ABSTRACT

OAS 01
Asthma Mechanisms

0180 | NEK7-NLRP3 binding is essential in the assembly of NLRP3 inflammasome in the pathogenesis of house dust mite-induced asthma

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Background: NLRP3 inflammasome, consisting of NLRP3, the adaptor protein ASC, and the protease caspase-1, is responsible for the production of active forms of IL-1β and IL-18. In this process, oligomerization of ASC is a key step in assembly/activation of inflammasome and, recently, NEK7, a serine and threonine kinase, has been also reported as an essential activator of the NLRP3 inflammasome. MCC 950 is a small-molecule inhibitor of NLRP3 inflammasome; however, molecular action mechanism of MCC 950 is not fully understood.

Method: We investigated the therapeutic effects of the MCC 950 on allergic airway inflammation and its action mechanism using house dust mite (HDM)-inhaled mice, particularly focusing on the NLRP3 inflammasome assembly process involving ASC and NEK7.

Results: Respiratory HDM exposure into mice led to the significant increases of pulmonary NLRP3, caspase-1, and IL-1β. Furthermore, levels of ASC oligomers were elevated in lung tissues of HDM-exposed mice and we observed the cytoplasmic co-localization of immunofluorescence intensities of NLRP3 and NEK7 in bronchoalveolar lavage (BAL) cells. Notably, treatment with MCC 950 significantly reduced the HDM-induced increases of ASC oligomerization and NLRP3-NEK7 colocalization in the lung of mice, and that ameliorated the HDM-induced increases of airway inflammatory cells infiltration, airway hyper-reactivity, and pulmonary T_H2 cytokines.

Conclusion: These results suggest that MCC 950 may have potential for treating HDM-induced allergic asthma partly through the regulation of HDM-induced ASC oligomerization and NEK7-NLRP3 binding in NLRP3 inflammasome assembly.

0224 | Potential role of mir-185-5P as feedback mechanism for controlling airway remodeling and smooth muscle contraction

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Background: Asthma is a chronic airway disease where breath difficulties, cough and airway obstruction occur due to an abnormal immune response, airway remodeling and smooth muscle contraction. In asthma a mélange of immune modulators including cytokines, mediators, exosomes and microRNAs shape the heterogeneity of the disease. We previously described that microRNAs are differentially expressed in eosinophils and serum from asthmatics compared to healthy individuals. The objective is to evaluate the function of one of these deregulated miRNAs: miR-185-5p, as a regulator of airway remodeling and muscle contraction.

Method: To study miR-185-5p role, we enhanced miR-185-5p expression using miR-185-5p mimic and also diminished miR-185-5p expression with miR-185-5p inhibitor in Bronchial Smooth Muscle Cells (BSMCs) and Small Airway Epithelial Cells (SAEC); next we studied gene expression and cellular functions. Gene expression and protein levels for periostin (*POSTN*), *CDC42*, and *RHOA* were analyzed by quantitative PCR and ELISA/Western Blot respectively. BSMC contractility was analyzed using cell-embedded collagen gels and measurement of intracellular calcium mobilization was performed using Fura-2 probe. Additionally, miR-185-5p and *POSTN* gene expression were evaluated in sputum cells from healthy (n = 3) and asthmatics (n = 5).

Results: MiR-185-5p overexpression downregulates periostin mRNA and protein levels in BSMCs and SAECs at 48 hours (*P* < .05). MiR-185-5p mimic decreases CDC42 and RHOA protein levels at 48 hours, consequently, miR-185-5p inhibitor increases CDC42 and RHOA quantity at 72 hours, evidencing that these contractile-related proteins are miR-185-5p targets. MiR-185-5p inhibition produced higher BSMCs contraction induced by histamine (79% size gel size *vs* 100% of scrambled control, *P* < .05). Calcium mobilization was not modified by miR-185-5p, showing that miR-185-5p role in BSMC

contractility is not via calcium waving but probably by regulating CDC42 and RHOA instead. In sputum cells miR-185-5p expression inversely correlates with *POSTN* expression (Pearson $r = -0.67$) and a reduction in miR-185-5p expression ($P < .01$) was seen in sputum cells from asthmatics compared to healthy.

Conclusion: We hypothesize that miR-185-5p is upregulated in asthmatic airways as a negative feedback loop to control airway remodeling through periostin secretion and smooth muscle contraction, evidencing the potential of miR-185-5p as therapeutic target.

0569 | The $\alpha_2\beta_1$ integrin, a collagen-binding receptor, may be involved in pathology of airway remodelling in asthma

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Background: Collagen deposits are important contributors of airway remodelling in asthma. Previously, we have demonstrated that $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins, important collagen receptors, are overexpressed on blood eosinophils and T-cells in asthmatics.

Aim: We investigated whether those collagen receptors might be also involved in pathology of airway structural changes in asthma.

Method: In 105 white adult asthmatics (53 with persistent airflow limitation) and 36 controls we determined computed tomography derived airway cross-sectional geometry of the right upper lobe apical segmental (RB1) and the right basal posterior (RB10) bronchus, performed bronchofiberoscopy with bronchoalveolar lavage (BAL) and endobronchial biopsy with measurement of reticular basement membrane (RBM) thickness, assessment of collagen I and IV deposits, as well as α_1 and α_2 integrin subunits in bronchial specimens by immunohistochemistry (IHC). We also analysed expression of α_1 and α_2 on blood and BAL CD4+ and CD8+ T-cells, eosinophils, and monocytes/macrophages using flow cytometry, as well as measured blood and BAL biomarkers, including interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, IL-23, interferon γ and periostin, together with circulating a metalloproteinase domain-containing protein (ADAM)33, and serum α_1 and α_2 subunits.

Results: Asthmatics had thicker RBM, but similar collagen I and IV accumulation in bronchial specimens, as compared to controls. Surprisingly, in asthma we documented an inverse relationship between RBM thickness and percentage of stroma showing collagen I reactivity. The score of α_1 and α_2 in IHC correlated inversely with RBM thickness and collagen I deposits. Increased α_2 in bronchial specimens was related to the thinner RB10 wall.

Persistent airflow limitation was characterized by raised circulating α_2, as well as higher α_2 expression on blood and BAL CD4+ T-cells, blood monocytes, BAL CD8+ T-cells and eosinophils, as compared to control. Expression of α_2 on blood eosinophils was inversely related to the RBM thickness and collagen I accumulation. Higher circulating α_2 was determined by increased blood levels of IL-6, IL-12p70 and ADAM33.

Conclusion: Although large observational studies are needed to verify this hypothesis, raised $\alpha_2\beta_1$ integrin on inflammatory cells and in bronchial mucosa may play a protective role against unfavourable airway structural changes, defined as prevailing RBM thickening or collagen I accumulation in asthma.

0933 | Age-related changes in innate immune response to in vitro rhinovirus infection in asthma patients

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Background: Asthma in elderly asthmatics may be challenging problem for clinicians because of different clinical manifestation than in younger age, which may be partially associated with age depended alteration of the immune response. The aim of the study was to investigate the impact of age on innate immune response to rhinovirus infection in asthmatics.

Method: Peripheral blood mononuclear cells (PBMCs) from 24 asthmatics (12 aged 30-50 years old and 12 above 65 years) and age-matched controls were isolated from whole blood and incubate with Rhinovirus 1b (RV1b). Cellular expression of toll-like receptors (TLR-3, TLR-5, TLR-7, TLR-8), RIG-I and NOD-2 receptors on monocytes was evaluated with flow cytometry. Analysis of expression of miRNA-106a, -126a, -146a, –19b, -22 and -142 was performed using the Real-Time PCR. In cultures supernatants INF-α, -γ, IL-6 and IL-17A levels were measured with flow cytometry while IFN-β and RANTES levels were determined with the use ELISA method.

Results: RV1b induced higher expression of TLR-5 on monocytes from asthmatics than from controls (%CD14+ cells: 29.5 ± 28.4 *vs* 11.3 ± 14.7; $P = .013$). Younger patients with asthma had lower levels of RIG-I positive cells than elderly (13.1 ± 27.7 *vs* 26.8 ± 28.8; $P = .048$). Following RV infection the expression of TLR-8 on monocytes decreased in younger asthmatics ($r = -0.64$, $P < .05$) and NOD-2 expression decreased in elderly subjects with asthma ($r = -0.64$, $P < .05$). After RV1b infection levels of INF-α and IL-17A in asthmatics were lower as compared to controls (64.6 ± 68.8 vs 120.4 ± 326.9 pg/mL; $P = .0032$ and 2 ± 3.2 vs 7.2 ± 9.5 pg/mL; $P = .023$, respectively). Lower level of INF-γ was found In elderly asthmatics as compared to the younger (499.1 ± 600.9 vs 2855.9 ± 3593.9 pg/mL; $P = .002$). IL-6 concentration was nearly ten times higher in younger asthmatics than in age matched controls (10583.3 ± 22543.2 vs 1191.2 ± 2321.7 pg/mL; $P = .0046$). Age was negatively correlated with INF-γ ($r = -0.54$, $P < .05$) and positively with IL-17A ($r = 0.7$, $P < .05$).

In asthmatics the expression ($2^{\wedge}DCt$) of miRNA-106a after RV1b infection was positively correlated with INF-α and -γ ($r = 0.43$; and $r = 0.53$; $P < .05$). miRNA-146a was correlated positively with

INF-γ and negatively with Il-17A ($r = 0.64$; $P < .05$, $r = -0.51$; $P < .05$; respectively).

Conclusion: In elderly asthmatics as compared to younger asthmatics the innate immune response to rhinoviral infection is different and may be regulated by certain microRNAs. Polish National Science Centre grant no. 2013/09/B/NZ6/00746.

1059 | Analysis of inflammatory cytokines during stable asthma and asthma exacerbations in patients with moderate-severe asthma

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Background: Asthma exacerbations (AE) are responsible for most of the morbidity in asthma patients. Allergens and especially viral respiratory tract infections are common triggers for AE. Mouse studies suggest viral-associated AE are correlated with an increase in blood IL-25, IL-33 and TSLP. In humans an increase of blood anti-inflammatory cytokines such as IL-10 is described. We aim to gain insight into the immunological dynamics and their relation to viral etiology of moderate-severe exacerbations.

Method: The Breathe study is a double-blind RCT to determine the effects of the bacterial lysate OM-85 in patients with GINA 4 asthma and recurrent AE. Patients were randomized to either 2-year winter season OM-85 or placebo treatment and were seen 3-monthly and before the start of AE treatment. Patients with an AE during this study were included.

Nasopharyngeal swab medium was analyzed with viral PCR, blood leukocyte differentiation was analyzed with DxH, plasma cytokines were analyzed with ELISA and flow cytometry was used to analyze blood T-cell subsets and intracellular cytokines. Both treatment groups were analyzed as one, because of the absence of major differences between the groups. Data are shown in mean ± SD or median (IQR).

Results: Thirty-five patients with an AE, of which 72% had T2 high asthma, were included. ACQ increased during an AE. In 42% a virus was detected. Blood neutrophils increased during AE (3.70 ± 2.1 vs $5.30 \pm 1.7*10^9$/L, $P < .01$), no change in blood eosinophils was observed.

Plasma cytokine analysis showed an increase of IL-10 (1.95 (1.95-9.49) vs 5.61 (1.95;14.31), $P = .038$) and IL-25 (9.72 (3.66;25.96) vs 13.29 (3.66;36.48), $P = .023$) and a decrease of IFNλ (62.5 (62.5;343.7) vs 62.5 (62.5;314.3) $P = .039$).

Preliminary subgroup T-cell subset analysis showed a decrease of %CD4^{+} Th2 and Th17 cells (figure 1). Intracellular CD4^{+} T-cell cytokine analysis (N = 22) revealed an increase of IL-5 (0.79 (0.49;1.17) vs 1.93 (1.17;4.53), $P = .005$) and IL-9 (0.89 (0.50;2.65) vs 2.44 (0.91;3.01), $P < .001$) and a decrease of IFNγ (9.85 (6.75;14.93) vs 5.18 (3.62;9.28) $P = .004$). Subanalysis showed the increase of IL-5 and IL-25 were only present in viral-associated AE.

Conclusion: In this study 42% of AE were virus-associated. Moreover, the increase of IL-5 and IL-25 was only seen in virus-associated AE. In contrast, blood CD4^{+} Th2 and Th17 cells decreased during an AE. These conflicting results could be due to migration from systemic to more local, which should be studied in future studies.
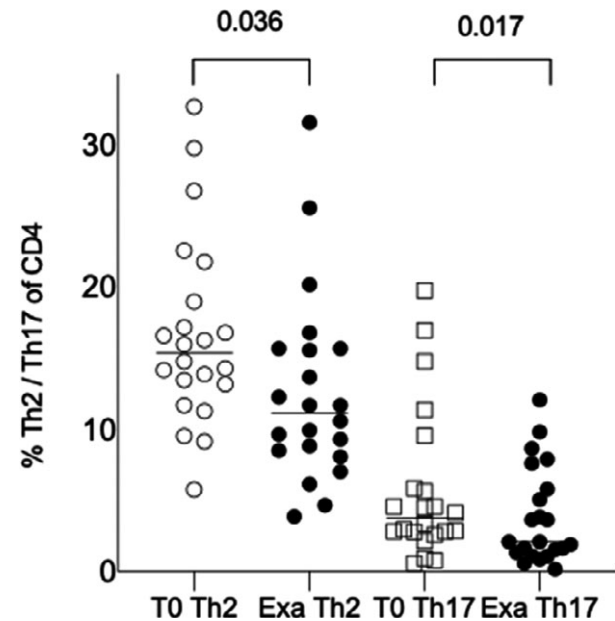
OAS 02
Biomarkers and Drugs of Allergic Rhinitis

0249 | MP-AzeFlu improves quality of life of patients with allergic rhinitis: A real-world study

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Background: Patients with poorly controlled allergic rhinitis (AR) experience nasal and ocular symptoms, and suffer from sleep disturbances, emotional problems, and activity impairment. In patients with more severe disease, AR is often associated with impaired quality of life (QOL). MP-AzeFlu (137 μg azelastine hydrochloride/50 μg

fluticasone propionate intranasal spray) is safe and effective, but the impact on QOL among patients with AR requires further real-world investigation. The main objective of this observational study was to evaluate changes in patient sleep quality and trouble with daily work and social activities after ~14 days of treatment with MP-AzeFlu in routine clinical practice.

Method: This multicenter, prospective, noninterventional, real-world study included patients with moderate-to-severe AR. Over approximately 14 days of treatment with MP-AzeFlu (1 spray per nostril twice daily), changes in overall AR symptoms, sleep quality, and trouble with daily work/school activities, social activities, and outdoor activities were evaluated using a visual analog scale (VAS). VAS scores were printed on a patient card, and ranged from "not at all troubled" (0 mm) to "extremely troubled" (100 mm).

Results: The mean VAS scores of overall AR symptoms among 1103 patients decreased 46 mm from severe (73.2 mm) at Day 0 to mild (27.2 mm) at Day 14. Following treatment with MP-AzeFlu for 14 days, mean VAS scores for impairment of sleep quality (−33.1 mm; $P < .0001$), daily work/school activities (−34.6 mm; $P < .0001$), social activities (−32.7 mm; $P < .0001$), and outdoor activities (−39.4 mm; $P < .0001$) significantly decreased from Day 0 (Table). Results were similar across populations, regardless of AR phenotype, comorbidity, age, and gender.

Conclusion: MP-AzeFlu not only relieves symptoms but also improves patient QOL, as illustrated by better sleep quality and less impairment of work/school, social activities, and outdoor activities after 14 days of treatment. These findings support the recommendation of MP-AzeFlu as first-line therapy in patients with moderate-to-severe AR.

Table. Time Course Effect of MP-AzeFlu on Quality of Life Outcomes

	Day 0 VAS (mm)	Day 7 VAS (mm)	Day 14 VAS (mm)
Overall AR symptoms	73.2	36.1*	27.2*
Impairment of sleep quality	55.2	29.8*	22.1*
Impairment of daily work/school activity	57.6	30.4*	23.0*
Impairment of social activity	55.1	29.0*	22.4*
Impairment of outdoor activity	64.4	32.6*	25.0*

AR indicates allergic rhinitis; VAS, visual analog scale.

* $P < .0001$.

0299 | Reformulating budesonide nasal spray (budesolv 10) multiplies clinical potency in grass pollen allergic patients

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Background: Budesonide, a poorly water-soluble corticosteroid, is currently marketed as a suspension. It was reformulated to enhance solubility and local concentration on the target mucosa. The novel aqueous formulation of the dissolved substance is named Budesolv and contains ~85% less corticosteroid than the marketed comparator. Budesolv showed increased bioavailability in preclinical models. Objective: Purpose of this randomised, double-blind, placebo-controlled, three way crossover pivotal phase III trial was to evaluate onset of action and non-inferiority of Budesolv 10 μg compared to Rhinocort® aqua 64 (= RA) in an environmental exposure chamber.

Method: Adult subjects with clinically proven grass pollen allergy received Budesolv 10 μg, the marketed comparator RA or placebo once daily for 8 days with a washout period of 3 weeks between treatment blocks. Allergic symptoms were induced by 6 h grass pollen challenges in the Vienna Challenge Chamber at days 1 and 8 of each treatment period. Primary endpoint was the mean total nasal symptom score (= TNSS; sum of obstruction, itch, sneeze and rhinorrhoea assessed on a categorical scale from 0 to 3) between 2 and 6 hours of allergen challenge on day 8. Secondary endpoints included onset of action, ocular and asthma symptoms and objective measures of nasal secretion and nasal obstruction. First dose was applied after 105 min of challenge to evaluate onset of action of either treatment. A 95% confidence interval (CI) was calculated for difference in means between active treatments. Non-inferiority was stated if the upper limit of the CI did not exceed 115% of the reference. Onset of action was defined as first time point when the TNSS difference between active treatment and placebo was $P < .05$.

Results: 75 patients concluded the study per protocol. The primary endpoint mean TNSS was met proving non-inferiority of Budesolv 10 μg compared to RA. A significant difference between Budesolv 10 μg and placebo was shown for TNSS at 2.45 h and for total asthma score 2 h after first dose. On day 8 Budesolv 10 μg was significantly superior to placebo for all parameters evaluated.

Conclusion: Non-inferiority of Budesolv 10 μg compared to Rhinocort® aqua 64 was shown on day 8 of treatment. An early onset of action was evident for Budesolv 10 μg only within 2.45 hours for rhinitis symptoms and 2 hours for asthma symptoms after initial dose on day 1.

0458 | MP-AzeFlu improves allergic rhinitis severity regardless of disease phenotype – a real-life study

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Background: Phenotyping allergic rhinitis (AR) by IgE sensitivity and presence of comorbidities may help characterize allergic diseases, provide a clinical framework to inform treatment decisions, and improve clinical trial design. MP-AzeFlu (137 μg azelastine hydrochloride + 50 μg fluticasone propionate intranasal spray) is a treatment for AR but the effectiveness of MP-AzeFlu on disease severity by AR phenotypes is unknown. This prospective, noninterventional study evaluated the effectiveness of MP-AzeFlu (1 spray in each nostril twice daily for 2 weeks) across novel AR phenotypes.

Method: Patients with moderate-to-severe AR according to ARIA classification with acute symptoms (visual analog scale [VAS] >50 mm) for whom MP-AzeFlu was prescribed for the first time were enrolled. AR subpopulations (ARPs) were assigned based on classifications from the MeDALL study and presence of comorbidities: specific IgE response to 1 allergen with no family history (ARP1); specific IgE response to > 5 allergens with family history (ARP2); nonallergic polyclonal IgE response with no family history (ARP3); intermediate AR phenotypes (ARP4); and AR with comorbidities (ARP5). AR symptoms over the previous 24 hours were documented using a single-line VAS (AR-VAS) with ratings from "not at all bothersome" (0 mm) to "extremely bothersome" (100 mm) at days 0, 1, 3, 7, and ~14.

Results: A total of 1103 patients with AR were included in the final analysis. Mean baseline VAS for AR symptom severity ranged from 70.3 to 75.1 mm (severe) across the different ARPs. The response rate (score less than 50 mm on at least one day) in the full analysis set was 86.6%. In the subpopulations, the response rates ranged from 79.3% to 89.6%. From day 1 through the last day of the study, mean AR-VAS scores decreased across all subpopulations. Mean reduction in VAS score from baseline to the last day ranged from −47.9 mm to −40.9 mm across phenotypes, resulting in final AR-VAS scores of 27.2 mm to 30.1 mm (mild).

Conclusion: MP-AzeFlu was associated with reduced severity based on VAS scores from baseline in the general study population and in all ARPs. These results support the effectiveness of MP-AzeFlu for moderate-to-severe AR, regardless of IgE response, family history of allergy, and presence of comorbidities.

Assessment of overall symptoms of AR (VAS [mm]) - Line plot of mean course Safety population
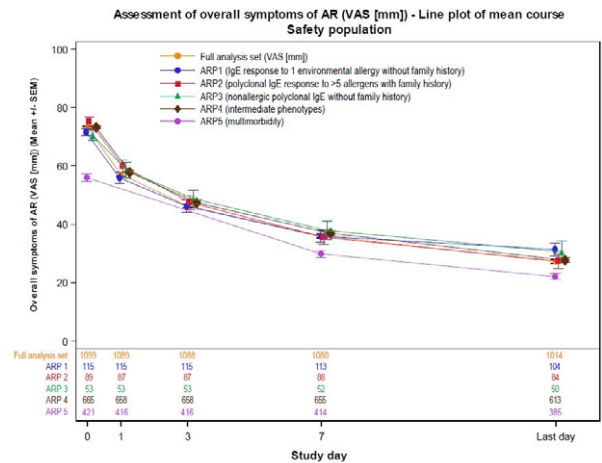
0583 | Probiotic NVP-1703 alleviates perennial allergic rhinitis by inducing IL-10 expression: A 4-weeks, multi-center, double-blind, randomized, placebo-controlled clinical trial

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Background: Probiotic NVP-1703, a mixture of *Bifidobacterium longum* IM55 and *Lactobacillus plantarum* IM76, had effect on ovalbumin- or house dust mite allergen-induced allergic rhinitis (AR) in mice. The present study examined the efficacy and safety of NVP-1703 in perennial AR volunteers

Method: Adult volunteers with perennial AR for ≥ 2 years were randomly assigned to take NVP-1703 (1×10^{10} CFU with maltodextrin 2 g/day, n = 47) or placebo (maltodextrin 2 g/day, n = 48) for 4 weeks. The primary outcome was changed in total nasal symptom score (TNSS). Rhinitis control assessment test (RCAT), blood eosinophil count, allergen-specific IgE, and immunological parameters in serum or urine were assessed at baseline and week 4.

Results: The changes in TNSS from baseline at week 1, 3, and 4 were −0.47 ± 0.20, −1.25 ± 0.33, and −1.69 ± 0.39 in the NVP-1703 group, respectively, and 0.10 ± 0.17, −0.40 ± 0.21, and −0.64 ± 0.27 in the placebo group, respectively (P = .033, 0.031, and 0.029, respectively). RCAT increased from baseline by 3.83 ± 0.73 and 2.21 ± 0.67 in the NVP-1703 and placebo groups, respectively, at 4 weeks (P = .049). NVP-1703 treatment significantly reduced specific IgE to *Dermatophagoides farina* compared with the placebo group (P = .033). The changes of IL-10 from the baseline showed a significant difference between groups (P = .047). The ratios of IL-10/IL-4, IL-10/IL-5, and IL-10/IL-13 were significantly increased in the NVP-1703 group but decreased in the placebo-treated group (P = .046, 0.088, and

0.018, respectively). NVP-1703 treatment reduced urinary PGF2α and LTE4 levels.

Conclusion: These findings suggest that NVP-1703 can be a treatment option of perennial AR.

0689 | Evaluating the real-life effect of MP-AzeFlu on asthma control in patients with allergic rhinitis and asthma in UK primary care

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Background: MP-AzeFlu (azelastine/fluticasone propionate in one spray) is the most effective allergic rhinitis (AR) treatment available. Its effect on asthma control in co-morbid patients is unknown. We examined whether MP-AzeFlu altered asthma control in patients with AR and asthma.

Method: This pre-post historical cohort study, carried out using the Optimum Patient Care Database, included patients aged ≥ 12 years from UK general practice with active asthma in the year before MP-AzeFlu initiation. Active asthma was defined as a recorded diagnosis, with ≥ 1 prescription for reliever or controller inhaler in the year prior to and including the initiation date. The primary endpoint was change in number of acute respiratory events (i.e. an exacerbation or an antibiotic course for a respiratory event) between baseline and outcome years. Study outcomes were assessed by Wilcoxon signed rank test or McNemar's test.

Results: 1,188 patients were included (average age: 47 yrs; 58.4% female). Most were prescribed intranasal corticosteroids (60.4%) and Inhaled corticosteroid (ICS)/long-acting β_2-agonist (59.3%) at baseline. MP-AzeFlu initiation was associated with significantly fewer acute respiratory events (P = .013). Significantly more patients had well-controlled asthma in the year after MP-AzeFlu initiation (P = .004). These asthma control benefits were noted despite significant reduction in short-acting β_2-agonist (SABA) dose (P < .001), fewer patients requiring > 2 SABA puffs/week (P < .001) and the average ICS dose remaining stable (64.0% of patients) or reduced (20.4% of patients).

Conclusion: MP-AzeFlu initiation is associated with reduced acute respiratory events, improved asthma control and reduced SABA and maintenance ICS dose in patients with AR and asthma.

OAS 03
Mechanisms of Gastrointestinal Food Allergy and its Management

0303 | Short-chain fatty acids restore esophageal barrier function of human esophageal epithelial cells after IL-13-induced barrier impairment

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Background: Eosinophilic esophagitis (EoE) is an emerging food allergen-driven chronic inflammatory disease of the esophagus. Evidence of abnormal epithelial cell proliferation and dilated intercellular spaces suggest that a compromised esophageal epithelial barrier potentially contributes to the pathogenesis of EoE. Active food components that restore barrier function and increase immune fitness may be a promising tool in the dietary management of EoE. Short-chain fatty acids (SCFAs) are such components with immune-modulating capacities. We investigated the potential barrier-restorative effects of the three most abundantly produced SCFAs – butyrate, propionate, and acetate – on an IL-13-compromised barrier of EPC2-hTERT (EPC2), a human esophageal epithelial cell line.

Method: EPC2 were cultured on semi-permeable membranes under air liquid interface (ALI) conditions to induce epithelial stratification. After 3 days of ALI culture with or without IL-13, stratified EPC2 were left untreated or were treated with SCFAs for 4 days. Esophageal epithelial barrier integrity was monitored by measuring transepithelial electrical resistance (TEER), paracellular flux, and RNA transcripts for tight junction (TJ) complex proteins and pro-inflammatory mediators. To investigate possible SCFA targets, ALI-cultured IL-13-treated EPC2 were exposed to agonists and antagonists of GPR41, GPR43, GPR109a or histone deacetylase (HDAC).

Results: Butyrate and propionate, but not acetate, enhanced the barrier function of ALI-cultured IL-13-treated EPC2 as demonstrated by an increase in TEER (2.9-fold), a decrease in paracellular flux (5.4-fold), an increased expression of the type II keratin *KRT78* (2.4-fold) and TJ complex proteins *CLDN7* (5.1-fold) and *FLG* (10-fold), and a decreased expression of the pro-inflammatory mediators *CAPN14* (3.5-fold) and *CCL26* (2.6-fold). GPR antagonists did not abolish the barrier-restorative effects of butyrate and propionate. In contrast to GPR agonists, an HDAC antagonist induced similar effects in IL-13-treated EPC2 as these SCFAs.

Conclusion: Butyrate and propionate restored the integrity of the IL-13-compromised esophageal epithelial barrier. Our data suggest that these beneficial effects may be independent of GPRs, but may involve the HDAC inhibiting properties of these SCFAs. Butyrate and propionate may have the potency to support the dietary management of EoE.

0334 | Oral tolerance induction for cow's milk allergy prevention using polymeric nanoparticles loaded with beta-lactoglobulin derived peptides

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Background: 2-4% of infants are affected by cow's milk allergy (CMA), which persists in 20% of cases in adult life and enhances the risk of developing other allergic diseases later in life. Thus, early intervention for CMA prevention is important.. To prevent early life CMA, 2 selected 18-AA peptides derived from the major allergen beta-lactoglobulin (PEP3 and 4) were loaded into PLGA nanoparticles (NP) for oral delivery [1, 2]. Encapsulation of the peptides aims not only to protect the orally administered peptides from gastrointestinal digestion, but also to deliver the peptides across mucosal linings for effective oral tolerance induction.

Method: PEP3 or 4 were loaded into PLGA NP by double emulsion solvent evaporation method. In vitro, fluorescent labelled empty PLGA NP (Cy-5 NP) were co-incubated with human monocyte derived dendritic cells (moDC) for 20 hrs (n = 2), and cellular uptake was analyzed with FACS. In vivo, 3-week old female C3H/HeOuJ mice were given orally either PBS, whey, PEP3 and 4, a high/low dose of PEP3-and 4-NP, or PEP3 and 4 plus empty NP for 6 serial days, followed by oral sensitization with whey plus cholera toxin for 5 serial weekly treatments. 5 Days after the last sensitization, the mice were intradermally (i.d.) and orally challenged with whey. After 18 hours, splenocytes were isolated for FACS analysis and stimulation with anti-CD3 or whey for 2 or 5 days respectively, and cytokines were measured in the supernatant.

Results: PEP3-and 4- NP have encapsulation efficiency (EE) of ~70-90%, showing no burst release in PBS buffer (37°C, pH 7.4) from the polymer matrix. In vitro, FACS analysis showed ~83-98% of human moDC (n = 2) uptake of Cy5-NP and no influence on viability or %CD11c+HLA-DR+ among viable cells. In vivo, a high dose of PEP3- and 4-NP group showed a reduced acute allergic skin response than the whey-sensitized group, PEP3 and 4 group (P < .05) and low dose PEP3- and 4-NP group (P < .01). Anaphylactic shock score and body temperature showed a similar pattern. Ex vivo stimulation of splenocytes with anti-CD3 elicits a significant lower level of IL-13 and IL-10 in the high dose PEP3- and 4-NP group than the whey tolerant group (P < .001).

Conclusion: In vitro, Cy5-NP were taken up by human moDC in a dose-dependent manner. High dose of PEP 3- and 4- NP induced oral tolerance by prevention of CMA symptoms upon i.d challenge with whey. This proof of principle study allows us to further develop the concept of peptide-loaded PLGA NPs for CMA prevention.

0430 | Dupilumab normalizes expression of genes associated with fibrosis, remodeling, and barrier function in patients with eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic inflammatory disease characterized by barrier dysfunction, remodeling, and fibrosis. EoE patients have an altered esophageal transcriptome compared with healthy controls. Dupilumab (DPL), a fully human monoclonal antibody, blocks the shared receptor component for interleukin (IL)-4 and IL-13, key and central drivers of type 2 inflammation in multiple diseases. In a double-blind, placebo (PBO)-controlled, phase 2 study (NCT02379052), adults with active EoE were randomized (1:1) to receive 12 weeks of subcutaneous DPL 300 mg weekly (qw) or PBO; DPL significantly improved dysphagia and histological and endoscopic measures of disease with an acceptable safety profile. This analysis assessed the effect of DPL on the expression of genes involved in barrier function, fibrosis, and remodeling in patients enrolled in the study.

Method: Pinch biopsies were collected from proximal, mid, and distal esophagus at baseline (BL) and Week 12, and RNA was extracted for transcriptome genome sequencing from 41 patients with active EoE. Mean gene expression of the 3 esophageal regions for each patient at BL and Week 12 was examined. A relative change from BL of ≥ 2-fold, q < 0.05 was considered significant, following adjustments for multiple testing.

Results: The gene expression modulated by DPL 300 mg qw at Week 12, the DpxOme, included 1,302 genes, 513 of which were downregulated and 789 upregulated. The post-DPL treatment transcriptome more closely resembled the normal esophageal transcriptome compared with the published EoE and PBO-treated transcriptomes.

DPL normalized expression of genes associated with fibrosis, re-modeling, and barrier function (Figure). DPL downregulated the expression of collagen genes, including *COL4A3*, *COL4A4*, *COL4A6*, *COL8A2*, *COL14A1*, *COL21A1*, and periostin (*POSTN*), an extracellular matrix protein associated with fibrosis. DPL treatment also up-regulated filaggrin (*FLG*), desmoglein 1 (*DSG1*), *SPINK5*, *SPINK7*, and *SPINK8*, which are associated with an intact epithelial barrier. PBO treatment did not induce any significant gene expression changes.

Conclusion: In this phase 2 dupilumab study, EoE patients had altered BL transcriptomes, consistent with previously published data. Treatment with dupilumab normalized expression of genes associated with remodeling, fibrosis, and barrier function in adult EoE patients, in line with study findings of reduced symptoms and histological disease characteristics.
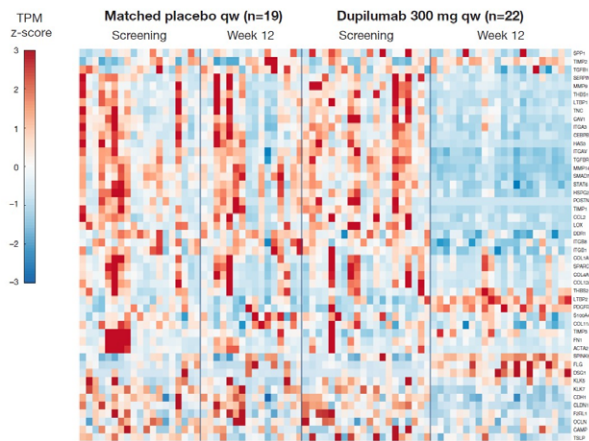
0959 | Serum levels of soluble interleukin-2 receptor and thymus and activation-regulated chemokine in non-IgE-mediated gastrointestinal food allergies

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Background: Non-IgE-mediated gastrointestinal food allergies (non-IgE-GI-FAs) is characterized by gastrointestinal symptoms: vomiting, diarrhea and bloody stools. Although its pathology is unclear, non-IgE-GI-FAs are associated with elevated serum level of proinflammatory cytokines. During searching for genes specifically associated with pathology of non-IgE-GI-FAs, we found that mRNA levels of interleukin (IL)-2 receptor (IL-2R) α and thymus and activation-regulated chemokine (TARC) were elevated in antigen-stimulated peripheral blood cells of non-IgE-GI-FAs patients. However, little is known about whether IL-2Rα and TARC are elevated at protein levels in serum of patients with non-IgE-GI-FAs and associated with its disease status.

Method: Five patients with non-IgE-GI-FAs were retrospectively recruited at Gunma University Hospital from 2011 to 2015, whose serum had been collected at the onset and remission phases, and stored. The patients were diagnosed based on the results of elimination and provocation test, and lymphocyte stimulation test. Serum samples from 4 healthy controls were also used. Soluble IL-2R (sIL-2R) and TARC concentrations were determined with enzyme-linked immunosorbent assay. Severity of the patients was graded according to the literature (Yagi et al. *Allergo Int* 2019). The study was approved by the ethics committee of Gunma University Hospital.

Results: The mean levels sIL-2R and TARC in symptomatic patients were 2531 and 2708 U/mL, respectively. Each of them was significantly higher than that of controls (491 and 376 U/mL) ($P = .016$ and 0.016, Mann–Whitney). Severe cases of non-IgE-GI-FA showed a trend of an increase in sIL-2R and TARC. The sIL-2R and TARC levels in symptomatic phase were higher than those in remission. Spearman analysis showed sIL-2R levels are correlated with the TARC levels ($R = 0.9286$, $P = .0022$).

Conclusion: The sIL-2R and TARC levels were elevated in patients with non-IgE-GI-FA. In addition, sIL-2R and TARC levels were higher in severe cases than mild and control cases. This indicates that they can be used as biomarkers for the disease severity and reflect the pathophysiology of non-IgE-GI-FAs. This suggested that the serum levels of both sIL-2R and TARC are correlated to the disease pathophysiology and severity.

1154 | Eosinophilic gastrointestinal responses during peanut oral immunotherapy in a randomized controlled trial

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Background: Oral immunotherapy (OIT) is promising for the treatment of food allergy; however, gastrointestinal side effects are common and eosinophilic esophagitis (EoE) is a potential complication. In a randomized controlled trial involving peanut OIT, we aimed to characterized eosinophilic gastrointestinal responses.

Method: Twenty adult subjects with peanut allergy were randomized to peanut OIT (n = 15) and placebo (n = 5); one additional subject withdrew before randomization. Serial gastrointestinal biopsies were obtained at baseline (n = 21, 0 weeks), following dose escalation (n = 10, 52 weeks), and maintenance (n = 12, 104 weeks). Endoscopic findings were characterized using the EoE endoscopic reference score (EREFS). Biopsies were assessed for eosinophils per high-power field (eos/hpf) and other pathologic features using EoE Histologic Scoring System (EoEHSS). Immunohistochemical staining for eosinophil peroxidase (EPX) was performed and quantified using automated image analysis.

Results: No subjects reported gastrointestinal symptoms at baseline; however, all subjects had dilated intercellular spaces and 3 participants had ≥ 15 eos/hpf (esophagus). Peanut OIT induced significant transient eosinophilic inflammation at 52 weeks in the proximal, middle, and distal esophagus; whereas no significant changes were seen in the placebo arm. These changes corresponded with

significant increases in EoEHSS scores and EPX deposition. Four subjects (57%) had new-onset or worsening eosinophilia (≥15 eos/hpf) during OIT and one met clinicopathologic criteria for EoE. Three OIT subjects (43%) also crossed histologic thresholds for eosinophilic gastritis and/or duodenitis. In most, OIT-induced gastrointestinal eosinophilia (GE) resolved by the end of maintenance therapy. Gastrointestinal symptoms were not clearly associated with GE.

Conclusion: Our findings show that peanut OIT induces transient GE, and less commonly EoE, that is not always associated with gastrointestinal symptoms.

1544 | Is IgA a piece of the EoE puzzle?

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Background: The development of Eosinophilic Esophagitis (EoE) in some patients undergoing oral immunotherapy (OIT) for IgE-mediated food allergy has raised concerns. There is a need for biomarkers to identify patients who are likely to develop EoE from OIT and who may benefit from other therapies. A baseline upper endoscopy (*BUE*) is the only method to assess esophageal eosinophilic infiltration (EEi), but it is not routinely performed prior to OIT. The pathogenic role of IgE and IgG4 in EoE has been proposed. However, there are no studies describing the role of IgA, a major mucosal antibody, in the pathophysiology of EoE. Additionally, antigen and epitope-specific IgE and IgG4 repertoires are associated with different phenotypes of food allergy, serving as a promising tool for diagnosis and prognosis of cow's milk allergy (CMA).

This study aim to investigate the differences in milk epitope-specific IgE, IgG4 and IgA antibody repertoires in patients with persistent CMA who developed EoE following milk OIT compared to those who did not.

Method: The bead-based epitope assay was used to quantitate IgE, IgG4 and IgA profiles to 66 sequential epitopes on 5 milk proteins in serum or plasma from 13 children (12 ± 7.6 years of age) with CMA who underwent milk OIT. Of those subjects, 6 developed EoE and 7 were desensitized (DS) with no evidence of EoE. Samples were collected at baseline and following OIT. A *BUE* was performed at baseline in all individuals to rule-out EEi prior to therapy. Mixed-effects models, adjusted for the OIT duration, were used to evaluate epitope-specific changes; *P*-values were corrected for multiple comparisons using Benjamini-Hochberg approach.

