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Current Opinion in Biotechnology

Gene delivery for immunoengineering

Sarah Y Neshat[1], Stephany Y Tzeng[1] and Jordan J Green[1,2,3,4,5]

A growing number of gene delivery strategies are being employed for immunoengineering in applications ranging from infectious disease prevention to cancer therapy. Viral vectors tend to have high gene transfer capability but may be hampered by complications related to their intrinsic immunogenicity. Non-viral methods of gene delivery, including polymeric, lipid-based, and inorganic nanoparticles as well as physical delivery techniques, have also been widely investigated. By using either *ex vivo* engineering of immune cells that are subsequently adoptively transferred or *in vivo* transfection of cells for *in situ* genetic programming, researchers have developed different approaches to precisely modulate immune responses. In addition to expressing a gene of interest through intracellular delivery of plasmid DNA and mRNA, researchers are also delivering oligonucleotides to knock down gene expression and immunostimulatory nucleic acids to tune immune activity. Many of these biotechnologies are now in clinical trials and have high potential to impact medicine.

Addresses
[1] Department of Biomedical Engineering and the Translational Tissue Engineering Center, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA
[2] Institute for Nanobiotechnology, Johns Hopkins University, Baltimore, MD 21218, USA
[3] Departments of Materials Science and Engineering and Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
[4] Departments of Oncology, Ophthalmology, and Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA
[5] Bloomberg~Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA

Corresponding authors: Tzeng, Stephany Y (stzeng1@jhmi.edu), Green, Jordan J (green@jhu.edu)

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Introduction

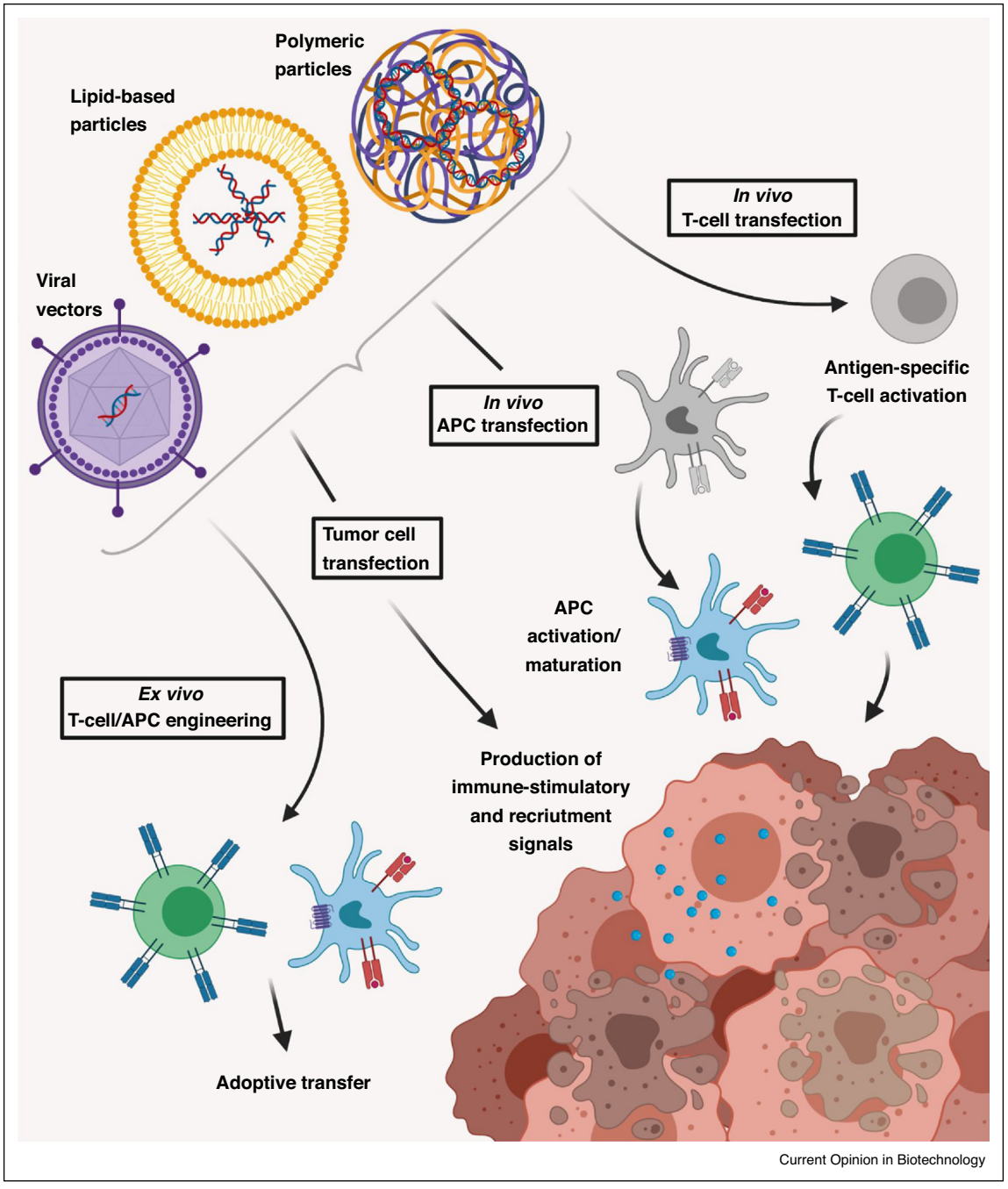
Gene delivery is increasingly being used to engineer the immune system in the laboratory and the clinic. Various biotechnologies have been developed for the delivery of nucleic acids to cells, both *in vivo* and *ex vivo*. To overcome challenges of *in vivo* manipulation, several *ex vivo* cell engineering technologies have advanced to the clinic [1]; however, delivery vehicles that can be administered directly to patients with delivery of the cargo efficiently to target cells *in vivo* also show great promise.

