



Nucleic acid vaccines and CpG oligodeoxynucleotides for allergen immunotherapy

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Purpose of review

Molecular forms of allergen-specific immunotherapy (AIT) are continuously emerging to improve the efficacy of the treatment, to shorten the duration of protocols and to prevent any side effects. The present review covers the recent progress in the development of AIT based on nucleic acid encoding allergens or CpG oligodeoxynucleotides (CpG-ODN).

Recent findings

Therapeutic vaccinations with plasmid deoxyribonucleic acid (DNA) encoding major shrimp Met e 1 or insect For t 2 allergen were effective for the treatment of food or insect bite allergy in respective animal models. DNA expressing hypoallergenic shrimp tropomyosin activated Foxp3⁺ T regulatory (Treg) cells whereas DNA encoding For t 2 down-regulated the expression of pruritus-inducing IL-31. Co-administrations of major cat allergen Fel d 1 with high doses of CpG-ODN reduced Th2 airway inflammation through tolerance induction mediated by GATA3⁺ Foxp3^{hi} Treg cells as well as early anti-inflammatory TNF/TNFR2 signaling cascade. Non-canonical CpG-ODN derived from *Cryptococcus neoformans* as well as methylated CpG sites present in the genomic DNA from *Bifidobacterium infantis* mediated Th1 or Treg cell differentiation respectively.

Summary

Recent studies on plasmid DNA encoding allergens evidenced their therapeutic potential for the treatment of food allergy and atopic dermatitis. Unmethylated or methylated CpG-ODNs were shown to activate dose-dependent Treg/Th1 responses. Large clinical trials need to be conducted to confirm these promising preclinical data. Moreover, tremendous success of messenger ribonucleic acid (mRNA) vaccines against severe acute respiratory syndrome coronavirus 2 must encourage as well the re-exploration of mRNA vaccine platform for innovative AIT.

Keywords

allergen-specific immunotherapy, CpG oligodeoxynucleotides, nucleic acid vaccines

INTRODUCTION

Conventional allergen-specific immunotherapy (AIT), based on subcutaneous or sublingual (SCIT, SLIT) repetitive administrations of allergen extracts, is effective not only to alleviate the clinical symptoms of allergic diseases but also to reprogram the Th2-biased allergic responses for the installation of long-term tolerance [1]. However, these treatments, despite their simplicity and cost-effectiveness, are by far optimal and commonly associated with several important issues including poor immunogenicity and standardization of allergen extracts, side effects [immunoglobulin E (IgE) or T-cell-mediated], duration (2–3 years for the development of tolerance).

The cloning of allergens together with the elucidation of their structure and the mapping of their B- and T-cell epitopes triggered the development of molecular approaches to AIT with higher safety and

efficacy [2^a]. Nucleic acid-based therapeutics, consisting in plasmid deoxyribonucleic acid (DNA) or messenger ribonucleic acid (mRNA) encoding antigens, could be viewed as extremely versatile vaccine platforms which combine multiple advantages: simple and fast design, reproducible and consistent manufacturing, stability and cost-effectiveness [3]. Remarkably, these strategies allowed the rapid

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KEY POINTS

- In animal studies, allergen-specific immunotherapy (AIT) based on plasmid DNA encoding allergen or CpG oligodeoxynucleotide (CpG-ODN) formulations promoted protective Th1 or Treg response.
- Recent preclinical data confirmed the efficacy of DNA vaccinations for the treatment of food or insect bite allergy and the need to formulate allergens with high doses of CpG-ODN to promote allergen-specific tolerogenic responses.
- Non-canonical CpG-ODN and methylated CpG motifs can display as well sustained immunosuppressive properties in *in-vitro* or animal studies.
- Efficacy of DNA- or CpG-based AIT identified in animal studies has yet to be translated to human trials.
- The potent immunogenicity and higher safety of mRNA vaccines over DNA vaccines together with the prodigious success of mRNA-based Covid-19 vaccines must prompt the design of mRNA-based AIT

clinical evaluation of nucleic acid vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-Cov2) to successfully tackle Covid-19 pandemic [4,5^{***},6^{***}].

