Chapter

Perspective Chapter: Liposome Mediated Delivery of Immunotherapeutics for Cancer

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Abstract

Tumors have complex properties that depend on interactions between epithelial cancer cells and the surrounding stromal compartment within the tumor microenvironment. In particular, immune infiltration plays a role in controlling tumor development and is now considered one of the hallmarks of cancer. The last few years has seen an explosion in immunotherapy as a targeted strategy to fight cancer without damaging healthy cells. In this way, long-lasting results are elicited by activation of an antitumor immune response, utilizing the body's own surveillance mechanisms to reprogram the tumour microenvironment. The next challenge is to ensure targeted delivery of these therapies for increased efficacy and reduction in immune-related adverse events. Liposomes are an attractive drug delivery system providing versatility in their formulation including material type, charge, size and importantly surface chemical modifications that confer their tumour specificity. These tunable properties make them an attractive platform for the treatment of cancer. In this chapter, we will discuss clinically approved immunotherapies and those undergoing clinical trials together with, recent liposomal approaches for enhanced specificity and efficacy.

Keywords: immunotherapy, liposomes, nanocarriers, systemic delivery, cancer

1. Introduction

Cancer cannot be considered a mass of isolated tumor cells, but instead, it relies on several interactions with the surrounding microenvironment. Indeed, in response to evolving environmental conditions and oncogenic signals from growing tumors, the tumor microenvironment (TME) continually changes during cancer progression, highlighting the need to consider its influence on metastasis as a dynamic process, and to understand how tumor cells drive the construction of their own niche [1, 2]. The TME stromal compartment comprises both nonmalignant cells such as fibroblasts, myofibroblasts, endothelial cells and immune cells as well as signaling molecules including growth factors, chemokines, cytokines, extracellular matrices

(ECMs) and matrix-degrading enzymes that act together to promote cancer progression and metastasis. Indeed, they all become educated by the tumor to acquire pro-tumorigenic functions [3]. Based on these considerations, in the last few years, several strategies to fight cancer have been developed to alter the TME and effectively reprogram it [4]. These include chemotherapy, targeted therapy, immunotherapy and combinations of these therapies. Chemotherapy elicits anti-cancer effects by acting on cancer cell survival and proliferation, but it can also affect the TME for instance by increasing anti-tumor immune cells. However, patients have poor tolerance and can develop strong drug resistance. Therefore, there is a need to reduce side effects to chemotherapy [4]. Targeted therapies for specific TME components or signaling pathways have become the key to suppressing cancer proliferation and invasion. For example, Lee et al. found that bortezomib (BTZ) and phenobarbital (PST) reduced the survival rate of cancer-associated fibroblasts (CAFs) by inducing caspase-3-mediated apoptosis, thereby inhibiting the proliferation of cancer cells in a breast cancer mouse transplantation model [5]. Another promising strategy is immunotherapy, therapeutics that utilize the body's immune system to reprogram or activate anti-tumor immunity to kill tumor cells, without damaging normal cells. For instance, it has been demonstrated that molecules usually expressed on activated T cells, such as the immune checkpoint proteins CTLA-4 and PD-1 play a crucial role in the immunosuppression observed in the TME [6]. For this reason, several monoclonal antibodies (mAbs) targeting CTLA-4, PD-1 or PD-L1 have been developed and tested in clinical trials for the treatment of several types of cancers [7–10].

However, the promise of providing long-lasting results where other therapies have failed has not yet been realized as they are faced with a number of challenges including immune-related adverse events due to low specificity in tumor cell targeting. The use of smart drug delivery systems such as liposomes could help overcome these challenges. This chapter will give an overview of the current immunotherapy landscape and the use of liposomes to directly deliver anticancer immune therapies to tumor sites.

2. Immunotherapies

Cancer immunotherapy focuses on modulation and use of the patient's own immune system or agents that activate or enhance the immune system's recognition and killing of tumor cells [3–5]. Modulating the immune system to target cancer is a successful treatment for some solid malignancies. However, some cancers are immunogenically cold [11]. This nomenclature is given to tumours that have fewer immune cells and decreased cancer antigen expression leading to an intrinsic resistance to immunotherapies. In these 'cold' malignancies, the TME acts as a cloak to mask cancer cells from host's immune system, even in the presence of novel immunotherapies (**Figure 1**). Several approaches including cell-based therapies, cytokines, oncolytic viruses and immune checkpoint inhibitors have been approved for clinical use by the Food and Drug Administration (FDA) or in clinical trials (**Table 1**).