Various types of nucleic acid have been delivered for immune applications. Here, we will discuss the delivery of plasmid DNA (pDNA) and mRNA to overexpress a gene of interest; oligonucleotides such as small interfering RNA (siRNA) and micro RNA (miRNA), which can knock down gene expression; and immunostimulatory nucleic acids that elicit a specific immune response. Differences in their physical, chemical, and immunological properties have been discussed in detail elsewhere [2]. Briefly, pDNA, the largest of these and generally >2 kb in size, is double-stranded and relatively stable to chemical degradation, but it must enter the nucleus. mRNA, while less chemically stable than pDNA, is smaller and acts in the cytoplasm, therefore not requiring nuclear entry. siRNA and miRNAs are generally only ~20 bp and are also active in the cytoplasm, while immunostimulatory nucleic acids, which include CpG sequences in pDNA and double-stranded RNA structures, may be active in various intracellular locations, including the cytoplasm or the endosomal compartment. Nucleic acids can activate the innate immune system, such as through sensing by Toll-like receptors (TLRs), with single-stranded (ss) RNA recognized by TLR-7 and -8, double-stranded (ds) RNA recognized by TLR-3, and unmethylated CpG sequences in DNA recognized by TLR-9 [3,4]. Other mechanisms of immune sensing include the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway and the absent in melanoma 2 (AIM2) pathway, which both detect cytosolic DNA [5], and dsRNA sensors like RIG-I-like receptors, reviewed here in detail [6]. Examples below will show that nucleic acids may also be engineered to enhance or minimize this effect.

As much of the recent progress in the field of gene delivery for immunotherapy has been for cancer applications, this review will focus on cancer immunotherapy but will also cover certain non-oncology applications. Major strategies that have been explored for cancer immunotherapy [7] center on increasing the immunogenicity of the tumor microenvironment, enhancing the ability of antigen-presenting cells (APCs) to be activated, improving the activation of T cells and other lymphocytes in the context of the tumor while lessening the effect of suppressive immune cells, and vaccinating the patient with a

tumor-specific antigen in order to generate a tumor-targeted immune response (Figure 1). Broadly, an overarching goal of immunoengineering is often to shift the balance of the immune response between activation and suppression, or, more specifically, between the T_H1-type, including cell-mediated cytotoxic behavior that can be used to combat cancer; T_H2-type, which leads to a humoral response against extracellular pathogens and parasites; and T_H17-type, which is important for inducing inflammation.

Figure 1



Broad gene delivery strategies for cancer immunotherapy.
Gene delivery can be accomplished using viral, lipid-based, or polymeric vectors, or a combination of various materials. These can be used to genetically engineer immune cells *ex vivo* for adoptive transfer, or they can modify tumor cells or immune cells directly *in vivo* to promote immune activation against the tumor. Some examples of gene delivery methods that can be used for tumor immunotherapy are shown here.

Viral gene delivery strategies for cancer immunotherapy

Viral vectors have historically been used for delivery of nucleic acids due to their effectiveness at transferring the nucleic acid payload to host cells. The most widely used vectors in recent years are retroviruses, including lentiviruses, which insert a gene of interest into the host genome; adenoviruses, which deliver an episomal, non-integrating DNA plasmid to cells; and adeno-associated viruses (AAV), which can only replicate in coordination with a second virus [8•]. For instance, Zhu *et al.* described an AAV vector for the delivery of the cytokine IL-27, which inhibits T_H17 and T_H2 responses [9]. In the B16-F10 murine melanoma model, they found that this strategy depleted suppressive regulatory T cells (Tregs), including at the tumor site, and led to enhanced efficacy of a cancer vaccine and anti-PD-1 checkpoint blockade therapy.

