Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Recent advances in development of nano-carriers for immunogene therapy in various complex disorders

Sanaz Shahgordi [1, 2], Fatemeh Oroojalian [3], Ezzat Hashemi [4], Maryam Hashemi [2, 5*]

[1] Immunology Department, Faculty of Medicine, Golestan University of Medical Science, Gorgan, Iran
[2] Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Department of Advanced Sciences and Technologies, North Khorasan University of Medical Sciences, Bojnurd, Iran
[4] Department of Neurology and Neurological Sciences, Stanford University, Stanford, CA, USA
[5] Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad, University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article type:
Review

Article history:
Received: Aug 16, 2021
Accepted: Jan 1, 2022

Keywords:
Gene delivery
Immunogene therapy
Immunotherapy
Nano-carriers

ABSTRACT

Immunotherapy is a novel preference for the treatment of various complex diseases. Considering the application of varying agents for suppression or activation of the immune system, immunogene therapy was confirmed to stand as a proper alternative for other immunotherapeutic strategies due to its capability in targeting cells with more specificity that leads to controlling the expression of therapeutic genes. This method facilitates the local and single-dose application of most gene therapies that result in the usage of high therapeutic doses with a low risk of systemic side effects while being cost-efficient in long-term administrations. However, the existing barriers between the administration site and cell nucleus limited the clinical uses of genetic materials. These challenges can be overcome through the promising method of exerting non-carriers with high stability, low toxicity/immunogenicity, and simple modifications. In this study, we attempted to review the potential of nanoparticle application throughout the immunogene therapy of different diseases including cancer, microbial diseases, allergies, inflammatory bowel disease, rheumatoid arthritis, and respiratory infections. We included the outline of some challenges and opportunities in regards to the delivery of genetic materials that are based on nano-systems through immunotherapy of these disorders. Next to the promising future of these vectors, more detailed analyses are required to overcome the current limitations in clinical approaches.

▶ Please cite this article as:
Shahgordi S, Oroojalian F, Hashemid, Hashemi M. Recent advances in development of nano-carriers for immunogene therapy in various complex disorders. Iran J Basic Med Sci 2022; 25:134-147. doi: https://dx.doi.org/10.22038/IJBMS.2022.59718.13245

Introduction

Immunotherapy is an effective strategy for preventing and treating various disorders including viral infections, allergies, cancer, and autoimmune diseases (1). The application of numerous agents was trialed for suppression or activation of the immune system such as modified dendritic cells, monoclonal antibodies, stem cells, peptides, nucleic acids (NAs), and nanoparticles (NPs) (2).

The procedure of gene therapy attempted to introduce exogenous NAs into specific cells for the modulation of gene expression, which could also counteract or replace a malfunctioning gene (3). Immunogene therapy is a novel preference for the treatment of various complex disorders and a proper alternative for other immunotherapeutic strategies. This method is capable of targeting cells with more specificity, which leads to controlling the expression of therapeutic genes. Consequently, this route facilitates the local and single-dose application of most gene therapies that result in the usage of high therapeutic doses with a low risk of systemic side effects, while being cost-efficient in long-term administration.

Therapeutic genetic materials are categorized into DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid) types with limited clinical uses due to the existing barriers between the administration site and cell nucleus. The delivery efficiency of genetic materials was attempted to be improved through the development of various gene carriers, which are required to have the potential of overcoming certain obstacles such as containing the ability to circulate in the bloodstream, remain undetected by the immune system, survive in the extracellular environment, successfully cross the cell membrane, protect the NAs from nuclease degradation, and release the functional form of NAs in the nucleus (4, 5).

The application of viral vectors with high transfection efficiency is extensively considered for gene delivery purposes. However, there are disadvantages to this approach such as irregular cytotoxicity and immune system stimulation, limited targeting of specific cell types, low DNA carrying capacity, inability to infect non-dividing cells (6), mutagenesis (7, 8), high risk of wild-type virus reversion (9, 10), and inflammation (11, 12). Therefore, nano-carriers (13) attracted the focus of many owing to their high stability, low toxicity, decreased immune responses, flexible designs, and simple modifications (14-16). On the other hand, the main challenge of nano-delivery systems is to develop a delivery vehicle with high cellular uptake efficiency and the ability to promote endosomal escape, inhibit its transference to lysosomes, and ensure its localization in appropriate cellular compartments (17).

*Corresponding author: Maryam Hashemi. Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-51-31801219; Email: hashemim@mums.ac.ir

NPs proved to stand as a promising method for overcoming the mentioned challenges (NPs) due to offering notable advantages such as the ability to prevent degradation, deliver high concentrations of the payload for targeting the desired site, and function as a controlled release system (18-20). All of the nano-carriers that were exerted in immunogene therapy, including polymeric NPs, lipid-based NPs, liposomes, carbon nanotubes, and gold NPs (3, 21, 22), could be designed to produce efficient long-term immune responses (23).

The objective of this study was to review the applications of nano-carriers, which are based on immunogene therapy, for the treatment of various diseases.

Genetic materials used in immunotherapy

Various genetic materials were applied in immunogene therapy including synthetic oligonucleotide (ODN), plasmid DNA, and RNA interference (RNAi).

Oligonucleotides

ODNs are known as short, single-stranded synthetic DNA molecules and according to several studies, they contain unmethylated CpG (Cytosine-Phosphate-Guanine) motifs that are capable of arranging potent immune systems, which consequently improve the obtained responses to vaccines, reduce allergic responses, and provide protective effects in immunotherapy and cancer therapy (24). Figure 1 depicts the action mechanisms of ODNs throughout the immune system.

Recognized by toll-like receptor 9 (TLR9) (25) in antigen-presenting cells (APCs), the activation of CpG ODN oligonucleotides requires the Myeloid differentiation primary response 88(MYD88)-IRF7 (interferon regulatory transcription factor) signaling, which leads to up-regulation of IRF and nuclear factor-κB (NF-κB). The activated APCs are able to stimulate the secretion of immunomodulatory cytokines, including interleukin-6 (IL-6), IL-1, IL-2, IL-10, IL-12, IL-18, Tumor necrosis factor-α (TNFα), and Interferon-α/β (IFN α/β) (26), that consequently activate the innate immune effector cells (e.g., natural killer cells [NK]). These cytokines provide the production of other immune-stimulatory cytokines and chemokines, as well as improve the adoptive immunity through the induction of Ag-specific T cells (27-29) and increase neutrophil migration, monocyte, and macrophage activation, and acute-phase protein production (30, 31). Another capability of CpG ODNs is to initiate the activation of B cells as an APC in order to produce IgM and cytokines such as IL-6 and IL-10. Additionally, IL-6 can facilitate the activation and differentiation of long-lived plasma cells for secretion of other kinds of antibodies such as IgG (32).

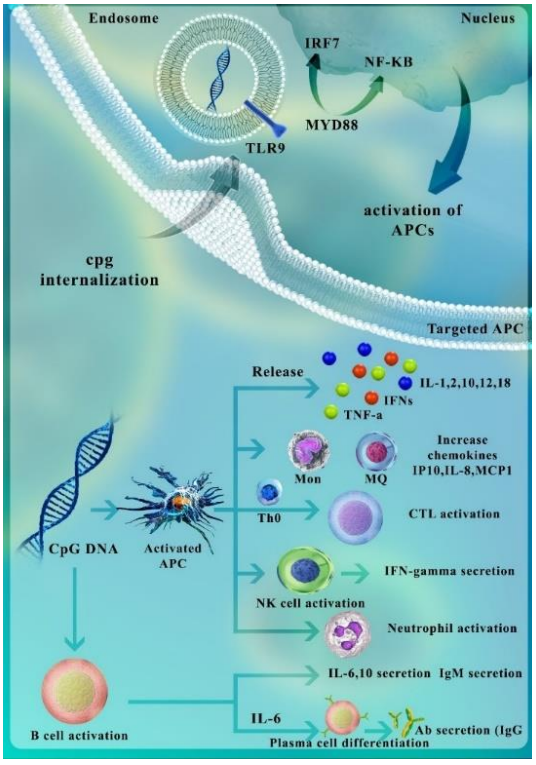

Figure 1. Mechanism of effects of ODNs on immune system
As CpG ODN oligonucleotides are internalized in APCs, activated APCs release various cytokines and chemokines, causing effector immune cell differentiation and activation; TLR: toll-like receptor, IRF: interferon regulatory transcription factor, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, Mon: monocytes, MQ: macrophage

According to recent studies, CpG formulations could inhibit immune responses through enzyme up-regulation of indoleamine-pyrrole 2, 3-dioxygenase (IDO), and T regulatory cell (Treg)-mediated suppression (33). The induction of general immune responses stands as the main difficulty of naked CpGs, which is caused by the release of inflammatory cytokines and systemic activation of lymphocytes (33). However, this issue can be surpassed by exertion of specific vehicles designed for carrying CpGs.

Plasmid DNA

Another candidate for immunogene therapy is plasmid DNA (pDNA) due to its ability in expressing particular antigens (34). The produced antigens can increase the antigen expression of traveling APCs towards draining lymph nodes that present the antigenic peptide-MHC complexes for T cell stimulation, which results in the generation of long-term cellular immunity. Furthermore, humoral immunity can be generated through this process since it initiates the antibody production cascade (35-38). The sequences of CpG throughout bacterial plasmid were exerted as potent adjuvants in pDNA-mediated vaccination (25, 39, 40). In addition to adaptive immunity, the unmethylated CpG oligonucleotide sequences can turn the functionality of pDNA into a potent innate immunity inducer. Figure 2 displays the immune cell modulation that involves the internalization of NPs, which carry the plasmids throughout the suppression of allergic reactions.

The internalized NPs, which carry plasmid DNA into the targeted APCs end up engulfed by endosomes and the plasmid DNA is translated to the intended protein to result in the activation of APCs. The activated APCs induce T helper cell (Th1) differentiation to suppress the Th2 cells through IFN-γ secretion and decrease the allergic cytokine secretions (IL-4 and IL-5). Moreover, these APCs can create Treg cells with the ability to suppress Th2 cells, as well as arrange the secretion of IgG2a and IgA from B cells through inhibitory cytokines, suppress mucus production, and inhibit other allergic immune cells such as basophils, mast cells, and eosinophils (41, 42).

