

Clinical Trials with Recombinant Allergens – Three Perspectives: Industry

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Introduction

The Guideline on the Clinical Development of Allergen Products for Specific Immunotherapy for the Treatment of Allergic Diseases (CHMP/EWP/18504) is now available and serves as the basis for clinical development of recombinant allergen products.

Recombinant proteins are an ideal basis for development of diagnostic and therapeutic preparations since they are molecularly defined and can be produced in high purity with consistent quality, thereby circumventing many of the difficulties associated with natural allergen extracts and their standardisation. For the first time in the development of allergen preparations it is possible to decide on the exact composition of a product and optimise the dose and relative amounts of all components. Such pharmaceutically designed therapeutic preparations include the most relevant allergens, by contrast with allergen extracts which contain substantial amounts of irrelevant non-allergenic material. They contain defined amounts of pure proteins in fixed relative amounts, preferably in equimolar concentrations.

Recombinant grass pollen allergens from the species *Phleum pratense* have already been used successfully for specific immunotherapy with those proteins considered to be the most relevant causes of sensitisation [1,2,3].

In order to reduce the risk of IgE-mediated therapy-induced side effects different strategies are being pursued to create hypoallergenic derivatives of the major birch allergen *Bet v 1*. Most recently *in vitro* studies have led to the development of a folding variant of recombinant *Bet v 1* (*rBet v 1-FV*) which exhibits hypoallergenic characteristics without the need for resorting to genetic engineering techniques [4]. In IgE-inhibition assays and in basophil activation tests it could be shown that IgE-binding activity was substantially reduced by comparison with the natural allergen.

Proliferation assays with representative *Bet v 1* specific T cell lines showed that the recombinant folding variant was at least as reactive as the natural and recombinant wild type molecules, and often more so.

Experience has shown that it is possible to use recombinant DNA technology to engineer allergen derivatives which combine the benefits of hypoallergenic characteristics with the advantages of recombinant proteins [4]. The reduced IgE-reactivity of the preparations confers a smaller risk for inducing IgE-mediated side-reactions and allows a maximum therapeutic dose to be achieved in a relatively short course of injections by comparison with the native allergen [5]. Here we present and discuss results from three clinical studies of allergen specific immunotherapy with recombinant allergen preparations, one using a mixture of five grass pollen allergens in a dose finding study with randomised double-blind placebo controlled design, and the others using a hypoallergenic derivative of the major birch pollen allergen *Bet v 1* in a randomised, open comparative trial with a native allergen extract and a large randomised double-blind placebo controlled trial.

Methods

A pre-seasonal, randomised, actively controlled phase II study was performed in birch pollen allergic patients suffering from rhinoconjunctivitis with or without asthma GINA grade I or II. The hypoallergenic recombinant folding variant of the major birch pollen allergen (*rBet v 1-FV*) adsorbed onto aluminium hydroxide was compared with an established native birch pollen preparation. 51 patients (24 *rBet v 1-FV* and 27 comparator) started therapy, receiving one course of pre-seasonal injections during the five months prior to the pollen season. The primary end-point was a combined symptom-medication score (SMS).

