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Polymeric Non-Viral Gene Delivery Systems for Cancer Immunotherapy

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Abstract:

Unlike viral vectors with undesired safety or immunogenicity concerns, biocompatible polymeric non-viral vectors as gene delivery systems are more promising in the gene or cell therapy. Lines of evidence have demonstrated that the rational design of polymeric non-viral gene vectors with optimal structure, charge density, biocompatibility and stimulus responsiveness could deliver therapeutic genes or gene vaccines (in terms of deoxyribonucleic acid DNA or ribonucleic acid RNA) into tumor-associated immunocytes (*i.e.*, macrophages, T cells or dendritic cells) in an effective and controllable manner for enhanced cancer immunotherapy. However, a timely and systematic summary of this subject is lacking. This review presents an overview of polymeric non-viral carriers with immune cell transfection ability and their potential applications in cancer immunotherapy; it also provides a tutorial for designing polymeric immune-cell genetic modification vector, although there are still few vectors in the product pipeline. With the rapid growth of immunotherapy in cancer treatment and knowledge accumulation in vector structure design, polymeric non-viral gene delivery systems might provide new hopes for tumor therapy.

1. Introduction

Immunotherapy aims to use the body's own immune system for tumor immunosuppression, in contrast to traditional cancer therapies in forms of surgical resection, chemotherapy, or radiotherapy.¹ Currently, cancer immunotherapy mainly consists of approaches to activate tumor-associated antigen release, to promote antigen take up, to activate immune cells, or to achieve T cells' tumor infiltration and tumor growth inhibition ability.² Since the first commercialized immunotherapy product in form of recombinant cytokine interferon- α (IFN- α) for leukemia treatment in 1986, current endeavors on cancer immunotherapy have been focusing on the investigations of monoclonal antibodies to block tumor immunosuppressive pathways (*i.e.*, programmed death 1 (PD-1)/programmed death ligand-1 (PD-L1) pathway), and tumor-specific T cell or antigen-presenting cell activation. Both methodologies can enhance the immune efficacy and shed new lights on tumor therapy.³

Besides antigen stimulation, immune cell activation might also be achieved by genetic modification or gene transfection, which might solve both hereditary and non-hereditary diseases at the molecular level. It is worth mentioning that the success of this strategy heavily relies on the approach of introducing a target foreign gene (also known as transgenic) into immune cells. The successful gene transfection can modulate the biology behavior or phenotype of immune cells (*i.e.*, macrophage, T lymphocyte, B cell, or dendritic cell).⁴ As a typical example, transgenic chimeric antigen receptor (CAR) T cell therapy has been achieved by modifying T cells with single-chain fragment variable (scFv), CD3 ζ signal domain, and costimulatory signal domains (*i.e.*, CD28, CD20, CD30, CD38, CD138, or CD137 (4/1BB)) by plasmid transfection approach. Food and Drug Administration of the United States (FDA) authorized CAR-T cell therapy for treating acute lymphoblastic leukemia in 2017,⁵ after its successful clinical trials. In this case, the effectiveness of this CAR-T cell therapy heavily depends on the rational design of efficient and safe gene delivery vectors, which transport CAR plasmids into T cells. Typical gene carriers are classified into viral and non-viral vectors.

The viral vector shows good transfection performance in immune cells, but it might experience adverse or undesired immune stimulation.⁶⁻¹⁰ Fortunately, recent polymeric non-viral vectors with controllable safety properties have been successfully utilized in immunotherapy. As a typical example, Zhang *et al.* constructed a polymeric vector to transport functional nucleic acid into immune cells, which endowed the cells with improved solid tumor targeting and infiltration abilities.³ This design has been proven to be an effective method for solid tumor treatment, indicating the importance of non-viral gene carriers in tumor immunotherapy.⁶ Lines of evidence have demonstrated that rationally designed polymeric non-viral gene vectors with optimal structure, charge density, biocompatibility and stimuli responsiveness are able to deliver therapeutic genes or gene vaccines (in terms of deoxyribonucleic acid DNA or ribonucleic acid RNA) into tumor-associated immunocytes (*i.e.*, macrophages, T cells or dendritic cells) in an effective and controllable manner for enhanced cancer immunotherapy, but a timely and systematic summary is lacking. Hence, as shown in **Scheme 1**, this review aims to provide an overview of recently reported polymeric non-viral carriers with immune cell transfection ability as well as their potential applications in cancer immunotherapy.

2. Structural Designs of Polymeric Non-Viral Gene Delivery Systems

In the past few decades, a number of delivery approaches (*i.e.*, physical methods, viral vectors, and non-viral vectors) have been successfully applied in cancer immunotherapy. In the case of a non-viral vector, it needs to overcome three barriers when transferring genes into immune cells. Firstly, in order to improve transfection efficiency, the vector should effectively cross the cell membrane. Secondly, it is necessary to protect the genes from the acidic environment in lysosomes before release. Finally, the gene should penetrate the nuclear membrane before its successful expression of target protein inside the nucleus.¹¹ Interestingly, polymeric delivery systems can be designed to promote the intracellular transportation of target genes and to prevent genetic substance degradation by lysosomes of immune cells. For

example, cationic polymeric delivery systems with nanostructure formation ability (*i.e.*, micelle, layer by layer assembly) have been reported to achieve high stability and successful endosome escape by proton sponge effect, indicating the importance of polymeric vector as optimal delivery systems.¹² In the following sections, the unique strengths and typical examples of polymeric delivery systems will be further discussed.

Compared with physical transfection with only *in vitro* feasibility or viral vectors with safety concerns, non-viral vectors have a number of advantages, in terms of low immunogenicity, no infection risk, a wide range of sources, and ease in functional modification. As the popular vectors in cancer immunotherapy, non-viral carriers are classified into liposomes, synthetic cationic polymers, natural polymers, and inorganic nanoparticles.¹³ As the first generation of non-viral vectors, cationic liposomes have been developed since 1987. They can form lipid complexes with genes by electrostatic force, which effectively protect and deliver nucleic acids into immune cells for immune response stimulation. Although lipid-based gene vaccines are biocompatible and easily functionalized, their immunogenicity is poor and unstable.¹⁴ Beside lipid formulation, inorganic nanoparticles (*i.e.*, silicon, iron oxide, calcium phosphate, carbon nanotube) are also able to wrap therapeutic genetic molecules and achieve immunotherapy through endocytosis. Similar to liposomes, inorganic nanoparticles are easy to prepare and functionalize. However, their *in vivo* cycle time is short, and their stability is low.¹⁵ Interestingly, polymeric non-viral vectors (*i.e.*, natural chitosan), which mainly load target genes by non-covalent electric attractions, have been recently reported to be utilized for gene delivery application. In this regard, the structural characteristics (*i.e.*, charge density, hydrophobicity, chain compliance) of polymeric non-viral vectors might have a great influence on their gene-loading and transfection rates.¹⁶ Fortunately, polymers have advantages in terms of high transfection efficiency, low gene immunogenicity, low toxicity, biodegradability and good biocompatibility. They are able to complex with target genes to form nanospheres by self-aggregation, which are valuable for

constructing gene vaccines or nucleic acid adjuvants.¹⁷ Furthermore, it is worth noting that the cationic polymers with high gene loading ability to form nanoparticles or hydrogels are recently reported to induce a stronger and more durable immune cell reaction, which outperform other non-viral vectors and indicate a great application prospect in cancer immunotherapy. Herein, we aim to summarize the structure designs and applications of recently reported polymeric non-viral vectors in cancer immunotherapy. A compact list is shown in **Table 1**.

2.1. PLL-based polymer

Poly(L-lysine) (PLL) is a class of L-lysine homopolymer, containing 25~30 lysine residues. PLL was first discovered in 1977 when S. Shima and H. Sakai screened the Dragendo~Positive (DP) substance produced by an actinomycete. After analyzing the acid hydrolysate, it was confirmed that the DP substance was ϵ -PLL, which was approved by the FDA in 2003. PLL is one of the pioneer gene delivery cationic polymers for cancer immunotherapy, and it has been widely utilized in therapeutic applications.

Firstly, PLL contains a large number of hydrophilic amino groups, which can be used to construct water-soluble conjugated polymer brushes (WSCPBs) by coupling with other polymers. WSCPBs is a kind of polymers with conjugated main chains and multiple water-soluble side chains.¹⁸ As one of the gene delivery tools for antigen presentation cellular immunotherapy, WSCPBs have good photostability, photosensitivity and high fluorescence quantum efficiency. The existence of multiple water-soluble side chains (*i.e.*, PLL) reduces the aggregation of the conjugated skeleton.¹⁹ Based on these advantages, WSCPBs have been widely used in cell imaging, biosensor and anionic gene delivery.²⁰ As a typical example, Wang *et al.* used cationic polymer PLL as the side chain and poly (p-phenylene ethynylene) (PPE) as the conjugated main chain to prepare water-soluble WSCPBs. Owing to the unique brush structure within WSCPBs, PPE-PLL had both antigen gene loading ability and excellent

water solubility, which could deliver the ovalbumin (OVA) gene vaccine into dendritic cells (DCs) for successful immunotherapy²⁰ as shown in **Figure 1a**.

