The nasal symptoms were evaluated monthly for 3 months, with test score symptoms questionary 4 (TSSQ4) and auto-evaluation by scale Linker.

**Results:** We studied 94 AR patients (50% women and 50% men, range age between 5 and 40 years). Indoor antigens (mites, house dust and cockroach) were the main cause of allergy. Allergic patients had more eosinophiles and IgE than the healthy controls (P < 0.05). The number of CD8+ lymphocytes was slightly reduced in group 2 after treatment (P < 0.05), whereas the amount of IL-4 and IFN fwere increased in both groups (P < 0.005) and the amount of IL-10 was significantly increased in group 1 (P < 0.01) after treatment. Clinical evaluation was with initial TSSQ4 of 11.6 before handling and 5.1 (44%) after, with significant improvement (P < 0.0001) and Likert score was reduced 69% than the star the treatment.

**Conclusions:** The TF along with SLITAE in the treatment of patients with RA did not alter the clinical improvement induced by SLITAE alone for 3 months of treatment, but the combination increased production of IL 10 and production of IFNg.

---

**5 Posttreatment, Long-Term Clinical Efficacy of a 300 IR Sublingual Tablet of 5-Grass Pollen Allergen Extract in Adults With Grass Pollen Induced Allergic Rhinoconjunctivitis**

Alain Didier,1 Friedrich Horak,2 Margitta Worm,3 Hans-Jorgen Malling,4 Armeille Montagut,5 Patricia Rodriguez,2 Robert K. Zeldin,5 and Michèle Lheritier-Barrand.6 Respiratory Diseases Department, Ranque-Larrey Hospital, Toulouse, France; 4Allergy Centre Vienna West, Dptm. - Institute for Allergy Research, Vienna, Austria; 3Allergy-Centre-Charité, Charité - Universitätsmedizin Berlin, Berlin, Germany; 4National University Hospital, Copenhagen, Denmark; 5Stallergenes SA, Antony, France; 6Stallergenes, Antony Cedex, France.

**Background:** A 5-year study of adults with grass-pollen related rhinoconjunctivitis has demonstrated the sustained efficacy of discontinuous treatment with a 300 IR sublingual tablet of 5-grass pollen allergen extract, initiated 4 or 2 months before each pollen season and continued for its duration for 3 consecutive years. Here we report on the persistence of efficacy during the first of 2 post-treatment pollen seasons.

**Methods:** 633 adults were randomized to either placebo or one of 2 active groups receiving pre- and co-seasonal treatment for 3 pollen seasons starting each year either 4 months [4M] or 2 months [2M] prior to the pollen season. Patients were followed during the subsequent, treatment-free, grass pollen season. The primary endpoint for the Year 4 assessment of the post-treatment long-term efficacy was the Average Adjusted Symptom Score (AADSS, adjusting the Rhinoconjunctivitis Total Symptom Score for rescue medication usage) during the fourth pollen period. Secondary efficacy criteria included the Average Rescue Medication Score (ARMS) and the overall Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) score.

**Results:** Statistically significant differences compared to Placebo in the mean AADSS during the Year 4 pollen period were observed for both 300 IR (4M) and 300 IR (2M). The treatment effect for 300 IR (4M) was estimated as the difference in LS Means of −1.14 (95% CI, [−2.03 to −0.26], P = 0.0114), corresponding to a relative LS Mean difference from Placebo of −22.9%, whilst the treatment effect for 300 IR (2M) is estimated as the difference in LS Means of −1.43 (95% CI, [−2.32 to −0.53]), P = 0.0019, corresponding to a relative LS Mean difference from Placebo of −28.5%. The primary results were confirmed over the worst pollen period. Compared to placebo, the active treatment groups (4M and 2M) also showed a statistically significant LS Mean difference in ARMS (−24.6%; P = 0.00184 and −27.9%; P = 0.0082) and in overall RQLQ score (−32.8%; P = 0.0001 and −37.6%; P < 0.0001). No unexpected risk was identified in this study.

**Conclusions:** The post-treatment, long-term efficacy of 300 IR sublingual tablets of grass pollen allergen extract was demonstrated during the first of 2 post-treatment pollen seasons. This persistent improvement was clinically meaningful to patients.

