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REVIEW

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Nanocarriers for cutting-edge cancer immunotherapies

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Abstract

Cancer immunotherapy aims to harness the body's own immune system for effective and long-lasting elimination of malignant neoplastic tissues. Owing to the advance in understanding of cancer pathology and immunology, many novel strategies for enhancing immunological responses against various cancers have been successfully developed, and some have translated into excellent clinical outcomes. As one promising strategy for the next generation of immunotherapies, activating the multi-cellular network (MCN) within the tumor microenvironment (TME) to deploy multiple mechanisms of action (MOAs) has attracted significant attention. To achieve this effectively and safely, delivering multiple or pleiotropic therapeutic cargoes to the targeted sites of cancerous tissues, cells, and intracellular organelles is critical, for which numerous nanocarriers have been developed and leveraged. In this review, we first introduce therapeutic payloads categorized according to their predicted functions in cancer immunotherapy and their physicochemical structures and forms. Then, various nanocarriers, along with their unique characteristics, properties, advantages, and limitations, are introduced with notable recent applications in cancer immunotherapy. Following discussions on targeting strategies, a summary of each nanocarrier matching with suitable therapeutic cargoes is provided with comprehensive background information for designing cancer immunotherapy regimens.

Keywords Cancer immunotherapy, Nanocarrier, Nanoparticle, Multi-cellular network, Immunogenic cell death, Tumor microenvironment

Background

The major challenge of cancer therapy is its conflicting requirements. On the one hand, cancer treatment must specifically target the neoplastic cells to avoid off-tumor systemic toxicity. However, narrow targeting of specific genes and proteins can lead to evasion, particularly in the context of rapidly evolving heterogeneous cancers. On the other hand, approaches meant to activate multiple endogenous anti-cancer mechanisms, such as immunotherapy, are less specific and more likely to cause adverse systemic effects. There is no universal cure that meets all requirements, which necessitates carefully crafted plans relying on the synergistic effects of multiple treatments. Meanwhile, the advancement of immunotherapy has revolutionized cancer treatment by harnessing multiple effector components of the body's immune system to target and destroy cancer

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cells. The successes of various cancer immunotherapies, namely checkpoint inhibitors, adoptive cell therapy (ACT), cancer vaccines, cytokine therapy, monoclonal antibodies, and bacteriotherapy, demonstrate the dynamic and multifaceted nature of modern cancer treatment. By leveraging different mechanisms to enhance the immune system's natural capabilities, these therapies offer new hope and improved outcomes for patients battling various forms of cancer.

Successful cancer immunotherapies must ensure the (1) presence of immune effector cells at the tumor site, (2) penetrance of the cells into the tumor microenvironment (TME), (3) persistence of cells within the TME over an extended time, and (4) predominance of the immune response over the immunosuppression and low nutrient availability within the TME [1]. Many current immunotherapies are missing the ability to perform one or more of these essential functions. For example, while strides have been made to improve the persistence and predominance of CAR-T cells within the TME [2], there remain significant challenges to overcome, especially in the treatment of solid tumors. Cancerous cells persist because they evade immune detection or hijack immune regulatory systems to suppress immune functions within the TME [3]. Various strategies using cancer vaccines have been developed to turn so-called cold tumors, in which immune action is primarily absent, into hot, immunoreactive tumors [4]. However, current cancer vaccines still do not effectively improve the penetration, persistence, or predominance of immune effector cells within the TME and cannot respond to the dynamically evolving neoantigens. To overcome these limitations of individual immunotherapies, multiple approaches have been combined for their synergistic effects.

More recently, efforts to maximize the engagement of endogenous anti-cancer mechanisms by activating the multi-cellular network (MCN) within the TME have been proposed as a potential next step in cancer therapy in lieu of deploying direct killing of cancer cells only [1]. By activating various types of immune effector cells simultaneously, multiple anti-cancer cytotoxic mechanisms of action (MOAs) can be leveraged while effectively preempting the chance of developing treatment resistance. One promising strategy for engaging with MCN within the TME is to induce immunogenic cell death (ICD) in cancer cells, in which the dying cancer cells release proinflammatory factors such as damage-associated molecular patterns (DAMPs) that can secondarily recruit and activate immune effector cells.

Targeting cancer intrinsic mechanisms that amplify anti-tumor immune responses may be highly effective but may also require appropriate delivery systems. In that vein, nanocarriers have been an important part of cancer treatments since the FDA approved Doxil® in 1995 [5]. Traditionally, nanocarriers have been developed to provide site (tumor)-specific delivery of highly toxic payloads to kill neoplastic cells while preventing systemic damage to normal tissues and organs. Similarly, many immunotherapies, such as cancer vaccines, owe their success in large part to their nanoscale delivery methods [6]. Various nanocarriers have been exploited for tumor-specific delivery of antigens, adjuvants, and cytokines while reprogramming the immune cells in the TME, such as dendritic cells (DCs) [7], or modulating unfavorable mechanical or biochemical microenvironment of the TME, such as hypoxic or acidic conditions, mechanical barriers, and other immunosuppressive signals [8]. Nanocarriers have also been demonstrated to possess intrinsic adjuvant properties, and the degree of immune responses depends on the size and chemical properties of the nanocarrier [9, 10]. So far, many functionally and/or structurally distinct nanocarriers have also been explored as the vehicles to deliver ICD inducers with increased specificity and to improve the efficiency of ICD induction in vivo [11, 12].

An ideal nanocarrier should possess good biocompatibility, stability, and size consistency. In addition, it should be able to carry desired payloads with good loading capacity. Lastly, the nanocarrier should be capable of targeted delivery of payloads to specific organs, tissues, and cell types with potential tumor penetration and context-dependent intracellular release of multiple payloads [13]. Thus, in this review, we will consider the choice of nanocarriers with desired characteristics for modern cancer immunotherapies. We will first review the relevant therapeutic payloads. Then, we will survey various nanocarriers, the particles of external dimensions in the size range of 1–100 nm, and categorize them into types based on the material compositions and structures. We will also discuss the significance and methods of targeted delivery of nanocarriers. Last, we will discuss the advantages and disadvantages of each nanocarrier system for various therapeutic payloads and cargoes, the selection of nanocarriers for the desired cancer immunotherapy with a focus on clinical translation, the remaining challenges, and our perspective on the future of nanocarriers in cancer immunotherapy.

Payloads/Cargoes

The payloads or cargoes delivered by nanocarriers play crucial roles in cancer therapy by modulating the immune response, targeting cancer cells, and overcoming the limitations of conventional therapies [14]. The ideal nanocarrier for any given treatment depends not only on the therapeutic goal but also on the drug's form

and chemical properties. In this review, we categorize the therapeutic goals into three main categories: directly or indirectly boosting immune cell functions, counteracting the immunosuppressive effects of the TME, and inducing the ICD of cancer cells. It is worth noting that some therapeutic regimens fit more than one of these categories. From a biochemical viewpoint, cargoes employed in cancer immunotherapies generally fall into one of three categories: small molecule drugs, peptides and proteins, and nucleic acids. Each type of payload and delivery mechanism has unique challenges and advantages, making them suitable for different therapeutic strategies.

Payload purposes

- *Directly or indirectly boost endogenous immune cell effector function*

Proper immune function requires a delicate balance of signaling pathways to prevent either immune overreaction or underreaction, both of which can have fatal consequences. One common cancer immunotherapy approach upregulates immune functions to heighten activation. Another option is to activate the immune system in targeted TMEs against specific cancer cells that have previously evaded immune detection and attack.

Cytokines are small proteins, generally less than 30 kDa in size [15]. Cytokines that boost immune function, such as interleukin-2 (IL-2), interleukin-12 (IL- 12), and interferon-alpha (IFN-α), have been successfully employed in cancer treatments [16]. They can convert immunologically 'cold' tumors to 'hot' tumors and overcome the TME's immunosuppressive effects [4]. However, cytokines are famously pleiotropic, and systemic administration of these signal molecules leads to dose-limiting and sometimes fatal toxicity. Nanocarriers can enable the delivery of cytokines directly to the TME, altering the local immune landscape with minimal systemic effects [17]. Small molecule analogs or engineered cytokines have extensively been used in cancer immunotherapy [18].

Agonistic antibodies that target co-stimulatory receptors on T cells, such as OX40, CD137, and CD40, can directly enhance T cell activation and proliferation. Nanocarriers can deliver these antibodies to the tumor site, promoting specific and robust anti-tumor immunity. Studies have shown that nanoparticles carrying anti-OX40 antibodies can significantly increase the infiltration of activated T cells into tumors, leading to enhanced anti-tumor activity [19].

Cancer vaccines are a promising approach in nano-immunotherapy, explicitly training the immune system to elicit robust and targeted immune responses to *tumor-associated antigens (TAAs) or tumor-specific antigens* (also known as neoantigens) [20]. These antigens in the form of tumor lysates, proteins, or peptides are included as components of the vaccines. Other vaccines instead employ *messenger RNAs (mRNAs)* encoding for the translation of the antigens [21]. Some cancer vaccine trials also include mRNAs that encode proteins that stimulate the immune system, along with the TAA-encoding mRNA [22]. While broad interest in mRNA vaccines was spurred by their use in COVID-19 vaccines, mRNA-based cancer immunotherapies have been developed since 2009 [22] for various cancers, including melanoma, pancreatic cancer, and head and neck squamous cell carcinoma [23, 24]. *Adjuvants* are crucial components of cancer vaccines as they alert the immune system at the vaccine's site to promote immune cell recruitment and activation. A list of adjuvants currently approved for human use by the FDA and EMA can be found elsewhere [25]. Meanwhile, nanocarriers play a critical role in delivering cancer vaccines, enhancing their stability and targeting, thus improving the efficacy of each component. For example, adjuvants like *CpG oligodeoxynucleotides (ODNs)* delivered by nanocarriers are recognized by Toll-like receptor 9 (TLR9) on DCs and other antigen-presenting cells (APCs), which can significantly enhance the immune response against co-delivered tumor antigens [26]. Similarly, targeting other toll-like receptors by delivering respective agonists via nanoparticles has been extensively pursued to boost immune responses in the TME for cancer immunotherapy [27].

- *Overcome TME's immunosuppression*

Properly managing the immune landscape of the TME is the key to the success of cancer treatment. Tumors can block immune cell infiltration with physical barriers and employ various mechanisms to suppress immune functions within the TME [1]. Thus, converting an immunosuppressive or 'cold' tumor to an immuno-reactive 'hot' tumor is one of the main goals of cancer immunotherapies. Here, we focus specifically on therapies designed to overcome immunosuppression within the TME, though it should be acknowledged that there is some crossover with the category previously discussed. As in the previous section, tumor-specific targeting or controlled release is important because of concern for autoimmune reactivities associated with systemic treatments.

Some important immunotherapy targets are specific *immune checkpoints* such as Programmed Cell Death Protein-1 (PD-1) and Cytotoxic T-Lymphocyte

Associated protein 4 (CTLA-4), which suppress anti-cancer T-cell activity [28]. PD-1 signaling is an essential pathway for the prevention of autoimmune disease. Activation of this pathway can lead to apoptosis of antigen-specific T cells or suppress apoptosis of regulatory T (T_{reg}) cells. PD-L1, a ligand for PD-1, is often present on the surface of immuno-evasive cancer cells and effectively inhibits the responses of T cells against cancer [29].

The most common immune checkpoint therapies involve molecules that disrupt signaling pathways. *Immune checkpoint inhibitors (ICIs)* target immune checkpoint receptors or ligands. Examples already on the market include pembrolizumab (Keytruda) and nivolumab (Opdivo), antibodies against PD-1 that prevent PD-1/PD-L1 interactions. Other FDA-approved ICIs bind to the PD-L1 or CTLA-4 [30]. Atezolizumab (Tecentriq), targeting PD-L1, has shown efficacy in various cancers, including non-small cell lung cancer [31] and urothelial carcinoma [32]. Ipilimumab, which targets CTLA-4, has also been used to enhance T cell activation and proliferation, improving immune responses against various tumors [33]. Nanocarriers can enhance the delivery and efficacy of ICIs, such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies, to the TME, resulting in improved T cell activation and anti-tumor responses while minimizing unintended side effects due to off-target immune activation [34].

While other ICIs produce similar effects, they are chemically very different from antibodies. Small molecules called BMS molecules, named for their creator, Bristol-Meyers-Squibb, induce protein conformational changes, promote dimer formation, and disrupt PD-1/PD-L1 interactions [35, 36]. These molecules are much smaller than antibodies and hydrophobic, so their targeted deliveries require different strategies.

Interferon-gamma (IFN-γ) is a cytokine that not only promotes the activity of immune cells but also enhances MHC-I expression and antigen presentation of tumor, primarily through the JAK-STAT signaling pathway [37]. This ultimately suppresses the immune evasion by increasing the immunogenicity of neoplastic cells.

Indoleamine 2,3-dioxygenase 1 (IDO1) is an enzyme that degrades tryptophan, whose action is known to lead to the suppression of T-cell immunity against cancer and the promotion of T_{reg} cell development at the TME [38]. To suppress or silence such immunosuppressive genes, small nucleic acids such as small interference RNA (siRNA), short hairpin RNA (shRNA), or anti-sense oligonucleotides (ASO) can be delivered to the TME. For example, *siRNA against IDO1* was delivered using lipid-based nanoparticles [39], which effectively silenced the expression of IDO1, restored T-cell activity, and enhanced overall anti-tumor immunity [40]. A small-molecule drug *IDO1 inhibitor*, epacadostat, was also successfully employed for the same purpose. Epacadostat has been studied in combination with pembrolizumab for various cancers [41].

Furthermore, nanocarriers can deliver *molecules that modulate the tumor stroma* to reduce immunosuppression. Transforming growth factor-beta (TGF-β) is a cytokine commonly associated with immune evasion. Nanoparticles loaded with TGF-β inhibitors can specifically target the TME, reduce TGF-β signaling, and consequently enhance the infiltration and activity of immune cells like cytotoxic T lymphocytes (CTLs) in a preclinical model [42].

Moreover, *chemotherapeutic agents* can be employed to deplete or reduce the functions of T_{reg} cells and myeloid-derived suppressor cells (MDSCs) that contribute to immunosuppression in the TME. A combination of low-dose cyclophosphamide and anti-CD25 antibody was shown to reduce T_{reg} populations and boost neoantigen reactive T cells [43]. Similarly, a chemotherapeutic agent, gemcitabine, delivered in the form of a nanoparticle, could deplete the MDSCs, enhancing anti-tumor immunity against melanoma [44].

