LATE-BREAKING ABSTRACTS PRESENTED AT SCIENTIFIC SESSIONS AAAAI ANNUAL MEETING MARCH 4-7, 2016

The following abstracts were accepted for presentation after the deadline for the abstract supplement

L1 Potential Role of Gut Microbial Metabolites in Allergy Prevention in Children

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RATIONALE: Short-chain fatty acids (SCFAs) are metabolites produced by microbes in fermented foods or by microbes in the gut following fermentation of fibers. SCFAs have been shown to have anti-inflammatory properties in animal models. Our objective was to investigate the potential role of SCFAs in the prevention of allergic diseases among children and allergic airway-inflammation in mice.

METHODS: Measurement of SCFAs in fecal water were performed among a subset of 1 year old children (n=301) from a European birth cohort. Data on environmental factors and allergy were collected by questionnaires. We used ovalbumin (OVA) or house dust mite (HDM) sensitised mice to model allergic airway-inflammation.

RESULTS: In the birth cohort study, we observed a positive association between yogurt consumption in the first year of life and the fecal levels of butyrate. The children with the highest fecal butyrate levels had a significantly reduced risk of becoming sensitized to inhalant allergens, with a similar directional trend for asthma, atopic dermatitis and sensitization to food allergens. Oral administration of SCFAs to mice significantly reduced the severity of allergic airway-inflammation, both in the OVA and HDM models. All SCFAs tested reduced the total number of cells and eosinophils in bronchoalveolar lavages as well as reduced airway hyperresponsiveness. The single most effective SCFA was butyrate and oral administration of butyrate further reduced levels of Th2 cytokines in lung cells.

CONCLUSIONS: SCFAs, especially butyrate, protect against allergic airway inflammation and strategies designed to increase SCFA levels in children should be considered, both as a preventive and a therapeutic option.

L2 Associations of Early Life Exposures and Environmental Factors with Asthma Among Children in Rural and Urban Areas of Guangdong, China

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RATIONALE: Environmental factors may play important roles in asthma, but findings were inconsistent. The study was to determine the associations between early life exposures, environmental factors and asthma in urban and rural children in southeast China.

METHODS: A screening questionnaire survey was performed in 7164 children from urban Guangzhou and 6087 from rural Conghua. In the second stage, subsamples of 854 children (419 from Guangzhou, 435 from Conghua) were recruited for a case-control study including detailed questionnaire enquiring family history, early life environmental exposures, dietary habits, and testings including histamine airway provocation, skin prick test, and serum antibody analysis. House dust samples from 76 Guangzhou and 80 Conghua families were obtained to analyze levels of endotoxin, house dust mite and cockroach allergens.

RESULTS: The prevalence of doctor-diagnosed-asthma was lower in children from Conghua (3.4%) than Guangzhou (6.9%, $p<0.001$) in the screening survey. A lower percentage of asthma was found in rural compared to urban subjects (2.8% vs 29.4%, $p<0.001$) in case-control study. Atopy (odds ratio 1.91, 95% confidence interval 1.58-2.29), parental allergic diseases (2.49, 1.55-4.01), hospitalization before age 3 (2.54, 1.37-4.70), high milk product consumption (1.68, 1.03-2.73) and dust *Dermatophagoides farinae* 1 level (1.71, 1.34-2.19) were positively, while crop farming before age 1 (0.15, 0.08-0.32) and dust endotoxin level (0.69, 0.50-0.95) were negatively associated with asthma.

CONCLUSIONS: A variety of environmental factors were found to be associated with asthma. Parental allergic diseases, atopy, diet and early life exposures might explain the lower prevalence of asthma in the rural environment in southeast China.

L3 Austri, a Large Randomized Study in Adolescents and Adults with Asthma, Assessing the Safety and Efficacy of Salmeterol in Combination with Fluticasone Propionate Compared to Fluticasone Propionate Alone

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RATIONALE: Previous studies have shown an excess of serious asthma-related outcomes, including death, in subjects taking Long Acting Beta Agonists (LABAs). This study was designed to examine the risks and/or benefits of LABA therapy when added to an ICS in a combination inhaler in patients with asthma.

METHODS: A global, randomized, double-blind, parallel group study of asthmatic subjects ≥12 years; treated with salmeterol (SAL) and fluticasone propionate (FP) in combination (FSC) or FP alone for 26 weeks. The primary endpoint was time to first serious asthma-related event, the composite of death, intubation or hospitalization. To declare non-inferiority the hazard ratio of subjects with a serious asthma-related event with FSC compared to FP was <2.0 based on the upper bound of the 95% confidence interval (CI) on the estimate of the hazard ratio. The secondary endpoint was time to first asthma exacerbation requiring OCS.

RESULTS: Of 11,751 subjects randomized, 67 subjects experienced 74 serious asthma-related events with 34 and 33 subjects treated with FSC and FP, respectively. The FSC/FP hazard ratio was 1.029 (0.638-1.662) for time to first serious asthma-related event. Non-inferiority was achieved. There were no asthma-related deaths and 2 asthma-related intubations (both on FP). The FSC/FP hazard ratio for time to first asthma exacerbation was 0.787 (0.698-0.888).

CONCLUSIONS: There was no evidence of an increased risk of serious asthma-related events when SAL was used in a combination product with FP compared to FP. There was a significant reduction in risk of asthma exacerbations for FSC compared to FP alone.

L4 The Diagnostic Testing Accuracy of Urinary Leukotriene E4 in Determining Aspirin Intolerance in Asthma: A Systematic Review and Meta-Analysis

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RATIONALE: Urinary leukotriene E4 (ULTE4) may be a biomarker that distinguishes aspirin-intolerant asthma from other asthma subtypes. Specific Aim: to estimate the diagnostic testing accuracy of ULTE4 as a marker of aspirin intolerance in patients with asthma using previously published studies.

METHODS: We identified relevant clinical studies from a systematic review of English and non-English articles using MEDLINE, EMBASE, and CENTRAL. Articles were screened at the abstract and full text level by two independent reviewers. We included previously published studies which analyzed ULTE4 in human subjects with asthma who had been characterized as having or not having aspirin intolerance on the basis of a specified definition. Receiver operator characteristic (ROC) curves were constructed and area under curve (AUC) calculated for each method used to measure ULTE4 by comparing against the gold standard of a positive aspirin challenge.

RESULTS: The search strategy identified 867 potential articles, of which 86 were reviewed at the full text level and 10 met criteria for inclusion. The sensitivity, specificity, positive predictive value and negative predictive values of ULTE4 to determine aspirin intolerance in asthmatic subjects were 0.55, 0.83, 0.77, 0.65 (Amersham-EIA); 0.76, 0.79, 0.73, 0.82 (Cayman-EIA); 0.73, 0.81, 0.76, 0.79 (mass spectrometry) and 0.81, 0.80, 0.65, 0.90 (radioimmunoassay) at optimal threshold of 192, 510, 165 and 69 pg/mg Cr respectively. The diagnostic odds ratio for each methodology was 6.11; 12.27; 11.70; and 17.33 respectively.

CONCLUSIONS: This study defines the diagnostic testing accuracy of ULTE4 in determining aspirin intolerance in asthma.

L5 Factors Affecting Control and Adherence to One Year Treatment in Elderly Asthmatics in Turkey

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RATIONALE: The objective of this study was to investigate the factors that affected the control and the adherence to one year treatment of newly diagnosed elderly asthmatics(EA) living in different areas of Turkey, and to compare these with young asthmatics(YA) regarding different parameters.

METHODS: A total of 1116 newly diagnosed adult asthmatic patients from 122 secondary or tertiary centers of different geographic locations took part in the study, and a standard web-based questionnaire was applied from July-2012 to March-2014. Patients were divided into two groups as YA (age: 18-59) and EA (age≥60). The differences in biometric parameters, pulmonary functions, allergic status, comorbidities, first given therapies, one year control, and adherence to treatment were analyzed.

RESULTS: The age of 12.2% of the new-onset asthma patients was ≥60 years. Body mass index was found as 27.8 kg/m^2 for YA and 29.8 kg/m^2 for EA ($p<0.001$). The presence of any comorbidity was 66.2% and 52.2% in EA and YA, respectively ($p=0.003$). Combined inhaled steroid plus long acting beta2 agonists were the most frequently administered treatment (83.0% vs. 93.4% in YA and EA, $p=0.002$).

The asthma control during one year was not significant between groups. But the number of visits were elevated in EA than YA (1.60 vs 1.22, $p=0.011$). The adherence to therapy was not significant between groups. The adherence to therapy in EA was significantly correlated with the presence of hypertension ($p< 0.025$).

CONCLUSIONS: Our findings demonstrated that EA presented more comorbidities and the presence of hypertension increased adherence to asthma treatment in elderly asthmatics.

L6 Comparison of Omalizumab Therapy Effectiveness in Patients with Hypersensitivity to Non-Steroidal Anti-Inflammatory Drugs (NSAID) and Patients Who Tolerate NSAID (non-NSAID) – Polish Real Life Experience

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RATIONALE: Hypersensitivity to non-steroidal anti-inflammatory drugs (NSAID) is a distinct phenomenon of severe asthma, but may coexists with allergy. The aim of the analysis was to compare the effectiveness of omalizumab (OMA) in the treatment of severe allergic asthma in patients with hypersensitivity NSAID to patients who tolerate NSAID (non-NSAID).

METHODS: 38 patients started OMA therapy in the Polish program for the treatment of severe allergic asthma in Barlicki Hospital between 2013 and 2015 year. We prospectively evaluated OMA effectiveness recording changes in oral corticosteroids daily dose (OCS), annual numbers of asthma exacerbations, the Asthma Control Questionnaire (ACQ) score, and the Asthma Quality of Life Questionnaire (AQLQ) score in 16th week and 52nd week of therapy. At the baseline the positive history of hypersensitivity to NSAID was reported by 14 patients, 24 patients tolerated NSAID.

RESULTS: The baseline characteristic of study groups in respect of demographic data, anthropologic data and severity of asthma did not significantly differed between NASID and non-NASID ($P>0.05$). 4 patients (2/2 from NASID/non-NASID) stopped themselves the therapy due to subjective lack of benefit. In both groups we observed significant improvement in ACQ, AQLQ scale as well the reduction of exacerbations and the OCS dose in 16th and 52nd week ($P<0.05$). The improvement in asthma control parameters between study groups did not differed in 16th and 52nd week ($P>0.05$).

CONCLUSIONS: The OMA seems to be equally effective in patients suffering from severe allergic asthma independently of NSAID hypersensitivity status, but larger population study is required to confirm this observation.

L7 Role of Home Environmental Staphylococcus Aureus Bacterial Allergens in Childhood Asthma

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RATIONALE: *Staphylococcus aureus* (SA) may induce allergic (Th2-biased) inflammatory responses through secreted staphylococcal enterotoxin (SE) A-D superantigens. SA is known to exacerbate eczema and increasingly is implicated in asthma exacerbations. We quantified putative staphylococcal allergens in home dust using a bacterial genetic method, then we associated SA/SE exposures with respiratory symptoms among children with asthma.

METHODS: We measured SA (*femB*) and SEA-D genes in home dust extracts from the randomization visit (before treatment) in the completed Asthma Control Evaluation cohort (NCT00114413) using real-time PCR. We tested cross-sectional associations between dust exposures and self-reported respiratory symptoms in 245 inner-city children with asthma (~50% of the cohort) using linear and binomial regression modeling.

RESULTS: We identified SA genes in 189 (77%) of 245 homes, with prevalence of any SE gene detection as follows: SEA (60%); SEB (52%); SEC (51%); SED (63%). Among children with asthma, mean ACT score was 20.7 and mean days of symptoms in the prior two weeks were: wheeze/cough: 2.2; interference with activities: 1.2; sleep disruption: 0.6. Strong dust SEA detection, *i.e.* threshold cycle (Ct)≤35, was associated with worse ACT score [β=-1.46, p=0.01] and increased odds of having a symptom day for each of the two-week outcomes, *e.g.* [wheeze/cough OR 1.55, p<0.001]. SA and SEB-SED were variably associated or were not associated with respiratory symptom outcomes.

CONCLUSIONS: Home staphylococcal dust exposures (SA/SE) were common among inner-city children with asthma. Dust SEA detection consistently was associated with increased respiratory symptoms in this cohort. Longitudinal studies are needed to confirm and explicate this novel finding.

L8 Environmentally-Induced Epigenetic Changes Correlate with Race and Childhood Asthma Severity

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RATIONALE: Socioeconomic status, genetic predisposition and environmental factors contribute to asthma incidence and severity. Children with asthma who are economically disadvantaged likely live in substandard housing with potential indoor environmental exposures that may manifest through epigenetic mechanisms. We examined the association of global DNA methylation with socioeconomic status, asthma severity and race/ethnicity.

METHODS: Global DNA methylation was measured in peripheral blood of children with asthma between the ages of 2 and 17 yrs enrolled in the Kansas City Safe and Healthy Homes Program. Inclusion criteria included residing in the same home for a minimum of 4 days per week and total family income of less than 80% of the Kansas City median family income (MFI). A three-way mixed factorial ANOVA was used to analyze global DNA methylation. When appropriate, follow-up analyses were performed using independent-samples *t*tests and ANOVA models with Bonferroni corrections.

RESULTS: Our results indicate that overall, African American children with asthma had significantly higher levels of global DNA methylation than children with asthma of other races/ethnicities ($p = 0.029$). This difference was more pronounced when socioeconomic status and asthma severity were considered ($p = 0.042$). In children with persistent asthma from the lowest income families (<50% Kansas City MFI), significantly higher levels of global DNA methylation were observed in African American children compared to children of other races/ethnicities ($p = 0.05$).

CONCLUSION: Our study demonstrates a significant interaction effect among global DNA methylation levels with asthma severity, race/ethnicity, and socioeconomic status.

L9 Withdrawn

L10 Mepolizumab in COPD with Eosinophilic Bronchitis: A Randomized Clinical Trial

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RATIONALE: Chronic obstructive pulmonary disease (COPD) is associated with eosinophilic bronchitis in 10–20% of patients. Mepolizumab, an anti-interleukin-5 antibody, depletes blood eosinophils, sputum eosinophils and reduces exacerbations. We investigated if it had similar effects in COPD with airway eosinophilia.

METHODS: This was a double-blind, placebo-controlled, randomized single-centre study. Patients (40 – 80 years) with current moderate-to-severe COPD (post-bronchodilator $FEV_1/VC<70\%$; post-bronchodilator $FEV_1<60\%$ predicted) and current/ex-smokers (>10 pack-years) with sputum eosinophilia (≥3%) received monthly IV injections of mepolizumab 750 mg or placebo for 6 months.

