group 1 and 5 allergens in a 13 grass extract and to standardize the extraction method.

**Methods:** The grass pollens were extracted in isolation and pooled and also in combination and analysed using a variety of techniques including enzyme-linked immunosorbent assay (ELISA), liquid chromatography-mass spectrometry (LC-MS) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

**Results:** Gold-staining and IgE immunoblotting revealed a high degree of homology of protein bands between the 13 species and the presence of a densely stained doublet at 25 to 35 kD along with protein bands at approximately 12.5, 17 and 50 kD. The doublet from each grass species demonstrated a high level of group 1 and 5 interspecies homology. However, there were a number of bands unique to specific grasses consistent with evolutionary change and indicative that a grass mix immunotherapeutic could be considered broad spectrum.

**Conclusions:** SDS-PAGE and IgE immunoblotting showed all 13 grasses share a high degree of homology particularly in terms of group 1 and 5 allergens. IgE and IgG ELISA potencies were shown to be independent of extraction method.

### 10 Seasonal Versus Symptom-based Evaluation of a Depigmented Grass-Birch Allergoid

Oliver Pfaar, MD,1 Tilo Biedermann,2 and Angelika Sager, MD3. 1Center for Rhinology and Allergology, Department of Otorhinolaryngology, University Hospital Mannheim, Wiesbaden, Germany; 2Department of Dermatology, University Hospital, Tübingen, Germany; 3Medical Department, LETI Pharma GmbH, Witten, 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. The results of such clinical studies are highly dependent on the quantity and quality of pollen exposure and their measurements. To identify a pollen-independent efficacy assessment we compared the combined symptom and medication score (SMS) measured during the pollen season with the SMS defined by a minimum symptom score of the placebo group in a subcutaneous immunotherapy with a depigmented Grass-Birch allergoid over 2 years.

**Methods:** 269 (ITT) patients with confirmed rhinitis and/or rhinoconjunctivitis were treated during 2009 and 2010 in Germany, Romania, Poland, Lithuania, and Bulgaria. 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. In addition the SMS of the actively treated patients was analysed following the placebo treated patients eliciting a considerable symptom burden ≥2.

**Results:** The clinical results following the seasonal approach are given in the other abstract of our group. Taking all days with mean symptom score >2 in the placebo group as calculation basis, the combined SMS values were considerably lower for actively treated patients than for placebo treated patients. For both seasons, the differences between the treatment groups were highly statistically significant (median: 2009: 5.06 vs 7.97, 2010: 4.26 vs 6.43; ITT set) with P-values of 0.0038 and 0.003.

**Conclusions:** The results show that the efficacy assessment of specific immunotherapy might be better discriminated in relation to the actual symptoms of the placebo group rather than following the days of pollen exposure.

### Reference

1. Abstract # 3093: Biedermann T, Pfaar O, Sager A. "Comparison of efficacy and safety of a depigmented polymerized allergen extract of grass and birch with placebo in patients with type-I allergic rhinoconjunctivitis."

### 11 Efficacy and Tolerability of HDM Injective Immunotherapy With Monomeric Allergoid

Enrico Compalati, MD,1 Isabella Atzeni, MD,2 Sergio Cabras, MD,3 Paolo Fancello, MD,2 Giulio Gaspardini, MD,2 Rocco Longo, MD,3 Vincenzo Patella, MD,4,5 and Giorgio Tore, MD,6. 1Allergy & Respiratory Diseases Clinic, University of Genova, Genova, Italy; 2Servizio di Allergologia, Ospedale "San Gavino Monreale", ASL 6, Sanluri (CR), Italy; 3Casa di Cur A Madonna del Rimedio, Oristano, Italy; 4Divisione ORL, Ospedale, Cagliari, Italy; 5Servizio Territoriale di Allergologia, Azienda Sanitaria Provinciale, Vibo Valentia, Italy; 6Division of Clinical Immunology and Allergy, General Hospital, ASL SALERNO, Agropoli (SA), Italy; 7U.O. di Allergologia e Immunologia Clinica, Ospedale Generale, Agropoli (SA), Italy.

**Background:** Subcutaneous immunotherapy (SCIT) is an effective treatment of seasonal and perennial allergic rhinoconjunctivitis (AR) and其它 AR. In this prospective controlled study, SCIT with HDM carbamylated monomeric allergoids was tested over 2 years.

**Methods:** A preparation of 0.70 mL of 10 BU/mL containing modified extract with 50% Dermatophagoides pteronyssinus and 50% Dermatophagoides farinae (amount of major allergen: 4 μg of group 1 per milliliter) was delivered monthly for 12 months, following a 5-week build-up induction phase (0.10–0.20–0.30–0.50–0.70 mL), to 58 patients (60% males, mean age 25.1 ± 12.7) suffering from AR due to mites for at least 2 years, whereas 60 patients with similar baseline characteristics were observed as controls. All patients were allowed to assume traditional drug therapy for their condition. At the end of the study changes from baseline in symptoms scores, in number of days with drug assumption, in severity of AR (according to ARIA classification) were compared between the 2 groups; moreover an overall assessment of clinical efficacy and tolerability was based on patients’ and physicians’ judgements (unsatisfactory, mild, good, optimal).

**Results:** In respect to baseline both groups showed, after 1 year, an improvement in symptoms score (P < 0.001) with a significant difference in favour of SCIT group (P < 0.05). Days of drug intake were significantly lower in patients receiving SCIT (P < 0.05). The number of patients with severe AR decreased in the first group while no variation was observed in controls. The subjective clinical overall assessment was optimal in 31 cases and good in 24 according to physicians’ and patients’ judgements; similarly 38 patients judged tolerability as optimal and 18 as good, whereas according to physicians it was optimal in 37 patients and good in 19; in only 1 patient the treatment was considered unsatisfactory.

**Conclusions:** In this prospective controlled study, SCIT with HDM carbamylated allergoid was associated with a significant clinical benefit observed through objective and subjective outcomes; the traditional safety of monomeric allergoids was confirmed by the subjective judgements of tolerability.

### Allergen Structure 1

#### 12 Comprehensive Detection of Allergens in Grass Pollen Extracts by Mass Spectrometry

Steffen Augustin, PhD,1 Liane Mitulski,2 Oliver Cromwell, PhD,3 Gerald Reese, PhD,4 and Andreas Nandy, PhD5. 1Allergopharma Joachim Ganzer KG, Laboratory Molecular Biology, Reinbek, Germany; 2Allergopharma Joachim Ganzer KG, Reinbek, Germany; 3Research and Development; 4Allergen Research; 5Molecular Biology Research, Allergopharma Joachim Ganzer KG, Reinbek, Germany.

**Background:** More than 40% of type 1-allergic individuals suffer from hypersensitivity to grass pollen. Patients are treated traditionally with specific...