PROJECT FINAL REPORT

Grant Agreement number: 201871

Project acronym: FAST

Project title: TOWARDS SAFE AND EFFECTIVE IMMUNOTHERAPY OF PERSISTENT LIFE-THREATENING FOOD ALLERGIES

Funding Scheme :HEALTH-2007-1.4.3: Immunotherapy of human food allergies

Date of latest version of Annex I against which the assessment will be made: 30-06-2016

Name, title and organization of the scientific representative of the project's coordinator[1]:

Prof. Ronald van Ree

Professor of molecular and translational allergology

Dept. of Experimental Immunology, K0-130

Meibergdreef 9, 1105AZ, Amsterdam

The Netherlands

Tel: +31 (0)20 5666076

Fax:+31 (0)20 5669756

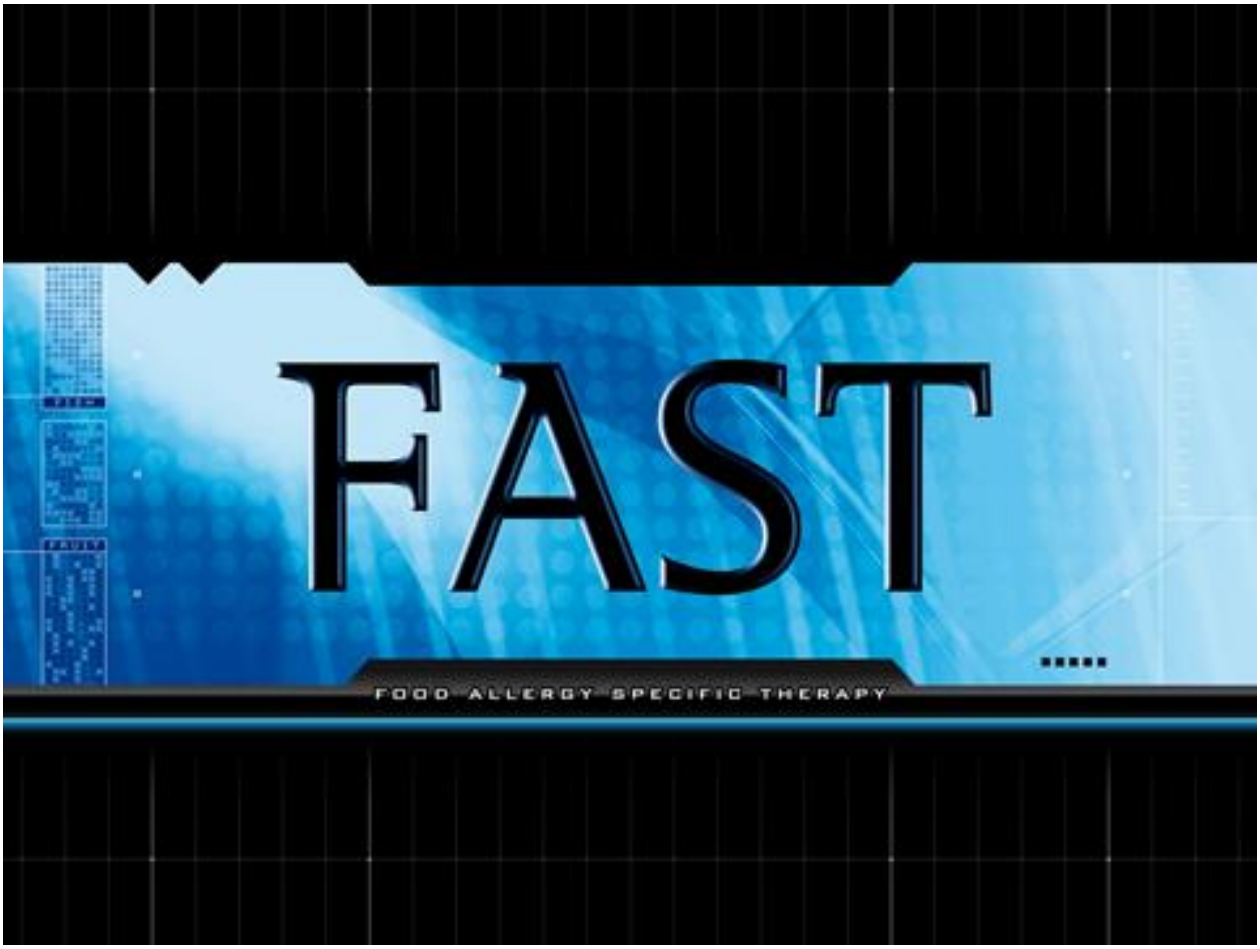
E-mail: r.vanree@amc.uva.nl

Project website[2] address: http://www.allergome.org:8080/fast/index.jsp

[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the grant agreement

.

4.1 Final publishable summary report



Executive Summary

Food allergy (FA) is a chronic, potentially life-threatening (anaphylactic shock) disease with a great impact on the quality of life of patients. To date there are no registered therapies to treat FA, leaving avoidance as the only option. In case of accidental exposure, rescue medication may be used. An effective and above all safe treatment is urgently needed. Therefore, many clinical trial programs, both investigator- and company-driven, are ongoing to develop FA immunotherapy. The first attempt dates back to the early 90s, but subcutaneous injection of aqueous peanut extract proved to be far too unsafe. Since then, focus was on developing immunotherapy via other routes generally regarded to be safer: oral, sublingual and epicutaneous. Most efforts are focused at peanut and to a lesser extent milk and egg allergy and all approaches aim to induce tolerance to the implicated food. In these approaches, foods are administered in their native allergenic form, solid (oral immunotherapy) or extracted (sublingual and epicutaneous) and consequently allergic side-effects during administration are inevitable. In case of oral immunotherapy the side-effects are frequent and often quite severe while sublingual and epicutaneous administration are quite well tolerated. Efficacy of oral immunotherapy is good but is lost in the vast majority of patients as soon as they stop therapy. The effect size of the better tolerated sublingual and epicutaneous approaches is much smaller. The problem of FA immunotherapy is finding the most appropriate route with the right balance between efficacy (high dose needed) and safety (low dose preferred). From treatment of hay fever and allergic asthma we know that the subcutaneous route is very effective, but these use native extracts that were too unsafe for application in FA. Therefore, to apply the effective subcutaneous route for FA safely, the FAST project set out to develop hypo-allergenic immunotherapy vaccines for the treatment fish and fruit allergy. Biotechnology was used to modify the major allergens of fish (parvalbumin) and fruit (lipid transfer protein, LTP) into hypo-allergenic safe vaccines. For the LTP, the project did not succeed to produce a suitable vaccine candidate in time for executing clinical trials during the project. Although 12 molecules were developed and tested extensively and two candidates actually show promising results in mouse models, none warranted GMP production, toxicity testing and clinical trials in humans during the project. For parvalbumin development of a candidate vaccine with a very significantly improved safety profile, produced according to state-of-the art pharmaceutical standards, was successful. The vaccine was tested in a first-in-man two-centre Phase I/IIa safety trial in 15 patients and proved to be safe and well-tolerated. Subsequently, it was tested in 41 patients in a multicentre Phase IIb trial in six countries across Europe for efficacy and further safety assessment. Again the vaccine proved to be very safe and well-tolerated with no severe side-effects at all. The treatment was accompanied by a very robust induction of protective IgG antibodies and a highly significant reduction of the skin prick test to fish. Efficacy was primarily assessed by measuring the threshold for fish in double-blind placebo-controlled food challenge. The target of a significant increase of the threshold was not reached. About half of the patients only had subjective symptoms, the other half (also) had objective symptoms. The patients with subjective symptoms showed a strong improvement on placebo that masked any potential beneficial effect of active treatment for the whole group. Separate analysis of patients with only objective symptoms, showed active treatment increased the threshold (although not significantly due to the low number of patients). Overall, despite not reaching the primary endpoint, the outcome of the clinical trial is very promising for patients with severe fish allergy: the vaccine is safe via the effective subcutaneous route and it induces a robust protective IgG response that translates into a reduction of skin reactivity. Although clinical improvement could not be established due to the mixed patient population with subjective and objective symptoms, the trend observed in the small group of patients with objective symptoms is very promising. New studies, preferably also in children, are therefore warranted.

Summary of project context and objectives

Project context

Although reliable figures are still largely unavailable, food allergy is thought to affect around 1-2% of adults and 4-8% of children, i.e. roughly around 10 million EU inhabitants. The clinical presentation of food allergy varies from mild local symptoms of the oral cavity, usually referred to as the oral allergy syndrome (OAS), to severe systemic reactions which can include life-threatening anaphylaxis. Moreover, the permanent threat of an anaphylactic shock has great impact on the quality of life of patients and their families. Most food allergies are life-long diseases. At present the only treatment for food allergy is avoidance, supplemented with rescue medication in cases where avoidance fails. Failure to avoid is the main cause of emergency room visits for anaphylaxis. Therefore, there is an urgent need to develop a treatment for food allergy with a view to curing the disease, making avoidance unnecessary or at the very least less significant. Allergen-specific immunotherapy (AIT) is a successful treatment of respiratory allergies. It is the only therapy that comes close to a cure by targeting the immunological basis of the disease. Subcutaneous AIT as a treatment for peanut allergy has been evaluated using an aqueous native peanut extract. This was done in the early 90s of the last century in the USA. Although a significant level of efficacy was demonstrated, anaphylactic side-effects were too frequent. One explanation of these frequent and severe side-effects may have been that, on top of the use of native allergen, the extract was not adsorbed to a depot like aluminium hydroxide (alum). Tragically, due to a mistake of the hospital pharmacy a patient on placebo received the full maintenance dose of this aqueous peanut extract and died. Obviously, the project was abandoned.

This set-back did not take away the awareness that a treatment for food allergies is urgently needed. However, since the experience in the early nineties resulting in a casualty, subcutaneous immunotherapy for food allergy became a no-go approach for a long time. Clinical investigators and later also companies turned their attention towards different routes of exposure that were considered less prone to severe side-effects than the subcutaneous route. These alternatives include the oral route (OIT), the sublingual route (SLIT) and the epicutaneous route (EPIT). Oral immunotherapy has been tested for peanut, milk and egg in randomized controlled trials and has been shown to be efficacious during treatment, but tolerance is lost in the majority of patients soon after treatment is stopped. Moreover, side-effects during treatment are unexpectedly frequent and severe. In a small group the side-effects included the spreading of food hypersensitivity to the oesophagus, i.e. the induction of eosinophilic esophagitis. Sublingual immunotherapy has also been tested for peanut and has proven to be efficacious but the effect size is clearly smaller than that achieved with OIT. On the other hand, the safety profile of SLIT compares favourably to that of OIT. The performance of epicutaneous immunotherapy is very similar to that of SLIT, with good safety and tolerability but quite moderate effect size. All three routes have in common that the end goal is self-administration at home. It is doubtful whether adherence will be sufficient in a home setting compared to well-controlled randomized clinical trials. Knowing that, after termination of treatment, tolerance disappears quite rapidly in many patients, at least for OIT, poor adherence in a home setting may introduce significant safety risks.

Against this background, there clearly is room for improvement to find a better balance between safety and efficacy. The target of the FAST project was to take the challenge to develop safe and effective immunotherapy of food allergy via the subcutaneous route. Obviously, with the experience in the past in mind, the vaccine should have significantly increased safety. The FAST project aimed to achieve that in three ways:

- The use of recombinant major allergens made hypo-allergenic to replace native allergenic extracts.
- The adsorption of the drug substance to aluminium hydroxide
- Administration by allergy specialists instead of home self-administration: better control of adherence.

Around the start of the FAST project, there was one development program ongoing in the USA that applied a mix of hypo-allergenic recombinant major allergens of peanut. The project was in the end abandoned because the administration via the rectal route unexpectedly gave too many side-effects as well. Whether this was caused by (a combination of) the route, the absence of a depot like alum, or by the quite original vehicle chosen by the investigators (probiotic bacteria expressing the hypo-allergens) is not clear. In the FAST project we chose not to work on peanut having multiple major allergens, but on two foods also associated with severe food allergies but mainly being caused by a single major allergen: fish with its major allergen parvalbumin and fruit with its major allergen lipid transfer protein (LTP). The reason for this was mainly that pharmaceutical development of multiple hypo-allergenic variants of major allergens per food would not be feasible within the financial and time constraints of an FP7 project.

