

DRUG PROFILE

Oralair Birch, a recombinant major birch pollen allergen tablet for sublingual immunotherapy of allergic rhinitis caused by birch pollen

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Oralair Birch is a dissolving tablet being developed for sublingual immunotherapy (SLIT) of allergic rhinitis caused by birch pollen allergy. Oralair Birch is being developed by Stallergenes SA and Canadian licensee Paladin Labs Inc. Oralair Birch is a recombinant protein that is synthesized from the DNA coding region of Bet v 1a, the major birch pollen allergen. During preclinical characterization, Oralair Birch had comparable structural and biological properties to the natural Bet v 1 allergen. However, Oralair Birch was more homologous than the natural Bet v 1 allergen, making a greater level of quality control possible. The administration of SLIT in tablet formulation provides a more uniform dose compared with liquid drops and better local application, which might enhance local uptake into dendritic cells of the sublingual submucosa and efficacy. Using skin prick testing, the performance of recombinant Bet v 1 was comparable to the natural Bet v 1 allergen. The results of a dose-finding phase IIb/III clinical trial of Oralair Birch were positive, with the primary endpoint met by all three tested doses. A confirmatory phase III trial was planned for 2011. Oralair Birch is a very promising treatment option for patients with birch pollen allergic rhinitis.

Introduction

During the last five decades, the global incidence and prevalence of allergic rhinitis has increased, with an estimated 500 million individuals affected in 2008 [1079175]. In 2001, data collated by the European Community Respiratory Health Survey suggested that approximately 35% of all European and Australasian adults have had allergic rhinitis [1085628]. The International Study of Asthma and Allergies in Childhood I (ISAAC I) and a follow-up study, ISAAC III, were the first worldwide efforts to construct a global map of the prevalence and prevalence trends of asthma, rhinitis and eczema in children [1079175]. From ISAAC I data, published in 1997, the global prevalence of rhinoconjunctivitis symptoms in a 6- to 7-year-old and in a 13- to 14-year-old population varied between 0.8 and 14.9% and 1.4 and 39.7%, respectively [1079175]. Allergic rhinitis is a risk factor for asthma, and ISAAC III data published in 2006 reported a prevalence of severe asthma symptoms in the aforementioned two age groups of between 0 and 20.3% and 0.1 and 16%, respectively [1079175], [1085633]. Furthermore, children with asthma aged between 3 and 11 years were likely to be monosensitized to an allergen and were at increasing risk of becoming polysensitized [1086292], [1086293]. In 2009, regional analyses of prevalence trends in the Asia-Pacific rim documented an increased prevalence of asthma and rhinitis symptoms in lower income countries [1085635]. This was reflected by an increasing cost burden that rose from US \$6.1 billion in 2000 to US \$11.2 billion in 2005 for the treatment of allergic rhinitis in the US [995008].

Therapeutic Oralair Birch

Originator Stallergenes SA

Licensee Paladin Labs Inc

Status Phase III Clinical

Indication Allergic rhinitis

Actions Allergen, Anti-inflammatory, Therapeutic vaccine

Technologies Biological therapeutic, Buccal formulation, Protein recombinant, Subcutaneous formulation, Systemic, Tablet formulation

Allergy (also called atopy) is an altered reaction of the immune system that occurs in genetically predisposed persons in response to indoor and outdoor allergens (ie, a generally proteinaceous and non-pathogenic substance that would not normally elicit immune reactivity) [1079175]. The immune response is aberrant because a protective T-helper cell type 1 (Th1) and antibody (IgM and later IgG) response is replaced with an allergic Th2 cell and antibody (IgE) response [1086115]. Cellular events are triggered that include antigen presentation to T-cells by APCs, such as dendritic cells, which result in T-cell-mediated activation of B-cells. The B-cells are stimulated to produce allergen-specific IgE antibodies, which bind to the surface of mast cells and basophils via a high-affinity receptor for the Fc region of IgE and causes allergic sensitization in the individual

[1086115]. A skin prick/puncture test (SPT) is the preferred technique for the diagnosis of IgE-mediated allergic sensitization [1085648]. Subsequent contact with the allergen leads to direct degranulation of the mast cells, which release histamine and other allergy mediators. If this reaction takes place in the nasal airways and paranasal sinuses, it gives rise to the classical symptoms of nasal allergy and sinusitis, including runny nose, pruritus, sneezing and nasal obstruction. This reaction may also occur in the eyes causing symptoms of allergic conjunctivitis, such as itchy/watery eyes. In later stages of the disease, inflammatory cells, such as eosinophils, lymphocytes and neutrophils, arrive at the locus of the allergic inflammation and can cause irreversible tissue damage [1079175], [1085649].