Secondly, the combination of PLL with immune control point inhibitors or photosensitive immune adjuvants into an active hybrid nanostructure not only improves the anticancer efficiency but also elevates the *in situ* photo-immune response.²¹ Based on this finding, many scientists successfully combined different anticancer approaches (*i.e.*, photodynamic therapy, or immunotherapy) to achieve enhanced tumor therapy.²² Recently, Wu *et al.* reported a novel photo/immune-active hybrid nanostructure, which was based on PLL cationic polymer, graphene quantum dot (GQD), and nucleic acid adjuvant oligodeoxynucleotides (ODN) containing cytosine-phosphate-guanine (CpG) motifs,²³ as shown in Figure 1b. Due to the photo-responsibility of GQD, this nanocomposite could release endogenous tumor antigen and promote the immune stimulation response in the tumor microenvironment for enhanced cancer growth inhibition, indicating its promising application in cancer immunotherapy.²⁴

Among various types of PLL polymers, carboxylate ϵ -poly(L-lysine) (CPLL) has been proved to have antifreeze properties, with potential application as the cryoprotectant to preserve natural killer (NK) cells. As NK cells are a class of lymphocytes that can kill virus-infected cells and tumor cells without pre-sensitizing, their preservation is valuable for cancer treatment. As a typical case, Assal *et al.* found that a non-toxic biological cryopreservation agent, made of CPLL and dextran, could be used in cryopreservation of human cytokine active NK cells. It is worth mentioning that the carboxyl groups on the surface of CPLL can form biconical or hexagonal ice crystals. Compared with circular flat ice crystals observed in cooling water, hexagonal ice crystals can effectively reduce the undesired damage to cell membrane²⁵ (Figure 1c). Experimental results showed that NK cells cryopreserved by CPLL generally have stronger tumor lethality and higher resistance to leukemia cells, which can be helpful for cancer immunotherapy.

2.2. PEI-based polymer

Poly(ethylenimine) (PEI) has high charge density due to the presence of primary amine, secondary amine, and tertiary amine. Because of this high charge density, PEI has been reported to be easier in complexing with gene than PLL as early as in 1995, although PLL is popular in presenting gene vaccines and protein.²⁶ Furthermore, PEI can deliver anionic nucleic acids and produce the endosomal escape effect (ion pump effect or proton sponge effect) in immune cells. This unique proton sponge effect promotes the lysosomes escape of genes and prevents them from undesired degradation by lysosomes.²⁷

In detail, although PLL has been utilized as a gene delivery vector to deliver small interfering ribonucleic acid (siRNA), its inaccessibility to the tumor environment has limited its accurate translation within an immune cell.²⁸ However, this problem can be alleviated by PEI formulations. For example, Melamed *et al.* compared the effects of nanoparticles on the uptake rate and cytotoxicity of immune cells under two PEI and siRNA assembly methods: (1) by using siRNA as nucleic acid center, PEI nanoparticles were wrapped around the core to form poly(ethylenimine)-coated spherical nucleic acids (PEI-SNAs) nanoparticles; (2) PEI and siRNA were randomly assembled into PEI-siRNA complexes. The experimental results showed that the uptake rate of PEI-SNAs on cells was faster and stronger than that of PEI-siRNA complexes, with less cytotoxicity, indicating that the nanostructure basis and surface chemical modification of non-viral vectors were very helpful to improve gene silence.²⁹

Furthermore, recent reports have shown that PEI exhibited better cellular uptake for more effectively delivering nucleic acids into immune cells, in comparison with other non-viral vectors.³⁰ However, the high toxicity of PEI is one of its disadvantages, which is caused by the high positive charge density on its surface. Although the increased molecular weight can be helpful to improve PEI's gene delivery efficiency in immune cells, it might also induce toxicity effect. In order to solve this problem, researchers are trying to design PEI based polymer with immune cell targeting ability and reduce the cytotoxicity of PEI by structural

modification or addition of neutralizing ion.³¹ Two typical examples of reducing the toxicity of PEI based polymers are shown as follows. Firstly, Kwak *et al.* designed a disulfide-cross-linked poly(ethylenimine) (CLPEI) with dermatan sulfate (DS) addition, which could neutralize PEI cations to reduce toxicity effect. This novel CLPEI/DS could load small interfering RNA with programmed death ligand-1 (siPD-L1). With DS, this CLPEI/DS showed targeting ability and its complex with RNA exhibited specific toxicity only to cancer cells expressing CD146 membrane receptor (*i.e.*, B16F10 melanoma cells), as shown in **Figure 2a**.³² Beside PEI modification by using sulfate, acylation of PEI is also an effective approach to reducing cytotoxicity.³³ Cheng *et al.* designed and synthesized stearyl poly(ethylenimine) (stPEI) as the core, which could form nanoparticles by double emulsion with plasmid and human serum albumin (HSA). Cheng *et al.* further connected this nanoparticle with siRNA or clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated protein 9 (Cas9) by non-covalent bonds to form composite nanoparticle, which could deliver multiple nucleic acids at the same time. More interestingly, the experimental results revealed that this composite nanoparticle had low cytotoxicity and high cellular uptake, indicating the importance of structure modification for PEI's toxicity reduction.³⁴

Due to the successful design of PEI with reduced toxicity and good transfection efficiency, recent endeavors have revealed that PEI based non-viral vector could be utilized in cancer immunotherapy. For example, Cheng *et al.* reported that the nanoparticles formed by PEI and oligodeoxynucleotides containing cytosine-phosphate-guanine motifs (CpG ODNs) nucleic acid adjuvant could promote the internalization of CpG ODNs nucleic acid adjuvant, which guided the simultaneous secretion of both IFN- α and IL-6 to kill cancer cells, as shown in Figure 2b.³⁵ Besides the design of using PEI to deliver CpG nucleic acid adjuvant alone, the combination of PEI and other enzymes can also be helpful in cancer immunotherapy. As a typical case, Guan *et al.* successfully utilized PEI to co-deliver CpG and antigen ovalbumin

(OVA) by electric attractions, which could effectively inhibit tumor growth with the combination of hyaluronidase (HAase) (Figure 2c). Positive PEI can enhance the uptake of this gene vaccine, promote the escape of endosomes, and deliver genes into the cytoplasm of dendritic cells. Furthermore, the combination of HAase can lead to degradation of hyaluronic acid in tumor intracellular matrix and increase the permeability of vaccine.³⁶

2.3. PBAE-based polymer

As a cationic polymer, poly (β -amino ester) (PBAE) has the potential to deliver targeted genes to the tumor microenvironment.³⁷ Compared with non-biodegradable PEI, the polyester skeleton of PBAE contains tertiary amine structure, which can degrade rapidly and increase the water solubility of PBAE.³⁸ Due to its biodegradability, PBAE has the advantages of low toxicity and low *in vivo* accumulation risk.³⁹ Recently, PBAE based nanoparticles have been widely reported to deliver plasmid deoxyribonucleic acid (DNA) as genetic substance into immune cells, although its transfection efficiency is not as stable as PEI.⁴⁰

Similar to PEI, single PBAE can be used to deliver genes, adjuvants, and vaccines. However, the transfection efficiency of PBAE can be easily affected by the buffer system, which significantly limits its application. In detail, Wilson *et al.* investigated the complexation buffering systems made of PBAE and plasmid DNA. PBAE could be directly resuspended in DNA solution to form nanoparticles by self-assembly even in DMSO. However, compared with the monovalent cationic sodium acetate (NaAc) buffer system, PBAE non-viral polymer vectors within divalent calcium acetate (CaAc_2) or magnesium acetate (MgAc_2) buffer showed better transfection efficiency. These buffer solutions could improve the effectiveness and reproducibility of PBAE for immune cell transfection, which might further improve the durability of these non-viral vectors in cancer immunotherapy.⁴¹ Furthermore, experimental results showed that PBAE based non-viral vector was not sensitive to the mixing ratio of PBAE and pDNA, which was different from previous PEI vectors and indicated its instability.⁴²

Although PBAE has a stability problem, it can be useful as a gene adjuvant delivery system due to its low toxicity and endosome escape ability. In detail, cancer immune vaccine therapy requires gene adjuvants to stimulate the tumor microenvironment and induces proinflammatory cytokine responses or type I interferons to exert antitumor effects. Compared with the high toxicity of cationic PEI polymers, PBAE shows much lower toxicity, which safely and effectively promotes the anti-tumor effect of adjuvants.⁴³ For example, Wilson *et al.* tried to deliver cyclic dinucleotides (CDNs) nucleic acid adjuvant to the cytoplasm of antigen-presenting cells by using PBAE based nanoparticles. Experimental results showed a strong immune response to CDNs, which proved that CDNs nucleic acid adjuvant loaded by PBAE could effectively resist its cytoplasmic retention.⁴⁴ Compared with naked CDNs nucleic acid adjuvant, the effect of nanoparticle vaccine formed by PBAE and CDNs on B16 melanoma exhibited a log-fold enhancement in immune response stimulation.⁴⁵ For the other case, similar to PEI, PBAE can easily form complexes with anion genes. It is worth mentioning that PBAE has better buffering ability and endosome escape performance. The presence of acid lysosomes has been proved to achieve the gene release from nanoparticles within a few hours during immune cell-mediated cell uptake process (**Figure 3a**).⁴⁶ Due to these advantages, Tsai *et al.* firstly synthesized PBAE by Michael addition reaction to form complex adjuvants with CpG, which showed the targeting effect and immune stimulation on tumor cells (**Figure 3b**).⁴⁷ Not limited to linear non-viral polymers, branched ones with small molecular weight or low cationic degree can also be used for cancer immunotherapy.⁴⁸ In order to increase the molecular weight or cationic degree, Wilson *et al.* designed branched poly(ester amine) quadpolymers (BEAQs) to replace the traditional linear PBAE. Experimental results showed that the plasmid DNA delivery efficiency of branched BEAQs was better than that of PBAEs, which was mainly due to the effective load volume increase by the medium bifurcation of PBAEs.⁴⁹

Although PBAE alone can be used as the vector of gene adjuvant, the rapid biodegradability of PBAE is not conducive to its continuous *in vivo* function. Hence, lots of efforts have been spent to improve the physical and chemical stabilities of PBAE structure, by conjugation with PEI, oligopeptide, or poly(caprolactone) (PCL). In the case of PEI conjugation, the non-biodegradability of PEI provides a strategy to compensate for the biodegradability of PBAE, while the existence of hydrolysis-degradable ester group of PBAE keeps its low molecular toxicity. Typically, Zhao *et al.* synthesized an amphiphilic polymer PEI-PBAE with low biodegradability and low toxicity to deliver minicircle DNA (mcDNA). The experimental results proved that this cationic polymer could condense mcDNA into nanoscale particles, with high gene delivery efficiency, longer *in vivo* duration, and non-toxicity within several years (Figure 3c).⁵⁰ In the case of oligopeptide conjugation, Fornaguera *et al.* reported the linkage of oligopeptide to the end of PBAE, which could form complexes with message RNA with specific targeting ability to antigen presenting cells and favor their immunotherapy application.⁵¹ Last but not least, the application of both Michael step-growth polymerization and ring-opening polymerization leads to the production of PBAE-PCL copolymers, which have been reported to show more stable structure after intravenous injection and several times higher delivery efficiency than that of PEI in spleen.⁵² Although there are many reports on the applications of PBAE, its clinical trials are still not ideal, indicating the importance of further investigation on its structure-function relation.

