

IVN201 – Therapy With a Construct Containing Functional Fusion Peptides

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Abstract

The new concept of immunotherapy with IVN201 against cat dander allergy combines two proprietary technology platforms: Intralymphatic immunotherapy (ILIT) with Modular-Antigen-Transportation (MAT) proteins.

Intralymphatic immunotherapy (ILIT) is the injection of immunotherapeutics directly into the lymph node. Lymph nodes contain a high density of antigen presenting cells together with interacting T cells, which are necessary for allergen tolerance development. Therefore, this site-directed route of administration requires fewer injections of much smaller doses of the allergen than conventional administration routes to induce a highly effective, disease modifying immune response.

ILIT was recently demonstrated in a clinical study to enhance safety and efficacy of immunotherapy and to reduce treatment time from 3 years to 8 weeks with only three injections, thereby significantly improving patient compliance. Patients subjectively perceived the intralymphatic injections less painful than a venous puncture.

IVN201 is a functional fusion peptide which is a tailor-made recombinant allergen for ILIT with *Fel d 1* as allergen module. It is designed to be rapidly taken up by antigen presenting cells in the lymph nodes and to improve the presentation of the allergen to the immune system. IVN201 was shown to induce allergen tolerance in a murine anaphylaxis model. Stimulation assays with basophil leukocytes isolated from allergic patients suggest an increased safety profile when compared to cat extract or recombinant *Fel d 1*. Therefore, MAT molecules are expected to be safer and more efficacious in inducing the desired immune response than recombinant allergens or allergen extracts in allergen immunotherapy when administered via the intralymphatic route.

Introduction

The general research and development activities in the field of allergy immunotherapy are focused on several key issues:

- Improvement of immune response to immunotherapy by adjuvants or by enhanced antigen presentation.
- Reduction of allergic reactions by chemical modifications (e.g. allergoids) or recombinant allergens or peptides without B-cell epitopes.
- Alternative routes of administration particularly sublingual and oral immunotherapy.

ImVisioN's concept for immunotherapy addresses all of these three improvement approaches: It is based on the induction of a highly efficient, disease modifying immune response via an increased presentation of a recombinant Modular-Antigen-Transportation (MAT) molecule with hypoallergic and immune-stimulatory properties that is administered intralymphatically.

Results and Discussion

Intralymphatic Immunotherapy (ILIT)

In order to achieve a therapeutic effect (i.e. to induce an immune response with disease modifying characteristics), immunotherapeutics have to be taken up by antigen presenting cells (APCs), which are mainly located in the lymph system. Therefore, ImVisioN applies an innovative route of administration by injecting MAT based immunotherapeutics directly into the lymph node of patients.

The injected lymph nodes, located in the subcutaneous fat of the upper outer quadrant of the groin, are on average only around 5 mm underneath the skin surface (Figure 1). The injection can easily be controlled by an ultrasound device.

