PROJECT FINAL REPORT

Grant Agreement number: 601763

Project acronym: BM4SIT

Project title: AN INNOVATIVE CAUSAL THERAPY FOR ALLERGY: SAFE AND RAPID INDUCTION OF AN ANTI-INFLAMMATORY IMMUNE RESPONSE USING A MUTANT HYPOALLERGEN AND VITAMIN D3

Funding Scheme: FP7-HEALTH-2013-INNOVATION-1/ HEALTH.2013.1.3-3: Safety and efficacy of therapeutic vaccines

Period covered: **from 01-02-2014** **to 31-01-2019**

Name of the scientific representative of the project's co-ordinator[1], Title and Organisation:

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.bm4sit.eu

[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.euabc/symbols/emblem/index_en.htm ; logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

Final publishable summary report

1. Executive summary

Treatment of hay fever caused by birch pollen is most commonly treated by symptomatic drugs. Allergen immunotherapy (AIT) is the only treatment that can achieve sustained efficacy by targeting the underlying mechanism, i.e. a causal treatment. The main reasons for the niche position of AIT are the risk of potentially severe allergic side-effects and the three to five years administration of monthly injections needed to achieve sustained efficacy. The BM4SIT project aimed at improving the safety and efficacy of birch pollen AIT, thereby making it a more attractive alternative to chronic lifelong symptomatic drug use. The approach chosen to achieve this is based on two pillars: 1) replacement of currently used birch pollen extract by a recombinant hypo-allergenic but immunogenic version of the major birch pollen allergen Bet v 1, the BM41 molecule; 2) addition of an immune-modulatory adjuvant vitamin D3 that promotes anti-allergic protective immunity. BM41 is expected to make the treatment less prone towards allergic side-effects, and more effective in stimulating production of protective allergen-specific IgG_4 antibodies. Vitamin D3 is thought to induce the anti-allergic immune response more rapidly. Together, both innovations are predicted to increase safety, enlarge the effect-size and more rapidly induce the protective immune response.

To evaluate the health economics perspective of AIT, the BM4SIT project participated in a systematic literature review coordinated by the European Academy of Allergy and Clinical Immunology (EAACI) in the frame of establishing new guidelines for AIT. The outcome of this process has provided health economic support for AIT, and these results are widely disseminated to relevant stakeholders.

During the project, mouse models were performed to obtain additional pre-clinical support for both innovations. Despite the fact that birch pollen allergy induced in mice differs from human allergy in the sense that the role of the major allergen Bet v 1 is far less dominant than in man, the experiments nevertheless provided further evidence for the increased safety and efficacy profile of BM41 compared to Bet v 1, and for the added value of vitamin D3 as adjuvant.

The potential added value of vitamin D3 in man was tested in two subsequent clinical trials. The first trial was a study in which the adjuvant was tested for safety, tolerability and immune-modulatory potency. The outcome was that it was safe and well tolerated. Several immune outcomes provided support for immune-modulation away from allergic inflammation. In a follow-up trial, a comparison was made between a current AIT product and the same product with vitamin D3. The clinical trial extending outside the BM4SIT contract was successfully completed in Amsterdam. Analyses of biological samples and of collected data could not be completed during the BM4SIT contract, due to the previous delay caused by the new toxicity studies necessary with BM41. The BM4SIT project achieved in producing a GMP-compliant BM41 vaccine for first-in-man testing. Before reaching that stage, two toxicity studies in rats were needed to establish a safe dose, the no-observed adverse-effect level (NOAEL). The established NOAEL was 40 µg per injection. To build in an extra safety margin, it was decided to use 20 µg in the first-in-man clinical trial.

A first-in-man clinical trial was designed in which BM41 was tested in a randomized double-blind placebo-controlled fashion, also including a third open comparator arm with a current AIT product. The clinical trial extending outside the BM4SIT contract was successfully completed in Odense. Analyses of biological samples and of collected data could not be completed during the BM4SIT contract, for similar reasons as mentioned above.

In summary, the BM4SIT project has successfully brought both the BM41 molecule and vitamin D3 to clinical testing. The outcome of these clinical evaluations is still unclear until additional funding can be secured.

2. A summary description of project context and objectives

Safer, better and quicker therapeutic vaccines

Allergy is the most prevalent chronic inflammatory disease, having great socio-economic impact. Chronic IgE-mediated allergies like allergic rhino-conjunctivitis (hay fever) and allergic asthma, induced by exposure to environmental allergens such as pollen and house dust mite, can in most cases be suppressed effectively by symptomatic drugs such as antihistamines, local and systemic corticosteroids and anti-leukotrienes. Chronic lifelong dependency on symptomatic drugs is reality for the vast majority of allergy sufferers, which in some cases, for example prolonged use of systemic corticosteroids, can be accompanied by undesirable side-effects.

Allergen-specific immunotherapy (AIT) is the only causal and effective treatment targeting the underlying immune mechanism in a more cost-effective way, suppressing symptoms effectively not only during treatment but even after its cessation. For achieving such sustained effects, however, years of monthly injections are required with the culprit of the allergic reaction, i.e. with the bioactive allergen, usually adsorbed to a depot like aluminium hydroxide (alum). Daily sublingual administration of allergen extract as drops or tablets for years is an alternative that is considered almost similarly effective.

These protocols are not only of long duration often leading to poor compliance, but can also be accompanied by (sometimes severe) allergic side-effects, having additional impact on adherence. For good efficacy compliance is however pivotal. The two hallmarks of effective SIT are the induction of allergen-specific non-inflammatory (protective) IgG_4 and IgA antibodies and of anti-inflammatory regulatory T-cells (Tregs) and the cytokines IL-10 and TGFβ (messenger molecules of the immune system). Together these immune players dampen the disease provoking effects of allergen-specific IgE antibodies and Th2 cells.

BM4SIT stands for "**B**et v 1 **M**utant for [**4**] **S**pecific **I**mmuno **T**herapy". BM4SIT aims at providing an escape from its current niche position by making the treatment safer, more effective and patient friendlier, i.e. developing a therapy with negligible side-effects but improved efficacy, achieved by less injections. The concept of BM4SIT is based on two innovations: replacing current natural allergen extracts by mutated recombinant allergens having been designed to be hypo-allergenic but hyper-immunogenic, and adding an adjuvant (immune-stimulatory compound) to support more rapid and effective induction of an anti-inflammatory protective immune response. The project focuses on birch pollen allergy, the third most common respiratory allergic disease in Europe. Birch pollen allergy is caused by a single major allergen, a protein called Bet v 1. This molecule is responsible for induction of allergic reactions virtually on its own. Current AIT is carried out with birch pollen extracts. It has been demonstrated that AIT can also be effectively performed with just its component, the major allergen Bet v 1 instead of the whole extract. In both cases, therapy is however performed with the unmodified culprit of the allergic reaction, the bioactive allergen. The concept of BM4SIT is to instead administer a mutant of Bet v 1, the BM41 molecule. BM41 was designed as a so-called "hypo-allergen", meaning that its safety profile is greatly enhanced by reduced recognition by IgE antibodies of birch pollen allergic patients. On top of that, the BM41 molecule was engineered to be highly efficient for the treatment of birch pollen allergy, as it has increased immunogenicity. Compared to the original allergen Bet v 1, it is expected to more vigorously induce protection. Thus, BM41 is designed to **reduce allergenic activity but at the same time enhance immunogenic properties**, i.e. AIT with a molecule with a negligible risk of allergic side-effects that more effectively induces a protective allergen-specific IgG_4 and IgA antibody response. Pre-clinical data have provided support for strongly reduced allergenicity by showing highly reduced recognition of BM41 compared to Bet v 1 by human IgE, and for significantly increased immunogenicity and efficacy in a mouse model for birch pollen allergy.

