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Engineered biological nanoparticles as nanotherapeutics for tumor immunomodulation

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Biological nanoparticles, or bionanoparticles, are small molecules manufactured in living systems with complex production and assembly machinery. The products of the assembly systems can be further engineered to generate functionalities for specific purposes. These bionanoparticles have demonstrated advantages such as immune system evasion, minimal toxicity, biocompatibility, and biological clearance. Hence, bionanoparticles are considered the new paradigm in nanoscience research for fabricating safe and effective nanoformulations for therapeutic purposes. Harnessing the power of the immune system to recognize and eradicate malignancies is a viable strategy to achieve better therapeutic outcomes with long-term protection from disease recurrence. However, cancerous tissues have evolved to become invisible to immune recognition and to transform the tumor microenvironment into an immunosuppressive dwelling, thwarting the immune defense systems and creating a hospitable atmosphere for cancer growth and progression. Thus, it is pertinent that efforts in fabricating nanoformulations for immunomodulation are mindful of the tumor-induced immune aberrations that could render cancer nanotherapy inoperable. This review systematically categorizes the immunosuppression mechanisms, the regulatory immunosuppressive cellular players, and critical suppressive molecules currently targeted as breakthrough therapies in the clinic. Finally, this review will summarize the engineering strategies for affording immune moderating functions to bionanoparticles that tip the tumor microenvironment (TME) balance toward cancer elimination, a field still in the nascent stage.

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1. Introduction

Malignant diseases are on the rise and present a global health problem. The probability of developing cancer increases with advancing age. The conventional management of cancer therapy has centered on the trifecta of surgical resection, chemotherapeutics, and radiation therapy with alternative treatment options, such as cryoablation, immunotherapy, and exploratory clinical trials for refractory, recurrent, or advanced diseases.[1] The advent of nanomedicine has achieved progress in providing critical options for patients and physicians facing the dilemma of viable treatment selections after standard care failure. The rise of nanoscience and nanotechnology in the 1980s significantly boosted the synthesis of laboratory-derived nanoformulations for medical purposes. Currently, nanomedicine is considered a viable treatment option, with FDA-approved and exploratory formulations available for clinical trials.[2]

The appeal of nanotechnology for drug delivery and imaging is the enormous potential of targeting the treatment agents to specific targets in the body, including the brain.[3] Other advantages of nanoencapsulation include the ability to confer controlled drug release and increased drug stability. Various inorganic and organic materials, such as silver and gold nanoparticles, have been used to fabricate advanced nanoformulations to achieve these end goals.[4,5] Organic materials such as liposomes, dendrimers, polymeric micelles, nanoemulsions, and carbon nanomaterials were extensively explored for drug delivery. The appeal of these various nanotechnology systems is that they can encapsulate both hydrophobic and hydrophilic drugs, increasing their stability and bioavailability to target tissues.[6] Despite their advantages and increased efficacy, toxicity issues plagued using inorganic nanomaterials. Their potential bioaccumulation and limited knowledge of their long-term effects on the body and the environment limit their clinical utility. Organic nanoparticle systems are limited by low drug-loading capacity, high local absorption, possible drug leakage, rapid elimination *via* the reticuloendothelial system (RES), and non-specific interactions with biological components, which limits their treatment efficacy.[7–11] Thus, the collective disadvantages have hindered the progress of synthetic formulations into clinical translations.

Nanoparticles are defined as small materials ranging from 1–100 nm. Although nanoparticles in biomedical research were used to describe various laboratory-derived synthetics, nanoparticles have existed in nature for millions of years, with sophisticated and precise assembly systems encoded in the genetic material of living organisms.[12] These biological nanoparticles (bionanoparticles) are derived and isolated from organic living systems. They utilize their complex assembly machinery to manufacture biological products that could be further engineered in the lab for specific functionalities. Bionanoparticles are pursued due to their diverse function in nature, biocompatibility, non-toxicity, and extended circulatory lifespan. The advent of bionanoparticles in the current landscape is attributable to the impedance of synthetic formulations to clinical fruition, despite the significant efforts, mainly because of their toxicity and biodistribution issues. Hence, bionanoparticles are considered a new paradigm for designing safe and reliable nanomedicine. The immune system is intrinsically able to recognize and eliminate malignant tumor cells. However, a cancerous mass evolved to hide from and escape immune surveillance. Adding immunomodulatory functions to bionanoparticle design, such as promoting M2 to M1

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macrophage polarization or inducing inflammatory redox reactions, could potentially enhance nanoparticle-based approaches for cancer therapy by remodeling the tumor microenvironment for immune-mediated tumor eradication. To achieve such an end, the research community has spent effort on two fronts: (1) developing biomimetics that are fabricated with biological molecules on their surface to mimic the immunomodulatory functions of natural biological constituents and (2) engineering bio-immune components to immunologically inert bionanoparticles. In this regard, biomimetic formulation can comprise biological components and synthetic materials. The added biological components to organic or inorganic nanoparticles are vital in the formulation as they confer biocompatibility, specificity, and increased circulation time.

In this review, we will place biomimetic nanoparticles engineered with biologically sourced components under the umbrella of bionanoparticles together with biologically sourced, self-assembling nanoparticle systems. The objectives of this review are threefold. Firstly, it aims to facilitate future bionanoparticle design by presenting the common immune-related aberrations induced in the tumor microenvironment (TME). Secondly, the review will focus on the most applied strategies for achieving nanoimmunomodulatory functions. Finally, the limitations of each approach and potential avenues in the field of bionanoparticle design will be discussed.

2. Tumor-induced aberrations of the immune environment

Genetic instability is an evolving hallmark of cancer, resulting in the expression of abnormal proteins foreign to the immune system.[13] Such foreign entities on the cancer cells serve as markers and immunogenic neoantigens that can spontaneously trigger $CD8^+$ T-cell responses, which involve direct killing of the target *via* granule and cytokine secretion. Hence, the immune system is critical in identifying nascent tumor tissues and responding appropriately to uncontrolled cell division.[14] The network of cell, molecular, and metabolic, effectors in the TME involved in tumor control and elimination is complex. Several deviations from the typical immune response can breed dysregulation, leading to molecular processes that drive oncologic pathologies. Recognizing the immune dysregulation within the TME is crucial for developing targeted therapies to disrupt the dynamics of interactions between cancer cells and the microenvironment, restore tissue homeostasis, and enhance antitumor immune responses. This section introduces and summarizes the cellular, molecular, and metabolic effectors involved in tumor-induced immune aberrations to provide a comprehensive view of this topic.

2.1. Immunosuppressive cells

2.1.1. Regulatory T-cells (Tregs). Regulatory T-cells (Tregs) are a distinct T lymphocyte cell subpopulation (cluster of differentiation, $CD4^+$) responsible for immunological self-tolerance. They are defined by the Foxp3 (Forkhead box p3) gene expression, a member of the forkhead/winged-helix family of transcriptional regulators. T lymphocytes develop in the bone marrow from a common lymphoid progenitor (Fig. 1). The progeny programmed to produce T-cells leave the bone marrow and migrate to the thymus.[15] Tregs protect the host from developing autoimmune diseases and allergies. However, in oncologic malignancies, Treg dysregulation prevents mounting an effective antitumor activity.[16] They are further classified into three subfractions based on the expression levels of distinct and definitive molecular markers: (i) Naïve Treg cells, $Foxp3^{lo}CD45RA^+CD25^{lo}$, (ii) effector Tregs, $FoxP3^{hi}CD45RA^-CD25^{hi}$, and (iii) non-Treg cells, $FoxP3^{lo}CD45RA^-CD25^{lo}$ (Fig. 2). Upon leaving the thymus, the Treg cells are naïve and possess weak immunosuppressive function. The naïve Treg cells can be activated into effector Treg cells (eTreg) following appropriate T-cell receptor (TCR) stimulation. eTreg cells are the immunosuppressive workhorse within the tumor microenvironment and are the final differentiation stage of the Treg lineage. Non-Treg cells are not immune suppressive but produce inflammatory cytokines such as interferon-γ (IFN-γ) and IL-17. Non-Tregs are the only subpopulation associated with a better clinical prognosis in the TME of colorectal cancers.[17]

Tregs possess multiple chemokine receptors (CCR4, CCR8, CCR10, CXCR3) and are targeted *via* chemoattractant chemokine gradients (CCL17, CCL22, CCL1, CCL28, CXCL0/10/11) secreted within the TME.[19] They can impair the maturation of antigen-presenting cells (APCs) *via* the expression of checkpoint molecules CTLA-4 and PD1 (discussed in Section 2.2), induced by the transcription factor BATF.[20] They also act as an IL-2 cytokine sink through the high expression of CD25 (IL-2 receptor α-chain), resulting in a limited amount of IL-2 in the TME for T-cell proliferation and activation.[21] Treg also contributes to the inhibitory immune environment by secreting anti-inflammatory cytokines such as IL-10 and TGF-β, aiding in tumor growth and metastasis.[22] The abundance of Tregs in the TME is also attributed to the metabolic pathways that breed a favorable environment for Treg proliferation, survival, and functions (discussed in Section 2.3).

2.1.2. Myeloid-derived suppressor cells (MDSCs). Myeloid cells are formed in the bone marrow, differentiated from common progenitors derived from hematopoietic stem cells (Fig. 1). Myeloid stem cells can differentiate to form either erythrocytes, leukocytes, or platelets.[23] Hence, they are vital components of the circulatory and immune systems and are involved in tissue remodeling and repair. The MDSCs are a heterogeneous population of cells with similar morphology to granulocytes and macrophages. They possess potent immunosuppressive activity, which contributes to the pathology of malignancies. Under physiological conditions, colony-stimulating factors (CSF) drive myelopoiesis and their subsequent differentiation into terminal cell types such as granulocytes and macrophages (Fig. 1). In cancerous conditions, neutrophils and monocytes are converted to MDSCs by the overproduction of the regulators and the activation of various molecular pathways, which has been previously discussed in various reviews.[24–26] MDSC development occurs in two phases. The conditioning and myeloid expansion first appears in the bone marrow and spleen. In the next phase,

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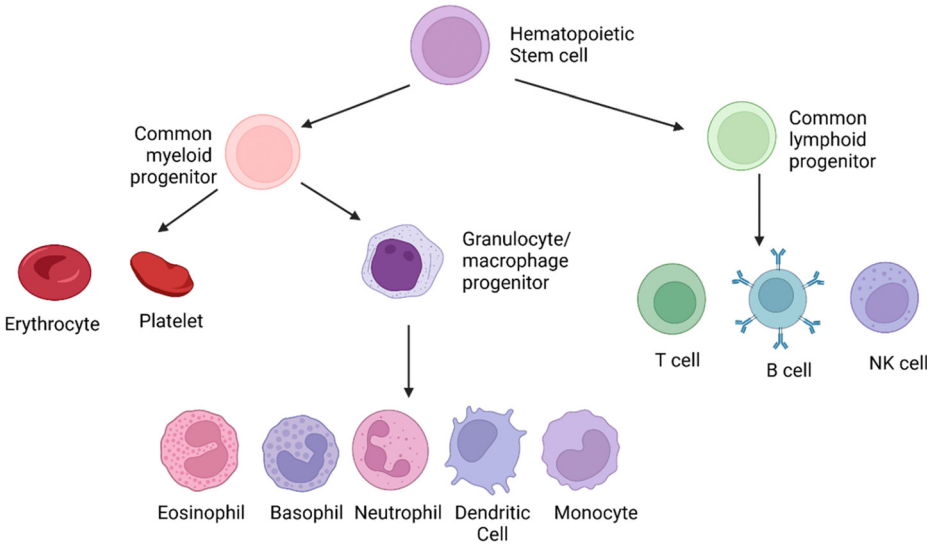

Fig. 1 The origins of the cellular elements of the immune system. The cellular components of blood, the adaptive and the innate immune system, arise from the hematopoietic stem cells in the bone marrow. These cells divide to produce two specialized cells, the common myeloid progenitor and the common lymphoid progenitor. The common lymphoid progenitor gives rise to T-cells ($CD4^+$, $CD8^+$, Tregs), B-cells, and the natural killer (NK cells). T-Cells undergo differentiation in the thymus, while B cells differentiate in the bone marrow. Though NK cells are derived from the common lymphoid progenitor, they lack antigen specificity, which is the hallmark of the adaptive immune response. The common myeloid progenitor will give rise to the leukocytes *via* the intermediary macrophage/granulocyte progenitor. The monocytes (activated to macrophages in the tissues), dendritic cells, and polymorphonuclear granulocytes (eosinophil, basophil, neutrophil) are formed from the intermediary progenitors. The polymorphonuclear granulocytes are distinct in appearance due to their irregularly shaped nuclei.[18] This image was created with BioRender.com.

conditioned pre-MDSCs neutrophils and monocytes are converted to pathologically activated MDSCs in the peripheral tissues, and the TME.[27] The accrual and expansion of MDSCs to the TME are facilitated by the CXCR2 and growth factor receptors on their surfaces that serve as a chemotaxis conduit by interacting with the ligands secreted by the tumor cells. The CXCR2 ligands secreted by the tumor and associated with MDSCs chemotaxis are CXCL5 CXCL2, CXCL1, and CXCL8 (IL-8).[28]

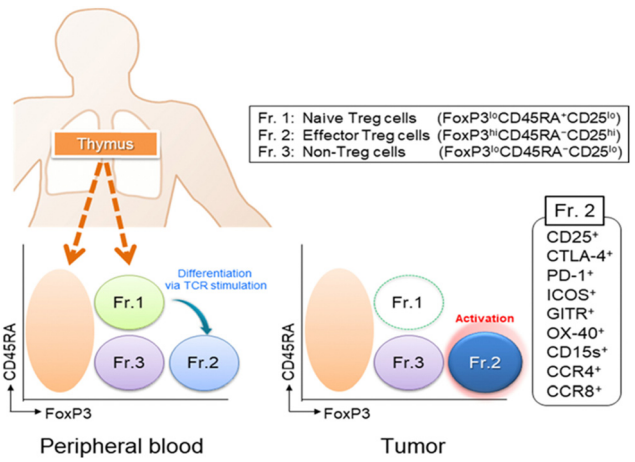

Fig. 2 Tregs are classified into three subfractions: Fr.1 naïve/resting Treg cells (nTreg), Fr.2 effector/activated Treg (eTreg), and Fr.3 non-Treg cells. Typical staining patterns of Treg subfractions in peripheral blood and tumor tissues. Treg cells are found in low frequencies in peripheral blood (1–5%) but are higher in the TME (10–50%). Naive/resting Treg cells are hardly detected in the TME. Reproduced from ref. 16 Copyright 2019 with permission from John Wiley & Sons.

MDSCs are further divided into monocytic (M)-MDSCs and granulocytic/polymorphonuclear (PMN)-MDSCs, defined by specific molecular markers on their surfaces Fig. 3. In mice, the MDSCs are identified functionally by their ability to suppress other immune cells due to the lack of phenotypic cell surface markers that distinguish classical neutrophils/monocytes from MDSCs. In humans, the MDSCs are characterized by the expression of specific markers, such as lectin-type oxidized receptor 1 (LOX1) for PMN-MDSCs and MHCII for M-MDSCs. However, MHCII is an inadequate determinant of M-MDSC in humans. Hence, efforts are ongoing to define the human MDSC subsets further, which will aid in exclusively identifying the suppressor cells in the population and benefit therapeutic strategies aimed at targeting MDSCs within the tumor milieu. MDSCs in the TME contribute to forming the premetastatic niche by facilitating the escape of tumor cells to the circulation and their subsequent engraftment by inducing immune suppression, matrix remodeling, and promoting angiogenesis.[27] In the circulatory system, neutrophil PMN-MDSCs escort the circulating tumor cells (CTCs) and protect them from recognition and killing by NK cells.[29] PMN-MDSCs also promote the extravasation of CTCs through the vasculature by trapping the CTCs in the microvasculature with an extracellular structure called neutrophil extracellular traps (NETs). NETs can also recruit tumor cells to the premetastatic niche *via* CDC25.[30] Data from both *in vitro* and *in vivo* studies suggest the presence of MDSC markers that correlate with cancer conditions in humans.[31,32] Translational studies have also demonstrated the predictive value of MDSCs in esophageal squamous cell carcinoma (ESCC) patients.[33] However, there is a shortage of

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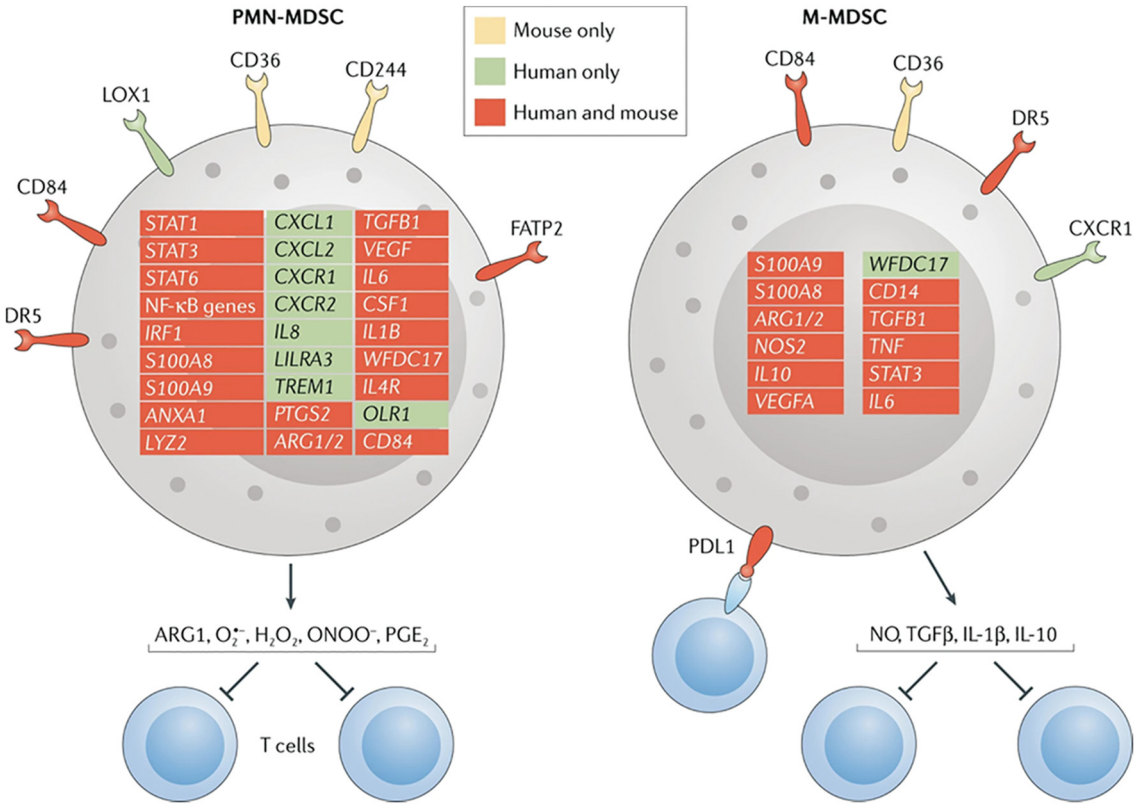

Fig. 3 Distinguishing between the MDSCs subtypes. This figure shows the genes and surface molecules that distinguish the PMN_MDSCs and M-MDSCs from classical neutrophils and monocytes. CXCR1, CXC-chemokine receptor 1; FATP2, fatty acid transport protein 2; LOX1, lectin-type oxidized LDL receptor 1; NO, nitric oxide; PGE2, prostaglandin E2. Reproduced from ref. 27 Copyright 2021 with permission from Springer Nature.

published clinical data on MDSC-targeted cancer therapy due to the lack of novel MDSC targets. Moreover, targeting MDSCs is limited by the heterogeneity of the MDSC populations with varying phenotypes and functions, which makes it a challenge to use specific markers and targets that can inhibit all MSDC subsets.

2.1.3. Tumor-associated regulatory dendritic cells (TADCs). Dendritic cells (DCs) are known for their ability to internalize antigens and present them to naïve T-cells to activate specific immune responses. They can extend the reach of the immune response beyond the initial activation site by migrating to the lymph nodes. The cells in the TME can release soluble factors such as IL-10 that transform immunocompetent DCs into immunosuppressive subtypes during malignancy.[34] After driving the functional switch of conventional DCs to TADCs, the TADCs exert their immunosuppressive effects and drive tumor progression by various molecular and metabolic pathways (Fig. 4). The tumors secrete galectin-1 (Gal-1), a small ~29 kDa protein that induces heparin-binding EGF-like growth factor (HB-EGF) production by the TADCs. HB-EGF is known for its ability to promote tumor progression and invasiveness.[35] High reactive oxygen species (ROS) in the TADCs promote intracellular lipid peroxidation, generating reactive byproducts that can trigger ER stress, leading to uncontrolled lipid accumulation. Abnormal lipid accumulation critically contributes to further DC malfunction by inhibiting the efficient loading of antigenic peptides onto MHC molecules, thereby impairing optimal antigen presentation to T-cells.[36]

The β-catenin/T-cell factor (TCF) pathway in TADCs facilitates the expression of vitamin A-metabolizing enzymes, which catabolize vitamin A to retinoic acid (RA).[37] RA induces Treg responses *via* Foxp3 activation and stabilizes the Treg phenotype.[38] Other metabolic enzymes upregulated in TADCs are those involved in the catabolism of amino acids, namely tryptophan and L-arginine. Upregulated IDO1 and IDO2 degrades tryptophan, reducing the tryptophan levels in the TME. Upregulation of L-arginase catabolizes the breakdown of L-arginine. The catabolic activities deplete these amino acids, essential for T-cell effector function and proliferation.[39–41] A separate mechanism also depletes L-arginine in the tumor milieu. TADCs overexpress NOS, and NOS requires L-arginine as a substrate to produce NO, which accumulates in the TME. NO can induce the conversion of $CD4^+CD25^-$ T-cells to $CD4^+CD25^+$ Treg cells, resulting in Treg-induced immune suppressiveness in the TME.[42] The expression of iNOS also suppresses DC differentiation into effector APCs.[43] Tumor-derived prostaglandin E2 (PGE2) can also induce DC-mediated T-cell tolerance. Additionally, PGE-2 affects DC activity by blocking IL-12 expression and inducing the expression of regulatory molecules in T-cells, specifically CD25 and IDO, that will deter their stimulation.[44,45] Functional proteins expressed on the surface of TADCs play various roles in inducing T-cell suppression. One of the surface

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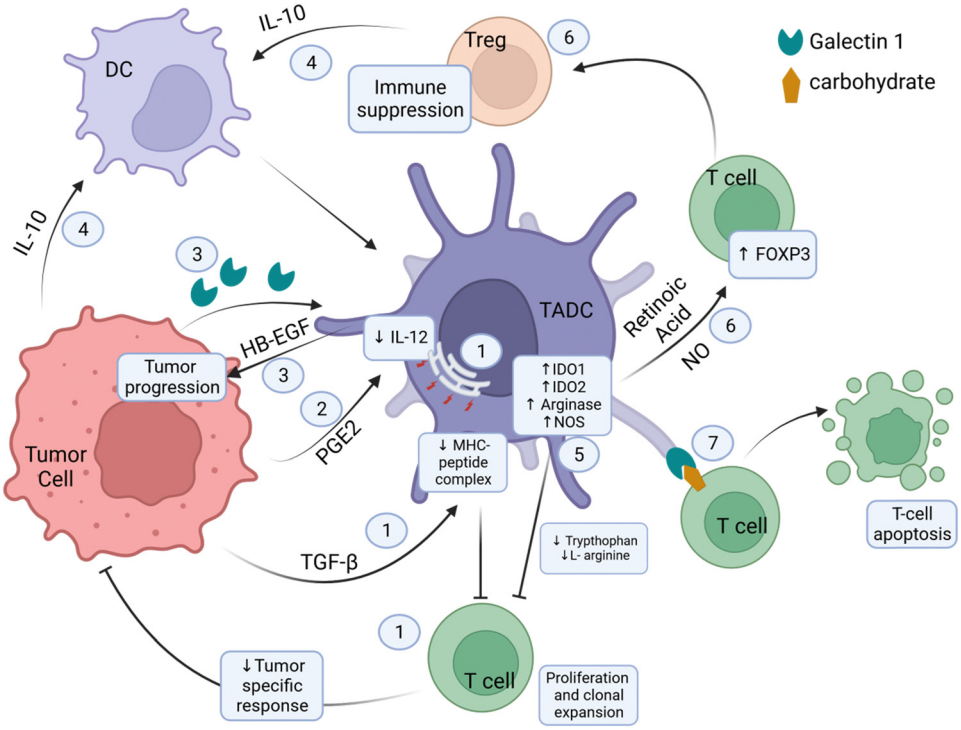

Fig. 4 Tumor-DC-T-cell dynamics in the TME. ① The combination of tumor-derived TGF-β and ER stress results in inefficient loading of antigenic peptides on the MHC molecules, reducing the antigen-presenting ability of the DC to the T-cells. Hence, T-cells are unable to activate and mount a tumor-specific immune response. TGF-β limits the ability of the TADC to migrate to the lymph nodes and present antigens to T-cells. ② Tumor-derived PGE2 blocks IL-12 expression, which is essential for T-cell activation. ③ Secretion of galectin 1 by tumor cells induces TADC to secrete HB-EGF, which promotes tumor progression. ④ Tumor cells and Treg cells secrete IL-10, inducing the polarization of DCs to TADCs. ⑤ Overexpression of IDO1, IDO2, and arginase depletes tryptophan and L-arginine, inhibiting T-cell proliferation and clonal expansion. ⑥ Overexpression of iNOS leads to NO build-up. NO and retinoic acid produced by the TADCs induce FOXP3 expression, leading to T-cell differentiation to Treg cells. Treg cells induce and maintain an immunosuppressive environment in the TME. ⑦ Galectin 1 on TADC interacts with the carbohydrates on the surface of T-cells to induce apoptosis. HB-EGF, heparin-binding EGF-like growth factor; FOXP3, forkhead box P3; PGE2, prostaglandin E2; TGF-β, transforming growth factor β; IDO, indoleamine-2,3-dioxygenase; NOS, nitric oxide synthase; NO, nitric oxide; MHC, major histocompatibility complex. This image was created with BioRender.com.

molecules is Gal-1, which can also be secreted and bind to surface carbohydrates on T-cells to mediate downstream effects of inducing T-cell apoptosis.[46] Gal-1 also promotes T-cell differentiation to Foxp3$^+$ Tregs.[47,48] TADCs also express inhibitory immune checkpoint ligand PD-L1 on their surface, which will engage surface PD-1 on T-cells, thereby transmitting inhibitory signals.[49]

Owing to its powerful antigen-presenting ability, the DCs were harnessed as a vaccination tool for cancer treatment in the late 1990s. *Ex vivo* preparations of tumor antigens-activated DCs were prepared and infused in patients. The first DC-based preparation (sipuleucel-T) for treating prostate cancer was FDA-approved in 2010.[50] Combining DC-based therapy with chemotherapy and checkpoint inhibition was also investigated to provide safer and more effective treatment outcomes.[51,52] A *meta*-analysis study of DC-based clinical trials confirms the clinical effectiveness of the regimen in improving mid-term survival for glioblastoma multiforme (GBM) patients and recurrence-free survival in HCC patients.[53,54] Despite being promising, DC-based cancer vaccines for cancer immunotherapy are associated with several limitations, such as inconsistent vaccine efficacy due to variations in DC quality and quantity, inefficient loading of tumor antigens into DCs, and the plasticity of the DCs which makes them a challenge for clinical use. These limitations underscore the need for further research and development to optimize the efficacy of DC-based cancer vaccines in clinical settings.

