

Development and preclinical evaluation of a vaccine targeting IL-4 and IL-13 for the treatment of allergic asthma

1 | DESCRIPTION OF INVENTION AND POTENTIAL APPLICATIONS

Asthma is the most prevalent chronic respiratory disease, affecting more than 300 million people worldwide.¹ Although asthma is a heterogeneous disease that presents with various phenotypes, around 50% of the patients suffer from type 2 inflammation, characterized by elevated levels of IgE, type 2 cytokines such as IL-4 and IL-13 and eosinophilia.¹ In recent years, several recombinant monoclonal antibodies (mAbs) targeting IgE, type 2 cytokines or their receptors have been approved for the treatment of asthma.² However, the use of these mAbs is limited by high cost and the need to perform injections over years to lifelong. Therefore, there is a clear need to improve current strategies in order to reach long-term effects.

Our patent covers the use of therapeutic vaccines, called IL-4/IL-13 kinoids, that induce neutralizing antibodies against IL-4 and IL-13 for the treatment of asthma and other allergic diseases.³ IL-4/IL-13 kinoids are produced by coupling recombinant IL-4 and IL-13 with a carrier protein such as diphtheria 'cross-reactive material 197' (CRM₁₉₇, a non-toxic mutant of diphtheria toxin used in a number of approved conjugate vaccines⁴), using a thiol-maleimide conjugation. To this end, recombinant IL-4 and IL-13 are first modified with N-γ-maleimidobutyl-oxysuccinimide ester (sGMBS, a maleimide-containing agent reacting with primary amines), and sulfhydryl moieties are introduced on the carrier protein using SATA (N-succinimidyl-S-acetylthioacetate). Functionalized proteins are then conjugated to generate the IL-4/IL-13 kinoid vaccines (Figure 1A). Upon injection with an adjuvant, such vaccine will induce a polyclonal antibody response against the carrier protein, but also against IL-4 or IL-13 (Figure 1B). It is thus expected to induce long-term beneficial effects in pathologies linked to aberrant IL-4 and/or IL-13 production, such as asthma and other chronic allergies.

2 | PATH TAKEN TO THE INVENTION

Prior work conducted in 2007 by the French biotech Neovacs using a mouse IL-4-keyhole limpet hemocyanin (KLH) conjugate vaccine demonstrated that active immunization against mouse IL-4 can partially reduce IgE levels and eosinophilia, with only minor effects on

mucus production in a mouse model of asthma.⁵ On the other hand, a recombinant mouse IL-13 peptide-based virus-like particle vaccine reduced mucus production, but not IgE levels.⁶ These results highlighted the fact that, although IL-4 and IL-13 share one receptor subunit (IL-4Rα), the two cytokines also play some key non-redundant functions in asthma. This was further confirmed in humans, with the superior efficacy of a mAb targeting both IL-4 and IL-13 pathways (ie dupilumab) over neutralizing mAbs against IL-4 or IL-13 alone.⁷

In 2017, we thus decided to develop kinoid vaccines targeting both IL-4 and IL-13 with the rationale that such vaccines would reduce key asthma features and thereby induce long-term protection. This project was set as a collaboration between the French National Institute of Health and Medical Research (Inserm) and the Institut Pasteur and Neovacs. To demonstrate the efficacy of IL-4/IL-13 kinoids, we first produced mouse surrogates of the vaccines. We found that mice vaccinated with mouse IL-4 and IL-13 kinoids in emulsion with a squalene oil-in-water emulsion adjuvant (SWE)⁸ developed a neutralizing antibody response against these cytokines, which was still observed in more than 60% of the mice one year after primary immunization. Antibodies generated upon vaccination were in a large majority of IgG1 isotype.

We then assessed the efficacy of IL-4/IL-13 kinoids in a clinically relevant mouse model of chronic asthma induced by the intranasal administration of house dust mite (HDM) extract. We observed a more pronounced reduction of IgE levels and lung mast cell numbers with the anti-IL-4 vaccine than with the anti-IL-13 vaccine. By contrast, airway hyperresponsiveness (AHR) to inhaled methacholine and mucus overproductions were reduced to a greater extent with the anti-IL-13 vaccine. Dual vaccination against IL-4 and IL-13 nearly abrogated all these key asthma features, confirming the clear benefit of targeting both cytokines. Importantly, the dual vaccination strategy was efficient using both prophylactic and therapeutic vaccination protocols.