RNA interference (RNAi)

RNA interference (RNAi) mediated by short interfering RNA (siRNA), short hairpin RNA (shRNA), and microRNA (miRNA) (43) is a natural cellular process that acts as a gene silencer throughout the eukaryotic system at transcriptional, posttranscriptional, or translational levels (44-46). In this process, siRNA molecules function as the key mediators for inhibiting target gene expressions. Owing to their high

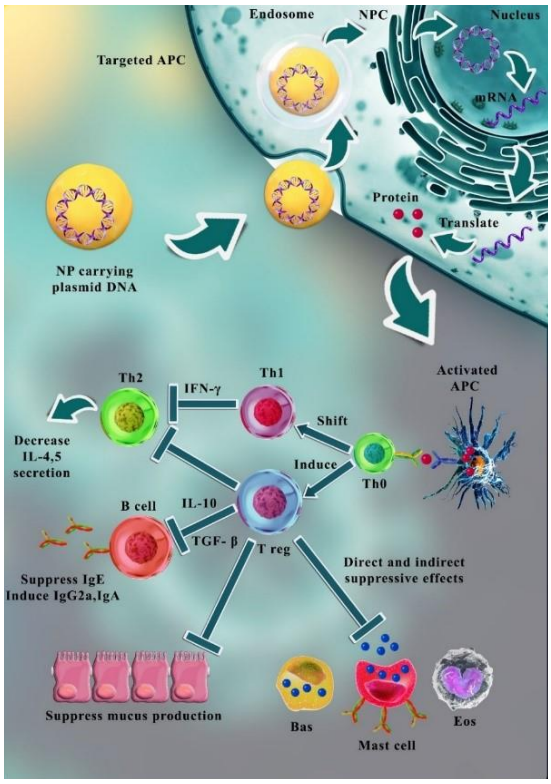

Figure 2. Immune cell modulation through internalization of NP carrying plasmids in suppression of allergic reactions

efficiency, these molecules are considered promising gene silencers and biotherapeutic agents for a wide array of diseases. In addition, siRNA can serve as the guide sequence for creating the cleavage of target-specific Messenger RNA (mRNA) through activation of highly conserved regulatory mechanisms (47). It has been strongly proven that siRNA can be recognized by endosomal (e.g., TLR 3, 7, 8) and cytoplasmic pathways such as retinoic acid-inducible gene I, melanoma differentiation-associated antigen 5, and RNA-activated protein kinase. This process leads to activation of various transcription factors, including NF-κB, interferon regulatory factor 3 (IRF-3), and IRF-7, and consequently result in IFN secretion. Furthermore, T cells, NK cells, and DCs can be also activated through this process (48).

Figure 3 demonstrates the silencing mechanism of programmed death-ligand 1 (PDL1) by PDL-1 siRNA which is a model for reduction of tumor cell survival. Accordingly, long double-stranded RNA (dsRNA) was cleaved through the dicer enzyme (endoribonuclease) next to the production of siRNA, which enabled the cells to assemble the RNA-induced silencing complex (RISC). siRNA becomes unwound upon incorporation of siRNA into RISC and forms a single-strand siRNA that binds to its PDL1 complementary mRNA and creates mRNA cleavage; considerably, this type of mRNA cannot be translated to PDL1 protein (49). Due to lack of PDL1 expression on tumor cell surfaces, immune cells with PD1 on their surface cause a reduction in tumor cell survival, which include cytotoxic T cells (CTL), macrophages (MQ), NK cells, and invariant natural killer T cells (iNKT) (50, 51).

Nano-carriers in immunogene therapy

Nowadays, various biodegradable and biocompatible NPs are exerted in the form of gene delivery vehicles. In addition to their applications as protective genetic materials against

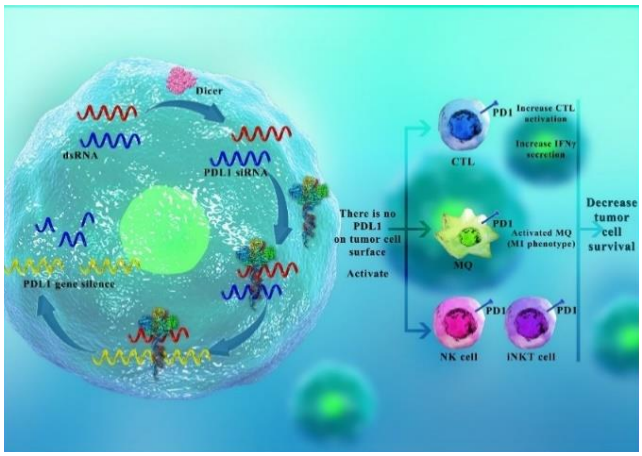

Figure 3. Silencing of programmed death-ligand 1 (PDL1) to decrease tumor cell survival

enzymatic degradation, nano-carriers can efficiently deliver therapeutic agents to the targeted cells. In the following section, we discussed the potential of nano-carriers in immunogene therapy of various diseases including cancer, microbial diseases, allergies, inflammatory bowel disease, rheumatoid arthritis, and respiratory infections.

Cancer

According to recent findings, the applied nano-carriers for gene delivery are capable of modulating immune responses and affecting the tumor microenvironment (TME). In this regard, several studies attempted to focus on this method for treatment of colon and ovarian cancers and melanoma. Figure 4 exhibits the immunomodulatory effects of NPs on cancer. It can be observed that the entrance of NPs containing various immune modulatory genes into the tumor environment leads to the occurrence of changes including the minimized infiltration of tumor-associated microphages (TAM), modulation of cellular immunity cells (e.g., CTL, Treg, and B lymphocytes), innate immune cell modulation (e.g., NK cells), and modulated secretion of soluble mediators from immune cells (e.g., cytokines and chemokines). All of these alterations may result in to tumor cell necrosis or apoptosis.

Table 1 displays some of the exerted nano-carriers for forming a complex with various genetic materials for cancer immunotherapy.

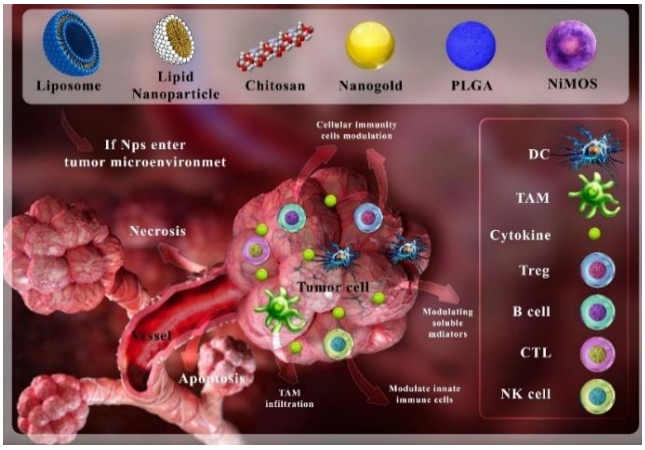

Figure 4. Immune modulatory effects of NPs in the tumor microenvironment

Melanoma

Melanoma has yielded numerous immunogenic epitopes such as tyrosinase-related protein (TRP-2) in the form of a tumor-associated antigens (52) and low levels of MHC-I (75). Application of MHC-I-dependent CTLs proved to be essential for suppression of tumor growth and inhibition

Table 1. Nanoparticles (NPs)-based immunogene delivery used in cancer treatment

Cancer type	Gene therapeutic agents	NPs	Functional mechanisms	Ref.
Melanoma	Tlr9 agonist Trp2 peptide	Liposome	High-activity CTL generation DC Activation	(52)
Melanoma	Tlr9 agonist Trp2 peptide	Lipid-calcium-phosphate (LCP)	Modest CTL response Moderate DC activation	(53)
Melanoma	Tlr9 agonist	Gelatinase NP	T Cell activation Immune response amplification	(33)
Melanoma	Hsp-70 gene	Magnetic cationic liposomes (MCL)	CTL activation Elicit Pro-inflammatory cytokines MHC-I Up-regulation	(54)
Melanoma	C-myc Antisense	Liposome	Immune Cell activation Increased CD69,86 expression Increased $CD8^+$ T Cells Increased IFN-γ, IgG1, and IgG2a secretion Induction of humoral immune responses Induction of balance between Th 1 and Th 2 Breaking self-antigen tolerance	(26)
Melanoma	Ovalbumin	Lipid NP	Increased $CD8^+$ T Cell levels Increased innate immune system pattern Recognition receptors Type I IFN up-regulation	(55)
Melanoma	Ovalbumin-TLR9 ligand	MgAl layered double hydroxide (MgAL-LDH)	Increased IgG2a/IgG1 ratio Induced Th 1/Th 2 balance Increased $CD8^+$ T Cell recruitment	(56)
Leukemia	PDL1-siRNA	Lipid NP	Increased $CD8^+$ T Cell proliferation CTL degranulation upon Ag re-stimulation	(57)
Leukemia	β-galactosidase	Chitosan	Increased MMP-9 levels No Pro-inflammatory and IL-10 changes APC activation	(58)
Leukemia	IL-12	Chitosan	Increased IL-12 and IFN-γ levels Modulated immune system	(59)
Leukemia	IL-12	LNP (lipid nanoparticles)	Increased IL-12 and IFN-γ levels Activated innate immunity Recruited monocytes, NK cells, and T cells Activated MYD88 and STING signaling pathways	(60)
Ovarian cancer	PDL1-siRNA	Polyethylenimine (PEI)	TLR 3, 5, 7 and MYD88 stimulation IFN-γ secretion Innate immune system activation Tumor-associated DC activation Silenced PDL-1	(50)
Ovarian cancer	PDL1-siRNA	PEI	PDL-1 knockdown Increased CAR-T Cell function	(51)
Ovarian cancer	miR-155 ds-RNA duplexes	PEI	DC activation Increased Ag presentation Produced Th 1 cytokines Tumor-reactive T cell expansion	(61)
Ovarian cancer	IL-12	Liposome	Prime antitumor immunity Increased $CD8^+$ T Cell levels Increased perforin-positive Cell Infiltration to TME	(62)
Lung cancer	Anti-VEGF siRNA	Gold NP	Decreased MQs in lung tissues No changes in cytokine levels or TLR activation Modulate TAM recruitment	(63)
Lung cancer	IL-12	PEI-DNA NP	Increased IL-12 and IFN-γ levels Activate antitumor immune responses	(64)
Lung cancer	IL-12	tumor-targeted micelleplexes	Increased IL-12, IFNγ and TNFα levels T4+, T+, and NK cell activation Switching M2 MQs into M1 MQs	(65)
Hepatocellular carcinoma	IL-12	PLGA DOTAP	Increased IL-12 and IFN-γ levels Induced immune responses NK cell activation	(66)
Hepatocellular carcinoma	PDL-1 IL-2	Tumor targeted lipid-dendrimer-calcium-phosphate (TT-LDCP NP)	Increased IL-2 level Decreased PD-L1 level Increased T $CD8^+$ activation and infiltration to TME	(67)
Hepatocellular carcinoma	IL-12	PAMAM	Increased IL-12 level	(68)
Pancreatic cancer	HIFα siRNA	Lipid polymer hybrid NP	Decreased IL-6 and IFN-γ Levels	(69)
Colon cancer	IL-12	DMP micelle	T cell infiltration to the tumor microenvironment Increased TNF-α levels	(70)
Colon cancer	IL-12	Cholesterol-conjugated PEGylated PAMAM	Stimulated TH1 differentiation	(71)
Colorectal cancer	CCL19	Folate-modified nanoparticle	Increased TNF-α and IFN-γ secretion Activated T cells Decreased T regulatory cells Decreased M2 macrophages Decreased MDSCs	(72)
Colon cancer	IL-15	Protamine-liposome system (CLPP)	Increased NK cells Activated $TCD8^+$ cells Increased TNF-α secretion	(73)
Brain cancer	TGFβ	Polybutyl cyanoacrylate nanoparticles	Increased T CD25+ cells Decreased TGFβ2 levels	(74)

of tumor recurrence. Down-regulation of MHC-I is a fundamental mechanism for the escape of tumor cells from T-cell-mediated anti-tumor immunity (76, 77).