Importantly, however, viral vectors often elicit a strong immune response. Not only can this have deleterious effects on the patient, but it may also reduce the efficacy of viruses, particularly upon repeated administration due to the formation of neutralizing antibodies. To address this, engineers have developed methods of coating viruses with polymers [8•] to prevent exposure of antigenic viral epitopes (Figure 2). Jung *et al.* used a hydrogel to encapsulate oncolytic adenoviruses, which are engineered to replicate only in tumor cells, and showed that encapsulation not only sustained the local release of virus in a hamster pancreatic cancer model but also reduced the animals' antiviral immune response, resulting in a >60% lower tumor burden compared to treatment with the adenovirus alone [10]. The use of polymer coatings can also improve viral transduction efficacy and allow co-delivery of therapeutics: for instance, an adenovirus encoding the pro-T_H1 cytokine IL-12 was coated with a copolymer of β-cyclodextrin and the cationic polyethylenimine (PEI) to enhance delivery up to 600-fold *in vitro* and co-deliver a small molecule inhibitor of the suppressive TGF-β in the B16 model [11].

Non-viral gene delivery strategies for cancer immunotherapy

Because of concerns about the safety of viral vectors, a wide range of non-viral delivery vehicles have been developed. State-of-the-art synthetic materials for gene delivery are reviewed in detail by Lostalé-Seijo *et al.* [12].

Lipids

Lipid-based nanoparticles or lipoplexes have been extensively researched for gene delivery, particularly in the case of mRNA and oligonucleotides. Amphiphilic lipids can form liposomal structures that encapsulate nucleic acids within the core; cationic lipids can associate with the negatively charged nucleic acid to form nanostructures. In one case, three immune-stimulatory genes encoded in mRNA were delivered directly to B16-F10 melanoma or MC38 colorectal tumors in mice using lipid nanoparticles, which caused tumor regression and long-term immunity to the tumor [13••]. Engineering the chemical structure of lipids can improve the efficiency to allow better and more specific delivery of siRNA and small single-guide RNA (sgRNA) to cells that are normally hard to transfect, including T cells [14]. Specifically, lipids with conformationally constrained tails were fivefold to 10-fold more effective at gene delivery than similar lipids with unconstrained tails.

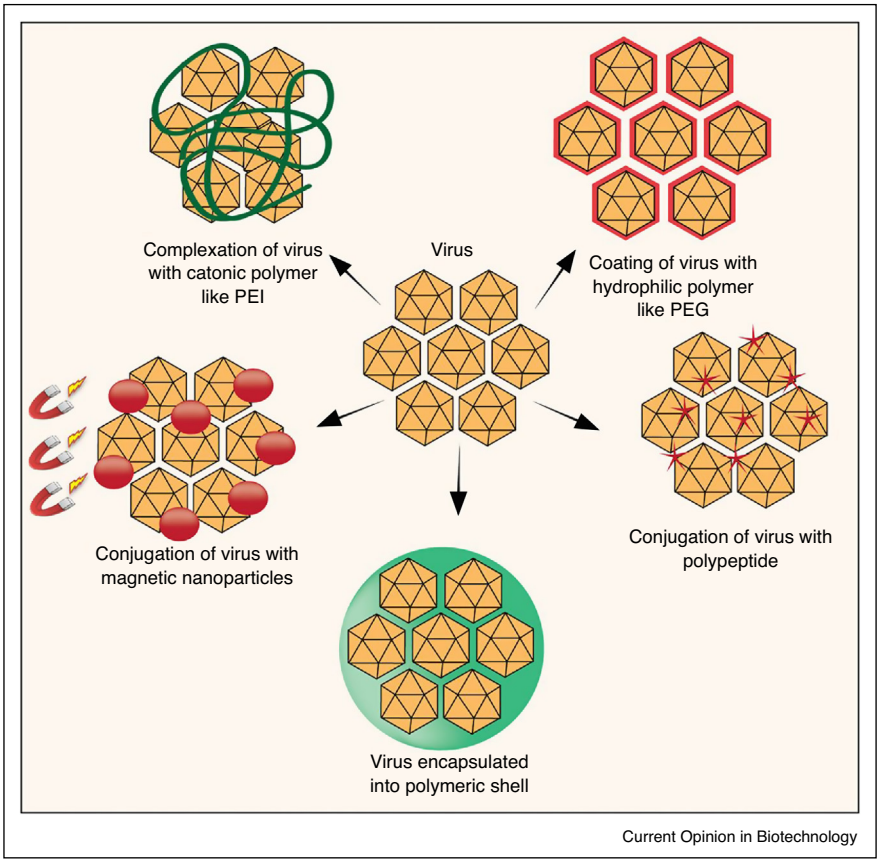
Several researchers have taken advantage of the immune-stimulatory properties of nucleic acids delivered by lipid nanoparticles. Among these are cyclic dinucleotides (CDNs), such as cGAMP agonists of stimulator of interferon genes (STING), which can increase the activity of APCs at the tumor site but, due to their high negative charge, require encapsulation to improve cellular internalization [15]. Some lipid structures, particularly those with amine-containing cyclic head groups, have also been found to intrinsically activate STING, and the use of these immune-stimulatory lipids to deliver tumor antigen-encoding mRNA resulted in effective vaccination against B16-F10 and ovalbumin-expressing B16 tumors, as lipids with cyclic head groups inducing nearly twofold higher antigen-specific cytotoxicity after injection [16•]. On the other hand, excessive activation of interferon type I (IFN I) via pathways like STING can be damaging if uncontrolled; therefore, some groups have engineered mRNA constructs to prevent IFN I induction while using lipid nanoparticles to co-deliver an adjuvant like monophosphoryl lipid A (MPLA) that triggers IFN I in a more controlled manner [17].