Switch of the immune system away from the allergic IgE antibodies to the production of protective IgG_4 and IgA antibodies and the induction of anti-inflammatory Tregs and cytokines (IL-10 and

TGFβ) normally requires chronic exposure to the antigen, hence the long duration of current AIT protocols. In BM4SIT, the concept of a hypo-allergenic but immunogenic mutant Bet v 1 will therefore be complemented by the use of an adjuvant to more **rapidly skew** the immune system **towards an allergen-specific non-/anti-inflammatory immune response**. The selected adjuvant is vitamin D3, which has been shown to promote active suppression of inflammation, both in vitro and in a mouse model. The novel adjuvant vitamin D3 is therefore expected to help to increase treatment efficacy by exploiting its anti-inflammatory properties, allowing earlier onset of treatment effect.
In summary, BM4SIT aims to improve the quality of life of patients with birch-pollen induced respiratory allergy. This shall be accomplished by the decreasing treatment burden (less injection with less side-effects), thus allowing an easier choice away from chronic symptomatic drug use to more curative AIT protocols.
Ergo, the **overall objective** of BM4SIT is:

- **a significantly safer (BM41) treatment of birch pollen allergy achieving higher efficacy (BM41) more rapidly (vitamin D_3)**

The major building stones of the approach are:

- **improved safety** by using the derivative of Bet v 1, BM41, with **hypoallergenic properties**
- **enhanced efficacy** by using the derivative of Bet v 1, BM41, with **enhanced immunogenicity**
- **more rapid efficacy** by using vitamin D_3 as adjuvant promoting **anti-inflammatory responses**

An effective consortium of scientific, clinical and industrial excellence
BM4SIT will be carried out by a **small product-focused multidisciplinary consortium**. The nucleus of the consortium is formed by an Austrian biotech SME (small to medium-sized enterprise), Biomay, bringing in the relevant background IP (patent) on BM41, essential for future protection and exploitation, and the industrial environment and expertise needed to bring academic knowledge from bench to bedside. This SME will be supported by two strong leading academic research groups in the field of molecular biology, proteomics and allergo-immunology, a GLP-accredited laboratory for pre-clinical toxicity studies and four leading European clinical research centres of which two qualify as SME. Together this consortium is perfectly suited to produce the proposed drug substances and drug products, complying with GMP (good manufacturing practice) regulations and GCP (good clinical practice) requirements needed for human clinical trials.
The product-focused, IP-protected consortium of BM4SIT aims at bringing BM41 and/or vitamin D3 closer to Phase III clinical trials (the last step before market authorization) via the stages of GMP production, toxicity studies, and Phase I and/or II clinical trials. The ultimate goal is to bring both innovations together, BM41 and vitamin D3, in a next generation immunotherapy for birch pollen allergy. Both innovations however also have stand-alone value. Replacement of birch pollen extracts used in current generation products by BM41 could in itself improve immunotherapy by increasing safety (hypo-allergenicity) and efficacy (increased immunogenicity). Addition of vitamin D3 to shorten the time needed to achieve efficacy can prove to be of added value in combination with current generation extract-based immunotherapy products, thus having value even if human testing provides no support for BM41. To be able to evaluate the potential of both innovations, the BM4SIT program has been built up to assess both innovations in an independent way, bringing them together when results are supportive. Vitamin D3 is therefore first tested for safety and tolerability, before combining it with an existing immunotherapy with market authorization. Together, these two studies will provide safety and tolerability data for subcutaneous administration of vitamin D3 (not the normal route of administration of vitamin D3) and will allow evaluating whether it has added value as an adjuvant by more rapidly inducing clinical improvement than currently available immunotherapies. BM41 will be a first-in-man evaluation and will therefore focus on safety and

tolerability. By comparison to both placebo and to a current extract-based product, the expected added value with respect to safety and surrogate markers for efficacy can be investigated. The original plan to perform a clinical trial combining both innovations had to be abandoned due to unexpected results from toxicity studies and the objection made by regulatory authorities that a first-in-man for BM41 should be a true smaller safety study and not a large efficacy study with a second innovation (vitamin D3), complicating interpretation of safety and efficacy data.
To develop a new AIT vaccine for birch pollen allergy, the BM4SIT program was divided into two tracks, the BM41 track and the vitamin D3 track, both in parallel complemented by additional mouse model work to obtain further pre-clinical support. The BM41 track consisted of three phases. In the first phase, the GMP production process for BM41 was developed and assays for quality control were set up and implemented. In the second phase, toxicity studies with BM41 were performed. In the third phase, first-in-man evaluation of BM41 was carried out. The vitamin D3 track consisted of two phases, running in parallel with the BM41 track. In phase one, subcutaneously administered vitamin D3 was tested for safety, tolerability and immune-modulatory characteristics. In phase two, the added value of using vitamin D3 as novel adjuvant in combination with an approved birch pollen AIT vaccine (Alutard SQ) was investigated.

Objectives throughout the project

- A GMP-like batch of BM41 and formulated drug products for pre-clinical toxicity studies and mouse model experiments to complete the pre-clinical evidence for first-in-man administration.
- A GMP batch of BM41 and of the formulated drug products for first-in-man evaluation of their performance in immunotherapy and skin testing.
- A full QA/QC protocol for BM41 drug products including a new potency assay, compliant with all regulatory and GCP requirements.
- Proven stability of the drug products using real-time stability studies.
- A successful outcome of the pre-clinical toxicity studies (acute and repeated) for the drug products.
- Well-characterized immune-skewing properties and proven safety of subcutaneously administered vitamin D_3.
- Proven safety of the BM41 drug products.
- Clinical evaluation (safety and pilot efficacy) of a of BM41 compared to a commercially available Immunotherapy treatment (Alutard).
- Clinical evaluation (safety and efficacy) of a commercially available immunotherapy treatment (Alutard) with or without vitamin D_3.

3. A description of the main S&T results

Production and quality control of BM41 products
Within BM4SIT the first aim was to produce the necessary allergen preparations: 1. The BM41 protein (drug substance) 2. The formulated drug products based on the BM41 protein: an aluminium hydroxide-adsorbed vaccine and an aqueous solution with glycerol for skin prick testing (SPT) 3. Placebos needed to perform placebo-controlled studies. These tasks were mainly performed by Biomay in the first 1,5 years of the project, in collaboration with subcontractors for formulation (Polymun) and controlled storage (ABF).