Xu *et al*. reported the usage of TRP-2-loaded lipid-calcium-phosphate (LCP) NPs in conjugation with CpG ODN (TLR-9 agonist). Accordingly, the surface of NPs was modified with mannose due to expression of its receptor on DCs. The prepared formulation consisted of 50 μg of TRP-2 antigen, and 20 μg of CpG was administered to melanoma-bearing female C57BL/6 mice. In comparison with the other groups, the obtained results were indicative of modest CTL response, mild DC activation signals, and modest antitumor activity (53).

In another study, the melanoma-bearing C57BL/6 mice were pretreated with Heat shock proteins (HSP-70) gene (total amount: 20 μg/mouse), which was combined with induced hyperthermia by magnetic cationic liposomes (MCLs), and succeeded in strongly arresting tumor growth; presumably, activation of CTL through MHC-I up-regulation could be the reason behind this achievement (54). *HSP-70* is also capable of acting as an extracellular protein with regulatory effects on monocytes eliciting proinflammatory cytokines for providing innate immunity (78).

According to the findings of Oberli *et al*. the administration of a lipid NP complex with unmodified mRNA-encoding ovalbumin (10 μg of mRNA/mouse) to melanoma-bearing C57BL/6 mice was resulted in blocking tumor growth through the induction of IFN-I up-regulation comparing with control group. IFN-I is an essential factor for $CD8^+$ T cell protective immunity. This data could be transfected to various immune cells such as DCs, MQs, Neutrophil (NEU), and B cells due to resulting in strong activation of CD 8^+ T cells. In this regard, the gathered evidence confirmed the effectiveness of this formulation as an immune system activator and modulator (55).

Another related research was performed and in which the exertion of MgAl-layered double hydroxide (LDH) with a similar chemical composition to Alum was applied in conjugation with TLR-9 ligand CpG and ovalbumin (OVA) for the treatment of melanoma in female C57BL/6 mice. The findings indicated that this nanoformulation induced an approximately 6-fold higher IgG2a/IgG1 ratio than Alum-CpG-OVA ($P<0.01$), which can strongly direct the immune responses towards Th1 (56). LDH is capable of effectively causing immune responses through several mechanisms including the efficient loading and protection of OVA and CpG, as well as increased uptake of these cargos by APCs and endosomal processing, in which the released CpG interacts with TLR-9 receptor on the surface of endosomes (79). The proposed formulation could also increase the recruitment of $CD8^+$ T-cell to TME and consequently block tumor growth. In the mentioned study, the application of MgAl-LDH with immune modulation can efficiently deliver Ag and CpG to APCs in order to provoke the immune system (56).

Li *et al*. reported that the administration of self-replicated IL-12 RNA along with lipid nanoparticles (LNP) to C57BL6/J and BALB/C mice (10 μg/mice) was lead to a higher IL-12 and IFNγ expression and recruited monocytes, as well as observed the presence of NK cells and T cells throughout the tumor microenvironment and reported the inducement of STING and MYD88 signaling pathway. Furthermore, the outcomes of *in vitro* assays on B16F10 tumor cells indicated the occurrence of innate immunity activation caused by the upregulation of TLR3, MDA5 and IFNI along with an increase in IL-12 gene expression. In total, this therapy could modulate tumor microenvironment and increase the rate of immunogenic cell death (60).

Leukemia

Leukemia is a malignant disease that involves the release of an increased number of immature or abnormal leukocytes into the bloodstream or bone marrow. DC vaccines function as T-cell immune response enhancers and stand among the optimal treatment options for leukemia.

A group of researchers conducted an study on human DC vaccine with an improved immunogenic potential by forcing siRNAs to target the incorporated PD-L genes into the lipid NPs (LNPs) in conjugation with Minor histocompatibility antigen (MiHA, a key Ag in the antitumor responses expressed by malignant cells) and mRNA electroporation. Moreover, Immature DCs (iDCs) were generated by culturing the isolated monocytes from peripheral blood mononuclear cells. Their findings pointed out the enhanced proliferation of Ag-specific $CD8^+$ T cells in comparison with the control group. However, these specialized DCs were not capable of expressing PDL-1, while the activated T cells remained in highly activated states. In addition, the expanded MiHA-specific $CD8^+$ T cells could be efficiently de-granulated upon Ag re-stimulation. In the mentioned study, LNPs were exerted due to their ability to efficiently encapsulate and deliver siRNA through the cell membrane (57). Moreover, considering the common usage of monocytes for the generation of DCs (79), NPs were covered by Polyethylene glycol (PEG) in order to take advantage of its transfection efficiency for human monocyte-derived DCs (57).

CAR-T (chimeric antigen receptor) cells are T lymphocytes engineered in laboratories to express the artificial receptors that would be targeted towards specific antigens in tumor cells.

According to another findings, the delivery of PDL-1-siRNA by the combination of PEI NPs with anti-PDL-1 antibody was results in knockdown of PDL-1 on epithelial ovarian cancer (EOC) cells, which consequently increases the functionality of chimeric CAR-T through higher IFN-γ secretion ($P<0.01$) and CD107a expression on the surface of CAR-T cells ($P<0.05$). CD107a is a lysosomal marker that involves the degranulation activity of CAR-T cells (51). However, CAR-T cell therapy has been undermined by various immunosuppressive mechanisms that are based on EOC cells, as well as the interaction between PD-1 and its ligand that leads to the hyporesponsiveness of T-cells (80-83). Therefore, this method can modulate the immune system through the blockage of PDL-1 on ovarian cancer cells.

Previous findings confirmed the essentiality of miRNAs for regulation of every immune cell type (84, 85). MiR-155 is basally expressed at low levels in B cells (85, 86), T cells (87), MQs (88), and DCs (85) and stands as a perfect example of miRNA functionality throughout the immune system (61). On the other hand, MiR-155 is expressed at high levels in human cancer cells (61). Signals activation (e.g., Ags) can rapidly increase the expression of miR-155 in leukocyte subsets that contain bone marrow DCs and MQs (85-87).

In a study in 2012, the encapsulated miR-155 ds-RNA duplexes (50 μg/mice) by PEI-based NPs were transmitted to C57BL/6 mice that suffered from ovarian cancer. Active miRNA can be bound to the miRNA recognition element on multiple mRNAs, resulting in simultaneously silencing

multiple target genes. Therefore, it is indicated that the treatment can reverse the tolerance activity of ovarian cancer-associated DCs and promote their potential to enhance antitumor immunity through enhancement of robust Ag presentation, production of Th1 cytokines (P<0.01), and expansion of tumor-reactive T cells. According to the mentioned study, the duplexes (double-stranded molecules of nucleic acid) were processed by tumor-associated DCs in order to generate mature miR-155 (61).

Lung cancer

Bronchogenic carcinoma is currently a worldwide highly prevalent malignant disease (64). The infiltrating leukocytes of malignant solid tumors are abundantly filled with MQs (63). The inflammatory and regulatory phenotypes of these tumor-associated MQs (TAM) are exhibited through their M2 markers (89). In fact, TAMs have been recently identified as the key mediators in cancer progression, metastasis, and resistance to therapy (90, 91).

M2 peptide was coupled with gold NPs (AuNPs) and used to deliver anti-VEGF siRNA (0.05 mg/kg) in the sample of BALB/c mice with lung cancer. According to the findings, this hybrid approach could reduce the number of existing MQs in lung tissues when compared with the results of the treated group with solitary AuNPs and siRNA. However, there were no changes observed in the cytokines at the mRNA level along with the lack of any TLR activation. It is notable that the mentioned study succeeded in targeting an angiogenic factor (VEGF) that was produced by inflammatory MQs and lung cancer cells and modulated the recruitment of TAMs (63). Therefore, these observations proved the capability of this therapy in modulating the immune cell infiltration into inflammatory sites.

It was shown that the combination therapy of cisplatin (CDDP) and pIL-12 (plasmid encoding IL-12) lead to achieving a higher rate of antitumor activity and cancer cells apoptosis. Besides, the delivery of this formulation to C57 mice bearing lung cancer cells ((50 μg/mice) resulted in a higher IL-12 expression, increased immune effector cells (T4+, T8+, and NK cells), conversion of M2 MQs into M1 MQs, and higher levels of IFNγ and TNFα. Therefore, this formulation proved to be capable of enhancing the immune responses in the tumor microenvironment (65).

Colon cancer

Men *et al.* introduced the application of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)- modified mPEG–PCL micelles (DMP) for the delivery of plasmid encoding IL-12 gene to colon carcinoma-bearing female BALB/c mice, which increased the level of IL-12 in treated mice in comparison with the control group (P<0.001) (70). IL-12 is an immunomodulatory cytokine with the ability to regulate the immune system through induction of immune cell proliferation (92). According to the obtained results, the proposed method could provide a strong T cell infiltration into the tumor microenvironment and increase the rate of TNF-α secretion from immune cells such as macrophages and neutrophils (70). Thus, it is indicated that successful expression of IL-12 in the tumor microenvironment leads to activation of immune cells and causes inducement of strong antitumor immune responses.

In conformity to another study, the delivery of cholesterol-conjugated PEGylated Polyamidoamine (PAMAM) complexed with plasmid encoding IL-12 into HT29 cells was resulted in increasing the production of IL-12 when compared with the control group (71). Doubtlessly, IL-12 initiated an immunomodulatory effect in this method through stimulation of TH1 differentiation, which augmented the antitumor activity of the immune system (93).

Considering the findings of Liu *et al.* the administration of folate-modified NPs containing Chemokine (C-C motif ligand) (CCL19) gene to female BALB/c mice with colorectal cancer caused a higher expression of CCL19 when compared with the control group (P<0.05). A higher level of CCL19 can intensify the expressions of IFN-γ (P<0.05) and TNF-α (P<0.05), activate lymphocyte T, decrease regulatory T cells, reduce M2 macrophage ($CD45^+CD11b^+F4/80^+CD206high$) polarization, and decrease the number of myeloid-derived suppressor cells (MDSCs) ($Gr1^+CD11b^+$) (72). In conclusion, this combination therapy can induce antitumoral activities by activating immune responses.