Results: At baseline, patients who developed EoE had *higher levels* of epitope-specific (*es*)IgA to 59 epitopes (89%), with no significant differences in esIgE or esIgG4 compared to DS subjects. While esIgA to 14 peptides increased in the DS group during OIT, the levels remained less than those in the EoE group, whose esIgA remained

unchanged following OIT. Both groups had increases in esIgG4, with more significant changes in the EoE than DS patients (55 vs 24 epitopes).

Conclusion: Epitope-specific IgA was significantly greater at baseline in patients who developed EoE during milk OIT and should be further investigated as a potential biomarker. Additionally, during OIT, esIgG4 increased more in the EoE group than in individuals who did not develop EoE.
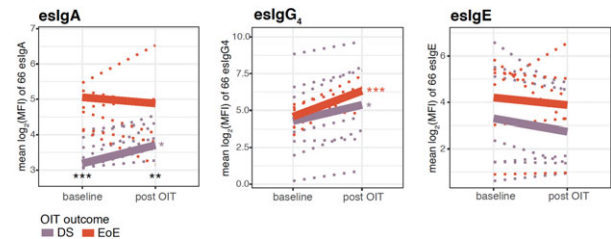
OAS 04
Microbiota as a Regulator Of Immune Responses

0170 | Modulation of the immune system in feto-maternal tissues by prebiotics supplementation during pregnancy: A future strategy for allergy prevention

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Background: Allergies are multifactorial diseases related to the dysfunction of the microbiota, epithelial barriers and the immune system leading to a failure in the establishment of immune tolerance. Pregnancy represents an optimal window of intervention in the regulation of the allergic process by modulating the immune and microbial systems of the fetus. Prebiotics can modulate the immune system, the microbiota and the intestinal barrier. A preclinical study carried out in our laboratory shows that prebiotics supplementation during pregnancy and lactation reduces the development of food allergy in offspring. The aim of this study was to understand the immunological processes of prebiotics administered during pregnancy on fetal and maternal tissues to highlight the potential establishment of a tolerogenic environment.

Method: Pregnant Balb/c mice received a standard diet or a diet enriched with prebiotics (GOS/inulin). After 18 days of gestation, the frequency of the different lymphoid and myeloid cell populations was determined in the different gestational (decidua, placenta, uterus), maternal (spleen) and fetal (intestine, blood) tissues. The effect of prebiotics on the frequency of hematopoietic stem cells from mother and fetus femur was also determined.

Results: Supplementation with prebiotics during gestation increases the frequency of CD19^{+}CD9^{+} and CD19^{+}CD25^{+} regulatory B lymphocytes in the placenta compared to mice on a standard diet (26.8%±4 and 12.8%±4 vs 13.3%±1.6 and 2.7%±0.6 respectively,

$P < .005$). These B cells are functional, characterized by their ability to secrete IL-10. They are also found in the fetus, in the intestine for CD19^{+}CD25^{+} B cells (8.6%±1.7 vs 1.4%±0.2 in prebiotic diet vs control respectively, $P < .005$) and in the bone marrow for CD19^{+}CD9^{+} B cells (63%±5 vs 20%±5 in prebiotic diet vs control respectively, $P < .001$). The rate of CD4^{+}CD25^{hi}FoxP3^{+} regulatory T cells is also increased in the placenta of mice supplemented with prebiotics compared to control mice (2.2%±0.3 vs 0.8%±0.1 respectively, $P < .001$). Prebiotics have no effect on the frequency of dendritic cells nor on the homeostasis of hematopoietic stem cells.

Conclusion: In conclusion, prebiotic supplementation during pregnancy leads to the establishment of a tolerogenic environment which could protect the fetus against future allergies.

0405 | Skin mycobiome sequencing reveals a high fungal diversity in patients with severe atopic dermatitis

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Background: Atopic dermatitis (AD) is a multifactorial, chronic relapsing inflammatory skin disease. Characteristics are an impaired skin barrier and an altered skin immune system, which often come along with predominant colonization by *Staphylococcus aureus*. The role of fungi, i.e. the mycobiome, remains poorly investigated although AD patients are frequently sensitized to *Malassezia*, the most abundant fungus on human skin. We aim to improve the understanding of the skin mycobiome in AD.

Method: Skin swabs of 11 AD patients and 11 healthy controls (HC) were taken from 4 skin sites (antecubital crease, glabella, vertex, and dorsal neck). To assess temporal shifts in the mycobiome, AD patients were sampled at 3 time points (0, 2 and 4 weeks). HC were sampled at 2 time points (0, 4 weeks). We assessed relative abundance of fungal genera and species by amplicon-based next-generation sequencing (NGS) of the fungal ITS1 region.

Results: The most abundant fungi at all skin sites were *Malassezia* spp. The species distribution was site-dependent with high abundances of *M. globosa* at the neck and *M. restricta* at the glabella and vertex, and overall lower abundance of *Malassezia* at the antecubital crease. Patients with severe AD tended to be more frequently colonized with non-*Malassezia* fungi such as *Candida*. In most HCs and patients with mild to moderate AD, the mycobiome was comparable between individuals and stable over time. In contrast, in severe AD the mycobiome was different between individuals and changed over time.

Conclusion: Patients with severe AD had a high intra- and interpersonal species diversity. We speculate that the impaired skin barrier in severe AD allows colonization with more different fungi than healthy skin. Vice versa, the altered mycobiome may cause activation of the skin immune system leading to inflammation and eczema. In the next step, we will correlate these results with the bacterial microbiome in the same samples.

0474 | Defining intestinal tissue pathology and microbiota/metabolome composition in mice carrying Rag1 hypomorphic mutations

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Background: Hypomorphic Recombination Activating Gene 1 (RAG1) mutations result in residual T- and B-cell development in both humans and mice and have been found in patients presenting with delayed-onset combined immune deficiency with granulomas and/or autoimmunity (CID-G/AI) and atypical severe combined immune deficiency (atypical SCID). Recent studies have shed light on how hypomorphic RAG1 mutations alter the primary repertoire of T and B cells, but less is known about their effect on immune dysregulation in target organs.

Method: In order to investigate the role of these mutations in determining intestinal disease, we are studying gut immunity and microbiota interplay in Rag1 mutant hypomorphic mice.

We evaluated a mouse model carrying the homozygous Rag1 mutation R972W (Rag1[w/w] mice), corresponding to the human mutation R975W described in patients with atypical SCID. Immunological characterization of the mouse model showed partial development of T and B lymphocytes.

Results: Analysis of intestinal pathology in Rag1[w/w] mice revealed severe spontaneous colitis. Interestingly, the most severe inflammatory phenotype was observed in female mice. Moreover, analysis of female mice at different ages showed that the inflammation becomes evident after 3 weeks of age, thus after the weaning time. The study of colon lamina propria T cells revealed a Th1/ Th17 phenotype that was confirmed by cytokine expression assay on colonic tissue.

In a preliminary set of experiments, we have crossed F1 X F1 Rag1[+/w] mice. F2 mice were co-housed by gender, irrespective of the genotype (+/+, +/w, or w/w littermates). Co-housing mice of different genotypes ensures exposure to the same microbes based upon coprophagy. We observed a severe restriction of microbial diversity in Rag1[w/w] mice as compared to co-housed Rag1^{+/+} and Rag1[+/w] mice, indicating a dramatic effect of the genotype. These abnormalities were much more pronounced in female Rag1w/w mice.

Conclusion: We are performing an integrative analysis to evaluate the impact of Rag1 mutations on fecal microbiome and metabolome composition. Moreover, we are testing two different therapies: vedolizumab, a monoclonal antibody directed specifically against the gut homing receptor $\alpha 4\beta 7$, and the Janus kinase (JAK) inhibitors, addressing the Th1/Th17 cytokine storm observed in this mouse model. Finally, we are currently studying patients with RAG1 hypomorphic mutations, focusing on the interplay between the immune system and the gut and skin microbiota.

1072 | Gut microbiota at the onset of food allergic milk-mediated reactions does not differentiate between allergic infants and non allergic controls

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Background: Gut microbiota plays an important role in the development of the immune system at early stages of life. Cow's milk allergy (CMA) is one of the most prevalent food allergy (FA) concerns among infant population. Its pathogenesis and the acquisition of oral tolerance are very complex processes and not completely known. Changes in the composition of gut microbiota (the so called dysbiosis) have been related to the development of FA. Since the primary colonization of gut microbiota occurs via maternal route, we hypothesized that there might exist a longitudinal influence, transmitted from mothers to offspring, in the composition of the gut microbiota that could be directly related to development of cow's milk allergy in infants.

Method: We recruited 126 patients. Faecal samples of 30 cow's milk-allergic and 12 non allergic infants (aged 0-6 months), as well as their respective mothers and grandmothers, were studied. Gut microbiota was profiled by 16S rRNA sequencing. DNA from faecal samples was extracted using the QIAamp DNA Stool Mini Kit. The V3-V4 regions of the 16S rRNA gene were amplified and sequenced using the MiSeq platform from Illumina. Taxonomy was assigned using the DADA2 implementation of the RDP classifier, using the DADA2 formatted RDP training set 16 release 11.5 from the DADA2 website. Statistical analyses were performed with R.

Results: Bacterial diversity was different among groups. It was higher in adults (mothers and grandmothers) than in infants, which is easily understood by the different type of diet among groups. Statistical analyses showed no significant differences between allergic and non-allergic groups, which means that there is no presence of a dysbiotic signature, transmitted from mothers to infants, that might predict the development of CMA.

Conclusion: Bacterial diversity increased significantly over time. There are no evidences suggesting that gut microbiota dysbiosis, at this stage of life (0-6 months), is related with the development of CMA. Changes in the composition of gut microbiota might be a consequence of a previous altered state of mucous membranes that determine the allergic phenotype beforehand. However, further longitudinal studies need to be done.

1404 | Modulation of T cell responses by a helminth immunomodulator from ascaris lumbricoides

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Background: Intestinal helminths may modulate the immune system of infected hosts, sometimes leading to immunosuppression. Isolation of helminth derived immunomodulators could be useful as anti-inflammatory agents. We have partially characterized a cystatin from *Ascaris lumbricoides* (Al-CPI) and demonstrated that it is able to strongly ameliorate experimental colitis and airways inflammation in mice and to inhibit human dendritic cell maturation. Here, we sought to evaluate the immunomodulatory effects of Al-CPI on T-cell responses.

Method: rAl-CPI was produced as a his-tag free recombinant product in BL21 (DE3) strain and purified by FPLC. Removal of endotoxin was confirmed by a quantitative Limulus amebocyte assay.

Peripheral blood mononuclear cells (PBMC) were isolated from 6 house dust mite allergic patients and 3 non-atopic controls. For T cell proliferation assays, PBMC were labeled with Cell Trace Violet and incubated with rAl-CPI 1 μM in the presence of *Blomia tropicalis* extract or CD-Mix (Anti- CD3/CD28/CD2). Live T helper cells (CD3+ CD4+) were identified by flow cytometry. Cytokine measurements (IFN-γ, IL-10 and IL-5) in cell culture supernatants were done by ELISA. Experiments were repeated using a CD4+ CD3+ T cell fraction purified by cell sorting.

Results: In PBMC cultures, rAl-CPI significantly suppressed CD4+CD3+T cell proliferation (16.7%, SD ± 9.3%, $P = .015$) induced by CD-Mix with a reduction of IL-5 ($P = .019$) and an increase of IFN-γ ($P = .037$) levels, without changes in IL-10 production. Also, rAl-CPI inhibited proliferation (57.5%, SD ± 5%, $P = .033$) of purified CD3+CD4+T cells stimulated with CD-Mix and reduced *B. tropicalis*-induced production of IL-5 and IL-10 from allergic patients PBMC ($P = .042$ and $P = .019$, respectively) but not in healthy controls.

Conclusion: Al-CPI has immunomodulatory effects on CD4+T cells that reduce the intensity of the allergic response, possibly by IL-10 independent mechanisms.

OAS 05
Targeted Treatment in Asthma

0818 | A post hoc analysis of dupilumab efficacy in asthma patients with comorbid allergic rhinitis and chronic rhinosinusitis/nasal polyps from the LIBERTY ASTHMA QUEST study

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Background: Dupilumab, a fully human monoclonal antibody, blocks the shared receptor component for interleukin (IL)-4 and IL-13, key and central drivers of type 2 inflammation in multiple diseases. In phase 3 LIBERTY ASTHMA QUEST (NCT02414854), add-on dupilumab 200/300 mg every 2 weeks (q2w) vs matched placebo significantly reduced severe exacerbations, improved pre-bronchodilator (BD) forced expiratory volume in 1 second (FEV$_1$), asthma control, and health-related quality of life (QoL) in patients with uncontrolled, moderate-to-severe asthma. Dupilumab was generally well tolerated. Comorbid allergic rhinitis (AR) and chronic rhinosinusitis with or without nasal polyps (CRS/NP) significantly contribute to disease burden in asthma patients and can complicate asthma management. The aim of this post hoc analysis was to assess the efficacy of dupilumab in patients with uncontrolled, moderate-to-severe asthma with comorbid AR and CRS/NP.

Method: Asthma patients with both comorbid AR and CRS/NP had a self-reported medical history of comorbidities. Rate of annualized severe exacerbations over the 52-week treatment period was analyzed using negative binomial models. Least squares (LS) mean change from baseline in pre-BD FEV$_1$ (L), asthma control (5-item Asthma Control Questionnaire [ACQ-5] scores [0 = totally controlled to 6 = severely uncontrolled]), and QoL (Asthma Quality of Life Questionnaire [AQLQ] global scores [7 = not impaired at all to 1 = severely impaired]) was analyzed using a linear mixed-effect model with repeated measures.

Results: 253/1,902 patients with uncontrolled, moderate-to-severe asthma had both comorbid AR and CRS/NP. Baseline demographics and disease characteristics were comparable across treatment groups. In this patient group, dupilumab 200/300 mg q2w combined vs placebo significantly reduced the annualized rate of severe exacerbations over the 52-week treatment period by 56% ($P < .0001$). At Week 12, dupilumab 200/300 mg q2w combined significantly improved pre-BD FEV$_1$ by 0.35 L and by 0.39 L at Week 52 (0.18 L and 0.21 L difference vs placebo; both $P < .001$). Dupilumab 200/300 mg q2w combined reduced ACQ-5 scores at Week 24 by 1.60 (0.41 vs placebo; $P < .01$) and improved AQLQ scores by 1.29 (0.32 vs placebo; $P = .01$) (Table).

Conclusion: Dupilumab treatment vs placebo in asthma patients with both comorbid AR and CRS/NP significantly reduced severe asthma exacerbation rates, improved lung function, asthma control, and health-related QoL.

Table. Efficacy of dupilumab and patient-reported outcomes in asthma patients with comorbid AR and CRS/NP.

	Combined placebo	Dupilumab 200/300mg q2w combined
	n = 86	n = 167
Annualized rate of severe exacerbations during the 52-week treatment period (95% CI)	1.199 (0.879–1.635)	0.523 (0.397, 0.690)
Relative risk vs placebo (95% CI)		0.437 (0.294, 0.649)
P value vs placebo		< 0.0001
	n = 86	n = 160
LS mean change from baseline in pre-BD FEV$_1$ (SE) at Week 12, L	0.16 (0.05)	0.35 (0.03)
LS mean difference vs placebo (95% CI), L		0.18 (0.08, 0.29)
P value vs placebo		0.0009
	n = 71	n = 137
LS mean change from baseline in pre-BD FEV$_1$ (SE) at Week 52, L	0.17 (0.05)	0.39 (0.03)
LS mean difference vs placebo (95% CI), L		0.21 (0.10, 0.33)
P value vs placebo		0.0002
	n = 82	n = 162
LS mean change from baseline in ACQ-5[a] (SE) at Week 24, L	−1.18 (0.12)	−1.60 (0.08)
LS mean difference vs placebo (95% CI)		−0.41 (−0.68, −0.14)
P value vs placebo		0.0030
	n = 81	n = 155
LS mean change from baseline in AQLQ[b] (SE) at Week 24	0.97 (0.10)	1.29 (0.08)
LS mean difference vs placebo (95% CI)		0.32 (0.08, 0.57)
P value vs placebo		0.0100

[a]ACQ-5 is a patient-reported measure of the adequacy of asthma control and change in asthma control that occurs either spontaneously or as a result of treatment. Scores range between 0 (totally controlled) and 6 (severely uncontrolled), with a change of 0.5 considered clinically relevant. [b]AQLQ is a patient-reported measure of asthma-specific health-related QoL. Higher scores indicate better health-related QoL; a global score is rated on a 7-point Likert-like scale (7 = not impaired at all to 1 = severely impaired), with a change of 0.5 considered clinically relevant.
CI, confidence interval; SE, standard error.

0875 | Dupilumab associated with lower reports of respiratory infections in moderate-to-severe asthma - safety analysis from the LIBERTY ASTHMA QUEST study

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Background: Dupilumab, a fully human monoclonal antibody, blocks the shared receptor component for interleukin (IL)-4 and IL-13, key and central drivers of type 2 inflammation which plays a role in multiple diseases. This pathway may play a role in helminth infection but is not known to be important in protection against non-helminthic infections. In phase 3 LIBERTY ASTHMA QUEST study (NCT02414854), add-on dupilumab 200/300 mg every 2 weeks vs placebo significantly reduced severe asthma exacerbations, improved pre-bronchodilator forced expiratory volume in 1 second, and was generally well tolerated in patients with uncontrolled, moderate-to-severe asthma. This post hoc analysis evaluated the effect of dupilumab on reported upper and lower respiratory infection rates in QUEST patients.

Method: Annualized rate of specific treatment-emergent (STE) infections at patient level (number of patients with ≥ 1 infection event per 100 patient-years [100PY]) and at event level (number of events per 100PY) were evaluated over the 64-week treatment-emergent safety evaluation period.

Results: Of 1,897 patients, 631/632 received dupilumab 200/300 mg, and 313/321 matched placebo. Annualized rates of reported STE infections at both the patient and event level were significantly lower with dupilumab vs placebo. Adverse event (AE) reporting of the high level term (HLT) "upper respiratory tract infections (URTIs)," "infections and infestations," and "upper respiratory infections" was reduced with dupilumab at the patient level (risk ratio [RR] 0.74, 0.81, 0.80) and event level (RR 0.73, 0.76, 0.77) (nominal $P < .01$ vs placebo). AE reporting of the HLT of "lower respiratory tract and lung infections" and "viral URTIs" was less common with dupilumab at event level (RR 0.73, 0.82; nominal $P < .05$ vs placebo); however, incidence was only numerically lower at patient level (RR 0.80, 0.89; all $P > .05$ vs placebo). RRs for AE reporting of "serious or severe infections and infestations" and "bacterial URTIs" were rare and not significant at 1.05 and 0.70 (patient level) and 1.23 and 0.61 (event level) (all $P > .05$) (Table).

Conclusion: Post hoc analysis demonstrated that reported rates of respiratory infections and overall infection rates were reduced in patients with uncontrolled, moderate-to-severe asthma treated with dupilumab vs placebo. Further studies are needed to prospectively confirm this finding and to better understand the mechanism of action of this finding.

Table. Annualized rate of specific treatment-emergent infections per 100 patient-year – safety population.

	Annualized rate of specific treatment-emergent infections per 100 patient-year		Risk Ratio (95% CI) vs placebo / P value
	Matching placebo (n = 634)	Dupilumab 200/300 mg q2w combined (n = 1,263)	Dupilumab 200/300 mg q2w combined
SOC: Infections and infestations			
nP (nP/100PY)	417 (121.534)	741 (98.017)	0.81 (0.72–0.92) 0.0008
nE (nE/100PY)	2,077 (330.779)	3,148 (250.501)	0.76 (0.72–0.80) < 0.0001
Serious or severe SOC: infections and infestations			
nP (nP/100PY)	11 (1.770)	23 (1.851)	1.05 (0.51–2.15) 0.9029
nE (nE/100PY)	11 (1.752)	27 (2.149)	1.23 (0.61–2.47) 0.5682
HLT: Lower respiratory tract and lung infections			
nP (nP/100PY)	100 (17.845)	165 (14.293)	0.80 (0.62–1.02) 0.0723
nE (nE/100PY)	167 (26.596)	243 (19.337)	0.73 (0.60–0.89) 0.0015
Upper respiratory infections[a]			
nP (nP/100PY)	329 (78.107)	552 (61.319)	0.80 (0.69–0.91) 0.0011
nE (nE/100PY)	601 (95.714)	921 (73.288)	0.77 (0.69, 0.85) < 0.0001
HLT: Upper respiratory tract infection			
nP (nP/100PY)	210 (41.457)	322 (30.431)	0.74 (0.62–0.88) 0.0007
nE (nE/100PY)	339 (53.988)	495 (39.389)	0.73 (0.64–0.84) < 0.0001
HLT: Viral upper respiratory tract infections			
nP (nP/100PY)	168 (32.089)	300 (28.154)	0.89 (0.74–1.08) 0.2444
nE (nE/100PY)	248 (39.496)	409 (32.546)	0.82 (0.70–0.96) 0.0162
HLT: Bacterial upper respiratory tract infections			
nP (nP/100PY)	10 (1.609)	14 (1.121)	0.70 (0.31–1.57) 0.3829
nE (nE/100PY)	14 (2.230)	17 (1.353)	0.61 (0.30–1.23) 0.1662

Categories listed in this table are not 'Preferred Terms'. [a]Upper respiratory infection is defined as HLT: upper respiratory tract infection, viral upper respiratory tract infection, bacterial upper respiratory tract infection. CI, confidence interval; HLT, MedDRA High Level Term; MedDRA, Medical Dictionary for Regulatory Activities; nE, number of events; nP, number of patients; q2w, every 2 weeks; SOC, MedDRA System Organ Class.

0879 | Seasonal variability of exacerbations in patients with severe, uncontrolled asthma and clinical benefits of tezepelumab: Results from the PATHWAY phase 2b study

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Background: In the PATHWAY phase 2b study (NCT02054130), tezepelumab (anti-TSLP) significantly reduced annualized asthma exacerbation rates (AAER) by up to 71% vs placebo in adults with severe, uncontrolled asthma. This analysis evaluated the effect of tezepelumab on exacerbations on a seasonal and weekly basis.

Method: Adults (18-75 years old) with severe, uncontrolled asthma were randomized to tezepelumab (70 mg every 4 weeks [Q4W], 210 mg Q4W or 280 mg every 2 weeks) or placebo for 52 weeks. AAER was estimated by season for the overall population, and the mean number of days with exacerbations per patient was summarized descriptively per season and over the year, for the overall population and patients with high (≥300 cells/μL) and low (<300 cells/μL) baseline blood eosinophil counts. Data from patients in the southern

hemisphere were transformed to align with northern hemisphere seasons. Data are reported for tezepelumab 210 mg and pooled tezepelumab dose groups.

Results: Overall, 550 patients were randomized to placebo (n = 138), or tezepelumab 70 mg (n = 138), 210 mg (n = 137) or 280 mg (n = 137). AAER was reduced by 64% (95% confidence interval [CI]: 22-83), 80% (95% CI: 41-93), 82% (95% CI: 48-94) and 67% (95% CI: 21-86) in the tezepelumab 210 mg group vs placebo in winter, spring, summer and autumn, respectively. In the pooled tezepelumab group, AAER was reduced by 63% (95% CI: 37-78), 70% (95% CI: 42-85), 77% (95% CI: 56-88) and 63% (95% CI: 34-79) vs placebo over the same seasons, respectively. In the placebo group, 1.5-3.7% of patients experienced ≥ 2 exacerbations over the seasons, vs 0.0-0.8% in the 210 mg group, and 0.0-0.5% in the pooled tezepelumab group. Figure 1 shows mean weekly exacerbation days per patient over the seasons. In the placebo group, the mean (standard deviation) numbers of days with exacerbations per patient were 2.5 (7.2), 1.6 (4.9), 1.9 (5.5) and 2.3 (7.4) during winter, spring, summer and autumn, respectively, vs 0.6 (2.8), 0.4 (2.0), 0.2 (1.6) and 0.7 (3.2), respectively, in the 210 mg group, and 0.9 (3.7), 0.5 (2.8), 0.5 (3.0) and 0.8 (3.3), respectively, in the pooled tezepelumab group. Fewer tezepelumab-treated patients experienced exacerbations throughout the seasons than placebo-treated patients, irrespective of baseline blood eosinophil count.

Conclusion: In patients with severe, uncontrolled asthma, tezepelumab treatment consistently reduced exacerbations across all seasons compared with placebo, irrespective of baseline blood eosinophil count.
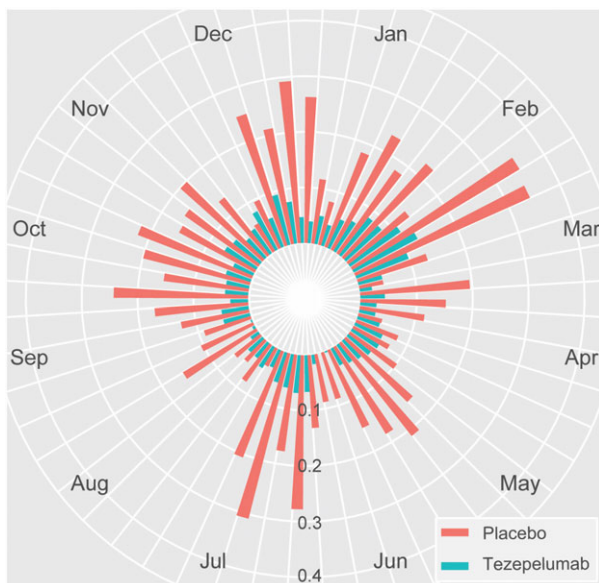
1103 | Fractional exhaled nitric oxide levels and eosinophil counts after cessation of tezepelumab: Results from the PATHWAY phase 2b study

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Background: In the phase 2b PATHWAY study (NCT02054130), tezepelumab significantly reduced exacerbations vs placebo and decreased inflammatory biomarker levels in adults with severe, uncontrolled asthma. This post-hoc analysis investigated changes in inflammation levels from baseline to end of treatment (week 52) and 12–16 weeks after the last tezepelumab dose (week 64).

Method: Adults with severe, uncontrolled asthma were randomized to tezepelumab (70 mg every 4 weeks [Q4W], 210 mg Q4W or 280 mg every 2 weeks) or placebo for 52 weeks. At baseline and weeks 20, 40, 52 and 64, patients were grouped by inflammation levels: low (eosinophils [EOS] <150 cells/μL and fractional exhaled nitric oxide [FeNO] <25 ppb), medium-low (EOS ≥ 150–<300 cells/μL or FeNO ≥ 25–<50 ppb or both), medium-high (EOS ≥ 300 cells/μL or FeNO ≥ 50 ppb, not both) or high (EOS ≥ 300 cells/μL and FeNO ≥ 50 ppb). Proportions of patients and median EOS and FeNO levels at each timepoint are reported for pooled tezepelumab doses and placebo for patients with data for all timepoints.

Results: Of 550 randomized patients, 188 had biomarker data for all timepoints. At baseline, 18%, 31%, 43% and 8% of tezepelumab-treated patients (n = 131) and 18%, 33%, 28% and 21% of the placebo group (n = 57) were in the low, medium-low, medium-high and high groups, respectively. Tezepelumab treatment over 52 weeks markedly increased the proportion of patients with low inflammation (47%) and slightly increased the proportion of patients with medium-low inflammation (33%). The proportions of patients with medium-high and high inflammation were markedly reduced after tezepelumab treatment (17% and 3%, respectively). In the placebo group, the proportions of patients in each inflammation group remained similar to baseline (Figure). At week 64, 37% and 39% of tezepelumab-treated patients were in the medium-low and low groups, respectively, vs 32% and 19% of the placebo group, respectively. Median EOS and FeNO levels were reduced from baseline to week 64 in tezepelumab-treated medium-high and high groups and remained relatively stable in medium-low and low groups. In comparison, smaller changes in median levels of EOS and FeNO were observed in most placebo groups. Results were similar when EOS and FeNO levels were analysed separately.

Conclusion: Tezepelumab treatment for 52 weeks reduced biomarkers of inflammation in a subpopulation of patients with severe,

uncontrolled asthma. Suppression of biomarkers persisted for 12–16 weeks after cessation of tezepelumab.
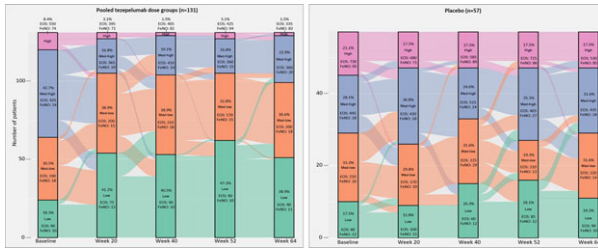
1105 | The effect of tezepelumab in patients with allergic asthma: Results from the PATHWAY phase IIb study

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Background: Tezepelumab is a human monoclonal antibody that blocks the activity of thymic stromal lymphopoietin. In the PATHWAY phase IIb study (NCT02054130), tezepelumab significantly reduced annualized asthma exacerbation rates (AAER) by up to 71% *vs* placebo, irrespective of baseline disease characteristics, and improved lung function, asthma control and health-related quality of life in adults with severe, uncontrolled asthma. This *post hoc* analysis evaluated the effect of tezepelumab in patients with allergic asthma who were eligible for omalizumab (OMA) treatment according to the European union (EU) label.

Method: Patients (18–75 years old) with severe asthma were randomized to receive subcutaneous tezepelumab (70 mg every 4 weeks [Q4W], 210 mg Q4W or 280 mg every 2 weeks [Q2W]) or placebo Q2W for 52 weeks. OMA-eligible patients (defined as having allergic asthma) were taking high-dose inhaled corticosteroids and had a positive (>0.35 fluorescence units) fluorescence enzyme immunoassay test for classic perennial aeroallergens (cockroach, cat dander, dog dander, *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*), a baseline total serum immunoglobulin (Ig) E level ≥ 30–≤1500 IU/mL, and an IgE and bodyweight combination within the range on the OMA EU label. AAER and forced expiratory volume in 1 second (FEV_1) were determined for patients eligible and ineligible for OMA treatment. Data are presented for tezepelumab 210 mg and pooled tezepelumab dose groups.

Results: Of 550 randomized patients, 92 and 453 patients were eligible and ineligible for OMA, respectively (5 unknown). OMA-eligible patients had higher total serum baseline IgE and fractional exhaled nitric oxide levels than OMA-ineligible patients. Compared with placebo, tezepelumab 210 mg reduced AAER over 52 weeks by 79% (95% confidence interval [CI]: 37–93) and 69% (95% CI: 42–83) in OMA-eligible and OMA-ineligible patients, respectively (Figure). For pooled tezepelumab doses, AAER was reduced by 76% (95% CI:

46–90) and 64% (95% CI: 45–77) in OMA-eligible and OMA-ineligible patients, respectively. Improvements in FEV_1 were observed in both eligibility groups, but point estimates were greater in OMA-eligible patients.

Conclusion: Treatment with tezepelumab reduced exacerbations and improved FEV_1 compared with placebo in patients with allergic and non-allergic asthma, further supporting its potential benefits in a broad population of patients with severe asthma.
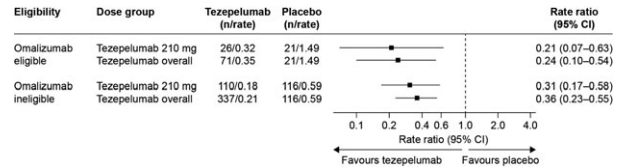
1175 | Effects of tezepelumab on asthma exacerbations, type 2 biomarkers, lung function and asthma control in patients with severe, uncontrolled asthma with and without nasal polyps: PATHWAY phase IIb study

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Background: In the phase 2b PATHWAY study (NCT02054130), tezepelumab (anti-thymic stromal lymphopoietin monoclonal antibody) significantly reduced annualized asthma exacerbation rates (AAER) in adults with severe, uncontrolled asthma. Nasal polyps (NP) are a comorbid, type 2 (T2) inflammatory condition present in up to 40% of patients with asthma and are associated with greater levels of T2 inflammation. This *post-hoc* analysis evaluated the effect of tezepelumab on AAER, inflammatory biomarkers, lung function and asthma control in patients with (NP+) and without (NP-) self-reported NP.

Method: Adults with severe, uncontrolled asthma were randomized to receive tezepelumab (70 mg every 4 weeks [Q4W], 210 mg Q4W, 280 mg every 2 weeks) or placebo (PBO) for 52 weeks. AAER was analysed and descriptive statistics were generated for blood eosinophil (EOS) count, interleukin (IL)-5, IL-13 and fractional exhaled nitric oxide (FeNO). Changes from baseline in Asthma Control Questionnaire (ACQ)-6 score and forced expiratory volume in 1 second (FEV_1) were also evaluated. Data are presented for tezepelumab 210 mg. Biomarker data are expressed as mean change ± standard deviation.

Results: Overall, 550 patients were randomized to PBO (n = 138) or tezepelumab 70 mg (n = 138), 210 mg (n = 137) or 280 mg (n = 137). NP+ patients represented 15.2% of the study population (NP+, n = 82; NP-, n = 458). Baseline blood EOS counts were elevated in

NP+ patients (NP+, 537.7 ± 367.8 cells/μL; NP-, 342.3 ± 345.3 cells/μL) with a greater proportion of NP+ patients having ≥ 300 cells/μL than NP- patients (NP+, 74.4%; NP-, 44.8%). In patients treated with PBO, AAER was higher in NP+ patients than NP- patients (NP+, 1.08, 95% confidence interval [CI]: 0.65-1.68; NP-, 0.68, 95% CI: 0.54-0.85). Compared with PBO, AAER was reduced by treatment with tezepelumab at week 52 to a similar extent in both NP+ and NP- patients (NP+, 0.29, 95% CI: 0.11-0.64; NP-, 0.19, 95% CI: 0.12-0.29). Blood EOS counts, serum levels of IL-5 and IL-13, and FeNO levels decreased, and ACQ-6 scores and FEV_1 improved in patients receiving tezepelumab *vs* PBO, irrespective of NP status (Table).

Conclusion: Tezepelumab reduced exacerbations and inflammatory biomarkers and improved FEV_1 and ACQ-6 score in patients with and without NP, supporting its efficacy in a broad population of patients with severe asthma.

	Biomarker	Placebo		Tezepelumab (210mg)	
		NP- (n=117)	NP+ (n=18)	NP- (n=112)	NP+ (n=23)
Baseline	Blood EOS (cells/μL)	367.5 ± 327.0	472.8 ± 350.2	346.1 ± 365.9	470.4 ± 262.0
	IL-5 (ng/L)	1.14 ± 1.88	1.19 ± 1.04	1.33 ± 3.02	1.88 ± 1.87
	IL-13 (ng/L)	0.05 ± 0.08	0.06 ± 0.05	0.05 ± 0.05	0.14 ± 0.19
	FeNO (ppb)	37.29 ± 40.84	39.91 ± 32.81	28.20 ± 26.68	48.02 ± 39.03
	ACQ-6 score	2.67 ± 0.65	2.51 ± 0.91	2.69 ± 0.78	2.85 ± 0.72
	Pre-BD FEV_1 (L)	1.80 ± 0.59	2.01 ± 0.60	1.81 ± 0.55	1.95 ± 0.73
Week 52	Blood EOS (cells/μL)	362.3 ± 308.5	405.3 ± 299.5	169.0 ± 139.0	240.0 ± 129.3
	IL-5 (ng/L)	1.00 ± 1.09	1.22 ± 0.92	0.44 ± 0.60	0.56 ± 0.36
	IL-13 (ng/L)	0.06 ± 0.10	0.06 ± 0.06	0.03 ± 0.03	0.03 ± 0.02
	FeNO (ppb)	34.56 ± 39.71	39.16 ± 30.20	24.33 ± 47.17	32.21 ± 27.10
	ACQ-6 score	1.72 ± 0.98	2.43 ± 1.46	1.44 ± 1.00	1.46 ± 0.93
	Pre-BD FEV_1 (L)	1.91 ± 0.70	1.89 ± 0.69	1.97 ± 0.58	2.21 ± 0.98
Change from baseline at week 52	Blood EOS (cells/μL)	−14.2 ± 217.1	−88.8 ± 272.2	−194.9 ± 357.4	−233.3 ± 224.2
	IL-5 (ng/L)	−0.15 ± 1.80	−0.01 ± 1.26	−0.82 ± 2.56	−1.73 ± 2.09
	IL-13 (ng/L)	−0.01 ± 0.10	0.00 ± 0.03	−0.03 ± 0.06	−0.08 ± 0.17
	FeNO (ppb)	−2.61 ± 25.15	10.65 ± 24.89	−4.05 ± 36.15	−9.43 ± 30.53
	ACQ-6 score	−0.95 ± 0.89	−0.03 ± 0.77	−1.16 ± 1.00	−1.35 ± 1.01
	Pre-BD FEV_1 (L)	0.11 ± 0.38	-0.13 ± 0.52	0.17 ± 0.39	0.36 ± 0.33

*Data are for pooled tezepelumab doses.
NP+ patients had self-reported NP.
Data are mean ± standard deviation.
ACQ, asthma control questionnaire; EOS, eosinophils; FeNO, fractional exhaled nitric oxide; IL, interleukin; NP, nasal polyps; pre-BD FEV_1, pre-bronchodilator forced expiratory volume in one second, ppb, parts per billion.
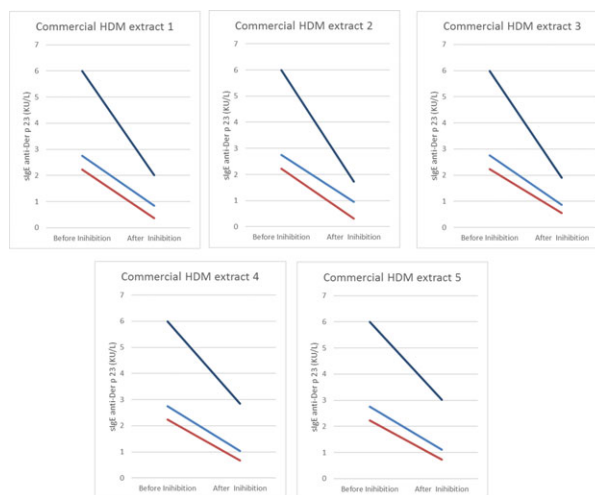
OAS 06
Component Resolved Diagnosis: New Insights

0450 | Der p 23 sensitization and impact on immunotherapy choice

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Background: House dust mite (HDM) induced allergic disease is a worldwide issue. The accuracy of the diagnosis has increased since the availability of component resolved diagnostics (CRD). However, HDM extracts for specific allergen immunotherapy (AIT) are not equally standardized and contain variable amounts of the main allergens Der p 1 and Der p 2. Importantly, extracts are not standardized for Der p 23, a major HDM allergen, which undermines the prescription of an adequate AIT for patients with a clinically relevant HDM allergy when Der p 23 is the main sensitizer

Objectives: To evaluate the IgE reactivity profiles to Der p 1, Der p 2, and Der p 23 in HDM allergic patients of an area of high prevalence of HDM sensitization (Barcelona, Spain).
To determine, through ImmunoCAP inhibition studies, if commercially available *Dermatophagoides pteronyssinus* (Dpt) extracts could be effective in patients mainly sensitized to Der p 23.