Cell-based immunotherapies manipulate or stimulate autologous immune cells that specifically target abnormal antigens expressed on the surface of tumor cells [52]. These include lymphocytes, macrophages, dendritic cells, natural killer cells and cytotoxic T lymphocytes (**Table 1**). However, induction of nutrient depletion and activation of negative immune regulatory pathways by cancer cells contribute to an immunosuppressive TME that compromises anti-tumor immune pathways and therefore

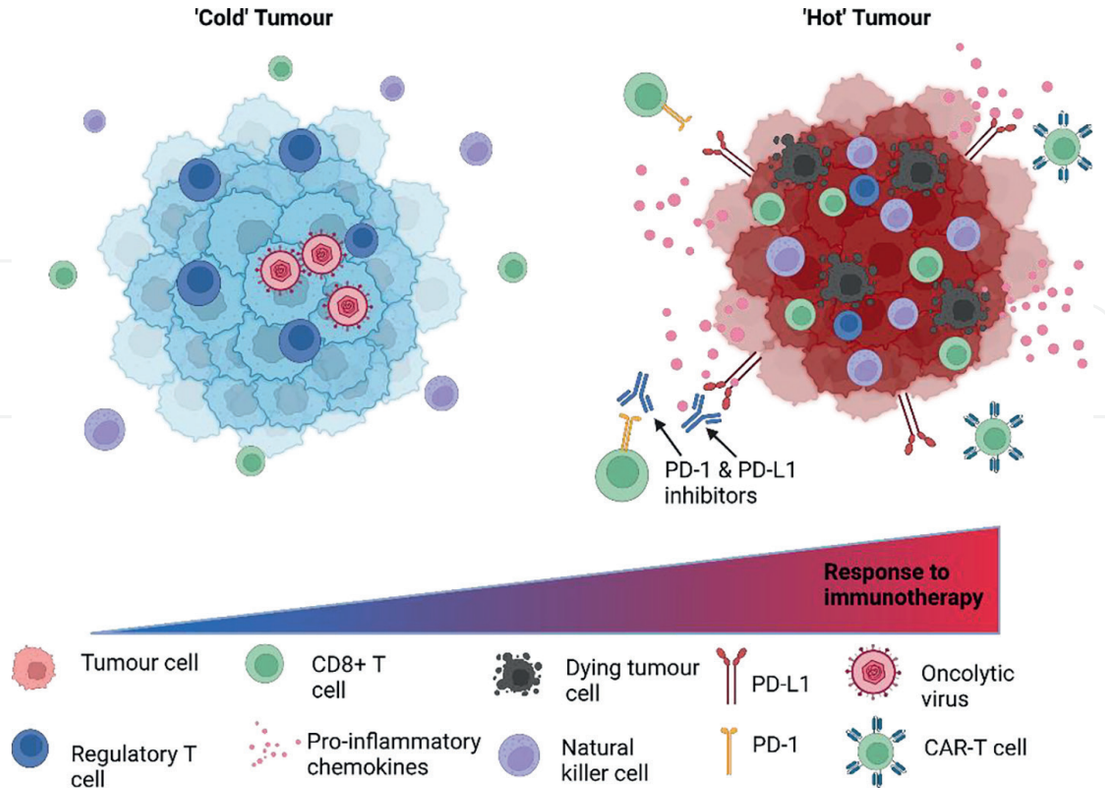

Figure 1.
Inflaming the cold tumour microenvironment using immunotherapies. 'Cold' tumors demonstrate an immunosuppressive environment with the exclusion of immune cells including Tregs, CD8+ T cells and natural killer cells from the TME resulting in poor prognosis and response to immunotherapy. 'Hot' tumor types demonstrate high immune cell infiltration and expression of pro-inflammatory markers. Immunotherapies inhibit tumor cells from deactivating T cells via PD-1, PD-L1 and CTLA-4 blockade and augment immune cell recruitment and activation via cytokine therapy to enhance tumor lysis. Created using Biorender.

the therapeutic effect of cell-based immunotherapy. This is seen in the stimulation of immunosuppressive Tregs and MDSCs [53] and patterns of expression of immune checkpoint inhibitors by activated T cells [8, 54]. Playing a crucial role in the immunosuppression observed in the TME, PD-1 and CTLA-4, through interaction with their ligands (PD-L1/PD-L2 and CD80/CD86 respectively), transmit inhibitory signals to T cells [55], thus suppressing effector T cell activation and function. Crucially, upregulation of PD-L1 and CTLA-4 on the surface of tumor cells has been detected in recent years [56] resulting in the development of mAbs targeting PD-1, PD-L1, PD-L2 and CTLA-4 for blockage of these immunosuppressive pathways [7, 8, 53–56]. Classified as immune checkpoint inhibitors, these mAbs have undergone a number of clinical trials for the treatment of several types of cancers [7–10, 12].

Manipulation of the TME by cancer cells is facilitated by cytokines and growth factors and it is well known that deregulated cytokine production and aberrant cytokine signaling can lead to altered cell growth, differentiation and apoptosis as well as the secretion of factors that foster cancer progression and immune evasion [57, 58]. Thus, cytokine therapy has been explored in the treatment of cancer to enhance anti-tumoral immunity [40, 59]. Currently three cytokines have been approved by the FDA for use in cancer patients: recombinant interleukin IL-2 (Proleukin; Chiron) and two variants of recombinant interferon alpha 2 called IFNα2a (Roferon-A; Roche) and IFNα2b (Intron-A; Merk & Co) (**Table 1**).