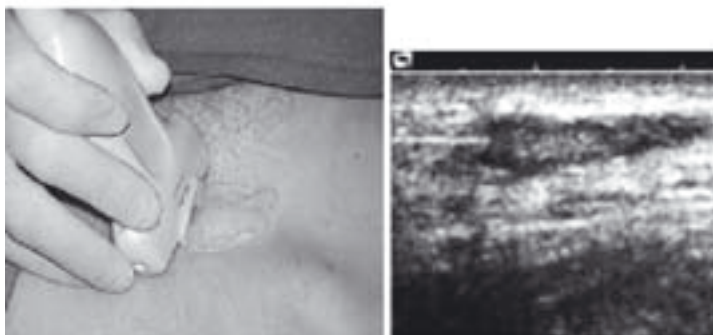


Fig. 1. *Intralymphatic injection under ultrasound guidance*

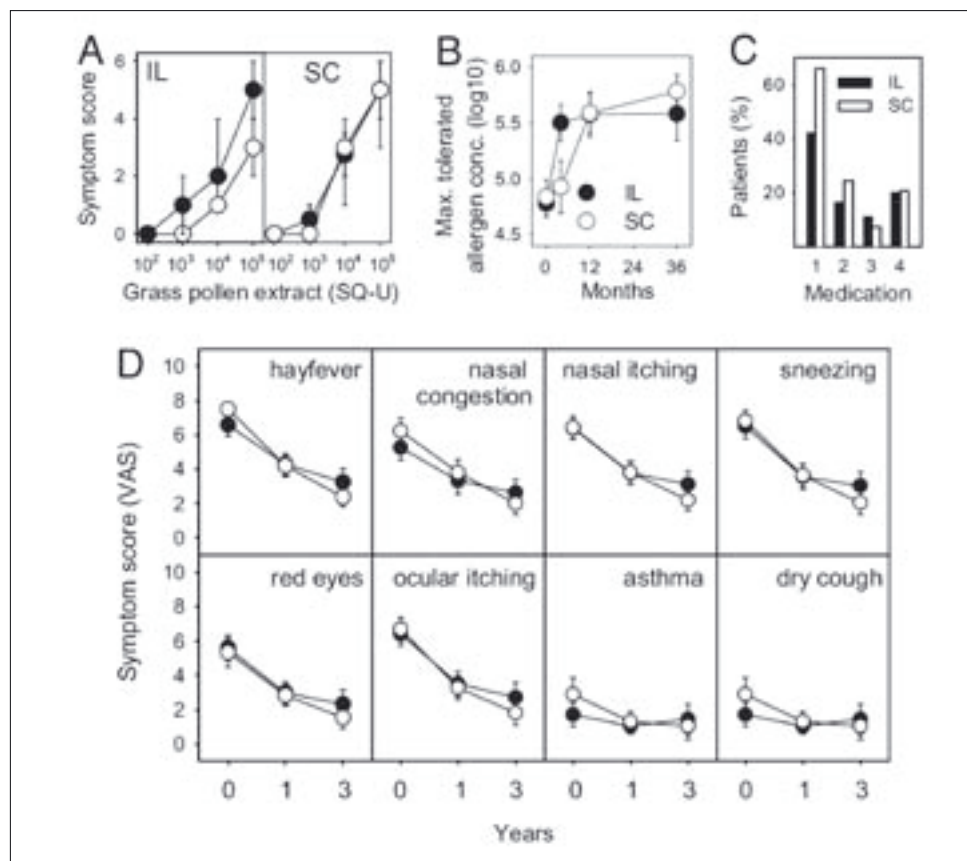


Fig. 2. Efficacy of intralymphatic (IL) versus subcutaneous (SC) immunotherapy. (A) Symptom scores during nasal provocation testing with grass pollen extract at baseline (●) and 4 months after initiation of treatment (○). (B) Maximal tolerated pollen concentrations (inducing scores ≥ 4) in nasal provocation tests with grass pollen extract at baseline and after 4 months, 1 year, and 3 years. (C) Use of rescue medication during first year of treatment: Antihistamine tablets (medication 1: desloratadine), nasal corticosteroid sprays (medication 2: mometasone furoate), asthma inhaler (medication 3: budesonide with formoterol), and antihistamine eye drops (medication 4: emadistinum). (D) Scores of symptoms of hay fever, congested nose, itchy nose, sneezing, red eyes, itchy eyes, asthma, and cough, at baseline, after 1 year, and after 3 years on a visual analogue scale ranging from 0 to 10.

Efficacy of intralymphatic immunotherapy

In a clinical study 165 patients, suffering from grass pollen induced rhinoconjunctivitis were randomized to receive 54 subcutaneous injections with grass pollen extract over 3 years (cumulative allergen dose of 4,031,540 standard quality units [SQ-U]) or 3 intralymphatic injections over 2 months (cumulative allergen dose of 3,000 SQ-U). Patients were evaluated after 4 months, 1 year and 3 years by nasal provocation and allergy symptom scores.

