Review

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Lipid-based nanoparticles for cancer immunotherapy

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Abstract: As the fourth most important cancer management strategy except surgery, chemotherapy and radiotherapy, cancer immunotherapy has been confirmed to elicit durable antitumor effects in the clinic by leveraging the patient's own immune system to eradicate the cancer cells. However, the limited population of patients who benefit from the current immunotherapies and the immune related adverse events hinder its development. The immunosuppressive microenvironment is the main cause of the failure, which leads to cancer immune evasion and immunity cycle blockade. Encouragingly, nanotechnology has been engineered to enhance the efficacy and reduce off-target toxicity of their therapeutic cargos by spatiotemporally controlling the biodistribution and release kinetics. Among them, lipid-based nanoparticles are the first nanomedicines to make clinical translation, which are now established platforms for diverse areas. In this perspective, we discuss the available lipid-based nanoparticles in research and market here, then describe their application in cancer immunotherapy, with special emphasis on the T cells-activated and macrophages-targeted delivery system. Through perpetuating each step of cancer immunity cycle, lipid-based nanoparticles can reduce immunosuppression and promote drug delivery to trigger robust antitumor response.

Keywords: cancer immunity cycle; cancer immunotherapy; lipid-based nanoparticles; macrophages; T cells

Introduction

Cancer has been a great threat to human health and life worldwide, with a predicted rise in the annual number of new cases from 18.1 million in 2018 to 29.4 million in 2040 resulting from ageing problem and population increase [1]. In the past decade, cancer treatment based on immunotherapy including immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR)-T cell therapy as well as antitumor nanomedicine has gained promising prospect both philosophically and practically [2, 3].

In solid tumors, majority of the successful cases have been achieved with ICIs, but the situation is developing at top speed, and different mechanisms of action (MoA) have already been uncovered or will probably arrive soon to the clinical practice [4]. Since an antibody targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) called ipilimumab was approved by the US Food and Drug Administration (FDA) for the treatment of metastatic melanoma in 2011 [5], several other ICIs emerged in the following decade (Table 1), mostly targeting programmed death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) (Table 1). In recent years, novel immune checkpoints such as lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and ITIM domain (TIGIT), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), V-domain immunoglobulin suppressor of T cell activation (VISTA), B7 homolog 3 protein (B7-H3), inducible T cell costimulatory, and B and T lymphocyte attenuator (BTLA) are available and promising options for combating solid tumors [6]. Clinical trials involving above immune checkpoints are currently going on in full swing, which have been summarized in another review [7].

Tumor immunotherapy with CAR-T cells has achieved tremendous successes in treatment of hematological malignancies [8]. Until October 2021, five CAR-T cell products have

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Table 1: ICIs approved by FDA.

Inhibitor	MoA	Year	Company	Commercial name	Approved indication
Ipilimumab	CTLA-4	2011	BMS	Yervoy	Metastatic melanoma
Pembrolizumab	PD-1	2014	Merck	Keytruda	Metastatic melanoma
					NSCLC or metastatic NSCLC
					Metastatic SCLC
					Metastatic HNSCC
					Hodgkin's lymphoma
					Primary mediastinal large B cell lymphoma
					Metastatic urothelial carcinoma
					Recurrent or metastatic cervical cancer
					Hepatocellular carcinoma
					Locally advanced or metastatic Merkel cell carcinoma
					Metastatic RCC
Nivolumab	PD-1	2014	BMS	Opdivo	Metastatic melanoma
					Late-stage NSCLC
					Late-stage SCLC
					Metastatic RCC
					Hodgkin's lymphoma
					HNSCC
					Urothelial carcinoma
					Metastatic colorectal cancer
					Hepatocellular carcinoma
Atezolizumab	PD-L1	2016	Roche/Genentech	Tecentriq	Locally advanced or metastatic urothelial carcinoma
					Metastatic nonsquamous NSCLC
					Metastatic triple-negative breast cancer
					Extensive-stage SCLC
Durvalumab	PD-L1	2017	AstraZeneca	Imfinzi	Locally advanced or metastatic urothelial carcinoma
					NSCLC
					Extensive-stage SCLC
Avelumab	PD-L1	2017	Merck/Pfizer	Bavencio	Metastatic Merkel cell carcinoma
					Locally advanced or metastatic urothelial carcinoma
					Locally advanced or metastatic RCC
Relatlimab	LAG-3	2022	BMS	N/A	In combination with nivolumab for metastatic melanoma

ICIs, immune checkpoint inhibitors; MoA, mechanisms of action; BMS, Bristol Myers Squibb; PD-1, programmed death protein 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T lymphocyte-associated protein 4; LAG-3, lymphocyte activation gene-3; NSCLC, non small cell lung cancer; SCLC, small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; RCC, renal cell carcinoma.

been approved by FDA for patients with relapsed and/or refractory B (R/R B) cell malignancies [9–13]. In children and young adults (<21 years old) with R/R B cell acute lymphocytic leukemia (ALL), tisagenlecleucel (the first CAR-T drug used in the world) produced an overall remission rate of 81 % in the patients in 3 months, with all 75 patients who responded to treatment becoming negative for minimal residual disease [14]. Additionally, the administration of tisagenlecleucel to adult patients with R/R diffuse large B cell lymphoma resulted in an overall response rate of 52 %, with 40 % of the patients achieving a complete response; the overall response rate of anti-CD19 (cluster of differentiation 19) CAR-T cells in clinical trials was greater than 80 % for patients with B cell ALL and non-Hodgkin's lymphoma [15].

Up till now, most cancer immunotherapeutic agents on the marker play their antineoplastic role through systematic routes, which may cause various adverse effects due to off-target toxicity, some of them even being lethal [16]. Recombinant cytokine interferon (IFN)-α was the first cancer immunotherapies approved by FDA for the treatment of hairy cell leukaemia in 1986. But the IFN-α has quickly lost its demand due to the rapid clearance and short half-life [17]. Another marketed cytokine for lymphocyte promotion was recombinant interleukin(IL)-2 in 1992. Although, it has leaded to complete response in some patient with melanoma and kidney cancer, the high dose for administration caused by its very short circulation time (about 12 min) resulted in undesired dose-related side effect. Once it encountered circulating effector lymphocytes, the lymphopenia occurred in a short time, and the vascular leak syndrome (VLS) and cytokine release syndrome was induced when it strongly bound to endothelial cells [18].

Thus, optimized pharmacokinetic profile or combination strategy is needed to reduce the required dose while alleviating the adverse effects. On the other hand, entailing these immunostimulatory recombinant cytokines the ability to target specific cell subsets or tumor sites through pharmaceutical method is also important to avoid off-target toxicity while increasing the therapeutic index. With the ground-breaking exploitation of checkpoint blockade, cancer immunotherapy has achieved great success in clinical practice and received more and more attention to date. Although the significant prolonged overall survival has been confirmed compared with chemotherapies, and a number of preclinical studies and clinical trials are ongoing to expand its efficacy in diverse tumor types, there are several inescapable limitations for the use of checkpoint blockade [17]. First, the systemic administration of these antibodies elicited broad immune related adverse events (irAEs) of skin, endocrine system and gastrointestinal tract even at the moderate dose. Due to the broader expression of CTLA-4 on T cells, and the depletion effect on regulatory T cells (Tregs), CTLA-4 inhibitor induced more severe autoimmune pathology compared to anti-PD-1/PD-L1 antibody [18]. Second, the low response rate in solid tumor and heterogeneous efficacy in different patients with the same tumor type hampered its application. These may be caused by the low infiltration of T cells or the deregulation of immune checkpoints [19]. Vaccine is another cancer immunotherapy strategy with great concern. There are a range of types of cancer vaccine, and the first marketed therapeutic cancer vaccine is a dendritic cells (DCs)-based vaccine (sipuleucel-T) in 2010 for prostate cancer. However, the limited efficacy leaded to the failure in clinical trials even though the high safety profiles. It is considered that the response could be enhanced by more efficient delivery to lymph nodes [20]. Meanwhile, it is worth noting that the ex vivo production process of CAR-T cell is not only technically sophisticated, but also time and money-consuming which limits its development. And the side effects, including neurotoxicity and cytokine release syndrome, and the poor efficacy in solid tumors are two other challenges of CAR-T cells therapy [21]. In summary, novel delivery technologies are in urgent need to overcome the shortcomings of these promising cancer immunotherapies mentioned above.

Encouragingly, with the rapid evolvement in nanotechnology, nanomedicine-based immunotherapeutic formulations can augment the antitumor efficacy while alleviating the off-target toxicities owing to their unique physicochemical properties and functionalized decorations (Table 3). Generally, Nanomedicine refers to synthetic formulations with the particle size on the nanometer scale, which originates from lipids, polymers, metals or inorganic materials [22]. Compared with traditional immunostimulatory molecules, nanoparticles can activate and perpetuate the immunity cycle more rigorously through releasing cargo drug in proper location and time under precise control [23]. Besides, nanoparticles can be easily loaded or coated with diverse therapeutics or functionalized ligands due to the high surface-area-to-volume ratio, which enabling them the capability of co-delivery and disease targeting.

In details, the advantages brought by nanoparticles can be concluded into following aspects. First, efficient accumulation in targeting sites rather than other organs is the key process to elicit therapeutic effect and reduce adverse effect. It is widely considered that the enhanced permeability and retention (EPR) effect of nanoparticles caused by the defective tumor vessels is a promoting factor for tumor targeting [22]. When the size of particles is among 10–100 nm, they are too large to penetrate normal blood vessels to enter healthy tissue or be cleared by the kidney, but can easily enter into tumor from dysfunctional vasculature [24]. However, studies have revealed that the EPR effect has high heterogeneity in individual patients, and there were only 0.7 % (median) nanoparticles actually reached tumors after systemic administration [25]. Therefore, other targeting strategies such as ligand modification or local injection is of vital importance. For lymph nodes targeting, which are the key sites for immune activation, nanoparticles also have priority than small molecular drugs. On the one hand, the fenestrated architectures are suitable for nanoparticles with proper size (5–50 nm) to penetrate when administered locally [26]. Second, nanoparticles with pathogen-like size are more easily taken up by antigen-presenting cells (APCs) than their soluble forms, which is crucial for cancer vaccines to activate T cells response. Furthermore, the uptake efficiency can be advanced by changing size, charge and hydrophobicity [23]. For the delivery of cancer vaccine, co-administration of antigen and adjuvant to the same DCs through nanotechnology is essential to trigger robust antigen response while preventing immunologic tolerance [27]. Through encapsulation, absorption or conjugation, diverse cargos can be loaded into the same preparation, and thus induce dramatic adaptive immune response. Third, sustained drug release and target engagement obtained by alerting pharmacokinetics (PK) profiles to prolong half-life was another advantage of advanced nanoparticles. For example, some well-designed nanoparticles can become antigen reservoirs to elicit prime and boost strategy after one injection [23]. And the bio-inspired nanoparticles are usually desired to extend the short half-life of the free counterparts due to their natural properties [28]. It is widely accepted that local therapy, including subcutaneous or intratumoral injection, can

achieve the efficient accumulation and retention of therapeutic drug on the favorable sites. However, systemic exposure of small molecular drug can occur even after intratumoral administration because of the high interstitial fluid pressure and leaky vasculature of tumor microenvironment (TME). Fortunately, this can be prevented by nanoparticles which can be trapped in the collagen-based extracellular matrix (ECM) due to its proper size [18]. Forth, safety considerations are indispensable in all kinds of immunotherapy which can be mitigated by using nanotechnology. Advanced nanoparticles can elicit sustained drug release and target engagement in the favor location, thereby minimizing undesirable systemic exposure and off-target toxicity.

Depending on these unique advantages, lipid-based nanoparticles, especially liposomes and lipid nanoparticles (LNPs), have successfully achieved clinical translation with high efficiency and low toxicity. In the past few decades, a list of lipid-based nanoparticles has been approved for cancer therapy by FDA [29]. As the first nanomedicine to achieve clinical translation, liposome has become a mature technology platform delivering a variety of drugs with desirable functions of controlled release, disease targeting and co-delivery for diverse disease type [30]. Another success is LNPs for the delivery of nucleic acid, which can enhance the cellular uptake and endosomal escape with the help of special lipids (especially ionizable cationic lipids). From hepatic targeting genetic disorder to virus infections and cancer immunotherapy, LNPs have showed promising potential for RNA-based therapeutics [31]. Table 2 summarized the representative lipid materials applied in lipid-based nanoparticles on market.

In this review, we discuss the different types of lipid-based nanoparticles and their applications in cancer immunotherapy.

Classification of lipid-based nanoparticles

Biomimetic lipid-based nanoparticles

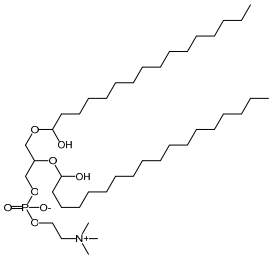
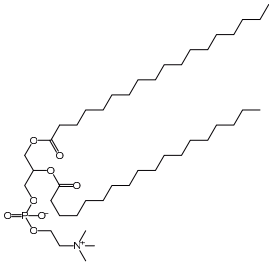
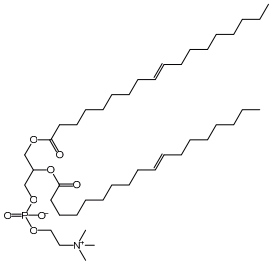
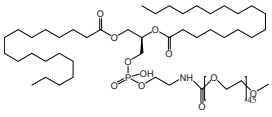
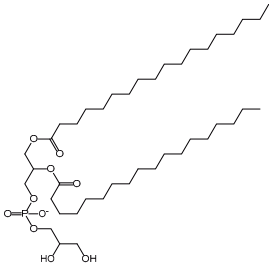
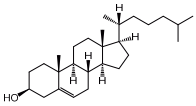
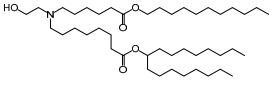
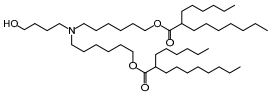
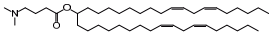
Bio-inspired drug delivery systems have received lots of attention recently, as they have optimal biocompatibility, disease targeting and immune stimulating capacity compared with synthetic carriers. Derived from bacterial membranes, cell membranes and extracellular vesicles, biomimetic lipid-based nanoparticles can be endowed with desired functions for the delivery of antigen proteins, RNA or immune modulators after bioengineered modification or cargo loading. In this section, we focus on the properties and multiple applications of lipid-based natural drug delivery vectors (Figure 1A–C).

Bacterial membrane-based nanoparticles

Bio-inspired drug delivery carriers from bacterial membrane include intact bacterial membrane (bacterial ghosts, BGs) and bacterial membrane-derived vesicles (BMVs). BGs are non-living empty cell envelopes produced by protein E-mediated cell lysis in Gram-negative bacteria, including *Escherichia coli K12* and *enterotoxigenic E. coli*, which loss all the cytoplasmic contents but save the unbroken inner membrane (IM) and outer membrane (OM) [32]. This non-living bacterial membrane still contains the intact surface morphology, structure and protein antigen composition of the living parent. The reserved pathogen-associated molecule patterns (PAMPs) such as lipopolysaccharides (LPS) confer it an adjuvant effect to activate and mature APCs. The upregulated release of IL-12 and tumor necrosis factor (TNF)-α of APCs by BGs enhance not only systemic immunity but also cellular and mucosal immunity [33]. Because of the enough inner space in BGs, a variety of antigenic peptides, nucleic acid and small molecule drugs can be loaded in OM, IM, periplasmic space or internal lumen of the cytoplasmic space, which means BGs can be an efficient delivery vehicle [32]. More interesting, the intact surface components provide BGs an intrinsic targeting ability to DCs, macrophages and tumor cells [34]. In addition, simple preparation, high stability, and low cost all make BGs an excellent delivery strategy.

Except for intact membrane, bacterial membrane-derived nanoscale particles, which are called BMVs, are also multifunctional platforms for drug delivery and cancer immunotherapy. Originating from membrane blebbing or cell lysis, BMVs are 20–400 nm nanoparticles which carry lipid, LPS, peptidoglycans, cytoplasmic proteins and nucleic acids [35]. In nature, BMVs can transport and distribute nutrients, toxin, antibacterial molecules and gene, thus play an important role in cellular metabolites, phage infection, horizontal gene transfer, and cell communication [36]. According to the parent bacteria and diverse formation routs, BMVs include a variety of types which differ in their structure and composition. Composed of IM, OM and peptidoglycan, Gram-negative bacteria can produce outer-membrane vesicles (OMVs) by cell membrane disturbance and blebbing, or self-assemble into outer-inner membrane vesicles and explosive outer-membrane vesicles by endotoxin-induced explosive cell lysis. The biggest difference is that the OMVs only have component from outer membrane but no cytoplasmic constituent. Gram-positive

Table 2: Summary of representative lipid materials applied in lipid-based nanoparticles on market.

Lipid	Structure	Payload	Product	Application	Advantage
HSPC		Doxorubicin	Doxil®/Caelyx™	Karposi's Sarcoma; Ovarian cancer; multiple myeloma	Improved accumulation in tumor; lower systemic toxicity
DSPC		Daunorubicin	DaunoXome®	Karposi's Sarcoma	Increased delivery to tumor; decreased side effects
DOPC		Cytarabine	DepoCyt©	Lymphomatous meningitis	Improved accumulation in tumor; lower systemic toxicity
DSPE-PEG 2000		Irinotecan	Onivyde®	Pancreatic Cancer	Increased delivery to tumor; decreased side effects
DSPG		Amphotericin B	AmBisome®	Fungal/protozoal infections	Reduced nephro-toxicity
Chol		Morphine sulphate	DepoDur®	Analgesia (post-operative)	Prolonged drug release
SM-102		Nucleoside modified spike mRNA	mRNA-1273	COVID-19 vaccine	Protecting RNAs and facilitating their cytosolic transport
AcuitasALC-0315		Nucleoside modified spike mRNA	BNT162b2	COVID-19 vaccine	Protecting RNAs and facilitating their cytosolic transport
DLin-MC3-DMA (MC3)		Transthyretin (TTR) siRNA	Onpattro®	TTR knockdown	Enhanced siRNA delivery; robust hepatic gene silencing

HSPC, hydrogenated soy phosphatidylcholine; DSPC, distearoyl phosphatidylcholine; DOPC, dioleoyl phosphatidylcholine; DSPE-PEG, polyethylene glycolylated distearoyl glycerol phosphoethanolamine; DSPG, distearoyl phosphatidylglycerole; Chol, cholesterol; siRNA, small interfering RNA.

Table 3: Summary of representative research on lipid-based nanoparticles for cancer immunity.

