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Review article

Biomaterial-based platforms for modulating immune components against cancer and cancer stem cells

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ABSTRACT

Immunotherapy involves the therapeutic alteration of the patient's immune system to identify, target, and eliminate cancer cells. Dendritic cells, macrophages, myeloid-derived suppressor cells, and regulatory T cells make up the tumor microenvironment. In cancer, these immune components (in association with some non-immune cell populations like cancer-associated fibroblasts) are directly altered at a cellular level. By dominating immune cells with molecular cross-talk, cancer cells can proliferate unchecked. Current clinical immunotherapy strategies are limited to conventional adoptive cell therapy or immune checkpoint blockade. Targeting and modulating key immune components presents an effective opportunity. Immunostimulatory drugs are a research hotspot, but their poor pharmacokinetics, low tumor accumulation, and non-specific systemic toxicity limit their use. This review describes the cutting-edge research undertaken in the field of nanotechnology and material science to develop biomaterials-based platforms as effective immunotherapeutics. Various biomaterial types (polymer-based, lipid-based, carbon-based, cell-derived, etc.) and functionalization methodologies for modulating tumor-associated immune/non-immune cells are explored. Additionally, emphasis has been laid on discussing how these platforms can be used against cancer stem cells, a fundamental contributor to chemoresistance, tumor relapse/metastasis, and failure of immunotherapy. Overall, this comprehensive review strives to provide up-to-date information to an audience working at the juncture of biomaterials and cancer immunotherapy.

Abbreviations: aAPCs, Artificial antigen-presenting cells; ABC, ATP-binding cassette; ACT, Adoptive cell treatment; AgNPs, Silver nanoparticles; ALDH, Aldehyde dehydrogenase; AML, Adult acute myeloid leukemia; APCs, antigen-presenting cells; AuNPs, Gold nanoparticles; CAFs, Cancer-associated fibroblasts; CAR-T, Chimeric antigen receptor-modified T cells; cGAMP, 2′,3′-cyclic GMP-AMP; CIK, Cytokine-induced killer; CNMS, Carbon-based nanomaterials; CNTs, Carbon nanotubes; CpG ODNs, Synthetic CpG oligodeoxynucleotides; CPP, Cell-penetrating peptide; CSCs, Cancer stem cells; CTLA-4, Cytotoxic T-lymphocyte associated protein-4; DCs, Dendritic cells; DOX, Doxorubicin; ECM, Extracellular matrix; EGF, Epidermal growth factor; EpCAM, Epithelial cell adhesion molecule; EPR, Enhanced permeability and retention; FAP, Fibroblast activation protein; FDA, Food and Drug Administration; FGF2, Fibroblast growth factor 2; FSP-1, Fibroblast-specific protein 1; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; HIFs, Hypoxia-inducible factors; ICBT, immune checkpoint blockade therapy; ICD, Immunogenic cell death; IDO-1, Indoleamine 2,3-dioxygenase 1; IFN, Interferon; IGFs, Insulin-like growth factors; IL, Interleukin; IMC, Immature myeloid cell; IRF, IFN regulatory factor; JAK, Janus kinase; LPS, Lipopolysaccharides; mAbs, Monoclonal antibodies; MDSCs, Myeloid-derived suppressor cells; MHC, Major histocompatibility complex; MNPs, Metallic nanoparticles; MPL, Monophosphoryl lipid A; MWNTs, Multi-walled nanotubes; NIR, Near-infrared; NK cells, Natural killer cells; NLRs, Nucleotide-binding oligomerization domain- (NOD-) like receptors; NPs, Nanoparticles; OVA, ovalbumin; OVT, Oncolytic virotherapy; PAMPs, Pathogen-associated molecular patterns; PD-1, Programmed cell death protein 1; pDCs, Plasmacytoid DCs; PDGF, Platelet-derived growth factor; PDGFR, Platelet-derived growth factor receptor; PD-L1, Programmed death-ligand 1; PEG, Polyethylene glycol; PEI, Polyethyleneimine; PLA, Poly(lactic acid); PLGA (or PLG), Poly(lactide-co-glycolic acid); PNP, Peptide nanoparticle; PRRs, Pattern-recognition receptors; RLRs, Retinoic acid-inducible gene I- (RIG-I-) like receptors; SCID, Severe combined immune-deficient; SDF1, stromal cell-derived factor 1; STING, Stimulators of interferon genes; SWNTs, Single-walled nanotubes; TAAs, Tumor-associated antigens; TACAs, Tumor-associated carbohydrate antigens; TAMs, Tumor-associated macrophages; TCL, Tumor cell lysate; TCR, T cell receptors; TDLN, Tumor-draining lymph nodes; TGF, Transforming growth factor; TILs, Tumor-infiltrating lymphocytes; TLR, Toll-like receptor; TME, Tumor microenvironment; Tregs, Regulatory T cells; VEGF, Vascular endothelial growth factor.

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Statement of significance

Cancer immunotherapy possesses incredible potential and has successfully transitioned into a clinically lucrative alternative to conventional anti-cancer therapies. With new immunotherapeutics getting rapid clinical approval, fundamental problems associated with the dynamic nature of the immune system (like limited clinical response rates and autoimmunity-related adverse effects) have remained unanswered. In this context, treatment approaches that focus on modulating the compromised immune components within the tumor microenvironment have garnered significant attention amongst the scientific community. This review aims to provide a critical discussion on how various biomaterials (polymer-based, lipid-based, carbon-based, cell-derived, etc.) can be employed along with immunostimulatory agents to design innovative platforms for selective immunotherapy directed against cancer and cancer stem cells.

1. Introduction

Our immune system is crucial in preventing the growth and spread of tumors. The process of effective anticancer immunity involves the identification of cancer cells by the innate and/or adaptive immune system, followed by their selective targeting (while preventing the development of immunological tolerance) and subsequent elimination. Cancer cells counter by downregulating tumor antigens, producing surface ligands that prevent T cell-mediated detection, and secreting immunosuppressive molecules [1]. Such mechanisms also lead to the failure of traditional cancer therapies.

In this context, immunotherapy is a rapidly developing area of biomedical research that utilizes the body's immune system to fight cancer. This approach trains a patient's intrinsic immune system (immune components) to recognize and target cancer cells (using tumor antigens and other tumor-associated macromolecules), making it a safer and more potent alternative to traditional cancer treatments such as chemotherapy, radiotherapy, and surgery [2]. In recent years, immune checkpoint blockers and chimeric antigen receptor-modified T cells (CAR-T) therapy have emerged as mainstream immunotherapy-based treatments, with numerous molecular adjuvants also being explored as part of cancer vaccines [3]. Despite its potential, there are still some challenges associated with immunotherapy. One of the major challenges is the current response rate of around 20%, which is largely due to tumor heterogeneity. In addition, immunotherapy can also cause various complications, including non-specific inflammation, autoimmune-like disorders, and severe toxicity-related disorders (such as neurotoxicity, macrophage activation syndrome, and cytokine release syndrome) [4].

The unsatisfactory results from conventional immunotherapies can be attributed to the functionally compromised state of immune cells in the tumor microenvironment (TME). The TME is a complex and dynamic network of cells, extracellular matrix, and various signaling molecules. Cancer cells, in coalition with cancer stem cells (CSCs), impart the characteristic "immunologically cold" nature of the TME wherein the immune cells get negatively modulated which renders them unable to mount an effective immune response. The key constituents of the tumor's "immune microenvironment" are dendritic cells (DCs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) [5]. These immune cells are desynchronized, leading to a pro-tumorigenic environment. Additionally, non-immune cells like cancer-associated fibroblasts (CAFs) are also influenced via molecular cross-talk. Such complicated interplay with immune cells allows cancer to form a tightly controlled domain where it can develop and spread uncontrollably. In such a scenario, there arises a need to orchestrate a selective modulation of immune components to alleviate the pro-tumorigenic effects. New immune adjuvants are being identified that can reinstate immune robust immune activity (as needed for DCs, TAMs, and MDSCs) or play a role in selective depletion (in the case of Tregs, and CAFs). Over the years, the utilization of functional biomaterials has brought remarkable breakthroughs in the domain of biology and medicine. Using appropriate formulation strategies, biomaterials can be fabricated into various functionalized platforms for application in cancer immunotherapy. Their use endows control over the release rate and release site of any externally delivered cargo, thereby overcoming the drawbacks associated with the systemic delivery of immunotherapy agents. In addition, biomaterials loaded with an appropriate adjuvant can serve as an artificial immune hotspot for specific enrolment and activation of immune cells.

The subsequent sections highlight existing strategies for cancer immunotherapy and how biomaterials (polymer-based, lipid-based, carbon-based, cell-derived, etc.) can be enarmed with immunostimulatory agents for functional alteration of immune components. The current knowledge has further been extended to discuss the possible eradication of CSCs that are crucial for tumor heterogeneity and cancer recurrence. Overall, this review aims to present detailed yet clear insights into the role of various immune components and how their biological interplay can be immunogenically modulated using biomaterials to combat cancer. By better understanding the current state-of-the-art, this review strives to ignite innovative ideas to transform cancer immunotherapy to the next level.

2. Cancer immunity and immunotherapy

2.1. Interaction of immune components with cancer

Cancer is caused by random genetic mutations leading to the uncontrolled growth of malignant cells. The process of tumor development and spread is dynamic and influenced by the interplay between intrinsic and extrinsic tumor-cell factors. Within the TME, different cellular components are recruited and activated to sustain tumor growth. While T cell infiltration is beneficial, the presence of other immune cells such as TAMs, Tregs, and MDSCs correlates with increased tumor growth and poor patient outcomes [6]. This bio-cellular communication between tumor and infiltrating immune cells is called "the immune contexture" [7].

MDSCs and TAMs negatively regulate innate and adaptive immune pathways. MDSCs are vital in tumor growth and progress by promoting immune privilege, remodeling TME, establishing a pre-metastatic niche, and interacting with tumor cells to promote angiogenesis and invasion [8]. Monocytes originate from myeloid progenitors in the bone marrow, infiltrate tumors via blood circulation, and then differentiate into macrophages. Based on their polarization status, macrophages are divided into M1 and M2 subtypes. Th1 cytokine interferon (IFN) and microbial compounds can activate M1 macrophages. Th2 cytokines like Interleukin-4 (IL-4), IL-10, and IL-13, in contrast, drive the differentiation of M2

macrophages [9]. M1 macrophages have tumoricidal effects in the presence of TAMs, whereas M2 macrophages promote carcinogenesis. Both M1 and M2 TAMs are plastic and reversible, and the TME regulates TAM functional polarization. MDSCs and TAMs impart anti-vascular endothelial growth factor (VEGF) resistance by secreting molecules that compensate for VEGF depletion and maintain angiogenesis. Furthermore, MDSCs also impart drug resistance to many anti-cancer drugs [10].

CAFs release cues that promote tumor formation and chemoresistance. In breast, lung, and pancreatic cancers, a higher incidence of CAFs in the tumor stroma is linked to poor clinical outcomes [11]. CAFs originate from local fibroblasts and get activated in response to growth factors and cytokines found in the TME (like transforming growth factor (TGF), fibroblast growth factor 2 (FGF2), and platelet-derived growth factor (PDGF)). Post activation, CAFs are involved in the extracellular matrix (ECM) formation and release of proteolytic enzymes such as matrix metalloproteinases and heparanase, resulting in ECM remodeling. Cumulatively, CAFs are a rich source of growth factors and cytokines, which promote tumor growth, angiogenesis, and treatment resistance [10].

Another important sub-class of the immune cell population within the TME is Tregs. They are a type of T cell that suppress immunological responses to maintain homeostasis and self-tolerance. Tregs suppress T cell proliferation and are critical for tumors in avoiding autoimmunity. CD4 T cell co-receptor and CD25, an IL-2 receptor component, are both expressed by natural Treg. As a result, Tregs are $CD4^+$ $CD25^+$ cells. The defining trait that controls natural Treg growth and function is the expression of the nuclear transcription factor Forkhead box P3. They suppress B cells/DCs by inhibiting $CD4^+$ and $CD8^+$ T cell activation, proliferation, and cytokine production [12]. Such multi-component interaction of tumors with immune cells creates an immunologically compromised state that substantially overwhelms the generation of any innate/adaptive antitumor immunity.

2.2. Suppression of immune components and cancer promotion

The immune system interacts with tumor cells through a process called immunoediting, consisting of 3 phases: Elimination, Equilibrium, and Escape (Fig. 1). The immune system destroys most tumor cells in the elimination phase, but some escape, leading to a latency phase, where equilibrium is established between tumor growth and immunological elimination. In this phase, cancer cells undergo genetic changes and may form a poorly immunogenic subpopulation of stem cells that outwit any immune recognition, thereby manipulating the entire immune system to promote their growth [13]. The final, escape phase, occurs when the more aggressive and less immunogenic cells form a clinically detectable tumor. The escape is driven by immunological tolerance (due to loss or alteration of antigen processing and presentation machinery) and CSCs-mediated recruitment of immune-suppressing cells in combination with the secretion of immunosuppressive molecules [14].

Advanced cancer often remains lethal despite early detection and treatment. While primary or early-stage cancer can be treated or cured with standard therapies and surgical resection, surgical interventions are not feasible in advanced stages. Traditional cancer therapies can leave behind dormant CSCs, resistant to treatment and leading to tumor relapse and metastasis. Chemotherapy resistance of CSCs is due to factors such as efficient DNA repair, high drug efflux pump expression, and complex interactions with the TME. After the tumors are formed, CSCs are in a dormant (G0) phase which makes their elimination difficult with conventional anti-cancer drugs that target proliferating cells. The undifferentiated state of CSCs is attributed to hypoxia, where cells exposed to hypoxia have downregulated differentiated markers, while genes involved in maintaining the stemness are upregulated. Hypoxia-inducible factors (HIFs) are the main factors that correlate with cancer stemness and hypoxia. In low oxygen conditions, HIF1α activates survival genes, while HIF2α binds to the promoter of Nanog and Oct-4, which are stemness-related genes. Transporter proteins like ATP-binding cassette (ABC) also contribute to drug resistance, as do signaling pathways like Hedgehog and Notch. Genes involved in cell death are dysregulated in CSCs, including the apoptotic gene p53, whose mutation or downregulation leads to inappropriate regulation of cell death. Understanding mechanisms underlying CSC resistance can aid in developing innovative strategies for CSC-specific targeting and elimination.

2.3. Biomaterials for enhanced modulation of immune components

2.3.1. Need for biomaterials in immunotherapy

While immunotherapy has brought about a paradigm shift in cancer management/treatment, there remains ample room for improvement. As mentioned earlier, current immunotherapy approaches carry some critical drawbacks, like limited efficacy (attributable to low antigen expression and constant immune-editing that imparts tolerance) and immune-related adverse events (owing to their non-localized/systemic nature). These drawbacks have prompted the broad investigation of biomaterials to find optimal solutions. In the context of cancer immunotherapy, the term biomaterial encompasses any biologically engineered material that is intended to shape and direct the outcome of a pro-immunogenic response directed toward cancer. These materials are specifically designed to interact with immune cells in a manner that promotes a therapeutically beneficial response [15]. By utilizing biomaterials, it is possible to enhance the safety and effectiveness of immunotherapies through precise targeting of immune cell subsets in TME or lymphoid tissues. This approach also allows modulation of the dosage, timing, and location of stimulation, resulting in improved outcomes. As we better comprehend the underlying cellular processes that govern immune response against cancer, the modularity and flexibility provided by biomaterials can be exploited to design the next generation of immunotherapeutic platforms [16]. It should be noted that even blank biomaterials (devoid of any immune modulatory molecules/drugs), by virtue of their chemical composition and structural/topological features (from nano to macro scale), can directly interact with the immune microenvironment. Albeit, they are not sufficiently immunogenic, thus requiring the presence of an externally loaded adjuvant to boost immune response [17]. Keeping this in mind, the prime focus of this manuscript is to highlight the advances made in the selective modulation of immune components by biomaterial-assisted delivery of immunomodulatory cargo.

2.3.2. Rationale and principles

From a clinical perspective, the development of an off-the-shelf prophylactic cancer vaccine would be revolutionary for cancer immunotherapy research. However, the sheer diversity of cancer and patient-specific antigen presentation alleles makes the concept of a prophylactic cancer vaccine impractical. The next best alternative is the development of therapeutic vaccines wherein immune adjuvant-loaded biomaterials have been extensively used to aid or enhance the adaptive immune response to combat pre-existing tumors. For maximum effectiveness, they should trigger specific immune responses against the right target, and the generated immune response must be robust enough to overcome the protective measures taken by cancer cells [18]. Regarding their practical applications, the biomaterials have the following use scenarios:

(i) *Self-adjuvanting bomaterials:* It is a class of materials that can stimulate an immune response without the need for additional

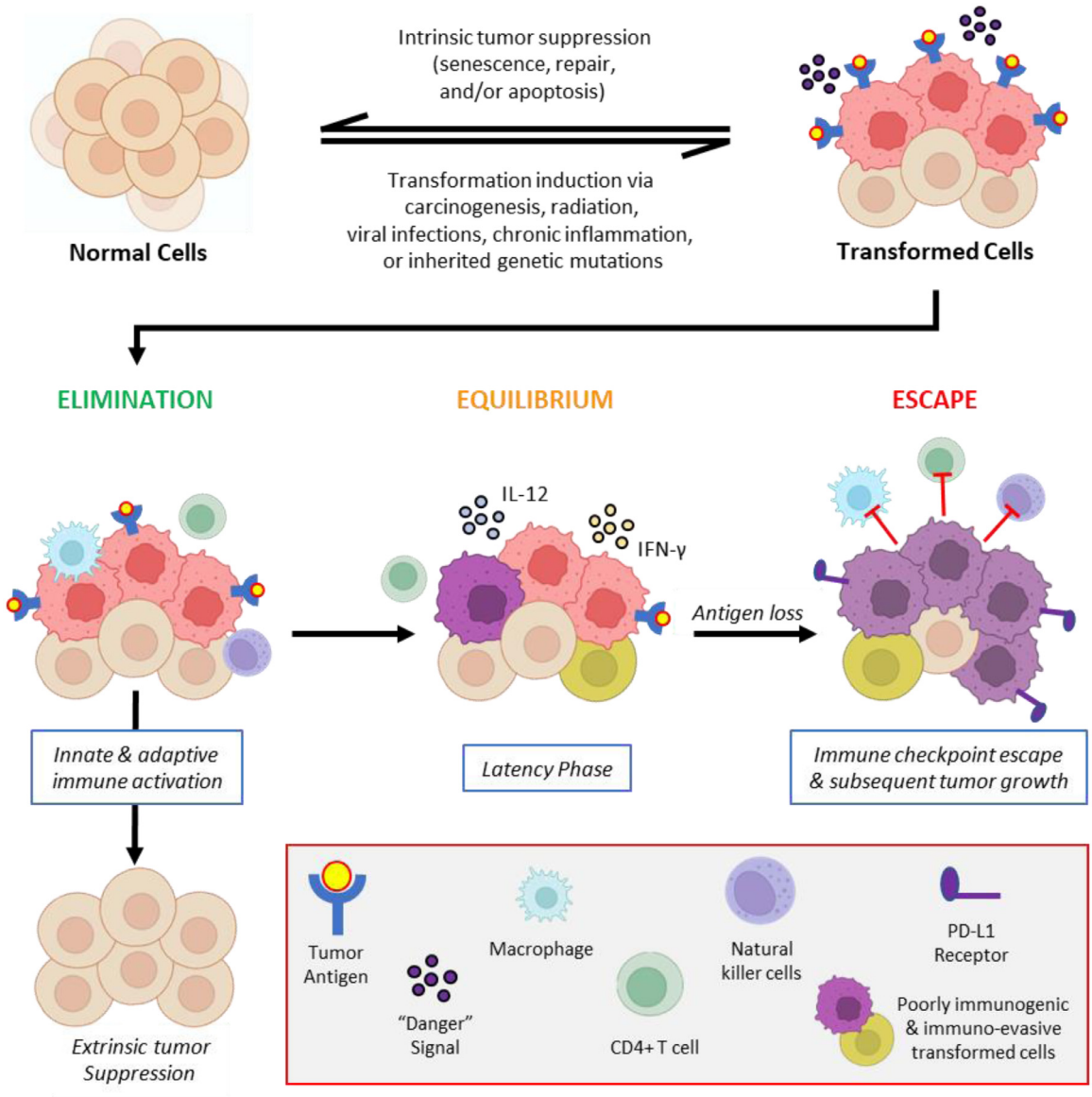

Fig. 1. Schematic illustration of the three E's of cancer immunoediting- Elimination, Equilibrium, and Escape. Exposure of normal body cells to carcinogens, radiation and/or viral infection, genetic mutation, or chronic inflammation leads to intrinsic tumor suppression and transformation into tumor cells. The extrinsic tumor suppressor factors, however, eliminate the transformed cells. Specific transformed cells might overpower the extrinsic tumor suppression and enter the equilibrium phase. Antigen and MHC loss in this phase helps the transformed cells escape the immune surveillance, leading to cancer establishment.

adjuvants. As their standalone efficiency is low, they are typically added to vaccines to enhance their effectiveness. They are designed to mimic the properties of pathogens, such as viruses or bacteria, by functioning as pseudo-antigens to the immune system in a way that elicits an immune response. They can either stimulate signaling pathways involved in the immune response or, more commonly, bio-mimic the natural invasion process of pathogens [19]. A common example is virus-like particles. While this topic is outside the scope of the current work, it has been extensively covered in other literature [20].

(ii) *Biomaterials as simple delivery vectors for the cocktail of immune modulators:* In this case, the primary function of the biomaterial used is to merely deliver loaded antigens/adjuvants efficiently in the vicinity of immune cells. The generation of any subsequent immune response is exclusively due to the biopharmaceutical and therapeutic attributes of the immune modulators. Here, instead of getting involved and interacting with immune systems, the biomaterial primarily functions as drug delivery vectors [21].

(iii) *Biomaterials as implantable/injectable scaffolds that generate an "immunogenic" depot for homing immune cells:* Biomaterials in the form of scaffolds can function as a biomimetic matrix that serves as artificial immune tissues for recruiting, housing, and programming host immunocytes. The scaffold's interconnected pores provide an environment that activates infiltrating dendritic cells, allowing them to process the antigens before moving to the lymph nodes to prime T cells specific to the antigen. By incorporating a sustained release attribute for the entrapped antigen/adjuvants, a long-lasting immune response can be secured. Additionally, the scaffolds can be positioned at specified spaces for regional therapies that reduce the toxicity related to systemic administration. Taking advantage of their adaptive shape, they can slot perfectly into irregular lesions (as formed by resection cavity), and localized adjuvant delivery can give desired immunotherapy outcomes at a lower dosage [22]. How-

ever, it should be noted that most of the scaffold-based systems are fabricated *ex vivo* and require either surgical placement in the body or large invasive needles for implantation (exceptions are "*in situ*"-forming injectable platforms like pH or redox-responsive hydrogels).

(iv) *Biomaterials as micro-/nanoparticulate systems that functionally interact and modulate the fate of immune cells:* Depending on the physiochemical attributes, such systems can either interact with the lymphatic system, get internalized by APCs, or directly target the compromised immune cell components within the TME to salvage them. In the case of biomaterial-based particulate systems, the major focus has been on the development of nano-sized carriers that can be loaded with immunostimulatory molecules. Unlike scaffolds, the size of nano-carriers can be tuned to be small enough that it reaches lymph nodes via lymphatic vessels from the injection site. By virtue of their physiochemical attributes (like hydrodynamic size, shape, and surface charge), they can directly interact with APCs and efficiently cross-present loaded antigens. A widely used approach is the surface engineering of nano-carriers that grants them the ability to directly target TME or facilitate direct interaction with the compromised immune cell populations [23]. As these surface engineering techniques will vary depending on the target immune cell, they are discussed in detail in later sections. Microparticles, although less diverse than their nano-sized counterparts, have unique applications. Their size and therapeutic attributes position them as an intermediate between systemically delivered nano-carriers and localized scaffolds. Rather than traveling toward tumors or immune cells, upon injection, they facilitate the recruitment of immune cells and parallelly provide controlled/sustained release of loaded constituents [24].

Increasing efforts are also being made to discover, design, and synthesize new immunomodulatory agents with higher selectivity and potency. Unlike conventional drug molecules, higher potency for an immunostimulatory agent doesn't necessarily translate to better therapeutic outcomes, as poor pharmacokinetic profile, susceptibility to biodegradation, and environmental factor-induced loss of pharmacological activity (in response to moisture, temperature, or pH, which can occur in the body or during storage) act as major roadblocks that limit their clinical translation. These underlying limitations emphasize the importance of implementing biomaterial-based delivery platforms tailored precisely for immunostimulatory payloads. Usually, the therapeutic effect of any immunostimulatory agent is highly specific for a particular cell subset. Their efficacy can be enhanced in such cases by employing biomaterials engineered with immune cell-specific targeting functionality. Such modifications can subsequently aid in improving bioavailability while reducing effective doses. In addition, using biomaterials can facilitate the co-delivery of multiple immunostimulants that preferably act via different immune pathways to generate a synergistic outcome [25].

The crosstalk of immune cell components within the immunosuppressive TME and the underlying mechanism of biomaterial-based approaches that can be employed to positively modulate them have been schematically represented in Fig. 2. The detailed mechanisms are discussed in later sections.

3. Current strategies for cancer immunotherapy

Before understanding the existing state-of-the-art in biomaterial-based immunotherapy platforms, it is necessary to comprehend the current clinically available modalities. A brief overview of the current cancer immunotherapy strategies and various classes of molecular adjuvants that can be incorporated with biomaterials for immune targeting/potentiation of their effect are discussed in Table 1 and Table 2, respectively.

4. Enhancing immunotherapy using bio-engineered platforms

The ensuing section provides an overview of the current state-of-the-art in biomaterials-based approaches for improving cancer immunotherapy. Special emphasis has been laid on understanding the key facets like composition, payload loading mechanism, and scope of functionalization for achieving target-specific delivery. Fig. 3 provides a schematic visual depiction and highlights the different classes of biomaterials discussed in this section.

4.1. Polymer-based platforms

Polymers are a well-documented class of biomaterials with outstanding potential as an immunostimulant delivery vehicle for cancer immunotherapy. Various polymer-based platforms have been explored for this purpose, including nanoparticles (NPs), microparticles, polymer-drug conjugates, and scaffold systems [49]. Popular polymers used to design biodegradable carriers include chitosan, dextran, polyethyleneimine (PEI), and co-polymers like poly(lactide-co-glycolic acid) (PLGA or PLG), poly(lactic acid) (PLA), and polyethylene glycol (PEG). Unique structural and chemical features of polymers allow optimal incorporation of immunostimulatory entities such as therapeutic proteins, enzymes, and cytokines via electrostatic attraction. As simultaneous encapsulation of both hydrophilic and hydrophobic molecules is possible, these platforms have also been explored for co-delivering immune adjuvants or in developing combinatorial cancer chemoimmunotherapy. Apart from contributing to payload encapsulation, the polymeric backbone can be chemically tailored to increase the availability of surface ligands that can be anchored with targeting entities. By varying the composition of constituents and degree of cross-linking, the release profile of the encapsulated payload can be significantly tweaked (from stimuli-responsive burst release to controlled/sustained release over a specified period) [50]. The following section will discuss some recent applications of polymer-based platforms for cancer immunotherapy.

By exploiting a polymeric system's ability to fabricate a stable multi-component system, Da Silva *et al.* developed a biodegradable and highly biocompatible PLGA-PEG nanoparticulate system for tumor localized and sustained release of doxorubicin (DOX), known to induce immunogenic cell death (ICD), two immune adjuvants (Poly I:C and R848) and one chemokine (MIP3α; CCL20). Additionally, the authors also explored the addition of IR-780 Iodide, a near-infrared sensitive dye, for monitoring the NP delivery via imaging. The system was made in an oil/water emulsion, using the solvent evaporation-extraction technique. Poly I:C, due to its hydrophilicity, showed rapid release from the polymeric matrix while other encapsulated compounds displayed a typical sustained release profile (Fig. 4a). The system showed potent therapeutic efficacy on account of the multi-component nature when examined in highly aggressive and treatment-resistant models like TC-1 lung carcinoma and MC-38 colon adenocarcinoma. Selective cytotoxicity towards cancer cells with a robust activation and maturation of DCs and MIP3α-mediated migration of the immune cell population (via chemotaxis) was reported [51]. Cancer vaccines based on a single antigen are inept in generating optimal APCs activation for inducing antigen-specific T cell response. To overcome this limitation, Sheikhzadeh *et al.* developed mannan-decorated PLGA NPs loaded with tumor cell lysate (TCL) and poly I:C for immunization of breast tumor-bearing Balb/c mice. The use of mannan (a natural ligand for mannose receptors expressed on the surface of DCs) as a targeting agent facilitated the enhancement in their dendritic

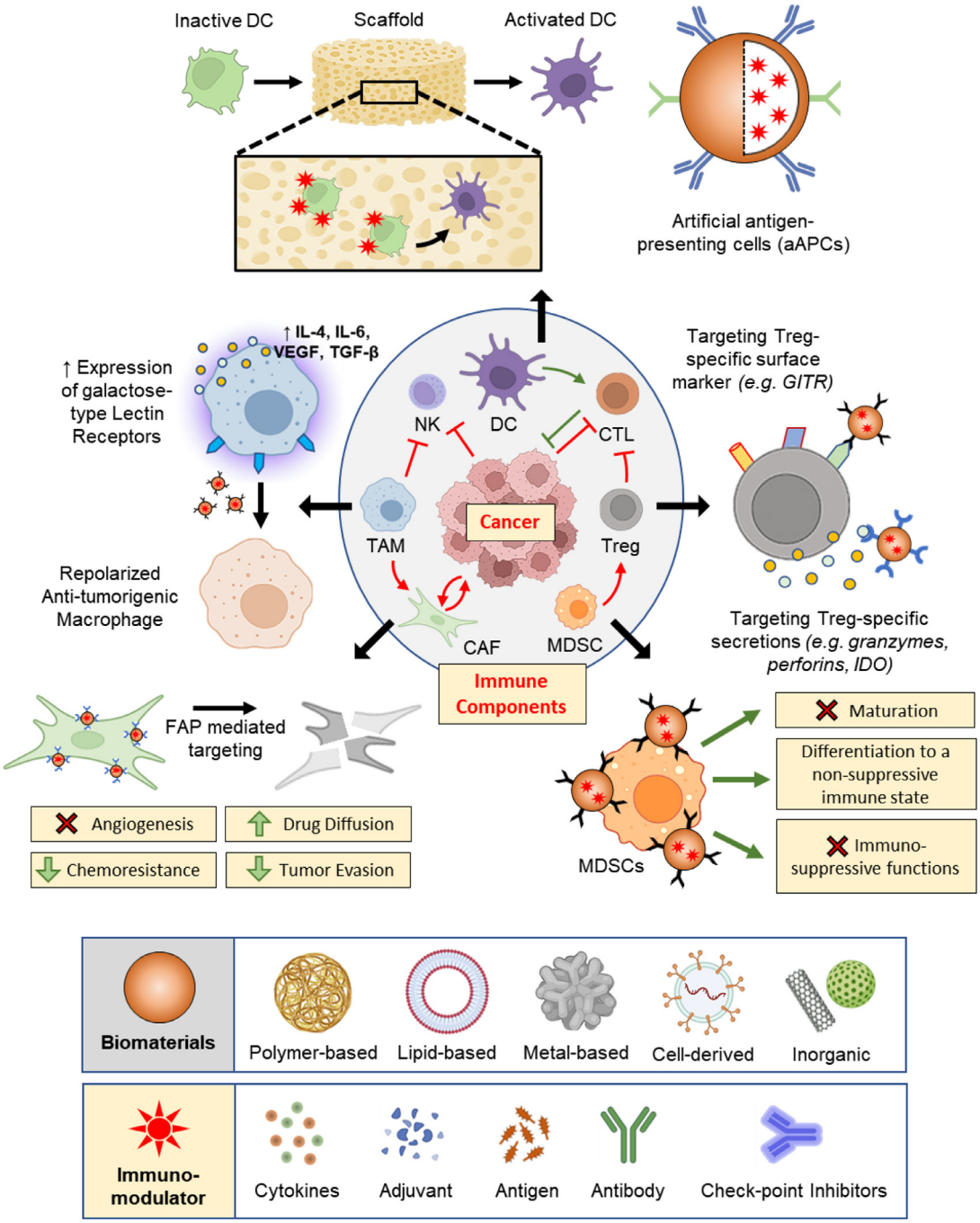

Fig. 2. Overview of biomaterial-based modulation of the immune-suppressive tumor microenvironment

uptake and was followed by the induction of robust immune response. As systemic exposure of loaded payload is minimized by mannan-mediated targeting, the side effects associated with the use of poly I:C like increased inflammatory cytokines, fever, and anorexia, were absent in rodents treated with the PLGA NPs. The benefits of the platform were highlighted by a significant decrease in tumor growth and metastases, with substantial infiltration of immune cells at the tumor site [52].