In another study, an experiment was conducted on the delivery of protamine-liposome system (CLPP)/IL-15 mRNA complex (20 μg protamine /20 μg liposome /10 μg IL-15 mRNA per mouse) to female BALB/c mice bearing colon tumor and resulted in increasing level of IL-15 when compared with the control group (P<0.05). This outcome can modulate immune responses by increasing the population of NK cells and activating T $CD8^+$, as well as inducing a high level of TNF-α, IFN- γ, and IL-12 (P<0.05) (73). Undoubtedly, this method is capable of inhibiting tumor cell growth through immunomodulation.

Other cancer types

According to recent studies, other types of tumors can be also modulated by the performance of immunogene therapy.

Hypoxia-inducible factor 1-α (HiF-1α) is a key transcription factor in tumor development, which is also known to play a fundamental role in hypoxia-mediated apoptosis (94). In a related study, Zhao *et al.* considered the exertion considered the exertion of lipid-polymer hybrid NPs for the co-delivery of HiF-1α siRNA (1.33 mg/kg) and gemcitabine (4 mg/kg) in female BALB/c mice with pancreatic cancer. Their findings indicated that the levels of IL-6 and IFN-γ were decreased to normal limits when compared with the group treated with free siRNA (69), which signified the possible modulation of immune responses that consequently reduced the inflammatory effects.

In another research, lipid NPs and chemokine receptor type 2 (CCR2) siRNA (0.5 mg/kg/day siCCR2) was administered for the treatment of colon cancer in a C57BL/6 and BALB/C mice, which resulted in silencing the CCR2 miRNA in inflammatory monocytes that consequently inhibited their migration to the inflammatory site. Moreover, it can be inferred that this method modulates the immune cell inducers of inflammation due to the existing $CD14^+CD16^-$ phenotype in the inflammatory monocytes, which can cause a rise in classical MQs that exacerbate inflammation (95).

A dual-targeted immune-gene therapy system was designed based on tumor-targeted lipid-dendrimer-calcium-phosphate (TT-LDCP) NPs for the *in vitro* and *in vivo* delivery of siRNA against immune checkpoint ligand PD-L1 and plasmid DNA encoding immunostimulatory IL-2 to hepatocellular carcinoma cell (HCC). This co-

delivery system resulted in significantly decreasing PD-L1 and increasing IL-2 expression in liver tumor cell lines in comparison with the performed treatment with solitary PD-L1 siRNA or IL-2 pDNA in TT-LDCP NPs. Furthermore, the observations indicated that the significant enhancement of tumoral CD8+ T cell infiltration and activation led to suppression of HCC progression (67).

According to another finding, administration of a TGF-β2 antisense oligonucleotides and a CMV-β-gal plasmid attaching to polybutyl cyanoacrylate nanoparticles (9.34 nmol/Fisher rat) combined with vaccination with F98 cells infected by New Castle Virus in rats bearing brain tumor, was lead to elevated levels of T CD25+ cells, this indicates the proliferation of activated IL-2 receptor-positive lymphocytes. The TGF-β2 levels were also significantly reduced (74). This evidence could confirm that this method may increase the survival rate.

It was reported that the delivery of plasmid encoding IL-12 gene in combination with PAMAM into mesenchymal stem cells (MSC), which resulted in increasing the expression of IL-12 from MSCs (68); increased levels of IL-12 can cause inducement of stronger tumor responses (96).

Viral and microbial diseases

According to the literature, immunogene therapy-based nano-carriers can be effective in the treatment of infectious diseases. Table 2 demonstrates the application of nano-carriers against microbial and viral diseases in immunogene therapy.

Piglet paratyphoid is recognized as one of the most severe epidemics caused by *Salmonella* infection (170). Plasmid-encoding IL-2 (6 pmol/mouse) were encapsulated in chitosan NPs for being exerted for the paratyphoid treatment of female BALB/c mice. According to the findings, the applied method increased the levels of IL-2, 4, 6 in peripheral blood when compared with the controls (P<0.05) (32). In addition, IL-2 was abled to stimulate T cell proliferation and its clonal expansion (102), while the activated T cells secreted Granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, and IL-4, which leads to APC proliferation and recruitment of more specific T cells to the infection site. On the other hand, IL-2 proved to be capable of activating the proliferation of Th2 cells which causes the releasing of IL-4, 5, 6 and stimulated B cell differentiation into mature antibody-producing plasma cells and memory cells, which also promotes the production of Ig-G, A, M (32). Therefore, these data confirmed the effectiveness of this method in significantly promoting cellular and humoral immunity.

Streptococcus pneumonia is considered a leading cause of invasive pneumonia. Pneumococcal surface antigen A (PsaA) is a lipoprotein that is highly conserved across its serotype (103). In a study, conducted in (2010), chitosan NPs encapsulated with PsaA DNA (50 μg/mouse) were used for the pneumococcal treatment of Specific-pathogen-free (SPF) female BALB/c mice. According to the results, the ratio of IgG1/IgG2a was balanced and a significant increase was induced in the expression of IL-17A and IFN-γ (P<0.01) (97). Recent related studies reported that IL-17 is mainly released from Th17 cells and has the ability to promote host immunity through the recruitment of neutrophils to mucosal sites and provoking their bactericidal potential (104, 105). Therefore, this method can be applied to efficiently modulate the immune system.

The liver maintains a tolerogenic environment against persistent viral infections, such as the *hepatitis C virus*, and often takes advantage of hepatic tolerance. PDL1-siRNA-cationic lipoid NPs (containing 0.5 mg of siRNA/kg) were exerte for the treatment of hepatic disease in C57BL/6 mice, which resulted in the occurrence of PDL-1 surface expression knockdown. This inducement increased the effector function of $CD8^+$ T cells and caused the release of granzyme B (GrB) from these cells that consequently improves their virus clearance potential. Furthermore, GrB secretion from the NK cells was enhanced without causing any changes in effector function (98). In viral hepatic infections, the high expression of PDL-1 can be observed on Kupffer cells and T cells, as well as NK, DC, and liver sinusoidal cells (106, 107).

Table 2. Nanoparticle-based immunogene delivery used in treatment of viral and microbial diseases

Microbial disease	Gene therapeutic agents	Nanoparticles	Functional mechanisms	Ref.
Paratyphoid	IL-12	Chitosan	Increased IL-2,4,6 T cell activation, and recruitment to the disease site Th2 cell proliferation Increased APC proliferation Increased GM-CSF and IFN-γ secretion Stimulated B cell proliferation Increased Ig G, A, M secretion	(32)
Pneumococcal disease	PsaA	Chitosan	Induced IgG1/IgG2a balance Increased IL-17A and IFN-γ expression	(97)
Hepatic disease	PDL1 siRNA	Lipid NP	PDL1 knockdown Increased $CD8^+$ effector T cells Increased granzyme B release from NK and CTLs No NK effector function changes	(98)
Mycobacterium Tuberculosis	HLA	Chitosan	Human DC maturation Increased CD 86,83,80 expression Increased IFN-γ secretion	(99)
RSV infection	NS1	Chitosan	Decreased goblet cell hyperplasia Decreased inflammatory cell infiltration Decreased IFN-α and β levels Increased IL-4 and IFN-γ secretion Increased cellular immunity Increased IRF-1 and STAT1 expression	(100)
RSV infection	RSV antigen	Chitosan	Increased IgG Increased nasal IgA Increased IFN-γ levels in lung High CTL generation	(101)

Table 3. Nanoparticle-based immunogene delivery used in allergy treatment

Allergy	Gene therapeutic agents	Nanoparticles	Functional mechanisms	Ref.
House dust mite allergy	Derp 1	Chitosan	Increased immune response Increased specific IgG2a and IgA	(41)
Allergic asthma	IFNγ	Chitosan	Decreased AHR symptoms Decreased eosinophils in BAL fluid Decreased mucosal metaplasia Decreased airway cellular infiltration Decreased IL-4,5 and IgE secretion Increased IFN-γ Activated STAT4 signaling pathway	(42)
House dust mite allergy	Derp1	Chitosan	Moderate increase in IgG2a levels Suppressed IgE secretion Decreased IL-4 and increased IFN-γ secretion	(115)
Peanut allergy	PCMVArah2	Chitosan	Decreased IgE secretion Decreased anaphylaxis symptoms Increased IgG2a and IgA secretion Deceased histamine levels	(116)
asthma	Thymulin	Poly β-Amino Ester (PBAE NP)	Deceased CCL11 and CXCL1 levels Increased CCL17 level Deceased IL-4 and IL-13 levels Decreased TGF-β level Increased M1 macrophages	(117)

In another study, the application of DNA plasmid encoding 8 HLA Mycobacterium tuberculosis(Mtb) epitopes, which were formulated in chitosan NPs (containing 25 μg of DNA per mouse), was considered for the treatment of HLA-A*0201/Kb female transgenic mice infected with M. tuberculosis. According to the findings, this method caused an increase in the surface levels of CD86, CD83, and CD80 which led to maturing of human DC (99). It was indicated by other studies that CD80 and CD86 are labeled as T cell costimulatory molecules, while CD83 has the potential of affecting the capacity of DC for stimulating T cells (108). In comparison with the outcomes of the intramuscular route, the application of this formulation was observed to increase the secretion of IFN-γ (99). Therefore, the capability of the proposed treatment method in modulating and activating immune responses was confirmed.

Respiratory syncytial virus (RSV) stands as the most common cause of lower respiratory tract viral infection that can result in inducement of bronchiolitis and otitis (109). According to the literature, nonstructural (NS) proteins, as well as F and G RSV proteins, have a fundamental role in disease progression (110, 111).

Plasmid NS1 siRNA gene complex with chitosan NPs was used for the treatment of BALB/c mice with RSV infection. In the course of the disease, NS1 was observed to suppress STAT1 (P<0.05), IRF-1 activation (P<0.01), and type I IFN secretion from the DCs (P<0.05), while NS1 proteins down-regulated the signaling of the IFN system by inactivating IRF-1 and STAT1. It can be concluded that this technique can decrease the goblet cell hyperplasia of bronchi and reduce the number of infiltrating inflammatory cells throughout the interstitial regions, while increasing IRF-1, STAT1, IFN-β, and IFN-α expression and causing the release of IL-4 and IFN-γ from $CD4^+$ and $CD8^+$ T cells; thereby, these occurrences result in enhancing the cellular immunity when compared with the control groups (100). In the mentioned study, the nano-formulation was observed to modulate the host immunity.

Chitosan nanospheres containing plasmid encoding all RSV antigen cDNAs were used for the treatment of BALB/c mice with RSV infection, which also resulted in increasing the neutralization of specific IgG and nasal IgA secretion, while increasing IFN-γ secretion in the pulmonary tissues and generating powerful CTL responses when compared with the controls (P<0.01) (101). The literature confirmed the role of RSV proteins as suitable targets for CTLs along with affirming the direct antiviral effects of IFN-γ, which are essential for stimulating the cytolytic activity of NK cells and CTLs (112, 113). These findings signify the potential of this method for being applicable in the treatment of RSV infections.