Class	Design	Mechanism	Cancer type	Properties and advantages	Ref.
Bacterial membrane-based nanoparticles	Using Gram-negative bacterial OMVs derived from genetically modified *Escherichia coli*	Inducing the production of anti-tumor cytokines CXCL10 and IFN-γ	CT26 B16BL6 4T1 MC38	Inducing long-term antitumor immune responses, specifically targeting the tumor tissue	[92]
	A versatile OMV-based vaccine platform through displaying tumor antigens on the OMVs surface by fusing with ClyA protein or employing a Plug-and-Display system	Innate immune response and antigen-specific T-cell-mediated anti-tumor immunity	Lung melanoma metastasis colorectal cancer	Simultaneously displaying multiple, distinct tumor antigens to elicit a synergistic antitumor immune response, personalized tumor vaccines	[39]
Cell membrane-based nanoparticles	Combining a BSA core covalently loading IFN inducer (ORY-1001) and T cell membrane functionalized by PD-1	Increasing IFNs and blocks IFN-induced immune checkpoint upregulation	4T1 B16F10 CT26	Targeting tumors that express PD-L1, triggering the internalization of PD-L1	[93]
	An artificial cytomembrane NVs obtained from DCs that presenting MHC-I -antigen complex, B7 co-stimulatory molecules and anti-PD-1 antibody	Improving antigen delivery to lymphoid organs and generating broad-spectrum T-cell responses that eliminate established tumors	Hep1-6-OVA B16F10 MC-38 LLC	The ability of antigen self-presentation and immunosuppression reversal, directly activating both native T cells and exhausted T cells, personalized cancer immunotherapy	[94]
Extracellular vesicles	Nuclei isolated from tumor cells were introduced into activated M1-like macrophages to form macrophage-tumor chimeric exosomes	Entering lymph nodes and priming T cell activation in both the classical manner and a unique “direct exosome interaction” manner	Lymphoma breast cancer melanoma cancers	High immunogenicity, high accumulation to lymph and tumor sites, ameliorating immunosuppression, personalized immunotherapy	[95]
	Anti-CTLA-4 antibody incorporated exosomes from BMDCs via a post-insertion technique	Enhanced binding to T cells in lymph nodes, effectively inducing T-cell activation, and improving the tumor homing of effector T cells	B16	Inducing a strong tumor-specific T-cell response, and increasing the ratio of effector T cells/Tregs within tumors	[96]
Liposomes	IL-2-Fc with extended PK and the F(Ab')2 fragments of anti-CD137 were coupled to PEGylated liposomes	IL-2 stimulates the proliferation and effector function of CTLs, while CD137 (4-1BB) is a T-cell co-stimulatory receptor	B16F10 A20 B cell lymphoma	Rapid accumulation in tumor sites, prolonged half-life, mitigating systemic toxicity by alerting biodistribution	[97]
	Loaded in liposomes, IOX1 was co-delivered with DOX liposome	Downregulating β-catenin and PD-L1, inhibiting P-gp to enhance DOX-induced ICD	CT26	Promoting T cell infiltration and activity, reducing tumour immunosuppressive, long-term immunological memory function	[98]
Lipid nanoparticles	Co-delivery of FAK siRNA, Cas9 mRNA and sgRNA via a multiplexed dendrimer lipid nanoparticle (siFAK + CRISPR-LNPs)	Reducing ECM stiffness and disrupting PD-L1 expression by CRISPR/Cas gene editing	ID8 A549 MYC-driven liver tumors	Increasing cellular uptake and tumor penetration, inhibiting tumor growth and metastasis	[99]
	Combining the co-stimulatory receptors mRNA (CD137 or OX40) formulated in LNPs with the corresponding agonist antibody	The delivery of costimulatory receptor mRNA to tumor-infiltrating T cells can enhance the antitumor effects of antibodies	A20 B16F10	Boosting the anti-tumor immune response to anti-PD-1 + anti-CTLA-4 antibodies	[100]
Lipid-based hybrid nanoparticles	A peptide vaccine delivery system formulated by LCP nanoparticles	IFN-γ production by Trp2-specific immune response	B16F10	Persistent antigen loading and stimulation, superior inhibition of tumor growth	[101]
	Peptide antigens were loaded on the BPs core, while stabilized by surface lipids including TLR4 agonist.	Triggering the proliferation and activation of DCs and innate-like γδT cells	B16F10	Recruit both innate and adaptive immunity	[76]

Table 3: (continued)

Class	Design	Mechanism	Cancer type	Properties and advantages	Ref.
	Plasmid coding PD-L1 trap was encapsulated in LPD composed with protamine core and cationic lipid layer	Potently binding to PD-L1 and block its signal pathway transiently and locally	CT26 MC38 B16F10 4T1	Preventing the upregulation of Th17 cells in spleen, improving the safety and tolerance of ICB therapy	[102]

ClyA, cytolysin A; I.V, intravenous injection; S.C, subcutaneous injection; I.T, intratumoral injection; OMV, outer-membrane vesicles; MHC, major histocompatibility complex; BSA, Bovine Serum Albumin; NVs, nanovesicles; PK, pharmacokinetics; IL-2, Interleukin-2; CTLs, cytotoxic T lymphocytes; IOX1, 5-carboxy-8-hydroxyquinoline; DOX, doxorubicin; P-gp, P-glycoproteins; ICD, Immunogenic cell death; FAK, focal adhesion kinase; sgRNA, single guide RNA; LNPs, lipid nanoparticles; CRISPR, clustered regularly interspaced short palindromic repeats; LCP, lipid-calcium-phosphate; IFN, interferon; BPs, bisphosphonates; TIL-4, Toll like receptor-4; LPD, lipid-protamine-DNA nanoparticle; ICB, immune checkpoints blockade; Trp2, phospho-tyrosinase-related protein 2; BMDCs, bone marrow-derived dendritic cells; Tregs, regulatory T cells; ECM, extracellular matrix.

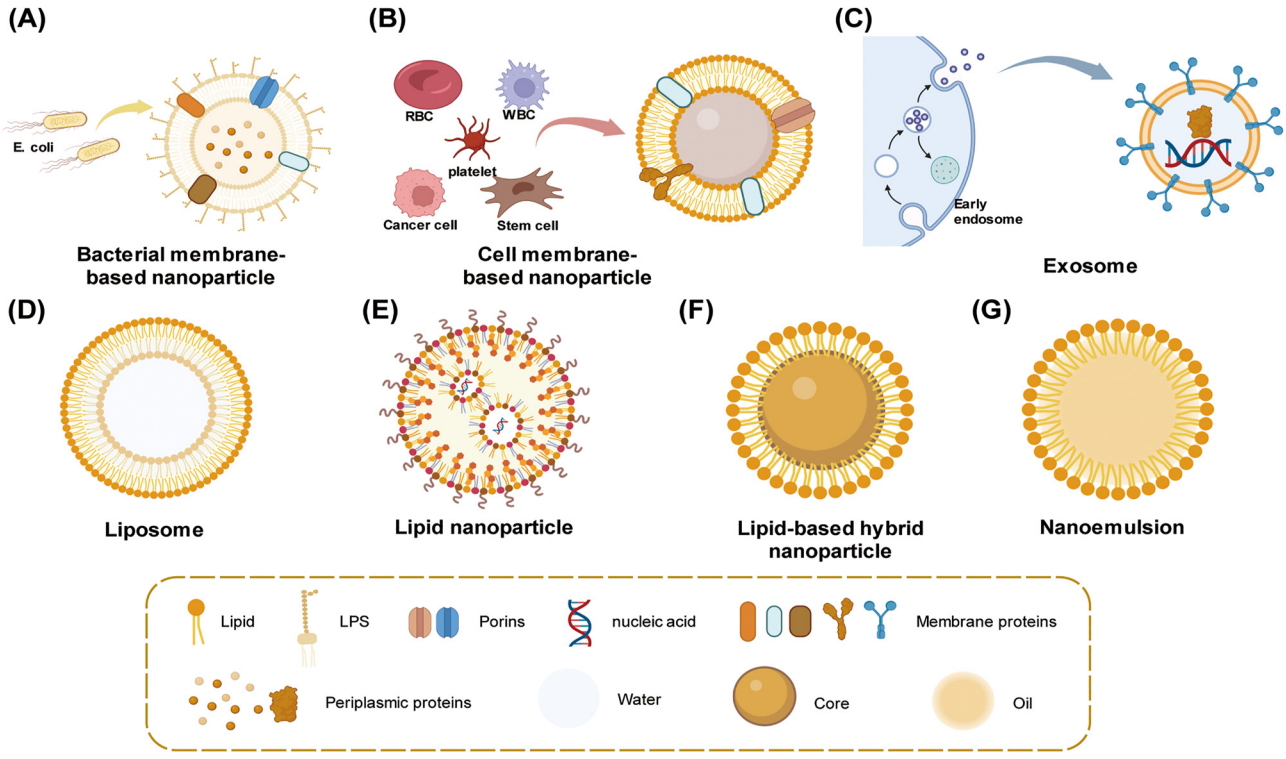


Figure 1: Illustration of different lipid-based nanoparticles for cancer immunotherapy. (A–C) are illustrations of representative biomimetic lipid-based nanoparticles, (D–G) are illustrations of representative non-biomimetic lipid-based nanoparticles. (A) BMVs which are originated from blebbing or cell lysis of *Escherichia coli*. (B) Cell membrane-based nanoparticle which are produced from different kinds of cells through co-extrusion, sonication, electroporation and infusion etc. (C) Exosomes which are derived from the inward budding of endosomal membrane. (D) Liposomes which are composed of lipid bilayer and inner water phase. (E) LNPs which are differs most from classical liposomes in that they do not display a lipid bilayer surrounding a hydrophilic core and efficient for mRNA delivery. (F) Lipid-based hybrid nanoparticles with core-shell-structure which are formulated by encapsulating a solid core with a lipid bilayer. (G) O/W nanoemulsions which are obtained by mixing lipids/oils and surfactants. WBC, white blood cell; RBC, red blood cell; LPS, lipopolysaccharides; BMVs, bacterial membrane-derived vesicles; LNPs, lipid nanoparticles.

bacteria, which only have cytoplasmic membrane and peptidoglycan, usually give rise to cytoplasmic membrane vesicles through “bubbling cell death” triggered by endotoxin [37].

Because of the lipid bilayer structure, BMVs can delivery hydrophobic drug through the lipid bilayer or hydrophilic drug by loading into the interior space or modified onto the exterior surface [38]. Passive encapsulation by physical or chemical methods and functionalization during biogenesis by genetically fusing proteins to cytolysin A (ClyA) or using “plug and play” system of SpyTag and SpyCather are all alternative strategies for loading cargo [39]. Besides the excellent delivery capacity, BMVs can serve as efficient vaccination strategies because of the diverse membrane

antigens and self-adjuvant properties. On the one hand, BMVs can induce protective humoral immune response by generating antigen-specific IgG/IgA antibody [40]. On the other hand, the rich and high variety of PAMPs (LPS, flagellin etc.) in BMVs can be recognized by pattern recognition receptors (PRRs) of innate immune cells such as Toll-like receptor (TLR), nucleotide-binding oligomerization domain-like receptor (NLR) and stimulator of interferon genes (STING), and thus activate nuclear factor kappa-B (NF-κB), TIR domain-containing adaptor inducing interferon-β (TRIF) and interferon regulatory factor 3 (IRF3) signaling pathway to prime adaptive immune system [35]. By encapsulating various antigens from bacterial, viral and cancer, BMVs can be applied as power vaccines preventing infections and tumor growth. For example, two OMV-based vaccines, MeNZB and Bexsero, which have been shown to be safe immunotherapy agents, have been approved by European Commission for the prevention of meningococcal group B infections [41, 42].

Cell membrane-based nanoparticles

Based on the different origin, cell membrane-based nanoparticles can be produced from red blood cells (RBCs), immune cells (macrophages or DCs or lymphocytes), cancer cells, stem cells, platelets or hybrid cells membranes. Cloaked by cell membranes, this kind of biomimetic nanoparticles usually have better pharmacokinetic behavior and targeting ability. Various methods can be used to prepare cell membrane-derived nanoparticles [43], including co-extrusion, sonication, electroporation and infusion etc.

Red blood membrane-coated nanoparticles (RBC-NPs) were a typical strategy for long-circulating drug delivery because of the “self-markers” (e.g., CD47 and acidic sialyl moieties) which prevent them from phagocytosis by mononuclear macrophage system (MPS) and enhance the biocompatible and non-immunogenic nature [44]. Besides, RBC-NPs showed better stability ether in phosphate buffer or serum than bare nanoparticles. However, red blood membranes lack targeting ligands, which means they need to be functionalized to get active targeting capacity in cancer therapy [28]. In order to preserve the intact structure and function of membranes, non-disruptive functionalization such as lipid insertion or avidin-biotin interaction-based insertion were established. In addition, by leveraging EPR effect and homotypic adhesive properties, RBC-NPs can target tumor via nanoscale and hemophilic adhesive domains without any modification [45]. Unlike RBCs, leukocyte cells possess active targeting capability of the inflammatory sites and cancer cells in addition to prolonged circulation time, because of the functional protein. For macrophage membrane-coated nanoparticles, they can not only actively target cancer cells through ligand-receptor interactions (e.g., α4 integrins and vascular cell adhesive molecule-1), but also home to inflamed endothelium in tumor sites through surface molecules (e.g., CD45, CD11a, glucans and CXCR2(chemokine (C-X-C motif) receptor 2)) [28]. For neutrophils and monocytes, they all show intrinsic targeting property of circulating tumor cells (CTCs) and inflammatory sites by adhesive molecular, so they are deemed as effective source of drug delivery vectors for preventing cancer metastasis [43]. Natural killer (NK) cells membrane-decorated nanoparticles can also selectively accumulate in tumor sites by overexpressed natural killer cell group 2D (NKG2D) in cancer cells.

Nanoparticles coated by cancer cell membrane have been proved to be an exciting strategy for cancer vaccines. On the one hand, with the help of exogenous adjuvant, tumor antigens on cancer cell membranes can be recognized and uptake by APCs and specifically activate adaptive immune [46]. On the other hand, cancer cell membrane-derived component can easily target to parent cancer cells owing to the homologous adhesive molecular on the surface (e.g., E-cadherin and galectin 3) [47]. Mesenchymal stromal cells (MSCs) have also become a versatile delivery system for cancer therapy because of the improved localization to tumor sites and paracrine-secretion capacities. By physical enucleation and bioengineered modification of chemokine (C-C motif) receptors (CCR-2, CCR-4) and adhesive molecules (P-selectin glycoprotein ligand-1), Wang et al. [48] developed “cargocytes” which retain cellular function and disease homing capacity. Systemically administered “cargocytes” delivering IL-10 can home to inflamed tissue and ameliorate acute pancreatitis effectively. Platelets, another nucleus-free cell which are important for wound healing and hemostasis after injury, have been conveniently conjugated with anti-PD-L1 monoclonal antibodies (aPD-L1) for post-surgical cancer immunotherapy. Taking advantage of the long lifespan and surgical wounds targeting of platelets, aPD-L1 showed enhanced antitumor efficacy while reducing off-target effects. The interactions between platelets and CTCs also entail the ability to prevent metastatic spread. In addition, platelets-derived chemokines released after activation such as soluble CD40 ligand (CD40L) can recruit and boost T cell immune response, and thus improve the objective response rate [49].

In order to integrate specific functions of diverse cells into one delivery vehicle, hybrid cell membrane-based vectors have also been constructed. For example, Chen et al. [50] combined *E. coli* cytomembrane and tumor cell membrane to co-delivery adjuvant and antigen. Based on the advantages in biocompatibility, targeting and immune activation

mentioned above, cell membrane-based nanoparticles turn out to be a great choice for cancer immunotherapy. However, the safety and heterogeneity of biomimetic vectors are challenges which cannot be ignored as for clinical translation.

Extracellular vesicles

Released from all kinds of cells, extracellular vesicles (EVs) are cells-derived membranous structures including exosomes and microvesicles [51]. BMVs mentioned above are one type of EVs, but in this section we only concentrate on EVs from eukaryotic cells. Categorized by origin, exosomes are 50–150 nm vesicles produced by the inward budding of endosomal membrane, while the microvesicles generated by outward budding of plasma have larger size ranging from 50–1000 nm (up to 10 μm) [52]. EVs are composed of various constituents of the original cells such as nucleic acid, metabolites, cytosolic, lipid and cell membrane protein, which play. a special role in waste removal, homeostasis maintenance and intercellular communication [53]. Because of the widely association between EVs and intracellular pathways, EVs are deemed as potential therapy of various diseases especially cancer progression.

Although tumor-derived EVs contribute to pro-tumor microenvironment by delivery Wnt 10b and epidermal growth factor receptor, they are also good source of tumor antigens [54]. Several clinical trials have demonstrated that tumor-derived EVs can stimulate APCs mature and T cell response, even though the antitumor efficacy has been limited [54]. DC-derived EVs, which are also called "Dexosomes" have been applied as cancer vaccine in a large number of preclinical and clinical research, due to the immunostimulatory factors and antigen present on the outer surface [55]. They can increase the cytolytic activity of NK cells in patients with NSCLC, although the responses were modest [56]. In addition, MSCs-derived EVs have been used in the therapy of breast cancer because of their immunomodulatory property. In order to increase the immune response, naïve EVs have been loaded with various therapeutic payloads such as oligonucleotides, microRNA (miRNA) and immune modulators [53]. Compared with cellular therapies and liposomes, EVs-based delivery systems have better biocompatibility, tolerance and pharmacokinetic profile due to the lipid/protein composition and nanoscale. Physical incorporation of functional RNA by electroporation or chemical agents is an effective approach to load cargo into EVs. Besides, overexpression functional proteins using bioengineering methods can not only load cargo, but also enrich targeting ligand for better distribution *in vivo* [57]. Based on above manipulations, MSC-derived EVs (termed iExosomes) delivering $KRAS^{G12D}$ small interfering RNA (siRNA) have been produced to suppress pancreatic cancer in phase 1 clinical trial [58]. Another engineered EVs from DCs containing antisense oligonucleotides (exoSTING) for stronger immune response have also been explored and are currently in phase II clinical trials.

Non-biomimetic lipid-based nanoparticles

Non-biomimetic lipid-based nanoparticles applied in cancer immunotherapy mainly include liposomes, LNPs, lipid-based hybrid nanoparticles and nanoemulsions (Figure 1D–G).

Liposomes

Liposomes, consisting primarily of phospholipids and cholesterol, are nanosized vesicles with demonstrated advantages in biocompatibility and enhanced targeted payload delivery with minimal toxicity. Amphiphilic phospholipids self-construct into a spherical lipid bilayer structure with their lipophilic tails, creating an environment for hydrophobic drugs to be encapsulated. On the other hand, hydrophilic heads of phospholipids assemble into an exterior surface and an aqueous core that can enclose hydrophilic agents [59]. Common components of liposomes are the neutral phospholipids such as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), hydrogenated soy phosphatidylcholine (HSPC), and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), which give the structures of lipid bilayer, and cholesterol, which enhances membrane stability. For the case of liposomes, negatively (1,2-dioleoyl-sn-glycero-3-phosphate (DOPA), 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS)) or positively (dimethyldioctadecy-lammonium (DDA), 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP)) charged lipids are used that can modulate liposome structure and surface properties.

Importantly, the encapsulation of therapeutics within different liposomal compartments allows safe and targeted drug delivery, since the liposomes can protect the enclosed cargos from degradation by the immune system while carrying them across biological membranes that the free drugs (nonencapsulated in liposomes) are often incompatible with. Liposomes, not only enabling the diverse molecules loading and delivery of both hydrophilic and lipophilic therapeutic agents while preserving their efficacy but also allowing surface modification for specific function [59, 60], are among the most successful nanotechnology-based drug products in cancer therapy [61]. Since PEGylated liposomal doxorubicin (DOX, Doxil®) became the first FDA approved nano-drug in 1995 [62], more than 10 liposomal drugs have been approved

by FDA for use in cancer therapy [63]. Building upon the success of liposomal drugs in chemotherapy, liposomes have been employed as one of the most attractive delivery vehicles in chemoimmunotherapy.

Lipid nanoparticles

Not all the lipid-based nanocarriers have a contiguous bilayer as liposomes. Exceptional examples are the LNPs which differs most from classical liposomes in that it does not display a lipid bilayer surrounding a hydrophilic core. The LNPs loading nucleic acid does not require electrostatic complexation to drive, nor does it require the balancing of constituent compound charges for efficient cell delivery [31]. Ionizable lipids are the key components of LNPs and the main determinant of its potency, such as DODAP, DLinDMA, SM102 and so on. Another important component is PEGylated lipids, plays an important role in prolonging circulation time and enhancing cellular uptake [64]. Other components of LNPs are similar with liposomes, include phospholipids and cholesterol.

