ABSTRACTS

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1637 | Peanut epitope-specific IgE binding can predict clinical peanut allergy

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Background: DBPCFCs remain the gold standard for clinical diagnosis of food allergy. However, this test requires significant resources and inherent risks. In addition to OFCs, physicians often rely on patients' allergen-specific IgE levels, which cannot consistently ascertain allergy status. Utilizing a cohort of 185 subjects from the CoFAR natural history study, we measured IgE and IgG4 binding to peanut epitopes in high risk children from 3 months to 10 years of age and determined their utility in predicting clinical peanut allergy.

Method: A Luminex-based assay was used to quantitate IgE and IgG4 antibody binding to 50 sequential epitopes found on Ara h1-3. Epitope-binding profiles (EBPs) were evaluated using plasma from subjects at baseline (n = 141), 2 years (n = 129) and approximately 5 years (or later) (n = 185). Machine learning algorithms were used to predict allergy status at the same time point. Only subjects with a peanut diagnostic category of confirmed, serologic, or not allergic were included in the analysis.

Results: Seventy-five percentage of the total 455 samples were randomly selected for model development (training) (n = 343) and the rest were kept aside to test model predictions (n = 112). We had previously identified the natural expansion of the epitope repertoire with age, especially for IgG4-epitopes. Age-specific models performed better than age-agnostic models. IgE-profiles were sufficient to predict allergy status, while models with only IgG4 did not perform well. Of the strategies evaluated, the random forest algorithm had the highest classification accuracy, with an average AUC >0.87 in cross-validation at baseline, reaching 0.99 and 0.95 at 2 and ≥5 years. The final IgE-based Age-specific model was then evaluated in the 'unseen' data. Allergy status at baseline was accurately classified in 24/35 patients (69% accuracy) with higher accuracy at 2 and ≥5 years (91% and 87%). Within the subset of patients with allergy diagnosis confirmed by OFC at ≥5 years, all 13 patients in the training set were correctly predicted, while prediction accuracy of the final model in the testing set (n = 10) was 80%.

Conclusion: Evaluation of the epitope repertoire is predictive of the peanut allergy diagnosis as defined by allergy history, peanut-specific IgE levels, and OFC. If confirmed in other studies, this assay may enable physicians to identify most infants with persistent peanut allergy without the need for an OFC.

1638 | Influence of the gut microbiome on IgE and non-IgE-mediated food allergies

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Background: The prevalence of food allergy (FA) in children has been increasing in last decade. Recent studies show changes in gut microbiome with FA. However, whether gut microbiome may differ between IgE and non-IgE-mediated FA is not defined. The aim of this study is to examine the intestinal microbiome composition in infants with IgE and non-IgE-mediated FA and healthy infants.

Method: Infants younger than 1-year-old, breastfed and diagnosed with FA by a physician were included in the study. DNA was isolated from stool samples of infants with non-IgE-mediated FA (n = 25) and IgE-mediated FA (n = 11) and healthy infants (n = 7). Whole genome shotgun sequencing was applied to identify the composition of microbial DNA (an average depth of 3.1 ± 0.8 million paired end reads and 0.9 ± 0.2 gigabase pairs).

Results: There were compositional differences among 3 different groups. Shannon index was significantly higher in IgE-mediated FA compared to non-IgE-mediated FA group (Kruskal-Wallis test,

$P = 0.034$). Even though β-diversity was similar, the Sparse Partial Least Square Discriminant Analysis (sPLS-DA) demonstrated that there were taxa-level differences among three groups. In species level, *Veillonella parvula* was in a significantly higher density in healthy infants compared to IgE and non-IgE-mediated FA groups. *Rahnella aquatilis* and *Lactobacillus salivarius* were significantly lower and *Treponema succinfaciens* significantly higher in IgE-mediated FA group compared to other groups. Additionally, Prevotella sp. oral taxon 299 was significantly lower in non-IgE-mediated FA group compared to others. Prevotella sp oral taxon 299 was related to mucus in stool whereas urticaria related species were Olsenall uli, Bactreoides thetaiotaomicron, Klebsiella variiocola, Rahnella aquatilis, Treponema succinfaciens, Ethanoligenens harbinenese.

Conclusion: Analysis of microbiome differences in FA patients may aid in the understanding of the disease process. The present data suggest that there are compositional variations mostly in species-level among infants with FA and healthy ones. Our results suggest that the gut microbiome has a stronger relationship to IgE-mediated than non-IgE-mediated FA. Further functional analysis of the microbiome may help better understand the changes seen in the gut microbiome in FAs and improve our knowledge in the disease etiopathology.

1639 | Use of home baked milk reintroduction in children with milk allergies

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Background: Current guidelines have recommended that in young children with cow's milk protein allergy, reintroduction can be achieved by the graded exposure, either at home or in hospital with supervision depending on severity, using a milk ladder. This should be reviewed every 6-12 months, with repeat skin prick testing if IgE-mediated. Reintroduction should be started with baked milk as it is less allergenic. We wanted to look at the effectiveness and safety behind the use of home baked milk re-introduction in our local paediatric group of children with milk allergy.

Method: We carried out a retrospective study in our local hospital, Sandwell Hospital, Children Outpatient Department, looking at paediatric patients whom underwent skin prick testing (SPT) and having positive SPT reaction towards milk from January 2015-January 2016. We identified 39 patients, for whom we looked to see if baked milk reintroduction has been discussed during clinic (or if dietitian referral has been made for this purpose). We then looked at the stages of baked milk containing foods which they have tolerated in 6-12 monthly follow up subsequently.

Results: The median age of patients whom had home baked milk re-introduction initiated was 18 months (most had wheal size in SPT for milk (in mm) of <8). Out of the 39 patients, 7 had unknown outcomes due to being lost to follow up, or due to be followed up

again. Out of the remainder 32 patients, 5 patients did not tolerate baked milk re-introduction and reported adverse effects. 27 patients (84%) tolerated baked milk re-introduction, with 12 patients achieving stage 4 type foods (indicating full tolerance to milk) by the end of 12 months. Adverse effects which were reported were not severe (not requiring hospital admissions).

Conclusion: Home baked milk re-introduction is effective as seen in our study results. Its use is also safe. Selection of patients according to their reactions towards milk is important. Dietician follow-up in the interim is useful for parents whom have any questions about baked milk re-introduction in between allergy clinic follow-ups.

1640 | Efficacy of dupilumab on components of a novel histological scoring system for active eosinophilic esophagitis in a randomized placebo-controlled phase 2 clinical trial

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Background: Eosinophilic esophagitis (EoE) is a chronic, type 2 immune-mediated disease. Dupilumab (DPL), an anti-interleukin (IL)-4Rα mAb that inhibits IL-4/IL-13, key drivers of type 2 inflammation, is approved for treatment of adults with inadequately controlled moderate-to-severe atopic dermatitis. In a phase 2 study (NCT02379052), DPL reduced dysphagia, improved esophageal eosinophil infiltration and endoscopic measures of disease and was generally well tolerated in adults with EoE. The EoE Histological Scoring System (HSS) is a recently validated instrument that assesses disease severity (grade) and extent (stage) scores of 8 features: eosinophil density, basal zone hyperplasia, eosinophil abscesses, eosinophil surface layering, dilated intercellular spaces (DIS), epithelial surface alteration, dyskeratotic epithelial cells (DEC), and lamina propria fibrosis. HSS provides a more comprehensive evaluation of EoE activity than the current use of eosinophil density only. This post hoc analysis reports DPL efficacy on EoE HSS in EoE patients.

