressure (vacuum) and the addition of heat allows the sublimation of the frozen water, preserving the physical form of biological compounds. The main problems of freeze-drying include their use with aqueous emulsion, long process times and high energy consumption by the vacuum pump [115].

4.3.3. Variosol

This is a solvent-free technology able to produce microparticles exploiting liquid or near-critical CO_2 properties. It is based on the capability of rapidly expanding liquid-dense CO_2 to adsorb heat (Joule-Thomson effect) while generating a pressure gradient, thus simultaneously inducing rapid cooling, controlled solidification and atomisation of fluid materials during spraying [116].

5. Clinical applications of lipid-based nanomaterials

The application of NPs in the treatment of various diseases have been extensively studied with a greater focus on cancer. The clinical approval of the first NP in the form of liposomal

doxorubicin (Doxil) in cancer treatment has paved the way for the approval of several other lipid-based formulations and many more are undergoing different phases of clinical development. Those in clinical trials are either already approved nanoformulations (e.g., for another cancer type or in combination with other therapies) or newly developed ones [117]. The clinical applications of lipid-based nanomaterials in cancer treatment that have been explored include chemotherapy [50], gene therapy [59], immunotherapy [118] and radiotherapy [119] delivery systems and some of them are highlighted here.

Talidox (TLD-1) is a new PEGylated liposomal formulation of doxorubicin developed by InnoMedica as an improved version of the already approved Doxil (Myocet and Caelyx/Doxil). TLD-1 was developed as a solution to the high incidence of Palmar-Plantar Erythrodysethesia (PPE; also known as hand-and-foot syndrome) associated with the use of Caelyx despite its ability to prevent doxorubicin-related cardiotoxicity (InnoMedica Talidox Brochure, 2018; retrieved from https://www.innomedica.com/wp-content/uploads/2018/04/Talidox.pdf). Preclinical studies demonstrated superiority of TLD-1 in comparison to free doxorubicin as well as Caelyx in terms of enhanced cytotoxicity and antitumour activity with reduced toxicity [29, 120]. This has prompted a Phase I trial in patients with advanced solid tumours (NCT03387917).

Annamycin is an improved new generation lipophilic anthracycline antibiotic characterised by avoidance of multidrug resistance and lack of cardiotoxicity in contrast to doxorubicin [121]. Moleculin Biotech, Inc. has developed a liposomal formulation of annamycin which is being studied in two different Phase I/II trials in patients with refractory or relapsed acute myeloid leukaemia (NCT03315039; NCT03388749).

The bioavailability of mytomycin C was enhanced by delivering it as a lipid-based prodrug in PEGylated liposomes known as Promitil® [122]. A stable liposomal formulation is produced

by encapsulating the prodrug within the bilayer which is only released as the active drug by the thioredoxin-thioreductase system which is overexpressed in tumours thereby improving tumour localisation [123]. Promitil, developed by Lipomedix Pharmaceuticals Inc., has been studied in a Phase I trial to treat patients with solid tumours (NCT01705002). A different Phase I study of Promitil combined with external beam radiotherapy is also being investigated in patients with solid tumours (NCT03823989).

Stimuli-responsive liposomes have also been investigated as a means to improve drug bioavailability. One example is a heat-responsive liposomal formulation of doxorubicin known as lyso-thermosensitive liposomal doxorubicin (LTLD) or ThermoDox developed by Celsion Corporation [124]. LTLD is PEGylated consisting of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxypolyethylene-glycol-2000, and is designed to release its payload at temperatures above 39.5 °C within seconds [125]. Following its administration by intravenous infusion, local heat is applied, which is beneficial in both maximising liposomal localisation in tumour sites due to increased vasculature and triggering doxorubicin release [125]. Local heating can be obtained by radiofrequency ablation (RAF), microwave hyperthermia or high-intensity focused ultrasound. ThermoDox has reached several clinical phases of study. The OPTIMA trial (completed Phase 3) studied the combination of LTLD with standardised RFA in treating non-resectable hepatocellular carcinoma (NCT02112656). Another study also combining LTLD with RFA has completed Phase I in cases of primary and metastatic liver tumours (NCT00441376). The TARDOX trial has completed Phase 1 and combines LTLD with focused ultrasound in patients with primary or secondary liver tumours (NCT02181075) and has shown promising results. The DIGNITY trial has completed Phase I/II evaluating LTD combined with microwave hyperthermia in treating local-regional recurrent breast cancer (NCT00826085). Currently patients with

