Accepted: 10 May 2018

DOI: 10.1111/all.13478

ORIGINAL ARTICLE

Experimental Allergy and Immunology

WILEY Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

Randomized controlled trials define shape of dose response for Pollinex Quattro Birch allergoid immunotherapy

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Funding information
This clinical study was entirely funded by Allergy Therapeutics (UK) plc.

Abstract

Background: The Birch Allergoid, Tyrosine Adsorbate, Monophosphoryl Lipid A (POLLINEX® Quattro Plus 1.0 ml Birch 100%) is an effective, well-tolerated short course subcutaneous immunotherapy. We performed 2 phase II studies to determine its optimal cumulative dose.

Methods: The studies were conducted in Germany, Austria and Poland (EudraCT numbers: 2012-004336-28 PQBirch203 and 2015-000984-15 PQBirch204) using a wide range of cumulative doses. In both studies, subjects were administered 6 therapy injections weekly outside the pollen season. Conjunctival Provocation Tests were performed at screening, baseline and 3-4 weeks after completing treatment, to quantify the reduction in Total Symptom Scores (as the primary endpoint) with each cumulative dose. Multiple Comparison Procedure and Modeling analysis was used to test for the dose response, shape of the curve and estimation of the median effective dose (ED_{50}), a measure of potency.

Results: Statistically significant dose responses ($P < .01$ & $.001$) were seen, respectively. The highest cumulative dose in PQBirch204 (27 300 standardized units [SU]) approached a plateau. Potency of the PQBirch was demonstrated by an ED_{50} 2723 SU, just over half the current dose. Prevalence of treatment-emergent adverse events was similar for active doses, most being short-lived and mild. Compliance was over 85% in all groups.

Conclusion: Increasing the cumulative dose of PQBirch 5.5-fold from 5100 to 27 300 SU achieved an absolute point difference from placebo of 1.91, a relative difference 32.3% and an increase in efficacy of 50%, without compromising safety. The cumulative dose response was confirmed to be curvilinear in shape.

KEYWORDS

allergen immunotherapy, birch pollen allergoid, cumulative dose, dose response curve

1 | INTRODUCTION

Efficacy of allergen immunotherapy (AIT) is related to the cumulative dose (CD) of the allergen or allergoid[1-8] for symptom control and immunological changes. No consensus exists on the best method to select the optimal effective and tolerable CD. Failure to select an effective dose is a major cause for the high failure rate of phase III pivotal studies.[9]

For AIT dose selection, the European Medicines Agency (EMA) suggests the use of allergen or pollen provocation tests,[10] with standardized titrated quantities of allergen used to elicit eye and/or nasal symptoms and ideally to select subjects with a threshold symptom score for a positive test. Efficacy of each dose regimen can be assessed without the influence of variable allergen exposure, in contrast to "field" studies with seasonal pollen counts[11,12] or perennial allergens.[13]

Choice of effective CD is often limited by systemic reactions such as anaphylaxis.[14] Dose regimens use tiny individual doses administered over a prolonged time, or hypoallergenic formulations such as allergoids,[15] peptides and recombinants[16-18] to allow quicker updosing and earlier achievement of CDs.

Dose response curves in studies of AIT products to date are either monotonic (exponential),[8,19] or nonmonotonic (higher dose less effective than the lower).[7,20] In either case, the ideal study design comprises a wide range of CDs and sufficient subjects to allow the dose response curve to be tested. Limitations of recent failed studies include restricted range of CDs, too few CDs or the use of pairwise comparisons of each CD with placebo.

Multiple Comparison Procedure and Modeling (MCP-Mod)[21] is an alternative method of analysis of dose response, best used with 4-10 active doses and 5-fold to 10-fold increases in CDs. It enables testing of the shape of the dose response curve (see Appendix S1) and controls the type 1 error rate, avoiding the false detection of a dose response. It has been qualified by the Committee for Medicinal Products for Human Use (CHMP)[22] and meets many of the International Committee on Harmonisation E4 requirements.[23]

The POLLINEX® Quattro plus 1.0 mL, subcutaneous immunotherapies (SCITs) currently offer short courses of injections that are effective and well tolerated.[24-34] They include POLLINEX® Quattro Plus 1.0 mL Birch 100% (PQBirch). Pollinex Quattro SCITs are listed in the current AIT European Academy of Allergy and Clinical Immunology (EAACI) guidelines with grade IA recommendation based upon data from double-blind placebo-controlled trials.[24-26,31-32] The PQBirch, currently available on a named patient basis, is under development for full registration.[18,35] We describe the results from 2 dose selection studies in which PQBirch was tested in subjects with seasonal allergic rhinoconjunctivitis due to birch pollen, with the aim of establishing a useful study design and selecting an optimal CD for use in phase III trials.

2 | METHODS

2.1 | Study design

Two randomized, double-blind, parallel group, multicentre studies were conducted in Germany, Austria (PQBirch203 and PQBirch204) and Poland (PQBirch203 only) between September 2013 and February 2016, outside birch and tree pollen season. PQBirch203 used a limited range (600 to 13 600 standardized units [SU] and 4 CDs for comparison). PQBirch204 was placebo-controlled with a wider range of CDs (up to 27 300 SU). Studies were conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The protocols were approved by the responsible competent and ethical bodies in each participating country. All subjects provided written informed consent before any study activity.