Since the first evidence on antiallergy effects mediated by vaccinations with plasmid DNA encoding allergens [7,8], numerous studies confirmed the induction of allergen-specific Th1 and Treg responses following nucleic acid administrations as well as treatment based on unmethylated CpG oligodeoxynucleotides (CpG-ODN) [9,10,11^{***}].

This review will not only present the few recent progress in the development of DNA vaccines and CpG-ODN for AIT but will also highlight the need to re-examine the mRNA vaccine platform in the context of allergic diseases.

DNA VACCINATION IN THE CONTEXT OF ALLERGIC DISEASES: MODE OF ACTION AND PAST PRECLINICAL STUDIES

Following intradermal (ID) or intramuscular (IM) injection, plasmid DNA encoding allergen is taken up by structural cells or directly by dendritic cells (DCs) (Fig. 1) [12]. The allergen is then expressed and processed into peptide by DCs for MHCI/MHCII presentation to antigen-specific CD4⁺/CD8⁺ T cells. Allergen-specific B cells are activated as well to trigger immunoglobulin G (IgG) antibody production. The DNA-induced immune response was shown to be early dependent on the cytosolic DNA sensing by Cyclic GMP-AMP synthase and the subsequent activation of the STING-TBK1 pathway [13]. Of note,

endosomal TLR9, a pathogen recognition receptor recognizing unmethylated CpG motifs, was initially considered as the key innate sensor of transfected DNA. However, similar immune responses were measured when DNA vaccines were administered in wild-type and TLR9-deficient mice [14].

A large collection of preclinical studies investigated the prophylactic or therapeutic properties of plasmid DNA encoding major house dust mite, latex, bee venom, peanut, cockroach, pollen allergens in animal models of the corresponding allergic sensitizations. Overall, the main data, shown in key review papers, highlighted the capacity of DNA plasmid encoding allergens to prevent or revert the Th2-biased parameters of the allergic response (as airway eosinophilia, specific IgE and Th2 cytokines as IL-4, IL-5) through the development of allergen-specific Th1 responses characterized by interferon (IFN) γ release and induction of specific IgG antibodies [9,10,15]. Some studies evidenced as well the ability of DNA vaccines to activate Treg cells as well as IL-10/TGF- β but mainly when co-administrated with protein allergen [16–18] (Fig. 1).

As the blocking capacity of allergen-specific IgG is correlated with the efficacy of AIT [19], optimization of the allergen expression or secretion could maximize the antibody response induced by plasmid DNA. In that context, the inclusion of a leader peptide upstream to the allergen coding sequence as well as *in-vivo* electroporation (EP) for DNA uptake optimization drastically amplified the level of antibody response [20,21]. In contrast, overexpression/secretion of native allergen could cause side effects by cross-linking preexisting IgE on the surface of basophils and mast cells or induce *de novo* IgE sensitization (in the context of immunoprophylaxis for healthy individuals). However, the risk of IgE-mediated side effects by DNA immunization is minimal as the amount of translated antigen is at least 3–5 orders of magnitude lower than the amount of protein injected during the course of AIT [22]. Hypoallergenic DNA vaccines were generated based on leader-less plasmid DNA, the disruption of IgE binding epitopes or the fusion with targeting sequences (Ubiquitin, lysosomal-associated membrane protein-LAMP) leading to the rapid degradation of the expressed allergen [22,23]. Unfortunately, these modifications could drastically decrease the IgG blocking antibody response.