RESULTS: A total of 18 patients were recruited (8 in active-arm; 10 in placebo).1 patient in the placebo group withdrew after randomization. Mepolizumab reduced sputum eosinophils (baseline 11% to 0.3% at 6 months in active arm vs 9.4% to 1.7% in placebo arm, $p<0.05$) and blood eosinophils (0.69 at baseline to 0.02 at 6 months in active-arm vs 0.36 to 0.28 in placebo-arm, $p<0.05$). There were no significant changes in the secondary outcome measures: lung function (FEV_1, FVC, SVC, FEV_1/SVC, FEV_1/FVC, TLC, RV, RV/TLC and DLCO), exacerbation rates and Quality of life scores; no significant treatment effects on airway-wall area %, lumen area, parametric response maps or relative areas of the CT density-histogram. However, the CRQ mean dyspnea domain score change was clinically meaningful (>0.5 units).

CONCLUSIONS: Mepolizumab does not improve lung function and exacerbation rates in COPD with eosinophilia. This suggests that although eosinophils are a predictor of response to treatment with corticosteroids, unlike in asthma, they may not directly contribute to luminal obstruction in COPD.

L11 Role of R213G Polymorphism in Airway HYPER-Responsiveness

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RATIONALE: The R213G polymorphism (rs1799895) in EC-SOD (Extracellular superoxide dismutase) protects smokers from developing COPD by releasing the EC-SOD from extracellular matrix into extracellular fluids such as plasma and epithelial lining fluid (ELF). The high levels of EC-SOD in ELF suggest a potential role for mitigating oxidative stress and airway hyperresponsiveness.

METHODS: C57BL/6 R213G knock-in mice were sensitized and challenged with ovalbumin (OVA) or saline. Airway hyperresponsiveness (AHR) was measured with flexiVent. Broncheoalveolar lavage fluid (BALF) was used for cell counts. Cytokines in supernatant from BALF were assayed using a V-plex assay from MSD.

RESULTS: Airway resistance (R) was significantly increased in wild-type (WT) OVA mice ($N=6$) compared to the saline mice ($N=9$, $p<0.0001$), but not in R213G heterozygotes (HETs) OVA mice ($N=7$). However, homozygotes (HMs) OVA mice ($N=4$) showed higher R at 25 ($p<0.05$) and 50mg/ml ($p<0.001$) methacholine than the saline mice. Total number of BALF cells increased in WT OVA compared to the saline group ($p=0.0017$). IL-4, IL-5, IL-6, TNF-α, and IFN-γ were increased in WT OVA ($p<0.01$) but not in HETs or HMs compared to the saline group. However, IL-1β and KC/GRO were higher in HMs and WT OVA compared to the saline group ($p<0.05$).

CONCLUSIONS: The R213G polymorphism appears to be protective in AHR and both Th1 and Th2 cytokines were suppressed. Maximal protection was observed in the heterozygotes, suggesting that both high ELF and tissue antioxidant activity may be important in the AHR.

L12 Prostaglandin E2in Induced Sputum Following Oralaspirin Challenge in Asthma Patients with and without Aspirin Hypersensitivity

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RATIONALE: Induced sputum (IS) supernatant allows to measure lipid mediators of asthmatic inflammation in bronchial secretions. The specific role of endogenous bioactive prostaglandin E2(PGE2) in aspirin-induced asthma (AIA) is not well understood.

METHODS: To investigate theinfluence of aspirin on sputum supernatant concentration of PGE2 during aspirin challenge, using chromatography-mass spectrometry measurements in subjects with AIA ($n=26$) and aspirin-tolerant asthma (ATA, $n=17$), and healthy controls (HC, $n=21$).ISwas collected before and following oral aspirin challenge. Sputum differential cell count and sputum supernatant concentrations of PGE_2were assessed.

RESULTS: Aspirin precipitated bronchoconstriction in all AIA subjects, but in none of the ATAand HC. Phenotypes of asthma based on the sputum cytology differed between the groups. The IS specimens were mainly eosinophilic in AIA and paucigranulocitic in HC. In ATA group non phenotype based on the sputum cytology was dominant. At baseline, mean sputum supernatant concentrations of $PGE_2$was higher in asthma patients independent of aspirin hypersensitivity as compared to HC. Following the challenge, PGE_2decreased in all study groups (ANOVA, $p<0,001$).However, this decrease was statisticallysignificant only in AIA patients($p=0.01$) and HC.A cumulativedose of aspirin had no effect on the magnitude of the PGE2 alterations.

CONCLUSIONS: PGE_2decreases significantly in AIA during the oral challenge.The results support theory on the inhibition of PGE2 biosynthesis as a trigger for bronchoconstriction mediated by cysteinyl leukotrienes in AIA.

L13 A New Pharmacological Approach for Asthma through Tissue-Specific Modulation of the GABA(A) Receptor

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RATIONALE: This study addresses the unmet need for an oral, safe, non-steroidal asthma treatment by targeting $GABA_A$ receptors ($GABA_AR$) in lung tissues. The hypothesis is that $GABA_A$Rs in inflammatory and airway smooth muscle (ASM) cell can be targeted by subtype-selective $GABA_AR$ agonists to tissue-selectively induce immunosuppression and ASM relaxation.

METHODS: A drug discovery approach identified $GABA_AR$ targets in lung cells by immunodetection, subtype selectivity by electrophysiology, preclinical characterization of active ligands using microsomes, S9, and blood plasma stability assays. Pharmacokinetic studies in mice are applied to identify *in vivo* stability and distribution. Murine pharmacodynamic models are used to quantify sensorimotor effects (rotarod), disease specific airway hyperresponsiveness, airway mucus production, and airway eosinophilia. Subtype-selective $GABA_AR$ ligands were evaluated for immune modulation using *in vitro* T-cell assays and ASM muscle relaxation with isolated ASM.

RESULTS: The α4 subtype-selective $GABA_AR$ ligand XHE-III-74EE showed high stability *in vitro* but a limited half-life *in vivo* due to rapid metabolism and clearance. Chronic administration of 20 mg/kg XHE-III-74EE successfully reduced airway hyperresponsiveness without inducing adverse CNS effects. Mucus hypersecretion was reduced for chronic and acute treatment. Similar results were observed for metabolite XHE-III-74A that exhibits α4 $GABA_AR$ subtype selectivity. XHE-III-74A significantly reduced eosinophilia, which is consistent with antiinflammatory suppressive effects in activated T-cells as measured by intracellular calcium release and IL-2 production. Both compounds were able to induce ASM muscle relaxation.

CONCLUSIONS: α4-Selective $GABA_AR$ agonists have a great potential as novel drug candidates for asthma to alleviate symptoms of airway hyperresponsiveness mediated by ASM constriction, hypereosinophilia, inflammation, and mucus overproduction.

L14 Role of Circulating ICOS+ Follicular Helper T Cells in the Pathogenesis of Birch Pollen Allergy

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RATIONALE: Production of antigen-specific immunoglobulins in tissues is controlled by follicular helper T (Tfh) cells, which are recognized as memory Tfh cells in peripheral blood. Recent studies have revealed that inducible T-cell co-stimulator (ICOS) and programmed death 1 (PD-1) are activation molecules in blood Tfh cells. However, the role of blood Tfh cells expressing such molecules in the pathogenesis of birch pollen allergy remains unknown.

METHODS: Patients with birch pollen allergy (n = 34) and healthy controls (n = 21) were recruited in this study. Expression of ICOS and PD-1 in blood Tfh cells from subjects in pollen and pollen-free seasons (i.e. before and after the periods with pollens) was examined by flow cytometry. Correlations between results of flow cytometry and clinical parameters were also analyzed.

RESULTS: Levels of $ICOS^+$ and/or PD-1^+ in blood Tfh cells in the patients were similar to those in the controls throughout the pollen-free seasons, whereas the percentages of $ICOS^+$ blood Tfh cells during the pollen season were temporarily increased in the patients compared to those in the controls. We also found that total symptom scores were significantly correlated with percentage of $ICOS^+$ blood Tfh cells. Moreover, differential levels of $ICOS^+$ blood Tfh cells in pollen- and pollen-free seasons were significantly correlated with those of birch pollen-specific IgE.

CONCLUSIONS: Our findings suggest that increase in $ICOS^+$ blood Tfh cells in response to exposure to birch pollen may underlie the pathogenesis of birch pollen allergy.

L15 Case Report of a Previously Unreported Type of DOCK8 Deficiency

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RATIONALE: Dedicator of cytokinesis 8 (DOCK8) deficiency is an autosomal recessive hyper-IgE syndrome characterized by recurrent bacterial, viral and/or fungal infections, atopic dermatitis and food allergies. Previous reports demonstrate either autosomal recessive or compound heterozygosity defects within the DOCK8 gene.

METHODS: Genetic sequencing evaluation of DOCK8, SPINK5, STAT3, and TYK2 were performed by GeneDx.

RESULTS: The patient presented with severe atopic dermatitis, eczema herpeticum, and severe food allergies. Immune evaluation showed decreased NK cell function, absent CD45+ total lymphocyte and CD3+ T cell responses to Tetanus toxoid and a serum IgE of 15,828 kU/L. Targeted comparative genomic hybridization revealed a heterozygous defect, c.624-12 T>A, a variant of unknown significance in the DOCK8 gene.

CONCLUSIONS: The c.624012 T>A variant is a previously unreported mutation that is likely responsible for the findings in this patient. This is the first reported case of this heterozygous mutation and may be clinically useful in the diagnosis and treatment of severe atopic dermatitis that does not fit the established criteria for previously reported hyper-IgE syndromes.

L16 Immune Phenotype in Children with Mitochrondial Disease

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RATIONALE: Mitochondria contributes to metabolic processes important for cellular growth and function. Defects in mitochrondrial function might negatively impact immune development and responses. Interestingly, there have only been a few publications reporting on increased rate of infections in certain patients with mitochrondial disease. In this clinical retrospective study, we performed immune analysis on 70 pediatric patients diagnosed with mitochrondial disease defined by definitive Walker criteria. The majority of patients lack a history of life-threatening infections.

METHODS: From our mitochrondia cohort, we selected all patients diagnosed as definitive based on the Walker criteria. Seventy patients were identified, 16 with Leigh, 6 with depletion, 2 with SANDO, 1 with NARP, 1 with MELAS and the rest with unknown syndrome. We performed a laboratory retrospective review, documenting all commercial immune results.

RESULTS: Immunoglobulin and IgG subclass levels were within normal range for >90% of patients. Lymphocyte subset data was present for 44 patients. Although the CD45RO absolute count was within the age-specific normal range, the vast majority (65/71, 92%) of the values were in the lower third of the normal range. However, the %CD45RO was below the lower threshold for normal values (n=60, 85%). Conversely, the CD45RA values were on the upper threshold of normal. Most patients have protective titers to tetanus, diphtheria and pneumococcus.

CONCLUSIONS: Most patients with mitochondrial disease do not have perturbed immune development except for reduced CD45RO memory lymphocytes. The clinical significant of this result is unclear, but it suggests that mitochondrial function might be necessary for optimal immune memory development.

L17 Evaluation of Serum Levels of Osteopontin and IgG Anti-Osteopontin Autoantibodies As Potential Biomarkers of Immune Activation in Patients with Allergic Diseases

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RATIONALE: Osteopontin (OPN) is a pleomorphic cytokine known to influence a wide range of immune cells; high OPN and IgG anti-OPN autoantibodies (AutoAbs) levels are associated with an increased risk of autoimmune lymphoproliferative syndrome, multiple sclerosis and systemic lupus erythematosus. We aimed to verify if serum levels of OPN and IgG anti-OPN AutoAbs may qualify as biomarkers of an activated immune response also in allergic patients.

METHODS: Serum OPN levels were measured by ELISA test (Human Osteopontin Duoset, R&D Systems, for OPN detection; "in-house" kit for anti-OPN AutoAbs). A series of 121 adult patients affected by asthma, allergic rhinitis (AR), Hymenoptera venom allergy (HVA), food allergy (FA), allergic contact dermatitis (ACD) and IgE-mediated hypersensitivity to beta-lactams (IEHB) was studied. 116 healthy subjects served as controls.

RESULTS: OPN serum levels were significantly higher in cases in comparison to controls (p=0.0010 by the Mann-Whitney test). Statistically higher levels were found in asthma (p=0.0269) and FA (p=0.046) groups in comparison to controls. Prevalence and titers of serum IgG anti-OPN AutoAbs were significantly lower in cases with respect to controls (p<0.0001). Lower levels of AutoAbs versus controls were found in patients with HVA (p<0.0001), AR (p=0.0009), ACD (p=0.0011) and asthma (p=0.0013), but not in FA group (p=0.0575). Patients with IEHB presented heterogeneous results for OPN and anti-OPN AutoAbs.

CONCLUSIONS: Serum OPN levels may represent a novel, potentially useful biomarker for allergic asthma and, interestingly, for food allergy.

L18 Patient-Reported Outcomes (PROs) in Patients Receiving Omalizumab (OMB): A Systematic Literature Review

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RATIONALE: To summarize clinical trial and real-world evidence describing the magnitude and duration of impact of OMB as add-on therapy on PROs in patients with moderate to severe allergic asthma.

METHODS: Systematic literature review (MEDLINE/EMBASE) was conducted to identify studies of OMB in pediatric/adolescent/adult patients with moderate to severe allergic asthma. Outcomes of interest included measures of self-reported asthma control, asthma-specific and general quality of life assessments/questionnaires, and patient symptom reports.

RESULTS: 25 randomized controlled trials (RCTs) and 34 non-randomized studies (NRSs) were included. Among 8 RCTS reporting the Asthma Quality of Life Questionnaire (AQLQ) overall score, statistically significant improvements favoring OMB versus placebo/control, were documented in 5 studies; at 52 weeks, mean/median changes from baseline in domain and overall scores ranged from 1.01-1.33 for OMB and from 0.8-0.98 for placebo ($P<0.01$). At 20–52 weeks, proportions of patients with a minimally important difference (MID) in AQLQ improvement (≥0.5 points from baseline) ranged from 57.5%–78.8% with OMB and from 22.2%–69.8% with placebo/control. Statistically significant improvements in mean Asthma Control Test (ACT) scores from baseline to post study were found in 12 of 22 NRSs, ranging from 9.4-17.28 at baseline to 17.4-22.5 at 8 months to 6 years. Seventeen of 22 NRSs reported achievement of a MID in ACT (≥3 points from baseline) for patients treated with OMB.

CONCLUSIONS: Results from this systematic literature review confirm that OMB-treated patients with moderate to severe asthma achieve clinically meaningful improvements in PROs, which are observed across both RCTs and observational studies.

L19 Epicutaneous Allergen Exposure Dose Determines Manifestation of Allergic Airway Disease in Mice

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RATIONALE: As cat allergies are associated with severe asthma in children, we sought to determine whether the application of cat dander to barrier-disrupted skin could play a role in the development of allergic asthma.