Tyrosine kinase inhibitors (TKIs) are another important category of small-molecule drugs in cancer immunotherapy [45]. Originally in cancer therapy, TKIs have been known to disrupt signaling pathways critical for tumor growth and metastasis by targeting certain kinases. More recently, some TKIs have been shown to enhance the anti-tumor activity of immune cells by reducing the suppressive function of MDSCs and T_{reg} cells within the TME, making them valuable in combination with other immunotherapies [46]. Small molecule inhibitors like ibrutinib, idelalisib, and venetoclax have significantly transformed the treatment of chronic lymphocytic leukemia (CLL) by targeting malignant cell survival and modulating immune cell activity [47].

Epigenetic modifiers are usually small molecules that can alter epigenetic mechanisms that can remodel the anti-tumor immunity within the TME [48]. Several agents targeting epigenetic enzymes, such as DNA methyl transferase (DNMT), histone deacetylase (HDAC), and EZH2, have been approved by the US FDA for treating diverse cancers.

Focal Adhesion Kinase (FAK) Inhibitors, like Defactinib (VS- 6063), are being explored for their ability to disrupt the TME and enhance immune infiltration. FAK not only drives cancer cell migration and survival but also recruits immunosuppressive cells to tumors, exacerbating poor outcomes. Inhibiting FAK

can remodel the tumor immune landscape, enhancing CD8+ T-cell responses and synergizing with PD-1 blockade for more effective cancer treatment [49]. Early-phase trials are investigating their efficacy in solid tumors, including mesothelioma, ovarian, and pancreatic cancers. By targeting the FAK pathway, these inhibitors aim to reduce tumor metastasis and improve patient responses to other forms of immunotherapy [50].

The colony-stimulating factor 1 receptor (CSF-1R) inhibitor is approved for tenosynovial giant cell tumors and is being investigated in Phase I/II trials for other cancers to modulate the TME and enhance immune responses [51].

It is important to note that the strategy of employing suitable nanocarriers has not been extensively pursued for all these therapeutic cargoes. Thus, there is considerable room for improvement in the targeted delivery of these cargoes using suitable nanocarriers to enhance their efficacy and reduce side effects.

- *Induction of immunogenic cell death (ICD)*

ICD is a process in which dying cancer cells become a source of antigens, which can stimulate robust anti-tumor immune responses. Thus, it not only directly eradicates tumor cells but also enhances the overall immunogenicity of the TME, making it a powerful strategy in cancer immunotherapy [1]. ICD is characterized by releasing DAMPs such as calreticulin, ATP, and HMGB1, which can initiate strong immune responses by recruiting and activating DCs and other APCs [52]. Nanocarriers can be engineered to deliver a variety of agents that induce ICD, thereby converting tumors into a vaccine-like entity [12].

Chemotherapeutic agents are traditional cancer treatments designed to kill rapidly dividing cells, including cancer cells. Many chemotherapeutic drugs also have immunomodulatory effects [53]. Chemotherapeutic agents like doxorubicin and oxaliplatin have been shown to induce ICD when delivered effectively to tumor sites. Nanocarriers enhance the delivery of these agents, ensuring that a sufficient concentration reaches the tumor while minimizing systemic toxicity. As an example, paclitaxel induces the release of pro-inflammatory cytokines and enhances the expression of MHC-I molecules on tumor cells. This facilitates the recognition and destruction of cancer cells by CTLs [53, 54].

Radiopharmaceuticals deliver radiation directly to cancer cells, leading to their destruction. Some of these agents also have immunomodulatory properties. Radium-223 used primarily for metastatic prostate cancer, emits alpha particles that cause localized DNA damage in tumor cells. The resulting cell death can release DAMPs, which enhance the recruitment and activation of immune cells to the TME [55]. Iodine-131, commonly used in thyroid cancer treatment, not only destroys cancerous thyroid cells but also stimulates an immune response by releasing TAAs [55]. This dual effect can improve the body's ability to recognize and attack residual cancer cells. More efforts to use the unique pharmacokinetic characteristics of nanocarriers for the targeted delivery of radiopharmaceuticals are actively taking place [56].

Photosensitizers are used for photodynamic therapy (PDT) that can also induce ICD [57]. Nanoparticles made of polymers conjugated with photosensitizers can accumulate in tumors and, upon irradiation with light of a specific wavelength, produce reactive oxygen species (ROS) that kill cancer cells and trigger ICD [58]. Recent advancements in nanocarriers used in PDT have been reviewed elsewhere [59]. It is worth noting that the use of PDT is limited because light cannot penetrate more than a centimeter into the tissue.

Payload structure

The payloads and cargoes discussed above, according to their functions in cancer immunotherapy, can also be categorized according to their physicochemical identities and properties, such as size, hydrophobicity, charge, polarity, and biological stability. These eventually determine the feasibility and need for employing certain nanocarriers for their deliveries.

Small-molecule drugs, characterized by their low molecular weights, are an essential part of cancer immunotherapy. These drugs can directly target cancer cells or modulate the immune response to enhance the body's ability to fight tumors. Their ability to attune immune responses, target specific signaling pathways, and enhance the effectiveness of other therapeutic modalities often leads to improved patient outcomes [60]. Often, the hydrophobicity of drugs significantly influences their solubility, stability, and off-target effects. Hydrophilic drugs (e.g., cytarabine) dissolve well in biological fluids but may struggle to cross lipid membranes, limiting bioavailability. In contrast, hydrophobic drugs (e.g., paclitaxel) require formulations to improve their solubility and risk accumulating in lipid-rich tissues such as liver and adipose tissues, leading to systemic toxicity. There are amphiphilic drugs (e.g., doxorubicin, irinotecan) that can interact with diverse biological environments but may partition into unintended compartments, causing off-target effects like cardiotoxicity. Nanocarriers help to overcome these issues by protecting the drugs from degradation, improving bioavailability, and ensuring targeted delivery,

thus enhancing therapeutic efficacy and minimizing side effects [61–64]. Their small size allows for easy encapsulation within various types of nanocarriers.

Proteins and peptides are at the forefront of cancer immunotherapy, offering novel and effective ways to harness the immune system against cancer. Proteins and peptides are advantageous over small molecules in terms of flexibility and ease of modification, low immunogenicity, solubility in aqueous solutions, and inexpensive cost of production. While larger than small molecules, they are often small enough to allow good biodistribution and biocompatibility while also potentially being highly selective. Target specificity and minimal toxicity are benefits of therapeutic peptides. Peptide-based cancer vaccines, tumor-targeting peptides, and cell-penetrating peptides can be used to design antigens or adjuvants for vaccine development. The proteins in this category include monoclonal antibodies, checkpoint inhibitors, cytokines, and components in cancer vaccines mentioned in the previous section. Important entities in this category worth mentioning include: i) protein-drug conjugates (PDCs), antibody–drug conjugates (ADCs), degrader-antibody conjugates (DACs), and ii) proteins that are used for gene-regulation and gene-editing such as zinc finger nucleases (ZFNs) [65], transcription activator-like effector nucleases (TALENs) [65], and clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) [66] or other ribonucleoproteins (RNPs). The unique aspects of nanoparticles designed for the delivery of polypeptides are reviewed in detail elsewhere [67, 68].

Nucleic acids have emerged as powerful tools in cancer immunotherapy due to their ability to modulate gene expression and induce robust immune responses. Various types of nucleic acid payloads offer unique mechanisms for targeting cancer cells. The significant advantage of nucleic acid cargos in cancer immunotherapy is that pleiotropic payloads that span multiple mechanisms of action can be delivered and thus result in more powerful anti-cancer activity. Nanocarriers play a critical role in overcoming fundamental limitations in clinical translatability by protecting therapeutic nucleic acids from degradation by nucleases while overcoming multiple biological barriers, ensuring that they reach the intended target [69]. Several recent reviews focus on the delivery of nucleic acids using different nanoparticle moieties for cancer therapy [70–73], in general [74–76], or for improving CAR-T therapy specifically [77–79]. While mRNA-based transfections are generally considered transient, new technologies based on self-amplifying RNA sequences can prolong the effects significantly when desirable [80–82], or a combined delivery of gene editing RNPs such as *CRISPR/Cas9* [69] can enable permanent genetic engineering. Here we will further introduce various nucleic acid cargoes used in cancer immunotherapy.

Deoxyribonucleic Acid (DNA)-based cancer immunotherapies encompass DNA vaccines and gene therapy approaches. DNA vaccines involve the introduction of *plasmid DNA (pDNA)* encoding tumor antigens, which are expressed by host cells to elicit an immune response. Gene therapy can involve the delivery of therapeutic genes to modify the TME or enhance immune cell function [83]. *pDNA* is generally larger than mRNA cargoes, ranging from 2 kb to over 10 kb, posing challenges for efficient delivery and cellular uptake [84]. Viral vectors, such as adenoviruses and lentiviruses, are often used to deliver pDNA, though concerns about immunogenicity and insertional mutagenesis exist [85]. As a representative example, a clinical trial study (NCT03491683) evaluated the safety, efficacy, and immunogenicity of INO-5401 and INO-9012 (two different synthetic DNA plasmid encoding hTERT, WT-1, PSMA and IL- 12). The combination therapy showed robust immune responses and improved overall survival, particularly in MGMT-unmethylated patients [86].

Antisense oligonucleotides (ASOs) are short (18–25 bases), synthetic single-stranded DNAs that are designed to bind to specific complementary sequences of mRNA. This binding can induce (i) degradation of target mRNA by RNase H, (ii) translational inhibition by obstructing ribosome binding, or (iii) alteration of the pre-mRNA splicing pattern by influencing spliceosome assembly, all of which can lead to the downregulation of target gene expression [87]. To overcome their intrinsic limitations, such as easy degradation in circulation, rapid renal clearance, and aberrant immunostimulation, various nanocarriers have been proposed for their effective administration and targeted deliveries [87, 88].

mRNA used in the formulation of cancer vaccines is typically 1–3 kilobases (kb) in length, which facilitates easier delivery compared to larger nucleic acids. However, mRNA is inherently unstable and prone to degradation by ribonucleases, necessitating the use of lipid nanoparticles (LNPs) or other delivery vehicles for protection and efficient cellular uptake [89]. Other options for mRNA protection, such as polymeric micelles and nano-hydrogels, are also being actively pursued [22].

Small interfering RNA (siRNA) technology involves short, double-stranded RNA molecules that mediate gene silencing through the RNA interference (RNAi) mechanism [90]. siRNA molecules are typically 20–25 nucleotides in length, making them smaller and more easily deliverable than mRNA or plasmid DNA. However, as with other nucleic acids, their delivery method must

avoid degradation and ensure efficient cellular uptake [90]. Nanoparticles and conjugations to target ligands are commonly employed strategies to overcome these hurdles. For instance, siRNA therapies targeting critical oncogenes like KRAS-G12D in pancreatic cancer and PLK1 in liver cancer are being evaluated in Phase II/III trials [91]. These therapies utilize polymers and lipid nanoparticles to ensure targeted delivery and uptake, aiming to suppress tumor growth by inhibiting specific gene mutations. Similarly, trials targeting vascular endothelial growth factor (VEGF) aim to inhibit angiogenesis in various cancers, including ovarian cancer, by delivering siRNA via lipid-based nanoparticles [92].

Short hairpin RNA (shRNA) plasmids generate RNA interference within cells, similar to siRNA, but are encoded within a plasmid for stable expression. These plasmids are used to knock down genes that suppress immune responses or promote tumor growth [93]. The size of shRNA plasmids varies depending on the promoter and other regulatory elements but is generally larger than siRNA alone. Viral vectors [94] or non-viral delivery systems like nanoparticles [95] are employed to deliver shRNA plasmids efficiently.

Long non-coding RNAs (lncRNAs) are a diverse group of RNA molecules longer than 200 nucleotides that regulate gene expression at various levels, including chromatin remodeling, transcription, and post-transcriptional processing [96]. In cancer immunotherapy, lncRNAs can modulate immune cell differentiation and function, potentially enhancing anti-tumor responses.

Circular RNAs (circRNAs) are a novel class of endogenous RNAs with a covalently closed circular structure, making them highly stable and resistant to exonucleases. CircRNAs can act as miRNA sponges, regulating gene expression and immune responses [97]. Their unique structure provides an advantage in stability over linear RNAs. However, the delivery of circRNAs remains a challenge due to their size, which can range from a few hundred to several thousand nucleotides [98].

Transfer RNA-derived small RNAs (tsRNAs) have recently been identified as regulatory molecules with potential roles in cancer immunotherapy. These small RNAs can modulate gene expression and immune responses, although their exact mechanisms are still under investigation. The size of tsRNAs is similar to siRNA, typically around 18–22 nucleotides, making them relatively easy to deliver. However, their stability and efficient targeting remain significant challenges [99].