RECENT FINDINGS PUBLISHED IN THE LAST TWO YEARS

A search on PubMed with the keywords ‘DNA and vaccine’ pinpointed 3031 articles covering the period 2019 to May 2021. Surprisingly, only three

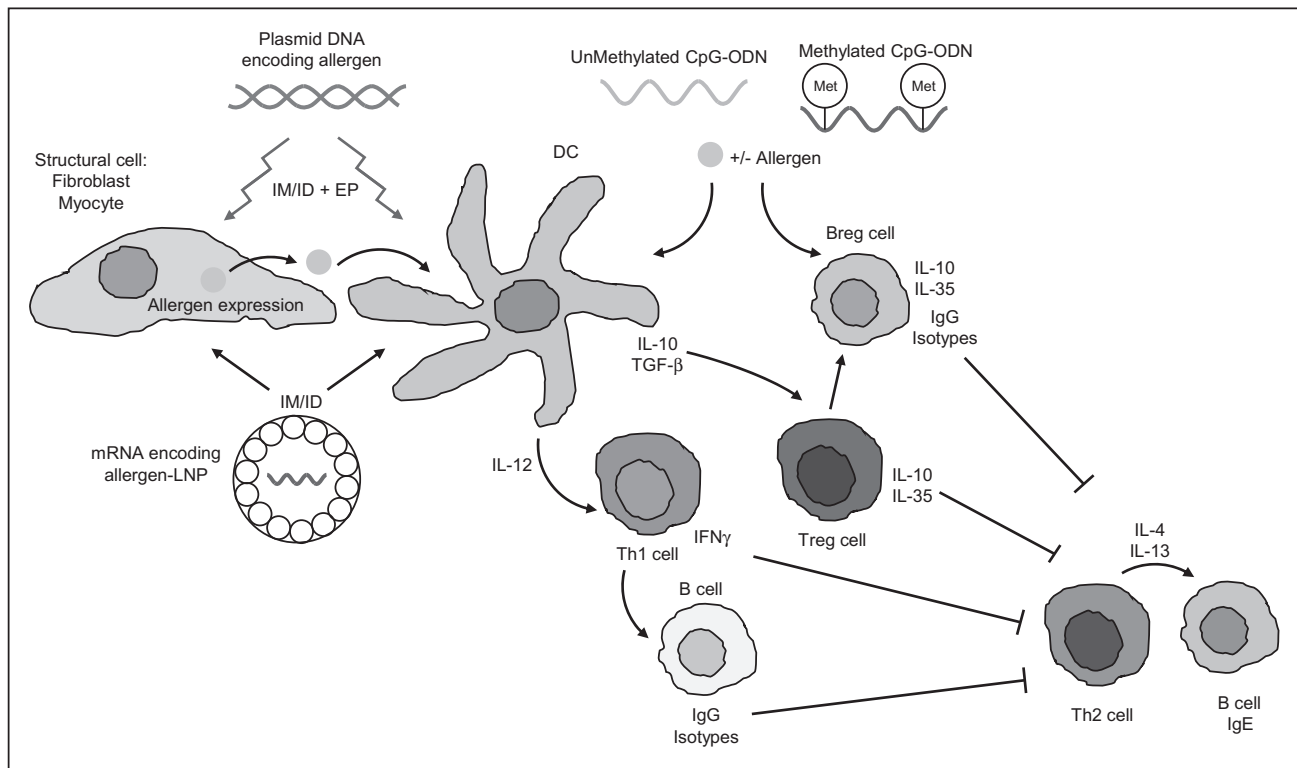


FIGURE 1. Immune responses developed by AIT based on DNA/mRNA encoding allergens or CpG-ODN formulations. Plasmid DNA encoding allergens are delivered by *in-vivo* EP whereas mRNA vaccines can be administrated by conventional IM/ID injections when appropriately formulated with LNPs or other carriers. The allergen directly expressed by DCs or by structural cells is subsequently processed by DCs for antigen presentation to Th cells. The cytokine environment (IL-12, IL-10, TGF- β) promote the Th1/Treg differentiation as well as the induction of potent allergen-specific blocking IgG antibodies by activated conventional B and Breg cells. Allergens adjuvanted with unmethylated CpG-ODN at low or high dose can skew DC differentiation toward a pro-Th1 or tolerogenic phenotype respectively. Unmethylated CpG-ODN can also mediate directly the induction of Breg cells. Moreover, methylated CpG-ODN could represent a new strategy to trigger immunosuppressive Treg cells. AIT, allergen-specific immunotherapy; CpG-ODN, CpG oligodeoxynucleotides; ID, intradermal; IM, intramuscular; LNPs, lipid nanoparticles.