The starting point of this project part was a genetic sequence corresponding to the amino acid desired sequence of BM41. The sequences were provided by the University of Salzburg (inventor of the BM41 molecule and partner in the project, PLUS) and after (codon) optimization of the BM41 sequence for expression in *E. coli* this was sub-cloned into an expression vector (pET-28b(+)) and expressed in a suitable *E. coli* strain. Using their GMP facilities and testing different fed-batch fermentation systems, Biomay created a so-called Master Cell Bank (MCB), which can be used as

the basis for further testing and development. Subsequently, an upstream- (fermentation and high-pressure homogenization) and downstream- process (chromatography, ultra/diafiltration excluding formulation) was tested and performed in order to implement the development process for the recombinant protein BM41 in the GMP facility. A pre-GMP batch was manufactured for toxicity testing. A flow chart of the GMP manufacturing is shown in Figure 1.

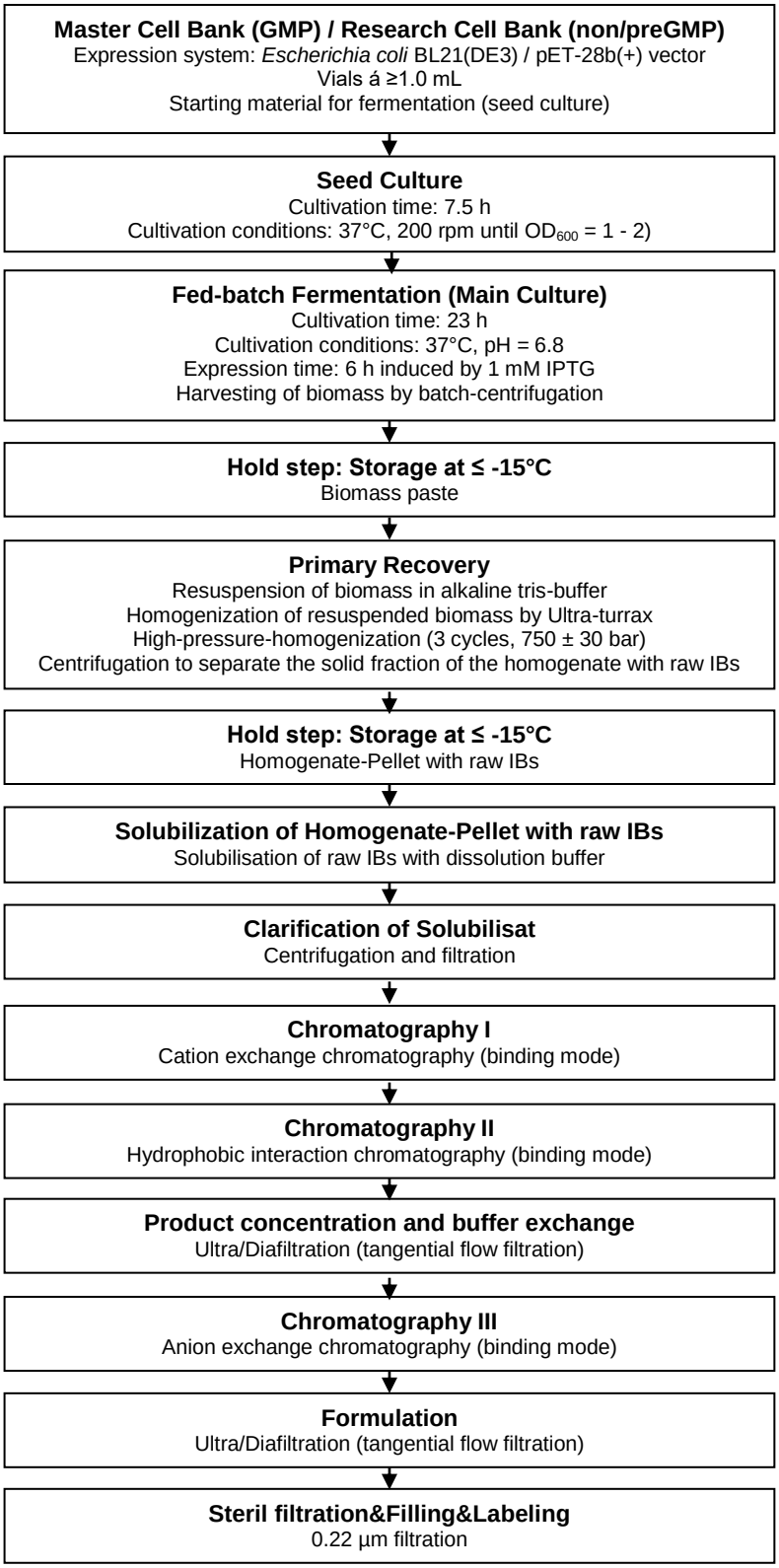

Figure 1. Flow chart of BM4 (BM41) manufacturing process.

PLUS generated and developed standard operating procedures (SOPs) for indirect ELISA as well as a sandwich ELISA to be used for the detection and quantification of BM41. Additionally, a tool box for in depth characterization of the BM41 drug substance was developed; a combination of spectroscopic/spectrometric, thermodynamic, and immunochemical methods to be used for quality control, structural characterization, and for monitoring clinically relevant epitopes during process development, manufacturing of pre-GMP material, and formulation development of the BM41 drug product. The correctness and chemical integrity of BM41 was determined by mass spectrometry (MS, intact mass measurement, peptide mapping) and by amino acid analysis. Aggregation status was investigated using HPLC-SEC, Dynamic Light Scattering (DLS), and Fourier-Transform Infrared (FTIR) spectroscopy. Folding characteristics were determined using Circular Dichroism (CD), FTIR, and one-dimensional 1H-NMR (1-D 1H-NMR). Stability was assessed by a combination of several techniques: CD, FTIR, 1-D1H-NMR, and Differential Scanning Fluorometry (DSF, ThermoFluor) (Pantoliano et al. 2001) for thermal stability; MS for chemical stability; ELISA, Direct Alhydrogel Formulation Immunoassay (DAFIA; Zhu et al. 2009), mediator release and the degradome (Egger et al. 2011) assays for monitoring the integrity of immune epitopes.
An ICH conform stability testing protocol was set up. Real-time and accelerated stability studies were started and ran for the remainder of the project. Until the end of BM4SIT no parameters assessed were out of specs.
In the second year of the project, Biomay and PLUS worked together to finalize the processes and the GMP-compliant BM41 manufacturing process (including stability testing) was successfully established. A GMP batch for formulation of the clinical trial IMP was produced. Formulation was outsourced to Polymun as subcontractor.

Health economics of AIT

In the second and third year of the project a literature review on the health economics of AIT was performed (by EBHC) and published. In addition, questions related to health economics were included in 5 systematic reviews on AIT, i.e. for asthma, rhino-conjunctivitis, food allergy, venom allergy and prevention of allergic diseases. Ten peer-reviewed papers were published for each of the five areas: a methods paper with the protocol and a results paper with the outcomes of the systematic reviews. Each of these reviews highlights the available health economics outcomes. The whole series of ten papers in which the outcome of the health economics investigations are reported were a collaboration with the AIT Guidelines project of the European Academy of Allergy and Clinical Immunology (EAACI). These are major publication outcomes of BM4SIT, both in the form of peer-reviewed papers in high-impact allergy journals and as books, with high impact dissemination to all relevant stakeholders. In this project, scientists, clinicians, patients, regulators and industry have been and are involved. Through the active collaboration with EAACI, the largest scientific society in the world in the field of allergy, the dissemination of the outcome of this task is very effective and broad.