The main groups of allergens can be found in house dust mite feces, pollens, cat dander, molds and cockroaches (the bodies, saliva and feces contain allergens that are of particular importance in crowded urban areas) [1085655]. Seasonal allergic rhinitis (also called hay fever and pollinosis) is caused by pollens from trees, grasses and weeds. Tree pollen from the birch tree (*Betula verrucosa*) has become a problem in some European cities, with up to 20% of the population being sensitized [1085655]. Although the birch pollen season normally lasts for only 4 to 8 weeks, with a peak usually between late March and mid May, the season can be intense with pollen counts up to 9000 grains/m³ [1079279]. Birch pollen contains multiple proteins, but only a small fraction of them have an allergenic potential and can be called allergens. These allergens are sequentially classified (eg, Bet v 1, followed by Bet v 2 and Bet v 3, and so forth). In the DNA coding of each of these allergens, mutations may lead to several natural variants known as isoforms. As such, for Bet v 1, there exist Bet v 1a, Bet v 1b and so on. If an allergen elicits the production of allergen-specific IgE in more than 50% of the people allergic to that substance, it is defined as a major allergen; the major birch pollen allergen is Bet v 1 [1079175]. Analysis of different allergenic proteins has highlighted that some plant proteins are highly evolutionarily conserved, even between phylogenetically distinct plants [1085656]. The Bet v 2 birch allergen (also called profilin) is a highly conserved and ubiquitous allergenic protein with a similar molecular appearance to other proteins found in pollens from trees (ie, trees in the birch family, such as silver birch, alder and hazel) and also fresh fruits and vegetables [1085656]. Patients who are allergic to Bet v 2 are normally allergic to several pollens or plant-derived substances because of allergenic cross-reactivity [1085657].

Medical treatments available for allergic rhinitis can be subdivided into rescue and controller medications [1079175], [1085649]. Rescue medications are used to rapidly relieve the acute symptoms of allergy; rescue medications include antihistamines, which block the effects caused by histamine, and decongestants, which

counteract the vasodilatation that is one of the causes of nasal obstruction. Controller medications reduce the allergic inflammatory condition of the tissues. Controller medications include leukotriene inhibitors, which block the action of the leukotrienes (the principal chemo-attractant for eosinophils, which prime the individual for further allergic responses), chromones, which stabilize mast cells and also have mild anti-inflammatory action, and corticosteroids, which are potent inhibitors of inflammatory cell action [1079175], [1085649]. However, these treatment options only control allergy symptoms and do not cure allergic disease.

Allergen-specific immunotherapy (IT) is a medical treatment that alters the way the immune system reacts towards the clinical tolerance of a specific allergen [1085661], [1085664]. This is accomplished by repeated vaccination of a patient by subcutaneous injection (SCIT) or by sublingual drops or tablets (SLIT) with a gradually augmented dose of allergen (build-up phase), followed by a maintained dose of allergen for at least 3 years (maintenance phase) [1085664]. During the first weeks of IT, an augmented regulatory T-cell (also called suppressor T-cells, these cells suppress immune system activation) response can be detected with a concomitant increase in IL-10 and TGF β that reduces the Th2 cell activity (ie, immune-regulation), to make place for a more balanced Th1 to Th2 cell response. After approximately 12 months of IT, an increase in IFN γ and specific IgG4-blocking antibodies is elicited, and the level of Th2 cell-derived cytokines is reduced [1079195], [1079199]. After a complete course of IT, symptoms of rhinoconjunctivitis and the use of rescue and controller medications are reduced for several years [1079180]. Moreover, the tendency for allergic patients to demonstrate an increasing number of new allergic sensitizations is strongly reduced, as demonstrated by house dust mite IT in children with allergic rhinitis and mild asthma and by IT with relevant allergens in patients with allergic rhinitis and/or intermittent asthma [1079182], [1079183]. Furthermore, in children with seasonal allergic conjunctivitis at risk of developing asthma, IT with standardized allergen extracts of grass and/or birch reduced the number of children who developed asthma after treatment termination [822691], [1079194].

SCIT was first applied in 1911 by Noon [1085685] and Freeman [1085686] as a prophylactic inoculation with natural allergens against seasonal allergic rhinitis. The mainstay of SCIT treatment is still the administration of natural allergen extracts with or without an adjuvant. However, because the source material has natural variation, the precise allergen composition of a natural allergen extract also varies. The allergenic variation of a natural allergen extract is dependent on multiple phenomena, including the time of harvest, plant life-cycle, climate, contaminating vegetation and immune-modulating substances from culture (ie, endotoxins). As a consequence, SCIT with natural birch pollen

extracts has been reported to induce new allergic sensitizations [1079283]. Apart from the variations found in allergen extracts, the two main drawbacks of SCIT are the invasive administration route and the rate of potentially fatal systemic adverse reactions [1079264], [1079267], [1079268], [1079269].

Mucosal-associated lymphoid tissue drives immune responses towards tolerance, so the natural immunogenic response in the oral cavity is to tolerate allergens. Moreover, the allergen delivered by SLIT onto the surface of the sublingual mucosa will only be absorbed slowly; therefore, it is harder to elicit an adverse reaction with SLIT [1079270], [1079271]. As a consequence, SLIT is generally better accepted and leads to less side effects than SCIT, although the latter is still considered somewhat more effective [1088131]. Clinical trials conducted with SLIT during the last 20 years have demonstrated that efficacy of SLIT requires much higher doses of allergen than with SCIT [1079272], [1079273]. In a clinical trial that assessed the efficacy of SLIT (Staloral) versus SCIT (Phostal), the cumulative dose of allergen received by SLIT-treated patients was 219-fold greater than the dose received by SCIT-treated patients [1082482].