Polymers

Given the intrinsic immunogenicity of some lipid carriers, polymers can serve as an alternative for nucleic acid delivery, though some polymers may also have adjuvant activity [18]. In addition to mRNA and small nucleic acids, cationic polymers are also used often for the delivery of pDNA, usually forming nanoparticles via self-assembly with nucleic acids. The versatility of polymer chemical and physical structures provides a wide range of properties that can be optimized. For instance, *ex vivo* transfection of T cells with mRNA and DNA with up to 25% and 18% efficiency, respectively, has been accomplished by tuning the branching architecture of the delivery vehicle, a co-polymer of poly(2-hydroxyethyl methacrylate) (pHEMA) and poly(2-dimethylaminoethyl methacrylate) (pDMAEMA) [19].

In many cases, *in vivo* transfection is preferred in order to avoid *ex vivo* processing and cell culture steps. Cationic PEI has been studied for decades for gene delivery purposes, though modifications are often necessary to improve nanoparticle stability and biodistribution *in vivo*. For instance, a poly(ethylene glycol) (PEG)-modified PEI nanoparticle was complexed with pDNA encoding small hairpin RNA

Figure 2



Strategies for improving the translatability of viral gene delivery.
Viruses are effective gene delivery agents but must contend with safety challenges as well as their intrinsic immunogenicity. Several methods have been devised to overcome this, including coating viruses with polymers, blocking neutralization sites using polypeptides, and physically encapsulating the viruses to isolate them from immune cells. Reprinted with permission from Rajagopal *et al.*, 'Polymer-coated viral vectors: hybrid nanosystems for gene delivery', *J Gene Med* 20(4):e3011, Copyright 2018, John Wiley and Sons [8•].

(shRNA) to knock down expression of PD-L1 in tumor cells. Local injection of hyaluronidase into the tumor improved nanoparticle accumulation after systemic administration by approximately twofold, leading to PD-L1 knockdown in the tumor [20]. Polyrotaxanes, consisting of four-arm PEG threaded with the cationic polysaccharide rings α-cyclodextrin, can deliver IL-12 pDNA to MC38 tumor cells after systemic administration by taking advantage of the stealth properties of PEG to achieve good pharmacokinetics [21]. Another PEG-modified material, cationic trimethyl chitosan, delivered siRNA against VEGF and PIGF to macrophages in breast cancer models, utilizing a mannose ligand to target macrophages and repolarize them to an immune-stimulatory phenotype [22]. This led to >90% tumor inhibition due to combination knockdown of both VEGF and PIGF *in vivo*. Polymersomes, described as amphiphilic block co-polymers that self-assemble into liposome-like structures with an aqueous core, were also used to deliver CDN as a STING agonist and improved survival in a B16-F10 model after intratumoral injection [23].

The toxicity of high-molecular-weight PEI is often limiting. Less toxic low-molecular-weight PEI can be cross-linked with degradable linkages to form a larger polymer with greater transfection efficacy while preserving the biocompatibility of the low-molecular-weight form. When functionalized with galactose to target the liver, a cross-linked PEI co-polymer was able to deliver IL-15 pDNA to tumor cells with significantly lower polymer-mediated cytotoxicity and twofold to threefold higher gene delivery *in vitro*, leading to improved survival in an orthotopic murine ML-1 hepatocellular carcinoma model *in vivo* [24]. In a similar vein, biodegradable polymers are commonly used to reduce toxicity. Poly (beta-amino ester)s (PBAEs) are cationic, hydrolytically degradable polymers that have been used to deliver CpG ODNs, agonists of Toll-like receptor 9 (TLR-9), which upregulates production of proinflammatory cytokines [25], or CDN as a STING agonist [26] by local intratumoral delivery in mouse melanoma models. Another PBAE was also used to deliver pDNA encoding

immune-stimulatory genes locally to tumors, resulting in slowed tumor progression and long-term survival in B16-F10 and MC38 models [27•]. Smith *et al.* further modified PBAE/pDNA nanoparticles by coating them with a targeting ligand, allowtransfection of T cells after intravenous injection. This led to *in situ* generation of leukemia-specific chimeric antigen receptor (CAR) T cells, with nearly 6% of the circulating T cells CAR^+ within days [28••], and the same group showed that a similar strategy could be used to deliver mRNA to T cells, inducing a memory phenotype in tumor-specific T cells as well as demonstrating proof-of-concept *in situ* gene editing [29]. Other biodegradable polymers, like charge-altering releasable transporters, deliver mRNA encoding the ovalbumin antigen to a mouse along with CpG as an adjuvant, allowing for successful vaccination of the mouse against ovalbumin-expressing A20 lymphoma after subcutaneous or intravenous delivery [30], with up to 40% of mice considered cured of established tumors. The chemical tunability of synthetic polymers provides many opportunities to optimize their properties for gene delivery and immune engineering.