Conjunctival Provocation Test (CPT)[36] (see Appendix S2) was performed at the first visit and repeated at subsequent visits to confirm eligibility and establish the baseline score prior to randomization. Study treatment was administered, and post-treatment CPT was performed 3-4 weeks (+/−3 days) after the last injection. In both studies, primary endpoint was change in baseline to post-treatment TSS recorded following CPT with the allergen concentrations eliciting the TSS ≥6 adjusted for reference eye score at the confirmatory CPT. The secondary endpoint for both studies was number of additional allergen concentration steps required to elicit a positive CPT post-treatment.

In PQBirch203, a further secondary endpoint was amount of Immunoglobulin G (IgG), IgG4 and IgE before and after treatment, whilst in PQBirch204, allergen-specific IgE was recorded and descriptively analysed.

2.2 | Study subjects

Eligible male and female subjects were 18-60 years of age, with a clinical history of moderate to severe symptoms of seasonal allergic rhinoconjunctivitis due to birch pollen exposure that required repeated use of antihistamines, nasal steroids and/or leukotriene modifiers for at least the last 2 years. Additionally, they had a positive skin prick test for birch ≥3 mm, a birch-specific IgE ≥class 2 (by an ImmunoCap test) and a positive CPT at screening (Total Symptom Scorev [TSS] ≥6, adjusted for reference eye score).

Key exclusion criteria were the presence of symptoms upon exposure to any other allergens that were unavoidable during the trial period, moderate to severe asthma, AIT within the past 5 years, completed or ongoing anti-IgE therapy, pregnancy, chronic or malignant diseases, drug or alcohol abuse and psychiatric disorders.

2.3 | Sample size estimation

2.3.1 | PQBirch203 study

It was calculated that 32 subjects in each dose group (128 subjects in total) would have 89% power to detect a difference in means of

−0.9 assuming a common standard deviation of 1.0 (*t* test with a 0.025 two-sided significance level). Assuming a 10% dropout rate, the study included 35 subjects per dose group.

2.3.2 | PQBirch204 study

The sample size was calculated to allow for sufficiently accurate estimation of the median effective dose (ED_{50}). Assuming equal baseline values, and with a mean post-treatment TSS in the placebo group of 6.1 points, a difference of 1.34 in mean post-treatment TSS change from baseline signified a 22% improvement.

Simulations revealed that, with an assumed ED_{50} of 1095 SU, and an assumed effect of 3.0 for the 27 300 SU dose, a sample size of 45 subjects in each dose group would yield a 90% confidence interval (CI) of length 2440 SU for the ED_{50}.

2.4 | The CPT allergen provocation test

Birch pollen allergen CPT material was supplied by Laboratorios LETI S.L (see Appendix S2) and administered at concentrations of 0.3, 1.0, 3.0 and 10 histamine equivalent potency (HEP)/mL. Additionally, the original reconstituted allergen solution of 30 HEP/mL was used post-treatment, if a subject tested negative to all other concentrations[36] (see Appendix S2). Aqueous diluent served as the negative control. Screening CPT was performed to identify the concentration giving a positive test. At the next visit, subjects were retested to confirm the result. If a positive test result was not achieved at the previously used concentration, the allergen challenge dose was raised to the next available concentration(s) until a TSS ≥6 was achieved and retested at a subsequent visit. Only a reconfirmed test could provide a baseline TSS and allergen concentration.

Four eye symptoms after challenge with the allergen or negative control were recorded as subject and investigator reported outcomes (mixed patient-reported outcomes measures [PROMs]) using a severity rating scale as follows: 0 = absent, 1 = mild, 2 = moderate and 3 = severe. Subjects independently scored the eye symptoms of itching and irritation, followed by co-assessment of eye tearing in conjunction with the investigator and scoring of eye redness by the investigator independent of the subject.[36] This questionnaire (Culture-Independent Assessment of the Conjunctival Provocation Test [CIA-CPT©]) was conducted 10 minutes after application of negative control or allergen.

The CPT was only conducted on subjects with no visible and/or reported eye symptoms or complaints on the day of the assessment and no current antihistamine treatment.

2.5 | Study medications and treatment schedules

PQBirch was administered as 6 subcutaneous injections at 7 (+/-) 1-day intervals.

In the PQBirch203 study, subjects received 1 of 4 CDs (600, 1550, 5100 or 13 600 SU), each achieved with individual injected doses of 1.0 mL containing 3500, 2000, 800, 300 or 150 SU of PQBirch or placebo. Subjects enrolled in the 13 600 SU dose group received 6 injections of the study drug, whilst subjects enrolled in the 600, 1550 and 5100 SU dose groups received 2 initial injections of placebo (1.0 mL of 2% w/v L-tyrosine) to maintain the blind, followed by 4 injections of study drug.

In the PQBirch204 study, subjects received either placebo or 1 of 6 PQBirch CDs (5000, 5100, 15 000, 15 300, 20 100 or 27 300 SU), each reached with individual injected doses of 1.0 mL containing 6000, 2400, 2000, 900, 800 or 300 SU PQBirch or placebo. Nearly identical CDs (5000 vs 5100 SU and 15 000 vs 15 300 SU) were given as either 4 or 6 active injections. The blind was maintained for subjects on regimens consisting of 4 active injections by 2 initial placebo injections.

Subjects were randomized to receive one of the CD treatments or placebo. All treatments appeared identical, with a 4-digit randomization number on the packaging of each study medication.

For both the PQBirch203 study and PQBirch204, eligible subjects were randomly assigned to dosing regimens in a double-blind manner either by the Interactive Web Response System (IWRS), PQBirch203 and integrated into the eCRF for PQBirch204. The randomization scheme was developed by the contract research organization (CRO) for each study. The systems provided the Investigator with the randomization number of the investigational medicinal product (IMP) administered, and the CRO was automatically informed about randomization of new subjects.