Evaluation of patients by nasal provocation testing showed that 3 low-dose intralymphatic injections within 8 weeks significantly increased tolerance to the grass pollen allergen already within 4 months by almost a factor of 10 (Figure 2A). At this time, the subcutaneous group did not show any increase in tolerance. The increased tolerance of intralymphatic immunotherapy was long lasting and equivalent to that achieved after standard immunotherapy for 3 years with 54 subcutaneous injections (Figure 2B). The rapid achievement of a therapeutic effect by intralymphatic immunotherapy was further indicated by a significantly lower usage of antihistamine tablets as rescue medication during the first year of treatment compared to patients in the subcutaneous treatment group (Figure 2C). No significant differences were seen for other medications.

Intralymphatic and subcutaneous immunotherapy comparably ameliorated allergic symptoms (Figure 2D). Patients were asked to score the symptoms of hay fever, congested nose, itchy nose, sneezing, red eyes, itchy eyes, asthma, and cough, at baseline, after 1 year, and after 3 years on a visual analogue scale ranging from 0 to 10. Both intralymphatic and subcutaneous immunotherapy significantly ameliorated subjective allergy symptoms. Amelioration in the two patient groups was not significantly different.

Safety of intralymphatic immunotherapy

Although injection into a lymph node sounds painful, it is not. The capsule of a lymph node is poorly innervated and the patient cannot feel the needle puncturing the lymph node. In a survey of the 54 patients receiving the intralymphatic immunotherapy, the intralymphatic injection proved equally or even less painful than intravenous puncture (Figure 3 left; [1]).