Allergies

Allergic diseases or type I hypersensitivity refer to the illnesses that are mediated by the IgE antibody that is produced by the atopic individual after being exposed to safe environmental allergens (114). The oral route is considered as a common track of gene delivery in allergy treatments (41, 115, 116), which provides poor efficacy due to the acidic pH of stomach and gastrointestinal tract enzymes (41). However, specific nano-carriers, such as cationic polymers, were utilized in an attempt to overcome these obstacles. Table 3 presents data on the application of immunomodulatory nano-formulation that contained genetic materials.

In the work of Chew *et al.* plasmid DNA containing Derp1 cDNA (50 μg) was encapsulated in chitosan NPs to be orally administered to female BALB/c mice that were allergic to house dust mite (Derp1). According to their outcomes, the immune responses against Derp1, as well as the production of specific IgG2a and IgA throughout the systemic circulation, was increased in the sample animals. They also reported the ability of chitosan to facilitate immunity induction by crossing the mucosal epithelial barrier to cause the occurrence of chitosan-DNA complex uptake by M cells and provide translocation of molecules to the underlying lymphoid organs in which APCs are located (41). Therefore, the effectiveness of this method in oral gene delivery was confirmed.

In another study, researchers administered chitosan-IFN-γ pDNA NPs (CIN) (25 μg/mouse) to allergic BALB/c mice. Their results included the observance of attenuated airway hyper-responsiveness (AHR) (P<0.01), significant reduction in the number of eosinophils in the Bronchoalveolar lavage fluid (P<0.01), decreased mucus cell metaplasia, cellular infiltration into the airways, and significant reduction in IL-4, 5 and IgE (P<0.05). However, the experiment also caused an increase in the secretion of IFN-γ, while the induced immune modulation via the CIN involved the Signal transducer and activator of transcription (STAT) 4-dependent (42). Moreover, the uptake of CIN

occurred through the means of MQs and epithelial cells, both of which play a fundamental role in asthma immunomodulation (118). CIN therapy can facilitate the induction of IFN-γ expression in epithelial cells and lead to immunomodulation. It is also notable that other Th1 cytokines (e.g., IL-12) were also involved in this process and displayed the ability to activate STAT4 (42). Based on these findings, it is inferred that mucosal therapy can decrease the allergen that is induced and established by AHR.

In another research, chitosan NPs that containing plasmid DNA and encoded house dust mite allergen Derp1 (100 μg/mouse) was conducted in allergic BALB/c mice. Their findings reported the inducement of a moderate increase in IgG2a levels (P<0.01), along with the suppression of IgE responses (P<0.05), a decrease in IL-4 (P<0.01), and an increase in IFN-γ (P<0.01) when compared with the groups treated with the allergen alone (only NPs and phosphate-buffered saline). IgG2a and IgE isotypes reflect the type of T cell responses (115) and in conformity to previous discoveries, high levels of IgG2a in conjugation with low levels of IgE can propose the shift of immune responses from Th2 toward Th1 (119). Therefore, it is indicated that this method is effective for the modulation of immune responses towards allergies.

On the same note, chitosan NPs that were composed of plasmid DNA and encoded dominant peanut allergen gene (pCMVArah2; 50 μg of DNA per mouse) for the treatment of peanut allergy in AKR/J mice. According to their results, the IgE levels and anaphylaxis symptoms were decreased, while IgG2a and IgA levels faced an increase when compared with the control group. In addition, they observed a reduction in the levels of histamine plasma. This method can activate mucosal immunity and systemic immunity (116) since the generation of mucosal immunity can protect the antigens' entry to the systemic immune system and also lead to unresponsive behaviors toward food allergens (120). Considering these data, the proposed method could be presumed as an effective technique for the modulation of anaphylactic responses (116).

Thymic nonapeptide (thymulin or serum thymus factor) is capable of preventing the cascades of inflammatory and fibrotic responses in a mouse model of allergic asthma (121). In this regard, PBAE NPs (Poly (ethylene oxide)-modified poly (beta-amino ester), consisted of thymulin expressing plasmids (50 μl of NP that contained 50 μg of plasmid per mouse) were applied for the treatment of asthmatic female BALB/c mice (117). In comparison with the control, the results of the target group displayed a decrease in the levels of CCL11 and CXCL1, which are responsible for recruitment of eosinophil and neutrophil (P<0.05) (122). An increase in the level of CCL17 (P<0.05) as a fundamental factor in T regulatory transmigration (123) causes a decrease in IL-4 and IL-13 (P<0.05) levels that involves the suppression of Th2 responses, a decrease in TGF-β level (P<0.05), and an increase in M1 macrophages (P<0.05) (117). Therefore, this method can effectively suppress allergic responses through immunomodulation.

Inflammatory bowel disease (IBD)

Inflammatory bowel disease (IBD) is an inflammatory condition caused by dysregulation of mucosal immunity to the luminal antigens derived from the intestinal microflora (124, 125). IBD has two main phenotypes including Crohn's disease (CD) and ulcerative colitis (UC). The condition of CD mostly affects the end of the small intestine and the beginning of the colon, while UC only affects the colon (126). Table 4 exhibits the application of nano-carriers against IBD in immunogene therapy.

In a related study, the application of nanoparticles-in-microsphere oral system (NiMOS) was reported for the delivery of pDNA-encoding IL-10 (100 μg/mouse) throughout the treatment of UC in BALB/c mice. The obtained results were indicative of increased IL-10 levels (P<0.05) and decreased TNFα, IL-12, IL-1β, and IFN-γ. Furthermore, colitis inflammation was detected in histological examinations when compared with the control group. About IBD, the levels of Th1 cytokines (e.g., TNFα, IL-12, IL-1β, and IFN-γ) were up-regulated in order to enable IL-10 to modulate Th1-derived cytokines. Their other findings reported reduced levels of monocyte chemoattractant protein-1 (MCP-1) and Macrophage inflammatory protein-1α (MIP-1α) without mentioning any observance of direct action in between these chemokines and IL-10 (127). Therefore, it can be inferred that this transfection and oral gene delivery is an effective option for IBD treatments.

In another study, the conjugation of NiMOS NPs with TNF-α siRNA were utilized for utilized the conjugation of NiMOS NPs with TNF-α siRNA for the treatment of UC in BALB/c mice. The obtained results were indicative of a decrease in TNF-α when compared with the control group (P<0.05) (126). According to related evidence, this cytokine has a fundamental functionality throughout modulation of inflammatory conditions in IBD (130). Furthermore, this study reported achievement of a lower expression of GM-CSF, MIP-1α, and MCP-1, which are accountable for regulating cell infiltration into the disease site and seem to be directly correlated with inflammation. Moreover, the solitary usage of NiMOS can cause immunomodulation effects and moderately down-regulate IL-1β (P<0.001), IL-5 (P<0.001), MIP-1α, and GMCSF (126). Based on these data, this formulation can be effective and applicable for the treatment of IBD.

Relatively, TNF-α siRNA-polyethyleneimine noncomplex (100 μg/kg) was used in C57BL/6 mice with IBD. The obtained results included the inducement of a

Table 4. Nanoparticle-based immunogene delivery used in IBD treatment

IBD	Gene therapeutic agents	Nanoparticles	Functional mechanisms	Ref.
IBD	IL-10	NiMOS	Decreased IL-10,12,1β and TNFα and IFN-γ secretion Decreased colitis inflammation Decreased MCP-1 and MIP-1α expression	(127)
IBD	TNF-α siRNA	NiMOS	Decreased TNF-α secretion Decreased GM-CSF, MIP-1α, and MCP-1 expression Down-regulated IL-1β and IL-5 secretion Regulated cell infiltration to the inflammatory site	(126)
IBD	TNF-α siRNA	PEI	Decreased TNF-α levels	(128)
IBD	TNF-α siRNA	PLGA	Decreased TNF-α levels	(129)

Table 5. Nanoparticle-based immunogene delivery used in RA treatment

Rheumatoid arthritis	Gene therapeutic agents	Nanoparticles	Functional mechanisms	Ref.
RA	Anti-TNFα dicer substrate siRNA	Chitosan	Decreased TNF-α and IFN-I release from MQs Non-induction of innate immune response Decreased inflammatory cell infiltration	(135)
RA	TNF-α siRNA	Liposome	Decreased TNF-α secretion Decreased IL-6 and MCP-1 locally Increased IL-10 secretion Induced balance between Th 1 and Th 2 through increased IL-4 and decreased IFN-γ	(136)

significant decrease in TNF-α levels when compared with the control group (P<0.01) (128), and also displayed the ability of nano-complex in mediating inflammation in IBD.

In another study, researchers loaded TNF-α siRNA into the galactosylated PLGA NPs and co-delivered this formulation with IL-22, which was embedded in chitosan/alginate hydrogel (20 μg/kg of siTNF and 50 μg/kg of IL-22), to the FVB male mice with UC. The essential functionality of IL-22 in mucosal healing is undeniable. Considering the induced decrease in TNF-α expression as a result of this procedure (P<0.05), it is evident that the blockade of TNF-α expression leads to inhibition of IL-22 production (129), which signifies the ability of this co-administration to facilitate the recovery of UC through immunomodulation.

Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic inflammatory disease that is associated with joint destruction caused by the synovial infiltration of Th1 cells (131). As an important cytokine in RA, TNF-α is involved in inflammatory conditions (132) and therefore, its targeting at the RNA level can stand as an effective therapeutic approach for the treatment of RA (133, 134). Table 5 represents the application of immunogene therapy by the usage of various nano-formulations in RA treatment.

In a related study, chitosan NPs that contained the unmodified anti-TNF-α dicer substrate siRNA (DsiRNA) (2.5 μg) were transfected to DBA/mouse with RA. The results of this method included a decrease in TNF-α (P<0.01) and IFN type I (P<0.05) from the MQs when compared with the untreated groups, which led to observing the lack of innate immune responses (135). During the acute and chronic phases of arthritis, the activated peritoneal MQs release inflammatory cytokines such as IL-1,6 and TNF- α (137). In this regard, the proposed method can be considered as a novel strategy for RA treatment due to succeeding in reducing joint destruction and inflammatory cell infiltration (135).

In conformity to another study, the application of cationic liposome, containing TNF-α siRNA, in DBA/1 mice with RA caused a decrease in TNF-α release throughout the knee joint and led to the local reduction of IL-6 and MCP-1 while increasing the secretion of anti-inflammatory cytokines (e.g., IL-10) when compared with the control group. Therefore, this method was confirmed to be effective in RA treatment since TNF-α suppression can significantly balance the induced changes between Th1 and Th2 in the draining lymph node by causing an increase in IL-4 secretion while decreasing the IFN-γ levels (136).