Objectives

The overall objective of FAST was **the development of effective and safe immunotherapy food allergy.** This overall objective was to be reached for the two chosen foods, fish and fruit, by a series of sub-objectives:

1) Development of recombinant major allergens made hypo-allergenic by biotechnological means.
2) Development of pharmaceutical GMP-compliant production of recombinant hypo-allergens
3) Formulation of the drug substance as a depot by adsorption to aluminium hydroxide to further increase safety
4) Pre-clinical toxicity testing in laboratory animals
5) Characterization of fish and fruit allergic patients from 6 and 3 European countries, respectively
6) Phase I/IIa clinical evaluation for safety of the novel drug products
7) Phase IIb clinical evaluation
8) Detailed immunological characterization of fish and fruit allergic patients, before and after treatment

To achieve these objectives the project was organized around three scientific themes and management:

1) **Allergens** covering the first 4 sub-objectives
2) **Clinical studies** covering sub-objectives 5-7
3) **Immunology** covering the last sub-objective
4) **Management** covering all objectives

The consortium partnership was multi-disciplinary to cover molecular biology and protein chemistry, pharmaceutical science and QA/QC, clinical allergology and immunology.

Main S&T results

The project started a number of activities in parallel from each of the three scientific themes:

Theme "Allergens"

Pre-clinical development of a series of hypo-allergenic candidates for fish parvalbumin and peach lipid transfer protein.

Theme "Clinical studies"

Clinical characterization of fish allergic patients and fruit allergic patients from across Europe

Theme "Immunology"

Identification of dominant T-cell epitopes of parvalbumin and lipid transfer protein.

Theme "Allergens"

In the development of suitable vaccine candidates three major criteria were evaluated:

- Hypo-allergenicity
- Immunogenicity
- Stability

For fish parvalbumin the parvalbumin of carp, Cyp c 1, was chosen. Natural purified Cyp c 1 (nCyp c 1) and wild-type recombinant Cyp c 1 (rCyp c 1) were used as control allergenic reference molecules to assess hypo-allergenicity, immunogenicity and stability of two candidate hypo-allergenic variants:

1) Glutaraldehyde-modified rCyp c 1 (GA-rCyp c 1)
2) Mutant rCyp c 1 (mCyp c 1)

The concept of the first candidate GA-rCyp c 1 was based on the technique of glutaraldehyde modification of allergen extracts that has been used for decades by some companies to make their extracts safer (=hypo-allergenic) for immunotherapy. The second concept of mCyp c 1 is based on the observation that the calcium-binding muscle protein parvalbumin loses most of its allergenicity if it is depleted for its calcium. The hypothesis now was that by mutating the calcium-binding site, a molecule would be created that cannot bind calcium anymore and thereby loses its IgE-binding capacity, i.e. becomes a hypo-allergen.

For fruit LTP, peach LTP (also known as Pru p 3) was chosen. Natural purified nPru p 3 and wild type recombinant rPru p 3 were used as control allergenic reference molecules to asses hypo-allergenicity, immunogenicity and stability of 5 candidate hypo-allergenic variants:

1) Cysteine-mutant, cysPru p 3
2) Reduced and alkylated rPru p 3, RA-Pru p 3
3) Surface mutant, surPru p 3, in which a reported surface-exposed IgE epitope was mutated
4) Glutaraldehyde-modified rPru p 3
5) Recombinant strawberry LTP, rFra a 3

The concept behind preventing formation of the 4 disulfide bridges of Pru p 3 is quite simple (candidates 1 and 2). The molecular structure of lipid transfer proteins is heavily dependent on these disulfide bridges. By destroying those, the loss of 3D structure is expected to affect IgE binding. The third candidate is based on published reports in which a dominant surface exposed IgE epitope was identified. Mutation of the key amino acids of this epitope was expected to decrease IgE binding. The concept of glutaraldehyde-modification is already explained above for Cyp c 1. Finally, the idea behind including rFra a 3 was that strawberry is rarely causing true food allergic reactions, but contains an LTP that is quite homologous to the strongly allergenic peach LTP.

The choice of variant was mainly driven by expectations of impact on allergenicity. The other two parameters were important and evaluated but did not really influence the choice of vaccine candidates. Of course in general very significant structural changes can be expected to have a negative impact on protein stability and consequently possibly also on immunogenicity.

Hypo-allergenicity was assessed in two ways:

1) Competitive IgE-binding assay, using native/wild-type allergen on solid phase and as reference inhibitor.
2) Basophil histamine release (BHR) as a measure of the biological activity of IgE

Stability was assessed by SDS-PAGE/Coomassie staining, gel filtration and reverse phase high performance liquid chromatography (HPLC), fixed angle light scattering and mass spectroscopy (MALDI-ToF) of proteins stored at different temperatures (accelerated stability studies). In addition, CD spectroscopy was performed to judge conservation of secondary structure elements.

Immunogenicity was assessed by immunizing laboratory animals (rabbits and mice) with the candidate vaccines.

Fish parvalbumin: selection of candidate hypo-allergen

For fish parvalbumin, both candidate hypo-allergens, GA-rCyp c 1 and mCyp c 1, proved to be very hypo-allergenic, stable and immunogenic. Since glutaraldehyde modification is les well-defined from a pharmaceutical perspective, the better defined mutant molecule mCyp c 1 was chosen for further pre-clinical development. Figures 1-4 illustrate hypo-allergenicity, immunogenicity and stability of mCyp c 1.

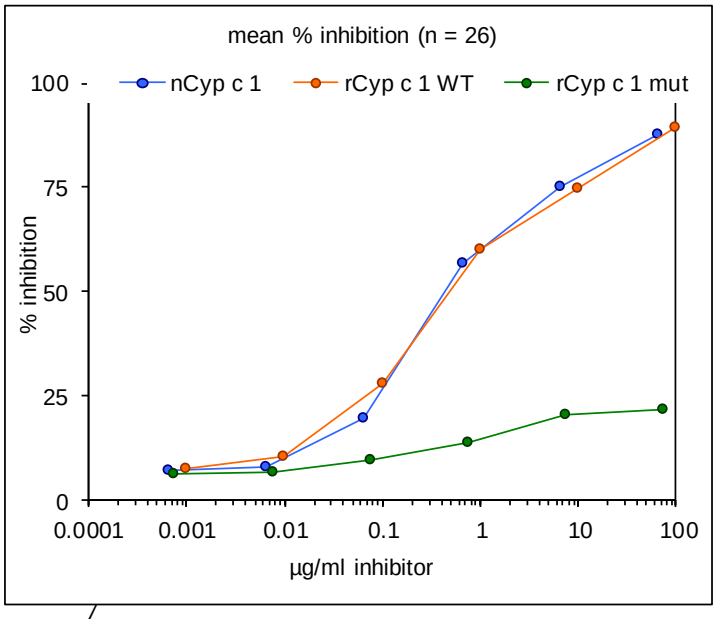
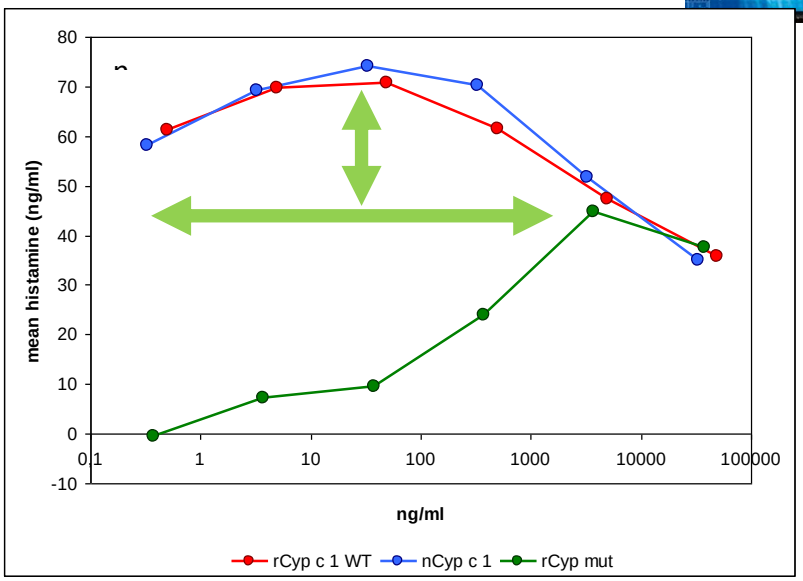


Figure.1 Hypo-allergenicity assessed by ImmunoCAP inhibition:
rCyp c 1mut (mCyp c 1) is hardly capable of achieving any inhibition

Figure.2 Hypo-allergenicity assessed
by basophil histamine release (BHR): rCyp c 1mut (mCyp c 1) is around a 1000-fold less biologically active.



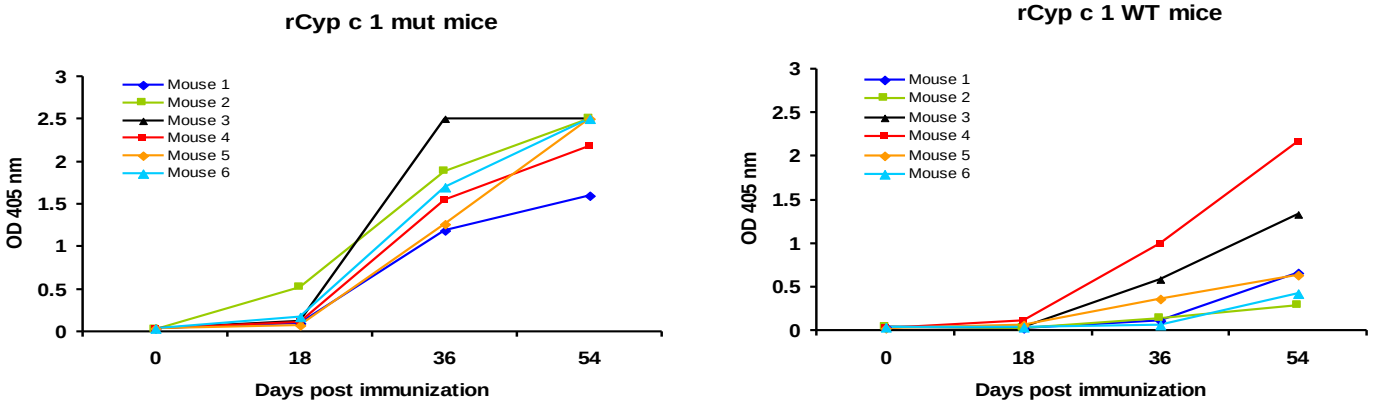
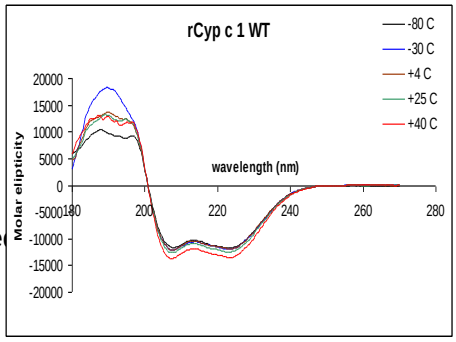
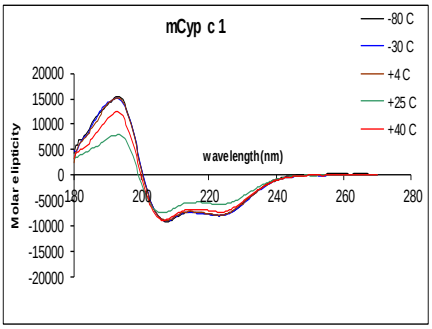
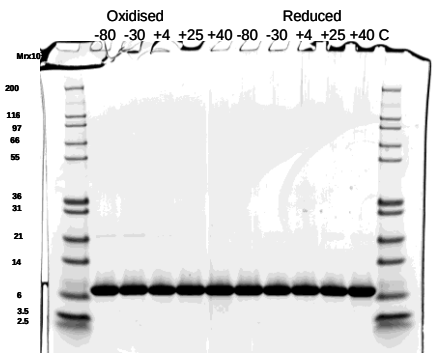


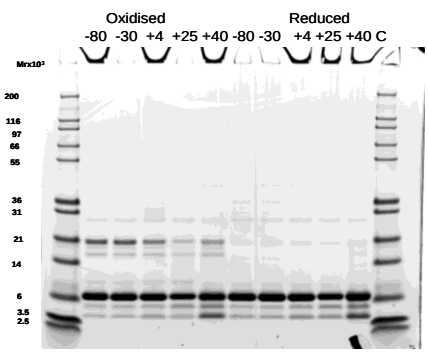
Figure. 3. Immunogenicity as assessed by immunization of mice. Serum IgG binding was measured to WT rCyp c 1. As can be seen, mice immunized with mCyp c 1 (left panel) have a stronger response than those immunized with wildtype (WT) rCyp c 1. This confirms good immunogenicity of the hypo-allergen.