---

**6 Escherichia coli Heat-Labile Enterotoxin (LTS61K) Modulates Dendritic Cell Function and Attenuates Airway Inflammation in Mouse Model of Allergic Asthma**

Jiu-yaow Wang, MD, PhD,1 Yu-Shen Hsu Hsu, PhD,2 and I-Ping Lin, MSc.2
1College of Medicine, National Cheng Kung University Hospital Tak, Taichung, Taiwan; 2Development Center for Biotechnology, Taipei, Taiwan; 3College of Medicine, National Cheng Kung University, Tainan, Taiwan.

**Background:** Escherichia coli heat-labile enterotoxin (LT) with different mutant forms has been used as adjuvant for vaccines due to its ability to enhance immune response to specific antigen in vivo. We hypothesize that LTS61K or LTS61K mixed with dust mite allergen, Der p, (LTS61K/Der p) can modulate dendritic cells (DCs) s’ functions thus alleviate allergen-induced airway inflammation.
Methods: Two protocols (ie, preventive and therapeutic protocol) were designed to evaluate the effects of LTS61K in Der p sensitized and challenged mouse model of asthma.

Results: Both intranasal inoculations with LTS61K or LTS61K/Der p decreased allergen-induced airway inflammation and alleviated systemic T\textsubscript{H}2-type immune response. In addition, bronchoalveolar lavage (BAL) fluids and sera from LTS61K/Der p treated mice have higher concentrations of Der p-specific IgA than those of other groups. In the in vitro study, bone marrow-derived dendritic cells (BMDCs) and DC cell line, DC2.4 cells stimulated with LTS61K/Der p both secreted pro-inflammation cytokines IL-6 and TNF-a. In contrast, after LTS61K treatment, only BMDCs decreased production of IL-6 and TNF-a as well as decreased maturation. Furthermore, we found that pre-treatment BMDC with LTS61K inhibited Der p-induced NF-kB translocation which might explain the delayed maturation and decreased productions of IL-6 and TNF-a in LTS61K pre-treated BMDCs. Intratracheally adoptive transferred with LTS61K- or LTS61K/Der p-primed DC2.4 cells or BMDCs into Der p-sensitized mice decreased inflammatory cells infiltration and T\textsubscript{H}2-type chemokines in BAL fluids and alleviated airway inflammation.

Conclusions: Our results show that LTS61K may influence DCs maturation and its cytokine production. On the other hands, LTS61K/Der p may induce more Der p-specific IgA production to decrease allergic T\textsubscript{H}2 cytokine responses and alleviate airway inflammation in murine model of asthma. These finding suggested that LTS61K may have clinical application as an immune-modulator effect on the diseases of allergy and asthma.

ALLERGEN IMMUNOTHERAPY 2

7 Comparison of Efficacy and Safety of a Dipigmented Polymerized Allergen Extract of Grass and Birch with Placebo in Patients With Type-I Allergic Rhinoconjunctivitis

Angelica Sager, MD, Tilo Biedermann, and Oliver Pfära, MD. 1 Medizinische Klinik Universitatsklinikum Ernst von Bergmann, Witten, Germany; 2 Department of Dermatology, University of Tuebingen, Tuebingen, Germany; 3 Center for Rheology and Allergology, Department of Otorhinolaryngology, University Hospital Mannheim, Wiesbaden, Germany.

Background: The safety and efficacy of specific immunotherapy (SIT) with depigmented and polymerized allergen extracts of pollen is well documented in several clinical trials. We investigated efficacy and safety of an extract containing 2 taxonomically non-related pollen species (birch and grass) in a subcutaneous immunotherapy over 2 pollen seasons in co-sensitized allergic patients with rhinitis and/or rhinoconjunctivitis with or without allergic asthma.

Methods: 269 (ITT) patients with confirmed rhinitis and/or rhinoconjunctivitis were treated during 2009 and 2010 in Germany, Romania, Poland, Lithuania, and Bulgaria. For each patient a 1-day build-up phase applying 0.2 mL and 0.3 mL of 1000 DPP/mL allergen extract was applied. During the remaining 18-month period maintenance 500 DPP were administered in 4 to 6 weeks intervals. Patients were randomised to the treatment groups on a 2:1 basis (175 verum: 94 placebo). The main parameter in this study was the combined symptom and medication score during the birch and grass pollen season 2010 over 7 weeks. Secondary parameters were symptom score, medication score, IgE, IgG4 as well as quality of life.