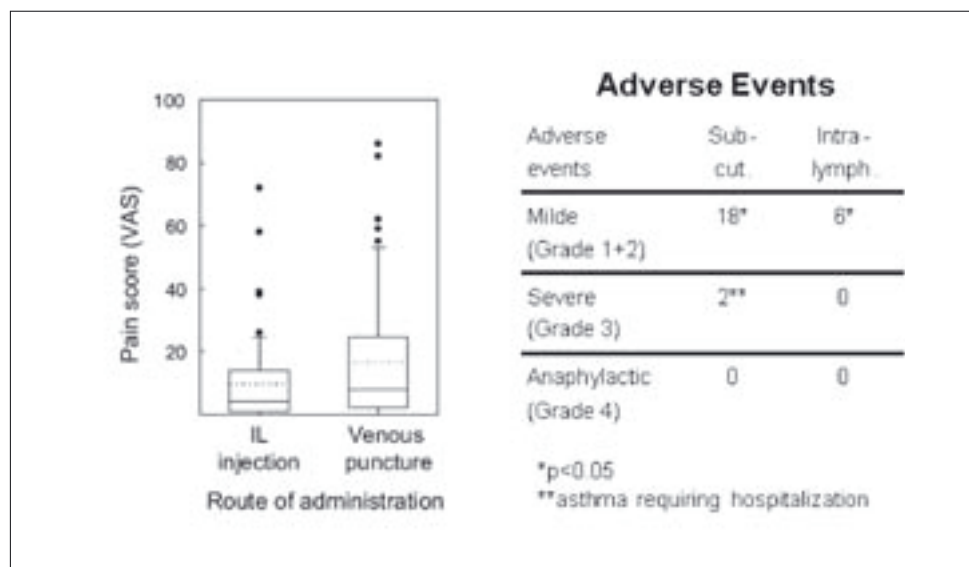


Fig. 3. Pain visual analogue score analysis and adverse events

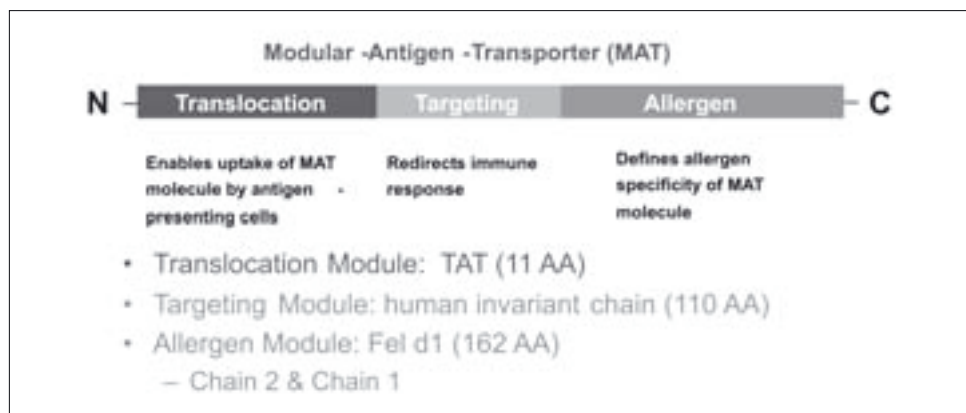


Fig. 4. Composition of MAT fusion protein

In the same study conducted by Senti [1], where intralymphatic treatment (3 injections) was compared to the classical subcutaneous route of administration (54 injections) of grass pollen extract in a three year longitudinal study design, intralymphatic immunotherapy was safer than subcutaneous immunotherapy, since it caused significantly fewer allergic adverse events (Figure 3 right).

In conclusion, intralymphatic immunotherapy enhances safety, induces an earlier onset of efficacy of allergen immunotherapy and reduces treatment time from 3 years to 8 weeks.

IVN201, a Modular-Antigen-Transportation (MAT) Molecule for ILIT

MAT molecules as therapeutic allergens are designed to compensate for the deviation of the immune response towards a Th2 response in allergic patients by boosting selectively the T helper cell type 1 (Th1) (protective) responses against an antigen structure. IVN201 MAT fusion protein consists of three functional elements (Figure 4):

- A translocation module (TAT) which enables the MAT molecule to pass through the cell membrane and to get into antigen presenting cells (APC).
- A targeting module (invariant chain) which directs the processing of the MAT molecule within the APC and which improves the presentation of the allergen and its components to the immune system via the MHC class II pathway.
- A module containing the allergen (*Fel d 1*) to which the patient is allergic and which determines the specificity of the induced immune response.

Figure 5 illustrates the current hypothesis for the mode of action of MAT molecules [2]. A limiting step for effective antigen presentation is the uptake of the antigen or allergen by APCs. **TAT** as a short arginine rich peptide possesses the intrinsic ability for a fast and efficient transduction of coupled proteins into potentially all cell types. Using this strategy, extracellular proteins can be converted into cytoplasmic proteins.

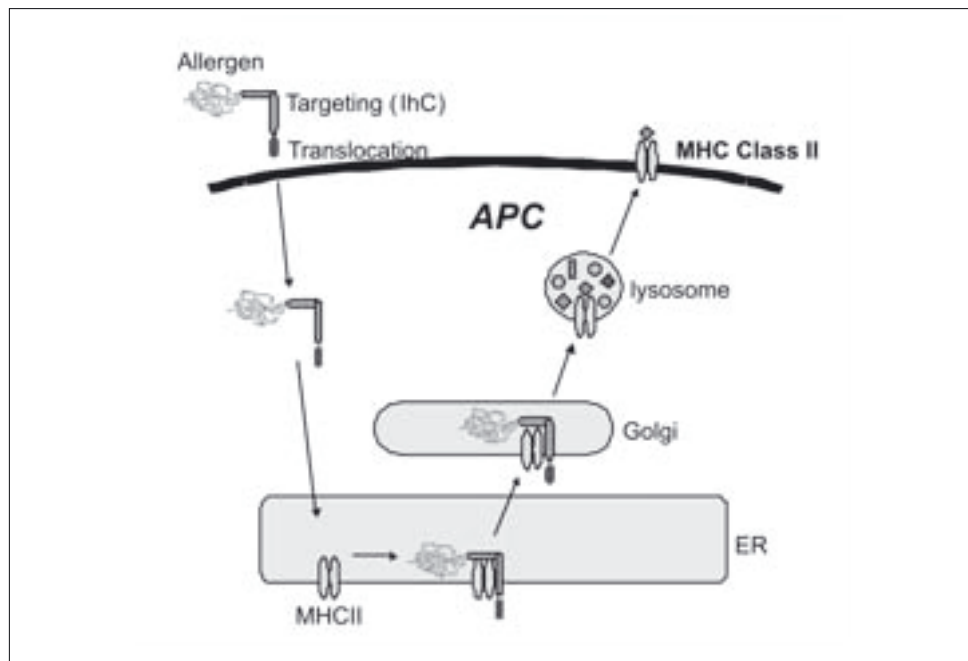


Fig. 5. *Proposed mechanism: MAT processing in the antigen presenting cell (APC)*