Post-treatment CPT was performed 3-4 weeks after completion of treatment.

2.6 | Statistical analysis

2.6.1 | Study PQBirch203

The analysis of the primary efficacy variable (change from baseline to post-treatment in TSS following CPT) was performed using an analysis of covariance (ANCOVA) with dose group and grade of birch pollen sensitivity as class variables and the baseline TSS as covariate. An a priori strict semihierarchical test procedure was applied to the hypotheses starting with the highest to the lowest dose, using a Bonferroni adjustment to control the type 1 error rate. Thus, the 2 highest CDs (13 600 and 5100 SU) were first tested against the lowest dose (600 SU) in parallel, using a significance level of alpha/2 (0.025). The next lower dose was tested against the next lowest dose only if both tests were statistically significant.

The primary efficacy analysis was conducted on the modified Full Analysis Set (mFAS), which included all subjects who had received the full CD to which they had been randomized and who had baseline and post-treatment TSS values. Sensitivity analyses were performed on the Full Analysis Set (FAS) and Per Protocol Set (PPS).

Amongst others, the number of additional concentration steps (using the categories 0, 1, 2, 3 and 4 steps) performed until a positive CPT was achieved or "no positive test result post-treatment". Differences in birch-specific IgE, IgG and IgG_4 values pre- and post-

TABLE 1 Demographics and baseline characteristics PQBirch204 and PQBirch203 (modified Full Analysis Set)

Study	Dose	N	Sex, N (%)	Race, N (%)	Age [y]		Height (cm)	Weight (kg)	Birch pollen–related asthma		Baseline TSS	
			Female	Caucasian	Mean (SD)	Range	Mean (SD)	Mean (SD)	Without current therapy, no. (%)	With current therapy, no. (%)	Mean (SD)	Range
PQBirch204 (N = 370)	Placebo	49	19 (39)	49 (100)	37 (12)	19-59	175 (9)	74 (14)	3 (6)	9 (18)	7.8 (1.3)	6-12
	5100 SU	44	29 (66)	41 (93)	36 (12)	18-57	172 (10)	74 (17)	2 (5)	8 (18)	8.0 (1.5)	6-12
	5000 SU	50	28 (56)	48 (96)	35 (11)	19-57	172 (9)	75 (17)	0 (0)	9 (18)	7.5 (1.2)	6-11
	15 300 SU	50	24 (48)	47 (94)	37 (12)	20-59	174 (9)	80 (16)	1 (2)	5 (10)	7.5 (1.3)	6-11
	15 000 SU	51	28 (55)	51 (100)	36 (13)	19-59	173 (10)	79 (20)	3 (6)	8 (16)	7.5 (1.3)	6-11
	20 100 SU	48	20 (42)	47 (98)	34 (12)	18-56	175 (8)	76 (14)	1 (2)	7 (15)	7.1 (1.1)	6-10
	27 300 SU	54	29 (54)	48 (89)	35 (12)	19-59	171 9)	75 (15)	1 (2)	6 (11)	7.6 (1.4)	6-12
PQBirch203 (N = 143)	600 SU	38	20 (53)	36 (95)	33 (10)	20-54	174 (9)	74 (17)	5 (13)	1 (3)	7.6 (1.4)	6-11
	1550 SU	34	17 (50)	33 (97)	34 (10)	22-52	175 (9)	73 (12)	7 (21)	0 (0)	7.6 (1.5)	6-12
	5100 SU	35	22 (63)	34 (97)	36 (11)	18-58	172 (10)	74 (16)	7 (20)	0 (0)	7.3 (1.3)	6-12
	13 600 SU	36	22 (61)	35 (97)	37 (12)	22-59	172 (9)	72 (16)	6 (17)	2 (6)	7.4 (1.2)	6-11

N, number of subjects; SD, standard deviation; SU, standardized units; TSS, Total Symptom Score.

TABLE 2 Multiple Comparison Procedure and Modeling analysis PQBirch204 alone, all 6 dose groups (modified Full Analysis Set)

Model	Multiple contrasts procedure step		Modelling step		
	t Statistic	Adjusted *P*-value	AIC	Normalized weight for model averaging	ED_{50} [SU]
E_{max}	3.105	0.0016	1636.9	0.2461	3138.1
Linear in log-dose	3.215	0.0011	1634.9	0.6658	2470.6
Logistic	2.930	0.0031	1638.9	0.0881	3475.3
Model averaging					2723.4

AIC, Akaike information criteria; ED_{50}, median effective dose; E_{max}, maximum possible effect for the agonist; SU, standardized units.

treatment for each dose group and by visit were analysed as secondary efficacy endpoints on an exploratory basis. The Wilcoxon rank sum test was used to compare the dose groups pairwise.

2.6.2 | Study PQBirch204

The primary efficacy variable was the change in TSS following CPT, from baseline to post-treatment, using the mFAS.

The MCP-Mod methodology was used to test for a dose response trend using the placebo, and 5100, 15 000, 20 100 and 27 300 SU treatment arms, and to estimate the dose response shape. The multiple contrast tests for the dose response were calculated based on an ANCOVA with dose group as class variable and the baseline TSS as covariate (see Appendix S1).

Review of published dose response studies enabled preselection of 3 models in the statistical analysis plan: a maximum possible effect for the agonist (E_{max}) model, a logistic model and a linear in log-dose model; model averaging was used to estimate the shape of the dose response curve. The final estimated dose response shape was a weighted mean of the estimations from the 3 candidate models, where, depending on the Akaike information criteria (AIC) of each candidate model, models with a better fit were given more weight. This was then used to estimate the ED_{50} as a measure of potency.