metastatic breast cancer are being recruited in a Phase I trial to study LTLD combined with magnetic resonance guided high intensity focused ultrasound (MR-HIFU) and cyclophosphamide as part of the i-GO study (NTC03749850). LTLD and MR-HIFU will also be investigated in a Phase I study currently recruiting children with relapsed and refractory solid tumours (NCT02536183).

Polymeric micelles have successfully reached clinical trials as delivery vehicles for chemotherapeutic drugs. Genexol PM developed by Samyang Biopharmaceutical Corporation is a novel polymeric micellar formulation of paclitaxel [126]. Genexol PM has been investigated in many clinical trials including pancreatic cancer either alone (Phase II; NCT00111904) or combined with gemcitabine (Phase I/II: NCT00882973), urothelial cancer (Phase II; NCT01426126), in non-small cell lung cancer combined with cisplatin (Phase II; NCT 01023347) and with gemcitabine (Phase II; NCT01770795), in ovarian cancer combined with carboplatin (Phase I; NCT0087725 and Phase II; NCT01276548), in advanced head and neck cancer combined with cisplatin (Phase II; NCT01689194). A novel sustained-release micellar formulation of cisplatin (NC-6004) was developed by NanoCarrier Co., Ltd. to overcome cisplatin-associated toxicities [127]. NC-6004 has been studied in combination with gemcitabine in cases of pancreatic cancer (Phase I/II; NCT00910741 and Phase III; NCT02043288) and solid tumours (Phase I/II; NCT02240238) and in combination with fluorouracil (5-FU) and cetuximab to treat head and neck cancer (Phase I/II: NCT03109158).

Significant progress in gene delivery was achieved with the first lipid-based nanomaterial, Onpattro, to be approved in cancer treatment and many more are in the pipeline. SynerGene Therapeutics, Inc. have developed cationic liposomal formulations; SGT-94 delivering plasmid DNA encoding the tumour suppressor gene RB94 [128] and SGT-53 delivering plasmid DNA encoding the normal human wild type p53 suppressor gene [129]. Cationic liposomes, composed of DOTAP and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), are

conjugated with an anti-transferrin (TfR) single-chain antibody fragment and target tumours by binding to TfR which is overexpressed in cancer cells [130]. A Phase I study with SGT-94 in patients with solid tumours has been completed (NTC01517465). A Phase Ib trial of SGT-53 combined with docetaxel in patients with solid tumours has also been completed (NCT00472613). An ongoing Phase 1 trial is investigating SGT-53 alone and in combination with topotecan and cyclophosphamide in children with recurrent or refractory solid tumours (NCT02354547). Patients with metastatic pancreatic cancer are also being recruited for a Phase II study combining SGT-53 with gemcitabine/nab-paclitaxel (NCT02340117).

The delivery of the tumour suppressor gene TUSC2(FUS1), which is inactivated in 100% of small cell lung cancer and 82% of non-small lung cancer cases, was investigated in the treatment of lung cancer by complexing the gene with cationic liposomes consisting of DOTAP and cholesterol [131]. This NP delivery system, known as ONCOPREX® by Genprex, Inc., was originally developed in collaboration with the University of Texas MD Anderson Cancer Centre and the National Institute of Health (Genprex website retrieved from https://www.genprex.com/technology/oncoprex-nanoparticle-delivery-system/). The synergistic effect of this liposomal formulation with erlotinib is currently being investigated in an ongoing PhaseI/II trial in cases of Stage IV non-small cell lung cancer (NCT01455389).