Conclusion and future perspective

Immunogene therapy is potentially considered an effective approach for the treatment of various complex disorders due to being capable of targeting cells more specifically and providing control over the expression of therapeutic genes. On the other hand, the local and single-dose application of most gene therapies results in the exertion of high therapeutic doses with a low risk of systemic side effects. In this case, the U.S. Food and Drug Administration (FDA) has reviewed and confirmed all comments about clinical trial of immunogene therapy in combination with AstraZeneca's Tagrisso® in patients with late-stage non-small cell lung cancer (NSCLC) whose disease progressed after treatment with Tagrisso in Genprex, Inc. (138). Various gene delivery systems have been developed to improve the delivery efficiency of genetic materials. Most of the applied carriers in human clinical trial phases for immunogene therapy include different types of viral vectors. The combination of AdCD40L (an adenovirus carrying CD40 ligand gene, a key activator of adaptive immunity) in phase I/II study (NCT01455259) with cyclophosphamide chemotherapy was exerted for patients with metastatic malignant melanoma (139). In addition, clinical-grade chimeric antigen receptor (CAR) T cells were engineered by viral vectors and established to mediate tumor rejection. There are important limitations due to uncontrolled responses as a consequence of constitutive expression of the CAR molecules on the surface of T cells (140). So, more standard manufacturing processes are required for immunogene T-cell therapies (141).

Viral vectors were also exerted for the development of clinical trials with critical inflammatory cytokine gene therapies for cancer treatments.

There are reports on the application of nano-systems in clinical or preclinical studies for the delivery of therapeutic genes. Nano-carriers offer prominent advantages such as safety, high targeting potential, low toxicity, cost-efficiency, and easy preparation. However, several factors can possibly affect the immune responses of NPs such as the composition, size, surface chemistry, transfection efficiency, cytotoxicity, and exposure route of carriers.

Most of the immunogene therapy studies by the usage of NPs were conducted with animal models, which provide a strong preclinical basis for human clinical approaches. Therefore, further clinical trials are required in order to clarify the therapeutic efficacy and possible toxic effects of exerted nanosystems in immunogene therapy.

Acknowledgment

The authors are thankful to Mashhad University of Medical Science (MUMS). No funding to declare.

Authors' Contributions

MH Supervised the research; SSH and FO Draft manuscript preparation; SSH and EH Critically revised the paper. SSH, FO, EH and MH Read and approved the the final version to be published.

Conflicts of Interest

The authors declare that there are no relevant financial or

non-financial competing interests to report.

References

1. Pusuluri A, Wu D, Mitragotri S. Immunological consequences of chemotherapy: Single drugs, combination therapies and nanoparticle-based treatments. J Control Release 2019;305:130-154.
2. Smith AJ, Oertle J, Prato D. Immunotherapy in cancer treatment. Open J Med Microbiol 2014;4:178.
3. Oroojalian F, Charbgoo F, Hashemi M, Amani A, Yazdian-Robati R, Mokhtarzadeh A, *et al*. Recent advances in nanotechnology-based drug delivery systems for the kidney. J Control Release 2020;321:442-462.
4. Naldini L. Gene Therapy Returns to Centre Stage 2015;526:351-360.
5. Zhou Y, Zhang C, Liang W. Development of RNAi technology for targeted therapy—a track of siRNA based agents to RNAi therapeutics. J Control Release 2014;193:270-281.
6. Zhang X, Godbey W. Viral vectors for gene delivery in tissue engineering. Adv Drug Deliv Rev 2006;58:515-534.
7. Cherkassky L, Grosser R, Adusumilli PS. Regional Gene Therapy for Cancer. J Cancer Ther 2020;55-71.
8. Heilbronn R, Weger S. Viral vectors for gene transfer: current status of gene therapeutics. Drug Deliv.2010;143-170.
9. Fischer A, Hacein-Bey-Abina S. Gene therapy for severe combined immunodeficiencies and beyond. Exp Med 2020;217:e20190607.
10. Ugwu GC, Egbuji JVI, Okanya LC, Omeje JN, Eyo JE. Gene therapy, physiological applications, problems and prospects-a review. Anim Res Int 2019;16:3367-3392.
11. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. Nat Rev Genet 2020;21:255-272.
12. Shirley JL, de Jong YP, Terhorst C, Herzog RW. Immune Immune responses to viral gene therapy vectors. Mol Ther 2020;28:709-722.
13. Deng W, Fu M, Cao Y, Cao X, Wang M, Yang Y, *et al*. Angelica sinensis polysaccharide nanoparticles as novel non-viral carriers for gene delivery to mesenchymal stem cells. Nanomed: Nanotechnol Biol Med 2013;9:1181-1191.
14. Kommareddy S, Amiji M. Poly(ethylene glycol)–modified thiolated gelatin nanoparticles for glutathione-responsive intracellular DNA delivery. Nanomed: Nanotechnol Biol Med 2007;3:32-42.
15. Pathak A, Patnaik S, Gupta KC. Recent trends in non-viral vector-mediated gene delivery. Biotechnol J 2009;4:1559-1572.
16. Chuan D, Jin T, Fan R, Zhou L, Guo G. Chitosan for gene delivery: Methods for improvement and applications. Adv Colloid Interface Sci 2019;268:25-38.
17. Pridgen EM, Langer R, Farokhzad OC. Biodegradable, polymeric nanoparticle delivery systems for cancer therapy. Nanomedicine 2007;2:669-680.
18. Ozpolat B, Sood A, Lopez-Berestein G. Nanomedicine based approaches for the delivery of siRNA in cancer. J Intern Med 2010;267:44-53.
19. Bayat S, Rabbani Zabihi A, Amel Farzad S, Movaffagh J, Hashemi E, Arabzadeh S, Hahsemi M. Evaluation of debridement effects of bromelain-loaded sodium alginate nanoparticles incorporated into chitosan hydrogel in animal models. Iran J Basic Med Sci 2021; 24:1404-1412.
20. Rezaei M, Hosseini SN, Khavari-Nejad RA, Najafi F, Mahdavi M. Fast antibody responses by immuno-targeting and nanotechnology strategies versus HBsAg vaccine. Iran J Basic Med Sci 2021; 24:545-555.
21. Fontana F, Liu D, Hirvonen J, Santos HA. Delivery of therapeutics with nanoparticles: what's new in cancer immunotherapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2017;9:e1421.
22. Rashidi A, Omidi M, Choolaei M, Nazarzadeh M, Yadegari A, Haghierosadat F, *et al*., editors. Electromechanical properties of vertically aligned carbon nanotube. Adv Mat Res 2013;332-336.
23. Singh MS, Bhaskar S. Nanocarrier-based immunotherapy in cancer management and research. ImmunoTargets and Therapy 2014;3:121-134.
24. Klinman DM. Immunotherapeutic uses of CpG oligodeoxynucleotides. Nat Rev Immunol 2004;4:249.
25. Fu J, Cai J, Ling G, Li A, Zhao J, Guo X, *et al*. Cationic polymers for enhancing CpG oligodeoxynucleotides-mediated cancer immunotherapy. Eur 2019;113:115-132.
26. De Jong S, Chikh G, Sekirov L, Raney S, Semple S, Klimuk S, *et al*. Encapsulation in liposomal nanoparticles enhances the immunostimulatory, adjuvant and anti-tumor activity of subcutaneously administered CpG ODN. Cancer Immunol Immunother 2007;56:1251-1264.
27. Gao Q, Hong J, Xiao X, Cao H, Yuan R, Liu Z, *et al*. T cell epitope of arginine kinase with CpG co-encapsulated nanoparticles attenuates a shrimp allergen-induced Th2-bias food allergy. Biosci Biotechnol Biochem 2019;84:804-814.
28. Gunawardana T, Ahmed KA, Goonewardene K, Popowich S, Kurukulasuriya S, Karunarathana R, *et al*. CpG-ODN induces a dose-dependent enrichment of immunological niches in the spleen and lungs of neonatal chicks that correlates with the protectivei mmunity against *Escherichia coli*. J Immunol Res 2020; 2020:2704728.
29. Jahanban-Esfahlan R, Seidi K, Majidinia M, Karimian A, Yousefi B, Nabavi SM, *et al*. Toll-like receptors as novel therapeutic targets for herpes simplex virus infection. Rev Med Virol 2019;29:e2048.
30. Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol. 2002;20:709-760.
31. Mavi SA, Modarressi MH, Mohebali M, Shojaee S, Zeraati H, al. e. Assessment of the immunogenicity and protective efficiency of a novel dual-promoter DNA vaccine, harboring SAG1 and GRA7 genes, from RH strain of Toxoplasma gondii in BALB/c mice. Infect Drug Resist 2019;12:2519.
32. Yang Y, Chen J, Li H, Wang Y, Xie Z, Wu M, *et al*. Porcine interleukin-2 gene encapsulated in chitosan nanoparticles enhances immune response of mice to piglet paratyphoid vaccine. Comp Immunol Microbiol Infect Dis 2007;30:19-32.
33. Bourquin C, Anz D, Zwiorek K, Lanz AL, Fuchs S, Weigel S, *et al*. Targeting CpG oligonucleotides to the lymph node by nanoparticles elicits efficient antitumoral immunity. J Immunol. 2008;181:2990-2998.
34. Kawakami S, Higuchi Y, Hashida M. Nonviral approaches for targeted delivery of plasmid DNA and oligonucleotide. J Pharm Sci. 2008;97:726-745.
35. Xu Y, Yuen P-W, Lam J. Intranasal DNA vaccine for protection against respiratory infectious diseases: the delivery perspectives. Pharmaceutics. 2014;6:378-415.
36. Pishdadian A, Varasteh A, Gholamin M, Roozbeh Nasiraie L, Hosseinpour M, Moghadam M, Sankian M, Lung-derived innate cytokines: New epigenetic targets of allergen-specific sublingual immunotherapy. Iran J Basic Med Sci 2016; 19:64-71.
37. Havenar-Daughton C, Carnathan DG, Boopathy AV, Upadhyay AA, Murrell B, Reiss SM, *et al*. Rapid germinal center and antibody responses in non-human primates after a single nanoparticle vaccinei mmunization. Cell Rep 2019;29:1756-1766. e1758.
38. Liu MA. A comparison of plasmid DNA and mrna as vaccine technologies. Vaccines 2019;7:37-57.
39. Gupta J, Pathak M, Misra S, Misra-Bhattacharya S. CpG enhances the immunogenicity of heterologous DNA-prime/protein-boost vaccination with the heavy chain myosin of Brugia malayi in BALB/c mice. Parasitol Res 2019;118:1943-1952.
40. Wu H-M, Xie Q-M, Zhao C-C, Xu J, Fan X-Y, Fei G-H. Melatonin biosynthesis restored by CpG oligodeoxynucleotides attenuates allergic airway inflammation via regulating NLRP3 inflammasome. Life Sci 2019;239:117067.
41. Chew JL, Wolfowicz CB, Mao H-Q, Leong KW, Chua KY. Chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen, Der p 1 for oral vaccination in mice. Vaccine 2003;21:2720-2729.