Amongst others, secondary endpoints analysed were the comparison of the effect of treatment with nearly identical CDs of PQBirch when administered as 4 vs 6 injections using an ANCOVA model, and the number of additional concentration steps (using the categories 0, 1, 2, 3 and 4 steps) performed until a positive CPT was achieved or "no positive test result post-treatment".

2.7 | Safety analysis

2.7.1 | Safety endpoints

All adverse events (AEs) reported during the course of the study were coded using the Medical Dictionary for Regulatory Activities (versions 16.1 and 18.1) using the primary system organ class. Local and systemic AEs, expected serious adverse drug reaction, suspected unexpected serious adverse reactions and AEs of special interest (defined as occurrences of new onset autoimmune disease and neuroinflammatory disease) were recorded. In both studies, safety endpoints were analysed using the safety data set (all treated subjects).

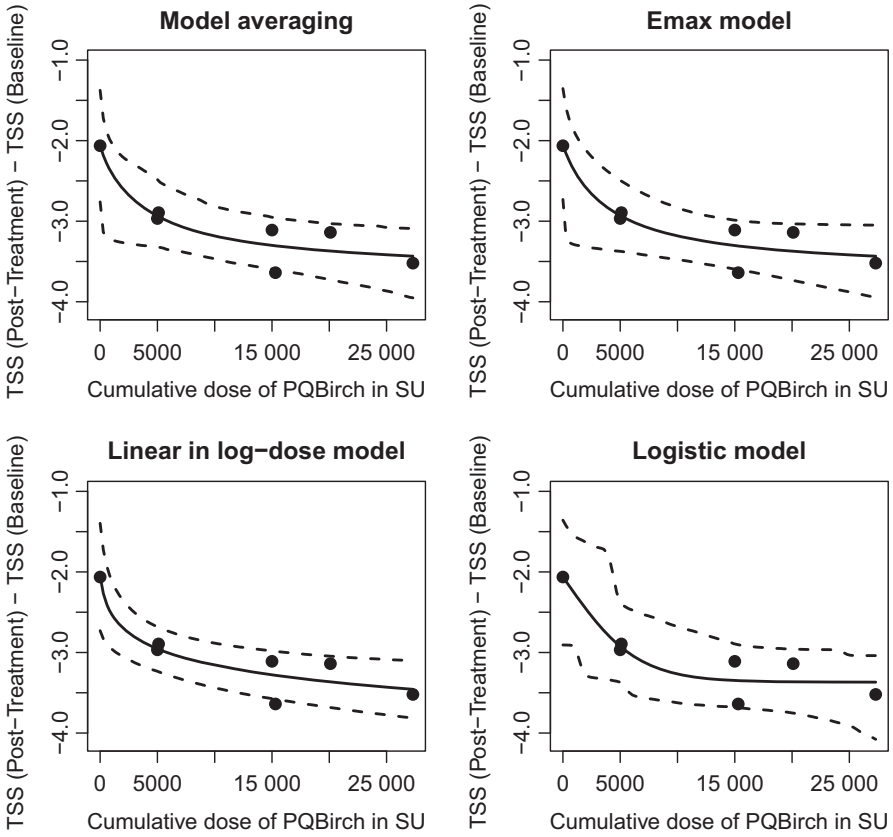

FIGURE 1 Multiple Comparison Procedure and Modeling results from PQBirch204 alone, all 6 dose groups (modified Full Analysis Set). The a priori specified models from Multiple Comparison Procedure and Modeling showing estimated dose response curves with 95% confidence intervals (represented by dashed lines) and actual means for cumulative doses from the model average; E_{max}, linear in log-dose and logistic models. Differences between post-treatment and baseline Total Symptom Scores in the modified Full Analysis Set were used in the calculations. E_{max}, maximum possible effect for the agonist; SU, standardized unit; TSS, Total Symptom Score

2.7.2 | Extensions of planned analyses

As PQBirch203 and PQBirch204 had the same inclusion/exclusion criteria, CPT procedure and primary efficacy endpoint, the results of both studies were pooled together. In both studies, the differences in each dose group vs placebo were estimated using ANCOVA, with post-treatment TSS as an outcome variable and the baseline TSS and unique dose group identifier as explanatory variables. The modelling step of the MCP-Mod was used to test the shape of the dose response curve. The PPS was used to estimate the relative and point differences between the individual CDs and placebo.

3 | RESULTS

In the PQBirch203 study, 174 subjects were enrolled and screened, and 149 were randomized to receive study medication, including 37 randomized into dose group 13 600 SU, 37 randomized into dose group 5100 SU, 36 randomized into dose group 1550 SU, and 39 randomized into dose group 600 SU. The main reason for ineligibility was violation of the inclusion or exclusion criteria. One subject requested to be withdrawn, and 8 subjects were not randomized as the recruitment target had been achieved.

In the PQBirch204 study, 461 subjects were enrolled and screened, and 371 were randomized to receive study medication, including 1 subject who had been randomized into the 27 300 SU dose group despite being a screening failure and was not treated.