METHODS: In 4-6 week old female mice (BALB/c, C57Bl/6 and mice transgenic for the human HLA DRB1*0401), cat dander extract (CDE) was applied (1.5, 15 or 150 μg) to a shaved area on their back for 10 days after tape stripping. Mice were then administered intranasal challenges of CDE to localize the response to the lungs. Eosinophilia was determined by Wright-Giemsa staining of the bronchoalveolar lavage fluid (BALF) and hematoxylin and eosin staining of lung sections. Airway resistance was measured through a nebulized methacholine challenge.

RESULTS: Mice exposed to 15 μg CDE on the skin showed increased eosinophils in the BALF and peribronchial tissue (BALB/c: 2.3 ± 1.8 x 10^4 eosinophils and 0.262 ± 0.257 eosinophils/mm^2 respectively) compared to naive mice (BALB/c: 0.02 ± 0.04 x 10^4 eosinophils in BALF and 0 eosinophils/mm^2 in the peribronchial tissue; $p < 0.05$). Airway resistance was also increased. Intriguingly, eosinophilia and airway resistance were markedly reduced in mice that received 150 μg CDE on the skin (BALB/c: 0.5 ± 0.4 x 10^4 eosinophils in BALF and 0.07 ± 0.05 eosinophils/mm^2 in the peribronchial tissue). These trends were observed in all three strains.

CONCLUSIONS: Although epicutaneous exposure to cat dander on barrier-disrupted skin can lead to allergic airway disease, at a high dose of cat dander on the skin these features of disease are attenuated.

L20 Analysis of Home Dust for Allergens Related to *Staphylococcus Aureus*

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RATIONALE: The bacterium *Staphylococcus aureus* (SA) is known to induce allergic inflammatory responses, including through secreted staphylococcal enterotoxin (SE) A-D superantigens. SA is known to exacerbate eczema; a growing body of evidence suggests SA exposure may exacerbate a related disease, atopic asthma. While methods are established to quantify home environmental allergen exposure, corresponding methods for SA/SE assessment have not yet been validated. We adapted a method for home dust SA/SE detection and applied it in INHALE study homes of inner-city adults with asthma.

METHODS: We conducted laboratory experiments to optimize sample processing and real-time PCR methods for genetic assessment of SA (*femB*) and SEA-D, based on published primers. We applied this method to dust and dust extract from 21 homes. We compared results from bacterial gene assessment to culture-based results from the same homes.

RESULTS: The Biostic® Bacteremia DNA Isolation Kit (MoBio Laboratories) with 50mg raw dust and using 9μl isolated DNA for qPCR assessment performed equally or better than alternative methods. Application to INHALE homes demonstrated that while 10 (48%) of 21 homes were culture-positive for SA, all had detectable SA genes. Prevalence of SE detection in cultured SA isolates was 0% but in raw dust was: SEA 33%; SEB 76%; SEC 62%; SED 24%. Dust extract and raw dust demonstrated strong SA gene correlation (*femB*, Pearson's coefficient 0.80), but weaker correlations for SE genes.

CONCLUSIONS: Compared to culture-based assessment, bacterial gene-based testing of home dust was more sensitive for staphylococcal (SA/SE) exposures. Staphylococcal exposures may be common among inner-city adults with asthma.

L21 Risk Factors for Childhood Peanut Allergy in a Large Birth Cohort Study: Growing up in New Zealand

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RATIONALE: The prevalence of IgE-mediated food allergy is increasing worldwide. However the prevalence of childhood food allergy and early life determinants remain unclear. We determined the prevalence of peanut allergy at age 2 years and both perinatal and postnatal factors associated with the risk of peanut allergy, within a contemporary New Zealand (NZ) birth cohort study.

METHODS: *Growing Up in New Zealand* is an ethnically and socio-economically diverse cohort made up of 6853 births from 2009-2010 (11% of all births in NZ over this period). Between late pregnancy and when the children were 2 years old information was collected on child characteristics and their environments. Prevalence of peanut allergy was determined by parental report of doctor diagnosis. Multivariable logistic regression was used to describe the early life factors associated with the presence of peanut allergy.

RESULTS: By age 2 years, 162 (2.6%, 95% CI 2.2-3.0%) cohort children were identified as peanut allergic. The odds of having peanut allergy were increased for boys (OR=1.59, 95% CI 1.13-2.26), children diagnosed with eczema since 9 months (OR=10.72, 95% CI 7.26-16.31), children whose mother had a history of atopic disease (OR=1.40, 95% CI 1.00-1.97), or whose mothers identified as being of Asian ethnicity (OR=2.27, 95% CI 1.48, 3.43).

CONCLUSIONS: This is the first study to determine prevalence in a diverse NZ cohort and identify key early determinants. In particular the increased likelihood of a peanut allergy in children born to mothers who identified as Asian may be related to discrete biological and environmental factors, further investigation is needed.

L22 Increased Cis-to-Trans Urocanic Acid Ratio in the Skin of Chronic Urticaria Leads to the Enhancement of Mast Cell Degranulation

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RATIONALE: Increased filaggrin expression was positively correlated with urticaria severity in our previous study. However, the role of filaggrin breakdown products (FBP) in the pathogenesis of CU has not been studied.

METHODS: FBP, including pyrrolidone carboxylic acid (PCA) and urocanic acid (UCA) were quantitated in stratum corneum (SC) samples collected from volar forearm regions (6 consecutive tapes) employing UPLC-MS/MS from 10 CSU, 10 atopic dermatitis (AD) and 10 normal subjects. *In vitro* effects of *cis*- and *trans*-UCA on human mast cell degranulation were assessed by beta-hexosaminidase release assay using LAD2 cells.

RESULTS: With normalization by protein content, total amount of FBP and PCA content was significantly decreased in lesional (21.56±20.2 and 16.70±15.4 ng/mg protein, respectively, $P<0.01$) AD skins as compared to NC (63.86±21.6 and 49.14±16.3). However, those were not significantly different in CSU lesions (44.54±31.2 and 34.48±23.6) compared with NC. *Trans*-UCA, the primary isomer of the UCA in NC, was significantly decreased in CSU and AD. The proportion of *cis*-UCA was significantly higher in CSU skin (0.44±0.24, $P<0.01$) compared with AD (0.14±0.20) and NC (0.10±0.12). Both TEWL and pH were significantly increased in AD lesions compared with CSU lesions. *Cis*-UCA dose-dependently enhanced the IgE- and calcium-mediated degranulation of LAD2 cells ($P<0.001$), which was not observed with *trans*-UCA.

CONCLUSIONS: FBP deficiency in AD was confirmed in the association with a significant increase in TEWL and pH in AD. Increased ratio of *cis*-to-*trans*-UCA, and decreased epidermal pH in CU can be associated with CU pathogenesis. *Cis*-UCA could contribute to the pathogenesis of CU by enhancing mast cell degranulation.

L23 Multicenter Study of Food Induced Anaphylaxis in Korean Infants

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RATIONALE: Food induced anaphylaxis in young age group is increasing. We aimed to analyze clinical characteristics of anaphylaxis in Korean infants.

METHODS: A retrospective medical record review was performed on infants (0~2 years old) diagnosed with anaphylaxis between 2009 and 2013 in 23 tertiary hospitals in South Korea

RESULTS: 363 anaphylaxis cases (66.9% male) were identified. Cutaneous symptoms (98.6%) were the most common symptoms followed by respiratory (83.2%), gastrointestinal (29.8%), and neurologic (11.6%). Cardiovascular symptoms were rare (7.7%). 338 cases (93.1%) of anaphylaxis was induced by foods. 185 cases (51.0%) of anaphylaxis occurred within 30 minutes after offending food exposure. The most common trigger food was milk (44.3%) followed by egg (22.0%), walnut (8.3%), wheat (7.7%), peanut (4.8%), other nuts (3.0%), and fish (2.1%). The median value of specific IgE (sIgE) by immunoCAP to milk was 6.80 (range 0.37 ~ 427.00) kU_A/L. 51.7% of infants under 12 months of age and 55.9% of infants aged 12 months and over had their symptoms even under the levels of milk-sIgE diagnostic decision points. The median value of egg-sIgE was 10.40 (range 1.03~100.00) kU_A/L. 93.2% of egg-induced anaphylaxis cases had egg-sIgE levels above diagnostic decision points.

CONCLUSIONS: Milk was the most common trigger food of anaphylaxis in Korean infants. Half of the cases of anaphylaxis occurred within 30 minutes after exposure. Even in very low level (0.37 kU_A/L) of milk-sIgE, anaphylaxis could occur and more than half of the infants with milk anaphylaxis showed milk-sIgE levels under the diagnostic decision point.

L24 Proteomic Profiling of Atopic Dermatitis, Psoriasis, and Contact Dermatitis Patients

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RATIONALE: Atopic dermatitis (AD), psoriasis (PS), and contact dermatitis (CD) are common inflammatory skin diseases characterized by significant barrier disruption and systemic inflammation. Transcriptomic profiling has identified unique epidermal signatures as well as common inflammatory pathways. Given the systemic nature of the diseases, this study profiled the proteomic signatures in serum from subjects with AD, PS, and CD compared to healthy donor controls.

METHODS: Serum was collected from 20 subjects with moderate-to-severe AD, 20 subjects with CD, 12 subjects with moderate-to-severe PS, and 10 healthy controls with no history of skin disease. Protein expression was evaluated by SOMAscan™, Singulex®, and multiplex technology. Expression in AD, CD, and PS serum was compared to healthy controls for statistical significance (fold change ≥ 1.5 and false discovery rate < 0.05) and lists compared between diseases to identify unique proteomic signatures.

RESULTS: This study identified 7 proteins (Up Regulated: C5a, PARC, LBP, CRP, ILT-4; Down Regulated: CAMK2B, Carbonic anhydrase 6) that were similarly modulated in all inflammatory skin diseases compared to healthy controls. Additional comparisons with serum from healthy controls revealed significant modulations in a total of 25, 5, and 64 proteins in subjects with AD, PS, and CD, respectively. Protein signatures were further refined by comparing between inflammatory skin diseases. This resulted in a unique signature of increased IgE, CCL17/TARC, and CCL22/MDC in AD; which significantly correlated ($p<0.05$) with disease severity.

CONCLUSIONS: This study suggests unique proteomic signatures in the sera may potentially distinguish between inflammatory skin diseases despite similar epidermal barrier disruption and epithelial inflammation.

L25 Efficacy and Safety of Crisaborole Topical Ointment, 2%, a Novel, Nonsteroidal, Topical, Anti-Inflammatory, Phosphodiesterase Inhibitor in 2 Phase 3 Studies in Children and Adults with Mild-to-Moderate Atopic Dermatitis

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RATIONALE: Phosphodiesterase 4 (PDE4) enzyme is overexpressed in inflammatory cells of patients with atopic dermatitis (AD); this leads to disease exacerbation. Here, we present safety and efficacy from 2 multicenter, double-blind, vehicle-controlled phase 3 studies of identical design in patients with mild-to-moderate AD (NCT02118766 and NCT02118792) treated with the novel, nonsteroidal, topical, anti-inflammatory investigational PDE4 inhibitor Crisaborole Topical Ointment, 2%.

METHODS: Patients ≥2 years old with mild-to-moderate AD were randomized 2:1 to receive crisaborole or vehicle twice daily with evaluation on Days 8, 15, 22, and 29. Primary and secondary efficacy endpoints analyzed AD disease severity with the Investigator's Static Global Assessment (ISGA). Supportive efficacy endpoints examined time to improvement in pruritus, severity of pruritus, and signs of AD.

RESULTS: Studies 1 and 2 enrolled 503:256 and 513:250 crisaborole/vehicle patients, respectively. At Day 29, more crisaborole-treated patients achieved ISGA success than those treated with vehicle (study 1: 32.8% vs 25.4%, $P=0.038$; study 2: 31.4% vs 18.0%, $P<0.001$) with a greater percentage of "almost clear/1" or "clear/0" ISGA scores (study 1: 51.7% vs 40.6%, $P=0.005$; study 2: 48.5% vs 29.7%, $P<0.001$). Success in ISGA and improvement in pruritus were achieved earlier with crisaborole than vehicle ($P<0.001$ vs vehicle). A greater proportion of crisaborole-treated patients achieved success for all clinical signs of AD by Day 29. Treatment-related adverse events were infrequent, transient, and mild/moderate in severity.

CONCLUSIONS: Two Phase 3 studies demonstrate that Crisaborole Topical Ointment, 2%, represents a novel, safe, and efficacious treatment for children and adults with mild-to-moderate AD.

L26 The Negative Impact of Persistent Penicillin Allergy Labeling

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RATIONALE: Although 8-20% of patients have penicillin allergy labels (PAL), less than 1% of the population are truly allergic. The extent to which a PAL persists in the EMR despite documented penicillin tolerance is currently unknown.

METHODS: The synthetic derivative (de-identified version) of the electronic medical record (EMR) was mined for patients >18 years or older with >3 visits linked to Vanderbilt ambulatory care from January 2000 to August 2014. Key outcomes including antibiotic utilization and presence of C. difficile infection were compared between cases with (n=11,504) and controls (n=31,084) without PAL. Cases were examined for the persistence of the PAL despite documented tolerance. Categorical variables were analyzed by Pearson Chi-squared test and continuous data by Wilcoxon signed rank test.

RESULTS: Most PAL (67%) were already labeled upon entry into the EMR, and 96% remained persistently labeled. Cases were more likely to develop C. difficile infection (1.2% vs 0.9%, p=0.001). The proportion of prescription encounters for levofloxacin (15% vs 12%), vancomycin (5% vs 4%), clindamycin (8% vs 4%), and aztreonam (1% vs <0.1%) were overrepresented in PAL cases versus unlabeled controls (all p<0.001). Of 11216 PAL, 4321 (39%) had EMR documentation of having received and tolerated a penicillin, however despite this 4045/4321 (94%) retained the PAL.

CONCLUSIONS: In this largely ambulatory population, PALs persist within the EMR despite proven tolerance and are associated with higher risk antibiotic treatments and C. difficile infection. Major reform of the EMR to utilize systematic approaches of documenting, reconciling, and removing the PAL is urgently needed.

L27 Skin Testing for the Diagnosis of Severe Perioperative Anaphylaxis to Clindamycin

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RATIONALE: Clindamycin hypersensitivity reactions (HSRs) are rare with an incidence of 0.4%. Mild cutaneous type I HSRs are most common, and severe HSRs are extremely rare with only three prior case reports of anaphylaxis. Clindamycin skin testing has low sensitivity in mild to moderate HSRs but has not been evaluated in severe HSRs.

METHODS: Skin prick and intradermal tests were performed with rocuronium, propofol, midazolam, ondansetron, and clindamycin.