Oncolytic viruses (OVs), as a new therapeutic agent, offer a two-pronged attack mechanism. Their direct tumour killing is afforded in the first place by specific viral

Immunotherapy type	Drug name	Mechanism	Phase	Tumour type	References
DCs-based Vaccine	Sipuleucel-T	Patients' APCs activated by PAP and GM-CSF	Approved by FDA	Advanced prostate cancer	[12–14]
CAR-T cell therapy	Kymriah	Patient's T cells are engineered to target a protein called CD19	Approved by FDA	B-cell acute lymphoblastic leukaemia	[15, 16]
CAR-T cell therapy	Yescarta	Patient's T cells are engineered to target a protein called CD19	Approved by FDA	Large B-cell lymphoma	[17]
NK cell therapy	oNKord	NKs generated *ex vivo* from umbilical cord blood progenitor cells.	Phase I clinical trial	Acute myeloid leukaemia	[18–21]
Immune checkpoint inhibitor	Ipilimumab	Anti-CTLA-4 mAb	Approved by FDA	Unresectable or MM	[10, 22–27]
Immune checkpoint inhibitor	Nivolumab	Anti-PD-1 mAb	Approved by FDA	NSCLC, MM, HL, SCCHN, MUC	[28–31]
Immune checkpoint inhibitor	Pembrolizumab	Anti-PD-1 mAb	Approved by FDA	NSCLC, MM, HL, SCCHN, MUC	[32–35]
Immune checkpoint inhibitor	Durvalumab	Anti-PD-L1 mAb	Approved by FDA	MUC	[36]
Immune checkpoint inhibitor	Avelumab	Anti-PD-L1 mAb	Approved by FDA	Metastatic Merkel carcinoma	[37]
Immune checkpoint inhibitor	Atezolimumab	Anti-PD-L1 mAb	Approved by FDA	NSCLC, MUC	[38]
Immune checkpoint inhibitor	CA-170	Anti-PD-L1/ PD-L2 and VISTA mAb	Phase I clinical trial	Lymphomas and solid cancers	[10, 39]
Cytokine	Proleukin	IL-2	Approved by FDA	Metastatic melanoma, RCC	[40, 41]
Cytokine	Roferon-A	IFN-α2a	Approved by FDA	HCL, CML	[40, 41]

Immunotherapy type	Drug name	Mechanism	Phase	Tumour type	References
Cytokine	Intron-A	IFN-α2b	Approved by FDA	AIDS-related Kaposi's sarcoma, melanoma, FL, multiple myeloma, HCL, CIN	[40, 41]
Oncolytic virus	T-Vec (Herpes simplex virus)	Cancer cells killing and GM-CSF expression for APCs recruitment	Approved by FDA	Advanced melanoma	[42–44]
Oncolytic virus	JX-594 (Vaccinia virus)	Cancer cells killing and GM-CSF expression	Phase I clinical trail	Melanoma, HCC	[45, 46]
Oncolytic virus	CG0070 (Adenovirus)	Cancer cells killing (viral replication under the control of Rb)	Phase I clinical trail	Non-muscle invasive urothelial cancer	[47–49]
Oncolytic virus	Reolysin (Reovirus)	Cancer cells killing (viral replication under the control of Ras)	Approved by FDA	Malignant glioma, metastatic breast cancer	[50, 51]

Table 1.
Main immunotherapeutic agents approved by the FDA or in clinical trials for cancer treatment.

replication within cancer cells resulting in oncolysis. This provides self-amplification and release of viral progeny for infection of neighbouring tumour cells. Oncolysis also releases tumor antigens and following uptake by antigen presenting cells (APC), indirectly induces a systemic anti-tumor immunity through both innate and adaptive immune pathways [42, 43, 60, 61].

As therapeutic agents they also offer versatility via genetic modification to maximise their features. They can be engineered to increase tropism towards specific cancers via capsid insertion of ligands for enhanced tumor cell binding [43, 62, 63]. Additional transgenes can be inserted for expression of proteins designed to further amplify immune activation at the tumor site. Moreover, strategies to improve selective replication in cancer cells and hence their safety, include the deletion/insertion of tissue- or cell type- specific promoters to induce gene expression in tumor cells [64]; or the placement of viral genes under the control of tissue specific elements. Despite these attractive properties, successful use of OVs in the clinic to date, have been limited to direct tumor injection as systemic delivery results in rapid clearance whilst in circulation, thus preventing tumor targeting. For these to be used more widely in the clinic, strategies are needed to protect the virus in the blood stream so that tumors in inaccessible locations can be treated [65]. Whilst in the last decade immunotherapy

has become a viable treatment option for some cancers, for many patients this is still limited due to its low response rates as a monotherapy [66].

Combination therapy is instead a treatment modality that combines two or more therapeutic agents to fight cancer. It is probably the most effective approach because it targets key pathways in a characteristically synergistic or an additive manner, reducing drug resistance and providing therapeutic anti-cancer benefits, such as reducing tumor growth and metastatic potential, arresting mitotically active cells, reducing cancer stem cell populations, and inducing apoptosis [67]. However, obtaining these achievements is complicated and an easier and more promising approach could be the use of nanotechnology. Indeed, the use of nanomedicine has several advantages such as the early diagnosis of disease and the combination of different therapeutic agents for overcoming cancer resistance [68]. Moreover, nanoparticles can be fabricated with unique characteristics including their material type, size, shape, charge and surface chemical modifications for tunable optimization [69]. Indeed, changing nanoparticles physical and chemical properties has an important effect on their kinetics of internalization, biodistribution, cellular uptake, immunogenicity and loading efficiency [70, 71] making them the most promising platform for biomedical applications [69].