IT with one major allergen or a recombinant protein overcomes the problems of natural variations inherent in natural allergen extracts. Recombinant proteins have been developed that represent major allergens from trees and grasses including from timothy grass pollen (*Phleum pratense*; Phl p 5), hazel (*Corylus avellana*; Cor a I) and birch pollen (*B verrucosa*; Bet v 1) [1085702], [1085704], [1085705]. Recombinant Bet v 1 (rBet v 1) has demonstrated similar IgE-binding capacity compared with natural Bet v 1 (nBet v 1) in immunoblot and ELISA analyses [1079299]. Moreover, the proliferation of allergen-specific T-cell clones and histamine release from basophils (both derived from patients with birch pollen allergy) induced *in vitro* by rBet v 1 was equivalent to nBet v 1 [1079299].

Hypoallergenic variants of rBet v 1 (defined by low IgE-binding capacity) for SCIT are being evaluated in clinical trials. Results were recently presented from a double-blind, placebo-controlled, randomized clinical trial that assessed a hypoallergenic rBet v 1 trimer and a mixture of two rBet v 1 fragments (Allergopharma Joachim Ganzer KG), in patients (n = 124) with birch allergy [996067]. Although there was a trend for improvement in the combined rhinitis symptom-medication score, no statistically significant differences were demonstrated in comparison with placebo [996067]. More interesting were the results presented from an ongoing phase III, randomized, double-blind, placebo-controlled, multicenter clinical trial (ClinicalTrials.gov identifier: NCT00554983) assessing rBet v 1-FV, an aluminum hydroxide-adsorbed hypoallergenic rBet v 1 derivative (Allergopharma), in patients (n = 228) with birch pollen allergy. The rBet v 1-FV demonstrated reduced IgE reactivity and

decreased capacity to activate basophils compared with nBet v 1 and rBet v 1 *in vitro* [1079334]. Patients received 8 weekly injections in the build-up phase and a maintenance dose of 80 µg administered at 6-week intervals. After 18 months of maintenance treatment, the active group had a 32% reduction in the combined symptom-medication score, with no serious adverse events. Systemic and local reactions were more frequent in the active group. The major advantage of this treatment variant was the fast build-up phase compared with a conventional IT build-up phase of 20 to 30 injections [996067].

Stallergenes SA and Canadian licensee Paladin Labs Inc are developing Oralair Birch, an rBet v 1a in a SLIT tablet formulation for the treatment of allergic rhinitis caused by birch pollen [756466], [1042410]. At the time of publication, positive results from a phase IIb/III trial had been reported [1042410]. The development of Oralair Birch will be reviewed here.

Synthesis

An *Escherichia coli* strain BL21 (DE3) protein expression system was used to express the rBet v 1a protein [653290]. A cDNA (corresponding to amino acids 1 to 483 of the wild-type Bet v 1a protein) was amplified and subcloned using restriction enzyme recognition sites (*NdeI/EcoRI*) into the multiple cloning region of a pMW172 expression vector (pMW172-Bet v 1a), which contained an ampicillin resistance gene. *E coli* transformed with the pMW172-Bet v 1a vector were grown in a 5-l bioreactor and then stored frozen in vials. YPN culture media (200 ml) containing yeast extract (1%), vegetable peptone (2%), sodium chloride (NaCl; 2%) and ampicillin (100 µg/ml) was inoculated with a thawed vial of *E coli* and incubated overnight at 37°C. This culture was used to inoculate a 5-l bioreactor of YPNKG culture media containing yeast extract (1.5%), vegetable peptone (0.66%), NaCl (1.5%) and potassium dihydrogen phosphate (0.3%) that had been supplemented with glucose (2%) in the absence of ampicillin. The *E coli* culture was fermented in the bioreactor at 37°C with stirring (300 to 1000 rpm) and aeration (20% oxygen at 750 ml/min) at pH 7 (maintained by the addition of 5 M sodium hydroxide) for 4 h to obtain log phase bacterial growth. The pMW172 expression vector was controlled by a bacteriophage T7 promoter; thus, protein expression from *E coli* was induced by the addition of isopropyl-β-D-thiogalactoside (50 ml of a 0.1 M solution). T7 RNA polymerase was provided by a prophage λ under the conditional control of a *lacUV5* promoter. After 3 h of continued fermentation, the bioreactor culture was supplemented with glucose (100 ml of a 50% solution) and was harvested after 4 h. The *E coli* were collected by centrifugation (3000 rpm for 30 min) and stored as cell pellets at -20°C. Thawed (4°C) *E coli* pellets were pooled, resuspended in 1 l of lysis buffer containing tris(hydroxymethyl)aminomethane (50 nM), NaCl (50 nM) and ethylenediaminetetraacetic acid (1 mM) at pH 8 and