RESULTS: A 60-year-old woman with leakage of bilateral breast implants presented for capsulectomy with removal and replacement of implants. Anesthesia was induced with midazolam, fentanyl, propofol, and rocuronium. She had an unknown childhood penicillin allergy and was given clindamycin in the operating room prior to induction and ondansetron at the time of induction for nausea. Immediately after induction, she became difficult to ventilate with no response to sevoflurane or albuterol nebulizer. Upon intubation, she was persistently hypoxemic and bradycardic. She developed linear urticaria on her bilateral extremities and went into PEA arrest. ACLS was administered for 8 minutes prior to ROSC. After resuscitation, serum tryptase and histamine were obtained and were 106 ng/mL and >9.99 ng/mL, respectively. She recovered and was evaluated in the Allergy Clinic two months later. Repeat serum tryptase was 3.5 ng/mL, and latex IgE was <0.35 kU/L. Skin testing was negative to rocuronium, propofol, midazolam, and ondansetron. Skin prick test was positive to clindamycin with a 10 mm wheal and 35 mm flare.

CONCLUSIONS: Clindamycin hypersensitivity can cause life-threatening anaphylaxis. Skin testing is useful for diagnosis in severe type I HSRs.

L28 Antibiotic Allergy De-Labeling: Teaching an Old Dog New Tricks

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RATIONALE: Antibiotic allergy labels (AAL) significantly impact antibiotic prescribing and may lead to the inappropriate use of broad spectrum antibiotics which creates a public health concern. Infectious disease (ID) physicians from the Emerging Infections Network (EIN) of the Infectious Diseases Society of America (IDSA) were surveyed to determine their views, access and use of antibiotic allergy testing (AAT).

METHODS: A 10-item online survey was distributed by the EIN in September 2015 to 1172 members practicing adult ID, 323 pediatric and 24 both. Two reminders were sent to non-respondents.

RESULTS: Of 736/1,545 (48%), only 43% had skin prick/intradermal testing (SPT) available and 30% were either unaware of options or had none available. Although 78% overall suggested that a negative test would lead to AAL removal, those with > 15 years experience were significantly less likely to remove AAL (P<0.001). Most felt AAL removal would aid antibiotic selection (95%), appropriateness (92%), safety (74%) and antimicrobial-stewardship (AMS) (82%). Although 68% overall advocated incorporation of AAT into AMS, those with < 15 years experience were significantly more likely to support this (p=0.006). In settings of a remote reaction history, point-of-care testing (40%) was preferred to antibiotic desensitization (7%).

CONCLUSIONS: ID physicians perceive inadequate access to AAT services. Less experienced physicians were both more likely to view AAT as a means to remove AAL and advocate its incorporation into AMS. A generational shift appears to be occurring that should support AAT as a tool to improve antibiotic appropriateness.

L29 The Use of Drug Desensitization Protocols at a Pediatric Institution

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RATIONALE: Protocols for adults to achieve immunologic IgE and non-IgE induction of temporary drug tolerance (drug desensitization) have been well described. Use of these protocols is recommended only when administration of the drug is essential and requires close collaboration between Allergists, nursing and pharmacy staff. Application of these protocols to pediatric patients is further challenging due to variations in patient weights, target doses and minimum volumes required to infuse drugs. We have established pediatric protocols based on adult guidelines at our pediatric tertiary care center to perform antibiotic desensitizations via a 12 step, 4 syringe method. We describe these protocols and their success.

METHODS: We conducted a retrospective chart review of all patients who had drug desensitization performed between 1/1/2013 and 7/15/2015 under the supervision of a Pediatric Allergist using standardized desensitization protocols and reviewed their outcomes.

RESULTS: In the given period, 5 patients underwent desensitization using the protocol involving 5 different antibiotics (ceftriaxone, ceftazidime, linezolid, ertapenem, oxacillin). Three of the 5 subjects were female and the mean age was 12 years (range of 3 -19 years). All 5 patients tolerated the desensitization procedure and subsequent dosing of the drug to complete the full therapeutic course.

CONCLUSIONS: Dose calculation for the various steps of drug desensitization is challenging in a pediatric population where there is a need for customized dosing. This procedure is cumbersome and prone to human error. The pediatric protocols established at our institution have been utilized with success and can potentially be applied to use for other agents

L30 Early Introduction of Dietary Egg Reduces Egg Sensitization at 12 Months of Age in Infants at Risk of Allergic Disease

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RATIONALE: Epidemiological evidence suggests delayed introduction of dietary egg may promote rather than protect from egg allergy in infants at risk of allergic disease, as has been recently shown for peanut. We examined whether introduction of dietary egg between 4-6 months of age would reduce sensitisation to egg, in infants at risk of allergy.

METHODS: We conducted a randomised controlled trial in infants with at least one first degree relative with allergic disease. Infants were randomised at 4 months of age and included where egg-white (EW) skin prick test (SPT) was <2mm. Infants were randomised to receive pasteurised raw whole-egg powder or rice powder from introduction of solids until 8-months of age, with all other egg excluded. Diets were liberalised at 8-months. Primary outcome was EW-SPT ≥3mm at 12 months of age and analysed using Chi-Square test. IgG4/IgE were analysed by non-parametric tests.

RESULTS: 319 infants were randomised to egg (n=165) and rice (n=154). 14 infants reacted to egg within one-week of introduction despite egg-SPT <2mm at randomization. 254 infants were assessed at 12 months of age. Loss to follow up was similar between groups. Sensitisation to EW at 12 months was 20% and 11% in infants randomised to rice and egg powder, respectively, (OR=0.46, 95% CI 0.22 – 0.95, p=0.03). IgG4-EW, ovalbumin and ovomucoid and IgG4/IgE ratios were higher in patients randomized to egg (p<0.0001 for each) at 12 months.

CONCLUSIONS: Early introduction of whole-egg into the diet of high risk infants reduced sensitisation to EW at 12-months of age.

L31 Correlation of Negative Tree Nut Skin-Prick Tests and Successful Tree Nut Food Challenges Among Peanut-Allergic Children

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RATIONALE: Children with peanut allergy are regularly instructed to avoid all tree nuts. However, children with peanut allergy are likely not allergic to all tree nuts. In our cohort of peanut anaphylaxis patients undergoing oral immunotherapy (OIT), we sought to determine the correlation of tree nut skin prick testing (SPT) results and likelihood of successfully passing a tree nut challenge.

METHODS: **S**kin-prick testing was performed to peanut and tree nuts (macadamia, pine nut, coconut, hazelnut, brazil nut, cashew, pecan, walnut, pistachio, almond) in 27 patients with known peanut allergy. The probability of negative SPT (wheal <3mm) for each nut was determined.

RESULTS: All patients demonstrated positive peanut allergy diagnostics in skin test, component testing or food challenge. Only 15.4% of patients were SPT positive to peanut alone. Macadamia, pine nut, and coconut SPT had a probability of negative SPT of 0.97, 0.97, and 0.91 respectively. The odds ratio for this group having a negative SPT (compared to a negative SPT) was 46.22. For hazelnut, brazil nut, and cashew the probability of a negative SPT was 0.81, 0.77, and 0.73, respectively. Pecan, walnut and pistachio had odds ratios of 0.68, 0.68, and 0.64, respectively. All patients with macadamia, pine nut and coconut negative SPT subsequently passed 9 gram food challenges without OIT.

CONCLUSIONS: Despite current recommendations to avoid all tree nuts for peanut allergic patients, the majority of patients with peanut allergy will have negative skin tests and food challenges to certain tree nuts, especially macadamia, pine nut, and coconut. This pattern was seen despite most patients having multiple nut sensitizations.

L32 Withdrawn

L33 Cor a 14 Specific Ig E Best Distinguishes Between Hazelnut Allergic and Tolerant Patients

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RATIONALE: Several hazelnut (HZNT) allergens have been identified to date including Cor a8 (LTP), Cor a9 (11S globulin) and Cor a14 (2S albumin). The aim of the study was to determine the importance of these allergens in component resolved diagnosis in HZNT allergic patients.

METHODS: Forty-four children suspected to have fruit, nut and/or legume allergy were selected. Patients were classified as allergic if they had presented at least 2 reactions unequivocally related to HZNT ingestion in the last 2 years. Patients were defined as tolerant if they consumed HZNT on a regular basis. Clinical questionnaire, skin prick test (SPT), serum total and specific IgE and MIA-ISAC IgE (Thermo Fisher Scientific, Uppsala, Sweden) were performed.

RESULTS: Sixteen patients (11 males) were defined as allergic and 28 (15 males) tolerant. HZNT-SPT wheal size (mm) (median 7.75;IQR:4-12 vs.2.5; IQR:0-9.5,p=0.000) and HZNT-sIgE (kU/L) (median 14.45;IQR:1.98-370 vs 0.82;IQR:0.02-14.3, p=0.000) were significantly greater in allergic than in tolerant children. Both positive Cor a9-sIgE and Cor a14-sIgE were significantly more frequent in allergic patients (75.00% vs. 14.28%,p=0.000 and 75.00% vs 10.71%,p=0.000, respectively). Cor a9-sIgE values were significantly higher in allergic children whether by means of ImmunoCAP (median 4.38 kU/L; IQR:0.26-16 vs. 0.02 kU/L; IQR:0-0,21, p=0.000) or MIA-ISAC (median 0.14 ISU;IQR:0-2.2 vs. 0 ISU;IQR:0-0, p=0.000), as were Cor a14-sIgE values (median 4.97 kU/L;IQR:0.39-20.4 vs. 0.02 kU/L;IQR:0.01-0.09, p=0.000). This was not found for Cor a8. ROC curves were constructed for the three allergens showing Cor a14 the best diagnostic performance (AUC:0.925,95% CI:0.847-1,p=0.000).

CONCLUSIONS: Cor a14 is the best discriminating allergen in the diagnosis of HZNT allergic patients.

L34 Resolution of Severe Near Fatal Food Allergy Following Hematopoietic Stem Cell Transplantation

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RATIONALE: Previously in the literature, it has been described that patients who underwent hematopoietic stem cell transplantation (HSCT) have acquired asthma, allergic rhinitis and even food allergy from their donors. To date, no papers have reported complete resolution of severe food allergy subsequent to HSCT. Here we present a 6 year old female with HyperIgE syndrome, severe persistent asthma and multiple severe near-fatal food allergies who underwent HSCT due to acute mixed myeloid and T-cell leukemia (AML) with subsequent complete resolution of her food allergies.

METHODS: Skin prick testing (SPT) (Greer), Total IgE and ImmunoCAP testing by Seattle Children's laboratory,

RESULTS: Patient's total IgE prior to her diagnosis of AML was 22,500IU/mL. Subsequent to AML chemotherapy treatment, patient's IgE had reduced to 883. Food ImmunoCAP immediately prior to transplant revealed numerous positives including wheat(80), peanut(23.7) and milk(65.3). Patient underwent non-myeloablative chemotherapy with fludarabine, cyclophosphamide and total body irradiation with a mismatch cord transplant. At day +80, patient had 100% donor chimerisms but food ImmunoCAP testing while decreased remained positive. At one year post-transplant, ImmunoCAP testing revealed negatives(<0.35) to all tested foods, which was confirmed with SPT.

CONCLUSIONS: We believe that this is the first reported case to prospectively examine an individual with multiple severe food allergies through their post-HSCT course. The steady decrease in food specific IgE over the year post transplant likely indicates the gradual destruction of peripheral IgE memory B-cells through graft versus host disease. Subsequent oral food challenges confirmed clinical tolerance to each of the foods that had previously provoked anaphylaxis.

L35 Population-Based Study Suggests Strong Genetic Association Between Eosinophilic Esophagitis and Asthma

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RATIONALE: Significant similarities exist between the pathogenesis of eosinophilic esophagitis (EoE) and atopic diseases. Studies have shown an increase in allergic disorders in those with EoE and in their first-degree relatives (FDR) but not distant relatives. Excess familial clustering of a disease in distant relatives would suggest a genetic contribution.

METHODS: Utilizing the Utah Population Database (UPDB), we compared EoE patients (n=4009), their FDR, second-degree relatives (SDR), and third-degree relatives (TDR), and their spouses against matched controls (n>100,000) to evaluate possible links between EoE and atopic diseases. The UPDB links genealogy information for the state of Utah to inpatient and outpatient electronic health records. Atopic disease was identified using ICD-9 coding and defined as presence of anaphylaxis, atopic dermatitis (AD), asthma, allergic conjunctivitis (AC), and/or allergic rhinitis (AR). Cox logistic regression was used for analysis.

RESULTS: EoE probands, as well as their FDR, SDR and TDR had increased risk of asthma (OR 3.95 95% CI (3.62-4.31); p<2e-16, OR 1.49 95% CI (1.42-1.57); p<2e-16, OR 1.13 95% CI (1.09-1.18); p = 3.28e-09, OR 1.08 95% CI (1.04-1.12); p =0.00026, respectively). In addition, other select atopic diseases, specifically anaphylaxis, AC, AR and AD were also increased. Spouses and sibling-spouses of EoE probands did not show an association.

CONCLUSIONS: In this novel Utah population-based study, we observed evidence of significant familial clustering of asthma and atopic diseases in distant relatives of EoE probands, suggesting a strong genetic component. Studies of high-risk families with an excess of asthma and atopic diseases in EoE probands may facilitate identification of disease-causing genes.

L36 Molecular Sensitization Pattern Profile in Proton Pump Inhibitor-Responsive Esophageal Eosinophilia Vs Proton Pump Inhibitor-Nonresponsive Eosinophilic Esophagitis (EoE) in Adult Patients

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RATIONALE: Recent studies showed that proton pump inhibitor-responsive esophageal eosinophilia (PPI-REE) may be an EoE variant. Component resolved diagnosis (CRD) allows to distinguish primary sensitization from sensitization due to cross-reactivity. The aim of this study was to describe the allergen sensitization profile in PPI-REE and PPI non responsive EoE (PPI-nREE) by means of CRD.

METHODS: Eighty two patients with confirmed EoE and CRD diagnosis were included. Specific IgE antibodies against 103 and 112 different allergen components were measured by ImmunoCAP MIA-ISAC (Thermofischer Diagnostics, Uppsala, Sweden) in 36 and 46 patients, respectively.

RESULTS: Most patients were male (78%) and the mean age was 34.9 ± 12.3 years. Twenty-six patients had PPI-REE, 35 PPI-nREE and response to PPI was not confirmed in 23. No significant differences were found between groups regarding sex (p=0.142) and age (p=0.876). Peripheral eosinophilia was statistically significantly higher in PPI-nREE than in PPI-REE (median: 420 vs 235 cel/mm^3; p=0.025)(range: 40-970 vs 50-710 cel/mm^3), whereas there were no significant differences regarding serum total IgE, eosinophilic cationic protein or tryptase. Besides, no significant differences in allergen sensitization were found between PPI-REE and PPI-nREE, except regarding to *Can f 5* sensitization (p=0.018), which showed higher IgE levels in the PPI-REE group.

CONCLUSIONS: We found no statistically significant differences regarding to serum total IgE, tryptase, eosinophilic cationic protein and allergen sensitization between PPI-REE and PPI-nREE, except the higher levels of *Can f 5* in the PPI-REE group. Peripheral eosinophilia was higher in PPI-nREE.