Method: Patients with HDM respiratory allergy candidates to receive AIT were tested for specific IgE against Dpt complete extract, Der p 1, Der p 2, and Der p 23 using ImmunoCAP 1000®. ImmunoCAP inhibition studies were performed incubating the patient's sera with four different commercial HDM extracts. The inhibition rate was calculated by the formula: (1-(patients' Dpt sIgE inhibited with the Dpt extract/ patients' Dpt sIgE without inhibition))x100.

Results: A total of 124 patients were included. The frequency of IgE binding to the three main HDM allergens was 81.5% for Der p 2, 67.7% for Der p 1, and 66.7% for Der p 23. sIgE Der p 23 levels were significantly lower than Der p1 and Der p 2.
Seven patients were "mono"-sensitized to Der p 23 (negative sIgE for Der p 1 and Der p 2) and ImmunoCAP inhibition studies were performed in 3 of them (those with higher Der p 23 sIgE titters) yielding inhibition rates for Der p 23 that ranged from 49% to 71%.

Conclusion: Our study demonstrates sensitization to Der p 23 in 66.7% of the patients which is in line with results from other European countries (Austria 70%, France 80%, Italy 87%).
We detected a 5.6% of Der p 23 mono-sensitized patients in our population, which may consequently be under risk of an ineffective AIT. Nevertheless, *in vitro* inhibition assays suggested that all the commercial HDM extracts tested contain Der p 23 in different amounts and could be adequate for a successful treatment. However, further studies in larger cohorts are necessary.

0877 | Molecular sensitization profiles and clinical characteristics of seasonal allergic rhinitis in seven mediterranean countries: The @IT2020 multicenter study

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Background: In the Mediterranean region, many patients with Seasonal Allergic Rhinitis (SAR) are poly-sensitized, making the precise prescription of allergen-specific immunotherapy (AIT) particularly challenging. Molecular IgE-testing can be a useful tool to improve the diagnostic precision in poly-sensitized patients.

Objective: To describe molecular sensitization profiles and clinical characteristics of SAR-patients in 9 Mediterranean cities.

Method: 815 patients between 10 and 60 years of age, suffering from SAR were included in the @IT2020 multicenter study in nine cities - Oporto (POR), Valencia (VAL), Marseille (MAR), Rome (ROM), Messina (MES), Tirana (TIR), Athens (ATH), Istanbul (IST), Izmir (IZM). Clinical questionnaires, skin prick tests (SPT) and serum IgE analyses with a customized immunoblot multi-parameter test were performed.

Results: 209/348 (60%) children and 353/467 (76%) adults suffered from moderate-severe, persistent SAR (ARIA classification) starting at 7 ys (median; IQR 6) and 18 ys (median; IQR 14), respectively. Asthma prevalence ranged from 14% (TIR) to 47% (ATH, IZM). Urticaria, asthma unrelated to pollen, and atopic dermatitis were the most common comorbidities. Poly-sensitization (IgE to major allergens of 3 or more pollen) was observed in 418 of 815 (51%) participants, who were most often sensitized to grass, cypress and olive pollen. The allergenic molecules most frequently recognized by IgE were Phl p 1/Phl p 5 in POR, ROM, TIR, IST, IZM, Cup a 1 in MAR), Ole e 1 in VAL, Cyn d 1 in ATH and Par j 2 in MES. The prevalence of serum IgE to panallergens (profilins, nsLTP and polcalcins) ranged from 7.5% in MAR to 59,6% in ROM.

Conclusion: The clinical characteristics and molecular sensitization profiles of SAR-patients in the @IT2020 study show a wide heterogeneity in different regions of Southern Europe. Our results suggest that local aerobiological and epidemiological scenarios must be considered when designing diagnostic tools, trials and guidelines for SAR and SAR-related AIT prescription.

0897 | Expect the unexpected: Allergic reactions to horse chestnut are triggered by sensitization to Art v 1 from mugwort pollen

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Background: Allergic reactions upon skin contact and accidental swallowing of horse chestnuts (*Aesculus hippocastanum*) during handcrafting were reported in day cares. We aimed to identify the underlying allergenic molecule and respective pollen source responsible for primary IgE sensitization.

Method: Austrian horse chestnut/weed pollen allergic patients were tested for skin reactivity using fresh horse chestnut and commercial mugwort pollen extract. IgE reactivity to horse chestnut and mugwort pollen was determined in immunoblot and ELISA. A 10-kDa protein was purified from horse chestnut and structurally analysed by mass spectrometry and circular dichroism. IgE reactivity and cross-inhibition potential to purified allergens was determined by ELISA.

Results: Patients with adverse reactions to horse chestnut were diagnosed positive for the allergen source. Approximately half of mugwort pollen allergic patients also presented positive reactivity to horse chestnut in prick-to-prick tests. Of the 24 horse chestnut positive sera, 56% were positive in ELISA using an in-house horse chestnut extract. An IgE reactive protein migrating at 10 kDa in immunoblots was identified as antimicrobial protein AhAMP1 using mass spectrometry. This defensin-like protein demonstrated 58-60% sequence identity and high structural similarity to allergenic defensin-proline linked proteins from weed pollen, i.e. Art v 1. Based on results obtained by circular dichroism, purified natural AhAMP1 is a highly thermostable protein with full capacity to renature upon heat treatment. All horse chestnut sensitized patients' sera were tested positive to AhAMP1 and Art v 1, also showing highly correlating IgE levels ($r = 0.891$). IgE inhibition ELISA revealed that Art v 1 potently inhibited binding to immobilized AhAMP1. In contrast, the inhibitory capacity of the horse chestnut defensin was only 35% suggesting that primary sensitization occurred through Art v 1 from mugwort pollen.

Conclusion: AhAMP1 from horse chestnut represents a novel potent allergen due to its structural stability and IgE cross-reactivity with Art v 1. Thus, mugwort pollen allergic patients are at risk to develop allergic symptoms against horse chestnuts. In addition, recent use of horse chestnuts as active ingredients in cosmetics, medicinal lotions and washing agents might trigger unforeseen and so far difficult to assign adverse reactions.

1075 | New allergens derived from Ara h 1 and Jug r 2: molecular characterization of the cross-reactivity between the N-terminal leader sequences

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Background: The vicilins from peanut and walnut are considered major allergens and are translated with leader sequences (LS) that are cleaved before yielding the mature protein. These LS were thought to be degraded and unstructured, but previous work suggested they might contain immunoreactive epitopes, and possibly be a source of cross-reactivity despite very low overall sequence identity, typically less than 20%.

Method: Linear IgE epitopes were identified using microarray data generated by printing 15-mer peptides offset by 5 amino acids on glass slides. IgE binding by peanut and walnut allergic sera was detected with a fluorescently-labeled antibody. Western blots and mass spectrometry were used to show the presence of the LS in peanut and walnut seeds. The NMR structures of the Ara h 1 LS (A1LS), and the three fragments of the Jug r 2 LS (J2LS-1, 2, & 3) were determined and the IgE binding sites were modeled on the structures.

Results: The epitopes with the highest degree of IgE binding were clustered within regions that were near cysteine residues. Of the peanut allergic patients tested, 96% showed IgE binding to those epitopes even if they recognized no other epitopes in the A1LS. By comparing immunodominant epitopes from peanut and walnut exclusive allergic patients, there are patterns in the cross-reactivity for J2LS-3, which may be diagnostic of the sensitizing allergen.

The NMR structures of all the LS showed 4 of the cysteine residues are disulfide bonded and hold together two parallel alpha helices, in contrast to predictions that these regions might be disordered. The structures of all 4 LS are strikingly similar, which may explain the observed cross reactivity.

Conclusion: The results indicate that cysteine residues known to confer high structural stability to allergens may also coincide with areas of increased IgE binding frequency and intensity in Ara h 1 and Jug r 2 LS. The leader sequence contains multiple immunodominant epitopes and appears to be important for cross-reactivity and nut allergy.

1280 | Seafood allergy in the Asia-Pacific – current advances in diagnosis and management

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Background: Seafood allergy affects up to 6% of adults and children worldwide. The evolutionary and taxonomic diversity of consumed seafood species, particularly in the Asia-Pacific region, poses a challenge for reliable diagnostics. Detailed understanding of seafood allergy prevalence, responsible species and their allergen repertoire is urgently required for improvement of patient management.

Method: The prevalence of seafood allergy was determined in Vietnam using a cross-sectional population-based study in over 25,000 adult and pediatric participants. The IgE reactivity to different seafood species was investigated in over 220 Australian and 50 Vietnamese participants. 120 adult and 100 paediatric patients were skin-tested to 10 representative species. IgE reactivity was analyzed to raw and heated extracts of 91 seafood species. SPT preparations from six different manufacturers, covering over 20 seafood species, were analyzed for the presence and abundance of allergens. The transcriptome of shrimps, barramundi, cod and tuna were analyzed for all putative allergens using the WHO/IUIS Allergen Nomenclature and AllergenOnline.

Results: Prevalence of doctor-diagnosed food allergy in Vietnam was higher in children (6.7%) than adults (5.6%), leading by crustacean (>50%), followed by fish and mollusk. Under-reported allergens were identified and characterized, including collagen and tropomyosin in several fish species. Most patients allergic to bony fish seem to tolerate cartilaginous fish. Heating of shellfish extracts increased their IgE binding and basophil activation. Transcriptomic analysis revealed up to 40 unreported allergens in different shrimp species, including heat shock protein, chymotrypsin, and beta-enolase. Commercial SPT solutions demonstrated variable levels of seafood allergens.

Conclusion: The prevalence of seafood allergy in Vietnamese and Australian children and adults seem is higher than previously reported from the Asia-Pacific region, implying different eating habits contributing to sensitization patterns. The largest cohort of seafood-allergic individuals demonstrated distinct, previously unreported allergens. Some pan-allergens, including parvalbumin, tropomyosin

and arginine kinase, induced considerable immunological and clinical cross-reactivity. Our studies lead to better component-resolved diagnostic methods and urgently needed individual management and treatment of seafood allergy, including component-resolved immunotherapy.

OAS 07
Functional Genomics in Respiratory Diseases: Allergy and Beyond

0108 | Genome-wide association studies on individuals from han chinese background reveal novel genes as potential risk factors for allergic rhinitis

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Background: Allergic rhinitis (AR) affects about 30% of the world's population and causes poor quality of life and a heavy economic burden due to ineffective therapies. Genetic factors play a significant role in the manifestation of AR, but current data are limited. In this study, we used the genome-wide association study (GWAS) approach to identify genotypic markers that were risk factors in the development of AR within individuals of Han Chinese background.

Method: The protocols used in this study have been approved by IRB of the National University of Singapore (NUS07-023, NUS10-343) and samples were collected with written consent from participants. GWAS was carried out on 2146 cases (AR) and 2039 controls (non-AR) of Han Chinese individuals to identify potential single nucleotide polymorphisms (SNPs) associated with AR. Functional predictions were then performed using bioinformatics tools.

Results: Of the 5,215,687 SNPs (genotyped and imputed), 86 SNPs were identified to be potentially associated with AR in Han Chinese individuals. These SNPs were further refined and clustered into 6 quantitative trait locus (QTL) regions, which contained eleven genes that were associated with risk of AR. Only two genes (SMAD2 and BMPR1B) have been previously shown to be associated with allergic rhinitis and asthma respectively, while the remaining 9 genes were novel in their association with AR. We identified 31 SNPs in SMAD2 associated with AR. In comparison to previous reports, only one of these SNPs was linked to cardiorespiratory risk factors (within the British population) and asthma (within the European and Australian populations). The functions of the remaining 30 SNPs in AR are novel and remain to be investigated.

Conclusion: We identified 86 SNPs, clustered into 6 QTL regions, as the candidate genotypic markers that are risk factors of AR in Han Chinese population. These SNPs were located within eleven genes, of which the majority of the SNPs and genes identified were novel in their links to AR. A better understanding of the functionality of the SNPs on these genes would improve our knowledge on the

underlying mechanisms that lead to the development of AR. These genes could be targeted for early diagnostics or targeted therapies in the future.

0301 | Shaping lifelong immune health: A network-based strategy to predict and prioritize markers related to immune interventions in early life

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Background: A healthy immune status is strongly conditioned during early life stages. Insights into the molecular drivers of early life immune development are prerequisite to identify strategies to enhance immune health. In this study a network-based strategy was used to predict and prioritize markers to assess effects of early life immune modulation

Method: An inventory of relevant literature (till Jan. 2019) regarding 6 immune developmental periods (1st/2nd/3rd trimester of gestation, birth, newborn (0-28 days), infant (1-24 months)) was made using Scopus and PubMed. The text mining tool INDRA extracted and structured relevant entities (genes/proteins/metabolites/ processes/diseases) from the full texts resulting into 6 early life-immune causal networks. These 6 networks were denoised using GeneMania, enriched with data from DisGeNET and Gene Ontology, inferred missing relationships and added expert knowledge to generate information-dense early life immune networks. Finally, the 6 networks were subjected to the PageRank centrality algorithm to identify the key genes in the networks

Results: In total 829 articles were considered relevant after screening of which INDRA extracted resp. 2101, 3234, 3654, 1568, 2917 and 1487 unique relationships between entities, resulting in 6 large causal early-life immune networks each covering a different early life period. Gene enrichment steps further increased the number of gene-bioprocess and gene-disease relationships (range 5816-16082 unique relationships). In addition, the inference steps added 1529-3343 relationships to the early life immune networks.

PageRank analysis not only confirmed the central role of the usual suspects (chemokines, cytokines, other immune regulators (e.g. *CD55*, *FOXP3*, *GATA3*, *CD79A*, *C4BPA*), but also identified less obvious key marker candidates (e.g. *CYP1A2*, *FOXK2*, *NELFCD*, *RENBP*). Comparison of the different early life periods, resulted in the prediction of 11 key early life genes overlapping all early life periods (*TNF*, *IL6*, *IL10*, *CD4*, *FOXP3*, *IL4*, *NELFCD*, *CD79A*, *IL5*, *RENBP* and *IFNG*), and also genes that were only described in certain early life period(s)

Conclusion: Here we describe a promising network-based approach that provides a science-based and systematical way to explore the functional development of the early life immune system in time. This systems approach aids the generation of a testing strategy of early life immune modulation by predicting the key candidate markers during different phases of early life immune development.

0901 | FCER1A promoter variants confers susceptibility to allergic asthma by modulating immune subsets classical monocytes and pDC

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Background: Genetic variants on chromosome 1 have been identified as harboring variants associated with IgE levels and recently as an allergy susceptibility locus. However, the candidate gene at this locus has not been validated.

Method: In this study, we use data from a mega-eQTL consortium and 2 independent functional cohorts to validate the candidate gene for this susceptibility locus and identify relevant cell subsets. mRNA gene expression data and genotype for SNPs from eQTLgen consortium was analyzed for eQTL associations. For the Singapore Systems Immunology cohort (SSIC), FCER1A protein levels in various immune subsets was determined by flow cytometry and genotyping data obtained from Illumina Omni2.5 chip. Gene expression data from SSIC and BAMSE cohort were extracted from whole transcriptome chips and associated with asthma phenotype.
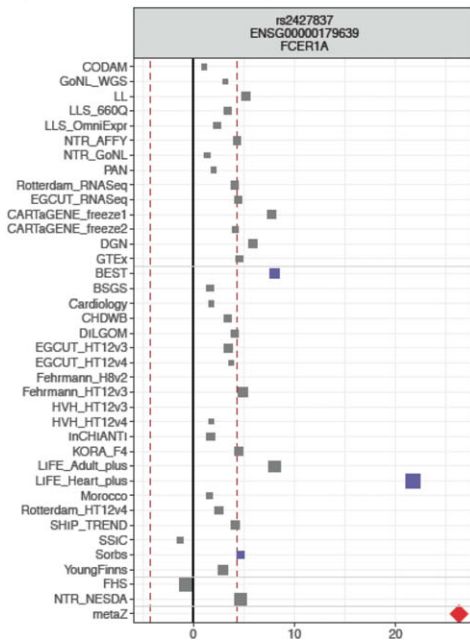
Results: We first used data from the eQTLgen consortium with gene expression in whole blood from 31,430 samples across 35 cohorts along with the genotype for rs2427837 to establish that *FCER1A* gene had the strongest association (Figure 1A). We then wanted to validate this association at the protein level using flow cytometry. We used the Singapore Systems Immunology Cohort (SSIC) (N = 135) and found significant association of FCER1A promoter SNP 2427837 to FCER1A protein levels in monocytes and not to basophils, eosinophils or lymphocytes (Figure 1B-E).We then validated this finding by analyzing additional samples with a more comprehensive flow cytometry panel that allowed to gate out the four major FCER1a-expressing immune cell subsets monocytes, plasmacytoid DC (pDC), myeloid DC (mDC) and basophils. We found association only to classical monocytes and pDC, however with opposite trends ($P = .0006$, $P = .03$ respectively). We also show FCER1A mRNA levels to be significantly differential for asthma phenotype in BAMSE and SSIC cohort ($P = .0086$, $P = .04$ respectively).

Conclusion: Our study validates the candidate gene for this chromosome 1 allergy susceptibility locus as FCER1A and the relevant subsets as classical monocytes and pDC. We propose genetic regulation
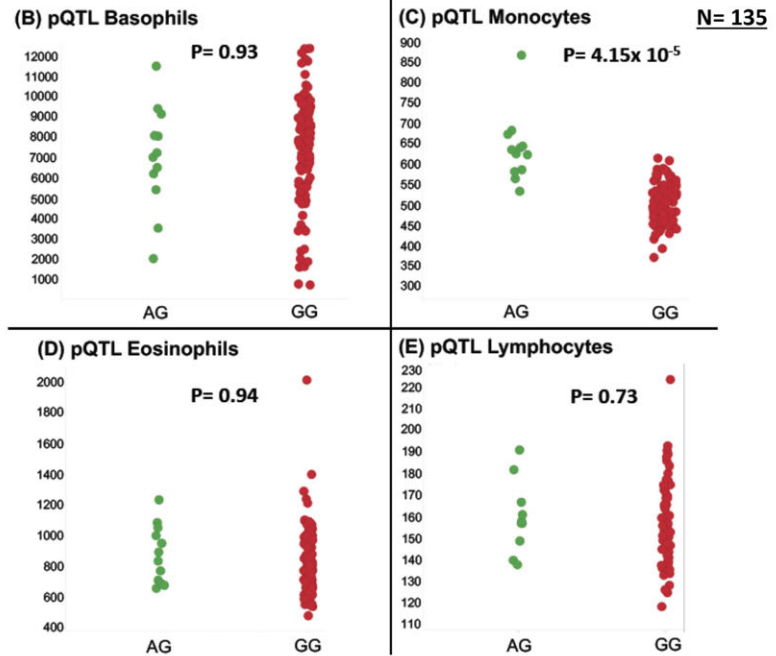
in immune subsets control asthma susceptibility. However, further studies aiming at combining the genetics and immune characterisation of FCER1A in cell subsets would help elucidate the contribution of each factor to disease. This might also help in providing targeted therapy and fits in line with the overall aim of treating every patient through a personalized approach.

(A) eQTLGen Figure (35 cohorts, N = 31430)

Association of rs2427837 genotype to FCER1A protein levels in SSIC

(B) pQTL Basophils — P= 0.93

(C) pQTL Monocytes — N= 135 — P= 4.15x 10⁻⁵

(D) pQTL Eosinophils — P= 0.94

(E) pQTL Lymphocytes — P= 0.73

1122 | Personalised immunoinflammatory phenotypes of children with acute viral bronchiolitis

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Background: A subset of infants are hyper-susceptible to acute/severe viral bronchiolitis(AVB), for reasons incompletely understood. Studies in infants have been limited and mainly restricted to circulating cells, and systems-level studies of the underlying mechanisms are urgently required.

Aim: Characterise cellular/molecular mechanisms underlying infant AVB.

Method: PBMC and nasal scrapings were obtained from infants(<18mths) and children(>18mths-5 years) during AVB and post-convalescence. Immune response patterns were profiled by multiplex analysis of plasma cytokines, flow cytometry, and transcriptomics (RNA-Seq). Molecular profiling of group-level data utilised a combination of upstream regulator and coexpression network analysis, followed by individual subject-level data analysis employing personalised N-of-1-pathways methodology.

Results: Group-level analyses demonstrated that infant PBMC responses were dominated by monocyte-associated hyper-upregulated type1 interferon signalling/pro-inflammatory pathways (drivers: TNF, IL6, TREM1, IL1B), *vs* a combination of inflammation (PTGER2, IL6) plus growth/repair/remodelling pathways (ERBB2, TGFB1, AREG, HGF) coupled with Th2 and NK-cell signalling in children. Age-related differences were not attributable to differential steroid usage or variations in underlying viral pathogens. Nasal mucosal responses were comparable qualitatively in infants/children, dominated by interferon types 1-3, but the magnitude of upregulation was higher in infants (range 6-48-fold) than children (5-17-fold). The most complex/intense response profiles were observed in infants manifesting febrile symptoms. N-of-1-pathways analysis confirmed differential upregulation of innate immunity in infants and NK cell networks in children, and additionally demonstrated covert AVB response sub-phenotypes that were independent of chronological age.

Conclusion: Dysregulated expression of interferon-dependent pathways following respiratory viral infections is a defining immunophenotypic feature of AVB-susceptible infants, particularly those who develop fever at infection, and is also observed in a subset of children. Susceptible subjects appear to represent a discrete subgroup who cluster based on (slow) kinetics of postnatal maturation of innate immune competence.

1537 | **Mechanisms of airway remodeling unique to eosinophilic asthma: Insights from differential gene co-expression analysis**

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Background: Heterogeneity of asthma makes search for targeted treatment against airway hyperresponsiveness and remodeling difficult. Gene co-expression analysis may elucidate differences in pathogenesis of specific asthma phenotypes. We conducted a systems biology approach study to establish a list of genes differentially co-expressed between eosinophilic and non-eosinophilic asthma patients and infer their possible role in the disease.

Method: A group of N = 40 non-smoking asthma patients (N = 20 presenting as eosinophilic asthma, i.e. >= 1% eosinophils in bronchoalveolar lavage fluid at the time of sample collection). None of the patients had active neoplastic disease, hyperthyroidism, heart failure, autoimmune disease (e.g. Churg-Strauss syndrome), hepatic or renal disease.

Peripheral blood of the patients was evaluated for mRNA expression using hybridization to cDNA microarray Human MI ReadyArray™ and signals were detected with Innopsys ® Mapix software, v. 6.0.1, resulting in expression profiles of 33519 gene products. Genomic data were analyzed with Bioconductor v.3.7. software of the R environment v.3.5.0. Differential co-expression was established using CoXpress R plugin. Biological interpretation of gene co-expression patterns was supported by Gene Ontology (GO), KEGG (Kyoto Encyclopedia of Genes and Genomes) and DO (Disease Ontology) databases through visual analytics platforms Cytoscape and NetworkAnalyst 3.0.

Results: 32 genes with the lowest differential gene co-expression *P*-value were chosen for subsequent analyses. Among them, candidate genes which regulation and action may differentiate airway remodeling mechanisms in eosinophilic asthma as compared to non-eosinophilic asthma bear significance in the following areas: cytomegalovirus infection (e.g. ATP1B1), Th2 differentiation (e.g. CLC, PSG2), cell proliferation and migration (RAPH1), production of inflammatory mediators (SRPRB), exocytosis (CABP5), smooth muscle contraction (OR52I1), deposition of extracellular matrix (RECK), myofibroblast differentiation (CCT7), angiogenesis (GPI).

Conclusion: Genes bearing known significance in asthma pathogenesis along with candidate genes of tentative role in the disease were established in regard to a condition cognizable in clinical practice.

OAS 08
Atopic Dermatitis: New Insights

0277 | **Sleep patterns and development of children with atopic dermatitis**

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Background: Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin disease that begins in early childhood. Sleep problems have increased in children with AD because of itching and scratching which interfere with falling asleep and maintenance of sleep. Sleeping is necessary for the cognitive and behavioral development of the child. The aim of this study was to evaluate sleep patterns and the development of children with AD at an early age.

Method: A total of 80 children aged 0-36 months with AD were evaluated. The sleeping pattern of the participants was evaluated with the Turkish version of the Brief Infant Sleep Questionnaire (BISQ), and the developmental characteristics of the patients were evaluated with the International Guide for Monitoring Child Development (GMCD).

Results: The median age (IQR) of the patients was 6 (4.25-9) months, 63.7% of them were male. According to the BISQ questionnaire, 50% of the patients with AD had sleep problems. Sleep problems were more frequent in patients with moderate-severe AD (*P* = .024). There was a positive correlation both between the SCORAD index and the number of night wakings(*P* = .009 *r* = 0.289)and between the SCORAD index and nocturnal wakefulness time (*P* = .002 *r* = 0.347). Male sex (OR: 3.78, *P* = .024, 95% CI, 0.083-0.837), patients with AD who were in the first 3 months of diagnosis(OR: 3.56, *P* = .020, 95% CI, 1,220-10,431) and the moderate-severe AD (OR: 5.09, *P* = .005, 95% CI, 1,649-15,748) were determined as risk factors for sleep problems. In 40 patients with sleep problems, only 65% of them were described to have sleep problems according to their mothers' statements. Totally, 12.5% of the patients needed developmental support. Developmental delay was higher in patients with sleep problems (*P* = .037). Multiple siblings (OR: 14,381, *P* = .019, 95% CI, 1,557-132,871) and the presence of sleep problems (OR: 8,011, *P* = .024, 95% CI, 1,764-36,387) were found to be risk factors for developmental delay.

Conclusion: Sleep patterns and development of children with AD may be affected at an early age. Mothers' perception was good but insufficient in detecting sleep problems. Boys with moderate-severe

AD within the first 3 months after the onset of symptoms were at increased risk of sleep problems. Children with AD who have multiple siblings and sleep problems should be evaluated for developmental delay and monitored closely in terms of developmental delay, particularly in the area of gross motor skills.

0379 | Anti-inflammatory activities of IL-37b on atopic dermatitis via regulating AMPK-mTOR and intestinal metabolites-dependent autophagy

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Background: Interaction between eosinophils and dermal fibroblasts is essential for allergic inflammation in atopic dermatitis (AD). Autophagy plays a vital role in regulating immunity via distinct intracellular signaling mechanism.

Method: Human eosinophil and dermal fibroblast co-culture with AD-related cytokine IL-31/IL-33 stimulation, and MC903-induced AD murine model were employed to investigate the anti-inflammatory mechanism of regulatory IL-1 family cytokine IL-37, by using the perspective of molecular biology, intestinal bacterial diversity and metabolites.

Results: IL-37 inhibited IL-6, CXCL8, CXCL9 and CXCL10 production *in vitro*, and upregulated autophagy-related LC3B and AMP-activated protein kinase (AMPK) and down-regulated mammalian target of rapamycin (mTOR) expression *in vitro* and *in vivo*. In CRISPR/Cas9 human IL-37b knock-in mice, IL-37b could also decrease ear tissue swelling and the expression of inflammatory cytokines and chemokines including IL-4, CCL2, IL-17A and transforming growth factor-β, itching sensation and eosinophil infiltration. Furthermore, IL-37b could indirectly enhance *in vivo* autophagy by regulating intestinal metabolites such as 3-methyladenine, adenosine monophosphate and 2-hydroxyglutarate, and suppress inflammation by inhibiting innate immune response via the modulation of intestinal metabolite cholic acid and gut microbiota.

Conclusion: In summary, IL-37b could significantly ameliorate eosinophils-mediated allergic inflammation by enhancing its autophagy and suppressing inflammatory response via regulating intestinal bacteria and their metabolites in AD. Results therefore suggest IL-37 is a promising anti-inflammatory cytokine for AD treatment.

0429 | Long-term dupilumab treatment for 100 weeks reduces serum TARC and total IgE in patients with moderate-to-severe atopic dermatitis

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Background: Thymus and activation-regulated chemokine (TARC) is expressed by Th2 cells and is implicated in the pathogenesis of atopic dermatitis (AD). In most patients with moderate-to-severe AD, TARC and total IgE concentrations are elevated and correlate with disease severity. Dupilumab, a fully human monoclonal antibody, blocks the shared receptor component for interleukin (IL)-4 and IL-13, key and central drivers of type 2 inflammation in multiple diseases. Here we assess the impact of long-term dupilumab treatment on TARC and IgE concentrations in adults with moderate-to-severe AD enrolled in a phase 3 open-label extension (OLE) trial.

Method: LIBERTY AD OLE (NCT01949311) assessed long-term safety and efficacy of dupilumab 300 mg weekly in adults with moderate-to-severe AD who had previously participated in controlled dupilumab clinical trials (parent studies). We present TARC and total IgE data from a subset of patients treated for at least 100 weeks. Data were analyzed descriptively based on available samples at individual timepoints.

Results: At the cut-off date for this analysis (December 1, 2018), 288 patients had both TARC and IgE assessments for 100 weeks of treatment: 57 dupilumab-naive patients; 226 re-treated patients (≥ 13 weeks between last dupilumab dose in parent studies and first dose in the OLE); and 5 patients with continuous treatment (data not shown). Median values of TARC and total IgE at the baseline of the parent studies and the OLE study are shown in the Table. The median percent reduction in TARC concentration from baseline of parent study for dupilumab re-treated and dupilumab naive was −73.5% and −64.3% at Week 2, reaching −84.7% and −91.1% at Week 100, respectively (Table). The median percent reduction in total IgE was −45.1% and −5.1% at Week 4, reaching −88.1% and −84.6% at Week 100, respectively (Table). The safety profile was consistent with previously reported safety data for dupilumab.

Conclusion: Long-term dupilumab treatment, up to 100 weeks, led to a substantial and sustained reduction in TARC and total IgE, both in patients previously treated with dupilumab and in dupilumab-naive patients.

TARC and total IgE assessment in patients with both TARC and IgE data available at Week 100

	Dupilumab re-treated (n = 226)	Dupilumab naive (n = 57)
Median (IQR) TARC at baseline of parent study, pg/mL	2,281.6 (1,147.2 to 6,069.0)	2,781.1 (1,155.1 to 11,917.0)
Median (IQR) TARC at baseline of OLE, pg/mL	1,393.5 (694.2 to 2,907.1)	1,830.1 (853.7 to 6075.3)
Median (IQR) percent change from baseline of parent study in TARC at Week 2 of OLE	−73.5% (−84.7% to −51.0%)	−64.3% (−88.4% to −45.3%)
Median (IQR) percent change from baseline of parent study in TARC at Week 100 of OLE	−84.7% (−94.0% to −69.6%)	−91.1% (−96.2% to −62.5%)
Median (IQR) IgE at baseline of parent study, kU/L	2,959.0 (447.0 to 10,000.0)	4,160.0 (754.0 to 10,000.0)
Median (IQR) IgE at baseline of OLE, kU/L	1,478.0 (281.0 to 4,225.0)	2,357.4 (359.8 to 5,781.1)
Median (IQR) percent change from baseline of parent study in IgE at Week 4 of OLE	−45.1% (−56.7% to −17.6%)	−5.1% (−30.5% to 33.1%)
Median (IQR) percent change from baseline of parent study in IgE at Week 100 of OLE	−88.1% (−93.3% to −80.8%)	−84.6% (−91.4% to −78.9%)

IQR, interquartile range.

1115 | Worldwide survey shows that atopic dermatitis is associated with a high disease burden in adolescents

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Background: Information on the disease burden of atopic dermatitis (AD) in adolescents is lacking. This survey describes patient-reported AD burden across severity strata worldwide.

Method: This cross-sectional, web-based, self-report survey of children aged 0.5 to < 18 years was conducted in 18 countries in 5 regions. Parents were invited to participate without knowledge of the survey topic; parents and children responded. Quotas were set for age, sex, region (and urban/rural split in some countries) and a weighting adjustment applied to obtain a representative population for each country. We present disease burden results from adolescents (12 to < 18 years) here. Adolescents were categorized as having AD if: they self-reported ever being diagnosed with AD (eczema) by a physician AND ever had an on and off itchy rash for ≥ 6 months AND had this rash in the past 12 months AND it was located in the elbow folds, behind the knees, in front of the ankles, under the buttocks or around the neck, ears or eyes. Severity of their AD in the past week was rated by patient global assessment (PtGA) as clear/mild, moderate or severe. Adolescents reported the impact of their AD on itch, sleep and pain in the past 24 hours using numerical rating scales (0 = none to 10 = most severe). Health-related quality of life (HRQoL) in the past week was assessed using the Children's Dermatology Life Quality Index (CDLQI) (0 = no effect to 30 = largest effect). Adolescents also reported atopic comorbidities and days missed from school in the past 4 weeks for AD-related reasons.

Results: Among 3078 adolescents with diagnosed AD, 56.2% had clear/mild AD, 37.9% moderate AD, and 5.7% severe AD. Overall mean±SD (median) scores by AD severity (clear/mild / moderate / severe) were 9.5 ± 7.5 (9)/14.7 ± 6.9 (15)/21.3 ± 7.6 (22) for CDLQI; 3.8 ± 2.7 (4)/6.0 ± 2.3 (6)/7.6 ± 2.4 (8) for itch; 3.5 ± 2.8 (3)/5.5 ± 2.6 (6)/7.2 ± 2.4 (8) for sleep impact and 3.6 ± 2.8 (3)/5.6 ± 2.4 (6)/7.3 ± 2.5 (8) for pain (Table). Most adolescents reported ≥ 1 atopic comorbidity (93.0%/95.1%/98.3%). Many reported missing ≥ 1 school day in the past 4 weeks (71.7%/86.7%/87.0%) for mean±SD (median) of 4.7 ± 5.6 (3)/8.0 ± 7.1 (6)/12.1 ± 8.7 (10) days (Table). Regional data are shown in the Table.

Conclusion: AD is a multidimensional condition that affects HRQoL in adolescents. Disease burden reported by adolescents with moderate or severe AD was substantial across multiple domains, including CDLQI, symptoms (itch, sleep, pain), atopic co-morbidities and missing school.

	Self-report by adolescents with clear/mild / moderate / severe AD[a]					
	Europe[b]	North America[c]	Latin America[d]	Eurasia/Middle East[e]	Asia[f]	Overall
n (unweighted base)	650 /368 /52	214 /164 /35	495 /332 /46	246 /187 /23	167 /92 /4	1772 /1143 /160
CDLQI (past week) (0 = no effect on HRQoL to 30 = largest effect on HRQoL)	7.0 ± 7.0 (5) /13.7 ± 7.1 (13) /20.0 ± 6.8 (20)	8.0 ± 8.1 (4) /13.8 ± 7.5 (13) /21.8 ± 7.1 (23)	10.1 ± 6.8 (10) /15.4 ± 6.6 (16) /22.8 ± 6.4 (24)	12.7 ± 8.0 (11) /15.1 ± 6.3 (15) /22.8 ± 6.4 (24)	4.9 ± 5.6 (3) /11.1 ± 7.5 (11) /22.8 ± 8.6 (30)	9.5 ± 7.5 (9) /14.7 ± 6.9 (15) /21.3 ± 7.6 (22)
Itch intensity (last 24 hours/today) (0 = no scratching/itching to 10 = worst scratching/itching possible)	3.2 ± 2.6 (3) /5.6 ± 2.2 (6) /7.7 ± 1.7 (8)	3.3 ± 2.7 (3) /5.7 ± 2.4 (6) /7.8 ± 1.8 (8)	3.9 ± 2.7 (4) /6.0 ± 2.4 (6) /7.6 ± 2.5 (8)	4.6 ± 2.7 (5) /6.2 ± 2.2 (6) /6.9 ± 3.1 (8)	3.1 ± 2.6 (3) /5.6 ± 2.4 (6) /7.7 ± 3.2 (10)	3.8 ± 2.7 (4) /6.0 ± 2.3 (6) /7.6 ± 2.4 (8)
Sleep quality (previous night) (0 = best sleep possible to 10 = worst sleep possible)	2.8 ± 2.6 (2) /5.2 ± 2.3 (5) /7.4 ± 1.7 (8)	2.9 ± 2.7 (2) /5.4 ± 2.5 (5) /7.0 ± 2.0 (7)	3.8 ± 2.7 (4) /5.8 ± 2.6 (6) /7.8 ± 2.3 (8)	4.4 ± 2.8 (4) /5.5 ± 2.6 (6) /6.1 ± 3.1 (7)	2.4 ± 2.3 (2) /4.7 ± 2.8 (5) /8.2 ± 2.2 (10)	3.5 ± 2.8 (3) /5.5 ± 2.6 (6) /7.2 ± 2.4 (8)
Pain intensity (last 24 hours/today) (0 = no pain to 10 = worst pain possible)	3.0 ± 2.7 (2) /5.4 ± 2.2 (6) /7.2 ± 1.9 (7)	2.9 ± 2.9 (2) /5.3 ± 2.3 (5) /7.5 ± 1.4 (8)	3.8 ± 2.8 (4) /6.0 ± 2.4 (6) /7.6 ± 2.5 (8)	4.4 ± 2.8 (4) /5.6 ± 2.5 (6) /6.3 ± 3.4 (7)	1.9 ± 2.4 (1) /4.7 ± 3.0 (5) /6.6 ± 2.8 (7)	3.6 ± 2.8 (3) /5.6 ± 2.4 (6) /7.3 ± 2.5 (8)
≥1 atopic comorbidity[g]	84.8 /89.2 /98.7	91.2 /91.2 /95.5	94.8 /97.0 /100	97.3 /99.7 /97.2	96.3 /90.2 /100	93.0 /95.1 /98.3
≥1 school day missed for AD-related reasons (past 4 weeks)	53.7 /79.6 /87.5	53.9 /71.3 /79.3	82.4 /94.0 /89.5	87.5 /95.3 /90.2	32.4 /54.5 /79.7	71.7 /86.7 /87.0
School days missed for AD-related reasons (past 4 weeks)	3.4 ± 5.4 (1) /7.3 ± 7.6 (5) /11.0 ± 9.5 (8)	3.3 ± 4.8 (1) /6.4 ± 7.4 (4) /10.2 ± 8.3 (10)	5.4 ± 5.1 (4) /9.0 ± 6.9 (7) /15.1 ± 8.5 (17)	6.3 ± 6.4 (4) /8.1 ± 6.4 (6) /8.6 ± 6.1 (8)	1.9 ± 4.6 (0) /4.8 ± 7.1 (2) /12.7 ± 10.9 (12)	4.7 ± 5.6 (3) /8.0 ± 7.1 (6) /12.1 ± 8.7 (10)

Data are mean±SD (median) or %.

[a] By PtGA during the past week.

[b] France, Germany, Italy, Spain, United Kingdom.

[c] Canada, United States.

[d] Argentina, Brazil, Colombia, Mexico.

[e] Israel, Kingdom of Saudi Arabia, Russia, Turkey, United Arab Emirates.

[f] Japan, Taiwan.

[g] Asthma, allergic rhinitis, allergic conjunctivitis, seasonal allergies, food allergies, chronic rhinosinusitis, nasal polyps, allergic urticaria, eosinophilic esophagitis or atopic keratoconjunctivitis.

1127 | Genetic polymorphisms of CD14 and TLR-4 and utility of probiotic therapy in adults with atopic dermatitis

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Background: The risk of atopic dermatitis development can be associated with genetic polymorphisms of the CD14 and TLR-4 genes. Probiotics may modulate chronic inflammation in atopic skin.