2.1.4. Tumor-associated macrophages (TAMs). Macrophages are cellular components of the innate immune system. They specialize in engulfing and eliminating particles larger than 0.5 μm in diameter, such as apoptotic cells, microorganisms, and foreign entities, *via* a process called phagocytosis.[55] Like all immune cells, macrophages are derived from a pluripotent hematopoietic stem cell in the bone marrow and leave the bone marrow into the circulatory system in an inactivated stage called the monocytes (Fig. 1). Monocytes will migrate to the tissues, differentiating into resident tissue macrophages such as the Kupffer cells in the liver and Langerhans cells in the skin. Generally, the macrophages are accrued to the TME *via* chemotaxis from the periphery and undergo phenotype switching to TAMs in response to external stimuli.[56] Recent evidence shows that tissue-specific embryonic-derived resident macrophages are the source of TAMs for certain tumors.[57] The significance of the differences in monocyte origin and their distinct roles in tumor development is currently unknown.

TAMs in the TME are divided into two polarization states, namely M1 and M2 types. A coordinated network of stimuli,

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signaling pathways, transcriptional, and post-translational factors tightly control the polarization process.[56,58] M1 macrophages are defined by the expression of iNOS and the generation of ROS.[59] Due to their stimulation by infection and inflammatory events, the M1 phenotype possesses strong antigen presentation *via* MHCII expression and produces high levels of inflammatory cytokines IL-12 and IL-23.[59] M2 phenotypes migrate and are activated in healing tissues that require debris removal, angiogenesis, injury repair, and tissue reconstruction.[60] M2 macrophages are characterized by the upregulated expression of scavenger receptors, mannose receptors, dectin-1, DC-SIGN, and chemokine receptors (CCR2, CXCR1, and CXCR2). M2 macrophages express arginase, producing ornithine and polyamines *via* the arginase pathway.

In the TME, TAMs are generally polarized to the M2 phenotype, which enhances tumor survival by facilitating tissue regeneration and remodeling. However, the M1 and M2 macrophages are present in the TME proportionate to the balance of pro and anti-inflammatory markers in the tumor milieu (Fig. 5). Tumor-derived cytokines such as IL-4, IL-10, TGF-β, macrophage-CSF (MCSF), and CSF-1 have been implicated in M2 phenotype polarization.[61] Other factors released by the tumor cells that are M2-polarizing include osteopontin and sonic hedgehog (SHH) protein.[62] These factors drive M2 polarization by facilitating monocyte chemotaxis and activating molecular pathways (Hedgehog, Hh) that recruit TAMs and MDSCs for immune modulation.[63,64]

M2 phenotype TAMs create a hospitable environment for tumor survival by releasing factors that promote tumor progression and inhibit the effector immune cells. Tumor-proliferating factors expressed by M2 TAMs include the epithelial growth factor (EGF) and other ligands of the epithelial growth factor receptor (EGFR) family, the platelet-derived growth factor (PDGF), TGF-β1, and the basic fibroblast growth

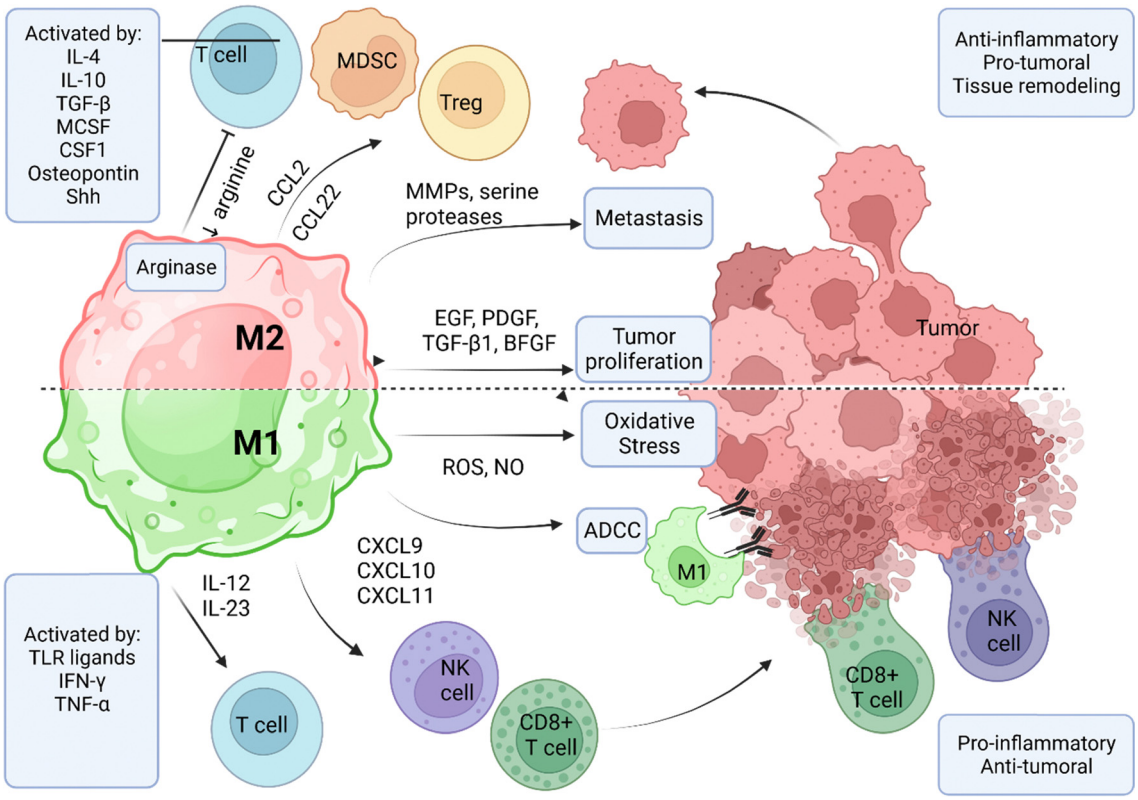

Fig. 5 Functional differences between M1 and M2 TAM phenotypes and their role in the tumor microenvironment. M1 TAMs are activated by TLR ligands or pro-inflammatory cytokines such as TNF-α and IFN-γ. M1 TAMS are characterized by high production of inflammatory cytokines, ROS, and NO in the TME, leading to tumor oxidative stress and DNA damage. Inflammatory cytokines CXCL9, CXCL10, and CXCL11 recruits $CD8^+$ T-cells and NK cells. These specialized killer cells can directly eliminate the tumor by secreting pro-apoptotic factors. Production of IL-12 and IL-23 facilitates T-cell activation, proliferation, and tumor-specific immune response. M1 TAMS can directly participate in tumor cell killing *via* the ADCC process, where their Fcγ receptors will interact with the Fc portion of tumor-specific antibodies bound to the tumor surfaces. Anti-inflammatory cytokines and factors such as IL-4, IL-10, TGF-β, and osteopontin activate M2 TAMS. M2 TAMS facilitates tumor progression by the secretion of proliferative and survival factors such as EGF, PDGF, TGF-β1, and BFGF. The secretion of MMPs and serine proteases enables the breakdown of the extracellular matrix to facilitate tumor metastasis. M2 TAMs secrete CCL22 to create a chemoattractant gradient Treg accrual and activation. Secretion of CCL2 facilitates the recruitment of MDSCs into the tumor milieu. Overexpression of arginase depletes L-arginine from the TME, inhibiting T-cell proliferation and clonal expansion. IL, interleukin; TGF-β, transforming growth factor β; MCSF, macrophage colony-stimulating factor; CSF-1, colony-stimulating factor 1; Shh, sonic hedgehog protein; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor α; MMP, matrix metalloproteinases; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; BFGF, basic fibroblast growth factor; ROS, reactive oxygen species; NO, nitric oxide; ADCC, antibody-dependent cellular cytotoxicity. This image was created with BioRender.com.

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factor (BFGF).[65] M2 TAMs facilitate metastasis *via* releasing enzymes and factors that degrade the components of the extracellular matrix, such as MMPs and serine proteases, thereby facilitating the migration of tumor cells into the circulatory system.[66] Most importantly, M2 TAMs contribute to the immunoediting of the TME by suppressing T-cell function and inhibiting $CD8^+$ T-cell proliferation facilitated by arginase expression.[67] Finally, M2 TAMs can recruit Treg and MDSCs to the TME by secreting CCL22 and CCR2 ligands.[68–70] The activation of M1 macrophages is vital for controlling tumor burden *via* three classical immune-mediated processes: (i) indirect killing by the accrual of other immune cells that can lyse and kill the cancer cells, (ii) direct cytotoxicity by the release of harmful products such as ROS, and (iii) antibody-dependent cellular cytotoxicity (ADCC). M1 phenotype activation is induced by stimuli such as toll-like receptor (TLR) ligands and pro-inflammatory cytokines.[58] M1 phenotypes release inflammatory cytokines in the TME, such as CXCL9, CXCL10, and CXCL11, that recruit and activate $CD8^+$ T-cells and natural killer cells.[71] M1 TAMs kill tumor cells by releasing cytotoxic factors such as ROS and NO, which cause cell death *via* oxidative stress and DNA damage.[72] M1 macrophages play a crucial role in ADCC by recognizing and eliminating antibody-bound target cells by interacting with the Fc fragment of antibodies *via* their Fc gamma receptors (FcγR). The receptor interaction triggers phagocytosis of the antibody-bound tumor cells, resulting in their elimination and removal.[73]

The M1 to M2 phenotype ratio is a proxy indicator for the inflammatory-to-anti-inflammatory factor balance. Various clinical studies have indicated that the M1 to M2 ratio correlates with improved survival, an indication to justify the ratio as a prognostic marker and to target TAM polarization for cancer therapy.[74–77] Pre-clinical studies in mice suggest that targeting TAM activation *via* MHC I molecules,[78,79] or reprogramming TAMs into antitumor M1 phenotype might achieve better success in the clinic due to the positive activation effects on antitumor M1-like functions.[80,81]

2.1.5. Other immunosuppressive cells in the TME

Regulatory B-cells. Human B cells derive from the common lymphocyte progenitor lineage in the bone marrow, where they undergo V(D)J recombination to become immature/naïve B-cells expressing B-cell receptors (BCRs) of the IgM/IgD isotype. After encountering their cognate antigen, B-cells undergo affinity maturation and class switch recombination (CSR) to develop into memory B cells or antibody-producing plasma cells with isotypes IgG/A/E. B-Cells can exert anti-tumor functions by activating the complement system and generating neoantigen-specific antibodies that bind to NK cells and macrophages *via* their Fc receptors.[82] The accrual of these effector cells to antibody-targeted tumor cells facilitates recognition and subsequent attack for elimination. DCs and B-cells can recognize these antibodies and internalize the neoantigens for presentation to $CD4^+$ T-cells and $CD8^+$ T-cells. Finally, B-cells release various cytokines and cytotoxic effector molecules that propagate and regulate immune responses.[83] Studies using single-cell RNA sequencing (scRNA seq) of tumor biopsies revealed B-cell populations spanning many states and isotype expression in a cancer-dependent fashion.[84] Generally, an unswitched naïve-like state and a switched state with a memory-like phenotype are observed with rare populations of germinal center cells and plasma cells.[82] However, the exact role of these B-cell states and the antigens they react to remain elusive. These tumor-infiltrating B-cells (TIL-Bs) are usually associated with a positive response and promote anti-tumor immunity *via* antigen presentation to T-cells.[82]

Several protein surface markers studies observed regulatory B cells (Bregs) expressing the IgA isotype and secreting IL-10, IL-35, and TGF-β. Bregs are the antithesis of tumor-infiltrating B-cells and are similar to Tregs. Bregs exert strong immunosuppressive functions, but unlike Tregs, which are synonymous with FOXP3 expression, Bregs do not present with a uniform surface marker or transcription factor expression.[85] Bregs can exert immunosuppressive effects from a distance by producing antibodies that circulate to the tumor and engage the Fc receptors on macrophages and mast cells, thereby facilitating a pro-tumorigenic state.[86] B-Cells can utilize the PD-1/PD-L1 immune checkpoint pathway and express PD-L1 to suppress $CD8^+$ T-cell responses in the TME. Finally, the most commonly reported Breg immunosuppressive mechanism in human studies is the secretion of IL-10.[86] $IL\text{-}10^+$ Breg cells were detected by immunohistochemistry or flow cytometry in various human cancers.[87,88] Breg and Treg expression are also strongly correlated in these studies, suggesting a possible interaction between these two immunosuppressive subtypes.[88] Another critical regulatory cytokine secreted by Breg cells is IL-35, which is functionally divergent from $IL\text{-}10^+$ Bregs in that IL-35 limits memory differentiation while IL-10 suppresses effector cell functions.[85] Nevertheless, these Breg subtypes showed overlapping ability to induce inhibitory receptors such as PD-1 and TIM-3.[85]

There is congregating evidence that tumor-infiltrating B-cells are prominent in anti-tumor responses. The presence of TIL-Bs is associated with the accrual of effector T-cells, NK cells, and myeloid cells in the TME of 'hot tumors,' indicating active antigen recognition and diverse effector functions. However, manipulating TIL-Bs for immunotherapy presents a limitation because their effector functions rely heavily on the nature of their cognate antigens. Hence, ongoing efforts to fully understand their antigenic profiles with methodologies complementing scRNA-seq, enabling the mapping of clonotypes to phenotypes and antigen-specificities. Immunotherapy strategies targeting TIL-B activation (cytokines, antibodies, and vaccines) or antibody-induced Breg depletion were investigated in disease models to delineate the molecular mechanisms involved in treatment efficacy.[85,86]

Cancer-associated fibroblasts (CAFs). CAFs are activated fibroblastic cells in the TME that present a distinct phenotype from the latent fibroblasts in normal tissues. CAFs can originate from bone marrow-derived precursors/mesenchymal stem cells (MSCs) or tissue-resident fibroblasts, adipocytes, and pericytes.[89] Various factors are involved in cancer-induced

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fibroblast reprogramming, including epigenetic changes, microRNA expression, metabolic determinants, and oxidative stress.[90] Generally, CAFs are divided into three functional groups: tissue remodeling (myofibroblastic CAFs), mutual signaling with other cells within the TME, and immune regulation. Currently, there is no single definitive marker that can be used as a determinant hence, both positive and negative markers are used to identify and distinguish latent fibroblasts from CAFs.

Profiling CAF populations across malignancies using scRNA-seq and flow cytometry established the current knowledge of CAF phenotypes and functions. Biffi *et al.* summarized the phenotypic and functional yield of these studies in their review article, which profiled CAFs in pancreatic ductal adenocarcinoma (PDAC), breast cancer, lung cancer, melanoma, colon cancer, and head and neck cancer.[90] In general, both myofibroblastic (α smooth muscle actin (SMA)-high) and non-myofibroblastic (αSMA-low) CAF populations are present across all cancer types.[90–93] Myofibroblastic CAFs are associated with extracellular matrix (ECM) signature, whereas non-myofibroblastic CAFs are typically secretory and inflammatory.[94] ECM deposition of CAFs plays distinct roles in the TME, including provision of nutrients, obstructing drug delivery, which leads to hypoperfusion and elevated interstitial fluid pressure, and supporting tumor growth.[90] Non-myofibroblastic CAFs create an immunosuppressive TME by preventing T-cell activity and accrual through the secretion of immunosuppressive ligands such as TGF-β and CXCL12.[90] CAFs can recruit monocytes, differentiate macrophages, and polarize them into the pro-tumorigenic M2 subtype.[95] Additionally, CAFs can recruit immunosuppressive cell populations such as the MDSCs into the tumor stroma. Interestingly, antigen-presenting CAFs (apCAFs) express MHCII proteins but do not express co-stimulatory molecules, which are essential in the induction of T-cell activation and clonal expansion. Hence, it was hypothesized that apCAFs may act as a decoy receptor to detain T-cells and inhibit their response.

Recent studies in PDAC highlighted CAF subtypes that displayed tumor-restraining properties.[90] The genetic depletion of αSMA cells and the deletion or pharmacological intervention of sonic hedgehog (SHH) of the hedgehog (Hh) pathway led to reduced survival in preclinical and clinical studies.[96,97] The results indicate a tumor-restraining role for these cells. However, genetic approaches will affect other αSMA and SHH-expressing populations, so the assumption that CAF subtypes were involved must be cautiously approached. However, the heterogeneity and diverse roles of CAFs in tumor burden control underscore the need for a unified classification system that can aid in the development of targeted therapies.[98]

2.2. Immune checkpoint molecules

2.2.1. Programmed death ligand-1 (PD-L1) and PD-1. In the last decade, unprecedented clinical achievements in immunotherapies targeting the immune checkpoint axis of PD-L1 (B7-H1 or CD274) and PD-1 (programmed cell death protein 1 or CD279) have generated interest in the nanotechnology community. PD-1 is a type 1 transmembrane glycoprotein approximately 50–55 kD in size that contains a single extracellular N-terminal IgV-like domain, a hydrophobic transmembrane domain, and a cytoplasmic tail containing two tyrosine-based structural motifs.[99] PD-1 is expressed on activated T-cells, natural killer (NK) cells, B lymphocytes, macrophages, dendritic cells (DCs), and monocytes and is highly overexpressed on tumor-specific T-cells.[100]

PD-L1, the ligand binding to PD-1, is a 33 kDa type 1 transmembrane glycoprotein with immunoglobulin domains in its extracellular region. PD-L1 is expressed in various cell types, including lymphocytes, lung tissues, vascular endothelium, mesenchymal stem cells, islet cells, astrocytes, neuronal cells, and keratinocytes.[101,102] PD-1 and PD-L1 are crucial immune checkpoint molecules. Their interaction induces inhibitory signaling pathways that fine-tune the activation of effector T-cells during antigen presentation (Fig. 6). However, PD-L1 is also notably overexpressed in tumor cells, contributing to the evasion of immune system-mediated eradication. Its interaction with the PD-1 receptor, expressed on activated T-cells, can suppress T-cell responses, resulting in T-cell anergy, inhibition of cytokine expression, and T-cell apoptosis.[103] Thus, overexpression of PD-L1 on tumor cells is one of the essential strategies for evading immune response and eradication. Genomic alterations (amplification or translocation), aberrant signaling pathways (inflammatory and oncogenic signaling), and post-transcriptional/translational modifications (miRNA, methylation, phosphorylation, glycosylation) contributed to the oncogenic overexpression of PD-L1 on tumor and immune cells. The details of such mechanisms have been extensively reviewed by Yadollahi *et al.*[104] Under normal conditions, the interaction of PD-1 with PD-L1 results in a signaling cascade that protects healthy host tissues by promoting Treg development and inhibiting self-reacting T-cells.[105] PD-L1 could also be upregulated during multiple inflammatory signaling pathways to restrain T-cell hyperactivity.[106] However, the interaction of PD-1 with overexpressed PD-L1 on tumor cells suppresses normal antitumor immunity, protecting the cancerous cells from the host response.

The pathways activated by the PD-1/PDL-1 interaction are widely studied and extensively reviewed by Patsoukis *et al.*[107] The cytoplasmic tail of PD-1 contains an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM).[108] The tyrosine residues in these two domains are phosphorylated after PD-1/PD-L1 interaction, leading to the recruitment and activation of Src homology region 2 (SH2) domain-containing tyrosine phosphatase-2 (SHP-2) and the homologous SH2 domain-containing tyrosine phosphatase-1 (SHP-1). These phosphatases will then dephosphorylate CD28, T-cell receptor (TCR), and other costimulatory molecules, inhibiting the signal transduction that leads to T-cell proliferation and cytokine secretion. This sequence of events is known as the canonical "*trans*" interaction, whereby the interacting PD-L1 and PD-1 are expressed on two different cell types (Fig. 7). However, the molecular interplays vary depending on the co-expression status of PD-L1 and PD-1. When PD-L1 and PD-1 are co-expressed in an APC or cancer cells, it will result in a *cis* level

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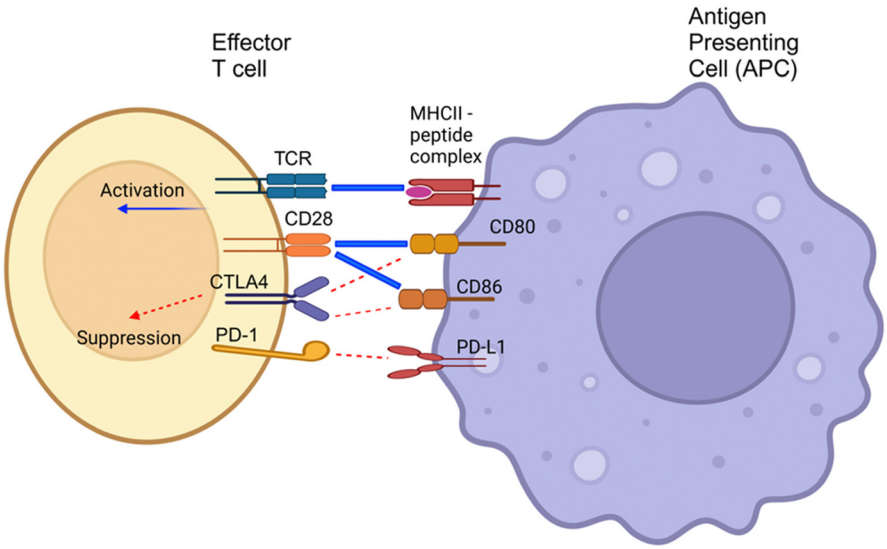

Fig. 6 The activation of T-cells by antigen-presenting cells. T-Cell activation requires two activation signals (blue lines). The priming of the T-cell is initiated by the recognition of the MHC I or II molecules, complexed with an antigen peptide. A co-stimulatory signal is generated by the interaction of CD28 molecules with either CD80 (B7-1) or CD86 (B7-2). The activation signals will induce T-cell activation and responses such as proliferation and secretion of cytokines. The degree of activation can be modulated by additional stimulatory (*e.g.* ICOS-ICOSL, not represented in the schematic) or inhibitory signals. Inhibitory signals are mediated by immune checkpoint molecules, PD-L1/PD-1 and CTLA-4. CTLA-4 binds to CD80 or CD86 with greater affinity than CD28, inhibiting its co-stimulatory function. TCR, T-cell receptor; MHCII, major histocompatibility complex II; CTLA4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1. This image was created with BioRender.com.

of interaction where the PD-L1 and PD-1 expressed within the same cell interact and diminish the ability of the PD-L1 to bind to PD-1 expressed on T-cells in *trans*.[109] Hence, co-expressing APCs or cancer cells fails to induce an immunosuppressive effect on PD-1-expressing T-cells. Understanding the mode of PD-1/PD-L1 expression is very important in treatment strategies using PD-1 blockade.