L37 A Trial of an Oral CRTH2 Antagonist in Antihistamine-Refractory Chronic Spontaneous Urticaria

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RATIONALE: Chronic spontaneous urticaria (CSU) skin lesions show degranulated mast cells and infiltration by CRTh2-bearing leukocytes. Our prior work demonstrated altered blood basophil and eosinophil surface CRTh2 expression in CSU. We sought to evaluate the safety and efficacy of the oral CRTh2 antagonist AZD1981 in CSU.

METHODS: Antihistamine-refractory adult CSU subjects were recruited for a Phase II study involving 4 weeks of double-blind, placebo-controlled treatment with AZD1981. Subjects completed daily hive and itch scoring and disease activity surveys. We examined PGD_2-induced eosinophil shape change, blood total leukocyte histamine content, CBC differentials, and CRTh2 expression on blood basophils, eosinophils, and ILC2s at baseline and after treatment.

RESULTS: Thirty-six subjects were screened and 22 subjects completed the study. Weekly itch scores were significantly lower 1 week following active treatment with AZD1981 (9.5 to 7.2, n=12, p=.0264). PGD_2-induced eosinophil shape change (10-7 M PGD_2) was significantly reduced at the end of treatment (26.9 to 5.1 net MFI, n=12, p=.0005) but was similar in the placebo group (10.44 to 7.81 MFI, n=8, p=.8438). CBC eosinophil percent significantly increased with active therapy (3.17% to 4.43%, n=12, p=.0396). No SAE's were reported.

CONCLUSIONS: This is the first study of an oral CRTh2 antagonist patients with antihistamine-refractory CSU. AZD1981 treatment led to reductions in patient reported itch, reduced PGD_2-induced eosinophil shape change, increased basophil CRTh2 expression, and increased blood eosinophils in CSU subjects. These results provide evidence supporting the role for this pathway in CSU.

L38 BCX7353, a Potent Inhibitor of Plasma Kallikrein, Shows Sustained Maximal Enzyme Inhibition When Dosed Orally Once Daily: Results from a Phase I Trial in Healthy Subjects

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RATIONALE: Plasma kallikrein is a proven target in the treatment of hereditary angioedema (HAE). A first-in-human study evaluated the pharmacokinetics, pharmacodynamics and safety of plasma kallikrein inhibitor BCX7353.

METHODS: Healthy subjects (n= 94 enrolled, n = 92 completed) received single (10, 30, 100, 250, 500 or 1000mg) or multiple (125, 250, 500mg x7 days or 350mg x14 days), once-daily (QD) oral doses of BCX7353 or placebo. Drug levels were measured in serial post-dose samples and plasma kallikrein enzyme activity was measured in a specific bioassay. Safety was evaluated by clinical and laboratory monitoring.

RESULTS: BCX7353 exposure increased slightly greater than proportionally with increasing dose. The half-life of BCX7353 was 50-60 hours, and accumulation in AUC_{tau} was approximately 4-fold after dosing to Day 7 or 14. Kallikrein inhibition was highly correlated to plasma concentrations, r=0.916. On Day 7, at doses ≥250mg QD, plasma concentrations were within or above the target therapeutic range and inhibition of plasma kallikrein was maximal and sustained throughout the dosing interval. Two subjects discontinued the study for gastrointestinal adverse events (AEs). One subject had a diffuse maculopapular rash that resolved with oral steroids. There were no serious AEs, and the maximum tolerated dose was not reached.

CONCLUSIONS: Once daily BCX7353 has a generally well tolerated safety profile and provides sustained potent and maximal plasma kallikrein inhibition. Plasma concentrations met or exceeded the predicted therapeutic range over a 24 hour dosing interval. Clinical studies with HAE patients are planned to assess the efficacy of BCX7353 in reducing the occurrence of attacks.

L39 House Dust Mite Major Allergens Contributes Significantly to Specific IgG4 Response during Allergen Immunotherapy

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RATIONALE: Allergen-specific IgG4 increases during allergen specific immunotherapy (AIT). In this study, specific IgG4 against the individual major allergens of dust mites during AIT was investigated.

METHODS: Patients of allergic rhinitis (n=52) sensitized to dust mite were treated with subcutaneous immunotherapy using standardized *D. pteronyssinus* (Dp) extract. Patients with allergic rhinitis (n=14) sensitized to dust mites who received medications alone were controls. Specific IgE and IgG4 against Dp, *D.farina*(Df) and corresponding major allergens of group 1 (Dp1 and Df1) and group 2 (Dp2 and Df2) were measured before AIT, 6 months and 12 months later.

RESULTS: Combined symptom and medication scores significantly decreased in immunotherapy group. Specific IgG4 against Dp1, Df1, Dp2 and Df2 allergens increased significantly during AIT (Dp: 0.3, 0.99, 2.72; Dp1: 0.16, 0.67, 2.04; Dp2: 0.10, 0.49, 1.49; Df: 0.38, 1.04, 2.62; Df1: 0.12, 0.37, 1.01; Df2: 0.1, 0.44, 1.27. before AIT, 6 months and 12months later respectively, mgA/L). Of the correlations between dust mite extract IgG4 and the individual subgroup allergen IgG4, it was shown that there were strong correlations in terms of both concentrations (after 12 months: Dp-Dp1: r=0.99; Dp-Dp2: r=0.93; Df-Df1: r=0.93; Df-Df2: r=0.95) and levels of increase (after 12 months: Dp-Dp1: r=0.69; Dp-Dp2: r=0.59; Df-Df1: r=0.90; Df-Df2: r=0.77) (P<0.0001 for all). With the same testing instrument (UniCAP system), Df1 and Df2 IgG4 contributed 87% to Df specific IgG4 response, whereas Dp1 and Dp2 contributed 130% to Dp IgG4 response at the 12 months of AIT.

CONCLUSIONS: Our findings underscores the importance of major allergens in AIT standardization and design.

L40 Altered TGF-î² Signalling in Inflammatory Nasal Polyps Drive Remodelling in Crswnp

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RATIONALE: Dysregulation of TGF-b and activin signalling play fundamental roles in lower airways remodelling. This study focuses on characterising remodelling changes seen in CRSwNP and accompanying alteration in TGF-b signalling.

METHODS: Immunohistochemical staining was performed on inferior turbinate and nasal polyp biopsy specimens measuring TGF-beta$_1$, activin-A and its receptor ALK-4, and phosphorylated SMAD$_2$in subjects with CRSwNP (n=10) and healthy controls (n=19). Staining for D2-40 and CD34 were used to define lymphatic and vascular remodelling; smooth muscle actin and HSP-47 to study collagen synthesis and myofibroblast transformation. Matrix metalloprotease7/9 with their inhibitor TIMP-1 were enumerated. Basement membrane thickness was defined using Siris red stain.

RESULTS: Basement membrane zone was markedly thinned in both polyp and turbinates of CRSwNP ($p<0.01$ versus controls). Turbinates show increased lymphatic and vascular remodelling with polyps nearly devoid of glands and possessing very little blood vessels as demonstrated by differences in total CD31/ D2-40 cell counts ($p<0.01$, $p=0.01$), blood vessels ($p<0.01$, $p=0.02$), vessel size ($p<0.01$, $p=0.04$), or vascularity ($p=0.02$, $p=0.25$). HSP-47 expression is elevated in polyps ($p=0.09$) whilst SMA is increased in turbinates ($p=0.03$). MMP7/9: TIMP-1 ratios are elevated in turbinates. TGF-b expression is increased in polyps ($p<0.01$) with ALK-4 elevated in polyps and turbinates ($p=0.02$ and $p=0.03$). Activin levels tend to be higher in polyps CRSwNP ($p=0.08$).

CONCLUSIONS: This data demonstrates remodelling alterations in both polyps and turbinates of CRSwNP. It is possible that dysregulated TGF-b signalling in inflammatory polyps drives chronic changes in turbinate architecture thereby resulting in characteristic remodelling in this nasal disease.

L41 Real-Life Study on the Effect of Micronized Cellulose Powder As Add-on to Intranasal As-Needed Treatment of Subjects with Pollen Allergic Rhinitis

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RATIONALE: The use of symptom relievers on demand is the most common approach in real life for treating exacerbations of allergic rhinitis. We have demonstrated previously that commercially available micronized hydroxyl-propyl-methyl-cellulose powder (HPMC) applied after local decongestant significantly enhances its action in subjects with persistent allergic rhinitis. This study investigated whether this beneficial effect of HPMC translates into clinical benefits in a real life setting.

METHODS: Thirty-six symptomatic seasonal allergic rhinitis patients (25 male, median age 31 years) were instructed to treat their bothersome symptoms locally with intra-nasal xylometazoline and/or azelastine and/or mometasone, or, if symptoms persevered, with oral bilastine or prednisone. Patients were randomized to "seal" the effect of each local application with one puff of either HPMC or placebo (lactose powder). They completed diaries with symptom scores (0-3), and medications (1 score for any drug application). Objective measurements of Peak Nasal Inspiratory Flow (PNIF), measure of the level of nasal congestion, and Exhaled Breath Temperature (EBT), surrogate marker of airway inflammation, were made before and after treatment.

RESULTS: Combined Symptom and Medication Scores (CSMS) were significantly ($P=0.03$) lower in the HPMC group, 90 ± 9 vs. 122 ± 12, (mean $\pm$ SEM). Following treatment PNIF increased in the HPMC arm by 60% vs. 31% in the placebo one. The before vs. after treatment differences were in favor of the HPMC for both PNIF ($P=0.01$) and EBT ($P=0.007$).

CONCLUSIONS: In real life intra-nasal HPMC applied following local rescue medications decreased symptoms and reduced nasal congestion/ inflammation in subjects with symptomatic allergic rhinitis.

L42 Distinct and Common Gene Expression Profiles of Nasal Polyp Tissues in Eosinophilic and Non-Eosinophilic Chronic Rhinosinusitis

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RATIONALE: Chronic rhinosinusitis (CRS) can be classified into two groups: CRS with (CRSwNP) and without (CRSsNP) nasal polyps. CRSwNP is reportedly characterized by massive eosinophil infiltration and type 2 inflammation. However, some CRSwNP patients, especially Asians, show much less eosinophil infiltration. To clarify the molecular characteristics of these nasal polyps, we investigated the comprehensive gene expression profiles of CRSwNP in Japanese patients.

METHODS: Nasal polyp tissues from adult patients with CRS with eosinophilic polyps (ECRS; n=13, tissue eosinophil count >70 HPF) and CRS with non-eosinophilic polyps (NECRS; n=10, tissue eosinophil count <70 HPF) were diagnosed on the basis of the JESREC Study (Allergy. 2015 Aug; 70(8):995-1003.). Those and nasal mucosa biopsy specimens from age-matched control subjects (n=7) were analyzed by a microarray system to determine their comprehensive gene expression profiles.

RESULTS: Expression of type 2- and eosinophil-related genes (IL13, IL5, IL1RL1, CLC, CCL26 and CCL23) was increased in ECRS compared with the controls, and the results were comparable to those for CRSwNP in Western countries. In contrast, expression of type 1- and neutrophil-related genes (CSF3, CXCL10, IL8, IFNG and IL1B) was increased in NECRS. A primary component analysis revealed three distinct clusters, reflecting ECRS, NECRS and controls. However, expression of monocyte/macrophage- and lymphocyte-related genes (CCL18, MARCO, F13A1, CD209 and IL2RA) was increased in both ECRS and NECRS.

CONCLUSIONS: The characteristic gene expression profiles indicated the existence of at least two separate CRSwNP endotypes in Japanese patients. Their shared gene expression profiles may help understand the pathogenesis of nasal polyps.

L43 IgG4 Drives M2 Macrophages to Cortisol, Lcn-2 and IL-10 Release: Implications in Maintenance of Tolerance and Allergen Immunotherapy

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RATIONALE: M2 macrophages play a role in resolving inflammatory responses: macrophages are a prominent source of i) cortisol, and ii) of human lipocalin-2 (LCN-2) having a glucocorticoid-responsive element in its promoter. We addressed whether macrophages are a source of IL-10 and whether IgG antibodies have an impact in regulating them, for understanding allergen immunotherapy (AIT).

METHODS: Primary macrophages from healthy PBMCs or monocytic cell line THP-1 were differentiated into M2 macrophages by M-CSF and LPS, and for further sub-differentiation with IL-4/IL-13 (M2a), or with IgG immunoglobulins (M2b). The supernatants were analyzed in radioimmunoassay for cortisol, or by ELISA for LCN-2 and IL-10. Alternatively, Bos d 5 was co-incubated with these supernatants, either loaded or emptied from its ligand by dialysis against deferoxamine, as controlled by Prussian Blue staining.

RESULTS: Prussian Blue staining detected iron in M2b > and M2a, but not in M2c macrophages. Only IgG4, but not IgG1 immune complexes rendered M2b macrophages capable of secreting significant levels of cortisol, LCN-2 and IL-10. When ligand-emptied Bos d 5 was incubated to the M2b supernatants, it decreased the free levels of cortisol and LCN2.

CONCLUSIONS: Alternatively activated macrophages, are differentially regulated by IgG classes: Only IgG4 is leading to cortisol, LCN-2 and IL-10 secretion. Moreover, exogenous unloaded lipocalin allergens may lower the levels of bioavailable cortisol, and LCN-2 and IL-10 release. Our data unravel a novel mechanism of how IgG4, being a hallmark in AIT, is able to regulate M2 macrophages towards a tolerogenic phenotype.

L44 Facilitated Allergen Binding (FAB) Is a Meaningful Immunological Biomarker for Monitoring Immediate Clinical Efficacy in Short-Term Peptide Allergen Immunotherapy

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RATIONALE: Short-term peptide-allergen-immunotherapy is a novel approach for treating allergic rhinoconjunctivitis. We investigated whether changes in mucosal reactivity to allergen exposure after this short-term therapy are detectable in the immunological parameters of sIgG4 and the functional blocking antibody response measured by facilitated allergen binding (FAB).

METHODS: Data was collected from a DBPC dose-finding study in 198 patients who received placebo or a peptidase-hydrolysate of grass-pollen peptides at 5 visits over 4 weeks at cumulative doses of up to 370µg (EudraCT-No:2013-005445-37). Conjunctival allergen challenge was used as a surrogate marker of efficacy before and after immunotherapy. We have shown that this parameter has a predictive value for patients' symptoms and medication needs during the pollen season. Serum samples were taken to determine sIgG4 and FAB.

RESULTS: Patients exhibiting diminished reactivity and tolerating an at least 10-fold higher concentration of the conjunctival challenge solution showed a significantly greater increase in FAB (20.01% ± 16.706) than patients who did not improve ($p = 0.01$); The immediate change in sIgG4 observed in the improved patients was not significant ($p = 0.233$). Also, patients showing no reaction to the highest conjunctival allergen concentration had significantly higher FAB values ($p = 0.034$) than patients who still reacted to one of the allergen challenge dilutions; the immediate induction of sIgG4 found in the non-reacting patients was not significant ($p = 0.797$).