homogenized (10,000 rpm for 15 min). Homogenized *E. coli* were then disrupted at 1200 bar and the cell lysate produced was treated with nuclease (10 IU/ml) at room temperature for 1 h and then centrifuged (8000 rpm for 60 min at 4°C). The rBet v 1a protein was precipitated from the supernatant by overnight incubation at 4°C in the presence of salts (0.76 M NaCl and 0.65 M sodium phosphate monobasic). After further centrifugation (18,000 rpm for 20 min) and filtration, the rBet v 1a protein was affinity purified via hydrophobic interaction chromatography with a phenyl-sepharose column. The rBet v 1a protein then underwent diafiltration via tangential flow filtration (Proflux M12 system/Pellicon 2 ultrafiltration module) at 4°C against tris-HCl buffer at pH 8. This was followed by a further filtration step prior to diethylaminoethyl-sepharose anion-exchange chromatography at room temperature. The rBet v 1a protein was then concentrated and diafiltered via tangential flow filtration against NaCl (155 mM) buffer to obtain a final concentration of 1 mg/ml. The purified rBet v 1a protein (Oralair Birch) was sterilized with a final filtration (Mini Kleenpak DFL filter; 0.22 µm) step and stored at -80°C [653290]. The concentration of endotoxin in the aluminum hydroxide-absorbed vaccine formulation was < 0.05 endotoxin units/ml [1043690].

Preclinical development

The proteins rBet v 1 and nBet v 1 (derived from birch pollen extract) were purified to clinical grade and characterized in parallel; rBet v 1 and nBet v 1 had low endotoxin content (< 0.02 and 0.03 endotoxin units/µg, respectively) [653290]. In an analysis with SDS-polyacrylamide gel electrophoresis, rBet v 1 resolved mainly as an 18-kDa protein and nBet v 1 resolved as an 18- to 20-kDa mixture of proteins that were also evident in a birch pollen extract comparator; rBet v 1 had greater estimated homogeneity (ie, was > 95% free from contaminating proteins) compared with nBet v 1 (75 to 80%). The rBet v 1, nBet v 1 and birch pollen extract exhibited equivalent (immunoblot) reactivity against IgE obtained from patients with birch pollen allergy (pooled sera) and Bet v 1-specific murine mAbs, indicating the correct, natural folding of the recombinant protein. An analysis using circular dichroism indicated that rBet v 1 and nBet v 1 contained similar α -helical secondary structures, but rBet v 1 had a greater β -strand secondary structure content compared with nBet v 1. In an investigation of protein thermodynamics, rBet v 1 was demonstrated to be folded in a configuration that was similar, but more compact and stable, compared with nBet v 1. The rBet v 1 and nBet v 1 proteins induced equivalent activation of basophils (determined by histamine release from granulocytes obtained from patients with birch pollen allergy) and proliferation of rBet v 1-specific T-cell lines (generated from rBet v 1-stimulated PBMCs obtained from patients with birch pollen allergy) [653290].

Batches of an rBet v 1.0101 protein isoform produced in accordance with GMP were characterized using physiochemical and ELISA methods under the aegis of

the Biological Standardization Program of the European Directorate for the Quality of Medicines & Healthcare (EDQM) [1043571], [1043573], [1043679], [1043687]. The quality of multiple rBet v 1.0101 protein batches was consistent (ie, in appearance, clarity, color, purity, rBet v 1 protein content, pH, DNA content, host cell protein content and endotoxin content). Mass spectrometry-based analyses indicated that rBet v 1.0101 was 159 amino acids long and had 96.2% sequence identity, and a theoretical average molecular weight (17,439.6 Da) that coincided with a major Bet v 1a pollen allergen entry in the Swiss-Prot protein sequence database (primary accession number: P15494) [1043571], [1043573], [1043679], [1043687]. Characterization using orthogonal separation techniques demonstrated that the rBet v 1.0101 protein was > 99.9% homogeneous. In a dynamic light scattering analysis, the rBet v 1.0101 protein contained monomeric molecules that were stable in tests performed up to 3 months after storage at 4°C. An analysis using circular dichroism indicated that the α -helical and β -strand secondary structures of rBet v 1.0101 were typical of other clinical-grade rBet v 1 preparations (see the reference for details [653290]). The rBet v 1.0101 protein exhibited allergenic potency (ie, contained relevant IgG epitopes and dose-dependently activated basophils) that was equivalent to an nBet v 1 protein in functional assays. This characterization demonstrates that GMP-grade rBet v 1.0101 was highly similar to nBet v 1 and it was selected by the EDQM as an international reference material for product characterization and stability studies required for the clinical application of allergenic drug candidates, such as Oralair Birch [1043573].