The prognostic value of PD-L1 and PD-1 in various cancers has been well reported.[110] PD-L1 expression has been associated with poor clinical outcomes in colorectal cancer,[111] gastric cancers,[112] head and neck cancers,[113] and breast cancer.[114] However, PD-L1 upregulation was associated with better outcomes in an aggressive subtype of breast cancer.[115] Such an observation could result from the *cis* PD-1/PD-L1 interaction that suppresses PD-1 signaling. Hence, PD-1/PD-L1 interaction modes must be factored in for checkpoint blockade therapy eligibility. Clinical trials have demonstrated that PD-L1 or PD-1 blockade therapy successfully abrogates the inhibitory effects, restoring T-cell antitumor activities and functions.[116] Tang *et al.* meticulously studied the data presented from 99 clinical trials to find that preoperative anti-PD-1/PD-L1 combined therapy, particularly with chemotherapy, could achieve better treatment response rates and reduce the number of immune-related adverse events compared to PD-1/PD-L1 monotherapy or dual immunotherapy.[117]

2.2.2. Cytotoxic T-lymphocyte antigen 4 (CTLA-4). CTLA-4 (CD152) is a member of a family of immunoglobulin-related receptors that inhibits T-cell function. In contrast, other immunoglobulin-related receptors such as CD28 and inducible T-cell costimulator (ICOS) stimulate T-cell activities.[118] CTLA-4 is expressed on T-cells, including $CD4^+$ T-cells, $CD8^+$ T-cells, and Treg cells, contributing to their suppressive functions and maintenance of immune tolerance.[119] CTLA-4 is also expressed in monocytes and neoplastic cells. Expression of CTLA-4 is regulated by transcription factors FoxP3, nuclear factor of activated T-cells (NFAT), and nuclear factor-κB (NF-κB).[120] CTLA-4 and CD28 are structurally similar despite their opposing functions. The structure comprises type-I integral membrane proteins composed of a single immunoglobulin variable domain (IgV), a transmembrane segment, and a cytoplasmic tail bearing various signaling motifs.[121]

The role of CTLA-4 in antitumor immunity occurs at the junction between T-cells and APCs, where the innate immune cells present antigenic molecules to adaptive immune effector cells. The interaction between the two components enhances immune response by instructing the recognition of patterns for destroying cells expressing the antigenic molecules, continued surveillance, and memory for long-lasting protection.[122] CTLA-4 and CD28 share binding capabilities with two ligands expressed on APCs: CD80 (B7-1) and CD86 (B7-2).[118] CTLA-4 interacts with both ligands with higher affinity to CD28, thereby acting as an antagonist of CD28-mediated co-stimulation of T-cell activation, proliferation, and cytokine secretion (Fig. 6).[118] The expression of CTLA-4 is primarily localized and stored in intracellular vesicles due to the rapid constitutive endocytosis of CTLA-4 from the plasma membrane. CTLA-4 is rapidly recruited to the cell surface from vesicle storage and new gene expression upon T-cell activation (Fig. 8).[118] However, sustained overexpression of CTLA-4 is often induced in tumor-infiltrating T-lymphocytes, contributing to the progression of both solid and hematological cancers *via* aberrant signaling pathways.[123–125]

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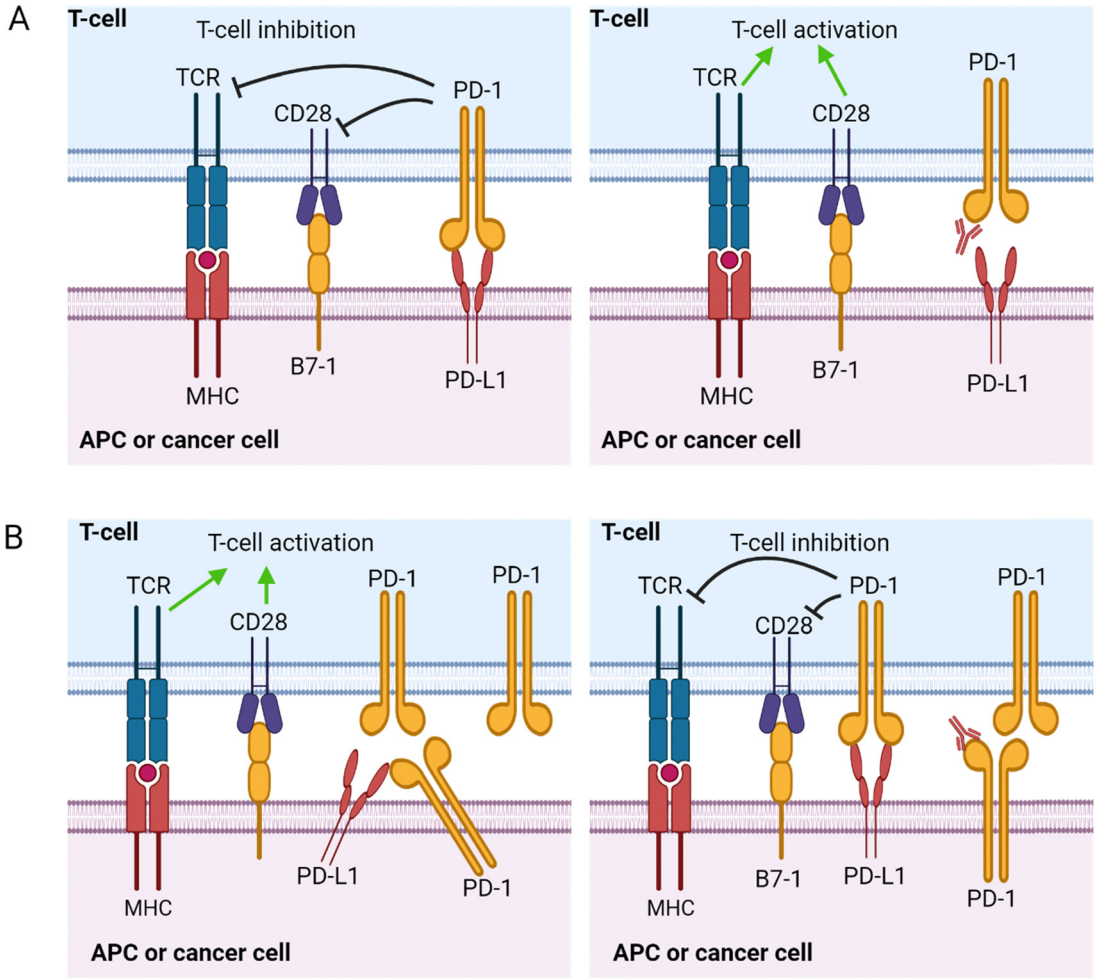

Fig. 7 The *trans* and *cis* interaction modes of PD-1 and PD-L1. (A) The canonical PD-1/PD-L1 interaction is a *trans*-mode interaction. PD-L1 expressed on APCs or tumor cells interacts with PD-1 expressed on T-cells in *trans*. The PD-1/PD-L1 interaction will attenuate the activation of T-cells mediated by the TCR/MHC and B7-1/CD28 stimulation pathways. Checkpoint blockade in *trans* mode will abrogate the inhibitory signaling of PD-1/PD-L1 interaction and restore T-cell activation. (B) *Cis* mode interaction occurs when PD-1 and PD-L1 are co-expressed in tumor cells or APCs. Co-expressed PD-1 and PD-L1 will interact, preventing the interaction of PD-L1 on APCs/tumor cells to PD-1 on the T-cells in *trans*. Checkpoint blockade in *cis* interaction mode reactivates inhibitory PD-1/PD-L1 interaction in *trans*. This image was created with BioRender.com.

During tumorigenesis, CTLA-4 decreases the T-cell activation by various molecular pathways. After TCR engagement, CTLA-4 expression and recruitment to the membrane were immediately increased, and it competes with the CD28 costimulatory molecule for the CD809/CD86 ligands, leading to a weakened immune response. The interaction of CTLA-4 with the CD80/CD86 molecules in circulating APCs can induce the activity of indoleamine-2,3-dioxygenase (IDO), which leads to catabolization of tryptophan, which is essential for T-cell proliferation, and the generation of inhibitory tryptophan metabolites[125] (Fig. 8). Additionally, the signalosome interaction between serine-threonine protein phosphatase 2 A (PP2A) and (SHP-2) activates the downstream PI3K/Akt pathway, which helps to sustain T-cell anergy and immune tolerance without inducing antigen-induced cell death.[126] The signalosome also inhibits the NF-κB and cyclin D pathways, which are essential regulators of T-cell activation and proliferation.[127–129]

CTLA-4 is constitutively expressed on FoxP3$^+$ positive cells, unlike conventional T-cells, which express CTLA-4 only after activation, indicating its specific association with Treg cells.[130] CTLA-4 blockade treatment with monoclonal antibodies led to the depletion of Treg cell populations in the TME. Studies analyzing the prognostic value of CTLA-4 in cancer treatment yield controversial outcomes.[131] Ipilimumab is the first anti-CTLA-4 human monoclonal antibody that was first FDA-approved for treating advanced melanoma patients with a considerable therapeutic effect in renal cell carcinoma.[132,133] However, anti-CTLA-4 antibody treatment exhibited a higher incidence of immune-related adverse events (irAEs) than those recorded in PD-1 blockade studies.[134] Hence, combinations of CTLA-4 blockade with other therapies, such as chemotherapy, radiotherapy, and double checkpoint blockade (CTLA-4 plus PD-1), were investigated to reduce the severity of irAEs with favorable risk-benefit profiles[135,136]

2.2.3. Other immune checkpoint molecules. Immune checkpoint blockade (ICB) therapy has transformed the clinical management of malignant disease in the last decade. Encouraged by the success of anti-PD-1 and anti-CTLA-4 immunotherapies,

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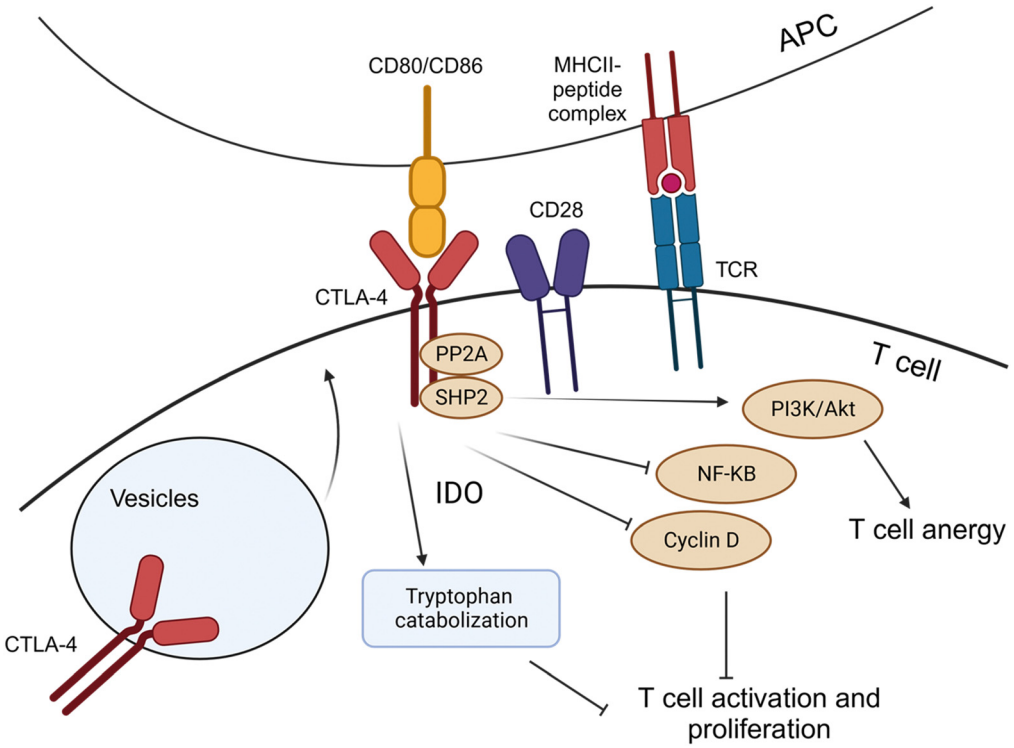

Fig. 8 CTLA-4 is usually stored in intracellular vesicles and is rapidly recruited to the cell surface following T-cell activation. CTLA binds to CD80/86 at a higher affinity than CD28, displacing the molecule from the co-stimulatory complex. The interaction with its ligands induces CTLA-4 activation, mediated by the signalosome that includes PP2A and SHP-2. CTLA-4 activation activates the PI3K/Akt pathway to sustain T-cell anergy without immunogenic cell death. NF-KB and cyclin D pathways were inactivated, resulting in the inhibition of T-cell activation and proliferation. Additionally, indoleamine-2,3-dioxygenase (IDO) activity catalyzes the breakdown of tryptophan, increasing the intracellular levels of tryptophan degradation products, which are effector T-cell inhibitors and induce Tregs. This image was created with BioRender.com.

other immune checkpoint candidates were screened and explored for their potential as ICB targets. T-Cell immunoglobulin-3 (Tim-3), lymphocyte activation gene-3 (Lag-3), and TIGIT (T-cell immunoglobulin and ITIM domain) represent the next generation of checkpoint molecules that are currently explored in clinical trials. This section will briefly discuss their characteristics, function, and role in malignant diseases.

Tim-3. TIM-3 was identified 12 years ago as a cell surface molecule selectively expressed on IFN-producing T-cells.[137] Now, the expression of Tim-3 has been demonstrated on Treg cells, DCs, NK cells, and monocytes. Tim-3 expression is encoded by the Tim family of genes associated with immune-mediated diseases such as asthma and allergy.[138] Binding of Tim3 with Tim-3 ligands (galactin-9: Gal-9, high-mobility group protein: B1HMGB1, carcinoembryonic antigen cell adhesion molecule 1: CEACAM-1, phosphatidylserine: PtdSer) initiate a signaling cascade that results in the activation of nuclear factor of activated T-cells (NFAT) and nuclear factor kB (NF-κB) of the TCR signaling pathway in a normally regulated immune reaction.[139] However, in malignant diseases, Tim-3 expression marks dysfunctional or exhausted $CD8^+$ T-cells, which is exacerbated when Tim-3 is doubly expressed with PD-1.[140] The first Tim-3 monoclonal antibody, sabatolimab, blocks the binding of Tim-3 to ligands Gal-9 and PtdSer to restore T-cell function by obstructing Tim-3 mediated exhaustion. A phase I/Ib clinical trial of sabatolimab alone and with anti-PD-1 showed that the combination treatment was well-tolerated with preliminary signs of anti-tumor activity.[141]

Lag-3. Lag-3 was discovered in 1990 as a molecule upregulated on activated T-cells and a subset of NK cells.[142] Structurally, Lag-3 resembles CD4 co-receptor and binds to MHCII with higher affinity than CD4.[142] Another Lag-3 ligand is LSECtin, a member of the DC-SIGN family of molecules. Lag-3 is a negative regulator of T-cell receptor signaling, but it promotes Treg cell-mediated suppression. The opposing effects of Lag-3 engagement beg the question of the Lag-3 mediated signaling on different effector T-cell subsets to achieve its immunosuppressive effects. At present, Lag-3-related signaling events remain unclear. The known Lag-3 intracellular interaction is cross-linking with CD3 to inhibit calcium flux, T-cell proliferation, and cytokine production.[143] Relatlimab is the first commercially developed anti-LAG-3 mAb currently being investigated with the PD-1 inhibitor, nivolumab, to treat unresectable or metastatic melanoma in a phase 2/3 randomized trial. The combination of the two checkpoint inhibitors enhanced progression-free survival than anti-PD-1 therapy alone without any new adverse events.[144]

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TIGIT. TIGIT was identified in 2009 in a genome-wide search for costimulatory or inhibitory molecules on activated T-cells.[145] TIGIT is widely expressed in regulatory, memory, and activated T-cells, and its expression levels are upregulated after lymphocyte activation.[145] There are five TIGIT ligands: CD155 (also known as PVR), CD112, CD113, Nectin4, and Fab2.[139] The binding of TIGIT to its ligands activates immunosuppressive pathways *via* the interference of T-cell co-stimulation signaling mediated by CD226 or CD96.[139] Currently, a phase I anti-TIGIT therapy with IBI939 antibodies is currently ongoing in China for patients with leukemia and solid tumors.[146] The investigating team has yet to publish their results.

2.3. Immunosuppressive metabolites

Metabolic pathways release soluble mediators in the tumor microenvironment that influence and shape the immunobiology of many tumor types. This section lists the critical metabolic pathways that lead to profound immunosuppression of effector cell responses.

Kynurenine pathway (KP). Tryptophan is an essential amino acid critical for protein synthesis and proliferation. The KP is the main pathway for tryptophan metabolism, which is crucial for the formation of bioactive compounds involved in physiological functions such as serotonin, indoles, kynurenines, and nicotinamide adenine dinucleotide (NAD^+).[147] Tryptophan cannot be synthesized and must be obtained from the diet. The ingested tryptophan is catabolized in the KP by two primary rate limiting enzymes: indoleamine-2,3-dioxygenase (IDO) and tryptophan dioxygenase (TDO).[147] IDO and TDO are expressed by a multitude of tumor types, such as glioma, bladder, breast, melanoma, ovarian carcinoma, and hepatic carcinoma, with high levels of expression associated with decreased patient survival and rapid tumor progression.[147] High IDO and TDO activities lead to the depletion of tryptophan in the tumor microenvironment with subsequent accumulation of tryptophan metabolites, particularly kynurenines (kynurenine, kynurenic acid and 3-hydroxykynurenine), in the local environment.[148] These metabolites may result in T-cell death or anergy at high concentrations or induce the reprogramming of T-cell responses to the regulatory phenotype at lower concentrations. Additionally, the local depletion of tryptophan presents a key 'death by starvation' mechanism involving the imbalance of tryptophanyl tRNA synthases towards uncharged forms and subsequent activation of the amino acid starvation-sensing response pathway, which leads to T-cell anergy and cell death.[148] IDO is also expressed by various components of the immune system, which serves to limit inflammatory immune response and protect the tissues from inflammatory damage.[147] Hence, inhibition of IDO activity is one of the clinical strategies utilized to overcome immunosuppression.[148] However, limited clinical effectiveness has dampened the enthusiasm for IDO inhibition therapy.

Glucose metabolism. Cancer cells exhibit increased glucose uptake due to the proliferative and energy demands. Rather than oxidative phosphorylation, cancer cells undergo glycolysis in the presence of oxygen, leading to the accumulation of lactate and an acidic microenvironment. This event is known as the Warburg effect and is regulated by many enzymes.[149] The accumulation of lactate facilitates the assembly of an immune escape network involving the differentiation of immunosuppressive effector cell subtypes, including TAMs, Tregs, and MDSCs—responses, enabling the escape of tumor cells from immune surveillance and response.[150]

Fatty acid oxidation (FAO). Tumor cells and immune cells compete for nutrients in the TME. To cope with the acidified and hypoxic environment, tumor cells undergo metabolic reprogramming and increase their energy uptake for survival. Glucose in cancer cells is used for anabolic processes such as ribose production, protein glycosylation, and serine synthesis.[151] Glutamine is a critical alternative nutrient source that can provide reduced NADPH for lipid synthesis and the Krebs cycle. Fatty acids are another relevant nutrient source that can provide NADPH. Besides tumor cells, MDSCs in the TME displayed enhanced fatty acid uptake and oxidation, leading to increased immunosuppressive capacity.[152] This immunosuppressive effect is further influenced by the type of dietary fatty acid, with unsaturated fatty acids being particularly potent.[153] The mechanism behind this immunosuppression involves the regulation of glucose and glutamine metabolism, mitochondrial depolarization, and the generation of pro-inflammatory or pro-resolving lipid mediators through fatty acid oxidation.[154] Other cellular components of the immune system also displayed metabolic reprogramming and utilized the FAO pathway for sustenance and maintenance of immunosuppressive subtypes.[153]

3. Current status of nano-immunotherapy for cancer

Cancer immunotherapy has become a research hotspot during the last two decades. However, current cancer immunotherapy strategies remain inundated with challenges and limitations. A subset of patients fails to respond to the therapy, relapsing after initial treatment. Additionally, the immune-related adverse events that follow are potentially debilitating and life-threatening. Hence, there has been a recent budding pursuit of developing nanotechnology and nanoengineering platforms to narrow the gap in cancer immunotherapy strategies. Examining the repertory of nanoformulations engineered for cancer immunotherapy currently investigated in human clinical trials will provide insights into the strategy, the investigative and evaluation process, and possible avenues for further research endeavors.

Due to its FDA approval status, clinical success, and the maturity of the technology, liposomes are the predominant material of choice for developing immunomodulatory formulations for cancer therapeutics. Liposomal platforms are potentially versatile, able to encapsulate various pharmacological agents, and can be functionalized for active targeting of specific tissue sites. An immunotherapy agent for cancer treatment should ideally target tumor-associated antigens (TAAs) to drive the development of a tumor-specific immune response.

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The most widely investigated liposomal formulation with TAA targeting in the clinic is Tecemotide, formerly known as L-BLP25 or Stimuvax®, which targets adenocarcinomas that express mucin-1 (MUC-1), a member of the membrane-bound 0-glycoprotein mucin.[155] FixVac is an RNA–liposome complex formulation comprising RNA encodes for tumor-associated antigens, NY-ESO, and MAGE-3. FixVac is currently investigated in a phase I trial as a potential immunotherapy for melanoma. Interim analysis detected strong $CD4^+$ and $CD8^+$ T-cell responses after vaccination with FixVac.[156] The study was completed recently, but the investigative team has not published the results. Lipovaxin-MM is a liposome-based nanotechnology formulated to treat malignant melanoma. It targets DC activation to harness their potent antigen-presenting ability. Very little treatment efficacy was demonstrated when the vaccine was used to treat a small group of patients with malignant myeloma. No humoral or cellular response to the vaccine was observed, indicating that immunosuppressive mechanisms induced by the tumor may have negated the effect of the vaccine.[157]

Due to careful ethical considerations and calculated risk-to-benefit ratio, exploratory formulations are investigated in advanced, refractory, or recurrent malignancies, which are often a dilemma to manage due to the lack of alternative treatment options. Observing beneficial responses, such as increased overall survival and tolerable toxicity profiles in these clinical cases, are signs of further potential, especially in early malignancies or as preventative measures. Research endeavors should also focus on developing similar biomimetic or bionanoparticle platforms for cancer therapy. For example, exosomes are being investigated in the clinic as prognostic markers (https://www.clinicaltrials.gov). One clinical study was conducted to test the efficacy of a tumor vaccine comprising tumor-antigen-loaded DC-derived exosomes on patients with unresectable NSCLC (ClinicalTrials.gov ID: NCT01159288). However, the results were not published. The outcomes of these studies could provide insights into developing efficient strategies for cancer vaccination and immunotherapy. Nevertheless, the liposome-based cancer immunotherapy studies in the clinic have generated some critical insights that could help direct future growth areas. Table 1 depicts a simple SWOT analysis of the current nano-immunotherapy landscape in the clinic. Based on the study, bionanomaterials have considerable potential to impact this subspecialty of the oncology field.

4. Strategies for functionalizing bio-nanoparticles for tumor immunomodulation

The tumor microenvironment is complex, shaped intricately by the growing tumor that exhibits heterogeneity, increasing the challenge of standard therapies to provide a complete response. Modulating the immune response towards cancer termination inflammatory phenotypes has significantly impacted clinical practice with the advent of checkpoint inhibitors and CAR-T-cell therapy. Using bionanoparticles to modulate the immune response is a relatively new yet rapidly evolving field with significant potential for clinical translation. Bionanoparticles, such as cell membrane-derived nanoparticles, exosomes, and albumin-based nanoparticles, have garnered attention for their ability to modulate immune responses and deliver therapeutic agents to tumors. The prospect for bionanoparticles in cancer immunotherapy is optimistic, with continued efforts to refine their therapeutic potential and advance their translation into clinical practice. This section focuses on the most utilized bionanoparticles engineered to stimulate an anticancer immune response.