Toxicity studies

In the meantime, we proceeded with the pre-GMP batch with the following step in the project before BM41 could be used in clinical trials; the pre-clinical toxicity testing. To establish pre-clinical toxicological safety of BM41 drug products, partner 8 (UTU) performed the toxicity studies, complying with the necessary GLP regulations. The evaluation included both acute toxicity and repeated toxicity protocols (6 months) necessary to obtain permission to enter into human clinical trials. To develop a new immunotherapeutic product, most companies have followed the vaccine guidelines (CPMP/SWP/465/95). However, as we intend to treat a chronic disease and aim at monthly administrations of the maintenance dose for the subcutaneous immunotherapy and additionally we use a recombinant hypo-allergen, we also had to take into account the guideline for biotechnology-

derived pharmaceuticals (ICH/S6-CPMP/ICH/302/95). Taking these guidelines together, we decided to perform acute toxicity studies in two species, a rodent and a non-rodent. Upon established safety in the acute toxicity studies in both species, the repeated dose toxicity studies was performed in rodents only.

The acute toxicity studies were completed and no adverse effects were observed in rabbits and rats. While we were waiting for the results of the repeated dose toxicity studies in rats, we already prepared all documents needed for submission of the first in man clinical trial with BM41 (protocol, manual of procedures, patient information sheet and informed consent form, investigational medicinal product dossier (IMPD), including the preliminary toxicity data, investigators brochure (IB) and all administrative contracts for the sites and insurance). Approval was obtained in NL, the Danish and German authorities had comments, of which a substantial amount related to the preliminary toxicity data. However, when the repeated dose toxicity studies in rats became available, a number of adverse effects were observed that were possibly test item (BM41) related, in some cases only with the high dose, in some also with the low-dose:

- A reversible weight loss in female rats
- Some effects in immune cellular composition
- Some differences in clinical (bio)chemistry outcomes
- Some differences in (absolute and in some cases relative) organ weights
- Some (minimal/mild) histopathological effects in liver (increased mitosis, hepato-cellular necrosis) en in bone-marrow (depletion of erythroid compartment)

Overall, the toxicity studies therefore did not deliver a no-observed adverse effect level (NOAEL), necessary for proceeding into human studies. This was quite unexpected, having the experience with other recombinant allergens that were found safe in toxicity studies at similar concentrations (e.g. in the FAST project, another FP7 project with a hypo-allergenic recombinant allergen, coordinated by P1 as well and studies of Allergopharma with a recombinant Bet v 1 that went up to 320 µg per injection). Nevertheless, this meant that an extra toxicity study at lower concentrations was needed before we could go into a first-in-man study.
New repeated dose toxicity studies were immediately started in September 2017 with two lower dosages, i.e. with 40 µg per injection and 20 µg per injection. These dosages would still allow the use of 40 µg per injection in humans if this dose turned out to be the NOAEL in rats (~300 times lower body weight). The studies were performed successfully and resulted in an established NOEL of 40 µg. The final report of the toxicity studies was delivered in the summer of 2018, right in time to be included in the regulatory and ethical application for the first-in-man study with BM41 in Odense (DK), to be started at OUH in September 2018.

Due to the one-year's delay caused by the repetition of the toxicity studies, the original aim to perform a first in man clinical trial with BM41 ± vitamin D3 with a 1 year duration became impossible within the frame of this project. In addition, regulatory authorities in Germany and Denmark clearly expressed reservations against the design of the original study as being too complicated (two innovations introduced in a single study: difficult interpretation) and too much focused on efficacy instead of safety as is common in first-in-man studies. Therefore, we had extensive discussions with the external advisory board of the BM4SIT project and the project officer (PO) from Brussels how to proceed. Supported by the external advisory board, we proposed a new strategy to address both original innovations of the project separately in two smaller and shorter clinical trials; one Phase I randomized double-blind-placebo-controlled clinical trial with BM41 or placebo in (open) comparison to a standard IT, Alutard SQ and a second Phase II clinical trial with commercially available standard IT (Alutard SQ) with or without addition of vitamin D3 (Zemplar).

This way forward was accepted then by the PO. While the toxicity studies were running we prepared all documents needed for submission of both trials.

Mouse model studies

Throughout the project, we worked on gathering further pre-clinical support in mouse model studies for the strategy to apply a combination of BM41 and a vitamin D3 analogue in immunotherapy. Several studies have been performed investigating the efficacy of BM41 SCIT, also in combination with Zemplar. The experiments are extensively described in the third reporting period, so here we describe the background and conclusion, presented as three major questions.

Question 1: Can immunotherapy be accelerated by co-administration with Zemplar?

Background: Vitamin D3 has been recognized to contribute to immunological tolerance via induction of IL-10 production by regulatory T cells, resulting in the activation of regulatory B cells that are responsible for production of protective IgG_4 antibodies. It is not yet clear whether co-administration of vitamin D3 to immunotherapy will enhance its efficacy, in particular whether it will speed up the onset of efficacy. In a mouse model of birch pollen immunotherapy, birch pollen (BP) allergic mice were treated with two weekly BP subcutaneous immunotherapy injections containing Zemplar (synthetic biologically active vitamin D3 analogue paricalcitol, 10 ng) or not, and efficacy was compared to eight weekly BP subcutaneous immunotherapy (BP SCIT) injections without Zemplar. The aim was to investigate whether co-administration of vitamin D3 to two BP SCIT injections would yield the same result as seen after 8 BP SCIT injections without vitamin D3.

Conclusion: This concentration of vitamin D3 did not accelerate BP SCIT. This can be due to the concentration vitamin D3. This dose was chosen based on earlier publications showing an effect for 10 ng vitamin D3 on a suboptimal concentration of ovalbumin immunotherapy. It can be that a higher concentration is needed as BP extract contains more potentially Th2-stimulating cofactors than purified ovalbumin.

Question 2: Can co-administration with Zemplar potentiate BM41 immunotherapy?

Background: In earlier reported studies, we investigated the efficacy of BM41 immunotherapy in BP allergic mice. These studies showed that BM41 SCIT was able to suppress eosinophilia and local IL-5 production (in lungs), two hallmarks of allergic disease of the lungs, but was not as effective as SCIT with BP. This under-performance may be explained by a disadvantage of the mouse model used for studying birch pollen allergy and immunotherapy. We chose to sensitize mice to birch pollen extract to mimic sensitization in real (human) life. When mice are sensitized to birch pollen, their IgE response however turns out to be directed mostly to other allergens in birch pollen than to Bet v 1. In humans the anti-birch pollen IgE response is for >80% dominated by Bet v 1, but in mice this does not exceed around 20%. So, where a therapy targeted at Bet v 1 using a Bet v 1 mutant (BM41) can be expected to cover most of the human IgE response against birch pollen, in mice this may be insufficient. A mouse model in which sensitization is performed with Bet v 1 instead of birch pollen extract may better serve its purpose to evaluate the efficacy of BM41 (see question 3). In this study, we investigated whether co-administration of 10, 100 or 1000 ng vitamin D3 (Zemplar) would potentiate the BM41 SCIT in BP sensitised and challenged mice.