| | Distal esophageal region | | | | Mid esophageal region | | | | Proximal esophageal region | | | |
| | LS mean (SE) change in grade score | | LS mean (SE) change in stage score | | LS mean (SE) change in grade score | | LS mean (SE) change in stage score | | LS mean (SE) change in grade score | | LS mean (SE) change in stage score | |
	PBO (n = 21/3)	DPL (n = 22/1)	PBO (n = 21/3)	DPL (n = 23/0)	PBO (n = 20/4)	DPL (n = 22/1)	PBO (n = 20/4)	DPL (n = 23/0)	PBO (n = 22/2)	DPL (n = 22/1)	PBO (n = 22/2)	DPL (n = 23/0)
Total score	−0.3 (0.46)	−6.2 (0.45)***	−0.1 (0.47)	−5.4 (0.45)***	−1.6 (0.63)	−7.8 (0.61)***	−2.0 (0.56)	−6.5 (0.54)***	0.8 (0.61)	−4.9 (0.60)***	0.4 (0.50)	−4.5 (0.49)***
Basal zone hyperplasia	−0.0 (0.15)	−2.0 (0.15)***	−0.2 (0.23)	−1.2 (0.23)**	−0.4 (0.17)	−2.4 (0.16)***	−0.7 (0.27)	−1.7 (0.26)**	0.1 (0.18)	−1.4 (0.18)***	0.0 (0.21)	−1.3 (0.21)***
Eosinophil density	−0.0 (0.12)	−1.5 (0.12)***	−0.1 (0.15)	−2.2 (0.15)***	−0.5 (0.15)	−2.0 (0.15)***	−0.8 (0.18)	−2.6 (0.18)***	−0.1 (0.15)	−1.4 (0.14)***	−0.0 (0.17)	−1.6 (0.17)***
Eosinophil abscesses	0.1 (0.12)	−0.4 (0.11)**	0.0 (0.07)	−0.3 (0.07)***	−0.1 (0.13)	−0.6 (0.13)**	−0.1 (0.07)	−0.4 (0.07)***	0.2 (0.10)	−0.2 (0.10)***	0.2 (0.07)	−0.2 (0.07)***
Eosinophil surface layering	−0.3 (0.11)	−0.7 (0.11)**	−0.1 (0.09)	−0.5 (0.08)**	−0.1 (0.15)	−0.7 (0.15)**	−0.1 (0.07)	−0.4 (0.07)***	0.5 (0.18)	−0.4 (0.17)***	0.1 (0.07)	−0.2 (0.07)***
Dilated intercellular spaces	−0.2 (0.08)	−0.5 (0.08)***	0.0 (0.12)	−0.3 (0.12)	−0.2 (0.12)	−1.0 (0.12)***	−0.0 (0.16)	−0.6 (0.16)**	−0.0 (0.11)	−0.6 (0.12)***	−0.1 (0.17)	−0.6 (0.17)*
Epithelial surface alteration	−0.1 (0.16)	−0.8 (0.16)**	−0.1 (0.15)	−0.7 (0.15)**	−0.3 (0.19)	−1.1 (0.19)**	−0.2 (0.14)	−0.7 (0.14)**	0.2 (0.17)	−0.6 (0.17)***	0.1 (0.12)	−0.4 (0.12)**
Dyskeratotic epithelial cells	0.1 (0.07)	−0.2 (0.06)*	0.1 (0.07)	−0.1 (0.06)**	−0.1 (0.06)	−0.1 (0.05)	−0.1 (0.05)	−0.1 (0.05)	−0.1 (0.03)	−0.2 (0.03)	−0.1 (0.03)	−0.1 (0.03)

LS, least squares; N, number of patients/imputed patients; qw, once weekly; SE, standard error. LS means and P values were based on analysis of covariance (ANCOVA). Missing data were imputed using multiple imputations.

*P < 0.05, **P < 0.01, ***P < 0.001 vs PBO.

Method: 47 adults with active EoE were randomized (1:1) to 12-week treatment with subcutaneous DPL 300 mg or placebo (PBO) weekly. At Week 12, both grade and stage scores (scale 0–3; 0 = normal) were assessed for 7 features of the HSS (total scores 0–21 for proximal, mid, and distal regions) in esophageal biopsies. Lamina propria fibrosis measures were excluded as unevaluable in >50% of biopsies.

Results: DPL improved total scores and all components of the EoE HSS in EoE patients vs PBO except for stage and grade scores for DEC in the mid and proximal regions and stage score for DIS in the distal region (Table).

Conclusion: Dupilumab significantly improved both the severity and extent of most histological features of EoE measured by the HSS. The results support the reliability of the HSS as an activity measure for adults with EoE.

1641 | Dietary management of adult eosinophilic esophagitis: A pilot feasibility study of an elemental plus few foods diet versus the six food elimination diet

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Background: An elemental diet is highly effective but rarely used for clinical treatment of adult eosinophilic esophagitis (EoE) due to poor compliance. Combining an elemental diet with a few low-risk foods has the potential to increase palatability whilst maintaining high efficacy.

Method: In this feasibility study adults with EoE were randomised to either an elemental plus few foods diet (FFD, six low-risk foods eg lamb, broccoli, butternut squash, coconut, quinoa, millet) or a six-food elimination diet (SFED, avoidance of milk, wheat, egg, soya, fish and nuts) for six weeks. Outcome measurements included eosinophil counts (esophageal biopsies), eosinophil cationic protein (ECP-esophageal string, saliva), symptom scores (PEESS 2.0), quality of life (EoE-QoL-A), BMI/weight loss, nutrient intake (food diary) and adherence (questionnaire, food diary).

Results: Thirteen participants were recruited with eight randomised to SFED and five to FFD. Four (31%) withdrew, two in each arm, and nine underwent post-intervention biopsies. One out of six patients (17%) in the SFED group had a histological response (eosinophil count below 15 post intervention) versus one out of three (33%) in the FFD arm. There were no significant changes in ECP or overall symptom scores, although there was significant improvement in dysphagia subscale scores (P = 0.046). BMI was significantly

reduced (P = 0.041; 95% CI 0.054-1.506) as well as quality of life (P = 0.040; 95% CI 0.035-1.097), in particular the eating/diet impact sub-score (P = 0.026; 95% CI 0.175-1.925). Baseline diet quality was poor for three out of five participants (SFED n = 2, FFD n = 1) but did improve during the interventions. Adherence was acceptable for eight out of nine participants (89%), although there was missing data.

Conclusion: To our knowledge this is the first randomised trial of dietary intervention in adults with EoE, piloting the novel combination of elemental and FFD. The study presented many challenges, in particular recruitment and retention. Neither intervention showed high efficacy rates seen in other studies, despite intensive dietetic input. However the sample size was small and no UK outcome data exists for comparison. Future studies should focus on nutritional parameters and consider supportive strategies to overcome potential adverse effects on QoL and nutrition, for instance regular dietetic input. Further research into less invasive biomarkers such as the esophageal string test would also be beneficial.

1642 | Skin prick test to raw milk is superior to commercial milk extract and milk specific IgE for identifying clinically relevant sensitisation in adults with eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic immune-mediated disease, isolated to the esophagus, characterised by eosinophil-predominant inflammation on biopsy. Milk is known to be the most common trigger. However, data on allergy tests including skin prick tests (SPT) or specific immunoglobulin-E (sIgE) to guide dietary treatment is limited. These tests are routinely performed in EoE patients referred to our adult allergy department.

Method: To assess if SPT to a commercial milk extract or raw milk is more effective in identifying clinically relevant milk sensitisation, a retrospective case review was undertaken. Only EoE patients with milk sensitisation (either with SPT and/or sIgE) were included. All patients underwent testing before dietary treatment that included milk elimination (six-food or test directed food elimination diet). Responses were monitored by repeat esophageal biopsy, which is defined as full response (eosinophil count per high power field of less than 5), partial response (eosinophil count of 5-15 or total of 50% reduction) or no response.

Results: Twenty-four EoE patients with milk sensitisation were identified. One patient was excluded due to non-compliance with the treatment plan. Of 23 patients, 14 (61%) had either partial or full response following a dietary intervention that included milk elimination. Responders to diet had significantly greater wheal sizes with raw milk than non-responders (mean (±SE) = 4.7 ± 0.8 mm [n = 14]

vs 1.3 ± 0.7 mm; *P* = 0.005). No significant differences were seen in SPT size to milk extract (*P* = 0.63) or milk IgE titres (*P* = 0.47). In the responder group 13 of 14 patients had a positive SPT (≥3 mm) to raw milk vs 2 of 8 tested in the non-responder group (*P* = 0.001). Sensitivity and specificity of a raw milk SPT were 0.87 and 0.86, respectively.

