Final Report Summary - FAST (TOWARDS SAFE AND EFFECTIVE IMMUNOTHERAPY OF PERSISTENT LIFE-THREATENING FOOD ALLERGIES)

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 the 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.

Project Context and Objectives:
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.
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
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.

Project Results:
Main S&T results (this is readable in the attached pdf...)
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 assess 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.
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 less 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°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...
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 40°C 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 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 dose 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
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 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.
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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.

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.
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 IgG4 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

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<th>Active Ν=19</th>
<th>Placebo Ν=13</th>
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<td>Differences (post-pre) Mean(SD) Median(IQR) Mean(SD) Median(IQR) p-value</td>
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<td>slG G (kU/L) cod</td>
<td>1.61 (1.05) 1.58 (0.65 2.31) 0.01 (0.15) 0.01 (-0.02 0.05) 0.001</td>
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<tr>
<td>slG G (kU/L) rCyp c 1</td>
<td>4.32 (2.81) 3.62 (1.91 6.44) 0.005 (0.21) 0.02 (-0.05 0.03) 0.001</td>
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<tr>
<td>slG G (kU/L) rCyp c 1 mut</td>
<td>3.36 (2.79) 2.82 (0.96 4.4) -0.003 (0.06) 0.01 (-0.02 0.02) 0.001</td>
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<tr>
<td>slG G4 (kU/L) cod</td>
<td>3226.5 (3677.2) 1070.1 (428.8 5782.9) -146.78 (376.6) 1.5 (-20.6 17.7) &lt;0.001</td>
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<tr>
<td>slG G4 (kU/L) rCyp c 1</td>
<td>7188.2 (6896.4) 6492 (1138.2 11140.1) -170.9 (443.2) -21.5 (-62.3 13.7) &lt;0.001</td>
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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 IgG4, to fish allergen. There are no significant changes in clinical reactivity, however in the post-hoc
serum IgG4, 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 IgG4 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 IgG4 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).

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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)
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. IgG4 responses in actively treated and placebo patients against cod fish extract (A), rCyp c 1 (B) and mCyp c 1 (C).

Potential Impact:
4.2 Use and dissemination of foreground (this is readable in the attached pdf...)

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

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<th>Publisher</th>
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<th>Year of publication</th>
<th>Relevant pages</th>
<th>Permanent identifiers (if available)</th>
<th>Is/Will open access provided to this publication?</th>
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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 a multicenter Phase IIb clinical trial across Europe Stavroulakis G, et al. in preparation
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 Main leader Title Organisation Date, Place Type of audience 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
11 Conference presentation P1 The Choice of Hypo-allergens for Fish and Peach to Develop Food Allergy...
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/ GA2LEN Food Allergy Training Course Vienna, August 2012 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 Clínico 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 presentation P1 Food allergy specific immunotherapy (FAST) FAAM February 2013, Nice, France Scientific community ~50 EU
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
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 immunotherapy (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 Immunologia Clinical y Allergia, Puerto Vallarte, Mexico Mexican and other Spanish-speaking allergologists about 200 Latin America
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
Medical conference P10
Hypoallergenic molecules for Allergen Specific Immunotherapy October 13-15, 2016 Food Allergy & Anaphylaxis Meeting (FAAM), Rome, Italy Global allergologists about 200 Global

Workshop P10 Research Workshop, Allergy Dpt, 2nd Pediatric Clinic, UoA 12 April 2013 Athens Greece Scientific Community (higher education, Research) 100 persons Greece

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

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

Conference P10 6th International Symposium on Molecular Allergology 19-21 November 2015 Lisbon Portugal Scientific Community (higher education, Research) 300 persons International

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

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

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 International

Flyers P10 Research Workshop, Allergy Dpt, 2nd Pediatric Clinic, UoA 12 April 2013 Athens Greece Scientific Community (higher education, Research) 100 Greece

Flyers P10 20th Workshop, Allergy Dpt, 2nd Pediatric Clinic, UoA 8-10 May 2015 Pelion Greece Scientific Community (higher education, Research) 100 Greece

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

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

Conference, invited plenary talk P17 International Symposium on Molecular Allergology 5-7 December 2013 Vienna, Austria Scientific Community 350 International

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

Conference, oral presentation P17 Symposium of the PhD program Immunity in Cancer & Allergy 30 June – 1 July 2014 Salzburg, Austria Scientific Community 90 Austria

Conference, poster presentation P17 PMU Science Get Together 3 June 2014 Salzburg, Austria Scientific Community 150 Austria

Conference, poster presentation P17 Annual Congress of Austrian Allergy and Immunology Society, Salzburg, Austria 6-8 November 2014 Salzburg, Austria Scientific Community 350 National/International

PhD thesis Stephanie Eichhorn P17 PhD degree at the University of Salzburg, Austria September 2016 Salzburg, Austria Scientific Community Civil Society
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 Nachwuchswissenschaftlerinnen 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 Community

74 Conference P8 “Fish hypersensitivity: clinical manifestations and fish-specific IgE-sensitization” poster discussion session EAACI Congress June 2016 Vienna, Austria Scientific Comunity

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 Community

76 Conference P8 Allergy to fish-Immunotherapy –oral presentation, clinical cases presentation XVI AAIK, June 2017 Łódź, Poland Scientific Community

77 Conference P2 Young scientists association (YSA) PhD Symposium June 2017 Vienna, Austria Scientific Community

Section B (Confidential 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: 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 Prelu 3 mimicks Gabriela Gadomskaie, Fatima Ferreira, Peter Lackner
Part B2

Type of Exploitable Foreground Description
of exploitable foreground Confidential
Click on YES/NO Foreseen embargo date Exploitable product(s) or measure(s) Sector(s) of application
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
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 c1. 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 c1 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 c1) and/or by other parties to which the Background IP on mCyp c1 is licensed out.

GMP process for mCyp c1 drug substance
Biomay has developed a GMP process including all QA and QC protocols for the production of the mCyp c1 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 c1 drug product
Biomay and HAL Allergy have developed a GMP process including all QA and QC protocols for the production of the mCyp c1 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.
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 next 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
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.

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