42. Kumar M, Kong X, Behera AK, Hellermann GR, Lockey RF, Mohapatra SS. Chitosan IFN-γ-pDNA nanoparticle (CIN) therapy for allergic asthma. Genet Vaccines Ther 2003;1:3-13.
43. Hou Z, Han Q, Zhang C, Zhang J. Perspectives on RNA Interference in Immunopharmacology and Immunotherapy. J RNAi Gene Silenc 2016:285-309.
44. Kawasaki H, Taira K. Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. Nature 2004;431:211.
45. Morris KV, Chan SW-L, Jacobsen SE, Looney DJ. Small interfering RNA-induced transcriptional gene silencing in human cells. Science 2004;305:1289-1292.
46. Bowers K. RNA interference-mediated inhibition of ESCRT in mammalian cells. The ESCRT Complexes 2019;1998:305-318.
47. Lichtenberg SS, Tsyusko OV, Palli SR, Unrine JM. Uptake and bioactivity of chitosan/double-stranded RNA polyplex nanoparticles in Caenorhabditis elegans. Environ Sci Technol 2019;53:3832-3840.
48. Mansoori B, Mohammadi A, Shir Jang S, Baradaran B. Mechanisms of immune system activation in mammalians by small interfering RNA (siRNA). Artif Cells Nanomed Biotechnol 2016;44:1589-1596.
49. Wittrup A, Lieberman J. Knocking down disease: a progress report on siRNA therapeutics. Nat Rev Genet 2015;16:543-552.
50. Cubillos-Ruiz JR, Engle X, Scarlett UK, Martinez D, Barber A, Elgueta R, *et al*. Polyethylenimine-based siRNA nanocomplexes reprogram tumor-associated dendritic cells via TLR5 to elicit therapeutic antitumor immunity. J Clin Invest 2009;119:2231-2244.
51. Teo PY, Yang C, Whilding LM, Parente-Pereira AC, Maher J, George AJ, *et al*. Ovarian cancer immunotherapy using PD-L1 siRNA targeted delivery from folic acid-functionalized polyethylenimine: strategies to enhance T cell killing. Adv Healthc Mater 2015;4:1180-1189.
52. Jerome V, Graser A, Muller R, Kontermann RE, Konur A. Cytotoxic T lymphocytes responding to low dose TRP2 antigen are induced against B16 melanoma by liposome-encapsulated TRP2 peptide and CpG DNA adjuvant. J Immunother. 2006;29:294-305.
53. Xu Z, Ramishetti S, Tseng Y-C, Guo S, Wang Y, Huang L. Multifunctional nanoparticles co-delivering Trp2 peptide and CpG adjuvant induce potent cytotoxic T-lymphocyte response against melanoma and its lung metastasis. J Control Release 2013;172:259-265.
54. Ito A, Matsuoka F, Honda H, Kobayashi T. Heat shock protein 70 gene therapy combined with hyperthermia using magnetic nanoparticles. Cancer Gene Ther 2003;10:918-925.
55. Oberli MA, Reichmuth AM, Dorkin JR, Mitchell MJ, Fenton OS, Jaklenec A, *et al*. Lipid nanoparticle assisted mRNA delivery for potent cancer immunotherapy. Nano Lett 2017;17:1326-1335.
56. Yan S, Rolfe BE, Zhang B, Mohammed YH, Gu W, Xu ZP. Polarized immune responses modulated by layered double hydroxides nanoparticle conjugated with CpG. Biomaterials 2014;35:9508-9516.
57. Hobo W, Novobrantseva TI, Fredrix H, Wong J, Milstein S, Epstein-Barash H, *et al*. Improving dendritic cell vaccine immunogenicity by silencing PD-1 ligands using siRNA-lipid nanoparticles combined with antigen mRNA electroporation. Cancer Immunol Immunother 2013;62:285-297.
58. Chellat F, Grandjean-Laquerriere A, Le Naour R, Fernandes J, Yahia L, Guenounou M, *et al*. Metalloproteinase and cytokine production by THP-1 macrophages following exposure to chitosan-DNA nanoparticles. Biomaterials 2005;26:961-970.
59. Kim TH, Nah JW, Cho MH, Park TG, Cho CS. Receptor-mediated gene delivery into antigen presenting cells using mannosylated chitosan/DNA nanoparticles. J Nanosci Nanotechnol 2006;6:2796-2803.
60. Li Y, Su Z, Zhao W, Zhang X, Momin N, Zhang C, *et al*. Multifunctional oncolytic nanoparticles deliver self-replicating IL-12 RNA to eliminate established tumors and prime systemic immunity. Nat Rev Cancer 2020;1:882-893.
61. Cubillos-Ruiz JR, Baird JR, Tesone AJ, Rutkowski MR, Scarlett UK, Camposeco-Jacobs AL, *et al*. Reprogramming tumor-associated dendritic cells *in vivo* using miRNA mimetics triggers protective immunity against ovarian cancer. Cancer Res 2012;72:1683-1693.
62. Suzuki R, Namai E, Oda Y, Nishiie N, Otake S, Koshima R, *et al*. Cancer gene therapy by IL-12 gene delivery using liposomal bubbles and tumoral ultrasound exposure. J Control Release 2010;142:245-250.
63. Conde J, Bao C, Tan Y, Cui D, Edelman ER, Azevedo HS, *et al*. Dual targeted immunotherapy via in vivo delivery of biohybrid RNAi-peptide nanoparticles to tumor-associated macrophages and cancer cells. Adv Funct Mater 2015;25:4183-4194.
64. Rodrigo-Garzon M, Berraondo P, Ochoa L, Zulueta JJ, Gonzalez-Aseguinolaza G. Antitumoral efficacy of DNA nanoparticles in murine models of lung cancer and pulmonary metastasis. Cancer Gene Ther 2010;17:20-27.
65. Sun Y, Yang J, Yang T, Li Y, Zhu R, Hou Y, *et al*. Co-delivery of IL-12 cytokine gene and cisplatin prodrug by a polymetformin-conjugated nanosystem for lung cancer chemo-gene treatment through chemotherapy sensitization and tumor microenvironment modulation. Acta Biomater. 2021;128:447-461.
66. Diez S, Navarro G, de ICT. In vivo targeted gene delivery by cationic nanoparticles for treatment of hepatocellular carcinoma. J Gene Med 2009;11:38-45.
67. Huang K-W, Hsu F-F, Qiu JT, Chern G-J, Lee Y-A, Chang C-C, *et al*. Highly efficient and tumor-selective nanoparticles for dual-targeted immunogene therapy against cancer. Sci Adv 2020;6:eaax5032.
68. Azimifar MA, Salmasi Z, Doosti A, Babaei N, Hashemi M. Evaluation of the efficiency of modified PAMAM dendrimer with low molecular weight protamine peptide to deliver IL-12 plasmid into stem cells as cancer therapy vehicles. Biotechnol Prog. 2021;37:e3175.
69. Zhao X, Li F, Li Y, Wang H, Ren H, Chen J, *et al*. Co-delivery of HIF1alpha siRNA and gemcitabine via biocompatible lipid-polymer hybrid nanoparticles for effective treatment of pancreatic cancer. Biomaterials 2015;46:13-25.
70. Men K, Huang R, Zhang X, Zhang R, Zhang Y, He M, *et al*. Local and systemic delivery of interleukin-12 gene by cationic micelles for cancer immunogene therapy. J Biomed Nanotech 2018;14:1719-1730.
71. Pishavar E, Oroojalian F, Ramezani M, Hashemi M. Cholesterol-conjugated PEGylated PAMAM as an efficient nanocarrier for plasmid encoding interleukin-12 immunogene delivery toward colon cancer cells. Biotechnol Prog 2020;36:e2952.
72. Liu X, Wang B, Li Y, Hu Y, Li X, Yu T, *et al*. Powerful anticolon tumor effect of targeted gene immunotherapy using folate-modified nanoparticle delivery of CCL19 to activate the immune system. ACS Cent Sci. 2019;5:277-289.
73. Lei S, Zhang X, Men K, Gao Y, Yang X, Wu S, *et al*. Efficient colorectal cancer gene therapy with IL-15 mRNA nanoformulation. Mol Pharm. 2020;17:3378-3391.
74. Schneider T, Becker A, Ringe K, Reinhold A, Firsching R, Sabel BA. Brain tumor therapy by combined vaccination and antisense oligonucleotide delivery with nanoparticles. J Neuroimmunol 2008;195:21-27.
75. Zoller M, Strubel A, Hammerling G, Andrighetto G, Raz A, Ben-Ze'ev A. Interferon-gamma treatment of B16 melanoma cells: opposing effects for non-adaptive and adaptive immune defense and its reflection by metastatic spread. Int J Cancer. 1988;41:256-266.
76. Melief CJ. Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. Adv Cancer Res. 1992;58:143-175.