Lipid-polymer hybrids

Lipid-based and polymer-based delivery systems can be combined to take advantage of properties from both materials. Folate-modified methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) and dioleoyl-3-trimethylammonium propane (DOTAP) nanoparticles encapsulating pDNA encoding the CC-motif chemokine ligand 19 (CCL19) were designed to transfect folate receptor-expressing tumor cells in CT26 colon cancer models and induce expression of CCL19 to modulate dendritic cell (DC) and lymphocyte interactions [31]. The authors describe advantages over certain CAR T therapies, such as augmenting the favorable anti-tumor immune response while avoiding the detrimental cytokine release syndrome (CRS). A similar hybrid concept has been used in combination with chemotherapy drugs. In one particular design, oxaliplatin (OxP) chemotherapy is delivered systemically to cause immunogenic cell death (ICD) in parallel with locally delivered DNA encoding a PD-L1 trap fusion protein via lipid-protamine-DNA (LPD) nanoparticles that function selectively within the tumor, thus resulting in minimized immune-related adverse effects, for CT26, B16-F10, and 4T1 tumor models (Figure 3) [32]. The combination treatment with PD-L1 trap DNA and OxP was significantly more effective than either treatment alone, with approximately 20% increase in median survival in CT26-bearing mice. These LPD nanoparticles have also been implemented to simultaneously silence expression of HMGA1 (high mobility group protein A1) to increase T lymphocyte infiltration twofold to fourfold and induce PD-L1 trap expression to improve checkpoint inhibitor therapy in CT26 and 4T1 models [33].

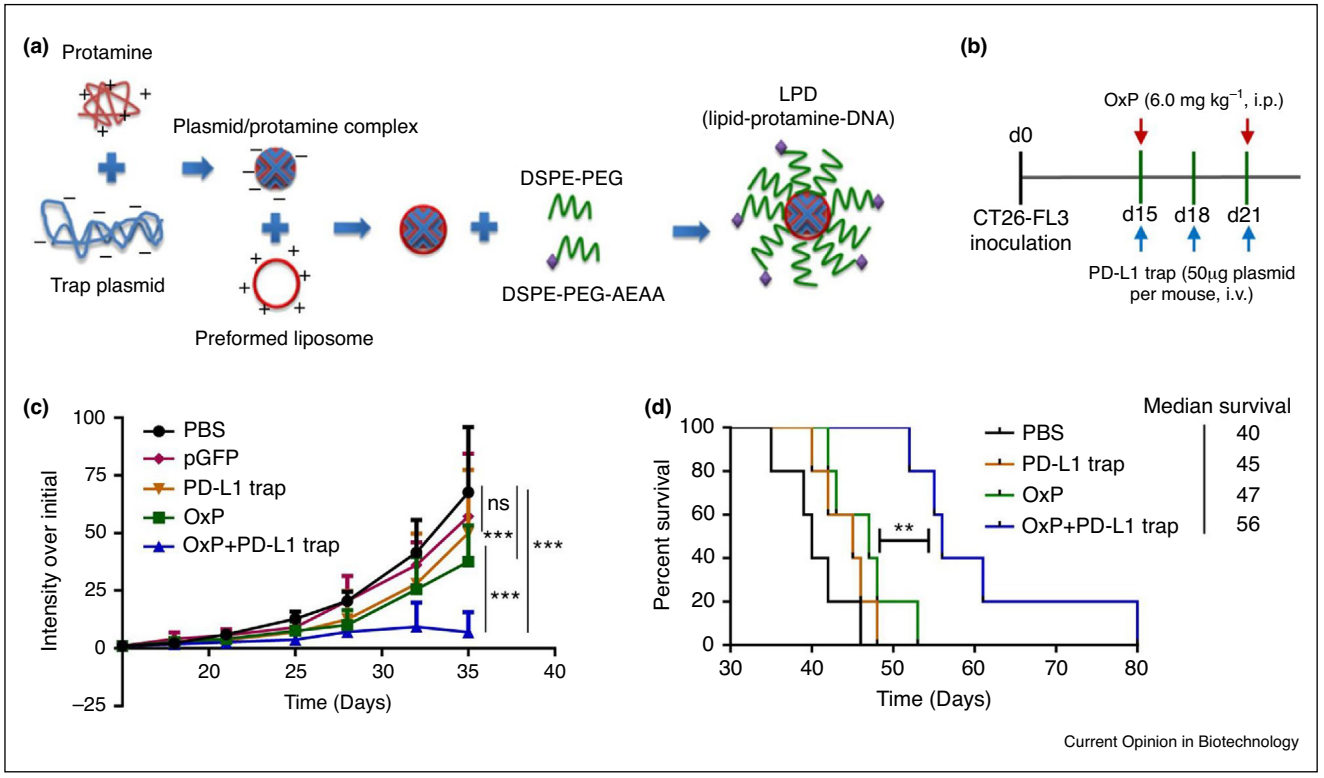
Alternatively, McKinlay *et al.* describe the generation of a combinatorial library to screen for hybrid-lipid charge-altering releasable transporters [34]. These specific materials are primarily effective for mRNA transfection in T-cell lines, primary T cells *in vitro*, and splenic *in situ*. The incorporation of oleyl and nonenyl lipid elements increased the transfection efficacy of T cells to 1–1.5% and that of B cells to 11%, while minimizing toxicity-mediated cell death compared to the previously published polymeric charge-altering releasable transporters, which have been reported to transfect <1% and 1–7% of T cells and B cells, respectively [34].