MTL-CEBPA developed by MiNa Therapeutics, Ltd. is the first-in-class small activating (saRNA) oligonucleotide delivered in the SMARTICLES® liposomal product [132, 133]. The NP formulation is designed to upregulate CEBPA (CCAAT/enhancer-binding protein alpha) expression in the liver, in the treatment of cirrhotic hepatocellular carcinoma [134]. The potential of MTL-CEBPA in acute and chronic and inflammatory diseases is also reported [135]. Promising results of a first-in-human Phase I trial in cases of advanced HCC using MTL-CEBPA alone has prompted the OUTREACH trial which is a Phase Ia/b trial currently investigating the combination of MTL-CEBPA with sorafenib in advanced HCC

(NTC02716012). The TIMEPOINT trial is recruiting patients with advanced solid tumours in a Phase Ia/b study combining MTL-CEBA with pembrolizumab (NCT04105335).

A neutral liposomal formulation consisting of DOPE was developed to deliver the EphA2-siRNA (EPHARNA) to target cancer cells overexpressing the EphA2 receptor [136]. A Phase I trial is currently recruiting to investigate the formulation in cases of advanced or recurrent solid tumours (NTC01591356).

The liposomal formulation BP1001, entrapping Grb2 antisense oligonucleotide, was developed by Bio-Path Holdings to downregulate the growth factor receptor-bound protein 2 (Grb2) responsible for tumour progression [137, 138]. A Phase I/b trial investigating BP1001 in patients with refractory or replaced acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), Philadelphia-chromosome-positive chronic myeloid leukaemia (CML) or myelodysplastic syndrome (MDS) has been completed (NTC01159028) with favourable results [137]. A Phase II trial is currently recruiting to investigate the combination of BP1001 with venetoclax plus decitabine in patients with AML (NTC02781883).

Cancer immunotherapy using lipid-based nanomaterials, including vaccine delivery, have also been explored [118, 139]. OncoQuest-CLL developed by XEME Biopharma Inc., is a liposomal product delivering a vaccine composed of whole-cell tumour lysates with IL-2 for the purpose of early intervention in asymptomatic chronic lymphocytic leukaemia (CLL) [140]. The patient's leukaemia cell lysate and a recombinant human IL-2 are delivered in multilamellar liposomes providing personalised cancer vaccines (XEME Biopharma website retrieved from https://www.xemebiopharma.com/personalized-vaccines). A Phase Ib trial to study the vaccine's safety and efficacy in patients with previously untreated CLL is ongoing (NCT01976520). A mRNA vaccine loaded liposomal product, W_ova1, against ovarian cancer is being investigated in Phase I of the OLIVIA trial in combination with carboplatin and

paclitaxel (NCT04163094). The IMMUNOCERV trial is a Phase IIA trial investigating the liposomal multipeptide vaccine (PDS0101) against human papilloma virus (HPV16 E6/E7) combined with cisplatin and radiation therapy in patients with Stage IB3-IVA cervical cancer (NTC04580771).

The applications of lipid-based materials in cancer treatment have also been explored in the delivery of radiotherapy. Brachytherapy by convection-enhanced delivery of liposomes entrapping the radionuclide rhenium-186 were investigated in the treatment of glioblastoma demonstrating promising preclinical results [119]. The liposomal product 186RNL, developed by Plus Therapeutics, is currently being studied in Phase I/II of the ReSPECT trial in patients with recurrent or progressive glioma (NCT01906385).

6. Clinically approved lipid-based NP medicines

The first approved product of nanomedicines by the FDA was Doxil® which was approved in 1995 for the treatment of ovarian cancer and AIDS-related Kaposi's sarcoma [60, 141-143]. Doxil®, a PEGylated liposomal doxorubicin was a liposomal formulation attached with polyethylene glycol (PEG) chains on the liposome surface (PEGylation) [141, 144, 145]. This formulation was produced to minimise the limitations of the conventional non-PEGylated liposomes regarding short circulating half-life due to rapid clearance by macrophages. Also, Doxil® was proven effective in the reduction of cardiotoxic side effects of doxorubicin (DOX) treatment and enhanced drug levels in malignancies when compared to free DOX [142, 144, 146]. Other liposomal formulations with DOX were designed to be more tolerable and more effective than free DOX such as Lipo-Dox® [142]. Moreover, Myocet®, co-encapsulation of DOX and cyclophosphamide in a liposome was approved by the European Medicines Agency (EMA) in 2000 and indicated for treatment of metastatic breast cancer [141, 142, 146, 147]. The PEGylated liposomal carrier was quickly adopted for the delivery of other drugs such as