Hexylamine (HEA), phenylpropylamine (PPA) and 4-mercapto-ethyl-pyridine (MEP) mixed-mode chromatographic (HyperCel) sorbents were investigated to optimize the purification of rBet v 1 protein during manufacturing [1043575]. Analyses established that rBet v 1 could be captured under physiological-like conditions (ie, low conductivity and neutral pH) from crude rBet v 1-expressing *E. coli* lysate (100 µl in a 96-well plate format at 3 mg/ml total protein content) with HEA and PPA sorbents, but only partially with the MEP sorbent. An investigation of desorption of rBet v 1 from HEA and PPA sorbents by charge repulsion during increasing acidity with sodium acetate buffer (50 mM at pH 5, 4.5, 4 and 3) demonstrated that desorption was optimal at pH 4 and pH 4 to 3, respectively. The capture of rBet v 1 was further investigated with prepacked resolute chromatography columns (HyperCel) containing MEP, HEA, and PPA sorbents; rBet v 1 was captured with HEA and PPA prepacked columns, but only partially with the MEP prepacked column, confirming previous results. The rBet v 1 was recovered from HEA and PPA prepacked columns with sodium acetate buffer (50 mM) and homogeneity was estimated with size-exclusion chromatography; rBet v 1 recovered from HEA prepacked columns was 85.2 (pH 5) and 58.7% (pH 4.5) homogeneous, and from PPA prepacked

columns was 85.7% (pH 4) homogeneous, compared with 9.3% homogeneity for the rBet v 1-expressing *E coli* lysate. Total rBet v 1 protein recovered from HEA prepacked columns was 78.3% compared with 74.1% from PPA prepacked columns. An rBet v 1-contaminating protein, which retained an N-terminal translation initiator methionine residue (Met-rBet v 1) was not retained by HEA and PPA prepacked columns at the same pH as rBet v 1; Met-rBet v 1 was recovered from HEA and PPA prepacked columns with sodium acetate buffer (50 mM) at pH 4 to 3 and 3 compared with pH 5 to 4.5 and 4 for rBet v 1, respectively [1043575].

Toxicity

There were no available toxicity data for Oralair Birch or rBet v 1 variants at the time of publication.

Metabolism and pharmacokinetics

At the time of publication, there were no available metabolism and pharmacokinetics data for Oralair Birch; however, there were metabolism and pharmacokinetics data available for SLIT with other major allergens. For example, a ^{123}I -labeled allergen from *Parietaria judaica* (Par j 1) was not detected systemically until the allergen was swallowed, when it was rapidly degraded and absorbed in the gastrointestinal tract. Most of the tracer was lost from the oral cavity after 2 h, but 2% of local radioactivity remained associated with the oral mucosa for up to 20 h after administration, even after rinsing the mouth [1079317], [1079319].

Clinical development

Prior to the development of the SLIT Oralair Birch, clinical trials with the recombinant molecule were conducted using an SPT (allergen placed on the forearm prior to a skin prick) to demonstrate immunogenicity and using a subcutaneous route of administration.

Skin prick test

A clinical trial assessed rBet v 1 and rBet v 2 (30 μl of 3 or 10 $\mu\text{g/ml}$ rBet) allergens in patients ($n = 58$) with rhinitis or respiratory complaints who had a positive SPT in response to a licensed birch pollen extract (Alustal) or to a mixed birch pollen extract (25% each of alder, birch, hornbeam and hazelnut pollen). Healthy control volunteers ($n = 7$) had no prior clinical history of allergy, and atopic control volunteers ($n = 8$) were monosensitized to either cat or mite allergens [1079301]. Patients in the birch-sensitized cohort had a positive SPT reaction to rBet v 1 (92%), rBet v 2 (19.6%) or to both allergens (13.7%). In the patients who reacted to the 3- or 10- $\mu\text{g/ml}$ rBet v 1 dose, a significant correlation was observed between the wheal diameters induced after an SPT with licensed birch pollen extract and with rBet v 1 ($p < 0.05$ for both doses). Significant correlation was also noted between the erythema induced after an SPT with birch pollen extract and with the 3- $\mu\text{g/ml}$ rBet v 1 dose ($p = 0.01$). Using immunoblotting, IgE antibodies against rBet v 1

or 2 were detected in the serum of 83 and 60% patients who had an rBet v 1- or 2-positive skin reaction, respectively. Healthy control volunteers did not react to rBet v 1 or 2 and serum IgE antibodies against rBet v 1 or 2 were undetectable. Patients ($n = 37$) with birch-sensitized rhinitis reacted to rBet v 1 and those who were monosensitized to birch did not react to rBet v 2; a reaction to rBet v 2 was associated with a reaction to other pollens, such as grass [1079301].

A clinical trial assessed rBet v 1, nBet v 1 and birch pollen extract in patients ($n = 25$) with birch pollen allergy; a control group ($n = 5$) were non-allergic to birch pollen [1085194]. After SPT with the allergens in evaluable birch pollen-allergic patients, the level of serum IgE antibodies, basophil activation (histamine release and basophil activation marker CD203c expression by allergen-stimulated PBMC *in vitro*) and skin responses were associated, but the magnitude of the each response was not well correlated. The lack of correlation was not explained by the levels of serum IgG or IgA antibodies against nBet v 1, or by IFN γ or IL-10 production by allergen-stimulated PBMCs *in vitro*; in fact, allergen-stimulated PBMCs from birch pollen-allergic patients secreted more IL-10 than allergen-stimulated PBMCs from the non-allergic control group. Allergen-stimulated PBMCs from patients with birch pollen allergy also secreted more IL-5 than allergen-stimulated PBMCs from the non-allergic control group [1085194].