Conclusion: Co-administration of 100 or 1000 ng vitamin D3 potentiated the suppression of eosinophilia and suppressed in particular IL-4 production and tempered the Bet v 1 IgE response. Although this result is based on a single experiment, the outcome is promising as support for the added value of vitamin D3. It also shows that, despite the focus of the IgE response in mice on other allergens than Bet v 1, BM41 did achieve significant reduction in Th2 inflammation.

Question 3: Can sensitisation to Bet v 1 in mice be enhanced?

Background: In BP-allergic patients the major allergen is Bet v 1, with IgE against Bet v 1 accounting for around 80% of the IgE response against birch pollen. On the other hand, in BP-sensitised mice the level of Bet v 1 specific IgE is low compared to the IgE response to BP extract (<20% of the response), showing that mice are more easily sensitised to other proteins available in the BP extract than to Bet v 1. This is not ideal as a model for pre-clinical evaluation of BM41 which targets the allergic response to Bet v 1.

To achieve more dominant sensitization to Bet v 1 in mice, an experiment was performed in which mice were sensitized with Bet v 1 alum instead of with BP alum. They were subsequently challenged intra-nasally with different concentrations of Bet v 1 instead of BP to boost the IgE response against Bet v 1. As a proof of concept, the effect of immunotherapy with wild-type Bet v 1 adsorbed to alum was investigated in these Bet v 1-sensitized mice.

These pilot experiments showed that sensitisation with 10 µg Bet v 1 alone instead of the complete BP (containing 12 µg Bet v 1) increased the Bet v 1 specific IgE response while the Bet v 1 specific IgG_1 response was similar compared with BP-sensitised mice. This increased IgE response was accompanied by an increased Bet v 1 specific Th2 cytokine production of IL-13 and IL-10. IL-4 and IL-5 production was similar to mice sensitised with BP. The recruitment of eosinophils was also comparable between BP sensitised and Bet v 1 sensitised mice in these experiments. Bet v 1 SCIT was shown to completely suppress the recruitment of eosinophils in these Bet v 1-sensitized mice.

Conclusion: Although these results are the outcome of a single experiment, it is tempting to speculate that a Bet v 1 sensitisation model would be more suitable to investigate the efficacy of BM41 SCIT, as possible IgE responses to other proteins than Bet v 1 in the BPE will not counteract the suppressed immune response to Bet v 1 by treatment with BM41.

Overall conclusion mouse model experiments:

In general, BM41 was shown to be safer, much more immunogenic than birch pollen extract (and Bet v 1). BM41 was shown to be effective in dampening allergic inflammation and this effect was increased by addition of the vitamin D3 analogue Zemplar. BM41 did however not prove more effective than BP, but this may be a flaw of the mouse model that is not dominated by sensitization to Bet v 1. A Bet v 1-driven sensitization model may prove to be more appropriate to obtain further support for the superior efficacy of BM41.

Clinical trials with vitamin D3

During the first 1.5 year of the project, we prepared the first clinical trial with the vitamin D3 analogue Zemplar (Abbvie); a Phase I study testing the safety of (off-label; intended use intravenous) subcutaneous administration of Zemplar in healthy and allergic volunteers, which was approved by the ethical/regulatory authorities in Amsterdam and the clinical trial including immunological analysis was successfully performed as a single centre study in The Netherlands in the following year of the project.

The primary objective was to evaluate whether 2.5 µg vitamin D3 analogue in multiple subcutaneously administered doses (n=4) induces a more favourable (read: anti-inflammatory) systemic immune modulation both in general parameters and allergen-specific responses in birch pollen allergic subjects, more resembling the response observed in gender- and age-matched non-allergic control subjects. To this end, the primary outcome of the study was the observed production of the anti-inflammatory marker cytokine IL-10, in response to polyclonal and allergen-specific stimulation, after 4 weekly injections with the vitamin D3 analogue Zemplar® compared to placebo. The primary outcome that we originally wanted to include in the protocol was more holistic in the sense that we wanted to study the impact of treatment on a broad spectrum of immune parameters to characterize the immune-skewing profile. The ethical committee insisted on having a measurable (quantitative) outcome, hence we chose IL-10. Having said that, as intended we included a broad

spectrum of immune parameters that would allow assessing the overall immune skewing potential of vitamin D3.

The following immune parameters were included as secondary outcomes:

- Comparison of ΔIL-10 between allergic subjects and healthy controls
- Cellular composition of PBMC with respect to Th1, Th2, Th17, Th22, and Treg cells, B cells, and antigen-presenting cells (APC)
- PBMC proliferation and cytokine production in response to allergen (Bet v 1)
- PBMC proliferation and cytokine production in response to polyclonal stimuli (αCD3/αCD28)
- Intracellular cytokine measurements in response to PMA/ionomycin
- IgE responses in serum to birch pollen
- Blood safety biochemistry/haematology parameters, urinalysis, vital signs and ECG, lung function will be determined at baseline and at the follow up visit.
- Safety and tolerability (local and systemic reactions / [serious] adverse events) of the vitamin D3 analogue Zemplar® administered via the subcutaneous route will be evaluated at each treatment visit and the follow up visit.

It is also noted that safety and tolerability were not chosen as the primary outcome of this Phase I study, although these were important outcomes for the study. The clinical investigators of the BM4SIT consortium considered it more likely to get approval for the Phase I study using an immunological parameter as primary outcome. For this outcome, the proposed power of 12 subjects per arm was considered appropriate. For safety/tolerability it is always quite difficult to power studies appropriately.
The observations during the trial provided support that Zemplar, used off-label (subcutaneously), is safe and well tolerated. We did notice a swelling reported by ~25% of the patients, which was significant in size but was only observed during the first and sometimes second injection. It was not considered a too big inconvenience by the subjects, also witnessed by the fact that none of the enrolled volunteers dropped out. We did not find an enhancement of IL-10 production nor of IL-10+ T cells (Figure 2), the primary outcome of the study, but we did observe an enhancement of anti-Th2 cell type cellular responses, characterized by increased IFNγ and IL-17 production (Figure 3A/B). Moreover, we observed the enhancement of a tissue-repair type of response characterized by IL-22 (Figure 3C/D). Interestingly, these changes were not observed in the healthy controls. The picture emerging is that vitamin D3 skewed the immune response towards the response observed in healthy controls, i.e. away from Th2. Overall, we concluded that treatment of allergic donors with Zemplar may be beneficial for reducing their allergic status, being a Th2-dominated immune status.
It needs to be stressed here that it is quite surprising that just 4 local injections of vitamin D3 had significant impact on the systemic immune system. The concept of using vitamin D3 as an adjuvant for immunotherapy is in fact supposed to work mainly locally in the micro-environment of the injection site. The idea is that antigen-presenting cells at the injection site will encounter allergen in the context of vitamin D3, and that the allergen-specific immune-response with as a result be skewed towards an anti-inflammatory response. The fact that even systemically these local injections seems to transfer an anti-Th2 effect is unexpected but not unwelcome.