Inorganic materials, physical transfection, and other methods

Inorganic or physical modifications can be applied to nanoparticles for increased stabilization or improved uptake by certain targeted cell populations. Calcium phosphate (CaP) has long been used for gene delivery due to its ability to encapsulate nucleic acid efficiently and dissolve intracellularly under the acidic conditions of the endolysosomal compartment, but it is limited by poor stability and lack of control in manufacturing, necessitating use of other materials to improve the properties of CaP [35]. Lipid-coated calcium phosphate (LCP) nanoparticles functionalized with mannose have been developed to target delivery of mRNA to dendritic cells (DCs) draining to the lymph node in triple negative breast cancer (TNBC) *in vivo* models as a nanovaccine [36]. The mRNA encodes the tumor-associated antigen mammary type mucin (MUC1). Transfected DCs express MUC1 and present it to 4T1 TNBC-specific cytotoxic T cells, which expand and, in combination with anti-CTLA-4 antibodies, leads to tumor infiltration, tumor growth inhibition, and memory [36]. LCP NPs have also been used to deliver mRNA encoding tyrosine related protein 2 (TRP2) and PD-L1 siRNA to B16-F10 melanoma models [37], resulting in transfection of DCs to induce them to present a tumor antigen while also down-regulating expression of a checkpoint molecule.

Alternatively, macrophages can be targeted using modified nanoparticles. CpG oligodeoxynucleotides (ODN) can be targeted to macrophages using mannosylated carboxymethyl chitosan/protamine sulfate/$CaCO_3$/ODN (MCMC/PS/$CaCO_3$/ODN), a polymer/inorganic nanoparticle hybrid whose features are designed to improve ODN encapsulation, macrophage uptake, and pH-mediated intracellular release [38]. CpG delivery to macrophages promotes expression of CD80, an activating co-stimulatory signal to lymphocytes, therefore inducing the anti-tumor M1 phenotype *in vitro* with RAW264.7 cells [38], measured by approximately twofold higher secretion of IL-12 and other inflammatory cytokines compared with macrophages treated with the common commercial transfection agent Lipofectamine® 2000. Another hybrid, peptide/hyaluronic acid/protamine/$CaCO_3$/DNA nanoparticles (PHNP), was developed to

Figure 3



Hybrid lipid-polymer materials can be used for pDNA delivery for tumor immunotherapy.
The cationic polymer protamine was used to condense pDNA for PD-L1 trap, then coated with PEGylated lipids for stability and targeting (LPD) **(a)**. These LPDs were injected into tumor-bearing mice along with systemic oxaliplatin (OxP) therapy **(b)**, and the combination of OxP and PD-L1 trap expression significantly inhibited tumor growth **(c and d)**. Adapted from Song *et al.*, 'Synergistic and low adverse effect cancer immunotherapy by immunogenic chemotherapy and locally expressed PD-L1 trap', *Nat Comm* 9:2237, Copyright 2018, Springer Nature [32].

target pDNA to J774A.1 macrophages and HeLa tumor cells *in vitro* [39]. The fusion protein promotes recognition by Fc receptors on macrophages internalization by tumor cells, while the hyaluronic acid (HA) interacts with CD44 found on both cell types. The pDNA encodes IL-12 to repolarize macrophages from anti-inflammatory M2 to anti-tumor M1, in addition to downregulating the CD47 'don't-eat-me' signal and upregulating co-stimulatory CD80 on tumor cells *in vitro* to reverse cancer-induced immunosuppression [39]. Arginine-coated gold nanoparticles (ArgNPs) have also been used to target macrophages and deliver CRISPR-Cas9 in order to edit out signal-regulatory protein α (SIRP-α), the inhibitory receptor for CD47, thus allowing fourfold greater phagocytosis of human bone osteosarcoma cells *in vitro* [40]. The cationic arginine allowed binding to the single guide RNA (sgRNA) for SIRP-α and the Cas9 protein, which was engineered to include glutamic acid tags at the C-terminus and nuclear localization signal (NLS) at the N-terminus.