Nanocarriers—delivery vehicles

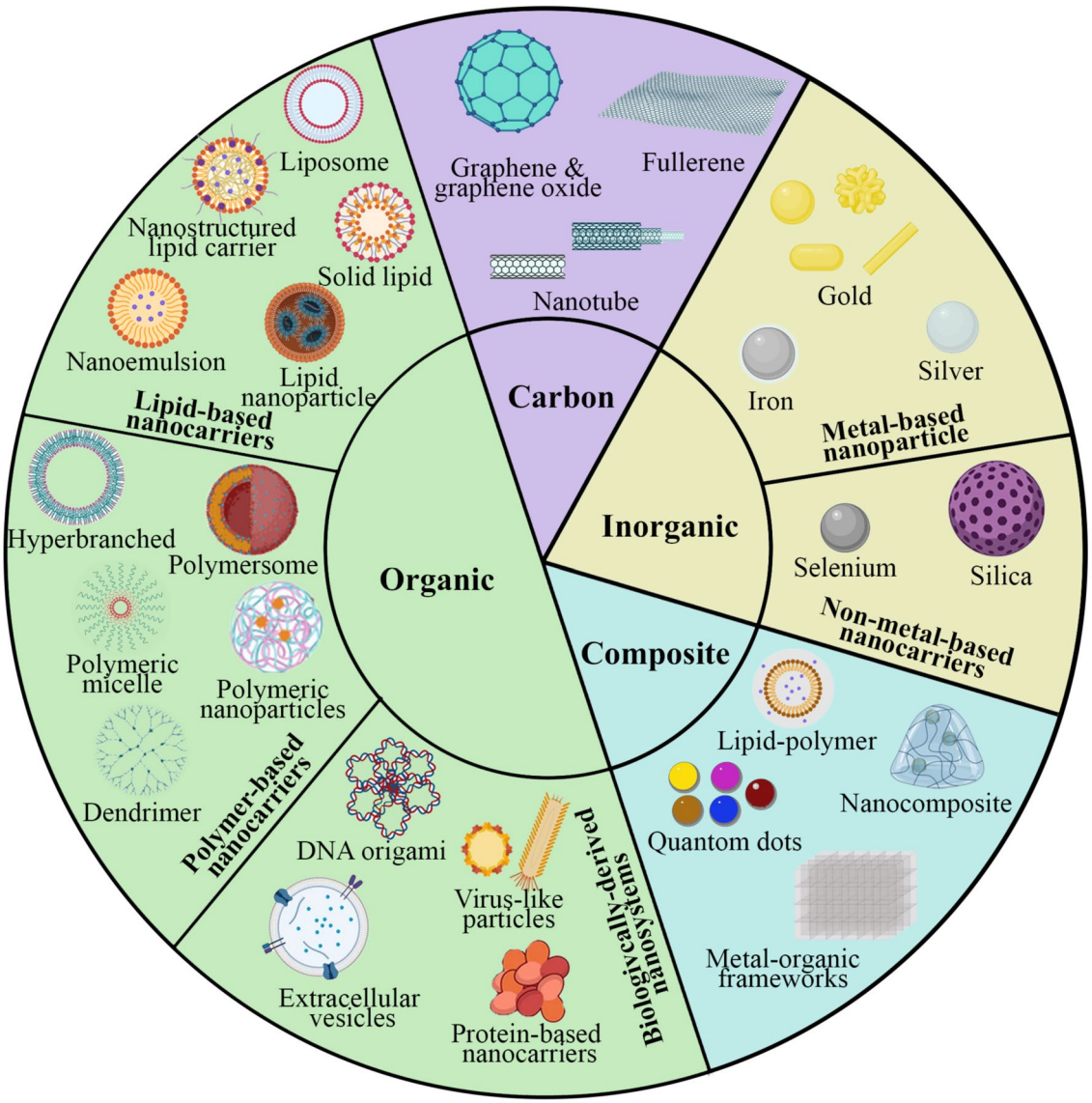
In this section, we will present the survey of various nanocarriers that can serve as effective delivery vehicles for the cargoes described above. As shown in Scheme 1, they are categorized into organic, carbon, inorganic, and composite nanocarriers. For organic nanoparticles, we will introduce various kinds of lipid-based, polymer-based, and biologically-derived nanocarriers. Carbon nanoparticles include fullerenes, carbon nanotubes, graphene, and graphene oxide. Inorganic nanoparticles can be sub-grouped to non-metal-based and metal-based nanoparticles. Lastly, we will introduce the composite nanocarriers that consist of various materials, including organic hybrid nanosystems, composites of inorganic and organic nanomaterials, metal–organic frameworks (MOFs), and quantum dots (QDs) as examples.

Organic nanocarriers

- *Lipid-based nanocarriers*

Lipid-based nanocarriers generally involve amphipathic molecules that self-assemble into nanostructures at a critical concentration and hold their structure because of hydrophobic interactions. While there is variation in chemical composition, they are generally categorized based on structure. The lipids may form a bilayer or a monolayer enclosing either an aqueous solution, a hydrophobic liquid interior, or solid particles. Possibly the most well-known example of lipid-based nanocarriers is the lipid nanoparticles used to deliver mRNA vaccines, which incorporate ionizable lipids to better encapsulate and release charged (anionic for nucleic acids in the physiological condition) cargoes [100]. It should be noted that there are some discrepancies in the terminology for lipid-based nanocarriers. In some papers, the term 'lipid nanoparticles' (LNPs) refers broadly to any lipid-based nanocarrier. Other papers apply the term LNP specifically to the LNPs formulated with ionizable lipids.

Other types of lipid-based nanocarriers include liposomes, lipid nanoemulsions, nanostructured lipid carriers, and solid lipid nanoparticles [101]. All of these nanocarriers have been extensively explored due to their high biocompatibility, biodegradability, versatility, and adaptability [102]. As of 2022, about half of nanopharmaceuticals developed as cancer treatments are lipid-based nanocarriers [101]. Lipid-based nanocarriers can increase circulation time, extend plasma half-life, and enhance tumor uptake when compared with traditional drug distribution methods [101]. They often use biocompatible and biodegradable components, many of which are generally recognized as safe (GRAS) by the FDA [103]. Additionally, these carriers improve pharmacokinetics and can reduce the systemic effects of toxic drugs. These carriers also offer versatility in terms of which drugs can be encapsulated and which cells or systems can be targeted. Targeting mechanisms include



Scheme 1 A comprehensive classification of nanocarriers for cancer immunotherapy. Adapted and modified from Ref. [132]

passive targeting via the enhanced permeability and retention (EPR) mechanism [103] or active targeting with chemical or stimulus-responsive targeting. Active targeting has been extensively explored with liposomes and LNPs.

A general limitation specific to lipid-based carriers is their tendency to spontaneously fuse with off-target cells [104], which makes efforts to introduce suitable shielding and targeting strategies in their formula very important (discussed in the targeting section). Other limitations include fast uptake from the reticuloendothelial system (RES), hydrolysis and oxidation of constituent phospholipids, and concerns over long-term toxicity. The addition of PEGylated lipids to the formulation of lipid-based nanocarriers is common since PEG has been found to reduce reticuloendothelial phagocytosis [105]. However, repeated dosages of PEG-containing lipids in conjunction with immunogenic vaccine components have been reported to induce anti-PEG humoral immunity which leads to accelerated blood clearance of future doses of PEG-containing LNPs [106]. Additionally, scale-up of manufacturing processes for nanomaterials is challenging, and mass-produced lipid-based nanocarriers run the risk of differing from their benchtop counterparts in terms of particle size or structure, polydispersity, stability, or encapsulation efficiency [101, 107]. Therefore, care must be taken to understand their limitations and

ensure excellent quality control when these systems are employed for the delivery of immunotherapeutics.

Liposomes are spherical colloid particles composed of phospholipids that form contiguous membrane bilayers capable of entrapping water-soluble materials in their aqueous core or hydrophobic chemicals within the bilayer. Cholesterol is also generally included for its ability to control membrane fluidity and thus improve liposome stability. The bilayer membrane can protect drugs from hydrolysis or oxidative degradation and, at the same time, minimize toxicity. They may have a single bilayer or be composed of multiple concentric bilayers in an onion-like configuration. Most clinically approved liposomes have diameters between 50 and 300 nm [102], while some have diameters on the micrometer scale [108]. Liposome stability in vivo can be a challenge, but surface modification, most notably using PEG, improves its stability [109] and immunoevasion. Depending on the phospholipids used, liposomes can exhibit different surface charges at physiological pH. Negatively charged liposomes are subject to opsonization that may facilitate their clearance by macrophages, while cationic liposomes and lipid nanoparticles tend to aggregate with negatively charged serum proteins, which in turn may indirectly increase their clearance by the mononuclear phagocyte system [102]. Liposomes improve the delivery and efficacy of chemotherapeutic agents such as doxorubicin and paclitaxel, and some formulations were approved to treat a variety of cancers [110]. Liposomes are also effectively used to deliver protein-based cargoes such as cytokines. The drug release profile heavily depends on the liposome formulation, and the off-target effect is still the remaining challenge [109].

Lipid nanoparticles (LNPs) have more complex structures than liposomes and include an ionizable lipid in their formulation. During the synthesis process, changes in pH cause ionization (and neutralization) of the ionizable lipids, which induces a conformational change in the LNP, accompanying encapsulation of anionic cargoes such as nucleic acids. While the ionizable lipids enable cargo encapsulation, neutral phospholipids and PEGylated lipids form the exterior, and they contribute to LNPs' endosomal escape and evasion from RES [100].

The success of mRNA vaccines for COVID-19 has further highlighted the potential of LNPs in nucleic acid delivery, demonstrating their stability and effectiveness in clinical applications [100]. Studies have also demonstrated that LNPs can effectively deliver mRNA to cancer cells, resulting in significant antitumor activity [100]. LNPs can effectively protect the genetic cargo from degradation. Their ability to bypass endosomal trapping (endosomal escape), principally through membrane inversion, however, remains poor, resulting in the delivery of only ~2% of nucleic acids to the cytosol of target cells [111]. Another noteworthy characteristic of LNPs is their tendency to accumulate in the liver upon systemic intravenous administration, which can lead to potential liver-targeting therapeutic strategies [112] once the concern of hepatotoxicity is resolved.

Lipid nanoemulsions are colloidal dispersions of two immiscible liquids, typically oil (lipid) and water, stabilized by a surfactant monolayer at the interface, forming droplets ranging from 20 to 200 nm in diameter. Like other lipid-based nanocarriers, lipid nanoemulsions are well-suited for delivering hydrophobic drugs and can protect cargoes from enzymatic degradation and offer controlled release. Compared with other lipid-based nanocarriers, lipid nanoemulsions offer less expensive manufacturing, but challenges include the potential toxicity of surfactants and relatively fewer opportunities for surface modification [105]. Lipid nanoemulsions have been explored as carriers for hydrophobic drugs, including chemotherapeutic agents such as paclitaxel [113] and doxorubicin [114], phytochemicals such as curcumin [115, 116], nucleic acids [117], and proteins.

While the previously discussed lipid-based nanocarriers are liquid at body temperatures and rely on amphiphilic interactions for stability, *solid lipid nanoparticles (SLNs)* include a core made of lipids with high melting temperatures. So, this solid core at body temperature offers improved encapsulation efficiency, stability, and drug-release properties compared to liposomes. Additionally, the manufacturing process is more straightforward and scalable than that of other lipid-based nanocarriers. However, the solid lipids in SLNs tend to crystalize during storage, which releases the drugs into the surrounding medium, thereby decreasing effective encapsulation efficiency [101, 118].

Nanostructured lipid carriers (NLCs) offer promising improvements over SLNs, most notably by solving the crystallization problem. NLCs have a core composed of a mixture of solid and liquid lipids, creating a more amorphous structure, which facilitates the retention of drugs within the core during storage and improves encapsulation efficiency. Another advantage of NLCs is the prospect of tunable drug release kinetics, enabled by manipulating the proportion of liquid to solid lipids in the core. Notably, they are being explored for oral administration, which is not done with liposomes or LNPs [101, 118].

- *Polymer-based nanocarriers*

Polymeric nanoparticles (PNPs) are uniformly dispersed, biocompatible, and typically biodegradable nanocarriers that are made with synthetic or natural

polymers. Biodegradable polymers commonly used include poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA) [119] copolymers, poly(ε-caprolactone) (PCL), poly(amino acids), and natural polymers like alginate, chitosan, gelatin, and albumin. The size, size distribution, and properties of PNPs widely vary and are potentially tunable due to the huge variety in chemical structure, molecular weight, and resulting physicochemical characteristics of different polymers. There are numerous examples of PNPs that have been used in cancer immunotherapies, and they are well summarized in another review [120]. Their major limitations are lack of natural specificity, toxicity of synthetic polymers, and rapid clearance by phagocytes. Poly(ethylene glycol) (PEG) is a polymer that has been the most widely used material that endows shielding and "stealth" character on various nanocarriers by coating the surface, so-called PEGylation. As mentioned above, however, PEGylation reduces their uptake by the targeted cells and is known to be immunogenic [121, 122]. For a long time, PNPs have been developed and clinically used as delivery vehicles for highly cytotoxic cancer drugs, such as paclitaxel [123].

Numerous PNPs have been explored for delivering proteins or peptides as nano-vaccines. While they have shown effectiveness and promising preclinical results, challenges remain in optimizing their use for protein and peptide delivery by enhancing particle stability, circulation time, and drug loading capacity [124]. However, they are a potential substitute for viral vectors since they can carry large-sized DNA constructs. In particular, positively charged synthetic polypeptides (e.g., poly-L-lysine) efficiently bind negatively charged nucleic acids and have been explored as gene delivery systems [125]. A special category is pH-responsive PNPs that change conformation as the particle transitions from the extracellular milieu to more acidic environments, such as the endosomal compartment (pH ~6.5) and the lysosomes (pH ~5). The conformational changes can cause the disassembly of the nanoparticles and release of the therapeutic cargo, resulting in their increased availability for intra-cellular processing [126]. Biodegradable PNPs are polyester structures that encapsulate the nucleic acid and undergo hydrolysis in the body, resulting in their slow release [127]. The use of cationic polymers like chitosan or polyethyleneimine (PEI) enhances the complexation with nucleic acids, improving their stability and transfection efficiency [128]. The ability to achieve targeted delivery and controlled release makes PNPs suitable for nucleic acid-based therapies in cancer immunotherapy. Several clinical trials have been initiated to explore the use of PNPs for nucleic acid delivery, with a significant focus on cancer immunotherapy [128, 129].

Dendrimers are synthetic, highly branched macromolecules with a tree-like structure, typically characterized by a central core, interior branches, and terminal functional groups, typically ranging in size from 1 to 50 nm, depending on the generation and the type [130, 131]. These structures provide a high degree of surface functionality and internal cavities. They are used for the delivery of proteins, nucleic acids, and small-molecule drugs [130–132]. For instance, a novel photothermal-triggered nanovaccine, G5-PBA@CuS/cGAMP, was developed by Shen et al. using PBA-functionalized G5 dendrimers, copper sulfide nanoparticles, and cGAMP. This advanced nanosystem effectively induced an antitumor immune response, inhibiting both primary and distal melanoma tumors by combining photothermal therapy and in situ tumor antigen absorption [133]. Polyamidoamine (PAMAM) dendrimers are among the most extensively studied, known for their biocompatibility and ability to encapsulate a wide range of therapeutic agents [134]. Researchers developed a cancer vaccine as an immunotherapy strategy using PAMAM dendrimer modified with guanidinobenzoic acid, demonstrating that it can effectively deliver protein antigens to DCs, enhancing antigen cross-presentation and inducing robust T-cell responses against tumors [135]. PAMAM and other amine-containing dendrimers are also well-suited for nucleic acid delivery due to their ability to form stable complexes with negatively charged genetic material. PAMAM dendrimers have demonstrated high transfection efficiency and low cytotoxicity in gene delivery applications [136]. *Hyperbranched polymers*, while similar to dendrimers in their highly branched nature, are less structurally defined and typically somewhat larger (10 to 100 nm in size). They offer advantages for drug delivery due to their ease of synthesis and functional versatility [130, 137], but this less-defined structure can lead to variations in loading and release profiles [138]. Many research studies have used dendrimers and hyperbranched polymers for nucleic acid delivery, which are reviewed elsewhere [139–141].