Abelcet® and Ambisome®, an amphotericin B to treat invasive fungal infections [144, 146, 148]. Amphotericin B is used for the treatment of invasive fungal infections and acts through interaction of drug and sterols in the cell membrane of susceptible fungi, with a resulting change in membrane permeability [142, 146]. The liposomal formulations were presented as AmBisome®from NeXstar Pharmaceuticals, Inc. (now Astellas Pharma, Inc.); lipid complexes such as Abelcet® from Enzon Pharmaceuticals (now Sigma Tau Pharmaceuticals, Inc) [142, 148].

In the 2000s, there has been a growing number of trials and approvals using liposomal delivery. Liposomes encapsulated with a photosensitiser for photodynamic therapy was introduced [148]. Visudyne®, a product of Novartis AG, Switzerland, is a unilamellar phospholipid vesicle based on DMPC and egg phosphatidyl glycerol (EPG) delivering verteporphin (VPF), a synthetic chlorine-like porphyrin [142, 144, 146]. Visudyne® is the first light-activated drug that is available for the treatment of patients with predominantly classic subfoveal choroidal neovascularisation due to age-related macular degeneration (AMD) [146, 148]. This formulation was designed to eradicate the abnormal blood vessels in the eye related to conditions such as wet macular degeneration [144]. Paclitaxel (PTX) is a well-established therapeutic drug used for ovarian cancer therapy. PTX in liposomal formulations such as Lipusu®, a formulation approved in China (Sike Pharmaceutical Co. Ltd.) have been developed [142]. Subsequently, more approved liposomal formulation products have become available for the treatment of cancer and different diseases. For instance, Exparel®, a bupivacaine liposomal injection form is a multivesicular liposome based on Depofoam™ technology which is used for pain control [146, 149]. Exparel® was developed by Pacira Pharmaceuticals, Inc. and FDA-approved for local surgical site injection for post-operative pain after maemorrhoidectomy and bunionectomy [142, 149, 150]. DepoDur®, liposome encapsulated morphine sulphate has been developed by SkyePharma, San Diego, CA for administration

before surgery or following clamping of the umbilical cord during a caesarean section [142, 146].

The first product based on a liposomal vaccine or virosome technology was Epaxal® which was patented by Crucell Berna Biotech, Switzerland. Epaxal® is a virosome-adjuvanted vaccine for hepatitis A (HAV) which has a high tolerability [142, 146]. This vaccine lacks aluminium salts and thiomersal that allows intradural or subcutaneous administration due to fewer adverse local effects comparing to conventional aluminium-adsorbed vaccines. Virosomes are spherical vesicles composed of phospholipids, lecithin, phosphatidylcholine and phosphatidylethanolamine that contains viral envelope glycoproteins intercalated in the phospholipid bilayer membrane. The lipid components of Epaxal® virosomes are (DOPE) and DOPC. The hepatitis A vaccine (HAV) vaccine Epaxal® is based on formalin-deactivated RG-SB strain HAV particles, which are combined to the surface of virosomes. The RG-SB HAV strain was produced on an MRC-5 human diploid cell culture and then the virus was purified from disrupted cells by ultrafiltration. HAV was deactivated by treatment with formalin and then attached to the virosome surface. This structure facilitates the delivery of the HAV antigen to immunocompetent cells owing to the properties of fusion-active glycoproteins [51]. A clinical trial study demonstrated more than 95% of subjects were protected from HAV infection for more than 20 years [146, 151]. Inflexal® V is also a virosomal-adjuvanted vaccine which contains influenza virus. It is produced from the double membrane of lecithin-phospholipid liposomes loaded with haemagglutinin surface molecules of influenza viruses after preparation of influenza surface glycoproteins, neuraminidase (NA) and hemagglutinin (HA) by detergent treatment. The superior immunogenicity of Inflexal® V has shown statistically significant improvement above conventional influenza vaccines [146, 152, 153]. This formulation was patented by the Crucell Berna Biotech, Switzerland and approved by EMA in 2008.