research papers on DNA vaccinations were published in the field of allergic diseases during the same period. Several reasons likely explain the paucity of new publications: (1) the vaccine potential of plasmid DNA encoding most of major allergens from different allergenic sources were previously evaluated in preclinical studies; (2) the lack of robust clinical data on the effectiveness of DNA vaccine against allergic diseases (See later); (3) the level of the IgG blocking antibodies which could be suboptimal for effective AIT.

Hypoallergenic DNA vaccine against shellfish allergy

A DNA vaccine was constructed to express a hypoallergenic form of shrimp tropomyosin (Met e 1), one major cross-reactive shellfish allergen [24]. In a murine model of shrimp tropomyosin sensitivity, therapeutic ID vaccinations with such DNA vaccine reduced allergic symptoms together with the Met e

1-specific IgE levels, intestinal Th2 cytokines expression and inflammatory cell infiltration in the jejunum. Depletion or adoptive transfer of Treg cells confirmed the key role of CD4⁺CD25⁺Foxp3⁺ Treg cells in the vaccine-induced reversion of the allergic response.

DNA vaccine against biting insect allergy

The development of DNA vaccine for skin allergy was limited so far to the prevention of biting insect allergy and consisted in a plasmid DNA expressing For t 2, a major allergen from the *Forcipomyia taiwana* biting midge [25]. Recently, vaccinations of midge-sensitized mice with DNA-For t 2 were shown to alleviate allergen-induced bouts of scratching [26]. For t 2-specific IgE and IgG1/IgG2a ratio, skin eosinophil infiltration, ID expression of pruritus-specific IL-31, as well as IL-4 and IL-13 production by splenocytes were decreased and in a TLR9 independent manner.

Plasmid DNA co-expressing house dust mite allergen Der p 2 and A20

Sustained activation of the transcription factor nuclear factor- κ B (NF- κ B) is central to the pathogenesis of many inflammatory disorders, including allergic asthma [27]. NF- κ B signaling is negatively regulated by the ubiquitin-modifying enzyme TNFAIP3 (TNF α -induced protein 3, also known as A20). Intratracheal administration of adenovirus encoding A20 attenuated allergic airway inflammation in mice [28].

A plasmid DNA co-expressing Der p 2 and A20 and encapsulated into Poly(lactic-co-glycolic acid) nanoparticles was recently tested in a Der p 2-induced allergic rhinitis mouse model [29]. Although the present study did not show any data on Der p 2 or A20 expression in airway mucosa following intranasal DNA vaccinations, Der p 2-induced nasal allergic inflammation was ameliorated by the treatment. The Der p 2-specific IgE, IL-4 and IL-3 serum levels dropped while the Der p 2-specific IgG1, IgG2a and IFN γ expression levels in the serum and splenic CD4⁺CD25⁺Foxp3⁺ Treg population were significantly increased.

CLINICAL EVALUATION OF PLASMID DNA-BASED ALLERGEN-SPECIFIC IMMUNOTHERAPY

No clinical data on plasmid DNA-based AIT was published over the past three years. Clinical evaluations of plasmid DNA encoding allergens were exclusively performed for the treatment of cedar pollen or peanut allergy by Immunomic Therapeutics in 2017 and 2018, respectively. To improve the immunogenicity of these vaccine candidates, the genes encoding allergen were fused with sequence encoding LAMP to optimize the MHCII presentation.