Polymeric Micelles are self-assembled colloidal structures that form when amphiphilic polymers, typically block copolymers, spontaneously organize in aqueous environments. The hydrophobic core of micelles can encapsulate hydrophobic drugs, while the hydrophilic shell ensures their solubility and stability in biological fluids. This unique structure makes micelles particularly effective in enhancing the bioavailability and therapeutic efficacy of various therapeutic agents [142, 143]. Micelles generally range in size from 1 to 200 nm, which is ideal for passive targeting of tumors through the EPR effect [143]. Despite some challenges related to stability and potential

toxicity, the advancements in polymeric micelle design and the success of mRNA-based vaccines underscore their potential in cancer gene therapy [144, 145]. Studies showed that micelles have the ability to co-deliver multiple drugs for chemo-immunotherapy. One study demonstrates a novel strategy using E-selectin-modified thermal-sensitive micelles to co-deliver doxorubicin and an A2A adenosine receptor antagonist, enhancing chemo-immunotherapy effects by targeting tumor sites with leukocyte hitchhiking and microwave-induced hyperthermia, leading to significant antitumor, anti-metastasis, and anti-recurrence effects [146]. Another study demonstrates that doxorubicin-loaded micelles demonstrated significant enhancement in antitumor efficacy for treating triple-negative breast cancer when they are combined with an ICI, anti-PD1 antibody [147]. The ability of micelles to deliver nucleic acids for cancer immunotherapy is reviewed comprehensively elsewhere [144, 148].

Polymersomes are vesicles formed from amphiphilic block copolymers, mimicking the structural and functional versatility of liposomes but with enhanced stability and loading capacity due to the robust nature of their polymeric membranes [149] and improved tunability due to the diversity of block copolymers available as potential components [150]. This stability translates to prolonged circulation times in the bloodstream, reduced premature release of the cargo, and enhanced delivery efficiency to the tumor site [151, 152]. Typically ranging from 10 to more than 1000 nm in size, their bilayer membrane structure and amphiphilic nature enable them to encapsulate a variety of therapeutic agents, thereby offering a multifaceted approach to cancer treatment [153]. Polymersomes can be formulated with stimuli-responsive block copolymers for the controlled release of therapeutic cargo. Moreover, the surface of polymersomes can be modified with targeting ligands, such as antibodies or peptides, to enhance their specificity for cancer cells, further improving their therapeutic efficacy and reducing off-target effects. Loading cargo into polymersomes involves various methodologies tailored to the nature of the therapeutic agent. Small molecule drugs are typically incorporated during the self-assembly process of the polymersome, ensuring they are encapsulated within the hydrophobic core [154]. The wide range of possible block copolymers used for polymersomes, ironically, presents an obstacle to clinical translation since many of them have not yet attained approval from regulatory agencies for clinical use. Polymersomes also suffer from a common limitation of nanoparticles: manufacturing scale-up. Despite these challenges, this nanocarrier shows promise for cancer immunotherapy, especially given the potential to tune structure and properties specifically for immune uptake, improve targeting through surface functionalization, and potential functionalities such as stimuli-specific cargo release [150]. A study developed pH-sensitive poly(2-(methacryloyloxy)ethyl phosphorylcholine-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC-PDMA) polymersomes that effectively encapsulated and delivered doxorubicin and paclitaxel specifically to cancer cells, exploiting elevated scavenger receptor expression, which enhances targeting and reduces off-target toxicity in head and neck squamous cell carcinoma [155]. Proteins and peptides are often loaded using passive encapsulation techniques, whereas the polymersome is formed in the presence of the therapeutic protein, trapping it within the aqueous core [156]. A study developed a polymersome that entrapped IFN-γ, which enhanced the anticancer activity of 5-fluorouracil by improving tumor targeting and modulating the TME by enhancing the CD4+ and CD8+ T cell populations, promoting the IL-12 secretion and suppressing the IL-10 secretion in colorectal cancer [157]. Since the nucleic acids are hydrophilic and negatively charged, they are usually incorporated into the aqueous core of polymersomes or into the nanostructure as they bind to the positively charged polymeric components. Previous research has illustrated that electrostatic interactions between cationic polymers and anionic cargo significantly enhance the encapsulation efficiency of proteins and nucleic acids. Asymmetric polymersomes made from triblock copolymers incorporating outer PEG, hydrophobic P(TMC-co-DTC), and cationic inner hydrophilic blocks like PDEA, PEI, or spermine, demonstrated high encapsulation efficiencies for proteins and siRNAs [150]. Reduction-responsive biodegradable chimeric polymersomes were developed to deliver cyclic dinucleotides as STING agonists. The results showed enhanced tumor retention and cytosolic delivery of the synthetic CDN ADU-S100, leading to better tumor repression and improved survival in B16 F10 melanoma-bearing mice [158]. Another study used pH-responsive imidazole-functionalized polymersomes to enhance the encapsulation of ovalbumin and CpG. This pH-responsive system also facilitated the reassembly of vesicles, significantly increasing CpG loading and enhancing biocompatibility by eliminating the need for harmful organic solvents [150].

- *Biologically-derived organic nanosystems*

Protein/peptide-based nanocarriers leverage the inherent biocompatibility, biodegradability, and functional diversity of proteins and peptides for the delivery of therapeutic cargo. These nanocarriers, typically ranging from 10 to 100 nm [159], can

encapsulate and deliver therapeutic agents directly to tumor sites, enhancing treatment efficacy while minimizing systemic toxicity. Proteins are the most diverse class of biomolecule, and protein-based nanocarriers vary widely in terms of stability, immunogenicity, loading capacity, and release properties depending on the composition. These modalities can avoid some limitations associated with nanoparticle-based systems, such as liver accumulation and immunogenicity, and they can be engineered to target specific tumor antigens, improving selectivity and reducing off-target effects [159–161]. Albumin nanoparticles have been FDA-approved to deliver several traditional cancer drugs, including paclitaxel [161]. Other recent studies have demonstrated the potential of protein-based nanocarriers in enhancing immune responses against tumors, such as by delivering ICIs or cancer vaccines [162, 163], showing promising results in preclinical and clinical settings [164–166]. Their ability to elicit potent immune responses while maintaining high stability and efficacy underscores their potential as versatile and powerful tools in the fight against cancer. Recently, researchers have explored the development of nanomedicine based on natural mussel foot proteins for tumor immunotherapy. The researchers designed a co-delivery system integrating an immunoadjuvant prodrug and a photosensitizer into this protein-based nanomaterial, leveraging the proteins bioadhesive properties for precise drug delivery. This nanomedicine not only promotes tumor photothermal ablation but also modulates the TME to overcome immunosuppression, enhancing the durability and effectiveness of antitumor immune responses [167]. In another study, researchers developed pH-responsive porphyrin-peptide nanosheets loaded with the IDO inhibitor NLG919, designed for dual applications in photodynamic therapy and immunotherapy. The nanosheets were shown to effectively target tumor cells overexpressing αvβ3 integrins, ensuring heightened cellular uptake. In an acidic tumor microenvironment, these nanosheets generated significant singlet oxygen, leading to potent photocytotoxic effects in HeLa cells. Moreover, following laser irradiation, treatment with nanosheets significantly boosted the adaptive immune response, resulting in the proliferation of CD8+ and CD4+ T cells [168]. In addition to small molecule drugs, these peptide- and protein-based nanocarriers can deliver different kinds of therapeutic nucleic acids, which are recently reviewed elsewhere [169, 170].

DNA origami, a pioneering approach in nanotechnology, utilizes the self-assembly properties of DNA to create intricate three-dimensional structures capable of delivering therapeutic agents. Typically ranging in size from 10 to 100 nm, these structures are designed by folding a long single-stranded DNA molecule into a desired shape with the aid of shorter staple strands [171]. This precise folding capability allows for the encapsulation and delivery of a variety of cargos, making DNA origami a versatile tool in cancer treatment. DNA origami can create nanocages or nanocarriers that securely hold different agents, releasing them specifically at the tumor site to maximize efficacy and minimize side effects [172, 173]. Proteins and peptides, integral to immune response modulation, can be effectively delivered using DNA origami. By incorporating protein-binding sites into the DNA framework, these nanostructures can present antigens or deliver immune checkpoint inhibitors directly to the TME. This targeted approach not only improves the therapeutic index but also enhances the immune system's ability to recognize and attack cancer cells [174]. A notable recent study addressed the challenge of efficiently delivering multiple immunological stimulators to enhance cancer immunotherapy by developing a DNA nanodevice. This nanodevice precisely assembles three immunological stimulators: doxorubicin to induce immunogenic cell death and enhance phagocytosis, exogenous double-stranded DNA (dsDNA) to activate STING signaling in APCs, and IL-12 and shPD-L1 transcription templates to regulate the TME. The device is targeted to tumors using cRGD peptide units on DNA origami and incorporates disulfide bonds for responsive release in the presence of tumor intracellular glutathione. The results demonstrated that the nanodevice promotes CD8+ and CD4+ T cell infiltration into tumors, generating a highly inflamed TME, effectively inhibiting tumor growth and preventing lung metastasis without systemic toxicity. This strategy holds promise for enhancing the effectiveness of cancer combination treatments [175].

Extracellular membranous vesicles (EMVs), also known as *exosomes* (20–150 nm) or *microvesicles* (100–1000 nm) [176] can be produced by "*farming*" cells in vitro [177–180]. Defined as naturally occurring lipid bilayer vesicles secreted by cells, they play a crucial role in intercellular communication by transferring bioactive molecules between cells, making them highly suitable for therapeutic delivery [181]. They are less toxic and less immunogenic than synthetic nanoparticles, demonstrating a better ability to cross biological barriers and evade mononuclear phagocytic systems, which results in better biodistribution [182]. The versatility of EMVs stems from their natural role in transporting various biomolecules, such as proteins and nucleic acids, as part of cellular communication processes. Studies also have shown the effective delivery of small-molecule drugs by EMVs to cancerous cells [183]. Their

ability to encapsulate and protect nucleic acids from enzymatic degradation is particularly beneficial for gene therapy applications [184]. A notable example is the use of exosomes to deliver siRNA targeting oncogenic KRAS in pancreatic cancer. This approach not only significantly reduced tumor growth but also prolonged survival in preclinical models [185]. The stability provided by the lipid bilayer membrane of EMVs ensures that nucleic acids remain intact and functional until they reach their target cells. A recent study explored the impact of exosome-delivered PD-L1 and CTLA-4 siRNAs on colorectal cancer progression and immune responses. Results show that these exosomes suppressed cell malignancy, inhibited tumor growth, and activated immune responses, with increased CD8+ T cell activity and cytokine expression, reducing tumor immune escape [186]. Furthermore, the surface proteins on EMVs can be engineered to target specific cell types, enhancing delivery precision. These vesicles can carry surface proteins and peptides, which can modulate immune responses or inhibit specific signaling pathways in cancer cells. Studies have demonstrated that engineered DC-derived exosomes loaded with peptides of melanoma-associated antigen MAGE could induce a robust immune response in patients with NSCLC, highlighting their potential in cancer vaccines [187].

Virus-like particles (VLPs) are non-infectious engineered viral capsids lacking their natural genome [188]. Expression of the viral proteins and their self-assembly can be conducted in engineered prokaryotic or eukaryotic cells or a cell-free expression system [189–191]. Initially, VLPs were approved by the FDA as a potent vaccine platform for vaccination of both infectious diseases and cancers. More recently, they have been drawing attention for their applications in targeted drug delivery and gene therapy [192]. Typically, VLPs range in size from 20 to 200 nm, making them ideal for navigating the biological milieu and targeting cancer cells effectively. Genetic engineering allows precise attachment of ligands such as antibodies to the surface of VLPs, making them potentially highly specific, similar to DAC/ADCs but capable of carrying much bigger payloads [193, 194]. Specific cancer cell targeting has been demonstrated with VLPs functionalized with antibodies such as anti-HER2 or anti-PSMA [188]. VLPs presenting the HPV16 E7 protein have shown potent immunogenicity, eliciting strong cytotoxic T lymphocyte responses and providing protection against HPV-associated tumors in preclinical models [195]. This highlights the potential of VLPs in not only delivering therapeutic proteins but also in activating the immune system against cancer. VLPs have been used to carry chemotherapeutics, nucleic acids, and proteins and peptides by electrostatic adsorption, passive encapsulation, genetic fusion, or conjugation chemistry [196–200]. Combining surface functionalization with the ability to load large therapeutic cargos makes VLP ideal for delivering complex therapeutics such as pleiotropic genetic payloads. For example, a study explored the use of bacteriophage MS2 VLPs loaded with mRNA encoding the PAP as a prostate-extensive antigen and granulocyte–macrophage colony-stimulating factor (GM-CSF). The results demonstrated that the VLPs induced robust antigen-specific immune responses and significantly inhibited tumor growth in vivo [201]. Another important characteristic of some VLPs is that they can naturally bypass endosomal trapping (endosomal escape) by fusing the lipids contained in the viral epitope with the phospholipids of the endosomes (membrane fusion mechanism) [202]. This significantly increases the endosomal escape and, therefore, the payload delivery to the cytoplasm. Humanization of VLPs has been studied to avoid sequestration and immunogenicity of the viral capsid. A VLP-based selective endogenous encapsidation for cellar delivery (SEND) based on PEG10, a retroelement from the human genome, has been described [203]. This protein is a homolog of a retroviral capsid and naturally binds mRNA, and when self-assembled into a VLP, it can deliver nucleic acid cargo. Any mRNA flanked by the PEG10-specific untranslated region (UTR) is automatically packaged in the SEND system. To increase cellular delivery, SEND was enhanced by pseudotyping the VLP with the vesicular stomatitis virus envelope protein G (VSV-G), which highly increased its efficiency. In addition, other mouse and human fusogenic transmembrane proteins were used to replace the VSV-G; in particular, human syncytin ERVW1 and ERVFRD-1 could successfully transfer genetic messages to target cells, creating a fully humanized gene transfer system [203].

Carbon-based nanoparticles

Carbon-based nanoparticles, a diverse group including fullerenes, carbon nanotubes (CNTs), graphene, and graphene oxide (GO), have emerged as promising tools for the delivery of therapeutics in cancer immunotherapy. These nanoparticles are characterized by their unique structural properties, such as high surface area, mechanical strength, and electrical conductivity. The stability of carbon-based nanoparticles is generally high due to their strong carbon–carbon bonds, which confer resistance to chemical and thermal degradation [204]. Carbon-based nanoparticles are often functionalized with various molecules for improved stability, dispersibility in physiological conditions, and conjugation and loading of therapeutic cargoes [205].