Due to the use of neutral lipids and passive encapsulation process, the use of liposomes for nucleic acid delivery is faced with some problems such as poor encapsulation. The advent of ionizable cationic lipids made a breakthrough. These pH-titratable lipids are positively charged at low pH but mostly are uncharged at the pH of blood. This feature confers on the lipid/LNPs the advantages of positive charge (the ability to complex RNA and the ability to interact with the endosomal membrane as part of the fusion process to release RNA into the cytosol of the cell), without the drawbacks (rapid elimination and poor tolerability) [65]. the use of PEGylated lipids can enhance cellular uptake via endocytosis thus became a critical design that contributed to the success of the current generation of LNPs [64, 66]. It was reported that utilization of LNPs to deliver mRNA resulted in strong activation of innate immunity [67].

LNPs are widely applicated in the field of nucleic acid delivery, and plenty of products have entered the stage of clinical trials. LUNAR® is an LNP platform developed by Arcturus Therapeutics, Inc. (San Diego, CA, USA) for RNA delivery which was named as lipid-enabled and unlocked nucleomonomer agent modified RNA, and was employed to encapsulate the human factor IX mRNA for treatment of hemophilia B in a preclinical setting [68]. Except for LUNAR®, a variety of RNA-LNP have now entered clinical trials. TKM-080301, which is composed of a siRNA encapsulated in LNPs that can target polo-like kinase 1 (PLK1), was produced by Arbutus Biopharma Corporation for the treatment of solid tumors and was involved in a phase I/II clinical study with promising safety and anti-tumor efficacy data [69, 70]. Another example is DCR-MYC, which was produced by Dicerna Pharmaceuticals (Lexington, Massachusetts MA, USA) and incorporates synthetic double-stranded RNA to target the *MYC* oncogene and suppress cancer progression. In addition, several LNP products under phase I or II clinical study for cancer treatment by delivering nucleic acids also include mRNA-2752 [71], EphA2 siRNA [72], and ALN-VSP02 [73].

Lipid-based hybrid nanoparticles

Lipid-based hybrid nanoparticles with different structures (e.g., core–shell-structured hybrid nanoparticles with either a lipid core or shell) are also attractive for cancer immunotherapy. Some inorganic nanoparticles with a lipid shell have been developed for efficient therapeutic loading, including lipid-coated biodegradable hollow mesoporous silica nanoparticles (dHMLB) [74], nano-Folox (formed by a nanoprecipitate ($C_{26}H_{35}N_9O_7Pt$) core and an aminoethyl anisamide-targeted PEGylated lipid shell) [75], CaBPs (calcium bisphosphonates) vaccine (formed by CaBPs core coting with DOPA, lipid A and polyethylene glycolylated distearoyl glycerol phosphoethanolamine (DSPE-PEG)) [76]. On the other hand, lipids as the core of hybrid nanoparticles can also be used for encapsulation of chemoimmunotherapy drugs. In a study using twin-like core–shell nanoparticles to target cancer cells and tumor associated macrophages (TAMs) for enhancing tumor-localized chemoimmunotherapy, the cationic lipid-based nanoparticles served as the core for loading different therapeutics [77]. Other lipid composite nanoparticles without shell-core structures have also been investigated in tumor immunotherapy studies. For example, cationic lipid-assisted nanoparticles were developed for the treatment of orthotopic pancreatic cancer by co-delivery of indoleamine 2,3-dioxygenase 1 (IDO1) siRNA and oxaliplatin (OXA). In the presence of cationic lipids, siRNA was successfully delivered to the tumor draining lymph nodes and tumor tissues [78].

The lipid-based hybrid nanoparticles retain the benefits of lipid-based nanoparticles yet provide a more flexible structure via introducing other functional components, which could be advantageous in cancer immunotherapy. However, this kind of delivery system faces the limitation of complex structure and tedious preparation process. This will hinder the clinical translation of these hybrid nanoparticles.

Nanoemulsions

Nanoemulsions are oil-in-water (O/W), water-in-oil (W/O) dispersion of two immiscible liquids stabilized using an appropriate surfactant, whose droplet diameter is normally

below 500 nm [79, 80]. The composition of nanoemulsion mainly includes lipids/oils and surfactants. Lipids/oils to be used in nanoemulsions are usually included resterified fractions derived from different oil. Vitamin E family [81, 82], oleic acid and ethyl oleate [83, 84] has been extensively used as a carrier in nanoemulsions. And surfactants, such as poloxamer family [85], sodium dodecyl sulfate [86], amphiphilic proteins like casein, can stabilize nanoemulsions by reducing interfacial tension, and prevent droplet aggregation [87]. This class of nanoparticles have higher solubilization capacity than simple micellar dispersions and greater kinetic stability than coarse emulsions [80]. Nanoemulsions have been used to increase drugs solubility, provide protection for cargos from harsh environmental factors (oxidation, pH, hydrolysis) [88], and protect target organs by enhancing permeability and retention effects [89]. Several nanoemulsions have been reported to perform direct lymphatic absorption, thus improving bioavailability and largely reducing the dose of drugs for liver transformation [90].

Nanoemulsions have been exploited as novel vaccine adjuvants, such as adjuvant MF59, AS03, AF03. Their development and application demonstrated the acceptable safety, enhanced immunogenicity and antigen retention ability [91]. Similar with lipid-based hybrid nanoparticles, the clinical translation of nanoemulsions is also limited. It is mainly resulting from the limitation of drugs that can be used to prepare nanoemulsions, the dosage constraint of surfactant and the high production cost.

Lipid-based nanoparticles for DCs and T cells associated tumor immunotherapy

It is well known that anti-tumor immune response is composed of a series of progressive events which occur iteratively [103]. Termed “cancer-immunity cycle”, this stepwise process is closely related to the activation and antigen presentation of DCs and priming, infiltration and killing process of T cells, both of which play a pivotal role in the whole cycle. Briefly, the “cancer-immunity cycle” includes the following steps [104]: (1) tumor-associated antigens released by necrosis or apoptosis through a range of treatments (e.g., immunogenic cell death (ICD), surgery), (2) presentation of tumor-associated antigens on major histocompatibility complex (MHC) molecules of DCs, (3) priming and activation of T cells by DCs in the draining lymph nodes, (4) trafficking of effector T cells to the tumor sites in response of chemokines and adhesion molecules, (5) infiltration of T cells into the deep parts of tumors, (6) recognition of cancer cells by the T cell receptors/MHC complex of effector T cells, (7) killing of cancer cells by effector T cells through the release of granzymes and perforin. However, this cycle can be impeded to varying degree according to the different suppressive TME such as PD-L1 expression or Tregs proliferation, which may cause immune escape or even treatment fails. Having the special physicochemical properties in nanoscale, nanomedicines have been widely used to perpetuate the cancer-immunity cycle. The biggest advantage of nanomedicines is that they can achieve the immunomodulation in the controlled and suitable time and location when properly designed, which can overcome the low efficiency and off-target toxicity of traditional drugs [22]. In this section, we focus on the application of lipid-based nanomedicine targeting DCs and T cells in the order of cancer-immunity cycle.

DCs targeting

Increasing immunogenicity or antigen release

A great proportion of unsatisfactory outcomes in cancer immunotherapy are due to the weak immunogenicity of tumor antigens, thus hindering the activation of APCs, such as DCs, and infiltrating of cytotoxic T lymphocytes (CTLs), which limiting the therapeutic efficacy of checkpoint blockade immunotherapy. Hence, a variety of lipid-based, cancer therapeutic and/or preventative vaccines have been developed to enhance the immunogenicity of tumor cells, which includes vaccines containing neoantigens and/or adjuvants, vaccines encapsulating plasmid DNA/RNA, vaccines that induce ICD of tumor cells, as wells as those that synergize with phototherapy, thermotherapy or chemotherapy [105].

Cancer vaccine

Wang et al. [95] produced a type of chimeric exosomes (aMT-exos) by introducing nuclei isolated from tumor cells into activated M1-like macrophages. The nano-sized chimeric vesicles were highly immunogenic and were able to accumulate in both lymph nodes due to size effect and tumor sites through “homing behavior”. aMT-exos entered lymph nodes and primed DCs activation in both the classical antigen-presenting immunostimulatory manner and a unique “direct exosome interaction” manner; they could also ameliorate the immunosuppressive TME at the malignant sites, where aMT-exos exhibited efficient inhibition of primary tumors, tumor metastases, and postoperative tumor recurrence for personalized immunotherapy. Xu et al. [101] developed a kind of lipid-calcium-phosphate

(LCP) nanoparticles to fulfill the codelivery of melanoma tumor-associated antigen, phospho-tyrosinase-related protein 2 (Trp2), and a commonly used adjuvant, CpG ODN, the former decorated with two phosphor-serine residues to increase its encapsulation. Compared with free Trp2 peptide/CpG, vaccination with LCP encapsulating *p*-Trp2 and CpG resulted in superior inhibition of tumor growth in both B16F10 subcutaneous and lung metastasis models. Qin and Li et al. [76] prepared a CaBPs coordination nano-vaccine system to co-deliver BPs, tumor antigens and adjuvant lipid A. In addition to adaptive immunity activation caused by antigen and adjuvant delivery, DCs and γδT cells were also stimulated by BPs, an inhibitor of farnesyl pyrophosphate synthase in the mevalonate pathway that could induce the intracellular accumulation of isopentenyl pyrophosphate. With innate and adaptive dual-immunity activation, the nanocarrier demonstrated remarkable and enduring tumor therapeutic and prophylactic efficacy, as well as favorable biocompatibility *in vivo* (Figure 2). Cong et al. [106] reported a cationic lipid nanoparticle encapsulating cationic liposome/DNA complexes (CLN/DNA) formulated with cholesterol, DOTAP, and DSPE-PEG2000, significantly increased the tumor cell death with high immunogenicity. Intratumorally injected CLN/DNA effectively facilitated the activation of DCs in the tumor-draining lymph node. Furthermore, both local tumor growth and distant tumor formation were markedly inhibited by T cell-mediated antitumor immune response.

Inducing immunogenic cell death

ICD is a form of cell death induced by immune stimulation, which is defined by chronic exposure of damage-associated molecular patterns (DAMPs) in the TME [107, 108]. During ICD process, DAMPs released by dying tumor cells can stimulate APCs (for example, DCs) maturation followed by naïve T cells activation and transformation into CTLs through cross-presentation process [109–111]. CTLs can instantly and directly attack tumor cells by secreting cytotoxic molecules, such as perforin, granzyme, IFN and TNF, enhancing tumor cell lysis or apoptosis [110, 111]. Thus, the research of novel and effective approaches for induction of ICD has become a hotspot for preferable cancer immunotherapy. It is worth noting that there are multiple types of DAMPs, among which, calreticulin (CRT), high mobility group protein B1 (HMGB1), and ATP are considered to be the hallmarks for the potential immunogenicity of ICD inducers.

Liposomes are widely used in ICD-based chemoimmunotherapy [112]. Modified liposomes containing specific proteins, antigens, or other biological substances can be applied to selectively deliver drugs for a particular tissue [113]. In order to increase the selectivity of liposomes at tumor sites and enhance the efficiency of ICD induction, many researchers have adopted active targeting strategies to modify liposomes [112]. For example, Liu et al. [114] employed thin film dispersion methods to prepare 17-AGG (a potential ICD inducing agent, 17-(allylamino)-17-demethoxygeldanamycin) loading liposomes in a tumor targeted manner to facilitate the T cell mediated immunotherapy as well as to reverse the immunosuppressive TME. Despite the fact that low dose 17-AAG liposomes demonstrated a limited therapeutic effect alone on 4T1 tumor, promising efficacy was observed when 17-AAG liposomes combined with checkpoint blockade immunotherapy.

Liposomes are able to deliver multiple components to achieve combined treatment of tumors. Yu et al. [115] used liposomes to deliver photosensitizers (IR780), OXA and PD-L1 inhibitors (BMS-1), which can induce innate and adaptive immune responses through photothermal therapy (PTT) and ICD to inhibit tumor growth and prevent metastasis. In this study, the modified liposome system prolongs the circulation time and increases the accumulation in the tumor, thereby enhancing the light thermal effects and reduced systemic toxicity. Moreover, PTT and OXA have a promising synergistic effect in ICD induction.

Nano-platform modified with biomimetic membrane can also be used to facilitate ICD of tumor cells. Li et al. [116] developed an *in situ* multifunctional vaccine based on BMVs (1-MT@OMV-Mal) to efficiently capture immunogenic tumor antigens released from tumor cells after local PTT. Maleimide groups (Mal) were decorated on the surface of 1-MT@OMV-Mal vaccine to facilitate antigen capture process, while inhibitor of IDO, 1-methyl-tryptophan (1-MT), was encapsulated into the vaccine to overcome the immune inhibition of IDO on tumor-infiltrating effector T cells, which led to remarkable inhibition on both primary and distant tumors in subcutaneous CT26 tumor-bearing model.

Improving DCs targeting and antigen presentation

In the prime process, antigen uptake and presentation by DCs in lymph nodes is crucial for the mature and differentiation of effector T cells. So, enhancing lymph nodes targeting by changing the size, charge or ligand modification of lipid-based nanoparticles or promoting antigen presentation by co-delivery strategy is of great important in cancer immunotherapy [117].

Controlling the particle size

Localized injection is the mostly used administration method of vaccine, which contribute to prolonged retention and lower system exposure. There have been two local cancer immunotherapies approved by FDA, bacille

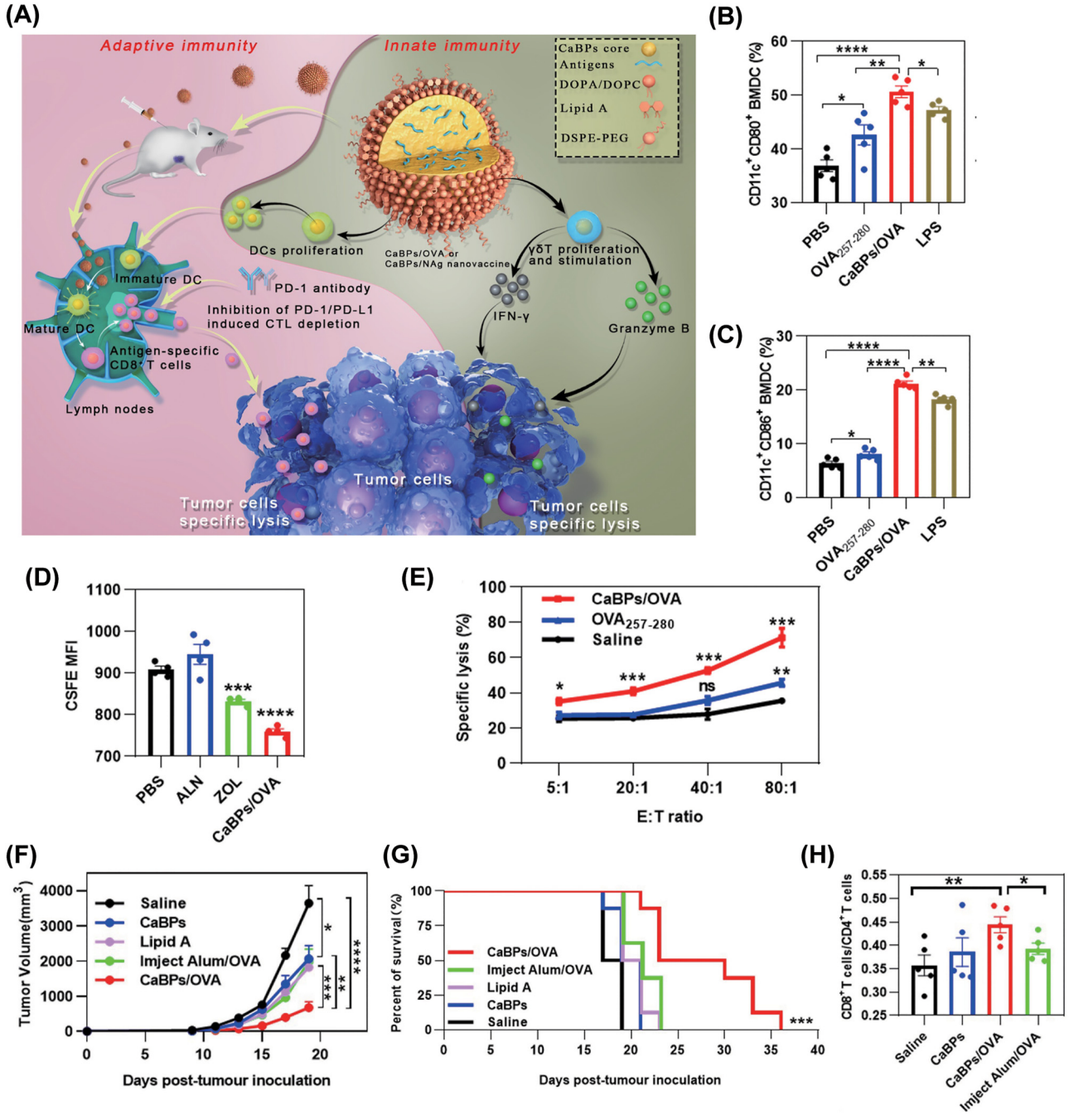


Figure 2: A calcium bisphosphonates (CaBPs) coordination nano-vaccine system co-delivering bisphosphonates (BPs), tumor antigens and adjuvant lipid A. (A) Scheme for antitumor immune mechanism of CaBPs nanovaccine. Expression of CD80 (B) and CD86 (C) on BMDCs after incubation with 400 μg mL^{-1} nanovaccine for 20 h. (D) ZOL and CaBPs/OVA induced the poliferation of BMDCs *in vitro*. (E) CTLs cytotoxicity to B16-OVA induced by CaBPs/OVA. (F, G) Tumor growth (F) and survival rates (G) of B16-OVA tumor-bearing C57BL/6 mice after the mice were inoculated subcutaneously on the right flank with 2 × 10*4 B16-OVA cells and vaccinated with the indicated formulations (equivalent to 2 μg OVA257-280 peptide, and 5 μg Lipid A per mouse) on days 3, 6 and 9. (H) The ratio of $CD3^+CD8^+$ and $CD3^+CD4^+$ T cells in splenocytes from mice vaccinated with the indicated formulations. CaBPs, calcium bisphosphonates; BPs, bisphosphonates. Reproduced with permission [76]. Copyright 2021, Elsevier.

Calmette–Guérin (BCG) bacteria for treatment of bladder cancer and engineered herpes simplex virus type 1 for the treatment of melanoma. For localized administration, particle size of nanomedicine partially decides its delivery fate. Subcutaneous injection of nanoparticles can target to lymph nodes preferentially with the size ranging from 5–50 nm which are too large to enter the blood vessels [26], while smaller particles/molecules (<5 nm/16–20 kDa) are more likely

to enter the blood capillaries. As for the larger particles (50–100 nm), they tend to be detained in the ECM and can only be delivered to lymph nodes by phagocytosis of DCs or macrophages [118]. Similarly, Xu et al. [119] has compared the different capacity to drain to lymph nodes of three yeast cell wall-derived formulations with small/middle/large size after intratumor injection. The results showed that the accumulation in lymph nodes was inversely related to the particle size, which can be explained by a percolation model. Another example to make use of the size effect of nanoparticles is an ultrasmall (10–20 nm) lymph-targeted nanovaccine. In an effort to exert the full effect of cell membrane disruption and immunomodulation of melittin, a scaffold composed of a high-density lipoprotein (HDL)-mimicking peptide and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was developed to load this medicine (α-peptide-nanoparticle). With the desired size of 10–20 nm, α-peptide-nanoparticle can efficiently drain to lymph nodes to activate DCs for the prime of adaptive immune, and promoted the recruitment of NK cells for inducing innate immune as well, thus it could elicit profound antitumor immunity both *in situ* and in distance. On the other hand, loaded on the phospholipid monolayer, the positive charge of melittin was partially shielded, leading to the reduced side effect of hemolysis [120].