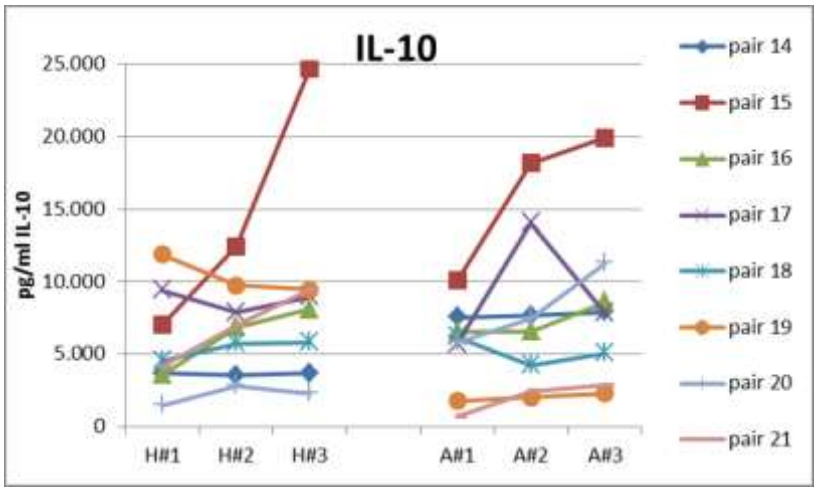

Figure 2. Allergic (A) and healthy (H) individuals were treated with Zemplar and PBMCs were isolated at various time points; baseline (#1), during Zemplar treatment (#2) and after Zemplar treatment (#3), were polyclonally stimulated with aCD3/CD28 for 48 hrs. Supernatants were harvested and analysed for production of IL-10 by Legendplex (biolegend) following the instructions of the manufacturer.

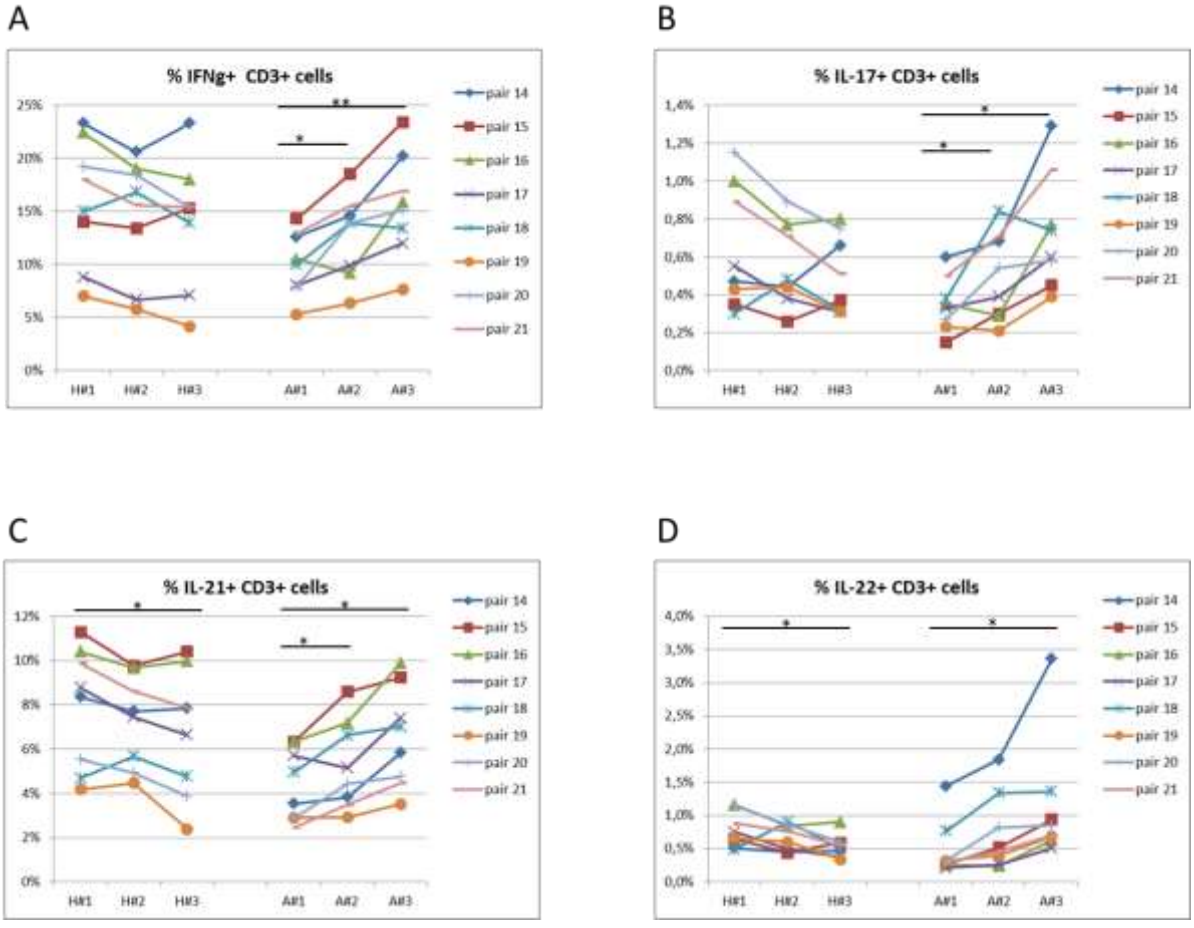

Figure 3. Allergic (A) and healthy (H) individuals were treated with VD3 and PBMCs isolated at various time points, baseline (#1), during VD3 treatment (#2) and after VD3 treatment (#3), were polyclonally stimulated with PMA/ionomycine for 6 hrs in the presence of Brefeldin A to allow determination of the intracellular expression of IFNγ (A), IL-17 (B), IL-21 (C) and IL-22 (D) by flowcytometry. *P < 0.05; **P < 0.01.

After the completion of this first study with Zemplar, all documents needed for submission of the next single center Phase II clinical trial in The Netherlands with commercially available standard IT

(Alutard SQ) with or without addition of vitamin D3 (Zemplar), were prepared and submitted to ethical committee and regulatory authorities. Approvals were obtained to start inclusion in September 2018. A total of 233 patients were contacted and 63 of those patients were screened. A total of 34 patients fulfilled all the inclusion criteria and were randomized. The last patient was included December 21st 2018, the last day according to protocol, bringing the total to 34 patients. In the course of the treatment 3 patients dropped out. Patients were treated and followed up until the end of BM4SIT and beyond. Last patient out was end of June 2019.

First-in-man clinical trial with BM41

All documents needed for submission of the first-in-man single center Phase I clinical trial in Denmark with BM41 in comparison to a standard IT, Alutard SQ, were prepared and approval was obtained from both regulatory authorities and ethical committee to start inclusion in September 2018. A total of 93 patients were contacted, 51 patients screened (screening stopped on the 7th of December 2018) and 47 patients finally randomized. There was a single drop-out in the course of the treatment due to an adverse event. In April 2019 all remaining 46 patients finished the study protocol (i.e. after termination of the contract of BM4SIT).

Ethical considerations

The ethical committee (members of the external advisory board) have evaluated the process of the ethics applications for the three clinical trials in BM4SIT. The conclusions from the ethical committee were positive, stating that the consortium has followed all necessary steps and has adequately answered to question raised by ethical and regulatory authorities. Monitors and DSMBs installed for the clinical trials have not identified any ethical deviations in the performance of the clinical trials.