MHC class II molecules loaded with antigen-derived peptides are successively exported to the APC surface to be presented to the T cell receptor. Peptides presented on the MHC II context are recognized by CD4⁺ T helper cells which provide the necessary help for antibody production to B cells. The **targeting module** of MAT molecules (the invariant chain, Ii) potentially assists specific targeting of the MAT proteins to the endoplasmic reticulum (ER) where MHC class II molecules assemble (alpha- and beta chain) and bind together with the invariant chain to build trimers. The presence of MAT proteins during the assembly of heterotrimers enhances the chance of the Ii-antigen in the MAT to be incorporated into the MHC class II complex and thus to be selectively transported to lysosomes where antigen loading of the MHC complex with degraded antigen fragments occurs. This should lead to an increased presentation of the allergen module of the MAT molecule in the MHC II context.

Immune response to MAT Immunotherapeutics ex vivo

First assessments of the efficacy of MAT immunotherapeutics have been performed using cell cultures derived from peripheral blood mononuclear cells (PBMC) isolated from blood of allergic patients [2]. The experiments show that the MAT molecules stimulate T cell proliferation in PBMCs of allergic patients at much lower (about 1/10 to 1/100) concentrations compared to the proliferation induced by the corresponding recombinant allergens. This indicates an increased antigen presentation to T cells with MAT immunotherapeutics.

In addition, cytokine release experiments applying MAT allergen molecules to PBMCs of allergic patients indicate a strong bias towards a Th1 type immune response (as indicated by $\text{INF}\gamma$ increase and IL-4 and IL-5 decrease) compared to the recombinant allergens. Perhaps most important, MAT turned out to be a strong inducer of IL-10, the regulatory cytokine considered to be essential for successful immunotherapy.

In vivo efficacy of MAT Molecules

An *in vivo* sensitization model for cat hair extract in mice was established at the University Hospital Zurich to evaluate the efficacy of immunization using the intralymphatic administration of different allergens [3]. Mice were sensitized by multiple weekly injections of cat dander extract over a period of eight weeks. After the sensitization period, the mice were treated by three intralymphatic injections of (I)