Conclusion: This small retrospective study suggests that SPT to raw cow's milk may have utility in identifying clinically relevant milk sensitisation in EoE patients. Further evaluation is warranted in larger prospective studies.

1643 | A double-blind, placebo-controlled phase I/II dose-finding study of viaskin milk in children and adolescents for the treatment of IgE-mediated cow's milk protein allergy (CMPA): Results from miles

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Background: CMPA is a serious disease impacting millions of patients worldwide with no treatment options. Viaskin is a novel, investigational immunotherapy, consisting of once-daily applications of an epicutaneous patch with allergen on intact skin. MILES is a multicenter, double-blind, placebo-controlled Phase I/II dose-finding study, designed to identify the optimal dosing and patient population for future development, and to evaluate the safety and efficacy of epicutaneous immunotherapy (EPIT) with Viaskin® Milk (VM), in children ages 2-11 and adolescents ages 12-17 with IgE-mediated CMPA.

Method: In MILES, 198 subjects with cow's milk specific IgE $\geq$10 kU$_A$/L, skin prick test wheal $\geq$6 mm and an objective reaction to $\leq$300 mg of CMP assessed by double-blind, placebo-controlled food challenge (DBPCFC) were randomized to one of three VM doses (150 μg, 300 μg or 500 μg) or placebo (1:1:1:1) for a 12-month double-blind treatment period. The main efficacy endpoint evaluated the percentage of patients who responded to treatment as assessed by pre-specified changes from baseline in DBPCFC cumulative reactive doses (CRD).

Results: In the Intent-to-Treat (ITT) population, response rates at Month-12 with VM were 36.7% (150 μg), 49.0% (300 μg) and 36.2% (500 μg) vs 30.2% for placebo. The greatest benefit was observed with the 300 μg dose, with a significant treatment effect in the Per-Protocol (PP) population (P = 0.027); these findings were consistent with ITT statistical trends. In children (2-11 years), the 300 μg dose showed a response rate of 57.9% vs 32.5% for placebo (P = 0.042, ITT; 62.5% vs 31.4%, P = 0.021, PP). Increases in CRD of 1322.4 mg compared to baseline were also significant in this age group as compared to placebo (P = 0.045, ITT; 1340.3 mg, P = 0.043, PP). Mean patient compliance across VM treatment groups was 97.2%. No serious adverse events related to treatment occurred. Overall, the discontinuation rate was 4.5% with a 1.5% dropout rate due to adverse events. Most subjects (98.9%) who completed the 12-month treatment period opted to continue treatment in the open label extension period.

Conclusion: At month-12, treatment with VM 300 μg in children 2-11 years of age resulted in a significantly greater response and improvement in CRD as compared to placebo, warranting future studies of VM 300 μg in this patient population. All doses of VM were safe and well-tolerated. Detailed results, including immunologic data, will be available for presentation.

1644 | A pre-coseasonal course with monomeric allergoid sublingual tablets is very well tolerated and reliefs the symptoms of seasonal rhinoconjunctivitis in hungarian patients allergic to ragweed pollen

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Background: A few studies suggested that a relatively short immunotherapy course beginning just before the start of the ragweed season may be effective. In the past the ragweed monomeric allergoid tablets determined a marked reduction in both symptoms and medication intake and a significant decrease in nasal reactivity to provocation test after one year. Aim of this trial is the evaluation of the efficacy and safety of a 21-weeks course of sublingual immunotherapy (SLIT).

Method: In this double-blind, placebo-controlled 1:1 randomized multicentre trial (EudraCT number: 2014-004431-38), ragweed monomeric allergoid tablets were administered at 2000 AU/day to adult subjects, with rhinoconjunctivitis retrospective symptoms score >8 (range 0-18) with/or without concomitant asthma controlled without continuous inhaled steroids, for 3-4 months before and 2 months during the 2016 ragweed pollen season. Primary objective was the Total Combined Score (TCS), sum of 6 daily Symptoms Score (dSS, 0-18) and daily Medications Score (dMS, 0-18) during the 15 days peak pollen period. Safety was assessed according to

GCP rules. Pollen counts were collected by Department of Air Hygiene and Aerobiology, Budapest.

Results: A total of 105 subjects entered the full analysis set. Compared to the symptoms in previous ragweed season, more than 78% of patients felt overall improved in both groups. The analysis of the primary efficacy endpoint showed that the TCS (AUC) for the peak pollen period was in favour of the active treatment by 13.3%. The average dSS score was 4.71 in active group and 5.10 in controls (average retrospective 13.9). The intake of antiallergic medications (AUC) was inferior in the active group compared to placebo (intergroup mean difference −24%). The number of well days (without intake of rescue medication and dSS ≤2) along the entire pollen season was superior in the active group (intergroup mean difference +16.9%). No serious adverse events occurred, only 1 moderate adverse drug reaction (glossitis) in active group, leading to discontinuation, was reported (1.9%).

Conclusion: A 21-weeks course of SLIT with ragweed monomeric allergoid tablets initiated 3-4 months before the pollen season showed a safety profile similar to placebo and better improved the rhinoconjunctivitis symptoms with a lower need of antiallergic medications.

1645 | Allergy immunotherapy provides long-term relief of birch family pollen-induced allergic rhinitis up to 6 years following treatment cessation: A real-world dataset analysis

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Background: Allergy immunotherapy (AIT) in the form of subcutaneous (SCIT) or sublingual immunotherapy (SLIT) is the only disease-modifying intervention for allergic rhinitis (AR) with long-term clinical efficacy. However, clinical evidence is currently available mainly for short-term treatment periods, and real-world evidence can further inform us with long-term observation and large, representative sample sizes. Here, we evaluated the effect of AIT after treatment cessation in patients with birch family pollen-induced AR and/or asthma (AIT group) versus a control group receiving symptomatic treatment only (non-AIT group).

Method: A retrospective, comparative cohort analysis of a German longitudinal prescription database covering 9 pollen seasons (Jan 2008–Feb 2017) assessed 6 AIT products indicated for both birch family pollen-induced AR and/or mild to moderate asthma: native pollen SLIT and SCIT (one each) and 4 allergoid SCIT preparations.

AIT users with AR medication at baseline had received ≥2 successive AIT seasonal treatment cycles, while AIT non-users had ≥3 prescriptions against AR in 3 seasons or the previous month; all patients were followed up for ≥2 years after treatment cessation. Severe asthma was an exclusion criterion. Patients in both groups were matched for index year, age group, gender, main indication at index date, number of seasonal cycles covered by treatment period, and baseline AR and asthma treatment prescriptions. Multiple regression analysis compared prescription data in the AIT and non-AIT groups as a proxy for clinical status and disease progression.

Results: The AIT and non-AIT groups comprised 9001 (6349/472 with AR/AR plus asthma) and 45 005 (31 745/2360 with AR/AR plus asthma) patients, respectively. At up to 6 years of follow-up, significantly more patients in the AIT vs non-AIT group were medication-free [65.4% vs 47.4%, respectively; odds ratio (OR) for AIT: 0.511, $P < 0.001$] with a highest rate of 74.4% (OR: 0.314, $P < 0.001$) for SLIT. AR treatment at the individual-patient level decreased to 16.4% compared to pre-index values in the AIT group, versus 45.6% in the non-AIT group. AR medication use was 28.6% points lower in the AIT group after adjustment for covariates than the non-AIT group ($P < 0.001$).

Conclusion: Real-world treatment using birch family pollen AIT of patients with AR and/or asthma was associated with significantly reduced AR progression and need for symptomatic medication up to 6 years after treatment cessation.

1646 | Evaluation of a standardized bakery product (SUTMEK) as a potential tool for baked milk tolerance and immunotherapy research studies

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Background: About 65%-80% of children with IgE mediated cow's milk allergy (CMA) can tolerate extensively heated milk (EHM). We have invested a mass fabrication of a test product containing milk protein baked at 180°C for 30 minutes (SUTMEK-milk) and a milk free placebo (SUTMEK-placebo) to carry out a standardized double-blind placebo controlled food challenge (DBPCFC) test in patients with CMA.

