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Tolamba™

TLR9 Receptor Agonist
Treatment of Allergic Rhinitis

AIC
Amb a 1-ISS-1018
Amb a 1-ISS-DNA

Amb a 1 ragweed pollen allergen linked to the ISS-DNA (immunomodulatory sequence of DNA) ISS-1018

EN: 280256

Abstract

Allergic diseases are a growing health concern in developed countries. Early studies suggested that DNA-based immunotherapy might be effective in the treatment of these diseases. Consisting of the principal ragweed pollen allergen, Amb a 1, and Dynavax's immunostimulatory sequences of single-stranded DNA (ISS-DNA), the Amb a 1-ISS conjugate (AIC, Tolamba™) is designed to target the underlying cause of seasonal allergic rhinitis caused by ragweed. *In vitro* and *in vivo* studies indicate that both antibody recognition and induction are highly sensitive to the number of linkages of ISS to the protein, with low ISS:protein ratios (2.0-3.0) inducing overall the highest antibody response. Preclinical studies indicate that Tolamba™ is effective in enhancing the Th1 immune response and suppressing the Th2 immune response. Tolamba™ proved safe and well tolerated in the treatment of ragweed allergy patients. Early clinical studies showed that patients treated with Tolamba™ experienced statistically significant clinical benefit. However, a 30-center, placebo-controlled study (DARTT) involving 738 ragweed allergic patients was recently discontinued because only minimal ragweed-specific disease was observed in the overall study population and no meaningful efficacy could therefore be measured.

Background

Allergic diseases are a growing health concern in industrialized countries. Despite advances in our understanding of the pathogenesis of allergic diseases and improvement in the treatment of these diseases, the prevalence of allergic diseases has dramatically increased during the last two decades. It has been suggested that a decreased Th1-biased immune response and an increased Th2-biased immune response due to improved public health practices, referred to as the hygiene theory, may account for this trend (1-4).

Introduced in the early twentieth century, allergen-specific immunotherapy has proved effective and has been widely practiced since its introduction. However, current immunotherapy is associated with limitations, including the need for frequent dosing, the need for relatively large doses and a risk of clinically significant allergic reactions (2, 5-7).

A growing number of studies suggest that DNA-based immunotherapeutics may be effective both in the treatment of allergic diseases and in the reversal of allergen-specific hypersensitivity by inducing a Th1-biased immune response and preventing Th2-biased responses. Recent studies indicate that conjugates of allergen and immunostimulatory oligodeoxynucleotides (ISS-ODN) may be more effective and safer than conventional immunotherapy (1, 2, 5, 8).

Dynavax's Amb a 1-ISS conjugate (AIC, Tolamba™) is a DNA-based immunotherapeutic candidate designed to target the underlying cause of seasonal allergic rhinitis caused by ragweed. The agent consists of the principal ragweed pollen allergen, Amb a 1, conjugated to Dynavax's 1018 immunostimulatory phosphorothioate oligodeoxynucleotide (sequence: 5´-TGACTGTGAACG-TTCGAGATGA-3´). Linking of Amb a 1 to an immunostimulatory sequence (ISS) ensures that both the allergen and the ISS are presented simultaneously to the same cells to produce a highly specific and potent Th1-biased immune response (5, 9).

Preclinical Pharmacology

In vitro and *in vivo* studies demonstrated that Tolamba™ was associated with enhanced Th1-biased immune responses and reduced allergenicity compared to Amb a 1 alone or mixed with free ISS. In sensitized mice, the conjugate significantly suppressed ragweed-

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induced pulmonary hyperresponsiveness. Tolamba™ also markedly increased anti-Amb a 1 IgG_{2a} titers, with a much smaller increase in IgG_1 titers, and it significantly increased interferon gamma (IFN-γ) levels in Amb a 1-stimulated splenocytes (10).

Further studies in mice examined the effect of the degree of conjugation on antibody responses. A mixture of Amb a 1 and ISS provided a very low antibody response, which was mainly IgG_1. In contrast, a conjugate with a high ISS:protein ratio (5.3-6.4) gave a similar IgG_{2a} response and a significantly lower IgG_1 response, a conjugate with an intermediate ISS:protein ratio (3.8-4.7) gave a significantly enhanced IgG_{2a} response, and a conjugate with a low ISS:protein ratio (2.0-3.0) gave the highest overall antibody titers, indicating a strong inverse relationship between antibody response and the degree of conjugation. Immunization with Amb a 1 produced a Th2 T-cell response associated with high levels of IL-5 and low levels of IFN-γ. Tolamba™, however, provided a Th1 response and enhanced IFN-γ levels while decreasing IL-5 levels; the IFN-γ response was independent of the degree of conjugation (11).