Apart from injectable particulate systems, various polymer-based scaffolds (for insertion at a subcutaneous site or tumor resection cavity) and microneedles have also been reported [53]. Ji *et al.* recently employed a polymer-based immune implant for post-operative (after surgical resection) *in situ* immunotherapy to prevent local recurrence or distal metastasis in colorectal cancer (Fig. 4b). Such an approach provides a unique window having direct access to the tumor site. The implant was formed by crosslinking freeze-dried oxidized dextran with -arm PEG-NH_2. It possessed ideal tissue adhesion properties and prolonged induction of immunity by releasing the encapsulated Resiquimod and anti-OX40 antibody in a sustained manner [54]. It is worth mentioning that several individual studies have concluded that polymers have their distinct intrinsic immunogenicity [55]. While such attributes can be useful in rare cases when the immune signal is weak, polymers having high immunogenicity are objectionable for the delivery of immunostimulatory agents. Since polymer immunogenicity is dependent on factors like molecular weight, surface charge, and rate

Table 1
Overview and mechanism of the current cancer immunotherapy strategies.

Approach	Overview	Mechanism	Ref.
Adoptive Cell Therapy (ACT)	It involves the isolation and transfer of tumor-specific T cells, which are modified (to recognize and attack cancer cells), expanded, and reinfused to specifically target cancer cells within the patient's body (while minimizing the harmful effects on healthy cells). The major subtypes of ACT include tumor-infiltrating lymphocyte (TIL) therapy, T cell receptor-engineered T cell (TCR-T) therapy and CAR-T cell therapy.	• Anti-tumorigenic TILs, isolated from surgically removed tumors are expanded *in vitro* using specialized techniques and culture conditions, followed by their re-infusion. • TCR-T and CAR-T cell therapy involve the transfer of genetically modified T cells (isolated from the patient's blood) having enhanced expression of specific T cells or chimeric antigen receptors that aid in attacking cancer cells.	[26]
Immune Checkpoint Blockade Therapy (ICBT)	In normal circumstances, immune checkpoints are accessory molecules that act as "brakes" on the over-activation of the immune system, thereby preventing unwanted damage to healthy cells. In cancer, tumor cells undergo "immune escape" by employing complementary ligands that interact and block the surface checkpoint proteins on T cells. ICBT involves the use of therapeutic interventions (against CTLA-4 or PD-L1), allowing robust immune activation to effectively recognize and terminate the cancer cells.	• CTLA-4 competes with the CD28 molecule to bind with CD80/CD86 ligands on the APCs. While the Programmed death-ligand 1 (PD-L1) ligand of tumor cells binds with the Programmed cell death protein 1 (PD-1) protein of T cells to suppress its activation. • Small molecule drugs or specifically designed monoclonal antibodies can competitively block checkpoint proteins, thereby promoting "immune elimination" by preventing the deactivating interaction of immune cells.	[27]
Cancer Vaccine Therapy	These are platforms that enable the immune system to specifically attack cancer cells using one or more tumor antigens and produce immune memory cells, which can be used to prevent tumor recurrence, as well as to treat the existing tumor. They can be categorized into two types based on their antigens loading method: traditional nanovaccines that introduce antigens into the body and *in situ* nanovaccines that extract antigens from active tumors. The physicochemical attributes of the nano-carriers can be tuned to achieve spatiotemporal control over the delivery of loaded immune modulators.	• Traditional nanovaccines focus on delivering the loaded antigens and adjuvants to lymphoid tissues, which are populated with various APCs vital for antigen cross-presentation • Key steps of *in situ* vaccination involves reaching the tumor site and inducing immunogenic cell death, resulting in the revelation/exposure of previously concealed tumor antigens, such as damage- or danger-associated molecular patterns. • In either scenario, DCs undergo maturation and activation in response to antigen uptake. Subsequently, the matured DCs present the antigens to the CD8+ T cells through the MHC molecules and cause T cell expansion. These antigen-specific T cells infiltrate the TME and exert cytotoxic effects against the tumor cells.	[28]

of degradation, a better understanding of the relationship between these physicochemical properties and immunogenicity is desirable [56].

4.2. Lipid-based nanocarriers

Over the past several decades, lipids have been exceedingly explored as building blocks to generate numerous nanocarriers like liposomes, solid lipid nanoparticles, lipid nano-capsules, and nanomicelles for the targeted delivery of chemotherapeutic and immunostimulatory agents [57]. Amongst these delivery systems, liposomes (along with their modified derivatives) are preferred by the research fraternity due to their distinct advantages like superior biocompatibility, biodegradability, low toxicity profile, and ease of preparation [58]. Initially discovered in the mid-1960s, liposomes are nanosized bilayer spheres prepared by the self-assembly of amphiphilic lipids, which generates an outer hydrophobic bilayer with an internal aqueous core. Depending on polarity, adjuvants can either be entrapped within the hydrophobic liposomal membrane or they can be loaded into the hydrophilic center (directly or via entrapment within an inner core material) around which the bilayer is formed. The thin-film hydration method is commonly utilized for liposome preparation due to the simplicity of the process, but for optimal results, it requires the temperature of the hydration buffer to be raised above the phase transition temperature of the lipid. This limits the usability in the case of thermolabile immune adjuvants, for which other milder methods like solvent vaporization or freeze-thawing are preferred [59]. The size distribution and lamellarity of the fabricated liposomes are controlled by employing size-reducing processes like membrane extrusion or sonication. Overall, the commercial scalability and ability to incorporate diverse molecules have led to >20 liposome-based formulations getting approved by global regulatory bodies, with multiple other formulations awaiting clinical approval [60].

Like their polymeric counterparts, lipid-based nanocarriers provide an increase in bioavailability for the loaded immunoadjuvants by curbing systemic exposure and enzyme-induced degradation. The physicochemical properties of any lipid-based system govern its performance as an immune modulator [61]. A size profile less than 100 nm facilitates enhanced permeability and accumulation within solid tumors (attributed to the leaky tumor vasculature and poor lymphatic clearance, "enhanced permeability and retention (EPR) effect"), while systems with a size less than 25 nm are more likely to be delivered to lymph nodes [62]. The surface charge also contributes to immune activation as nanocarriers with a net positive charge have better interaction with the negatively charged mucosal surface, which directly results in enhanced uptake by immune cells (vs. neutral or negatively charged systems) [63]. But using lipids that possess a positive charge at physiological pH and high concentration of cholesterol (commonly used as lipid bilayer stabilizer) causes adverse inflammatory events associated with complement activation. In contrast, using phosphatidylserine as a lipidic component enables apoptotic cells-like mimicking properties through which it regulates macrophage functions, resulting in anti-inflammatory effects [64]. For lipid-based immunotherapy, cell-specific targeting can be achieved by functionalizing the nanocarrier surface with ligands like antibodies, peptides, and aptamers. Apart from targeting, surface functionalization with PEG has been extensively explored to develop "stealth" liposomes having properties like prolonged systemic circulation, protection from opsonization, and enhanced biodistribution profile [65]. The following section will discuss recent trends and important advancements in lipid-based strategies for cancer immunotherapy.

Su *et al.* developed a liposomal system based on a cationic polymer-lipid hybrid nanovesicle (termed P/LNV liposomes) for concurrent delivery of an immunogenic cocktail (Fig. 5a). The payload consisted of anionic antigen epitopes, CpG, and 1-methyl-tryptophan (1-MT, an Indoleamine 2,3-dioxygenase 1 inhibitor; IDO-1). The system aimed to enhance the low antigenicity of the

Table 2
Different classes of molecular adjuvants that can be incorporated with biomaterials for immune targeting/potentiation of their effect.

Types of Immune Adjuvants		Overview	Mechanism	Refs.
Immune Stimulators	Alum	It enhances antigen presentation and activates Th2 effector response, which results in the production of cytokines (such as IL-4, IL-5, and IL-13) that stimulate the antibody production and activation of $CD4^+$ T cells.	• Alum operates as an antigen depot. It also functions as an irritant, attracting immune cells like neutrophils to the injection site. • NOD-like receptors (NLRs) identify alum through uric acid release or direct activation of the NLRP3/NALP3 inflammasome.	[29]
	M59	M59 is a squalene oil-based emulsion adjuvant stabilized with non-ionic surfactants tween 80 and span 85. M59 activates immune cells and facilitates their recruitment at the injection site. By traveling to the draining lymph nodes, it can initiate an adaptive immune response by activating lymphocytes.	• It specifically targets the NLR pathway to enhance the immune response. • By binding to NLRP3, M59 triggers the release of pro-inflammatory cytokines and other signaling molecules.	[30]
	Lipopolysaccharides (LPS)	LPS are made of a lipophilic phospholipid and a hydrophilic polysaccharide (lipid A). They are a class of bacterial outer membrane glycolipids from Gram-negative bacteria.	• TLR4 detects lipid A and drives DC toward Th1 immunity. • Binding of TLR4 leads to the production of cytokines such as IL-6, TNF and IL-1.	[31]
	Monophosphoryl lipid A	It is a synthetic lipid molecule that is prepared by modifying the LPS found in the cell wall of non-pathogenic Salmonella.	• It primarily functions as a TLR4 agonist. • It can stimulate the synthesis of IL-12 and IFN, which support Th1 responses.	[32]
	Flagellin	It is a protein that forms the filament (or "flagellum") in many bacteria, including Escherichia coli, which are used for motility.	• It generates an immune response by interacting with TLR5 present on the immune cell surface. • By the phosphorylation of NLRC4 and NAIP5, it can simultaneously target inflammasomes.	[33]
	Muramyl dipeptide (MDP)	It is a synthetic immunoreactive peptide consisting of N-acetyl muramic acid attached to a short amino acid chain of L-Ala-D-isoGln.	• Besides secretion of pro-inflammatory cytokines (TNF, IL-1, IL-6), it activates the NOD2 receptor which results in the release of nitric oxide and upregulation of adhesion molecules CD 11a, CD11b, CD11c/CD18, and CD54	[34]
	CpG oligodeoxynucleotides (CpG-ODNs)	They are short synthetic single-stranded DNA molecules containing CpG motifs. They have a high adjuvanticity and low reactogenicity.	• It works as a polyclonal activator, causing B-cells to multiply and develop into IgG-producing cells. • They indirectly activate monocytes and macrophages resulting in the production of pro-inflammatory cytokines. • Through the TLR-9 receptor, it activates the MyD88 pathway, Type I INF, and cytokine response.	[35]
	Imido-quinolines	Imido-quinolines (like Imiquimod and Resiquimod) are synthetic TLR agonists and potent dendritic cell activators with established anticancer activity.	• They activate the TLR-7/8 present on DCs and trigger downstream pathways, producing cytokines that stimulate APC activation. • They also utilize the TLR-independent pathway by interfering with adenosine receptor signaling pathways leading to increased pro-inflammatory activity.	[36]
	Polyinosinic:polycytidylic acid (Poly I:C)	Poly I:C is a synthetic double-stranded RNA molecule that is recognized by the TLR3 receptor as a sign of viral infection.	• Poly I:C promotes the Type I INF response and upregulates the anticancer activity by activating the melanoma differentiation-associated protein 5 cytoplasmic receptor or RIG-1 receptors. • It activates DCs, causing them to generate IL-12 and type I IFN while also increasing MHC-II expression.	[37]
	STING Agonists	They are small molecule analog of cyclic GMP-AMP (cGAMP) that acts as an agonist of the stimulator of IFN genes protein (STING; transmembrane protein 173) with potential immunoactivity properties.	• Upon activation of the STING pathway, a signaling cascade is initiated that results in the production of type I IFN and other cytokines, such as IL-6 and TNF-α.	[38]
Monoclonal Antibodies (mAbs)	Anti-CD47	They are designed to bind to the CD47 protein on cancer cells, thereby blocking the "don't eat me" signal and allowing the immune system to recognize and attack the cancer cells.	• Signal regulatory protein attaches to CD47 and triggers the signaling cascade that prevents phagocytosis. Anti-CD47 mAbs prevent this binding, thereby suppressing tumor development and metastasis.	[39]
	Anti-CD44	They are antibodies that bind to CD44, a cell surface receptor that is involved in critical cellular processes, including cell adhesion, migration, and survival.	• By blocking the function of CD44, the antibodies can interfere with cell adhesion, migration, and survival, leading to the inhibition of tumor growth and the reduction of cancer cell migration and invasion.	[40]
	Anti-EpCAM	These mAbs target EpCAM (epithelial cell adhesion molecules) that are involved in the formation and maintenance of epithelial tissues, and are often overexpressed in cancer	• Besides its adhesion function, EpCAM transmits various oncogenic signaling and gene expression pathways to the nucleus, the blocking of which has therapeutic benefits.	[41]

(*continued on next page*)

Table 2 (*continued*)

Types of Immune Adjuvants		Overview	Mechanism	Refs.
	Anti-IGF Receptor I	Anti-IGF-1R refers to antibodies that target the insulin-like growth factor 1 receptor, which is a transmembrane receptor protein that is involved in regulating cell growth and division.	• It functions by binding to the IGF-1R protein on cancer cells, blocking its activation and disrupting its ability to promote cancer cell survival and growth. • It also triggers apoptosis of cancer cells and inhibits their ability to migrate and invade healthy tissue.	[42]
	Anti-Frizzled	It refers to antibodies that target the Frizzled family of proteins, which are involved in the Wnt signaling pathway.	• It works b disrupting the Wnt signaling pathway (critical for cancer cell growth and chemoresistance). • This can inhibit cancer cell survival, and also stimulate the immune system to recognize and attack cancer cells	[43]
Antitumor Cytokines	IL-2	IL-2 is a pleiotropic cytokine that plays a significant role in the innate and adaptive immune response. It is an important growth regulator and mitogen of T cells.	• IL-2 activates and proliferates NK cells and T cells. • IL-2 receptor couples with the Janus kinases (JAK) and activates the transcription factors for the "Signal Transducer and Activator of Transcription" (STAT) protein.	[44]
	IL-7	IL-7 is known for its growth-promoting effects on progenitors of B cells. it contributes to host defence by regulating the development and homeostasis of immune cells, including T lymphocytes, B lymphocytes, and NK cells.	• IL-7 receptor binding leads to the phosphorylation of tyrosine residues, subsequently activating the JAK/STAT pathway, PI3, and SRC kinases. • IL-7 receptor is expressed on immature B cell progenitors; exposure of IL-7 to its receptor helps in B cell development.	[45]
	IL-15	IL-15 is involved in the activation, differentiation, and proliferation of T, B, and NK cells. It also supports the maintenance of memory $CD8^+$ T cells, thereby promoting long-term antitumor immunity. It also enhances the differentiation of DCs.	• By binding with its complementary receptor complex, IL-15 causes its activation which triggers JAK, leading to the phosphorylation of the receptor and the subsequent activation of the STAT proteins.	[46]
	INF-α	IFN-α is a type I IFN mainly involved in stimulating the immune responses against viral infections. It promotes the production of MHC class I molecules on tumor cells and induces the maturation of DCs.	• IFN-α induces tyrosine phosphorylation of STAT proteins, leading to IL-4 production and B cell activation. • IFN-α targets the induction of apoptosis in tumor cell lines through activation of the caspase cascade. • It also upregulates costimulatory and co-inhibitory receptors on cells, leading to tumor antigen expression activation.	[47]
	Granulocyte-macrophage colony-stimulating factor (GM-CSF)	GM-CSF is a 14-35 kDa protein that primarily derives from T cells and is variably glycosylated. It has a crucial role in regulating the growth and functions of granulocytes and cells of the macrophage lineage, from their earliest stages of development to maturity.	• GM-CSF promotes cell survival and proliferation via activation of JAK/STAT and NF-kB pathways.	[48]

peptide vaccine while simultaneously inhibiting the immunosuppressive TME, ultimately boosting the efficacy of cancer combination immunotherapy. The cationic liposomes loaded with hydrophobic IDO inhibitor 1-MT were electrostatically complexed with anionic peptide-modified epitopes (AE) (generated from MHC-I-restricted melanoma antigens and CpG), which cumulatively formed a typical tumor vaccine. Surface functionalization with AE resulted in enhanced uptake of P/LNV liposomes by DCs followed by their maturation. Increased percentage of $CD86^+$ and activated DCs resulted in a potent cytotoxic T-lymphocyte response against B16-OVA tumor cells. C57BL/6J mice treated with P/LNV liposomes displayed a strong cancer-specific T cell response with increased infiltration of $CD8^+$ T cells in the tumor and draining lymph nodes [66]. By co-loading immune adjuvants that have an independent mechanism of generating an anti-tumor immune response, lipid-based nanocarriers can be harnessed to generate a response via multiple immune pathways. Haung *et al.* reported spherical nucleic acid, prepared using liposome (composed of 2-Dioleoyl-sn-glycero-3-phosphocholine) as nanoparticle core around which a shell of highly oriented and densely packed immunomodulatory oligonucleotides was formed. For optimal attachment onto the liposomes, oligonucleotides were conjugated to the 3′-terminal of cholesterol. Two unique oligonucleotides that activate independent TLR 9 signaling pathways (each in a sequence-specific fashion) were simultaneously conjugated onto the liposomal core. The platform showcased efficient trafficking in DCs which resulted in the activation of the TLR9 signaling pathway and infiltration of the tumor antigen-specific T lymphocytes. By controlling the stoichiometric ratio of conjugated oligonucleotides, the authors reported control over crucial cell signaling and regulatory processes (Fig. 5b) [67].

Although lipid-based immunotherapy shows great promise, some critical limitations still exist. Firstly, the use of organic solvents and harsh processing conditions are prominent during fabricating, which can cause the denaturation of heat-labile immune adjuvants [68]. Secondly, the adjuvant loading capacity is affected by lipids' inherent electronegativity [69]. And finally, delivery systems made using lipids exhibit poor stability when stored for a prolonged period. Addressing these challenges with an outcome-driven approach can lead to better clinical translation.

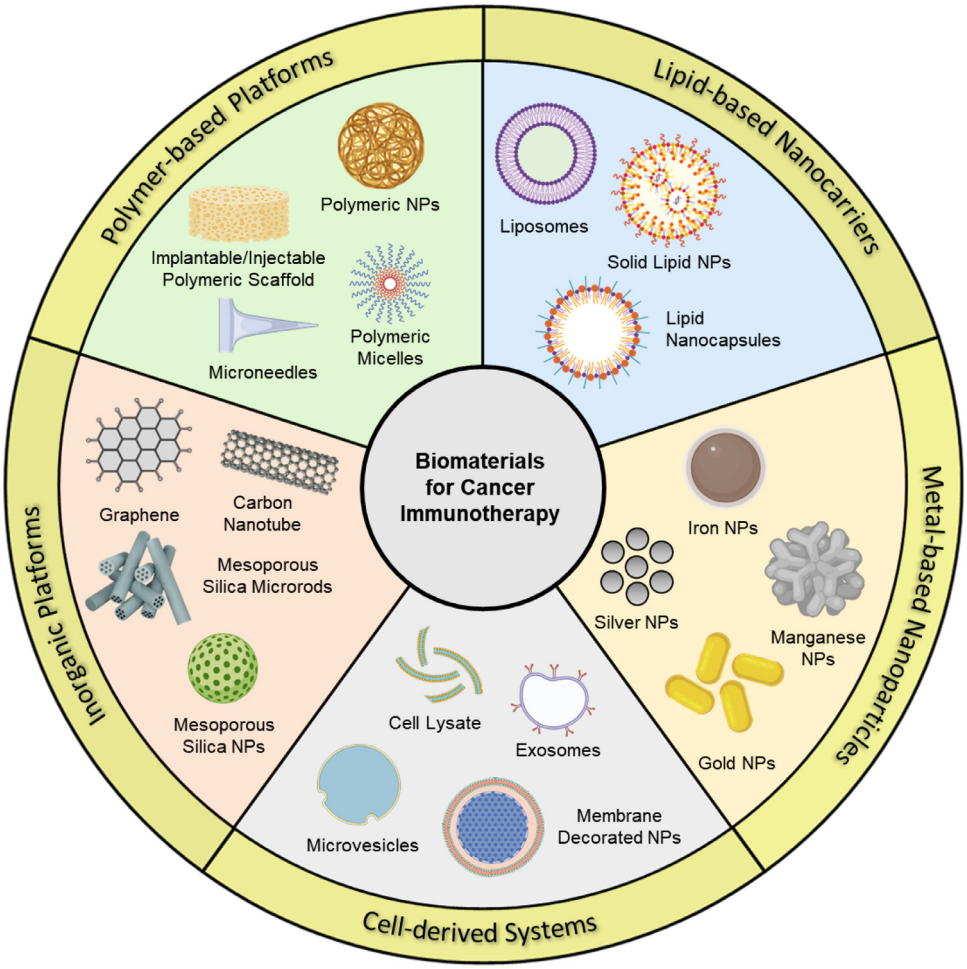

Fig. 3. Different classes of bio-engineered platforms for application in cancer immunotherapy. Depending on their physicochemical properties and fabrication approach, biomaterials can be bestowed with well-defined characteristics and functionalities for incorporating various immune modulators.

4.3. Inorganic biomaterial-based platforms

4.3.1. Carbon-based nanocarriers

By virtue of attractive physical (thermal, optical, and electronic), chemical, and mechanical properties, carbon-based nanomaterials (CNMs) have generated substantial interest in developing cancer theranostics platforms. Under the umbrella of carbon-based biomaterials, graphene, graphdiyne, fullerenes, carbon nanotubes, and carbon quantum dots have been widely used as multifunctional delivery vectors for chemotherapeutic drugs and immunomodulatory cargo [70]. With a size profile ranging from 1 nm to 1 µm, CNMs possess a high surface area to mass ratio that allows higher loading of drugs/immune adjuvants. A key advantage of CNMs is their tunable surface chemistry which can be readily modified/functionalized to enhance biocompatibility and water solubility, prolong systematic circulation time, and impart active tumor-targeting properties. Dangling bonds, located in large numbers at the edge and the defective sites can be subjected to covalent (adding hydroxyl groups, carboxyl groups, or amino groups) or noncovalent (grafting of amphiphilic molecules like chitosan, bovine serum albumin or PEG derivatives) modifications [71]. While CNMs are capable to achieve passive tumor localization via the EPR effect, active tumor targeting can be accomplished by conjugating ligands for common receptors which are overexpressed in tumor/vasculatures (e.g., folate receptors, epidermal growth factor receptors, integrin receptors, transferrin receptors, etc.). A distinct feature of CNMs is their strong optical activity in the near-infrared (NIR) range. Due to the graphitic carbon structure, CNMs absorb electromagnetic waves in the NIR-I (750-1000 nm) and NIR-II (1000-1700 nm) windows. This allows the fabrication of unique combinatorial platforms that can integrate cancer immunotherapy with photothermal therapy [72]. The following section will discuss prominent CMNs systems and their recent applications in cancer immunotherapy.

Graphene is a two-dimensional (2D) sp^2-hybridized allotrope of carbon of one atom thickness, having a sheet-like assembly with a honeycomb structure. Multiple graphene-based derivatives have been synthesized (including graphene oxide, reduced graphene oxide, graphene nanoribbons, and graphene nanoplatelets) and explored in cancer theranostics [73]. The synthesis techniques to generate graphene and its derivatives includes mechanical exfoliation, liquid-phase exfoliation, and epitaxial growth. Immune adjuvants can be attached to graphene surfaces by simple surface adsorption techniques like covalent bonding, hydrophobic interaction, and electrostatic interaction [74]. Wang *et al.* reported aluminum oxyhydroxide-modified graphene oxide nanosheets (GO-AlO(OH)) as a carrier to deliver immune antigens while simultaneously overcoming alum's innate limitation in eliciting cell-meditated immunity, which can help potentiate its effectiveness as an immune adjuvant (Fig. 6a). Antigen-loaded GO-AlO(OH) nano complexes were formulated by the incorporation of model antigens using a facile mixing/adsorption approach. The system enabled cellular uptake and cytosolic release of antigens along with DC maturation, thereby producing higher antigen-specific IgG titers

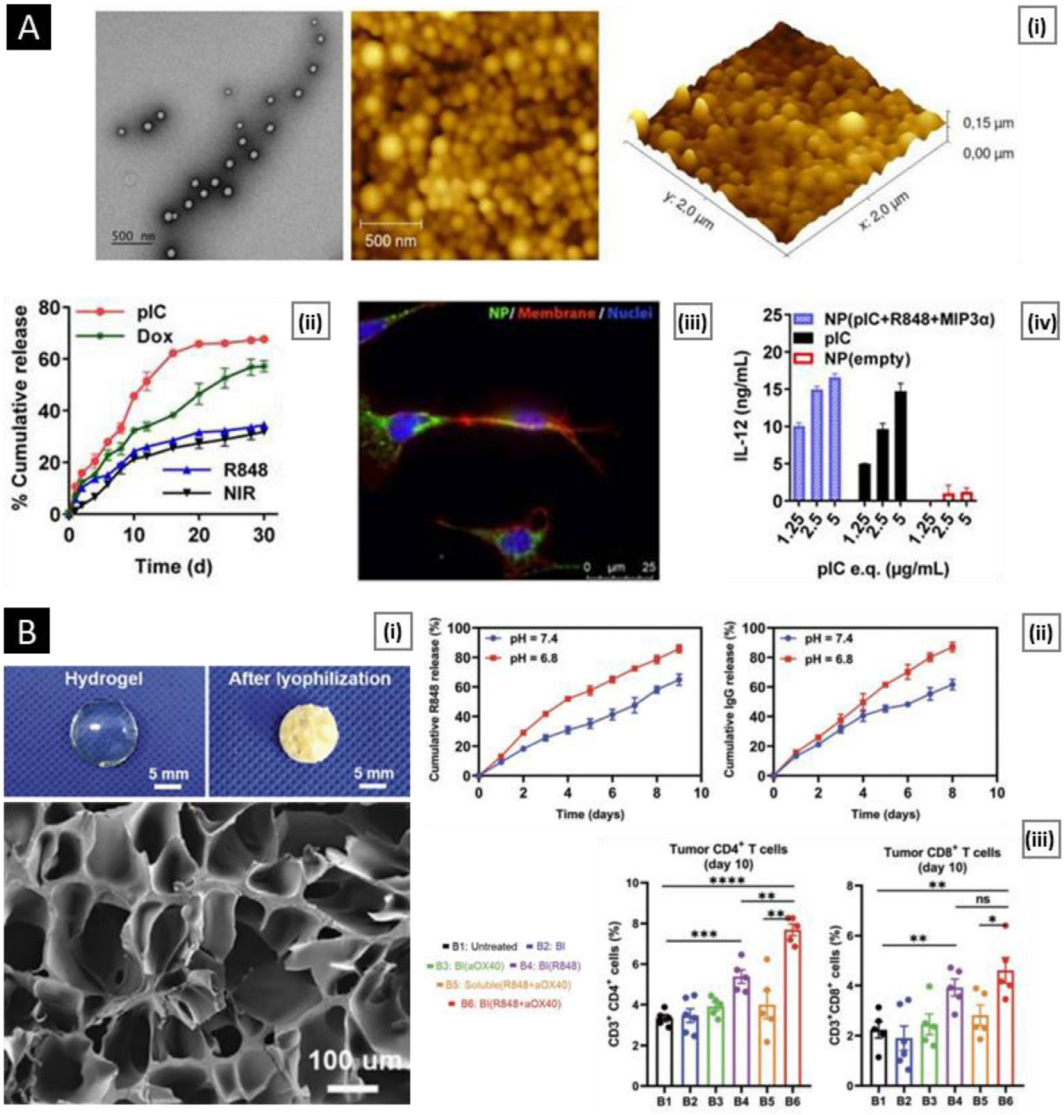

Fig. 4. Polymer-based Bio-engineered Platforms for Cancer Immunotherapy. **(A)** Biodegradable PLGA-PEG nanoparticles for co-delivery of DOX and immune adjuvants. Here, sub-figure (i) shows a representative morphology image of empty NPs obtained by TEM and atomic force microscopy (2D/3D). Sub-figure (ii) shows the release kinetics of encapsulated constituents. Sub-figure (iii) shows NPs uptake by TC-1 cells after 2 hours of incubation (shown by fluorescence microscopy; Red: cell membrane; purple: cell nucleus; green: NIR dye). Sub-figure (iv) shows the activation of DCs measured by the secretion of IL-12p40 upon 48 hours of incubation with NPs. **(B)** Biopolymer immune implant co-loaded with R848 and anti-OX40 antibody for preventing postoperative colorectal tumor relapse and metastasis. Here, sub-figure (i) showcases a picture of the implant before and after lyophilization (scale bar: 5 mm) and a corresponding SEM image (scale bar: 100 µm). Sub-figure (ii) shows the *in vitro* release of R848 and IgG from the implant in PBS buffer (pH 7.4 or 6.8). Sub-figure (iii) depicts the tumoral levels of $CD4^+$ and $CD8^+$ T cells (on day 10) in treated mice. Adapted with permission from [51] (Copyright 2019, Ivyspring International Publisher) and [54] (Copyright 2020, Wiley-VCH), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that aid to induce robust $CD4^+$ and $CD8^+$ T lymphocyte responses. Additionally, the authors demonstrated the potential of GO-AlO(OH) nano complexes as a personalized approach for cancer vaccine by using tumor cell lysate as adjuvant, which led to significant inhibition of tumor growth in the E.G7-OVA tumor-bearing mice [75].