2.4. PLGA-based polymer

Poly(lactide-*co*-glycolide) (PLGA) nanoparticles, with low immunogenicity, low toxicity, biodegradability and biocompatibility, are also popular pharmaceutic vectors.⁵³ Typically, PLGA is utilized as a gene vaccine vector to enhance the delivery efficiency of antigens. Compared with antigen-coated latex beads or soluble antigens, PLGA can significantly increase the delivery of major histochemical compatibility class I complexes and activate T cells for interleukin-2 (IL-2) secretion.⁵⁴ Furthermore, the gene vaccine adjuvant

made of hepatitis C virus genotype 1b (HCV1b) envelope protein 2 (E2) and PLGA was utilized as a microsphere carrier to construct gene vaccine adjuvant containing E2 envelope glycoprotein of hepatitis C virus, which showed obvious therapeutic effect by antibody neutralizing.⁵⁵ More interestingly, the size or shape modifications of PLGA microspheres to match HCV1b-E2 recombinant protein can increase the protein carrying capacity or stability, which is helpful in CD8⁺ T cells stimulation or T cell mediated immune response induction.⁵⁶

It is worth mentioning that the incorporation of PLGA with high safety in polyplex improves local transfection and expression of Toll-like receptor (TLR) agonist gene vaccine in animals, which outperforms that mediated by cationic liposome. Here are two typical examples of the combination of PLGA and TLR agonist gene vaccines. The first example was from Han *et al.*, who used PLGA as a vector to co-encapsulate polyinosinic-polycytidylic acid sodium salt as the auxiliary agent and OVA gene vaccine as the antigen to form PLGA nanoparticles. The usage of PLGA vector could effectively enhance the delivery of auxiliaries or antigen to DCs and promote DC maturity (as shown in **Figure 4a**). This novel PLGA/OVA/TLR gene vaccine could stimulate abundant OVA-specific CD8⁺ T cells production after stimulating DCs, which exhibited ideal antitumor effect in TC-1 or EG.7 tumor bearing mice.⁵⁷ The another case was the delivery of toll-like receptor TLR 7/8 specific agonist gene vaccine with PLGA polymer. In detail, the rapid *in vivo* elimination of the gene vaccine after injection limited its therapeutic application. Kim *et al.* found that the delivery of TLR 7/8 by PLGA formulation could obviously increase the gene vaccine circulation stability and promote the production of CD8⁺ T cells.⁵⁸ Furthermore, the addition of bicarbonate within PLGA could achieve pH responsive release of TLR 7/8 agonist in lysosomes, as carbonate would be destroyed under acidic conditions and lead to polymer shell breakage as well as effective release of pharmaceuticals (as shown in Figure 4b).⁵⁹

Besides direct blood vessel injection, PLGA has also been widely used in oral, subcutaneous, or nasal allergen delivery systems because of its good biocompatibility and

biodegradability.⁶⁰ In comparison with free antigens, PLGA wrapped antigens have been proved to be superior in inducing mucous membrane and systemic immunity, which is important for allergic immunotherapy.⁶¹ For example, Salari *et al.* wrapped recombinant chenopodium album allergen with PLGA, which could significantly increase the specific expression of antigen immunoglobulin G type 2a (IgG2a) without disturbing immunoglobulin E (IgE) or immunoglobulin G type 1 (IgG 1) expressions (Figure 4c). More interestingly, it has been reported that PLGA facilitated immune vaccine delivery can enhance the immune response of T helper 1 (Th1) and require less allergen dose for effective sublingual immunotherapy.⁶²

Last but not least, PLGA can also be shaped into three dimensional (3D) porous scaffolds for cancer immunotherapy. For example, the low delivery ability of PLGA to semi-innate immune cells can be improved by implementation into 3D scaffolds.⁶³ The specific implementation methods include blending with chitosan⁶⁴, blending with poly (vinyl alcohol), coating by collagen⁶⁵, or surface functionalization with arginine-glycine-aspartic acid (RGD).⁶⁶ Typically, Yang *et al.* prepared 3D porous PLGA scaffolds by surface modification of multifunctional carbon nanotubes, solvent casting, and salt immersion. Interestingly, this structure modification of 3D PLGA scaffolds with carbon nanotubes incorporation significantly improved the surface hydrophilicity, which could effectively load targeted DNA for DCs activation and achieve successful cancer immunotherapy.⁶⁷

2.5. Dendrimer

Recently, the unique structure and physicochemical properties of dendrimer (a highly branched and symmetrical branched polymer) have attracted more and more attention. As early as 1985, the concept of dendrimer was put forward by Newkome *et al.* from California Institute of Technology and Tomalia *et al.* from Dow Chemical Company. Compared with the linear polymeric non-viral vectors, dendrimers (*i.e.*, polyamidoamine (PAMAM) or polyacrylic acid sodium (PAAS)) have high reproducibility and precision controllability of

structure in the synthesis process.⁶⁸ The synthesis process of dendrimers is usually based on a central core, followed by the connection of one or more radial branches to increase the degree of bifurcation step by step, and ended with functional groups on the outermost branches.⁶⁹ For example, PEG can be utilized as functional ligands to enhance the flexibility of dendrimers, so as to improve the efficiency of delivering genes to immune cells.⁷⁰ More interestingly, dendrimers can recognize DCs through multiple receptors (*i.e.*, C-type polyelectin receptor CD205, mannose receptor (MR or CD206), dendritic cell-specific intercellular adhesion molecule 3 grabbing non-integrin or DC-SIGN, as well as lectin 1/2) and further internalize DCs to promote further immune response (**Figure 5a**).⁷¹

Recently, dendrimers are widely reported to show vaccine delivery and macrophage activation abilities. For example, in 2017, Chang *et al.* verified that dendrimer and guanidinobenzoic acid (DGBA) conjugation showed high protein or peptide binding ability, which could effectively destroy endosomes in antigen-presenting cells and deliver target genes into the cytoplasm of DCs.⁷² In 2019, Xu *et al.* used DGBA modified PAMAM dendrimer as an effective vector to deliver OVA gene vaccine and CpG adjuvant, as tumor vaccine. This vector could effectively deliver protein antigen to DCs and showed excellent inhibition effect on the growth of B16-OVA melanoma (**Figure 5b**).⁷³ Furthermore, dendrimers are also suitable for NK cell-based cancer immunotherapy. To the best of our knowledge, it is extremely tedious to conduct the *in vitro* rapid expansion of NK cells, which limits NK cell-based cancer immunotherapy. To solve this problem, Poupot *et al.* reported that the usage of dendrimer based on poly(phosphorhydrazone) with terminal conjugation of amino-bis(methylene phosphonate) (ABP) could effectively promote the amplification of NK cells, upon the addition of this dendrimer into the culture medium of peripheral blood monocytes that were isolated from patients with multiple myeloma and healthy volunteers (**Figure 5c**). This finding has demonstrated that poly(phosphorhydrazone) dendrimers might play an important role in NK cell-related cancer immunotherapy.⁷⁴ Beside NK cells, M1 type

(anti-tumor type) macrophages can also be regulated by dendrimer facilitated transformation of M2 type (tumor growth-promoting type) macrophage.^{75,76} For example, cationic carbon-silane dendrimers were utilized to facilitate M1 macrophage phenotype transformation, because this dendrimer formulation could induce gathered collagen or IL-10 production increase and STAT3 expression reduction.⁷⁷ Furthermore, PAMAM dendrimers were also reported to deliver metalloproteinase plasmids into M2-type macrophages for macrophage polarization activation, indicating the importance of cationic dendrimer in cancer immunotherapy.⁷⁸

3. Targeted and tumor environment responsive gene delivery into immunocytes

The therapeutic effect of cancer immunotherapy relies on the design of polymeric non-viral vectors to deliver target genes for engineering immune cells. Ideal polymeric vectors should be capable of effectively penetrating the cell membrane, to protect the genes from the lysosome's destructive acidic environment, and to release the loaded genes from connotative lysosomes before cellular nuclear entry. By satisfying the above requirements, the construction of effective and safe gene vectors to transport genetic substances into the nucleus or cytoplasm of immune cells through blood circulation can greatly enhance the success of cancer immunotherapy or gene therapy.⁷⁹

Unlike the viral vector with concerns of undesired immune responses, non-viral carriers have the advantages of low toxicity, simplicity in the preparation process, large gene loading capacity, and specific immune cells accumulating ability after conjugating with targeting ligands. Polymeric non-viral vector nanoparticles are very attractive vectors in gene delivery because easy structure modification can be done to promote their interaction with antigen-presenting cells and to enhance immune response.⁸⁰ Furthermore, polymeric nanoparticles have also been reported to directly activate immune pathways by inflammatory activation (*i.e.*, TLRs, inflammasomes, interferon regulatory factor (IRF)-dependent signaling, or MyD88 signaling pathways), without antigen-presenting cells (as shown in **Figure 6a**).⁸¹

Figure 6b summarizes the typical immune cells (*i.e.*, T cells or T lymphocytes, macrophages, dendritic cells and inflammatory monocytes) in tumor microenvironments as well as their unique surface receptors (*i.e.*, mannose receptor, galactose-type lectin receptor, dectin-1 in macrophages; mannose receptor or DEC-205 in dendritic cells; CD3⁺ or CD8⁺ in T cells), which might be utilized as targets for polymeric non-viral vector delivery.⁶

Based on the membrane structure of immune cells, functionalization of cationic polymeric non-viral vectors with ligands that have binding ability to immune cell membrane receptors can help to overcome the biological obstacles in the gene transfection process. This strategy can enhance intracellular endocytosis, achieve endosome escape of genetic substances, and realize the effective nuclear entry of target gene before translation into functional proteins (**Figure 7a**). Besides the strategy of cellular membrane receptor binding, polymeric vectors can also be conjugated with the cell-penetrating peptides, which facilitate the target gene's intracellular infiltration.⁸² Furthermore, cationic polymeric vectors are able to interact with immune cells with the negatively charged membrane, which is favored for immunocytes' uptake of genetic substances. It is also worth mentioning that the utilization of hydrophobic or amphiphilic polymeric vectors might increase the stability of polyplex, prevent the degradation of nanoparticles by lysosomes in the immune cells, destroy the intima, prevent extracellular circulation, and promote gene transfection in the cytoplasm. Last but not least, polymeric non-viral vectors can also be designed with tumor-environment responsiveness to either internal (*i.e.*, pH, redox environment, enzyme activity) or external stimulations (*i.e.*, light, temperature, ultrasound, magnetic fields) (**Figure 7b**), indicating the versatility of polymeric vectors.⁷⁵ The applications of polymeric non-viral vectors in transfecting immune cells are summarized in **Table 2**.