In 2012, Marqibo®, a vincristine (VCR) sulfate nanoliposomal injection form produced by Talon Therapeutics, Inc. USA was FDA-approved for the treatment of adult patients with Philadelphia chromosome-negative (Ph^-) acute lymphoblastic leukemia (ALL) [141, 146, 148]. VCR is a semi-synthetic chemotherapeutic agent that is encapsulated in an aqueous interior core of sphingomyelin/cholesterol nanoliposomes to overcome the dosing, pharmacokinetic, and pharmacodynamic limitations of non-liposomal VCR [146, 148]. Marqibo® has proved to be safe and shown tolerability, with enhanced vincristine cell uptake, penetration, and concentration in tissues and organs with fenestrated vasculature or involved in the MPS [141, 142, 146].

Since 2015, three new liposomal formulations have been approved for clinical use by FDA. Onivyde® (irinotecan liposome injection), a product produced by Merrimack Pharmaceuticals Inc. was approved by the FDA and the EMA in 2015. Onivyde® is a PEGylated nanoliposomal hydrochloride irinotecan formulation for the treatment of metastatic pancreatic adenocarcinoma (mPAC) in combination with 5-FU and leucovorin (LV) in patients previously treated with gemcitabine-based therapy [142, 143, 148]. In general, there are still very few options for these patients and no consensus of care has been established. It was shown that patients given Onivyde® in addition to 5-FU/LV were shown to have a higher survival rate [142, 146]. Moreover, CPX-351 (Vyxeos™), FDA-approved in 2017 is also a liposomal product delivering cytarabine and daunorubicin for the treatment of acute myeloid leukemia (AML) [142, 148, 154]. Liposomal daunorubicin has proved to be effective with a low cardiac toxicity profile in an increased anthracycline dose in older patients, children, and adolescents. It also increases the plasma half-life and leads to drug accumulation within the bone marrow [142].

The most recently approved liposomal drug carrier is ONPATTRO® (2018) or Patisiran [142]. ONPATTRO® is a siRNA-delivering liposome developed and marketed by Alnylam, for silencing of a specific gene responsible for expression of transthyretin (TTR), which can cause hereditary transthyretin amyloidosis (hATTR) [142, 143, 155]. Thus, Patisiran comprises a TTR mRNA-specific siRNA formulation [155, 156] It is also the first clinically approved example of an RNAi therapy-delivering NP that can be administered intravenously. It is also the first therapeutic RNAi approved by the FDA [143, 155]. Clinical data have shown a potent and sustained knockdown of TTR expression. The patients who received the RNA had better outcomes on measurement of polyneuropathy including muscle strength, sensation (pain, temperature, numbness), reflexes and autonomic symptoms including blood pressure, heart rate and digestion when compared to those receiving the placebo infusions. However, it still has side effects and there has been little evidence of safety concerns about platelets, renal function or liver enzyme elevations [142]. Additionally, it was suggested that patisiran may stop or possibly reverse the progression of hATTR [142, 157].

From this evidence, liposomal drug delivery has become a useful tool for clinical medicine and this platform will continue to evolve into next generation nanomedicines with improvements in nanotechnology. Liposomal formulation products that have been approved for clinical use are shown in Table 1.

Table 1. Approved liposomal formulation products for clinical use.

Trade name	Active agent	Indications	Status
CPX-351 (Vyxeos™)	Daunorubicin and cytarabine	Acute myeloid leukemia	Approved by FDA in 2017
Onivyde	Irinotecan, fluorouracil and folinic acid	Pancreatic adenocarcinoma	Approved by FDA in 2015
Marqibo	Vincristine	Non-Hodgkin's lymphoma and leukemia	Approved by FDA in 2012
Exparel	Bupivacaine	Pain management	Approved by FDA in 2011
Mepact	Mifamurtide	Osteosarcoma	Authorised for use in the European Union
Genexol-PM	Paclitaxel	Breast, lung and ovarian cancer	Approved in Korea and marketed in Europe in 2007
Lipusu	Paclitaxel	Gastric, ovarian and lung cancer	Approved by FDA in 2005
Lipo-Dox	Doxorubicin	Breast and ovarian cancer	Approved by FDA in 2012
Myocet	Doxorubicin + cyclophosphamide	Metastatic breast cancer	Approved by EMEA in 2000
Visudyne	Verteporphin	Ocular histoplasmosis	Approved by FDA in 2000
Abelcet	Amphotericin B	Invasive fungal infection	Approved by FDA in 1995
Doxil	Doxorubicin	Kaposi's sarcoma	Approved by FDA in 1995
AmBisome	Amphotericin B	Invasive fungal infection	Approved by FDA in 1997
ONPATTRO®	Patisiran (siRNA)	Hereditary transthyretin amyloidosis	Approved by FDA in 2018