IM immunizations with 2 or 4 mg of naked cedar pollen allergen Cry j 2-LAMP DNA were well tolerated in Japanese red cedar/mountain cedar-sensitized patients [30].

Four months after the fourth administration, conversion of cedar pollen-specific prick tests from positive to negative was observed in a majority of sensitized patients. However, the effects of DNA vaccination on Cry j 2-specific allergic response were unfortunately not evaluated. In a follow-up study, some participants from the previous trial received a 2 mg booster dose to examine the long-term safety. Conversion from skin positivity to skin negativity was also obtained for unrelated allergens, suggesting a possible bystander suppressive effect. Specific IgE titers remained unchanged, and no significant development of specific IgG was observed. The absence of changes in the allergen-specific humoral

response likely results from the very low exposure of patients to native allergen as the LAMP fusion partner diverts the synthesized protein into the MHCII/Lysosomal compartments. Phase I studies testing safety, tolerability and immunogenicity of ARA-LAMP-vax, a single multivalent peanut Ara h 1, h 2, h 3 LAMP DNA, in peanut-allergic patients were initiated (clinicaltrials.gov identifier NCT02851277), but the results were not disclosed yet.

CpG OLIGODEOXYNUCLEOTIDE AS ADJUVANT OR NON-SPECIFIC TREATMENT

Unmethylated CpG-ODN are immunostimulatory molecules that strongly activate endosomal TLR9. In humans, TLR9 is highly expressed in plasmacytoid DCs and B cells but is detected as well in alveolar macrophages and lung resident DCs. Low dose of CpG-ODN stimulates a TLR9-MyD88-NF κ B-AP-1-IRF7 pathway to mediate potent inflammatory responses characterized by the production of TNF α , IL-12, IL-1 β , IL-6, and IL-23 [31]. In contrast, CpG-ODN in high amount activates a TLR9-TRIF-non canonical NF κ B-IRF3 cascade to mediate the expression of type 1 IFN but also anti-inflammatory molecules such as IL-10, TGF- β and IDO [32]. Consequently, such induction of tolerogenic DCs by CpG treatment at high dose could represent a critical event for the development of Tregs and B regulatory (Breg) cells during AIT protocol (Fig. 1). Whether CpG-ODN needs to be used in combination with allergens or in allergen-free formulation remains to be more extensively investigated. It is reasonable to think that the tolerance induction with allergen-free CpG-ODN treatment would be short-lived and nonspecific due to the absence of immune memory.

As similarly observed with plasmid DNA, the clinical testing of CpG-ODN for the treatment of allergic diseases was not recently updated and remains limited to ragweed allergen Amb a 1 conjugated to type B CpG-ODN as well as QbG10, a Q β bacteriophage-based virus-like particle loaded with type A CpG-ODN [11^{***},33]. Despite promising Phase II efficacy data on the reduction of rhinitis symptoms with Amb a 1-CpG, subsequent Phase III failed to show positive outcome. Quite similar disappointing results were obtained with QbG10: significant positive effects on mild-to-moderate persistent but controlled allergic asthma in Phase II trial but no benefits of QbG10-based Phase IIb immunotherapy for the treatment of moderate to severe allergic asthma.

A fresh study confirmed the dose-dependence of tolerance induction elicited by formulation with CpG-ODN. In a preclinical model of cat allergy, co-administrations of endotoxin-free major cat

allergen Fel d 1 with high doses of B-type CpG-ODN reduced Th2 airway inflammation and bronchial hyper-responsiveness, specific IgE [34[■]]. This AIT up-regulated as well the levels of specific IgG1, IgA and IgG2a. The Fel d 1-specific immune tolerance was dependent on the engagement of plasmacytoid DCs (pDCs), Bregs, the induction of GATA3⁺Foxp3^{hi} double positive Treg population as well as early anti-inflammatory TNF/TNFR2 signaling cascade in natural killer and Breg cells. This report highlighted the importance of endotoxin-free allergen preparation for the tolerance development. Indeed, residual trace amount of LPS could activate TLR4 and then impair the TLR9 tolerogenic signaling [35].