Figure. 4. Stability as assessed by CD spectroscopy (upper panels) and SDS-PAGE (lower panels). Both wildtype and mCyp c 1 are very stable upon evaluation after six months storage up 40 ^{0}C









Peach lipid transfer protein: selection of candidate hypo-allergen

For the 5 variants of lipid transfer protein tested results were more diverse with respect to hypo-allergenicity. The glutaraldehyde-modified was not included in the analysis because it proved to have solubility problems. Of the 4 remaining candidate hypo-allergens, figure 5 clearly illustrates that the two variants without disulfide bridge formation (cysteine mutant and the reduced and alkylated rPru p 3) were extremely hypo-allergenic. The surface mutant and strawberry LTP had far less hypo-allergenic properties.

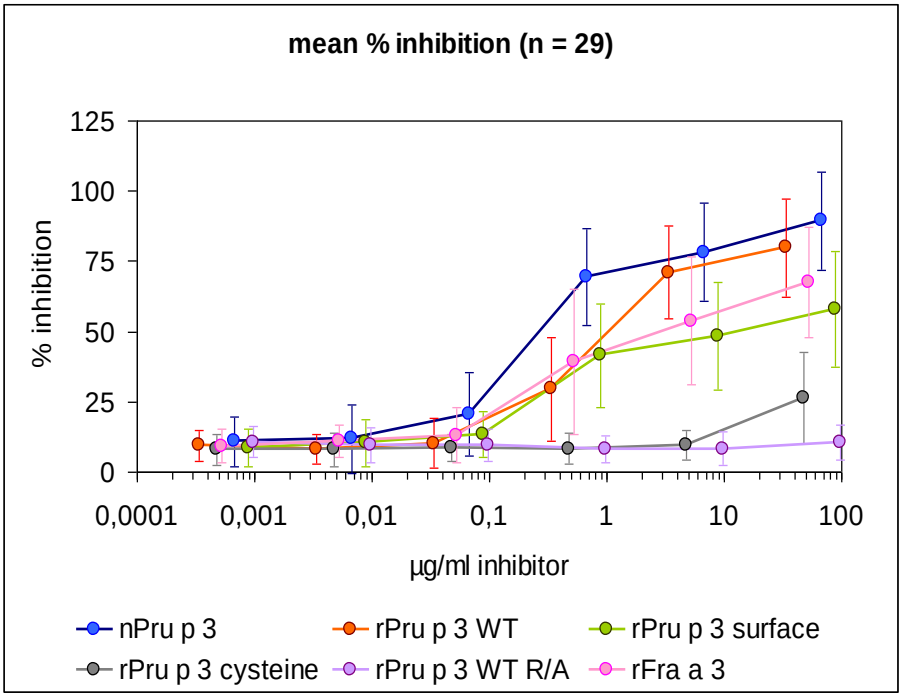


Figure.5. ImmunoCAP inhibition of candidate hypo-allergenic LTPs

Unfortunately, the cysteine mutant had severely reduced stability properties (figure. 6) the same was true for the reduced and alkylated version (not shown). As a consequence, immunization of mice and rabbits with these molecules did not induce any significant IgG responses.

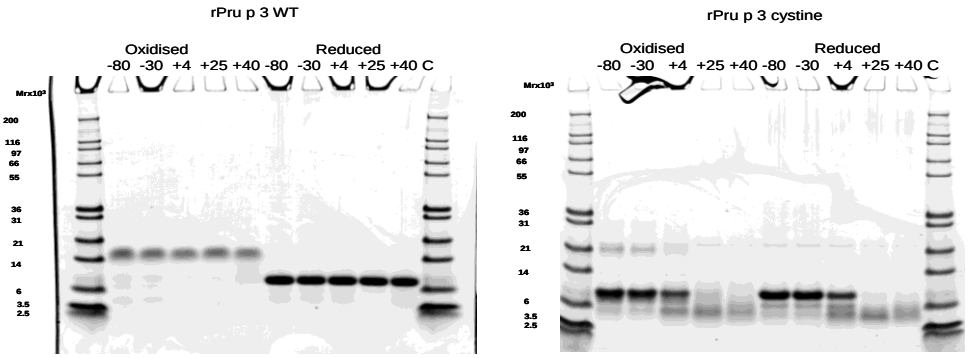


Figure.6. Stability of cysteine mutant LTP assessed by SDS-PAGE. Already at 4^0C breakdown is apparent upon 3 months storage.

Based on these observations, none of the 5 tested candidate hypo-allergens were considered suitable for further development of a vaccine. It was then decided to test an additional series of 5 new mutants and variants. Three of these five molecules had insufficient quality of production and purification and could not reliably be assessed. The two remaining molecules (Pru p 3-C1 and Pru p

3 -BAC3) were an improvement over all the other molecules when combining hypo-allergenicity, immunogenicity and stability but it was decided that the combined characteristics did not warrant full toxicity studies, GMP production and human clinical studies. Moreover, due to the delays that these extra evaluations had given, the time remaining in the project was insufficient to continue with the peach arm of the project. Thus we decided to transfer funds and efforts to focus completely on the promising vaccine candidate for the fish arm of the project.

Toxicity testing of mCyp c 1 vaccine candidate

A pre-GMP batch of the selected fish parvalbumin vaccine candidate mCyp c 1 was then tested in rabbits and mice for acute toxicity. In acute toxicity a single high dose is administered. Acute adverse effects are monitored and animals are kept for two weeks to assess whether late adverse effects were encountered. No adverse effects were reported. This acute toxicity study was the followed by a repeated dose toxicity study in which mice were subjected to essentially the same administration protocol as envisaged for human studies, i.e. 6 months of high dose repeated administrations. Again, also in the repeated dos toxicity studies, no adverse events were reported that were linked to the vaccine. In summary, the judgment was that the candidate vaccine is expected to be safe.

GMP production of mCyp c 1 drug substance and drug product

A single batch of GMP drug substance was then prepared. This batch was stored for future formulation of two batches of drug product, for the planned clinical trials in the theme “Clinical studies”: a first in man Phase I/IIa safety and tolerability study and a subsequent Phase IIb efficacy study. All GMP documentation and QA/and QC were established. These were used to characterize the drug substance and drug product, and were the basis of the product part of the IMPD for applications for permission from ethical committees and competent authorities for performance of both clinical trials. The IMPD for both drug substance and drug product were finalized in time for these applications.

With this the theme “Allergens” was completed.

Theme “Clinical studies”

Multi-centre diagnostic study

In parallel with the first activities in the theme “Allergens” (production and purification of candidate hypo-allergens for fish and fruit), all six clinical centres in Iceland, Denmark, Poland, Spain, Italy and Greece enrolled 5 to 6 fish allergic patients per centre and the three clinical centres in Spain, Italy and Greece in addition enrolled 8 to 10 peach allergic patients. The aim of this study was to evaluate both fish allergic patients with sensitization to parvalbumin and peach allergic patients with sensitization to LTP by double-blind placebo controlled food challenge (DBPCFC), the golden standard of diagnosis of food allergy. Patients with a positive DBPCFC to fish or to peach and sensitization to parvalbumin or LTP, respectively, were asked to donate blood. Serum of these patients with confirmed fish or peach allergy was used for evaluation of hypo-allergenicity of candidate molecules in the theme “Allergens”. This clinical study was performed successfully by all the clinical centres and made the activities in theme “Allergens” complete.

Phase I/IIa first-in-man study with mCyp c 1

The core of the theme “Clinical studies” was of course the two subsequent human clinical trials. After establishing safety in pre-clinical toxicity studies and GMP production of mCyp c 1 drug substance and drug product, a protocol was written for the first two-centre Phase I/IIa clinical trial. This trial was planned to take place at the allergy clinics in Odense and Copenhagen in Denmark.

Inclusion criteria were:

- case history of fish allergy
- positive DBPCFC within max 2 years
- SPT > 3mm and/or ImmunoCAP >0.7 kU/L
- ImmunoCAP rCyp c 1 >0.35 kU/L
- FEV1 > 80%
- age 18-65 yrs
- informed consent

Exclusion criteria were:

- severe anaphylaxis due to fish intake
- uncontrolled asthma
- ongoing allergic disease requiring medication
- serious systemic diseases such as cardiovascular or immune disease
- severe hypertension and treatment with beta-blockers
- severe psychiatric disease
- planned or actual pregnancy

The original protocol was a randomized double-blind placebo-controlled trial with three dosage arms (8 patients each). Due to inclusion problems there was a substantial delay and for reasons of stability of the IMP, we decided to bring it down to two dosage arms (illustrated in figure 7).

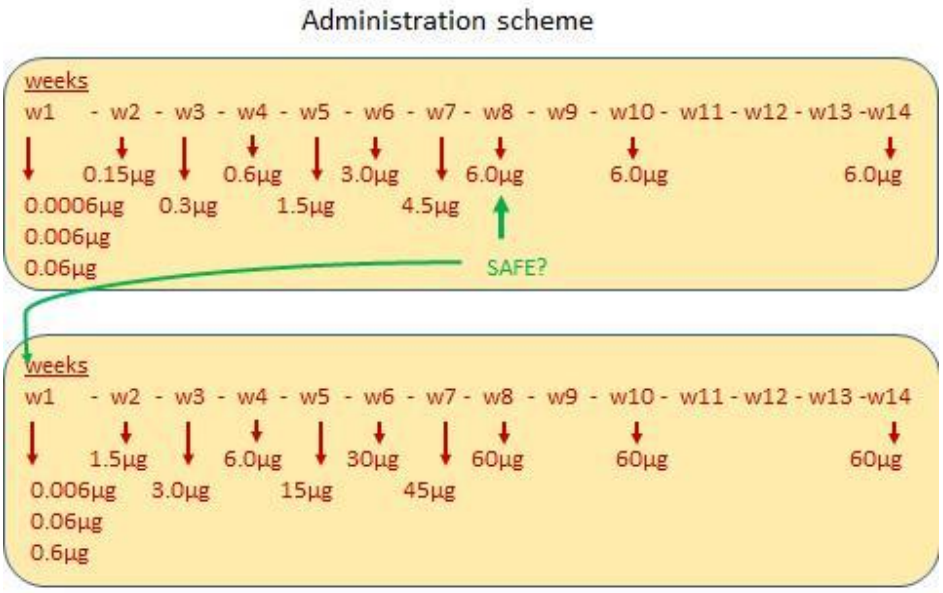


Figure 7. The two dosage arms in the Phase I/IIa trial with mCyp c 1.

The protocol, IMPD, Investigators Brochure, Informed Consent Form, Patient Information Sheet, and all other documentation were submitted to the Ethical Committee and Competent Authorities in Denmark. After some questions and answers back and forth permission was obtained to start the study. The inclusion proved to be difficult, but in the end the two dosage arms were completed in respectively 7 and 8 randomized patients.

After de-blinding, it turned out that in the first arm 2 patients had received placebo and 5 active treatment up to the planned maintenance dose of 6 µg. In the second arm, one patient dropped out and this turned out to be a placebo. Of the remaining seven, six received active treatment up to the planned maintenance dose of 60 µg. In neither of the two arms any severe adverse events related to the vaccine were reported. Only mild to moderate local reactions were reported that could be attributed to the vaccine. The overall conclusion from the first-in-man Phase I/IIa study was that the vaccine is well tolerated and safe. This gave the project the green light to start developing the protocol for the Phase IIb study.