However, it is noted that this size range for better lymphatic uptake is not constant especially for the flexible particles such as liposome and microemulsion. For example, 150–200 nm liposome displayed more substantial lymph uptake and antigen response than smaller ones (65 nm) [121]. Unlike localized administration, effective delivery of nanoparticles to lymphatic system by intravenous injection has lots of challenges. Tseng et al. [122] successfully established a lymphatic system-targeted LCP nanoparticle. In order to ensure the accurate and uniform size of the LCP, sucrose gradient centrifugation was used to removing the liposomes or micelles made by the excess lipid. Like the localized injection, LCP with small size (~25 nm) showed more obvious lymphotropism. Moreover, studies suggested that the enhanced tissue penetration and atypical lymphatic accumulation came from not only the small size but also the PEGylated modification and negative surface charge.

Changing the surface charge

Therefore, changing the surface charge to negative by using phosphatidylserine-based lipid is an effective way to increase the retention in lymph nodes [117]. A typical example is the RNA-lipoplexes (RNA-LPX) which can target DCs systematically by adjusting to negative charge through the change of lipid to RNA ratio without any functionalization. This negative charged RNA-LPX induced the release of IFN-α after the efficient uptake by DCs and macrophages, and thus triggered significant effector and memory T cell response when encoding viral or neo-antigens for tumor. In addition, this universally applicable vaccine has been tested in a phase I clinical trials for the treatment of melanoma and turned out strong immune response [123].

Ligand modification

Apart from taking advantage of the special size and charge effect of lipid-based nanoparticles, the surface modification also works well for lymph targeting. On the one hand, the PEGylation of cationic liposome can reduce the retention in the injection site, while increasing the accumulation of lymph nodes and augmenting the immune response [122, 124]. On the other hand, active modification with associated ligands tends to be a promising method for lymph node targeting as well. These efficient ligands include IgG, CD11c antibody, CD11b antibody, CD40 antibody and mannose [118, 125]. In summary, lymph targeting through ligand modification or physicochemical properties control is a promising strategy for the development of cancer immunotherapy, such as vaccine and STING agonists.

Co-delivery strategy

In general, co-delivery of antigen and adjuvant to APCs is essential to trigger robust antigen-specific T cell response. Otherwise, low efficacy of immunotherapy or even immunological tolerance may occur when antigen is delivered in the absence of adjuvant [23]. Therefore, a personalized cancer vaccine has been developed based on a nanodisc platform composed of synthetic high density lipoprotein (HDL). Phospholipids and apolipoprotein A1-mimetic peptides are main components of this nanodisc. When simply mixed with cysteine-modified antigen peptide and cholesterol-modified CpG, this pre-formed HDL nanodisc can co-deliver antigen/adjuvant with ultrasmall size in less than 2 h. Compared with free Ag peptides + CpG, this nanodisc improved cargo delivery to lymph nodes and prolonged antigen presentation to APCs, thus it induced much more CTLs responses and tumor inhibition. So, this safety, easily manufactured and patient-tailored platform may be a powerful approach for cancer vaccine [126]. Except for antigen/adjuvant co-functionalization on the surface of nanoparticles, antigen/adjuvant co-encapsulation in different layers of nanoparticles with core shell structure is also a good choice. Zhou et al. [127] formulated a biomimetic nanovaccine with poly-(D, L-lactide-co-glycolide) (PLGA) core and DMPC membrane loading Imiquimod (R837) and antigen (αOVA) respectively. Apolipoprotein E3 (ApoE3)

was further incorporated into the DMPC membrane which markedly enhanced uptake by DCs. Unlike other decoration of ligand targeting lymph node, this nanoparticle entered in DCs through micropinocytosis rather than receptor-mediated endocytosis. This ApoE3-engineered nanoparticle prevented lung metastasis potently and exerted preferable antitumor efficacy on B16-OVA tumor when combined with immune checkpoint inhibitor.

T cells targeting

Enhancing T cells infiltration

In cancer-immunity cycle, the activated effector T cells would traffic to and infiltrate into the tumor bed to exert the cancer cell killing effect. T cell infiltration plays an essential role in cancer immunity. However, high stomal density and compact structure of solid tumor as well as the immune suppressive microenvironment are the two main obstacles impeding lymphocytes entering the tumors. Immune checkpoints blockade (ICB) therapy and adoptive T cell therapy (ACT) are promising in cancer immunotherapy and insufficient infiltration of T lymphocytes is the common challenge they both faced with. Previous studies found that the elimination of ECM and reversal of immune suppressive microenvironment of tumor were beneficial to enhance T cell infiltration and consequently improve antitumor effects [128, 129].

Elimination of ECM

The dense tumor structure not only hinders the penetration of nanomaterials, but also becomes a barrier for immune cells to play their roles. The mechanical properties of tumor cells, stromal cells and the tumor ECM can be modulated by targeting focal adhesion kinase (FAK) in tumor tissue. In addition, inhibition of FAK activity can also regulate the tumor immune environment, leading to $CD8^+$ cytotoxic T cells infiltration [130–133]. Recently, a multiplexed dendrimer LNP was reported for target tumor issue and co-delivery FAK siRNA, Cas9 mRNA and single guide RNA (sgRNA). This delivery system successfully decreased the contractile force and membrane tension properties of tumor cells and ECM stiffness by FAK inhibition, and consequently result in improving CRISPR gene-editing efficacy and antitumor effects due to the enhanced infiltration of LNPs and $CD8^+$ T cells into deep tumor [99].

PTT is often involved into the combination strategies of tumor immunotherapy due to its dense ECM disruption effects in TME by the locally near infrared (NIR)-applied hyperthermia [134]. Tan et al. [135] constructed the cationic thermosensitive LNPs to combine PTT with ICB therapy. This delivery system introduced cationic NIR photosensitizer IR-780 iodide modified lipid components, thermosensitive lipid DPPC and PD-1/PD-L1 inhibitor BMS202 has been shown to significantly reduce cancer-associated fibroblasts (CAFs) and reshape the distribution of tumor infiltrating lymphocytes (TILs) in deep tumors after laser irradiation. In particular, the thermosensitive lipid component changes the permeability upon temperature increase, enabling photothermal controlled drug release. For better efficacy of PTT in cancer treatment, more approaches are added into the delivery system. It was proved previously that degradation of matrix fibers by applying collagenase increased T cell infiltration into tumor [136]. Zhan et al. [137] also described a thermosensitive liposome with surface modification of bromelain, an ECM-degrading enzyme, loaded with ferrous sulfide (FeS_2) nanoparticles as both NIR-II photothermal converters and Fenton catalysts, and 2′3′-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) as the STING agonist. The results suggested that this ECM-degrading nanoagonist induced enhanced effector T cell infiltration. It is also worth noting that lipids in this study were also used to enable the controlled release in response to thermal conditions. These studies suggested that lipid-based nanoparticles are beneficial for the design of conditionally responsive controlled release drug delivery systems, which have the potential to be developed as multifunctional delivery platforms in combination therapy strategies. CAFs are one of the stromal components in the TME of solid tumors, can build up and remodel ECM structure, forming a physical barrier that impedes the delivery of therapeutic agents and tumor infiltration of cytotoxic $CD8^+$ T cells [136, 138]. In a study conducted by Yang's group, Calcipotriol, which can significantly weaken the capacity of CAFs to support tumor growth [139], was co-delivered with photosensitizer indocyanine green (ICG) via tumor cell-derived microparticles to improve the efficacy of PTT by reducing ECM. This particle vector holds potential as tumor-targeted drug-delivery carriers due to great stability, high biocompatibility, low immunogenicity, and target-homing capability. The results indicated that tumor accumulation and penetration of ICG as well as the infiltration of $CD8^+$ T cells were enhanced to generate strong PTT efficacy and antitumor immunity [140].

The dense ECM of the tumor is also associated with transforming growth factor-β (TGF-β) [141]. Another study delivered both EDTA and ICG via liposomes. This system used EDTA to reduce TGF-β expression in tumor tissues, thereby reducing ECM and increasing tumor penetration of liposomes, while increased infiltration of $CD8^+$ T cells was also observed in tumor tissues [142].

Reversal of immunosuppressive TME

Effective immunotherapy should transform a "cold" tumor with low immune cell infiltration into a "hot" tumor with high immune cell infiltration including T cells. "Cold" tumors are in immunosuppressive microenvironment, including tumor abnormal vasculature, abnormal chemokine secretion, suppressive immune cells increasing etc., which is one of the main obstacles of T cell infiltration in cancer immunotherapy [128]. Currently, many studies have been devoted to reversing the immunosuppressive microenvironment to improve tumor immune response and thus improve the effect of immunotherapy.

Studies have demonstrated that chemokines CCL2, CXCL12 and its receptor CXCR4 play important roles in tumor immunity suppression. Tumor hypoxia promotes immunosuppressive chemokine expression such as CXCL12, CCL2 and CXCL8 to inhibit tumor immunity and promote tumor growth [143–145]. Abnormal increase of CCL2 and CXCL12 could attack immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs) and TAMs, thus suppress T cell activation and infiltration. Therefore, blocking of CCL2 or CXCL12-CXCR4 axis becomes a promising strategy for reserving tumor immunosuppression. Huang's research group described an LCP nanoparticle delivery system for delivering the plasmid DNA of an engineered *PD-L1 trap* and *CXCL12 trap* to the nucleus. The galactose or mannose conjugated to DSPE-PEG2000 on the surface were added to assist in reticuloendothelial system (RES) evasion and DCs or hepatocyte uptake. The outcomes of this study provided evidence that a transiently expressed *pCXCL12 trap* gene immunotherapy can drastically reverse tumor immune suppression and significantly enhance $CD8^+$ T cell infiltration [146]. Thereafter, this group used liposome-protamine-DNA (LPD) nanoparticle platform to delivery plasmids encoding IL-10 and CXCL12 traps, and this delivery also successfully utilized IL-10 trap and CXCL12 trap greatly, allowing trap proteins to accumulate in perivascular cells and enhanced T cell infiltration [147]. With the same materials, they also developed targeted LPD nanoparticles to locally "trap" CCL2, which is the key mediator secreted by tumor associated adipocytes, can also ameliorate the immunosuppressive TME. The nanoparticles surface was grafted with PEG and functionalized with the targeting ligand aminoethyl anisamide to reduce organ accumulation and improve tumor delivery efficacy [148]. Except for CXCL12, its receptor CXCR4, which is highly upregulated both on cell surface and cytoplasm in tumor tissues, is also an efficiency target for the same purpose. Another group developed a CXCR4-targeted liposomal formulation for delivering AMD3100, an anti-CXCR4 antagonist, and enhancing its therapeutic efficacy. Particularly, AMD3100, acting as a targeting moiety and a dual blocker capable of inhibiting CXCR4 activation, is not only encapsulated into the liposome but also coated on the surface. The result indicated that the liposomal-AMD3100 formulation showed better pharmacodynamic profile in $CD3^+$ T cells infiltration than free drug [149]. These studies demonstrated the potential of lipid-based nanoparticles in the regulation of local chemokines for remodeling both immune and stromal microenvironment and subsequently enhancing T cell infiltration.

The aberrant architecture of tumor vasculature, which is a substantial mechanism to exclude T cells from the tumor vicinity, plays an important role in TME and becomes a well-established aspect in tumor immunity suppression. An imbalance between proangiogenic and antiangiogenic signaling is the essential reason of vascular abnormalities. It is proven that tumor cells secret vascular endothelial growth factor (VEGF) to trigger angiogenic processes through binding to VEGF receptor (VEGFR) on endothelial cells. Anti-angiogenic inhibitors and other chemotherapy, which can inhibit tumor angiogenesis, were used to sensitize tumor blood vessels for immune cells infiltration in many published papers [150, 151]. Recently, lipid-based nanoparticles have been used as delivery systems in tumor vascular normalization strategies.

Endothelial cells synthesize nitric oxide (NO) to not only mediates angiogenesis but also maintains vascular homeostasis and endothelial function and the creation of perivascular NO gradients could contribute to normalizing vessels, resulting in improved response to anti-tumor treatment [152, 153]. However, most NO-delivery agents are facing the problems including short half-life, low bioavailability and poor tumor targeting, thus a sustained-release system that can extend the half-life. Bioinspired lipoproteins are successful NO delivery vehicles that can realize controlled release and have developed into multifunctional platforms combining physical barriers remodeling to tumor vascular normalization. Wang et al. [154] designed a stroma-cell-accessible bioinspired lipoprotein system (S-LFO), which contained reduction-sensitive NO donor conjugate of furoxans-OXA (FO) in its lipid layer, to facilitate CTLs infiltration in tumors. Targeting peptide HC8 was included in S-LFO to promote its access to epithelial cell, CAFs, and TAMs in tumors, and FO would responsively release NO in reductive intracellular environments to exert the tumor vessel normalization effect and disrupt the stromal barriers. The results showed that S-LFO treatment produced a 2.96-fold increase of $CD8^+$ T cells. The calcium phosphate (CaP) liposome has also been developed as a delivery system that combines multiple mechanisms. Chen's group reported a nano-sapper based on two mechanisms to enhance CTLs infiltration, which synergistically breaks the physical

obstacles and increases the recruiting signals. This sapper introduced CaP technique due to its efficient *in vivo* transfection and delivery of small molecules possesses phosphate groups to realize the simultaneously delivery of phosphate of α-mangostin, a natural small molecule that can reverse the abnormal activated CAFs and decrease collagen deposition, and the plasmid encoding LIGHT, a pleiotropic inflammatory cytokine which can normalize the intratumoral vessels, as well as facilitates T cell recruitment. This strategy effectively reduced collagen deposition, normalized the intratumoral vasculatures, paved the road for the CTLs infiltration, thus reshaped TME [155].

Regulating gene expression of key proteins in tumor immunosuppressive environment is also an important means to enhance tumor immunoreactivity. In studies applying this strategy, lipid-based nanoparticles have also been utilized to efficiently deliver nucleic acid sequences for silencing genes or expressing trap proteins. Huang's group used a lipid-protamine-hyaluronic acid (LPH) nanoparticle system to selectively silence high mobility group protein A1, which is reported to contribute to the immunosuppressive microenvironment in the tumor [156], and used cationic LPD nanoparticles to deliver plasmid DNA encoding for the Wnt family member 5A (Wnt5a) trap, which can disable the Wnt5a, a signaling protein inducing tumor fibrosis and hindering T-cell infiltration [157]. The results showed that the tumor immunosuppressive environment was reshaped and T-cell infiltration was enhanced in both studies. $CD8^+$ T cells tend to become apoptotic when exposed to a low pH environment. Thus, the acidic microenvironment caused by excess lactic acid produced by high rates of aerobic glycolysis in tumor is also an important part of its immune suppression [158]. Wang's group utilized an *in vivo* optimized vesicular cationic lipid-assisted nanoparticle to deliver siRNA that can systematically knockdown lactate dehydrogenase A in tumor cells, thus realize the neutralization of tumor acidity and increased infiltration with $CD8^+$ T cells and NK cells [159]. The above strategies for regulating specific biomolecule in tumor tissue can effectively improve the efficacy of cancer immunotherapy such as ICB therapy.

Other studies have used lipid-coated nanoparticles or liposomes to co-deliver multiple therapeutic agents to systematically modulate the TME. In a study conducted by Zhang's group, all-trans retinoic acid (ATRA), DOX and IL-2 were co-encapsulated by a dHMLB for chemo-immunotherapy [74]. Additional examples include a nanoplatform developed by Wang's group, which is based on palmitoyl ascorbate-liposome loaded with Mn-doped $CaCO_3$ nanoparticles and carbonic anhydrase inhibitor SLC-0111 [160], and a liposome loading with immunotherapy adjuvants CpG and tumor growth inhibitor BMS-202 simultaneously [161]. The reversing of tumor immunosuppressive microenvironment was observed in all these studies, including the reduction of suppressive immune cells and the enhancement of immunotherapy efficacy. Notably, a marked increase in effector T-cell infiltration was a common feature of the results of this type of study.

Improving proliferation and differentiation of T cells

In the drainage lymph nodes, antigens and co-stimulators presented on the surface of APCs can prime and expand T cells to effector and memory T cells which play a crucial role in antitumor immunotherapy. Therefore, cytokines, immunomodulatory factors or ICB which promote this process of T cells proliferation and differentiation have the potential to perpetuate the cancer-immunity cycle. However, the free stimulators usually lead to inevitable systemic toxicities and cytokine storm because of the lack of selectivity. Thus, loading the stimulators in a nanocarrier is of vital importance.

Co-stimulatory molecules and cytokines

Co-stimulatory molecules, such as CD137 (also termed as 4-1BB) and OX40 (also termed as CD134), are positive factors for the expansion and differentiation of T cells which interact with their receptors on T cells. However, the low expression of this co-stimulatory receptors in tumor infiltrating T cells greatly weakened the therapeutic efficacy of their antibody [162]. So, Li et al. [100] combined the co-stimulatory receptors mRNA with the corresponding agonist antibody to solve this conundrum and enhance cancer immunotherapy. Inspired by the cell membrane, a library of phospholipid derivatives containing multiple hydrophobic tails and ionizable tertiary amines groups were established, and a lipid-based mRNA delivery system were formulated when adding DOPE, Cholesterol and DMG-PEG2000. This combination therapy substantially improved the antitumor activity compared to anti-OX40/anti-CD137 antibody alone. Besides, this strategy showed remarkably high complete response rate in B16F10 tumor model in combination with ICIs (anti-PD-L1/anti-CTLA-4). Importantly, the mRNA delivery system can exert compatible efficacy when administered by diverse routs (i.t. or i.v.). The metastasis of tumor in the lung was dramatically suppressed by the systemic administration of OX40 mRNA, anti-OX40 antibody, anti-PD-L1 antibody, and anti-CTLA-4 antibody.

In addition to immunostimulatory antibodies, some cytokines are also powerful antitumor therapeutics. IL-2 is one of them, which can trigger the effector function of

cytotoxic T cells and NK cells, and has been approved for the therapy of metastatic melanoma and renal cancer. However, the clinical efficacy was hampered by cytokine storm and VLS at high dose which may be caused by the activation of circulating lymphocytes [163]. To this end, IL-2-Fc with extended PK and the F(Ab')2 fragments of anti-CD137 were coupled to PEGylated liposomes for systemic administration. With the help of the EPR effect, this bi-functional liposome showed rapid accumulation in tumor sites due to the prolonged half-life when compared to the free cytokines and antibody. Although, the immunoliposomes demonstrated equivalent immunostimulatory and antitumor activity to the parent drug combination such as T cells priming and granzyme expression, and they markedly mitigated systemic toxicity by alerting biodistribution [97]. Nevertheless, the immune response triggered by IL-2 may be thwarted by immunosuppressive factors including TGF-β, which promoting tumor growth and immune escape. Park et al. [164] co-encapsulated soluble IL-2 and hydrophobic TGF-β receptor-I inhibitor into a core–shell nanoparticle for the safe and sustained combination delivery. Cyclodextrin after modification was used to solubilize the small molecule inhibitor, and this complex was further loaded into a biodegradable polymer matrix together with IL-2 before being coated by a PEGylated liposomal layer. This nanoscale liposomal polymeric gels demonstrated potent immunotherapeutic effect in melanoma mouse models after administered intratumorally or intravenously. The upraised number of NK cells and $CD8^+$ T/Treg ratio suggested that this combination therapy stimulated both innate and adaptive immune response to exert synergistic anti-tumor effects.