Gene therapy-based immune engineering for other applications

Although many recent advances related to gene therapy have been focused on cancer, delivery technologies have been developed for other applications as well, including infection, autoimmune disorders, and allergy. Genetic vaccines are particularly advantageous when compared to traditional peptide-based vaccines given the ability to stimulate at lower quantities, maintain antigen expression, bypass HLA restriction, and expand to both humoral and cellular immunity responses [41]. Recent vaccine developments have incorporated gene delivery using both polymer and lipid nanoparticles. A Zika virus (ZIKV) vaccine formulated with the full natural DNA sequence of ZIKV premembrane and envelope protein (prM-E) within a tetrafunctional PEO/PPO/ethylene diamine amphiphilic block copolymer NP has elicited antigen-specific serum IgG, neutralizing antibodies, and protection upon intramuscular challenge [42]. Similarly, an intradermal LNP vaccine contained N(1)-methylpseudouridine mRNA encoding viral surface antigens, such as ZIKV prM-E, influenza virus hemagglutinin (HA), and HIV-1 envelope (Env) [43]. The vaccine was shown to establish an antigen-specific $CD4^+$ T-cell response in addition to an increase in B cells and plasma cells to generate humoral memory and high affinity neutralizing antibodies when compared to unmodified mRNA

Table 1

Select clinical nucleic acid delivery programs for immune engineering

Responsible company/lab	Phase	Target application	Delivery vehicle	Nucleic acid	Therapeutic agent	ClinicalTrials.gov identifier
ModernaTx., Inc.	Phase 1	Solid Tumors	Lipid NP	mRNA	mRNA-4157 & Pembrolizumab	NCT03313778 [51]
ModernaTx., Inc.	Phase 1	Advances/Metastatic Solid Tumor Malignancies or Lymphoma	Lipid NP	mRNA	mRNA-2416 & Durvalumab	NCT03323398 [57]
ModernaTx., Inc.	Phase 1	Relapsed/Refractory Solid Tumor Malignancies or Lymphoma	Lipid NP	mRNA	mRNA-2752 & Durvalumab	NCT03739931 [58]
Henry Ford Health System	Phase 1	Prostate Cancer	Oncolytic Adenovirus	DNA	Ad5-yCD/ mutTKSR39rep-hIL12 (IL12)	NCT02555397 [59]
University of Pennsylvania	Phase 1	Pleural malignancies (metastatic pleural effusions or pleural mesothelioma)	Adenovirus	DNA	Interferon-beta (BG00001, Ad. hIFN-β, interferon-beta (hIFN-β) gene	NCT00299962 [60]
Arthrogen	Phase 1	A Single Dose Clinical Trial to Study the Safety of ART-I02 in Patients With Arthritis	AAV	DNA	Interferon-beta (hIFN-β) gene under NF-κB control	NCT02727764 [54]
National Institute of Allergy and Infectious Diseases (NIAID) and ModernaTx., Inc.	Phase 1	SARS-CoV-2 (Coronavirus infection)	Lipid NP	mRNA	mRNA-1273	NCT04283461 [55]
University of Pennsylvania and Adaptimmune	Phase 1	HIV Infection	Lentivirus	RNA	α/6-gag-TCR modified T cells; WT-gag-TCR modified T cells	NCT00991224 [61]
ModernaTx., Inc.	Phase 2	Melanoma	Lipid NP	mRNA	mRNA-4157 & Pembrolizumab	NCT03897881 [52]
The Methodist Hospital System	Phase 2	High-risk Prostate Cancer	Viral vector (Herpes simplex virus)	DNA	ADV/HSV-tk	NCT03541928 [62]
Roswell Park Cancer Institute	Phase 2	Adult Solid Neoplasm	Retrovirus	RNA	NY-ESO-1 TCR/ TGFbDNRII-transduced TILs	NCT02650986 [63]
National Cancer Institute (NCI)	Phase 2	Glioblastoma Non-Small Cell Lung Cancer Ovarian Cancer Breast Cancer Gastrointestinal/ Genitourinary Cancer	Retrovirus	RNA	Individual Patient TCR-Transduced PBL (iTCR)	NCT03412877 [49]
Huazhong University of Science and Technology	Phase 3	Hepatocellular Carcinoma	Adenovirus	DNA	ADV-TK	NCT03313596 [64]

vaccines. Interestingly, Yan *et al.* describes a subcutaneously injected scaffold loaded with ovalbumin (OVA) mRNA-lipoplexes constructed from chitosan-alginate gel [44]. While a more traditional vaccine formulation consisting of the ovalbumin protein elicited a stronger short-term humoral immune response, the mRNA-lipoplex-based scaffold vaccine elicited greater T-cell proliferation within secondary lymphoid organs. IFN-γ secretion as a result of the mRNA-lipoplex scaffold was also greater than that due to protein, naked mRNA, or mRNA-lipoplex immunizations by two- to threefold [44]. Additionally, anti-viral vaccines have been engineered with self-amplifying mRNA (SAM) encapsulated in lipid nanoparticles and show an induced type 1 IFN

response locally when compared to TLR7 agonists [45•]. These studies have led to interest in exploring the use of SAM mRNA for nanoparticle-delivered immunotherapies [46].