3.1. Dendritic cell

Dendritic cells (DCs) are a subset of antigen-presenting cells and can be used as an important target for tumor bioimmunotherapy. The characteristics of DCs include activation

of innate immunity, acquired immune response, and unique antigen presentation, which make DCs-based tumor immunogene therapy superior to other immunotherapy methods.⁸⁰ To the best of our knowledge, DC is the most powerful cell type to activate silent initial T cells, by gene vaccine capture from apoptotic tumor cells and the major histochemical complex (MHC) development for T cell's recognition of tumor surface.⁸¹ Hence, DCs are used as “sentinel” for antigen presentation in cancer immunotherapy,⁸² and the binding of activated T cells (or effector T cells) produces the immune effect to kill cancer cells, which is accompanied by simultaneous tumor antigen release as well as continuous immune cycle activation to enhance tumor inhibition ability.^{83,84}

In this DC-based immune response process, the activation of DCs is the most challenging issue, which is mainly due to the following reasons. Firstly, antigens and adjuvants are instable. The major components of most antigens or adjuvants are nucleic acids, proteins, or polypeptides, which can be easily degraded due to the *in vivo* environment with powerful enzymes before their successful delivery into DCs. Moreover, the concentrations of the remaining antigens and adjuvants are not sufficient to activate DCs. Secondly, antigens and adjuvants are not able to target secondary lymphoid organs, and the accumulation in other organs or cells might induce undesired toxicity. Thirdly, antigens and adjuvants cannot be effectively delivered into the DCs, thus limiting their binding with MHC in the DCs. Fourthly, antigens and adjuvants are unable to function simultaneously in DCs.⁸⁵ Therefore, the design of ideal vector systems to effectively solve the above issues can be important in DC-based cancer immunotherapy.

Interestingly, a series of non-viral vectors with high transfection efficiency and high specific immune response have been utilized in cancer immunotherapy.⁸⁶ As early as 2004, Chamrathy *et al.* synthesized a polymeric gene vector with peptide conjugation, which significantly promoted the transfection of reporter gene coding plasmid into primary DCs.⁸⁷ After that, polymeric non-viral vectors and lipid complexes with tumor-related gene vaccine

loading become popular designs in cancer immunotherapy. As a typical example in 2016, RNA-based liposome complexes were constructed by BioNTech company. This formulation could protect mRNA from degradation by extracellular ribonuclease, and effectively deliver mRNA into DCs. More interestingly, phase I clinical trial results carried out by Kranz *et al.* represent that liposome complexes have high safety to normal cells or organs as shown in **Figure 8a**.⁸⁸ Although the delivery of mRNA by liposome complexes is inspiring, the transfection performance difference between *in vivo* and *in vitro* cases, as well as the long-term safety issue, are still under investigation. In 2017, a team in Texas University designed an acid-sensitive polymer, which could deliver tumor-associated antigens to DCs. More importantly, this acid-sensitive polymer maintained high therapeutic stability in colon cancer, melanoma, or HPV-related tumors both *in vivo* and *in vitro*, as shown in Figure 8b, which might be promising as tumor vaccines.⁸⁹ The above two examples provide breakthroughs in the usage of polymeric non-viral vectors for successfully delivering antigens to DCs. However, it is also worth mentioning that the understanding and design of polymeric non-viral vectors should be further investigated to increase therapeutic efficiency or mitigate undesired safety concerns.

3.2. T lymphocytes

Leukocytes, especially T lymphocytes, are important effector cells in cancer immunotherapy. The traditional T lymphocyte activation is realized by DC antigen presentation or the complex antigen release from tumor sites. As a direct attacking approach, many T lymphocyte based therapy pathways are of great interest to researchers (*i.e.*, regulating immune checkpoint to improve T cells' immune response, or promoting cytokine presentation to stimulate cytotoxic T lymphocytes).⁹⁰ In this case, polymeric non-viral vectors can effectively protect immune antigens from degradation, with improved T cell accumulating and activation ability.⁹¹

3.2.1. Immune checkpoint modulation

There are two main pathways for tumor cells to escape host immune systems. Firstly, tumor cells can down-regulate their surface protein expression in the form of major histocompatibility complex (MHC) class I, which inhibits the recognition and attack of immune cells.⁹² Secondly, tumor cells might also up-regulate the expression of immunosuppressive molecules,⁹³ which impairs the entry of immune cells into the tumor microenvironment.⁹⁴ Therefore, blocking the above immunosuppressive pathways of tumor cells is crucial for anti-cancer immunotherapy.

As a typical example, the protein knockout of the PD-1/PD-L1 immunosuppressive pathway by siRNA gene transfection has been proved to be effective in cancer treatment.⁹⁵ In detail, although adoptive T cell immunotherapy shows great potential in treating epithelial ovarian cancer (EOC), the interaction between PD-L1 from EOC cell and PD-1 from T cell causes undesired immunosuppression and significantly limits the therapeutic progress.⁹⁶ Based on this, a complex consisting of folic acid (FA) functionalized PEI polymer and PD-L1 siRNA was designed by Teo *et al.* This formulation could efficiently block the PD-1 and PD-L1 pathways to combat immunosuppression of T cells, as shown in **Figure 9a**. In this case, the FA/polymer/siRNA targeting system is effective in knocking out the PD-1/PD-L1 pathway and promoting T cell recognition towards ECO cells. Besides the PD-1/PD-L1 pathway, transforming growth factor- β (TGF β) mediated tumor microenvironment is also a key factor of immunosuppression.^{97,98} However, TGF β R1 inhibitors are highly toxic to normal cells because TGF β signaling is necessary for normal cells.⁹⁹ Fortunately, polymeric non-viral vectors with targeted drug delivery ability have been successfully utilized to encapsulate TGF β R1 inhibitors. For example, Schmid *et al.* used PLGA-PEG polymer to encapsulate TGF β R1 inhibitor SD-208, which could significantly reduce the cytotoxicity to normal cells and restore the immune function of effector T cells.¹⁰⁰ More interestingly, PLGA-PEG polymer can also be used to load PD-1 targeting system and release TLR in the tumor microenvironment to motivate the rapid growth of tumor invasive CD8⁺ T cells.¹⁰¹ Last

but not least, the delivery of tumor-targeted immunosuppressive factor siRNA by polymer non-viral vectors shows a good modulation effect in tumor invasive CTL. Typically, Li *et al.* loaded CTL-associated molecule-4-siRNA nanoparticles (NPs_{CTLA-4}) in polymeric vectors, which could obviously promote T cells' activation and inhibit tumor reproduction in melanoma mice, as shown in Figure 9b.¹⁰² In short, the application of immune checkpoint modulation based on non-viral vectors becomes a popular approach for cancer immunotherapy.

3.2.2. CAR-T cell therapy

Based on the significant therapeutic effect of adoptive T cell therapy, CAR-T cells with CD19 expression have been approved by FDA for the treatment of lymphocytic leukemia. As a typical case of cell therapy and gene therapy, the CAR (*i.e.*, CD19) gene-mediated T-cell can lead to direct tumor cell identification, a delay in lymphatic function failure, and a significant increase in the response or survival time of patients. Furthermore, the combination of the functional domains (*i.e.*, CD4 or CD8) can be beneficial to long-term amplification or low toxicity of CAR-T cells.¹⁰³

It is worth mentioning that traditional CAR-T therapy has a number of limitations (*i.e.*, complex preparation process, high cost of treatment, increased risk of chemotherapy). Hence, a novel approach to simplify the CAR-T preparation process or to provide patients with more compliant treatment solutions becomes important. As a typical case in 2017, Smith *et al.* explored an *in vivo* polymeric vector for rapidly programming CAR-T cells. In this case, anti-CD3 sequence as T cell-targeting molecule, peptide-containing nuclear localization signals (NLS), and microtubule-associated sequences (MTAS) could be simultaneously incorporated into a cationic PBAE nanoparticle, to achieve robust CAR production in T cells as shown in **Figure 10a**.⁴ Beside *in vivo* approaches, polymeric vectors can also be valuable as alternatives to traditional physical electroporation and virus-mediated transduction. For example, Moffett

et al. successfully designed a new CAR-T preparation method which delivered mRNA-coated nanoparticle into stem cells or T cells from the patients *in vitro* (Figure 10b).¹⁰⁴

3.3. Macrophage

Macrophages or tumor-associated macrophages (TAMs) in the primary and secondary tumor microenvironment can be favored for the angiogenesis of tumor cells, which improves cancer metastasis. In detail, TAMs are mainly divided into M1 macrophages and M2 macrophages according to different activation approaches in the tumor microenvironment. As a typical species, M1 macrophage is endowed to present antigen to the T cells to activate tumor cellular immune response, which can promote the growth of the other immune cells (*i.e.*, T cells) and kill tumor cells by high-expression of nitric oxide (NO), reactive oxygen species, interleukin 23 (IL-23), or interleukin 12 (IL-12). M2 macrophages can also present antigens, but they cannot realize the immune stimulation effect of T cells.¹⁰⁵ Hence, the macrophage based cancer immunotherapy is mainly divided into: (1) Enhancing the ability of macrophage antigen presentation; (2) Inducing TAM to transform to M1 macrophage.¹⁰⁶