4.1. Targeting tumor influx *via* cell membrane biomimetics

A pivotal area of research focuses on using cell membrane biomimetics to target tumor influx and enhance antigen presentation, thereby overcoming the immune evasion tactics that tumors employ. This section examines the latest advancements in the design and application of biomimetic nanoparticles derived from various cellular membranes, including those of tumor cells, immune cells, and blood cells, each offering unique mechanisms to modulate the tumor microenvironment and potentiate the immune response. Tumor cell membrane-coated nanoparticles are at the forefront of this research, with studies demonstrating their ability to mimic the antigenic profile of tumors and stimulate specific immune responses.[158–160] These biomimetic platforms have been engineered to deliver

Table 1 A SWOT analysis based on currently available nanoparticles for clinical immunotherapy

Strength	Weakness
• Inventory of mature technologies for innovation *e.g.* peptides, humanized monoclonal antibodies, checkpoint inhibitors • Human-derived bionanoNPs have a better safety profile • Microbial and viral-like particles have intrinsic immune modulating abilities	• Unclear plans for transitioning laboratory-developed technologies to the clinic • Resource limitations (*e.g.* lack of suitable TAAs and distinct immune surface markers)
Opportunities	**Threats**
• Developing computational analysis and AI base technologies that can strategize and predict probability of efficacy • Developing nanovaccines for preventative measure and early-stage malignant disease • Unmet needs in hematological malignancies after patients fail CAR-T cell therapy	• Debilitating and severe iRAEs • Risk –averse regulatory environment

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costimulatory signals, activate antigen-presenting cells, and elicit robust $CD8^+$ T-cell responses, showing promise in preclinical and clinical settings. Integrating these nanoparticles with other therapeutic modalities, such as photodynamic, sonodynamic, and photothermal therapies, further amplifies their immunomodulatory effects.[161–163]

Exploring immune cell membrane-coated nanoparticles, particularly those derived from macrophages and T lymphocytes, represents another transformative approach. These nanoparticles are designed to target and disrupt immunosuppressive pathways within the tumor microenvironment, induce immunogenic cell death, and enhance the infiltration and activity of cytotoxic T lymphocytes, thereby restoring the tumoricidal functions of the immune system.[164–167] Lastly, the section examines the innovative use of blood-derived membrane biomimetics, including those from red blood cells and platelets, which have been shown to improve drug delivery, evade immune clearance, and modulate the tumor microenvironment. These strategies have demonstrated efficacy in inducing immunogenic cell death, reprogramming immunosuppressive cells, and synergizing with checkpoint blockade therapies to inhibit tumor growth and metastasis.[168–171]

4.1.1. Tumor cell membrane. Efforts have focused on developing tumor vaccines and enhancing cancer immunotherapy through various tumor membrane biomimetics strategies (Table 2). Genetically engineered antibody-anchored membrane nano-vaccines have been designed to overcome the lack of costimulatory signals in whole-tumor cell vaccines.[163,172,173] The antibody-anchored cell membrane nanovaccine (nano-AAM) was constructed by anchoring an anti-CD40 single variable chain fragment (scFv) into the tumor cell membrane (Fig. 9A). These nano-vaccines effectively activate costimulatory pathways and enhance antitumor efficacy in both ''hot'' and ''cold'' tumor models. A tumor antigen can be incorporated into the membranes for a tumor-specific immune response. In their study, Li *et al.* employed ovalbumin (OVA) as a model tumor antigen and demonstrated significant cross-presentation of the OVA peptide with MHCI complex after co-culturing bone marrow-derived cells with NANO-OVA. *In vitro*, NANO-AAM/CD40 promoted DC maturation and T-cell activation. Similarly, MC38 tumor cell lysates can be loaded in the core structure, further boosting immune responses and improving antitumor efficacy against malignant MC38 tumors in mice. The team showed the construct is also efficient in cold immunosuppressive tumor models.[162] Combining tumor membrane-based vaccine immunotherapy and immune checkpoint blockade is another utilized approach. Zhao *et al.* fabricated a novel hybrid membrane nanovaccine comprising mesoporous silica nanoparticle (MSN) as a delivery carrier, R837@HM-NPs. MSN is functionalized with imiquimod R837, a TLR7 agonist for immune cell activation, and coated with hybrid cell membranes obtained from dendritic cells and cancer cells (Fig. 9B). Combined with anti-PD-1 blockade therapy, R837@HM-NPs successfully promoted tumor regression in subcutaneously implanted 4T1 breast cancer tumors in mice. The combination therapy facilitated the increase of the $CD8^+$/Treg ratio in the excised tumor and increased the secretion of IL-6, IL12-p40, and TNF-α in the TME.[158] Similar use of an immune adjuvant with the cell membrane coating technology provided immunity against immune checkpoint-resistant cancer.[174–176] Cell membrane-camouflaged liposomes and neuropeptide-loaded liposome combinations are suggested as a platform for personalized cancer vaccine therapy (Fig. 9C). These two separate nanovaccines are used together in a ''prime and boost'' strategy. The cancer cell membrane-camouflaged liposomes with R848 (Resiquimod, TLR7 and TLR8 Agonist) primes the M2-like macrophages and DCs. Then, the liposomes with neopeptides and R848 will boost the immune response by stimulating macrophages, DCs, and T-cells, thereby generating treatment outcomes. The ''prime and boost'' combination effectively inhibited tumor growth in two subcutaneous mice tumor models (B16F10 and TC-1) and increased $CD8^+$ T-cell and DC infiltration in the TME. Additionally, a concomitant increase in the mature DC population was observed in the draining lymph nodes, indicating migration of the DCs from the tumor to the lymphatic system for antigen presentation.[177]

Combining cancer cell membranes and inorganic immune adjuvants is a strategy utilized to elicit a robust and long-lasting immune response. For instance, cancer cell membranes are coated onto layered double hydroxide nanoparticles. These nanoparticles efficiently target antigen-presenting cells and inhibit immune escape, stimulating antigen-presenting cell maturation and tumor-specific $CD8^+$ T-cell responses. This approach has shown significant suppression of tumor growth *in vivo*.[178] Similarly, Gan *et al.* engineered tumor cell membrane-enveloped aluminum phosphate nanoparticles, stimulating tumor-specific $CD8^+$ T-cell immunity.[179] These studies underscore the advantages of using tumor cell membrane-coated nanoparticles to enhance immune cell-mediated cancer immunotherapy.[173,174,180]

Other immune adjuvants, such as CpG oligonucleotides (ODNs), can be utilized to enhance the immune response of the membrane nanoparticles. Johnson *et al.* developed an acute myeloid leukemia (AML) cell membrane-coated nanoparticle (AMCNP) vaccine platform with CpG oligonucleotides adjuvant-loaded into the nanoparticles and coated with leukemic cell membrane material (Fig. 10A). The AMNCPs can be recognized by immature antigen-presenting cells (APCs), leading to their maturation and activation. These APCs can then activate circulating T-cells to elicit an adaptive response. AMNCPs vaccination protected mice against AML challenge *via* intravenous injection of C1498 cells. A rechallenge model tested AMNCPs as an immune adjuvant after a chemotherapy session. The AMNCPs vaccination group received a long-lasting immunity against leukemia re-challenge, with mice surviving up to 12 weeks post-re-challenge. In contrast, mice given C1498 whole cell lysate survived only up to 3 weeks post-re-challenge.[159]

Another pivotal aspect in these investigations involves amalgamating membrane coating biomimetics with other techniques for synergistic cancer immunotherapy, such as photodynamic therapy (PDT), sonodynamic treatment (SDT), or photothermal therapy (PTT).[160,161,181–184] For example, Wang *et al.* described the formation of a DNA nanomedicine

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Table 2 List of tumor, immune, and blood cell-based membrane for cancer therapy and immunomodulation

Membrane type	Source	Application	Targeting	Immunomodulation mechansim	Animal model	Ref.
Breast cancer	4T1 cell line	Drug delivery	Tumor cells	Trigger ICD	Subcutaneous	181
Breast cancer	4T1 cell line	Drug delivery	Tumor cells	Increase the penetration of TILs	Metastatic	175
Breast cancer	4T1 cell line	Drug delivery	Mitochondria	Induce ICD	Orthotopic	182
Breast cancer	4T1 cell line	Antigen-presenting	Tumor cells	Trigger ICD	Orthotopic and residual	160
Breast cancer	Tumor tissue	Antigen-presenting	Dendritic cells	DC activation and cytokine production	Metastatic	172
Breast cancer	4T1 cell line	Drug delivery	Tumor cells	Increse the infiltration of CTLs	Subcutaneous	195
Breast cancer	Tumor tissue	Antigen-presenting	Tumor cells	Upregulate immunomodulatory cytokines	Metastatic	174
Colon cancer	CT26 cell line	Antigen-presenting	Macrophages	Trigger $CD8^+$ T lymphocyte responses	Subcutaneous	178
Colon cancer	MC38-OVA cell line	Drug delivery	Tumor cells	Stimulate human dendritic cells and T lymphocytes	Subcutaneous	177
Colon cancer	MC38 cell line	Antigen-presenting	Dendritic cells	Stimulate immune checkpoints	Transgenic	162
Leukemia	C1498-OVA cell line	Antigen-presenting	Tumor cells	Enhance T lymphocyte responses	Metastatic	159
Lung cancer	LLC cell line	Drug delivery	Dendritic cells	Increase the T lymphocyte infiltrations	Orthotopic	184
Melanoma	B16-F10 cell line	Drug delivery	Tumor cells	Increase co-uptake of tumor antigen	Immunogenic	179
Melanoma	B16-F10 cell line	Drug delivery	Tumor cells	Induce dendritic cell maturity	Subcutaneous	176
Melanoma	B16-OVA cell line	Antigen-presenting	Dendritic cells	Stimulate the maturation of dendritic cells	Subcutaneous	173
Melanoma	B16 cell line	Drug delivery	Tumor cells	Increases the numbers of M1 macrophages	Subcutaneous	196
Melanoma	B16F10 cell line	Drug delivery	Tumor cells	Cause PD-L1 DNA sequence breaks	Subcutaneous	183
Melanoma	B16-F10 cell line	Antigen-presenting	NK cells	Activate NK cells	—	180
NSCLC	H460 cell line	Drug delivery	Tumor cells	Re-activate lymphocyte cells	Subcutaneous	197
Prostate cancer	Tumor tissue	Drug delivery	Dendritic cells	Enhance $CD8^+$ T lymphocyte and NK cell infiltration	Subcutaneous	198
Macrophage	RAW264.7 cell line	Drug delivery	Tumor cells	Induce ICD	Subcutaneous	199
Macrophage	RAW264.7 cell line	Antigen-presenting	Tumor cells	Induce ICD	Subcutaneous	164
Macrophage	RAW264.7 cell line	Drug delivery	Tumor cells	Promote lymphocyte infiltration	Immunogenic	165
Macrophage	Blood	Drug delivery	Tumor cells	Induce CTLs infiltration	Subcutaneous	185
MSCm	Tissue	Drug delivery	Tumor cells	Promote T lymphocyte infiltration	Subcutaneous	200
T lymphocyte	CTLL-2 cell line	Antigen-presenting	Tumor cells	Activate cytotoxic T lymphocytes	Subcutaneous	186
T lymphocyte	CTLL-2 cell line	Antigen-presenting	Tumor cells	Activate cytotoxic T lymphocytes	Subcutaneous	187
T lymphocyte	Blood	Drug delivery	Tumor cells	T lymphocytes activation	Subcutaneous	167
Red blood cell	Blood	Drug delivery	Tumor cells	Recruit dendritic cells	Metastatic	171
Red blood cell	Blood	Drug delivery	Tumor cells	Escape uptake by macrophages	Subcutaneous	168
Red blood cell	Blood	Drug delivery	Dendritic cells	Induce ICD	Subcutaneous	188
Platelet	Blood	Drug delivery	Tumor cells	Release aPD-1 antibody	Subcutaneous	189
Platelet	Blood	Drug delivery	Tumor cells	Repolarize macrophages	Metastatic	192
Platelet	Blood	Drug delivery	Tumor cells	Enhance cytotoxic T-cell infiltration	Subcutaneous	190
Platelet	Blood	Antigen-presenting	Tumor cells	Enhance leukemia cells targeting	Orthotopic	194
Platelet	Blood	Drug delivery	Tumor cells	Enhances local immune activation	Metastatic	191
Platelet	Blood	Drug delivery	Tumor cells	Sensitize effective ferroptosis	Metastatic	170

Abbreviation: APCs, antigen presentation cells; CTLs, cytotoxic T lymphocytes; ICD, immunogenic cell death; LLC, Lewis lung carcinoma; MSCm, mesenchymal stem cell membranes; NK, natural killer; NSCLC, non-small cell lung cancer; TILs, tumor-infiltrating lymphocytes.

composed of PD-L1 aptamers and CpG nanoparticles (PCTL, Fig. 10B). A photosensitizer (TMPyP4) was inserted into the DNA structure. The PD-L1 aptamers are hidden by the presence of pHLIP-modified cDNA, rendering the formulation non-immunogenic. Under localized irradiation, the ROS generated will damage the nanostructure and release the internal DNA immunomodulators, providing a stimuli-responsive and spatiotemporal aspect to the nanomedicine. PCTL alone did not exhibit any antitumor activities *in vivo* but significantly contributed to tumor regression of subcutaneous B16F10 melanoma tumors after irradiation, yielding a prolonged survival time of 71% at 40 days post-treatment. The PCTL plus irradiation group also exhibited marked DC maturation in the tumors with a concomitant increase of $CD8^+$ T-cell activation.[183] These methodologies harness nanoparticles' photodynamic or photothermal effects to trigger immunogenic cell death and liberate tumor-associated antigens, subsequently activating immune cells and fostering antitumor immune responses. Tang *et al.* demonstrated that tumor cell membrane-targeted photosensitive dimers facilitated highly efficient immunogenic cell death (ICD) in tumor cells, propelling cancer immunotherapy.[161] Furthermore, Chen *et al.* designed cancer cell membrane-coated nanoparticles co-loaded with photosensitizer and TLR7 agonist, synergistically enhancing tumor immunotherapy.[163] A formulation comprising TLR9 agonist encapsulated with membrane particles extracted from cells pre-induced with doxorubicin (DM@NPs) was formulated and tested in a subcutaneous lung cancer model in mice (Fig. 10C). The complex generated a significant antitumor response that induced the accrual of activated T lymphocytes in the spleen and tumor lesions.[184] These examples highlight the importance of targeting immune influx and improving antigen presentation to overcome immune evasion. The immune response can be enhanced by utilizing tumor cell-derived membrane coating biomimetics. These findings provide valuable insights into the development of personalized cancer vaccines and the potential for combination therapies to improve the efficacy of cancer immunotherapy.

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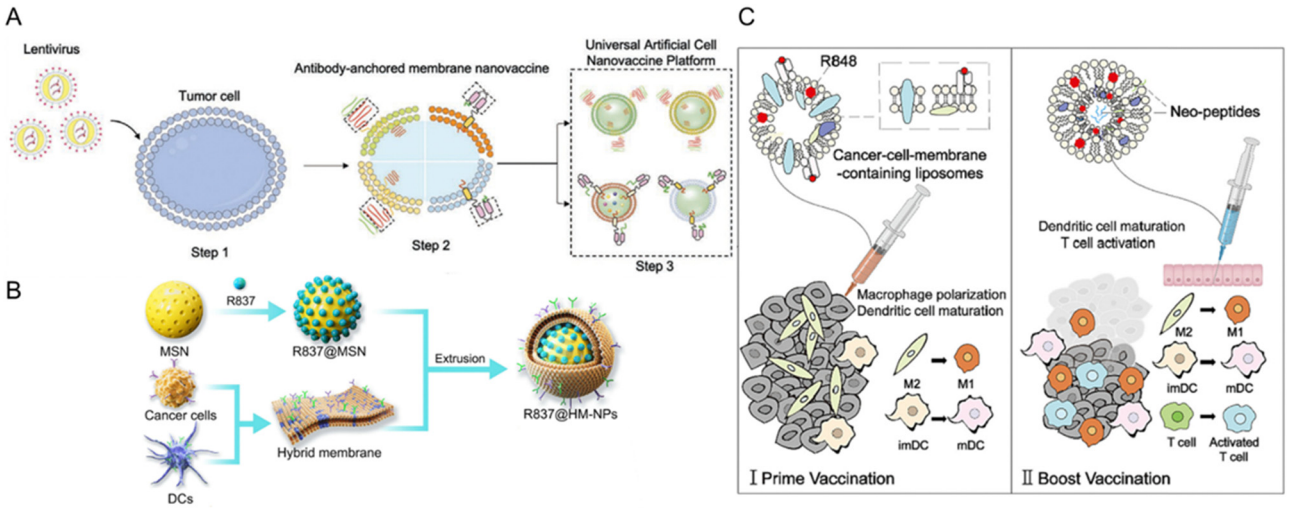

Fig. 9 Types of tumor cell membrane-derived nanovaccines fabricated for tumor immunomodulation. (A) Universal nanovaccine platform based on tumor cell-derived components and antibodyanchored membrane (nano-AAM). Reproduced from ref. 162 Copyright 2023 with permission from John Wiley & Sons. (B) A nanovaccines comprising mesoporous silica nanoparticles (MSN) and hybridized membrane components from cancer cells and DCs. MSN is functionalized with imiquimod R837, a TLR7 agonist for immune cell activation (R837@HM-NPs). Reproduced from ref. 158 Copyright 2022 originally published by and used with permission from Dove Medical Press Ltd. (C) Cell membrane-camouflaged liposomes and neopeptide-loaded liposomes for personalized cancer vaccine therapy. The cancer cell membrane-camouflaged liposomes with R848 (Resiquimod,TLR7 and TLR8 Agonist) primes the M2-like macrophages and DCs. Then, liposomes with neopeptides and R848 will boost the immune response by stimulating macrophages, DCs, and T-cells, thereby generating treatment outcomes. Reproduced from ref. 177 Copyright 2023 with permission from Elsevier.

4.1.2. Immune cell membrane. The application of immune cell membrane-coated nanoparticles represents a transformative approach in cancer therapy, addressing the challenges posed by the immunosuppressive tumor microenvironment (Table 3). Membrane particles from immune cells can potentiate the immune environment toward a favorable antitumor response. For example, specialized macrophage membrane-coated mesoporous silica nanoparticles have been engineered to target tumors by obstructing the adenosinergic A2A receptor (A2AR) pathway on Treg cells. Wen *et al.* devised the macrophage membrane-coated mesoporous silica nanoplatform containing catalase, doxorubicin, and R848 encapsulated in the mesoporous silica core (Fig. 11A). The nanoplatform can actively target the tumor site *via* ligand binding of the macrophage membrane to the A2AR of T regulatory (Treg) cells. The Treg cells can further be blocked by catalase, which releases

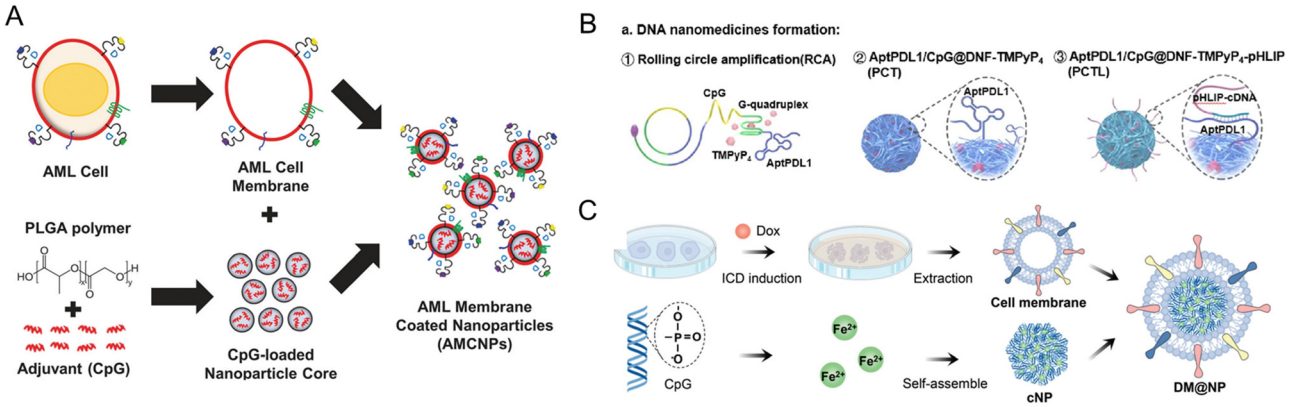

Fig. 10 Strategies for generating tumor cell membrane-coated nanoparticles for targeted delivery. (A) CpG oligonucleotides were encapsulated in poly(lactic-*co*-glycolic acid) (PLGA) polymer nanoparticle cores and loaded into isolated acute myeloid leukemia cell membranes equipped with membrane-associated MHCI restricted antigens (AMCNPs). Immature APCs can recognize the AMCNPs and induce immune activation. Reproduced from ref. 159 Copyright 2021 originally published by and used with permission from Springer Nature. (B) Formation of a DNA nanomedicine composed of PD-L1 aptamers and CpG nanoparticles (PCTL). A photosensitizer (TMPyP4) is inserted into the DNA structure. The PD-L1 aptamers are hidden by the presence of pHLIP-modified cDNA, rendering the formulation non-immunogenic. Reproduced from ref. 183 Copyright 2022 with permission from American Chemical Society. (C) Schematic illustration of the preparation of DM@NP. Tumor cells were pre-induced with doxorubicin to generate membrane particles with damage-associated molecular patterns (DAMPs) from immunogenic cell death. The cell membrane particles coat iron(II)-CpG nanoparticles, an immune adjuvant, and TLR9 agonists. Reproduced from ref. 184 Copyright 2023 with permission from John Wiley & Sons.

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Table 3 List of fused membrane for cancer therapy and immunomodulation

Membrane type	Source	Application	Targeting	Immunomodulation mechanism	Animal model	Ref.
Cancer cell and DC	4T1 cell line and BMDCs	Drug delivery	Tumor cells	Blockade immune checkpoints	Recurrent	158
Cancer cell and bacteria	4T1 cell line and *E. coli* bacteria	Drug delivery	Tumor cells	Enhance ICD	Metastatic	203
Cancer cell and RBC	ID8 cell line and blood	Drug delivery	Tumor cells	Activate cytotoxic T lymphocytes	Metastatic	169
Cancer cell and RBC	4T1 cell line and blood	Drug delivery	Tumor cells	Deplete tumor-associated macrophages	Subcutaneous	205
Cancer cell, RBC and macrophage	4T2 cell line, blood, and marrow	Antigen-presenting	Tumor cells	Polarize macrophages	Metastatic	204
Cancer cell and DC	4T1 cell line and BMDCs	Drug delivery	Tumor cells	Express immunological molecules	Bilateral	202
Cancer cell and DC	4T1 cell line and BMDCs	Drug delivery	Tumor cells	Activate cytotoxic T lymphocytes	Subcutaneous	201
Macrophage and thylakoid	RAW264.7 cell line and spinach	Drug delivery	Tumor cells	Induce TAM polarization	Subcutaneous	166

Abbreviation: BMDCs, bone marrow-derived dendritic cells; DCs, dendritic cells; ICD, immunogenic cell death; RBC, red blood cell.

oxygen, alleviating the hypoxic TME that activates the A2AR pathway in Treg cells. Once endocytosed in tumor cells, the platform releases doxorubicin to induce immunogenic cell death. R848 will stimulate DC maturation, leading to lymphocyte infiltration and activation in the TME. The macrophage membrane MSN platform significantly inhibited the growth of subcutaneous liver tumors *in vivo* and immunopotentiate the TME by decreasing the Treg population, increasing the $CD8^+$ population, and increasing cytokine secretion (IFN-γ and TNF-α).[164] Li *et al.* designed a similar macrophage membrane-coated nano gemcitabine (MNGs) for synergistic cancer immunotherapy (Fig. 11B). The system has an acidic pH drug-releasing capacity to potentiate lymphocyte infiltration and synergize with anti-PD-L1 therapy. MNGs were combined with PD-L1 checkpoint

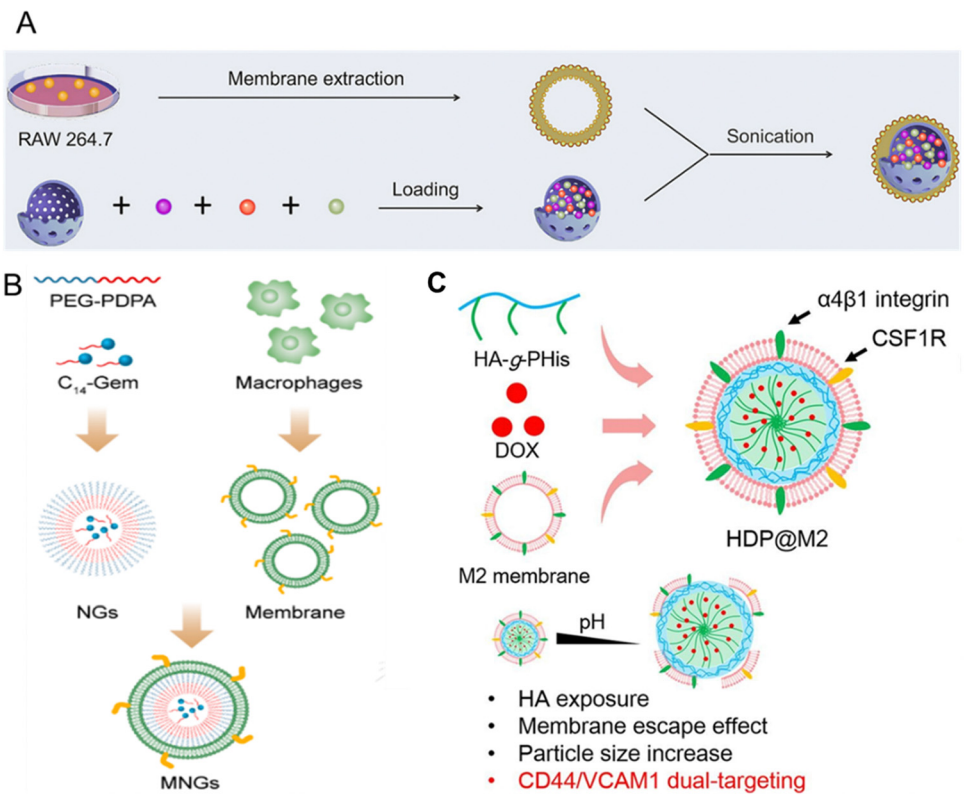

Fig. 11 Macrophage-coated nanoplatform. (A) Macrophage membrane-coated mesoporous silica nanoplatform containing catalase (purple dot), doxorubicin (orange dot), and R848 (green dot) encapsulated in the mesoporous silica core. Once endocytosed in tumor cells, the platform releases doxorubicin to induce immunogenic cell death and R848 to induce DC maturation, lymphocyte infiltration, and activation. Reproduced from ref. 164 Copyright 2022 with permission from Elsevier. (B) Macrophage membrane-coated nano gemcitabine (MNGs) for synergistic cancer immunotherapy. The system has an acidic pH drug-releasing capacity to potentiate lymphocyte infiltration and synergize with anti-PD-L1 therapy. Reproduced from ref. 165 Copyright 2023 with permission from American Chemical Society. (C) Tumor-associated macrophage (TAM) membrane-camouflaged nanoparticle for delivery of doxorubicin (DOX). Hyaluronic acid (HA)-*g*-poly(histidine) was synthesized and loaded with DOX in the inner core of the micelle. TAMs membrane was coated on the surface of the co-polymer to form HDP@M2. Reproduced from ref. 185 Copyright 2022 with permission from Elsevier.