- *Fullerenes*

Fullerenes are spherical carbon molecules typically around 1 nm in diameter [206]. They are extremely hydrophobic and must be mixed with solubilizing agents or undergo covalent modification to enable aqueous solubility. Covalently modified fullerenes, including hydroxylated and aminated fullerenes [207], have inherent free radical scavenging capabilities and can enter tumors due to the EPR effect and decrease intratumoral levels of ROS. This antioxidant effect can promote tumor vasculature normalization, polarize tumor-associated macrophages toward the antitumor M1 phenotype, and inhibit tumor metastasis [208]. Fullerenes can carry small-molecule drugs, including traditional chemotherapeutic and immunotherapeutic drugs, and catalase, an enzyme that alleviates tumor hypoxia, within the fullerene's hydrophobic interior, on their surface by conjugation or as part of a hybrid nanoformulation with biocompatible polymers [208, 209]. Covalently functionalized tetraamino fullerenes were first used for gene delivery in 2000 [210], with further progress in subsequent studies, including the proven efficacy of tetra(piperazino) fullerene epoxide (TPFE) as an in vivo transfection agent, with higher rates of transfection in the liver, kidney, and spleen when compared to Lipofectin [211, 212]and the development of simplified synthesis processes [207, 213]. Recent research addressed the challenge of non-hepatic delivery of siRNA by developing a fullerene-based nanocomplex for pulmonary delivery via inhalation. This study was focused on delivering PD-L1 siRNA to treat metastatic lung cancer in a mouse model utilizing a nanocomplex of TPFE, siRNA, and an anionic water-soluble polymer, with particles averaging 91 nm in diameter. This formulation effectively inhibited tumor progression without causing significant toxicity or adverse events. This innovative approach demonstrates the potential of fullerene-based nano-complexes for siRNA delivery in treating various pulmonary diseases [214]. Fullerenes have demonstrable abilities to cross biological barriers, including the blood–brain and blood-placental barrier, which can be an advantage or a disadvantage depending on the cargo and intended target. Overall, fullerenes exhibit both inherent anticancer activity and potential as a drug delivery platform, but more extensive research about delivery routes and drug release profiles is necessary before clinical trials of this nanocarrier [209].

- *Carbon nanotubes*

Carbon nanotubes, ranging from 1 to 100 nm in diameter and up to several micrometers in length, have been extensively studied for drug delivery due to their ability to penetrate cell membranes [215]. Their high aspect ratio allows for the attachment of multiple drug molecules, providing a high payload capacity. For instance, in-vitro studies have shown that CNTs loaded with different small-molecule drugs can target cancer cells, showing in-vitro anti-neoplastic activities with comparatively lower cytotoxicity of non-cancerous cells. Additionally, functionalized CNTs have shown promise in specifically targeting the lymphatic system and have proven capable of crossing the blood–brain barrier [216]. However, safety and general biocompatibility issues must be addressed before successful clinical translation is possible [217]. Specifically, CNTs can be cytotoxic, carcinogenic, and cause organ and neurological damage. Efforts to address these challenges through changes in manufacturing processes, CNT geometry and surface functionalization are ongoing [216].

The straightforward surface functionalization of CNTs has facilitated their application as gene delivery vectors, enabling the transport of various genetic materials [218]. A recent study presents a novel immunotherapy strategy for gastrointestinal cancer by sequentially delivering a plasmid encoding for OX40L and siRNA against PD-L1 using cationic polymer brush-grafted carbon nanotubes. This approach effectively upregulated the stimulatory checkpoint OX40L on DCs and downregulated the inhibitory checkpoint PD-L1 on tumor cells and DCs. This sequential reprogramming significantly enhanced DC maturation and CD8+T cell infiltration in tumors, leading to an augmented local antitumor response and improved T cell infiltration in tumor-draining lymph nodes in a mouse model [219].

- *Graphene and graphene oxide (GO)*

Graphene and graphene oxide (GO) are typically less than 10 nm thick and vary in lateral dimensions, offering a large surface area for the adsorption and conjugation of therapeutic agents [220, 221]. GO, in particular, is more biocompatible than CNTs, while less expensive to manufacture. However, this material is also much more in the early stages of development as drug carriers [221]. The two-dimensional structure of GO facilitates the loading and delivery of genetic material such as siRNA and DNA. Recent research has highlighted the use of GO for the delivery of siRNA targeting PD-L1 [221, 222]. A notable study explored a novel approach to mRNA-based cancer immunotherapy by developing an injectable hydrogel composed of GO and PEI. The composite

hydrogel was designed to generate and release mRNA of ovalbumin and the adjuvant R848 over 30 days after subcutaneous injection in mice. The results indicated a significant increase in antigen-specific CD8+ T cells, effectively inhibiting tumor growth. Additionally, the production of antigen-specific antibodies was induced, which prevented metastasis [223].

Inorganic nanomaterials

- *Non-Metal-based Nanoparticles*

Silica nanoparticles (SNPs), which range from 50 to 200 nm in size, demonstrate several favorable attributes, including ease of synthesis, stability, tunable pore size, ease of surface functional adaptation, biodegradability, biocompatibility, and manufacturing scalability [224, 225]. The large surface area and pore volume of SNPs allow for high drug-loading capacities [224]. This capability has been demonstrated in studies where SNPs delivered small molecular drugs directly to tumor cells. The encapsulation within SNPs not only improved the solubility of drugs but also facilitated their sustained release, leading to prolonged therapeutic effects and reduced systemic toxicity [226]. Additionally, the mesoporous structure of SNPs can be functionalized to enhance the stability and bioavailability of proteins and peptides for cancer immunotherapy [227, 228]. For example, a recent study explored the use of mesoporous silica nanoparticles (MSNs) as carriers for antigen peptides in cancer immunotherapy. The researchers conducted in-vitro experiments showing that MSNs successfully accumulate in mouse DCs, where they localize to the cytosol. In-vivo experiments further demonstrated that mice treated with MSN vaccines incorporating OVA peptides showed prolonged survival after being implanted with OVA-expressing lymphoma cells. Additionally, the treated mice exhibited OVA-specific immune responses, including the production of IgG antibodies and cytotoxic T lymphocytes, indicating activation of both humoral and cellular immunity [229]. Research has also shown that SNPs can be loaded with nucleic acids through weak non-covalent interactions and effectively deliver nucleic acids targeting oncogenes, resulting in significant gene silencing and tumor growth inhibition [230, 231]. In one study, a novel polyethylenimine-modified porous silica nanoparticle (PPSN) was designed for localized delivery of IL-2 cytokine mRNA in cancer immunotherapy. Results showed that intra-tumoral injections of cytokine mRNA-loaded PPSNs led to high protein expression within tumors, triggering immunogenic cancer cell death without off-target mRNA translation or systemic toxicity. Moreover, combining this approach with an immune checkpoint inhibitor enhanced anticancer responses and inhibited distant metastases in murine models [232]. SNPs are prone to bioaccumulation, but this is being addressed through surface modification including PEG-ylation and through varying other factors such as size, shape and route of administration. Other obstacles to clinical translation include synthesis concerns such as maintaining the uniform morphology while reducing the size and the need for further pharmacokinetic testing [226].

Selenium nanoparticles (SeNPs) have gained attention in the field of cancer therapy due to their antioxidant activity, biocompatibility, and ability to modulate cellular redox states [233]. SeNPs typically range from 75 to 200 nm in size. They exhibit high chemical and thermal stability, which is crucial for maintaining the integrity of the therapeutic cargo during delivery. Their stability also ensures a sustained release [233]. While SeNPs can carry various therapeutic agents, selenium's intrinsic capabilities, such as antioxidant and anti-inflammatory properties and health benefits as a micronutrient, make SeNPs particularly interesting materials for immunotherapy applications. A study investigates a novel approach to treating malignant pleural effusion in lung adenocarcinoma by addressing the immunosuppression of NK cells caused by selenium deficiency within the TME. The researchers developed functionalized lentinan selenium nanoparticles (LET-SeNPs) to replenish selenium levels, enhance NK cell quantity, restore their functionality, and activate them through the TrxR1-IL18RAP-pSTAT3 pathway. This activation led to effective lung cancer cell elimination and reduced pleural effusion. Combining LET-SeNPs with CAR-NK cell therapy further boosted the anti-tumor effects, showcasing a promising strategy to enhance NK cell-based immunotherapy [234]. While addressing selenium deficiency boosts the immune response, selenium has a narrow therapeutic window, and overdose can lead to adverse health effects [233]. Additionally, SeNPs can serve as nucleic acid carriers with some functionalization. For instance, researchers explored the systemic delivery of mRNA by SeNPs coated with chitosan and functionalized with folic acid for targeted cancer therapy. The SeNPs effectively protected mRNA from degradation and showed low cytotoxicity in various cell lines. Notably, SeNPs exhibited moderate cytotoxicity in colorectal cancer cells, likely due to selenium-induced apoptosis. The targeted delivery was particularly effective in nasopharyngeal (KB) cells with overexpressed folate receptors, as evidenced by significant transgene expression, highlighting the potential of SeNPs in mRNA-based cancer immunotherapy [235]. Key

challenges include optimizing synthesis, ensuring long-term safety through carefully managed dosing, and clinical translation [236].

- *Metal-based Nanoparticles*

Some metal nanoparticles (MNPs) themselves, without cargo, are being utilized for cancer treatments. Their catalytic properties can be used to disrupt the TME by ameliorating hypoxic conditions or increasing the prevalence of reactive oxygen species (ROS). Some MNPs can be administered in conjunction with laser irradiation or a magnetic field to induce controlled hyperthermia, which can result in ICD at temperatures around 65 °C. Meanwhile, MNPs are also useful as delivery systems of therapeutic cargoes, and careful tuning of MNP size, composition, and shape can improve targeting or result in synergistic interactions with the cargo. For example, some MNPs are prone to be taken up by the DCs and are thus being explored as carriers for drugs intended to modulate DC-based anticancer activity. All of these innovations are reviewed more extensively elsewhere [237]. MNPs range in size from 1 to 200 nm, enabling them to exploit the EPR effect for targeted drug delivery within the TME. In addition to the MNPs introduced below, MNPs based on other metals such as copper, platinum, palladium, and ruthenium are also being studied for their anticancer effects [237].

Gold nanoparticles (AuNPs) offer unique optical, electronic, and surface properties that make them ideal carriers for various therapeutic agents. One of the key applications of AuNPs in cancer immunotherapy is the delivery of small-molecule drugs. These drugs, such as doxorubicin, are typically loaded onto AuNPs through surface conjugation, often via thiol or amine linkages [238, 239]. Proteins and peptides are usually attached to AuNPs through physical adsorption or covalent binding, depending on the desired stability and release profile [239]. Nucleic acids are typically loaded onto AuNPs through electrostatic interactions or covalent bonding [238]. For example, AuNPs loaded with DOX and siRNA targeting the HER2 gene achieved significant gene silencing and inhibited tumor growth, illustrating the potential of AuNPs for breast cancer treatment [240]. Challenges include optimizing targeting, understanding biodistribution, and ensuring clinical safety and efficacy through rigorous studies [241].

Silver nanoparticles (AgNPs) exhibit remarkable antibacterial, antifungal, and anticancer properties. Their efficacy in cancer therapy is primarily attributed to their ability to induce oxidative stress, leading to the generation of ROS that can damage cellular components, ultimately triggering apoptosis in cancer cells. AgNPs induce cancer cell death through consistent mechanisms involving endocytosis, lysosomal fusion, and the release of silver ions, which disrupt cellular homeostasis and trigger apoptosis, regardless of variations in size, shape, or capping material [242]. This apoptosis-inducing capability is beneficial when applied to cancer cells, but detrimental if the AgNPs accumulate in healthy tissue [243, 244]. While passive targeting through the EPR effect increases tumor-specific AgNP accumulation, the efficacy of EPR varies from patient to patient. Thus, active targeting through tumor-specific ligands and Ag-containing NPs designed for pH dependent release have been explored to minimize off-target toxicity [242]. By delivering anti-cancer cargos, these nanoparticles are considered dual effectors, synergistically combining the intrinsic cytotoxic effects of the AgNPs with the co-delivered drug. AgNPs can carry small molecule drugs, proteins, peptides, and nucleic acids by conjugation onto the surface via different covalent and non-covalent bonding or electrostatic interactions, ensuring their stability and bioavailability [245]. AgNPs conjugated with siRNA have been shown to silence oncogenes, thereby inhibiting tumor growth and proliferation effectively. Additionally, the inherent cytotoxicity of AgNPs can synergize with gene therapy, enhancing the overall anticancer effect. The ability of AgNPs to penetrate cell membranes and accumulate within tumor tissues further supports their potential as a delivery vehicle for nucleic acids in cancer therapy. However, for successful clinical translation, any AgNP-based therapy strategy must address the general cytotoxicity, counteract nonspecific accumulation, and comprehensively study long-term effects of AgNPs [246].

Iron nanoparticles (FeNPs), especially superparamagnetic iron oxide nanoparticles (SPIONs), are promising for delivery in cancer therapy due to their magnetic properties, biocompatibility, and functionalization versatility. The magnetic properties of FeNPs enable their use in magnetic resonance imaging (MRI) and magnetic hyperthermia, providing dual functionality for both diagnostic and therapeutic purposes [247, 248]. Magnetic hyperthermia, the phenomenon of generating heat when subjected to an alternating magnetic field, can be exploited to kill cancer cells directly or to enhance the efficacy of chemotherapeutic agents. Small molecule drugs can be loaded via surface adsorption or encapsulation within a polymeric shell surrounding the iron or iron oxide core [248, 249]. In a study focused on developing a targeted drug delivery system for treating fibrosarcoma, a rare and aggressive cancer, SPIONs were synthesized using pulsed laser ablation in liquid. These SPIONs were then coated with paclitaxel, chitosan, and PEG and further functionalized with folate receptors to target cancer cells.

Nanoparticles exhibited favorable physical properties and effectively induced apoptosis in fibrosarcoma cell lines. In-vivo studies using tumor-bearing mice demonstrated that these nanoparticles significantly reduced tumor size, improved survival rates, and modulated immune responses [250]. Another study presents the development of SPIONs decorated with an anti-VEGF peptide, HRH, for targeted delivery of paclitaxel to tumors. In-vitro tests revealed high loading efficiency, sustained drug release, and significant anti-proliferative activity against A549 lung adenocarcinoma cells, accompanied by reduced VEGF-A secretion. In-vivo results demonstrated 76.6% tumor regression in a lung tumor xenograft mouse model, enhanced PTX half-life, and extended plasma circulation time [251]. A study developed a novel immunotherapy strategy for pancreatic cancer by a combination of gemcitabine loaded in dextran-coated iron oxide nanoparticles with siRNA targeting PD-L1. This combination therapy allows for both therapeutic intervention and noninvasive monitoring using MRI. In a murine model of pancreatic cancer, this therapy significantly reduced tumor growth, achieving a 90% reduction in tumor volume within two weeks and significantly extended survival [252]. Challenges include optimizing safety, biocompatibility, and targeting specificity, with ongoing research needed for clinical translation [253, 254].