A non-canonical unmethylated CpG site is characterized by the absence of consensus GACGTT and GTCGTT in the DNA sequence [11[■]]. Non-canonical CpG-ODN derived from the fungus *Cryptococcus neoformans* (ODN112) was previously shown to trigger in bone marrow-derived dendritic cells a strong TLR9-dependent IL-12p40 production [36]. A new study using ovalbumin (OVA)-induced asthma mouse model evidenced that ODN112, administered during the OVA sensitization (OVA + Alum), could prevent the induction of the specific allergic response [37[■]]. Methylated CpG motifs typically found in human genomic DNA are described as non-inflammatory molecules. Strikingly, a first report demonstrated that calf thymus DNA as well as synthetic methylated CpG-ODN display potent immunosuppressive properties through the induction of Foxp3⁺ Treg cells [38]. A similar methylated CpG site capable to activate the *in-vitro* Treg cell differentiation was identified in the genomic DNA from the probiotic *Bifidobacterium longum subsp. Infantis* [39[■]]. Animal studies are required to confirm the immunosuppressive capacity of methylated CpG-ODN for the treatment of allergic diseases

mRNA VACCINES AGAINST ALLERGY

The second nucleic vaccine platform tested in AIT consisted in messenger RNA (mRNA) encoding allergen [3]. Conventional mRNA is obtained from the *in-vitro* transcription (IVT) of genes encoding antigen and contain typically a 7mG cap at the 5' end and a polyA tail at 3' end. The coding sequence is flanked by untranslated region elements to increase the mRNA stability and thus the antigen expression.

Similarly to plasmid DNA vaccines, exogenous mRNA, once transfected into cells, activates a series of innate immune sensors as endosomal TLR3, TLR7, TLR8 and cytosolic MDA5 or RIG-1 [3,40]. However, these innate immune sensing of mRNA triggers potent production of type I interferons which inhibits antigen expression and lead to the

degradation of mRNAs. Taken together, these events elicit weak adaptive immune response. These issues can be circumvented by the purification of IVT mRNA and the introduction of modified nucleosides (as pseudouridine). Moreover, the formulation of modified mRNAs with lipid nanoparticles (LNPs) not only stabilize mRNA by the prevention of the enzymatic degradation but increase the *in-vivo* delivery into cells [40,41].

mRNA vaccine platform displays several advantages over the conventional plasmid DNA-based vaccine candidates, at least in terms of safety and efficacy (Table 1). mRNA cannot be integrated into genome once transfected into cells. Moreover, mRNA is degraded readily by cellular RNAses. Contrary to plasmid DNA encoding antigens, mRNAs do not contain additional genetic sequences. Consequently, the induction of antivector antibodies is absent. It must be pointed out that no study reported the induction of anti-mRNA antibodies so far.

Whereas plasmid DNA is typically produced in *Escherichia coli* following simple fermentation process, manufacturing mRNA by IVT is a chemical reaction free of animal or cellular components. Due to the poor immunogenicity of plasmid DNA in humans, sustained immune responses triggered by DNA vaccines were mostly obtained in clinical trials combining high doses (several mg of purified plasmid DNA) and *in-vivo* EP delivery technologies. Vaccination with mRNAs formulated with carrier molecules as LNPs does not need sophisticated instruments as electroporator for the vaccine delivery. Moreover, the strong immunogenicity of mRNA-LNPs-based vaccine drastically reduces the human dosing as judged by the administered doses of Pfizer/BioNTech or Moderna SARS-Cov2 mRNA vaccines (30 µg and 100 µg respectively, both given as a prime-boost regimen) [5[■],6[■]].