Carbon nanotubes (CNTs) belong to a sub-category of fullerene derivatives that have found diverse applications in biomedical engineering. Depending on the number of the carbon atom sheet, CNTs can be categorized into single-walled nanotubes (SWNTs, approximate diameter of 0.4-2 nm) and multi-walled nanotubes (MWNTs, approximate diameter of 10-100 nm) [76]. The common approaches to generate CNTs include chemical vapor deposition, electric arc method, and laser deposition method. CNT structure allows coupling with different molecules, which can be used for modulating CNT carrier properties or to achieve targeted delivery [77]. Xia *et al.* developed a nano-vehicle based on MWCNTs conjugated with a cell-penetrating peptide H3R6 (with terminal NH_2). The NH_2 group interacts with the carboxyl group of the MWCNTs, forming H3R6-MWCNTs (MHR). The CpG interacts with the positively charged segment of MHR to form an MHR-CpG complex. This platform enabled higher CpG uptake and surface hydrophilicity, thus resolving the long-term safety and toxicity issues. Further, it protected CpG from enzymatic degradation of nucleic acids and allowed its safe transportation into cells. MHR improved the immunogenicity of CpG (in both humoral and cellular immune pathways), as demonstrated by the augmented $CD4^+$ T cells, $CD8^+$ T cells, TNF-α, and IL-6 expression [78]. In a recent study, Li *et al.* developed cationic polymer brush-modified CNTs for delivering siRNA in cancer immunotherapy. By employ-

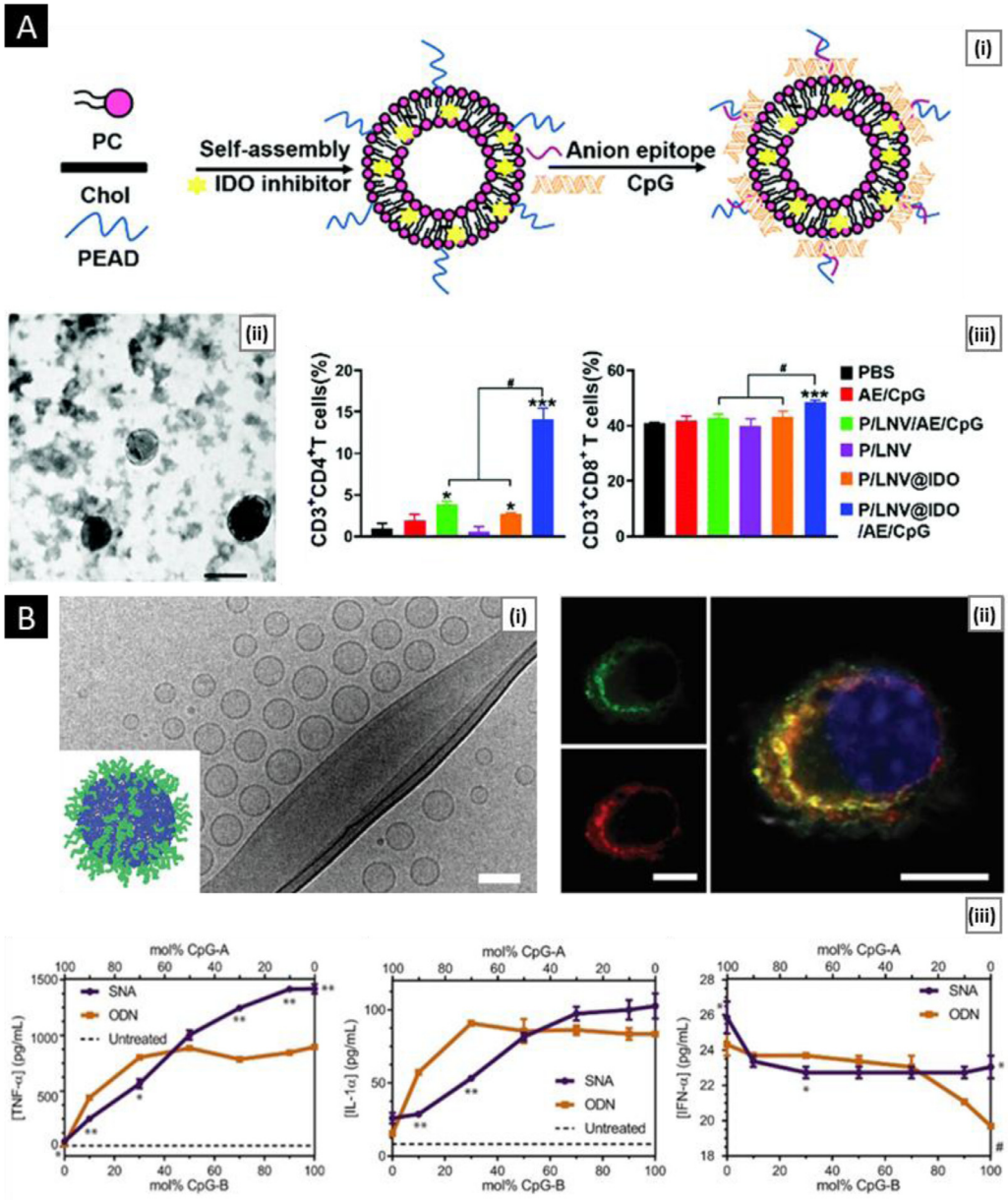

Fig. 5. Lipid-based Nanocarriers for Cancer Immunotherapy. **(A)** Cationic polymer-lipid hybrid nanovesicle (P/LNV liposomes) loaded with tumor vaccines and IDO inhibitors. Here, sub-figure (i) shows the schematic illustration of the assembly of P/LNV liposomes. Sub-figure (ii) shows the TEM image of drug-loaded (IDO/AE/CpG) P/LNV liposomes. Sub-figure (iii) shows a plot depicting the percentage of $CD3^+CD4^+$T cells in the tumor and the infiltration of $CD3^+CD8^+$ T cells in draining lymph nodes (in treated mice). **(B)** Liposome-based spherical nucleic acid construct for sequence-specific immune activation. Here, sub-figure (i) shows the cryo-TEM image of oligonucleotide functionalized liposome with a schematic representation of the constructs (Scale bars: 100 nm). Sub-figure (ii) shows representative confocal microscopy images of the nano-platform, 30 min after uptake by JAWS II cells (Color assignments: fluorescein-CpG-A (green), Cy5-CpG-B (red), nucleus (blue), actin (gray); Scale bars: 5 μm). Sub-figure (iii) shows the increase in cytokine and IFN production following intracellular localization after 24 h of treatment. Adapted with permission from [66] (Copyright 2021, Royal Society of Chemistry) and [67] (Copyright 2020, American Chemical Society), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ing the concept of surface-initiated atom transfer radical polymerization, cationic poly(2-dimethylaminoethylmethacrylate) brushes having mussel-inspired polydopamine chemistry were grafted onto CNTs surface. By changing the density of the bromo initiator moieties and polymerization period, the authors achieved precise control over parameters like density and length of the polymer chains which directly influenced the binding with siRNA. The platform was then explored as a carrier for the delivery of siRNA PD-L1. The CNT hybrids outperformed commercial transfection reagent in cell uptake assay (performed using fluorescent siRNA) while also displaying high efficiency (>60%) for knocking down PD-L1 in B16-F10 cells (Fig. 6b) [79].

4.3.2. Silica-based biomaterials

Silica (also known as silicon dioxide) is a naturally occurring inorganic material that is a key component of sand and rocks, making it the most abundant mineral on the planet. Known for its high strength, low density, high thermal stability, and chemical inertness, silica has been a subject of great interest in the field of material sciences. Owing to its outstanding attributes as a biomaterial (like biocompatibility, non-toxicity, ease of sterilization, and capability to be tailored to use-specific requirements), silica is used for diverse medical applications, including as a scaffold for tissue engineering, as a drug delivery vehicle, and as a material for dental/bone implants [80]. Mesoporous silica nanopar-

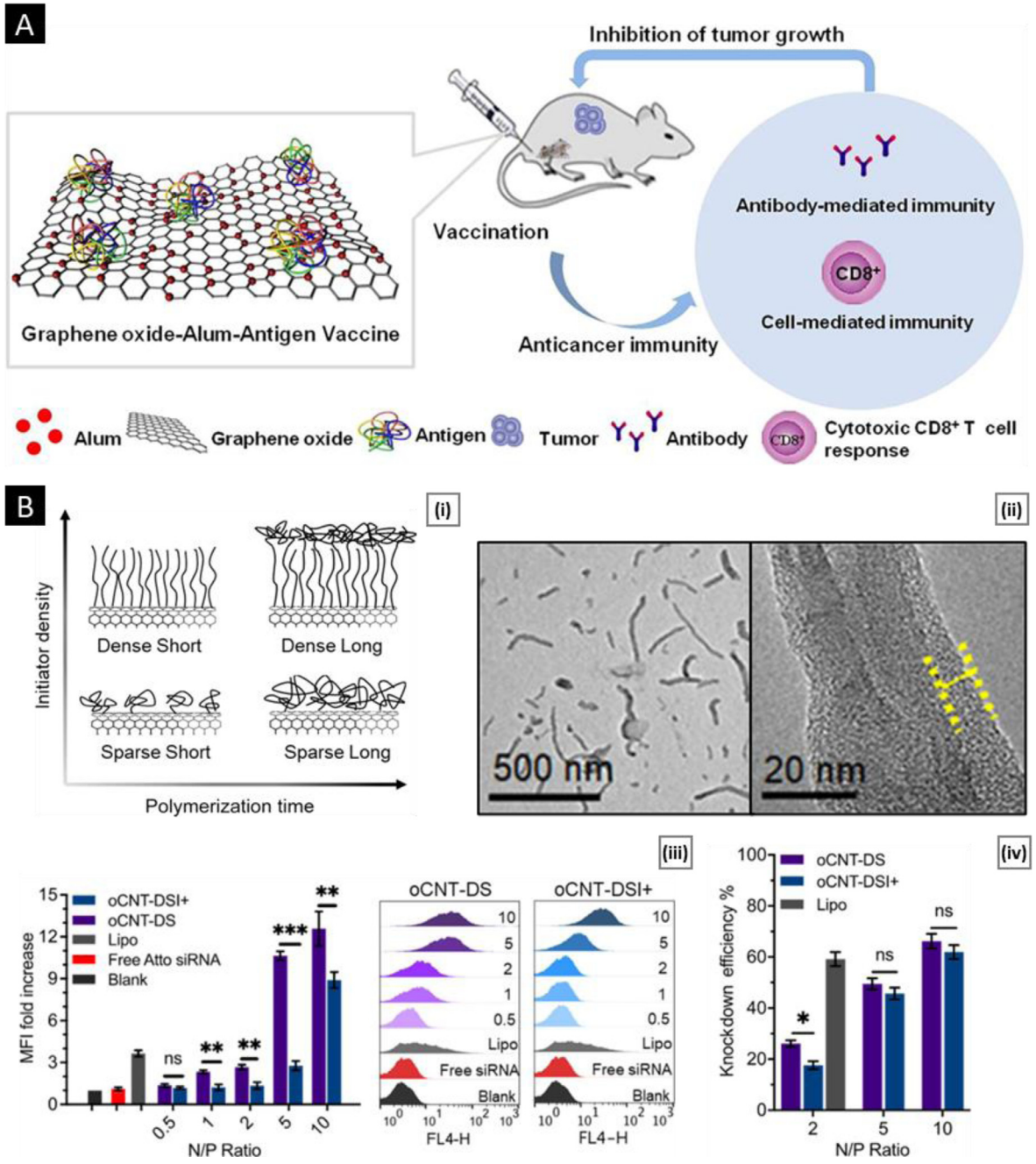

Fig. 6. Carbon-based Nanocarriers for Cancer Immunotherapy. **(A)** Schematic illustration of aluminum oxyhydroxide-modified graphene oxide nanosheets for targeting the delivery of immunostimulatory CpG oligonucleotides against prostate cancer. **(B)** Cationic Polymer Brush-Modified CNTs for siRNA Delivery. Here, sub-figure (i) shows a schematic depiction of different architectures of cationic polymer grafted on CNTs. Sub-figure (ii) shows representative TEM and HRTEM of oCNT-DS (dense short coating on CNTs); the highlighted area in HRTEM marks the polymer brush coatings. Sub-figure (iii) shows the siRNA uptake in B16-F10 cells at different N/P ratios (from 0.5 to 10, vs. Lipofectamine). Sub-figure (iv) shows the PD-L1 knockdown efficiency (quantified according to the mean fluorescence intensity). Adapted with permission from [75] (Copyright 2019, Elsevier) and [79] (Copyright 2021, American Chemistry Society), respectively.

ticles (MSNs) and mesoporous silica microrods (MSRs) are two of the most widely explored silica-based platforms in cancer immunotherapy research.

MSNs are silica-based nanostructures with a solid framework and a well-defined atomic-level arrangement of mesopores, that results in a large functional surface area (>1000 m^2/g). Originally reported in the early 1990s, it wasn't until the last decade that considerable investigation into the biomedical potential of MSNs was undertaken. They have distinctive features such as high chemical/biological stability, exceptional biodegradability, and readily programmable pore size (ranging from 2 to 50 nm) [81]. The four major methods to fabricate MSNs are the template-directed methodology, the sol-gel approach, the microwave-assisted technique, and chemical etching. Particle size and pore volume are the key factors that influence the usage of MSNs and they can be easily modulated/controlled by varying the silica source and operating parameters of the reaction mixture (like pH, temperature, and concentration of surfactant) [82]. MSNs have been utilized effectively as carriers for a variety of payloads, including small-molecule drugs or therapeutic macromolecules like proteins, DNA, and RNA. Depending on the type of cargo and the intended release profile, the pore size can be altered. In general, MSNs with large pore di-

ameters are favored for the transport of macromolecules, whereas MSNs with tiny pore sizes facilitate controlled drug release [83]. In addition, several pore morphologies and textures (hexagonal, cubic, concentric, radial) have been reported. Controlling the shape of pores affords an additional means of modulating release kinetics [84]. Since MSNs are predominantly made of a silicon dioxide matrix, they are susceptible to hydrolysis. An OH-mediated nucleophilic attack from water (as present in any physiological fluid) results in the formation of ortho-silicic acid, which is excreted via urine. In this scenario, surface modification is not required to enhance cytocompatibility; rather, functionalization tactics are intended to facilitate targeted or external stimuli-responsive payload delivery [85].

MSRs can be best defined as rod-shaped derivatives of silica having a high-aspect-ratio (with length in micron-scale) that possess a unique mesoporous structure that forms cylindrical end-to-end inner channels. The fabrication of MSRs involves a multistep process that involves the surfactant-assisted arrangement of a silica precursor into microrods having uniform nano-sized pores [86]. Briefly, the process initiates by the formation of a spherical self-assembled composite between a non-ionic surfactant (usually triblock copolymer) and a silica precursor (usually tetraethyl orthosilicate). As the silica condensation progresses, the change in molecular packing parameters initiates a configurational transition of spherical aggregates into cylindrical micelles. At this stage, the constituents exist in a liquid crystal phase. Subsequently, the polymerization process is initiated by adding a catalyst (such as hydrochloric acid) to the mixture. This causes the precursor to grow denser with time and finally separate from the water phase by forming the final mesostructured [87]. While MSNs are majorly used as carriers for the development of nano-vaccines (by delivering antigenic information to APCs), MSRs have a unique application in cancer immunotherapy. Upon administration, MSRs can transform into a porous three-dimensional scaffold formed by random stacking of rod-shaped particles. Such an *in situ* scaffold can promote the infiltration of immature DCs where they come in direct contact with the immunomodulators (loaded within mesopores), transforming them into mature antigen-presenting DCs that migrate to the lymphoid organs to provoke antigen-specific adaptive immune responses mediated by T cells. Additionally, this concept has also been explored for the *ex vivo* expansion of T cells by using cytokine-loaded MNRs [88].

The following section will discuss some recent applications of MSNs/MSRs-based strategies for cancer immunotherapy. Yang *et al.* reported red light-responsive, self-destructive MSNs (Se-MSN-PEG) by inserting reactive oxygen species (ROS)-cleavable diselenide-bonds into the silica framework. While stimuli-sensitive linkers are commonly used to create smart MSNs, most approaches place them on the particle surface, constraining responsiveness and drug release efficiency. To obtain high co-loading of methylene blue (photosensitizer) and DOX, the authors inserted ROS-cleavable linkers within the matrix of mesoporous silica. When irradiated with red light, methylene blue generated ROS that cleaved the diselenide-bridged silica backbones, resulting in the matrix degradation-based release of MB for further ROS generation. This cascade triggered DOX release, which synergized photodynamic treatment to boost ICD. In mice with 4T1 breast tumors, the platform elicited a robust anti-tumor immune response, while subsequent administration of PD-1 checkpoint blockers led to substantial abscopal effects and metastasis suppression. The platform also conferred long-term anti-tumor immunity, as shown by the increased production of TNF-α and IFN-γ in the sera of Se-MSN-PEG-treated mice (subjected to tumor rechallenge study) (Fig. 7a) [89]. In a different study, Chen *et al.* reported a strategically bio-engineered MSN-based immunotherapeutic nanocarrier for cyclic diguanylate monophosphate (cdG) delivery. The silica backbone was functionalized with Rhodamine B isothiocyanate (for imaging), PEG (for prolonged blood circulation), and N-trimethoxysilylpropyl-N, N, N-trimethylammonium chloride which contains a quaternary ammonium group (for inducing positive charge). The negatively charged cdG was loaded within the MSN complex via electrostatic interaction. RAW 264.7 cells treated with this system displayed enhanced secretion of IL-6, IL-1β, and IFN-β along with expression of phospho-STING (Ser365) protein (demonstrating that the inhibition of tumor growth was achieved by the STING-dependent signal pathway). The system also enhanced the infiltration of leukocytes, including $CD11c^+$ dendritic cells, $F4/80^+$ macrophages, $CD4^+$ T cells, and $CD8^+$ T cells within the TME, resulting in dramatic tumor growth inhibition in 4T1 breast tumor-bearing Balb/c mice [90].

Researchers at Mooney Lab were one of the first to report the immunotherapeutic potential of MSRs. By using high aspect ratio MSRs, they developed a vaccine platform that spontaneously assembles to form 3D scaffold in the body after subcutaneous injection. Sustained release of the encapsulated GM-CSF attracted immune cells (including immature DCs) to the macropores formed by a random distribution of MSRs at the injection site (Fig. 7b). With time, the number of recruited $CD11c^+$ DCs increased, and exposure to CpG-ODN and OVA stimulated them to transform into OVA-specific activated DCs. These activated DCs then migrated to lymph nodes where interaction with naïve T cells lead to the exertion of a systemic OVA-specific CTL and Th1/Th2 antibody response. The platform was able to prophylactically prevent EG7-OVA tumor growth in mice models, a testament to its ability to induce a strong cellular and humoral response [91]. Taking the platform further, the group modified MSRs with cationic polyethyleneimine that allowed delivery of immune modulatory glycoproteins (like E7 peptide). This platform was able to eliminated established E7-expressing TC-1 carcinoma in 80% of mice after a single vaccination, followed by the persistence of immunological memory for over 6 months. The platform also displayed a synergistic therapeutic effect when applied with anti-CTLA4 therapy [92]. Besides cancer vaccines, MSRs can also aid in ACT by serving as a tool for the rapid generation of generate therapeutically functional T cells. APC-mimetic MSRs were prepared by loading IL-2 in mesopores and coating T cell cue-bearing liposomes on the MSR surface. In T cell culture, the platform outperformed commercial microbeads for ex vivo T cell expansion by promoting substantially higher expansion rates of murine and human T cells in both polyclonal and antigen-specific ways [93]. These findings demonstrate the extensive variety of silica as a biomaterial for application in cancer immunotherapy.

4.4. Metal-based nanoparticles

Metallic nanoparticles (MNPs) are popular for use in nanomedicine due to their size, shape, and surface charge control capabilities. The chemistry involved in making MNPs is straightforward and allows numerous options for modifications with drugs/ligands. These process parameter-related attributes facilitate their fine-tuning to maximize the therapeutic outcomes of immunotherapy [94]. Their high surface-to-volume ratio can be exploited for modification with genetic materials, adjuvants, antigens, and tumor-targeting ligands. The use of MNPs is associated with ICD and the release of tumor antigens by the metal's inherent bioactivity. Moreover, MNPs undergo rapid uptake by APCs due to their high density. So, a better anti-tumor cytotoxic T cell response can be expected when immune adjuvants are delivered using them [95]. Additionally, some MNPs possess outstanding thermal and magnetic properties, which can be exploited to develop nanoparticle-mediated platforms for photothermal therapy, photodynamic therapy, and magnetic hyperthermia therapy. These

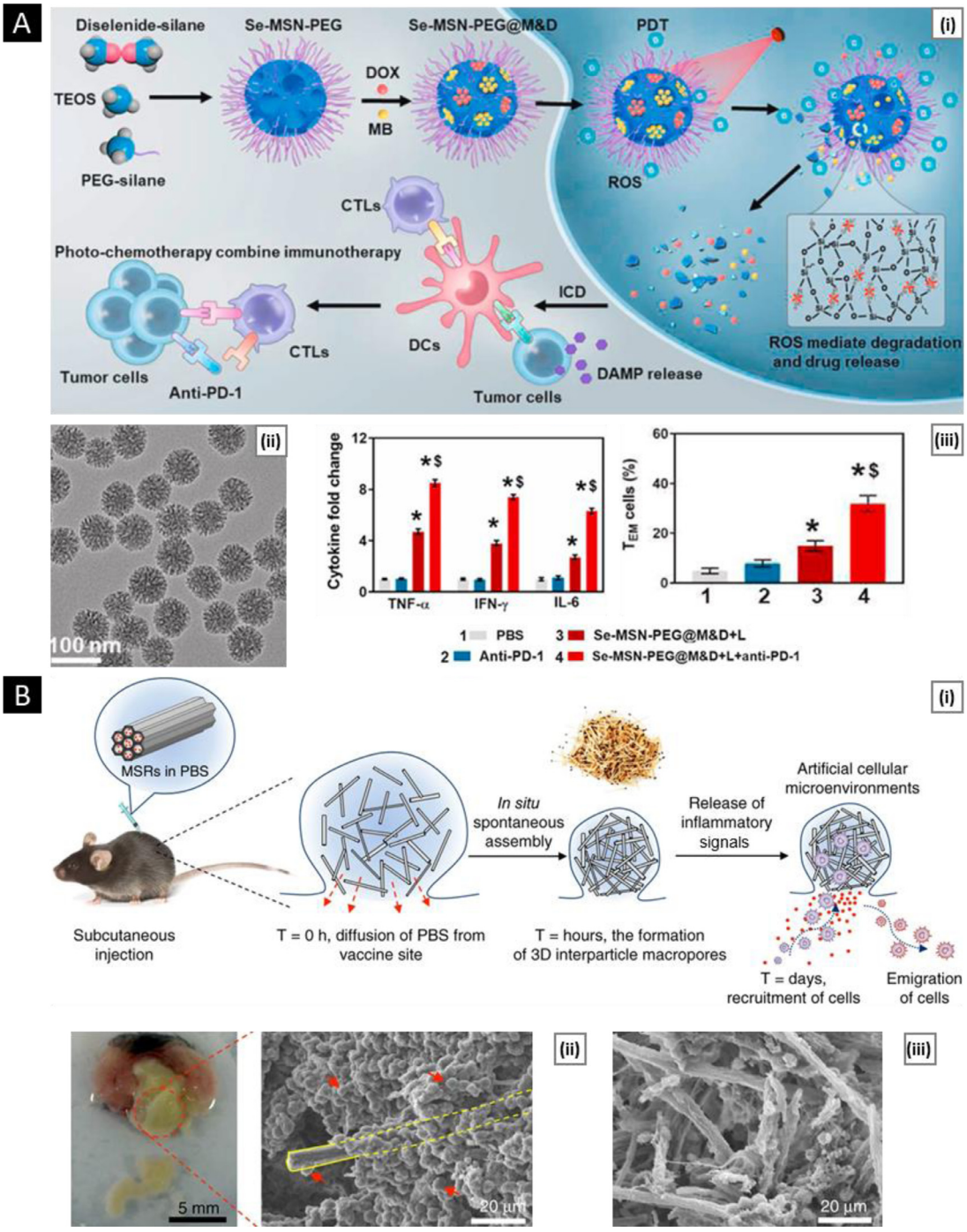

Fig. 7. Silica-based Bio-engineered Platforms for Cancer Immunotherapy. (A) MSNs as a platform for efficient and safe cancer chemo-photo-immunotherapy. Here, sub-figure (i) depicts the synthesis of Se-MSN-PEG with red light-triggered cascading drug release and amplification of ICD. Sub-figure (ii) shows the representative TEM-images of drug co-loaded Se-MSN-PEG. Sub-figure (iii) depicts the serum pro-inflammatory cytokines levels in each group and the percent of effector memory T cells (post-tumor rechallenge). (B) MSR scaffold for in situ immune cell manipulation. Here, sub-figure (i) depicts the schematic cascades for injectable MSR cancer vaccine. Sub-figure (ii) shows the surgically isolated MSR scaffold (post-injection) and a representative SEM photomicrograph indicating the high number of recruited immune cells (yellow outline represents a visible MSR, red arrows indicate cells). Sub-figure (iii) shows an SEM image of the MSR scaffold after the removal of most recruited cells. Adapted with permission from [89] (Copyright 2022, Elsevier) and [91] (Copyright 2014, Springer Nature), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

therapies can be utilized to modulate various internal TME factors by generating ROS, elevating hypoxia, and depleting glutathione. Particles prepared from metals like Gold, Copper, and Molybdenum exhibit localized surface plasmon resonance through which they produce heat upon irradiation with a NIR laser. Such thermal ablation in tumors can lead to protein denaturation, cell membrane lysis, apoptosis, and eventually cancer cell death. Multiple studies have concluded that immunotherapy can benefit from the immunogenically "hot" microenvironment created by MNP-mediated thermal ablation of tumors [96]. The rationale for using MNPs (apart from drug delivery vectors) in cancer immunotherapy and some drawbacks are briefly highlighted in Table 3.

Besides the above-discussed MNPs, other metal derivatives like zinc-based NPs (having an inherent ability to directly acti-

Table 3
MNPs in cancer immunotherapy.

Type of MNP	Rationale for use in cancer immunotherapy	Drawbacks	Refs.
Iron	• Various iron oxides like magnetite, hematite, and maghemite have been explored as magnetic nanocarriers for targeting lymphatic vessels. • They preferentially accumulate in TAMs where they can modulate their reprogramming and biological functions.	• As iron oxide core bears high surface energy and chemical reactivity, it needs to be encapsulated by polymers, lipids, or proteins to prevent rapid opsonization. • Excessive intra-tumoral accumulation can cause undesirable oxidative stress and DNA damage. • Cellular and molecular toxicity in vital organs like the heart and liver is reported.	[97]
Gold	• Gold nanoparticles (AuNPs) can be prepared in various shapes and sizes by different chemical, physical, or eco-friendly biological methods. • AuNPs with a size of 5-50 nm show dose-dependent up-regulation in IL-1β, IL-6, and TNF-α. • Their localization at tumor sites enhances the infiltration of immune cells and the tumor immunotherapy efficacy.	• Long-term toxicity of AuNPs is reported as they undergo negligible degradation in the body. • The pharmacokinetic and histocompatibility parameters of AuNPs highly vary based on their size/shape profile and surface modification, leading to variation in therapeutic outcomes	[98]
Silver	• Silver nanoparticles (AgNPs) possess admirable intrinsic anticancer and antibacterial properties. • Ag^{+} cations can modulate tumor-promoting cytokines, aid in controlling oxidative stress, and decrease cancer cell proliferation by suppressing ATP levels.	• Long-term low-level systemic exposure to AgNPs can provoke oxidative stress and hinder critical mitochondrial functions. • AgNPs have poor stability and generate hard aggregates when stored for a prolonged period.	[99]
Aluminum	• Multiple Al-based immune adjuvants have received FDA approval. • Al-based NPs selectively enhance antibody-mediated immune responses and induce differentiation of $CD4^{+}$ T cells into helper T cells.	• While the use of various Al-based adjuvants (like alum, aluminum phosphate, and aluminum potassium sulfate) has high safety profile, Al-based NPs are prone to undergo aggregation in the physiological environment. • Besides, they are susceptible to cause neurotoxicity by accumulating in brain	[100]
Manganese	• Manganese oxide NPs (having a hollow or solid core) possess superior drug loading and magnetic properties. • Mn^{2+} ions can directly activate innate/adaptive immunity when selectively delivered to the immune cell population. • Recently, manganese-based carriers are being explored for activation of cGAS and STING for the enhancement of cGAMP production, thus promoting the production of $CD8^{+}$ T cells.	• Without proper surface modification, manganese oxide NPs undergo self-aggregation and leach out Mn ions. These aggregates, when accumulated in tissues, can potentially lead to toxic effects. • Extensive investigations are still needed to under the molecular mechanism behind the synergistic immune effects of manganese-based nanomaterials	[101]

vate innate/adaptive immunity and induction of multiple inflammatory cytokines) and calcium-based NPs (involved in the proliferation of thymocytes, differentiation/maturation of immature CD4/CD8 cells, and cGAS-STING pathway) have been explored as candidates for immunoadjuvant delivery in cancer immunotherapy [102,103].

The following section will discuss some recent applications of MNPs-based strategies for cancer immunotherapy. Sungsuwan *et al.* utilized surface-functionalized magnetic iron oxide NPs as carriers for developing glycoconjugate-based anticancer vaccines. Unlike normal cells, cancer cells display distinct carbohydrate structures on their surfaces celled tumor-associated carbohydrate antigens (TACAs). These carbohydrate antigens can be exploited as discriminatory antigens for cancer immunotherapy, but their weak immunogenicity creates challenges in eliciting strong anti-TACA immune responses. To overcome this limitation, the authors coated magnetic iron oxide NPs with phospholipid-functionalized TACA glycopeptides via simple hydrophobic–hydrophobic interactions, which eliminates any need for covalent linkages/interaction. The large surface area of iron oxide NPs allowed several copies of glycopeptides to be attached, leading to multivalent binding and enhanced interactions with antibody-secreting B cells. Mice immunized with the NPs generated strong antibody responses resulting in tumor cell death through complement-mediated cytotoxicity [104]. In a different study, Cai *et al.* used mucin-1 (MUC1) glycoprotein as an immunogen. A three-component system was obtained by immobilizing a chimeric peptide (composed of a MUC1-derived glycopeptide sequence and T cell epitope P30 sequence) on PEGylated AuNPs. Analysis of the antisera obtained after treatment with the nanocarrier showed significant Th1 and Th2-mediated immune responses directed to the glycopeptide antigen [105]. Besides single metal, hybrid MNPS having a bimetallic or trimetallic alloy composition are also reported. Through such an approach, different metals can be combined to generate a synergistic therapeutic effect while simultaneously addressing any drawbacks of metals on an individual level [106].

4.5. Cell-derived systems

Cell-derived systems, alias cellular biomaterials, are materials that are composed of or made with the use of cells. As an upcoming class of biomaterials, various cell-derived systems have been identified, designed, and modified to produce creative tools that can serve as self-adjuvating vaccines or as vectors for the delivery of therapeutic/immunomodulatory cargo. Given that cells are involved in a vast array of biological processes, they are an inherently rich source of natural targeting ligands, functional modulators, and antigenic materials [107]. Based on these features, cells (and their components) can be employed to invoke immune activation and provide a long-term immune memory effect for inhibiting cancer recurrence. From an application viewpoint, cell-derived systems can be broadly sub-categorized into bioengineered whole tumor cells, extracellular vesicles (EVs), and isolated cell membranes.

Whole tumor cells have been extensively studied for their utilization as autologous cancer vaccines. They are prepared by the *ex vivo* modification of surgically isolated tumor cells (usually via X-ray irradiation) aimed to render them non-proliferative and non-tumorigenic. Post-modification, the immunogenically and metabolically active cells are delivered back to the patient to generate potent tumor-specific cellular and humoral immune responses. The presence of a broad repertoire of tumor antigens decreases the probability of “immune escape” through antigen loss, whereas the autologous nature allows the development of highly patient-specific vaccines without the need for mutational profiling. Additionally, they are target-independent and facilitate the recruitment of a broad response with the involvement of multiple immune cell populations [108]. While cells form tumor cell lines or from an appropriate donor (allogenic) can also be utilized, autol-

ogous cells are clinically preferred as they impart robust immunity on account of carrying the entire antigenic profile of the patient's tumor. Their immunogenicity can be enhanced by altering them via retroviral or adenoviral transduction to express/secrete immunomodulatory molecules (like GM-CSF) [109]. Whole tumor cells can be converted into cell lysate by subjecting them to repeated freeze-thawing. By doing so, the cells retain their immunogenicity but are now available in a more feasible form that can be readily co-incorporated (with other immune modulators) into a delivery system to yield complex cancer vaccines. Ye *et al.* reported a transdermal microneedle patch composed of hyaluronic acid loaded with tumor lysate from B16F10 melanoma, melanin, and GM-CSF. The platform provides a steady intracutaneous release of antigenic cell lysate and stimulated the immune system via the comprehensive network of lymphatic vessels in the dermis. The photosensitizer (melanin), upon irradiation with a near-infrared laser, generated localized heat that induced the release of inflammatory cytokines to appeal to the DCs and other types of immune cells, facilitating the production of the immune substrates. The heat also enhanced blood and lymphatic flow, thereby inducing B16F10-specific immune activation by promoting the migration of APCs and T cells [110]. It is reported that lysate generated from cells subjected to ICD is enriched with various biomolecules of the "damage-associated molecular pattern" category (like calreticulin, and heat shock proteins), which further synergizes their immune activity [111].