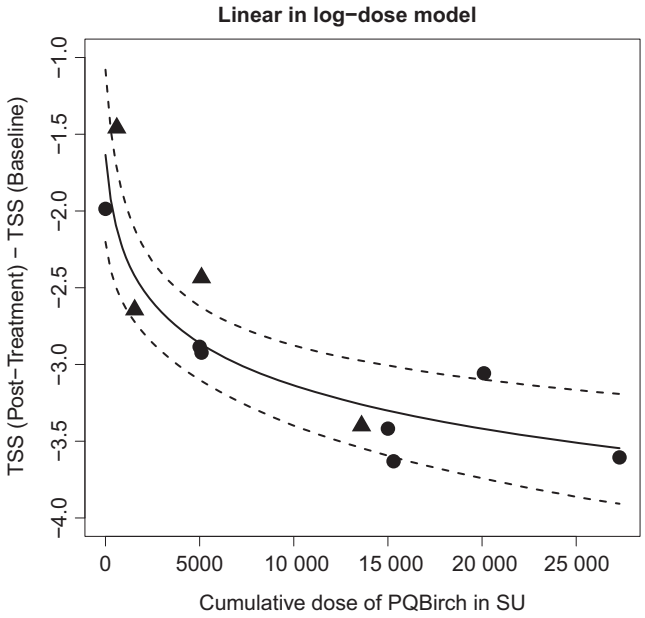

FIGURE 2 Multiple Comparison Procedure and Modeling pooled analysis of PQBirch203 and PQBirch204, absolute difference between cumulative doses, Total Symptom Score (post-treatment) – Total Symptom Score (Baseline) in points (Per Protocol Set). The a priori specified model linear in log-dose from Multiple Comparison Procedure and Modeling showing estimated dose response curves with 95% confidence intervals (represented by dashed lines) and actual means for cumulative doses from the model average, triangles from PQBirch203 and circles PQBirch204. SU, standardized unit; TSS, Total Symptom Score

TABLE 3 Multiple Comparison Procedure and Modeling pooled analysis PQBirch203 and PQBirch204, estimated differences and improvements to placebo from linear in log dose response curve (Per Protocol Set)

Dose	Number of subjects	Estimated TSS (post-treatment) – TSS (baseline)	Estimated TSS (post-treatment)	Estimated absolute improvement to placebo[a]	Estimated % improvement to placebo[a]
Placebo	**44**	−1.63	5.91	0	0.0%
600 SU	**36**	−2.12	5.43	−0.48	−8.1%
1550 SU	**33**	−2.42	5.13	−0.78	−13.3%
5000 SU	**43**	−2.86	4.68	−1.23	−20.7%
5100 SU	**39**	−2.87	4.68	−1.23	−20.8%
5100 SU	**34**	−2.87	4.68	−1.23	−20.8%
13 600 SU	**34**	−3.26	4.28	−1.63	−27.5%
15 000 SU	**43**	−3.30	4.24	−1.67	−28.1%
15 300 SU	**45**	−3.31	4.24	−1.67	−28.3%
20 100 SU	**40**	−3.42	4.12	−1.79	−30.2%
27 300 SU	**46**	−3.55	4.00	−1.91	−32.3%

SU, standardized units; TSS, Total Symptom Score.
White shaded rows: dose groups PQBirch204; grey shaded rows: dose groups PQBirch203.
The bold numbers in column 2 are the number of patients in each treatment group.
[a]Based on absolute post-treatment values, contrasting the single-dose groups to placebo.

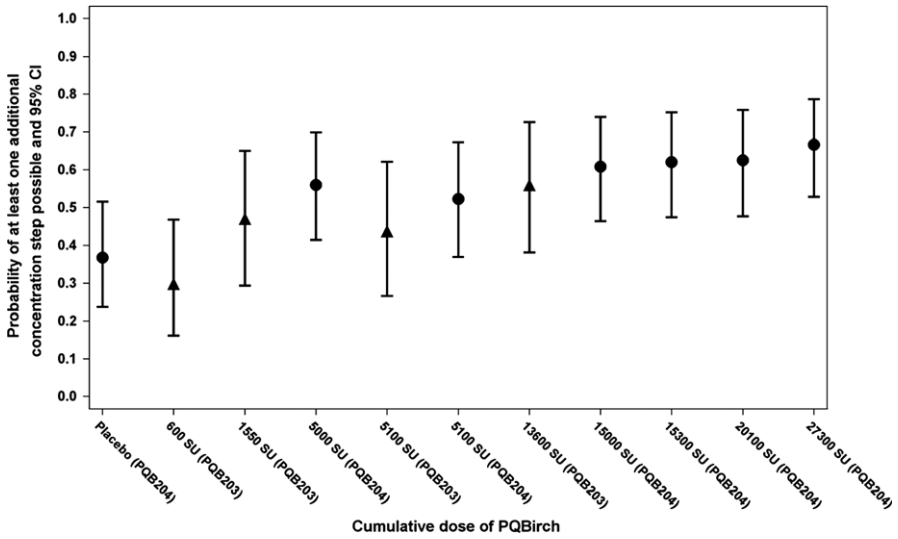

FIGURE 3 Probability of at least one step up in allergen concentration for all cumulative doses for the combined PQBirch203 and PQBirch204 studies together with the 95% confidence intervals. For each cumulative dose, Conjunctival Provocation Tests are shown as the proportion of patients who required at least one more allergen concentration compared with the pretreatment to achieve a Total Symptom Score of ≥6. Triangles represent the mean values for PQBirch203, and circles represent the mean values for PQBirch204. CI, confidence intervals; SU, standardized units

Of the 370 subjects who received treatment, 54 were randomized into the placebo group, 49 into dose group 5100 SU, 53 into dose group 5000 SU, 52 into dose group 15 300 SU, 55 into dose group 15 000 SU, 51 into dose group 20 100 SU and 56 into dose group 27 300 SU. The main reasons for ineligibility were screening failure (53 subjects) and failure to meet randomization criteria (25 subjects). Four subjects requested to be withdrawn, 1 subject was lost to follow-up, and 7 subjects were ineligible for other reasons.