Mice immunized with Amb a 1 or a non-ISS-Amb a 1 conjugate developed an IgG_1 antibody response, but no IgG_{2a} antibodies, whereas animals immunized with Tolamba™ mounted an antigen-specific IgG_{2a} response; a mixture of Amb a 1 and ISS-ODN was less effective. Spleen cells from Tolamba™-immunized mice showed significantly enhanced IFN-γ secretion and no IL-5 secretion. Other experiments involved priming of animals with Tolamba™ followed by boosting with Amb a 1 in alum. Boosting resulted in increased IgG_{2a} titers and it also enhanced the IgG_1 response; however, animals primed with Tolamba™ followed by boosting with Amb a 1 in alum showed a significantly reduced IgE response compared to animals primed with Amb a 1, Amb a 1 in alum or a non-ISS-Amb a 1 conjugate. Again, splenocytes from the Tolamba™-primed animals showed the highest IFN-γ levels and the lowest IL-5 levels. In animals primed with Amb a 1 in alum followed by 3 injections of Tolamba™ or Amb a 1 (controls), increased IgG_{2a} titers were only seen in the Tolamba™-immunized mice; IgG_1 titers increased in both groups but IgE titers increased only in the controls. In animals subsequently challenged with Amb a 1, IgG_{2a} titers again increased in the Tolamba™-immunized animals and only a small increase in IgE titers was seen in these animals. As above, splenocytes from Tolamba™-immunized animals displayed the highest levels of IFN-γ and the lowest levels of IL-5 (9).

The effects of Tolamba™ were also investigated in other animal species. In rabbits, injections of 10 μg of Tolamba™ induced a mean anti-Amb a 1 IgG antibody titer of 106,000 U/ml, whereas injections of 10 μg Amb a 1 alone or Amb a 1 mixed with 50 or 500 μg free ISS did not increase anti-Amb a 1 IgG titers above baseline. Similar results were found in studies carried out in cynomolgus monkeys; the animals that received two doses of 50 μg Tolamba™ developed high anti-Amb a 1 IgG responses, whereas injection of Amb a 1 alone did not induce a detectable antibody response; no IgE antibodies were detected (9, 12).

Studies in peripheral blood mononuclear cells (PBMCs) obtained from ragweed allergy patients indicated that the direct linkage of ISS to Amb a 1 dramatically enhances the ability to modify antibody and T-cell responses to the antigen. Amb a 1 incubation was associated with a Th2-biased response, with high levels of IL-4, IL-5 and IL-13 and low levels of IFN-γ. Treatment of the cells with the conjugate (high, intermediate and low ISS:protein ratios), however, resulted in a Th1 response, with significant reductions in IL-4, IL-5 and IL-13 secretion and a significant increase in IFN-γ secretion. Tolamba™ retained its effects on cytokine production even in the presence of Amb a 1. Furthermore, compared to Amb a 1 stimulation, Tolamba™ was associated with significant reductions in IL-1α, IL-4, IL-5 and IL-8 mRNA and enhancement of IL-2, IL-15 and IFN-γ mRNA. Tolamba™ was also more potent than Amb a 1 in stimulating antigen-specific T-cell proliferation (11, 13, 14). Similar results were obtained in nasal explants from ragweed-sensitive subjects (15).

Much higher concentrations of Tolamba™ were required to reduce histamine release to a similar extent as Amb a 1 *in vitro* in basophils from ragweed allergy patients, indicating less allergenicity (9, 11, 12, 16).

Safety

Findings from clinical trials in subjects with ragweed allergy demonstrated that Tolamba™ given by weekly s.c. injections in doses up to 30 μg was safe and well tolerated, with no serious treatment-related adverse events or laboratory abnormalities (17-19, 21-27).