CONCLUSIONS: After 4 weeks of peptide allergen immunotherapy, the immediate appearance of FAB can be correlated with a meaningful clinical parameter of therapeutic efficacy.

L45 Safety of STG320 Sublingual Tablets of House Dust Mite Allergen Extracts in Subjects with HDM-Associated Allergic Rhinitis: Results of a Pooled Analysis

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RATIONALE: Safety of house dust mite (HDM) sublingual tablets (STG320) for the treatment of HDM-associated allergic rhinitis (AR) with or without intermittent asthma has been assessed in seven DBPC clinical trials. The pooled safety data are presented here.

METHODS: Subjects (5-64 years) with medically confirmed HDM-associated AR were randomized to receive placebo or STG320 at doses from 100IR to 1,500IR. Adverse events were monitored and analyzed descriptively.

RESULTS: 2,407 subjects (1,718 adults, 443 adolescents, 246 children) comprised the Safety Set including 627 (26%) with intermittent asthma at enrollment. 1,571 participants received at least one dose of active treatment and 836 received placebo. 64% of actively-treated subjects and 20% of placebo-recipients reported treatment-emergent adverse events (TEAEs) suspected to be drug-related. These were mostly consistent with mild or moderate application-site reactions [e.g., throat irritation (23%), oral pruritus (17%), mouth edema (14%), ear pruritus (12%)] and mainly reported over the initial 4 weeks. Percentages of subjects with drug-related TEAEs were similar in those with and without asthma in active (59% and 66%) and placebo (19% and 20%) groups. Four subjects reported serious drug-related TEAEs (3 active: eczema, pharyngeal edema and dyspnea, and one placebo: urticaria). 123 (8%) and 24 (3%) subjects in active and placebo groups, respectively, discontinued mainly as a result of application-site reactions (e.g., mouth or lip edema). There were no reports of anaphylaxis and no epinephrine use.

CONCLUSIONS: Pooled safety data from the rhinitis program demonstrate the favorable safety profile of HDM sublingual tablets in subjects receiving any dose of active treatment.

L46 Immunological Effects of Treatment with STG320 Sublingual Tablets of House Dust Mite Allergen Extracts in Subjects with HDM-Associated Allergic Asthma

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RATIONALE: A clinical development program investigating the efficacy and safety of house dust mite (HDM) sublingual tablets (STG320) in adults with HDM-associated allergic asthma is ongoing. Here we present immunological data from a phase II study evaluating the effect of three doses of STG320 compared to placebo.

METHODS: This DBPC, dose-ranging study enrolled subjects (18-50 years) whose asthma was partly controlled [Asthma Control Test™ (ACT) 16-19] while receiving asthma therapies consistent with GINA treatment Steps 2 to 4. Eligible subjects were to have a positive skin prick test to HDM and HDM-specific serum IgE ≥0.7 kU/L. Participants were randomized to receive 100IR, 500IR or 1000IR of STG320 or placebo, daily for about 13 months. The primary endpoint was the ACT score after the treatment period. HDM-specific IgE and IgG_4assessed before and after treatment and fold-changes were analyzed descriptively in each group. Safety data were analyzed descriptively.

RESULTS: Of 386 randomized subjects, 344 were included in the analysis (100IR: 88, 500IR: 87, 1000IR: 81, placebo: 88). The primary endpoint was not met. At baseline, HDM-specific IgE and IgG_4 were similar in the four treatment groups. After treatment, HDM-specific IgE increased by 1.5- to 2-fold in the active groups, and was unchanged in the placebo group. Changes in IgG_4 increased with the dose, from 3-fold (100IR) to 6-fold (1000IR), and were unchanged for placebo. No unexpected adverse events were observed.

CONCLUSIONS: In this study, the HDM sublingual tablet showed a dose-dependent effect based on its immunological activity.

L47 Clinical Development Strategy for Unmet Need in Grass Subcutaneous Immunotherapy

Prof. Tim Higenbottam; Allergy Therapeutics.

RATIONALE: Grass MATA MPL is an immunotherapy treatment currently in late stage development that addresses the unmet need in the USA of a standardised immunotherapy for seasonal grass allergic rhinitis. This immunotherapy is a modified extract of sweet grasses adsorbed to a depot adjuvant complex containing MCT (micro crystalline tyrosine) and MPL (Monophosphoryl lipid A®) that requires fewer injections than traditional SCIT. To address the challenges in optimal dose evaluation with attention to safety and efficacy, a clinical development model is presented.

METHODS: A complimentary battery of phase I to III studies has been conducted to evaluate the relationship of allergen, MCT and MPL combinations in field and EEC (Environmental Exposure Chamber) studies. The effectiveness of allergen immunotherapy is thought to correlate to cumulative dose and the benefit of MCT + MPL has been previously demonstrated with the latter improving efficacy by ~25%. Optimal allergen dose finding was assessed in a combination of field and EEC studies.

RESULTS: A comparative assessment of up dosing of grass MATA MPL in different clinical arms has been completed. The relationship of cumulative dose is presented with consideration of monotonous and non-monotonous dose response including the relevance of dose response plateau and translation to TSS reduction. Ultra-short course treatment (4-6 injections) is compared with prolonged treatment therapy showing increased patient adherence with shorter courses.

CONCLUSIONS: The product and clinical development strategy for Grass MATA MPL is presented describing a process to address many of the variables contributing to optimal product efficacy for treatment of grass allergic US patients.

L48 Eassi Survey: European Multicentre Prospective Study to Collect Systemic Adverse Reactions Due to Allergen Immunotherapy: Pediatric Population Results

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RATIONALE: Systemic adverse reactions (SAR) due to Allergen Immunotherapy (AIT) still represent one of its major drawbacks preventing a more extensive use of this etiologic treatment. The objective of this EAACI-supported survey was to collect SAR due to aeroallergen AIT in real life practice.

METHODS: Data was centrally collected with an online database, and gathered through three different questionnaires: DQ: doctor questionnaire (filled in only once by each participant doctor), PQ: patient questionnaire (one per patient-treatment) and RQ: reaction questionnaire (one per reaction). Harmonized MedDRA terminology for SAR due to AIT was used.

RESULTS: Three countries (France, Germany and Spain), 95 doctors and 1578 pediatric patient-treatment were recruited, mean age 11.7 years(+/-SD 3.9), 59.1% (932) males. Allergic asthma and rhinitis/rhinoconjunctivitis was the AIT indication in 56.1% (886) patients, allergic rhinitis/rhinoconjunctivitis without asthma in 38.3% (604), asthma alone in 5.2% (82) and conjunctivitis alone in 0.4% (6) patients. Monoallergen AIT composition was 49% mites, 25.8% grass, 8.7% tree, 4.6% *Alternaria*, 0.8% epithelia, 0.6% weeds and 10.5% were mixtures. Subcutaneous AIT (SCIT) was used in 71.4% (n=1127). A total estimation of 19.669 and 131.550 doses of SCIT and sublingual AIT (SLIT) were given, and 29 SAR (79.3% SCIT) were recorded in 24 patient-treatments, 3 were anaphylaxis and only 1 was severe. SAR were more frequent in up-dosing (79.3%) but milder (82.6% mild) than in maintenance (33.3% mild) (p= 0.023). The use of natural extracts compared to allergoids was associated with higher risk of suffering SAR (OR=8.4, 95%CI: 1.9-36.5).

CONCLUSIONS: AIT showed to be a safe treatment with a low rate of SAR and even lower ratio of severe SAR.

L49 Molecular Fingerprinting of Complex Allergoids

Dr Matthew Heath, Mr. Andrew Bell, Dr. Murray Skinner, Mr. Alan D. Bullimore; Allergy Therapeutics.

RATIONALE: Targeted reduction in IgE reactivity of native allergen extracts to produce allergoids via covalent cross-linking is beneficial in producing safe and efficacious immunotherapies. We present techniques to demonstrate the presence of the relevant allergens in allergoid preparations by tandem mass spectrometry and the molecular fingerprint of those allergoids by high performance-size exclusion chromatography (HPLC-SEC).

METHODS: The polymerization profile of sweet grass allergoids was determined by HPLC-SEC, from which separate size fractions of allergoids were collected. Proteomic analysis of each corresponding fraction was purified, subject to tryptic digest and analysed via tandem mass spectrometry. Once the peptide had been identified, it was compared to protein databases such as NCBI or SwissProt, from which the sequence identity was assessed.

RESULTS: HPLC-SEC highlighted the spread of allergens/allergoids pre- and post-modification. The HPLC profiles of the allergoids showed a decrease in retention time (increase in molecular weight) after modification (i.e. polymerization). A greater number of allergens are identified from tandem mass spectrometry (proteomic) analysis as the predicted molecular weight range of each fraction decreases.

CONCLUSIONS: Native and modified extracts are not two discrete preparations but are instead a formula of native and modified allergens, within which IgG reactive epitopes are present. Proteomic analysis confirmed the presence of allergens from multiple grass species. This work demonstrates that IgG epitopes remain in an allergoid formulation while IgE epitopes are attenuated, allowing safe administration of a higher strength product in fewer doses.

L50 Patterns of Interferon Regulatory Factor 1 (IRF1) Expression By Respiratory Epithelial Cells Reveal Non-Redundancy of Type I Versus Type III Interferons

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RATIONALE: Types I and III interferon (IFN) are co-expressed by respiratory epithelial cells (REC) in response to viral infection, and stimulate neighboring REC to express a set of interferon stimulated genes (ISG) through shared signaling pathways. Whether types I and III IFN have non-redundant functions in anti-viral defense is unknown. Because transcription factors dictate cellular phenotype and function, we hypothesized that ISG that are transcription factors (TF-ISG) mediate non-redundant functions of types I or III IFN.

METHODS: We treated BEAS-2B human REC with increasing doses of IFN-beta or IFN-lambda1 alone or together, and measured expression of TF-ISG and a set of "canonical" ISG by qRT-PCR and western blot.

RESULTS: Alone, IFN-beta and IFN-lambda each induced expression of the canonical ISG and a subset of TF-ISG. By contrast, while IFN-beta alone induced *IRF1* expression, it was poorly induced by IFN-lambda1 alone. Saturating doses of the two IFNs together did not enhance peak ISG transcript expression greater than either alone. Western blots revealed that while IFN-beta alone induced early and transient IRF1 expression, it was lower but sustained (through 24h) after IFN-lambda1 alone. In contrast to transcripts, saturating doses of the two IFNs together enhanced expression of IRF1 protein at 2h, 4h, and 24h greater than either of them alone.

CONCLUSIONS: In REC, IRF1 is expressed early and relatively selectively in response to IFN-beta alone, and protein expression was enhanced after treatment with both IFNs together. IRF1 may mediate non-redundant qualitative functional responses of REC to types I and III IFN.

L51 IgE Cross-Linking Directly Modulates Degranulation and Tslpr Induction upon Food Allergen Challenge

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RATIONALE: Recent data reveals that IgE-cross-linking upregulates thymic stromal lymphopoietin receptor (TSLPR) expression on isolated basophils in a small cohort of allergic asthma patients. Both IgE and non-IgE signaling pathways facilitate basophil activation, yet it is unclear whether food allergens leads to basophil activation and TSLPR expression solely through IgE:FceRI signaling complexes. We hypothesize IgE-mediated signal transduction pathways are necessary for degranulation, type 2 cytokine IL-4 secretion, and TSLPR induction.

METHODS: Heparin-treated whole blood from 12 double-blind placebo-controlled food challenged (DBPCFC) confirmed food allergic patients were treated with IgE-stripping designed ankyrin-repeat protein (DARPin) molecules, E2_79 (monovalent) and bi53_79 (bivalent), and assessed for basophils activation by degranulation markers CD63 and CD203c upon allergen challenge. The basophils were further assessed for TSLPR induction upon nut allergen challenge.

RESULTS: Treatment with DARPin molecules perturbs IgE binding to high affinity FceRI on primary basophils with minimal disruption of FceRI expression on the plasma membrane. Treatment with DARPins significantly reduced CD63 percentages by >70% and CD203c levels by 58% after IgE cross-linking and food allergen challenge. Furthermore, DARPins abrogate TSLPR induction in primary basophils.

CONCLUSIONS: We demonstrated that IgE cross-linking upon food allergen challenge is essential for degranulation as determined by CD63+ and CD203c kinetics. DARPin treatment further impairs TSLPR induction upon IgE cross-linking and allergen challenge. Our data delineates IgE-mediated functions in basophils and a novel pathway for TSLPR induction upon food allergen challenge.

L52 Novel Noninvasive Biomarker for Eosinophilic Esophagitis (EoE)

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RATIONALE: The field of EoE research has expanded greatly in the understanding of disease pathogenesis including esophageal fibrosis; however, there has been a significant delay in identifying reliable predictive EoE specific non-invasive biomarkers. Herein, we propose a panel of noninvasive biomarkers for disease progression and diagnosis.

METHODS: A flowcytometer analysis to detect $CD274^+$ and $CD274^-$ eosinophil subsets and qPCR analysis to detect mRNA levels of IL-18Rα, CD274 (PDL1), VIP, CD101 in normal and EoE patients blood and biopsies.

RESULTS: We recently discovered two eosinophil subtypes in the blood of normal and EoE patients that will be identified by $CD274^+$ and $CD274^-$. The $CD274^+$ eosinophil increases in EoE patients as disease progresses and most eosinophil accumulated in esophageal biopsies of EoE patients are $CD274^+$. In addition, we found that mRNA levels of IL-18Rα, CD274 (PDL1), VIP (eosinophil chemoattractant), CD101 (T regulatory cells suppressor) significantly increases in blood and esophageal biopsies of EoE patients compare to normal and GERD patients. Additionally, the mRNA levels of IL-18Rα, CD274, VIP, CD101 correlates well with blood eosinophilia that significantly reduces in improved EoE patients.

CONCLUSIONS: We first time show eosinophil two subset and only $CD274^+$ eosinophil increases in the blood of EoE patients. Furthermore, blood and tissue mRNA levels of IL-18Rα, CD274, VIP, CD101 increases and correlates with the eosinophils of blood and biopsies, respectively. Taken together, induced $CD274^+$ eosinophils and indicated panel of molecules will be the novel noninvasive biomarkers for EoE, which even differentiate EoE from GERD.

L53 IL-33 Induces Eicosanoid Formation in Mast Cells By a Novel COX-1-Dependent Mechanism

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RATIONALE: Mast cells (MCs) are involved in allergic and inflammatory reactions; they release potent mediators such as prostaglandin $(PG)D_2$, thromboxane $(TX)B_2$ and cysteinyl leukotrienes (cysLTs) after activation. Interleukin (IL)-33 is an effector molecule of Th2 responses, and an agonist for mast cell activation, though the roles of mast cells and their eicosanoids in IL-33-dependent immune responses are not known.