Demographic and baseline data were analysed for the mFAS which included 143 subjects in the PQBirch203 study and 370 subjects in the PQBirch204 study (see Table 1). Females accounted for 39%-66% of the subjects; ages ranged from 18 to 59 years. The primary analysis set was the mFAS for both studies.

3.1 | PQBirch203 results

The difference in TSS change from baseline to post-treatment was 1.88 (P = .0019) between the 13 600 SU and 600 SU dose groups and 1.01 (P = .0919) between the 5100 SU and 600 SU dose groups, which confirms a significant dose response. No further

testing for statistical significance was allowed as the statistical test procedure was predefined to be hierarchical with respect to the first 2 comparisons. These results were confirmed in the FAS and the PPS. Addition of other ANCOVA models (eg, age, investigational site and country as covariates or factors) did not alter the results.

Secondary efficacy variables were in line with the dose response observed with the primary efficacy variable as seen with increases in IgG, IgG_4 no change in IgE and a rise in the IgG4/IgE rations with dose (see Figure S1).

3.2 | PQBirch204 results

The MCP-Mod analysis (mFAS) confirmed the statistically significant dose response for all 3 candidate models ($P < .0011$ to .0031, see Table 2). As a measure of potency of the PQBirch, the averaged model estimated the ED_{50} to 2723 SU. The linear in log-dose model showed the best fit (see Table 2 and Figure 1). Similar curves were estimated with the FAS and PPS.

Similar efficacy was seen with nearly identical CDs when administered over 4 or 6 weeks (or injections).

3.3 | Combined results of PQBirch203 and PQBirch204

Pooling of the data of the 2 studies increased the number of CDs, enabled increased precision in estimation of the dose response curve and its CI and showed the close correspondence of the mean values from the studies.

Whilst the same MCP-Mod methodology was deployed, the PPS was used to illustrate the relative and absolute differences between the CDs and the placebo. The linear in log-dose model for the dose response curve is shown in Figure 2. The estimated relative and absolute differences with respect to change in TSS from baseline and to post-treatment TSS are shown in Table 3. The highest CD of 27 300 SU achieved an absolute difference from placebo of 1.91 and a percentage difference of 32.3% in the post-treatment TSS.

3.4 | Allergen concentration required to elicit a positive CPT post-treatment in the PQBirch203 and 204 studies

In both studies, approximately 50% of subjects on active doses (≥1500 SU) required at least 1 further CPT concentration of allergen post-treatment to achieve a TSS of ≥6 (see Figure 3). There were in PQBirch203; 0 subjects on 600 SU CD who failed to achieve a TSS >6 with the highest CPT allergen concentration, 2 subjects with 1550 SU, 1 subject with 5100 SU and 1 on 13 600 SU. With the PQBirch204 study, 1 subject with placebo failed to achieve a TSS >6 with the highest CPT allergen concentration, 0 on either 5100 or 5000 SU, 1 on 15 300 and 2 with 15 000 SU, 1 on 20 100 and 1 on 27 300.

3.5 | Safety

3.5.1 | Results of PQBirch203 and 204 studies combined

A summary of subjects with treatment-emergent adverse events (TEAEs) across all dose groups is presented in Table 4. Very few subjects experienced a serious treatment-emergent adverse drug reaction (TEADR), and none of the serious TEADRs were related to CDs. Most AEs occurred within 24 hours. of the injections were mild to moderate. Most TEAEs were local, self-limiting and associated with injection-site reactions.

In PQBirch203, TEAEs classified as systemic reactions were experienced by 6 subjects overall; 2 subjects (5.0%) in the 600 SU dose group (conjunctivitis, cough, dyspnoea and pruritus generalized), 2 subjects (5.6%) in the 1550 SU dose group (hypertension, 2 events of allergic rhinitis in the same subject, vomiting; all possibly related) and 2 subjects (5.4%) in the 5100 SU dose group (lacrimation and rhinorrhoea considered related to treatment and urticaria considered possibly related to treatment). No systemic reactions were observed in the 13 600 SU dose group. Subjects recovered from all systemic AEs without sequelae.

In the PQBirch204 study, TEAEs classified as systemic reactions were experienced by 2.0% (20 100 SU dose group) to 15.1% (5000 SU dose group) of subjects in the active dose groups and in 11.3% of subjects in the placebo group. The active dose groups and the placebo group had similar percentages of subjects with at least 1 systemic reaction within 24 hours. of injection, with only 1 moderate (cough, 5000 SU dose group) and 1 severe (eye pruritus, 5100 SU dose group) systemic reaction during the whole study.

Considering both PQBirch203 and PQBirch204, the percentage of TEAEs occurring with each injected dose was over 50% in all dose groups compared with 15%-17% in the placebo group, but there was no dose relationship (see Figure S2).

Of specific interest, the compliance for all the CDs in both studies was >85% (see Figure S3).