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blockade therapy to investigate the possible treatment synergy in three subcutaneous tumor models in mice (4T1, PANC02, CT-26). The combined treatment exhibited enhanced antitumor response in all three tumor models, with the 4T1 model exhibiting the best treatment response. MNG plus PD-L1 blockade also restored the functions of $CD8^+$ T-cells and NK cells in the 4T1 tumors.[165] Tumor-associated macrophage (TAM) membrane-camouflaged nanoparticle for delivery of doxorubicin (DOX) was fabricated by Du *et al.* (Fig. 11C). In this formulation, hyaluronic acid (HA)-*g*-poly(histidine) was synthesized and loaded with DOX in the inner core of the micelle. TAMs membrane was then coated on the surface of the co-polymer to form HDP@M2. In the acidic TME, the particle size will swell and possess a ''membrane escape effect,'' exposing the HA residue. HA will target tumor cells *via* CD44 receptor-mediated endocytosis and facilitate entry of DOX in the cytosol. The α4β1 integrin on the macrophage membrane could also selectively bind to vascular cell adhesion molecule-1 (VCAM-1) on tumor cells, and CSF1R on the TAM membrane can neutralize CSF1 to abrogate the immune suppression in the TME. *In vivo*, HDP@M2 significantly inhibited the growth of subcutaneous 4T1 tumors, increased the IFN-γ secretion of $CD8^+$ T-cells, and significantly lowered the M2/M1 macrophage ratio in the TME.[185]

T lymphocyte membrane-decorated nanoinducers also offer a breakthrough solution in the tumor immunomodulation domain. T-lymphocyte membrane-derived exosomes are used to encapsulate ORY-1001, a potent and selective lysine-specific histone demethylase 1A (LSD1) inhibitor (OPEN, Fig. 12A). OPEN can be recognized by PD-L1 expressing cells due to the PD-1 on the surface of the T-lymphocyte membrane and upregulate intratumoral interferons and interferon-stimulated genes, such as MHCI that could enhance neoantigen presentation. Enhancing intratumoral interferons will block the immunosuppression mechanism and increase immune cell recruitment.[186] In another study by Li *et al.*, a PBA-modified T-cell membrane was used for cloaking the RCM to generate tumor microenvironment-responsive nanoparticles (Fig. 12B). RCM is a redox-sensitive hyaluronic acid grafted copolymer with antitumor drug curcumin (CUR) incorporated in the core. In the acidic TME, PBA dissociates, releasing the T-cell membrane debris and exposing HA for tumor-targeting. T-cell membrane debris binds with PD-L1 on the tumor cells and blocks immune checkpoint suppression, resulting in $CD8^+$ T-cell activation and infiltration for immunotherapy. The RCM will be endocytosed *via* HA interaction with CD44 and will release CUR for tumor killing *via* the breakage of disulfide bonds in the redox environment. These nanoparticles enhance cytokine release and induce immunogenic cell death, elevating $CD8^+$ T-cell levels and providing an effective immune-chemotherapy approach for melanoma.[187] Ma *et al.* integrated CAR-T-cell membranes onto silica nanoparticles for targeted delivery to HCC tumors. CAR-T-cell membrane, specifically recognizing $GPC3^+$ HCC cells, was used to coat mesoporous silica containing IR780 dye for PDT and PTT applications (Fig. 12C). This nanomaterial exhibited photothermal and targeting abilities, providing an alternative strategy for treating HCC, a disease with limited treatment options.[167] In summary, immune cell membrane-coated nanoparticles have shown great potential for tumor immunomodulation. These nanoplatforms actively target tumors, inhibit immunosuppressive pathways, induce immunogenic cell death, enhance immune cell infiltration, and restore tumoricidal functions. They offer a promising strategy for improving the efficacy of cancer immunotherapy,

4.1.3. Erythrocyte membrane. The utilization of blood cell membrane-camouflaged biomimetics has been explored for building cancer immunotherapy platforms (Table 2). A hybrid membrane comprising an ovarian cancer cell membrane and an RBC membrane was used to coat ICG-loaded magnetic nanoparticles to generate a Fe_3O_4-ICG@IRM nanoparticle platform (Fig. 13A). The complex is used for the synergistic PTT-immunotherapy of ovarian cancer. ID8 tumor antigens direct the platform towards ID8 tumors *via* homologous homing, and the tumor antigens on the IRM can induce antitumor immune response. *In vivo*, PTT with Fe_3O_4-ICG@IRM induced tumor regression in an antigen-specific manner, as observed by the lack of antitumor effects in the distant B16F10 implanted melanoma tumors in the opposite flank. Most importantly, the PTT platform induced a potent immunomodulation activity in the draining lymph nodes and the spleen. Increased $CD4^+$ and $CD8^+$ lymphocytes were observed, along with an increase in the percentage of activated DCs and macrophages.[169] A study by Lin *et al.* used a bacterial glucan, curdlan, as an immunomodulatory agent and erythrocyte membrane as a cloaking mechanism. Doxorubicin was loaded on the curdlan to provide added chemotherapeutic potential (Fig. 13B). The ICUR-DOC@RBC system displayed M2 macrophage reprogram capability *in vitro* and significantly inhibited the growth of A-375 cells subcutaneously implanted *in vivo*. The immunohistochemistry analysis of the tumor lesions corresponded with *in vitro* observations with reduced percentage of M2 TAMs markers.[168]

Another approach using erythrocyte membrane involves camouflaging DNA-functionalized upconversion nanoparticles (UCNPs) with red blood cell membrane for targeted breast cancer chemotherapy and immunotherapy (Fig. 13C). UCNPs were modified for doxorubicin loading followed by membrane coating to yield HR-UCAD. HR-UCAD induced immunogenic cell death of 4T1 cells *in vitro*, releasing factors that induces the migration and activation of DC2.4 cells. *In vivo*, the formulation is efficiently inhibited tumor growth with concomitant increase in the percentage of $CD8^+$ and $CD4^+$ T-cell populations.[188] Another PTT platform with erythrocyte membrane utility involves use of biomimetic black phosphorus quantum dot formulation coated with eryhtrocyte membrane (BPQD-RMNV, Fig. 13D). Combined with immune checkpoint blockade (anti PD-1), BPQD-RMNV induced regression of innoculated 4T1 breast cancers in mice, inhibited the growth of distant tumors, and increased infiltration of $CD8^+$ T-cells in the tumor lesions.[171] These studies exemplify the feasibility of erythrocyte membrane as a cloaking tool to increase the circulation time of various nanoparticle platforms without inducing severe toxicity.

Recent studies highlight the innovative use of platelet-derived membrane-coated nanoparticles (PMCNPs) in cancer therapy (Fig. 14). A novel delivery method employing PMCNPs

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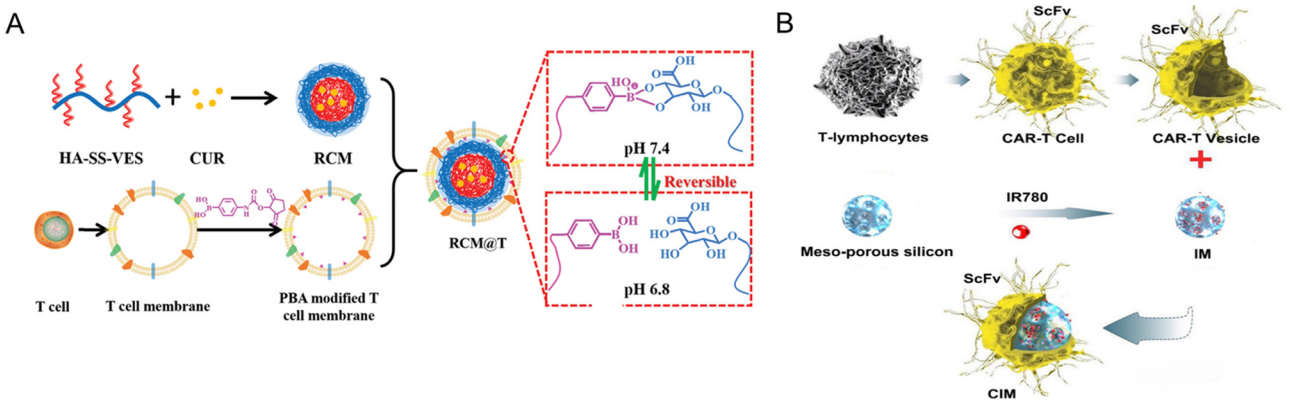

Fig. 12 T-cell-coated nano platform. (A) A redox sensitive hyaluronic acid grafted copolymer (HA-grated-disulfide bond-vitamin E succinate, shortened as HA-SS-VES) was incorporated with antitumor drug curcumin (CUR) to prepare RCM. A PBA-modified T-cell membrane was used to cloak the RCM and generate tumor microenvironment-responsive nanoparticles. In the acidic TME, PBA dissociates, releasing the T-cell membrane debris and exposing HA for tumor-targeting. T-Cell membrane debris binds with PD-L1 on the tumor cells and blocks immune checkpoint suppression, resulting in $CD8^+$ T-cell activation and infiltration for immunotherapy. The RCM will be endocytosed *via* HA interaction with CD44 and will release CUR for tumor killing *via* the breakage of disulfide bonds in the redox environment. Reproduced from ref. 187 Copyright 2021 with permission Royal Society of Chemistry. (B) CAR-T-cell membrane, specifically recognizing $GPC3^+$ HCC cells, was used to coat mesoporous silica containing IR780 dye for PDT and PTT applications to treat HCC. Reproduced from ref. 167 Copyright 2020 originally published and reused with permission from Ivy Spring International Publisher.

conjugated with anti-PD-1 antibodies, enhancing tumor-specific thrombosis and improving drug delivery, thus enhancing therapeutic efficacy in breast cancer models.[189] Another study focused on modulating the immunosuppressive tumor microenvironment by targeting lactate metabolism using PMCNPs coated with metal–organic frameworks, resulting in enhanced immunogenic cell death and tumor growth inhibition.[190] The potential of intratumoral immunotherapy was demonstrated using platelet-cloaked nanoparticles, showcasing tumor regression and metastasis inhibition.[191] Similarly, PMCNPs were utilized for pancreatic cancer therapy, encapsulating ferroptosis inducers to disrupt tumor vasculature and inhibit cancer

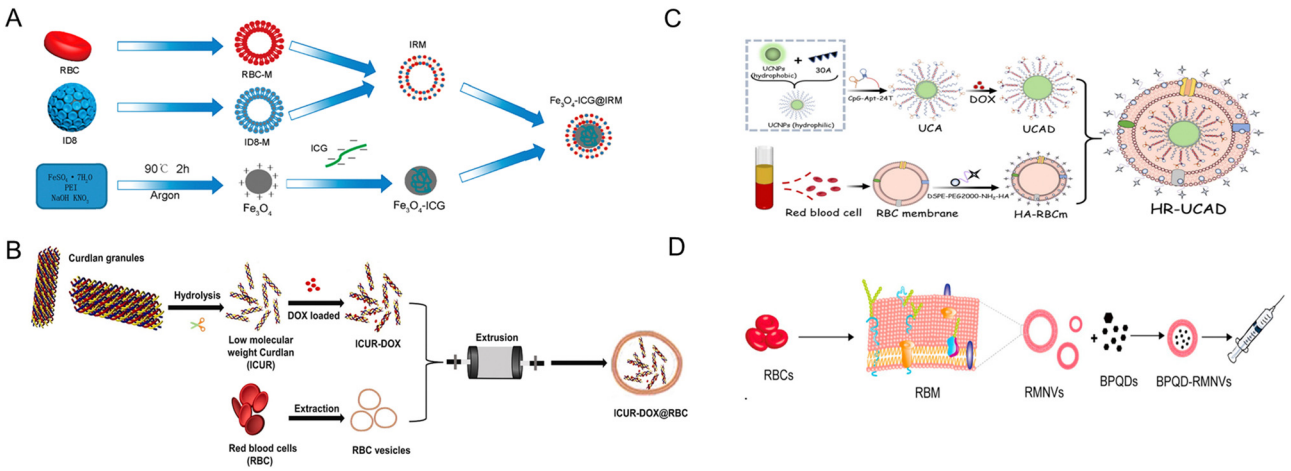

Fig. 13 Red blood cell (RBC)-derived bio-nanoparticles. (A) A hybrid membrane comprising ID8 (ovarian cancer) cell membrane (ID8-M) and RBC membrane (RBC-M) was used to coat ICG-loaded magmetic nanoparticles ((Fe_3O_4-ICG@IRM)). The complex is used for the synergistic PTT-immunotherapy of ovarian cancer. ID8 tumor antigens on the surface can direct to ID8 tumors *via* homologous homing and the tumor antigens on the IRM can induce antitumor immune response. Reproduced from ref. 169 Copyright 2021 with permission from American Chemical Society. (B) Doxorubicin was encapsulated in the helical structure of the curdlan, a microbial glucan. RBC membrane was then used to wrap the outer layer of the DOX-curdlan using the coextrusion method to generate ICUR-DOX@RBC NPs. Curdlan can interact with dectin-1 receptors on macrophages to induce pro-inflammatory cytokine production and induce M2 → M1 polarization. Reproduced from ref. 168 Copyright 2022 with permission from Elsevier. (C) RBC membrane-camouflaged DNA-functionalized upconversion nanoparticles (UCNPs) for targeted breast cancer chemotherapy and immunotherapy. UCNPs were modified with a 30-mer poly A oligonucleotide hybridized with 24-mer poly T of CpG-aptamer to form a duplex. Dox was introduced into the base pairs of the duplex and the UCNPs@CpG-Apt/DOX was encapsulated within the erythrocyte membrane to form HR-UCAD. Reproduced from ref. 188 Copyright 2023 with permission from Royal Society of Chemistry. (D) A biomimetic black phosphorus quantum dot formulation coated with eryhtrocyte membrane for the PTT of breast cancer (BPQD-RMNV). This formulation was combined with immune checkpoint blockade (anti PD-1) to cure innoculated breast cancer in mice. BPQD-RMNV displayed accumulation in tumor *in vivo* and increased infiltration of $CD8^+$ T-cells in the tumor. Reproduced from ref. 171 Copyright 2019 with permission from Elsevier.

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progression.[192] PMCNPs camouflaged with magnetic nanoparticles were reported for ferroptosis-enhanced cancer immunotherapy, improving the efficacy of immunotherapy and inducing tumor-specific immune responses.[170] Additionally, Yan *et al.* and Chen *et al.* utilized PMCNPs for combined chemo-immunotherapy, demonstrating significant inhibition of tumor growth and metastasis in melanoma and acute myeloid leukemia models, respectively.[193,194] These studies collectively underscore the potential of PMCNPs in targeting tumors and modulating the tumor microenvironment for enhanced cancer therapy.

4.1.4. Fused cell membrane. The fusion of cell membranes has emerged as a novel strategy to enhance the efficacy of cancer immunotherapy, as evidenced by a series of studies exploring the potential of hybrid biomimetic nanoparticles in augmenting antitumor immune responses (Table 3). These studies have collectively demonstrated that the fusion of tumor cell membranes with other cell types, such as erythrocytes, dendritic cells, and macrophages, can lead to the development of innovative therapeutic platforms with improved targeting capabilities, immune system modulation, and therapeutic outcomes.

One approach involves the combination of cancer cell membranes with erythrocyte membranes to create hybrid coatings for nanoparticles, which exhibit immune evasion and homologous tumor-targeting properties. This strategy has been successfully applied to ovarian cancer, where the hybrid membrane-coated nanoparticles activated specific immunity and exhibited synergistic photothermal-immunotherapy effects, leading to the activation of cytotoxic T-cells and reduction of regulatory T-cells.[169] A similar concept has been applied using cytomembranes of fused cells derived from dendritic cells (DCs) and cancer cells. This fusion results in nanoparticles that mimic antigen-presenting cells (APCs), displaying tumor antigens and co-stimulatory molecules, which can activate T-cells and induce a powerful antitumor immune response.[201] The same group further expanded this approach by engineering nanoplatforms from the cytomembranes of hybrid cells derived from cancer and DCs, which showed significant antitumor effects in mouse models.[202]

Another innovative strategy is the use of hybrid membranes combining tumor cell membranes with bacterial outer membranes. This approach has been shown to enhance radiosensitivity and trigger antitumor immune responses in breast cancer, thereby amplifying the effects of radiotherapy and immunotherapy.[203] Additionally, hybrid cellular membrane nanovesicles have been engineered to amplify macrophage immune responses against cancer recurrence and metastasis by blocking the CD47-SIRPα signaling axis and promoting macrophage repolarization within the tumor microenvironment.[204]

Moreover, pH-sensitive hybrid membrane-coated nanoparticles have also been developed to reprogram the tumor microenvironment and boost antitumor immunity. These

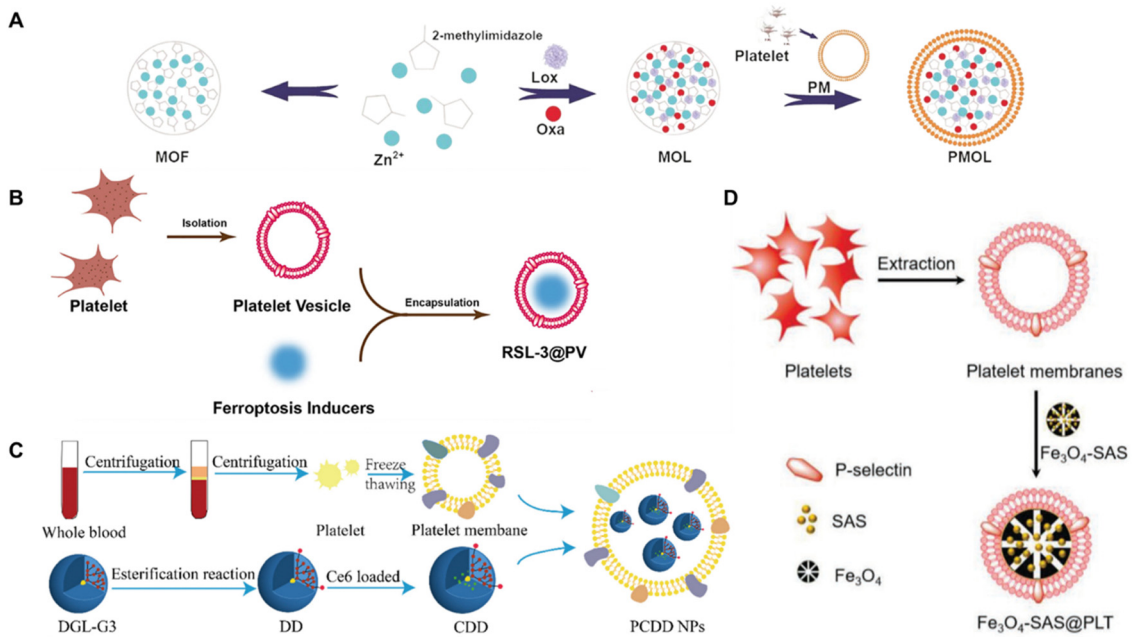

Fig. 14 Platelet-derived bio-nanoparticles. (A) Platelet vesicles (PVs) were used to encapsulate a ferroptosis inducer, RSL-3, generating RSL-3@PV nanoparticles. RSL-3@PV nanoparticles were used for treating pancreatic ductal adenocarcinoma (PDAC). Reproduced from ref. 192 Copyright 2022 originally published and reused with permission from frontiers. (B) Novel platelet membrane-coated nanoparticles (PCDD NPs) were constructed for combined chemo-photodynamic- and immunotherapy of melanoma. Reproduced from ref. 193 Copyright 2022 with permission from Royal Society of Chemistry (C) metal–organic frameworks were coated with platelet membranes (PM) for tumor site-specific delivery and rationally designed to carry lactate oxidase which catalytically consumed lactate, while oxaliplatin induced ICD. Reproduced from ref. 190 Copyright 2023 with permission from Elsevier. (D) Sulfasalazine (SAS)-loaded mesoporous magnetic nanoparticles (Fe_3O_4) were camouflaged with platelet membrane to generate Fe_3O_4-SAS@PLT nanoparticles. The formulation can trigger ferroptotic cell death, inducing a tumor-specific immune response and repolarize macrophages from M2 → M1 phenotype. Reproduced from ref. 170 Copyright 2020 with permission from John Wiley & Sons.

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nanoparticles are designed to target the tumor microenvironment and escape from endo/lysosomes after endocytosis, leading to significant tumor inhibition and immune activation.[200] Additionally, erythrocyte-cancer cell hybrid membrane camouflaged pH-responsive copolymer micelles have been employed to selectively deliver a CSF-1R inhibitor to tumor-associated macrophages, resulting in TAMs depletion and reversal of the tumor immune microenvironment.[205] Lastly, hybrid membrane-coated nanoparticles have been utilized to regulate the immunosuppressive tumor microenvironment and enhance breast cancer immunotherapy by co-delivering immuno-metabolic adjuvant and immune checkpoint inhibitors.[206]

In conclusion, the fusion of cell membranes to create hybrid biomimetic nanoparticles represents a promising avenue for cancer immunotherapy. These platforms leverage the unique properties of different cell types to target tumors, modulate the immune system, and deliver therapeutic agents, thereby enhancing the overall therapeutic efficacy against various cancers. These studies highlight diverse approaches utilizing cell membrane-camouflaged nanoparticles for tumor immunomodulation. These biomimetic nanosystems aim to optimize drug delivery, prolong circulation, and induce immune responses for effective cancer treatment. Utilizing various cell membranes, such as red blood cells and platelet membranes, to encapsulate therapeutic agents has shown promising outcomes, including inhibiting tumor growth, reprogramming immune cells, inducing immunogenic cell death, and improving cancer immunotherapy outcomes.

4.2. Engineering exosomes for immunomodulation

This section delves into the multifaceted roles of exosomes derived from tumors, immune cells, bacteria, and other sources in modulating the immune landscape of cancer. Tumor-derived exosomes (TDEVs) have been identified as double-edged swords within the tumor microenvironment (TME), capable of both inducing immunosuppression that hampers the efficacy of CAR-T-cells and other immunotherapies and, conversely, being engineered to enhance antitumor immunity.[207–210] The narrative then shifts to the promising capabilities of immune cell-derived exosomes. These nanoscale vesicles, sourced from T-cells, macrophages, dendritic cells (DCs), and natural killer (NK) cells, are adept at antigen presentation and can be tailored to deliver specific therapeutic payloads.[211–214] Their role in priming the immune system and synergistic effects with existing therapies, such as immune checkpoint inhibitors, are highlighted.