Method: We studied children with IgE-mediated CMA between 13-48 months of age. Specific IgE to milk proteins were quantified. A DBPCFC with our bakery product was performed and factors determining reactivity to EHM were evaluated. We also tested applicability of SUTMEK products in baked milk oral immunotherapy in a pilot assessment.

Results: We studied 15 children (8 girls, 7 boys) with a median age of 26 months (range: 13-48 months). Nine (60%) patients tolerated a

challenge with EHM, while six (40%) were found reactive (anaphylaxis = 2, wheezing = 2, urticaria; n = 2). Specific IgEs to milk, alpha-lactalbumin and casein and the wheal diameter on skin prick testing were higher in the reactive group compared to the tolerant ones ($P = 0.001$, $P = 0.001$, $P = 0.002$ and $P = 0.048$, respectively). ROC curve analyses yielded the following cut-off values for specific IgEs that would predict a reactivity to EHM; milk: 25 IU/mL (AUC: 0.981), casein: 32 IU/mL (AUC: 0.983) and alpha-lactalbumin: 17 IU/mL (AUC: 0.981). Nine patients continued with daily consumption of SUTMEK-milk or -placebo for 6 months with success.

Conclusion: Our bakery products were successfully used in DBPCFC studies and qualified as an acceptable tool for use in the research of interventional tolerance induction. Specific IgE appears useful in determining children at high risk of reacting to EHM.

1647 | Potential treatment of pollen food syndrome using birch AIT: A study investigating the effect of the SQ tree SLIT-tablet on symptoms during an apple challenge

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Background: A large number of tree pollen allergic individuals develop local allergic symptoms against certain foods such as raw vegetables, fruit and nuts. The symptoms manifest as a condition called pollen food syndrome (PFS). AIT with birch allergen may be beneficial in the treatment of PFS due to cross reactivity between Bet v 1 and the Bet v 1 homologous allergens responsible for PFS. Here we report the results of an explorative food challenge with apple, following approximately 6 months of treatment with the SQ tree SLIT-tablet (allergen extract from birch) as part of the phase III trial TT-04 (EudraCT 2015-004821-15).

Method: The TT-04 trial was a randomised, DBPC, phase III trial to evaluate the efficacy and safety of the SQ tree SLIT-tablet in rhinoconjunctivitis induced by pollen from the birch homologous group. 634 subjects were randomised 1:1 to the SQ tree SLIT-tablet (12 DU) or placebo. A subset of trial subjects with PFS participated in an open apple challenge at the end of the trial (N = 124), that included ingestion of increasing amounts of apple (4, 8, 16, 32 and 64 g apple) administered 15 minutes apart. Assessments included PFS symptoms (rated by VAS, 0-10 cm) and global evaluation of efficacy with regards to PFS (ie improvement in PFS since entering the trial) and quantification of apple specific Mal d 1 antibodies during the trial.

Results: While PFS symptom scores were generally low in both treatment groups (≤ 1 cm the VAS scale) during the apple challenge, subjects that had been treated with placebo experienced a more pronounced increase in symptoms upon first ingestion of apple compared to subjects in the 12 DU group. For the global evaluation of efficacy, a higher proportion of subjects in the 12 DU group reported that their PFS symptoms had improved after having treatment compared to subjects in the placebo group (87% vs 64%, OR = 0.27, p = .0028). Treatment with 12 DU resulted in a rise in serum levels of apple (Mal d 1) specific IgE and IgG_4 with the same kinetics as seen for birch specific IgE and IgG_4. There were no severe local or systemic allergic reactions associated with the apple challenge.

Conclusion: Overall, data from the apple challenge in TT-04 show a trend towards an effect of treatment with the SQ tree SLIT-tablet on PFS symptoms. Furthermore, an increase in apple specific antibodies was observed suggesting that the SQ tree SLIT-tablet may have the potential to induce tolerance towards apple.

1648 | AIT has long-term benefits for patients with allergic rhinitis and/or asthma induced by birch family pollen: Refinement of real-world study methodology designed to increase robustness of findings

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Background: Allergy immunotherapy (AIT) ie. subcutaneous (SCIT) or sublingual immunotherapy (SLIT) is the only disease-modifying intervention for allergic rhinitis (AR) with long-term clinical efficacy. Real-world evidence on long-term AIT effectiveness can guide treatment practice and regulatory decision-making, but such data are sparse. This study evaluated the long-term effect of symptomatic medication ± AIT on AR progression, initiation of asthma medication use and asthma progression after stopping treatment in patients with *Betulaceae* (birch family) pollen-induced AR and/or asthma. Its design built on prior real-world analyses of the long-term benefit of grass pollen SLIT in allergic patients.

Method: A retrospective, comparative cohort analysis of a German longitudinal prescription database covering 9 pollen seasons (Jan 2008–Feb 2017) assessed 6 AITs indicated for birch family pollen-induced AR and/or mild to moderate asthma: native pollen SLIT and SCIT products (1 each), and 4 allergoid SCIT products. Patients were stratified into AIT or non-AIT groups and matched by index year, age group, gender, main indication at index date, number of seasonal

cycles while on treatment, and baseline AR and asthma treatment prescriptions. Severe asthma was an exclusion criterion. Multiple regression analysis compared prescription data in both groups as a proxy for clinical status and disease progression.

Results: AIT and non-AIT groups comprised 9001 (6349 AR/2180 asthma/472 both) and 45 005 (31 745 AR/10 900 asthma/2360 both) patients, respectively. Average (range) follow-up was 4.4 (2.0–6.6) (AIT) and 4.2 (1.8–6.1) (non-AIT) years. Both groups were well-matched; a key difference was a greater proportion of specialist prescribers in the AIT group vs general practitioners in the non-AIT group, reflecting German treatment practice. AIT was associated with reduced progression of AR and asthma [28.6% and 32.0% greater vs non-AIT, respectively; both $P < 0.001$] up to 6 years after stopping treatment, and decreased new use of asthma medication during treatment. The stringent matching process was designed to reduce confounding and wide differences in covariate distribution, help align groups by treatment period duration, and avoid any bias from inter-group differences in baseline treatment levels.

Conclusion: AIT had long-term benefits in patients with birch family pollen-induced AR and/or asthma. Prior real-world study methodology was refined to improve robustness of results.

1649 | Protein extract from ixodes ricinus induce strong basophil activation in red meat allergic patients

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Background: Red meat allergy is a specific type of food allergy where IgE antibodies are directed against the carbohydrate epitope α-Gal. Patients suffer from gastro-intestinal symptoms, urticaria, angioedema and/or anaphylaxis several hours after red meat intake. Today there is evidence for tick bites as a cause of IgE-sensitization to α-Gal. Since red meat allergic patients report an unusual long delay of symptoms, the mechanism behind the allergic reaction must be different from other food allergies. Here we investigated the relationship between allergenic activity against protein extract from the European tick *Ixodes ricinus* (*I. ricinus*) and α-Gal in red meat allergic patients.

Method: Sera from 32 red meat allergic patients IgE positive to α-Gal were analysed for IgE antibodies against protein extract from *I. ricinus* (by streptavidin ImmunoCAP). Heparinized venous blood from 14 red meat allergic patients, one healthy and one atopic control were stimulated with protein extract from *I. ricinus* and α-Gal for basophil activation analysis by flow cytometry. Immunoblots with sera from red meat-allergic patients and anti-α-Gal antibody was assessed for presence of the α-Gal epitope.

Results: Our data showed that 97% of Swedish red meat allergic patients have an IgE response to *I. ricinus* extract. There was moderate correlation between the IgE levels to α-Gal and *I. ricinus* extract ($\rho = 0.54$, $P < 0.0001$). In IgE-immunoblotting, a wide-range of protein bands (25-150 kDa) were identified by sera from red meat allergic patients and the presence of α-Gal in ticks was further supported by IgE inhibition using bovine thyroglobulin and monoclonal anti-α-Gal antibody. Thirteen of the 14 red meat allergic patients tested activated basophils when stimulated with *I. ricinus* and α-Gal. The median value of CD63-positive cells to *I. ricinus* extract was 34.9% and against α-Gal 20.9%. There was strong correlation between CD63-positive cells to *I. ricinus* extract and α-Gal ($\rho = 0.79$, $P < 0.0001$).