Phase IIb clinical trial with mCyp c 1

The difficulties encountered in the Phase I/IIa study in Denmark with inclusion made the consortium decide that extra clinical centres were needed. In Spain two extra sites next to Madrid were included (Malaga and Cordoba), and a site in Utrecht in The Netherlands was included. Together with the sites in Reykjavik, Odense, Copenhagen, Lodz and Athens this made up a total of 9 sites in 6 countries.

A protocol for a randomized double-blind placebo controlled trial was drafted with efficacy primary read-out. The primary endpoint was the change in threshold for fish protein in a double-blind placebo-controlled food challenge from baseline to post-treatment. The inclusion and exclusion criteria were the same as for the Phase I/IIa clinical trial. Figure 8 represents the outline of the study visits and timelines.

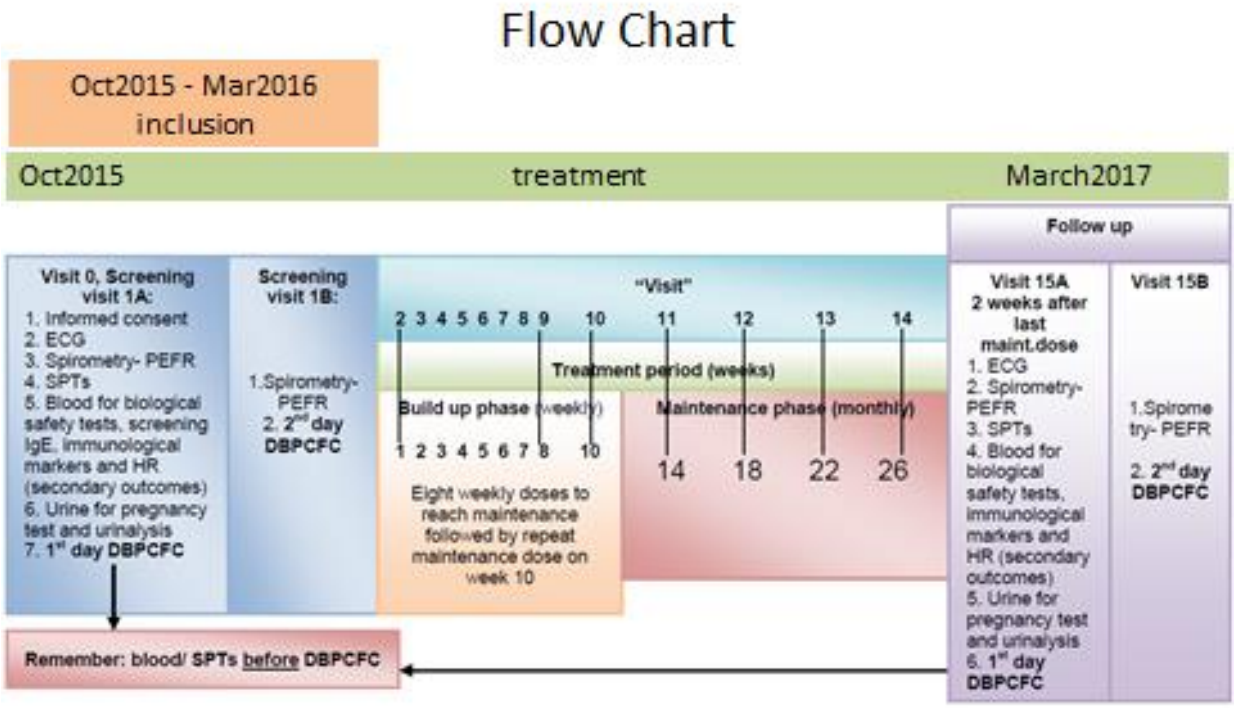


Figure 8. Outline of planned study visits and timelines

Special attention was given to the development of a challenge vehicle that could effectively blind the taste of fish. Figure 9 depicts an impression of the chicken based burger and the dosage scheme of the DBPCFC.

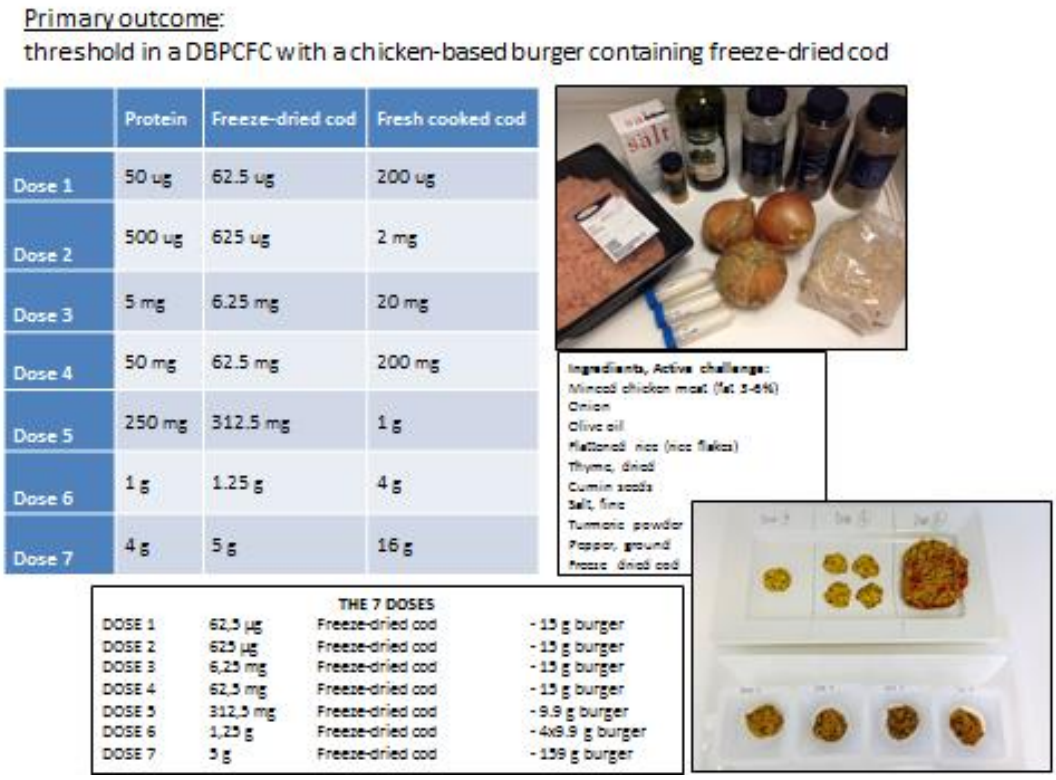


Figure 9. An impression of the chicken based burger and the dosage scheme of the DBPCFC.

The application to the authorities was done through the so-called voluntary harmonized procedure (VHP). After some back and forth questions and answers, permission was obtained in all six countries to perform the Phase IIb study in the 9 clinical centres.

The original target was to randomize 96 patients. This implied that each clinical centre had to enrol around 10-12 patients. As had already been experienced during the Phase I/IIa trial in Denmark, it proved to be extremely difficult to reach these numbers. The main reasons for the reluctance to participate were a general feeling amongst adults that they had adjusted their life to avoiding fish and that they did not feel the need to undergo a time-wise quite burdensome therapy (17 visits to the clinic in around 6 months). It is expected that this problem will not be so important in children, but at this stage of clinical development a study in children was from a regulatory perspective unfortunately not allowed. The time-burden was also a hurdle in another way, in particular in Greece and Spain: the economic crisis had made patients afraid of their jobs and they did not want to risk being away from their work too frequently to participate in a clinical trial. Finally, a smaller group was afraid of the possible risks of the new vaccine. A total of 698 patients were approached, of whom 108 signed informed consent. Of those 108 more than two third was a screening failure, mostly because their sIgE to Cyp c 1 was too low, or they were negative in the DBPCFC. In the end, the centres all together managed to randomize 41 patients that fulfilled all inclusion criteria. One of the consequences of the difficulty in randomizing patients was that the consortium decided to include

both patients with subjective and objective symptoms during challenge and patients with subjective symptoms only. Although the latter category was not listed in the exclusion criteria of the protocol, for obvious reasons it was the original aim of the consortium to only randomize patients that also had objective symptoms. This proved to be a very relevant issue when analysing the outcome of the clinical trial.

The study started in October 2015 and the last patient out was in March 2017. Database lock was in April 2017 and first statistical analyses were performed in May 2017. In the end, 32/41 patients were used for the final analyses. The other nine were either drop-outs or had incomplete data.

The primary endpoint of the clinical trial was not met. As a matter of fact, the increase in threshold showed even a trend to be larger in placebo-treated than in actively treated patients. This is illustrated in figure 10.

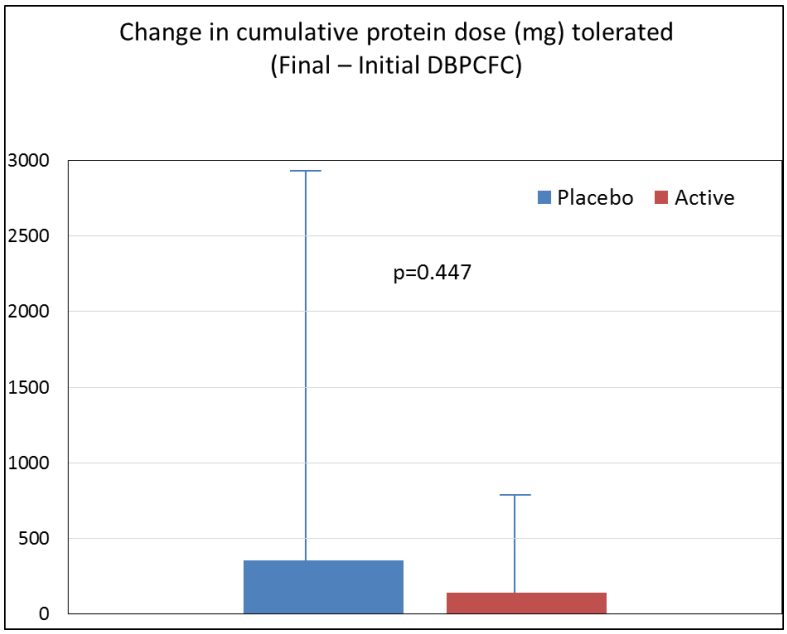


Figure 10. Changes in primary outcome: whole group.

However, in the post-hoc analysis in the sub-group of patients with objective symptoms in the entry DBPCFC (figure 11), there was a change from baseline in cumulative protein dose tolerated during the challenge between the active group compared to placebo; the geometric mean of the difference in cumulative protein dose tolerated during the challenge being 462.3 mg for the active group vs. 151.7 mg for the placebo group. However, this difference did not reach significance (p=0.564). On the other hand there was a statistically significant change from baseline in the sub-group of patients with subjective symptoms only in the entry DBPCFC, in favor of placebo (geometric mean of the difference in cumulative protein dose tolerated during the challenge was 17.2 mg for the active group vs. 603.6 mg for the placebo group, p=0.033). The post-hoc analysis has provided strong support for the original plan to only randomize patients with objective symptoms during challenge. The patients with subjective symptoms only turned out to be prone to a very strong placebo effect which probably has masked any significant treatment effect in the primary outcome for the whole group.