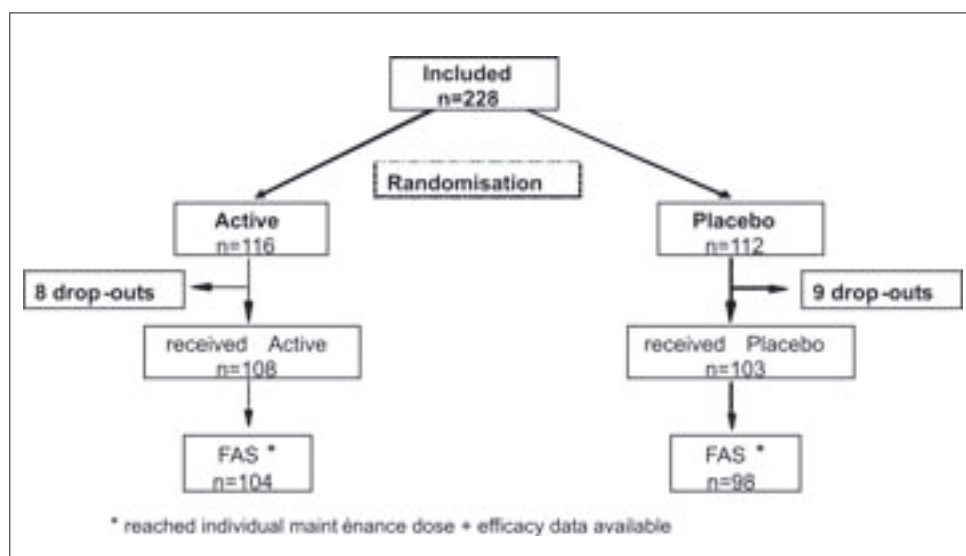


Fig. 1. *rBet v 1-FV DBPC study: Patient Flow.*

In a randomised, double blind, placebo controlled clinical trial 228 patients with rhinoconjunctivitis with or without asthma GINA grade I or II were randomised to the *rBet v 1*-FV preparation (n=116) or placebo (n=112) [Fig. 1]. After a baseline season birch pollen allergic subjects were treated on a perennial basis over a period of approximately 20 months encompassing two pollen seasons, with injection intervals being extended to 14, 28 and finally 42 days. The maintenance dose was reduced by 50% during the pollen seasons. Primary end-point was a combined symptom-medication score (SMS) [Fig. 2].

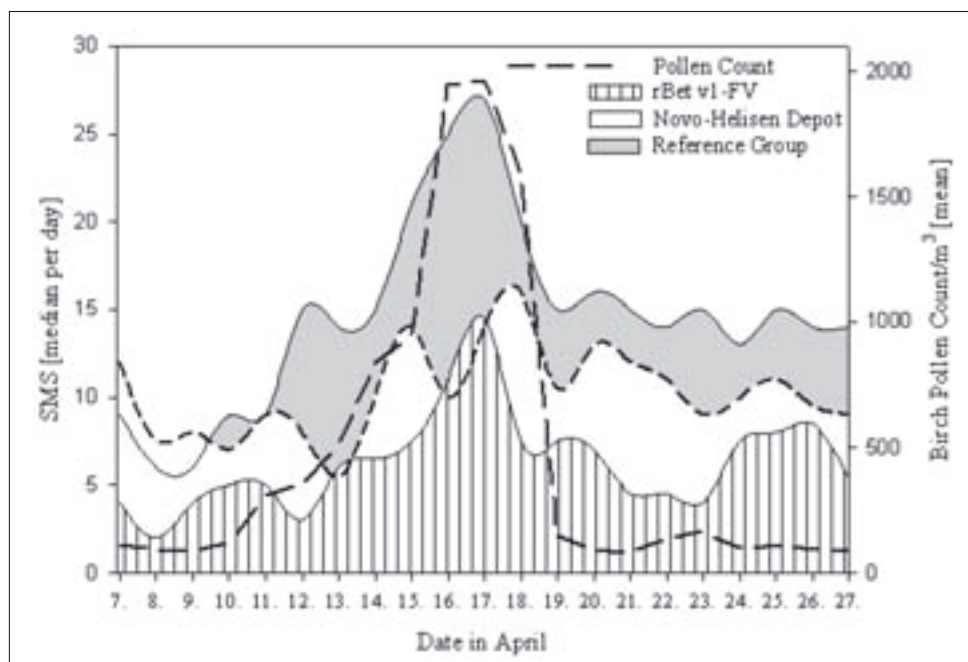


Fig. 2. Daily median SMS in the *rBet v 1*-FV and NHD groups and daily mean birch pollen count/m³. Additionally the time course of SMS of patients without SIT (n=53) from the same year and area is shown.

A cocktail of five grass pollen allergens, *Phl p 1*, *Phl p 2*, *Phl p 5a*, *Phl p 5b* and *Phl p 6*, adsorbed separately to aluminium hydroxide, combined in approximately equimolar amounts and formulated with a total protein concentration of 20 µg, 40 µg, 80 µg or 120 µg per maximum dose was used in a randomised, double blind, placebo controlled dose finding study. The patient flow is shown in Figure 3. Grass pollen allergic patients were treated for approximately 3 months prior to the grass pollen season. Primary endpoint in this study was the number of grade III and IV systemic reactions [6], the main secondary endpoint the early and late phase reaction in intracutaneous tests.