Subcutaneous immunotherapy

A phase II, double-blind, placebo-controlled, parallel-group-assignment, multicenter clinical trial (NCT00410930; DV08.01) assessed rBet v 1, nBet v 1 and a licensed birch pollen extract (Alustal) (0 to 15 μg of each allergen, SCIT administered weekly for 12 weeks, then 15 μg SCIT administered monthly for 2 years) in patients ($n = 147$) with a ≥ 2 -year history of birch pollen-related rhinoconjunctivitis, a positive SPT response (wheal > 3 mm diameter) to Bet v 1 and/or mild asthma (global initiative for asthma grade 1 to 2) [1043570], [1043592], [1043690]. Each Bet v 1 allergen was prepared as an aluminum hydroxide-absorbed vaccine; the placebo group was administered with histamine dihydrochloride to cause skin irritation for the purpose of blinding. Treatment with all three allergens significantly reduced the occurrence of rhinoconjunctivitis symptoms ($p = 0.0002$, 0.0024 and 0.0006 for rBet v 1, birch pollen extract and nBet v 1, respectively), skin sensitivity ($p < 0.0001$ for all three forms) and the use of rescue and controller medications ($p = 0.0011$, 0.0063 and 0.0025 for rBet v 1, birch pollen extract and nBet v 1, respectively) compared with the placebo group. Furthermore, after 1 to 2 years of treatment, the levels of Bet v 1-specific IgG and IgG subclasses (IgG1, IgG2 and IgG4) were increased in all treated groups compared with placebo. In contrast, after 1 to 2 years of treatment, no changes in Bet v 1-specific IgE, IgA or IgG3 were observed [1043690].

Sublingual immunotherapy

At the time of publication, two phase I, randomized, double-blind, placebo-controlled, parallel-group-assignment, single-center clinical trials to assess single-ascending-dose and multiple-dose Oralair Birch in patients with birch pollen-induced allergic rhinitis for ≥ 2 years were listed on the NIH clinical trials registry. The trials were undertaken outside of the birch pollen season when patients were symptom free. Trial NCT00889460 (VO58.07 DK) assessed Oralair Birch (12.5 to 100 μg SLIT, qd for 2 weeks except weekends) in ~ 55 patients and trial NCT00396149 (VO49.06 DK) assessed Oralair Birch (50 to 300 μg SLIT, qd for 2 weeks except weekends) in ~ 60 patients. A combined analysis of 112 patients from both trials has been reported [1016643], [1043685]. In the treated patient cohort ($n = 84$), birch and/or Bet v 1-specific IgE and IgG4 serum antibody titers increased by 82 and 36% from baseline, respectively [1016643].

A phase IIb/III, randomized, double-blind, placebo-controlled, parallel-group-assignment, multicenter clinical trial (NCT00901914; VO59.08) assessing Oralair Birch (12.5, 25 and 50 μg SLIT, qd for approximately 5.5 months) was ongoing in patients ($n = 483$) with symptomatic birch pollen-related allergic rhinoconjunctivitis. Treatment with Oralair Birch significantly reduced the average adjusted symptom score measured during the birch pollen season (~ 1 month) by approximately 25% over the whole birch pollen season and 30% at peak season compared with placebo ($p = 0.002$ to 0.03) [1042410].

At the time of publication, a phase III clinical trial of Oralair Birch was planned for 2011 [1042410].

Side effects and contraindications

In the phase II SCIT clinical trial that assessed rBet v 1, licensed birch pollen extract and nBet v 1 in 147 patients with birch pollen-related rhinoconjunctivitis, 3 patients in the birch pollen extract-treated group developed sensitization to Bet v 2 (measured by specific anti-Bet v 2 IgE); this was accompanied by an increased Bet v 2-specific IgG1 level compared with the rBet v 1-treated group [1043690]. No new sensitizations to Bet v 2 were developed in the rBet v 1- or nBet v 1-treated groups. Patients who were evaluable for safety ($n = 134$), reported a similar number of local and systemic reactions after treatment (all groups). Two patients in each of the three treatment groups experienced a treatment-unrelated serious adverse event; the events were appendicitis, endometriosis, hemorrhoids, hysterectomy, stroke and headache. A treatment-related serious adverse event, systemic anaphylactic reaction (urticaria with drop in blood pressure), was experienced by one patient in the nBet v 1-treatment group that required dose reduction, but did not cause the patient to discontinue the trial. Local reactions that occurred immediately after treatment, such as itching, pain, warmth or swelling, were reported equally by all groups. In contrast, local reactions that occurred later were most

often reported by the treated groups. The most frequent treatment-emergent adverse events reported ($\geq 10\%$) included rhinitis ($n = 13, 12, 16$ and 20 in the placebo, nBet v 1, rBet v 1 and birch pollen groups, respectively), sneezing ($n = 4, 1, 4$ and 4 , respectively), coughing ($n = 1, 0, 1$ and 5 , respectively), conjunctivitis ($n = 7, 12, 6$ and 17 , respectively), eye pruritis ($n = 14, 2, 4$ and 4 , respectively) and local reactions ($n = 7, 10, 48$ and 15 , respectively). A local reaction that was caused by histamine was reported by seven patients in the placebo group [1043690].