3. Liposomes

Traditionally known as liposomes, lipopolymers, solid lipid nanoparticles, nanostructured lipid nanoparticles, microemulsions and nanoemulsions, lipid nanoparticles are used primarily for the release of small molecules, peptides, genes and monoclonal antibodies [72]. Liposomes consist of spherical vesicles having one or more lipid layers containing an aqueous core. The structure of a conventional liposome allows the encapsulation of both hydrophilic and lipophilic agents in the lipid layers or in the internal compartment, respectively (**Figure 2**) [73, 74]. Depending on the water solubility of the payload, they can be encapsulated in the aqueous core (hydrophilic drugs) or in surrounding bilayer of the liposome (hydrophobic drugs) [75]. They are physically stable, and unlike other nanoparticles, they are not covalently bound. As a delivery system, LNPs offer many advantages, including simplicity of simulation, self-assembly, biocompatibility, high bioavailability, the ability to carry large payloads, and a range of physicochemical properties that can control their biological properties [76]. Lipid nanoparticles are the most common class of FDA-approved nanomedicine drugs (**Table 1**). Among them, the liposome-encapsulated form of Doxorubicin (Doxil) approved by the FDA in 1995 for the treatment of ovarian cancer and AIDS-related Kaposi's sarcoma can be considered the first success in this field [73, 74, 77, 78].

Other examples that need to be mentioned are liposomal daunorubicin (DaunoXome) for treatment of poor-risk acute leukemia [79], Liposome-encapsulated doxorubicin citrate (Myocet) for breast cancer therapy [80], the Liposomal cytarabine (DepoCyte) for the treatment of neoplastic meningitis [81], the vincristine sulfate liposome injection (Marqibo) for childhood and adult hematologic malignancies [82] and the irinotecan liposome injection (Nivyde) for the treatment of metastatic pancreatic cancer [83]. There are approximately 1862 clinical trials involving the use of liposomes in cancer therapy [84]. These liposomal formulations of chemotherapies were designed to overcome problems with severe side effects (nausea, fatigue, diarrhea, hair loss, disruption of mouth, pharynx mucosa, and bone marrow [85, 86]) as well as improvements in both the drug bioavailability at the tumor site

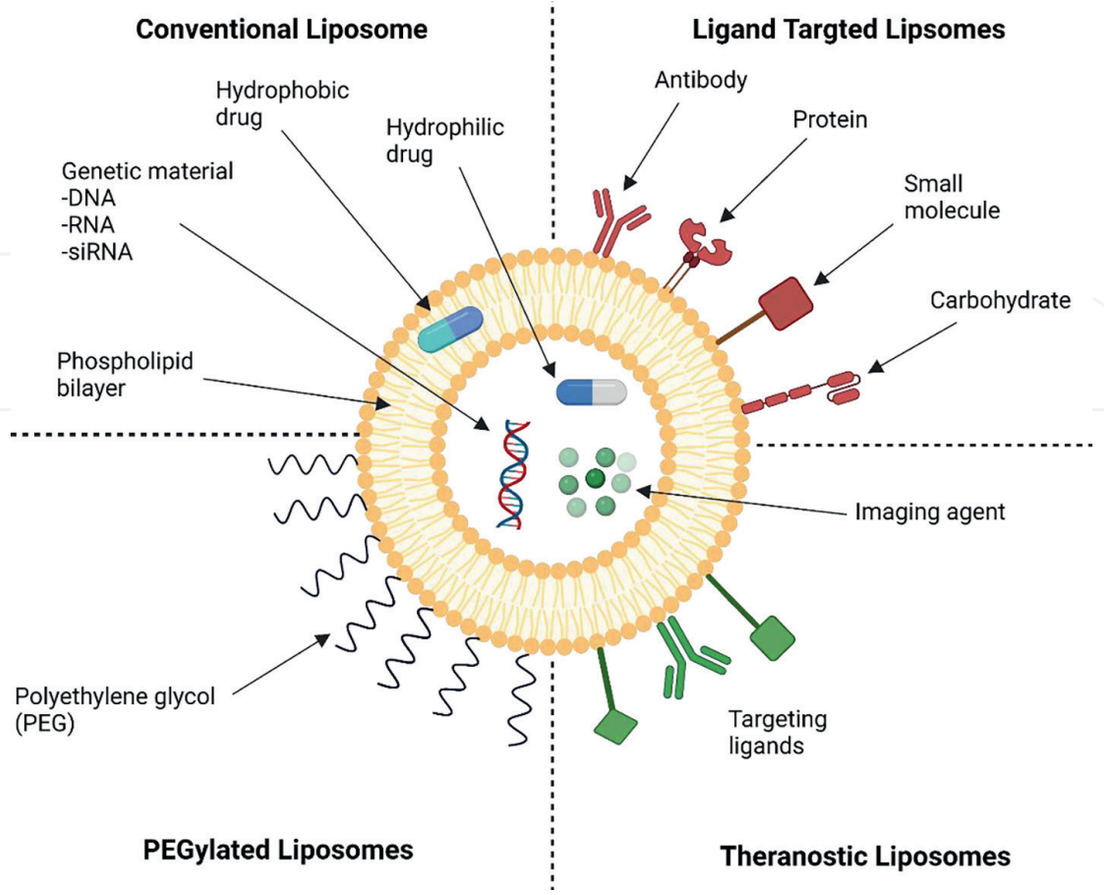

Figure 2.
Schematic of liposomes comprising outer phospholipid layer. PEGylated liposomes contain a layer of polyethylene glycol (PEG) on the surface of liposomes. Targeted liposomes contain a specific targeting ligand to target a cancer site. Multifunctional theranostic liposomes can be used for diagnosis and treatment of solid tumors.

and its pharmacokinetic properties in order to deliver the active drug molecules to the site of action, without affecting healthy cells.