We thus decided to move on and produce an anti-human IL-4/IL-13 vaccine. However, such human vaccine would be highly immunogenic in mice due to low interspecies similarities of IL-4 and IL-13. Thus, we used a novel mouse strain expressing human IL-4, human IL-13 and human IL-4Rα in place of the respective mouse proteins. Vaccination of these humanized mice with the human IL-4/IL-13 vaccine elicited an efficient neutralizing response against both human

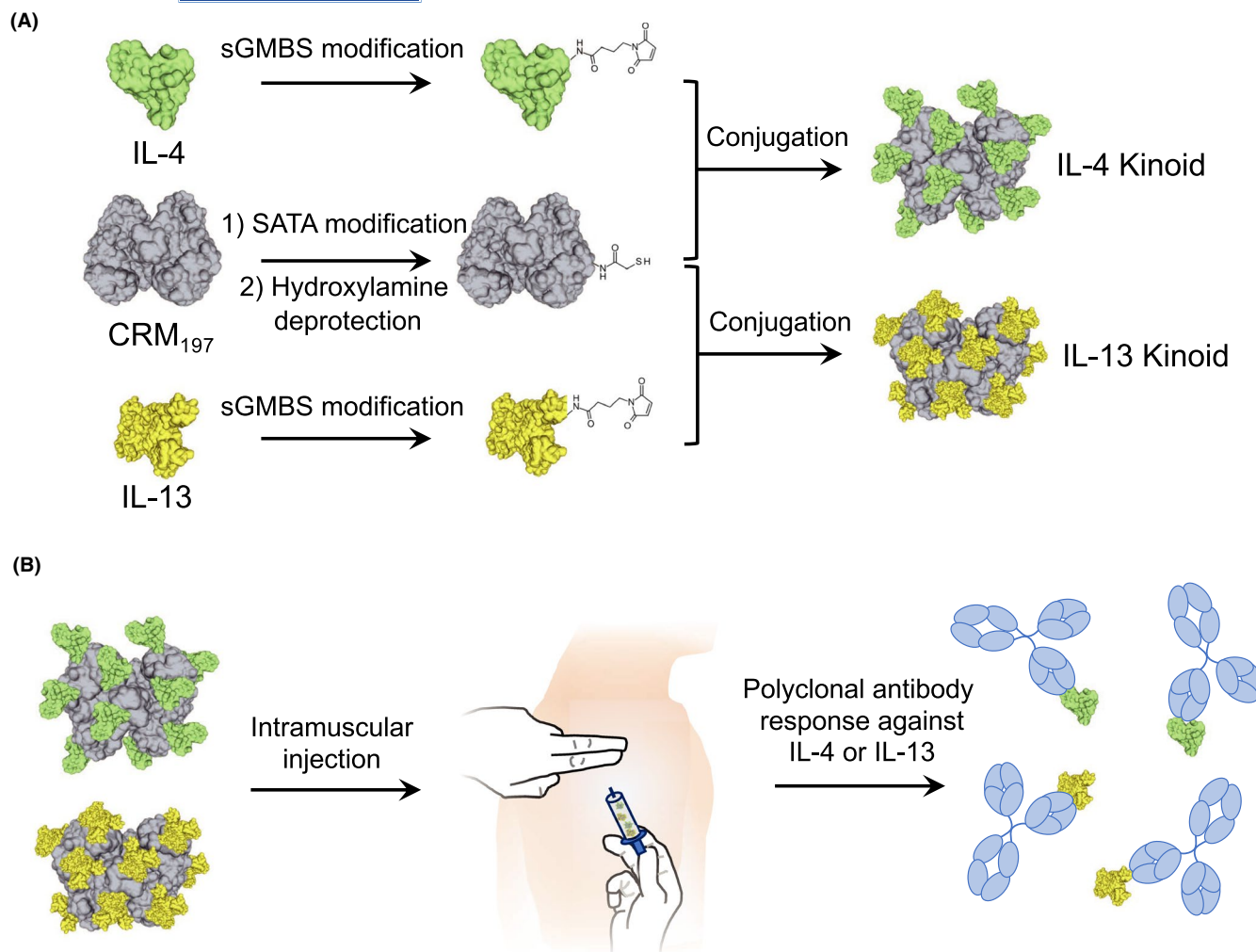


FIGURE 1 IL-4/IL-13 kinoids for the treatment of asthma and other allergies driven by IL-4 and IL-13. (A) Overview of the synthesis of IL-4 and IL-13 kinoid vaccines using a thiol-maleimide conjugation. (B) Immunization with IL-4/IL-13 kinoid vaccines together with an adjuvant will induce a polyclonal antibody response against IL-4 and IL-13, which should prevent their function in vivo and induce long-term protection against asthma and other chronic allergies

IL-4 and IL-13. The human vaccine also induced a marked reduction in circulating IgE, and IgE on the surface of blood basophils and skin mast cells, confirming its efficacy at desensitizing these cells.

Overall, we have demonstrated a promising effect of our patented vaccine for the treatment of allergic asthma. Unlike therapeutic mAbs, this effect is long-lasting, representing a clear benefit for the treatment of chronic diseases. These findings were published recently⁹ and, now, need to be confirmed in clinical trials. Interestingly, a kinoid targeting interferon alpha (IFN- α) was recently tested in a phase 2b study in 185 adults with active systemic lupus erythematosus. This kinoid induced a neutralizing response against IFN- α in 91% of treated patients, with a good safety profile.¹⁰ Although the strength and duration of the antibody response induced by the human IL-4/IL-13 kinoids will need to be determined in clinical trials, they may be similar to the responses observed with the IFN- α kinoids. Besides asthma, we also envision that this IL-4/IL-13 vaccine could have a beneficial effect in other allergic disorders, including atopic dermatitis, chronic