Conclusion: Tick extract induced a strong activation of basophils in red meat allergic patients pointing to the involvement of ticks in red meat allergy.

1650 | Comparison of a scanner based allergy lateral flow assay system for the determination of specific IgE with other in-vitro and in-vivo methods

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Background: Type I hypersensitivity is caused by allergen specific immunoglobulin E (sIgE) and thus sIgE represents a marker for modern allergy diagnosis. ALFA (Allergy Lateral Flow Assay) is a rapid test for the qualitative determination of sIgE in human serum, plasma or whole blood. The use of a special scanner system provides the opportunity of semi-quantitative interpretation of ALFA results within 20 minutes. The objective of the study is the evaluation of a rapid test for the semi-quantitative interpretation of sIgE compared with other in-vitro and in-vivo methods.

Method: Agreement between ALFA (Dr. Fooke Laboratorien GmbH) and ALLERG-O-LIQ (Dr. Fooke Laboratories)/ImmunoCAP® (Thermo Scientific) was investigated using 71 sera tested for specific IgE to Dermatophagoides pteronyssinus (d1), Dermatophagoides farinae (d2), timothy grass pollen (g6), birch pollen (t3) and hazel pollen (t4). Receiver Operating Characteristic (ROC) analysis and spearman correlations were performed for every single allergen separately and for all five allergens together. Skin Prick test results and/or nasal provocation results (Roxall) of more than 40 patients were compared to all three in-vitro methods.

Results: Excellent agreements were observed between ALFA results and in-vivo, ImmunoCAP® and ALLERG-O-LIQ results. Area under the curve (AUC) values were found at >0.95 compared to ImmunoCAP® and ALLERG-O-LIQ results. Agreements between ALFA and ImmunoCAP®/ALLERG-O-LIQ according to Spearman were found at 0.92/0.90 for d1, 0.92/0.88 for d2, 0.96/0.94 for g6, 0.95/0.93 for t3 and 0.91/0.92 for t4. AUC values of all three in-vitro systems compared in-vivo results were found >0.95. Compared to in-vivo results ALFA show a sensitivity of 0.96 (CI 0.92-0.98) and specificity of 0.85 (CI 0.74-0.92). Sensitivity and specificity between in-vivo results and ImmunoCAP®/ALLERG-O-LIQ were found at 0.95/0.88 and 0.81/0.90, respectively.

Conclusion: For the detection of sIgE, ALFA show results of high sensitivity and specificity when compared to ImmunoCAP®, ALLERG-O-LIQ and in-vivo results. AUCs of >0.95 indicates a nearly identical performance between ImmunoCAP®/ALLERG-O-LIQ and ALFA. The correlation for ALFA versus ImmunoCAP® and ALLERG-O-LIQ is also comparable with spearman's $\rho \geq 0.88$ for each tested allergen. The high sensitivity of the ALFA is supported by the Lateral Flow Assay Reader, especially for weak positive results.

1652 | Transcriptomic analysis identify molecular pattern of anaphylaxis

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Background: Anaphylaxis is a potentially life-threatening, rapidly progressing systemic allergic reaction, involving mast cell and basophils activation. However, the underlying mechanisms remain poorly understood. To better characterize the mechanisms leading to potentially lethal events, analysis of global transcriptional changes in peripheral blood human and mouse samples during anaphylaxis was performed.

Method: RNAseq based whole transcriptome characterization of total RNA from whole blood samples was performed at different time points in 15 patients with anaphylaxis presenting to the Emergency Department (at initial presentation, 7 days and 1 month later), and in 11 patients with anaphylaxis during double-blind placebo-controlled food challenges to peanut (before challenge, at anaphylaxis, and 2 to 4 hours later). Comparative RNAseq and pathway analyses of whole blood samples of 24 mice with different severity of IgE-mediated food-induced anaphylaxis (no anaphylaxis, mild anaphylaxis, severe anaphylaxis) was also performed to identify networks associated with IgE-mediated anaphylaxis severity. Extensive characterization of differential gene expression, cell-specific transcriptional alterations, analysis of alternative splicing patterns, and functional characterization of detected alterations was undertaken.

Results: Whole transcriptome expression analysis revealed overlapping alterations of gene expression during acute anaphylaxis/allergic reactions in humans and mice. However, specific species-dependent changes in expression were also seen. Major alterations revealed cellular movement and development, cell death and survival, signaling, interaction, and immune cell trafficking as well as inflammatory response, as the most important events taking place during anaphylaxis. Comparative analysis with expression signatures of immune cells identified changes in neutrophil, basophil, and dendritic cell populations during reactions.

Conclusion: These findings improve our understanding of biological mechanisms underlying anaphylaxis, with similarities and diversities in human and mouse, suggesting the involvement of distinct immune cells, and complex signaling changes, which reflect cellular movement and interaction during anaphylaxis.

1653 | Value of drug provocation test in non-immediate hypersensitivity reactions to betalactams in pediatric age

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Background: Betalactams (BLs) are the most frequent cause of antibiotic hypersensitivity in children. BLs induce immediate and non-immediate reactions. Diagnosis is based on a clinical history, skin testing and, if necessary, a drug provocation test (DPT). The aim was to analyze the clinical characteristics and to determine the value of DPT as a diagnostic method without performing intradermal test previously in a group of children with non-immediate hypersensitivity reactions (NIHR) to BLs.

Method: All children aged 1-14 years reporting NIHR to BLs from January to July 2017 were analyzed. Intradermal test was not performed. Diagnosis was confirmed by DPT, performed with a single dose followed by a 2 day/12 hours course at maximum dose at home.

Results: Sixty-seven patients were included, 50% were male and the median age 5 years (IR: 1-13). 44 (65%) had comorbidities: asthma, rhinoconjunctivitis and atopic dermatitis, being atopic dermatitis the most common. In a total of 44 cases (65.6%)amoxicillin was the culprit drug, in 22 (32.8%) amoxicillin-clavulanate and in 1 (1.5%) penicillin G. All patients took BLs for treating infectious diseases. The most usual symptoms were exanthema (46; 68.6%) cases, followed by urticaria (13; 19.4%) and urticaria-angiooedema (7; 10.4%). Only 6 (9%) patients were confirmed as being allergic, showing a delayed reaction during the course given at home after DPT.

Conclusion: After an allergological work-up, over 90% of the children evaluated were finally confirmed as non-allergic to BLs. Since all the reactions were non-immediate and took place during the 2 day course, DPT could be performed with a single dose, without health risk.

1654 | MicroRNA-146a is related to serum IgE levels in atopic dermatitis

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Background: Atopic dermatitis (AD) is a chronic and complex inflammatory skin disease. At least two AD subtypes have been described: allergic (characterized by Type-2-cell-mediated immunity

and increased immunoglobulin E [IgE]) and non-allergic (characterized by both Type-2-cell and Type-1/17-cell-mediated immunity and normal IgE levels). MicroRNA (miR) are small non-coding RNA molecules involved in genetic regulation. MiR-146a negatively regulates inflammatory responses during chronic skin inflammation, however, its role in the modulation of immune responses in AD is uncovered. As recently miR-146a was shown to promote IgE class switch in B cells in mice, we aimed to test whether there is association between miR-146a and increased IgE levels in AD.

Method: Serum samples from miR-146a$^{-/-}$ and wild-type C57BL/6J mice with MC903-induced AD-like inflammation were analysed (N = 8 mice/group) for IgE and cytokine levels. Additionally, 32 serum samples from AD patients were also analysed. Subjects were split into allergic (N = 22) and non-allergic (N = 10) according to IgE threshold of 150 IU/mL. MiR-146a relative expression was quantified by real-time PCR, IgE and human IL-12p40 by ELISA and mouse cytokines by Bioplex.

Results: MiR-146a$^{-/-}$ mice showed decreased IgE and increased IL-12p40 serum levels ($P < 0.001$), while there were no changes in other detected cytokines. Human miR-146a expression was not significantly different between allergic and non-allergic subgroups divided based on serum IgE level. However, we observed a negative correlation of serum miR-146a and IgE levels ($P < 0.05$) in allergic AD patients. In the allergic subgroup, miR-146a expression remained independently negatively associated with IgE ($\beta = -0.488$, $P < 0.05$) after adjusting for confounding variables as gender.