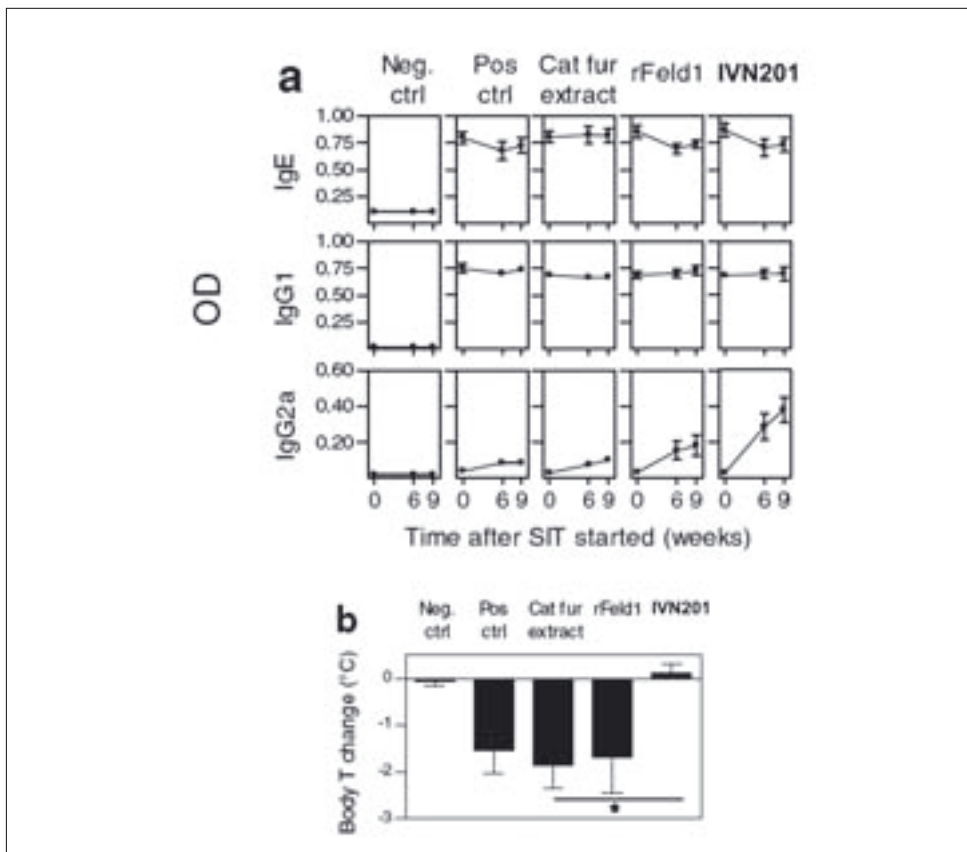


Fig. 6. Murine anaphylaxis model. (a) Cat allergen-specific IgG1 and IgG2a antibodies and total IgE analysed in sera from mice before ILIT (time point 0), as well as 6 and 9 weeks after the first ILIT injection. The negative control group was naive mice, and the positive control was a group of sensitized mice that did not receive ILIT. (b) Four weeks after the last ILIT injection, mice were challenged with 20 μg of cat hair allergen extract. The body temperature drop was measured before and 30 minutes after the challenge.

control solution, (II) recombinant *Fel d 1* (the major cat dander allergen), (III) Cat dander extract, or (IV) MAT-*Fel d 1* (IVN201). Finally, the mice were challenged by intraperitoneal injection of a high dose of cat hair extract and the temperature change within 30 minutes of challenge was measured. Drop of body temperature of about 1.5 to 2°C mimics natural systemic allergic or anaphylactic reactions in humans.

The Th2 response to allergen sensitization is indicated by a strong increase in allergen specific IgE and IgG1 antibodies (Figure 6a, upper and middle panel). The effect of allergen-specific immunotherapy on the animals is indicated by an increase of allergen specific IgG2a antibodies (Figure 6a, lower panel). The protective Th1-related IgG2a response was induced most effectively with MAT-*Fel d 1*.

After high dose allergen challenge the non-treated animal group showed clear anaphylaxis evidenced by a temperature drop of about 2°C (Figure 6b). Animals treated with MAT-*Fel d 1* (IVN201) did not show any drop of body temperature, while animals treated with recombinant allergen or cat hair extract showed a similar drop in body temperature as non-treated animals. The data of this experiment provide strong evidence that the immune response induced by MAT immunotherapy is effective in preventing allergic symptoms.

Safety of MAT molecules in ex vivo experiments

In an *ex vivo* study (IVN-CAT-001a) with blood from 10 patients allergic to cat dander, the safety of IVN201 in comparison to recombinant allergen and cat dander extract was assessed by using a Cellular Antigen Stimulation Test (CAST) [3]. In this test, the allergen is added to the white blood cell fraction derived from the blood of allergic patients, who are expected to have IgE bound to the surface of basophils. Upon contact with allergen, these basophils will degranulate, and the content of the granula, such as leukotrienes, will become measurable in the supernatant. It could be demonstrated that about a 100-fold higher concentration of MAT-*Fel d 1* (IVN201) is required to degranulate basophils when compared to recombinant allergen (*rFel d 1*)