Method: The A-896G of the TLR-4 gene and C-159T polymorphism of the CD14 receptor gene polymorphism were studied in 96 patients with atopic dermatitis. The control group included 90 non-atopic subjects. To evaluate the efficacy of the probiotics all patients were divided into three groups. The first group was selected from patients with CC genotype (C-159T) who received standard therapy and probiotics. The second group included patients with CC genotype who received only standard therapy. The third group included patients with TT genotype (C-159T) who received standard therapy and probiotics. The SCORAD and DLQI parameters were evaluated on Day 0, 14 and 28. The level of IL-4, IL-5, IL-10, TGF-β cytokines was determined on Day 0 and Day 28.

Results: In the control group the frequency distribution of genotypes (AA – 71 (78.9%), AG – 18 (20.0%), GG – 1 (1.1%)) was significantly different ($\chi2 = 7.46$, $P = .024$) from those with atopic dermatitis (AA – 58 (60.4%), AG – 36 (37.5%), GG – 2 (2.1%)). In the control group the frequency distribution of genotypes (CC – 20 (22.2%), CT – 48 (53.3%), TT – 22 (24.5%)) was significantly different from that with

atopic dermatitis (CC – 39 (40.6%), CT – 37 (38.5%), TT – 20 (20.9%), χ^2 = 7.45, P = .024). The addition of probiotics to standard treatment significantly increased the effectiveness of treatment of atopic dermatitis in adults with the exogenous form and CC genotype (C-159T), confirmed by clinical and immunological parameters.

Conclusion: The risk of atopic dermatitis development and efficacy of probiotics therapy are associated specific genetic polymorphisms of CD14 and TLR-4.

1477 | Maternal serum lipids at birth are associated with eczema at age 1, 4, 10 and 18 years in offsprings

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Background: Eczema may be related to a deficiency in lipids. Unsaturated fatty acids are also used to treat eczema. Using an untargeted metabolomic profiling, the aim was to identify lipids in maternal serum that are linked to the development of eczema in their offspring.

Method: Isle of Wight (IoW) is a three-generation birth cohort. We analyzed maternal serum samples from the F0-generation of IoW and assessed their association with eczema in the F1 children repeatedly measured at age 1~2, 4, 10 and 18 years. Untargeted chemical analyses for maternal serum specimen collected at birth using liquid chromatography-mass spectrometry (LC-MS), were conducted to identify fatty acid components of lipids and acidic lipophilic compounds (negative ion mode). We applied generalized linear mixed models with repeated measurements to identify lipid compounds that are associated with eczema while adjusting age, gender, history of maternal and paternal eczema. In addition, effects of maternal lipids on offspring eczema may differ over the age of the offspring. Hence, interaction effects between lipid compounds and age (1 to 18 years old) were included in the explanatory models. If the interaction effects were not statistically significant (P > .05), we only tested the main effect of lipids on eczema with regardless of time.

Results: After correction for degradation, 2,286 lipid compounds were identified in maternal serum at birth after adjusting multiple testing using False Discovery Rate (FDR) methods. 226 lipid compounds were discovered to be associated with eczema, of which 196 lipid compounds were differentially associated with eczema over time and 30 lipid compounds were related to eczema at all age 1~2, 4, 10 and 18. Of these candidate lipids, the following known lipids were statistically significant: SM (d40:1) and 5-a-pregnane-3-, 20-a, 21-triol 3-sulfate. 5-a-pregnane-3-, 20-a, 21-triol 3-sulfate is a neuroactive steroid that binds with GABA receptor, which is known to be related to eczema.

Conclusion: Maternal metabolism of lipids or its inheritance may affect the skin permeability and might thus alter the susceptibility to eczema.

OAS 09
Asthma Biomarkers

0083 | Involvement of IL-5/eosinophils in acquisition of steroid resistance in a severe asthma model

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Background: Although corticosteroid is a main therapy for asthma, it has been known that 5-10% of asthma patients are resistant to the steroid therapy. However, mechanisms underlying the acquisition of steroid resistance have been unclear. On the other hand, we have reported that when sensitized mice were challenged with a high dose of antigen, asthmatic responses such as development of airway resistance were resistant to dexamethasone treatment. Objective of this study is to elucidate whether IL-5/eosinophils are involved in the acquisition of steroid resistance using the murine model of severe asthma.

Method: OVA-sensitized BALB/c mice were intratracheally challenged with OVA at a dose of 5 or 500 μg/animal four times. Infiltration of eosinophils and neutrophils into the lung were assessed by bronchoalveolar lavage 1 day after the 4th challenge. Development of airway remodeling such as airway epithelial thickening, mucus accumulation and subepithelial fibrosis were histologically evaluated by periodic acid Schiff attaining and Masson trichrome staining 1 day after the 4th challenge. Dexamethasone (1 mg/kg) and/or anti-IL-5 mAb (TRFK-5, 50 μg/animal) was intraperitoneally administered during the multiple challenges.

Results: (1) Infiltration of eosinophils and neutrophils into the lung, and the development of airway remodeling in the 5 μg OVA-induced model were significantly suppressed by dexamethasone. However, those asthmatic responses in the 500 μg OVA-induced model were not inhibited by dexamethasone. (2) Under the treatment with anti-IL-5 mAb, in which the eosinophil infiltration was selectively and strongly reduced, the steroid resistance on the development of airway remodeling in 500 μg OVA-induced model was partly but significantly cancelled.

Conclusion: It was suggested that mechanisms associated with IL-5/eosinophils are involved in the acquisition of steroid resistance in the severe asthma of mice. Clinical effectiveness of anti-IL-5 antibody and anti-IL-5Ralfa antibodies may be associated with improvement of sensitivity to corticosteroids in asthma patients.

0428 | Metabolic biomarkers from a severe food-pollen allergy model classify uncontrolled asthmatic patients

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Background: In a previous study from our group, statistically significant metabolites were found to be altered on a model of severe allergic inflammation using metabolomics. Those metabolites are related to energetic metabolism, lysophospholipid and sphingolipid metabolisms. We hypothesize that these metabolites should also be altered in other allergic inflammatory diseases, such as asthma. Asthma is a multifactorial disease characterized by airway hyper-responsiveness, obstruction, remodelling, and inflammatory infiltration. Target metabolomics offers a novel approach to evaluate a set of previously selected metabolites.

Method: Patients with asthma and sensitized to HDM were recruited and classified into two groups, according to their response to treatment, as controlled with inhaled corticosteroids (ICS group; n = 5) and uncontrolled (UC group; n = 5) asthmatic patients. Sera were collected and samples were analysed using liquid chromatography coupled to mass spectrometry with a triple quadrupole analyser (LC-QQQ-MS). 28 metabolites were measured, encompassing organic acids, fatty acids, amino acids and peptides, sphingolipids, carnitines, and lysophospholipids.

Results: The concentrations obtained from the 28 metabolites were represented in a non-supervised model, which showed a clear separation between ICS from UC asthmatic patients. Additionally, we observed that one patient from the ICS group was clustered close to the UC patients. Surprisingly, the revision of the clinical history showed this patient was suffering from HDM-asthma for more than 40 years. This fact assesses the robustness of the model. Moreover, after discriminant analysis of the groups using multivariate PLS-DA and OPLS-DA models, the separation between groups was confirmed. Univariate analysis between groups showed that 11 out of 28 metabolites were significantly different, including Lactic acid, Pyruvic acid, L-carnitine, Phenylalanine, LPC 18:0 and LPC 18:1, amongst others.

Conclusion: Our results show that this set of metabolites has a great potential to be used in the stratification of patients in allergic inflammation-related diseases. These findings support the hypothesis that all the inflammatory diseases might have both common and specific features. Nevertheless, further studies are needed to validate these findings.

0672 | Does shifting from a risk to a protective gut microbiome provide protection against allergic airway disease after house dust mite (HDM) challenge?

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Background: Previously, we showed that germ free mice transplanted with fecal microbiota from 3-month-old children that developed persistent eczema had higher responses to house dust mite allergen (HDM) than mice with mouse microbiota ([Mo]microbiota). This human risk microbiota ([Hu]microbiota) also impacted overall elastic properties of the airways in the mice including resistance and elastance. Here, we hypothesized that 1) an allergy-associated gut microbiome can be replaced by a protective microbiome, and 2) that, once established, this transplant could significantly improve lung function and decrease allergic responses after HDM challenge.

Method: C57BL/6 mice carrying a human allergic risk microbiota ([Hu]microbiota) acquired vertically from their parents were given antibiotic (ABX) treatment for 1 week, then they were either transplanted with a fecal slurry from the "protective" mouse microbiota ([Mo]microbiota) or sham inoculated with sterile saline to allow natural recovery of their gut microbiome after antibiotics were removed. Four weeks after transplant, all mice except for saline controls were given HDM allergen and tested for lung function and allergic airway responses. Six experimental groups were used: 1) [Mo]microbiota (no ABX, no transplant, saline), 2) [Mo]microbiota (no ABX, no transplant, HDM), 3) [Mo]microbiota (ABX, no transplant, HDM), 4) [Hu]microbiota (no ABX, no transplant, saline), 5) [Hu]microbiota (ABX, no transplant, HDM), and 6) [Hu]microbiota (ABX, [Mo]microbiota transplant, HDM).

Results: The "protective" [Mo]microbiota transplant successfully replaced the [Hu]microbiota "risk" microbiome and partially restored lung resistance and compliance and decreased the number of inflammatory cells in the lungs. Yet, the [Mo]microbiota transplant did not reduce all types of inflammatory cells or decrease IgE levels that can promote allergic responses.

Conclusion: We accepted our hypothesis that an allergenic gut microbiome can be replaced by a protective microbiome. However, lung function and inflammatory indices responded differently to a "protective" gut microbiota transplant. More work is needed to understand the mechanisms and timing underlying improvements and lack of improvements in allergic responses after transplant.

OAS 10
Clinical Aspects and Mechanisms of Drug Allergy

0620 | Profile of a case series of patients with adverse reactions to iron at a tertiary hospital in Madrid, Spain

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Background: Intravenous (IV) and oral Iron therapy are commonly used in anemia due to iron deficiency or malabsorption. This treatment is considered a safe procedure, but adverse events comprising hypersensitivity reactions could develop, although the former seldomly occur. We aim to present a case series of 10 patients who had suffered adverse reactions to iron therapy.

Method: This is a retrospective case series study, which evaluates patients who were under treatment with iron between 1994 and 2019. Data were retrieved by the use of a metasearch engine (Excalibur®) for electronic health records, available at our hospital. The following variables were assessed: 1. Age; 2. Gender; 3. Iron formulation; 4. Administration route (IV/oral); 5. Latency period (early/late phase); 6. Symptoms; 7. Provocation test/formulation of iron tested; 8. Desensitization/formulation of iron used.

Results: The mean age of the patients who developed iron adverse reactions (IAR) was 45.5 years old. The ratio of IAR was 5:1 (Females: Males). Iron formulations that cause IAR were with oral ferrous sulfate (n = 5), intravenous sucrose iron (n = 3), intravenous carboxymaltose iron (n = 1) and oral gluconate iron (n = 1). 70% of the IAR were immediate and 30% were late phase reactions. 100% of late IAR were with ferrous sulfate (n = 3); therefore, all immediate IAR were caused by other formulations (n = 7). The symptoms described were urticaria (n:8), angioedema (n:1), abdominal pain (n:1) and fever (n:1). A total of 10 provocations were made, 8 of them with the iron involved in the initial reaction (3+/5−) and 2 of them with other iron formulations (2-). A successful desensitization was performed with ferrous sulfate in the patient with positive provocation to this formulation.

Conclusion: 1. Ferrous sulfate was the culprit iron formulation involved in most cases and accounts for all the late phase reactions in our series.

2. Up to 50% of patients finally were diagnosed as iron hypersensitivity.

3. All patients were able to receive treatment successfully, by desensitization and controlled negative provocation approaches against alternative iron formulations.

1349 | Proactive penicillin de-labeling in pregnant patients: Safety and outcomes

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Background: 10% of the population report a penicillin allergy; however, up to 90% of these individuals are not allergic. Over half of all pregnant women require penicillin-based antibiotics during delivery. Pregnant patients with unconfirmed penicillin allergies receive alternative antibiotics which are less effective and safe, resulting in higher surgical site infection rates, longer hospital stays and exposure of broad spectrum antibiotics to the neonate. Few studies have assessed the safety of penicillin allergy evaluation in pregnancy. Our study is the first to focus on proactive penicillin allergy assessment during pregnancy.

Method: Pregnant patients delivering at B.C. Women's Hospital and Health Center are referred to our dedicated interdisciplinary penicillin allergy clinic. The study is divided into two phases. Patients with a clear non-allergic history were de-labelled on history alone. Phase one: all patients are skin tested; if negative, they undergo oral challenge to amoxicillin. Phase two: patients are risk stratified based on a structured questionnaire. Low risk patients are given a direct oral challenge. Patients at risk for an immediate receive skin testing followed by direct oral challenge if negative. For both, patients with positive skin tests are not oral challenged.

Results: Between July 5, 2019 and January 10, 2020, 48 patients were assessed. In phase 1 (4 months), 23 patients received skin testing followed by oral challenge, with all 23 being de-labelled. One patient was de-labelled through history alone. Phase 2 (3 months), 16 received direct oral challenge and 7 received selective skin testing based on history with subsequent challenge. 1 patient had no testing performed, as she had tolerated amoxicillin. No patients had positive skin tests, acute reactions to oral challenge or delayed rashes. All 48 were successfully de-labelled.

Conclusion: Rates of true penicillin allergy in pregnant patients are low, similar to the general population. Both universal skin testing followed by oral challenge and selective skin testing are viable strategies to approach penicillin allergy de-labeling during pregnancy. Selective skin testing is more cost effective. Direct oral challenge of low risk pregnant patients is a safe option, and ongoing data are being collected for a direct benefit of changes in antibiotic prescribing during birth.

1526 | Flexible and rigid dendrimer-based nanostructures for cell effector studies in the context of amoxicillin allergy

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Background: Dendrimers are monodisperse synthetic carriers that can be perfectly characterised. Amoxicilloyl-dendrimer conjugates have proved to be recognised by amoxicillin specific IgE. These IgE-antigen complexes may induce effector cell activation and thus an immediate allergic response. Herein we designed well-defined rigid and flexible multivalent nanostructures of different sizes decorated with amoxicilloyl units. We hypothesise that the distance between epitopes may be relevant for the immune complex morphology and effector cell degranulation.

Method: Flexible nanostructures were synthesised introducing polyethylene glycol (PEG) linkers (of different lengths) between two dendrons, whereas rigid nanostructures involved the use of dendrimers. The conjugates were accomplished by reaction of PolyAMidoAMine dendrimers/dendrons with amoxicillin (AX). Their chemical structures and dimensions were studied by Nuclear Magnetic Resonance. Allergenic activity was determined using cell activation assays. Mouse-bone-marrow-derived mast cells were passively sensitised with a mouse anti-AX IgE monoclonal antibody. Likewise, human mast cells were passively sensitised with sera from 3 AX-allergic and 3 AX-tolerant subjects. The degranulation assays were performed by stimulation of mouse or human mast cells with amoxicilloyl-dendrimer conjugates and measuring β-hexosaminidase or histamine release, respectively. An optimised negative-stain electron microscope was used to study the morphology of the antibody-nanoarchitecture complex.

Results: The rigid construct based on G_2-PAMAM with sixteen end valency did not result in a substantial marker release. However, the PEG-spaced architectures (containing PEG of molecular weight 6000, 8000 and 10000) displaying the same amoxicilloyl units as G_2-PAMAM have shown responses of greater than 15% β-hexosaminidase or 50% histamine release. Likewise, the larger generations of the rigid structures with 32, 64 and 128 end epitopes have also resulted in degranulation of sensitised cells. The immune complex shape was similar for the diverse nanostructures, showing the clustering of IgE according to the antibody:nanostructure ratio.

Conclusion: The results establish that besides the presence of the antigenic determinant, conjugate architectural features are important for cell degranulation. In fact, a cross-linking process involving a distance of 18 and 20 Å between epitopes of amoxicillin is optimal for effector cell activation.

OAS 11
Trends in Insect Venom Hypersensitivity

0601 | Adherence to hymenoptera venom immunotherapy: Real-life data from Austria

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Background: Hymenoptera venom allergy (HVA) is one of the most common causes of severe anaphylaxis in adults. Venom-specific immunotherapy (VIT) can reduce the risk for subsequent systemic reactions to hymenoptera venom. Adherence to VIT is of utmost importance for its effectiveness. The aim of this study was to evaluate the adherence to VIT in patients with hymenoptera venom allergy, and to identify the reasons that lead to premature discontinuation. Furthermore, we aimed to identify risk factors associated with non-adherence.

Method: In this cross-sectional questionnaire study, we enrolled 259 patients with HVA who had completed the buildup phase of VIT at our department between 2010 and 2016. All patients were informed in writing prior to a structured telephone interview. Data were collected on age, sex, culprit insect, tryptase concentration, degree of preceding sting reaction, preventive antiallergic medication during therapy, venom specific IgE concentration, duration of VIT, and reasons for premature discontinuation. Patients were considered as adherent to VIT when continued for at least 3 years or still ongoing at the time of the interview.

Results: One hundred ninety-five patients (75.3%) were treated with vespid venom, 51 patients (19.7%) received bee venom and 13 (5%) patients received both. Among those 195 patients (75.3%) completed the interview. Twenty-eight patients (14.4%) discontinued VIT before the 3-year treatment had ended. The most common causes for discontinuation were personal reasons followed by inconvenience, adverse reactions and lack of information about the foreseen duration of VIT. 52.3% (n = 102) of the study population underwent VIT over a time course of at least 3 years. 33.3% (n = 65) were still on treatment at the time of the interview. A patient age of less than 31 years was associated with premature discontinuation of VIT (P = .006; HR: 2.83; 95% CI: 1.35-5.97). On the other hand, frequent occupational and recreational exposure to hymenoptera was associated with a longer adherence to VIT (P = .011; HR: 0.16; 95% CI: 0.04-0.66).

Conclusion: Overall, adherence to VIT was high in this study population. Older patient age and frequent exposure to hymenoptera were associated with a longer adherence to VIT. Providing structured and understandable medical education, especially to younger patient populations, may be crucial to further increase patient adherence to VIT.

0647 | Venom immunotherapy in predominant Api m 10 sensitization: Does it work?

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Background: Honeybee sensitization profile might be of relevance for the success of venom immunotherapy (VIT). Some authors suggest than predominant IgE sensitization to Api m 10 can be a risk factor for treatment failure in honeybee VIT, since Api m 10 can be underrepresented in the extracts available for VIT and has demonstrated rapid degradation in venom extracts stored in solution.

Method: Patients who have been suffered a systemic reaction after a bee sting and diagnosed with *Apis mellifera* venom allergy were treated with *A. mellifera* VIT (Pharmalgen ALK-Abelló). Specific IgE (sIgE) against total bee venom and bee venom allergens (ImmunoCAP) was determined. VIT was administered using a build-up cluster schedule of 6 doses in 2 weeks interval (4+2). Systemic adverse reactions were recorded. VIT efficacy was assessed by outpatient honeybee sting challenge and/or field sting.

Results: Fifty-one patients were included: 43 man, 8 women with a median age of 46.2 years (IQR 35.6 - 54.6 years). Thirty-eight patients (74.5%) were sensitized to Api m 10. In half of them (37% of the total sample) the value of sIgE to Api m 10 was more than half the value of sIgE to the complete bee venom.

Only 3 patients developed a SAR with VIT and 15 out of 19 of those patients were stung (12 sting challenge, 3 field sting) without presenting any reaction, even though all of them were treated with the standard 100 µg dose.

Conclusion: We found no significant association between sIgE Api m 10 predominant sensitization and lack of efficacy of VIT. The use of freshly reconstituted therapeutic venom extracts could be a key point to avoid allergen losses and ensure treatment success in our patients.

0939 | The effect of specific Hymenoptera venom immunotherapy using the ultra-rush method on immunoregulatory properties of T and B Lymphocytes and serum cytokine concentration

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Background: The emergence of tolerance during Hymenoptera venom immunotherapy (VIT) using the ultra-rush method is a complex process associated with the simultaneous activation of several mechanisms at the T, B, and effectors cell level. Classical subcutaneous immunotherapy in the course of allergy to airborne allergens induces an anergy of specific T lymphocytes by demonstrating a decrease in T and B Lymphocyte proliferation under the influence of specific allergen stimulation. The immune mechanism of acquiring rapid tolerance to insect allergens during VIT has not yet been fully understood.

Method: We have analyzed concentration of 30 cytokines and chemokines in serum using Luminex system (e.g. IL-1b, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, G-CSF, IFN-g, IP-10, MCP-1, TNF-a, VEGF, TGF-b1, TGF-b2, TGF-b3), complement system (C3, C4, C5) and lymphocyte subpopulations using flow cytometry (CD3+, CD3− CD19+, CD3+CD4+, CD3+CD8+, CD3− CD16+CD56+, CD3+CD16+CD56+, CD3+HLA-DR, nTreg) of 40 patients with Hymenoptera venom allergy (III and IV Muller grade) classified to ultra-rush treatment in three-time points (before start of therapy, 2 and 6 weeks after treatment) as well as 40 volunteers as control group.

Results: We have found that VIT significantly influences the immune system by inducing changes in the complement system, cytokine secretion, and lymphocyte subpopulations. The study group at time 0 has a significantly higher percentage of T and B lymphocytes compared to the control group. What is more, the study group at time 0 is characterized by a decreased percentage of neutrophils. VIT caused a decrease in C3, C4, C5 activity in the study group. The study group is also characterized by decreased IL-4 and MCP-1 concentrations which did not change during the treatment. Patients with venom allergy have a higher percentage of CD3+CD4+helper T cells compared to the study group; however, the VIT leads to normalization of the number of these cells 2 weeks after the first dose of treatment. Finally, the decreased concentrations of G-CSF and TGF-beta2 in patients at time 0 has normalized in 2 weeks after treatment and remained at the same levels after 6 weeks.

Conclusion: Preliminary test results suggest that VIT has immunoregulatory properties influencing (normalizing vs control group) of G-CSF, TGF-beta2 concentration, and CD3+CD4+helper T cells percentage. Treatment does not influence the concentrations of IL-4 and MCP-1 for 6 weeks.

1136 | Development of a specific IgE immunoassay for Asian hornet venom (Vespa velutina)

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Background: Asian hornet (AH; *Vespa velutina*) is an Asian wasp which migrated to Southern Europe during the last decades. Several articles report attacks on people causing severe allergic reactions. It is important to identify the sensitizing species e.g. when selecting extracts for immunotherapy. If a specific IgE (sIgE) test is not

available for a certain species, a test from a related species is often used. sIgE tests are available for several species from the *Vespidae* family, but not for AH. We therefore set out to develop ImmunoCAP Asian hornet to investigate sensitization patterns using serum from wasp allergic subjects.

Method: A prototype ImmunoCAP Asian hornet was developed and the research use only test was utilized in a small study together with other insect venoms (yellow jacket, European paper wasp, European hornet, and white-faced hornet), insect venom components (Ves v 1, Ves v 5, and Pol d 5), and CCD (MUXF3) to compare sensitization patterns of wasp allergic subjects from Spain and Sweden.

Results: A prototype ImmunoCAP Asian hornet was developed according to conventional methods. The 3 Spanish serum samples revealed different sensitization patterns. One sample had highest sIgE concentrations ([sIgE]) for AH (32.60 kU_A/L) and European hornet (37.70), and lowest [sIgE] for European paper wasp (6.97 kU_A/L) and white-faced hornet (9.30 kU_A/L). The second sample had highest [sIgE] for yellow jacket (41.44 kU_A/L) and European paper wasp (30.20 kU_A/L), and lowest [sIgE] for European hornet (2.39 kU_A/L) and AH (7.85 kU_A/L), indicating different sensitizing species for the two samples. The third sample had positive, but low, [sIgE] for all species (average 0.32 kU_A/L). Comparison of [sIgE] in samples from 5 Swedish subjects showed in average a 10-fold higher [sIgE] (12.48 kU_A/L) for yellow jacket (*Vespula spp.*) compared to AH and European hornet (1.28 and 1.08 kU_A/L). This indicate that the Swedish subjects are sensitized to *Vespula*, which was further strengthened by the Antigen 5 results (average 1.9-fold higher Ves v 5 vs Pol d 5). Five samples had significantly increased [sIgE] against Ves v 5 and Pol d 5 as opposed to Ves v 1. In the remaining 3 samples the trend was reversed. For CCD, 7/8 samples were negative and one Spanish sample had detectable levels (0.11 kU_A/L).

Conclusion: Asian hornet (*Vespa velutina*) was successfully developed. The test can be used to facilitate investigation of sensitization patterns using serum samples from wasp allergic subjects.

1304 | Venom-induced anaphylaxis – phenotype and risk factors – data from the European Anaphylaxis Registry

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Background: Identifying the differences in phenotypes of anaphylaxis is crucial for future management guidelines and the development of a personalized medicine approach. The aim of this study was to compare symptoms, risk factors and management of venom-induced anaphylaxis with anaphylaxis triggered by other elicitors (i.e., food, drugs).

Method: In this multicenter, international, prospective, case-control study using data from the European Anaphylaxis Registry we compare 3612 cases of venom-induced with 3605 cases of gender- and age-matched non-venom-induced anaphylaxis. Patients with the diagnosis of moderate to severe anaphylaxis (according to Ring and Messmer grade II-IV) who gave informed consent to provide their clinical data for the Anaphylaxis Registry were included in the study.

Results: Venom-induced anaphylaxis more frequently involved more than three organ systems and was more frequently associated with cardiovascular symptoms, but intramuscular or intravenous epinephrine was administered significantly less often, in particular in patients without prior history of anaphylaxis. Baseline serum tryptase within the upper normal range (8-11.5 ng/mL) and absence of skin symptoms (i.e., urticaria or flushing) were more frequently associated with severe anaphylaxis.

Conclusion: Venom-induced anaphylaxis may often present with cardiovascular symptoms and without skin manifestations. Therapy should follow the international management guidelines, and epinephrine should be given more often. Upper levels of baseline serum tryptase (> 8 ng/mL) indicate patients at risk, who may require additional prophylaxis of future episodes. Indolent systemic mastocytosis or mast cell activation syndrome should be excluded and patients should be provided with two epinephrine auto-injectors for acute self-management.

OAS 12
Food allergy: Recent Research from Birth to Adolescence

0148 | Risk factors for cow's milk allergy in Europe: EuroPrevall birth cohort

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Background: Cow's milk is one of the commonest causes of food allergy in infancy. The aim of this study was to assess antenatal and postnatal risk factors for cow's milk allergy (CMA) development in the EuroPrevall birth cohort.

Method: In the pan-European EuroPrevall prospective, population-based birth cohort study, parental questionnaires were undertaken at 12 and 24 months or when parents reported symptoms in the study child. Children with suspected CMA were invited for clinical evaluation including skin prick testing, specific IgE assessment and double-blind, placebo-controlled food challenge (DBPCFC) as indicated. Each CMA case (positive DBPCFC or reported cow's milk-induced anaphylaxis) was allocated up to two age- and country-matched controls.

Results: 12,049 were recruited into the EuroPrevall birth cohort. 9,336 (77.5%) were followed until 2 years. 42 children were diagnosed with IgE-mediated and 13 with non-IgE-mediated CMA. They were matched with 66 and 17 controls, respectively. The following factors were associated with IgE-mediated CMA: lower birth weight (P = .031), higher SCORAD (P < .001), previous/current eczema or allergic rhinitis (P < .001), wheeze with upper respiratory infection (P = .029). Children with IgE-mediated CMA received their first solid food at an older age than controls [median 6.0 (IQR 5.0-6.0) vs 5.0 (4.0-6.0) months, P = .044]. Only eczema severity was an independent risk factor for IgE-mediated CMA. Additionally, age at introduction of cow's milk protein associated with the development of non-IgE-mediated CMA in children who received it at an older age than controls [9.0 (IQR 5-10) vs 6.0 (4.5-6) months, P = .032].

Conclusion: Our results suggest that, similar to peanut and egg allergy, eczema was strongly associated with IgE-mediated CMA development in very young children. The different risk factors for non-IgE-mediated food allergy may indicate that it is a different disease or we lack power. Greater understanding of risk or protective factors for the development of different types of CMA may facilitate better identification and enhance the diagnosis, help to design clinical trials and make recommendations for the prevention of the disease.

0790 | Exposure to the mycotoxin deoxynivalenol during pregnancy and lactation enhances the ovalbumin-specific allergic response in the offspring

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Background: The mycotoxin deoxynivalenol (DON) is one of the most prevalent contaminants of cereals and grain-based products

which can cause reproductive toxicity and immunotoxicity. Direct exposure to DON can facilitate allergic sensitization to whey in the adult mice. Therefore, considering the importance of immune development in early life, the present study focused on the effects of prenatal exposure to DON on the development of food allergy in the offspring at later stage.

Method: Pregnant C3H/HeOuJ mice received either control or DON-contaminated diets (12.5 mg/kg of diet) immediately after mating until the last week of lactation. Female offspring were fed a control diet after weaning and received once a week an oral gavage of either PBS (sham), ovalbumin (OVA) or a mixture of OVA and cholera toxin as an adjuvant (CT/OVA), for 4 weeks, starting 2 weeks after weaning. Acute allergic skin response (ASR) and shock symptoms were measured upon intradermal OVA challenge; and Immune cell population in spleen samples and OVA-specific plasma immunoglobulins were analyzed.

Results: The offspring of DON-exposed mothers had significantly higher ASR in the OVA and CT/OVA group, compared to the offspring of control mothers. Moreover, in the CT/OVA group born to DON-exposed mothers, more animals were observed to have shock symptoms after intradermal OVA challenge. In line with the clinical symptoms, the plasma level of OVA-specific IgE was significantly higher in the CT/OVA offspring born to DON-exposed mothers, compared to the CT/OVA offspring of non-exposed mothers, and there was a significant reduction in T helper 1 (Th1) population in the spleen of the offspring born to DON-exposed mothers.

Conclusion: Chronic exposure to DON-contaminated diet during pregnancy and lactation increases the susceptibility of the offspring to develop OVA-specific food allergy and intensifies the allergic responses. This may be in part explained by the reduction in Th1 cell population, which consequently induces more Th2-skewed immune responses.

1230 | Treatment satisfaction with AR101 oral immunotherapy for peanut allergy in a European paediatric population

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Background: Efficacy and safety of AR101, an investigational biologic drug for peanut oral immunotherapy (OIT), was evaluated in ARTEMIS (NCT03201003), a phase 3, randomised, double-blind, placebo-controlled trial in peanut-allergic subjects aged 4–17 years in Europe. Following approximately 9 months of treatment, the primary efficacy and safety endpoints of ARTEMIS were met. OIT is associated with allergic reactions that are typically mild-to-moderate in severity and reduce in frequency with ongoing treatment. As such, the assessment of subject-reported treatment satisfaction was an important exploratory endpoint in ARTEMIS.

Method: Eligible subjects aged 4-17 years with a clinical history of peanut allergy and supportive diagnostic tests were randomly assigned (3:1) to receive AR101 or placebo for approximately 9 months. Treatment Satisfaction Questionnaire for Medication (TSQM-9) was completed by subjects after trial exit and unblinding and consists

of 9 questions in 3 scales (Effectiveness, Convenience and Global Satisfaction). Individual scale scores are rated on a scale from either 1 to 7 or 1 to 5 and total scale scores range from 0 to 100; higher scores indicate greater satisfaction (Bharma et al. *Health Qual Life Outcomes.* 2009). Scores for the intention-to-treat population who received AR101 were calculated and summarised using descriptive statistics.

Results: 175 subjects enrolled in ARTEMIS (AR101 n = 132, placebo n = 43). 111 AR101-treated subjects (84.1%) completed ≥ 1 question on the TSQM-9. Means for individual TSQM-9 item scores generally showed that AR101-treated subjects reported moderate (<6) individual item scores and high total scale scores (59.76-75.07 [Table]).

Conclusion: Despite the known rigors of OIT, peanut-allergic subjects aged 4-17 years treated with AR101 in ARTEMIS report moderate-to-high levels of treatment satisfaction.

Parameter	AR101 (n = 132)
Subjects who completed the TSQM-9, n (%)	111 (84.1)
Total Scale Scores, LS mean (95% CI), n[a]	
Effectiveness scale	70.16 (64.74, 75.57), 115
Convenience scale	59.76 (55.41, 64.10), 114
Global Satisfaction scale	75.07 (69.12, 81.02), 114
Individual Item Scores by Scale, mean (SD), n[b]	
Effectiveness scale	
Medication prevents/treats condition	5.6 (1.55), 114
Medication relieves symptoms	5.4 (1.48), 114
Time for medication to start working	5.0 (1.36), 114
Convenience scale	
Easy-to-use medication	4.7 (1.29), 114
Ease of planning when to use medication	4.7 (1.32), 114
Convenience of taking medication	4.6 (1.44), 114
Global Satisfaction scale	
Confident medication is a good thing	4.1 (1.02), 114
Good things about medication outweigh the bad	4.1 (1.07), 114
Satisfied with medication	5.7 (1.35), 114

CI, confidence interval; LS, least squares; SD, standard deviation; TSQM-9, Treatment Satisfaction Questionnaire for Medication-9.
[a] Total scale scores ranged from 0 to 100; higher scores indicate greater levels of satisfaction.
[b] Level/degree of satisfaction, ease, confidence or agreement with statement were rated from 1 (lowest satisfaction) to 7 (highest satisfaction) for all items except "Confident medication is a good thing" and "Good things about medication outweigh the bad," which were rated on a scale of 1 (lowest satisfaction) to 5 (highest satisfaction).

1467 | Current practice in the diagnosis and management of paediatric eosinophilic oesophagitis in a multi-centre cohort in London

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Background: This multicentre retrospective cohort study assessed EoE diagnosis, management & outcomes within London paediatric gastro-allergy centres against current consensus standards.

Method: Data were anonymised from 4 centres. Inclusion criteria: Age ≤ 18 years, histologically confirmed EoE (≥15 eosinophils/hpf), diagnosed between April-November 2018

Results: 40 children were identified (75% M) with median diagnostic age of 9 years (range 0.9-16.3). 70% had other atopy: immediate food allergy 45%, asthma 45%, eczema 40%, rhinitis 23% & urticaria 8%. Of those tested (73%), 68% had positive IgE-tests to food & 38% to aeroallergens. At diagnosis all were symptomatic - upper GI symptoms (83%), dysphagia (63%), feeding difficulties (30%), lower GI (23%) & failure-to-thrive (23%). At initial endoscopy 72% had ≥ 4 oesophageal biopsies taken; macroscopic features were furrows (56%), exudates (28%) & trachealisation (6%); eosinophil counts ranged from 15-100/hpf; other EoE histological features seen in 66.7%.

First-line management: 15% had only an elimination diet (ED), 28% had only medical management (PPI only), and 57% having combined medical & dietetic strategies - PPIs + ED (n = 12), oral viscous budesonide (OVB) + ED (n = 5), PPI + OVB + ED (n = 2). Of EDs used: 2-food ED (15%), 4-food (13%) & 6-food ED (25%). Cow's milk (55%) & wheat (50%) were the commonest excluded allergens.

Within 6 months of diagnosis, 57% of children were reassessed. 48% had endoscopic reassessment, of which 97% of whom had > 4 biopsies from > 1 site. Histological remission (<15 Eos/hpf) was found in 40% and histological response in 53% of children. 70% had symptom response to initial treatment. 7.5% were lost to follow-up.

Second-line management: Adding/switching to OVB (25%), further ED (14%), increasing PPI dose (6%) or starting swallowed fluticasone (3%). 11% had treatment de-escalated. At second reassessment, similar histological (63%) & symptomatic response (73%) was seen. No oesophageal strictures were found. 48% were also seen by an allergist, 78% by a dietitian & 20% by a psychologist.

Conclusion: This study provides unique insight into current practice & challenges. Combined strategies were a common first-line approach with good histological/symptom response. The prevalence of atopy and ED as treatment suggests the need of a multi-professional approach. In future, a wider national study with additional parameters is planned.

OAS 13
Epidemiology: Clinical Trials - Real Life

0348 | Spatiotemporal characteristics of asthma emergency department presentations in diverse biogeographical and climatic regions

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Background: Sudden episodes of asthma exacerbation are often captured by hospital emergency departments (ED). Acute asthma presentations generally represent the severe proportion of asthma morbidity requiring urgent medical care. Understanding acute asthma determinants is required. It is hypothesised acute asthma determinants differs geographically. The study's objective was to identify acute asthma determinates across latitude and climatic regions.

Method: Six years of Australian routinely collected data (2012 to 2017) from 29 major public hospitals, was extracted from Queensland Health's Emergency Department Information Systems. Extracted data included episode level asthma or asthma like emergency department presentation diagnoses. Descriptive statistics were used to analyse asthma presentation data. A time series multiplicative seasonal decomposition was performed to determine seasonality and trends.

Results: The study period consisted of 2,195 days with a total of 61,411 Queensland asthma ED presentations. The 6-year average daily incidence rate was 257 asthma ED presentations per 100,000. The highest burden of asthma occurred from May to August. Children (3 to 17) showed a higher incidence rate compared to adults (>17), with males experiencing a higher burden compared to females in the pre-teenage years (3 to 12). During adolescence (>12 to 17), an inversion was observed where the female burden exceeded males. The Wide Bay region (lowest socioeconomic advantage) showed the highest burden and the Gold Coast (highest socioeconomic advantage) the lowest (average daily IR over 6 years: 450 per and 161 per 100,000 people respectively). Socioeconomic status appears to have an association with acute asthma presentations. Observed trends, timing and strength of seasonality varied between climates and latitudes.

Conclusion: Overall in this dataset an increasing trend of acute asthma presentations appeared, with a strong seasonal component peaking across winter months. As opposed to adults, children demonstrated seasonal peaks outside the winter period In February, May and November. November childhood peaks coincided with spring and presumably seasonal allergen exposure. Patient cohort characteristics (age, sex, regional socioeconomic status and place of birth) may explain large variation observed in acute asthma presentations, within and between different climatic regions. The most northern and tropical monsoonal climatic region, Cairns, had the weakest seasonal acute asthma variation.

0444 | Dupilumab reduces severe exacerbations in patients with uncontrolled, moderate-to-severe allergic (atopic) asthma regardless of presence of perennial aeroallergen-specific IgE: LIBERTY ASTHMA QUEST

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Background: Dupilumab, a fully human monoclonal antibody, blocks the shared receptor component for interleukin (IL)-4 and IL-13, key drivers of type 2 inflammation, including IgE-mediated allergic inflammation in asthma. In phase 3 LIBERTY ASTHMA QUEST (NCT02414854), add-on dupilumab 200 mg and 300 mg every 2 weeks (q2w) vs placebo significantly reduced severe asthma exacerbations and improved pre-bronchodilator forced expiratory volume in 1 second in patients with uncontrolled, moderate-to-severe asthma. Treatment effects were greater in patients with elevated type 2 biomarkers (blood eosinophils ≥ 150 cells/μL or fractional exhaled nitric oxide ≥ 25 ppb) at baseline (BL). Dupilumab was generally well tolerated in patients with uncontrolled, moderate-to-severe asthma. Type 2 inflammatory asthma represents a large proportion of the overall population of asthma patients, many of whom show IgE-mediated allergic (atopic) inflammation. The aim of this analysis was to assess the effect of dupilumab on severe exacerbations in patients with uncontrolled, moderate-to-severe asthma with evidence of allergic (atopic) asthma, defined as total serum IgE ≥ 30 IU/mL and ≥ 1 perennial aeroallergen-specific IgE ≥ 0.35 IU/mL at BL.