Summary

Overall, the project has faced many challenges and due to the need to perform the toxicity studies again with lower doses, was delayed for a year. Even though the consortium has managed to come up with alternative strategies and has succeeded in obtaining approval for the two alternative clinical trials in two countries that allow evaluation of both innovation pillars of the BM4SIT project, i.e. of BM41 and of vitamin D3. The next important step, besides running these trials, was to obtain approval for an amendment to redistribute the tasks and budget, but more importantly, to obtain an extension of eight months in order to be able to complete the clinical trials, perform extensive laboratory tests and analyse these results. Unfortunately, this request for an extension was denied by the European Commission. Because we as a consortium, judged it to be unethical not to finalize the trials, we have succeeded to complete the two clinical trials started during the BM4SIT contract but running on beyond the contract end. We performed these activities on budget provided by the respective consortium partners. Unfortunately, we will not be able to (immediately and or completely) perform the extensive laboratory tests and analyse these results, because funding is not available anymore n. This makes it hard to assess the success of the project with respect to reaching the original aims and to evaluate the potential impact (see next section).

4. The potential impact

The first-in-man Phase I clinical trial with BM41 aims to establish whether BM41 is safe in humans and possibly safer than current extract-based products. Very important secondary outcomes, i.e. IgE and IgG antibody responses against BM41 and against the native allergen Bet v 1, as well as a proxy for protection monitored in two functional cellular assays (BAT and FAB), will be compared to those parameters observed in response to standard treatment with an extract-based registered product. These analyses will allow us to assess whether the strong immunogenicity of BM41 compared to its native homolog Bet v 1 (in extracts) observed in pre-clinical animal studies, is confirmed in human allergic subjects. A confirmation of safety and enhanced immunogenicity will pave the way towards Phase II efficacy trials. Increased safety and efficacy will beyond doubt make immunotherapy for birch pollen more attractive to patients. Without a formal analysis being possible within the contract duration, we can already conclude that BM41 was safe and well-tolerated and induced. We are convinced that we will find funding to perform the laboratory analyses on the biological samples to confirm te superior immunogenicity of BM41.

The second clinical trial aimed to evaluate the added value of adding vitamin D3 (Zemplar) to an existing registered product for treatment of birch pollen, hay fever and asthma (Alutard SQ). The expectation is that vitamin D3 will skew the immune response more effectively and more rapidly towards a protective anti-inflammatory response. The immune skewing is the primary endpoint. If a more rapid onset of a therapeutic effect is confirmed (secondary outcome), this will pave the way to perform further studies. These may be either Phase III studies using registered immunotherapy treatments with vitamin D3 or Phase IIb studies combining BM41 and vitamin D3. The latter is dependent on the final outcome of the first-in-man study with BM41. For the laboratory analyses of the second clinical trial to establish the added value of vitamin D3, we are currently searching for alternative funding. It is very likely that we have found a solution in a nationally funded project. We are there for optimistic that we can deliver the impact that was originally foreseen.

Overall, due to the delays caused by the toxicity studies, the consortium was unable to complete the laboratory analyses for both clinical trials. The consortium is nevertheless optimistic that funding will be found to perform laboratory and statistical analyses and finally deliver its originally predicted impact to make immunotherapy more effective, safer and less burdensome to patients, making it a more attractive alternative to chronic symptomatic drug use.

5. The address of the project public website

The project website (**http://bm4sit.eu/**) is live in four languages: English, German, Dutch and Polish.

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	*Allergen immunotherapy for insect venom allergy: a systematic review and meta-analysis.*	*Dhami S*	*Allergy*	*March 2017*	*Wiley*		*2017*	*pp. 342-365*	*doi: 10.1111/all.13077*	yes
2	*Allergen immunotherapy for IgE-mediated food allergy: a systematic review and meta-analysis.*	*Nurmatov U*	*Allergy*	*August 2017*	*Wiley*		*2017*	*pp. 1133-1147*	*doi: 10.1111/all.13124*	yes
3	*Allergen immunotherapy for insect venom allergy: protocol for a systematic review.*	*Dhami S*	*Clin Transl Allergy*	*February 2016*	*Springer Nature*		*2016*		*doi: 10.1186/s13601-016-0095-x*	yes
4	*Allergen immunotherapy for IgE-mediated food allergy: protocol for a systematic review.*	*Dhami S*	*Clin Transl Allergy*	*July 2016*	*Springer Nature*		*2016*		*doi: 10.1186/s13601-016-0113-z*	yes
5	*Allergen immunotherapy for allergic asthma: protocol for a*	*Dhami S*	*Clin Transl Allergy*	*February 2016*	*Springer Nature*		*2016*		*doi: 10.1186/s13601-016-*	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.

	systematic review.								*0094-y*	
6	*Allergen immunotherapy for allergic rhinoconjunctivitis: protocol for a systematic review.*	*Dhami S*	*Clin Transl Allergy*	*March 2016*	*Springer Nature*		*2016*		*doi: 10.1186/s13601-016-0099-6*	yes
7	*Allergen immunotherapy for allergic asthma: A systematic review and meta-analysis*	*Dhami S*	*Allergy: European Journal of Allergy and Clinical Immunology*	*Vol. 72/Issue 12*	*Blackwell Publishing*	*United Kingdom*	*01/12/2017*	*1825-1848*	*10.1111/all.13208*	yes
8	*Health economic analysis of allergen immunotherapy for the management of allergic rhinitis, asthma, food allergy and venom allergy: A systematic overview*	*Asaria, M*	*Allergy: European Journal of Allergy and Clinical Immunology*	*Vol. 73/Issue 2*	*Blackwell Publishing*	*United Kingdom*	*01/02/2018*	*269-283*	*10.1111/all.13254*	yes
9	*Allergen immunotherapy for the prevention of allergy: A systematic review and meta-analysis*	*Kristiansen, M*	*Pediatric Allergy and Immunology*	*Vol. 28/Issue 1*	*Blackwell Munksgaard*	*Denmark*	*01/02/2017*	*18-29*	*10.1111/pai.12661*	yes
10	*Allergen immunotherapy for allergic rhinoconjunctivitis: A systematic review and meta-analysis*	*Dhami, S*		*Vol. 72/Issue 11*	*Blackwell Publishing*	*United Kingdom*	*01/11/2017*	*1597-1631*	*10.1111/all.13201*	yes

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	Type of activities[5]	Main leader	Title	Date	Place	Type of audience[6]	Size of audience	Countries addressed
1	*Development and maintenance of the project's website*	*Lorenz Aglas (PLUS)*		*27 July 2015 - ongoing*	*www.BM4SIT.eu*	*patients, clinicians, other interested people*	*world-wide*	world-wide
2	*invited lecture*	*Lorenz Aglas (PLUS)*	*The birch pollenome - novel perceptions on birch pollen sensitization and therapeutic strategies*	*2018*	*Institute of Environmental Medicine, UNIKAT-T / IEM in Augsburg, Germany*	*clinicians, researchers*	*conference participants*	*Germany*
3	*oral presentation/interview*	*Lorenz Aglas (PLUS)*	*In vivo induction of IgG antibodies towards Bet v 1 and associated food allergens by a hypoallergenic birch pollen allergy AIT vaccine candidate*	*2018*	*"NextGeneration ÖGAIng Meeting 2018" by the Austrian Society of Allergy and Immunology at the "Schutzhaus zur Zukunft" in Vienna, Austria*	*clinicians, researchers*	*conference participants*	*Austria*
4	*oral presentation*	*Lorenz Aglas (PLUS)*	*The hypoallergenic birch pollen allergy AIT candidate BM4 induces cross-reactive IgG antibodies towards Bet v 1 and associated food allergens*	*2018*	*EAACI 2018 in Munich, Germany*	*clinicians, researchers*	*conference participants*	*Europe/world-wide*
5	*invited lecture*	*Fatima Ferreira (PLUS)*	*Immunotherapy of birch pollen allergy with a highly immunogenic hypoallergen: Pre-clinical studies*	*2017*	*SIICA 2017 in Bari, Italy*	*clinicians, researchers*	*conference participants*	*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.