3.1 Liposomes and chemotherapy

The mechanism of action of Doxil is based on the use of sterically stabilized (composed of high Tm phospholipids and cholesterol), PEGylated nano-liposomes to prolong drug circulation time and allow efficicent extravasation via the EPR effect. Additionally, stable loading of doxorubicin (DOX) as well as DOX release at the tumor target is provided by a transmembrane ammonium sulfate gradient [78]. Unlike Doxil, the Myocet liposome does not have a PEG coating, but it seems to have less cardiotoxicity. It is approved in the European Union and in Canada for the treatment of metastatic breast cancer in combination with cyclophosphamide, but it has not been approved by the FDA for use in the United States [87].

Another anthracycline to utilize the advantages of liposomal packaging is daunorubicin. DaunoXome contaisn an aqueous solution of the citrate salt of daunorubicin encapsulated within lipid vesicles composed of a lipid bilayer of distearoylphosphatidylcholine and cholesterol [88]. By protecting the entrapped compound from chemical and enzymatic degradation, DaunoXome increases its biocompatibility and bioavailability by reducing uptake by normal tissues and minimizing protein binding respectively. It is FDA approved to treat AIDS related Kaposi's sarcoma. It is also commonly used to treat specific types of leukemia and non-Hodgkin lymphoma [88].

Another example of lipid-nanocarrier for chemotherapeutic agents is Depocyte, a liposomal formulation of cytosine arabinoside (Ara-C) which is a cytosine analog with arabinose sugar that kills cancer cells by interfering with DNA synthesis [89]. Ara-C has a short plasma half-life, low lipophilicity, stability and limited bioavailability. DepoCyte consists of multivesicular lipid-based polymeric liposomal carriers composed of cholesterol, glycerol trioleate, triglyceride, phospholipids that increase the Ara-C half-life and consequently in the treatment of lymphomatous meningitis [90].

Vincristine (VCR) is a vinca alkaloid that is thought to work by interfering with cancer cell growth during mitosis and it used for treatment of hematologic malignancies and solid tumors. Its main challenge is that it has a diffuse distribution and tissue binding that can limit drug efficacy and generate several side effects [82]. To overcome this, VCR has been encapsulated in sphingomyelin/cholesterol liposomes to produce a vinCRIStine sulfate liposome injection called Marqibo [82]. It is specifically indicated for the treatment of adults with Philadelphia chromosome-negative (Ph-) acute lymphoblastic leukemia in second or greater relapse or whose disease has progressed following two or more anti-leukemia therapies.

The topoisomerase I inhibitor Irinotecan is another example of how lipid carriers can increase chemotherapy efficacy and reduce toxicity. Irinotecan is indeed a drug currently used in the treatment of multiple solid tumors, such as metastatic colorectal cancer (mCRC), small-cell lung cancer, non-small-cell lung cancer, gastric cancer, and cervical cancer [91]. The main challenges in irinotecan usage are the acute toxicities caused by it and its fast elimination that can strongly limit its clinical applications [92, 93]. For this reason, the liposomal formulation Onivyde has been developed to improve the pharmacokinetics and reducing host toxicity. Onivyde was approved by the US Food and Drug Administration (FDA) in October 2015 as a combination regimen for patients with gemcitabine-based chemotherapy-resistant metastatic pancreatic cancer [91].

Considering all these advancements, it is clear that liposomes have overcome the limitations of conventional chemotherapy by improving drug bioavailability and stability and minimizing their side effects by site-specific targeted delivery. This success has paved the way for the use of liposomal agents in the field of cancer immunotherapy together with additional modifications of the liposomal surface, facilitating their active targeting to tumors. Whilst passive targeting relies on the EPR effect for accumulation of liposomes within tumors, active targeting is obtained by linking to liposomes membrane specific ligands that bind specific antigens on cancer cells [75] (**Figure 2**). Next, we describe these modifications in the context of liposomal delivery of immunotherapeutics.

3.2 Liposomal immunotherapies

Improving CAR-T cell therapy against solid tumors has recently adopted the use of lipid nanoparticles in order to address the issues surrounding the presence of an immunosuppressive TME that can decrease the treatment efficacy [71]. A recent study by Zhang and colleagues showed promising results in a model of murine breast cancer to overcome this obstacle using infusions of lipid nanoparticles coated with the tumor-targeting peptide iRGD and loaded with a combination of a PI3K inhibitor to block immunosuppressive tumor cells activity and α-GalCer (an iNKT cell activator). The investigators demonstrated a switch in the TME from immunosuppressive to stimulatory thereby enabling tumor-specific CAR-T cells to home to the

tumor, undergo robust expansion and trigger tumor regression [94] during a 2 week therapeutic window. This strategy has been applied to a number of immunotherapies (**Table 2**) to assist in their delivery, efficacy and safety as follows.