As mentioned earlier, monocytes, DCs and macrophages are widely used as APCs, which can also increase the immune stimulation effect of T cells by macrophage antigen presentation. However, the toxicity, poor immunogenicity, and low transfection efficiency of current vectors greatly limit macrophage antigen presentation process. It's worth mentioning that the painless and noninvasive polymeric microneedles (MNs) become useful designs in macrophage activation.^{107,108} For example, Duong *et al.* designed MNs array by using a polyelectrolyte trigger layer made of pH-sensitive polymer and heparin. *In vivo* experimental results showed that MNs could effectively realize the antigen presentation of macrophages to T cells and stimulated immune responses, as shown in **Figure 11a**.¹⁰⁹

Recently, macrophage toxicity to tumor cells has been recovered by switching TAM to M1 thanks to polymeric vector-based formulations. In the tumor microenvironment, most types of TAM are M2 macrophages, which can promote tumor vascular growth, metastasis

and modulate immunosuppression to produce malignant diseases.¹¹⁰ Hence, switching the TAM phenotype from M2 macrophages to M1 macrophages through nucleic acid therapy can be an important strategy for cancer immunotherapy.^{111,112} Shukla *et al.* proved that the presence of massive ROS around macrophages could degrade nucleic acids and further endow ineffective gene therapy.¹¹³ Based on the above findings, Zhang *et al.* utilized biodegradable star-typed polyasparagine polymer modified with folic acid or mannose to successfully transfect plasmid DNA refractory cells (*i.e.*, macrophages). Experimental results showed that the addition of dendrimers improved the transfection efficiency of plasmid DNA to macrophage by plasmid protection, which facilitated the switch of TAM to M1 macrophage.^{114,115} More interestingly, M2 macrophage phenotype transformation can also be achieved by polymeric vectors.¹¹⁶ For example, Tran *et al.* used hyaluronic acid (HA) and PEI to form non-viral vectors for loading miR-223 expression plasmid DNA, and achieved M1 macrophages to M2 phenotype transformation as shown in Figure 11b, indicating the importance of polymeric vectors in regulating macrophage phenotype.^{117,118}

4. Conclusion and Perspectives

In view of the safety and mutagenicity concerns of viral gene vectors, polymeric non-viral vectors become popular choices for immunocyte transfection. More importantly, recent works have shown that polymeric vectors with rational designs on structure, charge density, biocompatibility, and stimuli responsiveness can achieve safe, efficient, and accurate transfection of therapeutic genes in immunocytes for cancer treatment, indicating the feasibility of this novel approach. In the next decade, further development might focus on the simplification of polymeric non-viral vectors, exploration of novel intracellular transportation approaches, design of controllable or triggered gene transfection vectors, and acceleration of clinical trials. With these efforts, the technology of polymeric non-viral vector transfection in engineering immune cells might be closer to clinical practice and bring new hopes cancer patients receiving immunotherapy.

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References

- [1] D. N. Khalil, E. L. Smith, R. J. Brentjens, J. D. Wolchok, *Nat. Rev. Clin. Oncol.* **2016**, *13*, 273.
- [2] C. A. Klebanoff, S. A. Rosenberg, N. P. Restifo, *Nat. Med.* **2016**, *22*, 26.
- [3] E. Zhang, J. Gu, H. Xu, *Mol. Cancer.* **2018**, *17*, 7.
- [4] T. T. Smith, S. B. Stephan, H. F. Moffett, L. E. McKnight, W. Ji, D. Reiman, E. Bonagofski, M. E. Wohlfahrt, S. P. Pillai, M. T. Stephan, *Nat. Nanotechnol.* **2017**, *12*, 813.
- [5] S. Yang, C. E. Vervaert, J. Burch, J. Grichnik, H. F. Seigler, T. L. Darrow, *Int. J. Cancer.* **1999**, *83*, 532.
- [6] E. B. Harrison, S. H. Azam, C. V. Pecot, *Front. Pharm.* **2018**, *9*, 307.
- [7] X. Chen, Z. Chen, B. Hu, P. Cai, S. Wang, S. Xiao, Y.-L. Wu, X. Chen, *Small* **2018**, *14*, 1703164.
- [8] P. Cai, W. R. Leow, X. Wang, Y.-L. Wu, X. Chen, *Adv. Mater.* **2017**, *29*, 1605529.
- [9] X. Chen, Y.-K. Qiu, C. Owh, X. J. Loh, Y.-L. Wu, *Nanoscale* **2016**, *8*, 18876.
- [10] H. Cheng, X. Fan, C. Wu, X. Wang, Y.-L. Wu, *Macromol. Rapid Commun.* **2019**, *40*, 1800207..

- [11] Y.-L. Wu, N. Putcha, K. W. Ng, D. Leong, C. T. Lim, S. C. Loo, X. Chen, *Acc. Chem. Res.* **2013**, *46*, 782.
- [12] K. L. Kozielski, Y. Rui, J. J. Green. *Expert Opin. Drug Delivery* **2016**, *13*, 1475.
- [13] T. M. Allen, P. R. Cullis, *Adv. Drug Delivery Rev.* **2013**, *65*, 36.
- [14] S. Naskar, K. Koutsu, S. J. Sharma, *J. Drug Targeting* **2019**, *27*, 379.
- [15] Y.-L Wu, H. Yin, F. Zhao, J. Li, *Adv. Healthcare Mater.* **2013**, *2*, 297.
- [16] M. Foldvari, D. W. Chen, N. Nafissi, D. Calderon, L. Narsineni, A. Rafiee, *J. Controlled Release* **2016**, *240*, 165.
- [17] X. Liu, X. Chen, M. X. Chua, Z. Li, X. J. Loh, Y.-L. Wu, *Adv. Healthcare Mater.* **2017**, *6*, 1700159.
- [18] Y. Zhang, Z. Zhang, C. Liu, W. Chen, C. Li, W. Wu, X. Jiang, *Polym. Chem.* **2017**, *8*, 1672.
- [19] X. Zhen, C. Xie, K. Pu, *Angew. Chem. Int. Ed.* **2018**, *57*, 3938.
- [20] C. Wang, P. Sun, G. Wang, P. Yuan, R. Jiang, W. Wang, W. Huang, Q. Fan, *ACS Appl. Bio Mater.* **2018**, *1*, 1972.
- [21] X. Duan, C. Chan, W. Lin, *Angew. Chem. Int. Ed.* **2019**, *58*, 670.
- [22] D. Ma, Q. M. Lin, L. M. Zhang, *Biomaterials* **2014**, *35*, 4357.
- [23] C. Wu, X. Guan, J. Xu, Y. Zhang, Q. Liu, Y. Tian, S. Li, X. Qin, H. Yang, Y. Liu, *Biomaterials* **2019**, *205*, 106.
- [24] S. K. Rajendrakumar, S. Uthaman, C. S. Cho, *Biomacromolecules* **2018**, *19*, 1869.
- [25] R. E. Assal, L. Abou-Elkacem, A. Tocchio, S. Pasley, S. Matosevic, D. L. Kaplan, C. Zylberberg, U. J. Demirci, *Adv. Sci.* **2019**, *6*, 1802045.
- [26] L. Liu, Z. M. Zong, Q. Liu, S. S. Jiang, Q. Zhang, L. Q. Cen, H. Yao, *Biomaterials* **2018**, *184*, 20.
- [27] S. Yamano, J. Dai, S. Hanatani, *Biomaterials* **2014**, *35*, 1705.

- [28] K. Xue, X. Wang, P. W. Yong, Y.-L. Wu, Z. Li, X. J. Loh, *Adv. Ther.* **2019**, *2*, 1800088.
- [29] J. R. Melamed, N. L. Kreuzberger, R. Goyal, E. S. Day, *Mol. Ther.-Nucl. Acids.* **2018**, *12*, 207.
- [30] A. P. Pandey, K. K. Sawant, *Mater. Sci. Eng.* **2016**, *68*, 904.
- [31] B. Singh, S. Maharjan, T. E. Park, T. Jiang, S. K. Kang, Y. J. Choi, C. S. Cho, *Macromol. Biosci.* **2015**, *15*, 622.
- [32] G. Kwak, D. Kim, G. h. Nam, S. Y. Wang, I. S. Kim, S. H. Kim, I. C. Kwon, *ACS Nano.* **2017**, *11*, 10135.
- [33] B. Thapa, S. Plianwong, K. R. Bahadur, B. Rutherford, H. Uludağ, *Acta Biomater.* **2016**, *33*, 213.
- [34] W. J. Cheng, L. C. Chen, H. O. Ho, H. L. Lin, M. T. Sheu, *Int. J. Nanomed.* **2018**, *13*, 7079.
- [35] T. Cheng, J. Miao, D. Kai, H. Zhang, *ACS Biomater. Sci. Eng.* **2018**, *4*, 1013.
- [36] X. Guan, J. Chen, Y. Hu, L. Lin, P. Sun, H. Tian, X. Chen, *Biomaterials* **2018**, *171*, 198.
- [37] A. Mangraviti, S. Y. Tzeng, K. L. Kozielski, Y. Wang, Y. Jin, D. Gullotti, M. Pedone, N. Buaron, A. Liu, D. Wilson, *ACS Nano.* **2015**, *9*, 1236.
- [38] H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin, D. G. Anderson, *Nat. Rev. Genet.* **2014**, *15*, 541.
- [39] P. Mastorakos, E. Song, C. Zhang, S. Berry, H. W. Park, Y. E. Kim, J. Hanes, *Small.* **2016**, *12*, 678.
- [40] S. Min, Y. Jin, C. Y. Hou, J. Kim, J. J. Green, T. J. Kang, S. Cho, *Adv. Healthcare Mater.* **2018**, *7*, 1800052.
- [41] D. R. Wilson, M. P. Suprenant, J. H. Michel, E. B. Wang, S. Y. Tzeng, J. J. Green, *Biotechnol. Bioeng.* **2019**, *116*, 1220.