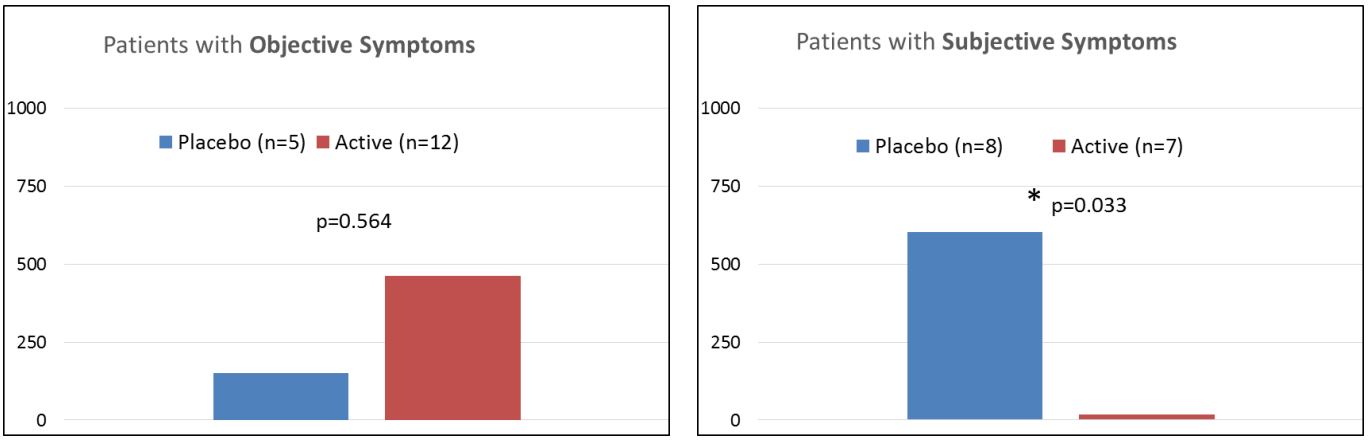


Figure 11 Changes in primary outcome (DBPCFC): patients with objective vs subjective symptoms

Having said that, there are very promising results in the secondary outcomes. First of all, an objective in vivo surrogate marker for clinical improvement, the skin prick test (SPT) showed a highly significant decrease in the actively treated group that was not seen in the placebo group (figure 12).

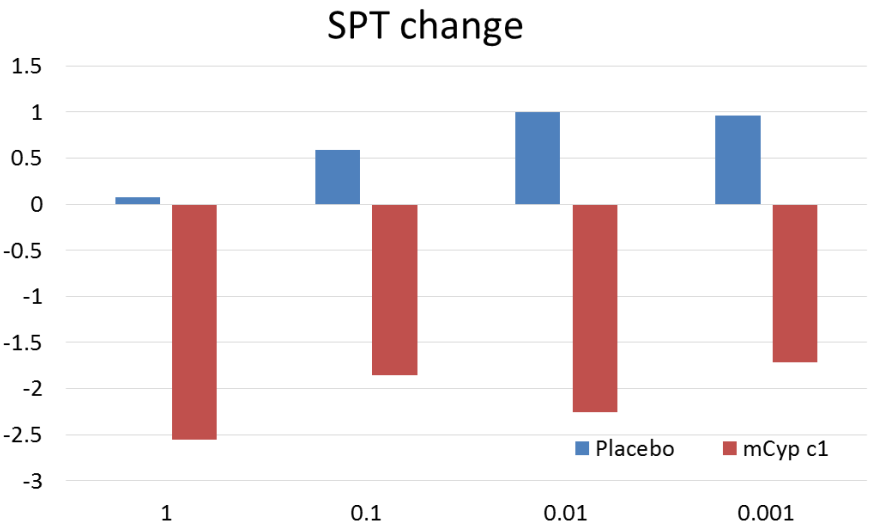


Figure 12. Changes in SPT reactivity

Furthermore, serological analyses (details in the section on theme “Immunology”), showed a very robust induction of protective IgG and particularly the IgG_4 subclass (table 1).

Importantly, the Phase IIb clinical trial confirmed the excellent safety and tolerability profile observed in the phase I/IIa clinical trial. No severe adverse events related to the vaccine were reported. Only local side effects were observed.

Table 1. Change in serological markers

	Active N=19		Placebo N=13		
Differences (post-pre)	**Mean(SD)**	**Median(IQR)**	**Mean(SD)**	**Median(IQR)**	**p-value**
sIgG (kU/L) cod	1.61 (1.05)	1.58 (0.65, 2.31)	0.01 (0.15)	0.01 (-0.02, 0.05)	**0.001**
sIgG (kU/L) rCyp c 1	4.32 (2.81)	3.62 (1.91, 6.44)	0.005 (0.21)	0,02 (-0.05, 0.03)	**0.001**
sIgG (kU/L) rCyp c 1 mut	3.36 (2.79)	2.82 (0.96, 4.4)	-0.003 (0.06)	0.01 (-0.02, 0.02)	**0.001**
sIgG$_4$ (kU/L) cod	3226.5 (3677.2)	1070.1 (428.8, 5782.9)	-146.78 (376.6)	1.5 (-20.6, 17.7)	**<0.001**
sIgG$_4$ (kU/L) rCyp c 1	7188.2 (6896.4)	6492 (1138.2, 11140.1)	-170.9 (443.2)	-21.5 (-62.3, 13.7)	**<0.001**
sIgG$_4$ (kU/L) rCyp c 1 mut	3769.4 (4137.5)	1725 (844.9, 7389.3)	-11.5 (20.6)	0 (-20.3, 0.9)	**<0.001**

In conclusion, a 4-month subcutaneous immunotherapy with rCyp c 1 mutant is safe and well-tolerated. The vaccine is able to reduce SPT wheal size, while robustly increasing protective serum IgG, in particular serum IgG$_4$, to fish allergen. There are no significant changes in clinical reactivity, however in the post-hoc analysis, results were skewed significantly by an (unexpected) response to placebo of patients with subjective symptoms only. Overall, despite the fact that the primary outcome is negative, we are confident that there is ground suggesting that the molecule is a promising for the treatment of fish allergy.

Theme "Immunology"

In the first part of the project, dominant T-cell epitopes of parvalbumin and lipid transfer protein were identified. This was relevant to evaluate whether mutations applied to candidate hypo-allergens did affect dominant T-cell epitopes. The analysis demonstrated that this was not the case.

The most important task of the theme "Immunology" was to monitor serological and cellular changes in response to treatment with the parvalbumin vaccine. In serology, the focus was on IgG and in particular IgG_4 against mCyp c 1, wildtype rCyp c 1 and whole fish extract. In particular the latter two responses are important to establish whether the vaccine induced IgG protective IgG antibodies that do not only recognize the original immunogen (mCyp c 1), but do cross-react to the native parvalbumin, both recombinant rCyp c 1 and natural represented in cod extract. Cross-reactivity to cod extract is not only relevant to establish cross-reactivity to natural parvalbumin but also to parvalbumins of a different fish (carp versus cod). In addition, specific IgE responses were also monitored. IgE responses against wildtype rCyp c and against cod extract did not change after therapy. Only IgE against mCyp c 1 showed a significant increase. This increase can theoretically have significance for side-effects (no proof for that was found), but has no relevance for exposure to fish in real life because fish will not contain an equivalent of the mutant. Figures 13-15 show the IgE response and demonstrate that very robust protective IgG and IgG_4 responses are induced against mCyp c 1 that strongly cross-react to rCyp c 1 and to cod parvalbumin in cod extract.

With respect to cellular responses, we focused on establishing by basophil histamine release whether biological activity of IgE is inhibited by the therapy. These analyses were performed outside the project's lifetime and are still ongoing. First preliminary observations however support that basophil histamine release is inhibited in actively treated patients and not in placebo-treated patients.

As can be seen in the figures above, all immunoglobulin responses increase in the actively treated group but not in the placebo. The only exceptions are IgE against cod fish extract and against rCyp c 1 (native parvalbumin). By expressing in fold-increase the robustness of in particular the IgG4 response becomes apparent (table 2).

	IgE			IgG			IgG_4		
fold increase	**cod**	**rCyp c 1**	**mCyp c 1**	**cod**	**rCyp c 1**	**mCyp c 1**	**cod**	**rCyp c 1**	**mCyp c 1**
mean	1.2	1.6	17.8	7.2	11.6	18.4	3903.2	13294.7	25992.9
median	1.0	1.4	4.1	4.8	8.3	12.3	21.3	60.8	90.9

Table 2 fold increase of antibody responses in the actively treated patients

Overall the immunological analyses give a very favourable picture of the performance of the mCyp c 1 vaccine.

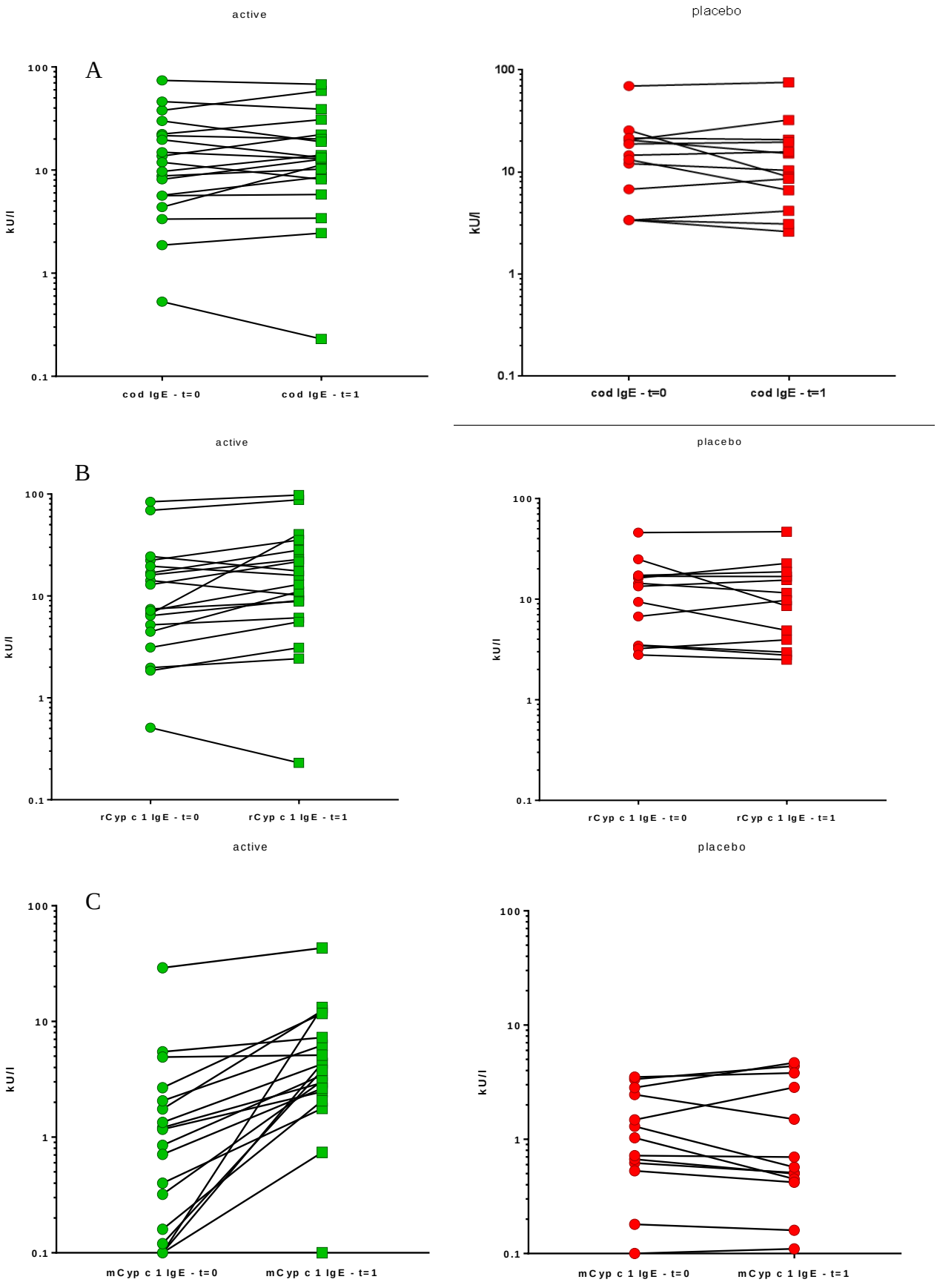


Figure 13. IgE responses in actively treated and placebo patients against cod fish extract (A), rCyp c 1 (B) and mCyp c 1 (C)