EVs are heterogenous lipid-bilayer bound vesicles that are discharged into the extracellular environment by cells (including diseased cells like cancer) and function as natural carriers of intercellular information/materials. They can be classified into three main sub-classes based on their biogenesis and size distribution: microvesicles (MVs; 100-1000 nm in diameter), exosomes (30-150 nm in diameter), and apoptotic bodies (typically 1-5 microns) [112]. MVs are formed by shedding or outward budding of the plasma membrane while exosomes are formed by the inward budding of intracellular endosomes (released by exocytosis). Apoptotic bodies are released by dying cells during the later stages of apoptosis. MVs are rich in cytosolic proteins and lipids, while exosomes contain a unique set of membrane and cytosolic proteins, RNA, and microRNA. Apoptotic bodies are mostly composed of cell debris, hence their biomedical use is limited. [113]. The unique composition and structure of EVs endow them with the ability to play a critical role in cancer, by modulating the immune system and promoting tumor growth and progression. Cancer cell-derived EVs primarily contribute to tumor development by: [i] increasing immune evasion (by promoting the activation/proliferation of Tregs that subsequently inhibit the activation of effector T cells), [ii] aiding metastasis (EVs contain molecules like matrix metalloproteinases and laminin-binding integrins, that facilitate the spread of cancer to distant sites; the presence of pro-angiogenic factors, such as VEGF, promote angiogenesis to support the growth and progression of new tumors), [iii] imparting drug resistance (via drug resistance-associated molecules, such as ABC transporters, which can pump out chemotherapy drugs), and [iv] maintaining an immunosuppressive TME (via molecules like TGF-β, which can inhibit the DCs activation and production of pro-inflammatory cytokines) [114].

Due to their high biocompatibility and permeability across biological barriers, EVs have been investigated extensively as potential carriers for enhancing the effectiveness and specificity of cancer immunotherapy. While they can be sourced from various somatic fluids, EVs originating from cancer/CSCs or immune cells provide better clinical outcome on account of their physicochemical attributes (like unique molecular cargo, and lipid-membrane modifications) that grants them homologous tumor targeting properties. Techniques like density gradient ultracentrifugation and solubility precipitation (as in commercial reagents like ExoQuick®) are employed to isolated purified EVs from source cells. Subsequently, they can be loaded with drugs/immunomodulatory molecules using loading strategies like electroporation, sonication, or freeze-thawing. Additionally, the EVs membrane can be chemically modified to externally anchor drugs or to achieve controlled delivery by integrating a stimulus-response module [115]. The following section will discuss recent examples of engineered EVs-based cancer immunotherapy.

In a first-of-its-kind study, Chen *et al.* attempted to develop EVs as direct agents for immune checkpoint therapy. By using genetic engineering techniques (CRISPR/Cas9) to modify the MDA-MB-231 cell line, the authors harvested EVs that overexpressed a high-affinity variant human PD-1 protein (havPD-1 EVs), while simultaneously knocking out intrinsic PD-L1 and beta-2 microglobulin (to reduce the side effects of tumor-derived EVs on the immune system). The havPD-1 EVs reduced the proliferation of PD-L1 overexpressing cancer cells, induced cellular apoptosis, and efficiently block PD-L1 mediated T cell suppression. Additionally, any antibody/complement-dependent cytotoxicity was absent. Treatment with havPD-1 EVs having a breast tumor-homing effect resulted in robust anti-tumor activity in both preventative co-implantation and therapeutic xenograft tumor models reconstituted with human T cells. Loading the havPD-1 EVs with Senaparib, a poly(ADP-ribose) polymerase inhibitor, showed an increase in efficacy that was superior to the clinically standard anti-PD1 monoclonal antibodies [116]. Huang *et al.* reported an *in situ* DC-primed vaccine prepared by loading human neutrophil elastase (ICD inducer) and hiltonol (TLR3 agonist) into α-lactalbumin-engineered breast cancer-derived exosomes (HELA-Exos). The platform possessed a profound ability to specifically induce ICD in breast cancer cells on account of enhanced targeting provided by α-lactalbumin (a breast-specific immunodominant protein). Adequate exposure to tumor antigens and Hiltonol following HELA-Exo-induced ICD of cancer cells activated type one conventional DCs (cDC1s) in situ and cross-primed tumor-reactive $CD8^+$ T cell responses, leading to potent tumor inhibition in a poorly immunogenic triple-negative breast cancer mouse xenograft model and patient-derived tumor organoids (Fig. 8a) [117].

An intriguing and unique sub-category of EVs that holds tremendous promise in the field of cancer immunotherapy is bacterial outer membrane vesicles (OMVs). They are spherical, non-replicative, bilayer structures (20-250 nm in diameter) released primarily by Gram-negative bacteria. They are generated by the process of outer membrane blebbing, which is a form of endocytosis that results in the formation of vesicles containing both outer membrane and periplasmic content. OMVs contain two essential components for use as vaccines: bacteria-derived antigens and various pathogen-associated molecular patterns (PAMPs, like lipopolysaccharide, lipoprotein, peptidoglycan, etc.) [118]. The particulate nature and substantial presence of PAMPs as innate composition grant OMVs intrinsic immunostimulatory properties. Furthermore, their capacity to accumulate in lymph nodes (due to their small size) and their manufacturing scalability (via bacterial fermentation), make OMVs attractive candidates as vaccine vectors that can stimulate humoral immunity [119]. The research group led by Dr. Guangjun Nie, through their innovative studies and cutting-edge approaches, has made significant contributions to exploring bacterial OMVs in cancer immunotherapy.

In their initial work, the group combined genetic engineering with "Plug-and-Display" technology to yield bioengineered bacterial OMVs that can serve as a versatile antigen display platform for tumor vaccination. By utilizing the C-terminal of Cytolysin A (a surface scaffold protein) as an anchor site, the authors successfully displayed exogenous tumor antigens on OMVs' surface. Delivery of tumor antigen with self-adjuvating OMVs can impart a

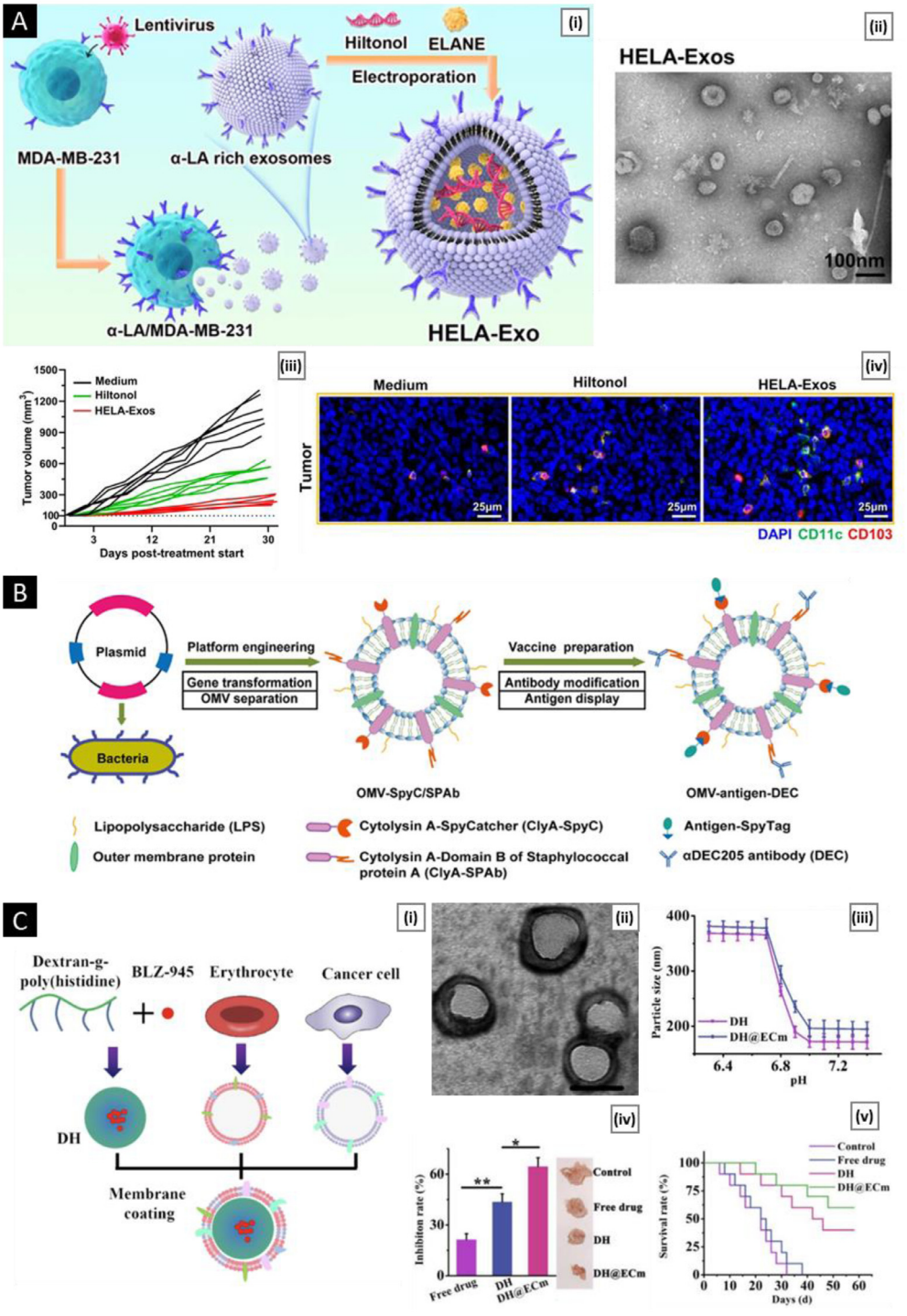

Fig. 8. Cell-derived Bio-engineered Systems for Cancer Immunotherapy. **(A)** HELA-Exo as an *in-situ* DC-primed vaccine for breast cancer. Here, sub-figure (i) depicts the schematic illustration of preparing drug-loaded HELA-Exo from genetically engineered MDA-MB-231 cells. Sub-figure (ii) shows its representative TEM image. Scale bar, 100 nm. Sub-figure (iii) depicts the change in tumor volume in response to HELA-Exo treatment (vs. control). Sub-figure (iv) shows the HELA-Exo-induced intratumoral accumulation of cDC1s/CD8^{+} T cells in Balb/c mice with orthotopic breast cancer. **(B)** Schematic of OMV platform engineering and vaccine preparation. **(C)** Erythrocyte-cancer cell hybrid membrane camouflaged pH-responsive copolymer micelle to target TAMs for cancer immunotherapy. Here, sub-figure (i) shows a schematical overview of the preparation of DH@ECm. Sub-figure (ii) TEM images of DH@ECm, Scale bar, 200 nm. Sub-figure (iii) shows the particle size variation of DH@ECm (vs. uncoated control) in different pH. Sub-figure (iv) represents the tumor inhibition rate with corresponding tumor images (of different formulations). Sub-figure (v) represents the survival rate of different formulation groups. Adapted with permission from [117] (Copyright 2022, Springer Nature), [121] (Copyright 2022, Elsevier), and [131] (Copyright 2020, Elsevier), respectively.

robust antigen-specific anti-tumor immune response on account of rapid antigen display combined with efficient antigen processing and presentation to DCs. The use of "Plug-and-Display" technology allows the vector and antigen to be synthesized separately, requiring only a simple combination procedure before immunization. The authors hypothesized that such modular design allows the establishment of a neoantigen library in advance, from which appropriate antigen combinations can be selected based on use scenario, which may reduce the production time and realize the bedside preparation of tumor vaccines for individual patients in the future [120]. Taking this platform further, OMVs decorated with DC-targeting αDEC205 antibody (OMV-DEC) were reported. It is commonly observed that strong immune adjuvants in any nanoparticle-mediated vaccination platform cause rapid maturation of DCs, thereby limiting their uptake capacity and subsequent generation of an antigen-specific immune response, a phenomenon called "maturation-induced uptake obstruction" (MUO). For OMVs, the MUO phenomenon is very critical due to the presence of TLR4 on DCs surface that gets activated by lipopolysaccharides (during DC recognition and uptake). To overcome this, the Fc fragment of antibody for αDEC205 (a type I C-type lectin receptor that is highly expressed on the DCs surface) was conjugated with OMVs isolated from genetically engineered bacteria that expressed Cytolysin A fused with domain B of Staphylococcal protein A (Fig. 8b). αDEC205 antibody endowed OMVs with an ectopic uptake pathway, independent of the maturation state. This resulted in greater uptake of OMVs by DCs, leading to increased antigen presentation and subsequent CTL and memory T cell activation, ultimately efficiently impeding metastasis in a pulmonary melanoma model [121]. In a recent study, the group reported an OMV-based controllable two-way adaptor platform, in which a CD47 nanobody is fused onto OMV surface, with the outer surface PEG layer containing di-selenide bonds to form PEG/Se@OMV-CD47nb. The surface PEG/Se layer endows the nanoparticles with radiation-triggered controlled release of OMV-CD47nb, mitigating the side effects observed from the intravenous injection of naked OMVs and enabling the precise release of OMV-CD47nb at the tumor site, thereby increasing the safety window of intravenous injection of OMV-based formulations. As a two-way adaptor, OMV-CD47 simultaneously binds to both TAMs and tumor cells, inducing TAM sensitization and CD47 blockade that synergistically promote robust phagocytosis of tumor cells by TAMs [122].

Cell membranes represent the last major sub-class of cell-derived systems. They are primarily employed to grant any accompanying nanocarrier system with "biomimetic" properties. The cell membrane (composed of phospholipids and glycolipids) is a selectively permeable barrier that surrounds all living cells, separating the internal cellular components from the external environment. It plays a crucial role in maintaining the cell's structural integrity and also controls the to-and-fro movement of important molecules/ions. For use in cancer immunotherapy, an ideal delivery system must protect the loaded therapeutic cargo from systemic degradation/immune uptake while simultaneously serving as a tool for specified *in vivo* interaction (that indirectly aid in targeted cargo release) [123]. In this context, coating nanocarriers with isolated cell membranes works as a simple top-down approach wherein the above-mentioned attributes can be incorporated into any nanocarrier (regardless of its inherent properties) without any extensive/complex surface modification. A widely used membrane isolation approach involves incubating the source cells with a hypotonic lysis buffer followed by their controlled disruption (with a Dounce homogenizer). The resulting cell lysate is subjected to differential ultracentrifugation to yield a purified cell membrane-rich pellet. Any nanocarrier can be coated by appropriate co-extrusion with the membrane fragments [124]. Based on the type of source cell, the isolated membrane will possess functional surface receptors and cell-specific markers/ligands that can modulate the *in vivo* fate of nanocarriers. For example, cancer cell-derived membranes display unique self-targeting towards homologous tumor cells (accredited to the presence of Thomsen-Friedenreich antigens, N-cadherin, and galectin-3 on the cell surface), red blood cells and platelet-derived membranes can be used to make non-immunogenic stealth carriers as CD47 surface marker help in escaping clearance from the immune system, and membranes derived from inflammatory/immune cells have chemotactic properties through which they selectively migrate to the immunogenically active TME [125]. Some recent examples of utilizing cell membranes as a biomaterial as discussed below.

Wu *et al.* reported an *in vitro* NK cell activation strategy by encapsulating Fe_3O_4@SiO_2 magnetic nanoparticles in tumor cell-derived membranes with embedded tumor-specific antigens. Serving as an *in vitro* antigen-presenting platform, the system having the presence of cancer-specific antigens on the surface effectively stimulated NK cells by enhancing the expression of surface activating receptors and boosting anti-tumor function through the secretion of soluble cytotoxic effectors [126]. T cell membranes contain several distinct markers involved in the induction of immune response, including T cell antigen receptors, human leukocyte antigen antigens, and IL receptors. To utilize these characteristics, Li *et al.* developed a TME-responsive (redox-sensitive) platform composed of a phenylboronic acid-modified T cell membrane that encapsulates a hyaluronic acid/vitamin E succinate/curcumin conjugate (RCM@T). Along with functioning as an outer protective shell, the T cell membrane also acts as a PD-1 "antibody" to selectively bind the PD-L1 of tumor cells. Upon intravenous administration into the bloodstream, RCM@T accumulates at the tumor site (via EPR-mediated passive targeting) and in response to the acidic pH the platform exerts a "membrane escape effect" which exposes the drug-loaded core. In B16 tumor-bearing mice, the authors observed that the presence of T cell membrane fragments led to a substantial increase in the level of $CD8^+$ T cells and serum cytokines (vs. cell membrane-free negative control) [127].

While nanocarrier coating with a single membrane can facilitate site-targeting and systemic immune evasion, the use of a hybrid membrane coating technique can provide a more versatile design scheme for delivering immunomodulatory agents. It involves the use of a composite multifunctional membrane, prepared by the fusion of two or more individual membranes that are derived from unique source cells. Characteristic attributes like prolonged systemic circulation time with minimal immune cell interaction, as seen in membranes sourced from erythrocytes, platelet, or leukocytes, can be combined with the homologous targeting ability of cancer cell-derived membranes [128]. Additionally, membranes from DCs or bacterial vesicles can be used to exploit their adjuvant-like properties. Before coating the nanocarriers, individual membranes can be fused by subjecting them to repeated freeze-thawing, ultrasonic treatment, or by employing microfluidic-based techniques [129]. Over the past few years, hybrid membrane technology has found some interesting usage in cancer immunotherapy. With an aim for targeted blockade of the metabolic support of CAFs to cancer cells, Zang *et al.* developed a biomimetic nanocarrier comprising solid lipid nanoparticles (loaded with paclitaxel and glycolysis inhibitor PFK15) coated with a hybrid membrane of cancer cells and activated fibroblasts. The system can dual-target tumor cells and CAFs. Owing to glycolysis inhibition, cancer cells lost the critical energy supply needed for survival and proliferation from CAFs. The authors also observed an increase in chemosensitivity in cancer cells resulting in optimal antitumor effects. Additionally, a reduction in lactate production ameliorated the immunosuppressive nature of TME [130]. Wang *et al.* designed a hybrid membrane cloaked pH-sensitive micelle (DH@ECm) based on RBCs and cancer cell membranes for targeted depletion of TAMs.

The micellar core consisted of copolymer dextran-grafted-poly histidine loaded with BLZ 945 (a CSF-1 receptor inhibitor). In response to the acidic TME, the system exerted a "membrane escape effect" to enable recognition and internalization by TAMs (via dextran-CD206 receptor), followed by *in vitro* TAMs depletion. In 4T1 tumor-bearing mice, the system efficiently reversed the tumor immune-microenvironment marked by elevation in $CD8^+$ T cells levels and a 64.5% tumor inhibition rate (Fig. 8c) [131]. In a different study, Hou *et al.* fused macrophage and thylakoid membranes to coat hollow mesoporous Prussian blue nanoparticles with mannose decoration and hydroxychloroquine adsorption. The outer hybrid membrane served multiple purposes. The macrophage-derived component granted tumor-specific localization and lowered the *in vivo* reticuloendothelial system uptake. The thylakoid-derived component helps in alleviating hypoxia (via O_2 generation with membrane explosion followed by mannose ligand exposure in TME). As the Prussian blue core degrades, it releases iron ions and hydroxychloroquine that cumulatively induce M2-type macrophages to differentiate into M1-type [132].

5. Biomaterial-mediated immune modulation

5.1. Immunogenic modulation of DCs

DCs, the most powerful APCs, are critically involved in regulating humoral and cellular immune responses. Considering their potential, great efforts have been made to utilize biomaterial-based platforms to engineer DCs for use in cancer immunotherapy [133]. The majority of this research focuses on the development of implantable or injectable systems to positively modulate DCs (by enhancing their functional abilities, promoting endogenous DC recruitment, or circumventing the TME to facilitate systemic antitumor immunity) (Fig. 9a) [134]. By using biomaterials, pill-sized scaffolds (loaded with multiple immune adjuvants, biological factors, or cellular components) can be designed for implantation at the tumor site (via a minor surgical procedure). Based on their matrix porosity, such scaffolds enable control over the release profile of entrapped bioactive agents, which can be tailored to recruit immune cells within the immediate vicinity [135]. PLG, due to its favorable attributes (like biocompatibility, tailorable rate of biodegradation, and ease of chemical modification) has been extensively employed as an implantable scaffold [136].

Ali *et al.* explored PLG to fabricate brain implants to counter intracranial glioma. The macroporous structure of the polymeric implant produced a sequential release of the adjuvant loaded. Based on their molecular size, GM-CSF releases first, which helps recruit DCs. Eventually, these DCs undergo immune activation when the scaffold subsequently releases CpG and immunogenic tumor lysate. Direct implantation in brain tissue resulted in 90% long-term survival ($>$ 100 days) of rats bearing intracranial glioma tumors (Fig. 9b) [137]. To address some drawbacks of polymeric implants (like an obligation for surgical procedure and use limited to peritumoral insertions), injectable immunotherapeutic systems based on hydrogel/cryogels have come into the picture. These "*in situ*" self-assembling systems are far less invasive than implants, hence eliminating any unwanted tissue damage associated with surgical insertion. Their viscoelastic nature allows for better occupation within/around biological tissues, which directly contributes to better interaction with tumors [138]. Injectable Cryogel/Hydrogel platforms based on alginate have been extensively studied by the Mooney lab and others [139,140].

Apart from the implant/injectable systems, a feasible yet effective approach for direct targeting of DCs involves the use of NPs with ligands that can selectively interact with the diverse DC-specific surface-expressed receptors (like CD40, CD11c, mannose receptors, Fc receptors, TNF-α family receptor, and the C-type lectin receptor family) [141]. In a first-of-its-kind comparative study, Cruz *et al.* designed ovalbumin and TLR-3/7 loaded PEGylated polymeric NPs decorated with different antibodies specific to DCs surface receptors to establish optimal targets to achieve DC-specific delivery (Fig. 10a). All surface targets were evaluated based on their efficiency in activating DC and elicit a potent $CD8^+$ T cell response. Compared to non-targeted NPs, all antibody-decorated NPs showed efficient targeting and internalization by DC. Amongst all receptors, CD40-targeting led to a small but significantly better binding and uptake capacity with the highest production of IL-12 under experimental *in vitro* conditions. The study also emphasized that the type and potency of loaded immune adjuvants are as important as the selection of targeting ligands in achieving robust DC activation and subsequent T cell response [142]. In a recent study, Watanabe *et al.* reported the development of a new synthetic adjuvant containing a liposome conjugated with a DC-targeting Toll-like-receptor ligand and a pH-sensitive polymer for enhancing the cross-presentation of co-delivered tumor antigen. Surface modification with TLR2 ligand and CD11c-binding proteins contributes to enhanced incorporation within DCs. The pH-sensitive polymer releases the loaded tumor antigen upon exposure to low pH (during DCs uptake). Immunization of tumor-bearing mice with the system significantly enhanced antigen-specific cytotoxicity with complete tumor remission. Additionally, vaccination significantly enhanced cytotoxicity, targeting not only the vaccinated antigen but also the other antigens of the tumor cell [143].

5.2. Targeting Main Immune Components within Immunosuppressive TME

As discussed previously, TAMs, MDSCs, and Treg cells are the three pivotal immune cells that produce an immunosuppressed TME. For targeting the TME, the design of any biomaterial-based platform should take into consideration the spatial and temporal pattern of immune adjuvant delivery so that their therapeutic effect can be maximized. For successful targeting of TAMs, MDSCs, or Tregs, an important step for any delivery platform is evading opsonization and removal by the reticuloendothelial system (RES). This can be achieved by precisely controlling the size distribution profile or by PEG modification. Next, intracellular delivery of immune adjuvants in a bioactive state is critical. This can be achieved by using lipid or polymers-based carriers that respond to low pH and undergo endosomal escape. Lastly, control over the temporal patterns can be achieved by finetuning system parameters like adjuvant loading technique, degradability of components, and overall system physiochemical properties. The following section provides a brief overview of targeting avenues pertaining to TME constituents.

5.2.1. Reprogramming tumor-associated macrophages

As a biological response to cancer-associated cytokines, monocytes in the TME differentiate into M2 macrophages. Unlike their M1 counterparts, which play an antitumor role (by releasing nitric oxide, ROS, and TNF and by secreting pro-inflammatory cytokines, like IL-1 and IL-2), M2-polarized macrophages produce high levels of pro-tumorigenic chemical intermediates like IL-6, IL-4, IL-10, VEGF, and TGF-β that aid in tumor angiogenesis and suppression of adaptive immune responses, resulting in tumor growth and survival [144]. Hence, therapeutic interventions to repolarize M2 macrophages provide a lucrative opportunity to modulate TME directly. This has mostly been achieved by targeting TAM-associated chemical cues.

Gunassekaran *et al.* used M1 macrophage-derived bioengineered exosomes for suppressing tumor growth by reprogramming TAMs into M1-like macrophages exploiting the overexpressed IL-4 receptors as the delivery target. The exosomes

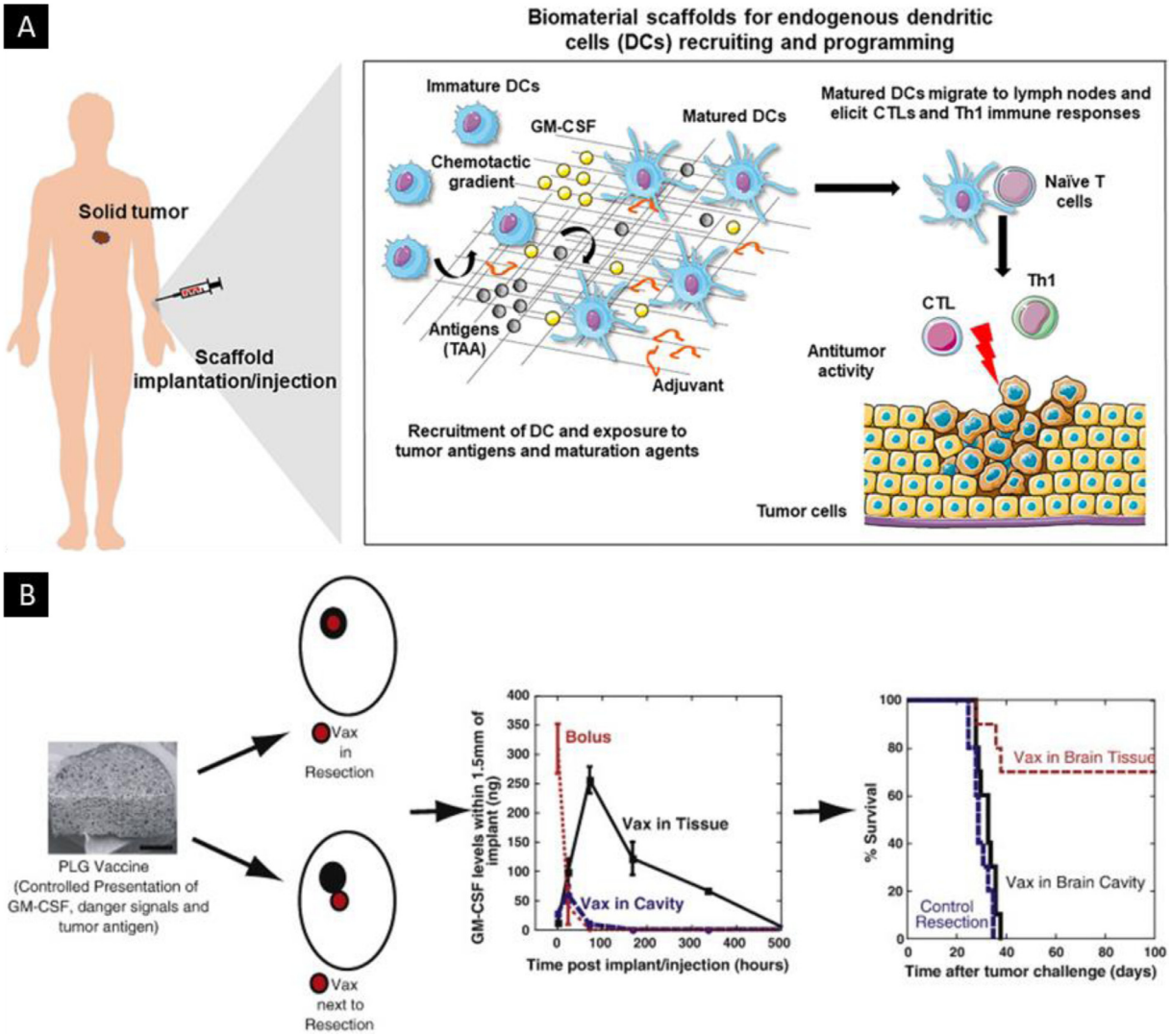

Fig. 9. **(A)** Schematic describing the process of DCs maturation after the subcutaneous implantation or injection of biomaterial-based scaffold loaded with a chemotactic agent, an adjuvant, and a source of tumor antigens. **(B)** PLG-derived brain tissue implant for intracranial glioma tumors. Direct implantation of PLG vaccines within brain tissue produced significant GM-CSF gradients for prolonged periods, which was not detected after implantation in resection cavities. The vaccination efficacy correlates with GM-CSF gradient formation, as evident from the survival outcome of rats bearing intracranial glioma tumors. Adapted with permission from [134] (Copyright 2019, Springer Nature) and [137] (Copyright 2011, Elsevier), respectively.

(transfected with NF-κB p50 siRNA and miR-511–3p) were surface-modified with IL4R-binding peptides. Systemic administration of the engineered exosomes showed effective TAM targeting with reduced tumor growth, downregulated target genes, and decreased levels of M2 cytokines [145]. Huang *et al.* employed galactosylated cationic dextran polymer (that can selectively bind with macrophage galactose-type lectin receptors expressed by TAMs) to fabricate a stable nano-complex composed of pH-sensitive PEG-histidine-modified alginate for co-delivery of CpG ODN, anti-IL-10 ODN, and anti-IL-10RA ODN (Fig. 10b). After tail vein administration, the complex showed localized accumulation in F4/80+ macrophages. TAMs phenotype reversal was characterized by suppressed expression of IL-10 and IL-10 receptors. Alternatively, the expression of pro-inflammatory cytokines and M2-like genes was significantly increased [146].

5.2.2. Modulating myeloid-derived suppressor cells

MDSCs can be described as a heterogenous immature myeloid cell (IMC) population located within the TME that undergoes transcriptional activation and differentiation in response to chronic exposure to tumor-associated pro-inflammatory cytokines. In humans, the MDSCs can be distinguished based on various myeloid cell markers like $CD11b^{+}$, $CD33^{+}$, $HLA\text{-}DR^{low/-}$, and negative for lineage-specific antigens [147]. MDSCs can be modulated by several approaches, which include blocking their development into mature cells, differentiating MDSCs into a non-suppressive immune state, or depleting/inhibiting the immunosuppressive functions [148]. Biomaterials can be utilized to facilitate the targeted/controlled release of immune modulators to achieve therapeutic goals using the above-mentioned approaches.