Composites nanocarriers

It is worth noting that some of the previously mentioned examples are *composite-based nanoparticles*, or *nanocomposites*, combining the benefits of multiple materials to enhance delivery efficiency and therapeutic outcomes [255]. These nanocomposites typically integrate organic and inorganic components, such as polymers, lipids, metals, semiconductors, and ceramics, to create multifunctional carriers with improved stability, targeting, and controlled release properties [256]. The combination of materials can prevent biological cargo from enzymatic degradation and immune recognition, ensuring their stability and activity until they reach the target site. Functionalization with targeting ligands allows precise delivery to cancer cells, enhancing uptake through receptor-mediated endocytosis. Additionally, nanocomposites can be engineered to release their payloads in response to specific stimuli within the TME, such as acidic pH or enzymes, maintaining therapeutic levels and minimizing off-target effects. The inclusion of imaging agents or therapeutic drugs alongside nucleic acids further supports their multifunctional use in simultaneous therapy and diagnostics [256].

- *Hybrid organic nanosystems*

Lipid-polymer nanohybrids (LPNHs) represent a sophisticated class of nanocarriers that have emerged as potent delivery vehicles for cancer immunotherapy, combining the advantages of both lipid and polymeric nanoparticles. LPNHs typically comprise a polymeric core encapsulated within a lipid shell, creating a unique structure that can effectively carry and protect various therapeutic agents [257]. The lipid shell of LPNHs facilitates efficient interaction with cell membranes, enhancing cellular uptake and intracellular delivery, while the polymeric core provides structural integrity and controlled release properties [258]. The size of LPNHs generally ranges from 50 to 200 nm, which is optimal for exploiting the EPR effect [259]. LPNHs can deliver a broad range of therapeutic agents. The hydrophobic interior of the lipid layer can effectively solubilize and stabilize hydrophobic drugs, while the hydrophilic polymer core can protect sensitive biomolecules like proteins and nucleic acids from degradation and promote their intracellular delivery [258]. This dual protection mechanism has improved the pharmacokinetics and bioavailability of small molecular drugs and proteins, leading to better therapeutic outcomes. Park et al. developed a hybrid LPNH to deliver a hydrophobic TGF-β inhibitor and IL- 2 to the TME for metastatic melanoma treatment, resulting in significant delay in tumor growth and increased survival time in tumor-bearing mice, highlighting the efficacy of hybrid nanocarriers for multi-drug delivery [260]. Finally, the efficacy of LPNHs in nucleic acid delivery has been demonstrated in various preclinical models [261]. The polymeric core can efficiently encapsulate and condense nucleic acids, while the lipid shell facilitates cellular uptake and endosomal escape. Hybrid nanoparticles composed of a cationic copolymer and lipid layers have been shown to efficiently deliver siRNA to knock down oncogenes in pancreatic cells, leading to significant tumor metastasis inhibition [262]. Similarly, folate-targeted cationic lipopolymer nanoplexes designed for the co-delivery of miRNA and a drug have exhibited potent antitumor activity in breast cancer models [263].

- *Organic–inorganic composites*

Numerous nanocarriers have been reported as composite structures of inorganic nanoparticles and organic polymers. For example, magnetic nanocomposites composed of superparamagnetic iron oxide nanoparticles (SPIONs) and polyethyleneimine (PEI) were developed. SPIONs exhibit magnetic targeting capabilities, facilitating precise delivery to tumor sites, while PEI enhances siRNA loading and protection, leading to significant gene silencing efficiency in vitro and

in vivo and reducing tumor growth in a mouse model of liver cancer [264]. Another example explored the use of a pH-sensitive hydrogel nanocomposite for delivering the anti-cancer drug quercetin. The nanocomposite, made of polyacrylic acid, agarose, and Fe3O4@SiO2 particles, demonstrated enhanced drug release control via a double emulsion method. The system with high drug loading and encapsulation efficiency significantly increased apoptosis of U- 87 MG glioma cells [265].

- *Metal–organic framework (MOF)*

MOFs consist of metal ions coordinated with organic linkers to create highly porous and customizable architectures. The unique structural features of MOFs, including tunable pore sizes ranging from 2 to 50 nm and large surface areas, provide a stable environment that enhances the bioavailability and stability of delicate therapeutic agents [266]. Their high loading capacity and controlled release properties were demonstrated by a study MOF known as ZIF-8 for delivery of doxorubicin, leading to enhanced cytotoxicity against cancer cells [267]. The therapeutic agents are incorporated into the pre-formed MOF structure or covalently into its linkers [268], while proteins/peptides and nucleic acids are typically loaded in or onto MOFs through covalent bonding or noncovalent interactions such as hydrogen bond and electrostatic interaction. Additionally, the surface of MOFs can be functionalized with targeting ligands, enabling specific binding to cancer cell receptors and facilitating targeted delivery. Upon reaching the TME, MOFs can respond to specific stimuli, such as acidic pH or enzymatic activity, to trigger the controlled release of their nucleic acid cargo. This targeted and responsive delivery system not only enhances therapeutic efficacy but also reduces off-target effects, thereby embodying a new paradigm in precision oncology that optimizes treatment outcomes while minimizing adverse effects on healthy tissues [269, 270].

- *Quantum dot (QD)*

QDs are small nanoparticles, ranging from 2 to 10 nm, which usually have a core–shell composite structure of semiconductor layers that allows them to fluoresce in a variety of colors based on their size and composition. This characteristic makes QDs excellent tools for imaging and tracking in biological systems, providing a dual function of therapeutic delivery and diagnostic monitoring [271]. Both hydrophilic and lipophilic drugs can be encapsulated or conjugated onto the surface of QDs through hydrophobic interactions or covalent bonds. While proteins and peptides can be linked to QDs via bioconjugation techniques, nucleic acids are typically loaded onto QDs through electrostatic interactions or covalent bonds. QDs can target specific receptors on cancer cells, facilitating internalization and subsequent endosomal escape for effective payload release [272]. Theranostic nanoparticles that combine carbon dots (CDs) with anti-PD-L1 antibodies were developed for enhanced diagnostics and therapy for triple-negative breast cancer. The CD-antibody conjugate facilitated more effective internalization of anti-PD-L1 antibodies into cancer cells, significantly boosting their cytotoxic effects and inhibiting cell viability and proliferation [273]. Another study used Ag2S QDs as a nucleic acid carrier. Long single-stranded DNA sequences containing PD-L1 aptamers and C-rich palindromic sequences were synthesized using rolling circle amplification. The resulting QDs specifically targeted and illuminated tumors with high PD-L1 expression, serving as effective molecular probes. Additionally, the QDs exhibited strong photothermal properties due to their high NIR-II absorption. The polyvalent PD-L1 aptamers on the QDs block the inhibitory PD-L1 signal on T cells, enabling a combined photothermal and immune checkpoint therapy. This approach enhances the biological stability and anti-bleaching of Ag2S QDs, creating a robust theranostic platform that effectively targets and treats PD-L1 high-expressing tumors in both in-vitro and in-vivo settings [274]. Toxicity of QDs depends on the formulation. Heavy metal-based QDs can exhibit poor renal clearance and risk heavy metal toxicity, even when coated with a more biocompatible material. Carbon-based QDs offer greatly improved safety profiles, at the cost of decreased quantum efficiency and manufacturing challenges. While quantum dots are establishing a niche in cancer diagnostics, they also have potential for success in drug delivery provided ongoing efforts to address toxicity and targeting efficiency are successful [275, 276].

Targeting

Need for precise targeting

Regardless of either conventional cancer therapies or modern cancer immunotherapies, non-specific delivery of highly toxic cargoes to healthy cells causes many underlying side effects and toxicities, which become one of the main reasons why so many promising cancer therapies in basic research cannot be successfully translated into clinics. Thus, deliberate strategies using nanocarriers are crucial to delivering these therapeutic

cargoes to the intended targets while minimizing their non-specific deliveries to their healthy counterparts [13, 177, 224].

Levels of targeting

Before therapeutic cargoes reach their intended targets, they confront various challenges and biological barriers to overcome, including RES, uptake by other immune cells, endothelial cell layer, and other physical barriers of tissues that prevent penetration, inactivation by biological molecules (*e.g.,* degrading enzymes), and cytoprotective mechanisms that prevent them from functioning on specific subcellular organelles [277]. The therapeutic index of payloads is often compromised by poor tumor accumulation, inefficient cellular internalization, or inaccurate subcellular localization [259, 278]. Thus, the strategies for targeted delivery of nanocarriers and their cargo can be designed at three different levels, targeting i) tumor tissues, ii) tumor cells, and iii) intracellular organelles. Here, we will briefly describe the strategies of targeting by categorizing them into passive-targeting, active-targeting, stimuli-responsive delivery, and biomimetic designs for targeting (Scheme 2).

Strategies of targeting

- *Passive Targeting*

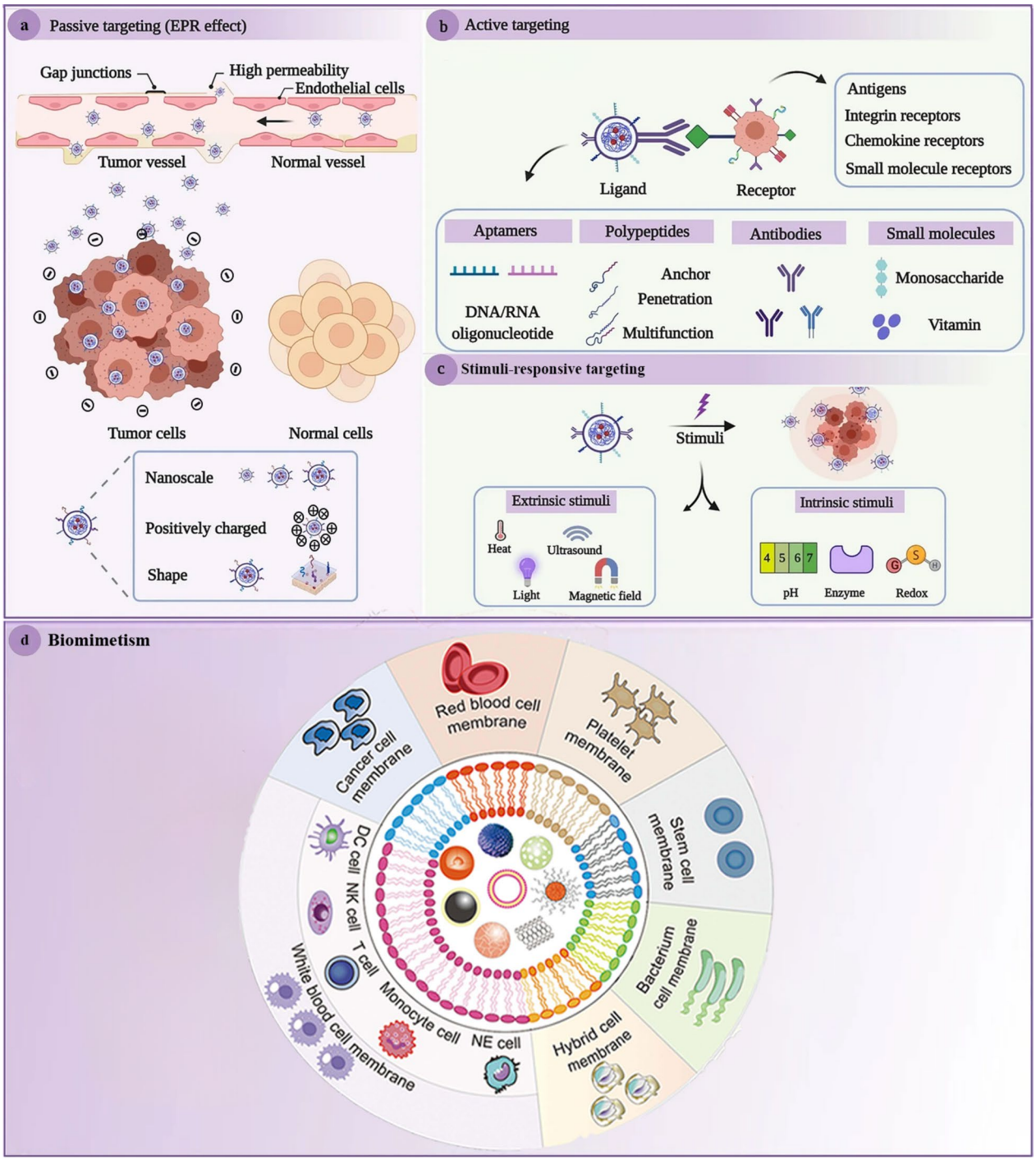
Targeting methods that do not employ specific molecular (e.g., receptor-ligand) interactions can be considered passive targeting strategies. Among such strategies, the EPR effect is a critical phenomenon that endows unique tumor tissue targeting capability in using nanocarriers for cancer immunotherapy [259, 278]. The EPR is a pathophysiological phenomenon by which macromolecular compounds or nanocarriers of sizes above a threshold size which is easily cleared (usually 40 kDa or a hydrodynamic radius of 5–6 nm) will progressively accumulate in tumor vascularized tissues. This is due to the combination of two structural features of rapidly growing tumor tissues. First, intratumoral angiogenesis creates defective and leaky endothelial fenestrations within tumor neo-vasculature, which allows easier extravasation of macromolecules and nanocarriers to tumor tissues (enhanced permeability). Second, the ineffective lymphatic drainage and lack of interstitial fluid transport within the tumor tissue retain the macromolecules and nanocarriers in the interstitium (retention). For this reason, the most important characteristic of nanocarriers for successful passive targeting is the size, where diameters from 50 to 150 nm are considered optimal.

However, targeting via the EPR effect is not always successful in clinical practice since its strength varies depending on the type and location of tumor tissues and faces challenges due to dense extracellular matrix, high interstitial fluid pressure levels, and other factors that arise from individual tumor heterogeneity and complexity. In addition, the EPR is a slow process that requires the nanoparticle to remain in circulation for an extended time. Thus, passive systems require surface modulation to avoid elimination and clearance by RES.