Method: The adjusted annualized rate of severe exacerbations during the 52-week treatment period in the intention to treat (ITT) population was derived by negative binomial analysis categorized by the following perennial aeroallergens: combined molds (*Aspergillus fumigatus*, *Alternaria tenuis/alternata*, or *Cladosporium herbarum/hormodendrum*), combined dust mites (*Dermatophagoides farina/pteronyssinus*), cat dander, dog dander, or German cockroach.

Results: Dupilumab vs placebo significantly reduced severe exacerbations in patients with allergic (atopic) asthma (n = 1,083) by 33–51% regardless of the presence of perennial aeroallergen-specific IgE (Figure). No difference was observed in patients sensitized to 1 vs ≥ 2 allergens at BL (*P* = .8727). Dupilumab reduced severe exacerbations by 42% (95% CI 0.392–0.867; *P* = .0078 vs placebo) in patients with 1 allergen sensitization at BL (29% each treatment group) and by 41% (0.457–0.769; *P* < .0001 vs placebo) in patients with multi-allergen sensitizations at BL (71% each group).

Conclusion: Dupilumab vs placebo significantly reduced the rate of severe exacerbations in patients with uncontrolled,

moderate-to-severe allergic (atopic) asthma, regardless of the presence of perennial aeroallergen-specific IgE.

Figure. Adjusted annualized rate of severe exacerbations in patients by specific IgE levels.
[a]Combined molds include: Aspergillus fumigatus, Alternaria tenuis/alternata, or Cladosporium herbarum/hormodendrum.
[b]Combined dust mites include:Dermatophagoides farina/pteronyssinus. ***P < 0.001, **P < 0.01, *P < 0.05 vs placebo.
CI, confidence interval.
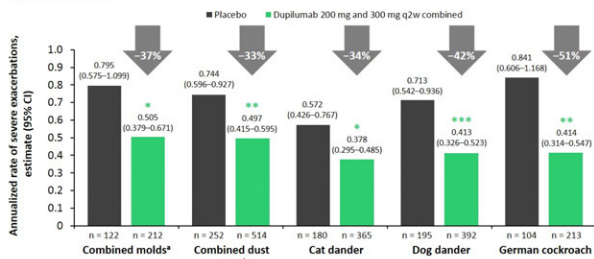
0698 | Real-world clinical outcomes for patients in the UK with severe, atopic eosinophilic asthma treated with benralizumab

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Background: A substantial percentage of severe eosinophilic asthma (SEA) patients have evidence of both atopic and eosinophilic airways inflammation and meet eligibility criteria for both anti-IgE (omalizumab) and anti–IL-5Rα/anti–IL-5 (benralizumab and mepolizumab) therapy. However, it is unknown whether such patients benefit from benralizumab and whether there is any difference in clinical response between anti-IgE eligible and ineligible patients. This preliminary analysis, part of AstraZeneca's XALOC real-world evidence program, describes real-world effectiveness of benralizumab in asthmatic patients with an overlapping atopic and eosinophilic phenotype.

Method: Retrospective medical records review of SEA patients treated with benralizumab at five specialist centres in the UK was performed. Eligibility for anti-IgE therapy was defined as sensitisation to one or more perennial aero-allergens and a total IgE measure between 30–1500 UI/mL. Clinical outcomes including annualised exacerbation rate (AER), oral corticosteroid (OCS) use, and patient-reported outcomes (PROs) following benralizumab initiation were described for anti-IgE–eligible and ineligible patients at 16, 24, and 48 weeks after first benralizumab dose, compared with baseline.

Results: 108 patients treated with benralizumab were included in this analysis. 67% were female, mean age was 49.6 years. 44% had received prior biologic therapy for severe asthma. 22% were deemed eligible for anti-IgE therapy. No substantial differences in baseline characteristics were observed between the two subgroups. At baseline, AER was 4.9 and 4.4 for non-atopic and anti-IgE therapy eligible patients, respectively, and 67% vs 58% of patients required maintenance OCS. At 48 weeks, AER declined by 84% for non-atopic and 75% for anti-IgE eligible patients. For patients receiving maintenance OCS, 33% and 45%, respectively, were able to completely stop OCS by 48 weeks. Substantial improvements in PROs were also observed regardless of phenotype. All outcomes were also consistent at 16 and 24 weeks.

Conclusion: In a real-world setting, benralizumab led to significant reductions in exacerbations and OCS use, and improved PROs in SEA. The clinical effectiveness of benralizumab is independent of co-eligibility of anti-IgE therapy, highlighting the central role of the eosinophil in asthma immune dysfunction and exacerbation pathogenesis.

		Baseline	16 weeks	24 weeks	48 weeks
Annualised Exacerbation Rate	Non-atopic				
	Number analysed	42	42	42	33
	AER	4.9	0.6	0.5	0.8
	Absolute difference estimates vs. baseline	n/a	-88%	-90%	-84%
	Eligible for anti-IgE therapy				
	Number analysed	24	24	24	19
	AER	4.4	1.4	1.2	1.1
	Absolute difference estimates vs. baseline	n/a	-68%	-73%	-75%
Oral corticosteroids use	Non-atopic				
	Number analysed	42	42	42	33
	% of patients on OCS	67%	52%	52%	45%
	Absolute difference estimates vs. baseline	n/a	22%	22%	33%
	Eligible for anti-IgE therapy				
	Number analysed	24	24	24	19
	% of patients on OCS	58%	42%	50%	32%
	Absolute difference estimates vs. baseline	n/a	28%	14%	45%
PRO: ACQ-6	Non-atopic				
	Number analysed	45	31	31	25
	Mean ACQ-6 Score	3.3	2.3	2.6	2.2
	Absolute difference estimates vs. baseline	n/a	-31%	-22%	-32%
	Eligible for anti-IgE therapy				
	Number analysed	23	17	23	17
	Mean ACQ-6 Score	3.5	2.0	2.3	2.8
	Absolute difference estimates vs baseline	n/a	-41%	-33%	-20%
PRO: AQLQ(S)-12	Non-atopic				
	Number analysed	35	29	27	24
	Mean AQLQ Score	3.5	4.0	3.9	4.1
	Absolute difference estimates vs. baseline	n/a	16%	11%	19%
	Eligible for anti IgE therapy				
	Number analysed	18	18	21	15
	Mean AQLQ Score	3.3	4.2	4.1	4.3
	Absolute difference estimates vs. baseline	n/a	26%	25%	31%

0719 | Three-year follow-up of the 2016 Melbourne Epidemic Thunderstorm Asthma patients: Insight into symptoms, healthcare seeking, and medication compliance

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Background: Three years have passed since the unprecedented Epidemic Thunderstorm Asthma (ETSA) in Melbourne, Australia in November 2016. Those who presented to Eastern Health Emergency Departments (EDs) have been followed every year since, providing longitudinal insight into post-ETSA natural history.

Method: Utilising the same standardised questionnaire as in 2017 and 2018, patients were contacted by phone, email, or mail in December 2019.

Results: 69% (n = 115) of patients who had given consent in 2018 for follow up had been contacted with 4 declining to respond. Of respondents (n = 111), 21% described having no asthma symptoms in the last 12 months, (c.f. 20% in 2017); 43% had infrequent symptoms (< 1/month), c.f. 57% in 2017; 15% had frequent symptoms (> 1/month, but < 1/week), c.f. 15% in 2017; and 22% had persistent

asthma (symptoms ≥ 1/week), c.f. 28% of patients in 2017. Urgent healthcare utilisation for asthma, including emergency visits to the General Practitioner (GP), EDs and overnight hospitalisations were reported by 15% of patients in 2019 compared to 18% in 2017. Non-urgent GP reviews for asthma were reported by 31% compared to 41% in 2017. Only 19% of patients had been prescribed asthma preventer medication before the 2016 ETSA, while 36% of patients have been prescribed one since then. However, of those prescribed a preventer, only 41% of patients reported compliance of ≥ 5 days per week during the month of November (c.f. 50% in 2017), while 30% did not use their preventer medication at all in November (c.f. 22% in 2017).

Conclusion: A significant proportion of patients continue to have on-going asthma symptoms 3 years after the ETSA, with a similar spread of symptom frequency compared to 2017. There has been a reduction in urgent and non-urgent healthcare utilisation, while preventer medication prescription has increased. Despite this, preventer adherence rates have remained low. These trends provide insight into the need for improved asthma planning and education for long-term symptom control.

OAS 14
Novel Mechanisms of Inflammation

0046 | Macrophages produce histamine through the interaction with antigen-specific Th2 cells

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Background: Antigen specific Th2 cells play an important role in the pathogenesis of allergic rhinitis. Recent studies have proved that some types of macrophages, such as M2 macrophages, are related to allergic diseases. In this study, we revealed that macrophages produce histamine through the interaction with antigen specific Th2 cells. Furthermore, we found that histamine produced from macrophages is involved in the nasal early phase reaction in mice model.
Method: We developed bone marrow-derived macrophages (BMDM) from WT and histidine decarboxylase-deficient $Hdc^{-/-}$ mice, which cannot synthesis histamine. DO11.10 mice-derived naive CD4 T cells, which express an OVA323-339-specific T-cell receptor, were differentiated into Th2 cells (OVA-Th2 cells) *in vitro*. Then, we co-cultured BMDM and OVA-Th2 cells with or without OVApep, and measured histamine concentration in the culture supernatant. To investigate the role of histamine production in macrophages *in vivo*, we created mice model, which were adoptively transferred with *in vitro* differentiated OVA-Th2 cells, then nasally challenged with OVA. In addition to counting the sneezing numbers, we analyzed nasal infiltrations of eosinophils and OVA-Th2 cells using a flow cytometry.

Results: In the presence of OVApep, the *in vitro* co-culture of WT-BMDM and OVA-Th2 cells produced 4 times higher amounts of histamine than that of without OVApep. In contrast, the amounts of histamine produced from co-culture of $Hdc^{-/-}$-BMDM and OVA-Th2 cells with OVApep were markedly decreased. Moreover, OVA challenge elicited sneezing responses in OVA- Th2–transferred mice, but not non-transferred mice *in vivo*. OVA challenge failed to elicit sneezing in OVA- Th2 transferred $Hdc^{-/-}$ mice. When we depleted macrophages from OVA-Th2–transferred and OVA–challenged mice by intraperitoneal injection of clodronate-containing liposomes, it completely abrogated OVA–induced sneezing responses in the OVA-Th2 transfer model.

Conclusion: We identified that macrophages interact with antigen specific Th2 cells through antigen, and then produce histamine. This novel histamine production pathway seems to be involved in the nasal early phase reaction, and that implies a target to treat allergic rhinitis.

0070 | Low antigen doses trigger B-cell igE class switching in tissue associated lymphoid structures but not in regional lymph nodes

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Background: We have previously shown that house-dust mite specific IgE production in young (1-8 years) allergic patients is not associated with allergen-specific IgG_4, IgG or IgA_1 production and, consequently, it may be triggered outside of the germinal centers and secondary lymphoid organs. The aim of this study was to clarify the hypothesis that B-cell IgE class switching triggered by low chronically administrated antigen doses may occur mostly in tissue associated lymphoid structures (TLS) and may not be occur in the germinal center B-cells.
Method: Female 6-8 weeks old BALB/c mice were immunized with low (100 ng) and high (10 ug) doses of OVA repeatedly for 4-5 weeks 3 times a week in the foot pad which is free of TLS and in the withers which is enriched due to the presence of subcutaneous fat with special TLS called fat-associated lymphoid clusters. OVA-specific IgG_1, IgG_{2a} or IgE production were determined by ELISA after 14th immunization. To detect specific germline and postswitch ε and $\gamma1$ transcripts, reflecting ongoing Ig switch, in mice withers adipose tissue (W) and axillary lymph nodes (LN) was analyzed by quantitative PCR (qPCR). Detection of B-cell functional markers *Bcl6*, *Ebi2*, *Aicda* was also determined by qPCR.
Results: Specific IgE production was induced only in mice immunized in the withers region but not in food pad and mostly by low but not high antigen doses. IgG_1 was the major IgG subclass and was induced mostly by high doses at a comparable level in mice immunized in foot pad or in withers. Expression of B-cell activation markers in

regional LN (*Bcl6, Ebi2, Aicda*) was triggered only by high dose, which indicates that low antigen doses were not delivered effectively in the secondary lymphoid organs (SLO). Expression of these markers was also induced in W and in the case of *Aicda* was significant only in low-dose group. Expression of germline ε and $\gamma 1$ transcripts was detected only in W, and mostly in low-dose group. However, post-switch ε transcripts appeared both in W and LN which indicate the migration of some cell after activation from TLS to SLO.

Conclusion: Altogether, specific IgE production is induced in TLS by chronically administrated low doses of the antigen which is not effectively delivered into SLO.

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0521 | Celiac disease causes epithelial disruption of the oral mucosa

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Background: We have previously shown that allergic disease progression is associated to mucosal barrier disruption. We wanted to assess whether these alterations are found in other mucosa-associated immune-related pathologies. Celiac disease (CD) is a chronic systemic autoimmune disease characterized by an immune-triggered enteropathy upon gluten intake. CD manifests itself with multiple forms and its diagnosis can be complicated. Several extraintestinal manifestations have been associated with CD, including, affection of the oral mucosa, i.e. aphthous ulcers. We hypothesize that oral mucosa is affected in CD and could be used as a diagnostic method.

Method: Eleven CD patients were recruited: 5 *de novo* diagnosed, and 6 under Gluten Free Diet (GFD) for at least 3 months. CD patients were included only when histological classification Marsh III (villi atrophy), positive Anti-Transglutaminase Antibodies (ATA) and DQ2 + allele of the histocompatibility complex (HLA) were observed. Two biopsies of the cheek lining were taken from each patient, one was embedded in RNA later for Real Time-qPCR and the other in paraformaldehyde (PFA) for immunohistochemical studies. A control group of non-celiac non-allergic subjects was included in the study (n = 8).

Results: We observed a significant decrease in protein expression levels of epithelial junctional complexes in both groups of CD patients. Transcript levels of occludin, periplakin and claudin were significantly increased in GFD group when compared to *de novo* diagnosed patients. FoxP3/CD3 ratio was significantly increased in both groups of CD patients when compared to the control group; among CD patients, this increase was significantly higher in *de novo* diagnosed patients. CD3+and CD8+intraepithelial lymphocytes (IEL) tended to be higher in the *de novo* diagnosed patients; however, these lymphocytes lacked gamma delta TCR expression. No differences were found in IFNγ, IL-15 or perforin transcript levels.

Conclusion: CD causes oral mucosa disruption. The molecular mechanisms of this damage are not equivalent to intestinal findings; however, IEL and FoxP3 increased recruitment are found in both types of mucosa. Avoidance of gluten contributes to healing of the oral mucosa. Overall, our findings suggest that oral mucosa could be a potential target for future treatment and diagnosis of CD.

1130 | IRF5 regulates airway macrophage metabolic responses to viral challenge

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Background: Airway macrophages (AMs) are strategically located at the interface between the external and internal pulmonary environment and form the first line of defence against inhaled pathogens and allergens. Increasing evidence suggests that alterations in AM metabolism underpin phenotypic changes in AMs. Interferon regulatory factor 5 (IRF5), a transcription factor involved in the induction/expression of pro-inflammatory cytokine responses to microbial and viral infection, is a master regulator of macrophage phenotype. However, the role of IRF5 in reprogramming macrophage metabolism in response to viral infection is not yet known. This research aimed to determine the role of IRF5 in controlling AM phenotype via alterations in metabolism in response to viral challenge.

Method: To investigate the role of IRF5 in controlling AM metabolism, inflammation and disease pathology in response to viral infection, we infected wild type (WT) and *Irf5*$^{-/-}$ mice with influenza. Furthermore, we identified overlapping regions of open chromatin across immune cell types and IRF5-DNA interaction sites. For this purpose, open chromatin regions (OCRs) as determined by ATAC-Seq as part of the Immunologic Genome Project were overlaid with published IRF5 ChIP-Seq data from murine LPS-stimulated bone marrow derived macrophages (BMDMs) to identify IRF5-DNA interaction sites. Moreover, the metabolic profiles of BMDMs and primary AMs after toll-like receptor (TLR)-stimulation were assessed using an extracellular flux assay.

Results: Influenza-infected *Irf5*$^{-/-}$ mice had enhanced type 2 immunity, characterised by augmented eosinophilia and increased numbers of T_H2 cells compared to WT. Overlap between IRF5-DNA interaction sites determined via ChIP-Seq data and detected

OCRs revealed that IRF5 co-localises at OCRs associated with pro-inflammatory and metabolic genes. Delineating the metabolic profiles of BDMDs and Primary AMs revealed a reduction of mitochondrial consumption and extracellular acidification rate upon TLR3 stimulation in $Irf5^{-/-}$ AMs but not BMDMs compared to WT controls. Thus, indicating a reduction in the overall metabolic capacity of $Irf5^{-/-}$ AMs but not BMDMs from $Irf5^{-/-}$ mice compared to WT. **Conclusion**: In summary, our data reveal a critical role for IRF5 in the regulation of macrophage metabolic phenotype after TLR activation in response to viral infection.

1277 | Human skin mast cells express photoreceptors, and blue light inhibits their degranulation

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Background: Allergic skin reactions are driven by dermal mast cells (MCs) and exhibit circadian differences. In human skin, circadian rhythms may be regulated by cryptochromes (CRYs) and opsins (OPNs), photoreceptors (PRs), which were recently identified to be expressed in a number of human cutaneous cell types. Whether human skin MCs express these receptors is currently unknown
Method: We obtained MCs from human skin (breast, foreskin, eyelids) and used also cultured CD34-positive peripheral blood stem cell-derived MCs (PSCMCs) and LAD2-MCs. MCs were analyzed for the expression of CRY1 and OPN 1-3 by qRT-PCR and Western Blot as well as for the effects of irradiation with blue light on activation via IgE/anti-IgE or cortistatin, a MRGPRX2 agonist.
Results: We found expression of the blue light sensitive *CRY1*, *OPN1 MW* (medium-wave-length), *OPN2* and *OPN3* in LAD2-MCs, cultured MCs from breast skin and foreskin as well as freshly isolated MCs from breast skin and eyelids, with the exception of *OPN2* in eyelid MCs. *CRY1* expression was the highest overall, and its presence was confirmed at protein level in cultured as well as freshly isolated MCs from breast skin and foreskin. PSCMCs, however, did not show expression of any of the investigated PRs. Importantly, fresh and cultured skin MCs were sensitive to blue light irradiation (at 453 nm) showing a dose-dependent reduction in their degranulation after exposure to 2 or 30 J/cm^2 blue light as measured by β-hexosaminidase release.
Conclusion: Our results demonstrate, for the first time, that human skin MCs express photoreceptors, i.e. CRY1 and OPN1-3, and blue

light, which activates these receptors, inhibits MC degranulation. These findings may explain, at least in part, circadian differences in allergic skin reactions and may be relevant for the development of photoreceptor-targeted treatments for patients with MC-driven skin diseases such as chronic spontaneous or inducible urticaria.

OAS 15
Cutting Edge Insights into Allergen Immunotherapy Mechanisms

0371 | Allergoids conjugated to mannan drive monocyte differentiation into tolerogenic dendritic cells by promoting metabolic reprogramming

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Background: Allergen-specific immunotherapy (AIT) is the only treatment with potential long-lasting disease-modifying effects for allergic diseases, but it still faces problems related to efficacy, security, duration and patient compliance. Glutaraldehyde-polymerized grass pollen allergoids conjugated to non-oxidized mannan (PM) represent next generation vaccines for AIT targeting dendritic cells (DCs) that promote the generation of forkhead box P3 (FOXP3)$^+$ regulatory T (Treg) cells. The aim of this study was to investigate the impact of PM over the monocyte differentiation process into DCs.
Method: Monocytes were differentiated with IL-4 and GM-CSF in the absence or presence of PM into human monocyte-derived DCs (hmoDCs) or PM/hmoDCs, respectively. The expression of tolerogenic markers, histone deacetylases and cytokine signature were determined by qPCR or ELISA. Allogeneic cocultures of PM/hmoDCs with naïve CD4$^+$ T cells were performed to analyze T cell polarization. The Warburg effect was determined photometrically by quantifying the OD at 570 nm. Lactate concentrations were quantified by colorimetric L-Lactate Assay kit. Mitochondrial mass and mitochondrial membrane potential were measured by MitoTracker Green FM and MitoTracker Red CMXRos, respectively.
Results: HmoDCs generated in the presence of PM showed a significantly lower cytokine production after LPS stimulation (TNF-α, IL-6 and IL-1β), higher IL10/TNF-α, IL-10/IL-6 and IL-10/IL-1β ratios, and higher expression of the tolerogenic molecules *PDL1*, *IDO*, *SOCS3* and *IL10* than conventional hmoDCs. PM/hmoDCs also displayed a higher capacity to induce FOXP3$^+$ Treg cells than conventional hmoDCs. PM/hmoDCs produced less lactate and displayed increased mitochondrial mass and mitochondrial membrane potential than hmoDCs generated in the absence of PM, suggesting that PM favours mitochondrial oxidative phosphorylation during hmoDCs differentiation. PM/hmoDCs also expressed lower levels of histone deacetylases genes (HDACs and SIRTs) than conventional hmoDCs, suggesting that epigenetic regulation might well also contribute

to the maintenance of the properties imprinted by PM during the hmoDCs differentiation process.

Conclusion: Allergoids conjugated to non-oxidized mannan modulate monocyte differentiation promoting tolerogenic DCs by mechanisms depending on metabolic reprogramming and epigenetic modifications, which might also contribute to the generation of healthy immune responses to allergens induced by these next-generation vaccines.

0438 | Fully functional PIPEline IgE, IgG1 and IgG4 against the major birch pollen allergen Bet v 1: Proof of concept in cellular assays

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Background: PIPE (Polymerase Incomplete Primer Extension) cloning is a rapid method for recombining variable and constant antibody region sequences and thus allows simple creation of antibodies against several targets (Ilieva et al., 2017). In this study we present PIPE-cloned IgE, IgG$_1$ and IgG$_4$ targeting the major birch pollen allergen Bet v 1.

Method: PIPE-cloned IgE, IgG$_1$ and IgG$_4$ vectors sharing the same variable region against Bet v 1 (Levin et al., 2014) were expressed using the Expi293F system and purified via affinity chromatography. Purity, size and correct assembly were confirmed via SDS PAGE, SEC-MALS and CD spectroscopy. Specificity was tested in a dot blot, ELISA (all isotypes) and ISAC112 microarray (IgE only). Antibody functionality and blocking capabilities were tested using the human mast cell line LAD2 and an RBL-SX38 degranulation assay. LAD2 cells were primed with IgE and FcεRI expression measured after 4 days of exposure via flow cytometry. For the determination of degranulation, RBL-SX38 cells were stimulated with Bet v 1 after sensitisation with anti-Bet v 1-IgE and dose-dependent β-hexosaminidase release detected using a fluorescent substrate. In the blocking experiments, stimulation was carried out in the presence of the respective IgG antibodies.

Results: SDS PAGE, SEC-MALS and CD spectroscopy confirmed the integrity of all produced antibodies. They furthermore bound Bet v 1 specifically and exclusively, as was shown with a dot blot, ELISA and the ISAC112 microarray. Incubation of LAD2 cells with IgE significantly increased FcεRI expression in a concentration-dependent manner. Allergen-induced, FcεRI-mediated degranulation of RBL-SX38 cells was also concentration-dependent and could be inhibited by epitope blocking by both IgG$_1$ and IgG$_4$ in a similar manner.

Conclusion: In this study we demonstrate the functionality of these antibodies in cellular assays, but also these models can prove useful in future for the study of Bet v 1 modifications and their allergenicity. Our findings may thus shed light on the cellular mechanisms underlying birch pollen allergy.

0743 | Dermatophagoides pteronyssinus subcutaneous immunotherapy induced allergen specific IgG4 can be detected in saliva and strongly correlated with serological IgG4

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Background: This study aims to detect allergen-specific IgG4 induced by *Dermatophagoides pteronyssinus* (DP) subcutaneous immunotherapy (SCIT) in saliva samples, in order to determine if salivary IgG4 can be used as an alternative marker to serological IgG4.

Method: 310 DP-allergic rhinitis and/or asthma patients were recruited for this study. 289 patients were included in the allergy immunotherapy (AIT) group and received DP-SCIT for 1 year. 21 patients were included in the control group and received 1 year of symptom treatment only. DP, Der p 1 and Der p 2 specific IgE in serum, specific IgG4 in both serum and saliva were measured at 0, 4, and 12 months of DP-SCIT. The correlation between salivary and serological IgG4 were investigated.

Results: During treatment with DP-SCIT, the allergen specific IgG4 in both serum and saliva increase significantly. After 1 year of treatment, DP-, Der p 1- and Der p 2-specific IgG4 levels in serum increased 29, 57, and 20 folds, respectively; while the increase of salivary IgG4 was 5.4, 4.1, and 1.8 folds, correspondently. No IgG4 changes in the control group were observed in either serum or saliva. The detection rate of salivary DP-specific IgG4 was 11% before AIT and 76% after 12 months of AIT. A strong correlation between salivary and serum IgG4 was observed after AIT 4 months (DP: $r = 0.534$, Der p 1: $r = 0.496$, Der p 2: $r = 0.363$; all $P < .001$) and

12 month (DP: r = 0.585, Der p 1: r = 0.631, Der p 2: r = 0.577; all P < .001). The correlation became stronger over the treatment time. The ratio between serum and salivary specific IgG4 levels was 100-300 before treatment, and 1200-1600 after 12 months of AIT.

Conclusion: It is possible to detect a kinetic change in major allergen-specific IgG4 levels in the saliva of most patients. The specific IgG4 detected in saliva was closely related to the IgG4 in serum. Salivary allergen specific IgG4 could be an alternative marker of serological IgG4 in the late phase of AIT.

0806 | Grass pollen sublingual immunotherapy treatment induces transcriptomic and metabolic changes over time

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Background: The prevalence of allergic diseases and their severity have increased worldwide in last decades. Sublingual administration of *Phleum pratense* allergy immunotherapy (SLIT) tablets is one of the most recent approaches of tolerance induction in patients with allergic rhinoconjunctivitis, with or without asthma. We have documented an immunomodulation effect of SLIT-tablets for at least 2 years after stopping a 3-year treatment; however, the mechanisms responsible for these effects remain unknown. Our aims in this work were first to identify the immunological mechanisms underlying SLIT and second to identify novel biomarkers useful for follow up and efficacy measurement.

Method: 22 grass pollen-allergic patients were enrolled in a double blind randomized controlled clinical trial for 2 years based on the administration of either placebo or immunotherapy treatment using a standardized grass SLIT-tablet. Serum samples and PBMCs from all the patients were used for metabolomic and transcriptomic analysis. Serum samples were analysed using Liquid Chromatography-Mass Spectrometry (LCMS) and Gas Chromatography-Mass Spectrometry (GCMS) techniques. Gene expression profile of all the samples was analysed using the GeneChip WT PLUS Reagent Kit and R software. Pathway analysis was carried out using IPA software.

Results: Both transcriptomic and metabolic analyses showed that poly-sensitized subjects presented a higher degree of inflammation than mono-sensitized patients prior to immunotherapy treatment. Interestingly, mono-sensitized patients presented a significant

down-regulation of mast cell activation pathways, that was not observed in poly-sensitized after 2 years of SLIT. Finally, transcriptomic changes induced by immunotherapy over time in mono-sensitized patients treated with immunotherapy pointed to an increase in their inflammation state.

Conclusion: Overall results showed that there are specific features that can characterize each allergic phenotype. Moreover, immunotherapy has a significant effect in pathways related to inflammatory response as demonstrates the down regulation of mast cells activation in mono-sensitized patients; however, as previously described, 3-year treatment is needed to consolidate a regulatory immune response. Our data support that clinical effect during is produce by effector cell desensitization and that peripheral improvement is achieved by prolonged AIT in the third year of intervention.

1206 | Grass pollen sublingual immunotherapy drives the generation of functional IL-10-producing innate lymphoid cells that are associated with clinical benefit: An RDBPC study

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Background: Innate lymphoid cells (ILCs) with the capacity to produce IL-10 have been recently reported. However, their role in allergic diseases remains unexplored. Here, we aimed to phenotype and identify the function of IL-10-producing ILCs (ILC10) in the blood of seasonal allergic rhinitis patients and following allergen immunotherapy (AIT).

Method: Peripheral blood mononuclear cells were collected from non-atopic controls (NAC,n = 18), grass pollen (GPA,n = 18), house dust mite (HDMA,n = 6) allergics and grass pollen subcutaneous immunotherapy-treated patients (GP-SCIT,n = 16) in a cross-sectional study. ILCs were isolated by FACS sorting or magnetic isolation. IL-10 production was induced by stimulation with IL-2/7/33/retinoic acid. Furthermore, in an RDBPC study, induction of ILC10 was evaluated following grass pollen sublingual (GP-SLIT,n = 13) and placebo (PL-SLIT,n = 12) by flow cytometry and single-cell transcriptomics and protein expression using feature barcoding technology (CITE-Seq; 10x Genomics). IL-10 protein levels were measured by ELISA.

Results: Immunophenotyping of ILC10 revealed that they were confined within KLRG1^{+}ILCs compared to KLRG1^{-}ILCs ($P < .001$). Expression of *CTLA4* and *HELIOS* were upregulated in IL-10^{+}KLRG1^{+}ILCs compared to IL-10^{-}KLRG1^{+}ILCs ($P < .05$). The proportion of IL-10^{+}KLRG1^{+}ILCs was dysregulated in GPA (47.5%, $P < .01$) and HDMA (19.6%, $P < .001$), compared to NAC (as 100%). However, GP-SCIT-treated group showed a restoration of these regulatory cells (79.1%, $P < .05$) and levels of secreted IL-10 were also restored ($P < .05$) compared to GPA, which correlated with clinical symptoms ($r = -0.6687$, $P < .05$). Moreover, in an RDBPC time-course study of GP-SLIT, IL-10^{+}KLRG1^{+}ILCs were increased at 12- ($P < .01$) and 24-months ($P < .001$) compared to PL-SLIT. The proportion of IL-10^{+}KLRG1^{+}ILCs in SLIT-treated patients correlated closely with clinical symptoms ($r = -0.6298$, $P < .05$). Clonal analysis of KLRG1^{+}ILCs also demonstrated that ILCs from GP-SLIT have a higher capacity to become IL-10-producers compared to PL-SLIT ($P < .05$). These observations were confirmed by single-cell CITE-Seq approach which provided a landscape overview of the differential gene expression profile of these ILCs following AIT.

Conclusion: For the first time, we show that functional IL-10^{+}KLRG1^{+}ILCs are induced following grass pollen SCIT and SLIT treatment and correlated closely with clinical symptoms. Insight into the innate immune components may provide novel biomarkers for monitoring the efficacy of AIT (SCIT and SLIT).

1330 | **IL-10+T follicular helper cells (CXCR5+PD-1+CD4+TFH10) promote immune tolerance and regulate b cell function following allergen immunotherapy: A proof-of-concept cross-sectional study**

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Background: T follicular helper (CD4^{+}CXCR5^{+}PD-1^{+};T_{FH}) cells are a subset of T cells that produce interleukin (IL)-21 and promote differentiation, proliferation and clonal expansion of IgE[+]B cells in the presence of IL-4. T_{FH} cells have been shown to produce IL-10. We hypothesized that IL-10^{+}T_{FH} cells are dysregulated in patients with seasonal allergic rhinitis (SAR) compared to non-atopic controls (NAC) and are restored following AIT, administered subcutaneously (SCIT) and sublingually (SLIT). Furthermore, IL-10^{+}T_{FH} cells have differential effects in regulating B cell functions when exposed to IL-4 and IL-21.
Method: In a proof-of-concept cross-sectional study of AIT, PBMCs were isolated from NAC (n = 13), SAR (n = 13), SCIT- (n = 10) and SLIT (n = 8)-treated groups. Circulating IL-10^{+}T_{FH} cells were enumerated

by flow cytometry. Isolated naïve and memory B cells were cultured in the presence of CD40L, IL-4, IL-21 and IL-10 for up to 14 days, and their role in regulating B cell functions were investigated by qPCR and flow cytometry.
Results: Total symptom scores were higher in SAR compared to NAC($P < .05$). SCIT and SLIT-treated groups exhibited reduced symptoms compared to SAR(both, $P < .05$). The proportions of IL-10^{+}T_{FH} cells were lower compared to NAC($P < .05$) and were restored following SCIT and SLIT(both, $P < .05$). IL-4 and IL-21, cytokines that are secreted by T_{FH} cells, promoted germinal centre reaction, class-switch recombination (CSR) and plasma cell differentiation in naïve and memory B cells cultures as indicated by upregulation of *BCL6*, *AICDA*, *PRDM1* and *XBP1* expressions(all, $P < .05$). *PRDM1* was upregulated following stimulation of naïve B cells with IL-4, but not IL-21 stimulation in the presence of IL-10($P < .05$). Furthermore, in the presence of IL-10, IL-4 but not IL-21 and dual IL-4/IL-21 stimulation resulted in high proportions of IgE^{+}CD27^{hi}CD38^{+} and IgE^{+} plasmablasts and CD138^{+} plasma cells. There was an increase in εGLT expression in naïve and memory B cell cultures in response to IL-4 stimulation which was downregulated in the presence of IL-10($P < .05$). No changes were observed in response to IL-21 stimulation, with/without IL-10. In addition, IL-10 suppressed tIgE levels in the presence of IL-4 in naïve and memory B cell cultures.
Conclusion: For the first time, we that showed IL-10^{+}T_{FH} cells are dysregulated in SAR and the differential effects of IL-4 and IL-21 in regulating B cell functions. Moreover, our study provided further insights on the role of IL-10 in regulating IgE responses by B cells.

OAS 16
Atopic Dermatitis: Pathogenesis and Treatment

0084 | **Evolution of skin microbiota and its relationship to eczema development in Chinese infants**

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Background: Eczema is the commonest chronic skin disease in children, and microbes such as staphylococci are abundant on their skin. A small birth cohort of Irish babies suggested that skin microbiota at 2-month predicted eczema occurrence at 12 months old age. Nonetheless, such data were not replicated in other populations. There were limited data on the evolution of skin microbiota in Asian infants. This prospective study investigated longitudinal changes in

skin microbiota during infancy and whether early-life exposures and skin microbiota might predict development of infantile eczema.

Method: This SMART Baby cohort recruited 120 healthy term Chinese newborns regardless of family history of allergic diseases. Their mean (SD) birth weight was 3119 (444) grams. Subjects were prospectively evaluated for eczema and allergies at 1, 6 and 12 months. Skin hydration (SH) was measured at antecubital and volar aspects of left forearm as well as upper back, and flocked skin swabs were then collected at the same sites, during study visits. V1-V3 regions of 16S rDNA in genomic DNA extracted from skin swabs were sequenced using Illumina MiSeq PE300. Bioinformatics analysis was performed with QIIME2.

Results: Subjects' SH at 1-month was lower among those with moderate-to-severe eczema at 12 months (44.9 ± 35.4) than those without eczema (80.1 ± 28.2) (P = .045). Besides, emollient use at 1-month predicted less eczema during infancy (52.7% *vs* 65.5%; P = .024). There were significant temporal variations in skin microbiota at 1-month and 6-month. Skin microbial compositions at upper back were different from those at left forearm. Shannon diversity index over left volar forearm at 1-month was lower among infants with persistent eczema (P = .004) but not in those with transient eczema (P = .440). The relationship between eczema and skin microbial biodiversity might be modified by mode of delivery, peripartum antibiotic as well as early-life feeding practice, pet keeping and passive smoking. There was no association between infantile eczema and skin microbiota at 6-month.

Conclusion: Biodiversity of skin microbiota at 1-month is associated with persistent eczema during infancy, supporting that neonatal skin microbial composition may predict eczema development. Such relationship is modified by a number of perinatal factors and early-life exposures. Skin microbiota at 6-month is not associated with infantile eczema. (funded by Health and Medical Research Fund [06170466]).

0501 | The role of metalloproteinase 9 in the pathogenesis of atopic dermatitis in children

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Background: To study the role of metalloproteinase 9 (MMP9) and the polymorphic variant *-8202A>G* of the MMP9 gene in the pathogenesis of atopic dermatitis (AD) in children.

Method: 54 children with AD were examined. A mild course of the disease was detected in 16.67%, a moderate course in 61.11% of the examined, and a severe course in 22.22% of patients. The control group consisted of 122 healthy children. Serum MMP-9 levels were determined by enzyme-linked immunosorbent assay using Cloud-Clone Corp.® test systems (USA). The polymorphic variants of the studied genes were determined by the method of allele-specific polymerase chain reaction using SNP-express reagent kits. An analysis

was made of the relationship of the association of the *-8202A>G* polymorphism of the *MMP9* gene with the risk of developing AD.

Results: An analysis of the results showed that the values of MMP9 in serum (573.75 ± 113.71 pg/mL) in patients with AD exceed the values in the control group (307.39 ± 42.39 pg/mL) [P = .025]. At the same time, there were no statistically significant differences in patients with varying severity (P ≥ .05). Analysis of the distribution of alleles and genotypes by the *-8202A>G* polymorphism of the *MMP9* gene revealed statistically significant differences between the control group and patients suffering from AD (P < .001). Moreover, the predominant allele in both groups is the *A*-allele (among patients with AD - 84.6%, in the control group - 50.8%). It was established that among children with AD, the *A/A* genotype prevails with a frequency of 69.2%, while in the group of healthy children the frequency of this genotype is 3 times lower (P < .001). It is important to note that in children with the *A/A*-genotype, the risk of AD developing is increased by 7.55 times (OR = 7.55 [95% CI - 2.97 - 19.21, P < .001]). Evidence of the effect of the mutant *A*-allele on the expression level of this protease is an increase in serum MMP9 values in homozygotes for the *A*-allele compared with carriers of heterozygous polymorphism.

Conclusion: It was found that the concentration of MMP9 in the blood serum of patients with atopic dermatitis exceeds the parameters in the control group. Moreover, the *8202A>G* polymorphism of the MMP9 gene is significant in the pathogenesis of atopic dermatitis in children. In homozygous carriers of the A-allele, the risk of developing skin manifestations of allergy is increased by more than 7 times.

0511 | The therapeutic and preventive effect of sublingual immunotherapy in mite-sensitized children with atopic dermatitis: A randomized, open, parallel-group study

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Background: Allergen immunotherapy with house dust mite (HDM) has been shown to improve eczema in patients with atopic dermatitis (AD). However, there are less data regarding therapeutic effect of sublingual immunotherapy (SLIT) on eczema and preventive effect on the development of respiratory allergy in children with AD. We aimed to evaluate the therapeutic and preventive effect of SLIT with HDM allergen extracts in HDM-sensitized children with AD.