3.2.1 Liposomes and ICI's

The development of immune checkpoint inhibitors (ICIs) has been a major breakthrough in cancer immunotherapy. However, only a small percentage of patients exhibit durable responses under monotherapy and their increasing use has led to the discovery of immune-related adverse events (irAEs) including myopathy [112], immune-related myasthenia gravis (irMG) [113] and pneumonitis [114] to name a few. Whilst BMS-202 (a small molecule inhibitor of PD-1/PD-L1) loaded liposomes have inhibited tumor growth in a model of triple negative breast cancer (TNBC) [115] and pancreatic cancer when combined with photothermal therapy [96], there is now a trend towards combination therapy (**Table 2**). For example, amplification of the therapeutic potential of DOX-loaded biomimetic hybrid nanovesicles (DOX@LINV) (synthesized by fusing artificial liposomes with tumor-derived nanovesicles, facilitating both targeted delivery of DOX to tumor tissue and eliciting effective immunogenic cell death response to improve the immunogenicity of the tumor) by combination treatment with aPD-1 antibody prolonged survival of B16F10 tumor-bearing mice by 33% [116]. Additionally, the utilization of PD1/PD-L1 mAbs as surface ligands for enhanced tumor targeting of nanoparticles is an emerging strategy whereby PD-L1 targeted DOX [117] and catalase [118] immunoliposomes are promising candidates for melanoma immunotherapy.

3.2.2 Liposomes and antibodies

One of the most notorious targets for interventional antibody therapy is the Human Epidermal growth factor Receptor 2 (HER2) which is involved in important stages of growth and cell differentiation and is overexpressed by HER2 positive breast cancer cells. Targeting HER2 positive cancers can be achieved by coating liposomes with an anti-HER2 monoclonal antibody [119] and in recent years, several targeted therapy options for HER2-positive breast cancer has been developed including Pertuzumab (Perjeta), Trastuzumab (Herceptin), Tucatinib (Tukysa), Neratinib (Nerlynx), Margetuximab (Margenza), DS-8201 (Enhertu), and Ado-trastuzumab emtansine or T-DM1 (Kadcyla) [120]. In particular, Herceptin was FDA approved in 1998 for the treatment of HER2-positive breast cancers [121] and has been studied extensively since including using various nano delivery systems. For example, Elamir et al., functionalized calcein and Doxorubicin-loaded pegylated liposomes with Herceptin and utilized Low-Frequency ultrasound for their controlled release to enhance uptake by cancer cells in vitro, paving the way for in vivo studies [119].

Anchoring antibodies to the surface of liposomes to enable targeted delivery (**Figure 1**) can also be performed within the circulation. For instance, in 2004 van Broekhoven et al. targeted DCs through anti-DEC-205 or anti-CD11c mAbs located on the surface of liposomes containing tumor antigens (B16 melanoma antigens or lipopolysaccharide), thus inducing potent anti-tumor immunity both in vitro and in vivo [122, 123].

Another molecule overexpressed by cancer cells is the vascular endothelial growth factor (VEGF) that increases angiogenesis for enhanced tumor growth. Indeed, it binds two VEGF receptors (VEGF receptor-1 and VEGF receptor-2) on vascular

Immunotherapy type	Delivery platform	Tumour type	Reference
Immune checkpoint inhibitors			
PD-L1	Cerasome nanoparticle loaded with Paclitaxel and decorated with PD-L1	Breast, colon	[95]
BMS-202 (PD-1/PD-L1 inhibitor)	BMS-202 loaded thermosensitive liposomes	Pancreatic	[96]
Monoclonal antibodies			
Intravenous immunoglobulin	PEGylated nanoliposome encapsulating the antibody	Colorectal	[97]
Anti-EGFR antibody	Porphyrin containing liposomal cersaome decorated with Cetuximab	Colorectal carcinoma	[98]
HER2	HER2 targeted PEGylated liposome	Metastatic breast cancer	[99]
Oncolytic viruses			
Oncolytic Adenovirus	Liposome-cloaked oncolytic adenovirus conjugated to tumour homing E.coli	Lung	[100]
Oncolytic Adenovirus	CCL2-coated liposomes for monocytic cell delivery	Prostate	[101]
Cancer vaccines			
Epitope vaccine	Mannose decorated liposomes activate DC maturation for enhanced cytotoxic T lymphocyte response	Metastatic breast cancer	[102]
LAG3-Ig + P5 tumour antigen	PEGylated liposome bearing surface conjugated LAG3-Ig and P5 tumour antigen	Breast	[103]
Synthetic long peptides	Liposome loaded with tumour specific synthetic long peptides	Lung, melanoma	[104]
Combination treatments			
Tumour vaccine of antigen epitopes + IDO inhibitor	Lipid hybrid nanovesicle-based liposomes containing tumour vaccine and immune checkpoint inhibitor	Melanoma	[105]
Anti-PD-L1 + Docetaxel	Liposome co-loaded with PD-L1 antibody and Docetaxel	Melanoma	[106]
siRNA-PD-L1 + Imatinib	Liposomal co-delivery of siRNA-PD-L1 and Imatinib	Melanoma	[107]
Interleukin-2 (IL-2) + anti-PD-L1 + Imiquimod	C25 antibody modified liposomes containing a combination of treatments attached to the surface of T regulatory cell	Melanoma	[108]
Other immune system modulators			
Interferon-gamma (IFN-γ)	PEGylated liposomes containing IFN-γ	Colon	[109]