6	*invited lecture*	*Laurian Jongejan (AMC)*	*Immunotherapy of birch pollen allergy with a highly immunogenic hypoallergen: Clinical study design*	*2017*	*SIICA 2017 in Bari, Italy*	*clinicians, researchers*	*conference participants*	*Italy*
7	*oral presentation*	*Esther de Jong (AMC)*	*Vitamin D treatment enhances anti-inflammatory and anti-Th2 pathways in allergic individuals but not in healthy controls*	*2017*	*EAACI 2017 in Helsinki, Finland*	*clinicians, researchers*	*conference participants*	*Europe/world-wide*
8	*poster presentation*	*Lorenz Aglas (PLUS)*	*Development of an enzyme-linked apta-sorbent assay (ELASA) for the quality control of a birch pollen immunotherapy vaccine*	*2016*	*Aptamer Congress in Oxford, the United Kingdom*	*clinicians, researchers*	*conference participants*	*Europe/world-wide*
9	*oral presentation*	*Lorenz Aglas (PLUS)*	*Development of an aptamer-based tool for quality control of a birch pollen immunotherapy vaccine*	*2016*	*EAACI 2016 in Vienna, Austria*	*clinicians, researchers*	*conference participants*	*Europe/world-wide*
10	*poster presentation*	*Lorenz Aglas (PLUS)*	*Immunologic evaluation of the hypoallergenic birch pollen AIT vaccine candidate BM4 during toxicity testing*	*2016*	*EAACI 2016 in Vienna, Austria*	*clinicians, researchers*	*conference participants*	*Europe/world-wide*
11	*invited lecture*	*Fatima Ferreira (PLUS)*	*Immunotherapy of birch pollen allergy with a highly immunogenic hypoallergen*	*2019*	*Hospital da Luz Setúbal in Lisbon, Portugal*	*clinicians, researchers*	*conference participants*	*Portugal*
12	*poster presentation*	*Lorenz Aglas (PLUS)*	*Assessment of pre-clinical blocking activity of a hypoallergenic birch pollen vaccine candidate*	*2019*	*EAACI 2019 in Lisbon, Portugal 2019*	*clinicians, researchers*	*conference participants*	*Europe/world-wide*
13	*Interview*	*Fatima Ferreira (PLUS)*	*Heimat großer Töchter und Söhne*	*26th of October 2018*	*ORF (Austrian TV)*	*TV audience*	*TV audience*	*Austria/German-speaking countries*
15	*published interview (online)*	*Fatima Ferreira (PLUS)*	*Wenn die Pollen fliegen" (engl. "When the pollen fly")*	*2nd of February, 2018*	*Internet https://www.lungen-magazin.eu/wenn-die-pollen-fliegen/*	*patients, clinicians, other interested people*		*Austria/German-speaking countries*
16	*Article*	*Fatima Ferreira and Lorenz Aglas*	*"Neuer Wirkstoff gegen Birkenpollenallergie" (engl. "New vaccine for the treatment of birch pollen*	*9th of March, 2018*	*published in PULS MAGAZIN, Das Salzburger Magazin für Medizin, Gesundheit und Freizeit*	*patients, clinicians, other interested*		*Austria/German-speaking countries*

		(PLUS)	*allergy")*		*www.pulsmagazin.at*	*people*		
17	*Interview*	*Ronald van Ree (AMC)*	*Could hacking the immune system cure allergies?*	*24th of January 2019*	*in HORIZON - The EU Research and Innovation Magazine*	*patients, clinicians, other interested people*		*Europe/world-wide*

Section B

Due to the fact that a contract extension was denied, it was impossible to finish the analyses for both clinical trials. Theses analyses are absolutely essential to be able to judge whether new foreground has been generated to support or extend existing IP.

Part B1
There were no applications for patents, trademarks, registered designs, etc. Reason: unfinished analyses.

Part B2
There is no exploitable foreground. Reason: unfinished analyses.

Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	601763
Title of Project:	BM4SIT
Name and Title of Coordinator:	Prof. Ronald van Ree

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'*	***Yes***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	NO
• Did the project involve patients?	YES
• Did the project involve persons not able to give consent?	NO
• Did the project involve adult healthy volunteers?	YES
• Did the project involve Human genetic material?	YES
• Did the project involve Human biological samples?	YES
• Did the project involve Human data collection?	YES
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	NO
• Did the project involve Human Foetal Tissue / Cells?	NO
• Did the project involve Human Embryonic Stem Cells (hESCs)?	NO
• Did the project on human Embryonic Stem Cells involve cells in culture?	NO
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	NO
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	NO
• Did the project involve tracking the location or observation of people?	YES
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	YES
• Were those animals transgenic small laboratory animals?	YES
• Were those animals transgenic farm animals?	NO

• Were those animals cloned farm animals?	NO
• Were those animals non-human primates?	NO
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	NO
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	NO
DUAL USE	
• Research having direct military use	NO
• Research having the potential for terrorist abuse	NO

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	5	4
Experienced researchers (i.e. PhD holders)	11	7
PhD Students	2	2
Other	56	29

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	none
Of which, indicate the number of men:	

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project? | | no

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

	Not at all effective				Very effective
❑ Design and implement an equal opportunity policy	○	○	○	○	○
❑ Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑ 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: Gender was evaluated in the enrolment of volunteers into the clinical trials in the project

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)?

○ Yes: master students participated in the performance of laboratory tests
PhD students / clinicians doing their specialty education participated in performance of the clinical trials

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

X Yes- please specify Website

F Interdisciplinarity

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

○ Main discipline[7]: Medical Sciences (all three disciplines)

○ Associated discipline[7]: | ○ | Associated discipline[7]:

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)* | ○ | 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

[7] 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
- ○ Yes - in implementing the research agenda
- ○ 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)
- ○ 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?	**15**
To how many of these is open access[8] provided?	**12**
How many of these are published in open access journals?	**4**
How many of these are published in open repositories?	**0**
To how many of these is open access not provided?	**3**
Please check all applicable reasons for not providing open access:	
X publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[9]: ……	

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).*		**0**
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	X	In small & medium-sized enterprises
X	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

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:* **15**

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

Difficult to estimate / not possible to quantify	❑

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?

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 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?

❑	Press Release	X	Coverage in specialist press
❑	Media briefing	X	Coverage in general (non-specialist) press
❑	TV coverage / report	X	Coverage in national press
❑	Radio coverage / report	X	Coverage in international press
❑	Brochures /posters / flyers	X	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]