Method: In this single center, randomized, open, parallel group trial, 60 children (aged 5-17 years) with a chronic course of AD (Scoring Atopic Dermatitis [SCORAD]> 8) and sensitization to HDM were randomized to receive SLIT (n = 30) or not (n = 30) during 12 months. SCORAD, Visual analogue scale (VAS) scores, and Investigator global Assessment (IGA) were compared between two groups, and specific IgE and IgG4 to HDM were measured at baseline and 12 months

after treatment. We assessed whether new asthma or allergic rhinitis develops during study period.

Results: The SLIT group had a significantly persistent reduction of SCORAD and IGA during total study period compared with the baseline, but control group did not. No significant differences were detected in VAS scores of both groups after 12-month treatment. After 1 year of treatment, specific IgE to HDM was not decreased, whereas IgG4 to HDM increased significantly only in SLIT group. Incidence of respiratory allergy during study period was 10.5% (2/19) in SLIT group, and 17.6% (3/17) in control group without significant difference.

Conclusion: SLIT for 1 year with HDM improved AD in HDM-sensitized children. However, there is no preventive effect on the development of respiratory allergy. It suggests that SLIT may be a treatment option in children with AD who are sensitized to HDM.

1545 | Increased bathing during infancy is associated with impaired skin barrier function, increased atopic dermatitis and reduced skin prick sensitisation to peanut and egg

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Background: Environmental exposures influence skin barrier function and may impact on the development of atopic dermatitis (AD) and food sensitisation during infancy. Aim: To determine whether bathing frequency during infancy is associated with skin barrier dysfunction, AD and food sensitisation.

Method: An observational analysis was conducted amongst children in the EAT Study cohort. Skin barrier function was assessed through the measurement of transepidermal water loss (TEWL) before each participant underwent examination for AD and skin prick testing at three and 12 months of age. Parents were surveyed at 3 and 12 months, asking whether their child had developed dry skin and about the regularity of their chosen bathing and moisturising skin care routines.

Results: Each additional bath per week at 3 months of age was independently related to skin barrier dysfunction (TEWL ≥ 15 g/m2*h; adjusted Odds Ratio (aOR) 1.21 (1.13-1.30), $P < .001$) after adjustment for family history of AD, filaggrin mutation inheritance, parental report of dry skin and frequency of emollient application. When treating bathing as a binary exposure, bathing more than weekly was associated with significantly increased chance of having developed AD on examination at 3 months (aOR 1.69(1.05-2.71), $P = .03$) when compared to bathing up to weekly. This relationship with AD was lost at 12 months of age, however skin prick sensitisation to peanut (≥3 mm; aOR 0.22(0.08-0.61), $P = .004$) and egg (≥3 mm; aOR 0.43(0.19-0.97), $P = .04$) were significant reduced with increased bathing even when correcting for presence of AD at 3 months.

Conclusion: Higher bathing frequency during infancy is associated with an increase in TEWL, examined eczema at 3 months, lesser peanut and egg skin prick sensitisation at 12 months. Prescribing bathing routines may constitute a worthwhile part of an intervention to prevent allergic disorders.

OAS 17
Novel Perspectives on Diagnosis and Management of Food Allergy

0190 | Allergen-blocking antibodies: A novel concept of peanut-allergy immunotherapy

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Background: Peanut allergy is an IgE-mediated disease with hypersensitivity to peanut (PN) proteins. At current, there is no immunotherapy available for these patients. The binding of PN allergens to IgE-FcεRI complexes, on the surface of mast cells and basophils, causes cellular degranulation that triggers symptoms of allergy. In the current project, we aim to develop and test a novel PN immunotherapy based on allergen-specific human monoclonal anti-PN antibodies (mAbs).

Method: Sera from PN allergic patients were screened for PN-specific IgE and IgG by ImmunoCAP and ELISA. From selected patients, B cells were isolated, and mAbs against PN allergens were molecularly cloned. The allergen specificity and binding affinity were tested by ELISA and surface plasmon resonance. The antibody function was tested *in vitro* with leucocytes from PN-allergic patients in `. Immunotherapeutic efficacy was performed in C3H mice sensitised with PN allergens by multiple low-dose intraperitoneal injection of peanut allergen extract. Mice received anti-peanut mAbs by intravenous injection at different time points before allergen-provocation by systemic injections of high-dose PN allergen extract. The anaphylaxis was monitored by clinical scoring and body temperature drop.

Results: We identified patients with anti-PN IgGs and were able to recover B cells and to clone mAbs with high affinity for different PN proteins. The mAbs inhibited basophil activation and leukotriene secretion *in vitro*. When sensitised mice were challenged with a mixture of anti-PN mAbs and PN allergen extract, no clinical signs of anaphylaxis were observed, while a challenge with PN allergens extract caused strong anaphylactic reactions and temperature drops. When sensitised mice were treated by passive immunization with anti-pN mAbs 1-14 days before the challenge, the anaphylaxis could be fully abrogated.

Conclusion: In closing, functional anti-PN IgG mAbs could be cloned from PN-allergic patients, and full protection against allergic anaphylaxis could be obtained by immunotherapy in PN-allergic mice. The results may pave the way for new clinical options for patients with difficult or untreatable allergies as well as for patients with seasonal allergies where allergen exposure is perennial.

0372 | Need for more than one dose of adrenaline to treat anaphylaxis: A systematic review and meta-analysis

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Background: The European Medicines Agency recommends all patients at risk of anaphylaxis carry 2 adrenaline auto-injectors at all times, in contrast to EAACI guidelines. The need to treat anaphylaxis with more than one dose of adrenaline has not been systematically evaluated.

Method: We undertook a systematic review and meta-analysis to assess the proportion of anaphylaxis reactions reported in the literature which were treated with at least 2 doses of adrenaline. We searched for relevant papers (cohort studies/registry reports and case series with ≥ 10 cases of anaphylaxis in MEDLINE, Embase, Web of Science or Cochrane, between January 1946 and July 2019, and also reviewed recent conference abstracts. Data were screened and extracted in duplicate and synthesized for meta-analysis using a generalised linear mixed model. Study registration: PROSPERO CRD42017069109.

Results: 77 datasets were included, representing over 20,000 anaphylaxis cases reported in the literature. Estimates of the proportion of anaphylaxis reactions treated with 2 + doses of adrenaline ranged from 3.9% [95%CI 2.1 to 7.1] for food challenges in hospital, to 7.3% [95%CI 5.2 to 8.0] for community reactions. There was significant heterogeneity in the data which was not affected by study design or definition of anaphylaxis used. 10.9% [95% CI 8.5 to 13.8] of patients given adrenaline for food-anaphylaxis demonstrate an incomplete response as assessed by a healthcare professional.

Conclusion: Around 10% of patients receiving adrenaline for anaphylaxis have a suboptimal response to a single dose of adrenaline, as assessed by a healthcare professional. The ramifications of these data on policy require further evaluation.

	Anaphylaxis (all cause, community)	Food-induced (community)	Food challenge (in hospital)
Anaphylaxis (study-defined)	7.3% [5.2 to 8.0]	6.6% [4.8 to 9.0]	3.9% [2.1 to 7.1]
Anaphylaxis (RS/CVS only)	7.8% [5.8 to 10.6]	N/A	6.1% [2.9 to 12.6]
Anaphylaxis [(sd)] 2nd dose given by HCP	6.7% [5.2 to 8.7]	7.0% [4.5 to 7.8]	4.5% [2.6 to 7.7]
Anaphylaxis [(sd)] treated with 1 + dose ADR	13.5% [11.5 to 15.8]	11.2% [8.7 to 14.5]	8.1% [5.6 to 11.7]
Anaphylaxis [(sd)] treated with 1 + dose ADR, 2nd dose given by HCP	12.8% [10.6 to 15.4]	10.9% [8.5 to 13.8]	8.3% [5.6 to 12.1]

Data are % reactions [95% CI].
ADR, Adrenaline; HCP, healthcare professional; RS/CVS, respiratory/cardiovascular symptoms; sd, study defined.

1362 | Efficacy and safety of epicutaneous immunotherapy (EPIT) for peanut allergy in subjects with and without concomitant food allergies

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Background: The efficacy and safety of EPIT in peanut-allergic children with a 250-μg peanut patch (VP250) have been studied in placebo-controlled randomized trials. It is important to understand whether concomitant food allergies (CFA) affect the treatment response or safety outcomes of peanut-allergic children treated with VP250.

Method: PEPITES was a Phase 3, randomized, double-blind, placebo-controlled trial of VP250 in children 4–11 years of age (n = 356). Post hoc analysis explored differences in efficacy and safety among peanut-allergic children with and without CFA at study entry.

Results: At baseline, 206 (58%) children had CFA and 150 (42%) children were solely peanut-allergic and were not avoiding additional foods.

Improvement in predefined primary outcome (based on changes in peanut eliciting dose following 12 months VP250 in the treatment group vs placebo group) was consistently in favor of VP250 irrespective of whether children had CFA or isolated peanut allergy at study entry. The VP250 response rate was 33.3% and 38.1%, respectively, and the placebo response rate was 16.9% and 9.4%, respectively (interaction P = .17).

Serious treatment-emergent adverse events (TEAEs) assessed as related to VP250 by investigators occurred in 1 participant (0.7%) with CFA and 2 (2.1%) participants with isolated peanut allergy. Likewise, TEAEs considered to be VP250-related by investigator assessment,

which led to permanent study discontinuation, occurred in 2 (1.4%) participants with CFA and 2 (2.1%) allergic to peanut alone. Local application site reactions were noted in 58.2% of participants randomized to VP250 with CFA and 56.7% of participants randomized to VP250 with isolated peanut allergy.

Conclusion: In peanut-allergic children aged 4-11 years who were treated with EPIT for peanut allergy, improvement in the predefined primary outcome measure over the 12-month treatment period was in favor of VP250, irrespective of whether they were allergic only to peanut or had CFA at study entry. The safety and tolerability profiles were similar in peanut-allergic children randomized to VP250 with peanut allergy alone and peanut allergy with CFA.

OAS 18
Diagnosis and Management of Ocular Allergy and Allergic Rhinitis

0522 | A controlled naturalistic approach to the study of allergic conjunctivitis (AC) in an environmental exposure chamber (EEC)

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Background: AC affects > 1-billion worldwide and increasing, yet many sufferers remain underserved by available medications. The EEC allows for controlled yet naturalistic airborne allergen exposures in which subjects rate their symptoms real-time. The aim of these analyses was to assess patients' ocular symptom development profiles in the EEC towards study of AC.

Method: Seventy ragweed allergic subjects with 2-year history and positive ragweed SPT were studied in the EEC. Subjects were administered saline drops in each eye and exposed to airborne ragweed pollen (mean exposure: 3500 ± 500 pollen grains/m^3) in the EEC for 90 minutes. Approximately every 10-mins, subjects self-rated ocular itching (9-point scale, 0-4, 0.5 increments) and tearing (4-point scale, 0-3, 1.0 increments) on an e-diary and staff assessed ocular redness (9-point scale, 0-4, 0.5 increments). Subjects with conjunctival redness of ≥ 2 in at least one ocular quadrant and ocular itching ≥ 2.5 were considered adequately allergic. Descriptive statistics and AUC for each symptom were calculated. Pearson correlation analyses of SPT wheal *vs* symptoms were evaluated.

Results: As expected, significant itching developed more rapidly than ocular redness. Mean scores in the last timepoint for itching, redness and tearing were 3.26 ± 0.66, 2.46 ± 0.53, and 2.16 ± 0.56 respectively. Mean AUC 0-90 min for itching (151.8 ± 55.7), redness (182.9 ± 33.6) and tearing (109.4 ± 38.5) were different related to differences in response profiles with redness highest due to higher redness at baseline, slow development with low-grade persistence throughout. Non-significant correlations were observed for wheal *vs* symptom severity and wheal *vs* onset of itching ($r = 0.058$, $P = .633$)

with a weak correlation between wheal and onset of redness ($r = 0.297$, $P = .012$).

Conclusion: These results provide insight into the profiles of ocular allergy symptom development that are consistent with their underlying mechanisms, with faster itching and slower, persistent ocular redness, due to early and late phase mediators, respectively. Lack of skin test correlation with ocular symptom magnitudes confirms SPT is not a good predictor of AC severity. The ability to provide a controlled yet naturalistic allergen exposure allows for both prophylactic and on-symptom drug testing. Taken together, the EEC approach is an excellent setting for the study of AC patients and novel putative AC medications.

0539 | Underdiagnosed and growing epidemic of ocular allergy (OA) - diagnostic tools and role of allergen immunotherapy in severe OA

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Background: Diagnosis of OA is usually based on history and clinical examination, with aid of in vivo or/and in vitro tests to identify specific allergen, in recalcitrant patients to topical treatment.

To evaluate the role of diagnostic tools and allergen immunotherapy in management of OA.

Method: 518 patients presenting with varying grades of ocular allergy, presenting to a tertiary care ophthalmic centre, were included in this study. The patients were evaluated for severity of allergy and detailed history of systemic allergy if present was taken. Concurrent tear samples and ocular wash was collected in the severe cases of ocular allergy who were not on topical steroids or immunomodulatory medications at that time. These patients were also evaluated for concomitant allergic disorders like Allergic Rhinitis (AR) and lower airway involvement, asthma in adults or Multi-trigger wheeze in the paediatric population. Investigations included serum Immunoglobulin (IgE) levels and skin prick test to a panel of common aeroallergen indigenous to the geographical area were done. Appropriate avoidance measures were advised and patients with severe OA, recalcitrant to topical therapy, were started on Sublingual Immunotherapy, after due consent from the patient and/or the parents. History of steroid dependence or steroid overuse was also taken.

Results: The patients were divided into groups based on severity of ocular allergy. They were further grouped based on the association of systemic allergy like AR, asthma or skin allergy. 20% of patients were found to be having severe ocular allergy or recalcitrant to topical therapy. Tear samples showed increased levels of inflammatory molecules like Interleukin (IL) 4 and 15 and IgE. No direct correlation was found between tear IgE and Serum IgE in these patients. 46 of these patients also had keratoconus requiring treatment. In the

patients who had severe recalcitrant ocular allergy, systemic therapy with sublingual immunotherapy (SLIT) was found to be useful in controlling the disease and associated complications, thereby improving the Quality of Life(QoL).

Conclusion: A stepwise algorithmic and multi-disciplinary approach, including ophthalmologist and allergist, is useful in these cases. Proper patient selection for Allergen Immunotherapy, along with appropriate allergen avoidance measures, will help improve QoL in severe OA.

0594 | Treatment of severe allergic conjunctivitis with antolimab indicated improvement of ocular signs and symptoms and reduction of severity of comorbid atopic diseases in a phase 1b open-label study

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Background: Severe allergic conjunctivitis (AC) presents with debilitating ocular symptoms and a risk to vision loss. AC is driven primarily by mast cells and eosinophils. Antolimab (AK002) is an anti-Siglec-8 monoclonal antibody that selectively depletes eosinophils and broadly inhibits mast cells. KRONOS was an open-label Phase 1b clinical study of antolimab in patients with chronic atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis (VKC), and perennial allergic conjunctivitis (PAC). This analysis evaluated the effect of antolimab on AC symptoms as measured by a patient-reported outcome (PRO) questionnaire and on AC signs and symptoms as measured by an investigator assessment tool.

Method: Moderate-to-severe patients with AC and history of corticosteroid use were enrolled to receive six monthly doses of antolimab on a dose escalation schedule (0.3, 1, 1 or 3, 1 or 3, 1 or 3, 1 or 3 mg/kg). Symptoms of AC were assessed with a daily patient-reported outcome (PRO) symptom questionnaire (Allergic Conjunctivitis Symptom Score (ACS): itching, light sensitivity, eye pain, foreign body sensation, and watering eyes) and a monthly investigator assessment of ocular signs & symptoms (OSS: itching, redness, tearing, and chemosis). Clinical activity of antolimab on comorbid atopic dermatitis, asthma, and/or rhinitis was assessed by a daily PRO questionnaire.

Results: Twenty-nine patients were enrolled from Mar-2018 to Nov-2018 (AKC n = 13, VKC n = 1, PAC n = 15), 87% of whom had atopic comorbidities. By 2 weeks post-last dose, median ACS for AKC, VKC, and PAC groups improved by 74%, 87%, and 71%, respectively. Consistent with these results, median OSS for AKC, VKC, and PAC groups improved by 71%, 86%, and 83%, respectively. There was substantial improvement in allergic comorbidities, with 65%, 72%, and 69% reduction of symptoms of atopic dermatitis, asthma, and

rhinitis, respectively. There were no drug-related serious adverse events (AE), and the most common treatment-emergent AE was mild-to-moderate infusion related reactions (IRRs: 16.7% IRR rate on first infusion, and 0.7% IRR rate on subsequent infusions).

Conclusion: Antolimab was well-tolerated and substantially improved symptoms of severe AC as measured by both a PRO and an investigator assessment. In addition, antolimab substantially improved symptoms of comorbid atopic conditions. Antolimab may be a promising treatment for severe AC as well as atopic dermatitis, asthma and other atopic conditions.
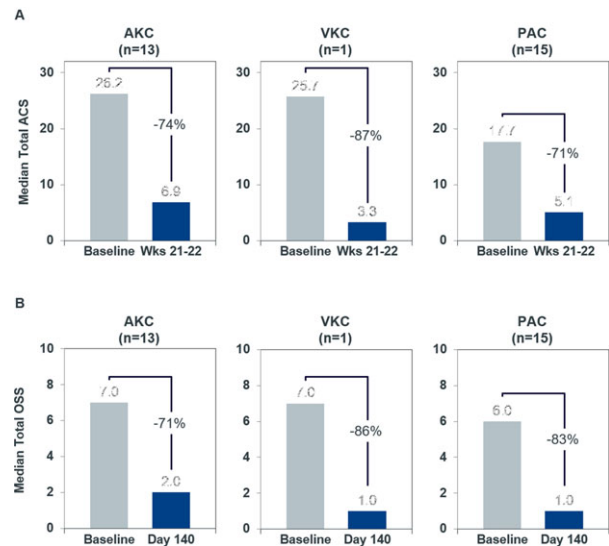
0663 | Efficacy of allergen immunotherapy for allergic rhinoconjunctivitis assessed by conjunctival allergen provocation–a real-life study

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Background: Conjunctival allergen provocation tests (CPT) are used to assess efficacy of allergen immunotherapy (AIT) in clinical trials; however, real-life studies regarding its use in daily practice are lacking. Patient reported satisfaction regarding AIT has been rarely evaluated in association with objective measures.

Aim: To assess the efficacy of AIT through CPT and evaluate patients' satisfaction through the "Satisfaction Scale for Patients Receiving Immunotherapy" (ESPIA questionnaire), in patients with rhinoconjunctivitis treated with Dermatophagoides pteronyssinus (Dp) and/or grass pollen AIT.

Method: Prospective study with patients with rhinoconjunctivitis from an allergy department of a University Hospital that performed CPT before and following at least 1 year of AIT with Dp and/or

grass pollen (*Dactylis, Festuca, Lolium, Phleum and Poa)*. CPT were performed with Leti®, allergen extracts, at tenfold increasing concentrations. Total ocular symptom score (TOSS) was assessed and a positive result was considered if TOSS ≥ 5. Results of CPT before and after starting AIT were compared. A cross-cultural translated version of ESPIA questionnaire was applied.

Results: A total of 29 CPT were performed in 20 patients (14 with Dp and 15 with grass pollen), 70% female, median age of 15 years, 60% children and half had a concomitant medical diagnosis of asthma. One quarter of patients were treated only with grass pollen, 15% with only Dp, and 60% with mixed (Dp and grass pollen) AIT. The median duration of AIT treatment was of 21[12;23] months.

The concentration that elicited a positive CPT with Dp increased in 93% of patients (up to 100 times higher); with grass pollen, the concentration increased in 40% of patients and decreased in 20%. The 3 patients with increased ocular reactivity to grass pollen were being treated with a mixed AIT.

Patients reported an ESPIA median score with grass pollen, mixed and Dp AIT of 64 [47;76]; 64[53;70]and 63[54;-], respectively.

Conclusion: CPT may be useful to assess efficacy of AIT in real life. However, larger samples with longer follow-up studies are needed to confirm these results. Furthermore, different CPT protocols, namely using two-fold concentration increase, may yield different results. Questionnaires of symptoms, satisfaction and quality of life should be used as an add-on tool to assess AIT efficacy.

0900 | Molecular sensitization predictors of allergen-specific immunotherapy efficacy in patients with perennial allergic rhinitis with sensitization to house dust mites

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Background: Allergic rhinitis (AR) and asthma are the most common allergic disorders worldwide. Aeroallergens are the causative factors in the pathogenesis of these disorders. Sensitization to aeroallergens differs in various countries and regions. Identification of the most common aeroallergen sensitization is crucial in the diagnosis and management of AR and asthma. Allergen-specific immunotherapy (AIT) is the only available treatment that can induce specific immune tolerance to allergens. However, the treatment course lasts at many months, with no reliable method to predict treatment response. This study assessed a treatment response to AIT based on sensitization patterns.

Method: 110 patients who had undergone 18 month of standard house dust mite AIT with sublingual forms of house dust mite allergens (D. pteronyssinus 50%, D. farinae 50%) were assessed. During the study, patients were divided into two groups: Group 1 - patients with sensitization only to major components of the house

dust mite (Der p1, Der p2), n = 74; and Group - patients with sensitization to the major and minor components of dust mite (Der p1, Der p2, Der p6, Der p10), n = 36. Clinical characteristics, skin-prick test responses, and treatment response were evaluated at months 12 and 18. Effective AIT was defined as a 50% reduction in average adjusted symptom score (AAdSS) from baseline at the end of the second year of immunotherapy.

Results: The overall efficacy rate at the end of 12 months of the AIT was 52.7% in Group 1 and 25.6% in Group 2. Age, sex, asthma, body mass index, smoking history, and aeroallergen categories were not associated with differences in efficacy of AIT. Meanwhile, efficacy data at month 12 (odds ratio [OR], 5.850; $P = .003$), month 18 (OR, 7.476; $P < .001$) in Group 1, and month 12 (OR, 4.130; $P < .004$), and month 18 (OR, 8.716; $P < .000$) in Group 2.

Conclusion: Efficacy of AIT at months 12 and 18 is strongly associated with sensitization to major components of the house dust mite (Der p1, Der p2). Efficacy of AIT can potentially be predicted before AIT treatment by assessment of Der p6 and Der p10 sensitization which reduces the overall efficacy at 1 year.

1202 | Personal Allergy Tracker (PAT): Description of a study protocol utilizing m-Health technology for monitoring allergic rhinitis. Can mobile technology improve control?

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Background: Lack of information concerning the nature, intensity, and duration of symptoms, is a common problem for physicians treating allergic rhinitis (AR). Patients may find such symptoms quite bothersome but also trivial to observe. Mobile Health (m-health) solutions can improve both monitoring and control of allergic rhinitis. The purpose of this study is to create an integrated, crowdsourcing e-Health /m-Health system for monitoring AR using smartphone and smartwatch sensors and applications.

Method: This prospective multicentered study includes patients with AR aged between 18 and 55 years old and healthy controls. Initially, subjects have provided data for the development and fine-tuning of two types of algorithms; for detection of gestures indicative of AR and conjunctivitis (i.e. allergic salute, rubbing of eyelids), and for detection of distinctive changes in voice during AR.

Subsequently, patients with AR will be monitored for 3 months through a smartphone app that will be able to record gestures via a connected wristband and voice changes, both attributed to AR, based on the previously developed algorithms. Data from gestures and voice recordings will be compared with responses to AR symptom questionnaires presented to the patients from their mobile app on a daily basis.

The effect of the system on AR outcomes will be then evaluated by comparing AR symptom control between two subgroups, the first receiving alerts to increase AR treatment when signals from gesture and voice change recognition are suggestive of escalation of symptoms' severity; the other receiving sham alerts. Both subgroups will follow action plans based on ARIA guidelines.

Conclusion: M-Health technology may help patients and physicians better understand AR's impact on everyday life and improve adherence to treatment through closer monitoring and self-management. Employment of m-Health services to a common chronic disease like AR will probably benefit patients, doctors, and health systems by optimizing control and reducing health costs.

OAS 19
Novel Innate Immune Mechanisms

0258 | Multiple myeloma exosomes suppress natural killer cells: Modification by omega-3 fatty acids

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Background: Multiple myeloma (MM), the most common malignancy of plasma cells, is still regarded as incurable due to frequent relapses. Considering previous findings that exosomes released from tumor cells including MM play a critical role in tumor survival and progression, in the present work we checked as to whether exosomes released from a myeloma cell line (L363) can affect the function of natural killer cells. We also checked whether the treatment of L363 cells with sub-lethal doses EPA and DHA, two polyunsaturated fatty acids (PUFA) for which various anti-cancer properties have been previously explained, can alter the release and function of MM-derived exosomes.

Method: Exosomes were isolated from EPA/DHA-treated and –untreated L363 culture supernatants and characterized using dynamic light scattering, electron microscopy and flow cytometry (CD81 and CD63). NK-92 cells were then treated with these exosomes either alone or in co-culture with K562 cells and their K562 killing function was assessed using flow cytometry. The degranulation marker CD107a, NKG2D and IFN-γ were assessed using flow cytometry and ELISA.

Results: Pre-treatment with MM-derived exosomes significantly reduced the cytotoxic function of NK cells against K562 cells, indicating an immunosuppressive role for these particles. Interestingly, exosomes from EPA/DHA-treated MM cells had a weaker immunosuppressive effect. NKG2D expression was reduced following treatment with MM exosomes, explaining a possible mechanism responsible for the observed suppressive effect. On the contrary, MM exosomes could stimulate both the production and release of IFN-γ, delineating a dual role of these exosomes on NK effector functions. Moreover, we found that EPA/DHA-treated L363 cells could release higher amounts of "less suppressive" exosomes compared to the untreated cells.

Conclusion: Our results showed that exosomes released from MM cells play a role in the immunosuppressive microenvironment of MM and that treatment with EPA/DHA can, at least partly, inhibit the immunosuppressive effects of MM exosomes. These findings provide some evidence on the noxious role of MM-exosomes in compromising the NK-mediated anti-myeloma immune response as well as the beneficial microenvironment-modifying effects of PUFAs in the context of MM. Further studies are warranted to explore the precise mechanisms involved.

0321 | Mesenchymal stem cells ameliorate long term pulmonary complications induced by sulfur mustard

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Background: Sulfur Mustard as a chemical warfare agent has major long-term complications in the lungs. There is a lack of effective medical care for victims due to the poor understanding of SM-immunopathogenesis mechanisms in the lungs. Here, we investigate the therapeutic effect of adipose-derived mesenchymal stem cells (AD-MSC) and MSC-derived conditioned medium (CM-MSC) on pulmonary injury induced by CEES, an SM analog.

Method: C57Bl/6 mice initially received CEES and were then treated with either AD-MSCs or CM-MSC. The immunophenotypical analysis of surface markers of alveolar macrophages and regulatory T-cells was carried out by flow cytometry and the concentration of cytokines were also determined by ELISA. The functional changes in the lungs was also checked using SPECT and histopathology.

Results: The injection of either AD-MSC or CM-MSC following CEES administration reduced histopathologic changes in the lung that were also confirmed by SPECT imaging. AD-MSC and CM-MSC administration reduced the levels of pro-inflammatory cytokines and affected the balance between M1 and M2 macrophages. Accumulation of macrophages with the predominance of the M1 phenotype was seen in response to CEES exposure that was reduced by MSC administration. AD-MSCs and CM-MSC caused a marked reduction in the CD86- and CD206-expressing macrophages such that this modulating effect in the M1-subset was much more pronounced compared to M2. Moreover, AD-MSC and CM-MSC treatment induced a modulating effect on T-regs in the lymph node after CEES exposure.

Conclusion: Our findings show that the therapeutic effect of MSCs in CEES pulmonary -long term complications is mediated by restoring the balance between M1/M2 alveolar macrophages and by maintaining homeostatic conditions in the lung, suggesting an effective therapeutic approach for improving sulfur mustard-induced pulmonary symptoms.

0796 | Extreme prematurity strongly imprints circulating Natural Killer- and γδ T cells, which are further influenced by sepsis

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Background: Preterm neonates with extremely low gestational age and birth weight (ELGAN/ELBW) are highly susceptible to infection, long-term complications and mortality. Impaired innate immune defense and less developed adaptive immunity may account for susceptibility to infection in those preterm infants. Little is known about the composition and contribution of Natural Killer (NK) cells and non-conventional T cells in clinical onset of sepsis in ELGAN/ELBW infants.

The aim of the present study was to evaluate the phenotype, frequency and functional characteristics of NK-, γδ T-, mucosa associated invariant T (MAIT)- and NKT cells in a cohort of ELGAN/ELBW neonates in comparison with full term infants and with different manifestations of the septic syndrome.

Method: Peripheral blood mononuclear cells (PBMC) and plasma were collected at 14, 28 days and after 36 + 0 PMW from 79 ELBW premature infants, participating in a randomized double-blind placebo-controlled study of probiotic supplementation. As a control, PBMCs and plasma from 29 full-term (FT) infants at 14 days of age were used. Non-conventional T cell populations were analyzed by multi-color flow cytometry.

Results: NK cell frequencies were markedly lower in 14-day old ELGAN/ELBW preterm neonates compared to the full-term neonates and they also had a clearly elevated CD56 [dim:high] ratio. Conversely, γδ T- and NKT cell frequencies were notably higher in 14-day old ELGAN/ELBW neonates than in full-term neonates. The γδ T-cells from ELGAN/ELBW neonates also had a markedly higher expression of the gut homing CCR9 receptor. Further, while the frequency of γδ T-cells with a naïve phenotype were reduced in the ELGAN/ELBW group, γδ T-cells with effector- and effector memory phenotypes were increased compared with full-term neonates. MAIT cell populations were similar in the two groups. Sepsis with an onset before 14 days of life associated with alterations in the NK cell- and γδ T cell-populations at day 14 of life. While the CD56^{+} NK cell frequency was further reduced, the proportion of the γδ T cell-population was higher, with an increased effector:naïve cell ratio in the preterm neonates with sepsis with an onset before day 14 compared with preterm infants without sepsis or sepsis onset after day 14.

Conclusion: The results show that prematurity gives a strong imprint on several both NK cells and unconventional T cells that is further enhanced in cases with early-onset sepsis.

0841 | Metabolic reprogramming and autophagy mediate the tolerogenic effects induced by the synthetic cannabinoid WIN55,212-2 in human dendritic cells

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Background: The endocannabinoid system (ECS) is a complex signalling network involved in many physiological processes. Different studies reported the contribution of the ECS in modulating immune responses. Human dendritic cells (DCs) express cannabinoid receptors 1 and 2, CB1 and CB2, respectively, but how these receptors regulate immune responses in DCs remains poorly understood. Here, we employed the synthetic cannabinoid WIN55,212-2 to elucidate the molecular mechanisms by which cannabinoids modulate immune responses in human DCs.

Method: Cocultures of human monocyte-derived DCs (hmoDCs) or total DCs with naïve CD4^{+} T cells, ELISA, western blot and flow cytometry were performed to study WIN55,212-2 effects in human

DCs. Warburg effect, lactate production, glucose uptake and mitochondrial function were analysed in hmoDCs to assess metabolic status.

Results: The synthetic cannabinoid WIN55,212-2 promoted a tolerogenic phenotype in hmoDCs as determined by a significantly lower cytokine production upon stimulation with LPS and higher capacity to polarize $CD4^+$ naïve T cells into functional $CD4^+CD25^+CD127^-$ $FOXP3^+$ regulatory T (Treg) cells. Supporting these data, similar responses were observed in total DCs. Mechanistically, we showed that WIN55,212-2 reduced Warburg effect, lactate production and glucose uptake whereas increased mitochondrial function in LPS-activated hmoDCs. Similarly, WIN55,212-2 abolished LPS-induced NF-kB, MAPKs and mTOR activation and promoted autophagy induction in hmoDCs. 3-methyladenine (3-MA), a specific autophagy inhibitor, significantly impaired the capability of LPS/WIN55,212-2-treated hmoDCs to induce Treg cells as well as the metabolic reprogramming imprinted by WIN55,212-2 in LPS-stimulated hmoDCs.

Conclusion: The synthetic cannabinoid WIN55,212-2 endorses the development of tolerogenic human DCs by mechanisms depending on metabolic reprogramming and autophagy induction.

1456 | CD45RO marks activated corticosteroïd resistant ILC2s as present in severe asthma and chronic rhinosinusitis with nasal polyps

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Background: Group 2 innate lymphoid cells (ILC2s) are type-2 effector cells involved in tissue homeostasis and defense responses against parasites. ILC2s have also been implicated in immunopathology associated with type-2 immunity, including chronic rhinosinusitis with nasal polyps and asthma. Importantly, under certain conditions ILC2s can develop resistance to corticosteroids, the main therapy for type-2 inflammatory diseases. How ILC2 activation, tissue localization and steroid sensitivity are related remains unclear.

Method: In this study, we investigated the transcriptional and phenotypical differences between type-2 inflamed nasal polyps (NP) and healthy peripheral blood (PB) ILC2s using microarray and flow cytometry. Furthermore we measured the activation status of ILC2s in PB of asthma patients.

Results: NP ILC2s displayed a more activated phenotype compared to PB ILC2s. Interestingly, while resting PB ILC2s expressed CD45RA, NP ILC2s mostly expressed CD45RO. The activated $CD45RO^+$ NP ILC2 phenotype can be induced *in vitro* by stimulating PB ILC2s with type-2 inflammatory cytokines. Strikingly, the frequency of circulating $CD45RO^+$ ILC2s is increased in asthma patients and correlated strongly with asthma disease severity and a requirement for higher

corticosteroid dosages. *In vitro* studies confirmed the differential steroid sensitivity of $CD45RA^+$ and $CD45RO^+$ ILC2s, as steroid resistance was strongly induced after differentiation of $CD45RA^+$ to $CD45RO^+$ ILC2s prior to dexamethasone treatment.

Conclusion: Our data identify an inflammatory ILC2 subset marked by $CD45RO^+$ that is linked to severe type-2 inflammatory disease and steroid resistance. Insights into the innate immune components may provide novel biomarkers for monitoring the responsiveness to treatment of type-2 inflammatory diseases.

(KG and EKvdP contributed equally, RS and SMB co-supervised this study).

OAS 20
Novel Allergens and Mechanisms of Food Allergy

0134 | Epitope mapping of the major allergen 2S albumin from pine nut

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Background: Allergy to pine nut has been described to be particularly severe and has been characterized by a high rate of monosensitized patients. In this study, an analysis of the IgE-binding epitopes of the major allergen 2S albumin from pine nut (Pin p 1) using sera from patients with clinical allergy to pine nut was carried out in order to deepen into the allergenic characteristics of Pin p 1 and to study its differential features with other 2S albumins.

Method: A library of 51 peptides consisting on 15-meric peptides, with an overlap of 12 amino acids, covering the complete sequence of Pin p 1 was incubated with individual sera from patients with clinical pine nut allergy. IgE-binding was detected and a heatmap displaying fluorescence intensities was created. A study of similarities of the IgE-binding epitopes of Pin p 1 with other peptides from allergens was performed. Finally, a predictive 3D model of Pin p 1 was built in order to localize the described epitopes in its structure.

Results: Three main regions of the sequence of Pin p 1 containing 5 epitopes were mainly recognized by sera IgE from patients with pine nut allergy. Although Pin p 1 had low similarity with other 2S albumins, the IgE-binding epitopes described here showed important similarities with epitopes of allergens such as Ara h 2, Ara h 6, or Ber e 1. Importantly, Pin p 1 epitopes were found to be well-exposed in the protein surface, which suggests a facile access for IgE-binding to the structure of Pin p 1 which is known to be highly resistant to heat and enzymes.

Conclusion: Our study contributes to the growing area of characterization of allergens' epitopes that will allow going one step forward in the molecular knowledge of allergens for their further use in Component-Resolved Diagnosis.

0172 | **Peanut 2S albumin Ara h 7: Purification from peanut, and initial biochemical and immunochemical characterization**

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Background: The 2S albumin protein family constitute the majority of allergenicity in peanut. From this protein family, only Ara h 2 and Ara h 6 have been characterized. So far, Ara h 7 has not been purified from peanut and is only available in recombinant form. Here, we purified and characterized Ara h 7 from peanut in its native form, i.e. taking into account possible post-translational modifications.

Method: Using extraction, fractionation, and chromatography, two Ara h 7 isoforms were isolated. Mass spectrometry was used for identification and assessing purity, and for characterizing post-translational modifications. SDS-PAGE, absorbance spectroscopy and far-UV CD spectroscopy were used to further investigate biochemical characteristics. IgE-binding was investigated using a serum pool from donors allergic to peanut who had a strong sensitization to the other peanut 2S albumins, Ara h 2 and Ara h 6.

Results: Two Ara h 7 isoforms were purified from peanut. These proteins eluted as single peaks during the last chromatography step, and appeared as single bands on SDS-PAGE. One of the purified proteins corresponds to IUIS 7.02 (NCBI: RYR43594.1, Uniprot: B4XID4). The second protein is similar to IUIS 7.02, but contains three amino acid substitutions. Both of the identified Ara h 7 isoforms appear to have a post-translation cleavage near the N-terminus, have 4 disulfide bonds per molecule, and contain hydroxyproline. Within a purified fraction, some diversity of intact masses could be explained by post-translational proteolysis at the N-terminus. Secondary structure composition of both Ara h 7 isoforms is highly comparable to that of Ara h 2 and Ara h 6. Both Ara h 7 isoforms bind IgE, and Ara h 7 is capable of inhibiting the binding between Ara h 2 and IgE, suggesting at least partially cross-reactive IgE epitopes. In crude peanut samples, no evidence of IUIS isoforms 7.01 and 7.03 was observed. It is likely that these isoforms are a result of frameshift sequencing errors and do not exist in peanut, and that two variants of Ara h 7.02 make up the complement of Ara h 7 in peanut seed.

Conclusion: Two Ara h 7 isoforms exist in peanut and have been purified for the first time. These purified proteins resemble characteristics of other peanut 2S albumins, and are at least partially IgE-cross-reactive with Ara h 2. This suggests that Ara h 7 can be a relevant peanut allergen too.

0880 | **Functional differences in peanut-specific IgE can explain discrepancies between IgE titres and allergic reactions to peanut**

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Background: IgE mediates allergic reactions to peanut; however, peanut-specific IgE (sIgE) titres do not equate to clinical peanut allergy. Functional differences between IgE of peanut sensitised but tolerant (PS) and peanut allergic (PA) individuals may be important. We looked to analyse characteristics of peanut-specific IgE and assess their influence on effector cell activation in peanut allergy.

Method: 100 children being assessed for peanut allergy were studied (50 PA, 40 PS, 10 non-allergic). IgE specificity was determined using ImmunoCAP. Specific activity was defined as the ratio of allergen-sIgE to total IgE. Avidity was measured by quantifying sIgE to peanut in the presence of NaSCN. IgE diversity was estimated within 112 allergens (Whole-diversity/WD) and 6 peanut allergens (Peanut-diversity/PD) using ImmunoCAP ISAC. Mast cell activation tests (MAT) were performed using patients' plasma on LAD2 cells. Statistical analyses were performed using SPSS v14 and Prism v7.