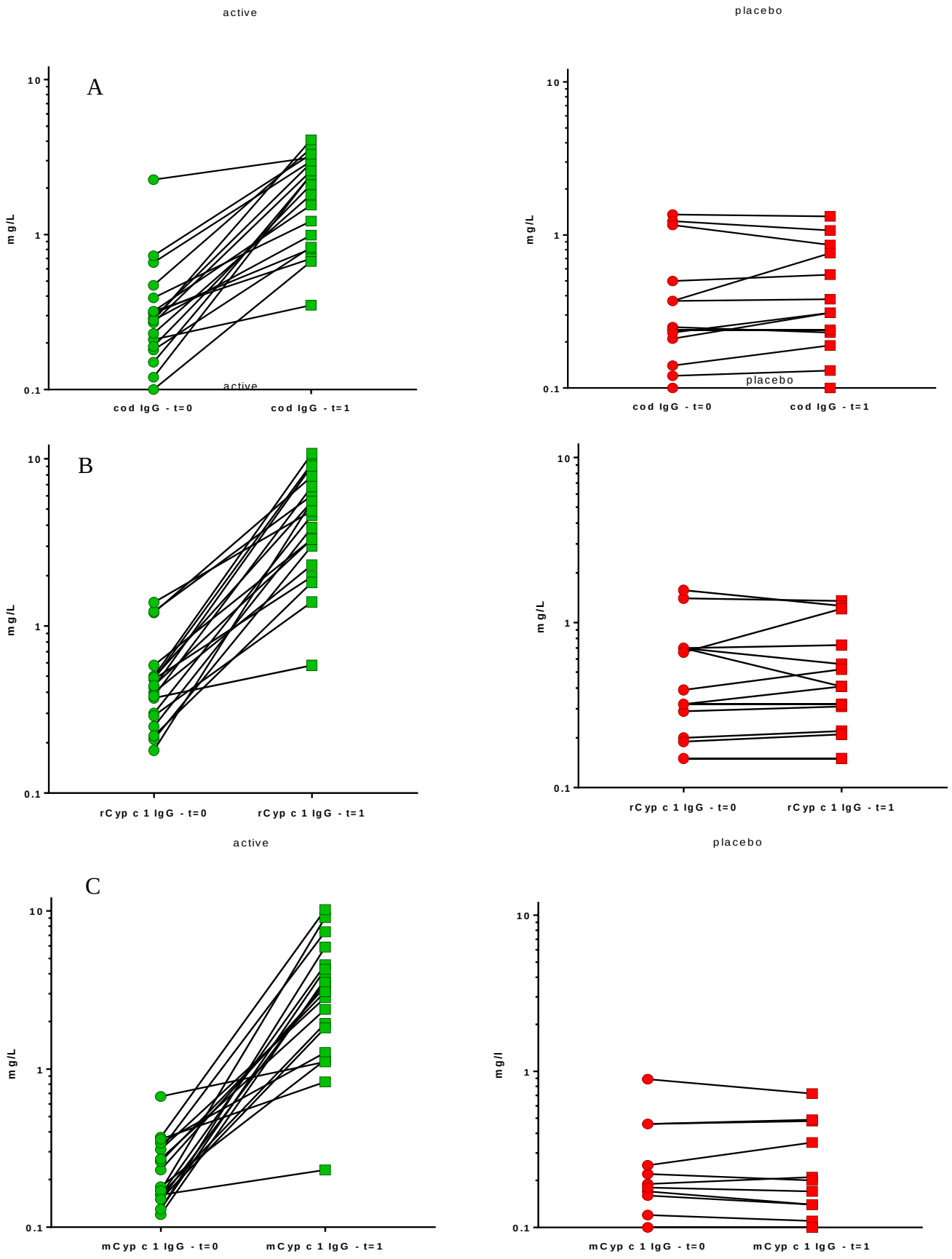


Figure 14. IgG responses in actively treated and placebo patients against cod fish extract (A), rCyp c 1 (B) and mCyp c 1 (C)

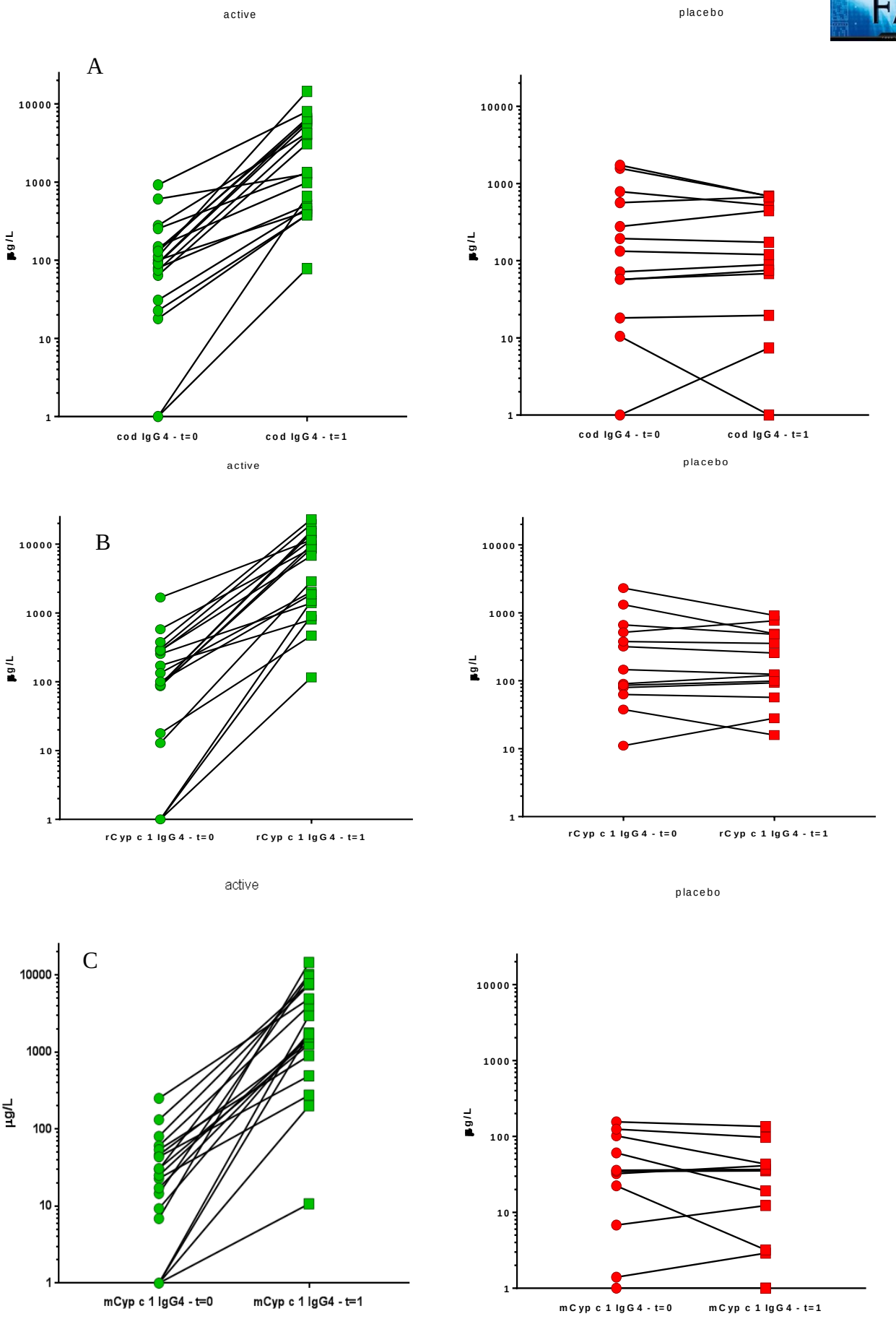


Figure 15. IgG_4 responses in actively treated and placebo patients against cod fish extract (A), rCyp c 1 (B) and mCyp c 1 (C).

4.2 Use and dissemination of foreground

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[3] (if available)	Is/Will open access[4] provided to this publication?
1	Development of a hypoallergenic recombinant parvalbumin for first-in-man subcutaneous immunotherapy of fish allergy.	Zuidmeer-Jongejan, L et al	Int Arch Allergy Immunol.	monthly	Karger		2015	166(1):41-51	DOI: 10.1159/000371657	yes
2	FAST: towards safe and effective subcutaneous immunotherapy of persistent life-threatening food allergies	Zuidmeer-Jongejan, L et al	Clin Transl Allergy.	monthly	BioMed Central		2012	9;2(1):5-14	doi:10.1186/2045-7022-2-5	yes
3	In vivo allergenic activity of a hypoallergenic mutant of the major fish allergen Cyp c 1 evaluated by means of skin testing	Douladiris et al.	J Allergy Clin Immunol.	monthly	Elsevier		2015	Aug;136(2):493-5	doi.org/10.1016/j.jaci.2015.01.015	yes

[3] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[4] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

	A general strategy for the generation of hypoallergenic molecules for the immunotherapy of fish allergy.	Swoboda et al.	J Allergy Clin Immunol.	monthly	Elsevier		2013	Oct;132(4):979-81	doi: 10.1016/j.jaci.2013.04.027	yes
4	Modified allergens and their potential to treat allergic disease.	L. Jongejan & R. van Ree	Curr Allergy Asthma Rep.	monthly	Springer		2014	Dec;14(12):478-488	DOI 10.1007/s11882-014-0478-9	yes
5	Food allergies: the basics.	Valenta et al.	Gastroenterology	monthly	Elsevier		2015	148:1120-1131	doi: 10.1053/j.gastro.2015.02.006.	yes
6	Hypoallergenic molecules for subcutaneous immunotherapy	Jongejan, L.	Expert Rev Clin Immunol.	monthly	Taylor and Francis		2016	12(1):5-7	DOI: 10.1586/1744666X.2016.1103182	yes
7	Blocking antibodies induced by immunization with a hypoallergenic parvalbumin mutant reduce allergic symptoms in a mouse model of fish allergy.	Freidl, R	J Allergy Clin Immunol.	monthly	Elsevier		2017	Jun;139(6):1897-1905.e1.	doi: 10.1016/j.jaci.2016.10.018.	yes
8	Allergy to fish-report of 5 cases	Drewnik A.	Alergia Astma Immunologia	December vol. 21	Mediton	Lodz	2016	206-2011	ISNN – 2083-2834	yes
9	Fish allergy	Drewnik A	Alergia Astma Immunologia	June vol.21	Mediton	Lodz	2016	88-	ISNN – 2083-2834	yes
10	Bioinformatics design of a hypoallergenic Pru p 3 for AIT	GG/SE	in preparation							
11	Safety and tolerability of hypoallergenic fish-allergen (mCyp c 1) subcutaneous immunotherapy – a phase I/IIa study	Malling, HJ et al	in preparation							
12	Safety and efficacy of immunotherapy with a recombinant hypoallergenic fish parvalbumin assessed in	Stavroulakis G, et al.	in preparation							

	a multicenter Phase IIb clinical trial across Europe									
13	Phase IIb clinical trial with a recombinant hypoallergenic fish parvalbumin: serum antibodies and their biological activity		in preparation							
14	Description of fish allergic patient population from Iceland, Denmark, Poland, Spain, Italy and Greece, including threshold data for reactions to fish.	Fernandez Rivas et al	in preparation							
15	Description of peach allergic patient population from Iceland, Denmark, Poland, Spain, Italy and Greece, including threshold data for reactions to fish.	Fernandez Rivas et al	in preparation							
16	Identification of T-cell epitopes of fish parvalbumin		in preparation							
17	Identification of T-cell epitopes of fish parvalbumin		in preparation							
18	Development of a mouse model for peach allergy		in preparation							

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

NO.	Type of activities[5]	Main leader	Title	Organisation	Date, Place	Type of audience[6]	Size of audience	Countries addressed
1	Conference presentation	P1	The FAST project	annual congress of the patient organization EFA (European Federation of Asthma and Allergy Associations)	Rome, April 2009	Scientific community	~500	EU
2	Conference presentation	P1	The FAST project	annual convention of the Australian Institute of Food Science and Technology	Brisbane, July 2009	Scientific community	~500	Australia
3	Press conference	P1,P4, P8, P10	joint press conference about FAST and EuroPrevall	EAACI congress	Warsaw, June 2009	Civil Society, Policy makers, Medias		EU
4	Interview	P1	The FAST project	Dutch radio (BNR)		Civil Society, Medias		
5	Conference presentation	P4	The FAST project	4th International Symposium on Molecular Allergology, ISMA	Munich October 2010	Scientific community	~100	EU
6	Poster	P3	The FAST project	Food allergy and anaphylaxis meeting (FAAM)	Venice, February 2011	Scientific community	~50	EU
7	Conference presentation	P1	The FAST project	FAAM	Venice, February 2011	Scientific community	~50	EU
8	Conference presentation	P1	"Allergenic Molecule-based Diagnosis: How it Drives Therapy Decision-making"	Allergy Drug Discovery and Development Meeting	San Diego, California, USA January 2012	Scientific community	~100	USA
9	Workshop	P7	Impact of Molecular Allergology on specific immunotherapy decision making	IDI-IRCCS Centre for Molecular Allergology	Rome, October 2012	Scientific community	~100	Italy
10	Conference presentation	P1	The FAST project	Italian Society for Allergology	Otranto, September, 2011	Scientific community	~100	Italy

[5] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[6] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias ('multiple choices' is possible.