Bacterial membrane vesicles, particularly outer membrane vesicles (OMVs) from Gram-negative bacteria, are presented as novel and potent tools for tumor immunomodulation. The section discusses how these OMVs can be harnessed to enhance the infiltration and activation of tumor-specific T-cells, demonstrating synergistic effects with checkpoint blockade therapies.[215–217] Lastly, the section examines exosomes derived from alternative sources such as plasma, red blood cells, and stem cells, underscoring their potential as biomarkers for immunotherapy outcomes and as vehicles for targeted drug delivery. These exosomes are poised to reshape the tumor microenvironment and augment the efficacy of immunotherapeutic strategies.[218–220]

4.2.1. Tumor-derived exosomes (TDEVs). Numerous studies have explored the impact of TDEVs on immune cells and their potential as therapeutic targets in cancer immunotherapy (Table 4). A consistent finding in these investigations is the ability of naturally occurring TDEVs to induce immunosuppression within the TME. TDEVs have been shown to impede the proliferation, migration, and functionality of CAR T-cells, diminishing their effectiveness against solid tumors (Fig. 15A).[207] Furthermore, TDEVs carrying the CD19 antigen have been identified as suppressors of CD19-targeted CAR-T-cells, resulting in treatment failure.[221] This is accompanied by elevated levels of immune checkpoint markers and T-cell exhaustion, reducing the cytotoxicity of CD19-CAR T-cells.[222–224] TDEVs also polarize macrophages toward an immunosuppressive state, induce T-cell exhaustion, and promote the expansion of regulatory T-cells (Tregs) and myeloid-derived suppressor cells (MDSCs).[225–228] This collectively creates an immunosuppressive environment within the tumor, inhibiting the antitumor immune response. Hence, the use of TDEVs is contentious and requires careful manipulation to generate therapeutic value.

On the contrary, engineering efforts have explored and opened the potential of TDEVs to enhance cancer immunotherapy.[229,230] Zhang *et al.* engineered HEK293T-derived EVs to present the CD19 antigen as the CAR target (Fig. 15B). *In vitro* evaluation demonstrated that the CD19-EVs activated the CAR-T cells in an antigen-specific and dose-dependent manner resulting in the expansion and cytokine secretion of the co-cultured CAR-T cells. *In vivo*, adoptive cell transfer of the CD19-EVs led to the significant regression of subcutaneously implanted Raji tumor cells in mice along with increased CAR-T population in the excised tumors. Excessive cytokine secretion is one of the limitations of CAR-T cell therapy. To ensure that the CD19-EVs did not induce cytokine release syndrome (CRS), serum levels of IFN-γ, IL-2 and TNF-α were measured. No obvious increase in serum cytokine was observed, except for the obvious increase in IFN-γ. This study demonstrates the utility of TDEVs as an antigen presentation vehicle, without the tumor promoting and CRS risks. Furthermore, it also demonstrated that tumor cells themselves can be utilized to reprogram the tumor microenviroment, *via* sufficient immune activation resulting in immunogenic cell death. Exosomes from the immunologically terminated tumors can hypothetically contain optimum EV cargo for TME reprogramming (Fig. 15C).

The use of standard treatment modality methods that can induce immunogenic cell death is another solution to generating TDEVs with therapeutic value. In these cases, TDEVS are used to encapsulate treatment agents that can be delivered to the tumor site and promote immunogenic cell death. Wang *et al.* fabricated exosomes loaded with sonosensitizers and immune adjuvants to stimulate anti-tumor immunity, namely $Exo^{Ce6+R848}$ (Fig. 16A).[231] $Exo^{Ce6+R848}$ was constructed by simple co-incubation of chlorin e6 and R848 (TLR7 and TLR 8 agonist). After intratumoral injection to the tumor site, ultrasound irradiation was performed two hours later. The treatment

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Table 4 List of tumor, and immume cell-derived exosome for cancer therapy and immunomodulation

Exosome type	Source	Application	Targeting	Immunomodulation mechansim	Animal model	Ref.
Bladder cancer	MB49 cell line	Antigen-presenting	Macrophages	Inhibit macrophages differentiation	Subcutaneous	226
Breast cancer	A549 cell line	Drug delivery	Tumor cells	Blockade immune checkpoints	Metastatic	230
Breast cancer	4T1 cell line	Drug delivery	Tumor cells	Induce ICD	Orthotopic	209
Breast cancer	4T1 cell line	Antigen-presenting	Tumor cells	Increase cytokines secretion	Subcutaneous	235
Breast cancer	4T1 cell line	Drug delivery	Tumor cells	Promote macrophage polarization	Subcutaneous	228
Colon cancer	CT26 cell line	RNA cargo	T lymphocyte	Increase T lymphocyte infiltrations	Subcutaneous	238
Colon cancer	MC38 cell line	Antigen-presenting	Tumor cells	Blockade immune checkpoints	Transgenic	233
Colon cancer	MC38 cell line	Antigen-presenting	T lymphocyte	Increase M2-like macrophages	Subcutaneous	226
CLL	EHEB cell line	Antigen-presenting	T lymphocyte	Impair T-Cell activation	—	238
Glioblastoma	Body fluids	Antigen-presenting	T lymphocyte	Inhibiti T lymphocyte cycle	Orthotopic	250
HNSCC	Tumor tissue	Antigen-presenting	Macrophages	Increase cytokines secretion	Subcutaneous	251
Leukemia	NALM6 cell line	Antigen-presenting	T lymphocyte	Activate CAR-T Cells	Subcutaneous	221
Lymphoma	Nalm-6 cell line	Antigen-presenting	T lymphocyte	Reprogram T lymphocytes	NA	222
Leukemia	HL-60 cell line	Antigen-presenting	T lymphocyte	Inhibiti T lymphocyte proliferation	—	252
Melanoma	B16 cell line	RNA cargo	T lymphocyte	Induce T lymphocyte responses	Transgenic	236
Melanoma	B16 cell line	Antigen-presenting	T lymphocyte	Blockade immune checkpoints	Metastatic	234
NSCLC	MRC-5 cell line	RNA cargo	Tumor cells	Induce immunosuppression	Subcutaneous	237
Neuroblastoma	9464D cell line	Antigen-presenting	Tumor cells	Sensitize tumors to dinutuximab	Transgenic	253
OSCC	Tumor tissue	Antigen-presenting	Lymph nodes	Show the distinct immunosuppression	Subcutaneous	254
Ovarian cancer	HeyA8 cell line	Antigen-presenting	Tumor cells	Induce T lymphocyte exhaustion	Orthotopic	223
Pancreatic cancer	MIA-PaCa-2 cell line	Antigen-presenting	Dendritic cells	Increase cytokines release	Engrafted	210
Pancreatic cancer	PDAC cell line	Antigen-presenting	T lymphocyte	Inhibit the efficacy of CAR-T Cells	Transgenic	207
Dendritic cell	DC2.4 cell line	Antigen-presenting	Tumor cells	Elicit T lymphocyte responses	Metastatic	213
Dendritic cell	Bone marrow	Drug delivery	Tumor cells	Induced T lymphocyte proliferation	Subcutaneous	212
Dendritic cell	Bone marrow	Antigen-presenting	Tumor cells	Induce susceptibility to anti-PD-1	Subcutaneous	255
Dendritic cell	DC2.4 cell line	Antigen-presenting	Tumor cells	Induce tumor-specific immune responses	Orthotopic	245
Dendritic cell	DC2.4 cell line	RNA cargo	Tumor cells	Induce anticancer immunity	Subcutaneous	214
Dendritic cell	Bone marrow	Antigen-presenting	T lymphocytes	Activate tumor-specific CTLs	Metastatic	256
Dendritic cell	DC2.4 cell line	Antigen-presenting	Tumor cells	Activate T lymphocyte	Subcutaneous	243
Macrophage	F4/80 cell line	Drug delivery	Tumor cells	Repolarize macrophages	Subcutaneous	211
Macrophage	RAW264.7 cell line	Antigen-presenting	Tumor cells	Promote macrophage polarization	Subcutaneous	241
Macrophage	RAW264.7 cell line	Drug delivery	Tumor cells	Induce M1 polarization	Subcutaneous	247
Macrophage	RAW264.7 cell line	RNA cargo	Tumor cells	Boost intratumoral immune activation	Subcutaneous	249
T lymphocyte	Spleen and blood	Antigen-presenting	Macrophages	Prime macrophages	Subcutaneous	257
T lymphocyte	Blood	Antigen-presenting	Tumor cells	Promote T lymphocyte migration	Transgenic	248
T lymphocyte	Blood	Antigen-presenting	Tumor cells	Target mesothelin	Subcutaneous	258
T lymphocyte	Blood	Antigen-presenting	Tumor cells	Block PD-L1 and scavenge TGF-β	Subcutaneous	242
T lymphocyte	Blood	Antigen-presenting	Tumor cells	Exhibit cytotoxic activity	Subcutaneous	244

Abbreviation: CLL, chronic lymphocytic leukemia; HNSCC, head and neck squamous cell carcinoma cells; CTLs, cytotoxic T lymphocytes; ICD, immunogenic cell death; MSCm, mesenchymal stem cell membranes; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; PD-L1, programmed death-ligand 1; PDAC, pancreatic ductal adenocarcinoma; TGF-β, transforming growth factor-beta.

significantly inhibited tumor growth and induced significant increase of Il-1β, IL-6, IL12, TNF-α, and IFN-γ in the tumor lysates. Conversely, the secretion of anti-inflammatory cytokines, IL-10 and TGF-β, was significantly reduced.[231] Similarly, Hu *et al.* developed doxorubicin-loaded hybrid nanovesicles comprising liposomes and tumor-derived exosomes (DOX@LINV) fused for combinational immunochemotherapy (Fig. 16B).[232] DOX@LINV delivered doxorubicin to the subcutaneous B16F10 subcutaneous tumor sites, eliciting immunogenic cell death. An analysis of the populations in the tumor draining lymph nodes (TDLNs) after immunochemotherapy revealed a decrease in the $Foxp3^+$ Treg, with a concomitant increase in $CD8^+$ and $CD4^+$ T-cell population and elevated granzyme B expression. Furthermore, DOX@LINV exhibited presence of mature DCs and T-cells in the, indicating mature DC migration and priming of T-cells *in vivo*.[232]

These studies highlight exosomes' capacity to deliver therapeutic agents to tumor sites, enhancing their anti-tumor effects. The ability of exosomes to modulate the immune response is commonly observed.[209,233] However, it is important to reiterate the contrasting nature of these studies according to the context of TDEVs exploration. Some studies explored exosomes' role in overcoming immunotherapy resistance.[233] Tumor-derived extracellular vesicles were found to counteract anti-PD-L1 antibodies, leading to therapy resistance.[227,234] Conversely, micrometer-sized tumor cell-derived vesicles acted as autologous cancer vaccines, enhancing systemic immune responses and potentially overcoming resistance.[208,209,235] Hence, exosomes studies should always be performed in a disease-specific context manner due to the innumerable tumor mechanisms that differ in each disease. Furthermore, naturally occurring TDEVs are useful when tumor cells were induced to undergo immunogenic cell death or are used as a nanoplatform as a vaccine and drug delivery capable of inducing immunogenic cell death, highlighting the importance of the death mechanism in generating therapeutic value. A recurring theme in these studies is the capacity of tumor-derived extracellular vesicles to carry tumor-associated antigens and interact with immune cells.[236,237] This interaction alters the phenotype and function of CAR-T cells, promoting the secretion of

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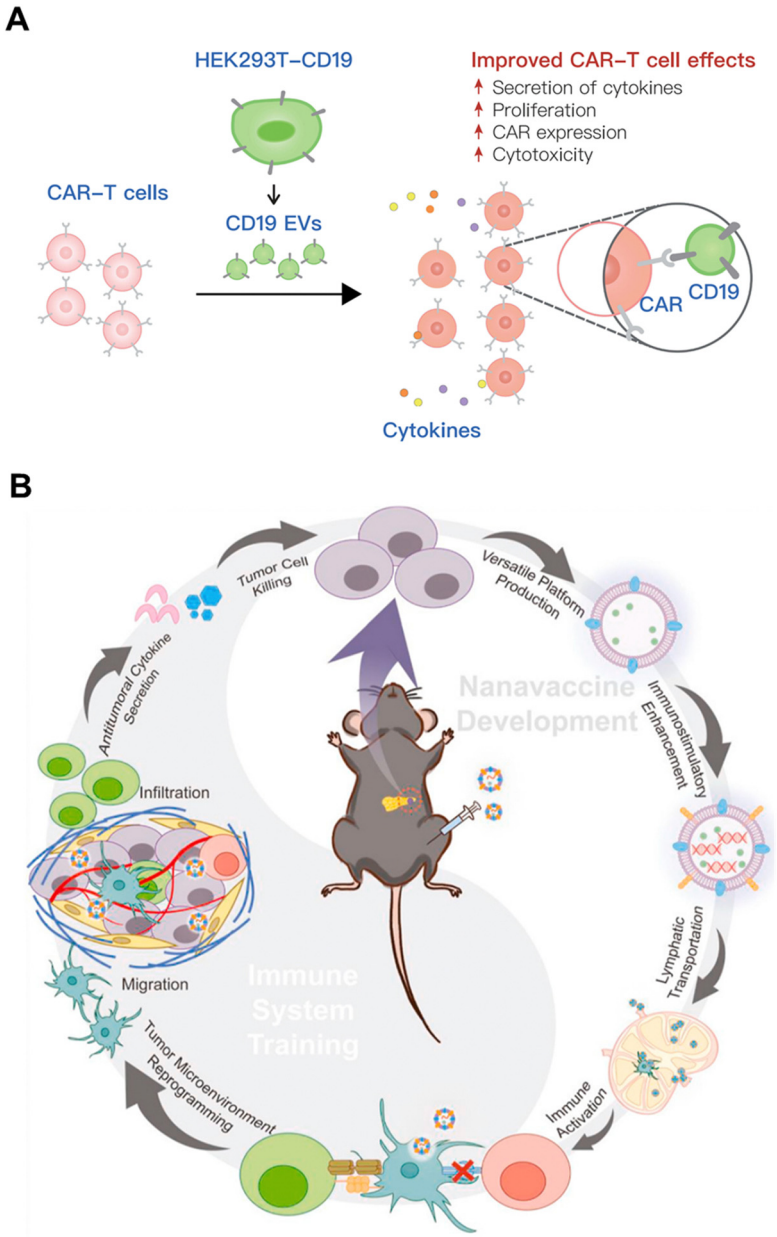

Fig. 15 Tumor-derived exosomes modulate the tumor microenvironment (A) EVs secreted by HEK293T cells stably expressed CD19 (CD19 EVs). The CD19 EVs were administered to CAR-T cells resulting in enhanced CAR T cell expansion and cytokine secretion, with elevated CAR expression leading to increased cytotoxicity. Reproduced from ref. 229 Copyright 2023 originally published by and used with permission from Dove Medical Press Ltd (B) exosomes derived from immunogenically dying tumor cells can be used to systemically activate the immune system and used for nanovaccine development. The systemically activated immune cells can home to the TME and reprogram the tumor milieu into a hot tumor state, training the immune system in the TME towards tumor eradication. Reproduced from ref. 208 Copyright 2022 with permission from Elsevier.

pro-inflammatory cytokines and upregulating activation-related genes.[236–238] In summary, while naturally occurring TDEVs impede immune responses, hindering CAR-T cell efficacy, engineered TDEVs have shown promise in enhancing immunotherapy, modulating immune responses, and potentially overcoming resistance. These findings underscore the dual role of exosomes in cancer immunomodulation and their significance in shaping effective therapeutic strategies.

4.2.2. Immune cell-derived exosomes. Exosomes, derived from various immune cells, have emerged as potent tools for cancer immunomodulation, showcased in diverse studies (Table 4). These nanoscale vesicles can be engineered to transport specific cargo, like proteins, cytokines, and immune checkpoint inhibitors, facilitating targeted delivery and immune response modulation. For example, T-cell-derived exosomes influence immune responses by antigen presentation.[239] A DC-based exosomal platform, mDexos, was incorporated with siBRAF siRNA *via* electroporation for a gene therapy-immunotherapy treatment combination (Fig. 17A). BRAF is a proto-oncogene linked to melanoma and some carcinomas involved in signaling pathways

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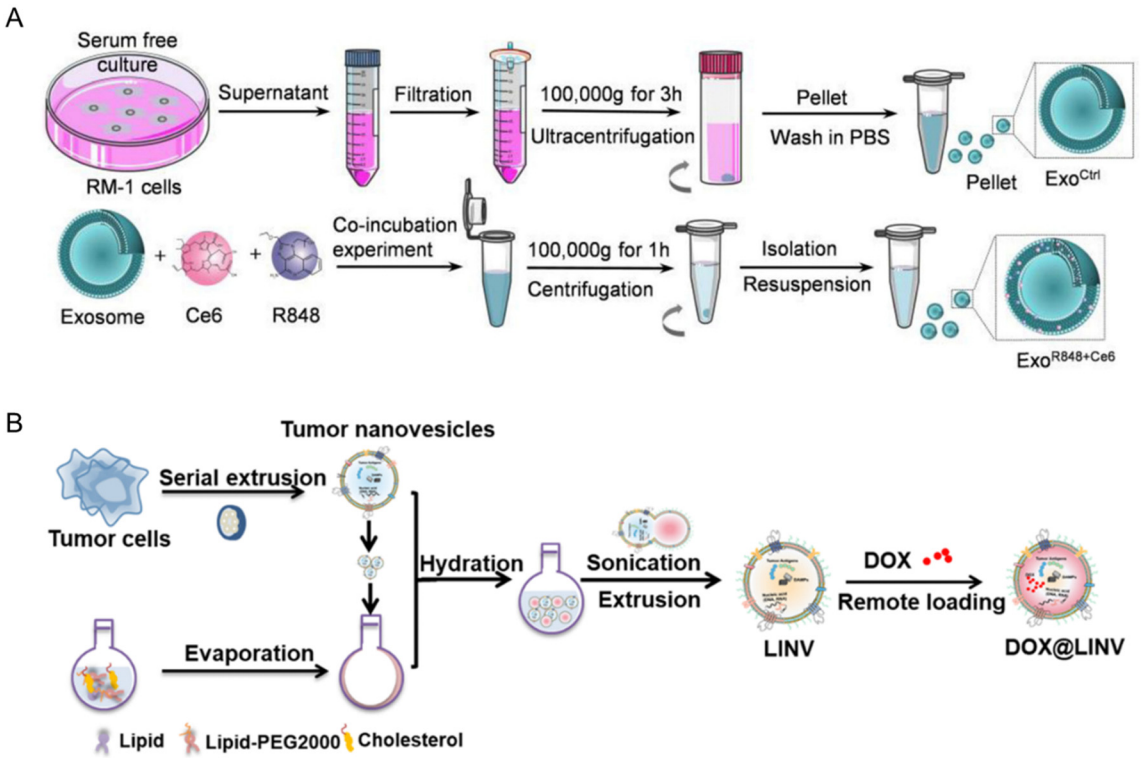

Fig. 16 Tumor-derived exosomes to enhance cancer immunotherapy. (A) $Exo^{Ce6+R848}$ was constructed by co-incubation of Ce8 and R848 with HEK293T cell-derived exosomes. $Exo^{Ce6+R848}$ can be engulfed by DCs and enhance their maturation after ultrasonic irradiation. These exosomes can also reprogram macrophages from M2 → M1 phenotype. Reproduced from ref. 231 Copyright 2022 originally published by and used with permission from Taylor & Francis Group. (B) DOX-loaded biomimetic hybrid nanoparticles were formed by the cofusion of tumor-derived nanovesicles with artificial liposomes (LIPs). The formed DOX@LINV platform was used for combination immunochemotherapy approach with the ability to target the tumor site *via* homologous homing and improve the immunogenicity of the tumor. DOX@LINV activated DCs and alleviate the immunosuppresive TME. Reproduced from ref. 232 Copyright 2021 with permission from American Chemical Society.

that lead to growth and survival.[240] The mDexos exhibited significantly greater inhibitory effect on BRAF expression, induced T-lymphocyte proliferation, and inhibited the growth of subcutaneous metastatic melanoma in mice.[212] Similarly, macrophage-derived exosomes have been engineered to deliver ferroptosis factors, RSL-3 to target tumor cells (Fig. 17B). The RSL-3-ExoM1 exhibited spontaneous homing to the 4T1 subcutaneous tumors inoculated in mice, inhibited tumor growth, and raised the percentage of M1 macrophages in the tumor lesions, reducing the M2 phenotype proportion. RSL3-ExoM1 treatment also promoted increased anti antitumorigenic cytokines (*i.e.*, IFN-γ and TNF-α) and decreased protumorigenic cytokines (*i.e.*, IL-10 and TGF-β) in the tumors.[241] Activated T-cells possess remarkable phenotypes for cancer immunotherapy but are limited in the TME due to immunosuppressive mechanism. Deriving exosomal platforms using T-cell membrane is a viable strategy to overcome the T-cell anergy associated with T-cell therapy approaches. In one example, Hong *et al.* fabricated T-cell-derived nanovesicles (TCNVs) *via* the serial extrusion method and characterized the EV system (Fig. 17C). TCNVs possess PD-1 protein and TGF-β receptor on their surface. Hence, they can block PD-L1 on cancer cells and scavenge TGF-β in the TME, preventing cytotoxic-T-cell exhaustion. TCNVs can also directly kill tumor cells *via* granzyme delivery. The antitumor effect of the TCNVs was demonstrated in an LLC (Lewis lung carcinoma) subcutaneous tumor model, displaying significant tumor growth inhibition and overall survival during 50 days of surveillance. Analysis of the tumor infiltrating lymphocytes form the excised lesions revealed increase granzyme B, IFN-γ, and TNF-α intracellular cytokine levels as well as decreased Treg populations. *In vitro*, TCNVs inhibited PD-L1 and TGFβ-mediated immune suppression of T-cells.[242]

Personalized cancer immunotherapies harness exosomes to deliver patient-specific neoantigens, provoking robust $CD8^+$ T-cell-mediated anticancer immunity.[214,243] Dendritic cell-derived exosomes loaded with neoantigens induce potent immune responses.[213] Furthermore, exosomes from chimeric antigen receptor T (CAR-T) cells maintain CAR expression, eliciting tailored tumor-specific immune responses and facilitating tumor eradication in preclinical models.[244] The exosome vaccines triggered tailored tumor-specific immune responses and tumor eradication in preclinical models.[245] Studies also explore innovative approaches.[214,246,247] Combining γδ-T-cell-derived exosomes (γδ-T-Exos) with radiotherapy overcomes radioresistance and preserves antitumor activities in immunosuppressive tumor microenvironments.[248] A photoactivatable silencing extracellular vesicle (PASEV) merges phototherapy with exosome-mediated small interfering RNA (siRNA) delivery against p21-activated kinase 4 (PAK4), an immune

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modulator involved in immune exclusion (Fig. 18A). PASEV elicited robust antitumor immunity against B16F10 subcutaneous melanoma tumors. *In vivo*, PASEV displayed PAK4 silencing and triggered potent antitumor immune response in the lesions and draining lymph nodes.[249]

Immune cell-derived exosomes for amplified CD47 blockade-based cancer immunotherapy is another strategic option. CD47 is found on the surface of many tumors and functions as a 'don't eat me' signal, shielding the tumor growth from immune cell prodding. A hybrid nanovesicles, hEL-RS17, derived from M1 macrophages and synthetic liposomes was formulated to target CD47 blockade (Fig. 18B). The formulation was decorated with RS17 peptide (for blocking CD47) metformin (an immunomodulator), shikonin (SK, a chemotherapeutic agent) and IR820 (a photosensitizer) for multi-approach treatment of metastatic melanoma. This platform represents a "all in one" nanoplatforms designed to tackle the signaling pathway effectors and induce immunogenic cell death *via* two separate mechanisms that does not exhibit cross-resistance. The platform exhibited potent antitumor efficacy against primary tumors in 4T1 breast tumor and B16F10 melanoma models in mice. An added benefit of lung metastasis inhibition is appealing as the immune response elicited involves protection from tumor recurrence and progression.[211]

A DC-based platform was engineered for precise solid tumor therapy *via* DC mediated antigen presentation (Fig. 18C). DC 2.4 cells were activated with ovalbumin as a proxy tumor antigen and was functionalized with anti-CD3 and anti-EGFR antibodies. The formulation functioned as CAR-T cell therapy system with MHC-antigen complexes for antigen presentation and CD86 co-stimulatory molecules for activation and expansion of T-cells. The formulation significantly inhibited the growth subcutaneous B16-Ova lung metastatic melanoma cells and curtailed the number of lung metastases foci *in vivo*. Significant increase of $CD4^+$ and $CD8^+$ T-cell population was observed in the tumor tissues *via* immunohistochemical staining.[243] In summary, immune cell-derived exosomes represent versatile immunomodulatory tools, offering targeted cargo delivery and profound immune response modulation. Through innovative strategies, they hold promise in reshaping cancer therapy, demonstrating their potential in personalized and combination treatments.