Results and Discussion

Recombinant hypoallergenic birch pollen allergen

***rBet v 1-FV* versus comparator:** Analysis of a combined SMS included 39 patients, (*rBet v 1-FV* $n=22$ and comparator $n=17$). After one pre-seasonal course of treatment the median of the daily SMS was 5.90 for the *rBet v 1-FV* group as compared with 12.48 for the comparator group during the three weeks encompassing the pollen season [Fig. 2]. The daily median score for a reference group of control patients receiving symptomatic treatment in a separate clinical study in the same geographic region was 14.67. The number and nature of the adverse events recorded during the study were comparable between the two groups. This is the first reported comparison between a hypoallergenic recombinant birch pollen major allergen (*rBet v 1-FV*) variant and an established SIT-preparation derived from an extract of natural pollen [5].

***rBet v 1-FV* DBPC phase III trial:** In the first phase III clinical trial 228 patients with rhinoconjunctivitis with or without asthma GINA grade I or II were randomised to the *rBet v 1-FV* preparation ($n=116$) or placebo ($n=112$) [Fig. 1]. During baseline season both groups were similar according to their SMS values. After 1½ years of perennial treatment there was a significant and clinically relevant reduction in median SMS per day in the active group compared to placebo ($p=0.0137$). Significant increases in both IgG1 and IgG4 birch pollen specific antibodies were observed for the *Bet v 1-FV* treated subjects, whereas no increase could be observed in the placebo group.

The combined symptom-medication score (SMS) was chosen as primary endpoint because this best reflects the very close relationship between symptom severity on one hand and the intake of medication on the other hand. A baseline season was included in the study design for the following reasons:

- Proof of comparability of the treatment groups in primary endpoint at start of therapy
- Change from baseline can be calculated for both active and placebo groups
- Immunological parameters (IgE, IgG): effect of the pollen season without intervention with immunotherapy can be calculated
- Calculation of Number Needed to Treat (NNT) – the average number of patients that need to receive treatment in order for one additional person to benefit from it.

Most of the immunotherapy trials conducted to date do not have a baseline season. However, the availability of this data provides the opportunity to precisely calculate the placebo effect. The placebo effect is dependent on the kind of medical intervention. In specific immunotherapy trials with a subcutaneous preparation and a baseline season the placebo effect has been shown to be 20–30%, as is the case in the phase III trial with *rBet v 1-FV* [7,8]. By contrast, specific immunotherapy trials with sublingual preparations and a baseline season have shown a placebo effect of 0–9% or even deterioration [9,10]. Therefore, the hurdle that has to be overcome in

order to show a significant effect compared to placebo seems to be much higher in subcutaneous immunotherapy trials.

Recombinant grass pollen allergens

Dose finding trial: A cocktail of 5 recombinant *Phleum* allergens was tested in a dose finding trial. 50 patients were randomized to placebo or 20 µg, 40 µg, 80 µg or 120 µg of total major allergen (n=10 in each group) [Fig. 3]. MedDRA was used for coding of adverse events and the severity of systemic effects was graded according to Tryba [6] which is comparable to the EAACI severity coding. Although these are some of the highest major allergen doses ever used in clinical trials with subcutaneous preparations, safety was very good and there were no drop-outs because of side effects. So this study showed that dose-ranging studies with SCIT products may provide limited safety information because of the slow increase to individually tolerated doses and the recombinant *Phleum* preparation seems to be very safe even in high doses. The early and late phase reactions after a titrated intra-cutaneous test were used to show efficacy. Both parameters were able to discriminate between the different doses of the recombinant *Phleum* preparation [11].