4 | DISCUSSION

In order to test the "shape" of the dose response curve, we confirmed the need to study a sufficient number and range of CDs with enough subjects. In the PQBirch203 study, the range of CDs was insufficient to determine the optimal dose. Increasing the CDs to include an over 5-fold increase above the current dose made it possible to define the optimal therapeutic dose. In the pooled analysis, the 27 300 SU dose achieved an increase in point difference of 1.91 relative to placebo when comparing the post-treatment values and a percentage difference of 32.3% without compromising safety. The current marketed dose of 5100 SU demonstrated efficacy and tolerability with a relative reduction in symptom score of 20.8%. Over 50% of subjects required an increase in allergen concentration in CPT after receiving AIT treatment to achieve the standard TSS ≥6 during the CPT. Minimal numbers of treated patients however failed to achieve the TSS >6 with the

TABLE 4 PQBirch203 and PQBirch204: Overall summary of treatment-emergent adverse events (Safety Set)

Study	Dose	N			Any TEAE	Any TEADR	Any severe TEAE	Any severe TEADR	Any serious AE	Any TEAE leading to study drug being discontinued	Any local AE within 24 hr of injection	Any mild or moderate local AE within 24 hr of injection	Any systemic AE within 24 hr of injection	Any mild or moderate systemic AE within 24 hr of injection
PQBirch204	Placebo	53	Ev	n	71	48	1	0	1	4	34	34	10	10
			Sub	N (%)	29 (54.7)	17 (32.1)	1 (1.9)	0 (0.0)	1 (1.9)	2 (3.8)	12 (22.6)	12 (22.6)	6 (11.3)	6 (11.3)
	5100 SU	49	Ev	n	188	161	7	5	1	6	125	125	9	4
			Sub	N (%)	37 (75.5)	34 (69.4)	3 (6.1)	1 (2.0)	1 (2.0)	2 (4.1)	33 (67.3)	33 (67.3)	4 (8.2)	3 (6.1)
	5000 SU	53	Ev	n	274	256	0	0	0	1	207	207	19	19
			Sub	N (%)	41 (77.4)	39 (73.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.9)	38 (71.7)	38 (71.7)	8 (15.1)	8 (15.1)
	15 300 SU	53	Ev	n	182	168	0	0	0	1	149	149	4	4
			Sub	N (%)	42 (79.2)	40 (75.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.9)	38 (71.7)	38 (71.7)	2 (3.8)	2 (3.8)
	15 000 SU	55	Ev	n	217	194	0	0	0	4	170	170	6	6
			Sub	N (%)	39 (70.9)	36 (65.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.6)	34 (61.8)	34 (61.8)	4 (7.3)	4 (7.3)
	20 100 SU	51	Ev	n	193	172	0	0	0	0	151	151	1	1
			Sub	N (%)	36 (70.6)	31 (60.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	29 (56.9)	29 (56.9)	1 (2.0)	1 (2.0)
	27 300 SU	56	Ev	n	288	265	0	0	1	2	234	234	11	11
			Sub	N (%)	47 (83.9)	46 (82.1)	0 (0.0)	0 (0.0)	1 (1.8)	2 (3.6)	43 (76.8)	43 (76.8)	5 (8.9)	5 (8.9)
PQBirch203	600 SU	40	Ev	n	124	91	0	0	2	2	73	73	4	4
			Sub	N (%)	37 (92.5)	26 (65.0)	0 (0.0)	0 (0.0)	2 (5.0)	1 (2.5)	24 (60.0)	24 (60.0)	2 (5.0)	2 (5.0)
	1550 SU	36	Ev	n	111	88	1	0	0	0	72	72	4	4
			Sub	N (%)	29 (80.6)	25 (69.4)	1 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	21 (58.3)	21 (58.3)	2 (5.6)	2 (5.6)
	5100 SU	37	Ev	n	141	114	1	1	0	2	102	102	3	3
			Sub	N (%)	26 (70.3)	24 (64.9)	1 (2.7)	1 (2.7)	0 (0.0)	1 (2.7)	23 (62.2)	23 (62.2)	2 (5.4)	2 (5.4)
	13 600 SU	36	Ev	n	159	137	1	0	1	0	123	122	0	0
			Sub	N (%)	29 (80.6)	26 (72.2)	1 (2.8)	0 (0.0)	1 (2.8)	0 (0.0)	23 (63.9)	23 (63.9)	0 (0.0)	0 (0.0)

AE, adverse event; Ev, events; n, number of events; N, number of subjects; Sub, subjects; SU, standardized units; TEADR, treatment-emergent adverse drug reaction; TEAE, treatment-emergent adverse event.

highest allergen concentration. Potency of PQBirch Allergoid was demonstrated with an estimated ED_{50} of 2723 SU (PQBirch204), approximately half the current dose of 5100 SU.

For selection of a therapeutic dose of AITs, it should be possible to use either allergen or pollen "challenge" studies, as advised by the EMA.[10] The CPT used in the PQBirch203 and PQBirch204 studies was first used to demonstrate efficacy of AIT by Noon in 1911.[37] It has been used to provide a diagnostic of specific allergy[38] and developed to provide a standardized method of allergen challenge suitable for dose selection studies.[39-41] Despite differences in how CPT is performed, all methods use a standard range of titrated allergen concentrations applied to the conjunctiva and PROMs. The CIA-CPT© uniquely allows both subject and investigator to contribute to scoring symptoms (a mixed PROM), and the test-retest reliability has been investigated in a pilot methodological trial.[36] Studying subjects individually avoids collusion and follows best practice for PROMs. In many aspects, CPT resembles the nasal provocation test,[42] as both tests directly add a known concentration of allergen to a responsive mucosa surface unlike pollen exposure intensity which varies. Both methods show concordance of symptom scores,[43] and post-AIT CPT results predict the efficacy of therapy during pollen seasons.[44]

In PQBirch204, the shape of the dose response curve showed that the highest dose of 27 300 SU was approaching a "plateau" (Figures 1 and 2). Nonmonotonic curves may occur with a sublingual immunotherapy (SLIT) and allergoid house dust mite SCIT.[7,8] Further assurance about the reproducibility of this method of analysis is confirmed by the overlapping of the mean TSS scores from the PQBirch203 on the PQBirch204 MCP-Mod dose response curves.