Results: sIgE levels to peanut ($P = .0019$), Ara h 2 ($P = .0001$), Ara h 3 ($P = .046$) and Ara h 6 ($P < .001$) were higher in PA than PS individuals; as were specific activity to peanut ($P = .0001$), Ara h 1 ($P = .004$), Ara h 2 ($P = .0001$), Ara h 3 ($P = .02$) and Ara h 6 ($P = .0001$). We were able to determine a range of avidities (7-72%), by measuring the % dissociation of sIgE-peanut binding across individuals with peanut sIgE ranging from 0.99-568 kU/L. The avidity of peanut sIgE in PA was higher than in PS individuals ($P = .0001$). Diversity variables were generated based on Shannon's diversity index with values ranging from 0-3.39. WD indices showed no significant differences between PA and PS individuals; however, PD was greater in PA individuals ($P < .001$). Positive correlation was reported when assessing IgE characteristics against mast cell activation (measured as %CD63 + LAD2 cells). The correlation was strongest for specificity to Ara h 2 ($r = 0.661$) followed by peanut specific activity ($r = 0.654$), PD ($r = 0.649$) and avidity ($r = 0.259$). A bioinformatics approach is being used to compute the contribution of individual IgE characteristics to allergen-induced mast cell activation.

Conclusion: IgE specificity, specific activity, avidity and diversity are distinct between PA and PS individuals and were correlated with mast cell response to peanut allergens. Improved understanding of the relative importance of these factors can further clarify

the discrepancy between peanut-sIgE titres and clinical reactivity to peanut.

0962 | The skin acts as the preferential sensitizing pathway in an in vivo model of anaphylaxis

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Background: Despite attempts to find a cure for food allergy, no definitive treatment has been approved. Although immunotherapy formulations seem promising, current strategies lack enough safety to be clinically validated, according to EAACI's Guidelines. This may relate with our still not fully understanding of the sensitization process, making it impossible to reverse the allergic condition once it has been established. So, our aim was to study how Pru p 3, as a model of food allergy, interacts with the mucosa-associated lymphoid tissue (MALT), analyzing the changes that lead to the consecution of a chronic type 2 state.

Method: An anaphylactic mouse model was developed using Pru p 3 in complex with its lipid ligand as the sensitizing agent. Mice were sensitized through three pathways (oral, nasal and epicutaneous), in an adjuvant-free model of the disease. After challenge, extreme phenotypes were characterized by H&E, immunofluorescence, flow cytometry and RT-qPCR. In addition, peripheral ILCs from human patients with peach allergy were cultured with or without the same allergen, analyzing the effects of this exposure.

Results: Epicutaneous sensitization (ES) induced the most severe phenotypes, in terms of drop in body temperature and leukocyte infiltration. Interestingly, this infiltration was not limited to the skin, but also localized through several parts of the MALT. Deepening in the skin, a thickened epidermis in ES-mice was observed, as well as a significant increase in the number of dermal dendritic cells (dDCs). Also, ES enhanced the expression of CD1d, a receptor needed for the recognition of Pru p 3's ligand, in keratinocytes. Finally, human ILCs cultured in vitro were shown to increase their expression of HLA-DR, CD80 and CD86 only upon exposure to Pru p 3 with its ligand, but not to Pru p 3 alone.

Conclusion: Our results suggest the skin is the main pathway in allergic sensitization to Pru p 3. Keratinocytes constitute the first sensors of the allergen, thanks to its recognition of its lipid fraction through CD1d, thus prompting an increase in the number of dDCs. We also reported that human ILCs can overexpress HLA-DR and costimulatory molecules after being cultured with Pru p 3 with its ligand. Given the infiltration found in several MALT regions and the antigen-presenting phenotype induced over ILCs, we hypothesize that not only has the skin a pivotal role in allergic sensitization, but also that the later cells could act as cross-talkers along the mucosa.

0974 | Mustard seed allergen Sin a 1 provokes epithelial inflammation and IL-33 release and may affect moDC activation

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Background: Sin a 1, a 2S albumin, is the major allergen from mustard seed but little is known about its sensitization pathway and its ability to interact both the intestinal epithelial cells (IECs) and underlying dendritic cells (DCs).

Aims: To evaluate the intrinsic capacity of Sin a 1 to interact with human IECs by stimulating inflammatory mediators and allergy driving IL-33 release as well as its effect on DCs activation in vitro.

Method: Sin a 1, a major allergen from mustard seed, was isolated from yellow mustard seed extract by chromatographic methods. Caco-2 cells were grown till 2-3 weeks post-confluence (TEER values were up to 500 $\Omega.cm^2$), and apically exposed to increasing concentrations of Sin a 1 for 24 h. On the other hand, HT-29 were exposed to increasing concentrations of Sin a 1, mustard seed extract or LPS for 24 h; then time-dose kinetics were performed with purified allergen and extract. Immature monocyte derived DCs (moDCs) were obtained from three different donors via differentiation of CD14 + cells with IL-4 and GM-CSF, and exposed to Sin a 1 in presence or absence of HT-29 cells. For this purpose, HT-29/moDC co-cultures were performed in transwell-plates and Sin a 1 was added apically for 48 h. After incubations, cell viability test (WST-1), Caco-2 barrier function (TEER and 4 kDa FITC-dextran permeability) and cytokine/chemokine detection by ELISA were performed. moDCs were collected to evaluate their phenotype by flow cytometry.

Results: Sin a 1 did not significantly affect Caco-2 barrier function as indicated by TEER vales and FITC-dextran permeability assays, however induced the secretion of DC chemoattractant IL-33 ($P < .05$) and CCL20 ($P < .01$). On the other hand, Sin a 1 dose-dependently increased the secretion of IL-33 ($P < .05$) and CCL20 ($P < .05$) by HT-29 at 24 h; preliminary data show that mustard seed extract and purified Sin a 1 induced the secretion of these mediators at 48-72 h, in combination with pro-inflammatory IL-8 and allergy linked CCL22 release. Sin a 1 exposure of moDCs in transwells in presence or absence of HT-29 tended to increase IL-8 and CCL22 concentrations. Expression of moDC activation markers CD80 and CD86 tended to increase when cultured in presence of HT-29 and Sin a 1.

Conclusion: Sin a 1 enhanced the release of inflammatory mediators and allergy associated IL-33 by IECs. Activation of moDC stends to be promoted by Sin a 1 exposed IEC, suggesting a role for epithelial activation in Sin a 1 induced allergic sensitization.

1217 | B cells responses during immunotherapy in allergic children compared to natural tolerance

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Background: Understanding the mechanisms of tolerance induction to food allergens is very crucial for the development of medical treatments in food allergies. The role of allergen-specific B cells in the induction of allergen tolerance remains unclear. Therefore, we aim to demonstrate the role of allergen-specific B cells and compare the differences of gene expression profiling in allergic children during oral immunotherapy and natural tolerance induction.

Method: Peripheral blood mononuclear cells were isolated from cow's milk allergic children and natural tolerance. αS_1-casein-specific and non-specific B cells were purified using dual-color staining with fluorescently labeled αS_1-casein allergen by flow cytometry. The immortalization of αS_1-casein specific B cells was transduced with a retroviral vector containing GFP, BCL6, and Bcl-xL and expanded by culturing with CD40L and IL-21. The Ultra Low RNA next-generation sequencing was performed for quantitative transcriptomics.

Results: After purification of allergen-specific B cells, we measured the Ag-specific Ig profile to confirm their specificity. Specific IgE, IgG1, and IgG4 production from culture supernatants of αS_1-casein positive B cells were significantly elevated compared to αS_1-casein negative cells, while total IgE, IgG1, and IgG4 levels were comparable. The in-depth analysis of gene expression showed significantly different αS_1-casein-specific B cells of allergic children before and after OIT compared to natural tolerance. The top 200 differentially expressed genes were shared between three groups. Within these shared significant genes, we identified roughly 30 tolerance-induced genes display similar gene expression patterns in allergic children after Food-AIT compared to natural tolerance. For example, gut homing marker genes including CCR6 and CXCR5 are upregulated, immunoregulatory genes including IL10RB and IGHG4 are upregulated, and allergic asthma and atopic dermatitis-related genes including MAPK6, SMC6, DIMT1, CKAP2, AXL, and LHFPL2 are downregulated. After Food-AIT proinflammatory cytokine machinery was shut down and tolerance related genes were upregulated.

Conclusion: Our data suggest that allergen-specific B cells in food allergic children compared to natural tolerance display clearly different gene signatures. Approximately 30 tolerance-induced genes were identified. The in-depth analysis of significant genes are suggesting the cells migrate to gut with the presence of immunoregulatory molecules and regulate the allergic-related genes.

OAS 21
Hereditary Angioedema: Quo Vadis?

0484 | Long-term safety and tolerability of Berotralstat (BCX7353) for Hereditary Angioedema (HAE) prophylaxis: APeX-S study results

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Background: Berotralstat is a novel oral once-daily highly selective inhibitor of plasma kallikrein in development for prophylaxis of HAE attacks. Berotralstat reduced HAE attack rates compared to placebo and was safe and generally well-tolerated in APeX-2 (NCT03485911), a 24-week, Phase 3 randomized, double-blind, placebo-controlled study. The ongoing open-label, parallel group APeX-S study (NCT03472040) evaluates the long-term safety of berotralstat.

Method: Subjects with HAE Type 1 or 2 were allocated to receive open-label berotralstat 150 mg (N = 127) or 110 mg (N = 100). Adverse events (AEs) were recorded throughout the study.

Results: Two hundred and twenty seven subjects took berotralstat (61.2% female, mean [range] age 40.3 [12 to 72] years). The mean (±SD) duration of treatment at the time of this analysis was 282.7 (±139.3) days (range: 11 to 540). 103 subjects completed 48 weeks of dosing (337 days). Overall, 206 (90.7%) subjects experienced a treatment-emergent AE (TEAE). No dose-related differences in rates of TEAEs were observed in the 150 mg and 110 mg dose groups. The most common AE was nasopharyngitis (30.8%). Nineteen subjects (8.4%) discontinued study drug due to any TEAE (10.2% and 6.0% in the berotralstat 150 mg and 110 mg groups, respectively). Three (1.3%) subjects experienced a serious AE assessed as related to study drug. The most frequent GI TEAEs were abdominal pain, diarrhea, nausea, and abdominal pain upper. These were generally mild to moderate, transient, occurred early in treatment and were self-limited. Only 6 subjects (2.6%) discontinued berotralstat due to a GI TEAE. Transaminase elevations (ALT or AST) > 3 × ULN were observed in 15 subjects (6.6%). All 15 subjects with these elevations had prior androgen use and 13 had discontinued androgens within 2 weeks of berotralstat initiation. These subjects were asymptomatic with no evidence of synthetic dysfunction or hepatitis. ALT values improved in all subjects, including those subjects who continued berotralstat. Benign delayed-type drug rash was uncommon (2 subjects, 0.9%).

Conclusion: Oral treatment with berotralstat 150 mg and 110 mg QD was safe and generally well-tolerated. No new safety findings emerged and no dose-related differences in TEAEs were observed. The safety profile of long-term dosing of berotralstat was generally consistent with the profile reported in previous studies.

1096 | Efficacy of lanadelumab is durable over time: Findings from the HELP Study and HELP OLE

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Background: Durable protection against hereditary angioedema (HAE) attacks is an important goal for many patients (pts) with C1-INH deficiency. Efficacy of lanadelumab was shown in the randomized HELP Study (NCT02586805) and confirmed in its open-label extension (OLE; NCT02741596). Findings from an analysis evaluating HAE attack rates in rollover pts in the OLE based on previous treatment in the HELP Study (through August 31, 2018) are presented.

Method: Eligible pts in the HELP Study were ≥ 12 yrs old with HAE type 1/2 and ≥ 1 attack within a 4-wk run-in period. Pts received lanadelumab 150 mg q4 wks, 300 mg q4 wks, 300 mg q2 wks, or placebo. Pts completing the HELP Study (through day 182) and continuing into the OLE (rollovers) received a single lanadelumab 300 mg dose until first attack (dose-and-wait period), and then 300 mg q2 wks thereafter (regular dosing stage). Nonrollover pts in the OLE (who had not previously participated in the HELP Study) received lanadelumab 300 mg q2 wks from day 0. Long-term efficacy was a secondary study objective of the OLE. Monthly attack rates were calculated during the HELP study and from the second dose in the OLE to the end of the treatment period for rollovers (regular dosing stage). For nonrollovers, attack rate was reported for the entire treatment period. In this analysis, the incremental reduction in monthly attack rates in rollover pts in the OLE based on previous treatment in the HELP Study was evaluated.

Results: 109 pts completed the HELP Study and entered the OLE (rollovers). At the time of the data-cut, 91.0% of rollover pts had completed at least 12 months in the OLE. A total of 109 rollover pts were included in this analysis. As shown in the Figure, lanadelumab efficacy was maintained over time, demonstrated by sustained reductions in HAE attack rates in pts previously treated with

placebo or lanadelumab in the HELP Study. At the end of the HELP Study/ start of OLE, mean attack rates/month ranged from 0.26 (active)—2.39 (placebo). For pts on lanadelumab treatment, in spite of the magnitude of attack rate reduction in the HELP Study, further reductions were still observed in the OLE, with mean attack rates ranging from 0.18—0.37 attacks/month. The efficacy profile of non-rollover pts was similar to efficacy in rollover pts with 2 yrs of cumulative study experience.

Conclusion: Findings from this analysis demonstrate durable efficacy of lanadelumab 300 mg every 2 wks in the prevention of HAE attacks over 2 years.
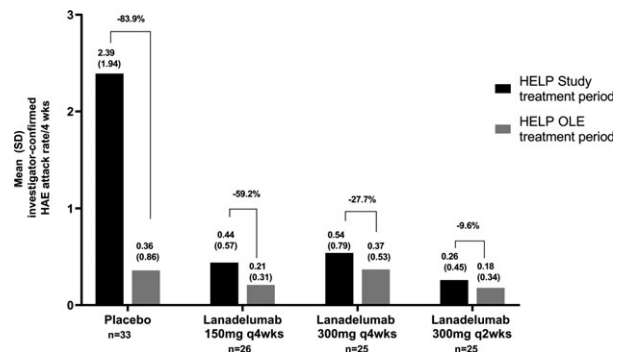
1118 | Long-term effectiveness and safety of icatibant for the on-demand treatment of hereditary angioedema attacks: 10 years of the Icatibant Outcome Survey

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Background: The Icatibant Outcome Survey (IOS; NCT01034969) is an ongoing international registry initiated in 2009 to monitor the effectiveness and safety of icatibant, a bradykinin B2 receptor antagonist, for the on-demand treatment of hereditary angioedema (HAE) attacks. In this analysis, data collected in IOS were used to document the 10-year long-term experience with icatibant use in patients with HAE due to C1 inhibitor deficiency (C1-INH-HAE; HAE type 1/2).

Method: Descriptive analyses are reported for patients enrolled in 13 countries between July 2009 and March 2019. Treatment

outcomes were analysed amongst patients with C1-INH-HAE who reported ≥ 1 icatibant-treated attack. Safety was assessed through reporting of icatibant-related adverse events (AEs) and serious AEs (SAEs).

Results: Since initiation, 1,052 patients with C1-INH-HAE have enrolled in IOS (59.9% female, 93.0% with type 1), including 39 paediatric patients. Overall, 5,995 HAE attacks in 549 patients were reported as treated with icatibant, with a mean of 11.5 attacks/patient (n = 523 with attack date).The proportion of patients self-administering rose from 25.0% in 2009 to 96.2% in 2018. Most treated attacks (5,818 with known location) were abdominal (50.2%); laryngeal (3.7%), cutaneous (31.3%) and multiple-location attacks (10.9%) were also treated. Most treated attacks (4,862 with known severity) were severe/very severe (45.2%) or moderate (43.6%); 11.2% were mild/very mild. Of 5,253 eligible treated attacks, median (range) time to resolution was 6.0 (0.0–99.5) hours, and median (range) attack duration was 9.0 (0.0–99.0) hours. A single dose of icatibant was used to treat > 90% of attacks. Icatibant was received by 618 patients, of whom 24 (3.9%) reported 75 possibly or probably treatment-related AEs; the most frequent were injection site erythema (29.3%) and asthenia (9.3%). Only 2 SAEs were considered icatibant-related by investigators (gastritis and angioedema), and no treatment-related deaths were reported.

Conclusion: Data collected during 10 years of IOS provide valuable insights into real-world experience with icatibant. Self-administration is now chosen by nearly all patients with C1-INH-HAE and symptoms resolve within a median time of 6 hours. Icatibant-related AEs were reported in < 4% of treated patients, with only 2 suspected treatment-related SAEs. Icatibant continues to be an effective and well-tolerated treatment option for adult and, where approved, paediatric patients with C1-INH-HAE.

1287 | Lanadelumab is well-tolerated and effective across patient subgroups: Findings from the HELP open-label extension study

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Background: Lanadelumab was well-tolerated and effectively reduced the rate of attacks in patients with hereditary angioedema (HAE) in the 26-week HELP study (2015-003943-20). The HELP open-label extension study (OLE; 2015-005255-27) evaluated lanadelumab over an extended period. We analysed the efficacy and safety of lanadelumab across subgroups of patient demographic and baseline characteristics.

Method: Patients were ≥ 12 years old with type 1/2 HAE. "Rollovers" continued into the OLE after completing HELP and received a single 300 mg lanadelumab dose on Day 0, then 300 mg Q2W after their first attack. "Nonrollovers" entered the OLE if they had a historical baseline of ≥ 1 attack/12 weeks, and received 300 mg Q2W starting on Day 0. Data herein were collected up to 31 Aug 2018.

Results: N = 212 patients received lanadelumab over a mean (range) of 19.7 (0-26.1) months during the OLE (n = 109 rollovers, n = 103 nonrollovers). The majority of patients were female (143 [67.5%]), white (198 [93.4%]), and had type 1 HAE (189 [89.2%]). Patients had a mean (range) age of 40.7 (12-76) years and BMI of 28.4 (16.9-55.0) kg/m^2. 125 (59.0%) patients used long-term prophylaxis, particularly C1-inhibitor replacement (113 [53.3%]), prior to entering the study, and 130 (61.3%) had a history of laryngeal attacks. Overall, the mean (SD) and median attack rate was reduced from 3.05 (2.66) and 2.00 attacks/month, respectively, at baseline to 0.26 (0.57) and 0.05 attacks/month, respectively, during treatment (mean 87.0% reduction; median 97.5%). The median attack rate reduction was consistent regardless of patient sex (98.6% reduction in males, 97.4% females); race (97.8% white, 95.9% non-white); HAE type (97.6% type 1, 97.4% type 2); age (97.4% <18 years, 97.4% 18-40 years, 98.4% 40-65 years, 92.0% ≥65 years); BMI (98.5% normal, 97.5% overweight, 97.1% obese); history of long-term prophylaxis use (97.2% C1-INH use, 97.1% no LTP); history of laryngeal attacks (97.1% yes, 99.8% no); and baseline attack rate (92.2% <1 attack/month, 100% 1-<2 attacks/month, 98.1% 2-<3 attacks/month, 96.5% ≥3 attacks/month). 202 (95.3%) patients reported a treatment-emergent adverse event (TEAE). 106 (50%) patients reported a TEAE related to treatment. The safety profile was comparable across all subgroups, with the most common treatment-related TEAE being mild injection site pain.

Conclusion: Lanadelumab was well-tolerated and effectively reduced attack rates over an extended treatment period across patient demographic and disease characteristics.

1451 | Results of a randomized, double-blind, placebo-controlled, phase 2 study, investigating the safety and efficacy of anti-factor XIIa monoclonal antibody garadacimab (CSL312) for prophylaxis of HAE

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Background: The activated factor XII (FXIIa)-driven contact pathway is essential for bradykinin production, a key mediator of hereditary angioedema (HAE). Garadacimab (CSL312) is a fully human IgG4 monoclonal antibody that effectively inhibits FXIIa at the origin of the contact cascade. This Phase 2 study (CSL312_2001; NCT03712228) aimed to study the safety, efficacy, and pharmacokinetics of prophylactic subcutaneous (SC) garadacimab in HAE.

Method: Eligible patients with type I/II HAE were randomized to receive placebo or 75, 200, or 600 mg SC garadacimab every 4 weeks for 12 weeks. One week prior to the first SC dose, an intravenous volume-matched loading dose of 0, 40, 100, or 300 mg was administered to the four groups, respectively. The primary endpoint was monthly HAE attack rate. Further endpoints included the reduction in attacks compared with the 4–8-week run-in or placebo, use of on-demand medication per month, and safety.

Results: Overall, 32 adult patients, with mean monthly attack rates of 5.17 during the run-in period, were randomized; of these, 56.25% were female, 90.63% were white, and 93.75% had type I HAE. The mean monthly attack rates were 4.24, 0.48, 0.05, and 0.40 for patients in the placebo, 75, 200, and 600 mg SC garadacimab arms, respectively. The mean percentage reductions in monthly attack rates in the garadacimab arms relative to placebo were 88.68%, 98.94%, and 90.50%. The percentage of patients experiencing at least one treatment-emergent adverse event (TEAE) with garadacimab was similar to placebo. All adverse events were non-serious and were determined to be mild or moderate. The most common TEAE related to the treatment (garadacimab and placebo) was mild to moderate injection site erythema (12.5%). All patients completed the study.

Conclusion: The study showed that monthly prophylactic SC treatment with garadacimab was well tolerated and effective in preventing attacks in patients with HAE. This study provides the first clinical evidence for the role of FXIIa in HAE.

Parameter	Placebo q4 wk (n = 8)	75 mg garadacimab q4 wk (n = 9)	200 mg garadacimab q4 wk (n = 8)	600 mg garadacimab q4 wk (n = 7)
N = 32				
Mean HAE attacks per month during run-in period, (median)	5.07 (4.57)	6.13 (6.30)	5.68 (5.67)	3.48 (2.95)
Mean HAE attacks per month during efficacy assessment period, (median)	4.24 (4.61)	0.48 (0.00)	0.05 (0.00)	0.40 (0.34)
Attack reduction (≥90%) vs run-in in responders, %	0.00	88.89	100.00	57.14
Patients free of HAE attacks, %	0.00	55.56	87.50	42.86
Mean number of treated attacks per month, (median)	3.98 (4.38)	0.44 (0.00)	0.05 (0.00)	0.15 (0.00)
Related TEAEs, n (%)	3 (37.50)	2 (22.22)	1 (12.50)	5 (71.43)
Injection site reaction, n (%)	2 (25.00)	1 (11.11)	1 (12.50)	4 (57.14)

HAE, hereditary angioedema; q4 wk, every 4 weeks; TEAE, treatment-emergent adverse event.

1475 | **Implementation of a novel DBS-based methodology to diagnose hereditary angioedema in subjects with recurrent abdominal pain of unclear etiology – the international EHA study**

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Background: Type 1/2 hereditary angioedema (HAE) is a rare autosomal dominant condition caused by variants in *SERPING1* gene encoding C1 inhibitor (C1-INH). Reduced levels of functional C1-INH (fC1-INH) result in bradykinin-mediated attacks of angioedema affecting the skin and the gastrointestinal/respiratory tracts. The non-interventional international EHA study (Epidemiological analysis for Hereditary Angioedema; NCT03558009) explores HAE prevalence in subjects with episodes of abdominal pain of unclear etiology through a novel and easy-to-use methodology based on Dried Blood Spot (DBS) biochemical and genetic analyses.

Method: At the time of data cut-off (01.12.2019), 611 subjects had been enrolled at 22 centres from 6 countries. In an initial screening step, levels of antigenic C1-INH (AgC1-INH) and C4 complement component (C4) were determined *via* tandem mass-spectrometry (MS/MS) from DBS samples. All samples with AgC1-INH and C4 levels below cutoffs (150 and 210 nmol/l, respectively), as well as 100 randomly selected samples with normal AgC1-INH and C4 values underwent *SERPING1* sequencing (Figure). Samples with identified *SERPING1* mutations were sent to an external licensed laboratory for HAE diagnosis confirmation: C1-INH activity as well as C1-INH and C4 protein levels were measured with classical spectrophotometric and chromogenic methods, respectively.

Results: Median age of the 611 patients was 33 years (range: 4-75) and 67% were female. Two patients had both low AgC1-INH and C4, 10 had low AgC1-INH only, and 7 had low C4 only. The following known pathogenic *SERPING1* variants were identified in 3 of these subjects: c.600dup (p.Lys201Glnfs*56), c.1397G>A (p.Arg466His), and c.1450C>T (p.Gln484*), resulting in a positive predictive value of 16%. In addition to the enrolled subject, the latter variant was subsequently found to also segregate in 9 additional affected family members over three generations suffering from recurrent abdominal pain who had not received HAE diagnosis before. None of the 100 randomly selected patients with normal AgC1-INH and C4 carried pathogenic *SERPING1* variants.

Conclusion: DBS-based evaluation of AgC1-INH and C4 followed by analyses of *SERPING1* proved to be a viable approach to detect HAE in subjects with unexplained abdominal pain. These findings confirm that patients with recurrent episodes of unexplained abdominal pain should be evaluated for HAE and provide support for a novel DBS-based approach to HAE screening and diagnosis in clinical practice.

DBS analysis algorithm
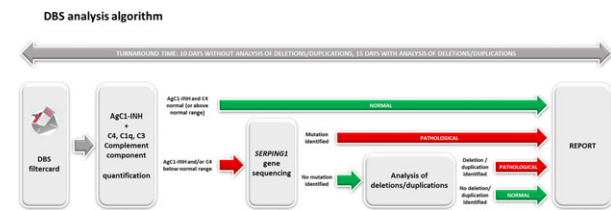
OAS 22
Prediction, Development and Natural Course of Allergic Diseases

0283 | **Household pet exposure in prenatal and early childhood and allergic diseases: A general population birth cohort study**

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Background: The association between household pet exposure and the incidence of allergic diseases remains controversial. This study aimed to determine the contribution of household dog and cat exposure in prenatal and early childhood to the development of allergic diseases in the Japanese general population.

Method: We obtained data from the Tokyo Children's Health, Illness, and Development (T-CHILD) general birth cohort study. Household dog and cat exposures were defined based on responses to parental questionnaires completed during pregnancy and at 6 and 18 months and 3, 6, and 9 years of age. We evaluated allergic diseases and aeroallergen sensitization at 5 and 9 years of age. Allergic diseases including current wheeze, eczema, and rhinitis were defined based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Serum-specific immunoglobulin E (IgE) for Can f 1, Fel d 1, Der f 1, and Cry j 1 levels were measured by ImmunoCAP ISAC, with sensitization defined as positivity for specific IgE (≥0.3 ISU). Associations of dog and cat exposures with allergic diseases and sensitization were analyzed by multiple logistic regression analyses.

Results: Of 1,550 children born into the cohort, we evaluated the outcomes of 1,196 and 907 at 5 and 9 years of age, respectively. Adjusted analysis showed that early-life exposure to a household dog decreased the risk of eczema development at 5 years of age (6-months: odds ratio [OR], 0.23; 95% confidence interval [CI], 0.08–0.63, $P = .004$; 18-months: OR, 0.34; 95% CI, 0.13–0.86, $P = .02$; 3-year: OR, 0.34; 95% CI, 0.14–0.88, $P = .03$). Household cat exposure at 18 months of age decreased the risk of rhinitis development at 5 years of age (OR, 0.45; 95% CI, 0.21–0.96, $P = .04$). Prenatal household dog or cat exposures were not significantly associated with

allergic diseases. Dog exposure and sensitization to Can f 1 and cat exposure and sensitization to Fel d 1 showed borderline significant positive associations, while no associations were observed for sensitization to Der f 1 and Cry j 1.

Conclusion: Our findings suggest that early-life dog or cat exposures are associated with decreased risks of eczema and rhinitis in Japanese children.

1101 | Development of a childhood asthma prediction model using machine learning approaches

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Background: The presentation of childhood asthma is highly heterogeneous in early life, with symptoms often being transient. With an objective diagnosis of asthma only made after age five, both over and under diagnoses in early life are common. Whilst numerous childhood asthma prediction models exist, mainly developed using regression-based methods, none have been widely implemented into clinical practice. Machine learning approaches for prediction in other disease areas have shown competitive, if not superior, performance compared to regression-based methods.

This study aimed to apply machine learning approaches to develop two prediction models (at infancy and preschool age) for asthma development at age 10.

Method: In 1536 children enrolled in the Isle of Wight Birth Cohort, data on clinical symptoms and environmental exposures were prospectively collected at 1, 2, 4 and 10 years. Recursive Feature Elimination (RFE) was used to identify the optimal subset of features from 39 and 54 candidate features associated with childhood asthma collected by age 2 (infancy model) and age 4 (preschool model), respectively. Individuals with complete data were split into a training and test set (ratio 2:1). Nine machine learning algorithms were compared (three support vector machines (SVM) with different kernel functions, decision tree, random forest, Naive Bayes classifier, multilayer perceptron (MLP), and weighted and unweighted K-nearest neighbours). Models were developed and hyper-parameters optimised on the training data using 5-fold cross validation, and predictive performance evaluated on the test set using area under the curve (AUC).

Results: Complete data were available for 490 and 373 individuals for the infancy and preschool models, respectively. RFE identified an optimal subset of 8 and 12 features of asthma at age 10 for the infancy and preschool model, respectively. Using complete predictor subset data, the best predictive performance was demonstrated by the MLP algorithm for the infancy model (AUC = 0.62) and the linear SVM for the preschool model (AUC = 0.78).

Conclusion: Two models to predict asthma development at age 10 were created, one for use at preschool age and another for earlier use in infancy. The preschool model demonstrated comparable performance to the best performing existing models. Addressing issues of class imbalances may further improve the performance of the models and future external validation is needed to assess the generalisability of the developed models.

1412 | Longitudinal trajectories of eczema severity, duration, and affected body-region predict risk of food allergy in combination with filaggrin gene mutations

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Background: The dual-allergen exposure hypothesis postulates that epicutaneous allergen sensitization through a dysfunctional skin barrier causes pediatric food allergy (FA). In order to assess the role of a broken skin barrier in the development of FA, we performed detailed longitudinal clinical assessments of the skin between 4 months and 6 years of life.

Method: Classifications ranging from healthy skin, to mild barrier dysfunction (e.g. dry skin), to severe eczema (e.g. SCORAD between 40 and 85) were assessed longitudinally. In a subset of participants, Filaggrin gene mutations were analyzed and detailed skin assessments of 50 specific regions of the body were tracked for 6 years in order to investigate eczema phenotypes. Furthermore, to characterize eczema phenotypes, we classified each body-region based on its potential for exposure to the environment. Allergy immune markers, including food specific IgE and SPT measurements were also collected at multiple time-points, and peanut allergy (PA) was assessed by oral food challenge.

Results: Longer eczema durations, higher eczema severity scores, and specific phenotypes of eczema (e.g. eczema-affected regions being those typically exposed to the environment) were positively associated with an increased risk of FA and allergic immune markers. Using a multivariable binary logistic regression model, significant effects were observed for duration and severity of eczema. For example, children that had mild eczema (SCORAD < 15) and an eczema duration of less than 4.5 months had approximately a 2% risk of peanut allergy (PA) in the first year of life; whereas, children with severe eczema (SCORAD > 40) that persisted for longer than 4.5 months had approximately a 10 times higher risk of PA (20%). Filaggrin gene

mutations interacted with eczema severity to produce a significantly (P < .05) higher risk of PA.

Conclusion: Using longitudinal skin assessments from well characterized cohorts, evidence for the dual-allergen exposure hypothesis is evaluated and shown to explain a large proportion of food allergy.

OAS 23
Biologicals and DARPins: New Mechanistic Insights and Novel Applications

0941 | Dupilumab suppresses serum total IgE levels across multiple atopic, allergic diseases

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Background: IgE is a systemic biomarker of allergic inflammation that is elevated in multiple type 2 inflammatory-mediated diseases including atopic dermatitis (AD), asthma, chronic rhinosinusitis with nasal polyps (CRSwNP), and eosinophilic esophagitis (EoE). Dupilumab, a fully human monoclonal antibody, blocks the shared receptor component of interleukin (IL)-4/IL-13, key drivers of type 2 inflammation. This analysis assessed the effect of dupilumab on serum total IgE in patients with AD (phase 3 Liberty AD SOLO 1 [NCT02277743], SOLO 2 [NCT02277769], Liberty AD CHRONOS [NCT02260986]), asthma (phase 3 Liberty ASTHMA QUEST, NCT02414854), CRSwNP (phase 3 Liberty NP SINUS-52, NCT02898454), and EoE (phase 2b proof of concept [PoC] study, NCT02379052).

Method: Serum total IgE levels were measured in adult patients with moderate-to-severe AD (total N = 2,119), uncontrolled moderate-to-severe asthma (N = 1,902), severe CRSwNP (N = 448), and EoE (N = 47). Median absolute values at end-of-treatment (EOT) and median percent change from baseline were assessed in patients receiving dupilumab or placebo. Changes from baseline are summarized by descriptive statistics.

Results: At baseline, total IgE levels in AD patients were higher vs patients with asthma, CRSwNP, or EoE. Dupilumab vs placebo significantly and consistently reduced total IgE across all studies (P < .0001). In dupilumab-treated patients, median reductions in total IgE were gradually progressive over time, with greater effects observed in longer treatment durations (47% at Week 16 and 77% at Week 52 in AD patients, 70% at Week 52 in asthma patients, 71% at Week 52 in CRSwNP patients, and 25% at Week 12 in EoE patients [all P < .0001 vs placebo]). In placebo-treated patients, total IgE showed minimal changes (increased from 0% to 8% in AD patients,

decreased by 4% in asthma patients, increased by 8% in CRSwNP patients, and increased by 8% in EoE patients) (Table).

Conclusion: Inhibition of IL-4 and IL-13 signaling by dupilumab treatment consistently reduced serum total IgE levels in patients across multiple type 2 inflammatory diseases (AD, asthma, CRSwNP, and EoE).

Type 2 disease and study (duration)	Median serum total IgE IU/mL (95% CI)		Median % change from baseline in serum total IgE (95% CI)	
	Placebo	Dupilumab	Placebo	Dupilumab
AD – SOLO 1 & 2 pooled[a,b]				
At baseline	n = 456 2849.0 (2174.0, 3817.0)	n = 465 2396.0 (2001.0, 3025.0)		
EOT (16 weeks)	n = 196 1,592.0 (927.0, 3022.0)	n = 366 1,107.0 (739.0, 1518.0)	n = 196 7.8 (2.1, 15.1)	n = 366 −46.5 (−48.4, −44.4) P < 0.0001
AD – CHRONOS[b,c]				
	n = 312 3477.0 (2471.0, 4757.0)	n = 110 4925.5 (3038.0, 10000.0)		
EOT (52 weeks)	n = 117 2257.0 (1327.0, 3711.0)	n = 83 951.0 (520.0, 1648.0)	n = 116 0.0 (−6.5, 0.0)	n = 83 −76.7 (−80.9, −72.7) P < 0.0001
Asthma – QUEST[d,e]				
At baseline	n = 318 178.5 (138.0, 213.0)	n = 626 174.0 (152.0, 200.0)		
EOT (52 weeks)	n = 251 176.0 (136.0, 217.0)	n = 482 48.5 (40.0, 55.0)	n = 248 −4.4 (−10.0, 0.5)	n = 478 −70.0 (−72.2, −67.6) P < 0.0001
CRSwNP – SINUS-52[e,f]				
At baseline	n = 150 139.0 (104.0, 172.0)	n = 148 172.50 (98.0, 157.0)		
EOT (52 weeks)	n = 128 144.00 (115.0, 191.0)	n = 140 29.50 (23.0, 40.0)	n = 128 7.5 (0.0, 14.6)	n = 139 −71.4 (−75.6, −67.4) P < 0.0001
EoE – PoC[h,g]				
At baseline	n = 24 126.5 (58.0, 254.0)	n = 23 67.0 (42.0, 220.0)		
EOT (12 weeks)	n = 19 159.0 (64.0, 388.0)	n = 23 53.0 (33.0, 147.0)	n = 19 8.3 (−6.9, 18.0)	n = 23 −24.8 (−33.2, −14.5) P < 0.0001

Differences between dupilumab and matched placebo in the change from baseline in biomarkers were analyzed using a rank ANCOVA model. Covariates included the corresponding baseline value and the 5 pre-specified intervention groups (age, sex, geographic region, baseline blood eosinophil strata, baseline ICS dose level for AD and asthma studies, and baseline total IgE, age, asthma/NSAID-ERD status, prior surgery history, and regions for SINUS 52 study). [b]Dupilumab 300 mg q2w vs placebo qw. [c]Serum total IgE levels were evaluated in the populations exposed to treatment and observed, defined as all patients in the safety population observed with censoring after rescue treatment use. [c]Dupilumab 300 mg

1022 | Effects of omalizumab on ASS intolerance in patients with aspirin-exacerbated respiratory disease (AERD)

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Background: Aspirin-exacerbated respiratory disease (AERD) comprises the triad of chronic rhinosinusitis with nasal polyps, asthma and intolerance to inhibitors of acetylsalicylic acid (ASS). This complex inflammatory disorder entails many therapeutic challenges and diminishes the patients' quality of life. Emerging literature indicates improvement of asthma and nasal polyps as well as a beneficial effect on ASS desensitization in AERD patients after treatment with omalizumab, an IgE inhibitory biological agent.

Method: To prospectively evaluate the efficacy of omalizumab in patients with AERD focusing on ASS tolerance after 24 weeks as determined by oral drug provocation testing. The primary endpoint was the maximally tolerated application rate, as measured by titrating 125 mg, 250 mg and 500 mg ASS before and after 24 weeks of omalizumab treatment. Secondary endpoints included i) the reduction of nasal polyps, as evaluated by total polyp score (TPS), computed tomographic scanning (Lund-Mackay score) and sino-nasal outcome test-20 (SNOT-20); ii) an improvement of allergic asthma (asthma control test (ACT), spirometry (FEV1%)) and iii) changes in related biomarkers (total IgE, serum ECP).

Results: After assessing 45 subjects, 36 patients were treated with OMA and 32 patients exposed to ASS. Before therapy 62.5% of the patients reacted with upper and 54.8% with lower respiratory symptoms to ASS provocation testing as opposed to 39.1% and 8.7%, respectively ($P < .05$) after 24 weeks of treatment. Nasal (TPS: −1.66, $P < .0005$; SNOT-20: −13.41, $P < .005$) symptoms were significantly reduced as compared to baseline, as confirmed by Lund-Mackay score. Interestingly, subjective asthmatic symptoms improved (ACT$p < 0.0005$), but no significant changes in spirometry (FEV1%) were observed. There was an increase in total IgE levels after 24 weeks (340.74 ± 41.4, $P < .0005$). When analyzing patients by co-occurrence of inhalation allergies we found no significant difference regarding the effectiveness of omalizumab. Adverse events were mild and included headache and joint pain.

Conclusion: Omalizumab induced ASS tolerance in the majority of patients after 24 weeks. In addition, patients showed a significant reduction of nasal polyps and an improvement of asthmatic symptoms. Omalizumab therefore displays a promising treatment option for patients with AERD. To date its mechanism of action in patients with AERD regarding ASS intolerance is unknown.