Sasso *et al.* reported monocytic MDSCs (a subset of MDSCs) targeted PEGylated lipid nano-capsules loaded with lauroyl-modified gemcitabine (termed GemC12-LNCs) for cancer immunotherapy. In mice bearing lymphoma and melanoma, the subcutaneous administration of GemC12-LNCs resulted in a significant reduction in Mo-MDSCs infiltrating into the spleen and tumor (in comparison with free gemcitabine). Unlike other immune cells, monocytic cells displayed substantial uptake of fluorochrome-labeled LNCs (in tumor-bearing mice as well as blood samples from healthy donors/melanoma patients). Low-dose treatment with GemC12-LNCs attenuated tumor-associated immunosuppression and enhanced the therapeutic efficacy of adoptive T cell therapy [149]. In a similar

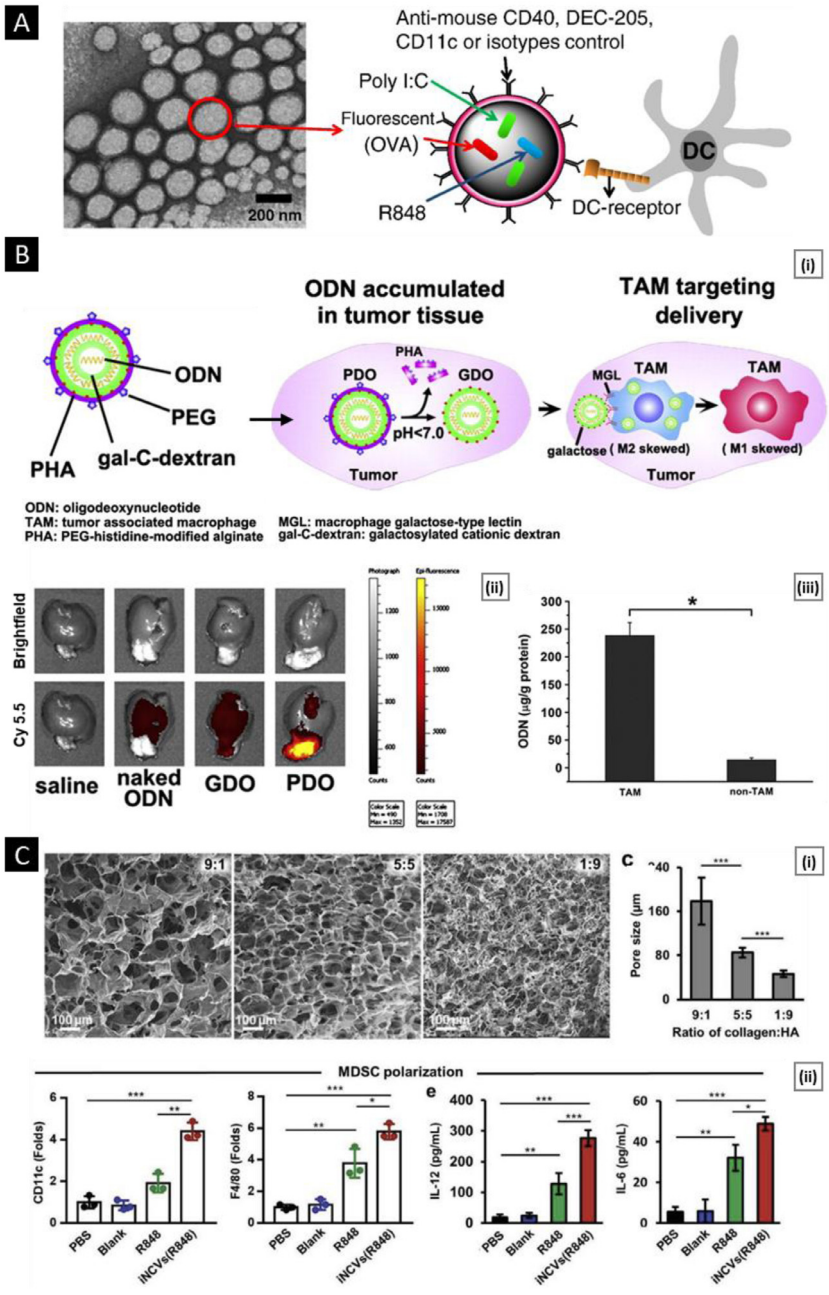

Fig. 10. Biomaterial-mediated Immune Targeting. **(A)** Schematic representation of PLGA NP vaccines targeting DC-specific receptors on mouse DCs along with TEM image. **(B)** pH-sensitive nano-complex composed of galactosylated cationic dextran polymer for selective binding with macrophage galactose-type lectin receptors expressed by TAMs. Here, sub-figure (i) provides a schematic overview of the platform's mechanism of functioning. Sub-figure (ii) shows the fluorescence imaging of the tumor at 6 h after Cy 5.5-labeled ODN administration, highlighting tumor-specific localization. Sub-figure (iii) shows the comparison of TAMs vs. non-TAM cells for ODN uptake. **(C)** Porous collagen/HA scaffold for co-delivery of R848, DOX, and ICB molecules (αPDL1/αPD1 antibodies) to induce an immunogenic tumor phenotype resulting in an enhanced ICB response. Here, sub-figure (i) shows the images from SEM analysis highlighting the morphology and corresponding pore size of collagen/HA scaffolds prepared using different collagen:HA ratios. Sub-figure (ii) shows the scaffold induced the polarization of MDSCs into tumoricidal APCs. Surface markers associated with DCs ($CD11c^+$) and macrophages ($F4/80^+$) were quantified via flow cytometry, whereas the production of the proinflammatory cytokines (IL-12 and IL was analyzed by ELISA. Adapted with permission from [142] (Copyright 2014, Elsevier), [146] (Copyright 2012, Elsevier), and [150] (Copyright 2019, Wiley-VCH), respectively.

study, Phuengkham *et al.* utilized a porous collagen/HA scaffold to fabricate cryogel co-loaded with resiquimod (within PLGA nanocarriers), DOX, and ICD inducers (αPDL1/αPD1 antibodies) to polarize MDSCs into anti-tumor APCs, while simultaneously serving as *in situ* cancer vaccine (Fig. 10c). By employing ice crystals during the crosslinking process, the authors achieved nano-sized pores that allowed the spatiotemporal modulation of TME by providing tumor localized and sustained release of immunomodulatory payload. The scaffold barred tumor recurrence and metastasis while prolonging long-term post-surgery survival in multiple murine tumor models, attributable to the robust elimination of MDSCs and TAMs [150].

5.2.3. Selective elimination of Tregs

With increased understanding regarding the role of intratumor Tregs and their effect on TME, approaches that can selectively deplete them are being explored for effective immunotherapy. Within the TME, Tregs possess some selective cell surfaces markers like CD15s, CD25, CD39, Cd103, CTLA-4, GITR, OX40, CCR4, and CCR8, which can be used to target them [151]. Apart from these

cell markers, Tregs secrete several molecules/enzymes involved in maintaining the immunosuppressive TME like granzymes, perforins, Indoleamine 2,3-dioxygenase (IDO), TGF-β, IL-10, and CTLA-4, which provide additional targeting avenues [152]. Along with enhancing the therapeutic outcomes, targeting helps in differentiating tumor-infiltrating Tregs from other Tregs important for their autoimmune role. Sacchetti *et al.* reported a PEG-modified SWCNT decorated with DTA-1 (mAb for anti-glucocorticoid-induced TNFR-related receptor, i.e., GITR) for targeting intra-tumoral Tregs. DTA-1 conjugation provides selective uptake into the cytoplasm of intra-tumoral Tregs (due to GITR overexpression) in comparison to non-Splenic Tregs. High Tregs infiltration within the TME indirectly helped in the tumoral localization of the PEG-SWCNTs platform [153]. Preceding studies have reported that indoleamine 2,3–dioxygenase (IDO) can increase the proliferation of Tregs [154]. To exploit this, Feng *et al.* employed TME-activatable PEGylated polymeric micelles containing Oxiplatin (a platinum-based anticancer drug) and NLG-919 (a potent IDO inhibitor). In response to acidic tumor pH, the PEG shell undergoes cleavage, causing a negative to positive charge shift on NP's surface. This helped in enhanced tumor accumulation followed by Oxiplatin mediated infiltration of cytotoxic T lymphocytes and NLG-919 mediated downregulates of IDO (causing a significant decrease in the Tregs population) [155].

5.3. Other immunomodulatory avenues

5.3.1. Artificial antigen-presenting cells (aAPCs)

aAPCs are bioengineered constructs that facilitate the activation and expansion of T cells. It functions by mimicking the interaction between biological APCs and T cells via presenting protein signals conjugated on their surface to stimulate T cells [156]. Conjugation of aAPCs with signals 1, 2, and 3 for antigen presentation, co-stimulation, and cytokine release, respectively, activates T cells. In signal 1, MHC of APC binds to T cell for antigen specificity. aAPCs bears antibodies to stimulate CD3T cells, leading to ligation of the TCR complex to trigger activation signaling within T cells [157]. The co-stimulatory signals constitute signal 2, whose molecules are upregulated on APCs, required for complete activation of T cell. Most aAPCs employ antibodies against CD28 as a co-stimulatory molecule [158]. Signal 3 involves the expansion and differentiation of T cells via the production and secretion of cytokines from APCs or T cells [159]. Steenblock *et al.* showed that there was a 3-4 fold increase in T cell expansion with IL-2 secreted from aAPCs compared to the equivalent amount of exogenous IL-2, while IL-7, IL-15, and IL-21 are few other cytokines that promote the better expansion of T cells [160].

Based on the synthetic and biomimetic biomaterials, aAPCs have been divided into:

(i) *Lipid-based aAPCs:* $CD4^+$ T cells were activated *in vitro* by Prakken *et al.* using MHC containing liposome which was followed by T cell proliferation and IL-2 secretion. The MHC-peptide complex gets pre-clustered on the membrane microdomain of APC even in absence of T cells for their enhanced activation [161]. Based on this, Ding *et al.* designed RAFTsomes, liposomes with membrane microdomains enriched with MHC complex-epitope, to stimulate the proliferation of $CD4^+$ T cells [162]. Based on similar results, *ex vivo* stimulation of human polyclonal T cells was performed by Giannoni *et al.*, where an artificial bilayer membrane was designed with microdomains of T cell ligands. Compared to soluble tetramers or uniformly distributed MHC on the artificial membrane, the pre-clustered MHC molecules triggered higher activation of T cells [163]. In a subsequent study, the same group achieved efficient interaction between T cells and aAPCs by incorporating an adhesive molecule, anti-LFA-1, in the microdomain, together with pre-clustered anti-CD28 and anti-CD3 antibodies. Higher expansion of polyclonal T cells was achieved along with *in vitro* activation of antigen-specific T cells [164].

(ii) *Polymeric aAPCS:* One of the earliest use of polymers to develop aAPCs were reported by Steenblock *et al.* where the authors reported efficient mimicking of physiological antigen presentation on a biodegradable microparticle constructed from PLGA. The microparticle core encapsulated IL-2 while the surface was equipped with various recognition and co-stimulatory ligands (anti-CD3, anti-CD28 antibodies, and peptide-MHC complex). The platform facilitated efficient polyclonal and antigen-specific T cell stimulation and expansion, by demonstrating that sustained release of IL-2 in the vicinity of T cell significantly improves the stimulatory capacity of these acellular systems (vs. exogenous addition of cytokine). A stable presentation of the surface ligand was observed for 20 days with a 45-fold enhancement in T cell expansion [165]. As natural APCs are not spherical, the shape of aAPCs is considered an important factor for the activation of T cells. Non-spherical PLGA-microparticles with increased contact area were designed to serve the purpose by Meyer *et al.* Their study showed that aAPCs with ellipsoidal shape showed higher in vitro T cell proliferation compared to spherical microparticles. When used in animal models, the ellipsoidal shape showed resistance to hepatic/splenic elimination which enhanced their pharmacokinetic properties. Compared to previously reported spherical aAPCs, their system generated a stronger immune response at an overall reduced protein dose [166].

(iii) *Inorganic aAPCs: Ex vivo* expansion of T cells has been performed by synthetic aAPCs with superparamagnetic components, such as magnetic particles, for separation from cells by an external magnetic field. Levine *et al.* covalently linked magnetic beads to anti-CD3 and anti-CD28 mAbs for *in vitro* expansion of $CD4^+$ T cells. Similar platforms for the *ex vivo* expansion of T cells later entered clinical trials [167,168]. Recently, Perica *et al.* used magnetic nano-aAPCs for binding to TCR which aggregated upon administration of a magnetic field, leading to TCR clustering and an increase in the expansion of T cells. Besides, the property of magnetic clustering can be combined with the direct trafficking of magnetic particles and particle-labeled cells in vivo [169]. Lee *et al.* reported Janus particles (having integration of two or more chemically discrepant composites into one structural system) that were magnetically responsive on one hemisphere and stimulatory to T cells on the other side. By manipulating the rotation and locomotion of Janus particles under an external magnetic field, the authors controlled the orientation of the particle-cell recognition and thereby the initiation of T cell activation [170].

5.3.2. Selective depletion of cancer-associated fibroblasts

Under normal circumstances, the fibroblasts (predominant cells of the stroma) secrete ECM, which provides a barrier against the advancement of cancer. The fibroblast cells, once ensconced in the TME, differentiate into proto-myofibroblasts, characterized by stress fibers. The mechanical tension generated in the ECM by proto-myofibroblasts activates TGF-β1, causing differentiation into myofibroblasts [171]. Identification of these myofibroblasts or CAFs are marked by the expression of alpha-smooth muscle actin (α-SMA) [172]. The stromal cells transform into CAF cells in presence of growth factors (VEGF and stromal-derived factor 1 (SDF-1)) and cytokines (IL-6 and TGF-β). The secretion of growth factors and cytokines and the expression of their respective receptors in CAFs augments angiogenesis, chemoresistance, tumor invasion and migration, and immune evasion [173]. The hypoxic condition and presence of ROS within the TME also abate this transformation [174]. The cellular origins of CAFs are illustrated in Fig. 11.

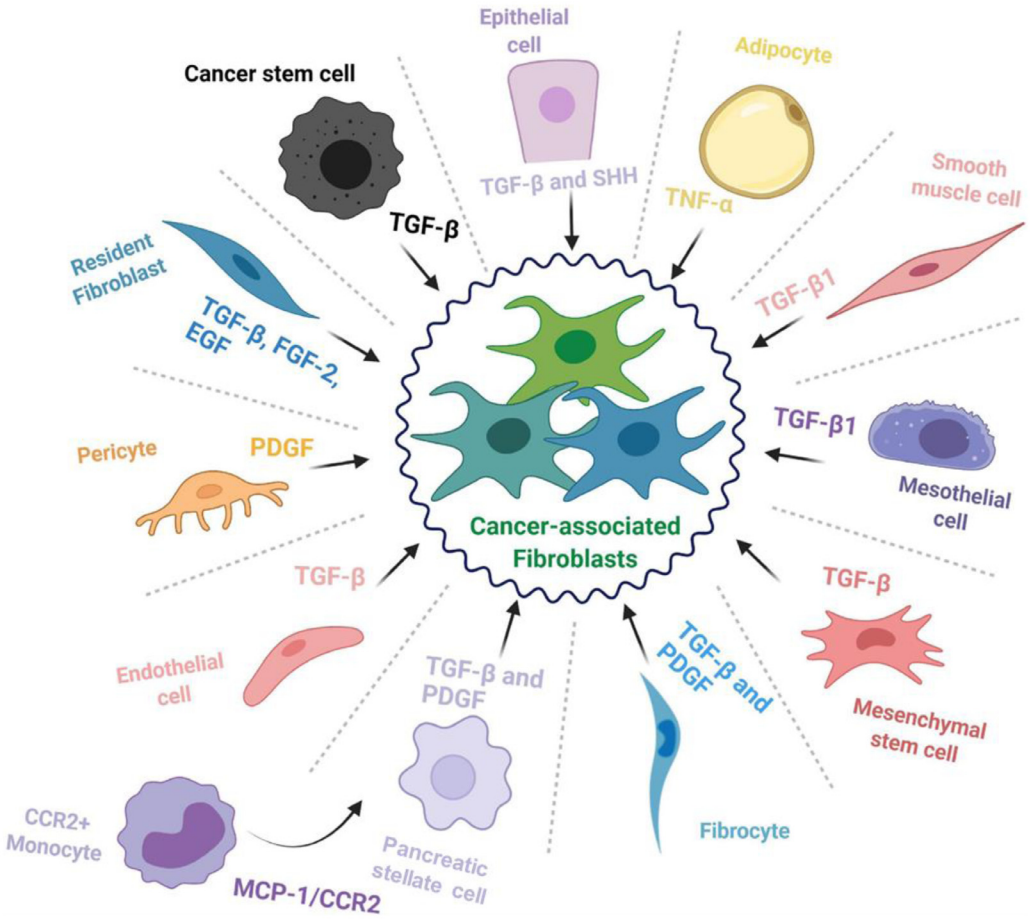

Fig. 11. Cellular origins of CAFs. Various processes can lead to the formation of CAFs from different cell types. Epithelial cells, through EMT transition, form CAF cells. Pericyte, smooth muscle, and adipocyte under transdifferentiation. Endothelial cells and mesenchymal cells in the presence of SDF-α and TGF-β, respectively, forms CAF. The normal fibroblast, known to inhibit tumors, in the presence of GFR, cytokines, hypoxia, certain miRNAs, or through an epigenetic switch, transforms to CAFs. Adapted with permission from [180] (Copyright 2021, Frontiers Media).

Cancer cells can induce the expression of inflammatory genes in normal fibroblasts through microRNAs (miRNAs) [175]. Exosomal delivery of miRNAs to target cells ensures the transformation of both local and distant fibroblasts. Fang *et al.* demonstrated the delivery of miRNA-1247 to fibroblasts via exosome in lung carcinoma. This brought about a transformation in the fibroblasts, promoting metastasis [176]. The switch from normal fibroblasts to CAFs is endorsed by several epigenetic regulators. Albrengues *et al.* showed that leukemia inhibitory factor (LIF), a member of the IL-6 superfamily, is one of the key contributors that initiates this epigenetic switch. This is followed by the activation of the JAK-STAT pathway which enhances the invasive behavior of the tumor [177]. Other cells, such as pericytes and adipocytes, can also be transformed into CAF cells through trans-differentiation [178,179].

Recent studies have highlighted that CAFs are heterogeneous, and α-SMA represents a subset of CAFs in the stromal cell population. Other CAF markers include PDGF receptor, fibroblast activation protein (FAP), podoplanin, meflin, and fibroblast-specific protein 1 (FSP-1). Independent researchers have identified two subpopulations of CAFs in pancreatic and other cancers. Myofibrotic CAFs (MyCAFs) are α-SMA expressing populations found residing within the tumor, while CXCL-12 and IL-6 expressing inflammatory fibroblasts (iCAFs) are found at the edge of the tumor nest [181]. As cancer progresses, CAFs secrete collagen, α-smooth muscle actin, fibronectin, and other proteins that alter the architecture ECM, which now helps in cancer sustenance. This brings about changes in the morphology of cancer cells, making them metastatic [182].

In recent years, biological hallmarks of CAFs have been explored to design nano-therapeutics to remodel the cancer TME and improve the therapeutic efficacy of chemotherapy. CAFs restrict the penetration of drugs in the tumor tissues by synthesizing and remodeling the ECM and maintaining a high tumor interstitial fluid pressure. Therapeutic strategies to eradicate CAFs could lead to the reduction of collagen in the ECM, resulting in improved drug diffusion and accumulation [183]. FAP can be exploited for specific targeting and drug delivery. Some researchers have constructed peptide-based NPs loaded with FAP-α antibodies to deliver siRNA to CAF cells. The nanoparticle system has been used in the TME of prostate cancer to downregulate the expression of CXCL12 in CAFs, which restricted the migration and invasion of tumor cells and significantly inhibited angiogenesis [184]. In a triple-negative murine breast cancer model, an angiotensin inhibitor, losartan, assembled with C16-N peptide hydrogel, was injected to inhibit the synthesis of collagen I by CAF [185]. The sustained release of the drug acted locally and enhanced the intratumoral penetration and accumulation of PEGylated DOX-loaded liposomes, which were administered as a combination therapy. Unlike single chemotherapy, C16-N/losartan together disrupted the CAF-controlled ECM to improve drug delivery, thereby suppressing primary tumor growth and its metastasis. CAF-destroying NPs have been used to selectively target CAF cells with chemotherapeutic drugs. For the treatment of prostate cancer, Ji *et al.* synthesized a dual-acting nanopar-

ticle that targets CAFs and enhances the cell penetration capability for improved drug delivery [186]. The nanoparticle comprises an amphiphilic cell-penetrating peptide (CPP) (C2KKG2R9) linked to a cholesterol monomer by a hydrophilic tail. This CPP-cholesterol moiety self-assembles into a core-shell peptide nanoparticle (PNP). This PNP is loaded with the anti-cancer drug DOX (PNP-D), which is modified with an anti-FAP monoclonal antibody to recognize and target CAFs. Recently, a peptide (C16-GNNQQNYKD-OH) has been synthesized that self-assemble into long filaments to form a hydrogel, entrapping losartan molecules. In the triple-negative breast cancer model, intratumor injection of this hydrogel inhibited the CAF-mediated synthesis of collagen I. The therapeutic efficacy was enhanced when combined with a chemotherapeutic drug [187].

5.3.3. Targeted delivery to tumor-draining lymph nodes

The metastatic spread of cancer from the primary tumor site to lymph nodes and distant organs involves many complex processes. The sentinel lymph node receives the first lymphatic drainage and constitutes the first line of defense against the metastatic spread by initiating an anti-tumor immune response. The presence of cancer cells in tumor-draining lymph nodes (TDLN) is a key prognostic factor in many malignancies [188]. For example, in oral or cervical cancer, lymphadenectomy provides overall survival benefits, whereas no such result is obtained in breast cancer and melanoma [189,190]. In recent years, it has been clear that for ICB to work, there should be a sufficient number of infiltrating T cells in the tumor site. In the TDLN, tumor-specific T cell responses are initiated when APCs present tumor-specific neoantigens to $CD8^+$ T cells for their effective priming [191]. In cancer, tumor-derived factors such as TGF-β, IL-6, VEGF, prostaglandins-E2, and extracellular vesicles, modify the function of TDLN. This results in the suppression of DC and activation of macrophages to the M2-phenotype, which prevents the cross-presentation of tumor antigens in TDLN [192]. Other alterations include an increase in lymphangiogenesis, remodeling of blood vessels, and increase in the secretion of cytokines and chemokines, which ultimately changes the composition and functioning of immune cells to create a “tumor-supportive” microenvironment or a “pre-metastatic niche”. Moreover, within the TDLN itself, tumor cells downregulate MHC-I molecules and upregulate immunosuppressive ligands to evade immune surveillance, eventually leading to metastatic growth [193]. Thus, for effective tumor-specific T cell response and prevention of metastatic spread, immune modulation of TDLN is required.

It has been observed that only a small fraction of systemically administered drugs reach TDLN. Immune-modulating agents applied locally, may be more effective in counteracting anti-tumor immune response in TDLN. Direct lymph node injection has been proposed for immunotherapeutic applications; however, it requires precise invasive surgical manipulations and involves a possibility of causing T cell sequestration/exhaustion at the injection site (instead of the desired systemic T cell response), rendering it unsuitable for the majority of biomaterial-based use scenarios. Lymph nodes have a continuous flow of lymph that is drained for the periphery. By incorporating biomaterial design principles that take advantage of interstitial flow (present in peripheral lymphatic capillary) and decreasing pressure gradient, facile strategies in the form of nano-/micro-particulate platforms can be crafted that can effectively reach the TDLNs after systemic injection [194].

Key physicochemical attributes that govern TDLN targeting can be categorized into:

(i) *Size and shape:* Particle size was one of the first investigated parameters for optimizing lymphatic targeting. Besides being an important determinant of many vital *in vivo* processes (like biodistribution, lymphatic uptake, and immune cell interaction), particle size can be effortlessly controlled by optimizing the method of preparation or changing the material used in the synthesis of the same, thus making it one of the most widely studied biomaterial attribute. Based on the findings of multiple extensive studies, it is now known that a lower hydrodynamic diameter favors transport to TDLNs [195]. Using fluorescent polypropylene sulfide NPs (size ranging from 20 to 100 nm), Reddy *et al.* showed that particles with size <50 nm can exploit lymphatic drainage and are readily transported to lymph nodes where they are processed by immature DCs (vs. larger 100 nm NPs). The conjugation of these NPs to ovalbumin (model antigen) triggered the humoral and cellular immune response in mice in a size-dependent manner [196]. By employing contrast agent-tagged dendrimers, Kobayashi *et al.* determined that there exists a lower size limit for preferential lymphatic drainage targeting. Below 8 nm, NPs pass through the endothelial cell junctions and get drained into the blood via absorption into capillary beds [197]. Upon reaching the TDLNs, the shape of NPs determines their extent of being recognized and internalized by macrophages. If the angle of contact between the particle and macrophage is small, the macrophage membrane forms an actin cup and ring structure that aids in particle internalization. But with a larger contact angle, the energy required to form the actin cup increases substantially which limits phagocytosis [198]. For sub-micron particles, the rate of shape-dependent internalization is in the order of disc-shape > spheroid > rod-shaped [199].

(ii) *Surface charge:* The influence of charge is a contentious topic as a negative (anionic) surface charge favors higher lymphatic uptake and enhanced lymph node retention whereas positive (cationic) charge-baring systems can interact better with immune cell populations within the TDLNs. As glycosaminoglycans (negatively charged) are the primary constituents of the interstitium, any cationic carrier systems will be trapped (by aggregating and forming a deposition at the injection site), hindering their movement toward lymphatic capillaries. On the contrary, the presence of a positive surface charge facilitates electrostatic binding with APCs resulting in their enhanced internalization [200]. It should be noted that the use of cationic biomaterials is associated with high cytotoxicity as they can lead to non-reversible disruption of negatively charged cell membranes [201]. In this context, emphasis can be laid on developing smart biomaterials that can maintain a neutral or anionic charge while traveling through TDLNs and undergo a premeditated transformation to a cationic charge upon interacting with APCs. Such a platform can hold great significance in improving the efficacy of immunotherapy by promoting antigen uptake and ameliorating cytotoxicity [202].

(iii) *Hydrophobicity:* In general, hydrophobic systems tend to accumulate in lymph nodes more efficiently than their hydrophilic counterparts due to their ability to interact with the lipid-rich environment of the lymphatic system. Systemically administered hydrophobic NPs also tend to have longer circulation times in the body, which can increase the chances of uptake by the lymphatic system [203]. Based on biomaterial class, hydrophobicity can be modulated by using amphiphilic polymers with different hydrophobic segments (for polymer-based biomaterials), by replacement of component with a more lipophilic alternative (for lipid-based biomaterials), or by chemical attachment/grafting of hydrophobic segments on the particle surface (for metal-based or inorganic biomaterials) [204]. Increasing the hydrophobicity can also provide immunological benefits by modifying the release profile of encapsulated antigens or immune modulators.

(iv) *Surface engineering:* Active targeting of biomaterials by conjugating their surface with targeting ligands is one of the most

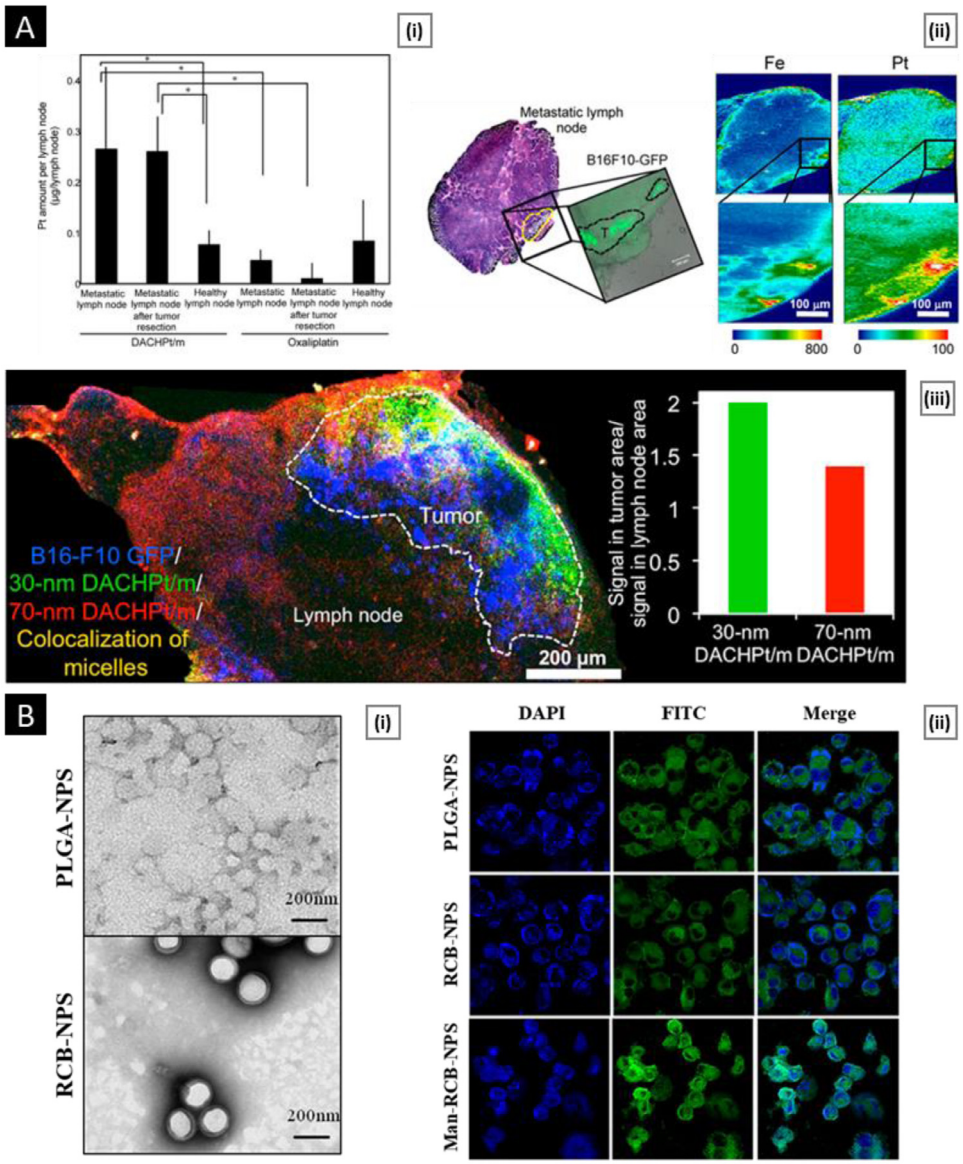

Fig. 12. Biomaterial-mediated Targeting of TDLNs. **(A)** Systemic Targeting of Lymph Node Using Size-Controlled Nanocarriers. Here, sub-figure (i) shows Pt accumulation in metastatic lymph nodes and healthy lymph nodes 24 h after injection of oxaliplatin or DACHPt/m (at 5 mg/kg). Sub-figure (ii) shows the micro-distribution of Pt and Fe (by μ-SR-XRF) in metastatic lymph nodes 24 h after injection of DACHPt/m (at 20 mg/kg). Sub-figure (iii) shows fluorescence microscopy of metastatic lymph node 24 h after co-injection of fluorescent-labeled 30 nm (green) and 70 nm (red) DACHPt/m (B16F10-GFP metastasis appears in blue and micelles colocalization in yellow) with the corresponding ratio of their fluorescence intensity. **(B)** Mannose-inserted erythrocyte membrane-encapsulated NPs for target APCs in the lymphatic organs. Here, sub-figure (i) shows the TEM images of normal PLGA-NPs membrane-encapsulated NPs. Sub-figure (ii) shows the confocal laser scanning microscopy images comparing the extent of cell uptake (PLGA-NPs vs. RBC-NPs vs. Man-RBC-NPs) in DC2.4 cells. Adapted with permission from [208] (Copyright 2015, American Chemical Society) and [209] (Copyright 2015, American Chemical Society), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

straightforward ways to modulate the immune response at a cellular level. This strategy facilitates the selective administration of immunomodulators to the desired subpopulation of immune cells, thereby eliminating non-specific toxicity. Each immune cells possess specific surface receptors that can be exploited by using complementary targeting ligands. Some of the widely explored targeting ligands are: (a) For macrophage targeting: Mannose, Hyaluronan, F4/80 antibody fragments [205], (b) For DCs targeting: Antibodies against DEC-205, CD209, CD40, and CD11c receptors [206], (c) To targeted T cells: CD4 targeting peptides, Antibodies against CD3 and Integrin receptors [207].

Several biomaterial-based platforms that employ the above-mentioned targeting strategies have been developed. Cabrel *et al.* demonstrated that sub-50 nm polymeric micelles (termed DACHPt/m) incorporating (1,2-diaminocyclohexane)platinum(II) (DACHPt, a platinum anticancer drug) could target lymph node metastases in a syngeneic melanoma model after systemic injection, even after removing the primary tumors, limiting the growth of the metastases. By comparing these micelles with clinically used DOX-loaded liposomes (Doxil, size: 80 nm), along with a 70 nm version of the micelles, the authors found that the targeting efficiency of the nanocarriers against lymph node metastases is associated with their size-regulated abilities to extravasate from the blood vasculature in metastases and to penetrate within the metastatic mass (Fig. 12a) [208]. Guo *et al.* developed erythrocyte-membrane-based PLGA NPs encapsulating antigenic peptide hgp100 MPLA. The authors modified the membrane with mannose to actively target APCs in the lymphatic organ. In addition, the

Table 4
Key CSC markers expressed in different human cancers.