Immunotherapy type	Delivery platform	Tumour type	Reference
Small immunostimulatory RNA	Liposomes containing immunostimulatory RNA	Melanoma	[110]
Interleukin-15 (IL-15)	Folate receptor targeted liposome containing IL-15 plasmid	Colon	[111]

Table 2.
Preclinical models of liposomal immunotherapeutics in development.

endothelial cells allowing tumor vasculature to grow exponentially thereby promoting cancer progression and metastasis [124]. Several agents, including antibodies and soluble receptor constructs, have been developed to target the VEGF system. The drug that is currently most widely used in the clinical practice to modulate VEGF-A is the humanized monoclonal antibody Bevacizumab, approved by the FDA and EMA for the treatment of metastatic colorectal cancer, non-small cell lung cancer, breast cancer and glioblastoma multiforme in combination with chemotherapy (**Table 1**) [125]. Several studies have been also conducted to improve bevacizumab efficacy and reduce its toxicity by using lipid nanocarriers. For instance, Kuesters and Campbell demonstrated that cationic pegylated liposomes that preferentially target the tumor vasculature, can be conjugated with bevacizumab and can increase its cellular uptake and tumor targeting in vitro [126]. Moreover, bevacizumab is extensively studied for ovarian cancer treatment since the combination of surgery and platinum-based chemotherapy is initially very effective in treating this cancer, but most patients will experience a recurrence because they acquire platinum resistance. To overcome these challenges, a phase II clinical trial (NCT04753216) is studying the combination of irinotecan liposome and bevacizumab in women with recurrent, platinum resistant ovarian cancer and the predicted results are that the liposomal encapsulation will enhance drug delivery and bioavailability, thereby improving efficacy and reducing toxicity [127]. These examples mentioned above, strengthen the idea that the use of therapy combination together with nanoparticles, in particular liposomes, as delivery systems, could strongly increase the cancer treatments efficacy, also overcoming drug resistance experienced by patients, and reduce their associated toxicities.

3.2.3 Liposomes and oncolytic viruses

The encapsulation of OVs inside lipid nanoparticles is another strategy that has demonstrated encouraging results in the last few years (**Table 2**). Acting as a protective shield, the phospholipid coating can hide viral epitopes thus reducing OV neutralization by pre-existing Abs upon systemic administration as seen by Chen et al. [128]. Not only that, but the efficacy of this encapsulated ZD55-IL-24 oncolytic adenovirus was demonstrated via inhibition of HCC proliferation and an enhanced anti-tumor immune response in vivo. Similarly, a separate study involvingliposome-encapsulated plasmid DNA of telomerase specific oncolytic adenovirus (TelomeScan) also recorded shielding from adenovirus-neutralizing Abs following intravenous administration into immune-competent mice compared to the naked virus together with potent anti-tumor effects on colon carcinoma cells both in vitro and in vivo [101, 129]. Shielding the viral epitopes from immunosurveillance has not only reduced their rapid clearance from the circulation but the addition of targeting ligands on the surface increases their

accumulation at target sites and reduces off-target side effects. Successful encapsulation of AD[I/PPT-E1A] into CCL2-coated liposomes were preferentially taken up by CCR2-expressing monocytes within the circulation thereby exploiting the recruitment of circulating monocytes by tumors for their targeted delivery [101]. This resulted in a significant reduction in tumor size and pulmonary metastases in pre-clinical model of prostate cancer at a viral titer 3 logs lower than AD[I/PPT-E1A] alone. Taken together, liposome-assisted delivery cannot only target OVs via the circulation to inaccessible tumors but reduction in concentration of virus required for efficacy provides additional safety and cost benefits.

3.2.4 Liposomes and immune-gene therapy

Liposomes have been studied in the field of cancer gene therapy for the targeting of genes involved in the development of cancer (**Table 2**). For example, the liposomal delivery of a stimulator of interferon genes (STING) agonist has augmented cytokine therapy. In a model of metastatic melanoma, investigators saw an increase in IFNγ production by tumor-associated APCs, leading to anti-tumor immunity enhancement and cancer regression compared to the free drug [130]. Further advancements in lipid nanotechnology for the delivery of gene therapy have developed strategies for controlled release, improved therapeutic loading and faster route to market as follows.