11	Conference presentation	P1	The Choice of Hypo-allergens for Fish and Peach to Develop Food Allergy Specific Immunotherapy (The FAST Project)	World Allergy Congress	Cancun, Dec 2011	Scientific community	~100	worldwide
12	Conference presentation	P1	The FAST project	EAACI congress	Geneva, June 2012	Scientific community	~100	EU
13	Poster	P1	Challenges and pitfalls; lessons learned in trying to develop a suitable toxicity program for food allergy specific immunotherapy using hypo-allergens (the FAST project)	EAACI congress	Istanbul, June 2011	Scientific community	~100	EU
14	Poster	P3	Blinding of fish – recipes developed for the FAST project	EAACI/ GA^{2}LEN Food Allergy Training Course	Vienna, August 2012	Scientific community	~50	EU
15	Conference presentation	P17	Generation of new molecules for treatment of LTP-related food allergy	ICA Symposium	July, 2012, Salzburg, Austria	Scientific community	~50	EU
16	Conference presentation	P17	Development of a hypoallergenic Pru p 3 variant and investigation of chestnut as cross-reactive allergen source	ICA Symposium	July, 2012, Salzburg, Austria	Scientific community	~50	EU
17	Conference presentation	P5	Immunoterapia con alérgenos recombinantes de alimentos	Seminar of Innovation in the Hospital Clinico San Carlo	February 2013, Madrid	Clinicians	~50	Spain
18	Master thesis Angelika Hörschläger	P17	Master degree at the University of Salzburg, Austria		October 2012, Salzburg, Austria	Scientific Community, Civil Society	-	-
19	Master thesis Stephanie Eichhorn	P17	Master degree at the University of Salzburg, Austria		November 2012, Salzburg, Austria	Scientific Community, Civil Society	-	-
20	Marie Andeßner Award Ceremony	P17	Generation of new candidate molecules for treatment of LTP-related food allergy and Plantago lanceolata: an important sensitizer for summer pollinosis		2014, Salzburg, Austria	Civil Society	70	national
21	Conference presentation	P2	Antibodies induced by immunization with a hypoallergenic mutant of the major fish allergen Cyp c 1 inhibit allergic symptoms in a mouse model of fish allergy.	FAAM	February 2013, Nice, France	Scientific community	~50	EU
22	Conference	P1	Food allergy specific immunotherapy	FAAM	February	Scientific	~50	EU

	presentation		(FAST)		2013, Nice, France	community		
23	Conference presentation	P5	Fish allergy across Europe: results of a multicentre study within the FAST project	EAACI	June 2013, Milano, Italy	Scientific community	~50	worldwide
24	Conference presentation	P1	Food allergy specific immunotherapy (FAST)	EAACI	June 2013, Milano, Italy	Scientific community	~50	worldwide
25	Conference presentation	P17	Development of hypoallergenic products for immunotherapy	ISMA	Dec 2013, Vienna, Austria	Scientific community	~50	EU
26	Conference presentation	P17	Development of a hypoallergenic and immunogenic Pru p 3 proline variant for treatment of peach allergy	ISMA	Dec 2013, Vienna, Austria	Scientific community	~50	EU
27	Workshop	P1	Food allergy specific immunotherapy (FAST)	HAL -sponsored symposium for local clinicians	Feb 2014, Barcelona, Spain	Clinicians	~50	Spain
28	Workshop	P1	Food allergy specific immunotherapy (FAST)	HAL -sponsored symposium for local clinicians	Feb 2014, Valencia, Spain	Clinicians	~50	Spain
29	Workshop	P10	Natural history of fish allergy -Food allergy specific immunotherapy (FAST)	FAST Research workshop	April 2014, University of Athens, 'P&A Kyriakou' Children's Hospital, Athens	Clinicians	~50	Greece
30	Poster	P2	Hypoallergenic allergen derivatives of Pru p 3 for immunotherapy of IgE-mediated peach allergy	FAAM	February 2013, Nice, France	Scientific community	~50	EU
31	Poster	P2	Hypoallergenic allergen derivatives of Pru p 3 for immunotherapy of IgE-mediated peach allergy	2nd Meeting of Middle-European Societies for Immunology and Allergology	October 2013, Opatija, Croatia	Scientific community	~50	EU
32	Conference presentation	P5		ALORA, Alergia Alimentaria de Origen Animal	Sept. 2014, Cadiz, Spain	Scientific community	~50	Spain
33	Conference presentation	P1	Updating developing and established ASIT		April 2014, Bologna, italy	Scientific community	~150	EU

34	Conference presentation	P1	Food allergen immunotherapy		Dec 2014, Mysore, India	Scientific community	~150	Asia
35	Conference presentation	P1	Desensitization and tolerance in Food allergy	EAACI	June 2014, Copenhagen Denmark	Scientific community	~500	EU
36	Conference presentation	P1	Molecular therapeutics	FAAM	Feb 2014, Dublin, Ireland	Scientific community	~150	EU
37	Conference presentation	P1	Molecular Allergology in allergy diagnosis and treatment		July 2014, Hangzhou, China	Scientific community	~150	Asia
38	Workshop	P2	Recombinants in Immunotherapy'	Co-organized by the ITMOs Immunologie, Hématologie, Pneumologie & the ITMO Circulation, Métabolisme, Nutrition, & the French Society of Allergology	April 2014, Paris	Scientific community	~50	EU
39	Poster	P2	Development of a protocol for oral tolerance induction to food protein in a mouse model of fish allergy	Allergy School e-PAD: EAACI Practical Allergy Diagnosis	August 2015, Moscow, Russia	Scientific community	~50	EU
40	Poster	P2	Development of a mouse model to study tolerance induction to the major fish allergen parvalbumin	European Congress of Immunology (ECI),	September 2015, Vienna, Austria	Scientific community	~50	EU
41	Medical conference	P16	#3302 Symposia: EAACI: Bringing Molecular Diagnosis and Treatment Closer to the Bedside: European Trials Immunotherapy with Modified Molecular Components: The Experience of FAST	February 20-24, 2015	2015 AAAAI Annual Meeting in Houston, TX	Global allergologists	about 300	Global
42	Medical conference	P16	Hypoallergenic molecules for subcutaneous immunoterhapy (SCIT)	June 6-10, 2015	EAACI 34th Congress, Barcelona, Spain	Global allergologists	about 300	Global
43	Conference	P2	ÖGAI symposium „50 years of B-lymphocytes"	December 2016	Vienna, Austria	Scientific community		Austria
44	Conference	P2	Conference on 'Immune mediated reactions to food'	February 2016	Teheran, Iran	Scientific community		Iran
45	Medical conference	P16	Immunotherapy for food allergy with hypoallergens	May 18-21, 2016	CMICA LXX Congreso Nacional de	Mexican and other Spanish-	about 200	Latin America

					Immunologia Clincal y Allergia, Puerto Vallarte, Mexico	speaking allergologists		
46	Conference	P2	3rd Training for Trainers of the International Network of Universities for Molecular Allergology and Immunology	June 2016	Vienna, Austria	Scientific community		International
47	Medical conference	P16	Hypoallergenic molecules for Allergen Specific Immunotherapy	October 13-15, 2016	Food Allergy & Anaphylaxis Meeting (FAAM), Rome, Italy	Global allergologists	about 200	Global
48	Workshop	P10	Research Workshop, Allergy Dpt, 2nd Pediatric Clinic, UoA	12 April 2013	Athens Greece	Scientific Community (higher education, Research)	100 persons	Greece
49	Workshop	P10	"Standardizing treatment in allergic diseases", 20th Workshop, Allergy Dpt, 2nd Pediatric Clinic, UoA	8-10 May 2015	Pelion Greece	Scientific Community (higher education, Research)	100 persons	Greece
50	Oral presentation to a scientific event	P10	Shanghai Oriental Pediatric Congress "Children Food Allergy: Food Avoidance and Intake in Early Life."	October 2015	Shanghai, China	Scientific Community (higher education, Research)	150	Shanghai, China
51	Conference	P10	6th International Symposium on Molecular Allergology	19-21 November 2015	Lisbon Portugal	Scientific Community (higher education, Research)	300 persons	International
52	Oral presentation to a scientific event	P10	Lecture to Singapore University – "Advances in the immunotherapy of food allergy"	February 2017	Singapore, Asia	Scientific Community (higher education, Research)	150	Singapore, Asia

53	Oral presentation to a scientific event	P10	Lecture to Singapore University – "How to achieve tolerance to food"	February 2017	Singapore, Asia	Scientific Community (higher education, Research)	150	Singapore, Asia
54	Conference	P10	European Academy of Allergy and Clinical Immunology (EAACI) Annual Congress	17-21 June 2017	Helsinki Finland	Scientific Community (higher education, Research)	500 persons	Inter-national
55	Flyers	P10	Research Workshop, Allergy Dpt, 2nd Pediatric Clinic, UoA	12 April 2013	Athens Greece	Scientific Community (higher education, Research)	100	Greece
56	Flyers	P10	20th Workshop, Allergy Dpt, 2nd Pediatric Clinic, UoA	8-10 May 2015	Pelion Greece	Scientific Community (higher education, Research)	100	Greece
57	Academic year report	P10	Academic year report 2013-2014	Sept 14	2nd Pediatric Clinic, University of Athens, 'P&A Kyriakou' Children's Hospital, Athens, Greece	Scientific community (higher education research), Faculty of NKUA, Hospital personnel, Industries	150	Greece
58	Academic year report	P10	Academic year report 2014-2015	Sept 15	2nd Pediatric Clinic, University of Athens, 'P&A Kyriakou' Children's Hospital, Athens, Greece	Scientific community (higher education research), Faculty of NKUA, Hospital personnel, Industries	150	Greece
59	Conference,	P17	International Symposium on Molecular	5-7 December 2013	Vienna,	Scientific	350	International

	invited plenary talk		Allergology		Austria	Community		
60	Conference, selected oral presentation	P17	Annual Congress of the European Academy of Allergy and Immunology	7-11 June 2014	Copenhagen , Denmark	Scientific Community	100	International
61	Conference, oral presentation	P17	Symposium of the PhD program Immunity in Cancer & Allergy	30 June – 1 July 2014	Salzburg, Austria	Scientific Community	90	Austria
62	Conference, poster presentation	P17	PMU Science Get Together	3 June 2014	Salzburg, Austria	Scientific Community	150	Austria
63	Conference, poster presentation	P17	Annual Congress of Austrian Allergy and Immunology Society, Salzburg, Austria	6-8 November 2014	Salzburg, Austria	Scientific Community	350	National/Inter national
64	PhD thesis Stephanie Eichhorn	P17	PhD degree at the University of Salzburg, Austria	September 2016	Salzburg, Austria	Scientific Community, Civil Society	-	-
65	Press release	P17	Universität Salzburg prämiert Nachwuchswissenschaftlerinnen	18 March 2014	Salzburg, Austria	Civil Society		
66	Article published in popular press	P17	Nachwuchswissenschaftlerinnen ausgezeichnet	18 March 2014	Salzburger Nachrichten, Austria	Civil Society		
67	Radio broadcast	P17	Radio Salzburg	19 March 2014	Salzburg, Austria	Civil Society		
68	Press release	P17	Neue Therapieansätze bei Pfirsichallergie	26 March 2014	Salzburg, Austria	Civil Society		
69	Article published in popular press	P17	Durchbruch bei Pfirsichallergien	27 March 2014	Salzburger Nachrichten, Austria	Civil Society		
70	Article published in popular press	P17	Preis für Nachwuchswissenschafterinnen	1 April 2014	Salzburger Nachrichten, Austria	Civil Society		
71	Article published in popular press	P17	Ihr Ziel ist eine Impfung gegen Allergien	27 March 2014	Salzburger Nachrichten, Austria	Civil Society		
72	Article published in popular press	P17	Erfolgreich Forschen	13 April 2014	Die Kronen Zeitung, Austria	Civil Society		