Cancer Type	CSC Marker	Refs.
Breast	$CD24^-$, $CD44^+$, $CD133^+$, $CD166^+$, $ALDH^+$, ESA^+, $EpCAM^+$	[217]
Cervical	$CD133^+$, $CD49f^+$, $CK\text{-}17^+$	[218]
Colon	$CD24^+$, $CD44^+$, $CD133^+$, $CD166^+$, $ALDH^+$, $EpCAM^+$	[219]
Esophageal	$CD44^+$, $ALDH^+$, Integrin $\alpha7^+$	[220]
Head & Neck	$CD44^+$, $CD133^+$, $BMI\text{-}1^+$	[221]
Liver	$CD24^+$, $CD44^+$, $CD49f^+$, $CD90^+$, $CD133^+$, $ALDH^+$, $ABCG2^+$	[222]
Lung	$CD44^+$, $CD87^+$, $CD90^+$, $CD133^+$, $ALDH^+$, $ABCG2^+$	[223]
Ovarian	$CD44^+$, $CD117^+$, $CD133^+$, $ALDH1^+$	[224]
Pancreatic	$CD24^+$, $CD44^+$, $CD166^+$, $ABCG2^+$, $ALDH^+$, $EpCAM^+$	[225]
Prostate	$CD44^+$, $CD133^+$, $\alpha2\beta1^+$, $ALDH^+$, $ABCG2^+$	[226]
Thyroid	$CD44^+$, $CD133^+$, $ALDH^+$, $SSEA^+$	[227]

peptide was conjugation with PLGA NPs via a redox-sensitive linkage that undergoes selective cleavage in the intracellular milieu, thereby increasing the anti-tumor immune response. The authors reported the observation of an antigen-depot effect from the administration site with enhanced retention in draining lymph nodes. Compared with other formulations after intradermal injection, the platform prolonged tumor-occurring time inhibited tumor growth and suppressed tumor metastasis in various melanoma models (Fig. 12b) [209]. Mottas *et al.* reported the delivery of immunostimulatory TLR7 ligands to TDLNs using AuNPs with approximately 5 nm hydrodynamic diameter coated with a mixture of 1-octanethiol and 11-mercaptoundecanesulfonic acid. The drug was loaded without modification through nonspecific adsorption into the shell, taking advantage of its amphiphilic nature. Upon subcutaneous injection into tumor-bearing mice, the drug-loaded particles rapidly transported to the TDLNs where they induced a local immune activation and fostered a cytotoxic T cell response that was specific for the tumor. Prominently, the particle-delivered TLR7 ligand blocked the growth of large established tumors and significantly prolonged survival compared to the free form of the drug [210].

6. Targeting cancer stem cells: the next big step in cancer immunotherapy?

6.1. Understanding CSCs

CSCs (also called stem-like cells or tumor-initiating cells) are a discrete sub-population of cancer cells located within the tumor niches. They are characterized by their unique self-renewing ability, high tumorgenicity, presence of specific surface markers, and resistance to conventional chemotherapy [211]. Towards the end of the 20th century, significant attention was laid to understanding and decoding the mechanisms of tumor heterogeneity. While the existence of CSCs was being continuously hypothesized and debated, the first experimental evidence was provided in the year 1994 when Lapidot *et al.* isolated human acute myeloid leukemia (AML) cells with stem cell marker phenotype, $CD34^+/CD38^-$. These cells, when transplanted to severe combined immune-deficient (SCID) mice, traveled to the bone marrow and in response to cytokine treatment, proliferated extensively generating a pattern of dissemination and leukemic cell morphology similar to that seen in the original patients [212]. Later in 2003, the presence of CSCs in breast cancer was first reported by Al-Hajj *et al.* [213]. Subsequently, the CSC model has been successfully established for its role in the initiation, progression, and metastasis of almost all other solid tumors [214]. As conventional radio-/chemotherapy is only effective on the tumor bulk, deep-seated and drug-resistant CSCs are primarily responsible for cancer relapse. From the perspective of CSCs-immunotherapy, efficient targeting can help overcome some of the clinical challenges associated with the long-term maintenance and progression of tumors. The identification and eradication are complicated considering the high plasticity of CSCs [215]. They are generally identified/isolated from the total cell population based on distinctive and specific cell surface biomarker expression using various methods like functional assays (e.g., ALDEFLOUR^{TM}, *in vitro* stem cell aggregates, organoid), side population discrimination assay and fluorescence-activated cell sorting (FACS) [216]. Table 4 highlights some key CSC markers expressed in different human cancers.

Apart from unique CSC markers, targeting of CSCs can also be achieved by activating the host immune system against TAAs that are preferentially and exclusively expressed by CSCs. These TAAs can be categorized into four distinct sub-groups: constitutively overexpressed tumor antigens (e.g., MUC1 and HER-2 in breast cancer; mesothelin in pancreatic cancer; PSMA and TPD52 in prostate cancer), tumor-activated cancer/testis antigens (e.g., MAGE, BAGE, GAGE, XAGE, SPANX, NY-ESO1), somatically mutated tumor antigens (e.g., neoantigens like MUM-1 and CDK4 in melanoma), and differentiation antigens (e.g., lineage-specific markers like CEA in colon cancer; PSA and PAP in prostate cancer; MART-1, Gp100, and Tyrosinase in melanoma) [228]. The following part will focus on establishing the need to target CSCs for successful immunotherapeutic outcomes and the application of biomaterials to achieve CSCs-targeting.

6.2. Significance of CSCs

Recent breakthroughs in the comprehension of the tumor immune microenvironment have highlighted the significance of the immunological contexture in influencing the therapeutic response and clinical prognosis of patients. A deeper understanding of the multi-directional crosstalk by which CSCs interact and dominate immune cells is of paramount importance for comprehending how their biomaterial-mediated selected targeting and subsequent ablation can aid in enhancing immunotherapy outcomes. While there are several factors determining the response to immunotherapy, CSCs are considered specifically important as in addition to efficiently avoiding identification by the immune system, they negotiate with immune cells and exploit them to establish an immunosuppressive, pro-tumorigenic niche. Moreover, CSCs thrive in such compromised environments to replenish their stemness features, thereby generating supplementary hindrance for immunotherapy by boosting tumor formation and progression. The following section delves into the significance of eradicating CSCs and reprogramming the TME (as enabled by biomaterial-based immunomodulatory platforms) by briefly highlighting the important CSCs-immune cell interactions that occur at the cellular and molecular level:

(i) *CSCs and DCs:* TME-associated CSCs inhibit the recruitment of DCs to the tumor site, impede their maturation, and stimulate their differentiation into immunosuppressive subtypes.

Table 5
Existing strategies for the immunotherapeutic targeting of CSCs. The table has been adapted and modified with permission from Badrinath *et al.* [242] (Copyright 2019, MDPI).

Type of immunotherapy	CSCs Targeting approach	Cancer model
Adoptive	CAR-T cells expressing EpCAM-specific chimeric antigen	Prostate Cancer
T	CAR-T cells targeting membrane-bound IL-15	Leukemia
cell	$CD8^+$ T cells specific for ASB4 (CSCs antigen)	Colon Cancer
therapy	CIK cells transduced with CAR T- cells against CD123	Leukemia
	CIK cells with NKG2D recognizing ligands	Nasopharyngeal Carcinoma
DC-	CSC lysate-pulsed DCs	Malignant Melanoma
based	DCs charged with Panc-1 CSC total lysate	Pancreatic Cancer
vaccine	DCs loaded with NANOG peptide	Ovarian Cancer
	DCs pulsed with $ALDH^{high}$ SCC7 specific CSCs	Squamous Cell Cancer
	$ALDH^{high}$ CSC-pulsed DCs	Metastatic Melanoma
Oncolytic	Oncolytic adenovirus targeting $CD133^+$ CSCs	Glioblastoma
virotherapy	Oncolytic vaccinia virus targeting $ALDH^{high}$ CSCs	Breast Cancer
	Oncolytic vaccinia virus targeting $CD44^+/CD117^+$ CSCs	Ovarian Cancer
	Oncolytic measles viruses targeting $CD133^+$ CSCs	Hepatocellular Carcinoma

CSCs secrete TGF-β or upregulate its signaling that subsequently interferes with DCs antitumor responses via different mechanisms like reduction of mature DCs, enrichment of tolerogenic DCs sub-population, or by downregulation of DCs-costimulatory molecules. In contrast, these immunosuppressive DCs get forced into a feedforward mechanism that triggers a chemokine-dependent signaling cascade, boosting the existing stemness by enhancing the self-renewing and metastatic properties of CSCs [229].

(ii) *CSCs and TAMs:* Several studies have established that CSCs alter the composition of intratumoral macrophages by attracting M2 macrophages or by inducing the polarization of both tissue-resident and recruited macrophages toward an anti-inflammatory M2 phenotype (via secretion of cytokines and growth factors) [230]. After recruitment to the TME, TAMs are deployed as a "niche" to support CSC growth. Infiltrating TAMs, by activating the NF-κB signaling pathway, secrete IL-1β, IL-6, IL-10, and TGF-β. These tumor-promoting cytokines bind to their receptors, further stimulating STAT3 activation in adjacent CSCs. This results in a vicious cycle of NF-κB activation as well as maintenance of CSCs' stemness [231]. TAM-derived TGF-β further promotes tumor invasion and metastasis by producing $EpCAM^+$CSCs through the induction of epithelial-to-mesenchymal transition. TAM-induced overexpression of the CD47 ligand (involved in binding to signal regulatory proteins on phagocytes) protects CSCs from cell-mediated phagocytosis [232]. Overall, CSCs-TAM communication generates immunosuppressive TME, which promotes CSCs' survival and impedes immunotherapy's ability to eradicate the tumor.

(iii) *CSCs and MDSCs:* As the chief TME constituents that cause immunosuppression, MDSCs are employed as a prognostic indicator for immunotherapy response and patient survival. They primarily reduce immunotherapy efficacy by secreting immunosuppressive cytokines and chemokines that complement the TME niche. Molecules like arginase-1 and nitric oxide grant MDSCs the ability to modulate tumor plasticity and T cell apoptosis [233]. Further, by serving as a middleman for CSCs, MDSCs impart immunosuppressive functions to macrophages, NK cells, and DCs via crosstalk. CSCs-mediated oncogenic mTOR signaling is responsible for MDSCs infiltration and accumulation in tumor sites [234]. Reciprocally, MDSCs improve the functionality of CSCs via several mechanisms that involve the upregulation of transcription factors and the production of tumor-supportive simulators that cumulatively increase the expression of stemness genes and CSC-associated properties [235].

(iv) *CSCs and Tregs:* CSCs alter the composition and functional properties of tumor-specific effector T cells and promote their expansion into pro-tumorigenic regulatory Tregs. CSCs-derived PD-L1, TGF-β, and specific chemokines (like CCL1 and CCL22) mediate the recruitment and infiltration of Tregs in TME. Subsequently, Tregs produce IL-17 and Prostaglandin E2 to promote self-renewal ability, stem cell markers, and epithelial-to-mesenchymal transition toward tumor progression and invasion. Tregs also guard CSCs from T cell killing by the differentiation of uncommitted $CD4^+$ T cells into Tregs [236].

In addition to the above-mentioned interactions with tumor immune components, CSCs significantly upregulate MHC-I molecules and NK cells-inhibitory ligands through which they escape NK cell-mediated clearance [237]. In addition to being highly efficient in evading the immune system, CSCs are notoriously chemoresistance. Their robust chemoresistance can be attributed to the rapid DNA repair ability combined with the localized overexpression of efflux transporters (particularly ABC transporters) and detoxifying enzymes (particularly by enhanced expression of aldehyde dehydrogenases) that work in synchronization to inactivate any externally administered chemotherapeutic drugs [238]. As the differentiated tumor cell (that constitute the bulk) are devoid of these drug-neutralizing abilities, they undergo apoptosis in response to cytotoxic drugs, giving CSCs a survival advantage that can lead to tumor recurrence (upon the termination of conventional chemo- and radiotherapy treatment). Lastly, by exploiting the ECM proteins, stroll support cells, blood vessels, and soluble factors that exist within the TME niche, CSCs promote rapid tumorigenesis that allows it to migrate to distinct sites where they colonize secondary tumor sites by differentiating into conventional tumor cells [239]. A direct product of rapid tumorigenesis is hypoxia, in response to which CSCs release various hypoxia-inducible factors that further stimulate neo-vascularization within the tumors and at distant metastatic sites [240].

To summarize, CSCs are the key culprits that reshape the TME by attracting immunosuppressive cell subsets and inhibiting effector T cells. In unison, the immune cells that interact with CSCs get compromised and are exploited by the mechanism discussed above to promote CSCs' harmful attributes like self-renewal, tumorigenicity, and metastasis. These findings emphasize the unique role of CSCs and the immense potential that lies in targeting them. Consequently, strategies leading to the targeted elimination of CSCs in addition to immunotherapeutic remodeling of immune cells can improve the clinical outcome for tumor patients.

6.3. Targeting CSCs

Current immunotherapy-based approaches to target CSCs are mainly limited to the use of bioengineered immune cells (like DC-based vaccines, $CD8^+$ T cells, NK cells, cytokine-induced killer (CIK)

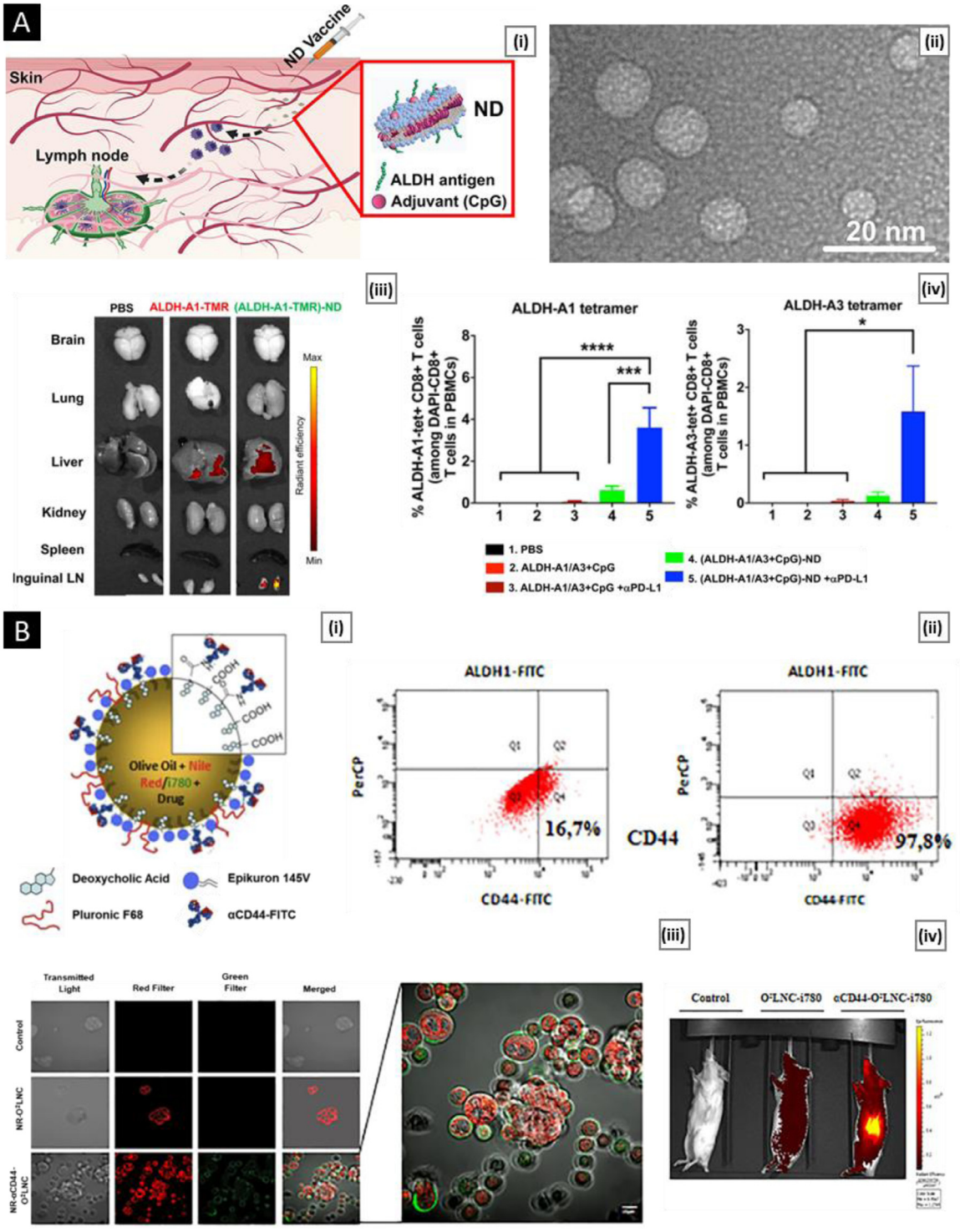

Fig. 13. Biomaterials-mediated Targeting of CSCs. (A) Adjuvant-loaded high-density lipid-based nano-discs to selectively target and reduce ALDH$^+$ CSCs. Here, sub-figure (i) provides a schematic overview of vaccination CSCs with nano-discs carrying ALDH epitopes. Sub-figure (ii) shows the representative TEM images of nano-discs. Sub-figure (iii) shows the fluorescence intensity of TMR-tagged ALDH-A1 peptides administered to C57BL/6 mice in nano-disc or soluble form. Sub-figure (iv) shows the ALDH-A1-specific and ALDH-A3-specific CD8$^+$ T cells response in treated tumor-bearing mice. **(B)** Anti-CD44-conjugated olive oil liquid nanocapsules for targeting pancreatic CSCs. Here, sub-figure (i) provides a schematic representation of αCD44-O^{2}LNC with major constituents. Sub-figure (ii) shows the representative DOT PLOTS showcasing the CD44 surface expression in BxPC-3 human PC cell line (monolayer) and cancer stem-like BxPC3 cells (secondary spheres). Sub-figure (iii) demonstrated the CD44-targeting of the developed platform via confocal microscopy images of secondary spheres (scale bar = 10 μm) incubated for 3 h with NR-O^{2}LNC and NR-αCD44-O^{2}LNC. Sub-figure (iv) is a representative image comparing the measured fluorescence of IR-780 iodide delivery via αCD44-O^{2}LNC (vs. O^{2}LNC and free form). Adapted with permission from [245] (Copyright 2020, American Chemical Society) and [252] (Copyright 2021, American Chemical Society), respectively.

cells, and $\gamma\delta$ T cells). More recently, oncolytic virotherapy (OVT) is emerging as a lucrative CSCs immunotherapy strategy. It involves the use of viruses that infect and replicate specifically in tumor cells causing direct cell lysis (associated with the release of TAAs, neoantigens, and DAMPs). The use of genetically modified viruses allows the induction of tumor-specific immunogenic cell death, which, when combined with cell lysis can synergize the induction of antitumor immunity [241]. Additionally, therapies that employ DC-based vaccines or OVT in combination with a checkpoint inhibitor (commonly PD-1 inhibitors) with have also been explored. A brief overview of various targeting strategies against CSCs has been highlighted in Table 5.

While the above-mentioned approaches are effective in countering CSCs, their clinical feasibility and translation are held back by their complexity, low response rates, and poor commercialization potential. In this context, functionalized biomaterials that can target CSC-specific markers (like ALDH, $CD44^+$, $CD90^+$) or CSC-associated signaling pathways can be potentially explored to develop off-the-shelf CSCs targeting immune-therapeutics. Although the clinical benefits of eradicating CSCs are highly lucrative, the domain of biomaterial-based targeted platforms for CSC-immunotherapy is relatively unexplored. Despite this, various applications of CSC-targeted chemotherapy have been reported over the past few years, which can be extended for immunotherapy if the anticancer cargo is to be replaced by suitable immunogenic molecules that can modulate CSCs. The subsequent section will discuss the current state-of-the-art CSCs-targeting.

Aldehyde dehydrogenase (ALDH) has been extensively studied as a functional biomarker of CSCs. In humans, the ALDH superfamily comprises 19 functional genes (subdivided into 11 families and four subfamilies) that express intracellular enzymes which are involved in the oxidation of aldehydes [243]. Apart from controlling various differentiation and metabolic pathways (by converting retinol to retinoic acid), ALDH serves a protective detoxifying role in CSCs by catabolizing aldehydes derived from pharmacological substrates. Enhanced tumorigenicity and chemoresistance observed in many cancers have been linked with high levels of CSC-associated ALDH activity [244]. H. Najafabadi *et al.* recently developed high-density lipid-based nano-discs (loaded with CpG as immune adjuvant) surface conjugated with ALDH antigen to selectively target and reduce $ALDH^+$ CSCs. The ALDH-A1 and ALDH-A3 epitope-bearing nano-discs were successful in enhancing antigen trafficking to lymph nodes which resulted in strong ALDH-specific T cell responses. To study the in vivo lymphatic delivery of the system, the authors subcutaneously immunized C57BL/6 mice with ALDH-A1 peptide fluorescently tagged with tetramethylrhodamine (TMR) in soluble or nano-disc form. The platform resulted in a significantly higher TMR signal in inguinal LNs compared with the soluble ALDH-A1-TMR group. The nano-discs, when combined with anti-PD-L1 therapy in multiple murine models, generated potent antitumor efficacy and prolonged animal survival (Fig. 13a) [245]. In a different study, Li *et al.* reported the use of decitabine, a DNA hypermethylation inhibitor, to overcome the ALDH-mediated drug resistance of CSCs. Polymeric NPs composed of biodegradable MPEG-b-PLA were loaded with decitabine (NP_{DAC}) and DOX (NP_{DOX}). *In vitro* studies showed that combination therapy with NP_{DAC} and NP_{DOX} significantly reduced $ALDH^{high}$ CSCs in MDA-MB-231 mammospheres. Systemic delivery of NP_{DAC} in a murine xenograft model increased caspase-9 expression, which increased the sensitivity of the bulk cancer cells (including CSCs) to DOX treatment [246].

While a wide variety of CSC-associated CD markers have been identified, CD44 and CD133 remain two of the most explored functional biomarkers as they are commonly found in various cancer types. CD44 is a multifunctional transmembrane glycoprotein encoded by the highly conserved CD44 gene [247]. It functions as a hyaluronic acid receptor, thereby serving as a signaling platform for multiple physiological and pathological functions (including cell adhesion, proliferation, growth, and migration) [248]. CD44v, a CD44 splice variant (isoform) has been identified to be the key regulator of CSCs' tumorigenic potential [35]. Multiple humanized IgG1 antibodies that target the extracellular hyaluronic acid-binding domain in CD44 isoforms are under advanced clinical trials [249]. CD44 produces IL-17 and IFN-γ while also regulating the survival and memory development of T helper type 1 (Th1) cells. Hence, CD44 targeting can generate an anti-tumor immune response [250]. Aries *et al.* reported selective targeting of CD44 surface receptors on CSCs using multi-functionalized iron oxide MNPs with anti-CD44 antibodies. The platform was used for intracellular delivery of gemcitabine, which was covalently immobilized on the MNPs surface. Using a CD44-negative non-tumorigenic cell line as a control, the authors demonstrated the efficient CD44 targeting endowed by an anti-CD44 antibody [251]. A recent study by Navarro-Marchal *et al.* reported the use of anti-CD44 antibody covalently coupled to lipid liquid nano-capsules for CSC-targeted delivery of lipophilic drugs (αCD44-O^2LNC). The final surface hydrophilicity of the platform favors the appearance of hydration repulsive forces allowing it to remain colloidally stable in saline solutions and typical cell-culture media. The *in vitro* results highlight the enhanced targeting activity and receptor-mediated binding mechanism of αCD44-O^2LNC with CD44 overexpressing pancreatic CSCs that led to roughly 4 times higher delivery of PTX when loaded in αCD44-O^2LNC (vs. free PTX). Lastly, the authors further demonstrated the pancreatic CSCs targeting and the non-invasive cell imaging/tracking ability of αCD44-O^2LNC using an orthotopic xenotransplant in vivo model (Fig. 13b) [252].

Originally discovered in human hematopoietic stem cells, CD133 is localized within the plasma membrane protrusions (e.g., in villi and cilia). While it interacts and binds with cholesterol, the exact biological function of CD133 has not been identified. Cancer cells expressing high levels of CD133 are more metastatic and resistant to chemo/radiation therapy [253]. Ni *et al.* reported salinomycin-loaded PEGylated PLGA NPs conjugated with CD133 aptamers (Ap-SAL-NP). Aptamer conjugation allowed for effective targeting and elimination of CD133-bearing osteosarcoma CSCs, evaluated using *in vitro* as well as *in vivo* models [254]. More recently, Kim *et al.* developed a dual-targeting immunoliposome to counter glioblastoma multiforme by selectively delivering temozolomide (TMZ) using angiopep-2 (a targeting ligand for low-density lipoprotein receptor-related protein 1) and anti-CD133 monoclonal antibody. Angiopep-2 helps in the transcytosis of blood–brain barrier, whereas anti-CD133 aids in specific delivery to glioblastoma stem cells (GSCs). The dual-targeting immunoliposome increased *in vitro* cytotoxicity by 425- and 181-folds in U87MG GSCs compared to free TMZ and non-targeted TMZ liposomes. The platform also helped in reducing the tumor size and increasing median survival time when administered to tumor-bearing mice (orthotopically-implanted brain tumor model) [255]. Although relatively unexplored, biomarker targeting can also be utilized to deliver small-molecule inhibitors that function by disrupting key CSCs developmental pathways (like Notch, Wnt/β-catenin, and Hedgehog) [256,257].

7. Concluding remarks

As evident from the numerous cutting-edge research investigations discussed in this review, biomaterials hold tremendous potential in enhancing the clinical outcomes of cancer immunotherapy. Conventional cancer immunotherapy strategies that include TCR-/CAR-engineered T cell therapy and ICB-based treatment modalities have already brought a paradigm shift in cancer management and revolutionized the way new cases of cancer are

treated in many developed countries. Considering their preliminary nature, the cost of establishing medical facilities that can administer these therapies is substantial; hence it confines the mass reach. In such a scenario, the advances made in the domains of biomaterial, nanotechnology, and material science can be synergized to develop advanced platforms that strategically deliver a wide variety of commercially available immunostimulatory agents. As discussed previously, immunostimulatory agents serve as unique tools for the immunogenic modulation of cancer. Their direct localization at the tumor site can serve as an *in-situ* antigen depot for the stimulation of immunogenic pathways. Moreover, their incorporation into the biomaterial-based delivery platform can be engineered to specifically target and alleviate the immunosuppressive effect of TME on key immune components. By doing so, the immunologically 'cold' nature of the TME can be eliminated. Some of the important benefits of developing biomaterial-based delivery platforms are their modular nature and straightforward application. Based on the physiochemical nature of the immunostimulatory cargo, the biomaterial can be modified/functionalized to control the *in vivo* distribution, thereby capitalizing on bioavailability while keeping in check unwanted systemic exposure and off-target side effects. With newer molecular adjuvants constantly being developed, emphasis must be laid on exploring such adjuvants that have site-specific yet potent immunotherapeutic effects. One such category is STING agonists, which generate a robust adaptive immune response by triggering the production of IFNs (type I) and proinflammatory cytokines/chemokines at a molecular level. Developing STING agonists with improved pharmacokinetic properties is a lucrative avenue for immunotherapy research. In cases where the use of a single agent is inadequate, biomaterials can be optimized and tuned to co-load different agents (preferably functioning via stand-alone as well as biologically independent pathways) for multistep immune targeting.

A major portion of this review focuses on utilizing various biomaterials like polymers, lipids, carbon-based nanocarriers (graphene, CNTs), MNPs, and cell-derived components for cancer immunoregulation. The niche advantages of the individual platform, along with their limitations, were briefly summarized. Apart from the direct delivery of immunostimulatory agents, how these biomaterials can be exploited to target the immune cell population and TME component has been extensively discussed. Over the past few years, an increasing understanding of tumor immunology and antitumor immune response has helped establish CSCs as a critical yet highly ignored component of TME. CSCs directly modulate chemoresistance, tumor relapse, and metastasis. CSCs, through their signaling pathways and cross-talk with other cellular components, contribute significantly to the creation and maintenance of an immunosuppressive TME. From an immunological point-of-view, dealing with CSCs is crucial as it directly induces genetic and nongenetic alterations responsible for immune evasion by means of reduced immune recognition, depletion of TAAs, and enhanced tolerance to cytotoxic effects of immunity. While noteworthy progress has been made in targeting CSCs via adoptive DC-based vaccine, T cell therapy, and oncolytic virotherapy, the utilization of biomaterial-based platforms for CSC-targeted immunotherapy is in its infancy phase. Few studies do exist that exploit CSC-specific surface markers for the targeted delivery of immune adjuvants, the majority of the literature available focuses on the delivery of chemotherapeutic drugs. Nonetheless, the abolition of CSCs has indirect pro-immunogenic benefits. As knowledge about CSCs' epigenetic profiles and functional signaling pathways increases, we as a scientific fraternity can expect new biomolecules that immunogenically regulate CSCs to be available in the near future.

While the juncture of biomaterials and cancer immunotherapy is bound to remain a hot research topic for the next few years, conscious emphasis must be made while developing these platforms for their efficient translation from academic laboratories into commercial products. At present, many roadblocks exist to any potential commercialization of biomaterial-based immunotherapeutics. Firstly, the designed platform should be suitable for large-scale production. The fabrication process should be reproducible while being as simple as possible. Any complex synthesis or quality-related issues would directly increase the cost of the end product. It is for the same reason that the majority of FDA-approved nanomedicines are confined to simple liposomes and polymeric NPs. Second, biological and safety-related issues come into the picture. Considering the heterogeneous nature of the disease, high patient-to-patient variability is observed in cancers of the same type. In such a scenario, the therapeutic outcomes in animal models may not replicate in a clinical setting considering the inherent differences in immune systems between laboratory animals and humans. A possible solution to this is the optimization of dose and administration routes using clinically relevant higher animal models. Additionally, care must be taken that the use of proper treatment controls, adequate randomization, and robust data analysis is implemented. Any significant late-stage alteration in the formulation demands additional experiments for safety assessment. The last roadblock lies in the form of inadequate support from global regulatory agencies. The lack of clear-cut regulatory and safety guidelines bottlenecks the timely development of novel nanotechnology-based medicines. This highlights a need for a global collaborative platform that can synchronize academic research bodies and research industries with regulatory bodies.

Moving forward, future research works can focus on identifying the most appropriate immune adjuvant based on the progression state of cancer. If such decisive information can be obtained, biomaterial-based platforms can be utilized for loading multiple immunomodulatory agents. The platform can be bioengineered to deliver these agents in a coordinated/programmed manner. Hypothetically, such a system can allow an on-demand release of the most effective immunomodulator (among several). As the treatment progresses, the system can switch to a different immunomodulator which can be more clinically appropriate depending on the observed therapeutic outcomes. While multi-component systems already exist, designing a platform that can distinguish and synchronize loaded cargo for release is extremely complex. Furthermore, as many biomaterials directly activate immunostimulatory pathways (in the absence of an auxiliary immune signal), fundamental investigations into biomaterial-immune cell interactions are the need of the hour to exploit the full potential of biomaterials. Overall, the use of biomaterial opens up varied and lucrative avenues for taking cancer immunotherapy research to the next level. Strategically implementation of biomaterial with immunostimulatory agents holds the potential to revolutionize cancer management and drastically enhance the quality of life of patients that get diagnosed with cancer.

Declaration of Competing Interest

The authors declare that they do have any conflict of interest.