The presentation of results as absolute and percentage differences between placebo and active treatment (see Table 3) is of particular value given the reported magnitude of the placebo effect in SCIT (15%-60%).[45] In the analysis of pooled data for this study, the placebo effect was 46% of the total change with the highest therapeutic dose.

An advantage demonstrated for allergoid SCIT was that the CD is more important in determining efficacy than the number of or time taken for injections, for example, 6 is no better than 4.

The TEAEs reported were mainly local, mild, related to injection-site reactions and short-lived (see Table 4). Finally, there was high compliance with all CDs (see Figure S3).

In summary, CPT combined with MCP-Mod and a wide range of CDs provides an effective means of selecting an optimal dose regimen for a SCIT product. The overlap of the data from the PQBirch203 and PQBirch204 studies demonstrates reproducibility as well as consistent immunological changes. PQBirch can be used at a higher CD using 6 injected doses to achieve 50% increase in efficacy, compared with the current effective dose without changing its safety. There were, in PQBirch203, increases in IgG, IgG_4, no change in IgE and a rise in the IgG4/IgE ratios with dose (see Figure S1).

ACKNOWLEDGMENTS

The study was entirely funded by Allergy Therapeutics. We wish to thank SynteractHCR for undertaking the project management of this study and Metronomia for the analysis of the results. We acknowledge the commitment and dedication of the subjects who enrolled in the study and wish to thank them along with the project management and the team who supported all the work. The study was only possible due to dedicated colleagues, who recruited patients, and their staff, who managed the tests. The authors would like to thank Scinopsis Medical Writing for their help with this manuscript which was financed by Allergy Therapeutics.

CONFLICT OF INTEREST

T. Higenbottam, D. Lee, M. Kramer, M. Skinner and B. Lees are full-time employees of Allergy Therapeutics (UK) Ltd. K. Gunawardena is a consultant working for Allergy Therapeutics (UK) Ltd. D. Wessiepe reports fees from Allergy Therapeutics Plc. to her employer Metronomia, during the conduct of the study. M. Worm reports that she has acted as the leading principal investigator for the PQBirch study and received financial consulting compensation for speaker activity from Bencard. S. Zielen received grants and personal fees from Allergy Therapeutics during the conduct of the study and receives grants and /or personal fees from bene-Arzneimittel GmbH, Biotest GmbH, Vifor Pharma Deutschland GmbH, ALK Arzneimittel, Novartis GmbH, Böhringer Ingelheim, Lofarma GmbH, IMS HEALTH GmbH & Co. OHG, GSK, Stallergen, Procter and Gamble and Allergopharma GmbH, outside the submitted work. R. Mösges receives grants and /or personal fees from ALK, ASIT biotech, Allergopharma, Allergy Therapeutics, Bencard, Leti, Stallergenes, Optima, Friulchem, Hexal, Servier, Klosterfrau, Bayer, FAES, GSK, MSD, Johnson & Johnson, Meda, Stada, UCB, Bitop AG, Hulka, Nuvo and Ursapharm; grants, personal fees and nonfinancial support from Lofarma; personal fees and nonfinancial support from Novartis; and nonfinancial support from Roxall, Atmos, Bionorica, Otonomy and Ferrero outside the submitted work. W. Aberer participated in well-controlled studies, regarding this and relating study products. O. Pfaar received grants from Allergy Therapeutics (UK) Ltd during the conduct of the study and receives grants and / or personal fees from ALK-Abelló, Allergopharma, Stallergenes Greer, HAL Allergy Holding B.V./HAL Allergie GmbH, Bencard Allergie GmbH/Allergy Therapeutics, Lofarma, Biomay, Nuvo, Circassia, Biotech Tools S.A., Laboratorios LETI/LETI Pharma, Novartis Pharma, MEDA Pharma, Anergis S.A., Sanofi US Services, Mobile Chamber Experts (a GA2LEN Partner), Pohl-Boskamp and Indoor Biotechnologies, outside the submitted work.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions: Margitta Worm was the co-ordinating Principal Investigator and principal author of the manuscript. Tim Higenbottam was the designer of the study, accountable for scientific content and conduct of the study according to good clinical practice. Oliver Pfaar was an Investigator and critical reviewer of the study and manuscript. Ralph Mösges provided advice and guidance on the conjunctival challenge and its

interpretation. Werner Aberer was the Austrian Principal Investigator and a critical reviewer of the protocol and manuscript. Kulasiri Gunawardena was the author of the study protocol. Dorothea Wessiepe was the principal advisor on the statistical methods used for analysis during the study. Denise Lee was the co-inventor of the method used for the conjunctival challenge, accountable for the conduct of the study and a critical reviewer of the manuscript. Matthias F. Kramer was the International Medical Director and provided the allergy medicine input to the study design. Murray Skinner was Chief Scientist and accountable for the quality control of the study. Bev Lees was Director of Operations and accountable for the production and quality of the products used in the study. Stefan Zielen was an Investigator and leading recruiter for the study, providing critical review of the study design and manuscript.

REGISTRATION

The studies are registered with the EU Clinical Trials Register, EudraCT number 2012-004336-28 (PQBirch203) and 2015-000984-15 (PQBirch204).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Worm M, Higenbottam T, Pfaar O, et al. Randomized controlled trials define shape of dose response for Pollinex Quattro Birch allergoid immunotherapy. *Allergy*. 2018;73:1812–1822. https://doi.org/10.1111/all.13478