In addition to infectious diseases, gene therapies have been used to address autoimmune disorders. An AAV vector (AAV5) has been used for single dose intra-articular delivery of the human interferon-β (hIFN-β) gene in patients with inflammatory arthritis, including rheumatoid arthritis (RA) [47]. This therapy is unique in that there is local transcriptional control of the hIFN-β transgene by an NF-κB promoter, therefore causing transgene expression only during states of flare-up inflammation. Recently, allergy immunotherapy has expanded with goals of provoking biased antigen-specific T_H1 responses. Microneedles coated with model antigen OVA co-formulated with STING agonist as an adjuvant induced the generation of OVA-specific serum IgG2a, signifying an enhanced T_H1 response [48]. When challenged with OVA, splenocytes produced higher IL-2 and IFN-γ T_H1 cytokines when compared to subcutaneous injections or microneedle delivery of alum formulations. Although the studies described above have been designed for viral vaccines and allergies, concepts can be applied to improve future developments of cancer immunotherapies.

Clinical translation of gene therapy-based immune engineering

Exciting translational methods and immune stimulation techniques are being investigated for clinical applications within the last few years, and some examples are shown in Table 1. For instance, researchers at the National Cancer Institute have developed a potential treatment for heterogeneous metastatic cancer by retrovirally transduced autologous peripheral blood mononuclear cells (PBMCs) to express neoantigen-reactive TCRs isolated from the patient to target shared oncogenes and multiple neoantigens with aims to address tumor escape [49,50]. Additionally, a Phase I trial led by ModernaTX, Inc. outlines the safety and tolerability of lipid nanoparticles encapsulating genetic material encoding tumor neoantigens while inducing neoantigen-specific T cells in 33 patients, whether given as a monotherapy or in combination with pembrolizumab, and it subsequently advanced to Phase II [51,52]. On the other hand, adenoviral-mediated delivery has been assessed in a completed Phase I clinical trial with patients with malignant pleural mesothelioma to induce production of IFN-α in the pleural fluid and serum [53]. Although the results of this heterogeneous pilot study were variable, disease stability or regression via scans and serum measurements of soluble mesothelin-related peptides (SMRP) was noted in five of the nine subjects described as younger patients with lower tumor burdens.

For applications outside of cancer immunotherapy, a Phase I clinical trial is currently ongoing to test AAV5 as a vector to induce NF-κB-regulated hIFN-β expression in patients with RA [54]. In response to the recent COVID-19 pandemic, ModernaTX, Inc. in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) are developing a lipid nanoparticle/mRNA-based vaccine for the novel SARS-CoV-2 coronavirus infection, currently in Phase I trials in healthy volunteers [55].

Conclusions

Immune responses can be engineered using gene delivery techniques to modulate the genetic composition of cells, enabling innovative cancer immunotherapy methods. Nucleic acid cargos of varying size can be optimized for nanoparticle encapsulation to upregulate or downregulate gene expression, leading to productive anti-tumor immune responses. Genetic material can be delivered via viral or non-viral approaches to immune cells, such as macrophages and dendritic cells, to activate or suppress activity. Nanoparticles are diverse in formulation, with each type of delivery material conveying advantages and disadvantages, which may be combined in hybrid formulations consisting of multiple material types. In addition, new cargos, such as self-amplifying mRNA, hold promise for efficient, next-generation vaccines, and nanostructures like polymersomes can allow for the delivery of combination immunotherapies. Recent research has shown the expansive applications of gene therapy to various immune-related disorders and diseases, including tumors, infectious diseases, autoimmune disorders, and allergy. Many of the current trials shown here are in early stages, but there are exciting developments that potentiate future clinical impact. It is important to note that gene-based therapies for immune engineering constitute a set of challenges that require further vetting of these platforms before entering further clinical stages, such as reproducibility, instability of genetic materials, production scale-up, transient off-target genotoxicity, and vector immunogenicity [56]. However, with well-funded companies increasingly sponsoring nucleic acid therapeutics and immune engineering approaches, there is great hope for the necessary advances that can achieve clinical efficacy while minimizing toxicity.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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While viral gene delivery is of high interest in the laboratory as well as in the clinic, it still faces several hurdles, some of which can be solved by combining viruses with other materials. This review covers many of the challenges to viral gene therapy as well as methods of overcoming them.

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