7. Lipid-based nanomaterials in diseases diagnosis

Nanotechnology is a useful approach that is quickly growing in several research fields, especially biomedical sciences. It provides various application forms of NPs which offer an opportunity to achieve better diagnosis, therapy and advanced multifunctional nanomedicines. NPs represent an innovative tool that incorporate molecular target ligands, active molecules and imaging agents for disease diagnosis and treatment assessment [144, 148]. Among the nanocarriers, lipid-based NPs are mainly attractive for biomedical use due to their helpful properties such as biocompatibility, ease of synthesis and encapsulation of multiple functional substances [158]. The application of lipid-based NPs in medical imaging and diagnosis has been vastly investigated in SLNs, nanostructured lipid carriers (NLCs) and liposomes [145, 158, 159].

SLNs consist mainly of solid lipid with relatively low toxicity and stabilised with surfactant. The most common lipids used to prepare SLNs include highly purified triglycerides, fatty acids and complex glycerides mixtures stabilised by a mixture of surfactants or polymers [158, 160, 161]. SLNs serve as a proficient and multipurpose colloidal drug carrier with useful features such as site-specific targeting, control of drug release, physical stability, ease of manufacturing and industrial scale-up. Importantly, it also has a capability to evade or minimise degradation of loaded drug [145]. SLNs have been employed as a pharmaceutical tool to transfer many classes of diagnostic agents such as superparamagnetic iron oxide, technetium-99 (^{99m}Tc), ^{64}Cu and quantum dots (QDs) [145, 158, 161]. Superparamagnetic iron oxide was encapsulated in SLNs and *in vitro* analysis showed a slow blood clearance of this formulation. *In vivo* magnetic resonance imaging (MRI) study also demonstrated the uptake of SLNs by the central nervous system (CNS) and SLNs were able to bypass the blood-brain barrier. This indicates favourable properties of SLNs for use as a CNS MRI contrast agent [145, 162, 163]. Moreover, it has been reported that SLNs could be radiolabelled with ^{64}Cu for positron emission tomography (PET)

imaging [164]. SLNs loaded with copper specific chelator-conjugated synthetic lipids were then radiolabelled with ^{64}Cu. Their stability was shown over 48 h and ^{64}Cu circulating in the blood was also observed 3 hours after injection. This indicates the potential of ^{64}Cu-SLNs for quantitative determination and *in vivo* PET imaging. In addition, SLN loaded with quantum dots can be used for theranostic applications. Co-encapsulation of paclitaxel-siRNA combination and quantum dots into SLNs showed anticancer activity on human lung carcinoma cells, meanwhile the strong fluorescence from quantum dots within SLNs indicated the visualisation of the intracellular translocation of SLNs in cancer cells [165]. This suggests the usefulness of SLNs as a multifunctional nanocarrier for both therapeutic and diagnostic tools.

NLCs have been developed as a novel improved generation of lipid NPs. The nanostructure of NLCs is a mixture of solid and liquid lipids with a lipophilic core that provides a suitable environment for encapsulation of hydrophobic drugs [158]. Due to their potential to improve the solubility of the lipophilic drug, NLCs are promising carriers for poorly water-soluble drugs. It has been evidenced that application of NLCs as a bioimaging agent delivery is used in medical imaging such as computerised tomography (CT) scanning, MRI, optical imaging and gamma scintigraphy for diagnostic applications [158]. For instance, QDs-loaded NLCs were successfully formulated and the cellular internalisation of QDs was conserved after NLC encapsulation. QD-NLCs exhibited the highest fluorescence intensity after cellular uptake and *in vivo* real-time monitoring revealed a persistent signal of labelled tumours for 24 h [166]. The experimental data indicates this lipid nanocarrier might be a promising delivery diagnostic NP.