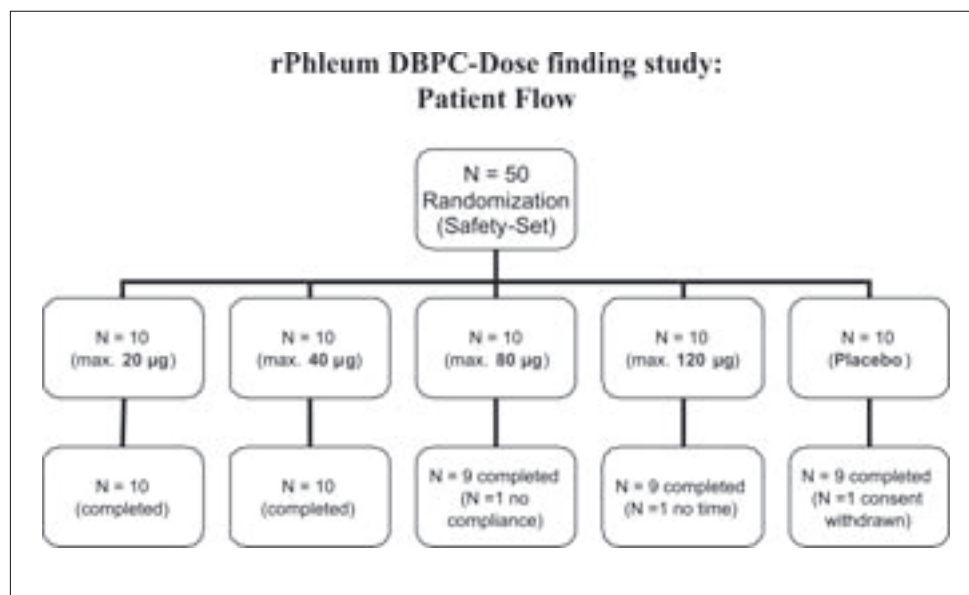


Fig. 3. rPhleum DBPC dose finding study: Patient Flow.

An important observation could be made when using the intracutaneous test. Testing at the end of treatment had to be stopped because of systemic allergic reactions after re-test. After un-blinding of the data it was found that all systemic allergic reactions had occurred in the placebo group and all placebo patients reacted [11]. This led to the conclusion that intracutaneous testing seemed to elicit an increase in sensitivity.

Conclusion

The cocktail of recombinant *Phleum* allergens and the recombinant birch pollen allergen derivative *rBet v 1*-FV have been shown to be well tolerated and clinically efficacious in the respective pollen allergies with or without asthma (GINA I or II). Recombinant DNA technology has thus delivered the prospect of a new generation of preparations for allergen specific immunotherapy with very encouraging first results. Although the regulatory hurdles seem to be much higher for these kind of therapies (2 pivotal studies in centralized compared to one in mutual recognition procedure for European marketing authorisations and more patients for safety), there is reason to hope that these innovative products will become available for use in the routine management of allergic disease in the near future.

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Discussion

Jacobsen: Actually, it is necessary to bring in the former presentation to discussion again. You mentioned that the symptom medication score is the optimal primary endpoint for this. But actually, we now got an open door to challenge objects and to measure provocation tests as outcome in studies. I tend to disagree with the possibility to use it and actually correlate it to clinical effects as such. But would it not be an idea to consider that, at least when you have your proof of concept, by using provocation tests to save some of the patients from being on placebo for many years and treatments that actually show up to be effective?

Narkus: I think it would make our life much easier, but knowing the data from a lot of studies where we used provocation tests, I think it is not that good as compared to symptom and medication or perhaps well-days or improvement. I have the impression that this might be much better. The problem is that it is more difficult to do. It more reflects what the patient really sees in daily life later on. Provocation tests would be much easier, but I really think to have it in a multicenter trial is extremely difficult, if you have it in a monocenter trial that is no problem at all. Then it can be done and then you will see very good results. But doing it in a big multicenter, multinational trial you sometimes have problems.

Kleine-Tebbe: I would say the upcoming guideline is designed to make it also a little bit easier in terms of drug development. Because, and that is the point, to save you some time in dose-finding studies, we could apply these provocation test models or challenge models, e.g. for the nose. But for the pivotal studies it is common to use a combined score. So I think it is already installed in the guideline that you can use, e.g., these provocation models for dose-finding studies.

Narkus: I totally agree. I thought it was meant in correlation to phase III studies. For phase III studies, I think, you cannot use it, but definitely for dose-ranging. The other point what I tried to say is that we all want to have dose ranges, because we think we need more information. But sometimes we think we will get more information out of these trials and perhaps do not really get it. So, it is not as easy as it looks if you write it down in guidelines.

Saloga: I found it very interesting that, after repeated intracutaneous testing in the placebo group, it will be kind of a normal patient group that we allergists might also test, mainly to increase sensitivity and even account for severe reactions. And I think that is an aspect that should be published separately for it is very important for the community to realize that.