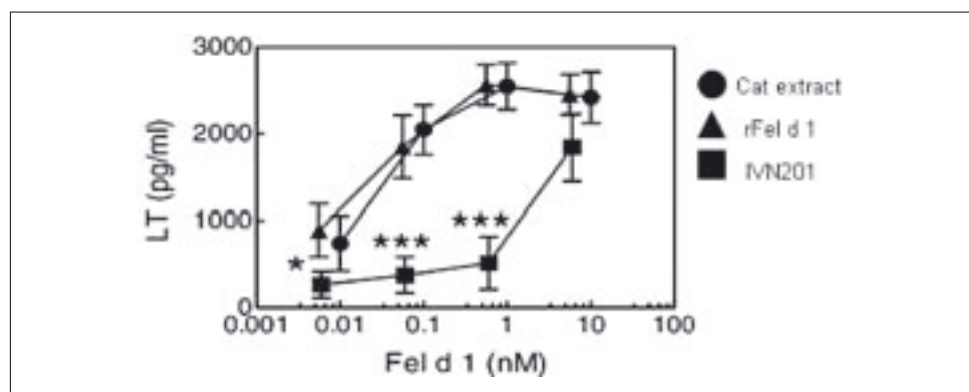


Fig. 7. Allergen concentration required to degranulate mast cells (CAST) measured as leukotriene release (LT)

and cat dander extract (Figure 7). An explanation for this improved safety profile of IVN201 might be that – due to the translocation module TAT – IVN201 is cleared from the extracellular space more rapidly than other allergens, i.e. before it can crosslink surface-bound IgE on the basophils, and that the *Fel d 1* in IVN201 is less well bound by *Fel d 1*-specific IgE in the blood of the patients. From these results it can be concluded that the use of IVN201 in allergic patients for allergen immunotherapy might be safer causing less or no systemic allergic adverse reactions.

In summary, the data of these *in vivo* and *ex vivo* preclinical experiments provide convincing evidence for the efficacy and safety of ImVisionN's concept for allergen-specific immunotherapy.

Acknowledgments

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Discussion

Cromwell: I noticed at the very end of your presentation, referring to the phase I clinical trial, that the first time you used aluminium hydroxide. Why is it necessary to include the alum together with your MAT technology?

Rose: We expect to get a kind of depot effect, which is necessary for the improvement of the immune response. We have tested this with and without alum and it clearly is better to use the alum also in the intranodal injection.

Cromwell: So how do you distinguish between the benefits achieved with alum as opposed to the new technology and have you controlled for that as well?

Rose: We did several experiments where we tested different constructs with the complete backbone, only with the Tat molecule or only with the recombinant *Fel d 1*. The complete construct, as we have used it, comes out better in these studies. All experiments done with alum-adsorbed allergen showed that this is the best way to do it.

Frew: You said that your justification for stopping at three injections was because of your antibody responses. We were discussing this yesterday as to how important that is for the clinical efficacy. Do you have any plans to look later on at whether a longer course of intranodal injections will either give you greater efficacy or more durability and effectiveness?

Rose: Yes, I think we have to do this in the further clinical development to see if a booster injection might be necessary after one year. But I think that will be the topic of the upcoming clinical studies.

Barber: Could you comment on the kind of preclinical safety data that you have to provide to the authorities before entering a phase I trial in humans with a completely new chemical entity with potentially unknown effects?

Rose: If you remember my slide on the preclinical development, it was a greater program which was demanded as compared to other biologicals, e.g., the mutagenicity and cytotoxicity studies had to be included. We also had to use rodent and non-rodent species in the study and altogether we followed the ICH guidelines S6 and M3.

Robinson: One rationale in your treatment is to induce or to augment the Th1-type response and you are showing, at least in your *in vitro* studies, the augmentation of interferon-gamma production. I wonder whether you have any safety concerns about that and whether you have used your pre-clinical mouse model to look at what actually happens, e.g. with an airway challenge. Do you see any development of a Th1-mediated pathology because, e.g., if this was an autoimmune meeting you might be actually looking at reducing Th1 responses?

Rose: We have not specifically addressed this up to now. It is included in the general safety data package, but it was not specifically addressed. I think as this is an allergen-specific Th1 shift and not a general shift to Th1; the danger is relatively low.