In the two phase I clinical trials assessing SLIT Oralair Birch in patients with birch pollen-related allergic rhinitis, data from evaluable patients demonstrated that Oralair Birch was well tolerated up to doses of 50 μg independent of dose titration [1016643], [1043685]. In contrast, incremental doses of $> 100 \mu\text{g}$ or single doses of 150 μg were not well tolerated. In total, 402 treatment-emergent adverse events (TEAEs) were reported. Of the patients ($n = 84$) who received Oralair Birch, 81% reported TEAEs that were SLIT-related. Of the reported TEAEs, 59% were mild and 3% were severe. In total, 16 patients withdrew from the trial. Of the patients receiving Oralair Birch, 10 withdrew from the trial because of TEAEs; the patients who withdrew had received the 50- ($n = 3$), 150- ($n = 5$), 200- ($n = 1$) or 300- μg ($n = 1$) doses of Oralair Birch [1016643]. There were no serious adverse events or deaths reported. The adverse events reported occurred within a short time scale at the site of tablet administration and resolved quickly. Commonly reported TEAEs and adverse events were rhinitis, throat irritation, pharyngeal edema, ear pruritis, oral mucosal blistering, swollen tongue, oropharyngeal pruritis and oral hypoesthesia [1016643], [1043685].

In the phase IIb/III clinical trial in patients with symptomatic birch pollen-related allergic rhinoconjunctivitis, Oralair Birch was well tolerated, particularly in the 12.5- and 25- μg dose groups [1042410].

Patent summary

Stallergenes' European patent EP-00278877 claims a grass pollen allergen that presumably covers Stallergenes' Oralair Grasses product. However, the claims in EP-00278877 are general and do not specify an allergen, so it may have relevance to Oralair Birch. Although the patent expired in January 2008, a supplementary protection certificate was granted in 2009 in France, and is due to expire in January 2013. This protection was presumably sought in response to the European launch of a grass allergen SLIT tablet product (Grazax) in 2006 and 2007.

Additional protection for Oralair Birch may be conferred from Biomay AG's Bet v 1 patent portfolio, which Stallergenes licensed in May 2003 [652916]. Biomay's Bet v 1 patent portfolio includes: WO-2009024208, WO-2007134350, WO-09410194 and WO-09202621.

WO-2009024208 claims specific mutant hypoallergenic forms of Bet v 1a for use in vaccines. WO-2007134350 claims the use of an antibody for passive immunization for the prevention and/or treatment of allergic reactions caused by an exposure to a birch pollen allergen, where the antibody binds to a Bet v 1 fragment, as well as a vaccine comprising the antibody. WO-09410194 claims recombinant forms of major allergens of certain tree species, including Bet v 1, and their uses for diagnosing tree pollen allergy and/or provoking tolerance to the allergen. WO-09202621 claims recombinant DNA molecules coding for allergens, including Bet v 1, as well as the allergens. Other applications from Biomay that may have some relevance to Oralair Birch include: (i) WO-03089009 claiming microparticles comprising a bead essentially consisting of a cross-linked carbohydrate (eg, aragose) and an allergen, in which Bet v 1a is briefly mentioned (however Timothy grass pollen is specifically claimed); (ii) WO-2005000274 claiming novel microspheres containing unspecified allergens, or DNA encoding allergens, characterized by high affinity to intestinal or nasal epithelial cells and their use for treating allergies; and (iii) WO-2007140505 claiming novel vaccine carrier fusion proteins.

Similarly, Stallergenes has made additional applications with some relevance to Oralair Birch, including: (i) WO-00192546 claiming a method for producing recombinant allergens in plant cells; (ii) WO-2006123230 claiming immunogenic compositions comprising an allergen, including Bet v 1, and a bacterium-derived adjuvant and/or a corticosteroid plus a vitamin D3-based adjuvant; (iii) WO-2008023233 claiming a synthetic particulate vector carrying an allergen, including Bet v1, for preventing or treating allergy, autoimmune disease or graft rejection; and (iv) claiming a mucoadhesive composition of chitosan particles loaded with an allergen, including Bet v 1.

Current opinion

Oralair Birch, a SLIT tablet containing the major birch pollen allergen rBet v 1a, in development by Stallergenes, appears to be a promising treatment option for allergic rhinitis in patients with birch pollen allergy. Allergen-specific IT provides a long-term solution for the treatment of allergic diseases as an alternative to traditional medical treatment that only controls allergic symptoms and inflammation. IT is a preferred treatment modality for patients with prolonged allergic disease because a complete, 3-year IT course has the potential to reduce allergic symptoms and the use of rescue and controller medication for years after treatment termination.

IT is mainly administered as SCIT and SLIT, with SLIT having a better safety profile. SLIT in liquid drops is mostly administered by means of droppers or in monodose vials [1079175]. The patients are instructed to maintain the liquid below the tongue for at least 2 min, which makes compliance with this method difficult,

especially in children. Sublingual tablets can be held under the tongue more easily and also ensure accurate dosing. Moreover, the local concentration of the allergen will probably be greater with a rapidly dissolving tablet than with liquid drops, which could improve allergen uptake by dendritic cells in the oral submucosa.