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References

[1] S. Jhunjhunwala, C. Hammer, L. Delamarre, Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion, Nat. Rev. Cancer. 21 (5) (2021) 298–312.
[2] M. Yang, O.T. Olaoba, C. Zhang, E.T. Kimchi, K.F. Staveley-O'Carroll, G. Li, Cancer immunotherapy and delivery system: an update, Pharmaceutics 14 (8) (2022) 1630.
[3] S. Zhu, T. Zhang, L. Zheng, H. Liu, W. Song, D. Liu, Z. Li, C.-x. Pan, Combination strategies to maximize the benefits of cancer immunotherapy, J. Hematol. Oncol. 14 (1) (2021) 156.
[4] S. Taefehshokr, A. Parhizkar, S. Hayati, M. Mousapour, A. Mahmoudpour, L. Eleid, D. Rahmanpour, S. Fattahi, H. Shabani, N. Taefehshokr, Cancer immunotherapy: challenges and limitations, Pathol. Res. Pract. 229 (2022) 153723.
[5] T. Fu, L.-J. Dai, S.-Y. Wu, Y. Xiao, D. Ma, Y.-Z. Jiang, Z.-M. Shao, Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic response, J. Hematol. Oncol. 14 (1) (2021) 98.
[6] T.A. Barnes, E. Amir, HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer, Br. J. Cancer. 117 (4) (2017) 451–460.
[7] D. Bruni, H.K. Angell, J. Galon, The immune contexture and immunoscore in cancer prognosis and therapeutic efficacy, Nat. Rev. Cancer. 20 (11) (2020) 662–680.
[8] M. Dysthe, R. Parihar, Myeloid-derived suppressor cells in the tumor microenvironment, Tumor Microenviron. (2020) 117–140.
[9] C. Li, X. Xu, S. Wei, P. Jiang, L. Xue, J. Wang, Tumor-associated macrophages: potential therapeutic strategies and future prospects in cancer, J. Immunother. Cancer 9 (1) (2021).
[10] Y. Ni, X. Zhou, J. Yang, H. Shi, H. Li, X. Zhao, X. Ma, The role of tumor-stroma interactions in drug resistance within tumor microenvironment, Front. Cell Dev. Biol. 9 (2021).
[11] T. Liu, C. Han, S. Wang, P. Fang, Z. Ma, L. Xu, R. Yin, Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy, J. Hematol. Oncol. 12 (1) (2019) 1–15.
[12] L. Rocamora-Reverte, F.L. Melzer, R. Würzner, B. Weinberger, The complex role of regulatory T cells in immunity and aging, Front. Immunol. (2021) 3566.
[13] R.D. Schreiber, L.J. Old, M.J. Smyth, Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion, Science (2011).
[14] M.M. Gubin, M.D. Vesely, Cancer Immunoediting in the Era of Immuno-oncology, Clin. Cancer Res. 28 (18) (2022) 3917–3928.
[15] P. Yousefpour, K. Ni, D.J. Irvine, Targeted modulation of immune cells and tissues using engineered biomaterials, Nat. Rev. Dis. Primers (2023) 1–18.
[16] S.J. Tsai, S.K. Black, C.M. Jewell, Leveraging the modularity of biomaterial carriers to tune immune responses, Adv. Funct. Mater. 30 (48) (2020) 2004119.
[17] A.D. Ariawan, J. van Eersel, A.D. Martin, Y.D. Ke, L.M. Ittner, Recent progress in synthetic self-adjuvanting vaccine development, Biomater Sci (2022).
[18] S. Mule, M. Handa, R. Shukla, in: Regulatory Landscapes in Approval of Cancer Vaccines, Hormone Related Cancer Mechanistic and Nanomedicines: Challenges and Prospects, Springer, 2023, pp. 325–347.
[19] B. Sun, T. Xia, Nanomaterial-based vaccine adjuvants, J. Mater. Chem. B 4 (33) (2016) 5496–5509.
[20] Z. Liao, J. Huang, P.-C. Lo, J.F. Lovell, H. Jin, K. Yang, Self-adjuvanting cancer nanovaccines, J. Nanobiotechnol. 20 (1) (2022) 1–20.
[21] Q. Xiao, X. Li, Y. Li, Z. Wu, C. Xu, Z. Chen, W. He, Biological drug and drug delivery-mediated immunotherapy, Acta Pharm. Sin. B 11 (4) (2021) 941–960.
[22] S. Han, J. Wu, Three-dimensional (3D) scaffolds as powerful weapons for tumor immunotherapy, Bioact. Mater. (2022).
[23] Y. Chao, Z. Liu, Biomaterials tools to modulate the tumour microenvironment in immunotherapy, Nat. Rev. Dis. Primers (2023).
[24] N. Cunningham, R. Lapointe, S. Lerouge, Biomaterials for enhanced immunotherapy, APL Bioeng. 6 (4) (2022) 041502.
[25] Y. Bo, H. Wang, Biomaterial-based in situ cancer vaccine, Adv. Mater. (2023) 2210452, doi:10.1002/adma.202210452.
[26] G. Ferrer, D. Álvarez-Errico, M. Esteller, Biological and molecular factors predicting response to adoptive cell therapies in cancer, J. Natl. Cancer Inst. 114 (7) (2022) 930–939.
[27] S.C. Wei, C.R. Duffy, J. Allison, Fundamental mechanisms of immune checkpoint blockade therapy fundamental mechanisms of immune checkpoint blockade therapy, Cancer Discov. 8 (9) (2018) 1069–1086.
[28] E. Grimmett, B. Al-Share, M.B. Alkassab, R.W. Zhou, A. Desai, M.M.A Rahim, I. Woldie, Cancer vaccines: past, present and future; a review article, Discov. Oncol. 13 (1) (2022) 1–17.
[29] T.R. Ghimire, The mechanisms of action of vaccines containing aluminum adjuvants: an in vitro vs in vivo paradigm, Springerplus 4 (1) (2015) 1–18.
[30] E.J. Ko, S.M. Kang, Immunology and efficacy of MF59-adjuvanted vaccines, Hum. Vaccines Immunother. 14 (12) (2018) 3041–3045.
[31] C. Pifferi, R. Fuentes, A. Fernández-Tejada, Natural and synthetic carbohydrate-based vaccine adjuvants and their mechanisms of action, Nat. Rev. Chem. 5 (3) (2021) 197–216.
[32] A. Hernandez, J.K. Bohannon, L. Luan, B.A. Fensterheim, Y. Guo, N.K. Patil, C. McAdams, J. Wang, E.R. Sherwood, The role of MyD88-and TRIF-dependent signaling in monophosphoryl lipid A-induced expansion and recruitment of innate immunocytes, J. Leukoc. Biol. 100 (6) (2016) 1311–1322.
[33] B. Cui, X. Liu, Y. Fang, P. Zhou, Y. Zhang, Y. Wang, Flagellin as a vaccine adjuvant, Expert Rev. Vaccines 17 (4) (2018) 335–349.
[34] K. Dzierzbicka, A. Wardowska, P.J. Trzonkowski, Recent developments in the synthesis and biological activity of muramylpeptides, Curr. Med. Chem. 18 (16) (2011) 2438–2451.
[35] C. Bode, G. Zhao, F. Steinhagen, T. Kinjo, D. Klinman, CpG DNA as a vaccine adjuvant, Expert Rev. Vaccines 10 (4) (2011) 499–511.
[36] G.M. Lynn, C. Sedlik, F. Baharom, Y. Zhu, R.A. Ramirez-Valdez, V.L. Coble, K. Tobin, S.R. Nichols, Y. Itzkowitz, N. Zaidi, Peptide–TLR-7/8a conjugate vaccines chemically programmed for nanoparticle self-assembly enhance CD8 T-cell immunity to tumor antigens, Nat. Biotechnol 38 (3) (2020) 320–332.
[37] S. Palchetti, D. Starace, P. De Cesaris, A. Filippini, E. Ziparo, A. Riccioli, Transfected poly (I: C) activates different dsRNA receptors, leading to apoptosis or immunoadjuvant response in androgen-independent prostate cancer cells, J. Biol. Chem. 290 (9) (2015) 5470–5483.
[38] Q. Chen, L. Sun, Z. Chen, Regulation and function of the cGAS–STING pathway of cytosolic DNA sensing, Nat. Immunol 17 (10) (2016) 1142–1149.
[39] M.E. Logtenberg, F.A. Scheeren, T.N Schumacher, The CD47-SIRPα immune checkpoint, Immunity 52 (5) (2020) 742–752.
[40] M. Hassn Mesrati, S.E. Syafruddin, M.A. Mohtar, A. Syahir, CD44: A multifunctional mediator of cancer progression, Biomolecules 11 (12) (2021) 1850.
[41] O. Gires, M. Pan, H. Schinke, M. Canis, P. Baeuerle, M. Reviews, Expression and function of epithelial cell adhesion molecule EpCAM: where are we after 40 years? Cancer Metastasis Rev. 39 (3) (2020) 969–987.
[42] V.P. Brahmkhatri, C. Prasanna, H.S. Atreya, Insulin-like growth factor system in cancer: novel targeted therapies, Biomed Res. Int. 2015 (2015), doi:10.1155/2015/538019.
[43] A. Koval, V. Purvanov, D. Egger-Adam, V.L. Katanaev, Yellow submarine of the Wnt/Frizzled signaling: submerging from the G protein harbor to the targets, Biochem. Pharmacol. 82 (10) (2011) 1311–1319.
[44] S.H. Ross, D.A. Cantrell, Signaling and function of interleukin-2 in T lymphocytes, Annu. Rev. Immunol. 36 (2018) 411.
[45] J. Lin, Z. Zhu, H. Xiao, M.R. Wakefield, V.A. Ding, Q. Bai, Y. Fang, The role of IL-7 in immunity and cancer, Anticancer Res. 37 (3) (2017) 963–967.
[46] J.C. Steel, T.A. Waldmann, J.C Morris, Interleukin-15 biology and its therapeutic implications in cancer, Trends Pharmacol. Sci. 33 (1) (2012) 35–41.
[47] C.M.L. de Padilla, T.B. Niewold, The type I interferons: Basic concepts and clinical relevance in immune-mediated inflammatory diseases, Gene 576 (1) (2016) 14–21.
[48] P. Bhattacharya, M. Thiruppathi, H.A. Elshabrawy, K. Alharshawi, P. Kumar, B.S. Prabhakar, GM-CSF: An immune modulatory cytokine that can suppress autoimmunity, Cytokine 75 (2) (2015) 261–271.
[49] T.M. Le, A.R. Yoon, T. Thambi, C.O. Yun, Polymeric systems for cancer immunotherapy: a review, Front. Immunol. 13 (2022) 826876.
[50] E.S. Lee, J.M. Shin, S. Son, H. Ko, W. Um, S.H. Song, J.A. Lee, J.H.J.A.H.M. Park, Recent advances in polymeric nanomedicines for cancer immunotherapy, Adv. Healthc. Mater. 8 (4) (2019) 1801320.
[51] C.G. Da Silva, M.G. Camps, T.M. Li, L. Zerrillo, C.W. Löwik, F. Ossendorp, L.J. Cruz, Effective chemoimmunotherapy by co-delivery of doxorubicin and immune adjuvants in biodegradable nanoparticles, Theranostics 9 (22) (2019) 6485–6500.
[52] S. Sheikhzadeh, N. Delirezh, R. Hobbenaghi, Mannosylated polylactic-co-glycolic acid (MN-PLGA) nanoparticles induce potent anti-tumor immunity in murine model of breast cancer, Pharmacotherapy 142 (2021) 111962.
[53] K. Bhatt, L.J. Eggermont, S.A. Bencherif, Polymeric scaffolds for antitumor immune cell priming, in: Engineering Technologies and Clinical Translation, Elsevier, 2022, pp. 63–95.
[54] G. Ji, Y. Zhang, X. Si, H. Yao, S. Ma, Y. Xu, J. Zhao, C. Ma, C. He, Z. Tang, Biopolymer immune implants' sequential activation of innate and adaptive immunity for colorectal cancer postoperative immunotherapy, Adv. Mater. 33 (3) (2021) 2004559.
[55] A.N Ilinskaya, M.A Dobrovolskaia, A. pharmacology, Understanding the immunogenicity and antigenicity of nanomaterials: Past, present and future, Toxicol. Appl. Pharmacol. 299 (2016) 70–77.
[56] B. Li, Z. Yuan, H.C. Hung, J. Ma, P. Jain, C. Tsao, J. Xie, P. Zhang, X. Lin, K. Wu, Revealing the immunogenic risk of polymers, Angew. Chem. Int. Ed. 57 (42) (2018) 13873–13876.
[57] A. Gao, X.L. Hu, M. Saeed, B.F. Chen, Y.P. Li, H.J. Yu, Overview of recent advances in liposomal nanoparticle-based cancer immunotherapy, Acta Pharmacol. Sin. 40 (9) (2019) 1129–1137.
[58] D. Guimarães, A. Cavaco-Paulo, E. Nogueira, Design of liposomes as drug delivery system for therapeutic applications, Int. J. Pharm. 601 (2021) 120571.
[59] D. Chatzikleanthous, D.T. O'Hagan, R. Adamo, Lipid-based nanoparticles for delivery of vaccine adjuvants and antigens: toward multicomponent vaccines, Int. J. Pharm. 18 (8) (2021) 2867–2888.
[60] V.V. Andra, S. Pammi, L.V. Bhatraju, L.K. Ruddaraju, A comprehensive review on novel liposomal methodologies, commercial formulations, clinical trials and patents, J. Bionanosci. 12 (1) (2022) 274–291.
[61] S. Mizrahy, I. Hazan-Halevy, D. Landesman-Milo, B.D. Ng, D. Peer, Advanced strategies in immune modulation of cancer using lipid-based nanoparticles, Front. Immunol. 8 (2017) 69.
[62] Y. Zi, K. Yang, J. He, Z. Wu, J. Liu, W. Zhang, Strategies to enhance drug delivery to solid tumors by harnessing the EPR effects and alternative targeting mechanisms, Adv. Drug. Deliv. Rev. (2022) 114449.
[63] W.H. Li, Y.M. Li, Chemical strategies to boost cancer vaccines, Chem. Rev. 120 (20) (2020) 11420–11478.

[64] F.Y. Glassman, R. Dingman, H.C. Yau, S.V. Balu-Iyer, Biological function and immunotherapy utilizing phosphatidylserine-based nanoparticles, Immunol. Invest. 49 (7) (2020) 858–874.
[65] M. Rastogi, R.N. Saha, A. Alexander, G. Singhvi, A. Puri, S.K. Dubey, Role of stealth lipids in nanomedicine-based drug carriers, Chem. Phys. Lipids 235 (2021) 105036.
[66] Q. Su, C. Wang, H. Song, C. Zhang, J. Liu, P. Huang, Y. Zhang, J. Zhang, W. Wang, Co-delivery of anionic epitope/CpG vaccine and IDO inhibitor by self-assembled cationic liposomes for combination melanoma immunotherapy, J. Mater. Chem. B 9 (18) (2021) 3892–3899.
[67] Z.N. Huang, L.E. Cole, C.E. Callmann, S. Wang, C.A. Mirkin, Sequence multiplicity within spherical nucleic acids, ACS Nano. 14 (1) (2020) 1084–1092.
[68] S.K. Yadava, S.M. Basu, M. Chauhan, K. Sharma, A. Pradhan, V. Remya, J. Giri, Low temperature, easy scaling up method for development of smart nanostructure hybrid lipid capsules for drug delivery application, Colloids Surf. B: Biointerfaces 190 (2020) 110927.
[69] F. Sciolla, D. Truzzolillo, E. Chauveau, S. Trabalzini, L. Di Marzio, M. Carafa, C. Marianecci, A. Sarra, F. Bordi, S. Sennato, Influence of drug/lipid interaction on the entrapment efficiency of isoniazid in liposomes for antitubercular therapy: a multi-faced investigation, Colloids Surf. B: Biointerfaces 208 (2021) 112054.
[70] L. Tang, J. Li, T. Pan, Y. Yin, Y. Mei, Q. Xiao, R. Wang, Z. Yan, W. Wang, Versatile carbon nanoplatforms for cancer treatment and diagnosis: strategies, applications and future perspectives, Theranostics 12 (5) (2022) 2290–2321.
[71] P.R. Riley, R.J. Narayan, Recent advances in carbon nanomaterials for biomedical applications: A review, Curr. Opin. Biomed. Eng. 17 (2021) 100262.
[72] S. Azevedo, R. Costa-Almeida, S.G. Santos, F.D. Magalhães, A.M. Pinto, Advances in carbon nanomaterials for immunotherapy, Appl. Mater. Today 27 (2022) 101397.
[73] A. Hussain, Y. Weng, Y. Huang, Graphene and graphene-based nanomaterials: current applications and future perspectives, Drug Delivery Using Nanomaterials (2022) 209–228.
[74] W. Cao, L. He, W. Cao, X. Huang, K. Jia, J. Dai, Recent progress of graphene oxide as a potential vaccine carrier and adjuvant, Acta Biomater. 112 (2020) 14–28.
[75] X. Wang, F. Cao, M. Yan, Y. Liu, X. Zhu, H. Sun, G. Ma, Alum-functionalized graphene oxide nanocomplexes for effective anticancer vaccination, Acta Biomater. 83 (2019) 390–399.
[76] E.N. de Carvalho Lima, R.S. Diaz, J.F. Justo, J.R. Piqueira, Advances and perspectives in the use of carbon nanotubes in vaccine development, Int. J. Nanomed. 16 (2021) 5411–5435.
[77] H.A. Hassan, S.S. Diebold, L.A. Smyth, A.A. Walters, G. Lombardi, K.T. Al-Jamal, Application of carbon nanotubes in cancer vaccines: Achievements, challenges and chances, J. Control. Release 297 (2019) 79–90.
[78] Q. Xia, C. Gong, F. Gu, Z. Wang, C. Hu, L. Zhang, L. Qiang, X. Ding, S. Gao, Y. Gao, Functionalized multi-walled carbon nanotubes for targeting delivery of immunostimulatory CpG oligonucleotides against prostate cancer, J. Biomed. Nanotechnol. 14 (9) (2018) 1613–1626.
[79] D. Li, M. Ahmed, A. Khan, L. Xu, A.A. Walters, B. Ballesteros, K.T. Al-Jamal, Tailoring the architecture of cationic polymer brush-modified carbon nanotubes for efficient sirna delivery in cancer immunotherapy, ACS Appl. Mater. Interfaces 13 (26) (2021) 30284–30294.
[80] T. Tieu, M. Alba, R. Elnathan, A. Cifuentes-Rius, N.H. Voelcker, Advances in porous silicon–based nanomaterials for diagnostic and therapeutic applications, Adv. Therap. 2 (1) (2019) 1800095.
[81] M. Manzano, M. Vallet-Regí, Mesoporous silica nanoparticles for drug delivery, Adv. Funct. Mater. 30 (2) (2020) 1902634.
[82] A.N. Frickenstein, J.M. Hagood, C.N. Britten, B.S. Abbott, M.W. McNally, C.A. Vopat, E.G. Patterson, W.M. MacCuaig, A. Jain, K.B. Walters, Mesoporous silica nanoparticles: Properties and strategies for enhancing clinical effect, Pharmaceutics 13 (4) (2021) 570.
[83] J. Tu, A.L. Boyle, H. Friedrich, P.H. Bomans, J. Bussmann, N.A. Sommerdijk, W. Jiskoot, A. Kros, Mesoporous silica nanoparticles with large pores for the encapsulation and release of proteins, ACS Appl. Mater. Interfaces 8 (47) (2016) 32211–32219.
[84] K. Möller, T. Bein, Talented mesoporous silica nanoparticles, Chem. Mater. 29 (1) (2017) 371–388.
[85] R. Huang, Y.W. Shen, Y.Y. Guan, Y.X. Jiang, Y. Wu, K. Rahman, L.J. Zhang, H.J. Liu, X. Luan, Mesoporous silica nanoparticles: Facile surface functionalization and versatile biomedical applications in oncology, Acta Biomater. 116 (2020) 1–15.
[86] Z. Li, J. Zhang, J. Du, B. Han, J. Wang, Preparation of silica microrods with nano-sized pores in ionic liquid microemulsions, Colloids Surf. A 286 (1-3) (2006) 117–120.
[87] W.A. Li, B.Y. Lu, L. Gu, Y. Choi, J. Kim, D.J. Mooney, The effect of surface modification of mesoporous silica micro-rod scaffold on immune cell activation and infiltration, Biomaterials 83 (2016) 249–256.
[88] T.L. Nguyen, Y. Choi, J. Kim, Mesoporous silica as a versatile platform for cancer immunotherapy, Adv. Mater. 31 (34) (2019) 1803953.
[89] Y. Yang, F. Chen, N. Xu, Q. Yao, R. Wang, X. Xie, F. Zhang, Y. He, D. Shao, W.F. Dong, Red-light-triggered self-destructive mesoporous silica nanoparticles for cascade-amplifying chemo-photodynamic therapy favoring antitumor immune responses, Biomaterials 281 (2022) 121368.
[90] Y.P. Chen, L. Xu, T.W. Tang, C.H. Chen, Q.H. Zheng, T.P. Liu, C.Y. Mou, C.H. Wu, S.H. Wu, STING activator c-di-GMP-loaded mesoporous silica nanoparticles enhance immunotherapy against breast cancer, ACS Appl. Mater. Interfaces 12 (51) (2020) 56741–56752.
[91] J. Kim, W.A. Li, Y. Choi, S.A. Lewin, C.S. Verbeke, G. Dranoff, D.J. Mooney, Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy, Nat. Biotechnol. 33 (1) (2015) 64–72.
[92] A.W. Li, M.C. Sobral, S. Badrinath, Y. Choi, A. Graveline, A.G. Stafford, J.C. Weaver, M.O. Dellacherie, T.Y. Shih, O.A. Ali, A facile approach to enhance antigen response for personalized cancer vaccination, Nat. Mater. 17 (6) (2018) 528–534.
[93] A.S. Cheung, D.K. Zhang, S.T. Koshy, D.J. Mooney, Scaffolds that mimic antigen-presenting cells enable ex vivo expansion of primary T cells, Nat. Biotechnol. 36 (2) (2018) 160–169.
[94] E.R. Evans, P. Bugga, V. Asthana, R. Drezek, Metallic nanoparticles for cancer immunotherapy, Mater. Today 21 (6) (2018) 673–685.
[95] A. Mohapatra, P. Sathiyamoorthy, I.K. Park, Metallic nanoparticle-mediated immune cell regulation and advanced cancer immunotherapy, Pharmaceutics 13 (11) (2021) 1867.
[96] J. Li, H. Ren, Y. Zhang, Metal-based nano-vaccines for cancer immunotherapy, Coord. Chem. Rev. 455 (2022) 214345.
[97] S. Chung, R. Revia, M. Zhang, Iron oxide nanoparticles for immune cell labeling and cancer immunotherapy, Nanoscale Horiz. 6 (9) (2021) 696–717.
[98] J.S. He, S.J. Liu, Y.R. Zhang, X.D. Chu, Z.B. Lin, Z. Zhao, S.H. Qiu, Y.G. Guo, H. Ding, Y.L. Pan, The application of and strategy for gold nanoparticles in cancer immunotherapy, Front. pharmacol. 12 (2021) 1430.
[99] P. Mathur, S. Jha, S. Ramteke, N.K. Jain, Pharmaceutical aspects of silver nanoparticles, Artif. Cells Nanomed. Biotechnol. 46 (1) (2018) 115–126.
[100] A.V. Skalny, M. Aschner, Y. Jiang, Y.G. Gluhcheva, Y. Tizabi, R. Lobinski, A.A. Tinkov, Molecular mechanisms of aluminum neurotoxicity: Update on adverse effects and therapeutic strategies, in: Advances in Neurotoxicology, Elsevier, 2021, pp. 1–34.
[101] B. Ding, J. Yue, P. Zheng, J. Lin, Manganese oxide nanomaterials boost cancer immunotherapy, J. Mater. Chem. B. 9 (35) (2021) 7117–7131.
[102] P. Sharma, J.B. Shin, B.C. Park, J.W. Lee, S.W. Byun, N.Y. Jang, Y.J. Kim, Y. Kim, Y.K. Kim, N.H. Cho, Application of radially grown ZnO nanowires on poly-l-lactide microfibers complexed with a tumor antigen for cancer immunotherapy, Nanoscale 11 (10) (2019) 4591–4600.
[103] S. Mathavarajah, J. Salsman, G. Dellaire, An emerging role for calcium signalling in innate and autoimmunity via the cGAS-STING axis, cytokine growth factor, Rev 50 (2019) 43–51.
[104] S. Sungsuwan, Z. Yin, X. Huang, Lipopeptide-coated iron oxide nanoparticles as potential glycoconjugate-based synthetic anticancer vaccines, ACS Appl. Mater. Interfaces 7 (31) (2015) 17535–17544.
[105] H. Cai, F. Degliangeli, B. Palitzsch, B. Gerlitzki, H. Kunz, E. Schmitt, R. Fiammengo, U. Westerlind, Glycopeptide-functionalized gold nanoparticles for antibody induction against the tumor associated mucin-1 glycoprotein, Bioorg. Med. Chem. 24 (5) (2016) 1132–1135.
[106] K.H. Huynh, X.H. Pham, J. Kim, S.H. Lee, H. Chang, W.Y. Rho, B.H. Jun, Synthesis, properties, and biological applications of metallic alloy nanoparticles, Int. J. Mol. Sci. 21 (14) (2020) 5174.
[107] Z. Meng, Y. Zhang, X. Zhou, J. Ji, Z. Liu, Nanovaccines with cell-derived components for cancer immunotherapy, Adv. Drug. Deliv. Rev. (2022) 114107.
[108] M. Qin, G. Du, N. Qiao, Z. Guo, M. Jiang, C. He, S. Bai, P. He, Y. Xu, H. Wang, Whole-cell-mimicking carrier-free nanovaccines amplify immune responses against cancer and bacterial infection, Adv. Funct. Mater. 32 (10) (2022) 2108917.
[109] S.A. Sadeghi Najafabadi, A. Bolhassani, M.R. Aghasadeghi, Tumor cell-based vaccine: an effective strategy for eradication of cancer cells, Immunotherapy 14 (8) (2022) 639–654.
[110] Y. Ye, C. Wang, X. Zhang, Q. Hu, Y. Zhang, Q. Liu, D. Wen, J. Milligan, A. Bellotti, L. Huang, A melanin-mediated cancer immunotherapy patch, Sci. Immunol. 2 (17) (2017) eaan5692.
[111] W.C. Hwang, D. Song, H. Lee, C. Oh, S.H. Lim, H.J. Bae, N.D. Kim, G. Han, D.S. Min, Inhibition of phospholipase D1 induces immunogenic cell death and potentiates cancer immunotherapy in colorectal cancer, Exp. Mol. Med. 54 (9) (2022) 1563–1576.
[112] J. Gao, X. Dong, Z. Wang, Generation, purification and engineering of extracellular vesicles and their biomedical applications, Methods 177 (2020) 114–125.
[113] S. Araujo-Abad, M. Saceda, C. de Juan Romero, Biomedical application of small extracellular vesicles in cancer treatment, Adv. Drug Deliv. Rev. (2022) 114117.
[114] C. Liu, Y. Wang, L. Li, D. He, J. Chi, Q. Li, Y. Wu, Y. Zhao, S. Zhang, L. Wang, Engineered extracellular vesicles and their mimetics for cancer immunotherapy, J. Controlled Release 349 (2022) 679–698.
[115] M. Ahmadi, M. Hassanpour, J. Rezaie, Engineered extracellular vesicles: A novel platform for cancer combination therapy and cancer immunotherapy, Life Sci. (2022) 120935.
[116] Y. Chen, L. Wang, M. Zheng, C. Zhu, G. Wang, Y. Xia, E.J. Blumenthal, W. Mao, Y. Wan, Engineered extracellular vesicles for concurrent Anti-PDL1 immunotherapy and chemotherapy, Bioact. Mater. 9 (2022) 251–265.
[117] L. Huang, Y. Rong, X. Tang, K. Yi, P. Qi, J. Hou, W. Liu, Y. He, X. Gao, C. Yuan, Engineered exosomes as an in situ DC-primed vaccine to boost antitumor immunity in breast cancer, Mol. Cancer 21 (1) (2022) 1–19.
[118] W. Huang, L. Meng, Y. Chen, Z. Dong, Q. Peng, Bacterial outer membrane vesicles as potential biological nanomaterials for antibacterial therapy, Acta Biomater. 140 (2022) 102–115.