In this vein, some nanocarriers were studied and designed for their tissue-specific tropism without employing specific ligands. Selective Organ Targeting (SORT) is a methodology that enables tissue-specific delivery of LNPs. By modifying the surface charge of LNPs, researchers have optimized their biodistribution to enhance the passive targeting and delivery of immunotherapeutic agents to specific organs such as the liver, lungs, and spleen [279]. These nanoparticles delivered mRNA and CRISPR-Cas9 components to epithelial cells, endothelial cells, B cells, T cells, and hepatocytes, showcasing the capability of SORT to effectively deliver therapeutic molecules for gene editing and protein replacement therapies. The compatibility of SORT with various gene editing techniques, such as mRNA, Cas9 mRNA/single guide RNA, and Cas9 ribonucleoprotein complexes, marked a significant advancement in targeted gene therapy [279]. A recent study further refined LNPs by altering lipid material structures and compositions for organ-targeted mRNA delivery [280]. By creating a combinatorial library of degradable-core ionizable cationic lipids and optimizing LNP compositions, the researchers enhanced organ-specific targeting, achieving better mRNA accumulation and translation in the lungs and liver. The study highlighted that removing cholesterol from LNPs reduced liver accumulation and increased delivery to the spleen. This study also explored three-component ionizable cationic lipid/permanently cationic lipid/PEG-lipid LNPs for enhanced pulmonary delivery, demonstrating superior stability, endosomal escape, and mRNA release profiles [280]. These innovations resulted in significant improvements in organ-specific cancer immunotherapy.

- *Active Targeting*

All strategies employing the conjugation of specific ligands on the nanocarriers can be regarded as active targeting. As mentioned in the previous sections, targeting ligands can be antibodies, antibody fragments, other proteins or small peptides, aptamers, carbohydrates, or small molecules. For specific



Scheme 2 A detailed representation of strategies for targeted delivery of nanocarriers in cancer therapy. **a** Passive targeting through the Enhanced Permeability and Retention (EPR) effect and how tumor vasculature and nanoparticle characteristics facilitate the targeting of TME. **b** Active targeting via ligands binding to specific cancer cell receptors. **c** Stimuli-responsive targeting, where the release of therapeutic cargo is activated by external or internal triggers. **d** Biomimetic approaches, employing cell membranes for camouflaging nanocarriers and improving targeting efficiency. Adapted and modified from Refs. [341, 342]

targeting of tumor tissues, various strategies have been developed, such as targeting receptors expressed by tumor vasculature or stroma (e.g., VEGFR, αvβ3 integrin receptor, part of TGFβ receptor complex CD105) or targeting tumor infiltrating immune-cells (e.g., monocyte and macrophage, neutrophil, T cell, stem cell, and red blood cells) [281]. Recently, the internalizing RGD (iRGD) peptide (CRGDKGPDC) has been widely explored as a ligand for effective tumor targeting and tumor penetration. First, its RGD sequence binds to αvβ integrins (αvβ3/αvβ5) overexpressed on tumors and tumor vasculature, which enables tumor-specific targeting. Within the TME, it is cleaved by local proteases, leaving the -RGDK sequence exposed at the C-terminus, a variant of the CendR motif, which binds to NRP- 1 (neuropilin- 1), inducing extravasation via transcytosis and deep tumor penetration [282].

For targeting specific cancer cells, antibodies or antibody fragments against tumor-associated antigens (TAA) (e.g., epidermal growth factor receptors (EGFR) [283], human epidermal growth factor receptor-2 (HER2), and prostate-specific membrane antigen (PSMA)) have been explored extensively. These TAAs can also be targeted by aptamers, single-stranded synthetic nucleic acids (both DNAs and RNAs) capable of self-pairing to acquire secondary structures that can predictably fold to complex tertiary structures that can specifically bind with high affinity to distinct biological targets [284]. Examples of cancer-specific aptamer binding are PSMA [285] and protein kinase 7 (PTK7) [286]. Aptamers are susceptible to degradation by nucleases in the bloodstream, which becomes one of their significant limitations, along with potential immunogenicity and difficulties in large-scale, cost-effective production. Protein ligands such as transferrin, high-density lipoprotein (HDL), lactoferrin, EGF, and various adhesion proteins (cadherins, integrins, and selectins) can also be used to target specific tumor cells. Meanwhile, various carbohydrates such as galactose, mannose, hyaluronic acid, beta-galactoside, and sialyl Lewis can be utilized as targeting ligands against their endogenous carbohydrate-binding proteins or domains (lectins) expressed dominantly in certain cancers. Lastly, small molecules such as folic acid, biotin, and ACUPA can be introduced as targeting ligands against their receptors on certain tumor cells.

Various therapeutic cargoes must be delivered to specific intracellular organelles for their maximized efficacy and minimized toxicity. For nuclear targeting, endogenous nuclear localization signals [287, 288], TAT peptide [289–291], dexamethasone [292–294], and AS1411 aptamer [183, 295] have been explored. For mitochondrial targeting, endogenous mitochondrial targeting signals [296], mitochondria-penetrating peptide [297, 298], triphenylphosphonium [299–301], DQAsomes [302, 303], and so-called Mito-Porter [304–306] have been developed and utilized. For endo/lysosomal targeting, endocytosis-mediated endo/lysosomal targeting strategies [307], the use of lysosomal sorting peptides [308–310], and morpholine [311, 312] have been widely explored. For targeting the endoplasmic reticulum (ER), ER retrieval signal [313] and pardaxin (FAL) peptide [314] were used. For targeting the Golgi apparatus, chondroitin sulfate [315, 316] and six-cysteine peptide (C6) [317, 318] have shown promising results.

- *Stimuli-Responsive Delivery*

For every level of targeting, nanocarriers' size, shape, charge, and ligand densities can contribute positively or adversely. Based on the unique physicochemical and metabolic characteristics of the TME as well as the MCN [74] composed of extracellular matrix, tumor cells, and intracellular organelles, active targeting strategies using *stimuli-responsive nanoparticles* have been developed [319]. These nanocarriers are designed to respond to specific internal (e.g., pH, redox condition, enzymatic activity) or external stimuli (e.g., temperature, light, magnetic field) [320–323]. As responses to these environmental stimuli, the nanocarriers would undergo the shrinkage of sizes [324] charge conversion [325–327], selective ligand exposure [328–330], and release of their therapeutic cargo in a controlled manner at the site of interest [289, 326]. This method significantly reduces systemic toxicity and improves therapeutic outcomes by concentrating the drug's action precisely where needed. As an example of internal stimuli, tumor tissues often exhibit an acidic microenvironment due to the Warburg effect, where cancer cells preferentially utilize glycolysis for energy production, even in the presence of oxygen, resulting in the production of lactate [17]. This characteristic acidic environment (pH 6.5–6.8) compared to normal tissues (pH 7.4) provides a basis for designing pH-sensitive nanocarriers. These carriers are typically constructed from materials that remain stable at physiological pH but undergo structural changes or degrade in acidic conditions, leading to the release of the encapsulated drug at the tumor site. The redox potential within cancer cells differs markedly from that in normal cells due to the elevated levels of glutathione (GSH), which is several folds higher in tumors [331]. Thus, redox-responsive nanocarriers are engineered with disulfide bonds or other redox-sensitive linkages that are cleaved

in the presence of high GSH concentrations, triggering drug release selectively within cancer cells [332]. Additionally, enzyme-responsive nanocarriers leverage the overexpression of specific enzymes in diseased tissues [333]. For example, matrix metalloproteinases (MMPs) are often overexpressed in tumors and inflamed tissues. Nanocarriers designed to be degraded by these enzymes can release their payload, specifically in the presence of the target enzyme, enhancing drug accumulation in the diseased site while minimizing off-target effects [334]. On the other hand, external stimuli-responsive systems rely on externally applied triggers such as temperature, light, or magnetic fields to initiate drug release. These systems offer precise temporal and spatial control over therapeutic cargo delivery [335].

Thermo-responsive nanocarriers are designed to release their drug load when exposed to hyperthermia, which can be externally induced. These carriers are often composed of polymers and lipid-based nanocarriers that undergo a phase transition at a specific temperature, typically slightly above body temperature (42 °C), allowing for localized drug release upon heating [336]. This method is particularly useful in conjunction with hyperthermia therapy for cancer treatment. Light-triggered drug release can be achieved using photo-responsive nanocarriers, which are activated by specific wavelengths of light. Near-infrared (NIR) light is particularly attractive because of its deep tissue penetration and minimal damage to surrounding healthy tissues. These nanocarriers can be designed with photosensitive molecules that change conformation or degrade upon light exposure, releasing the drug at the desired site [337]. Magnetic-responsive nanocarriers are engineered with magnetic nanoparticles that can be directed to the tumor site using an external magnetic field. Once accumulated at the target site, drug release can be triggered by applying an alternating magnetic field, which induces heat through magnetic hyperthermia or causes the carrier to deform and release its cargo [338, 339].

- *Biomimetism for Targeting*

Most synthetic nanoparticles are perceived as "*foreign*" materials by the body, resulting in off-target accumulation, particularly by the RES, and the generation of adverse responses. Due to recognition and sequestration by immune phagocytes, only negligible numbers of nanocarriers (roughly estimated to be 1%) reach the targeted site to perform their intended function. Taking advantage of natural cell membranes, the use of cell-derived surfaces has risen as an alternative to artificial coatings or encapsulation methods. Biomimetic technologies are based on the use of isolated natural components to provide autologous properties to the nanoparticle or cargo being encapsulated, thus, improving their therapeutic behavior [340, 341]. The main goal is to replicate the (bio)-physical properties of the source tissue, not only providing a stealthy character to the core but also taking advantage of homotypic properties [340]. By coating nanoparticles with cell membranes or other natural components, they can evade immune detection, leading to prolonged circulation times and reduced clearance [342]. This mimicry helps the nanoparticles blend in with the body's natural cells, avoiding rapid elimination by the immune system. Homotypic properties refer to the ability of the biomimetic nanoparticles to target and bind to cells of the same type from which the membrane or natural component was derived. For example, cancer cell membranes used to coat nanoparticles can help direct the drug-loaded nanoparticles to the same type of cancer cells in the body, enhancing targeted delivery with potentially lower doses and reduced side effects [340].

Nanoparticles can be functionalized with cell membranes either in their natural state or after genetic or chemical engineering to enhance specific properties. Engineering the membranes can involve adding targeting ligands or modifying surface proteins to improve homing to specific tissues or cells. This approach can be achieved through various forms of engineering, including chemical, genetic, and other advanced techniques. There have been four different strategies based on the target of biomimetic modification: 1. Leukocyte mimicking, which can improve the targeted delivery of therapeutics to diseased tissues, such as tumors or areas of chronic inflammation, leveraging the natural trafficking properties of leukocytes [343], 2. Cancer and cancer stem cell mimicking, which aims to leverage homing to tumors due to the homotypic binding properties [344], 3. Red Blood Cell (RBC) mimicking exploits the long circulation time and biocompatibility of RBCs [345], and 4. Biohybrid mimicking combines synthetic materials with biological components to create hybrid systems that leverage the advantages of both [346]. These systems can involve coating nanoparticles with various cell membranes or integrating cellular components to enhance targeting, delivery, and therapeutic efficacy.

Nanocarriers for clinical translation

In this section, we will assess our current understanding of clinical outcomes of nanocarrier based medicines, examine their potential for clinical translation in the future, and discuss current strategies in the field for improving their clinical translation. Clinical trials testing nanocarriers for cancer can be grouped into two categories: (1) nanocarriers used to enhance the killing of cancer cells, such as those used to deliver chemotherapies or enhance radiotherapies, and (2) nanocarriers designed to activate and potentiate anti-tumor immune responses.

Over multiple decades, clinical trials have been performed to understand the clinical benefit of using nanocarriers to improve the efficacy of chemotherapies [347, 348]. These include more advanced Phase II and Phase III trials testing the efficacy of chemotherapy (e.g., paclitaxel, doxorubicin) alone or delivered with a nanoparticle carrier. In these clinical trials, unfortunately, the patient outcomes were fairly evenly split between trials showing significant improvements using the nanocarrier and trials showing no significant improvements using nanocarriers [348]. Differences in patient population selection and treatment regimens may be leading to these discrepancies in the impact of nanocarriers on patient responses. Meanwhile, the intrinsic properties of nanocarriers have been explored in clinical trials to enhance existing cancer treatments using hyperthermia [349] and/or radiotherapy [350]. For example, NBTXR3 is a hafnium oxide nanoparticle that can induce apoptosis by generating ROSs in response to ionizing radiation. In a Phase II/III clinical trial, a pathological complete response (pCR) of 16% was seen in soft tissue sarcoma patients treated with NBTXR3 with radiotherapy compared to 8% pCR in patients with radiotherapy treatment alone [350]. Several Phase I-III clinical trials have been initiated to test NBTXR3 with radiotherapy alone or in combination with chemotherapies, checkpoint inhibitors, and targeted therapies in different cancer indications. Indeed, a current theme in the field is to determine how to enhance patient responses to nanocarrier-based medicines by combining them with other standard-of-care therapies in clinical trials. These current and future trials will further enhance our understanding of how to improve nanocarrier-based medicines and which combinations prove to be most efficacious in different contexts.

More recently, in the last 10 years, nanocarriers designed to activate immune responses to tumors have been explored in the clinic [351]. These include lipid nanoparticle-based cancer vaccines, which have been showing promising results. mRNA-4157 is a personalized mRNA cancer vaccine that resulted in a reduction in the risk of recurrence in melanoma patients when given in combination with the anti-PD1 checkpoint inhibitor pembrolizumab compared to pembrolizumab alone in a Phase II trial (KEYNOTE- 942) [352]. Autogene cevumeran (BNT122) is a personalized RNA neoantigen vaccine that induced de novo T cell responses to neoantigens in 8 of 16 pancreatic cancer patients in a Phase I trial [353]. Three years later, vaccine-induced T neoantigen-specific T cells could still be detected, and 6 of 8 patients with these de novo T cells remained disease-free, while 7 of 8 patients without an immune response to the treatment showed tumor recurrence [354]. These promising results have led to the initiation of further clinical trials testing these nanoparticle-based medicines in different cancer indications.