Oralair Birch is a recombinant allergenic protein and not a natural allergen extract. The advantages of a recombinant allergenic protein over a natural allergen extract are multifold and include the ability to mass-produce a vaccine to GMP standards with a high level of purity and homology. These advantages likely mean that a recombinant major allergen may be used to treat allergic patients with a smaller risk of causing the development of new allergic sensitizations than a natural allergen extract.

These theoretical advantages of Oralair Birch over natural pollen extracts are reflected clinically in the positive results from the phase IIb/III clinical trial, which demonstrated an improvement in the average adjusted symptom score of 25% over the whole birch pollen season compared with placebo. This compares well with the results presented from two clinical trials with SLIT grass allergen tablets; the average rhinoconjunctivitis scores reported by patients in active treatment groups demonstrated reduced rhinoconjunctivitis symptoms (16 to 27%) and reduced use of rescue and controller medication (28 to 46%) during the grass pollen season compared with placebo [1079274], [1079276]. The improvements in the Oralair Birch trial were slightly lower than those reported from a recombinant grass pollen SCIT clinical trial; the combined symptom-medication score was reduced by 39% compared with placebo [996067].

Birch pollen allergy is the most important tree allergy in Europe and, although the birch pollen season is short, the peak pollen count is usually high, which causes severe symptomatic allergic rhinitis and seasonal asthma. Worldwide, birch pollen allergy is probably among the most common allergies. Allergenic cross-reactivity between members of the alder tree family is high and cross-reactivity also occurs with certain food allergens (eg, hazelnut, apple and apricot). Therefore, the target population for Oralair Birch is extensive and could expand should the indications for Oralair Birch become broader in the future. If Oralair Birch is approved, it will probably be easier to gain approval for other recombinant allergen SLIT tablets such as Stallergenes' Oralair Mites, a phase III recombinant vaccine in sublingual tablet formulation that targets three mite allergens for the treatment of dust mite allergy [1004234].

A final indicator of the market potential of Oralair Birch is that Stallergenes has demonstrated continued growth since 2000 [994434], [1082700]. Stallergenes is a company with a proactive attitude that has earned credibility in the allergy community. Stallergenes has

expanded its market over the last couple of years, first in southern Europe and it now has commercial partners and distributors in various parts of the world. Moreover, the Stalair program for development and marketing of a range of sublingual tablets makes its future route clear: to meet the needs of allergic individuals worldwide [994425]. Stallergenes has established a partnership with Paladin for marketing and distribution in Canada and is openly looking for new partners in other territories [994434].

At the time of publication, there was only a press release disclosing the positive results from the phase IIb/III double-blind, placebo-controlled clinical trial of Oralair Birch. The global design of the trial and the number of patients enrolled appears adequate enough to demonstrate the primary efficacy endpoint for analysis of the results of the reduction in average adjusted symptom score, and the data disclosed do support a positive outcome. Analysis of these data should make it possible to select the optimum dose and to define the conditions for a confirmatory phase III trial that will be conducted with a view to filing a centralized MAA with the EMEA [1042410]. However, no

firm conclusions can be drawn until an in-depth analysis of the trial has been published.

In the EU, the regulation on the registration of IT products has become progressively more stringent [1088623]. As a consequence, registration and commercialization of new IT products has become a long and slow process. Reports from Stallergenes in 2003 mentioned 2007 as a target year for the registration of Oralair Birch [652931], but, at the time of publication, phase III trials had yet to be initiated. However, the registration of the Oralair Grasses tablet was rapid once positive phase III trial results had been published [994434], [1079276].

A potential to reengineer a recombinant protein vaccine, such as Oralair Birch, to improve the efficacy-safety balance has yet to be exploited. Indeed, there is potential to make an hypoallergenic variant of Oralair Birch or to combine Oralair Birch with immunomodulatory substances. Strong research in the areas of hypoallergenic recombinant birch pollen extracts [1079334], shuffled genes [1079337] and fusion proteins [1079339] are being pursued.

Deals

Biomay AG

In May 2003, Stallergenes and Biomay entered into a patent license agreement covering the gene encoding the Bet v 1a allergen used in Oralair Birch [652916].

Paladin Labs Inc

In January 2007, Stallergenes granted Paladin development, promotion and distribution rights in Canada to its Oralair therapies for grass, dust mite and birch allergies. Paladin would be responsible for clinical trials, registration, marketing, sales and distribution of the products in that territory. Financial terms were not disclosed [756466].

Solvay SA

In March 2009, Stallergenes entered into an exclusive collaborative agreement with Solvay SA, for the promotion and distribution of Stallergenes' products in Russia and the Commonwealth of Independent States. Financial terms were not disclosed [994425].

Development status

Developer	Country	Status	Indication	Date	Reference
Stallergenes SA	Europe	Phase III	Allergic rhinitis	31-OCT-08	1043041
Paladin Labs Inc	Canada	Discovery	Allergic rhinitis	09-JAN-07	756466

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