73	Conference: XII International Congress of Polish Society of Allergology	P8	„Fish Hypersensitivity –clinical profile of patients" –poster presentation	9-12.09.2015	Bydgoszcz, Poland	Scientific Comunity		
74	Conference	P8	"Fish hypersensitivity:clinical manifestations and fish-specific IgE-sensitization" poster discussion session	EAACI Congress June 2016	Vienna, Austria	Scientific Comunity	50	International
75	Conference	P8	Fish Hypersensitivity –clinical profile of patients"and specific IgE presence	XV Conference Allergy, Asthma Clinical Immunology (AAIK: Alergia, Astma, Immunologia Kliniczna), June 2016	Łódź, Poland	Scientific Comunity	500	Poland
76	Conference	P8	Allergy to fish-Immunotherapy –oral presentation, clinical cases presentation	XVI AAIK, June 2017	Łódź, Poland	Scientific Comunity	500	Poland
77	Conference	P2	Young scientists association (YSA) PhD Symposium	June 2017	Vienna, Austria	Scientific Comunity		Austria

Section B (Confidential[7] or public: confidential information to be marked clearly)
Part B1

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[8]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	no	-	EP13171445.3	Pru p 3 mimicks	Gabriele Gadermaier, Fatima Ferreira, Peter Lackner, Michael Wallner
Patent	no	13/04/2010	US7696314 B2 US 10/924,200	Hypoallergenic mutant polypeptides based on fish parvalbumin	Rudolf Valenta, Peter Valent, Susanne Spitzauer, Ines Swoboda, Biomay AG
Patent	no	27/06/2013	WO2013092953 PTC/EP2012/076553 EP2607376A1	Hypoallergenic allergen derivatives of Pru p 3 for immunotherapy of IgE-mediated peach allergy	Birgit Linhart, Antonia Gstöttner, Rudolf Valenta, Nikolaos Papadopoulos, Adriano Mari, Cristina Gamez, Ines Swoboda, Biomay AG
Patent	no	01/05/2014	US20140121356 A1 US 14/137,341	Hypoallergenic mutant polypeptides based on fish parvalbumin	Rudolf Valenta, Peter Valent, Susanne Spitzauer, Ines Swoboda, Biomay AG

[7] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[8] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Type of Exploitable Foreground[9]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application[10]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Commercial exploitation R&D results	Master Cell Bank for mCyp c 1	NO	NA		pharma industry	immediate		Biomay
Commercial exploitation R&D results	GMP process for mCyp c 1 drug substance	YES	NA		pharma industry	immediate		Biomay
Commercial exploitation R&D results	GMP process for mCyp c 1 drug product	YES	NA		pharma industry	immediate		Biomay HAL Allergy
Commercial exploitation R&D results	Results Phase I/IIa and Phase IIb clinical trials: vaccine is safe	NO	NA		medical / health care	3-5 years		FAST consortium
Commercial exploitation R&D results	Results Phase IIb clinical trial: vaccine has perspective for further testing	NO	NA		medical / health care	3-5 years		FAST consortium
Exploitation of results through innovation	Pru p 3 mutant C1	NO			pharma industry medical / health care	8-10 years	EP13171445.3	PLUS
Exploitation of results through innovation	Pru p 3 shuffled trimer BAC3	YES?			pharma industry medical / health care	8-10 years	WO2013092953 PTC/EP2012/076553 EP2607376A1	MUV

[9] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[10] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

The FAST project aimed at bringing two innovative vaccines for treatment of food allergy to the stage of evaluation in Phase IIb clinical trials. For fish allergy, the consortium succeeded in reaching that stage of clinical development, for fruit (peach) allergy this was not achieved. The project started out with a series of vaccine candidates for both allergies, i.e. 2 for fish allergy and 5 plus 5 extra (added in the course of the project) for fruit allergy. For fish, the selection process resulted in a single vaccine candidate to be further developed, i.e. mCyp c 1. At the start of the project, Biomay already owned a patent on this molecule and brought that Background into the project. The Consortium Agreement regulates how that Background can be used in the project, but more importantly also after the project in case the outcome is of Phase IIb trial being favourable enough to proceed with further clinical development (Phase III). To reach the stage of clinical evaluation in patients, steps had to be made on the pharmaceutical production side. In this process foreground was created.

Master Cell Bank

Recombinant proteins such as mCyp c 1 are produced in cultured cells, in this case *E.coli* bacteria. While in culture, these cells must be passaged regularly increasing the chance of genetic alteration, contamination, or loss of expression constructs as the cells divide. Thus, for optimal drug production, it is critical that cells are passaged as few times as possible and that the original high producing clone is not lost or altered. The purpose of a master cell bank is to create a resource of the original therapeutic-producing cell, cryopreserved in multiple vials to prevent genetic variation and potential contamination by eliminating the total number of times a cell line is passaged or handled during the manufacturing process. This Master Cell Bank was created by Biomay and is of high value for the further exploitation of the vaccine. It is the starting point of the production of the vaccine. In case the product is further developed, this resource may be used by Biomay (the owner of IP related to mCyp c 1) and/or by other parties to which the Bacgound IP on mCyp c 1 is licensed out.

GMP process for mCyp c 1 drug substance

Biomay has developed a GMP process including all QA and QC protocols for the production of the mCyp c 1 drug substance. Because the documentation linked to this process contains confidential information about company processes, this exploitable foreground is designated as confidential. Having said that, the information is essential for further exploitation of the novel vaccine for the treatment of fish allergy. In case other parties than Biomay will be involved in further clinical development of the vaccine, sharing of necessary information will be part of the licensing agreement. Like the Master Cell Bank, the GMP process and QA/QC protocols are essential elements for further clinical development to secure that future batches of drug substance will be comparable to the drug substance that was used in both FAST clinical trials.

GMP process for mCyp c 1 drug product

Biomay and HAL Allergy have developed a GMP process including all QA and QC protocols for the production of the mCyp c 1 drug product. Because the documentation linked to this process contains confidential information about company processes, this exploitable foreground is designated as confidential. Having said that, the information is essential for further exploitation of the novel vaccine for the treatment of fish allergy. In case other parties than Biomay and/or HAL Allergy will be involved in further clinical development of the vaccine, sharing of necessary information will be part of the licensing agreement. Like the

Master Cell Bank, the GMP process and QA/QC protocols are essential elements for further clinical development to secure that future batches of drug substance will be comparable to the drug substance that was used in both FAST clinical trials.

Results Phase I/IIa and Phase IIb clinical trials: vaccine is safe

By means of two clinical trials it has been convincingly demonstrated that the mCyp c 1 vaccine is very safe and well-tolerated. This is a true asset of the vaccine that was developed in FAST, compared to other immunotherapy treatments under development such as oral immunotherapy which is troubled by frequent and often quite severe side-effects. The documentation of its safety and tolerability is of pivotal value in further development of the novel vaccine. The results on the safety and tolerability of the mCyp c 1 vaccine will be in the public domain and will be used in future clinical development, such as in the clinical part of future IMPDs.

Results Phase IIb clinical trial: vaccine has perspective for further testing

By means of a Phase IIb clinical trial evidence has been obtained for efficacy of the mCyp c 1 vaccine. Although the primary endpoint of the study was not met, post-hoc analysis on subgroups of patients has provided a likely explanation why this endpoint was not met (placebo effect in patients with subjective symptoms only).Together with very positive outcomes of several secondary outcomes support efficacy of the vaccine. The outcome of the study warrants further clinical development and provides very useful leads for design of follow-up trials. The results of the efficacy trial will be in the public domain and will be used in future clinical development, such as in the clinical part of future IMPDs.

Pru op 3 mutant C1

This molecule came out of the second round of evaluations of candidate hypo-allergenic Pru p 3 variants. The molecule brings together acceptable hypo-allergenicity, immunogenicity and stability. The time constraints of the project did not allow further (in vivo) evaluation. It's characteristics do however warrant further exploration. The molecule has been patented. Especially for the Southern European market where fruit allergy linked to LTP is prevalent, the molecule could raise interest from industry (including one of the FAST partners: BIAL).

Pru op 3 shuffled trimer

This molecule came out of the second round of evaluations of candidate hypo-allergenic Pru p 3 variants. The molecule brings together acceptable hypo-allergenicity, immunogenicity and stability. The time constraints of the project did not allow further (in vivo) evaluation. It's characteristics do however warrant further exploration. The molecule has been patented. Especially for the Southern European market where fruit allergy linked to LTP is prevalent, the molecule could raise interest from industry (including one of the FAST partners: BIAL).

Overall impact of Foreground

The FAST project has delivered its major outcome, a vaccine for the treatment of fish allergy evaluated up to the stage of a first Phase IIb clinical trial, at a time where initiatives to develop immunotherapy for food allergy are really in the spotlight. Together the Foreground items related to fish allergy are a promising package to bring this treatment to the market in a subsequent Phase III trial. In the coming year, the commercial partner in FAST will discuss on possible nest steps to be taken. One of the avenues to be investigated is whether the vaccine would qualify as an orphan drug. This would make the follow-up program more

feasible and less burdensome and costly. The FAST project stands out with a number of unique features in the field of immunotherapy for food allergy. All current clinical development programs are focused on native allergenic drug substances and drug products The FAST vaccine is the only (safer) hypo-allergenic approach. In addition, the other initiatives all use products based on whole (processed) peanut source materials or extracts thereof. The FAST approach is based on a well-defined single molecule product. And last but not least, the FAST project has provided support for safe use of the subcutaneous route, a route for which there is broad consensus that it is very efficient and that it facilitates good adherence and close monitoring of safety (no self-administration at home). The FAST project may renew attention for the subcutaneous route, which was abandoned completely after the bad experience in the USA in the early nineties with an aqueous peanut extract. The hypo-allergenic depot vaccine of FAST has proven that safe subcutaneous immunotherapy is possible.

4.3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	201871
Title of Project:	TOWARDS SAFE AND EFFECTIVE IMMUNOTHERAPY OF PERSISTENT LIFE-THREATENING FOOD ALLERGIES
Name and Title of Coordinator:	Prof. Dr. Ronald van Ree / PhD

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***XYes 0No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	X
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	X
• Did the project involve Human data collection?	X
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	X
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	X
• Were those animals transgenic small laboratory animals?	
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	

DUAL USE	
• Research having direct military use	
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator		1
Work package leaders	3	2
Experienced researchers (i.e. PhD holders)	25	17
PhD Students	5	
Other	48	34

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**4**
Of which, indicate the number of men:	0

D Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project**?	X ○	Yes No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
X	Design and implement an equal opportunity policy	○	○	○	○	X
X	Set targets to achieve a gender balance in the workforce	○	○	○	○	X
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No (NA)

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

x Yes- please specify (Master and PhD students, several prizes awarded to students, Open Science Day)

○ No

Master/PhD students, open days, Lange Nacht der Forschung 22. April 2016

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

X No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

○ Main discipline[11]: 3.2

○ Associated discipline[11]:1.5

○ Associated discipline[11]:3.1, 3.3 (nursing)

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	○ X	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[11] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

○	No
○	Yes- in framing the research agenda
X	Yes - in implementing the research agenda, European Medicines Agency + national authorities
○	Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

○	Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
○	Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
X	No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**10**
To how many of these is open access[12] provided?	**All (after embargo has expired)**
How many of these are published in open access journals?	**0**
How many of these are published in open repositories?	**0**
To how many of these is open access not provided?	**0**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available x no suitable open access journal available x no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[13]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**4**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	X	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

[12] Open Access is defined as free of charge access for anyone via Internet.
[13] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
Difficult to estimate / not possible to quantify	x

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes x No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

x Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

x	Press Release	❑	Coverage in specialist press
❑	Media briefing	x	Coverage in general (non-specialist) press
❑	TV coverage / report	x	Coverage in national press
x	Radio coverage / report	❑	Coverage in international press
❑	Brochures /posters / flyers	❑	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	X	English
x	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]