Other methods are being employed using nanocarriers to enhance immune responses in the clinic. For example, nanoparticles are being used to induce the expression of chimeric antigen receptors (CARs) that target tumor antigens. A lipid nanoparticle MT-302 delivers CARs selectively to myeloid cells to enhance the recognition and killing of TROP2-expressing tumor cells to enhance adaptive immune responses. As another example, a lipopolymer-based nanocarrier, GEN- 1, is being used to locally deliver a DNA plasmid encoding IL-12, a cytokine that can reshape the TME, to promote antitumor immunity [355]. These cutting-edge nanocarrier-based medicines are incorporating advances in engineering and immunotherapy to test innovative combinations in the clinic. The results from these current and future trials will reveal valuable insights into the potential of nanocarriers in the clinic.

Conclusions—summary and recommendations

This review is intended to provide an overview of modern cancer immunotherapies leveraging various MOAs defined by the biological functions of the relevant therapeutic cargoes. These therapeutic cargoes of different physicochemical characteristics induce optimal efficacies without unnecessary side effects and toxicities only when delivered to desired tissues, cells, and intracellular organs. While such delivery is rarely achieved with the administration of bare therapeutic cargoes, prudent selection of suitable nanocarriers can enable precise delivery, minimal systemic clearance or degradation, and mitigated toxicities and side effects. This selection of nanocarriers should be accomplished by considering the biochemical

Table 1 Previous snapshot examples of nanocarriers for delivery of various cargoes for respective cancer immunotherapies

Nanocarrier	Size (nm)	Therapeutic Cargo	Types of cancer immunotherapy	Refs.
Lipid NPs	50–300	mRNA, SiRNA	ICD, Cancer vaccines, Intratumoral modulators of the TME	[100, 111, 112]
Liposomes	50–300	Both hydrophobic and hydrophilic small-molecule drugs Proteins, peptides Nucleic acids	ICD ICD, cancer vaccine, Intratumoral modulators of the TME Cancer vaccines, Intratumoral modulators of the TME	[102, 108–110]
Lipid nanoemulsions	20–200	Hydrophobic small molecular drugs With cationic lipids suitable for nucleic acids and peptides	ICD Cancer vaccine, Intratumoral modulators of the TME	[105, 113, 114, 117]
Nanostructured lipid carriers	10–500	Hydrophobic small-molecule drugs Proteins/peptides siRNA	ICD Cancer vaccine, Intratumoral modulators of the TME ICD	[101, 118]
Solid lipid nanoparticles	10–1000	Hydrophobic small-molecule drugs miRNA, siRNA	ICD ICD, cancer vaccine	[101, 118]
Polymer nanoparticles	Vast range from nano to micro	Small molecule drugs Large DNA	ICD Gene therapy, cancer vaccine	[121, 123, 125–129]
Dendrimers	1–50	Hydrophobic small-molecule drugs proteins	ICD, Cancer vaccine, Intratumoral modulators of the TME	[130, 131, 133–135, 139–141]
Hyperbranched polymers	10–100	Large proteins Large nucleic acids	ICD, cancer vaccine, Intratumoral modulators of the TME	[136–138, 140]
Polymeric Micelles	1–200	Both hydrophobic and hydrophilic small-molecule drugs Nucleic acids	ICD ICD, Cancer vaccine	[142–144, 144–148]
Polymersomes	10–100	Both hydrophobic and hydrophilic small-molecule drugs Proteins Nucleic acids	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine	[149, 150, 150–152, 154, 156–158]
Protein/peptide-based nanocarriers	10–100	Proteins/peptides Nucleic acids	ICD, Intratumoral modulators of the TME, cancer vaccine ICD, cancer vaccine	[159–170]
DNA origami	10–100	DNA Proteins	Gene therapy, cancer vaccine ICD, Intratumoral modulators of the TME, cancer vaccine	[171–175]
EMVs	20–150 (exosomes) 100–1000 (microvesicles)	Hydrophobic small-molecule drugs RNAi Surface proteins and peptides	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine, Intratumoral modulators of the TME	[176–187]
VLPs	20–200	Hydrophilic or hydrophobic small-molecule drugs Proteins Nucleic acids	ICD, Intratumoral modulators of the TME Cancer vaccine	[188, 193–200, 202, 203]

Table 1 (continued)

Nanocarrier	Size (nm)	Therapeutic Cargo	Types of cancer immunotherapy	Refs.
Silica	50–200	Hydrophobic small-molecule drugs Proteins and peptides Nucleic acids	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine, Intratumoral modulators of the TME	[224–232]
Selenium	75–200	Intrinsic features Hydrophobic small-molecule drugs mRNA	Intratumoral modulators of the TME ICD Cancer vaccine	[233–236]
Gold	10–100	Hydrophobic small-molecule drugs Proteins and peptides Nucleic acids	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine	[233–236]
Silver	1–100	Intrinsic features Hydrophobic small-molecule drugs Proteins and peptides Nucleic acids	ICD ICD Intratumoral modulators of the TME ICD, cancer vaccine	[242, 245, 246]
Iron	1–100	Hydrophobic small-molecule drugs Peptides Nucleic acids	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine	[247, 248, 248–254]
MOFs	2–50	Hydrophobic small-molecule drugs Proteins and peptides Nucleic acids	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine	[247, 248, 248–254]
Quantum dots	2–10	Both hydrophobic and hydrophilic small-molecule drugs Proteins and peptides DNA	ICD ICD, Intratumoral modulators of the TME ICD, gene therapy, cancer vaccine	[271–273, 275, 276]
Fullerenes	< 1	Hydrophobic small-molecule drugs siRNA	ICD ICD, Intratumoral modulators of the TME	[206, 209, 209, 211, 214]
Carbon nanotubes	1–100	Hydrophobic small-molecule drugs siRNA	ICD ICD, Intratumoral modulators of the TME	[215, 217–219]
Graphen/Graphen oxide	< 10	DNA, siRNA, mRNA	ICD, cancer vaccine	[220, 221, 221–223]
Lipid-polymer nanohybrid	50–200	Both hydrophobic and hydrophilic small-molecule drugs Proteins and peptides Nucleic acids except DNA	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine	[149, 150, 150–152, 154, 156–158]
Nanocomposites	Vast range from nano to micro	Both hydrophobic and hydrophilic small-molecule drugs Proteins and peptides Nucleic acids	ICD ICD, Intratumoral modulators of the TME ICD, cancer vaccine	[255, 256, 264, 265]

properties of the cargo as well as the physical and biochemical characteristics of biological barriers, the target site, and its microenvironment. Thus, we also provide a comprehensive overview of nanocarriers that have previously shown potential in cancer immunotherapies. Here, we summarize nanocarriers with different cargoes for cancer immunotherapies in Table 1. We also present generalized recommendations for each nanocarrier for delivering different types of cargo in Table 2.

The biocompatibility and safety of nanocarriers should be considered as the foremost requirement for the successful translation of these nanoparticles to clinics. For this reason, as introduced in the previous section,

Table 2 Suitability of nanocarriers with cargoes and functions

Nanocarrier type	Small molecule		Peptides and proteins	Nucleic acids	Endosomal escape	Intrinsic properties
	Hydrophobic	Hydrophilic				
Lipid Nanoparticle (LNP)	+	+ +	+	+ + +	+ +	• Extensively studied for mRNA delivery
Liposome	+ + +	+ + +	+ +	+		• Amphiphilicity • Softness and irregularity
Lipid nanoemulsion	+ +		+	+	+	• Easy manufacturing
Solid lipid nanoparticle (SLN)	+ +					• Better stability than liposome
Nanostructured lipid carrier (NLC)	+ +		+			• Solving crystallization problem of SLN
Polymeric nanoparticle	+ + +	+ +	+ +	+ +	+ +	• Wide variety of materials
Dendrimer/hyperbranched polymer	+ +	+	+ +	+ +	+ +	• High degree of functional groups
Polymeric Micelle	+ +	+	+	+ +	+	• Amphiphilicity
Polymersome	+ +	+ +	+ +	+ +	+	• High stability • High loading capacity
Lipid-polymer nanohybrid	+ +	+ +	+ +	+ +	+ +	• High stability • Good biocompatibility
Protein/peptide-based nanocarrier	+	+	+ +	+	+	• Good biocompatibility • Biodegradability
DNA origami	+		+	+		• Defined 3D structures
Extracellular membranous vesicle (Exosome)	+	+	+ +	+ +	+	• Low toxicity and immunogenicity
Virus-like particle (VLP)	+	+	+ + +	+ +	+ +	• Immunogenic • Defined structure and conjugation using genetic engineering
Fullerene (C60)	+ +			+ +	+	• High surface area • High mechanical strength • Electric conductivity • High stability • Photodynamic therapy (C60)
Carbon nanotube	+ +			+ +	+	
Graphene and GO	+ +	+	+	+ +	+	
Silica nanoparticle (SNP)	+		+ +	+ +	+	• Tunable pore size • Biocompatibility and biodegradability
Selenium nanoparticle (SeNP)	+		+	+	+	• Antioxidant activity
Gold nanoparticle (AuNP)	+		+	+	+	• Unique optical, electronic, and surface properties
Silver nanoparticle (AgNP)	+		+	+	+	• Generation of ROS
Iron nanoparticle (FeNP)	+		+	+	+	• Magnetic properties (MRI and magnetic hyperthermia)
Metal–organic framework (MOF)	+		+	+	+	• Tunable pore sizes and large surface area
Quantum Dot (QD)	+	+	+	+	+	• Fluorescence • Photothermal property
Nanocomposite	+	+	+	+	+	• Multi-functionality • Theranostic

Neutral/not adequately investigated yet

+ Adequate and good preliminary experimental data and preclinical trials, but still early in testing

+ + Excellent experimental results and clinical trials

+ + + Approved by a major regulatory body (such as FDA or EMA)

in many current and future clinical trials, a limited number of nanocarriers that have been previously approved by FDA or EMA, such as liposome (e.g., Doxil® [356]), lipid nanoparticles (e.g., mRNA vaccines [352, 357]), and polymeric nanoparticles made up of a few limited polymers such as PEG [358] and PLGA [359] are repeatedly employed. It is thus recommended that nanocarriers made of new synthetic lipids and polymers, as their components, need to be rigorously screened for their biocompatibility and toxicity in their

early development stages. In general, the inorganic and carbon-based nanocarriers are potentially more toxic than lipid- and polymer-based counterparts [360]. However, their intrinsic properties that enable unique theranostic functionalities make them very appealing candidates for future development [361, 362]. For all nanocarriers, developing scalable, reproducible and cost-effective manufacturing processes is an universal and ongoing challenge [363]. Along with this, we anticipate to advancement in establishing standardized protocols for assessing nanocarriers' safety and efficacy, as well as regulatory frameworks to support timely translation of novel and hybrid nanomedicine formulations.

As individual cancer immunotherapies with their distinct MOAs are proven effective, we envision that increased efforts to combine various immunotherapy strategies will emerge. Thus, nanocarriers that can effectively deliver multiple types of cargoes that possess pleiotropic functions may be highly desired in the future. Targeted delivery of multiple cargoes to different sites would demand further development of multifunctional, stimuli- or environment-responsive nanocarriers. Common to other complicated problems, the efforts to apply artificial intelligence and machine learning for designing, modeling, optimizing, and screening nanomedicine formulations have emerged and are being actively pursued [364–366]. Future research as combined efforts should be focused not only on the improvement of the safety and efficacy of the new nanomedicine formations but also on the optimization of manufacturing processes and the enhancement of regulatory alignment to achieve broader clinical translation.

Abbreviations

ACT	Adoptive cell therapy
ADC	Antibody–drug conjugate
APC	Antigen-presenting cell
ASO	Anti-sense oligonucleotide
Cas9	CRISPR-associated protein 9
CLL	Chronic lymphocytic leukemia
CNT	Carbon nanotube
CRISPR	Clustered regulatory interspaced short palindromic repeat
CSF- 1R	Colony-stimulating factor 1 receptor
CTL	Cytotoxic T lymphocyte
CTLA- 4	Cytotoxic T-lymphocyte associated protein 4
DAC	Degrader-antibody conjugate
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DNMT	DNA methyl transferase
EGFR	Epidermal growth factor receptor
EMV	Extracellular membranous vesicle
EPR	Enhanced permeability and retention
ER	Endoplasmic reticulum
FAK	Focal adhesion kinase
GO	Graphene oxide
GRAS	Generally recognized as safe
GSH	Glutathione
HDAC	Histone deacetylase
HDL	High-density lipoprotein
HER2	Human epidermal growth factor receptor- 2
ICD	Immunogenic cell death
ICI	Immune checkpoint inhibitor
IDO	Indoleamine 2,3-dioxygenase
LET-SeNP	Lentinan selenium nanoparticles
LNP	Lipid nanoparticle
LPNH	Lipid-polymer nanohybrid
MCN	Multi-cellular network
MDSC	Myeloid-derived suppressor cell
MMP	Matrix metalloproteinase
MNP	Metal nanoparticle
MOA	Mechanisms of action
MOF	Metal–organic framework
MRI	Magnetic resonance imaging
MSN	Mesoporous silica nanoparticle
NK cell	Natural killer cell
NLC	Nanostructured lipid carrier
NP	Nanoparticle
ODN	Oligodeoxynucleotide
PAMAM	Polyamidoamine
PCL	Poly(ε-caprolactone)
PD- 1	Programmed cell death protein- 1
PDC	Protein-drug conjugate
PDT	Photodynamic therapy
PEG	Poly(ethylene glycol)
PEI	Polyethyleneimine
PLA	Poly(lactide
PLGA	Poly(lactide-co-glycolide)
PNP	Polymeric nanoparticle
PPSN	Polyethylenimine-modified porous silica nanoparticle
PSMA	Prostate-specific membrane antigen
PTK7	Protein kinase 7
QD	Quantum dot
RBC	Red blood cell
RES	Reticuloendothelial system
RNP	Ribonucleoprotein
ROS	Reactive oxygen species
SEND	Selective endogenous encapsidation for cellar delivery
SeNP	Selenium nanoparticle
shRNA	Short hairpin RNA
siRNA	Small interference RNA
SLN	Solid lipid nanoparticle
SNP	Silica nanoparticle
SORT	Selective organ targeting
SPION	Superparamagnetic iron oxide nanoparticle
TAA	Tumor-associated antigen
TALEN	Transcription activator-like effector nuclease
TGF-β	Transforming growth factor-beta
TKI	Tyrosine kinase inhibitor
TLR	Toll-like receptor
TME	Tumor microenvironment
UTR	Untranslated region
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
VSV-G	Pvirus envelope protein G
VLP	Virus-like particle
ZFN	Zinc finger nuclease

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JH, PMA, and SB performed the majority of the article review and writing the manuscript. EH contributed to the study design. FMM and KHR conceptualized, structured, designed, and supervised the study. FMM and KHR edited and revised the manuscript. All authors read and approved the final manuscript.

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