- [42] C. Cekic, J. Linden, *Nat. Rev. Immunol.* **2016**, *16*, 177.
- [43] L. Corrales, L. H. Glickman, S. M. McWhirter, D. B. Kanne, K. E. Sivick, G. E. Katibah, K. Metchette, *Cell Rep.* **2015**, *11*, 1018.
- [44] D. R. Wilson, R. Sen, J. C. Sunshine, D. M. Pardoll, J. J. Green, Y. J. Kim, *Nanomedicine* **2018**, *14*, 237.
- [45] T. Lv, Z. Cheng, E. Zhang, H. Kang, Y. Liu, L. Jiang, *Small* **2017**, *13*, 1503402.
- [46] C. G. Zamboni, K. L. Kozielski, H. J. Vaughan, M. M. Nakata, J. Kim, L. J. Higgins, M. G. Pomper, J. J. Green, *J. Controlled Release* **2017**, *263*, 18.
- [47] S. J. Tsai, J. I. Andorko, X. Zeng, J. M. Gammon, C. M. Jewell, *Nano Res.* **2018**, *11*, 5642.
- [48] D. Zhou, Y. Gao, A. Aied, L. Cutlar, O. Igoucheva, B. Newland, V. Alexeeve, U. Greiser, J. Uitto, W. Wang, *J. Controlled Release.* **2016**, *244*, 336.
- [49] D. R. Wilson, Y. Rui, K. Siddiq, D. Routkevitch, J. Green, *Mol. Pharmaceutics.* **2019**, *16*, 655.
- [50] J. Zhao, P. Huang, Z. Wang, Y. Tan, X. Hou, L. Zhang, C. Y. He, Z. Y. Chen, *ACS Appl. Mater. Interfaces* **2016**, *8*, 19284.
- [51] C. Fornaguera, M. Guerra-Rebollo, M. Ángel Lázaro, C. Castells-Sala, O. Meca-Cortés, V. Ramos-Pérez, A. Cascante, N. Rubio, J. Blanco, S. Borrós, *Adv. Healthcare Mater.* **2018**, *7*, 1800335.
- [52] U. Capasso Palmiero, J. C. Kaczmarek, O. S. Fenton, D. Anderson, *Adv. Healthcare Mater.* **2018**, *7*, 1800249.
- [53] M. B. Heo, Y. Lim, *Biomaterials.* **2014**, *35*, 590.
- [54] A. Frede, B. Neuhaus, R. Klopffleisch, C. Walker, J. Buer, W. Müller, A. M. Westendorf, *J. Controlled Release* **2016**, *222*, 86.
- [55] H. Ma, C. He, Y. Cheng, D. Li, Y. Gong, J. Liu, X. Chen, *Biomaterials* **2014**, *35*, 8723.

- [56] H. Jiang, Q. Wang, X. Sun, *J. Controlled Release* **2017**, 267, 47.
- [57] H. D. Han, Y. Byeon, T. H. Kang, I. D. Jung, J. W. Lee, B. C. Shin, Y. J. Lee, A. K. Sood, Y. M. Park, *Int. J. Nanomed.* **2016**, 11, 5729.
- [58] H. Kim, D. Sehgal, T. A. Kucaba, D. M. Ferguson, T. S. Griffith, J. Panyam, *Nanoscale* **2018**, 10, 20851.
- [59] H. Kim, D. Sehgal, T. A. Kucaba, D. M. Ferguson, T. S. Griffith, J. Panyam, *Nanoscale* **2018**, 10, 20851.
- [60] H. Seok, J. Y. Noh, D. Y. Lee, S. J. Kim, C. S. Song, Y. C. Kim, *J. Controlled Release* **2017**, 265, 66.
- [61] J. S. Park, H. N. Yang, S. W. Yi, J. H. Kim, K. H. Park, *Biomaterials* **2016**, 76, 226.
- [62] F. Salari, A. R. Varasteh, F. Vahedi, M. Hashemi, M. Sankian, *Int. J. Immunopharmacol.* **2015**, 29, 672.
- [63] S. Y. Jeon, J. S. Park, H. N. Yang, H. J. Lim, S. W. Yi, H. Park, K. H. Park, *Biomaterials* **2014**, 35, 8236.
- [64] Y. Zhu, G. Liang, B. Sun, T. Tian, F. Hu, Z. Xiao, *Int. J. Nanomed.* **2016**, 11, 399.
- [65] C. Wang, Y. Ye, Q. Hu, A. Bellotti, Z. Gu, *Adv. Mater.* **2017**, 29, 1606036.
- [66] L. Kim, T. H. Yu, J. H. Jun, H. Yang, M. Y. Yang, M. J. Cho, Y. S. Nam, *Biomaterials* **2017**, 145, 223.
- [67] Y. Yang, S. Shi, Q. Ding, J. Chen, J. Peng, Y. Xu, *J. Biomed. Mater. Res., Part A* **2015**, 103, 1045.
- [68] A. J. Perisé-Barrios, J. L. Jiménez, A. Domínguez-Soto, F. J. de la Mata, A. L. Corbí, R. Gomez, M. Á. Muñoz-Fernandez, *J. Controlled Release* **2014**, 184, 51.
- [69] X. Du, B. Shi, Y. Tang, S. Dai, S. Z. Qiao, *Biomaterials* **2014**, 35, 5580.
- [70] E. Yuba, N. Sakaguchi, Y. Kanda, M. Miyazaki, K. Koiwai, J. V, *Vaccines* **2017**, 5, 41.
- [71] L. I. Moura, A. Malfanti, C. Peres, A. I. Matos, E. Guegain, V. Sainz, M. Zloh, M. Vicent, H. Florindo, *Mater. Horiz.* **2019**, 6, 1956.

- [72] H. Chang, J. Lv, X. Gao, X. Wang, H. Wang, H. Chen, X. He, L. Li, Y. Cheng, *Nano Lett.* **2017**, *17*, 1678.
- [73] J. Xu, H. Wang, L. Xu, Y. Chao, C. Wang, X. Han, Z. Dong, H. Chang, R. Peng, Y. Cheng, *Biomaterials.* **2019**, *207*, 1.
- [74] M. Poupot, C. O. Turrin, A. M. Caminade, J. J. Fournie, M. Attal, R. Poupot, S. Fruchon, *Nanomedicine.* **2016**, *12*, 2321.
- [75] Y. Li, J. Gao, C. Zhang, Z. Cao, D. Cheng, J. Liu, X. Shuai, *Top. Curr. Chem.* **2017**, *375*, 167.
- [76] M. J. Brun, E. J. Gomez, J. Suh, *J. Controlled Release* **2017**, *267*, 80.
- [77] B. Caffery, J. S. Lee, A. Alexander-Bryant, *Nanomaterials* **2019**, *9*, 105.
- [78] H. Y. Yoon, S. T. Selvan, Y. Yang, M. J. Kim, D. K. Yi, I. C. Kwon, K. Kim, *Biomaterials* **2018**, *178*, 597.
- [79] P. Rajagopai, S. Duraiswamy, S. Sethuraman, *J. Gene Med.* **2018**, *20*, 3011.
- [80] Y. Hu, B. Xu, Q. Ji, D. Shou, X. Xu, W. Liang, *Biomaterials* **2014**, *35*, 4236.
- [81] M. B. Heo, M. Y. Cho, Y. T. Lim, *Acta Biomater.* **2014**, *10*, 2169.
- [82] F. Veglia, D. Gabrilovich, *Curr. Opin. Immunol.* **2017**, *45*, 43.
- [83] S. Spranger, D. Dai, B. Horton, T. Gajewski, *Cancer Cell* **2017**, *31*, 711.
- [84] K. T. Byrne, A. L. Côté, P. Zhang, S. M. Steinberg, Y. Guo, R. Allie, W. Zhang, M. S. Ernstoff, E. J. Usherwood, *J. Clin. Invest.* **2011**, *121*, 1797.
- [85] B. Choi, H. Moon, S. J. Hong, C. Shin, Y. Do, S. Ryu, S. Kang, *ACS Nano* **2016**, *10*, 7339.
- [86] J. J. Green, J. H., *Nature.* **2016**, *540*, 386.
- [87] S. P. Chamarthy, L. Jia, J. R. Kovacs, K. R. Anderson, H. Shen, S. M. Firestine, W. Meng, *Mol. Immunol.* **2004**, *41*, 741.
- [88] L. M. Kranz, M. Diken, H. Haas, S. Kreiter, C. Loquai, K. C. Reuter, M. Meng, D. Fritz, F. Vascotto, H. Hefesha, *Nature* **2016**, *534*, 396.