Immune checkpoints blockade

In the ultima killing step of cancer immunity cycle, the immune checkpoints, PD-1 on T cells or the corresponding ligand (PD-L1) on cancer cells always prevent T cells from exerting cytotoxic function and thus induce immune escape. Therefore, lots of ICIs have been developed and approved by FDA for the treatment of non-solid tumor and showed excellent tumor growth inhibition, such as atezolizumab (PD-L1 inhibitor) and pembrolizumab (PD-1 inhibitor). However, limited clinical objective response rate (less than 20 %) and undesirable side effects (such as autoimmune disorders) occurred under the systemic administration of ICIs have largely impeded its clinical application [165]. Using delivery methods to solve the problems of anti-PD-1/PD-L1 therapy is a field which researchers are passionate about.

Platelets-based delivery system is an ingenious solution. On the one hand, platelets have relatively long life-span and innate targeting ability to surgical wounds and CTCs by which it can significantly improve the PK of loading cargo and prevent tumor metastases respectively. On the other hand, the platelet-derived chemokines (such as soluble CD40L) released after activation can mature and proliferate diverse lymphocytes [49, 166]. Inspired by these special properties of natural platelets, Wang et al. [49] constructed aPD-L1 conjugated platelets for the prevention of tumor recurrence after surgical. After intravenous administration, this biomimetic carrier targeted to surgical site and was activated to release platelet-derived microparticles (PMPs) which really exerted the immunomodulatory effect. Binding to DCs and cancer cells more greatly, PMPs demonstrated better preventative effect both in B16 melanoma and 4T1 breast cancer compared to the antibody alone. In addition, the upregulated expression of PD-L1 caused by the inflamed TME after platelets activation facilitated the efficacy of the anti-PD-L1 therapy. Based on the foundation of platelets strategy, Hu et al. [167] further decorated the aPD-L1 conjugated platelets on the membrane of haematopoietic stem cell (HSCs) to obtain the targeting ability for bone marrow for the treatment of acute myeloid leukaemia (AMK). With the help of the aPD-L1 delivery of platelets and homing capacity of HSCs, the growth and recurrence of AMK was dramatically suppressed. Furthermore, some combination therapy based on platelets-based aPD-L1 delivery system have depicted better immunotherapeutic efficacy. For example, aPD-L1 conjugated platelets, CAR-T cells and IL-15 nanoparticles were loaded into a hyaluronic acid hydrogel for the sustained activation of CAR-T cells [168]; Colony-stimulating factor 1 receptors (CSF1R) was co-delivered with aPD-L1 conjugated platelets through a biocompatible alginate-based hydrogel to deplete TAMs for more potent prevention of post-surgical carcinoma recurrence [169].

Except for small monoclonal antibody, engineered PD-L1 trap fusion protein which can potently bind to PD-L1 and block its signal pathway were also constructed. Through the genetic engineering, the trimeric PD-L1 trap protein was composed of PD-1 extracellular domain and a trimerization motif from cartilage matrix protein with much higher affinity to PD-L1 than PD-1 or anti-PD-L1 antibody. For better tumor targeting, the plasmid coding PD-L1 trap was encapsulated in an LPD composed with protamine core and cationic lipid layer. After injected intravenously, LPD accumulated in the tumor site and express PD-L1 trap protein locally and transiently. Because of that, this strategy prevented the upregulation of Th17 cells in spleen, which is the side effects of systemic anti-PD-L1 antibody therapy, while improving the safety and tolerance. Combined with OXA, this PD-L1 trap indicated potent therapeutic efficacy for the patient with ICB insensitive cancer type (non-hypermutated microsatellite-stable/mismatch repair proficient tumor) [102]. To overcome the poor penetration ability in

tumor of PD-1/PD-L1 antibody, Liu et al. [98] used a histone demethylase small molecular inhibitor, 5-carboxy-8-hydroxyquinoline (IOX1), to downregulate the expression of PD-L1 in tumor. Loaded in liposomes, IOX1 was co-delivered with DOX liposome to exert synergetic effect of increasing the intracellular uptake of DOX through the histone demethylase Jumonji domain-containing 1A/β-catenin/P-glycoproteins (P-gp) signaling. Thus, this combination therapy induced a long-term immunological memory by enhancing more T cells infiltration and overcoming multidrug resistance.

CTLA-4, a homologue of the CD28 receptor expressed on effector T cells and Tregs, is another ICB target which prevents T cells activation by competing with CD28 to bind to the co-stimulators on DCs (CD80/CD86) with much higher affinity [170]. Therefore, the anti-CTLA-4 therapy promotes the priming and proliferation of T cells in preclinical and clinical trials such as Ipilimumab. However, undesirable side effects such as irAEs brought by administrating CTLA-4-blocking monoclonal antibodies limited its application [171]. In case of that, the introduction of nanotechnology tends to be important. A PEGylated liposome encapsulating CTLA-4-blocking antibody has been formulated to prolong the circulation half-life and reduce organ toxicity when intravenously administrated. Except for the better biodistribution and accumulation in tumor, the liposome promoted greater effector T cells to Tregs ratio than free anti-CTLA-4 antibody, and thus increased the median survival time and tumor growth delay greatly [172]. In addition to being used alone for enhanced T cell activation, anti-CTLA-4 therapy can also be used in combination with vaccine. After conjugation with PEGylated lipid, anti-CTLA-4 antibody was incorporated into the lipid bilayer of the vaccine based on exosomes from bone marrow-derived dendritic cells (BMDCs) via a post-insertion technique. Within the size threshold for the optimal delivery to lymph nodes (5–50 nm), this functionalized exosome bound and activated T cells more effectively than unmodified ones after being injected subcutaneously. Thus, the strong antigen-specific T-cell response and remarkable CTLs/Treg ratio in TME lead to prominent tumor growth inhibition [96].

Combination therapy

Based on the positive immunomodulatory effect and relatedly low response rate of PD-1/PDL-1 blockade, the combination strategy with other immunostimulatory factors becomes an urgent need to expand the responsible cancer type. A tumor targeted gene cocktails therapy combining ICB (aPD-L1) with immunostimulatory cytokines (IL-2) was developed to treat advanced hepatocellular carcinoma (HCC), which was termed as tumor-targeted-lipid-dendrimer-calcium-phosphate (TT-LDP). The siRNA against PD-L1 and plasmid DNA encoding IL-2 was condensed in a dendrimer-CaP core which was further coated by a lipid bilayer containing an HCC targeting peptide (SP94). Importantly, the pH-sensitive CaP core and thymine-capped polyamidoamine played a major role in endosomal escape and nuclear acid delivery which enabled the stability and enhanced transfection activity. Besides gene delivery carrier, the special dendrimers also acted as an adjuvant by activate the STING pathway. Consequently, significant $CD8^+$T infiltration and HCC growth inhibition and metastasis suppression has been seen by the systemic administration of TT-LDP [173]. Another non-ignorable cytokine coordinating TME is IFNs, the deficiency of which usually leads to tumor progression. The co-delivery of IFN and aPD-1 was an excellent therapeutic choice to overcome the shortcoming of upregulating immune checkpoints after IFN treatment. Zhai et al. [93] created an artificial exosome composed of BSA core covalently loading IFN inducer (ORY-1001) and T cell membrane functionalized by PD-1 (Figure 3). With the help of PD-1 decoration, the upregulated PD-L1 in cancer cells by IFN was blocked, and the uptake of ORY-1001 was dramatically improved after intratumoural injection. Thus, this therapy induced 29-fold increase of the active CTLs in tumor site and remarkable suppressive effect in multiple tumor models. In addition to cytokines, the co-stimulator of T cell activation (CD28/B7) can also generate strong CTLs response in combination with ICB. A novel DC-derived nanovaccine with the ability of antigen self-presentation and immunosuppression reversal has been formulated through bioengineering technology. The membrane-localized anti-PD-1 antibody was obtained from plasmid vector expressed in DCs, which were infected with recombinant adenovirus consequently to present MHC-I-antigen complex and B7 co-stimulatory molecules. After sonication and ultracentrifuge, the DC membrane nanovesicles was prepared. Without the need of APC activation and antigen presentation, this vaccine can trigger potent CTLs response directly, while activating exhausted T cells by anti-PD-1 antibody as well [94].

IDO1 inhibitor

Except for immune checkpoint, IDO1, another immunosuppressive molecule, has also became a critical immunotherapeutic target. IDO1 can interfere with the proliferation of CTLs while upregulating Tregs instead by transforming L-tryptophan (Trp) to L-kynurenine (Kyn) and inhibiting the function of Trp in cancer cells and some innate immune cells. To rescue the immunosuppressive effect caused by IDO, Lu et al. [174] designed a lipid-conjugated prodrug of IDO1 inhibitor (indoximod, IND), and delivery it through two

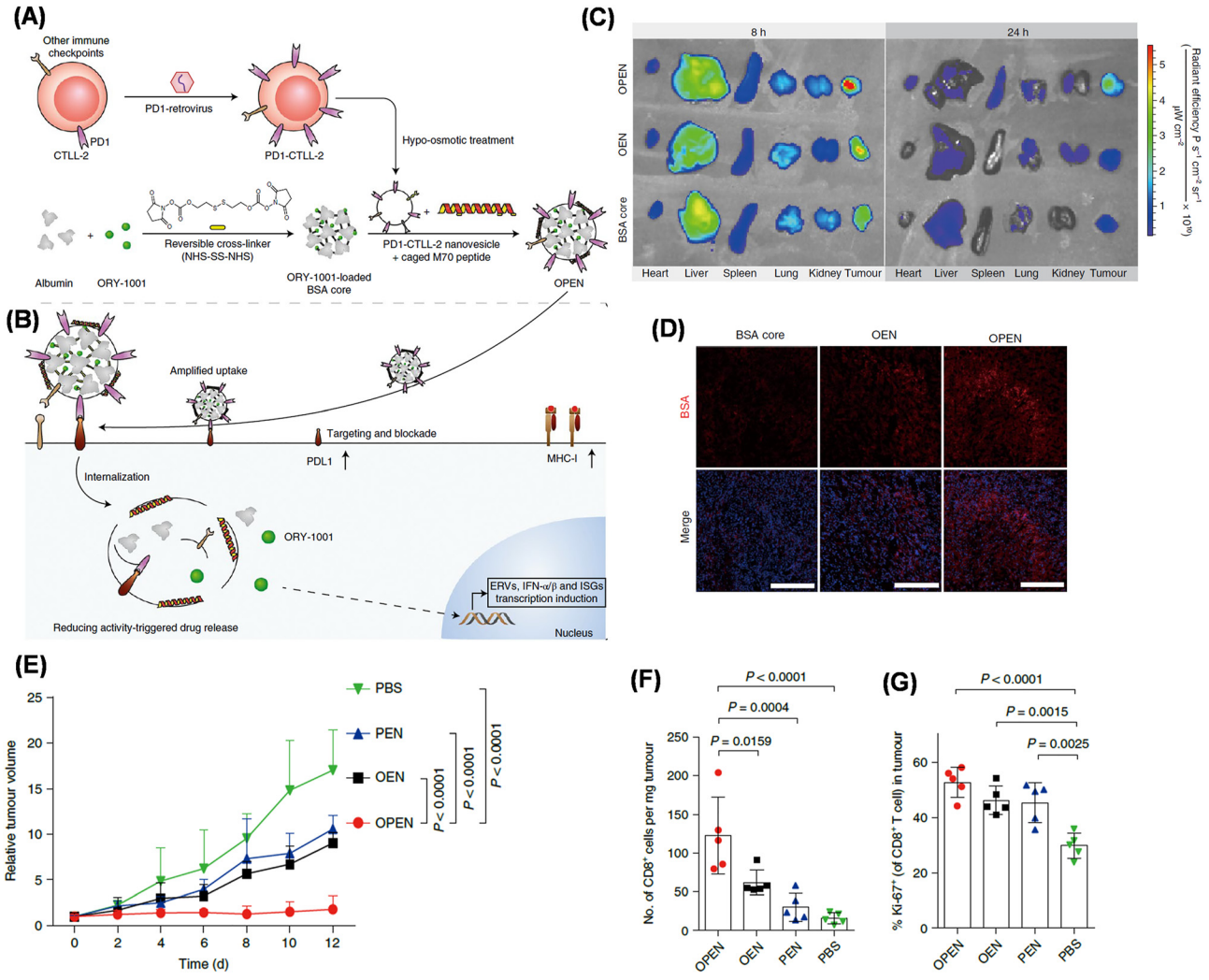


Figure 3: An artificial exosome composed of BSA core covalently loading IFN inducer (ORY-1001) and T cell membrane functionalized by PD-1 (A) Preparation of ORY-1001-loaded and PD1-overexpressing T lymphocyte membrane-decorated epigenetic nanoinducer (OPEN). (B) OPEN is expected to recognize and enter PD-L1-expressing cells and release ORY-1001 with the help of M70. ORY-1001 can upregulate the expression of IFNs, MHC-I and PD-L1. The upregulated PD-L1 is neutralized by subsequent OPEN. (C–D) Fluorescence images of major organs and tumors, as well as fluorescence images of tumor slices collected from mice receiving Cy5-labelled BSA core, OEN or OPEN. (E) Growth profiles of 4T1 tumors of mice receiving the indicated treatments. (F–G) Intratumoral densities of $CD8^+$ T lymphocytes (A) and the percentage and of Ki-67^+ cells among the $CD8^+$ T cells at seven days after the last injection. M70, macrolittin 70. ERV, endogenous retrovirus. Reproduced with permission [93]. Copyright 2021, Springer Nature.

lipids based nanocarriers. One soft nanoparticle was formulated by the self-assemble of the lipid prodrug, and another hard system was constructed by using a mesoporous silica core additionally. Dramatic tumor regression or eradication was achieved when combining IND prodrug with OXA for the treatment of pancreatic ductal adenocarcinoma. Another effective IND loading method is importing IND into a special liposome with the help of DOX which acting as a transmembrane-enabling agent. Under the acid pH, drug precipitates can be formed after the break of hydrazine linkage between DOX and IND which can be restricted within the liposome. Strikingly, IND-DOX liposome eliminated advanced orthotropic tumors including CT-26 and B16-F10 synergistically [175]. Zhang et al. [176] designed a multifunctional injectable hydrogel system loading siRNA, which silenced the expression of IDO1, and mitoxantrone (ICD inducer) through a nanoscale metal-organic framework. Further, this nanocarrier was co-extruded with the glioma-associated macrophage membrane for the homing ability to glioblastoma multiforme through α4β1 integrin. After injected into the surgical cavity, this multifunctional system reversed the “cold” tumors to a “hot” tumor immunity niche while tracing residual cancer cells and preventing post-surgical relapse efficiently.

Bring T cells and tumor cells closer

A bispecific T-cell engager (BiTE) is a recombinant bispecific protein that has two linked scFvs from two different antibodies, one targeting a cell-surface molecule on T cells (primarily CD3ε) and the other targeting a specific antigen on tumor cell surface [177, 178]. Different from natural antibodies, BiTEs can redirect T cells to specific tumor antigens and activate T cells directly by minimizing the distance between T cells and malignant cells [179]. Activated T cells secrete cytolytic perforin and granzymes (for example granzyme B) through immunological synapses [180, 181], which ultimately caused cancer cells lysis through membrane fusion and endosome-mediated endocytosis. Alhallak et al. [182] prepared nanoparticle-based BiTEs, which were liposomes decorated with antibodies simultaneously targeting CD3 and tumor specific antigens. Nanoparticles that targeted CD3 and multiple cancer antigens by conjugating multiple antibodies against multiple tumor antigens for T-cell engagement (nanoMuTEs) ware also developed through similar method. After being PEGylated, the half-life of nanoBiTEs reached up to 60 h, which enabled once-a-week administration instead of repeated infusion. Of note, nanoMuTEs showed greater efficacy in myeloma cells *in vitro* and *in vivo*, compared to nanoBiTEs targeting only one tumor antigen. Cheng et al. [183] developed CD3/PD-L1/mPEG trispecific antibodies as BiTEs to non-covalently bind chemodrug ganetespib-loaded PEGylated nanocarriers via anti-mPEG fragment in order to achieve synergistic antitumor effects through activating T cells to eliminate malignant cells, blocking the PD-1-suppression pathway, and boosting the antitumor efficacy of ganetespib.

Preparation and expansion of CAR-T

Preparation of CAR-T

CD19 CAR-T cell therapy is a very promising form of cancer immunotherapy and was approved by FDA for treatment of ALL and large B cell lymphoma [184, 185]. To produce CAR-T cell therapy, T cells of patients would be harvested, modified to express CD19-specific CAR, and reinfused into the patients. The CAR construct allows T cells to target and bind specific cancer cells to induce apoptosis and eradicate the cancer using the patient's own immune system [186]. Thus, this form of autologous therapy relies on *in vitro* cell engineering to produce CAR-T cells. However, the currently used method is to use viral delivery vectors to induce CAR expression, which is permanent and may lead to serious adverse side-effects. mRNA has been developed as a promising strategy for inducing transient CAR expression in T cells to alleviate the adverse effects. Compared to the most commonly used electroporation for mRNA delivery, LNP is a promising material due to the lower cytotoxic.

Recently, a library containing 24 ionizable lipids was established by Mitchell' research group to optimize LNPs formulation for mRNA delivery. And the screening results showed that 7 of them can enhance the capability of delivery in different degrees, revealing the influence of the selection of lipids on the transport efficiency. The highest-performing preparations among them can induce CAR expression with a level comparable and a cytotoxicity much lower than that of electroporation [187]. In another study of the same group, sequential libraries of ionized LNP formulation with different excipient compositions were screened in comparison to a standard formulation to improve mRNA delivery to T cells, with the top one providing a threefold increase in RNA delivery efficiency comparable to electroporation [188]. These studies demonstrated that mRNA delivery efficiency of LNPs could be greatly optimized by lipid or excipient selection with decreased cytotoxicity, while suggesting the great potential of LNP for CAR-T preparation *in vitro*. Furthermore, LNPs are also successfully used to engineering CAR macrophages *in vitro* [189], which further broadens the development prospect of LNPs in the mRNA transfection of cells.

Expansion of CAR-T

Expansion of functional T cells *in vitro* is a key step in the production of T cells for adoptive cell transfer, including CAR-T cells and remains a challenge. In the natural immune process, T cell activation and expansion can be achieved by the combination of stimulants on the surface of natural APC [190], but the high cost and complex process limit the clinical application [191, 192]. Engineered artificial APC (aAPC) has been developed as an alternative to overcome these limitations. In contrast to natural APC, aAPC has a well-defined composition and controlled, uniform signal expression. In addition, aAPC can be easily produced on a large scale [193]. To mimic the interaction between natural APC and T cells, lipid-bilayer coated particles were used to exploit aAPC. The activation of T cells induced by lipid-based aAPC has been widely verified.

Multiple studies have shown that the use of the lipid bilayer with pre-clustered MHC complex can successfully stimulate the proliferation of $CD4^+$ T cells *in vitro*, and it has been proved that the pre-clustered MHC complex on aAPC surface can improve the proliferation efficiency of T cells [194–196]. Solid particles such as silica particles or PLGA particles serve as the core or scaffold of the lipid bilayer to form the supported lipid bilayers systems, improving stability and drug loading capacity [46, 197, 198].