METHODS: Murine Bone Marrow-derived MCs (BMMCs) were stimulated with IL-33 and analyzed for eicosanoid production and level of cyclooxygenase (COX)1 and COX2 transcription over time. Wild type (Wt) mice were challenged intranasally with 4 doses of IL-33 (1 μg/day) and assessed for total (TCC) / differential cell count and lipid content in the bronchoalveolar lavage (BALs).

RESULTS: In BMMCs, IL-33 induces a robust release of PGD_2, TXB_2 and cysLTs. The response peaks within 3 h of stimulation and is accompanied by ERK phosphorylation and a sustained upregulation of COX2 transcript. Interestingly, both COX2 upregulation and eicosanoid production are completely suppressed by the selective COX1 inhibitor SC560. Intranasal IL-33 induces robust generation of PGD_2 and TXB_2, along with increases in eosinophils.

CONCLUSIONS: IL-33-dependent BMMC activation requires both COX1 and COX2. In this system, COX1 acts upstream of COX2 to mediate COX2 transcription, eicosanoids production and MAP Kinase activation. IL-33 is a robust inducer of mast cell-associated eicosanoids *in vivo*, which may participate in the recruitment of eosinophils.

L54 Development of a Germ-Free Murine Model for Prediction of Food Allergen Potency: Preliminary Studies Using Peanut Ara h1 and Ara h2 As Model Food Allergens

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RATIONALE: Novel food or protein sources are becoming increasingly common in our diets but have potential to sensitize consumers. A germ-free C3H/HeN mouse model for food allergy has shown promise for differentiating sensitization and elicitation profiles of known allergenic food proteins. The aim of this study was to determine if this mouse model can predict the potential potency of allergenic food proteins. Known peanut allergens, Ara h1 and h2, were used as model allergenic proteins with varying potency as reported by in vitro sera or basophil analysis from peanut-allergic individuals.

METHODS: Germ-free C3H/HeN mice were sensitized with 60μg Ara h1 (n=20) or h2 (n=18) by three weekly intraperitoneal injections (IP) with alum adjuvant, followed by IP challenge of 500μg of indicated protein. Thirty minutes post-challenge clinical scores were graded (0=no symptoms to 5=death) and body temperatures recorded. ELISA was used to measure presence of protein-specific IgE and mast cell protease in sera.

RESULTS: Germ-free mice sensitized with Ara h1 exhibited significantly less-severe clinical scores (mean=2) compared to mice sensitized with Ara h2 (mean=4) ($p<0.05$). Hypothermic responses post-challenge [average -2.5(SD=1.6) and -8.8(SD=0.9)°C, respectively ($p<0.05$)] correlated well with clinical scores.

CONCLUSIONS: Preliminary results based on clinical scores and hypothermia confirm that the germ-free C3H/HeN mouse model can differentiate between the potency of Ara h1 and h2 as reported in previous *in vitro* and *in vivo* analyses of human subjects. While further analysis of additional known allergens is needed, this model shows promise as a risk assessment tool for prediction of allergenicity of novel food proteins.

L55 Development of Multiple Features of Antigen-Induced Asthma Pathology in a New Strain of Mast Cell Deficient BALB/c-*Kit*$^{W\text{-}Sh/W\text{-}Sh}$ Mice

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RATIONALE: Genetically mast cell (MC)-deficient mice are used to identify and quantify the contributions of MCs to various biological responses *in vivo*, such as defense against venoms, parasite immunity and allergic inflammation. However, despite the fact that scores of genes have been identified as modifiers of allergic inflammation, most MC-deficient models have been available only on a single genetic background.

METHODS: We transferred the *Kit*$^{W\text{-}sh}$ allele onto the BALB/c background to generate BALB/c MC-deficient mice (BALB/c-*Kit*$^{W\text{-}sh/W\text{-}sh}$). We examined in BALB/c-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice models of allergic inflammation to which MCs substantially contribute in C57BL6-*Kit*$^{W\text{-}sh/W\text{-}sh}$mice.

RESULTS: BALB/c-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice have dramatically reduced numbers of MCs (0-2% of wild type) in all tissues examined. In addition, BALB/c-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice exhibited subtle hematologic differences compared to wild type mice, including splenomegaly with evidence of increased splenic hematopoiesis. In a model of acute allergic inflammation, IgE-dependent passive cutaneous anaphylaxis, both ear swelling and leukocyte infiltration were largely or entirely MC-dependent in BALB/c-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice. In contrast, in two different models of chronic allergic airway inflammation to ovalbumin or house dust mite, airway hyperresponsiveness, lung inflammation, and airway remodeling developed robustly in MC-deficient BALB/c-*Kit*$^{W\text{-}sh/W\text{-}sh}$mice.

CONCLUSIONS: These results support the conclusion that the importance of MC contributions in various models of allergic inflammation may be at least partially determined by genetic background.

L56 In Vitro Induction of Peanut-Specific Tr1 Cells

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RATIONALE: IL-10 producing type 1 regulatory T cells (Tr1) express the surface markers LAG3 and CD49b, can be induced *in vitro* and used as cell therapy to control undesired immune responses. Peanut allergy is a life-threatening condition with no curative treatment. Our aim is to induce peanut-specific Tr1s *in vitro*.

METHODS: Healthy controls (HC) and allergic patients undergoing peanut oral immunotherapy were included in this study. Mature (mDC) or tolerogenic (DC10) dendritic cells were differentiated as previously described (Pacciani et al., 2010) in the presence of the main peanut allergens Arah1 and Arah2. Autologous $CD4^+$T cells were co-incubated for 14 days with DC10 ('T10') or with mDC ('Tm') in the presence of absence of IL-10, respectively. We assessed by flow cytometry the expression of the Tr1 markers LAG3 and CD49b, of the gut-homing receptor GPR15, and the anergy of the T10 compared to the Tm upon restimulation with Arah1/2.

RESULTS: The percentages of $LAG3^+CD49b^+$ Tr1 cells were comparable in T10 cultures from patients (10.5%) and HC (9.4%). In both T10 cultures, the percentage of Tr1 was higher than in the control Tm culture. The $GPR15^+$ cells were enriched in the $CD45RA^-LAG3^+CD49b^+$ population compared to the $CD45RA^-$ population (19.3 vs 9.2% $p=0.03$). T10 from HC were anergic compared to Tm.

CONCLUSIONS: We successfully induced antigen specific $LAG3^+CD49b^+$ Tr1 cells from peanut-allergic patients and HC; those from HC were anergic. $GPR15^+$ cells were enriched in this population, suggesting their gut-homing capacity. Further studies are ongoing to assess the functional properties of Tr1 cells established from peanut allergic patients.

L57 Binding of the Active Vitamin A Metabolite Retinoic Acid to the Major Cows Milk Allergen Bos d 5 Down-Regulates T-Cell Responses

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RATIONALE: Recent research of our group has shown that the major cows milk allergen Bos d 5, a member of the lipocalin family, acts immunomodulatory depending on its load with siderophore-iron complexes. The aim of this study was to investigate whether Bos d 5 could influence Th1/Th2 immune reponses when complexed with the active Vitamin A metabolite retinoic acid (RA).

METHODS: Binding of RA to Bos d 5 was determined by autofluorescence quenching and ANS displacement assay. Activated PBMCs from 12 healthy donors were incubated with the milk allergen being "emptied" (apo-Bos d 5) or being loaded with RA (holo-Bos d 5). T-cell subsets (CD3+, CD4+, CD8+) were analyzed by FACS, cytokines (IFN-γ, IL-10, IL-13) measured by ELISA.

RESULTS: We calculated a dissociation constant of 1.7 μM and *in vitro* RA was able to dose-dependently displace ANS from Bos d 5. Incubation of PBMCs with apo-Bos d 5 for 48 hours significantly induced high IFN-γ, IL-13 and IL-10 levels whereas T-cell subsets remained unaltered. In contrast, stimulations with holo-Bos d 5 led to a significant decrease in CD4+ positive cells and to a pronounced decrease in all three cytokines. This phenomenon was dependent on the allergen-RA complex, as treatment with RA alone did not influence T-cell subsets or cytokine levels.

CONCLUSIONS: Our data suggest that holo-Bos d 5, with RA in its molecular pocket, has a pronounced immunosuppressive effect. We thus propose that proper loading of this major cows milk allergen may prevent subsequent allergic immune responses to it.

L58 The Skin Microbiome Differs with Age in Atopic Dermatitis

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RATIONALE: Pediatric and adult atopic dermatitis (AD) have different disease manifestations. The skin microbiome is thought to be critical in driving disease development. Whether the skin microbiome in young AD children is different from adults is unknown.

METHODS: We collected swabs from lesional and non-lesional skin of the volar forearm of 128 AD patients and 68 healthy subjects. We compared the skin microbiome of AD patients with healthy individuals in different age groups (2-12 and 13-62) using 16S rRNA gene sequencing. We analyzed correlations between the microbiome and age and investigated gene functions encoded in microbial genomes.

RESULTS: We found that the healthy skin microbiome was significantly different between young children and adults in microbial diversity and in relative abundance of prevalent bacterial genera. Compared to the diverse microbial community on healthy skin, AD skin microbiome was dominated by *Staphylococcus* species at all ages. Importantly however, shifts in the AD microbiome compared to the healthy microbiome were different between young children and adults. We identified distinct clusters of childhood-associated (represented by *Streptococcus*), adult-associated (*Propionibacterium* and *Corynebacterium*), and AD-associated skin bacteria. By analyzing 46 genomes representing major species in the clusters, we further identified specific functional profiles among these clusters.

CONCLUSIONS: Childhood-associated skin bacteria *Streptococcus* are replaced by adult-associated lipophilic commensals that associate with sebum production at puberty. Pathways unique to *Propionibacterium* and *Corynebacterium*, including porphyrin and chlorophyll metabolism, may provide additional protection for skin health in adults. Our findings suggest that pediatric and adult AD are driven by different microbial influences.

L59 Enhanced Efficacy and Confirmed Safety of a Two-Year Epicutaneous Immunotherapy (EPIT) Treatment of Peanut Allergy with Viaskin® Peanut: The Continuation of the Vipes Phase IIb Randomized Controlled Trial (RCT)

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RATIONALE: The 12-month VIPES RCT of EPIT using Viaskin® Peanut (VP) was continued as an open-label trial for an additional 24 months. We report results of the 12-month interim analysis.

METHODS: From 207 subjects completing the VIPES RCT (6–55 years), 171 (82.6%) entered the open-label extension. For this second year, 64.9% subjects initially treated with 50μg, 100μg, 250μg peanut protein (pp) i.e. VP50, VP100, VP250, or placebo were treated for 12 months with VP250. The remainder received VP50 or VP100 for 6 months before switching to VP250. Endpoint response was based on the proportion of successes, i.e. eliciting dose ≥10-fold above baseline or ≥1,000mg pp, at the 24-month DBPCFC.

RESULTS: The response rates after 24 months EPIT with VP250 were 69.7% (23/33) overall and 80.0% (16/20) in children 6-11 years, compared to 50% overall and 53.6% in children after 12 months VP250 EPIT. Adolescents/adults remained stable. In children, the peanut cumulative reactive dose after 24-months increased significantly compared to VIPES entry [mean(±SD)]: +1817.0(1853.9) mg pp; +983.3(1279.9) mg pp after 12-months. Children's median peanut-IgE decrease from baseline was -9% and -38% after 18 and 24 months; median peanut-IgG4 increase was +793.5% at 24 months. Mean(±SD) compliance was 94.8(±11.0)%; there were no serious AEs related to VP. Interestingly, the 12-month VP250 treatment of the ex-placebo group exactly reproduced the significant response rate in VIPES study with 50.0% (23/46) overall, 53.6% (15/28) in children.

CONCLUSIONS: The 24-month EPIT with VP250 is well accepted, safe and clearly enhances the 12-month therapeutic benefit overall and in children.

L60 The Efficacy of AR101, a Peanut-Derived Pharmaceutical for Oral Immunotherapy (OIT), Is Maintained and Tolerability Is Increased with Low-Dose Maintenance Therapy

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RATIONALE: AR101, a pharmaceutical for OIT, demonstrated robust efficacy in ARC001, a Phase 2, double-blind, placebo-controlled trial in 4-21 year olds. We now report results from the open-label continuation trial, ARC002.

METHODS: In ARC002, former ARC001 placebo subjects up-dosed to 300 mg/d of peanut protein as AR101, then underwent double-blind placebo-controlled food challenge (DBPCFC) after 2 more weeks of therapy. Those passing DBPCFC at 443 mg cumulative of peanut protein, were eligible to continue maintenance therapy for 12 additional weeks. Former AR101 subjects who up-dosed successfully in ARC001 entered ARC002's 12-week maintenance period directly. As all former ARC001 subjects underwent 12 weeks of open-label maintenance therapy with 300 mg/d AR101 in ARC002, the post-maintenance DBPCFC results from both groups were pooled.

RESULTS: All 26 ARC001 placebo subjects entered ARC002 and up-dosed over an average of approximately 22 weeks. Of these, 21 reached 300 mg/d AR101 (4 discontinuing from gastrointestinal AEs; 1 for scheduling issues), and 20 passed DBPCFC at 443 mg. Of 29 ARC001 subjects treated with AR101, 23 completed the study and 21 entered ARC002. Of the 40 subjects undergoing post-maintenance DBPCFC, 100%, 90%, and 60% tolerated a cumulative 443, 1043, 2043 mg of peanut protein, respectively. Only 2 subjects required single doses of epinephrine during the DBPCFC. AR101 showed improved tolerability during maintenance versus up-dosing, with reduced AE rates and no treatment-related discontinuations.

CONCLUSIONS: In ARC002, twelve weeks of AR101 maintenance at 300 mg/d resulted in 90% desensitization to ≥1043 mg of peanut protein, equivalent to ~4 peanuts, with improved tolerability.

L61 Efficacy and Safety of the SQ-House Dust Mite Sublingual Immunotherapy Tablet in North American Children and Adults: Findings from a Large Randomized, Placebo-Controlled Clinical Trial

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RATIONALE: SQ®–house dust mite (HDM) sublingual immunotherapy tablet (SLIT-tablet; MK-8237; Merck/ALK) has been demonstrated to have beneficial effects on allergic rhinitis and asthma outcomes, but previous trials were conducted in European subjects. This is the largest trial to assess the efficacy/safety of HDM SLIT-tablets in North American subjects with HDM allergic rhinitis with/without conjunctivitis (AR/C).

METHODS: In this double-blinded, multicenter trial (NCT01700192), 1,482 subjects (aged ≥12 years) with HDM AR/C with or without asthma were randomized to daily 12 SQ-HDM SLIT-tablet or placebo for up to 52 weeks. Subjects had a rhinitis daily-symptom score (DSS, 4 nasal symptoms, maximum=12) of ≥6, or ≥5 with 1 severe symptom, on 5 of 7 consecutive days before randomization. The primary endpoint was average total combined rhinitis score (TCRS), defined as rhinitis DSS plus rhinitis daily-medication score (DMS), during the last 8 weeks of treatment.