- [89] M. Luo, H. Wang, Z. Wang, H. Cai, Z. Lu, Y. Li, M. Du, G. Huang, C. Wang, X. Chen, *Nat. Nanotechnol.* **2017**, *12*, 648.
- [90] B. Şahin, J. Fife, M. B. Parmar, J. Valencia-Serna, H. Gul-Uludağ, X. Jiang, M. Weinfeld, A. Lavasanifar, H. Uludağ, *Biomaterials* **2014**, *35*, 9382.
- [91] K. T. Roybal, J. Z. Williams, L. Morsut, L. J. Rupp, I. Kolinko, J. H. Choe, W. J. Walker, K. A. McNally, W. Lim, *Cell* **2016**, *167*, 419.
- [92] Y. Xie, N. H. Kim, V. Nadithe, D. Schalk, A. Thakur, A. Kılıç, O. M. Merkel, *J. Controlled Release* **2016**, *229*, 120.
- [93] S. M. Geary, Q. Hu, V. B. Joshi, N. B. Bowden, A. K. Salem, *J. Controlled Release* **2015**, *220*, 682.
- [94] M. Li, M. Zhao, Y. Fu, Y. Li, T. Gong, Z. Zhang, X. Sun, *J. Controlled Release* **2016**, *228*, 9.
- [95] R. Monjezi, C. Miskey, T. Gogishvili, M. Schleef, M. Schmeer, H. Einsele, M. Hudecek, *Leukemia* **2017**, *31*, 186.
- [96] C. J. Maine, N. H. A. Aziz, J. Chatterjee, C. Hayford, N. Brewig, L. Whilding, A. J. George, S. Ghaem-Maghami, *Cancer Immunol. Immunother.* **2014**, *63*, 215.
- [97] P. Y. Teo, C. Yang, L. M. Whilding, A. C. Parente-Pereira, J. Maher, A. J. George, J. L. Hedrick, Y. Y. Yang, S. Ghaem-Maghami, *Adv. Healthcare Mater.* **2015**, *4*, 1180.
- [98] M. Pickup, S. Novitskiy, H. Moses, *Nat. Rev. Cancer* **2013**, *13*, 788.
- [99] K. Zhao, Y. Zhang, X. Zhang, C. Shi, X. Wang, X. Wang, S. Cui, *Int. J. Nanomed.* **2014**, *9*, 4609.
- [100] D. Schmid, C. G. Park, C. A. Hartl, N. Subedi, A. N. Cartwright, R. B. Puerto, Y. Zheng, J. Maiarana, G. J. Freeman, K. Wucherpfennig, *Nat. Commun.* **2017**, *8*, 1747.
- [101] L. J. Eggermont, R. Hammink, K. G. Blank, A. E. Rowan, J. Tel, C. Figdor, *Adv. Ther.* **2018**, *1*, 1800021.

- [102] S. Y. Li, Y. Liu, C. F. Xu, S. Shen, R. Sun, X. J. Du, J. X. Xia, Y. H. Zhu, J. Wang, *J. Controlled Release* **2016**, *231*, 17.
- [103] C. J. Turtle, L. A. Hanafi, C. Berger, T. A. Gooley, S. Cherian, M. Hudecek, E. Robinson, *J. Clin. Invest.* **2016**, *126*, 2123.
- [104] H. Moffett, M. Coon, S. Radtke, S. Stephan, L. McKnight, A. Lambert, B. Stoddard, H. Kiem, M. Stephan, *Nat. Commun.* **2017**, *8*, 389.
- [105] H. Yim, W. Park, D. Kim, T. M. Fahmy, K. Na, *Biomaterials* **2014**, *35*, 9912.
- [106] T. H. Tran, R. Rastogi, J. Shelke, M. Amiji, *Sci. Rep.* **2015**, *5*, 16632.
- [107] Y. Komohara, Y. Fujiwara, K. Ohnishi, M. Takeya, *Adv. Drug Delivery Rev.* **2016**, *99*, 180.
- [108] H. Kim, J. Kim, M. Lee, H. C. Choi, W. J. Kim, *Adv. Healthcare Mater.* **2016**, *5*, 1918.
- [109] H. T. T. Duong, N. W. Kim, T. Thambi, V. G. Phan, M. S. Lee, Y. Yin, J. H. Jeong, D. Lee, *J. Controlled Release* **2018**, *269*, 225.
- [110] Y. L. Luo, C. F. Xu, H. J. Li, Z. T. Cao, J. Liu, J. L. Wang, J. Wang, *ACS Nano* **2018**, *12*, 994.
- [111] P. Chen, X. Zhang, L. Jia, R. K. Prud'Homme, Z. Szekely, P. Sinko, *J. Controlled Release* **2014**, *194*, 341.
- [112] A. Singh, M. Talekar, A. Raikar, M. Amiji, *J. Controlled Release* **2014**, *190*, 515.
- [113] S. K. Shukla, P. Chaudhary, I. P. Kumar, F. Afrin, S. C. Puri, G. N. Qazi, R. Sharma, *Environ. Toxicol. Pharmacol.* **2006**, *22*, 113.
- [114] M. Cai, Z. Zhang, X. Su, H. Dong, Z. Zhong, R. Zhuo, *Polymer* **2014**, *55*, 4634.
- [115] Y. Zhang, Y. Wang, C. Zhang, J. Wang, D. Pan, J. Liu, F. Feng, *ACS Appl. Mater. Interfaces* **2015**, *8*, 3719.
- [116] E. Blanco, H. Shen, M. Ferrari, *Nat. Biotechnol.* **2015**, *33*, 941.
- [117] T. H. Tran, S. Krishnan, M. M. Amiji, *PLoS One* **2016**, *11*, e0152024.

[118] J. Schultze, *Curr. Opin. Pharmacol.* **2016**, 26, 10.

Scheme 1. Schematic illustration of recent endeavors on using non-viral polymeric carriers with favored biocompatibility and targeting ligands to design vector with their low immunogenicity, high specificity, high versatility, and high safety. Furthermore, polymeric non-viral vectors have been successfully applied in immunocyte modifications by transfecting genetic substrates (*i.e.*, plasmid DNA, PNA, mRNA, siRNA, or miRNA) into macrophages, T lymphocytes, or dendritic cells to activate the body's own immune responses for improved cancer immunotherapy.

Figure 1. Different applications of PLL-based polymers in cancer immunotherapy. (a) Synthetic schematic illustration of PPE-PLL@OVA. Reproduced with permission.²⁰ Copyright 2018, American Chemical Society. (b) Synthetic schematic illustration of PC@GCpD(Gd) photoimmunotherapeutic agent. Reproduced with permission.²³ Copyright 2019, Elsevier Ltd. (c) The structure of COOH-PLL, the schematic illustration of cryopreserving NK cells and evaluation of cryopreservation effect of NK cells. Reproduced with permission.²⁵ Copyright 2019, Wiley-VCH.

Figure 2. Different applications of PEI-based polymers in cancer immunotherapy. (a) Schematic illustration of siPD-L1/CLPEI/DS ternary complex composition and in vivo anti-tumor therapy mechanism.³² Copyright 2017, American Chemical Society. (b) Synthetic schematic illustration of PEI-CpG ODNs gene vaccine and schematic illustration of the cytokine production induced by gene vaccine in immune cells. Reproduced with permission.³⁵ Copyright 2018, American Chemical Society. (c) Schematic illustration of in vivo antitumor therapy with PEI/CpG/OVA gene vaccine combined with HAase.³⁶ Reproduced with permission. Copyright 2018, Elsevier Ltd.

Figure 3. Different applications of PBAE-based polymers in cancer immunotherapy. (a) Schematic illustration of PBAE/CDNs nanoparticle and in vivo anti-tumor therapy. Reproduced with permission.⁴⁶ Copyright 2017, Elsevier Inc. (b) Synthetic schematic illustration of PBAE/CpG DNA polyplex. Reproduced with permission.⁴⁷ Copyright 2018, Springer Nature. (c) Schematic illustration of amphiphilic PEI-PBAE(C₁₆)/mcDNA nanocomplex and transfection evaluation *in vitro/vivo*. Reproduced with permission.⁵⁰ Copyright 2016, American Chemical Society.

Figure 4. Different applications of PLGA-based polymers in cancer immunotherapy. (a) Schematic illustration of inhibiting tumor growth by PLGA/OVA NPs in vivo. Reproduced with permission.⁵⁷ Copyright 2016, Dove Medical Press Limited. (b) Schematic illustration of PLGA-TLR 7/8 gene vaccine and in vivo pH response anti-tumor mechanism. Reproduced with permission.⁵⁸ Copyright 2018, Royal Society of Chemistry. (c) Schematic illustration of PLGA/rChe a 3 allergen NPs and sublingual immunotherapy mechanism. Reproduced with permission.⁶² Copyright 2015, Elsevier B.V.

Figure 6. The immune response pathway and the various types of immune cells in the tumor microenvironment. (a) Schematic illustration of tumor inhibition pathway based on immune response in cancer immunotherapy. Reproduced with permission.⁷⁸ Copyright 2018, Elsevier Ltd. (b) Schematic illustration of various types of immune cells in tumor microenvironments as well as their unique surface receptors which might be utilized for targeting delivery. Reproduced with permission.⁶ Copyright 2018, Frontiers Media S. A.

Figure 7. A schematic diagram of the pathway of gene delivery by complex in immune cells. (a) Cell targeted delivery system: the complex enters the immune cells through endocytosis, and the endosomal escape of the complex leads to the entry of the gene into the nucleus. Reproduced with permission.⁷⁹ Copyright 2018, John Wiley & Sons, Ltd. (b) Environmental response delivery system: the release of genes in the nucleus through the response of internal- and external stimuli. Reproduced with permission.⁷⁵ Copyright 2017, Springer International Publishing Switzerland.

Figure 8. Comparison of lipid complexes and polymer nanoparticles in immunotherapeutic applications based on dendritic cells in cancer immunotherapy. (a) Schematic illustration of lipid complex loaded with encoding tumor-associated antigen proteins mRNA activating effect T cells through antigen presentation and evaluations of PD-10 and IFNα secretion in phase I clinical trial of human.⁸⁸ Reproduced with permission. Copyright 2016, Springer Nature. (b) Schematic illustration of promoting the secretion of cytokines by CD8⁺ T cells through antigen presentation by acid-sensitive polymer nanoparticles loaded with tumor-associated antigens, evaluations of IFNγ secretion and OVA-specific CD8⁺ cells amount proportion.⁸⁹ Reproduced with permission. Copyright 2017, Springer Nature.

Figure 9. The applications of immune checkpoint modification based on T cells in cancer immunotherapy. (a) Synthesis illustration of PEI-PEG polymer non-viral vector and evaluation of PD-L1 immune knockout. Reproduced with permission.⁹⁷ Copyright 2015, Wiley-VCH. (b) Schematic illustration of polymer nanoparticles loaded with NP_{siCTLA-4} mediated T cell activation and evaluation of in vivo anti-tumor therapy. Reproduced with permission.¹⁰² Copyright 2016, Elsevier B.V.