77. Melief CJ, Van Der Burg SH, Toes RE, Ossendorp F, Offringa R. Effective therapeutic anticancer vaccines based on precision guiding of cytolytic T lymphocytes. Immunol Rev. 2002;188:177-182.
78. Hulina-Tomašković A, Somborac-Bačura A, Rajković MG, Bosnar M, Samaržija M, Rumora L. Effects of extracellular Hsp70 and cigarette smoke on differentiated THP-1 cells and human monocyte-derived macrophages. Mol Immunol. 2019;111:53-63.
79. Kawai T, Akira S. TLR signaling. Cell Death Differ. 2006;13:816-825.
80. Abiko K, Mandai M, Hamanishi J, Yoshioka Y, Matsumura N, Baba T, *et al*. PD-L1 on tumor cells is induced in ascites and promotes peritoneal dissemination of ovarian cancer through CTL dysfunction. Clin Cancer Res. 2013;19:1363-1374.
81. Duraiswamy J, Freeman GJ, Coukos G. Therapeutic PD-1 pathway blockade augments with other modalities of immunotherapy T-cell function to prevent immune decline in ovarian cancer. Cancer Res. 2013;73:6900-6912.
82. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, *et al*. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A. 2007;104:3360-3365.
83. Maine CJ, Aziz NH, Chatterjee J, Hayford C, Brewig N, Whilding L, *et al*. Programmed death ligand-1 over-expression correlates with malignancy and contributes to immune regulation in ovarian cancer. Cancer Immunol Immunother. 2014;63:215-224.
84. Li QJ, Chau J, Ebert PJ, Sylvester G, Min H, Liu G, *et al*. MiR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell. 2007;129:147-161.
85. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, *et al*. Requirement of bic/microRNA-155 for normal immune function. Science. 2007;316:608-611.
86. Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, *et al*. Regulation of the germinal center response by microRNA-155. Science. 2007;316:604-608.
87. O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP, Taganov KD, Nicoll J, *et al*. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med. 2008;205:5855-5894.
88. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci U S A. 2007;104:1604-1609.
89. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4:71-78.
90. Cook J, Hagemann T. Tumour-associated macrophages and cancer. Curr Opin Pharmacol. 2013;13:595-601.
91. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. 2011;144:646-674.
92. Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nat Immunol. 2012;13:722-728.
93. Roupakia E, Markopoulos GS, Kolettas E. IL-12-mediated transcriptional regulation of matrix metalloproteinases. Biosci Rep. 2018;38: BSR20171420.
94. Luongo M, Brigida AL, Mascolo L, Gaudino G. Possible therapeutic effects of ozone mixture on hypoxia in tumor development. Anticancer Res. 2017;37:425-435.
95. Leuschner F, Dutta P, Gorbatov R, Novobrantseva TI, Donahoe JS, Courties G, *et al*. Therapeutic siRNA silencing in inflammatory monocytes in mice. Nat Biotechnol. 2011;29:1005-1010.
96. Hu Y-L, Miao P-H, Huang B, Zhang T-Y, Hu Z-J, Tabata Y, *et al*. Reversal of tumor growth by gene modification of mesenchymal stem cells using spermine-pullulan/DNA nanoparticles. J Biomed Nanotech. 2014;10:299-308.
97. Xu J, Dai W, Wang Z, Chen B, Li Z, Fan X. Intranasal vaccination with chitosan-DNA nanoparticles expressing pneumococcal surface antigen a protects mice against nasopharyngeal colonization by *Streptococcus pneumoniae*. Clin Vaccine Immunol. 2011;18:75-81.
98. Dolina JS, Sung SS, Novobrantseva TI, Nguyen TM, Hahn YS. Lipidoid nanoparticles containing PD-L1 siRNA delivered *in vivo* enter kupffer cells and enhance NK and CD8(+) T Cell-mediated hepatic antiviral immunity. Mol Ther Nucleic Acids. 2013;2:e72.
99. Bivas-Benita M, van Meijgaarden KE, Franken KL, Junginger HE, Borchard G, Ottenhoff TH, *et al*. Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of Mycobacterium tuberculosis. Vaccine. 2004;22:1609-1615.
100. Zhang W, Yang H, Kong X, Mohapatra S, San Juan-Vergara H, Hellermann G, *et al*. Inhibition of respiratory syncytial virus infection with intranasal siRNA nanoparticles targeting the viral NS1 gene. Nat Med. 2005;11:56-62.
101. Kumar M, Behera AK, Lockey RF, Zhang J, Bhullar G, de la Cruz CP, *et al*. Intranasal gene transfer by chitosan–DNA nanospheres protects BALB/c mice against acute respiratory syncytial virus infection. Hum Gene Ther. 2002;13:1415-1425.
102. Spolski R, Li P, Leonard WJ. Biology and regulation of IL-2: from molecular mechanisms to human therapy. Nat Rev Immunol. 2018;18:648-659.
103. Morrison KE, Lake D, Crook J, Carlone GM, Ades E, Facklam R, *et al*. Confirmation of psaA in all 90 serotypes of Streptococcus pneumoniae by PCR and potential of this assay for identification and diagnosis. J Clin Microbiol. 2000;38:434-437.
104. Curtis MM, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. Immunology. 2009;126:177-185.
105. Zhang Z, Clarke TB, Weiser JN. Cellular effectors mediating Th17-dependent clearance of pneumococcal colonization in mice. J Clin Invest. 2009;119:1899-1909.
106. Iwai Y, Terawaki S, Ikegawa M, Okazaki T, Honjo T. PD-1 inhibits antiviral immunity at the effector phase in the liver. J Exp Med. 2003;198:39-50.
107. Loke P, Allison JP. PD-L1 and PD-L2 are differentially regulated by Th1 and Th2 cells. Proc Natl Acad Sci U S A. 2003;100:5336-5341.
108. Lechmann M, Krooshoop DJ, Dudziak D, Kremmer E, Kuhnt C, Figdor CG, *et al*. The extracellular domain of CD83 inhibits dendritic cell-mediated T cell stimulation and binds to a ligand on dendritic cells. J Exp Med. 2001;194:1813-1821.
109. Abreo A, Wu P, Donovan BM, Ding T, Gebretsadik T, Huang X, *et al*. Infant respiratory syncytial virus bronchiolitis and subsequent risk of pneumonia, otitis media, and antibiotic utilization. Clin Infect Dis. 2019;71:211-214.
110. Li X, Sambhara S, Li CX, Ettorre L, Switzer I, Cates G, *et al*. Plasmid DNA encoding the respiratory syncytial virus G protein is a promising vaccine candidate. Virology. 2000;269:54-65.
111. Murphy BR, Collins PL. Live-attenuated virus vaccines for respiratory syncytial and parainfluenza viruses: applications of reverse genetics. J Clin Investig 2002;110:21-27.
112. Schwarz B, Morabito KM, Ruckwardt TJ, Patterson DP, Avera J, Miettinen HM, *et al*. Viruslike particles encapsidating respiratory syncytial virus M and M2 proteins induce robust T cell responses. ACS Biomater Sci Eng 2016;2:2324-2332.
113. Song X, Xiao H-T, Liao C-H, Li L, Kang Q-R, Jiang Y-C, *et al*. Natural products: the master regulators of antiviral cytokines. Curr Org Chem 2017;21:1805-1823.
114. Kay AB. Allergy and allergic diseases. NEJM. 2001;344:30-37.
115. Li G, Liu Z, Liao B, Zhong N. Induction of Th1-type immune response by chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen Der p 2 for oral vaccination in mice. Cell Mol Immunol 2009;6:45-50.
116. Roy K, Mao H-Q, Huang S-K, Leong KW. Oral gene delivery with chitosan–DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat Med

1999;5:387-391.
117. Da Silva AL, de Oliveira GP, Kim N, Cruz FF, Kitoko JZ, Blanco NG, *et al.* Nanoparticle-based thymulin gene therapy therapeutically reverses key pathology of experimental allergic asthma. Sci Adv 2020;6:eaay7973.
118. Tang C, Inman MD, van Rooijen N, Yang P, Shen H, Matsumoto K, *et al.* Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN-γ-dependent mechanism. J Immunol 2001;166:1471-1481.
119. Hajavi J, Hashemi M, Sankian M. Evaluation of size and dose effects of rChe a 3 allergen loaded PLGA nanoparticles on modulation of Th2 immune responses by sublingual immunotherapy in mouse model of rhinitis allergic. Int J Pharm 2019;563:282-292.
120. Lehrer RI, Ganz T. Biochemistry and function of monocytes and macrophages. Hematol 2001;6:865-869.
121. Da Silva AL, Martini SV, Abreu SC, Samary CdS, Diaz BL, Fernezlian S, *et al.* DNA nanoparticle-mediated thymulin gene therapy prevents airway remodeling in experimental allergic asthma. J Control Release 2014;180:125-133.
122. Isgro M, Bianchetti L, Marini M, Bellini A, Schmidt M, Mattoli S. The CC motif chemokine ligands CCL5, CCL11, and CCL24 induce the migration of circulating fibrocytes from patients with severe asthma. Mucosal Immunol 2013;6:718-727.
123. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. Immunity 2009;31:438-449.
124. Isaacs KL, Lewis JD, Sandborn WJ, Sands BE, Targan SR. State of the art: IBD therapy and clinical trials in IBD. Inflamm Bowel Dis 2005;11:S3-S12.
125. Korzenik JR, Podolsky DK. Evolving knowledge and therapy of inflammatory bowel disease. Nat Rev Drug Discov 2006;5:197-209.
126. Kriegel C, Amiji M. Oral TNF-α gene silencing using a polymeric microsphere-based delivery system for the treatment of inflammatory bowel disease. J Control Release 2011;150:77-86.
127. Bhavsar M, Amiji M. Oral IL-10 gene delivery in a microsphere-based formulation for local transfection and therapeutic efficacy in inflammatory bowel disease. Gene Ther 2008;15:1200-1209.
128. Laroui H, Theiss AL, Yan Y, Dalmasso G, Nguyen HT, Sitaraman SV, *et al.* Functional TNFα gene silencing mediated by polyethyleneimine/TNFα siRNA nanocomplexes in inflamed colon. Biomaterials 2011;32:1218-1228.
129. Xiao B, Chen Q, Zhang Z, Wang L, Kang Y, Denning T, *et al.* TNFα gene silencing mediated by orally targeted nanoparticles combined with interleukin-22 for synergistic combination therapy of ulcerative colitis. J Control Release 2018;287:235-246.
130. Mueller C. Tumour necrosis factor in mouse models of chronic intestinal inflammation. Immunology. 2002;105:1-8.
131. Arend WP. Physiology of cytokine pathways in rheumatoid arthritis. Arthritis Rheumatol 2001;45:101-106.
132. Park S, Le TT, Slejko JF, Villalonga-Olives E, Onukwugha E. Changes in opioid utilization following tumor necrosis factor inhibitor initiation in patients with rheumatoid arthritis. Rheumatol Ther 2019;6:611-616.
133. Kumar R, Dammai V, Yadava P, Kleinau S. Gene targeting by ribozyme against TNF-α mRNA inhibits autoimmune arthritis. Gene Ther. 2005;12:1486-1493.
134. Schiffelers RM, Xu J, Storm G, Woodle MC, Scaria PV. Effects of treatment with small interfering RNA on joint inflammation in mice with collagen-induced arthritis. Arthritis Rheumatol 2005;52:1314-1318.
135. Howard KA, Paludan SR, Behlke MA, Besenbacher F, Deleuran B, Kjems J. Chitosan/siRNA nanoparticle–mediated TNF-α knockdown in peritoneal macrophages for anti-inflammatory treatment in a murine arthritis model. Mol Ther. 2009;17:162-168.
136. Khoury M, Louis-Plence P, Escriou V, Noel D, Largeau C, Cantos C, *et al.* Efficient new cationic liposome formulation for systemic delivery of small interfering RNA silencing tumor necrosis factor α in experimental arthritis. Arthritis Rheumatol 2006;54:1867-1877.
137. Nissler K, Pohlers D, Hückel M, Simon J, Bräuer R, Kinne R. Anti-CD4 monoclonal antibody treatment in acute and early chronic antigen induced arthritis: influence on macrophage activation. ARD 2004;63:1470-1477.
138. Genprex Announces Initiation of its Phase 1/2 Acclaim-1 Clinical Trial for REQORSA™ Immunogene Therapy in Combination with Tagrisso® to Treat Non-Small Cell Lung Cancer Following FDA Review Yahoo finance2021 [Available from: https://finance.yahoo.com/news/genprex-announces-initiation-phase-1-123000637].
139. Malmström P-U, Loskog AS, Lindqvist CA, Mangsbo SM, Fransson M, Wanders A, *et al.* AdCD40L immunogene therapy for bladder carcinoma—the first phase I/IIa trial. Clin Cancer Res 2010;16:3279-3287.
140. Tristán-Manzano M, Justicia-Lirio P, Maldonado-Pérez N, Cortijo-Gutiérrez M, Benabdellah K, Martin F. Externally-controlled systems for immunotherapy: from bench to bedside. Front immunol 2020;11:2044-2061.
141. Xu J, Melenhorst JJ, Fraietta JA. Toward precision manufacturing of immunogene T-cell therapies. Cytotherapy 2018;20:623-638.