Alexander S Cheung et al. [199] described a system mimicking natural APC in which mesopore silica microrods are coated with a supported lipid bilayers, which can be functionalized (attached αCD3/pMHC, αCD28) to activate T cells and signaling, and the microbar acts as a carrier to continuously release soluble paracrine substance (IL-2) to stimulate T cell expansion. This system can effectively increase the expansion efficiency of primary mouse and human T cells *in vitro* by 2–10 times. Furthermore, this system supports the expansion of CD19 CAR-T cells with restimulation by 5 times more than DynadBeads (commercial expansion beads). This finding also demonstrates the great potential of lipid bilayers to induce T cell proliferation *in vitro*.

Lipid-based nanoparticles for macrophages associated tumor immunotherapy

TAMs are the most abundant non-tumor cells in the TME [200], usually including tissue-resident macrophages and myeloid-derived macrophages [201], which play a key role in the coordination of cancer-associated inflammation [202]. TME has a critical influence on the phenotype and function of TAMs [202, 203]. Therefore, macrophages are generally divided into two subsets according to their function in immunity: tumor killing (M1) or tumor promoting (M2) macrophages [204]. The classical M1 phenotype activated by IFN-γ or TNF-α can phagocytose pathogens while promoting inflammation and anti-tumor immunity trough mechanism such as inducible NO synthase [205]. However, alternatively activated M2 macrophages by Th2 cytokines (IL-4/IL-13) are responsible for angiogenesis and tissue repair, and thus exert anti-inflammatory and pro-tumor effects [206]. Unfortunately, most of the TAMs perform M2 phenotype driven by signals from TME, which create an immunosuppression microenvironment and attenuated anti-tumor efficacy. Therefore, re-educating TAMs to the pro-inflammatory M1 phenotype is of vital importance to modulate TME and enhance cancer immunotherapy. The current strategies consist of four aspects [202]: (1) inhibiting the recruitment and localization of macrophages at tumor sites, (2) TAM depletion, (3) polarization of TAM toward M1 phenotype, (4) blocking CD47-signal regulatory protein α (SIRPα) pathway to enhance phagocytosis. In order to avoid the systemic toxicity and improve limited immunotherapeutic effect of free therapeutics, delivery vectors are usually applied to release drug at appropriate time and place [22]. In this section, we will discuss lipid-based delivery system targeting TAMs.

Inhibiting the recruitment of macrophages

Signals which can recruit macrophages to tumor site mainly include chemokines (CCL2/CCL5), VEGF, CSF-1, and complement component [202]. Produced mainly by tumor cells and endothelial cells, CCL2/5 can lead to macrophage infiltration and enhanced metastasis [207]. Several monoclonal antibodies (mAb, CNTO 888 [208]) and small molecular antagonists (PF-04136309 [209]) have reached clinical phase for the treatment of pancreatic adenocarcinoma or prostate cancer. Nevertheless, these therapies showed negligible response and survival benefit, unless they were combined with conventional chemotherapy. This may be due to the systemic distribution and multiple endogenous ligands of the free drug [210]. Based on this, Liu and the co-workers established an innovative CCL2/CCL5 dual inhibitor (BisCCL2/5i) [210], which can specifically bind and neutralize CCL2 and CCL5 with high binding affinity. In order to improve the pharmacokinetic and mitigate toxicities, mRNA encoding BisCCL2/5i was delivered by Dlin-MC3-DMA-based LNPs for the treatment of liver malignancies. The LNP delivery system showed prolonged survival than the combined therapy of two neutralizing antibody by inhibiting the recruitment of macrophages and re-educating the M2-like TAM to M1 phenotype. When co-delivery with the mRNA encoding a trimeric PD-1 inhibitor, the BisCCL2/5i therapy can effectively alleviate liver metastatic of colon and pancreatic cancer, while inducing long-term immune memory. More importantly, systemic side effects and irAEs caused by increased Th17 cells were not observed in this co-delivery LNP.

Inhibiting the CSF-1/CSF-1R axis is also an attractive target to impede the recruitment of TAM precursor cells. Lots of small molecular inhibitors and antibodies have been developed in clinical trial, such as Pexidartinib (PLX3397; Plexxikon) [211] and Emactuzumab (RO5509554/RG7155; Roche) [212]. However no objective responses were observed when these inhibitors used alone [202]. Zhang et al. [213] established an M2-like TAMs dual-targeting LNPs (M2NPs) loading anti-CSF-1R siRNA by modifying a fusion protein of α-peptide (a scavenger receptor B type 1 targeting peptide) and M2 macrophage binding peptide. This strategy not only can reduce the M2-like TAMs in TME dramatically (52 %), but also can control tumor growth and prolong survival

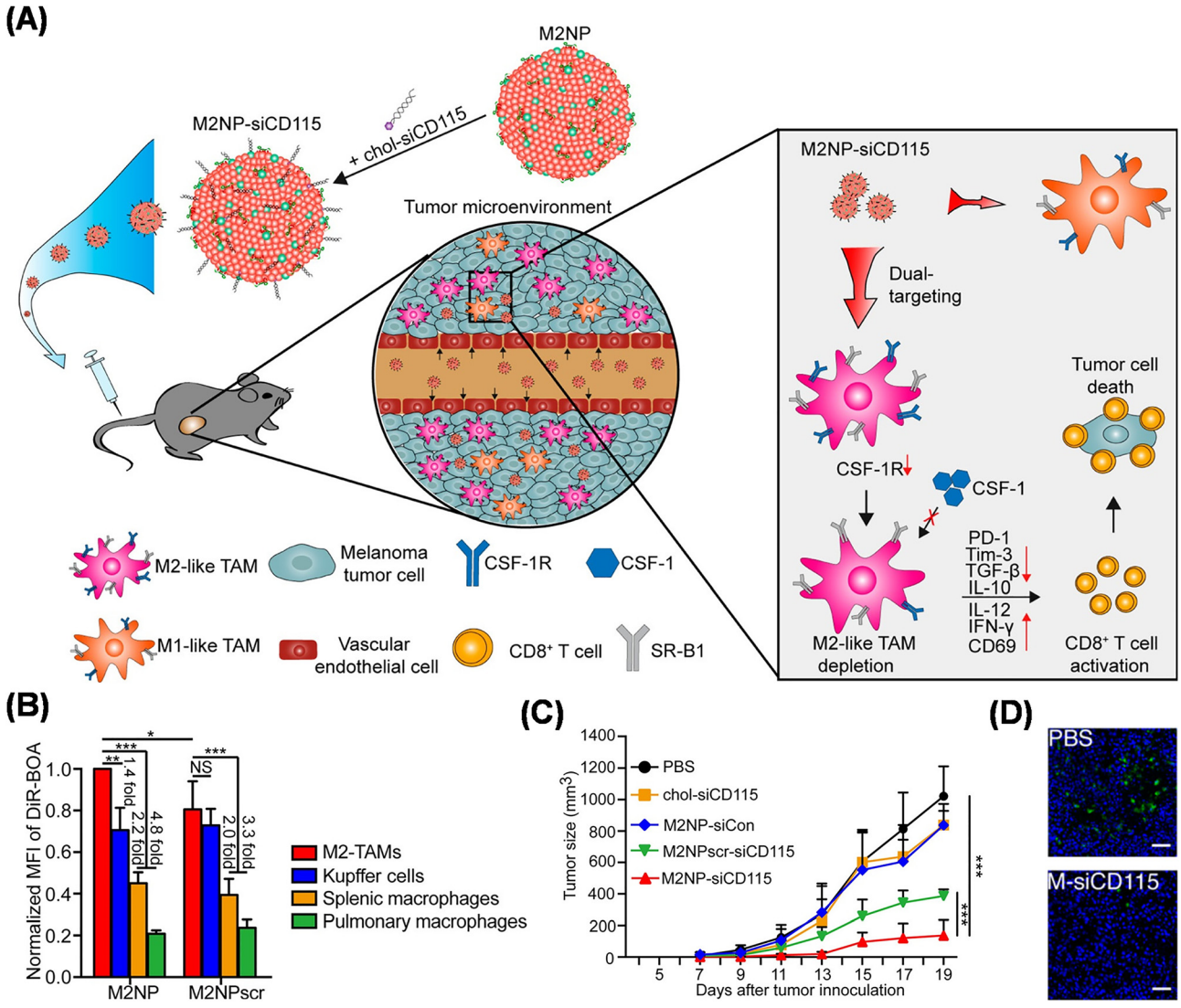


Figure 4: Dual-targeting nanoparticles delivering siRNA to TAMs for melanoma therapy. (A) M2NP-based delivery of siRNA for CSF-1R silencing and immune regulation via synergistic dual targeting of M2-like TAMs *in vivo*. (B) Comparison of the uptake of M2NPs by M2-like TAMs and macrophages in liver (Kupffer cells), spleen (splenic macrophages), and lung (pulmonary macrophages) at 48 h post injection (n=4 mice per group). The MFI values of DiR-BOA were normalized according to those of the M2-like TAMs in the M2NPs administration group. Data are presented as the mean ± SD (two-tailed *t*-test). (C) Tumor growth curves of B16 tumors in C57BL/6 mice treated with PBS, chol-siCD115, M2NP-siCon, M2NPscr-siCD115, or M2NP-siCD115, n=6 mice per group. (D) Representative immunofluorescence results for evaluating the presence of F4/80$^+$ TAMs after treatment with PBS and M2NP-siCD115. Scale bar: 50 μm. Green: Alexa Fluor 647 anti-F4/80, Blue: DAPI. * for p<0.05, ** for p<0.01, and *** for p<0.001. TAMs, tumor associated macrophages; M2NPs, M2-like TAMs dual-targeting LNPs; MFI, mean fluorescence intensity. Reproduced with permission [213]. Copyright 2017, American Chemical Society.

(Figure 4). In addition to suppressing recruitment, blocking CSF-1/CSF-1R pathway can contribute to reprogram TAMs. SIRPα blocker and CSF-1R inhibitor (BLZ945) were co-encapsulated in lipid bilayer through supramolecular assembly [214]. This bifunctional supramolecule can inhibit CSF-1R signaling more constantly and skew TAM toward M1 subset more effectively than small molecular inhibitors used in clinics. Therefore, blocking the recruiting signals for macrophages is a positive intervention for tumor immunotherapy.

Depleting TAMs

For the depletion of TAMs, clodronate/BPs liposomes and some small molecular drugs (Trabectedin, DOX) are used widely. BPs can regulate cholesterol synthesis and protein prenylation by inhibit farnesyldiphosphonate synthase. Several BPs (alendronate, risedronate and zoledronic acid) have been developed clinically for the treatment of glucocorticoid-induced osteoporosis [215]. In order to make full use of the high phagocytic ability of macrophages,

clodronate liposomes have been formulated to deplete TAMs or Kupffer cells by inducing apoptosis [216]. It has been reported that more than 50 % alveolar macrophages can be killed by clodronate liposomes when using a combination strategy of intratracheal and intravenous administration. Meanwhile, the tumor burden was significantly reduced compared to the vehicle group [217]. For fully reversing TME, clodronate liposomes are usually combined with other modulation methods. For example, Song et al. [218] developed a locally-delivered nano-liposome-bridged multidomain nanogel encapsulating gemcitabine and R837. Negatively charged clodronate liposomes can be loaded to this nanogel trough electrostatic interaction. By ICD, Toll-like receptor activation and TAMs depletion, the immunosuppressive TME was reshaped and the antitumor efficacy was enhanced.

In addition, chemotherapeutic drug Trabectedin which has anti-proliferative activity can kill monocytes or macrophages directly [200]. In order to reduce off-target toxicity and prolong circulation time, Trabectedin has been encapsulated within a TAMs-targeted liposome composed of DSPE-PEG, HSPC and cholesterol. This TAMs-targeted liposome showed the best therapeutic effect for a range of cancer types [219]. Besides, liposome modified by sialic acid-cholesterol conjugate was established for epirubicin delivery to eradicate TAMs in TME, which showed superior antitumor efficacy than other non-targeting liposomes [220]. So, directly depleting TAMs through liposome encapsulating toxic molecules to reverse suppressive immune microenvironment has been fully confirmed.

Repolarizing TAMs toward M1 phenotype

Tumors can recruit circulating monocytes and tissue-resident macrophages to the TME and subsequently induce them to M2 phenotype. By producing platelet-derived growth factor (PDGF), VEGF, TGF-β and so on, TAMs enhance tumor progression, angiogenesis, immunosuppression, invasion and metastasis [202, 221, 222]. Fortunately, TAMs are highly malleable [223]. Hence, reprogramming the pro-tumoral M2 macrophages to the pro-inflammatory M1 macrophages by lipid-based nanoparticles is promising in cancer immunotherapy. Yang et al. [224] developed a metformin-packaging mannose-modified macrophage-derived microparticle (Met@Man-MPs) to remodel the antitumor immune microenvironment by efficiently targeting M2 TAMs. As a consequence of the mannose, Met@Man-MPs were extremely accumulated in M2 TAMs of tumor sites, resulting in the polarization of M2 TAMs into immunostimulatory phenotype through adenosine 5′-monophosphate-activated protein kinase (MAPK)-NF-κB pathway. Furthermore, MPs derived from RAW264.7 macrophages entrapped matrix metalloprotein 9 (MMP9) and MMP14 proteins which could degrade tumor collagen to facilitate the infiltration of $CD8^+$ T cells into tumor regions (Figure 5). Due to the overexpression of mannose receptor CD206/MRC1, the mannose-mediated M2 TAMs-targeting strategy is widely used. Liu et al. [225] designed a chlorogenic acid-encapsulated mannosylated liposome to suppress G422 glioma tumor growth by the active targeting and repolarization of M2 TAMs via the signal transducer and activator of transcription 1 (STAT1) activation and the STAT6 inhibition.

In addition, membrane vesicles with desired properties have the potential to target M2 TAMs. Sathyanarayanan et al. [226] engineered an antisense oligonucleotide-inserted exosome (exoASO) for STAT6 targeting in TAMs and induce effective reprogramming of M2 TAMs into M1 phenotype. In contrast, OMVs are nonspecifically detected and phagocytized by macrophages owing to the high immunogenicity [36, 227]. Jiang et al. [228] reported a PTX and DNA damage response 1 (Redd1)-siRNA coloaded Gram-negative bacteria-derived OMVs (siRNA@M-/PTX-CA-OMVs) to regulate the TME and inhibit tumor growth. Compared with siRNA@ PTX-CA-OMVs, mannose-modified OMVs exhibited greater cellular uptake and tumor accumulation. In particular, siRNA@M-/PTX-CA-OMVs more significantly upregulated the ratio of M1 TAMs to M2 TAMs and the secretion levels of TNF-α. However, there was no obvious difference in relative tumor volume between the two groups. Besides the targeted delivery, some specific types of membrane vesicles showed unique anti-tumor effects [92, 229]. Yang et al. [230] developed irradiated tumor cell–derived microparticles (RT-MPs) which could eradicate tumors mainly through ferroptosis and reverse the immunosuppressive TME. After being treated with RT-MPs, BMDM-M2 cells induced by IL-4 showed M1 polarization via the activation of Janus kinase-STAT and MAPK pathways.

Liposomes and lipid-coated nanoparticles can deliver antibodies and plasmids or mRNA encoding proteins to increase the M1/M2 ratio. Shen et al. [231] fabricated an *IL-12* gene delivery system (APEG-LP/pIL12) that efficiently transfected both cancer cells and TAMs to produce IL-12. In KPC tumor models, the proportion of $IL12^+$ cells in APEG-LP/pIL12-treated tumors were about 10 or 3-fold than that of the Polyethylenimine (PEI) 25K/pIL12 or Lipo2000/pIL12 group. Moreover, the M1/M2 ratio increased significantly after the APEG-LP/pIL12 treatment.

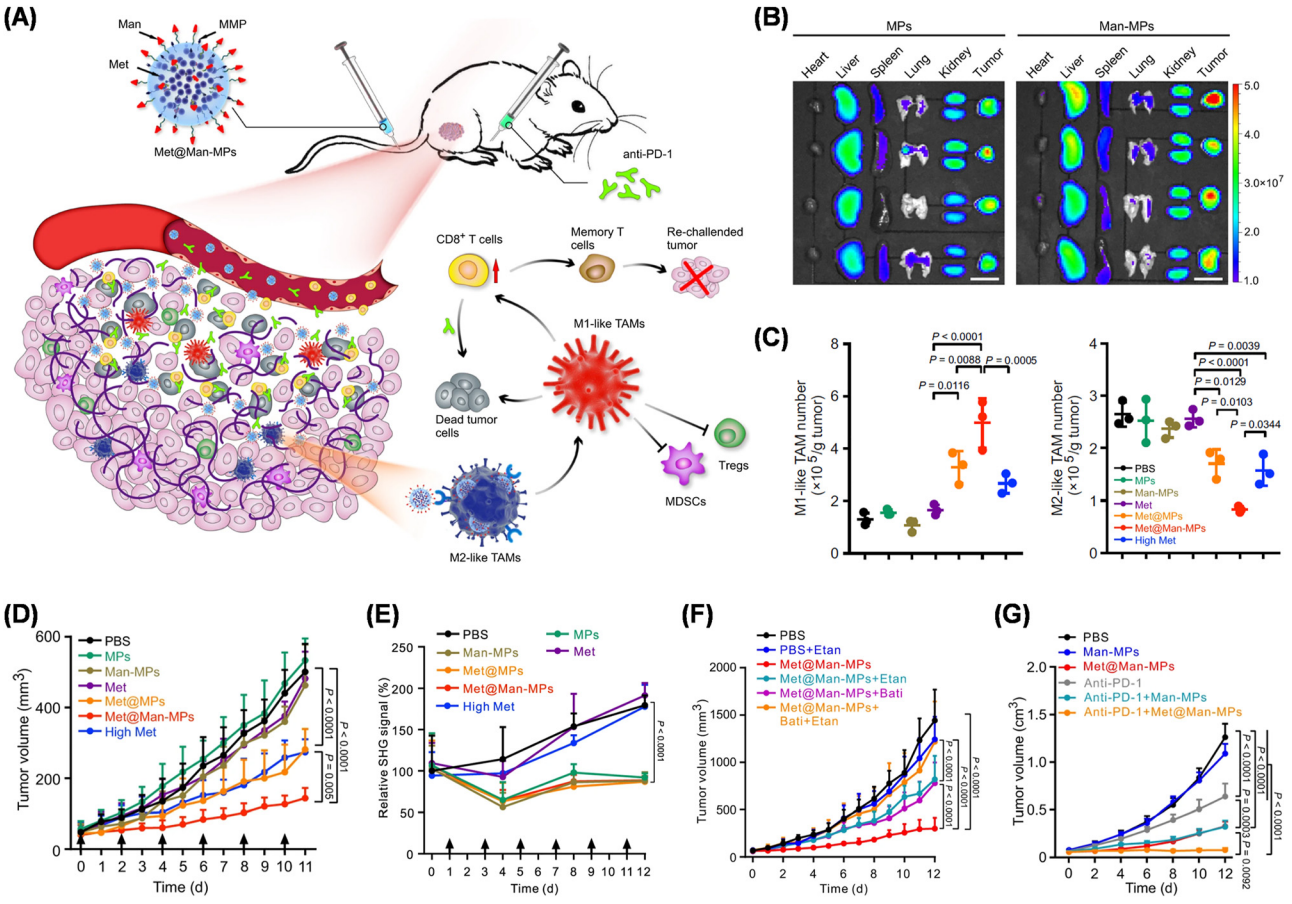


Figure 5: Metformin-packaging mannose-modified macrophage-derived microparticle (Met@Man-MPs) for hepatocellular carcinoma (HCC) therapy by repolarizing TAM toward M1 type. (A) Schematic of Met@Man-MPs as an efficient drug to boost anti-PD-1 therapy. Met@Man-MPs with MMP activity efficiently target to M2-like TAMs and degrade tumor collagen. (B) Ex vivo imaging of Cy5 in the organs and tumors of H22 tumor-bearing mice at 24 h after intravenous injection of Cy5-labeled MPs or Man-MPs at the dosage of 15 mg protein kg^{-1}. Scale bars: 1 cm. (C) The numbers of M1-like TAMs and M2-like TAMs in tumor tissues of H22 tumor-bearing mice after intravenous injection of PBS, MPs, Man-MPs, free Met, Met@MPs or Met@Man-MPs at the Met dosage of 10 mg kg^{-1}, or high dosage of Met at 100 mg kg^{-1} every two days for six times. Data are presented as mean ± SD (n=3 mice per group; one-way ANOVA followed by Tukey's HSD post-hoc test). (D) Tumor growth curves of H22 tumor-bearing mice. Data are presented as mean ± s.e.m. (n=5 mice per group; two-way ANOVA followed by Bonferroni's multiple comparisons post-test). (E) Relative SHG signal intensity in tumor tissues of H22 tumor-bearing mice after treatment. Data are presented as mean ± SD (n=10 fields for 3 mice). (F) Tumor volume of H22 tumor-bearing mice after intravenous injection of Met@Man-MPs pretreated with or without batimastat (0.6 mg mL^{-1}) at the Met dosage of 10 mg kg^{-1} every two days for six times, or/and intraperitoneal injection of etanercept at a dosage of 5 mg kg^{-1} every four days for four times. Data are presented as mean ± s.e.m. (n=6 mice per group). (G) Average tumor growth curves of H22 tumor-bearing mice after treatment at the anti-PD-1 antibody dosage of 100 μg per mouse and Met dosage of 10 mg kg^{-1}. Data are presented as mean ± s.e.m. (n=10 mice per group). Met, Metformin; Man, mannose; MP, microparticle; HCC, hepatocellular carcinoma. Reproduced with permission [224]. Copyright 2021, Springer Nature.