Conclusion: Low IgE and high IL-12p40 serum levels in miR-146a$^{-/-}$ mice indicate that miR-146a is needed for the production of IgE and associated with the regulation of Type-1/17-cell-mediated immune responses in mice. Negative association of miR-146a with serum IgE in allergic AD patients suggests that miR-146a might have capacity to limit Type-2-cell-mediated immune responses in AD. Further studies are needed to elucidate the possible mechanisms of miR-146a in AD etiopathology.

1655 | PIPE cloning for rapid generation of recombinant antibodies of different classes against the major birch pollen allergen Bet v 1

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Background: Modern cloning techniques are useful tools for tailoring monoclonal antibodies in research. As such a method, Polymerase Incomplete Primer Extension (PIPE) cloning allows simple exchange of variable immunoglobulin chains, thus enabling the rapid creation of antigen-specific antibodies of several classes. To further increase the specificity, the method can also be combined with site-directed mutagenesis (Ilieva et al. 2017). In this work, we aimed to design and produce Anti-Bet v 1-antibodies of the classes IgE and IgG_1 sharing the same variable regions.

Method: Plasmid vectors (pEX-A128, pVitro1) containing sequences from the Bet-v-1-specific M0418 variable region (Levin et al. 2014), kappa constant chain as well as IgE and IgG_1 constant chains were amplified using PIPE cloning. PCR products were digested using DpnI and the corresponding sequences ligated into one vector via self-assembly. The resulting constructs were then transformed into NEB® 10-beta *E. coli* cells. Clones were screened for variable regions using colony PCR and full antibody construct integrity was confirmed via sequencing. Antibodies were then expressed in the Expi 293 system. A dot blot was used to estimate antibody concentration after affinity chromatography and detect binding against Bet v 1.

Results: Agarose gel electrophoresis confirmed that the sizes of fragments obtained from PIPE cloning were in the expected range of ~333-4123 bp. Successful exchange of both variable regions was achieved in 70% (IgE), 100% (IgG_1) of the tested clones and 50% and 71.4% of the clones selected for sequencing had the correct antibody sequence (IgE, IgG_1, respectively). Subsequent expression in Expi293F cells yielded high antibody amounts. A dot blot conducted with IgE confirmed binding to Bet v 1.

Conclusion: PIPE cloning was successfully utilised to create IgE and IgG_1 antibodies sharing the exact variable regions to Bet v 1. It is therefore feasible for rapid generation of recombinant antibodies of several classes against the same allergen. In the future, antibody-class-dependent mechanisms in type I allergy can be studied using these antibodies.

1656 | Association of polymorphisms of Toll-like receptors 2 and 4 with elevated levels of specific IgE products in patients with allergic diseases

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Background: It is suggested that mutations in the genes encoding Toll-like receptors (TLRs) 2 and 4, as well as the sequence of proteins for the implementation of intracellular signals from these receptors (eg, IL1RL1, BPI, NOD1, NOD2, MAP3K7IP1) may be related with an increased level of IgE production and the development of allergic diseases (AD).

Method: Levels of allergen-specific IgE were determined using the Rolycheck immunoassay system (Germany). The polymorphisms of the TLR2 (rs5743708) and TLR4 (rs4986790, rs4986791) genes were investigated by PCR using specific oligonucleotide primers followed by restriction analysis.

Results: The most significant causative allergens were epidermal and everyday (cat, cat and horse epidermis, D. farinae, D. pteronyssinus). When comparing the frequency distribution of the three studied polymorphisms of the TLR2 and TLR4 genes in practically healthy individuals and patients with AD, the probable link between the presence of the polymorphic allele G of the TLR4 gene (rs4986790) with elevated levels of specific IgE was detected ($\chi2$ = 5.47; OsH = 4.84 (1.41-16.68), *P* = 0.019). The opposite data were obtained from the association of the presence of the polymorphic alleles A of the TLR2 gene (rs5743708) and the T gene of TLR4 (rs4986791) in the genotype of the presence of the TLR2 gene (rs5943998), in which no probable bonds were detected (*P* = 0.197 and *P* = 0.406, respectively).

Conclusion: The relationship between TLR2 (rs5743708) and TLR4 (rs4986790) polymorphism with elevated levels of specific IgE products in patients with AD allows to consider the presence of the above mononucleotide substitutions as an additional prognostic sign of individual susceptibility to these diseases.

1657 | Detection of phospholipases and their lipid metabolites in mastocytosis

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Background: Mastocytosis denotes a heterogeneous group of conditions defined by the expansion and accumulation of clonal tissue mast cells (MCs) in various organs and consequent release of MC-derived mediators that causes various effects. MCs release a variety of mediators such as phospholipases (PLs), a class of enzymes that catalyze the cleavage of membrane phospholipids and consequent release of lipid mediators such as diacylglycerol (DAG), endocannabinoids (EC) that activate different cells including MCs. To date, there are no data on the role of PLA_2, PLC, DAG and ECs in mastocytosis. The aim of this study was to analyze plasma levels of these mediators in patients with mastocytosis.

Method: We studied 25 patients with mastocytosis (median age 49 years; 44% males) and 37 healthy sex- and age-matched individuals (median age 32 years; 48% males). In our study we measured: plasma PLA_2 and PLC activities, DAG and ECs levels.

Results: PLA_2 and PLC activities were increased in patients with mastocytosis compared to controls [PLA_2: 2.1 (1.6-3.2) vs 1.3 (0.6-2.0) U/mL median values (interquartile ranges); PLC: 0.22 (0.15-0.3) vs 0.09 (0.04-0.14)]. To verify whether the increased activity of PLC led the DAG formation, we measured DAG (18:1/20:4 and 18:0/20:4) concentrations. DAG isoforms in patients with mastocytosis were higher than controls [DAG 18:1/20:4: 50.5 (21.5-100) vs 13.7 (7.9-19.5) pmol/mg of lipid extract; DAG 18:0/20:4: 374.2 (207.2-599.1) vs 118.1 (62.1-183.6)]. In the next group of experiments, we evaluated the concentration of ECs [2-Arachidonoylglycerol (2-AG), Anandamide (AEA) oleoyl ethanolamide (OEA) and palmitoyl ethanolamide (PEA)]. Despite the increase of DAG, 2-AG was not enhanced in patients compared to controls. AEA was decreased in patients with mastocytosis compared to controls [0.5 (0.3-0.7) vs 0.8 (0.5-1.6)], by contrast PEA concentration was increased [23.2 (16.2-44.5) vs 19.3 (9-30.2)]. OEA did not differ between the two groups. Interestingly, PLA_2, PLC and DAG levels correlated with severity of mastocytosis. Moreover, PLA_2 activity was increased in patients with more mediator-related symptoms whereas AEA was decreased.

Conclusion: In this study, we show that PLs and their lipid mediators, activators of mast cells, are increased in patients with mastocytosis and are correlated with diseases severity. Our results suggest that these mediators may become novel potential lipidic biomarkers of mastocytosis.

1658 | E1- and E2 epithelial cell models for allergy research

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Background: The airway epithelium is the first line of defense and is continuously exposed to environmental factors. By physical interaction of immune cells with the epithelium, epithelial cells can play a role in allergen specific immune responses. Cell lines are important tools to identify environmental risks and may also serve for mechanistic studies (eg, CRISPR/CAS), where immortalized cell systems are crucial. The key cytokines *IL-4* and *IFN-γ* are differentiating epithelial cells to E1 and E2 phenotypes. It is currently not known whether commonly used cell lines belong to either E1 or E2 phenotypes, and whether cell lines express the same the spectrum as primary cells.

Method: Primary bronchial epithelial cells (NHBE; n = 6) and Calu-3 cells were cultured with *IL-4* and *IFN-γ*. RNA was harvested after 6 hours and subjected to microarray gene expression profiling (8 × 60 K) and analyzed using Genespring Software.