4.2.3. Bacteria-derived exosomes. Research groups have successfully addressed the challenge of large-scale production of *E. coli* OMVs, demonstrating their potent anti-tumor effects in mouse models (Table 4). OMVs are usually highly immunogenic and can cause severe immune response such as sepsis, cardiomyopathy and pulmonary diseases.[259] Focused efforts on detoxifying the OMVs *via* biochemical processes prior to use as a therapeutic exosomal platform have yielded benefits. The purification of OMVs that carry fewer immunogenic bacterial cytosolic proteins is achievable *via* methods such as pH stress, temperature stress, andiron depletion.[260] These OMVs increased the infiltration and activation of specific $CD8^+$

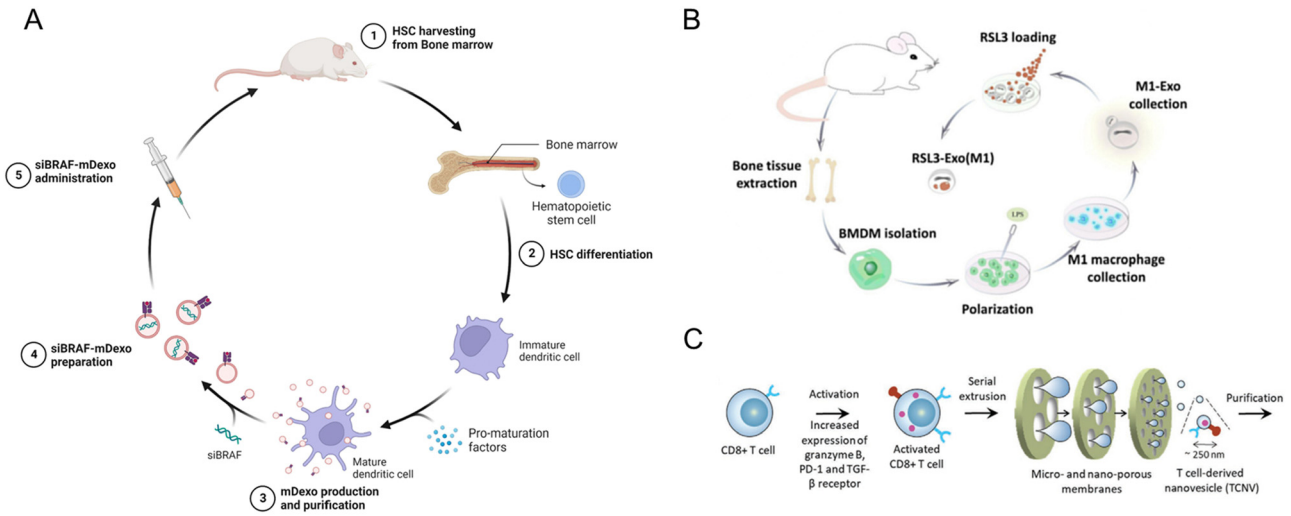

Fig. 17 Immune cell-derived exosomes. (A) Construction of DC-derived exosomes for co-delivery of gene therapy and immunotherapy factors, siBRAF-mDexos. Bone marrow cells from the fermus of healthy C57BL/6J mice were prepared for the isolation of hematopoietic stem and progenitor cells. The cells were left to differentiate in media containing 20 ng mL^{-1} IL-4 and 20 ng mL^{-1} GM-CSF for 5 days to generate immature DCs. The exosomes (mDexos) were isolated from the supernatant *via* gradient centrifugation. SiBRAF siRNA was incorporated into the mDexos *via* electroporation. Reproduced from ref. 212 Copyright 2023 originally published by and used with permission from Dove Medical Press Ltd. (B) Procedure for generating macrophage derived exosomes loaded with ferroptosis factors, RSL3-ExoM1. Bone marrow-derived macrophages were isolated from bone tissue of mice and stimulated by lipopolysaccharide to obtain M1-phenotype macrophages. The exosomes from the M1 macrophages were isolated, followed by M1-Exo production and collection *via* gradient centrifugation. RSL3, a ferroptosis inducer, was introduced into the exosomes *via* overnight shaking and excess unincorporated RSL-3 was removed by centrifugation. Reproduced from ref. 241 Copyright 2023 with permission from BMJ Publishing Group Ltd. (C) Preparation of T-cell-derived nanovesicles (TCNVs) for cancer immunotherapy *via* serial extrusion. TCNVs possess PD-1 protein and TGF-β receptor on their surface. Hence, they can block PD-L1 on cancer cells and scavenge TGF-β in the TME, preventing cytotoxic-T-cell exhaustion. TCNVs can also directly kill tumor cells *via* granzyme delivery. Reproduced from ref. 242 Copyright 2021 with permission from John Wiley & Sons.

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T-cells, including cancer antigen-specific stem-like $CD8^+$ T-cells expressing TCF-1 and PD-1.[215] Park *et al.* designed a synthetic SyBV bacterial exosomes fused with melanoma EVs as a cancer immunotherapy combined with PD-1 checkpoint inhibition (Fig. 19A). The synthetic SyBV bacterial exosomes were combined with melanoma EVs as an cancer immunotherapy combined with PD-1 checkpoint inhibition. The treatment induced tumor regression of subcutaneously implanted B16F10 or CT26 melanoma tumors in mice and elicited Th1 inflammatory cellular responses and humoral tumor-specific antibody production.[261] In fact, combining *E. coli* OMVs with anti-PD-1 antibody immunotherapy is an applied commonly used strategy resulted in synergistic effects, significantly inhibiting tumor growth and promoting the infiltration of activated cancer antigen-specific stem-like $CD8^+$ T-cells into the tumor microenvironment.[215,262,263]

Additionally, researchers utilized innovative strategies, such as modified *E. coli*-derived OMVs in versatile nanoplatforms, to enhance cancer immunotherapy. These modified OMVs facilitated targeted delivery, induced immunogenic cell death, and regulated the tumor environment. Bacterial OMVs-based *in situ* cancer vaccines and biomimetic hybrid nanoplatforms amplified antitumor immune responses and significantly inhibited tumors, offering potential clinical alternatives against tumors with reduced side effects.[203,216,264] In an exemplary work by Zhuang *et al.*, hybridized bacteria-plant hybrid vesicles (BPNs) can be generates by membrane fusion method comprising OMVs and thylakoid nanovesicles (NTs) prepared from spinach leaves (Fig. 19B). The efficacy of the BPNs in inducing tumor

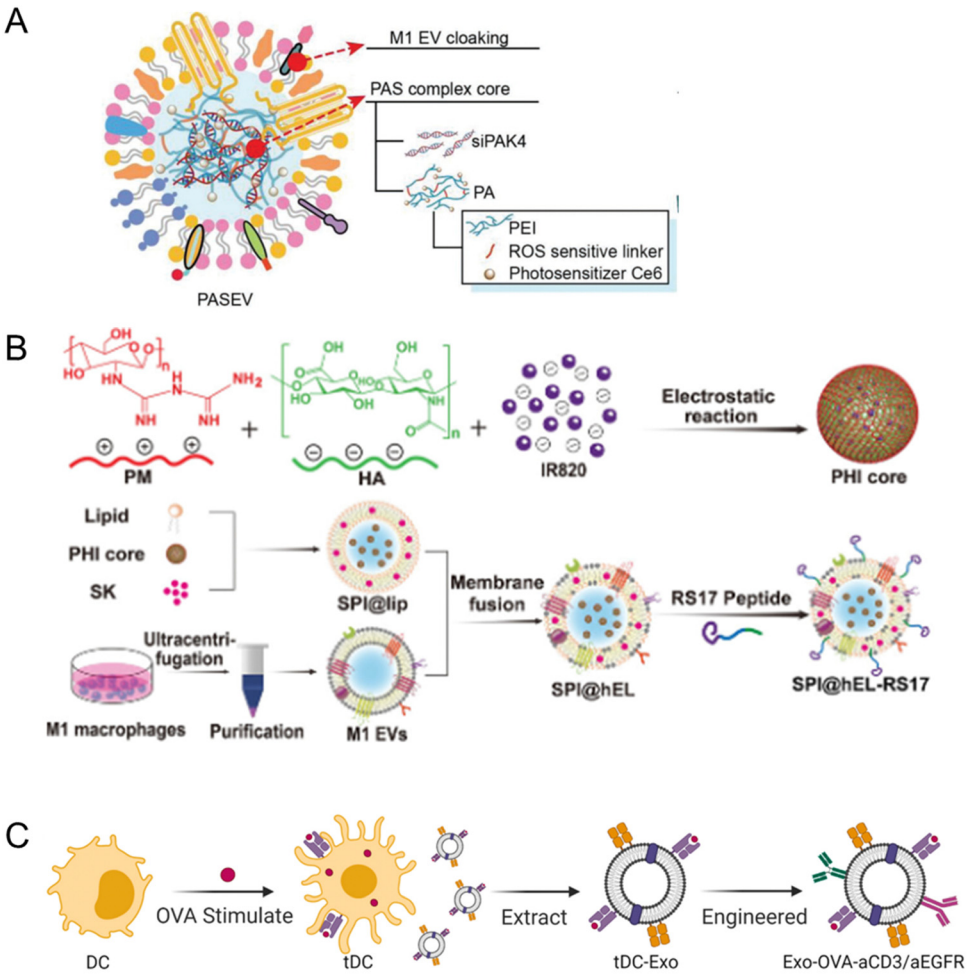

Fig. 18 Immune cell-derived personalized exosomes. (A) Photoactivatable silencing exosome sensitizes cancer immunotherapy (PASEV). Small interfering RNAs with, siPAK4 were assembled with a ROS-sensitive linker to form a nanocomplex core, camouflaged with EVs from M1 macrophages. Reproduced from ref. 249 Copyright 2022 with permission from John Wiley & Sons. (B) Immune cell-derived exosomes for amplified CD47 blockade-based cancer immunotherapy. Hybrid nanovesicles derived from M1 macrophages and synthetic liposomes. The formulation was decorated with RS17 peptide (for blocking CD47) metformin, shikonin, and IR820 (a photosensitizer) for a multi-approach treatment of metastatic melanoma. Firstly, the PHI nanoparticle was formed *via* electrostatic interactions between positively charged polymeric metformin (PM) and negatively charged IR820 and hyaluronic acid (HA). Then the SPI@hEL nanoparticles were formed by membrane fusion with M1 EVs. Reproduced from ref. 211 Copyright 2023 with permission from John Wiley & Sons. (C) Antibody-engineered exosomes from antigen-feeding dendritic cells for precise solid tumor therapy. DC 2.4 cells were activated with ovalbumin and the DC Exo-Ova was purified by gradient centrifugation. The Exo-Ova was functionalized with anti-CD3 and anti-EGFR antibodies *via* DSPE-PEG-NHS linker to generate Exo-OVA-aCD3/aEGFR. Reproduced from ref. 243 Copyright 2022 with permission from Elsevier.

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regression and immune modulation was performed in a xenograft CT26 subcutaneous tumor model in mice. The BPNs increase $CD8^+$ T-cells, reduce Treg populations, and elevated the M1/M2 macrophage ratio in the tumors.[264]

OMVs can also be utilized as a vaccination or adjuvant platform following a primary treatment. In one example, a multifunctional vaccine based on bacterial OMVs was designed for cancer immunotherapy after photothermal therapy (PTT). The OMV platform, termed 1-MT@OMV-Mal, was loaded with 1-methyl-tryptophan (1-MT), an IDO inhibitor (Fig. 19C). After a one-time ICG-based PTT of subcutaneous CT26 tumors in mice, the platform was used as an immunotherapy vaccine. The vaccination significantly reduced both treated primary tumor and untreated primary tumor volumes compared to PTT alone and induced a potent antitumor immune response involving $CD8^+$ T-cells, B-cells, and macrophages.[217] Overall, these studies demonstrate the promising potential of OMVs from Gram-negative bacteria in enhancing cancer immunotherapy through various innovative approaches and combinations with existing immunotherapies.

4.2.4. Exosomes derived from other sources. Erythrocytes can be used as a delivery vehicle for exosomal transfer (Table 5). In an example by Kim *et al.* EV-SIRPα designed to block the CD47 "don't eat me" signaling in tumors used avidity

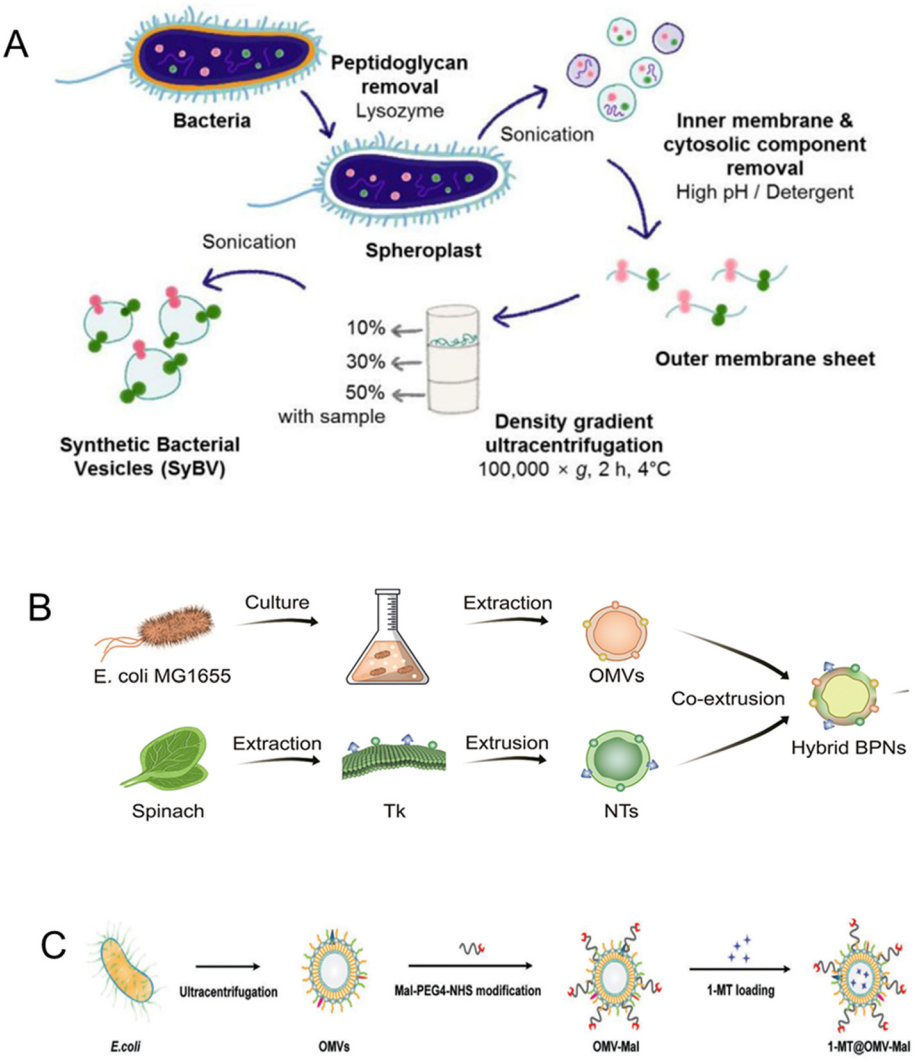

Fig. 19 Bacteria-derived exosomes. (A) Schematic diagram of the isolation of bacterial SyBV. *E. coli* cells were incubated with lysozyme to remove periplasmic components. They were then sonicated to disrupt the cell membranes. The outer membrane vesicles (OMVs) were collected from the interface layer of 10% and 30% iodixanol after buoyant density-gradient ultracentrifugation. Reproduced from ref. 261 Copyright 2021 with permission from John Wiley & Sons. (B) Phytochemically engineered bacterial exosomes for photodynamic effects promoted immunotherapy. The OMVs of *E. coli* MG1655 was extracted similarly to described in A. Thylakoid nanovesicles (NTs) were prepared from spinach leaves *via* the extrusion method. The OMVs and NTs were fused using membrane fusion method to obtain a bacteria-plant hybrid vesicles (BPNs). Reproduced from ref. 264 Copyright 2022 with persmission from American Chemical Society. (C) Schematic showing the synthesis of 1-MT@OMV-Mal. *E. coli* OMVs were isolated by multiple centrifugation and filtration steps. The maleimide (Mal) groups were modified on the surface of the OMV *via* a reaction between NHS ester in Mal-PEG4-NHS and the amine groups in the membrane proteins to prepare the OMV-Mal. The interior is then loaded with 1-methyl-tryptophan (1-MT, IDO inhibitor) to generate 1-MT@OMV-Mal. Reproduced from ref. 217 Copyright 2022 with permission from John Wiley & Sons.

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Table 5 List of bacteria-derived exosomes and other applications of exosomes for cancer therapy and immunomodulation

Exosome type	Source	Application	Targeting	Immunomodulation mechansim	Animal model	Ref.
Bacteria	*Escherichia coli*	Antigen-presenting	Tumor cells	Activate T lymphocyte cells	Subcutaneous	215
Bacteria	Gram-negative bacteria	Antigen-presenting	Tumor cells	Activate NK cells	Subcutaneous	263
Bacteria	*Escherichia coli*	Drug delivery	Tumor cells	Induce ICD	Subcutaneous	216
Bacteria	Mycobacterium bovis BCG	Antigen-presenting	Tumor cells	Induce cytokine responses	Orthotopic	272
Bacteria	*Escherichia coli*	Antigen-presenting	Tumor cells	Activate DCs	Metastatic	264
Bacteria	*Escherichia coli*	Antigen-presenting	T lymphocytes	Recruit DCs	Metastatic	273
Bacteria	Gram-negative bacteria	Antigen-presenting	T lymphocytes	Increase T lymphocyte infiltrations	Subcutaneous	217
Bacteria	Salmonella	Antigen-presenting	T lymphocytes	Increase T lymphocyte infiltrations	Metastatic	274
Bacteria	*Escherichia coli*	Antigen-presenting	Tumor exosomes	Induce T lymphocyte responses	Orthotopic	261
Bacteria	*Akkermansia muciniphila*	Antigen-presenting	T lymphocytes	Promote macrophage polarization	Subcutaneous	262
Liquid biopsy	Plasma	Antigen-presenting	Tumor cells	Predict ICI response	—	267
Liquid biopsy	Blood	DNA cargo	Tumor cells	Determinate circulating PD-L1	—	265 and 267
Liquid biopsy	Blood	Antigen-presenting	Tumor cells	Detect exosomal PD-L1	Subcutaneous	266
Liquid biopsy	Plasma	Antigen-presenting	Tumor cells	Predict immunotherapeutic outcomes	Subcutaneous	218
Red blood cell	Blood	Drug delivery	Tumor cells	Activate RIG-I pathway	Metastatic	219
Embryonic kidney cell	HEK293T cell line	Antigen-presenting	Tumor cells	Increase T lymphocyte infiltrations	Orthotopic	220
Mesenchymal stem cell	Bone marrow	RNA cargo	Tumor cells	Inhibit CD38 enzyme activity	Subcutaneous	268
Mesenchymal stem cell	Bone marrow	Drug delivery	Tumor cells	Activate dendritic cells	Subcutaneous	269
Mesenchymal stem cell	Bone marrow	Antigen-presenting	Tumor cells	Induce ICD	Orthotopic	271
Mesenchymal stem cell	Bone marrow	Antigen-presenting	Tumor cells	Promotes immune escape	Subcutaneous	270

BCG, bacillus calmette – guerin; DCs, dendritic cells; ICD, immunogenic cell death; ICI, immune-checkpoint inhibitors; NK, natural killer; PD-L1, programmed death-ligand 1.

difference to enable the piggy-backing of the EVs on red blood cells (Fig. 20A).[220] The EV-SIRPα induced a systemic T-cell mediated tumor-specific immune response in a B16F10 melanoma mouse tumor model. The treatment also exhibited anti-angiogenic effects *via* CD47 blockade with significant tumor growth inhibition. The use of plasma and red blood cell-derived exosomes are demonstrated by Bo *et al.* and Zhang *et al.*[265,266] Plasma-derived exosomes have been investigated as a potential biomarker for predicting and monitoring immunotherapeutic outcomes in various cancers. A proof-of-concept study by Bo *et al.* exemplifies the innovative use of EVs and CRISPR/Cas9 system to generate a fluorescence signal in the presence of PD-L1 positive lung tumors (Fig. 20B).[265] Such a platform is helpful as a diagnostic cum immunotherapy platform. However, the system is not yet tested *in vivo*, and it is a critical factor as PD-L1 positive cells are also present in healthy cell populations. *Ex vivo* applications can also be explored as the team performed a preliminary investigation using blood samples from 15 patients with lung cancer and 5 healthy controls, demonstrating the platform's potential for clinical diagnostic utility.[265] In another example, Zhang *et al.* identified correlations between circulating extracellular vesicles and responses to immune checkpoint inhibitors (ICIs) in gastric cancer patients, indicating a use of the exosomes as treatment prognostic markers.[218] Moreover, in non-small-cell lung cancer patients, high levels of TGF-β in exosomes were associated with poor responses to ICIs, indicating their potential as predictive indicators for treatment outcomes.[267] Additionally, exosomes derived from red blood cells (RBC-EV) are used to deliver RIG-I agonists as a potential immunotherapy strategy against cancer, leading to increased immune cell infiltration and potent tumor-specific $CD8^+$ T-cell immune response.[219]

Stem cell-derived exosomes represent a potent avenue in cancer immunotherapy, showcasing their ability to deliver therapeutic agents, reshape the tumor microenvironment, and enhance immunotherapeutic efficacy (Table 5). Recent studies underscore their potential applications. CD38 siRNA-loaded exosomes from bone marrow mesenchymal stem cells (BM-MSCs) were harnessed to counter immunosuppression in hepatocellular carcinoma, inhibiting tumor growth and metastasis.[268] Similarly, nanovesicles derived from BM-MSCs incorporated anti-PD-L1 antibodies for targeted drug delivery, resulting in robust immune activation and tumor ablation in photoimmunotherapy.[269] A dual delivery system from BM-MSCs was also demonstrated, triggering immunogenic cell death and enhancing therapeutic efficacy in cancer cell aggressiveness and immune evasion.[270] Zhou *et al.* fabricated OXA-MAL, a MSC-based exosomes functionalized with oxaliplatin prodrug on its surface to trigger immunogenic cell death. Additionally, the construct was loaded with galectin 9 siRNA to target the M2 phenotype mediated immunosuppression *via* the galectin 9/dectin 1 axis (Fig. 20C). *In vivo* efficacy testing showed an immune response centred on increased M1/M2 phenotype ratio in the tumor as well as Treg cell decrease and infiltration of $CD8^+$ T-cell in the tumor lesions.[271]

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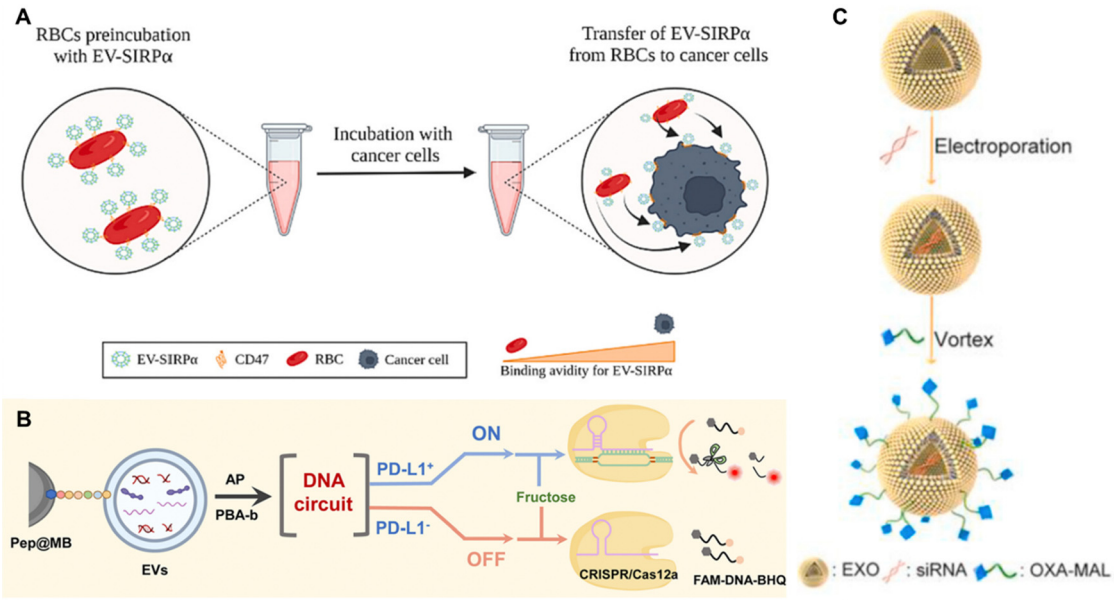

Fig. 20 Exosomes derived from other various sources. (A) EVs that express SIPRα, EV-SIRPα, is a RBC-derived exosomes that can hinder the CD47 "don't eat me" signaling present on tumor for cancer immunotherapy. Reproduced from ref. 220 Copyright 2022 with permission from Elsevier. (B) Circulating exosomes for lung cancer diagnosis and immunotherapy response prediction. A programmable DNA circuit will translate the presence of PD-L1 into the appearance of duplex DNA probes on the surface of the EVs to activate he *trans*-cleavage activity of CRISPR/Cas12a system, which finally produces a significant fluorescence signal. Reproduced from ref. 265 Copyright 2023 with permission from American Chemical Society. (C) Bone marrow mesenchymal stem cell (MSC)-derived exosomes for immunotherapy of pancreatic ductal carcinoma (PDAC). The exosomes from MSCs were isolated by gradient centrifugation and loaded with galectin 9 siRNA *via* electroporation. The surface of the exosome was modified with oxaliplatin (OXA) to trigger immunogenic cell death. Reproduced from ref. Copyright 2021 with permission from Elsevier.