Besides directly reprogramming the M2 macrophages, lipid-based nanoparticles are able to promote tumor blood vessel normalization and further exert its immunomodulating effects indirectly [232]. Tumor hypoxia is mainly caused by differences in function and morphology between tumor vessels and normal vessels. In addition, uncontrolled proliferation of tumor cells exacerbates oxygen and nutrient consumption, further reducing oxygen concentrations [233, 234]. In hypoxic tumors, hypoxia-inducible factor-1α and lactate promote the polarization of M1 TAMs to M2 phenotype [235]. Chen et al. [236] developed a DNIC-encapsulated lipid-coated PLGA nanoparticle (NanoNO) for the controlled release of NO. In orthotopic HCC models, although low-dose NanoNO had no effect on mean vessel density, it significantly improved the function and perfusion of tumor vasculature and decreased the hypoxic area. Low-dose NanoNO repolarized TAMs towards M1 phenotype *in vivo*, although DNIC did not reprogram macrophages *in vitro*. Moreover, tumor vessel normalization by NanoNO combined chemotherapy or immunotherapy augmented the antitumor immunotherapy.

Blocking “don’t eat me” signal

CD47, a receptor that ligates with SIRPα present on macrophages to downregulate the phagocytose, is usually expressed on the surface of all normal cells [237]. However, it is also overexpressed on kinds of tumor cells, activates the “don’t eat me” signal through binding to SIRPα, leading to immune escape from the MPS [238, 239]. Blocking CD47 or SIRPα could potentially enhance the anticancer immune response of macrophages. Therefore, CD47-SIRPα blockade is emerging as a new generation of ICB strategy in cancer therapy.

As promising carriers, Lipid-based nanoparticles were applied in CD47-SIRPα blocking strategies to transport nucleic acid [240], delivery therapeutic protein [241] and combined with other treatments [242]. Yang et al. [240] developed a systemic delivery strategy based on CD47 siRNA encapsulated in an LPH nanoparticle formulation, which led to an efficient knockdown of CD47 in cancer cells, to address the challenge of targeted delivery of siRNA-based therapeutics *in vivo*. Decreased expression of CD47 eventually led to growth inhibition of melanoma tumors and suppressed lung metastasis in a B16F10 murine melanoma tumor model. Hend Mohamed Abdel-Bar et al. [241] designed a stable nucleic acid-lipid particles (SNALPs) formulation for the simultaneous delivery of ICD inducing drug (DOX) with siRNA knocking down CD47, the SNALPs were able to induce surface calreticulin expression leading to a synergistic effect on macrophage-mediated phagocytosis of treated cells. Then they developed an LPH as a platform for the combinatorial delivery of siRNA (siCD47) and topoisomerase II inhibitor etoposide, and a superior therapeutic outcome can be observed in mice treated with combinatory therapy [242].

Another strategy is to use lipid-based nanoparticles to delivery CD47/SIRPα binding proteins, even CD47 itself [243, 244] or signaling pathway inhibitor [245] for blocking “don’t eat me” signal between cancer cells and macrophages. Anujan Ramesh et al. [243] demonstrated that a lipid-based phagocytosis nanoenhancer conjugated with anti-CD47 and anti-SIRPα antibodies can simultaneously block both CD47 and SIRPα and can effectively engage macrophage and cancer cell in close proximity. This dual-antibodies lipid-based nanoparticles significantly enhanced phagocytosis of cancer cells as compared to combination of free antibodies and provided a simple approach to improve anti-cancer macrophage immunotherapy. Cheng et al. [244] designed a hybrid therapeutic nanovesicles by fusing drug-loaded thermosensitive liposomes with gene-engineered exosomes, which could block the interaction through competitively occupying SIRPα by overexpressing CD47. The results demonstrated that the overexpression of CD47 on the surface of nanovesicles exhibited the long blood circulation and promoted the M1 macrophages-mediated phagocytosis of CT26 cells by blocking the interaction between CD47 and SIRPα.

The CD47-SIRPα interaction can also be blocked by Src homology region 2 (SH2) domain –phosphatases 2 (SHP2) inhibition, and the SHP2 inhibitor SHP099 has been shown to enhance macrophage phagocytosis (Figure 6) [245]. An LNP system loaded with amphiphilic R848-cholesterol (TLR7/8 agonist) and SHP099 in a predefined ratio has been designed by Vaishali Malik and his colleagues. The LNP-mediated co-delivery in the TAMs increased M2 to M1 repolarization and phagocytosis potential of macrophages in the TME [246]. Lipid nanoparticle delivery systems with multiple TAM reprogramming functions are also included the hybrid cell membrane nanovesicles displaying SIRPα variants with significantly increased affinity to CD47 and containing M2-to-M1 repolarization signals [247]. Besides, multifunctional immunoliposome that encapsulated the adrenergic receptor blocker carvedilol and connected the anti-CD47 and anti-PDL1 in series via a reactive oxygen species (ROS)-sensitive linker were also fabricated [248]. Furthermore, a paclitaxel loaded liposome could release anti-CD47 antibody triggered by MMP 2 which blocked the engagement of CD47 with SIRPα and promoted M2 TAMs repolarization into M1 TAMs [249].

Lipid-based nanoparticles for other immune cells associated tumor immunotherapy

NK cells

NK cells are the predominant subtype of group 1 innate lymphoid cells that could respond to viral infection and transformed cells [250, 251]. NK cells compose 5–15 % of peripheral blood lymphocytes and are universally found in bone marrow, spleen, liver and other tissues, but rarely localize in lymph nodes [251].

Following the recruitment to the sites under the gradient of pro-inflammatory chemokines produced by innate and adaptive immune cells, NK cells perform their antitumoral functions by three major mechanisms, namely “missing-self” mechanism, antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism and pro-inflammatory cytokines pathway [250]. ‘Missing-self’ mechanism is accomplished by cancer cells downregulating MHC-I

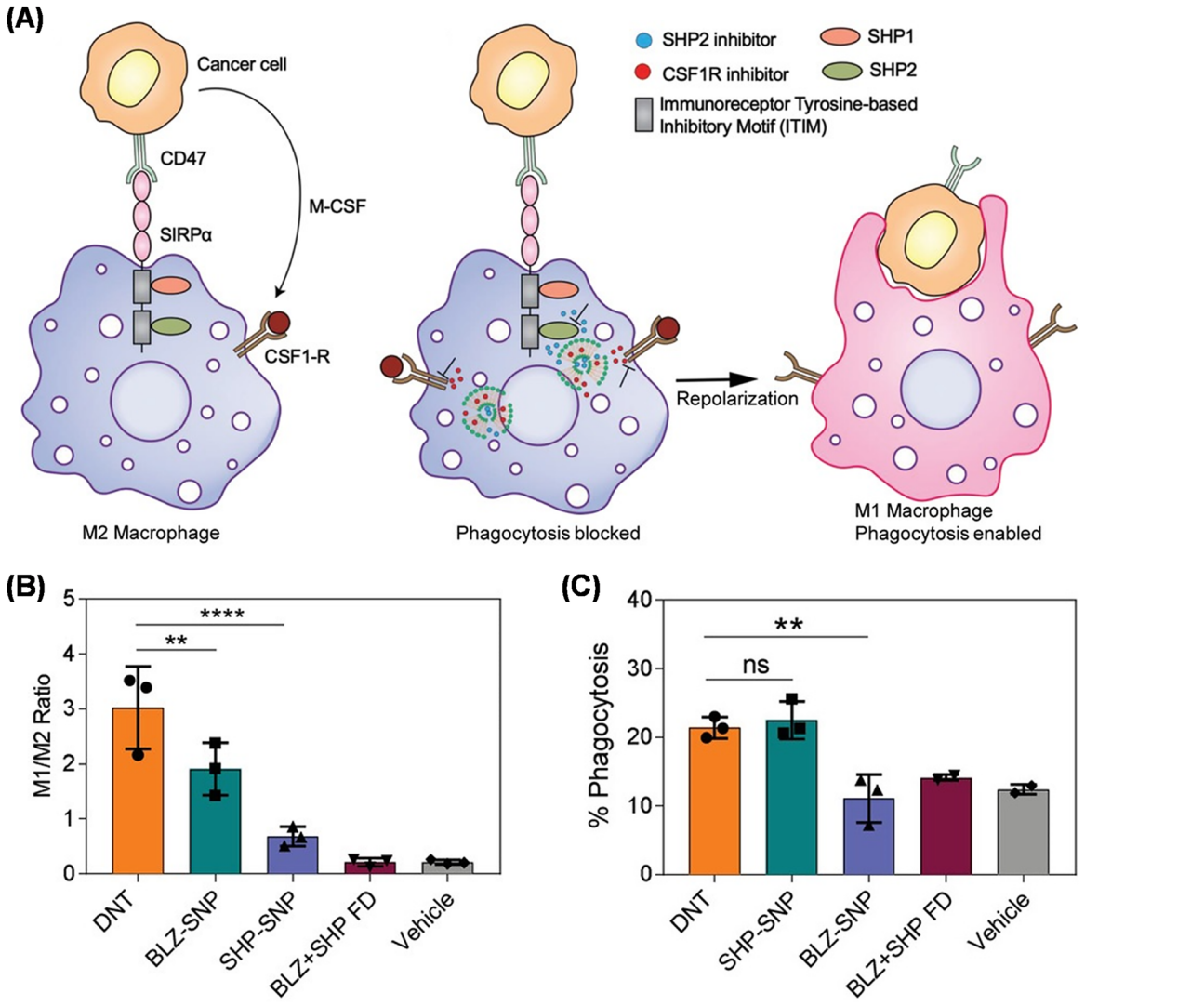


Figure 6: CSF1R-and SHP2-inhibitor-loaded nanoparticles (DNTs) enhance cytotoxic activity and phagocytosis in TAMs. (A) Schematics show deterministic codelivery of DNTs to the M2 polarized macrophage leads to concurrent inhibition of CSF1R and SHP2 which results in repolarization of macrophages to an antitumorigenic M1 phenotype while simultaneously increasing the phagocytic index. (B) RAW264.7 macrophages were polarized to M2 phenotype by treatment with IL-4 for 24 h, following which treatments were added in fresh medium for 48 h and sample was collected for FACS. Graphs demonstrating expression of M1/M2 ratio ($CD11b^+CD80^+$)/($CD11b^+CD206^+$) on macrophages as quantified from flow cytometry at 48 h following different treatments of 500×10^{-9} M concentration. Data shown are mean ± s.e.m. (n=3). (C) RAW264.7 macrophages were stained with cell trace far red and incubated with different treatments for 48 h. Following this, the cancer cells were stained with cell trace CFSE and cocultured with treated macrophages for 4 h and FACS was performed. Graphs show the percentage of macrophages that are phagocytic in a coculture of RAW264.7 with 4T1 breast cancer. Data shown are mean ± s.e.m. (n=3). Statistical analysis was performed with one-way ANOVA followed by Newman–Keuls post-test. ns, not significant; **p<0.01, ****p<0.0001. FACS, Fluorescence Activating Cell Sorter; CFSE, Carboxyfluorescein Succinimidyl Ester. Reproduced with permission [245]. Copyright 2019, Wiley-VCH.

molecules in order to escape from immune surveillance of cytotoxic T cells, since NK cell activation is partly suppressed by its inhibitory receptors binding to MHC-I. ADCC mechanism of NK cells is achieved through the systematic administration of therapeutic mAbs directed at tumor-associated antigens. Several of these mAbs, such as rituximab and trastuzumab, have proved evidence for the significance of NK cell-mediated ADCC, thus illustrating the therapeutic potential of NK cells. In addition to direct cytotoxicity, NK cells also generated its effects by means of pro-inflammatory cytokines, particularly of IFN-γ, TNF, granulocyte–macrophage colony stimulating factor (GM-CSF), as well as chemokines; these biologically active proteins possess great anti-proliferative, anti-angiogenic and pro-apoptotic effects on cancer cells, as well as the ability to enhance cytotoxic T lymphocyte responses.

Basically, there are several classical approaches boosting NK cells mediated anti-tumor cytotoxicity by lipid-based nanoparticles, which include enhancing the infiltration of NK cells to the TME or promoting the NK cell mediated recognition and cytotoxicity towards tumor cells [252].

Enhancing infiltration

Nakamura et al. [253] designed YSK05-liposomes encapsulating cyclic-di-gemcitabine monophosphate (c-di-GMP/YSK05-Lip) to efficiently deliver c-di-GMP, a ligand of STING signal pathway, to the cytosol of NK cells. The intravenous administration of c-di-GMP/YSK05-Lip into mice effectively induced the production of type I IFN as well as the activation of NK cells, increased the infiltration of NK cells in a lung metastasis mouse model, thus led to an NK cell dominating antitumor effect. As consequence, c-di-GMP/YSK05-Lip contributed to MHC-I nonrestricted antitumor immunity mediated by NK cells (Figure 7).

Promoting recognition

Liu et al. [254] developed a TGF-β inhibitor and selenocysteine co-delivering nano-emulsion system (SSB NMs) to overcome the therapeutic limitations caused by down-regulation of recognition ligands on the tumor cell surface. SSB NMs could markedly enhanced the lytic potency of NK92 cells by over 2 folds; a subtoxic dose of SSB NMs notably sensitized MDA-MB-231 triple-negative breast cancer cells to NK cells derived from clinical patients, leading to a more than 13 folds increase in cancer lysis. Mechanism research uncovered that the sensitizing effects relied on NKG2D signaling with the involvement of DNA damage response. SSB NMs also efficaciously suppressed TGF-β/TGF-β RI/Smad2/3 signaling, which thus enhanced NKG2D ligand expression on tumor cell membrane and stimulated NKG2D surface expression on NK92 cells, eventually resulting to the elevated immune response.

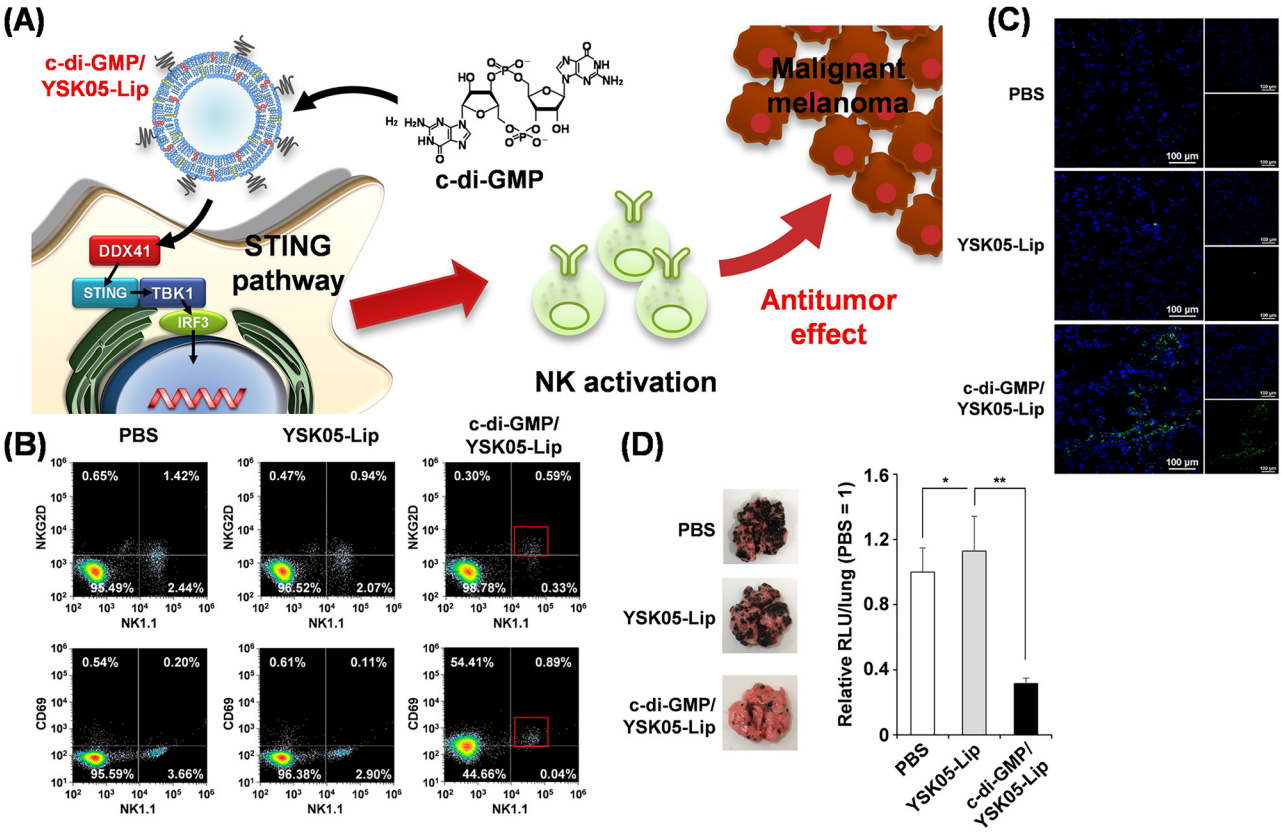


Figure 7: Liposomes loaded with a stimulator of interferon genes (STING) pathway ligand enhance cancer immunotherapy against metastatic melanoma. (A) c-di-GMP/YSK05-Lip efficiently deliver c-di-GMP to the cytosol of NK cells. (B) The $CD3^+$ cell population was excluded and the remaining cells were analyzed on NK1.1/NKD2G or NK1.1/CD69 plots. (C) CLSM images obtained by histological analysis of lung. Green fluorescence and blue fluorescence show NK cells (Cy5) and nuclei (Hoechst 33342), respectively. Bars=100 μm. (D) Pictures of the lungs collected on day 21 and quantitative analysis of lung metastasis by measuring luciferase activities. Luciferase activities are expressed as relative light units (RLU) per whole lung. The value of PBS treated group represent 1. Values are the mean ± SEM (n = 7–9, *p<0.05, **p<0.05). (E) The levels of IFN-β in serum was measured by ELISA. Data are the mean ± SEM (n=3, **p<0.01).STING, stimulator of interferon genes; Lip, Liposome; NK, natural killer, CLSM, confocal laser scanning microscope, RLU relative light units; IFN, interferon; ELISA, enzyme-linked immuno sorbent assay; GMP, gemcitabine monophosphate. Reproduced with permission [253]. Copyright 2015, Elsevier B.V.