Results: NHBE cells show a higher expression amplitude compared to Calu-3. While the overlap between Calu-3 and NHBE upon *IL-4*-stimulation was observed in only 28% of significantly regulated genes, *IFN-γ* showed uni-directional induction in 66% of the genes. Few *IL-4*-induced genes in NHBEs like *KAL-1* and *IL-24* and even less IFN-γ-induced Genes like *GLIS-3* and *OSR-1* were counter-regulated in Calu-3 cells. Key E1 and E2 cytokines such as *CXCL-9, -10, -11* and *CCL-26*, respectively, are induced in Calu-3 cells and provide a model for future research.

Conclusion: To summarize, Calu-3 cells appear as an E1-biased phenotype, which shows E1 gene regulation events similar to those observed in primary epithelial cells in response to *IFN-γ*. Additionally less than three percent of the induced genes are counterregulated.

1659 | Soluble FceRI modulates FceRI expression comparable to omalizumab

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Background: The soluble form of the high affinity IgE Fc-receptor (sFcεRI) is released upon receptor activation by antigen crosslinking. Once in circulation, sFcεRI forms complexes with circulating IgE and prevents IgE-loading to the receptor and basophil activation. Omalizumab, the humanized monoclonal anti-IgE antibody currently used as treatment in various diseases (allergic bronchial asthma, chronic idiopathic urticaria), has also been shown to prevent IgE-loading to the receptor. In addition, its long-term application decreases FcεRI expression on peripheral blood basophils and disrupts IgE:FcεRI complexes. Our aim was to investigate the long-term effect of sFcεRI on FcεRI expression and to compare this effect to omalizumab.

Method: MelJuSo (human melanoma-derived) cell line transfected with the trimeric or tetrameric isoform of FcεRI (αγ/αβγ), were loaded with chimeric IgE (cIgE) overnight, followed by pulses of sFcεRI (62.5 nmol/L) or omalizumab (62.9 nmol/L) once a day for three days. MelJuSo-ØØ was used as a negative control. Expression of surface FcεRI and bound cIgE were analysed by flow cytometry (n = 3). Competition between sFcεRI and omalizumab was assessed using different concentrations (0-1 μmol/L) in presence of cIgE. Formation of sFcεRI:cIgE complexes were detected by ELISA.

Results: Our results show that long-term application of both sFcεRI and omalizumab downmodulates surface FcεRI expression by 45% and 50% (on MelJuSo-αγ) and 20% and 39% (on −αβγ) respectively. We also demonstrate the capacity of both molecules to disrupt cIgE:FcεRI complexes on MelJuSo-αγ/αβγ cells by 91% and 92% respectively for sFcεRI, and 94% and 95% for omalizumab. Furthermore, our preliminary data show that omalizumab and sFcεRI are competitors for the same binding region on cIgE.

Conclusion: Long-term effect of sequential application of sFcεRI on MelJuSo shows a downmodulation of the FcεRI. It further disrupts surface IgE:FcεRI complexes. The effect was similar to omalizumab and thus might reveal an endogenous regulator of the high-affinity IgE receptor.

1660 | AllergoOncology: Anti-EGFR IgE triggers potent tumor cell killing by mast cells and monocytes

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Background: AllergoOncology at the interface of allergy and cancer, focuses on the role of Th2 mechanisms in tumors. In vitro and in vivo studies have shown that IgE-triggers remarkable tumoricidic responses by monocytes and macrophages. Due to the limitation of mouse models, we instead propose clinical studies in dog (Canis lupus familiaris) patients with spontaneously developing tumors. In contrast to rodents, dogs share a remarkably similar IgE biology with humans, as well as highly comparable genetic and molecular mechanisms of tumor development. This study evaluated the antibody-dependent immune responses of a canine anti-EGFR IgE (can225IgE-λ) in various ADCC and ADCP assays in dog and human cellular models.

Method: can225IgE-λ was expressed in Expi293F cells and purified using anti-dog IgE affinity column. Purified IgE was assessed for specificity, purity, FcεRI- and CD23-binding, by PAGE, western blots and flow cytometry. The functional potency of can225IgE-λ targeting various tumor cell lines was tested in a flow-cytometric ADCC and ADCP assay using human and dog monocytic and canine mast cells as effector cells.

Results: Purified can225IgE-λ was correctly assembled with a MW of 235 kDa and specifically recognized human and dog EGFR in western blots and flow cytometry. can225IgE-λ bound to human and canine FcεRI and to canine CD23 on various effector cells. can225IgE-λ triggered ADCC by human monocyte-like, and strong ADCC and ADCP by canine monocytic cells against EGFR-expressing tumor cells. Notably, EGFR+ tumor cells were able to crosslink membrane-bound can225IgE-λ on dog mast cells, resulting in a powerful cytotoxic tumor killing response.

Conclusion: can225IgE-λ is the first recombinant canine anti-EGFR IgE and is able to trigger potent tumor-killing response by monocytic cells, as well as by mast cells. This antibody may serve as a lead compound in trials with canine cancer patients for the clinical proof-of-concept of AllergoOncology. The study was supported by the Austrian Science Fund (FWF) grant W1205-B09 CCHD awarded to Prof. Dr. Erika Jensen-Jarolim.

1661 | Prevalence and manifestations of dry skin and associations to skin barrier in early infancy

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Background: Epidermal barrier dysfunction, measured by transepidermal water loss (TEWL), is a feature of atopic dermatitis (AD). Dry skin may be an early symptom of a dysfunctional skin barrier, but with limited documentation of prevalence and association to TEWL. The present aims were to assess the prevalence of dry skin and determine if TEWL was associated with dry skin at AD predilection sites in 3 months old infants.

Method: The study included 1143 infants attending the three-month follow-up investigation in the control arm of the randomized birth cohort: Preventing Atopic Dermatitis and Allergies in children (PreventADALL) (n = 2397). Children underwent skin examination and TEWL measurements (n = 1020) at the left lateral upper arm using open chamber DermaLab USB.

Results: Mean (SD; min, max) age, length and weight of the 1143 infants (46.6% girls) was 93.3 (8.2; 69, 138) days, 61.9 (2.3; 51.0, 79.9) cm and 6.3 (0.8; 4.2, 9.3) kg. Dry skin at one or more sites without AD was observed in 540 (47.2%) infants, 145 (12.7%) had signs of AD, while 458 (40.1%) had no signs of dry skin or AD (unaffected skin). Infants with dry skin on extensor parts of the extremities (extensors), but not the cheeks and without signs of AD (n = 98, 12.9%) had significantly higher mean (CI) TEWL (measured in $g/m^2/hour$): 7.8 (6.9, 8.8) than infants with unaffected skin 6.6 (6.3, 7.0) (P = 0.007). Infants with dry skin on extensors and cheeks had significantly higher TEWL of 9.5 (8.3, 10.6) compared to those with dry skin on extensors and not on cheeks (P = 0.035) (Table 1).

Conclusion: Nearly half of 3 months infants had signs of dry skin. Concurrent presence of dry skin on cheeks and extensor part of extremities was significantly associated with increased TEWL, indicating impaired skin barrier in these children possibly preceding development of AD.

1662 | Perturbation of stool short chain fatty acids profiles associated with distinct microbiome maturation in atopic eczema infants

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Background: The intestinal microbiota of an infant is a complex ecosystem that undergoes a succession of demographic changes shaped by several factors in the first year of life, and this has a strong influence on the maturation of the immune system. This effect is mediated, in part by short chain fatty acids (SCFAs) which are the products of dietary fibre fermentation by intestinal bacteria. The aim of this study was to evaluate and compare stool SCFAs and microbiota profiles between infants with atopic eczema and healthy controls.

Method: From the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort, a sub-cohort of 64 subjects categorized by clinical outcome at 18 months of age: (a) non-atopic (non-allergen sensitized) eczema (NAE) (n = 15), (b) atopic eczema (AE) (n = 14) and (c) healthy controls (n = 35), were selected for this retrospective analysis. A total of 164 stool samples were collected at week 3, months 3,

Table 1.