[119] X. Zhao, R. Zhao, G. Nie, Nanocarriers based on bacterial membrane materials for cancer vaccine delivery, Nat. Protoc. 17 (10) (2022) 2240–2274.
[120] K. Cheng, R. Zhao, Y. Li, Y. Qi, Y. Wang, Y. Zhang, H. Qin, Y. Qin, L. Chen, C. Li, J. Liang, Y. Li, J. Xu, X. Han, G.J. Anderson, J. Shi, L. Ren, X. Zhao, G. Nie, Bioengineered bacteria-derived outer membrane vesicles as a versatile antigen display platform for tumor vaccination via plug-and-display technology, Nat. Commun. 12 (1) (2021) 2041.
[121] J. Liang, K. Cheng, Y. Li, J. Xu, Y. Chen, N. Ma, Q. Feng, F. Zhu, X. Ma, T. Zhang, Personalized cancer vaccines from bacteria-derived outer membrane vesicles with antibody-mediated persistent uptake by dendritic cells, Fund. Res. 2 (1) (2022) 23–36.
[122] Q. Feng, X. Ma, K. Cheng, G. Liu, Y. Li, Y. Yue, J. Liang, L. Zhang, T. Zhang, X. Wang, Engineered bacterial outer membrane vesicles as controllable two-way adaptors to activate macrophage phagocytosis for improved tumor immunotherapy, Adv. Mater. 34 (40) (2022) 2206200.
[123] B.Q. Chen, Y. Zhao, Y. Zhang, Y.J. Pan, H.Y. Xia, R.K. Kankala, S.B. Wang, G. Liu, A.Z. Chen, Immune-regulating camouflaged nanoplatforms: A promising strategy to improve cancer nano-immunotherapy, Bioact. Mater. 21 (2023) 1–19.
[124] W. Lei, C. Yang, Y. Wu, G. Ru, X. He, X. Tong, S. Wang, Nanocarriers surface engineered with cell membranes for cancer targeted chemotherapy, J. Nanobiotechnol. 20 (1) (2022) 1–21.
[125] F. Raza, H. Zafar, S. Zhang, Z. Kamal, J. Su, W.E. Yuan, Q. Mingfeng, Recent advances in cell membrane-derived biomimetic nanotechnology for cancer immunotherapy, Adv. Healthc. Mater. 10 (6) (2021) 2002081.
[126] D. Wu, X. Shou, Y. Zhang, Z. Li, G. Wu, D. Wu, J. Wu, S. Shi, S. Wang, Cell membrane-encapsulated magnetic nanoparticles for enhancing natural killer cell-mediated cancer immunotherapy, Nanomed. Nanotechnol. Biol. Med. 32 (2021) 102333.
[127] X. Li, W. Zhang, J. Lin, H. Wu, Y. Yao, J. Zhang, C. Yang, T cell membrane cloaking tumor microenvironment-responsive nanoparticles with a smart “membrane escape mechanism” for enhanced immune-chemotherapy of melanoma, Biomater. Sci. 9 (9) (2021) 3453–3464.
[128] Y. Zhao, A. Li, L. Jiang, Y. Gu, J. Liu, Hybrid membrane-coated biomimetic nanoparticles (HM@ BNPs): a multifunctional nanomaterial for biomedical applications, Biomacromolecules 22 (8) (2021) 3149–3167.
[129] H.Y. Chen, J. Deng, Y. Wang, C.Q. Wu, X. Li, H.W. Dai, Hybrid cell membrane–coated nanoparticles: a multifunctional biomimetic platform for cancer diagnosis and therapy, Acta Biomater. 112 (2020) 1–13.
[130] S. Zang, K. Huang, J. Li, K. Ren, T. Li, X. He, Y. Tao, J. He, Z. Dong, M. Li, Metabolic reprogramming by dual-targeting biomimetic nanoparticles for enhanced tumor chemo-immunotherapy, Acta Biomater. (2022).
[131] Y. Wang, Z. Luan, C. Zhao, C. Bai, K. Yang, Target delivery selective CSF-1R inhibitor to tumor-associated macrophages via erythrocyte-cancer cell hybrid membrane camouflaged pH-responsive copolymer micelle for cancer immunotherapy, Eur. J. Pharm. Sci. 142 (2020) 105136.
[132] L. Hou, X. Gong, J. Yang, H. Zhang, W. Yang, X. Chen, Hybrid-membrane-decorated prussian blue for effective cancer immunotherapy via tumor-associated macrophages polarization and hypoxia relief, Adv. Mater. (2022) 2200389.
[133] A. Salah, H. Wang, Y. Li, M. Ji, W.B. Ou, N. Qi, Y. Wu, Insights into dendritic cells in cancer immunotherapy: From bench to clinical applications, Front. Cell Dev. Biol. 9 (2021) 1422.
[134] J. Calmeiro, M. Carrascal, C. Gomes, A. Falcão, M.T. Cruz, B.M. Neves, Biomaterial-based platforms for in situ dendritic cell programming and their use in antitumor immunotherapy, J. Immunother. Cancer 7 (1) (2019) 1–11.
[135] D.G. Leach, S. Young, J.D. Hartgerink, Advances in immunotherapy delivery from implantable and injectable biomaterials, Acta Biomater. 88 (2019) 15–31.
[136] S. Jin, X. Xia, J. Huang, C. Yuan, Y. Zuo, Y. Li, J. Li, Recent advances in PLGA-based biomaterials for bone tissue regeneration, Acta Biomater. 127 (2021) 56–79.
[137] O.A. Ali, E. Doherty, W.J. Bell, T. Fradet, J. Hudak, M.-T. Laliberte, D.J. Mooney, D.F. Emerich, The efficacy of intracranial PLG-based vaccines is dependent on direct implantation into brain tissue, J. Controlled Release 154 (3) (2011) 249–257.
[138] K. Lei, L. Tang, Surgery-free injectable macroscale biomaterials for local cancer immunotherapy, Biomater. Sci. 7 (3) (2019) 733–749.
[139] S.A. Bencherif, R.Warren Sands, O.A. Ali, W.A. Li, S.A. Lewin, T.M. Braschler, T.-Y. Shih, C.S. Verbeke, D. Bhatta, G. Dranoff, Injectable cryogel-based whole–cell cancer vaccines, Nat. Commun. 6 (1) (2015) 1–13.
[140] T.Y. Shih, S.O. Blacklow, A.W. Li, B.R. Freedman, S. Bencherif, S.T. Koshy, M.C. Darnell, D.J. Mooney, Injectable, tough alginate cryogels as cancer vaccines, Adv. Healthc. Mater. 7 (10) (2018) 1701469.
[141] M.K. Hossain, K.A. Wall, Use of dendritic cell receptors as targets for enhancing anti-cancer immune responses, Cancers 11 (3) (2019) 418.
[142] L.J. Cruz, R.A. Rosalia, J.W. Kleinovink, F. Rueda, C.W. Löwik, F.J. Ossendorp, Targeting nanoparticles to CD40, DEC-205 or CD11c molecules on dendritic cells for efficient CD8+ T cell response: A comparative study, J. Control. Release 192 (2014) 209–218.
[143] S. Watanabe, E. Yuba, T. Akazawa, V. Wijewardana, Y. Kakihara, A. Azuma, K. Hagimori, R. Kanegi, S. Hatoya, N. Inoue, Potent adjuvant effect elicited for tumor immunotherapy by a liposome conjugated pH-sensitive polymer and dendritic cell-targeting Toll-like-receptor ligand, Vaccine 40 (10) (2022) 1448–1457.
[144] J. Liu, X. Geng, J. Hou, G. Wu, New insights into M1/M2 macrophages: Key modulators in cancer progression, Cancer Cell Int. 21 (1) (2021) 1–7.
[145] G.R. Gunassekaran, S.M. Vadevoo, M.C. Baek, B. Lee, M1 macrophage exosomes engineered to foster M1 polarization and target the IL-4 receptor inhibit tumor growth by reprogramming tumor-associated macrophages into M1-like macrophages, Biomaterials 278 (2021) 121137.
[146] Z. Huang, Z. Zhang, Y. Jiang, D. Zhang, J. Chen, L. Dong, J. Zhang, Targeted delivery of oligonucleotides into tumor-associated macrophages for cancer immunotherapy, J. Control. Release 158 (2) (2012) 286–292.
[147] K. Mortezaee, Myeloid-derived suppressor cells in cancer immunotherapy–clinical perspectives, Life Sci. 277 (2021) 119627.
[148] X. Dai, L. Ren, M. Liu, H. Cai, H. Zhang, Q. Gong, Z. Gu, K. Luo, Nanomedicines modulating myeloid-derived suppressor cells for improving cancer immunotherapy, Nano Today 39 (2021) 101163.
[149] M.S. Sasso, G. Lollo, M. Pitorre, S. Solito, L. Pinton, S. Valpione, G. Bastiat, S. Mandruzzato, V. Bronte, I. Marigo, J.P Benoit, Low dose gemcitabine-loaded lipid nanocapsules target monocytic myeloid-derived suppressor cells and potentiate cancer immunotherapy, Biomaterials 96 (2016) 47–62.
[150] H. Phuengkham, C. Song, Y. Lim, A designer scaffold with immune nanoconverters for reverting immunosuppression and enhancing immune checkpoint blockade therapy, Adv. Mater. 31 (42) (2019) 1903242.
[151] Y. Ohue, H. Nishikawa, Regulatory T (Treg) cells in cancer: Can treg cells be a new therapeutic target? Cancer Sci. 110 (7) (2019) 2080–2089.
[152] H. Jonuleit, T. Bopp, C. Becker, Treg cells as potential cellular targets for functionalized nanoparticles in cancer therapy, Nanomedicine 11 (20) (2016) 2699–2709.
[153] C. Sacchetti, N. Rapini, A. Magrini, E. Cirelli, S. Bellucci, M. Mattei, N. Rosato, N. Bottini, M. Bottini, In vivo targeting of intratumor regulatory T cells using PEG-modified single-walled carbon nanotubes, Bioconjugate Chem. 24 (6) (2013) 852–858.
[154] M. Liu, Z. Li, W. Yao, X. Zeng, L. Wang, J. Cheng, B. Ma, R. Zhang, W. Min, H. Wang, IDO inhibitor synergized with radiotherapy to delay tumor growth by reversing T cell exhaustion, Mol. Med. Rep. 21 (1) (2020) 445–453.
[155] B. Feng, F. Zhou, B. Hou, D. Wang, T. Wang, Y. Fu, Y. Ma, H. Yu, Y. Li, Binary cooperative prodrug nanoparticles improve immunotherapy by synergistically modulating immune tumor microenvironment, Adv. Mater. 30 (38) (2018) 1803001.
[156] K.R. Rhodes, J.J. Green, Nanoscale artificial antigen presenting cells for cancer immunotherapy, Mol. Immunol. 98 (2018) 13–18.
[157] M. Oelke, C. Krueger, R.L. Giuntoli II, J.P. Schneck, Artificial antigen-presenting cells: artificial solutions for real diseases, Trends Mol. Med. 11 (9) (2005) 412–420.
[158] M. Schluck, L.J. Eggermont, J. Weiden, C. Popelier, L. Weiss, B. Pilzecker, S. Kolder, A. Heinemans, C. Rodriguez Mogeda, M. Verdoes, C.G. Figdor, R. Hammink, Dictating phenotype, function, and fate of human T cells with co-stimulatory antibodies presented by filamentous immune cell mimics, Adv. Ther. 5 (4) (2022) 2200019.
[159] S.C. Balmert, S.R. Little, Biomimetic delivery with micro-and nanoparticles, Adv. Mater. 24 (28) (2012) 3757–3778.
[160] E.R. Steenblock, T. Fadel, M. Labowsky, J.S. Pober, T.M. Fahmy, An artificial antigen-presenting cell with paracrine delivery of IL-2 impacts the magnitude and direction of the T cell response, J. Biol. Chem. 286 (40) (2011) 34883–34892.
[161] B. Prakken, M. Wauben, D. Genini, R. Samodal, J. Barnett, A. Mendivil, L. Leoni, S. Albani, Artificial antigen-presenting cells as a tool to exploit the immunesynapse, Nat. Med. 6 (12) (2000) 1406–1410.
[162] Q. Ding, J. Chen, X. Wei, W. Sun, J. Mai, Y. Yang, Y. Xu, RAFTsomes containing epitope-MHC-II complexes mediated CD4+ T cell activation and antigen-specific immune responses, Pharm. Res. 30 (1) (2013) 60–69.
[163] F. Giannoni, J. Barnett, K. Bi, R. Samodal, P. Lanza, P. Marchese, R. Billetta, R. Vita, M.R. Klein, B. Prakken, Clustering of T cell ligands on artificial APC membranes influences T cell activation and protein kinase C θ translocation to the T cell plasma membrane, J. Immunol. 174 (6) (2005) 3204–3211.
[164] R. Zappasodi, M. Di Nicola, C. Carlo-Stella, R. Mortarini, A. Molla, C. Vegetti, S. Albani, A. Anichini, A. Gianni, The effect of artificial antigen-presenting cells with preclustered anti-CD28/-CD3/-LFA-1 monoclonal antibodies on the induction of ex vivo expansion of functional human antitumor T cells, Haematologica 93 (10) (2008) 1523–1534.
[165] E.R. Steenblock, T.M. Fahmy, A comprehensive platform for ex vivo T-cell expansion based on biodegradable polymeric artificial antigen-presenting cells, Mol. Ther. 16 (4) (2008) 765–772.
[166] R.A. Meyer, J.C. Sunshine, K. Perica, A.K. Kosmides, K. Aje, J.P. Schneck, J.J. Green, Biodegradable nanoellipsoidal artificial antigen presenting cells for antigen specific T-cell activation, Small 11 (13) (2015) 1519–1525.
[167] B.L. Levine, W.B. Bernstein, M. Connors, N. Craighead, T. Lindsten, C.B. Thompson, C.H. June, Effects of CD28 costimulation on long-term proliferation of CD4+ T cells in the absence of exogenous feeder cells, J. Immunol. 159 (12) (1997) 5921–5930.
[168] N.M. Hardy, M.E. Mossoba, S.M. Steinberg, V. Fellowes, X.Y. Yan, F.T. Hakim, R.R. Babb, D. Avila, J. Gea-Banacloche, C. Sportès, B.L. Levine, Phase I trial of adoptive cell transfer with mixed-profile type-I/type-II allogeneic T cells for metastatic breast cancer, Clin. Cancer Res. 17 (21) (2011) 6878–6887.
[169] K. Perica, A. Tu, A. Richter, J.G. Bieler, M. Edidin, J.P Schneck, Magnetic field--induced T cell receptor clustering by nanoparticles enhances T cell activation and stimulates antitumor activity, ACS Nano 8 (3) (2014) 2252–2260.
[170] K. Lee, Y. Yi, Y. Yu, Remote control of T cell activation using magnetic Janus particles, Angew. Chem. Int. Ed. 55 (26) (2016) 7384–7387.

[171] P.J. Wipff, D.B. Rifkin, J.J. Meister, B. Hinz, Myofibroblast contraction activates latent TGF-β1 from the extracellular matrix, J. Cell Biol. (2007).
[172] A. Leask, D.J. Abraham, TGF-β signaling and the fibrotic response, FASEB J. (2004).
[173] L. Wang, L. Cao, H. Wang, B. Liu, Q. Zhang, Z. Meng, X. Wu, Q. Zhou, K. Xu, Cancer-associated fibroblasts enhance metastatic potential of lung cancer cells through IL-6/STAT3 signaling pathway, OncoTargets Ther. 8 (2017) 76116–76128.
[174] H.E. Ryan, M. Poloni, W. McNulty, D. Elson, M. Gassmann, J.M. Arbeit, R.S. Johnson, Hypoxia-inducible factor-1α is a positive factor in solid tumor growth, Cancer Res. (2000).
[175] N. Erez, M. Truitt, P. Olson, D. Hanahan, Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-κB-dependent manner, Cancer Cell (2010).
[176] T. Fang, H. Lv, G. Lv, T. Li, C. Wang, Q. Han, L. Yu, B. Su, L. Guo, S. Huang, D. Cao, L. Tang, S. Tang, M. Wu, W. Yang, H. Wang, Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer, Nat. Commun. (2018).
[177] J. Albrengues, T. Bertero, E. Grasset, S. Bonan, M. Maiel, I. Bourget, C. Philippe, C. Herraiz Serrano, S. Benamar, O. Croce, V. Sanz-Moreno, G. Meneguzzi, C.C. Feral, G. Cristofari, C. Gaggioli, Epigenetic switch drives the conversion of fibroblasts into proinvasive cancer-associated fibroblasts, Nat. Commun. (2015).
[178] S. Dulauroy, S.E. Di Carlo, F. Langa, G. Eberl, L. Peduto, Lineage tracing and genetic ablation of ADAM12 + perivascular cells identify a major source of profibrotic cells during acute tissue injury, Nat. Med. (2012).
[179] C. Jotzu, E. Alt, G. Welte, J. Li, B.T. Hennessy, E. Devarajan, S. Krishnappa, S. Pinilla, L. Droll, Y.H. Song, Adipose tissue derived stem cells differentiate into carcinoma-associated fibroblast-like cells under the influence of tumor derived factors, Cell. Oncol. (2011).
[180] P. Manoukian, M. Bijlsma, H.J.F.I.C. Van Laarhoven, D. Biology, The Cellular Origins of Cancer-Associated Fibroblasts and Their Opposing Contributions to Pancreatic Cancer Growth, Front. Cell Dev. Biol. 9 (2021) 743907.
[181] M. Nurmik, P. Ullmann, F. Rodriguez, S. Haan, E. Letellier, In search of definitions: cancer-associated fibroblasts and their markers, Int. J. Cancer 146 (4) (2020) 895–905.
[182] M.R. Junttila, F.J. De Sauvage, Influence of tumour micro-environment heterogeneity on therapeutic response, Nature (2013).
[183] A. Begum, R.H. McMillan, Y.T. Chang, V.R. Penchev, N.V. Rajeshkumar, A. Maitra, M.G. Goggins, J.R. Eshelman, C.L. Wolfgang, Z.A. Rasheed, W. Matsui, Direct interactions with cancer-associated fibroblasts lead to enhanced pancreatic cancer stem cell function, Pancreas 48 (2019) 329–334.
[184] Q. Ping, R. Yan, X. Cheng, W. Wang, Y. Zhong, Z. Hou, Y. Shi, C. Wang, R. Li, Cancer-associated fibroblasts: overview, progress, challenges, and directions, Cancer Gene Ther. (2021).
[185] V.P. Chauhan, J.D. Martin, H. Liu, D.A. Lacorre, S.R. Jain, S.V. Kozin, T. Stylianopoulos, A.S. Mousa, X. Han, P. Adstamongkonkul, Z. Popović, P. Huang, M.G. Bawendi, Y. Boucher, R.K. Jain, Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels, Nat. Commun. 4 (2013).
[186] T. Ji, Y. Ding, Y. Zhao, J. Wang, H. Qin, X. Liu, J. Lang, R. Zhao, Y. Zhang, J. Shi, N. Tao, Z. Qin, G. Nie, Peptide assembly integration of fibroblast-targeting and cell-penetration features for enhanced antitumor drug delivery, Adv. Mater. 27 (2015) 1865–1873.
[187] C. Hu, X. Liu, W. Ran, J. Meng, Y. Zhai, P. Zhang, Q. Yin, H. Yu, Z. Zhang, Y. Li, Regulating cancer associated fibroblasts with losartan-loaded injectable peptide hydrogel to potentiate chemotherapy in inhibiting growth and lung metastasis of triple negative breast cancer, Biomaterials 144 (2017) 60–72.
[188] R.H. Farnsworth, M.G. Achen, S.A. Stacker, The evolving role of lymphatics in cancer metastasis, Curr. Opin. Immunol. 53 (2018) 64–73.
[189] A.K. D'Cruz, R. Vaish, N. Kapre, M. Dandekar, S. Gupta, R. Hawaldar, J.P. Agarwal, G. Pantvaidya, D. Chaukar, A. Deshmukh, S. Kane, Elective versus therapeutic neck dissection in node-negative oral cancer, N. Engl. J. Med. 373 (6) (2015) 521–529.
[190] A. Zaal, R.P. Zweemer, M. Zikán, L. Dusek, D. Querleu, F. Lécuru, A.S. Bats, R. Jach, L. Sevcik, P. Graf, J. Klat, Pelvic lymphadenectomy improves survival in patients with cervical cancer with low-volume disease in the sentinel node: a retrospective multicenter cohort study, J. Gynecol. Cancer 24 (2) (2014) 303–311.
[191] D.S. Chen, I. Mellman, Oncology meets immunology: the cancer-immunity cycle, Immunity 39 (1) (2013) 1–10.
[192] R. van de Ven, J.J. Lindenberg, D. Oosterhoff, T.D. De Gruijl, Dendritic cell plasticity in tumor-conditioned skin: CD14+ cells at the cross-roads of immune activation and suppression, Front. Immunol. 4 (2013) 403.
[193] D. Jones, E.R. Pereira, T.P. Padera, Growth and immune evasion of lymph node metastasis, Front. Oncol. 8 (2018) 36.
[194] Y. Ding, Z. Li, A. Jaklenec, Q. Hu, Vaccine delivery systems toward lymph nodes, Adv. Drug. Deliv. Rev. 179 (2021) 113914.
[195] N. Singh, M. Handa, V. Singh, P. Kesharwani, R. Shukla, Lymphatic targeting for therapeutic application using nanoparticulate systems, J. Drug Targeting 30 (10) (2022) 1017–1033.
[196] S.T. Reddy, A.J. Van Der Vlies, E. Simeoni, V. Angeli, G.J. Randolph, C.P. O'Neil, L.K. Lee, M.A. Swartz, J.A. Hubbell, Exploiting lymphatic transport and complement activation in nanoparticle vaccines, Nat. Biotechnol. 25 (10) (2007) 1159–1164.
[197] H. Kobayashi, S. Kawamoto, M. Bernardo, M.W. Brechbiel, M.V. Knopp, P.L. Choyke, Delivery of gadolinium-labeled nanoparticles to the sentinel lymph node: comparison of the sentinel node visualization and estimations of intra-nodal gadolinium concentration by the magnetic resonance imaging, J. Controlled Release 111 (3) (2006) 343–351.
[198] J. Li, X. Jiang, H. Li, M. Gelinsky, Z. Gu, Tailoring materials for modulation of macrophage fate, Adv. Mater. 33 (12) (2021) 2004172.
[199] N. Kapate, J.R. Clegg, S. Mitragotri, Non-spherical micro-and nanoparticles for drug delivery: progress over 15 years, Adv. Drug. Deliv. Rev. 177 (2021) 113807.
[200] T. Nakamura, M. Kawai, Y. Sato, M. Maeki, M. Tokeshi, H. Harashima, The effect of size and charge of lipid nanoparticles prepared by microfluidic mixing on their lymph node transitivity and distribution, Mol. Pharmaceutics 17 (3) (2020) 944–953.
[201] E. Fröhlich, The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles, Int. J. Nanomed. 7 (2012) 5577.
[202] X. Li, E.C. Montague, A. Pollinzi, A. Lofts, T. Hoare, Design of Smart Size-, Surface-, and Shape-Switching Nanoparticles to Improve Therapeutic Efficacy, Small 18 (6) (2022) 2104632.
[203] A.S. Elz, N.L. Trevaskis, C.J. Porter, J.M. Bowen, C.A. Prestidge, Smart design approaches for orally administered lipophilic prodrugs to promote lymphatic transport, J. Controlled Release (2021).
[204] X. Ke, G.P. Howard, H. Tang, B. Cheng, M.T. Saung, J.L. Santos, H.Q. Mao, Physical and chemical profiles of nanoparticles for lymphatic targeting, Adv. Drug. Deliv. Rev. 151 (2019) 72–93.
[205] A. Mantovani, P. Allavena, F. Marchesi, C. Garlanda, Macrophages as tools and targets in cancer therapy, Nat. Rev. Drug Discovery (2022) 1–22.
[206] D. Corogeanu, S.S. Diebold, Direct and indirect engagement of dendritic cell function by antibodies developed for cancer therapy, Clin. Exp. Immunol. (2022).
[207] O. Kyrysyuk, K.W. Wucherpfennig, Designing cancer immunotherapies that engage T cells and NK cells, Annu. Rev. Immunol. 41 (2022).
[208] H. Cabral, J. Makino, Y. Matsumoto, P. Mi, H. Wu, T. Nomoto, K. Toh, N. Yamada, Y. Higuchi, S.J.A.N. Konishi, Systemic targeting of lymph node metastasis through the blood vascular system by using size-controlled nanocarriers, ACS Nano 9 (5) (2015) 4957–4967.
[209] Y. Guo, D. Wang, Q. Song, T. Wu, X. Zhuang, Y. Bao, M. Kong, Y. Qi, S. Tan, Z.J.A.n. Zhang, Erythrocyte membrane-enveloped polymeric nanoparticles as nanovaccine for induction of antitumor immunity against melanoma, ACS Nano 9 (7) (2015) 6918–6933.
[210] I. Mottas, A. Bekdemir, A. Cereghetti, L. Spagnuolo, Y.S. Yang, M. Müller, D.J. Irvine, F. Stellacci, C. Bourquin, Amphiphilic nanoparticle delivery enhances the anticancer efficacy of a TLR7 ligand via local immune activation, Biomaterials 190 (2019) 111–120.
[211] M. Najafi, B. Farhood, K. Mortezaee, Cancer stem cells (CSCs) in cancer progression and therapy, J. Cell. Physiol. 234 (6) (2019) 8381–8395.
[212] T. Lapidot, C. Sirard, J. Vormoor, B. Murdoch, T. Hoang, J. Caceres-Cortes, M. Minden, B. Paterson, M.A. Caligiuri, J.E. Dick, A cell initiating human acute myeloid leukaemia after transplantation into SCID mice, Nature 367 (6464) (1994) 645–648.
[213] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proc. Natl. Acad. Sci. 100 (7) (2003) 3983–3988.
[214] V. Tirino, V. Desiderio, F. Paino, A. De Rosa, F. Papaccio, M. La Noce, L. Laino, F. De Francesco, G. Papaccio, Cancer stem cells in solid tumors: an overview and new approaches for their isolation and characterization, FASEB J. 27 (1) (2013) 13–24.
[215] M. Najafi, K. Mortezaee, R. Ahadi, Cancer stem cell symmetry & plasticity: tumorigenesis and therapy relevance, Life Sci. 231 (2019) 116520.
[216] D. Zhang, D.G. Tang, K. Rycaj, Cancer stem cells: regulation programs, immunological properties and immunotherapy, Semin. Cancer Biol. (2018) 94–106.
[217] J. Dittmer, Breast cancer stem cells: Features, key drivers and treatment options, Semin. Cancer Biol. (2018) 59–74.
[218] G. Mendoza–Almanza, E. Ortíz–Sánchez, L. Rocha–Zavaleta, C. Rivas–Santiago, E. Esparza–Ibarra, J. Olmos, Cervical cancer stem cells and other leading factors associated with cervical cancer development, Oncol. Lett. 18 (4) (2019) 3423–3432.
[219] Z. Jahanafrooz, J. Mosafer, M. Akbari, M. Hashemzaei, A. Mokhtarzadeh, B. Baradaran, Colon cancer therapy by focusing on colon cancer stem cells and their tumor microenvironment, J. Cell. Physiol. 235 (5) (2020) 4153–4166.
[220] K. Liu, T. Zhao, J. Wang, Y. Chen, R. Zhang, X. Lan, J. Que, Etiology, cancer stem cells and potential diagnostic biomarkers for esophageal cancer, Cancer Lett. 458 (2019) 21–28.
[221] S.S. Yu, N.J. Cirillo, The molecular markers of cancer stem cells in head and neck tumors, J. Cell. Physiol. 235 (1) (2020) 65–73.
[222] T.K.W. Lee, X.Y. Guan, S. Ma, Cancer stem cells in hepatocellular carcinoma—from origin to clinical implications, Nat. Rev. Gastroenterol. Hepatol. 19 (1) (2022) 26–44.
[223] W.S. Heng, R. Gosens, F.A. Kruyt, Lung cancer stem cells: origin, features, maintenance mechanisms and therapeutic targeting, Biochem. Pharmacol. 160 (2019) 121–133.
[224] V. Keyvani, M. Farshchian, S.-A. Esmaeili, H. Yari, M. Moghbeli, S.R. Nezhad, M.R. Abbaszadegan, Ovarian cancer stem cells and targeted therapy, J. Ovarian Res. 12 (1) (2019) 1–11.

[225] A. Gzil, I. Zarębska, W. Bursiewicz, P. Antosik, D. Grzanka, Ł. Szylberg, Markers of pancreatic cancer stem cells and their clinical and therapeutic implications, Mol. Biol. Rep. 46 (6) (2019) 6629–6645.
[226] S. Skvortsov, I.I. Skvortsova, D.G. Tang, A. Dubrovska, Concise review: prostate cancer stem cells: current understanding, Stem Cells 36 (10) (2018) 1457–1474.
[227] V. Veschi, F. Verona, M. Lo Iacono, C. D'Accardo, G. Porcelli, A. Turdo, M. Gaggianesi, S. Forte, D. Giuffrida, L. Memeo, Cancer stem cells in thyroid tumors: From the origin to metastasis, J. Ovarian Res. 11 (2020) 566.
[228] R.K. Bright, J.D. Bright, J.A. Byrne, Overexpressed oncogenic tumor-self antigens: new vaccine targets, Hum. Vaccin. Immunother. 10 (11) (2014) 3297–3305.
[229] M. Sultan, K.M. Coyle, D. Vidovic, M.L. Thomas, S. Gujar, P. Marcato, Hide-and-seek: the interplay between cancer stem cells and the immune system, Carcinogenesis 38 (2) (2016) 107–118.
[230] C. Raggi, H. Mousa, M. Correnti, A. Sica, P. Invernizzi, Cancer stem cells and tumor-associated macrophages: a roadmap for multitargeting strategies, Oncogene 35 (6) (2016) 671–682.
[231] A. Sica, C. Porta, A. Amadori, A. Pastò, Tumor-associated myeloid cells as guiding forces of cancer cell stemness, Cancer Immunol. Immunother. 66 (2017) 1025–1036.
[232] X. Chen, M. Yang, J. Yin, P. Li, S. Zeng, G. Zheng, Z. He, H. Liu, Q. Wang, F. Zhang, Tumor-associated macrophages promote epithelial–mesenchymal transition and the cancer stem cell properties in triple-negative breast cancer through CCL2/AKT/β-catenin signaling, Cell Commun. Signal. 20 (1) (2022) 92.
[233] D. Bayik, J.D. Lathia, Cancer stem cell–immune cell crosstalk in tumour progression, Nat. Rev. Cancer 21 (8) (2021) 526–536.
[234] T. Welte, I.S. Kim, L. Tian, X. Gao, H. Wang, J. Li, X.B. Holdman, J.I. Herschkowitz, A. Pond, G. Xie, Oncogenic mTOR signalling recruits myeloid-derived suppressor cells to promote tumour initiation, Nat. Cell Biol. 18 (6) (2016) 632–644.
[235] N. Komura, S. Mabuchi, K. Shimura, E. Yokoi, K. Kozasa, H. Kuroda, R. Takahashi, T. Sasano, M. Kawano, Y. Matsumoto, The role of myeloid-derived suppressor cells in increasing cancer stem-like cells and promoting PD-L1 expression in epithelial ovarian cancer, Cancer Immunol. Immunother. 69 (2020) 2477–2499.
[236] E.N. Scott, A.M. Gocher, C.J. Workman, D.A. Vignali, Regulatory T cells: barriers of immune infiltration into the tumor microenvironment, Front. Immunol. (2021) 2282.
[237] H. Dianat-Moghadam, A. Mahari, R. Salahlou, M. Khalili, M. Azizi, H. Sadeghzadeh, Immune evader cancer stem cells direct the perspective approaches to cancer immunotherapy, Stem Cell Res. Therapy 13 (1) (2022) 1–12.
[238] H. Dianat-Moghadam, M. Heidarifard, R. Jahanban-Esfahlan, Y. Panahi, H. Hamishehkar, F. Pouremamali, R. Rahbarghazi, M. Nouri, Cancer stem cells-emanated therapy resistance: implications for liposomal drug delivery systems, J. Control. Release 288 (2018) 62–83.
[239] Y. Zhao, Q. Dong, J. Li, K. Zhang, J. Qin, J. Zhao, Q. Sun, Z. Wang, T. Wartmann, K.W. Jauch, Targeting cancer stem cells and their niche: perspectives for future therapeutic targets and strategies, Semin. Cancer Biol. (2018) 139–155.
[240] Z. Li, J.N. Rich, Hypoxia and hypoxia inducible factors in cancer stem cell maintenance, 2010 Diverse effects of hypoxia on tumor progression 21–30.
[241] W. Ban, J. Guan, H. Huang, Z. He, M. Sun, F. Liu, J. Sun, Emerging systemic delivery strategies of oncolytic viruses: A key step toward cancer immunotherapy, Nano Res. 15 (5) (2022) 1–17.
[242] N. Badrinath, S.Y. Yoo, Recent advances in cancer stem cell-targeted immunotherapy, Cancers 11 (3) (2019) 310.
[243] I. Ma, A.L. Allan, The role of human aldehyde dehydrogenase in normal and cancer stem cells, Stem Cell Rev. Rep. 7 (2) (2011) 292–306.
[244] M. Won, J.H. Kim, M.S. Ji, J.S. Kim, ROS activated prodrug for ALDH overexpressed cancer stem cells, Chem. Commun. 58 (1) (2022) 72–75.
[245] A. Hassani Najafabadi, J. Zhang, M.E. Aikins, Z.I. Najaf Abadi, F. Liao, Y. Qin, E.B. Okeke, L.M. Scheetz, J. Nam, Y. Xu, D. Adams, Cancer immunotherapy via targeting cancer stem cells using vaccine nanodiscs, Nano Lett. 20 (10) (2020) 7783–7792.
[246] S.Y. Li, R. Sun, H.X. Wang, S. Shen, Y. Liu, X.J. Du, Y.H. Zhu, W Jun, Combination therapy with epigenetic-targeted and chemotherapeutic drugs delivered by nanoparticles to enhance the chemotherapy response and overcome resistance by breast cancer stem cells, J. Control. Release 205 (2015) 7–14.
[247] L. Wang, X. Zuo, K. Xie, D. Wei, The role of CD44 and cancer stem cells, Cancer Stem Cells (2018) 31–42.
[248] A. Spadea, J.M. Rios de la Rosa, A. Tirella, M.B. Ashford, K.J. Williams, I.J. Stratford, N. Tirelli, M. Mehibel, Evaluating the efficiency of hyaluronic acid for tumor targeting via CD44, Mol. Pharm. 16 (6) (2019) 2481–2493.
[249] C.W. Menke-van der Houven, C. Gomez-Roca, C. van Herpen, A.L. Coveler, D. Mahalingam, H.M. Verheul, W.T. van der Graaf, R. Christen, D. Rüttinger, S. Weigand, M.A. Cannarile, First-in-human phase I clinical trial of RG7356, an anti-CD44 humanized antibody, in patients with advanced, CD44-expressing solid tumors, Oncotarget 7 (48) (2016) 80046–80058.
[250] B.J. Baaten, C.R. Li, M.F. Deiro, M.M. Lin, P.J. Linton, L.M. Bradley, CD44 regulates survival and memory development in Th1 cells, Immunity 32 (1) (2010) 104–115.
[251] A. Aires, S.M. Ocampo, B.M. Simões, M.J. Rodríguez, J.F. Cadenas, P. Couleaud, K. Spence, A. Latorre, R. Miranda, Á. Somoza, R.B. Clarke, Multifunctionalized iron oxide nanoparticles for selective drug delivery to CD44-positive cancer cells, Nanotechnology 27 (6) (2016) 065103.
[252] S.A. Navarro-Marchal, C. Griñán-Lisón, J.-M. Entrena, G. Ruiz-Alcalá, M. Tristán-Manzano, F. Martin, I. Pérez-Victoria, J.M. Peula-García, J.A. Marchal, Anti-CD44-conjugated olive oil liquid nanocapsules for targeting pancreatic cancer stem cells, Biomacromolecules 22 (4) (2021) 1374–1388.
[253] G.Y. Liou, CD133 as a regulator of cancer metastasis through the cancer stem cells, Int. J. Biochem. Cell Biol. 106 (2019) 1–7.
[254] M. Ni, M. Xiong, X. Zhang, G. Cai, H. Chen, Q. Zeng, Z. Yu, Poly (lactic-co-glycolic acid) nanoparticles conjugated with CD133 aptamers for targeted salinomycin delivery to CD133+ osteosarcoma cancer stem cells, Int. J. Nanomedicine 10 (2015) 2537–2554.
[255] J.S. Kim, D.H. Shin, J.S. Kim, Dual-targeting immunoliposomes using angiopep-2 and CD133 antibody for glioblastoma stem cells, J. Control. Release 269 (2018) 245–257.
[256] K. Song, M. Farzaneh, Signaling pathways governing breast cancer stem cells behavior, Stem Cell Res. Ther. 12 (1) (2021) 1–11.
[257] H.M. Zhou, J.G. Zhang, X. Zhang, Q. Li, Targeting cancer stem cells for reversing therapy resistance: Mechanism, signaling, and prospective agents, Signal Transduct. Target. Ther. 6 (1) (2021) 1–17.