Application of liposomes in diagnostic medical imaging is a new and rapidly growing field [158].The role of liposomes in imaging is to offer detectable differences between pathological and normal tissues and to permit appropriate imaging. They can also be applied to deliver diagnostic agents for gamma scintigraphy, ultrasonography and bioimaging [158]. Liposomes can be labelled with diagnostic agents including QDs, gadolinium (Gd) and manganese (Mn)

(both aqueous and chelated), radionuclides such as Ga, ^{111}In and ^{99m}Tc, iodine-based agents and even gases (echogenic liposomes) [158, 159, 167, 168]. Due to the flexibility of the liposome structure, it allows incorporation of imaging agents into either the bilayer or interior that makes them effective carriers for intensification of contrast. Liposomes can encapsulate a wide range of contrast agents to improve the contrast in *in vivo* visualisation [169-171]. Liposomes containing materials such as Gd have shown to be valuable for enhanced tumour diagnosis. Encapsulation or conjugation of Gd in liposomal formulation shows long retention time in the circulatory system, whereas pure Gd is quickly reduced in the intravascular plasma and dispersed extracellularly [172]. Furthermore, liposomes-encapsulating ^{64}Cu show unique imaging characteristics. Due to a protective barrier of the liposome membrane, it can prevent an exchange of ^{64}Cu with the biological environment, resulting in a high concentration of ^{64}Cu within the liposomes. Therefore, these liposomes provide non-invasive visualisation and quantification system appropriate for PET/CT imaging [173]. These applications reveal the increasing use of lipid-based nanomaterials in the field of disease and in particular cancer diagnosis as well as monitoring and treatment by enabling targeted delivery of therapeutic agents to the target tissues.

8. Lipid-nanoparticles toxicity

When designing lipid-based nanoparticles formulation, careful attention must be made for the potential toxic effects of these nanoparticles. While there are too many reports for the applications of these nanoparticles, the number of reports that address their toxic effects are limited.

The design of a specific lipid nanoparticles drug delivery system should be associated with careful evaluated of the cytotoxicity of these nanoparticles on different cell lines *in vitro* and on animal model *in vivo*. For example, solid lipid nanoparticles made from stearic acid or

dimethyl-dioctadecylammonium bromide (DDAB) were found to be non-toxic at concentrations around 0.01% [174]. Niosomes nanoparticles made of tween 85 and cholesterol and DDAB were found to be non-toxic at concentrations lower than 156 µg/ml [156]. It is worth mentioning that the IC_{50} will be different for the same formulation on different cell lines. This can be explained by the different uptake abilities by different cell lines which will result in different toxicities [175].

In several studies, the use of positively charged lipid nanoparticles is usually associated with higher toxicity compared to neutral particles. For example, higher hepatotoxicity with elevation in liver enzymes associated with weight loss were reported for mice treated with cationic nanoparticles compared to mice treated with neutral or anionic nanoparticles [176, 177].

Moreover, following intravenous administration of cationic nanoparticles into mice model, several immune responses were detected such as type I interferon response with elevated mRNA levels of interferon responsive genes in different subsets of leukocytes. An increase in the pro-inflammatory responses were also detected through the Th1 cytokines expression such as IL-2, IFN g and TNF a. This elevation was also higher for cationic nanoparticles compared to neutral or anionic particles [174, 176].

9. Conclusions

Different types of lipid-based nanomaterials can be effectively used to improve the diagnoses and treatment of various cancer types by enhancing the detection of cancer-specific biomarkers, imaging of tumours and their metastases, specific drug delivery to target cells, and real-time observation of treatment progression. Among these, liposomes represent the most widely used and studied lipid nanoparticles. Several lipid-based nanomaterial medications have been introduced to the market for these purposes and many others are yet to come after passing their clinical trials or the development stages which will significantly improve the outcomes of anticancer therapeutics and diagnostics.

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