4.3. Immunotherapy *via* multifunctional delivery using versatile albumin nanoparticles

Albumin is the most abundant protein in the blood and plays many roles in the body, including modulating the plasma oncotic pressure and transporting various endogenous and exogenous substances, such as hormones and drugs.[275] The protein has an intrinsic ability to target and accumulate in tumors due to enhanced passive uptake *via* the EPR effect. Albumin can also bind to specific surface receptors such as the secreted protein acidic and rich in cysteine (SPARC) and the 60-kDa glycoprotein (gp60) receptor. Uptake of albumin *via* these receptors can enhance the delivery of drugs to tumor cells. For example, the transport of nab-paclitaxel (nab-PTX, abraxane), an FDA-approved albumin-bound paclitaxel, was more efficient by 4.2 folds compared to standard paclitaxel solution.[276] Albumin possesses many functional groups that can be used for functionalization and for drug loading.[277] Due to its intrinsic role in transport biology, albumin-mediated delivery is stable and protects the cargo load from degradation. Research groups have spent efforts in formulating albumin-based compounds for immunotherapy due to its status as an FDA-approved bionanomaterial.

Albumin can be extracted from different sources such as ovalbumin from chicken egg and rat serum albumin. This section will focus on human (HSA) and bovine serum albumin (BSA), the most used protein bionanoparticle platforms, to assess the strategies used for endowing the albumin nanoparticle complexes with immunomodulatory properties. Albumin on its own is non- or mildly immunogenic exhibiting no cytotoxicity. Hence, it serves as a blank canvas for loading compounds that can elicit an immune response. The most applied strategy for immunomodulation with albumin is *via* the induction of ROS to trigger oxidative stress resulting in cell death and the use of checkpoint inhibition. ROS induced cell death is known to recruit and stimulate the immune system. Albumin nanoparticles were used as a photosensitizer delivery vehicle or assembled with zinc sulfide (ZnS) to trigger the formation of ROS. In most of these studies, phototherapy is a central treatment method to induce ROS buildup.

A formulation comprising human serum albumin HSA holding IR780 (a dye photosensitizer) and zinc sulfide (ZnS) was formulated by Yang *et al.* *via* self-assembly to generate IR780-ZnH@HSA complexes (Fig. 21A).[278] The zinc ions from ZnS can induce ROS of facilitated by generation of hydrogen sulfide (H_2S). IR780-ZnH@HSA was used as a photothermal therapy (PTT) and photodynamic therapy (PTT) effector that induces cells death *via* pyroptosis by activating the caspase-3-GSDME signaling pathway combined with anti PD-L1 therapy (aPD-L1). *In vivo* efficacy testing against a subcutaneous 4T1 mammary carcinoma model in mice showed that three doses of PDT/PTT and aPD-L1 combination therapy with IR780-ZnH@HSA controlled both primary tumor and distant metastases, indicating an immune response which was amplified systematically (Fig. 21B and C). Mice in the PDT/PTT with and without aPD-L1 exhibited better survival over 60 days. Most importantly, the treatment also induced increased $CD8^+$T-cell

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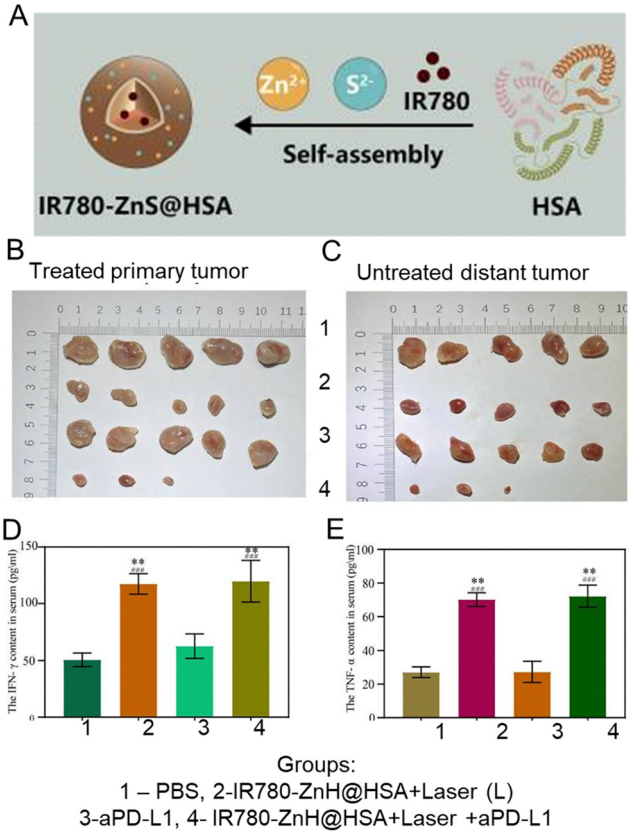

Fig. 21 The antitumor and immunomodulatory effects of IR780-ZnS@HSA nanoparticles (NPs). (A) A schematic of the fabrication strategy of IR780-ZnS@HSA nanoparticles (NPs). Photographs of (B) treated primary tumor, and (C) distant untreated tumors of 4T1 subcutaneous lesions after three phototherapy doses with IR780-ZnS@HSA nanoparticles and aPD-L1. Analysis of serum (D) IFN-γ and (E) TNF-α three days after various treatments. Data is represented as mean ± SD. $^{**}P < 0.01$. (n = 3). Reproduced from ref. 278 Copyright 2023 originally published by and used with permission from frontiers.

and decreased Foxp3 Tregs populations in the primary tumor. Elevated serum cytokine levels indicated a systemically activated immune response (Fig. 21D and E). A similar immune activation effect is observed by Cen *et al.* with their ZnS@BSA formulation designed to result in a ROS induced cell death *via* the release of Zn ions. The albumin nanoparticle combined with aPD-L1 treatment also promoted distant tumor growth inhibition and $CD8^+$ T-cell recruitment and activation in the primary tumor in a subcutaneous model of HCC with Hepa1-6 cells.[279]

Various BSA-based formulations carrying photosensitizers for PDT, such as MHI148 dye, chlorin e6, and TPA-Erdn, exhibited immunomodulatory mechanisms *via* increased $CD8^+$ or $CD4^+$ T-cell accrual to the tumor sites.[280–282] Hence, it is noteworthy that without checkpoint inhibition, ROS induced cell death that occurs sufficiently at a threshold above cellular tolerance point can edit the TME to a pro-inflammatory state *via* the release of dead tumor cell debris, essentially converting a cold tumor into a hot tumor state. Combining phototherapy with checkpoint inhibition enhances the treatment outcomes *in vivo*, adding another layer of immunomodulation guarantee in the package. Coating of albumin with gold nanoparticles (AuNPs) for as a photothermal agent is another viable strategy for generating antitumor immunity. Zhang *et al.* fabricated HSA@AuNPs that also deliver human melanoma peptide antigen $gp100_{25–33}$ (hgp100) as a PTT plus vaccination combination strategy, taking full advantage of the versatile binding sites of the HSA molecule.[283] Another Au–albumin complex, mPEG-GNRs@BSA/R837, was formed by preparation of mPEG (polyethylene glycol monomethyl ether with sulfhydryl end group)-Au nanorods (NRs) core followed by coating with BSA and loading with an immunoadjuvant imiquimod (R837) through electrostatic binding. PTT with mPEG-GNRs@BSA/R837 combined with anti-PD1 inhibitor provided long-term antitumor immunity (100 days surveillance) and inhibited tumor metastases in mice inoculated subcutaneously with metastatic melanoma cell line, B16F10. The release of inflammatory cytokines, TNF-α, IL-6, and IL-12 was significantly elevated in the serum three days after PTT and $CD8^+$ T-cells were detected in B16F10 tumor sections.[284] A similar complex of albumin-modified AuNRs for PTT triggered the activation of immature DCs in a contact-dependant manner between DCs and 4T1-Luc mammary tumor cells.[285] In summary, oxidative and heat stress are efficient methods to induce cellular death that can activate an antitumor immune response but such methods must be used with caution because mild levels of stress exposure can promote tumor progression.[286] Thus, it is necessary to utilize an exposure of the stress at levels beyond the cellular repair threshold, thereby directing the cellular fate towards death.

Checkpoint inhibition therapy has been gaining traction in the treatment of various advanced malignancies. Hence, efforts were focused on fabricating a single platform albumin-based nanomedicine bound with checkpoint inhibition molecules. Lai *et al.* fabricated a fexofenadine-loaded albumin nanoparticles functionalized with PD-L1 aptamers (PDL1-NP-FEXO) for the treatment of a subcutaneous colon carcinoma model in mice using CT26 cells. Fexofenadine is a H1-antihistamine and is known to stimulate the immune response by reducing M2 phenotype macrophages in the TME. The PDL1-NP-FEXO bind to PD-L1 positive MDA-MB-231 cells *in vitro* and significantly inhibited CT26 tumor growth *in vivo* without generating systemic toxicity.[287] A similar formulation was fabricated by Yao *et al.* with CTLA-4 aptamers (CTLA-4-NP-FEXO) which could bind CTLA-4 positive cells and improved antitumor immunity *in vivo via* lymphocyte activation.[288] Combination therapy was explored with HSA-PTX generated *via* albumin bound technology and then pooled with anti-PD-L1 monoclonal antibody through a pH-sensitive linker (Fig. 22A). The complex was termed PD-L1/PTX@HSA and was tested in a mouse subcutaneous model of mammary carcinoma with EMT-6 cells. Intravenous injection of the albumin complex was found to be distributed predominantly in the liver and kidneys with tumor site accumulation after 24 hours post intravenous injection. No organ toxicity was observed upon treatment completion. The treatment comprises five doses of the albumin complex, and mice were sacrificed on day 15 of the study, three days after the last treatment dose. Successful tumor growth inhibition was

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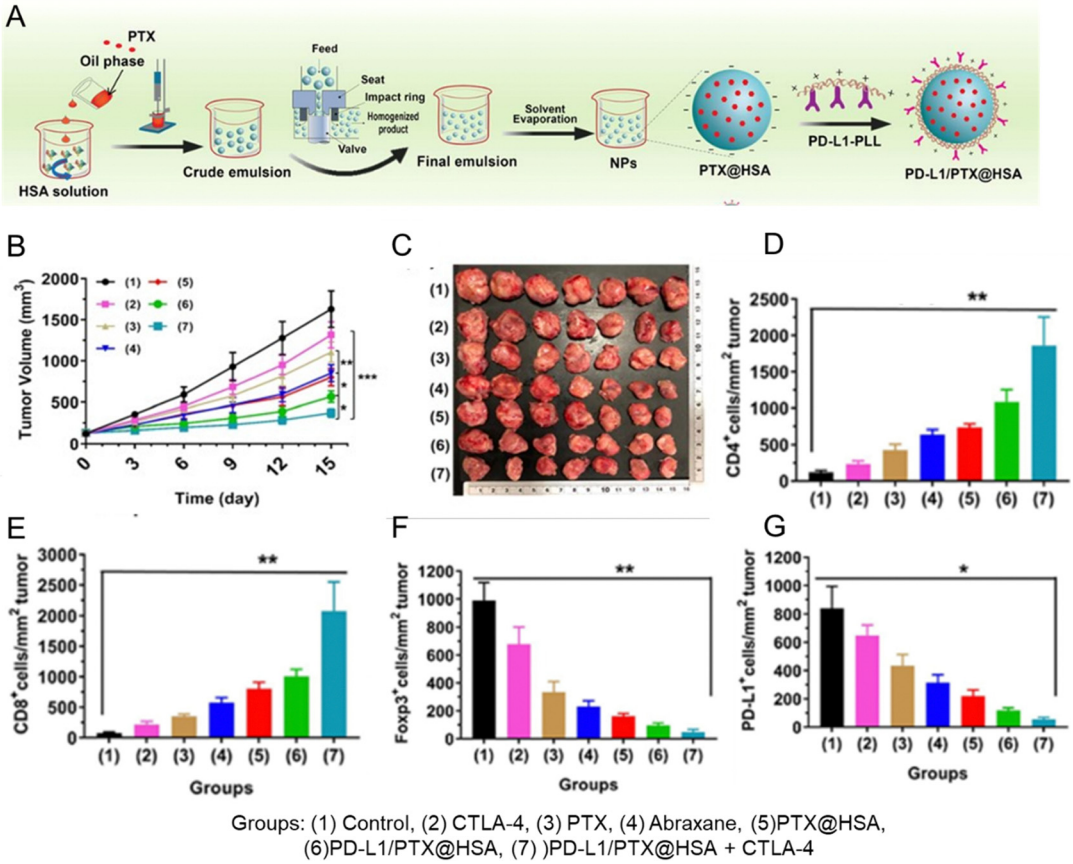

Fig. 22 Combination of chemotherapy and immunotherapy PDL1/PTX@HSA. (A) Schematic of the PDL1/PTX@HSA synthesis procedure. (B) 15-day examination of tumor volumes in each group. (C) Representative tumors excised after termination. Data are represented as means ± SD (n = 7). *P < 0.05, **P < 0.01, ***P < 0.001. Percentage of (D) $CD4^+$ T-cells, (E) $CD8^+$ T-cells, (F) $Foxp3^+$ Tregs, and (G) PD-$L1^+$ cells from immunohistochemistry analysis of the excised tumors after study period data are represented as means ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001. Reproduced from ref. 289 Copyright 2021 with permission from Elsevier.

observed along with the infiltration of $CD4^+$ and $CD8^+$ T-cells in the tumor, especially when combined with CTLA-4 inhibition (Fig. 22B-E). The infiltration of immunosuppressive $Foxp3^+$ and PD-$L1^+$ cell-types was significantly abrogated (Fig. 22F and G) signifying immunoediting towards an inflammatory antitumor environment.[289]

The versatility of the albumin macromolecule facilitates binding to various drugs and molecules. Ai *et al.* used BSA molecules as a nanocarrier for the delivery of CpG oligodeoxynucleotides (ODNs) to macrophages targeted *via* mannosylation of cationic BSA (ODN@MCBSA).[290] The mannose moiety functionalization of the BSA endows macrophage targeting through its interaction with the mannose receptor on the macrophages. The complex facilitated the polarization of macrophages to the M1 phenotype *in vitro*. Furthermore, the team demonstrated the enhanced secretion of pro-inflammatory cytokines such as IL-12, IL-6, and TNF-α after macrophage treatment with ODN@MCBSA *in vitro*. However, their formulation was used solely as a macrophage targeting agent to induce activation and polarization towards the M1 phenotype and did not exhibit any toxicity towards HeLa cells in vitro.[290] Another formulation aimed at overcoming the blood brain barrier (BBB) by functionalizing the albumin with a brain-targeting peptide sequence, ^{D}CDX (cgreirtgraerwsekf, D-form sequence), that can target the nicotininc acetylcholine receptors (nAChRs) expressed highly in the endothelial cells of the brain.[291] The functionalized albumin was used to co-encapsulate celastrol (CELA), and mTOR inhibitor, and LY2157299, a TGF-β receptor I (TGFβRI) inhibitor. CELA was reported to promote M2 to M1 macrophage polarization while the TGFβRI inhibitor can downregulate the effects of TGF-β in the tumor microenvironment, alleviating the tumor-induced immunosuppression. The ^{D}CDX-BSA NPs demonstrated efficient *in vivo* targeting to implanted orthotopic brain tumors in mice, revealing intracranial accumulation at 4.2 times higher than that of BSA-NPs group. Analysis of the immune environment in the glioma revealed a significant increase in the percentage of mature DCs and a decrease in the overall TAMs population.[291] Efforts to deliver inhibitors to metabolic actors responsible in tumor-induced immunosuppression, such as IDO and PI3Kγ, have also demonstrated success in modulating the TME *in vivo*.[292–294]

5. Limitations

The section discourse delves into the challenges of utilizing bio-nanoparticles for tumor immunomodulation, focusing on

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nuanced aspects crucial for therapeutic development. The choice of cell membrane source plays a pivotal role in dictating the system's efficacy, given the diverse interactions of various cell types with the immune system.[168,169] A paramount concern arises from the immunogenicity of cell membrane-based platforms, as these structures may be recognized and eliminated by the host immune system, potentially hampering their therapeutic effectiveness. Studies have explored innovative strategies, such as employing hybrid membranes like erythrocyte-cancer cell hybrids, to mitigate immunogenicity and amplify therapeutic outcomes.[167,205] Furthermore, the immunosuppressive milieu within tumor microenvironments poses formidable challenges. Tumor cells employ immune tolerance mechanisms, such as upregulating immune checkpoint molecules, impeding the effectiveness of cell membrane-based immunotherapies.[178,205] Despite efforts to bolster immune responses, these approaches encounter hurdles in overcoming the immunosuppressive microenvironment orchestrated by cancer cells. Combining cell membrane-based immunotherapy with immune checkpoint inhibitors exhibits promise in preclinical studies; however, further optimization is requisite for clinical translation.

In the realm of exosome-mediated immunomodulation, a fundamental obstacle lies in the incomplete understanding of the mechanisms underpinning this process. While existing literature underscores the immunomodulatory effects of exosomes derived from diverse cell types, the intricate mechanisms governing their interactions with immune cells and regulation of immune responses remain insufficiently elucidated.[208,209,232] It is imperative to delve deeper into these interactions, delineating the key molecular constituents orchestrating exosome-mediated immunomodulation. Moreover, researchers must consider the intricate immune microenvironment and the influence of diverse factors in the context of cancer immunomodulation, necessitating meticulous investigations. The precise biodistribution and targeted delivery of exosomes to tumor sites present significant challenges.[229,230,236] Despite some studies demonstrating the tumor-targeting capabilities of exosomes, enhancing strategies are indispensable to augment their accumulation and persistence within tumor tissues. This optimization is critical for ensuring the efficient delivery of therapeutic payloads, thereby maximizing the inherent therapeutic efficacy of exosomes. Sustained efforts in this domain are pivotal for advancing the potential applications of exosome-mediated immunomodulation in cancer therapy. Careful considerations must also be observed when using exosomes derived from tumor cells due to their involvement in cancer progression and therapy resistance.[295]

Tumor antigen heterogeneity poses a formidable obstacle for personalized cancer vaccines, as acquiring patient-specific antigens proves impractical. Exosome heterogeneity introduces challenges in treatment consistency, necessitating standardized approaches. The stability of cell membrane coatings emerges as a critical concern for long-term efficacy, urging further research efforts. Scalability issues plague both exosome and cell membrane production, as their production relies heavily on top-down approaches, demanding the development of efficient methods to meet the demands of therapeutic applications.[296,297] Safety concerns encompass potential autoimmune reactions, immune related adverse events, and off-target effects, mandating comprehensive studies to evaluate the safety profiles of these therapies.[134]

Albumin nanoparticles enjoy varying degrees of success as a drug delivery vehicle, transporting and releasing chemotherapeutic drugs in cancer therapy. Their low toxicity and biocompatibility also define the success of albumin as a carrier, as the carrier protein shows no toxicity to cancer or healthy cells. However, albumin is limited by its cargo loading ability, with issues in payload capacity and conjugation efficiency.[298] Without intrinsic immune potential, albumin is restricted in its role as a carrier and is limited in the selection of agents and pharmaceuticals that can be loaded into the structure. However, the albumin nanoparticle platform may offer the best chance of clinical translation due to their regulatory precedent. The prevalence of small sample sizes in existing research restricts the generalizability of findings. Undertaking larger-scale studies is imperative to validate the predictive and therapeutic potential of bionanoparticles in cancer immunomodulation. Addressing these multifaceted challenges through standardization, rigorous evaluation, and expansive studies is indispensable for propelling the field of cancer immunotherapy toward enhanced efficacy and broader applicability.

6. Conclusion and outlook

In this review, the authors comprehensively summarized the tumor-induced immune aberrations to enable a broad and general understanding of the challenges in tackling tumor-induced immune suppression using nanotechnology approaches. Furthermore, recent advances in fabricating membrane biomimetic bionanoparticles were discussed and organized according to membrane source. Fabricating bionanoparticles for immunotherapeutic purposes is still in a nascent stage, but developing rapidly, with huge potential to impact clinical practice. The addition of immunomodulation potential ups the ante by tipping the balance of a developed immunosuppressive environment, a hallmark of cancer, into an activated state equipped to recognize and mount a tumor-specific response. Various efforts on the bench provide a positive outlook with an excellent opportunity for growth and expansion. Currently, membrane coating and exosomes are the predominant strategies utilized to generate immunomodulating platforms for cancer therapy, with some focus on developing the FDA-approved albumin nanoparticle. Efforts in other areas can complement the progress in this field, such as developing computational and AI-based methods for modeling tumor-induced immune aberrations and using the model to predict outcomes of exploratory treatments. Other biological and bionanoparticle platforms that can be exploited for immunomodulation are listed below. Suggestions for combination approaches are also included for each platform in a point-by-point manner:

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• *Caged protein nanocarriers*. These nanoparticle complexes possess hollow structures with nearly monodispersed-sized distribution.[299] Some of the members of this family are naturally immunogenic, such as virus-based proteins, and they can be combined with checkpoint blockade to stimulate the immune response and subdue the immunosuppressive mechanisms induced by their structure.[300] They can also be functionalized with membrane proteins for tumor targeting.

• *Cytokines and peptides*. These proteins are potent stimuli that can recruit and activate innate and adaptive immune components. Customization and encapsulation of protein cocktails can be done to achieve the desired immune effect, such as the polarization of M2 to M1 phenotype macrophages or proliferation and clonal expansion of T-cells.[301] However, cytokines are generally pleiotropic, and the release of cytokines should be designed with stimuli responsiveness and spatially controlled in the TME.

• *Lipid rewiring*. The formation of lipid rafts and the accumulation of lipids are recently discovered mechanisms inducing dysfunction in antigen presentation and cytokine secretion. Lipid rewiring nanoparticles and blockage of lipid uptake using composite hydrogels have demonstrated the reactivation of immune cells and inhibition of immunosuppressive immune cells.[302,303]

• *Artificial antigen-presenting cells*. Synthetic artificial antigen-presenting cells were formulated for T-cell activation.[304] A biological-based platform can be fabricated with the MHC-peptide complex subunits and an immunostimulatory agent, such as viral-based nanoparticles or exosomes to generate a tumor-specific response and an inflammatory reaction conducive to tumor eradication.

• *RNA interference, mRNA technology, and gene editing techniques*. The structure-to-function relationship of biological molecules is well studied. Hence, bionanoparticles are amenable to modifications for assembly and functionalization *via* the genetic code, nucleotide, and protein chemistry. The fine-tuning of such modifications is achievable *via* genetic engineering techniques and mRNA technology. The encapsulation of messenger RNA (mRNA) in lipid nanoparticles is one of the most advancing strategies in this field, spurred by the success of the COVID-19 mRNA vaccine.[305] Similarly, RNA interference can be used to silence unfavourable phenotypes. Such approaches can also be attempted to generate exosomes with desirable cargo protein load at increased concentrations.

Nanotechnology applications in medicine have improved clinical management, from diagnostics to treatment and prognosis. Advances in bionanoparticle design for cancer therapy can draw inspiration and lessons from the journey of its synthetic counterparts. In a heavily regulated environment, the risk-to-benefit ratio of exploratory treatments is weighted carefully before approval for use in human cancer patients. Hence, most synthetic nanoplatforms fail due to biodistribution and toxicity issues. This risk is especially avoided to protect cancer patients whose systems are already compromised by the malignant disease. Better safety profiles are anticipated for bionanoparticle formulations. However, rendering immunomodulatory functions can be a double-edged sword, as the immune system is a powerful entity with a far-reaching influence on human physiology. As an example, debilitating and even fatal side effects were reported with the breakthrough CAR-T cell therapy despite its biological origin. Hence, careful considerations need to be made about the spatiotemporal manner of immune activation.

Certain cancers will benefit from a tissue-targeted treatment concept. Hence, adding tumor-targeting moiety and stimuli-responsiveness will help confine the activation in the TME, increasing treatment safety. Others, such as hematological malignancies, will require activation in the circulatory system, which presents more risks. Regardless, advanced nanotherapy design must consider the biological barriers to tissue-specific delivery and implement controls to circumvent the barriers, *e.g.*, endothelial barriers such as leaky tumor vasculature and extrinsic barriers such as high interstitial fluid pressure in tumors. Most importantly, rigorous pre-clinical studies done using orthotopic animal models that recapitulate the malignant disease in humans are pertinent for determining the efficacy, safety, toxicity, and long-term effects of the bionanoparticles before transitioning to clinical trials. Finally, multidisciplinary collaborative efforts and perspective input from material scientists, immunologists, pharmacologists, and oncologists are vital in advancing the development of bionanoparticles in the clinic. This development will be a boon for cancer patients for whom the standard of care and alternative therapies fail to provide a lasting cure.

Author contributions

Juwita N. Rahmat conceptualisation, writing – original draft, writing – review & editing; Jiayi Liu writing – original draft; Taili Chen writing – original draft; ZhiHong Li supervision; Yong Zhang conceptualisation, writing – review & editing, supervision.

Conflicts of interest

There are no conflicts of interest to declare.

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