Clinical Studies

A randomized, placebo-controlled phase I study conducted in mid-winter, when there was no exposure to ragweed or other pollens, examined the safety and efficacy of Tolamba™. A total of 19 subjects with ragweed allergy were randomized to receive 6 s.c. injections (0.06, 0.3, 1.2, 3.0, 6.0 and 12.0 μg) of Tolamba™ or placebo at weekly intervals. Subjects in the Tolamba™ group exhibited marked reductions in Th2 immunity (IL-5, CCL17 and CCL22) and increases in Th1 immunity (IFN-γ, CXCL9 and CXCL10) at 2 and 16 weeks after the last injection (18, 19). Another randomized study in 19 ragweed allergy patients indicated that Tolamba™ immunization prior to the ragweed season was associated with an increase in IL-10 production in antigen-stimulated PBMCs throughout the ragweed season (20).

In another study, 57 ragweed-sensitive patients with allergic rhinitis were randomized to receive Tolamba™ (n=28) or placebo (n=29). Six subcutaneous injections of Tolamba™ (0.06, 0.3, 1.2, 3.0, 6.0 and 12.0 μg) or placebo were administered at weekly intervals before the 2001 ragweed season. In terms of symptom scores or medication use, no differences were observed between

Tolamba™- and placebo-treated groups during the first ragweed season. However, during the second ragweed season, Tolamba™ decreased chest and nasal symptoms. Upon rechallenge following the first ragweed season, Tolamba™-treated subjects showed a significant reduction in eosinophils and IL-4 mRNA-positive cells, as well as an increase in IFN-γ-positive cells (21, 22).

The efficacy and immunogenicity of Tolamba™ were further evaluated in a randomized, double-blind, placebo-controlled phase II trial. Twenty-five adult patients with ragweed-induced seasonal allergic rhinitis were randomized to receive 6 weekly injections of Tolamba™ (0.06, 0.3, 1.2, 3.0, 6.0 and 12.0 μg) or placebo before the first ragweed season (2001) and were monitored during the next two ragweed seasons. During the first ragweed season, the Tolamba™-treated group reported better peak-season rhinitis scores, peak-season daily nasal symptom diary scores and mid-season overall quality-of-life scores than the placebo group, although the primary endpoint of change in albumin levels in nasal lavage fluid after nasal provocation was not met. Tolamba™ treatment transiently increased Amb a 1-specific IgG antibody levels but suppressed Amb a 1-specific IgE antibody levels. A reduction in the number of IL-4-positive basophils in Tolamba™-treated patients correlated with lower rhinitis visual analogue scale (VAS) scores. In the subsequent ragweed season, better peak-season rhinitis VAS scores and peak-season daily nasal symptom diary scores were again reported in the Tolamba™ group; Tolamba™ also suppressed the seasonal specific IgE antibody response (23).

Another double-blind, placebo-controlled phase II trial assessed the efficacy of Tolamba™ over two ragweed seasons. A total of 462 adults with ragweed allergy were randomized to receive 6 weekly doses (1.2, 3, 6, 15, 21 and 30 μg) of Tolamba™ (n=310) or histamine-containing placebo (n=152) prior to season 1 (2004); prior to season 2 (2005), patients in the Tolamba™ group were again randomized to receive either two weekly doses (3 and 30 μg) of Tolamba™ or placebo and patients in the placebo group continued to receive two weekly doses of placebo. Data indicated that Tolamba™ immunization was associated with a long-lasting improvement in hay fever symptoms, particularly when given only prior to the first ragweed season. Relative improvement compared to placebo in the total nasal symptom score (TNSS) in the Tolamba™ group during the first season was 15%, whereas relative improvement in the Tolamba™-placebo group during the second season was 28.5% and relative improvement in the Tolamba™-Tolamba™ group during the second season was 13.5% (24-26).

In 2006, Dynavax initiated a 2-year multicenter study (DARTT) involving 738 subjects with ragweed allergy who were randomized to one of three arms: the previously tested doses of 1.2, 3, 6, 15, 21 and 30 μg Tolamba™, higher doses of 3, 9, 30, 30, 30 and 30 μg or placebo. However, analysis of 1-year interim data indicated that no meaningful ragweed-specific allergic disease was observed in the study population, which made it impossible to measure the therapeutic effect of the therapy. In all three arms of the study, the change from baseline TNSS was not clinically significant and substantially lower than that observed in the previous trials. The trial was therefore discontinued and Dynavax is currently reassessing its clinical, regulatory and commercial strategy for the program (27, 28).

Source

Dynavax Technologies Corp. (US).

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