Results: During the 2010 season a statistically significant difference ($P = 0.0385$) was observed between treatment groups: in patients treated with the allergen extract the median time weighted AUC of the combined symptom and medication score was 5.70, in patients treated with placebo 7.07. This effect was predominantly due to the reduction of symptom score by 21% over placebo. The intake of rescue medication was very low during both seasons leading only to a 10% reduction (ns). Birch and phleum specific IgE did not change during the course of the study in both groups whereas respective IgG4 levels increased only in the verum group and remained nearly unchanged in the placebo group ($P < 0.0001$). Total QoL score was improved in verum patients ($P = 0.0254$). 5.4% of the patients in the verum group and 4.0% of the patients in the placebo group developed mild systemic reactions.

Conclusions: The results show that specific immunotherapy with a depigmented polymerized extract of 2 taxonomically non-related pollen (birch and grass) was effective and safe demonstrated by clinical and immunological parameter.

8 Allergenic Composition of Polymerized Allergen Extracts of Betula verrucosa, Dermatophagoides Pteronyssinus and Phleum pratense

Enrique Fernandez-Calda, PhD,1,2 Barbara Cases, PhD,1 Jose Ignacio Tudela, BS,2 Eva Abel Fernandez, BS,3 Miguel Casanova, MD, PhD,3 and Jose Luis Subiza, MD, PhD.1 1 Research & Development, Immunetek SL, Madrid, Spain; 2 Division of Allergy and Immunology, University of South Florida College of Medicine, Tampa.

Background: Allergenic extracts have been successfully used in the treatment of respiratory allergic diseases. They are modified allergen extracts that allow the administration of high allergen doses, due to their reduced IgE binding capacity. They maintain allergen-specific T-cell recognition. Since they are native allergen extracts that have been polymerized with glutaraldehyde, identification of the allergenic molecules requires more complicated methods. The aim of the study was to determine the qualitative composition of different polymerized extracts and investigate the presence of defined allergenic molecules using Mass spectrometry.

Methods: Proteomic analysis was carried out at the Proteomics Facility of the Hospital Nacional de Parapléjicos (Toledo, Spain). After reduction and alkyl-lation, proteins were digested with trypsin and the resulting peptides were cleaned using C18 SpinTips Sample Prep Kit; peptides were separated on an Ultimate nano-LC system using a MonoLich C18 column in combination with a precolumn for salt removal. Fractionation of the peptides was performed with a Probot microfraction collector and MS and MS/MS analysis of offline spotted peptide samples were performed using the Applied Biosystems 4800 plus MALDI TOF/TOF Analyzer mass spectrometer. ProteinPilot Software V 2.0.1 and the Paragon algorithm were used for the identification of the proteins. Each MS/MS spectrum was searched against the SwissProt 2010_10 database, Uniprot-Viridiplantae database and Uniprot_Betula database.

Results: Analysis of the peptides revealed the presence of native allergens in the polymerized extracts: Der p 1, Der p 2, Der p 3, Der p 8 and Der p 11 in D. pteronyssinus; Bet v 2, Bet v 6, Bet v 7 and several Bet v 1 isoforms in B. verrucosa and Phl p 1, Phl p 3, Phl p 5, Phl p 11 and Phl p 12 in P. pratense allergoids. In all cases, potential allergenic proteins were also identified, including ubiquitin, actin, Eenolase, fructose-bisphosphate aldolase, luminal-binding protein (Heat shock protein 70), calmodulin, among others.

Conclusions: The characterization of the allergenic composition of allergoids is possible using MS/MS analysis. The analysis confirms the presence of native allergens in the allergoids. Major allergens are preserved during polymerization.

9 A Proteomic Style Approach to Characterize a Grass Mix Product Reveals Potential Immunotherapeutic Benefit

Murray Skinner, PhD, Alan Bullimore, Nicola Swanc, MS, and Wemimo Alawode, PhD. Research & Development, Inmunotek R & D, Allergy Therapeutics, Worthing, United Kingdom.

Background: Grass allergy immunotherapies often consist of a mix of different grass extracts each containing several proteins of different physiochemical properties; however the subtle contributions of each protein are difficult to elucidate. This study aimed to identify and characterise the