rhinosinusitis with nasal polyps, food allergy or eosinophilic esophagitis, for which IL-4 and IL-13 also play a key role.

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CONFLICT OF INTEREST

Dr. Conde reports grants from French ANRT ('Association Nationale Recherche Technologie') CIFRE doctoral fellowship, personal fees from Neovacs stocks, during the conduct of the study; In addition, Dr. Conde has a patent WO2019228674 (A1) - 2019-12-04 'Immunogenic product comprising IL-4 and/or IL-13 for treating disorders associated with aberrant IL-4 and/or IL-13 expression or

activity' pending. Dr. Bruhns reports grants from French National Research Agency ANR-18-CE18-0023 grant 'AllergyVACS', grants from Neovacs s.a., during the conduct of the study; personal fees from Consultancy fee from Regeneron Pharmaceuticals, personal fees from Consultancy fee from Neovacs, outside the submitted work; In addition, Dr. Bruhns has a patent WO2019228674 (A1) – 2019-12-04 'Immunogenic product comprising IL-4 and/or IL-13 for treating disorders associated with aberrant IL-4 and/or IL-13 expression or activity' pending. Dr. Serra reports personal fees from BSA from Neovacs, personal fees from Bonus from Neovacs, other from Meeting paid by Neovacs, during the conduct of the study; In addition, Dr. Serra has a patent WO2019228674 (A1) – 2019-12-04 'Immunogenic product comprising IL-4 and/or IL-13 for treating disorders associated with aberrant IL-4 and/or IL-13 expression or activity' pending and V. Serra is the CSO/COO of Neovacs. Dr. Reber reports grants from French National Research Agency ANR-18-CE18-0023 grant 'AllergyVACS', grants from Neovacs s.a., during the conduct of the study; personal fees from Consultancy fee from Novartis, personal fees from Consultancy fee from Neovacs, outside the submitted work; In addition, Dr. Reber has a patent WO2019228674 (A1) – 2019-12-04 'Immunogenic product comprising IL-4 and/or IL-13 for treating disorders associated with aberrant IL-4 and/or IL-13 expression or activity' pending.

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