With their positive charge, cationic liposomes, can be used to easily encapsulate plasmid DNA (pDNA), messenger RNA (mRNA), or small interfering RNA (siRNA) via electrostatic interactions [131]. An important example is the T7 peptide modified core-shell nanoparticles (named as T7-LPC/siRNA NPs). The core-shell structure of T7-LPC/siRNA NPs enables them to encapsulate siRNA in the core and protect it from RNase degradation during circulation. Both in vitro and in vivo results show that this system can efficiently deliver the EGFR siRNA into breast cancer cells through receptor mediated endocytosis and down-regulate the EGFR expression [132]. Furthermore, plasmids can be encapsulated in lipid nanocarriers whereby a tumor-targeted liposomal nano delivery complex (SGT-94) carrying a plasmid encoding RB94, a truncated form of the RB gene, has shown promising results in metastatic genitourinary cancer in terms of selective tumor targeting and tolerability [133].

The first marketed RNA drug, Onpattro®, was launched by Alnylam Pharmaceuticals in 2018. Onpattro® comprises lipid nanoparticles (LNPs) prepared from ionizable lipids for siRNA encapsulation and delivery [134]. LNPs have since become the preferential vector for nucleic acid delivery. LNPs are constructed using phospholipids with ionizable lipids and other supporting phospholipids to complete the particle [76, 135]. A high degree of encapsulation is achieved by mutual adsorption of the nucleic acid's negative charge and the ionizable lipid's positive charge. When LNPs enter the body, the cytolysis mechanism mediated by low-density lipoproteins allows the nanoparticles to be successfully taken up by cells [136]. The endosome successfully releases the phagocytosed LNPs and transports them to the cytoplasm for expression, producing the corresponding protein.

These mRNA vaccines have gained a lot of attention due to their good safety profiles, successful preventative effects and rapid development of mRNA technology, making them very competitive [137]. Indeed, thanks to the prior optimization of mRNA and extensive basic research and testing of lipid nanoparticles, the mRNA vaccine against SARS-CoV-2 took less than a year from the publication of the virus sequence to the launch of the vaccine, and demonstrated an efficacy rate of over 90% [138]. This was previously unimaginable and unattainable. Application of this

technology for further optimization and improvement to CAR-T therapy has utilized lipid nanoparticles as a medium to target mRNA delivery to T cells and constructed CAR- T directly *in vivo* to treat heart failure symptoms in mice [139]. Upon delivery of mRNA to mice, large mRNA molecules are captured by T cells, allowing T cells to gain the ability to target cardiac fibroblasts specifically. The mRNA successfully encoded T cells in mice with heart failure, resulting in a significant reduction in myocardial fibrosis and heart repair to near normal size and function. The in vivo construction of CAR-T was accomplished through mRNA targeted delivery, and mRNA-LNP-targeted delivery is far less costly than traditional cellular therapies [140].

Other non-viral vectors for gene transfer into tumor cells is the use of lipoplexes (LPX) and micelleplexes and are proving promising in phase I/II clinical trials for advanced melanoma treatment. By protecting its' RNA payload from extracellular ribonucleases, these vectors improve cell uptake and hence gene expression. For example, in a B16-F10 murine melanoma tumor model, a micelleplex made of an acid-activatable cationic micelle, a photosensitizer and a small interfering RNA (siRNA) was able to inhibit PD-L1 resulting in inhibition of both primary tumour growth and formation of distant metastases formation compared to photothermal therapy alone [141]. This was achieved through its activatable composition, only switching "on" upon internalization in the acidic endocytic vesicles of tumor cells, demonstrating the versatility of these particles.

4. Route to clinic/challenges

Although nanoparticle drug delivery technology has now been extensively researched, the prevalence of nanomedicines is far below expectations [9, 56]. One of the main challenges is that the current processes used for liposome manufacturing suffers from many severe problems such as high costs of production related to multi-step batch processes, the need to use specialized tools and equipment for particle size reduction and limited batch sizes [77].

Polymeric materials were the most common delivery vehicles used by early scientists, such as polyethyleneimine (PEI), polyamine ester (PBAE), chitosan, etc. [142]. However, the application of polymeric materials has stalled at the pre-clinical trial stage [143]. In a study investigating PEI delivery of DNA to the lungs, the poor breakdown of PEI raised concerns regarding accumulation of the polymer as well as specific side effects, particularly for repeated treatment administration [144]. Most polymeric materials used for nucleic acid delivery require modification of fatty acid chains to improve their safety. A team of researchers developed a branched polyamine polymer for mRNA encapsulation and prepared polymeric RNA nanoparticles whereby these vaccine recipients successfully expressed antibodies against Zika and Ebola viruses [145]. Although LNPs have been used on a large scale (in particular how to achieve higher therapeutic efficacy as discussed) optimization of the production process is also required for successful translation of these formulations to the clinic. This includes optimization of the LNP production process, control of the LNP characteristics, shelf-life, regulatory considerations and cost effectiveness.

5. Conclusions

The possibility that immunotherapy could replace surgery and other forms of cancer treatment is being entertained for the first time. However, this is not all good news

as many of these immunotherapies are associated with potentially serious side effects, linked to inflammation in the bowel, lung, heart, skin and other organs. Liposomes display superiority as a delivery platform for cancer immunotherapy with the potential to overcome many of the challenges related to their systemic delivery and toxicity. However, for this to become reality, the less than satisfactory targeting efficiency of liposomes needs to be addressed to achieve improved clinical performance.

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Conflict of interest

The authors declare no conflict of interest.

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