RESULTS: Treatment with 12 SQ-HDM SLIT-tablet improved TCRS 17% vs placebo (95% CI: −25%, −10%). Improvements vs placebo in the secondary endpoints average rhinitis DSS, rhinitis DMS, total combined rhinoconjunctivitis score, and ARC symptoms assessed by visual analogue scale were 16%, 18%, 17%, and 16%, respectively. All nominal P-values were <0.001 vs placebo except rhinitis DMS. No treatment-related AEs meeting the ICH definition of serious were reported; 1 treatment-related systemic allergic reaction occurred (assessed as moderate) at first administration under medical supervision and was treated with epinephrine.

CONCLUSIONS: 12 SQ-HDM SLIT-tablet was well-tolerated and improved HDM ARC symptoms in adults and children. This was the first successful North American trial of a HDM SLIT-tablet.

L62 Pathogenic Autoantibodies in Patients with Severe Asthma and Sputum Eosinophils

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RATIONALE: An asthmatic airway with frequent degranulation accumulates immunogenic entities like peroxidases and autologous cellular materials, which can lead to breach of immune tolerance and generation of autoantibodies.

METHODS: Immunoprecipitated sputum immunoglobulins (IP-Igs) from moderate and severe asthmatics with eosinophilic, neutrophilic, and pleiotropic bronchitis were analysed for antibodies against eosinophil peroxidase (EPX) and anti-nuclear antibodies (ANAs). Eosinophils were labeled with IP-Igs and monoclonal anti-EPX antibodies, and examined by confocal and deconvolution microscopy. IP-Igs were assessed for inducing degranulation *ex vivo*. IL-5/eotaxin-2 (IL-5/hE2) double transgenic mice, IL-5 transgenic and wild type mice (n=3) were analysed for markers of eosinophil degranulation and autoantibodies.

RESULTS: Severe asthmatics with eosinophilic ($n=20$) and pleiotropic bronchitis ($n=18$) had detectable anti-EPX IgGs and ANAs in sputum samples, compared to neutrophilic ($n=13$), moderate-eosinophilic asthmatics ($n=13$) and healthy volunteers ($n=15$) ($p<0.001$). Significant binding of sputum IgGs to fixed and permeabilized eosinophils, along with co-localization with EPX immunostaining, confirmed the occurrence of autoantibodies to autologous eosinophilic cellular components. IP-Igs pooled from severe asthmatics ($n=5$) compared to healthy volunteers ($n=5$) induced eosinophil degranulation *ex vivo* (measured by lactose dehydrogenase and EPX release). Both anti-EPX IgGs and ANAs charted significant correlations with daily prednisone dose, free eosinophil granules and EPX content ($r>0.3$, $p<0.001$). Finally, IL-5/hE2 mice characterized by extensive eosinophil degranulation showed detectable airway anti-EPX IgGs (29±8 ng/μg protein) compared to IL5-transgenic (1.92±0.08), and wild-type (0.53± 0.09) mice.

CONCLUSIONS: We hereby report a sub-set of severe asthmatics with increased airway eosinophil 'activity' presenting with pathogenic autoantibodies against autologous eosinophilic cellular components.

L63 The Leukotriene E4 Receptor, GPR99 Mediates Mast Cell-Dependent Mucosal Responses to the Mold Allergen, Alternaria alternata

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RATIONALE: The mold aeroallergen *Alternaria alternata* triggers mast cell (MC) degranulation and the generation of cysteinyl leukotrienes (cysLTs). CysLTs act at three receptors, $CysLT_1R$, $CysLT_2R$, and GPR99, the recently identified receptor for the stable cysLT metabolite, LTE_4. GPR99 distribution and function in the respiratory mucosa is unknown.

METHODS: Wild-type (WT), MC-deficient (Mcpt5/DTA), FcRγ-chain-deficient (*Fcer1γ*$^{-/-}$), LTC_4-synthase-deficient (*Ltc4s*$^{-/-}$), *Cysltr1*$^{-/-}$, *Cysltr2*$^{-/-}$, and *Gpr99*$^{-/-}$ mice received a single intranasal (i.n.) dose of 0 or 30 μg *A.alternata*, and nasal goblet cell (GC) mucin content was assessed by Periodic acid-Schiff (PAS^+) staining after 1 hour. GPR99 expression in the nasal mucosa was assessed by RT-PCR in WT mice and by X-gal staining of tissue sections in *Gpr99*$^{-/-}$ mice.

RESULTS: *A.alternata* elicited GC mucin release in WT mice, as detected by a reduction in PAS^+ GCs. There was no detectable mucin release in *A.alternata*-treated Mcpt5/DTA, *Ltc4s*$^{-/-}$, *Gpr99*$^{-/-}$ mice and a reduction in *Cysltr1*$^{-/-}$ mice. By contrast, mucin release was intact in *Fcer1γ*$^{-/-}$ and *Cysltr2*$^{-/-}$ mice. GPR99 transcript was detected in the nasal mucosa of WT mice and transcript for *E.coli* β-galactosidase, inserted in the targeted deletion of *Gpr99*, was detected in *Gpr99*$^{-/-}$ mice. X-gal staining confirmed GPR99 expression in nasal epithelial cells. Finally, i.n. LTE_4 elicited GC mucin release in WT mice that was absent in *Gpr99*$^{-/-}$ mice.

CONCLUSIONS: These results demonstrate that GPR99 is expressed on murine respiratory epithelial cells and controls their secretory function. Moreover our results suggest that the innate immune response of respiratory epithelial cells to *A.alternata* is controlled, in part, through a MC-cysLT-GPR99 axis.

L64 Human Airway Epithelial Cells Express Functional IL-5 Receptors

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RATIONALE: Interleukin-5 (IL-5) is linked to asthma pathogenesis and exacerbations, presumably by promoting eosinophil production and function. We detected by microarray the IL-5 receptor alpha subunit (*IL5RA*) mRNA in differentiated airway epithelial cells and hypothesized that this receptor is functional in these cells.

METHODS: Airway epithelial cells obtained from 4 donor lungs were differentiated at air-liquid interface (ALI) and then incubated with recombinant IL-5 for 15 minutes, 1 hour, 6 hours, and 24 hours. Expression of the IL-5R α- and β-subunits was tested using qPCR and Western blot. Following incubation with IL-5 (10 ng/mL), cell lysates were analyzed for phosphorylation of downstream signaling molecules by Western blot.

RESULTS: Expression of the α-subunit of IL-5R was increased 18-fold in differentiated airway epithelial cells compared to undifferentiated monolayers. mRNA expression of the β-subunit was low in unstimulated ALI cells, but increased following incubation for 6 hours with IL-5. Protein expression of the α-subunit was confirmed in both treated and untreated differentiated airway epithelial cells. β-subunit protein expression was low but rapidly inducible by IL-5, suggesting re-localization within the cells. IL-5 stimulation (15-60 min) of ALI cells significantly increased phospho-ERK (mean fold increase=2.7, p=0.003, n=4) and phospho-AKT (mean fold increase=5.2, p=0.029, n=4), but not phospho-STAT5A.

CONCLUSIONS: Differentiated human airway epithelial cells express functional IL-5 receptors. The signaling molecules affected suggest that IL-5 may promote epithelial cell growth and proliferation. Collectively, these findings suggest that IL-5 affects airway physiology in asthma in part through effects on airway epithelial cells.

L65 Impairment of Autophagy in Pulmonary CD11c+ Cells Induces Corticosteroid-Unresponsive Airway Hyperreactivity

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RATIONALE: A significant proportion of asthmatic patients do not respond to steroid therapy and suffer from neutrophilic asthma with incompletely understood pathogenesis. Autophagy is an important intracellular organelle recycling pathway that has been implicated in asthma. We evaluated the role of autophagy in the pathogenesis of steroid-resistant neutrophilic asthma.

METHODS: We assessed the airway hyperreactivity (AHR) and inflammation, T cell response and DC profile in several autophagy impaired mouse models. We also generated a novel mouse model in which Atg5, a key gene in autophagy pathway, is specifically knocked out in $CD11c^+$ cells.

RESULTS: Our results show that induction of severe asthma impairs autophagy pathway in lung $CD11c^+$ cell. We found for the first time that house dust mite (HDM)-mediated induction of AHR and lung inflammation in $Atg5^{-/-}$ mice leads to neutrophilic steroid resistance asthma while in WT mice causes eosinophilic steroid-responsive asthma. Adoptive transfer of bone-marrow derived $CD11c^+$ cells from $ATG5^{-/-}$ but not WT mice is sufficient to mediate Th17-dependent neutrophilic asthma in WT recipients. Most importantly, we found that CD11c-specific $Atg5^{-/-}$ mice develop spontaneous AHR and neutrophilic lung inflammation. Lack of autophagy in $CD11c^+$ cells induces significantly higher level of key cytokines such as IL-1a, IL-1b and IL-23.

CONCLUSIONS: Our results provide novel insights into an important and previously unrecognized role of autophagy in asthma and suggest that inducing autophagy may affect pulmonary $CD11c^+$ cells function and therefore, may be considered as an attractive clinical target for future strategies of treatment and prevention of asthma.

L66 Ara h 1 Peptide Immunotherapy Protects Against Peanut-Induced Anaphylaxis in a Dose-Dependent Manner

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RATIONALE: Peptide immunotherapy, a disease-modifying treatment that uses short peptides representing major allergen T cell epitopes, has been shown to reduce symptoms of allergic rhinoconjunctivitis. This study evaluated the ability of peptide immunotherapy to protect against anaphylaxis in a murine model of peanut allergy.

METHODS: We identified a novel peptide from the major peanut allergen Ara h 1 that is recognized by C57Bl/6 mice. Mice were sensitized to peanut epicutaneously and treated 1 week later with 2 intraperitoneal injections of peptide, 1 week apart. We included 6 doses, ranging from 0.01 ug to 300 ug of peptide. Mice were subsequently challenged with whole peanut extract and evaluated for signs of anaphylaxis. They were monitored over a period of 40 minutes for clinical signs of allergic reaction, changes in rectal temperature, and vascular leakage.

RESULTS: Peptide immunotherapy provided significant protection against anaphylaxis in a dose-dependent manner. Mice that received 100 ug of Ara h 1 peptide exhibited the highest level of protection. Control mice treated with saline experienced a mean maximum temperature drop of 7.4°C, while mice receiving 100 ug of peptide experienced a drop of 2.0°C (p=0.01 vs control). Maximum mean clinical score was 4.0 in control mice, and 1.8 in treated mice (p=0.002). Mean hematocrit for control mice was 56.4%, and 48.9% for treated mice (p=0.16).

CONCLUSIONS: One T cell epitope-containing peptide from a single major peanut allergen can protect against anaphylaxis elicited by whole peanut extract challenge. Studies of peptide immunotherapy in clinical peanut allergy are warranted.

L67 Identification of Tr1 Cells and Other CD4+ T Cell Subsets in Humans Using Mass Cytometry: A Tool for Understanding Asthma

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RATIONALE: T cell subsets contribute to immune functioning and are critical for controlling allergic disease. We studied asthmatic and non-asthmatic children to investigate the contributions of various T cell subsets.

METHODS: Peripheral blood mononuclear cells (PBMCs) from healthy (n=10) and current asthmatic children (n=10) (based on Global Initiative for Asthma guidelines) were stained with 30 metal-conjugated antibodies for surface and intracellular targets. Plasma total IgE levels were measured. Pyrosequencing of the FoxP3 gene at 10 different CpG sites was also performed.

RESULTS: T cell subsets (Tr1, Treg, Th1, Th2, Th17, TCRgd) were identified using both Flow-Jo and 2 dimensional display using viSNE. Methylation at 4 CpG sites in the promoter region was negatively correlated with the percentage of Tr1 cells (CpG -146, p<.01; CpG-133, p<.01; CpG -127, p<.03; CpG -83, p<.02). IgE level negatively correlated with percentage of Treg cells (p<.05). In addition, there was a trend for asthmatics to have fewer Tr1 cells than healthy controls (p<.08).

CONCLUSIONS: This study is the first to our knowledge to identify of all of these T cell subsets using mass cytometry. Analysis at a single-cell levelmay be superior to flow cytometry, and elucidate more subtle findings, as reported here even with a limited sample size. These preliminary data indicate that asthmatics may have a reduced amount of Tr1 cells in comparison to non-asthmatics. Tr1 cells also correlate with FoxP3 methylation levels in the promoter region. Finally, IgE levels are inversely related to the number of Treg cells.

L9.1 Increased Nasal Plasmacytoid Dendritic Cells Are Associated with Recurrent Wheezing Following Severe RSV Bronchiolitis

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RATIONALE: Dendritic cells (DCs) play an important role in anti-viral responses and regulating differentiation of T cells. We hypothesized immune dysregulation of DC may contribute to recurrent wheezing in susceptible children following severe RSV bronchiolitis.

METHODS: From 2009 – 2013, we prospectively enrolled infants < 1 year of age hospitalized with their first episode of RSV-confirmed bronchiolitis into the RSV Bronchiolitis in Early Life 2 (RBEL-2) cohort. Nasal wash mononuclear cells were labeled for plasmacytoid DCs (pDCs): Lin^- (CD3, CD19, CD14, CD20, CD16, CD56), $CD123^+$, $HLA\text{-}DR^+$ and myeloid DCs (mDCs): Lin^- $CD11c^+$ $HLA\text{-}DR^+$ cells. Blood DC were isolated using Blood Dendritic Cell Enumeration Kit and labeled for pDCs, mDC1, and mDC2 by labeling with anti-BDCA-2, anti-BDCA1, and anti-BDCA-3 antibodies respectively.

RESULTS: 181 participant's (58% male, 47% Caucasian) mean age of entry was 4.3±3.0 months. After 38±19 months follow-up, 75 (41.4%) had recurrent wheezing (3 or more wheezing episodes) and 50 (27.6%) had no wheezing. The recurrent wheezing group had higher proportion of upper airway pDCs, compared to no wheezing (1.72 ± 2.22 vs. 0.78 ± 1.65 %, $p = 0.04$, adjusted $p = 0.016$) and were more likely to be African-American OR 6.1 (95%CI 1.7-26.0) in a multivariate model. There were no differences in nasal mDC and blood DCs between these groups.

CONCLUSIONS: Children with recurrent wheezing following severe RSV bronchiolitis had higher proportion of nasal pDCs which may reflect a heightened antiviral response in the airway leading to the subsequent development of asthma.

Disclosures for 2016 AAAAI Annual Meeting Late-Breaking Abstracts

Allekotte, Silke: Nothing to disclose, L44
Alvez, Ana: Nothing to disclose, L33
Arnold, Leggy: Nothing to disclose, L13
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