TEWL (g/m²/h) (Total n = 1008)	Unaffected skin (n = 411)	No dry skin cheeks & extensors (n = 79)	Dry skin cheeks (n = 161)	Dry skin extensors (n = 98)	Dry skin cheeks & extensors (n = 134)	Possible AD (n = 125)
Mean TEWL (CI 95%)	6.6 (6.3-7.0)	6.2 (5.4-7.0)	6.4 (5.7-7.1)	7.8 (6.9-8.8)	9.5 (8.4-10.6)	12.5 (10.9-14.0)
Min–Max	1.3-32.6	2.1-19.7	1.6-42.4	2.2-32.1	2.5-46.2	3.3-45.2

6 and 12. The levels of SCFAs (acetic, propionic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, caproic and 4-methylvaleric acids) were quantitated by liquid chromatography-tandem mass spectrometry. Microbiota profiling was performed using metagenomic sequencing. Longitudinal multivariate analysis and correlation network were employed to study the dynamic trends and interaction of SCFAs with bacterial group abundances, while adjusting for possible confounders.

Results: Longitudinal analysis revealed a unique metabotype characterized by reduced acetic, propionic and butyric acid levels in AE, but not NAE, compared to controls (adj $P < 0.05$) across all time-points. As such, the significant depletion of these SCFAs in AE occurred from 3 months onwards. Using correlation network for these 3 SCFAs and longitudinal analysis of microbiota profiles, the reduction in levels of acetic acid in AE was associated with reduced abundance of acetic-producing *Streptococcus* genus (*Firmicutes* phylum) compared to controls, whilst the decrease in butyric acid corresponded with a decreased abundance of butyric-producing *Firmicutes* bacteria which were predominantly made up of *Blautia*, *Clostridium*, *Faecalibacterium* and *Butyrivibrio* genera (adj $P < 0.05$). No dominant bacterial group influencing the level of propionic acid was determined.

Conclusion: Differences in the maturation of the gut microbiota is associated with perturbation of stool SCFAs and the development of AE in infants.

1664 | Larger household size is associated with an increased proportion of regulatory T cells in cord blood

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Background: The association between larger household and decreased allergic disease[1] has been replicated in numerous studies but the underlying mechanisms are unknown. We and others have found an association between decreased regulatory T cells at birth and subsequent allergic outcomes[2]. The aim of this study was to investigate the relationship between household size and regulatory T cells at birth.

Method: The Barwon Infant Study birth cohort (1064 mothers, 1074 infants) was recruited using an unselected, antenatal sampling frame. Demographic and environmental data were collected at enrolment. The proportion of $CD4^+$ $CD45RA^+$ $FOXP3^+$ regulatory T cells (nTreg) was measured in a subgroup (n = 363) by flow cytometry of fresh samples of cord blood collected at birth.

Results: Larger household size was associated with a higher proportion of nTreg in cord blood at birth (Table 1). The increase in nTreg per child in the household was greater than the increase per additional adult.

Conclusion: Larger household size during pregnancy, in particular the number of children, is associated with an increased proportion of nTreg in cord blood. Further studies are required to investigate the relationship between maternal microbial exposures during pregnancy and foetal immune development.

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Table 1 - The association between household size and nTreg at birth

Exposure Variable		Cord blood nTreg
Household size	% increase per additional member	3.8 95% CI (0.5, 7.3) P = 0.026
Birth order	% increase per additional sibling	3.7 95% CI (−0.1, 7.7) P = 0.059
Children in household during pregnancy	% increase per additional child	4.3 95% CI (0.6, 8.2) P = 0.023
Adults in household during pregnancy	% increase per additional adult	2.0 95% CI (−6.6, 11.5) P = 0.656

1665 | Are there differences in the innate response between bronchiolitis and pediatric recurrent wheeze?

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Background: Bronchiolitis is the most common lower respiratory tract infection in infants, being main cause of hospitalization of children younger than 1 year of age. Infants, who are affected with respiratory syncytial virus (VRS) or rhinovirus (HRV) bronchiolitis during the first months of life, frequently develop recurrent wheezing and asthma, but immune mechanism are not well established. The aim of this study was to analyse the innate immune response that characterise bronchiolitis (BQ) and recurrent wheezing (RW).

Method: Ninety-nine and seventy-two infants who were hospitalised with bronchiolitis or recurrent wheezing respectively, (October 2016 up to August 2017) were enrolled in the study; also 23

healthy infants were included. Nasopharyngeal aspirates (NPA) were processed. Type 2 innate lymphoid cells (ILC2) were evaluated by flow cytometry. A total of 28 cytokines and pro-inflammatory factors linked to innate immunity, inflammatory response and epithelial damage were evaluated in NPA supernatant by ELISA. Also, eleven different genes were analyzed in NPA cells by quantitative PCR (qPCR).

Results: Bronchiolitis group presented a significant increase of ILC2 percentage compared with RW group ($P < 0.05$). Likewise, ST2-positive ILC2 percentage was higher in BQ vs RW group ($P < 0.01$). At the gene level, *TLR3*, *IL33*, *IFNg*, *IL10*, and *FLG* (filaggrin) gene expression was significantly increased in BQ in relation to RW group (*TLR3*, *IL33*: $P < 0.001$; *IFNg*, *IL10*, *FLG*: $P < 0.05$). When we evaluated several cytokines and pro-inflammatory factors in NPA supernatant, we observed a significant increase of IFNg in BQ compared to RW group ($P < 0.05$), augmented levels of IL-10 in both pathologies compared to healthy population ($P < 0.001$). TSLP in BQ showed a higher level than RW and healthy groups. Elevated levels of periostin in BQ and RW groups compared to control were observed, although statistical significance was only observed in RW population vs healthy infants. Rest of molecules not showed statistically significant differences between both pathology groups.

Conclusion: Differential innate immune response and epithelial repair mechanisms have been observed between both pathologies; however, bronchiolitis and recurrent wheezing share belated mechanisms such us monocyte activation, vascular damage and fibroblast repair.

1666 | House dust mite sensitization and early onset atopic dermatitis precede shellfish sensitization at 5 years of age

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Background: Cross-reactivity to tropomyosin, the major allergen in shellfish and house dust mites (HDM), has been hypothesized to account for the high prevalence of shellfish and HDM sensitization in tropical climates, and higher rates of shellfish allergy in Asia. The pathogenesis of shellfish allergy is likely to be closely linked to HDM sensitization. However there are currently no prospective studies investigating the temporal relationship between primary HDM sensitization and shellfish sensitization. The route of sensitization (via cutaneous or inhalational routes) and the role of early onset atopic dermatitis (AD) have also not been investigated.

Method: 647 subjects from the GUSTO birth cohort completed skin prick testing (SPT) to HDM *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* at 18.36 and 60 months; and shrimp and crab at 60 months. AD status was determined via parental-reported physician diagnosis, and allergic rhinitis status through parental reports.

Results: The prevalence of HDM sensitization was 11.3% (n = 73) at 18 months, 22.4% (n = 145) at 36 months and 34.5% (n = 223) at 60 months. 24 (3.7%) children were shellfish-sensitized at 60 months, of whom 7 (29.2%) had been HDM sensitized at 18 months, and 12 (50%) had been HDM sensitized at 36 months. The combination of early onset AD by 6 months of age and HDM sensitization at 36 months [Adj OR 6.0, 95% CI (1.6–23.1), $P < 0.01$] as well as AD by 2 years and HDM sensitization at 36 months [Adj OR 5.8, 95% CI (1.8–18.3), $P < 0.01$] increased the risk of progression to shellfish sensitization by 5 years of age compared to HDM sensitization alone. Antecedent AD alone was not significantly associated with shellfish sensitization at 5 years. HDM sensitized rhinitis at age 36 months was also significantly associated with shellfish sensitization at age 5 years [Adj OR 4.3, 95% CI (1.3–14.0) $P = 0.02$]. However, rhinitis at 18 months, with or without HDM sensitization; and rhinitis at 36 months alone were not associated with the development of shellfish sensitization at age 5 years.

Conclusion: Longitudinal data from this study suggests that HDM is the primary sensitizer for shellfish in an Asian population. The presence of the defective skin barrier in AD may be the primary route of HDM sensitization predisposing to early shellfish sensitization, and subsequent allergy.