Biber et al. [255] engineered a kind of non-viral, lipid-based nanoparticles encapsulating siRNAs to target three intrinsic inhibitory NK cell signaling pathways critical for suppression of NK cell activity, namely SHP-1, Cbl-b, and c-Cbl. This lipid-based nano-carrier effectively and safely silenced *SHP-1* and *Cbls* genes in NK cells *in vitro* and *in vivo*, emancipated NK cell activity against HLA-matched cancer cells, and prolonged survival in humanized murine models.

Activating NK cells

Munich et al. [256] showed another way of targeting NK cells to facilitate immunotherapy by isolating exosomes from DCs (DCex). These DC-derived exosomes expressed multiple TNF superfamily ligands on their surface, which could effectively activate NK cells and stimulate them to secrete IFN-γ *in vitro* upon the interaction of DCex TNF with NK-cell TNF receptors.

Immunosuppressive cells

Myeloid-derived suppressor cells

MDSCs which can directly inhibit T cells in a cell-cell manner and include a heterogeneous population of immature cells, are important obstacles for natural antitumor immunity and immunotherapy. MDSCs also inhibit functional antigen presentation of DCs and drive macrophage differentiation to M2 type. Infiltrating MDSCs involve immunosuppressive microenvironment creation and suppress antitumor immune responses, and their depletion can restore effector T cells anti-tumor responses [257].

Liposomes targeting MDSCs have been used to enhance tumor immune response. Max Kullberg et al. [258] reported a drug delivery liposome that selectively targets polymorphonuclear(PMN) MDSCs. This system could conjugate with activated complement C3 through disulfide bonds after intravenous injection, then be internalize into PMN-MDSCs, which express the receptor for activated C3. This study demonstrated the MDSCs targeting potential of the liposomes *in vivo* in tumor-bearing mice.

Elimination of MDSCs by DOX or inhibition of MDSCs by sunitinib *in vivo* has been demonstrated [259–261]. For combination therapy based on MDSCs decrease, Jamshid Gholizadeh Navashenaq and his colleagues [262] designed a liposome system with P5 peptide to delivery Doxil®, and showed that it could boost the efficacy of immunotherapy so that it could be utilized in human epidermal-growth-factor receptor 2 (HER2) positive breast cancer treatment. Liposomes are also used as delivery systems for other drug loaded nanoparticles and as multiple delivery platforms to achieve programmed release of therapeutic agents. Xing et al. [263] reported a liposomal formulation of DOX-loaded small-sized polymeric nanoparticles (DOXNP) and free sunitinib in the aqueous cavity. The photosensitizer porphyrin–phospholipid on the liposomal membrane can increase the membrane permeability under NIR irradiation and release sunitinib first. Sunitinib exerts MDSCs inhibition and tumor immunosuppressive environment reversing effects by acting synergistically with subsequently released DOXNP. The outcome results indicated therapeutic immune enhancement.

Another multifunctional nanoplatform combined photodynamic therapy and MDSCs-targeting immunotherapy was also based on liposome. Ding et al. [264] established a liposome LIC for colon cancer treatment, which encapsulated phosphoinositide 3-kinase gamma (PI3Kγ) inhibitor IPI-549 and photosensitizer chlorin e6 (Ce6). While Ce6 caused ICD under laser irradiation, IPI-549 released by LIC inhibited PI3Kγ in the MDSCs to downregulate arginase 1 (Arg-1) and ROS, which can promote MDSCs apoptosis and reduce their immunosuppressive activity to $CD8^+$ T cells. This multifunctional system demonstrated high efficacy in colon cancer treatment (Figure 8). In addition to liposomes, other lipid nanoparticles are also used to deliver drugs with MDSC scavenging activity. Kong et al. [74] constructed a dHMLB with co-encapsulation of ATRA (with great potential for regulating MDSC-induced immunosuppression.), DOX and IL-2 for chemo-immunotherapy. And the LCP nanoparticle loading with GMP, an MDSC-depleting chemotherapeutic agent, was reported to improve the delivery efficiency of GMP and depleted MDSCs and Tregs [265].

Regulatory T cells

Tregs have been identified as important negative regulatory cells to suppress adaptive immune responses [266]. Tregs have high expression of CLTA-4 that can interact with CD80/86 on APCs to induce the production of IDO, which may play an important role in immune suppression [267]. Although the potential pro-tumor mechanisms of Tregs remain a challenge, it is well-established that infiltration of Tregs correlates with poor prognosis. Recent evidences regarding the role of intratumor Tregs have suggested that their selective depletion will be a desirable approach for efficacious immunotherapy [268]. Ursolic acid (UA) can regulate Tregs by inhibiting ATAT5 phosphorylation and IL-10 secretion, but its high hydrophobicity limits its application. Although it has been prepared as nanocrystals or loaded in nanoparticles, its half-life *in vivo* remains unsatisfactory. In order to overcome this obstacle, recently, Zhang

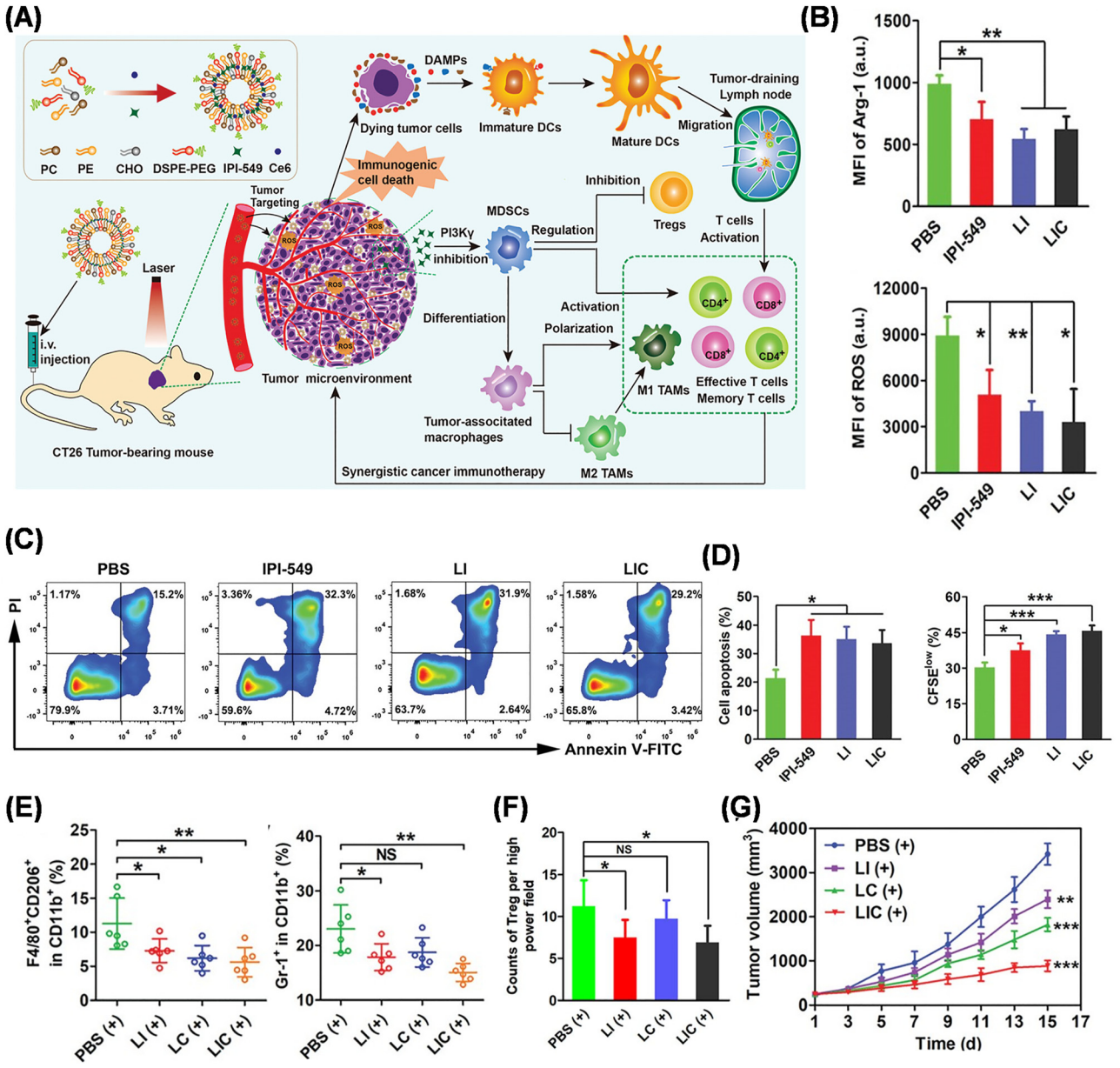


Figure 8: Multifunctional nanodrug mediates myeloid-derived suppressor cells (MDSCs)-targeting immunotherapy and synergistic photodynamic therapy of colon cancer. (A) Multifunctional nanodrug using liposome to co-encapsulate a photosensitizer Ce6 and a PI3Kγ inhibitor IPI-549 and the LIC-mediated colon cancer inhibition by synergistic immunogenic photodynamic therapy and MDSCs-targeting immunotherapy. (B) Statistical analysis of proportions of Arg-1 expression and ROS levels in MDSCs receiving various treatments (n=3). (C) Cell apoptosis determined by flow cytometry and statistical analysis of apoptotic rates in MDSCs receiving different treatments (n=3). (D) Statistical analysis of proliferation rates of $CD8^+$ T cells after being cultured together with nanodrug-pretreated MDSCs (n=3). (E) Statistical analysis of MDSCs ($CD11b^+Gr\text{-}1^+$) ratio and M2-like TAMs ($CD11b^+F4/80^+CD206^+$) ratio in $CD11b^+$ cells in different treatment groups (n=6). (F) Corresponding quantification analysis of Foxp3 expression in CT26 tumors in different treatment groups. (G) Tumor growth curves for mice receiving different treatments (n=6). Data are shown as mean ± SEM, *p<0.05, **p<0.01, ***p<0.001. MDSCs, myeloid-derived suppressor cells; PI3Kγ, phosphoinositide 3-kinase gamma; Ce6, chlorin e6. Reproduced with permission [264]. Copyright 2021, Wiley-VCH.

et al. [269] designed UA-liposomes that were generated by active loading assisted by hydroxypropyl-β-cyclodextrin (HP-β-CD) and enabled the formation of UA-Ca crystal structure inside the liposomes. This system achieved sustained release of UA, down-regulation of IL-10 secretion, and decreased the number of MDSCs and Tregs residing in tumor tissue after *in vivo* administration, ultimately more effectively preventing tumor growth (Figure 9).

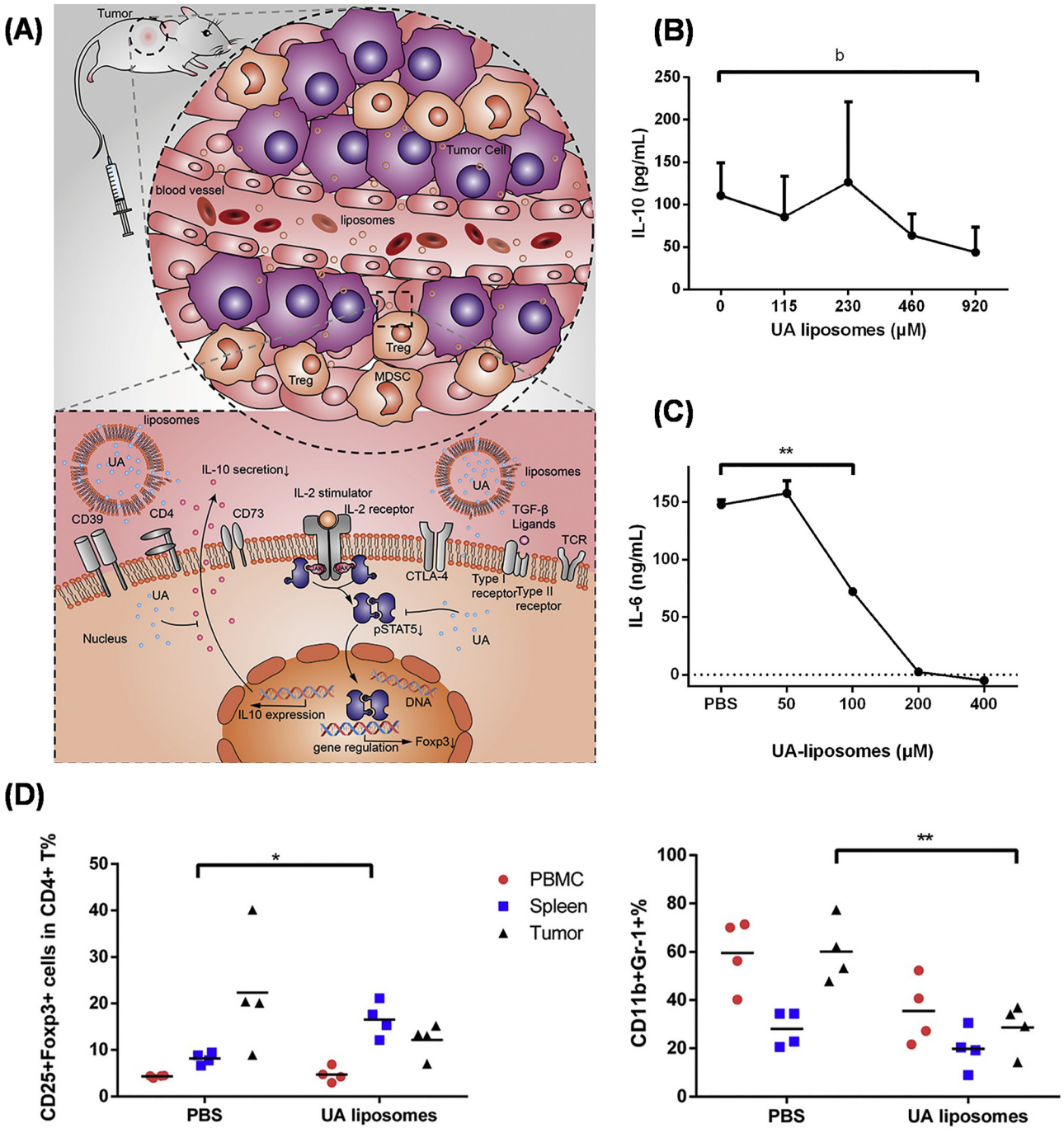


Figure 9: Liposome-based delivery of Ursolic acid (UA) for Tregs activities and modulating tumor microenvironment (TME) in cancer immunotherapy. (A) The proposed pharmacological mechanisms of UA-liposomes. UA-liposomes were injected and then extravasated through the leaky vasculatures at the tumor site. (B) IL-10 secretion from the mouse spleen $CD4^+$ T cells after treating with UA-liposomes. (C) Tumor volumes changes during treatments. (D) Summary of the percent of Tregs ($CD25^+Foxp3^+$) and MDSCs ($CD11b^+Gr\text{-}1^+$) changes after UA-liposomes treatment; Statistical analysis was done using Student's *t*-test (SPSS Statistics 18.0). *p<0.05; **p<0.01; *a*=0.09; *b*=0.077.UA, Ursolic acid; TME, tumor microenvironment. Reproduced with permission [269]. Copyright 2020, Elsevier B.V.

In addition, many relevant studies have proved that the downregulation of Tregs is conducive to enhancing the specific immune response, thus playing a more efficient role in cancer cell killing [264, 265, 270].

Conclusions

In this review, we described lipid-based nanoparticles and their application in cancer immunotherapy, especially those targeting T lymphocytes and macrophages. As the special examples discussed above, nanoparticles could yield robust antitumor immune effects through enhancing different steps of cancer immunity cycle when they were well-established by diverse functionalized delivery platforms. In a time-space controlled way, nanoparticles can be endowed with the properties of targeting, sustained release, and even stimuli-responsiveness, thus can exert better antitumor efficiency and reduce off-target toxicity than their free payloads. Due to the safety profile and easily modified quality of biocompatible and biodegradable lipid materials, nanoparticles formulated by various lipids achieved great success in clinical translation, especially liposomes and LNPs. Therefore, lipid-based nanoparticles have promising development in the area of cancer immunotherapy.

However, there is a huge disconnect between a large body of literature describing diverse nanoparticles for potential cancer immunotherapy and a handful of marketed once approved by FDA [271], which suggests that there are much more recognized or unknown challenges we need to surmount. For example, the tropism of nanoparticles for MPS make its efficiency and safety unclear [22]. Meta-analysis showed that only 0.7 % (median) of nanoparticles could be successfully delivered to solid tumors [25], and about 30–99 % of them would be absorbed and retained by the liver and spleen after administration [272], which may be the main reason for limiting the clinical translation of preparations for intravenous administration. Besides, highly reproducible fabrication method of relatively complexed nano-delivery system is also a great obstacle confronting the further development of nanoparticles [23]. The weak correlation between *in vitro* and *in vivo* effects, as well as small animal and large animal models is another dilemma we can't evade. On the one hand, novel research tools are in urgent need to permit more accurate and real analysis, such as cell activation state (mass cytometry) and cell type (multiplexed ion beam imaging) instead of conventional method, such as enzyme-linked immunosorbent assay (ELISA) and flow cytometry [23]. On the other hand, advanced ex vivo evaluation models can mimic human physiological environment in a more precise way, while avoiding time-consuming animal experiments. For example, transplantable lymphoid-like organoids can already be well-designed to obtain accurate anatomy and physiology, and the deposition of special cytokines, immune cells and matrix can be achieved precisely through 3D printing. This advance will contribute to our comprehensive understanding of the influence and fate of nanoparticles in the immune microenvironment [273]. Besides, low drug loading capacity and premature drug release *in vivo* of lipid-based nanoparticles need to be addressed before entering the clinical trials.

Although innovation in drug delivery technologies could overcome lots of challenges of cancer immunotherapies, only a small range of tumor types and patients do benefit from nanomedicines. For example, the high interstitial fluid pressure, dense ECM and compressed vasculature of solid tumors hinders T cell infiltration and drug accumulation. This reminds us that further understanding of tumor biology and the interaction between nanoparticles and tumor immune microenvironment guide the design of delivery system [17]. As for high heterogeneity of tumors, suitable biomarkers of response are needed to achieve more efficiency and fewer toxicities of nanomedicine [104]. Multifunctional and externally or internally triggered drug delivery system can be implemented according to the divers TME to stimulate robust response on demand. Besides, future fundamental investigations should focus on the biomaterial-immune cell interaction, especially the influence of physiochemical properties (size, shape, charge, hydrophobicity) of nanoparticles on immune response [17]. Finally, because of the complexed and interactive immune pathways, new strategies to leverage the different signaling synergistically or combination therapy may increase the percentage of patients who benefit from immunotherapy.

Lipid-based nanoparticles for cancer immunotherapy is thus a promising area worthy of further consideration and investigation. It is hoped that this review will prove essential in future innovations of drug delivery system for cancer immunotherapy.

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