Figure 11. The applications based on macrophages in cancer immunotherapy. (a) Schematic illustration of microneedles wrapped in the pH-responsive charge reversal copolymer for enhancing the antigen presentation ability of macrophages. Reproduced with permission.¹⁰⁹ Copyright 2017, Elsevier B.V. (b) Synthetic illustration of HA-PEI/miRNA nanoparticles to switch TAM into M1 macrophages and evaluation of the miR-223 expression. Reproduced with permission.¹¹⁷ Copyright 2016, PLoS.

Table 1. Various approaches of non-viral vectors for cancer immunotherapy. Porphyrin- PLL dendron-arginine, PP-PLLD-Arg; Matrix metalloproteinases-9, MMP-9; Propionic acid, PrA; (Polyethylene oxide)4-bis-amine end-capped poly(1,4-butanediol diacrylate-co-5-amino-1-pentanol), B4S5E5; Calcium phosphate, CaP; Hierarchical pores, HPSNs; Acetaldehyde, AC.

Polymers		Payload	Nanosystems		Cell model	Special advantages of polymers	Ref.
			Type	Size(nm)			
PLL-based	PPG-PLL	OVA	polymer brush	130	B16F10 tumors cells	Effective OVA delivery for DCs, strong immune response	21
	PP-PLLD-Arg	MMP-9 shRNA	complex	—	HNE-1 cells	Suited irradiation, low toxicity, light-response	23
	PLL-GCpD	CpG-Cy3	nanoparticle	190	EMT6 cancer cells	Dual-modal imaging, photoimmunotherapy	24
	COOH-PLL	Dextran	cryoprotectants	—	NK cells	Cryopreserved NK cell	26
	LA-PegPI	pDNA	nanoparticle	—	HepG2 or HeLa cells	Useful gene vector for threatening hepatocellular carcinoma	27
PEI-based	mTat-PEI	pDNA	complex	177	MC3T3-E1 cells	Significantly improve transfection efficiency	28
	PEI	CpG/OVA	nano vaccine	210.7	BMDCs	Powerful anti-tumor effect	29
	PEI-SNA	siRNA	polyplex	140	U87-MG glioma cells	Avoid being degraded by lysosomes and improve gene silencing ability	30
	LG - PEI /HG - PEI	DNA	polyplex	95-208	A549 cells	Avoid being degraded by lysosomes and improve transfection efficiency	42
	disulfide-linked PEI	siPD-L1/DS	complex	230-290	B16F10 Cells	Inhibition of melanoma growth through T cell-dependent mechanism	33
	PEI-PrA	pDNA	complex	150–200	MDA-231 and MCF-7 cells	Effective pDNA delivery in breast cancer	34
	stPEI-HSA	CRISPR/Cas9 or siRNA	nanoparticle	202.7	CT26 cells	Inhibit PD-L1 expression	35
	PEI	CpG ODNs	polyplex	160-380	RAW264.7 cells	Promote the internalization of CpG ODNs and induce cytokine secretion	36
PBAE-based	PBAE	DNA	nanoparticle	138	F98 glioma cells	High concentration at the tumor site	38
	PBAE-BPN	DNA	nanoparticle	100	HT22 cells	Mediate high-level transgene expression and rodent striatum	40
	B4S5E5 PBAE	pDNA	nanoparticle	200	B16-F10 cells	Improve tRNase gene's therapeutic effect	41
	PBAE	pDNA	polyplex	—	HEK293T and MDA-MB-231 cells	Represent transfection several folds within monovalent cations	42

PLGA-based	PBAE	CDNs	nanoparticle	110	B16-F10 cells	Deliver CDNs to cytoplasm to activate strong immune response	45
	PBAE	DNA	nanoparticle	157	HCC cells	Preferential DNA delivery to HCC cells	47
	PBAE	CpG DNA	polyplex	196-300	CD11c ⁺ cells	Polyplex interaction strengthens the binding response of CpG and TLRs	48
	highly branched PBAE	collagen VII (C7) DNA	polyplex	40-70	hADSC, HeLa and RDEBK cells	Significantly improve transfection efficiency	49
	BEAQs	pDNA	nanoparticle	50-100	HEK293T and ARPE-19 cells	Effective delivery of genes to refractory cells	50
	PEI-PBAE	mcDNA	nanoparticle	45.2	HEK293T cells	Mediate safe and effective gene delivery in vivo	51
	oligopeptide-end PBAE	mRNA	nanoparticle	140	HeLa, ARPE-19 and RAW264.7 cells	Stimulate immune responses to fight either infectious diseases or tumors	52
	PLGA	siRNA/R837	nanoparticle	146.7	EG7-OVA cells	Combine antigen-specific tumor therapy and immunomodulation	54
	CaP-PLGA-PEI	siRNA	nanoparticle	151.5	MODE-K cell	For local treatment of colonic inflammation	55
	PLGA-PEG-PLGA	PLK1shRNA/PEI-Lys/DOX	hydrogel	—	osteosarcoma Saos-2 cell	Enhance PLK1 silencing potency, higher apoptosis of tumor masses	56
	PLGA	OVA	nanoparticle	200	CD8 ⁺ T and EG.7 lymphoma cells	Deliver antigens or adjuvants to DCs	58
	PLGA	TLR 7/8 agonists	nanoparticle	210	B16F10-OVA and MB49 cells	Increase antigen presentation and the co-stimulatory molecule expression	59
	PLGA-PEI-MNs	pH1N1 DNA vaccine	polyplex	175	HEK293 cells	Induce strong humoral immune response	61
	PLGA-PEI	VEGF pDNA	nanoparticle	120	hMSCs	Cooperative promotion of angiogenesis	62
	PLGA	rChe a 3 allergen	nanoparticle	481	dendritic cells	Enhance significantly systemic Treg and Th1 immune responses	63
	PLGA-PEI	SOX9 protein/Rx2 siRNA	nanoparticle	128	hMSCs	High expression of extracellular matrix components	64
Dendrimer	PLGA/PEI/hyaluronic acid	pDNA	nanoparticle	193-207	HepG2 cells	Strongly inhibit the growth of tumor cells	65
	carbosilane dendrimers	siRNA-Nef	conjugate	—	PBMCs, CD4 ⁺ T cells, and monocytes	High inhibition of HIV-1 replication and decrease of phagocytic activity	69
	HPSNs-AC-PEI	pDNA	nanohybrid	160–250	HEK 293, HeLa and CHO cells	High gene loading rate and low cytotoxicity	70
	DGBA	OVA-CpG	nano vaccine	58–68	BMDCs	Superior prophylactic efficacy against B16-OVA melanoma	72

Table 2. Summary of treatment strategies based on various immune cells. Mannosylated-PEI₁₈₀₀-Cell-penetrating peptide, Man-PEI₁₈₀₀-CPP; Suppressor of cytokine signaling 1, SOCS1; Linoleic acid, LA; Caprylic acid, CA; Transferrin-PEI, Tf-PEI; Cytotoxic T lymphocyte-associated molecule-4 (CTLA-4)-siRNA, siCTLA-4; Hyaluronic acid, HA.

Targeted immune cells	non-viral vectors/Payloads	Therapeutic strategies	Special advantages of non-viral vectors	Ref.
Dendritic cells	Man-PEI ₁₈₀₀ -CPP/DNA	Maturing and activating DCs, trafficking into the spleen	Targeting DCs with mannose receptors for coupling CPP	84
	PLGA/ (OVA/SOCS1 siRNA)	Mature and activate DCs, various pro-inflammatory cytokine secretion, antigen presentation	Inhibition of SOCS1 gene expression is essential for enhancing antigen-specific antitumor immunity by DCs	85
	peptide YTYQGKL/DNA	Using peptide to mediate gene delivery in DCs	Peptide enhances the transfer of reporter gene coding plasmid in BMDC and JAWSII cells	91
	PEG-b-PR/OVA	BMDC uptakes OVA, Ag/MHC-1 presentation	Realize efficient cytoplasmic transmission of tumor antigen in DCs	93
	PEI-CA /siRNA, PEI-LA /siRNA	Block abnormal signal processing of CTCL cells	CDK18 and PI3K targets induced siRNA-mediated cell growth inhibition	94
T Lymphocytes	Tf-PEI/siRNA	Target ATCs in asthma via Tf receptor	Deliver selectively siRNA to ATCs	96
	PEG-FA/PD-L1 siRNA	Block PD-1/PD-L1 interactions by delivering PD-L1 siRNA	Target EOC cells which overexpressing folic acid receptors	101
	PEG-PLA/BHEM-Chol/siCTLA-4	Antigen presentation, CTLA-4 silenced, T cell activation and proliferation	CTLA-4-siRNA was delivered into T cell subsets of CD8+ and CD4+ subtypes at the tumor site	106
	PBAE/PGA/synthetic mRNA cargo	Isolation of patient cells, addition of nanoparticles to cell culture, selective binding, genetic programming, reinfusion of programmed cells into the patient	Complete dose-controlled delivery of macromolecules to HSCs or lymphocytes	108
Macrophage	HA-PEI/pDNA	Re-program the macrophages from M1 to M2 phenotype	Effective delivery of siRNA to lung cancer cells with high expression of CD44	110
	ssPEG-PEI-GO/pDNA	Biodegradation of GO by macrophage	Photothermal conversion produced by GO under NIR irradiation promoted endosomes escape	112
	PEG-b-PLGA/ (CRISPR/Cas9 plasmids)	Cas9 was expressed in macrophages, effective in the treatment of T2D while minimizing the off-target effect.	Effective encapsulating plasmid in macrophages overexpressing Cas9	114
	PLGA/microRNAs	Inhibition of macrophage fusion	Inhibition of foreign body giant cell formation in biomaterial implantation model	110

This tutorial summary includes the designs of polymeric immune-cell genetic modification vectors, an overview of currently reported polymeric non-viral carriers with immune cell transfection ability, and their potential applications in cancer immunotherapy. With the rapid growth of immunotherapy in cancer treatment, polymeric non-viral gene delivery systems might provide new hopes for cancer patients receiving immunotherapy. (55 words)

Keyword: gene delivery, non-viral vector, cationic polymer, immune cells

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Polymeric Non-Viral Gene Delivery Systems for Cancer Immunotherapy

ToC figure