Whereas the mRNA vaccine technology made a tremendous breakthrough with the Covid-19 pandemic, only two old reports described the immunogenicity and the vaccine prophylactic potential of mRNA encoding allergens [42,43]. ID vaccinations of naïve mice with conventional mRNA encoding major allergens (a panel of 29 pollen or mite allergens were tested) triggered allergen-specific Th1 responses characterized by low specific IgG1/Ig2a ratio, the absence of specific IgE. The Th1-biased specific immune response was maintained following sensitizations with the corresponding allergen. The induction of Th1-biased T cell memory was evidenced by the detection of IFNγ in lymphoproliferative assays together with the down-regulation of Th2 cytokines IL-4, IL-5 or IL-13 [42]. Immunoprophylaxis with mRNA encoding Phl p 5 pollen allergen prevented allergen-induced lung

Table 1. Comparison of plasmid DNA and mRNA vaccine

	DNA vaccine	mRNA vaccine
Safety	Possible integration into the genome DNA longevity into cells Long-term antigen expression Additional genetic sequences from the plasmid backbone. Possible induction of anti-plasmid DNA antibodies	No integration into the genome mRNA degradation by normal cellular processes Short-term antigen expression No additional genetic sequences (only coding sequence)
Efficacy	In most clinical trials, <i>in-vivo</i> electroporation required for efficient delivery of naked plasmid DNA (specific device) High Dose (1–4mg/dose) to induce appropriate immunogenicity in humans	Efficient delivery by formulations with carrier molecules (as LNPs) Low Dose (mRNA Covid-19 vaccines: 30–100 µg/dose)
Manufacturing	Production in bacteria Complex purification (contamination with animal/cellular components)	<i>In-vitro</i> transcription Purification more simple (contamination by nucleosides and double-stranded RNA)

inflammation in Phl p 5-sensitized mice intranasally challenged with the pollen allergen. Strikingly, the protective memory responses triggered by mRNA-Phl p 5 was robust as judged by the persistence of the antiallergy effects even after repeated allergen challenge over a period of at least 6 months [43].

CONCLUSION AND PERSPECTIVE

Despite three decades of promising preclinical efficacy data, AIT based on plasmid DNA encoding allergens failed to be successfully translated so far into viable clinical applications. This lack of translation is largely explained by the poor immunogenicity of DNA vaccines. Of note, only two clinical trials evaluated so far DNA vaccination for the treatment of allergic diseases and the efficacy data were not disclosed or too preliminary to bring strong conclusions. Additional trials will be essential to evidence the antiallergy effects of DNA vaccination in cohorts of allergic patients. It seems that, up to now, pharmaceutical companies from the AIT market are not so prone to take this path. Moreover, collaborations with biotech companies developing *in-vivo* EP (as INOVIO, ICHOR) would be critical as most of the successful clinical trials on DNA vaccines against infectious diseases were performed through this mode of DNA administration. AIT with high doses of unmethylated or methylated CpG-ODN look advantageous in terms of GMP production and cost. Moreover, ODN-based mono-immunotherapy could be broadly applied against any allergen-specific response. Again, consistent efficacy clinical trials are needed to provide robust conclusions on the immunosuppressive functions of CpG-ODN.

The success story of mRNA vaccines against SARS-Cov2 will be probably decisive to reconsider mRNA-based AIT. Moreover, key advantages of synthetic modified mRNA-LNP platform over DNA vaccines in terms of safety, efficacy or manufacturing are highly attractive to investigate mRNA vaccines for the treatment or the prevention of allergic diseases.

Finally, active immunizations with nucleic-acid (DNA/mRNA) encoding humanized monoclonal antibodies targeting Th2 cytokines or Th2 cytokines receptors could represent an emerging strategy to replace costly and repeated administrations of anti-Th2 monoclonal antibodies at high doses [44^a,45^a,46].

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Conflicts of interest

There are no conflicts of interest.

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