Narkus: Yes, definitely. We have seen it for the first time and in such a dramatic way that I think it is necessary to communicate it. Because I do not think that it is in the heads of the physicians.

Vieths: A comment on the dose-finding problem. I think at the level of EMEA and regulators this is considered very important and, in particular, if we keep in mind that dosage is often selected on the basis of the WHO recommendations. Keeping in mind what we learnt about the precision of major allergen assays and the fact that there is no generally accepted standard at the moment, I think it is still important to have at least a few studies where a proper dose-finding has been performed. So I appreciate very much what you have done.

Narkus: I totally agree that having some of these studies would make sense. But the question is: do we need it for every product, for every allergen, bearing in mind that we have a huge program to do and each development for all of us means 10 Million Euro for each product, we all know that.

Jacobi: I have a question or perhaps rather a comment regarding the use of base lines in clinical studies. I also used to be very fond of base lines, but I am certainly not any more. I think that it addresses two things. First of all, let us say two treatment arms or perhaps three or four treatment arms are equal before we start treatment. The randomization procedure is then introduced to ensure to have enough patients in the different arms. It is no different from the other therapeutic areas in which you are imposing treatment effects and in these areas you do not necessarily need a base line. But it is even more problematic with the base line in that pollen seasons vary from year to year and certainly the level of symptoms is influenced by pollen levels. So I think it is questionable whether you can really test the placebo effects by comparing a base line year with a subsequent year in which you have a placebo treatment. Changes from year one to year two could also easily be caused by variations in the pollen counts, not only absolute pollen counts but also the dynamics, that is: is there a steep increase in pollen counts or is there a slow decrease by the end of the pollen season? We can certainly see effects here. I would rather say that perhaps you should address the size of the placebo effect. In my mind it should rather be that you are having an open treated group as a control group and then a group that is given placebo and perhaps a group that is given extra treatment. Then you have three arms and you can actually see what the effect of taking a patient is and start treatment in that patient, whether it is placebo or active treatment.

Narkus: I think there are different ways going to the same goals, so to say. In our view, it was important to have at least a large amount of studies that really have base lines so that we can judge the different effects. Then, I would disagree with your remarks on the pollen counts. If they are different between the different years you will see that in the placebo group and in the active group as well. So I think that is no reason not to include a base line. I would rather like to see that the groups are really starting from the same level when they are going into treatment. And you cannot really be sure if you do not have the possibility to show that. But it might be with the dose-ranging studies that, if we have a set of studies with base lines, we can do some additional work in the future where we do not have it any more. But I think it gives very valuable, additional information.

Durham: I can follow that up just by saying that I think having a base-line year does several things. It allows proper stratifications so that you get comparable groups into the various arms of the study. The issue around the pollen counts is if you have a low pollen count in the base-line year, you may not get a very good estimate of the true disease severity of the patient. I think the other thing that is important, which you can get from the base-line year, is that you do not put into the eventual trial patients who thought they were suitable or who the center thought were suitable, but actually do not have sufficiently severe rhinitis to justify being in the trial. We have certainly seen that with some trials where we have gone straight into this, but at the end of the year, when you did construct the study, a lot of the placebo have not really had much in the way of symptoms. And that makes it very difficult to then judge the efficacy of the active treatment. So, I think there is an important issue when we do these trials that patients have sufficiently severe symptoms. Then,

when we come back and talk to our health authorities and other people that the trials were informative by treating the type of patients that we think we can justify the causes.

Robinson: It is a quick question about your dose-finding study, which I think is extremely interesting and brave. I just wanted to know, at the end of heading over all that interesting data, how did you interpret the optimal dose? If we are going to use challenge studies to do dose-finding, how do you then interpret the data from your challenge study and, actually, what can happen with the symptom medication scores?

Narkus: I think that is what I meant by saying dose-ranging studies do not always give you the answers you want. We thought we do these clinical trials as a dose-ranging, we will skip the 120 µg because that is not safe any more, and we have the separation in the clinical efficacy. But life is not that easy and especially not in subcutaneous treatment because the safety is very different from doing dose-ranging studies in sublingual treatment. So I think it is not so easy, we do not have an answer to this. The only possibility to go ahead is with a large trial with different dosages and doing it in a larger group of patients. We could have had that earlier, so to say, by not doing the dose-ranging.