1163 | Benralizumab in refractory hypereosinophilic syndrome effectively reduces eosinophil counts but does not always resolve all clinical symptoms

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Background: Hypereosinophilic syndrome (HES) is characterized by blood and tissue eosinophilia resulting in end-organ damage. HES can be primary clonal (in which the eosinophil is part of clonal hematopoiesis), secondary (due to eosinophilic stimulating cytokines) or idiopathic (I-HES). Treatment to reduce eosinophil counts and prevent organ dysfunction may consist of steroids, interferon alpha, hydroxyurea, or imatinib. Benralizumab is an anti-interleukin 5 receptor antibody that depletes eosinophils in blood and tissue and is effective in *PDGFRA* negative HES (*NEJM* 2019, Kuang et al.).

Method: 5 adults (4 with I-HES, 1 with secondary (lymphocytic variant) HES; age range 19-59 years; 4 male, 1 female) from our institution were treated with benralizumab at a monthly dose of 30 mg. They all were refractory or had contra-indications to conventional treatment, or steroids could not be reduced to an acceptable dose. Patients gave consent to off-label use of benralizumab. A mean of 4.8 injections of benralizumab were given (range, 2-7 injections), and the mean follow-up time after starting benralizumab was 3.8 months (range, 1-7 months).

Results: In 4/5 patients, eosinophils decreased to < 0.05x10^9/L within 1 month after the first injection. In 1/5 patients, eosinophils decreased from 12 to 1.5 x10^9/L after three injections. Most clinical symptoms (pulmonary complaints, heart failure due to a Loffler endocarditis, urticaria) disappeared within three months of treatment. However, in 2 patients with eosinophilic colitis secondary to the HES, the colitis was not resolved after 6 monthly benralizumab injections, although in one of these patients the prednisone dose could be tapered significantly after benralizumab was started. Side effects of benralizumab consisted of headache in 2/5 patients, and a herpes zoster infection in 1 patient. Furthermore, a thrombocytopenia emerged in a patient who was previously treated for Waldenstrom's macroglobulinaemia with fludarabine, and benralizumab was stopped. It is unlikely although not yet excluded that benralizumab was the cause of the thrombocytopenia.

Conclusion: Benralizumab is a new option to treat refractory HES in a safe and highly effective manner, especially to reduce eosinophils and resolve most clinical symptoms. However, we here present the novel finding that eosinophilic colitis secondary to HES does not respond well to benralizumab treatment, even though the eosinophilic count in peripheral blood normalized.

1172 | The role of IgE glycosylation patterns on its biological activity

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Background: Immunoglobulin E (IgE) is the most glycosylated antibody in humans. Specific carbohydrates have been described to be central for binding of IgE to its high-affinity receptor FcεRI on basophils. Upon allergen stimulation, these cells degranulate and release mediators causing allergic symptoms. It is well established that the therapeutic anti-IgE antibody omalizumab prevents binding of IgE to basophils and mast cells. We have previously reported that high concentrations of omalizumab lead to active desensitization of basophils. Further, we demonstrated that an engineered omalizumab-resistant IgE-Fc glycovariant may be used to replace the IgE-repertoire on the surface of primary human basophils when co-applied with omalizumab. This combination treatment significantly inhibits allergen-mediated basophil activation ex vivo. Here, we

characterize additional omalizumab-resistant IgE-Fc glycovariants, evaluate their ability to inhibit allergen-mediated basophil activation and investigate the potential mechanisms underlying IgE-Fc glycovariant mediated basophil inhibition.

Method: IgE-Fc glycovariants were characterized using ELISA and surface plasmon resonance measurements. The selected variants were tested on human basophils isolated from grass-pollen allergic individuals treated with different IgE-Fc glycovariants alone or in combination with omalizumab. Basophil activation was measured by flow cytometry. The gene and surface expression of potential inhibitory-receptors were analyzed by gene array or flow cytometry. IgE-Fc glycovariant mediated inhibition of basophil activation was investigated by blocking potential inhibitory receptors in the basophil activation tests.

Results: Several omalizumab resistant IgE-Fc glycovariants diminished basophil activation in a competition independent manner alone or in combination with omalizumab at low concentrations. Assessment of expression status of several immunoreceptors reveals a potential role of inhibitory carbohydrate receptors as IgE-Fc glycovariant interaction candidates. Blocking experiments revealed further insight into the IgE-Fc glycovariant mediated inhibitory effect on basophil activation.

Conclusion: Our data suggest that the glycosylation pattern on IgE is essential for its biological activity since IgE-Fc glycovariants may suppress primary basophil activation via interaction with inhibitory carbohydrate receptors. Moreover, addition of IgE-Fc glycovariants to omalizumab might represent an interesting combinatorial treatment approach.

1332 | Disruptive anti-IgE inhibitors for the treatment of allergic lung inflammation

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Background: Allergen-specific immunoglobulin E (IgE) is a key player in the pathophysiology of allergic asthma. It binds to the surface of airway mast cells via the high-affinity receptor FcεRI. Inhalation of the cognate allergen immediately leads to the degranulation of IgE-loaded mast cells and the induction of clinical symptoms. The therapeutic anti-IgE antibody omalizumab that prevents the binding of free serum IgE to FcεRI has proven efficient for the treatment of severe persistent allergic asthma. We recently described a novel class of disruptive anti-IgE inhibitors. These molecules not only suppress the binding of IgE to FcεRI but additionally remove FcεRI-bound IgE from allergic effector cells. Here, we describe the mode-of-action of such disruptive anti-IgE inhibitors and assess their efficacy in an allergic lung inflammation mouse model using double transgenic mice expressing the human immunoglobulin epsilon heavy chain (huIgε) and the human FcεRI alpha-chain (huFcεRIα).

Method: Double transgenic huIgε/huFcεRIα$^{+/+}$ mice were epicutaneously sensitized with ovalbumin in combination with the vitamin D analogue MC903. Prior to intranasal antigen challenge mice were treated with the disruptive anti-human IgE inhibitor DARPin bi53_79. Blood plasma and lung tissue were analyzed for total and allergen-specific IgE as well as mast cell-specific proteases. In addition, single cell suspensions from blood and lung tissue were assessed by flow cytometry and histological sections of skin and lung tissue were stained by immunohistochemistry.

Results: Increased total and allergen-specific IgE was detected in sensitized compared to control treated mice. Further, the number of basophils, eosinophils and mast cells in the lung were markedly augmented after sensitization. While basophil specific-*Mcpt8* expression in the lung was upregulated during sensitization, an increase of mast cell specific-*Mcpt1* as well as goblet cell specific mucin 5AC expression was only observed after intranasal challenge. Treatment with disruptive anti-human IgE inhibitor DARPin bi53_79 decreased systemic as well as local IgE levels and ameliorated the inflammatory phenotype in the lung.

Conclusion: We established an adjuvant free allergic lung inflammation model in humanized huIgε/huFcεRIα$^{+/+}$ mice. This experimental setup allows and facilitates the in vivo assessment of novel anti-human IgE drug candidates, such as disruptive anti-human IgE inhibitors.

1374 | A Siglec-8 antibody reduces substance P-induced inflammation by inhibiting MRGPR-mediated mast cell activation

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Background: The neuropeptide Substance P (SP) has been implicated in driving neurogenic inflammation, pain, itch, and chronic disease. The recent identification of MRGPRX2/*Mrgprb2* as a mast cell (MC)-specific SP receptor, and the potent activation of MCs by SP suggests that MCs are the key effector cell in SP-mediated inflammation. Siglec-8 is an inhibitory receptor selectively expressed on MCs and eosinophils. While Siglec-8 monoclonal antibodies (mAb) have been shown to inhibit IgE- and IL-33-mediated MC activation, the activity of a Siglec-8 mAb has not been evaluated in SP-mediated inflammation.

Method: Acute inflammation was induced by intraperitoneal (ip) injection of SP in Siglec-8 transgenic (tg), wild-type, and MC-deficient (c-Kit^{w-sh}) mice. Immune cells and cytokines were analyzed 3 hours post SP administration by flow cytometry and MSD, respectively. An anti-Siglec-8 or isotype-matched control mAb was dosed 1 hour before SP administration followed by immune cell and cytokine analyses as described above. Human skin tissue was enzymatically and mechanically digested into single cells followed by overnight incubation with SP in the presence of either a Siglec-8 mAb or isotype-matched control mAb.

Results: In vivo administration of SP rapidly induced mast cell degranulation, recruitment of neutrophils, and increased the expression

of pro-inflammatory cytokines in the peritoneal cavity of Siglec-8 tg mice. MC-deficient mice had significantly lower levels of SP-induced neutrophil infiltration and pro-inflammatory cytokines, compared to wild-type mice, suggesting MCs are key effector cells in SP-driven inflammation. Treatment with a Siglec-8 mAb decreased SP-induced degranulation of MCs and neutrophil influx in the peritoneal cavity. Siglec-8 mAb treatment also significantly decreased the level of inflammatory mediators, such as KC, MCP1, IL-6, TNFa induced by SP. Lastly, SP selectively activated human skin tissue mast cells and induced cytokine and chemokine production in ex vivo tissue that was reduced by a Siglec-8 mAb.

Conclusion: MCs are key effector cells in SP-mediated inflammation and treatment with a Siglec-8 mAb reduces inflammation induced by SP by inhibiting MRGPR-activation of MCs. An anti-Siglec-8 approach may have the potential to broadly treat mast cell and eosinophil-driven diseases associated with neurogenic inflammation, pain, and itch, such as irritable bowel syndrome, atopic dermatitis, and urticaria.

OAS 24
From Early Wheeze to Rhinitis and Asthma

0310 | Does probiotic microorganism lactobacillus reuteri prevents allergic rhinitis and rhinoconjunctivitis development in children

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Background: Allergic rhinitis (AR) and allergic rhinoconjunctivitis (ARC) are common chronic disorders in children, especially in developed countries. Studies performed in last few decades indicate that colonization of the gut early in life plays a substantial role in directing immune system development. Microbial exposure during the perinatal period is linked to the epigenetic regulation of genes involved in allergic inflammation, and it alters susceptibility to allergic diseases. Immune responses in the gut may modulate immune responses in distant target organs, including the nose. Unlike in other allergic diseases, the therapeutic effect of probiotics in allergic rhinitis has been primarily demonstrated, whereas their preventive effects have not been conclusively defined. The aim of this prospective study was to evaluate the efficacy of probiotic microorganism Lactobacillus reuteri (LR) in the prevention of the development of AR and ARC in 9 years old Slovene children.

Method: This prospective study included 316 maturely born infants with a positive history of parental allergy confirmed by allergy testing. According to the addition of LR in to the child diet children were divided in two groups: group A-201 infants exclusively breastfed for 4-6 months and group B-115 infants breastfed with addition of L. reuteri from fourth week of life for 12 weeks. Every child was followed up by the same paediatrician until it was 9 years old. The prevalence

of doctors diagnosed AR and ARC was observed. Data about frequency and duration of AR and ARC exacerbations were recorded. Statistical analysis was performed with PC using chi-square analysis with Yates' correction and paired t-test. P-values less than 0.05 were considered significant.

Results: At the age of 9 year the prevalence of AR and ARC in the total population was 19.6%. There was no significant difference between the prevalence of AR (10.4%) and ARC (9.2%), ($P > .05$). The prevalence of AR was significantly lower in group B (A: 13.9%; B: 4.3%), ($P = .01$). No significant between group difference in ARC prevalence was confirmed (A: 9.5%; B: 8.7%), ($P > .05$). Frequency and duration of AR exacerbations was significantly lower in group B ($P < .01$). No significant between group difference in frequency and duration of ARC exacerbations was observed ($P > .05$).

Conclusion: The study confirms that addition of LR to early child's diet has beneficial effect on the occurrence and course of AR but not of ARC.

0389 | Early life wheeze - risk factors for asthma in school age

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Background: One third of all toddlers are in need of medical care because of acute wheeze and many of these children develop asthma at school age. The aim of this study was to identify risk factors for asthma at school age in a group of children who experienced acute wheeze as toddlers.

Method: The study included 156 children; age 6-48 months, recruited from the emergency department at Astrid Lindgrens Children's Hospital, Stockholm, with acute wheeze (AW) and 101 age-matched healthy controls (HC). Children with AW were followed up after 2-3 months and thereafter annually until age 7 (n = 113). HC came for a second visit at age 7 (n = 54). The protocol included questionnaires, exhaled NO (FENO), blood sampling for cell count, specific IgE to food (fx5) and airborne allergens (Phadiatop) and Vitamin D measurement. Nasopharyngeal samples for viral detection were taken at the emergency visit. Asthma definition at age 7: >=5 days of reported breathing difficulties and/or medication with inhaled corticosteroids or leukotriene receptor antagonist >=5 days during the previous 12 months and/or an increase in FEV1 > 12% after bronchodilators.

Results: At age 7, 69.9% (n = 79) of AW had asthma and 1.9%, (n = 1) of HC ($P < .01$).

There were no differences between children with AW and HC regarding age at inclusion, age at the 7-year visit or gender.

Wheeze caused by rhinovirus (RV) at inclusion was more common among children that had asthma at age 7 (43.7% vs 21.2%, $P = .03$).

When adjusting for other viruses in logistic regression, RV had the greatest impact on asthma development at age 7 (OR = 3.6, 95% CI 1.3-9.8). Children with asthma at age 7 were more often hospitalized and spent longer time in hospital due to respiratory problems the first year following inclusion (*P* = .037). Children with asthma at age 7 had higher levels of FENO (*P* = .03), lower scores at asthma control test (*P* =<.01) and higher levels of blood eosinophils (*P* = .047).

There were no differences between children with or without asthma at age 7 regarding family history, atopic dermatitis and Vitamin D levels at inclusion, exclusive breastfeeding or allergic sensitization.

Conclusion: Rhinovirus induced acute wheeze in toddlers is a risk factor for developing asthma by the age of 7. These children were also hospitalized more frequently and needed more inpatient time. These findings indicate that nasopharyngeal virus sampling in acute pre-school wheeze is of importance to identify children who will develop subsequent asthma at school age.

0816 | FeNO, forced oscillation, or spirometry? Lung function testing in wheezy pre-schoolers and the prediction of asthma, a systematic review

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Background: Pre-school children with wheezing disorders represent a diagnostic and therapeutic challenge, consuming large amounts of healthcare resources. Most wheezing episodes are mild and transient, but some infants develop severe recurrent episodes which require hospital admission and further referral to specialist doctors for diagnosis and management. There exists an unmet need for a reliable method of differentiating between patients with transient wheeze and those who will develop asthma in later life. Lung function tests were proposed as one of the possible approaches.

Method: PubMed, EMBASE and Cochrane Library databases were searched (November 2019). We included studies which: 1) assessed the usefulness of various lung function tests in predicting asthma; 2) limited subjects to children of age 1-6 with wheezing disorders; 3) were written in English. The exclusion criteria were as follows: 1) reviews, case reports, or editorial comments; 2) studies conducted on animal models or *in vitro* systems; 3) studies concerned with monitoring of asthma treatment. Three researchers participated in data extraction and analysis.

Results: Of the initial 5326 findings, the vast majority of which were ineligible due to the broadness of the search strategy, 36 studies were deemed to fit the inclusion criteria. The lung function tests

applicable to patients from the defined population were found to be: exhaled breath condensate, fraction of exhaled nitric oxide (FeNO), impulse oscillometry (IOS), induced sputum, multiple breath washout, spirometry, volatile organic compounds, and whole body plethysmography.

Conclusion: Measuring FEV1 using spirometry is considered to be the golden standard, but for younger children spirometry may be impossible to perform. Nevertheless, other lung function and airway inflammation measurements including IOS, body plethysmography, multiple breath washout, and FeNO may be utilised in tertiary care units to provide insight into the risk of developing asthma in preschool wheezers. The importance of accurately measuring lung function lies in the avoidance of over and under treatment of pre-school wheeze. For most methods, however, there are, to date, no large-scale randomised clinical trials or longitudinal studies, which would help to establish their role in diagnosis and management of pre-school children with wheeze.

0993 | Effect of exercise on FENO levels in climate specific environment, in children with mild to moderate persistent asthma

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Background: Asthmatic patients often avoid physical activity and exercise due to fear of triggering exercise-induced bronchoconstriction (EIB), which has a major impact on their quality of life. Although exercise can provoke EIB, it has been suggested that specially designed and controlled training programmes could have a positive effect on exercise capacity, lung function and problems with breathlessness, resulting in better control of the disease. Fraction of exhaled nitric oxide (FeNO) is considered to be a non-invasive marker of airway inflammation in asthmatic patients, which is why we wanted to compare FeNO values before and after controlled physical activity and to see whether there could be a potential improvement in children with asthma.

Method: 110 children with mild to moderate persistent asthma were recruited in paediatric pulmonology, allergology and immunology clinic of Srebrnjak Children's Hospital. All of the children participated in pulmonary rehabilitation program (PRP) for 2 weeks, in climate specific environment at Island Lošinj, in the northern Adriatic Sea (Croatia). Physical program consisted of at least two hours of active exercise per day, including aerobic and anaerobic training, in combination with breathing exercises. In order to determine airway inflammation level, FeNO was measured on site using a portable FeNO device, at baseline and at the end of a 2 week program.

Results: There was a significant decrease in FeNO levels in participants 2 weeks after PRP, going from 27 ppb to 19 ppb (*P* < .001), highlighting improvement in airway inflammation. Also, there was an

improvement of running distance going from 903 ± 272 m at baseline to 968 ± 289 m 2 weeks after PRP (*P* < .001), suggesting improved exercise capacity even in short period of time.

Conclusion: Our results show that daily exercise could have a positive effect in children with asthma, by reducing the airway inflammation. This suggests that even a short-term program with specifically organised physical activity, under controlled conditions and in an allergen safe environment, could have a beneficial clinical effect and possibly be important factor in patients with poor response to prescribed medication. These findings highlight the need for better understanding of exercise effect on asthma control, and its potential clinical relevance.

1283 | Clinical profile of children with preschool recurrent wheeze from South India

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Background: Wheezing is one of the most common presenting features of 20-30% children under the age of 5 years with approximately one in three children develop at least one episode of wheeze prior to third birthday. Wheeze in the preschool age group is highly variable with a variety of etiological factors and triggers. We present the clinical profile of preschool children presenting with recurrent wheeze with respect to triggers and phenotypic classification.

Method: This is a prospective observational study conducted between the period of 2015-2017 at a tertiary care allergy referral centre from Chennai, India. The study population included preschool children who presented with recurrent wheeze (GINA 2016) requiring admission. Information was obtained from a detailed parent directed clinical history, examination and laboratory findings and the child was followed up until discharge. Comorbid conditions were noted. Children were classified as episodic viral (EVW), multi trigger (MTW) and atypical wheeze (AW) as per the Hong Kong respiratory consensus. Bronchoscopy was done in cases of suspected airway anomalies.

Results: 145/851 (17%) children admitted with wheeze fulfilled inclusion criteria. 42 children were infants. Episodic viral wheeze was the most common cause (38.63%), followed by Multi trigger wheeze (36.55%) and atypical wheeze (24.82%). Mean age of onset was lowest for AW (2.97 ± 2.82 months, *P* = .001) while MTW had a higher mean age of onset (8.75 ± 4.3 months). AW group had a longer hospital stay including ICU stay (9.17 vs 4.13 days for EVW). 41.6% children with AW had congenital airway anomaly most commonly bronchomalacia (20.6%). MTW children had higher incidence of maternal asthma (34% vs 30% in EVW, *P* = .001). Most common comorbidity associated with MTW was Allergic Rhinitis (30.3%), whereas it was GERD (39%) in AW (*P* = .001). MTW group had a higher peripheral eosinophilia (4.06%), *P* = .001) and total IgE (196 ± 20.09, *P* = .001) as compared to the other groups. Asthma Predictive Index

(API) was positive in MTW (66%) as compared to EVW (28.1%) (*P* =<.001)

Conclusion: Our study showed that episodic viral wheeze was the most common cause of recurrent wheeze in preschoolers. Atypical wheeze was associated with higher incidence of airway anomaly and longer hospital stay. Episodic wheeze was commonly associated with GERD. Multitrigger wheeze group had a higher incidence of maternal history of asthma, higher peripheral eosinophils, total IgE levels and positive API.

1344 | Healthcare use and asthma control among overweight/obese urban children sensitized and exposed to classroom allergens

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Background: The effect of body mass index (BMI) status on the relationship of allergic sensitization and allergen exposure on asthma morbidity in urban children is poorly understood.

Method: The School Inner-City Asthma Study enrolled students aged 4-13 years with asthma from 37 inner-city schools in the northeastern United States. Students had baseline determination of BMI percentile and allergen sensitization. Asthma-related healthcare use (HCU) and poor asthma control identified by any of the following in the past 4 weeks: shortness of breath more than twice weekly; asthma nighttime awakenings at least once; limitation in activity level; or use of rescue medication 2 or more times weekly, were monitored during the academic year. Vacuumed classroom dust samples, linked to enrolled students, were collected twice per year and analyzed for common indoor aeroallergens. We determined the relationship between allergic sensitization and increased allergen exposure on asthma outcomes by BMI stratification.

Results: A total of 279 predominantly Black (35%) or Hispanic (37%) students were included in analyses. Fifty percent were normal weight (5-84th BMI percentile, [NW]), 15% were overweight (>/=85-94th BMI percentile, [OV]), and 35% were obese (>/=95th BMI percentile, [OB]). Allergic sensitization to >/= 1 allergen was observed in 68% of enrolled students. Two-way interaction (BMI x exposure) analysis demonstrated that OV students had greater HCU with exposure to higher levels of classroom dog allergen (0.21 ± 0.07; interaction *P* = .004), cat allergen (0.20 ± 0.07; interaction *P* = .02), and mouse allergen (0.24 ± 0.07; interaction *P* = .03). Three-way interaction (BMI x sensitization x exposure) effects showed that OV and/or OB sensitized children had better asthma control with exposure to higher levels of classroom dust mite allergen (OV, interaction *P* = .004; OB, interaction *P* = .013) and cat allergen (OB, interaction *P* = .04), compared to NW sensitized children.

Conclusion: OV BMI status is associated with greater HCU with increasing levels of allergen exposure, independent of sensitization. However, compared to NW sensitized children, OV/OB sensitized children exhibited better asthma control with higher levels of exposure. Thus, the respiratory effects caused by OV/OB BMI status and inhalation of allergens are likely not mediated by IgE-induced inflammation, often linked to pediatric asthma. Further study of non-Th_2 mechanisms underlying this phenotype is needed.

Table 1. Association between Classroom Allergen Exposure and Healthcare Utilization Stratified by BMI Category*

	[1]Norm Weight Mean ± SE	[2]Overweight Mean ± SE	[3]Obese Mean ± SE	**P-value Overweight x exposure interaction	**P-value Obese x exposure interaction
Low Dust mite (Der f 1) (0.012 µg/mL)	0.17 ± 0.04	0.12 ± 0.06	0.11 ± 0.03	0.34	0.79
High Dust mite (Der f 1) (0.054 µg/mL)	0.21 ± 0.04	0.21 ± 0.07	0.15 ± 0.04		
Low Dog (Can f 1) (0.03 µg/mL)	0.24 ± 0.05	0.11 ± 0.04	0.15 ± 0.05	0.004	0.53
High Dog (Can f 1) (0.26 µg/mL)	0.18 ± 0.04	0.21 ± 0.07	0.14 ± 0.03		
Low Cat (Fel d 1) (0.09 µg/mL)	0.22 ± 0.04	0.15 ± 0.06	0.12 ± 0.04	0.02	0.13
High Cat (Fel d 1) (0.58 µg/mL)	0.17 ± 0.04	0.20 ± 0.07	0.14 ± 0.03		
Low Mouse (Mus m 1) (0.22 µg/mL)	0.19 ± 0.04	0.09 ± 0.04	0.14 ± 0.04	0.03	0.26
High Mouse (Mus m 1) (3.03 µg/mL)	0.21 ± 0.04	0.24 ± 0.07	0.11 ± 0.03		

[1] n =138.
[2] n =42.
[3] n =99.
* Results are from generalized estimating equations with robust variance estimates to account for multiple observations within subjects. Marginal means of the outcomes were calculated at the 25th and 75th percentiles of exposure.
** Independent variables included obese status (3-level), sensitization status (2-level), exposure (continuous), and all 2- and 3-way interaction terms. Models were adjusted for age, gender, race, controller medication use, home exposure, and season.

OAS 25
New Methodological Insights for Allergy Diagnosis

0929 | Biotin interference can cause false-negative IgE test results in patients with anaphylaxis

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Background: Numerous modern immunoassays (IA), which are designed for rapid and precise diagnosis, use biotin-streptavidin (B/SA) for analyte detection. Thus, depending on the design of the IA, test results may be susceptible to biotin interference. Recently, the intake of high doses of biotin has become quite common for medical and other reasons. As two major IA platforms for fully automated detection of sIgE use B/SA, we asked the question whether the correct IgE-based diagnosis of anaphylaxis using these two laboratory systems (B/SA-IA1*; B/SA-IA2**) could be hampered by the presence of biotin in patients' sera.

Method: We used 18 sera from patients with either challenge-positive anaphylaxis to cashew nut (n = 6) and peanut (n = 6) or with an anaphylaxis score ≥ grade II (Ring/Messmer) after a wasp sting (n = 6). Cashew nut (f202), peanut (f13) and wasp venom (i3) sIgE was measured in parallel comparing B/SA-IA1 and B/SA-IA2 in the presence of three biotin concentrations (ng/mL): 0, 184, 500. The latter values represent median biotin levels in human serum after taking medium or high doses of biotin, respectively. Samples were analyzed by standard procedures using B/SA-IA1, which has one, and B/SA-IA2, which has two incubation steps, at a sIgE cut-off of 0.35 kU/L.

Results: In B/SA-IA1, the addition of biotin to the patient serum led to a marked and concentration-dependent decrease in sIgE values in all patients. At 184 ng/mL, sIgE values were false negative in 11 out of 18 patients, while at 500 ng/mL biotin, sIgE values were false negative in 14/18 patients. Of the four patients not affected by biotin interference, three had sIgE values > 30 kU/L. The addition of biotin did not influence the results of B/SA-IA2, regardless of the sIgE level or the biotin concentration.

Conclusion: The diagnosis of anaphylaxis may be compromised in patients with increased biotin intake and lower sIgE values (<10 kU/L). In B/SA-IA1, excessive free serum biotin competes with biotinylated allergen-sIgE complexes for binding to streptavidin beads, thus leading to a reduced signal, false negative results and the possibility of a missed diagnosis of anaphylaxis. This is not the case in B/SA-IA2, where streptavidin microparticles and biotinylated allergen are coupled prior to the introduction of the patient sample, thus avoiding any interference by excessive biotin.

* B/SA-IA1, Immulite 2000, Siemens.

** B/SA-IA2, NOVEOS, Hycor.

0946 | Identification of optimal cut-off points for nasal volumes and symptoms score in the assessment of the outcome of the nasal allergen challenge

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Background: Nasal allergen challenge (NAC) is the gold standard for the diagnosis of allergic rhinitis (AR). In this study, we aimed to identify the optimal cut-off points for nasal volumes and total nasal-ocular symptom score (TNOSS) for discriminating positive and negative NAC responses.

Method: NACs were performed in 1985 subjects: 1165 AR and 369 non-allergic rhinitis (NAR) patients, and 361 healthy non-atopic control (HC) individuals. The NACs were monitored by the changes in nasal volumes (%Vol2-6 cm) measured by Acoustic Rhinometry (AcRh) and TNOSS. Receiver operating characteristic (ROC) curves were used for identifying the optimal cut-off points and for calculating the areas under the curves (AUC), sensitivity (SE), and specificity (SP). Positive and negative predictive values (PPV and NPV) and Likelihood ratios (LH+ and LH-) were also calculated. This study was funded by the Instituto de Salud Carlos III, Spanish Ministry of Science and Competitiveness (RD16/0006/0001).

Results: The optimal cut-off points for discriminating AR and HC were %Vol2-6 cm ≥ 24.48% and TNOSS ≥ 3.5 (AUC 1/0.944, $P < .001$; SE 99.7/67.1%, SP 100%/99.4%, PPV 100%/100%, NPV 98.9%/47%, LH+ */234.882, LH- 0/0.35 respectively). For differentiating AR and NAR, the optimal cut-off points were %Vol2-6 cm ≥ 24.40 and TNOSS ≥ 4.5 (AUC 1/0.880, $P < .001$; SE 99.7%/65.1%, SP 100%/91.6%, PPV 100%/96.1%, NPV 99.2%/45.4%, LH+ */7.745, LH- 0/0.38 respectively).

(*):LH+ cannot be calculated when SP = 100%.

Conclusion: In our study, the NAC-induced change of %Vol 2-6 cm (AcRh) was more sensitive, specific and predictive than TNOSS for discriminating AR, NAR and HC subjects. In rhinitis patients, the most accurate AcRh cut-off point for NAC positivity was a decrease ≥ 24.40%in %Vol 2-6 cm after the challenge, as compared to baseline.

1150 | Bet v 1 specific IgG4 from birch pollen allergic patients shows a more diverse repertoire than specific IgE

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Background: IgE specific to the major birch pollen allergen, Bet v 1, binds to conformational epitopes, which cannot be characterized by simple peptide mapping. Hence, most epitopes have not been identified, yet. By grafting epitope-sized surface patches of Bet v 1 onto a non-IgE-binding structural homologue we aimed to identify epitopes bound by IgE and IgG4 from birch pollen allergic patients.

Method: Based on a structural alignment, surface-exposed residues of the bacterial Bet v 1-related protein TTHA0849 from *Thermus thermophilus* were replaced by corresponding ones of Bet v 1 while preserving the hydrophobic core. Thereby, we created 14 chimeric proteins carrying partially overlapping Bet v 1-derived surface patches. Codon-optimized synthetic genes were expressed in *Escherichia coli* as 6xHis-tagged proteins and purified by metal chelate affinity chromatography. They were characterized via matrix-assisted laser desorption-ionization mass spectrometry, circular dichroism spectroscopy and dynamic light scattering. IgE and IgG4 binding were assayed by quantitative ELISA using sera from 20 Bet v 1-sensitized, birch pollen-allergic patients. This work was supported by the Austrian Science Fund (FWF): P 30936-B30.

Results: Until now, nine chimeras were expressed as soluble proteins with low levels of aggregation and secondary structure contents compatible with folded proteins. Both IgE and IgG4 bound to diverse subsets of epitopes in a patient specific manner. IgE from 50% of the patients recognized between 1 and 3 epitopes, while the other sera lacked IgE binding to all tested chimeric proteins. In contrast, 15 of 18 sera had IgG4 specific to 1-8 chimeras. The number of chimeras bound by IgG4 did not correlate with the amount of Bet v 1-specific IgG4. Bet v 1-specific IgG4 recognized significantly more epitopes than IgE (mean values 3.4 and 0.7; $P = .0008$). We did not find a correlation between the epitope binding patterns of IgE and IgG4.

Conclusion: By using structure based epitope grafting, we showed that the repertoires of conformational epitopes of Bet v 1 recognized by IgE and IgG4 were highly variable and isotype specific. Knowledge of the epitopes bound by allergen specific antibodies may aid in predicting symptom severity, cross-reactivity and efficacy of allergen immunotherapy.

1290 | Cow's milk allergy can be monitored through the degree of competition between specific IgE and IgG

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Background: Induction of specific IgG (sIgG) is often present during oral immunotherapy (OIT). The blocking action of sIgG on specific IgE (sIgE) depends on both the molar ratio and the respective avidities of sIgE and sIgG. However, monitoring the concentration of specific IgG (or of specific IgG$_4$) during OIT does not allow the estimation of the clinical success or failure of the treatment, which can only be assessed by an oral food challenge (OFC). As a follow-up to our previous work (Sereme Y. et al. *Allergy* 2019, 74:219-220), we study here the possibility of using the effect of IgG-depletion on allergen microarray results as an *in vitro* marker of OIT success or failure.

Method: Seven children (6 m. to 6 y.o.) allergic to cow's milk (CM) were studied before starting and after 9 to 36 months of OIT with cooked CM. The criteria for success (4/7 children) of OIT were: the non-reduction of the at-home daily CM intake and the doubling of the tolerated amount of milk between the initial and the final OFC. Three other children (4 m. to 7 y.o.) allergic to CM were followed up for 8 to 14 months without OIT. For the 10 children, sIgE, sIgG and sIgG$_4$, specific for anti-CM extract and anti-casein, were measured at start and end of the follow-up period. An ImmunoCAP ISAC allergen microarray was performed at the end of OIT, or follow-up without OIT, both on intact serum and on serum depleted from IgG. The effect of IgG depletion was quantified by post/pre-depletion ratios of sIgE, measured by ISAC.

Results: Between the start and the end of OIT, a moderate elevation of anti-CM and anti-casein sIgE, sIgG and sIgG$_4$ was present in most patients, but was in agreement with the success or failure of OIT for only 1 patient out of 7. At the end of OIT, the ISAC performed after IgG depletion showed an increase in the apparent concentration of anti-CM and / or anti-casein sIgE, only if OIT was a success (average: + 615%; median: + 192%). Conversely, sIgE levels were unchanged or decreased by IgG depletion if the OIT failed (mean: -9%; median: -15%). In the group of 3 children without OIT, we observed an average increase of + 98% (median: −26%) of anti-CM / casein sIgE with the IgG-depleted ISAC.

Conclusion: An allergen microarray, combined with the depletion of serum IgG, allows the quantification of the competitive effect exerted by IgG on the binding of sIgE to the allergen. In the context of CM OIT, this quantification is consistent with the OIT result in the majority of patients.

1445 | Environmental exposure chamber is a useful tool for better selection of AIT patients with HDM triggered rhinoconjunctivitis for assessment of potential biomarkers of allergen tolerance

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Background: The mechanism of allergen tolerance induced by allergen-specific immunotherapy (AIT) remains unclear. This might be due to poor stratification of patients that might benefit most of the treatment.

In this study the effect house dust mite (HDM) AIT on well characterized immune parameters associated with allergen tolerance were assessed in patients selected using allergen challenge in the Environmental Exposure Chamber (EEC).

Method: 50 patients with symptoms of allergic rhinoconjunctivitis were included in the study. Allergy to HDM was confirmed by skin prick tests (SPT), specific IgE (sIgE) and in EEC. Selected patients were treated with AIT against HDM. Allergen-specific CD4 + lymphocytes subpopulations were assessed by flow cytometry, at subsequent therapy time points. In addition, HLA genotyping was performed and the response profile to HDM was assessed.

Results: Very high negative correlation in Treg cell measured after 1 month of AIT and nasal symptoms assessed in EEC was observed ($P < .05$, $r = −0.9$). Patients with higher nasal symptoms showed higher number of Th2 cells before treatment ($P < .05$, $r = 0.9$).

Th1 lymphocyte response decreased during the course of AIT after 3 months of treatment. The response from Th2 GATA3+ and Th2 CCR4+ lymphocytes decreased during the course of AIT. The number of Th17 and Th22 lymphocytes was very variable during treatment. An almost six-fold increase in the number of Treg lymphocytes (Foxp3+) in the first month of AIT and a gradual decrease in the next five months was demonstrated. Immune balance was restored after 360 days of AIT.

Larger diameter for SPT and higher sIgE concentration using D.p. determines higher number of Treg cells after 1 month of AIT ($P < .05$, $r = 0.623$ and $r = 0.585$), while higher concentration of sIgE against D.f. correlates with the higher number of Treg lymphocytes 3 months after the start of AIT ($P < .05$, $r = 0.555$).

HLA DRB3*01:14 alleles were most commonly detected. In turn, patients with HLA DRB1 11*01 had the highest results of SPT and sIgE against HDM and the most concomitant allergies confirmed in both SPT and sIgE.

Conclusion: Rhinoconjunctivitis symptoms induced during exposure in EEC provide a plausible tool for a better patient stratification for AIT as assessed by strong specific Treg cell responses during AIT in HDM allergic subjects.

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In Vitro Diagnosis of Drug Allergy

0829 | Highly improved sensitive in vitro drug allergy test using an collection of new ß-lactam-protein antigens

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Background: ß-lactams (BL) are the most widely used antibiotics and today the first-choice drugs to control several bacterial infections. These molecules, acting as antigens when conjugated to intra or extracellular human proteins to become immunogenic, are able to trigger drug allergy through antigen-specific IgE or T-cell mediated mechanisms. Currently, *in vitro* diagnostic tests for drug allergy suffer from low sensitivity and specificity, probably because the IgE does not specifically recognize the antigens used and the differences between the reactivity profiles of patients make very difficult to have a constant response. Therefore, it is necessary to develop new *in vitro* drug allergy tests, which improves sensitivity and specificity as well as it presents multiplexing capacity to evaluate simultaneously different epitopes of BLs. For that, the characterization of new antigens is key to harmonize testing protocols and improve the clinical performances of the current in vitro tests.

Method: In this work, different proteins from human serum were conjugated to different antibiotics. This binding was accomplished through amino or carboxylic reactive groups present in BL's structures in order to study the influence of protein carrier in the epitope presentation. The performances of the conjugates were evaluated, analysing serum samples from 89 allergic patients and 76 negative controls using the DVD technology. This technology offers a low cost and portable disc drive as optical detector and a DVD which serves as surface to immobilize a wide set of determinants in microarray format, conferring multiplexing capacity.

Results: A new BL protein carrier presents improved immunorecognition capabilities, allowing to improve the analytical sensitivity of the *in vitro* test (0.1 UI/mL). The obtained results showed different immunorecognition patterns between patients. Consequently, it has been demonstrated the need to provide a wide collection of antigens for the same antibiotic, in order to improve the clinical sensitivity of the test.

Conclusion: The wide diversity of new BL-protein antigens obtained allows the *in vitro* determination of specific IgE in human serum, improving clinical sensitivity of the test by 50% when comparing with reference methods. DVD technology has proven to be useful to develop alternative *in vitro* allergy test, since it allows simultaneous detection of a wide repertoire of antigenic determinants using minimal sample volume.

0835 | The type of antigen presenting cells employed in the lymphocyte transformation test affects its effectivity in immediate allergic reactions to betalactams

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Background: One of the most used test to evaluate non-immediate allergic reactions to drugs is Lymphocyte transformation test (LTT), that allows determining proliferation of cells in response to the culprit drug. Nevertheless, its value in immediate allergic reactions to drugs is not well established. Different antigen presentation cells (APCs) can be used in LTT: non-professionals ASPCs such as B cells and monocytes, or professional APCs such as monocyte-derived dendritic cells (moDCs) or myeloid dendritic cells (mDCs). The use of professional APCs has demonstrated to improve LTT results in the evaluation of non-immediate reactions. Therefore, the main objective was to study LTT effectivity using pre-primed moDCs or mDCs as APCs, comparing them with traditional LTT, carried out with peripheral blood mononuclear cells (PBMCs) in immediate reactions to betalactams.

Method: Monocytes and mDCs were isolated from 10 clavulanic acid (CLV) and 10 amoxicillin (AX) allergic patients with immediate reactions, and from 10 tolerant subjects. moDCs were differentiated culturing monocytes in presence of GM-CSF and IL-4. Proliferation of different T cell subpopulations (CD3$^+$, CD4$^+$, CD8$^+$, CD4$^+$Th2 and Treg cells) were assessed by flow cytometry using Carboxyfluorescein succinimidyl ester (CFSE). Results were expressed as Proliferation Index (PI).

Results: Independently of the type of APCs tested, we observed higher proliferation values in both, AX and CLV patients compared with tolerant subjects. An increased sensitivity was observed when mDCS were used in AX patients (50% with positive proliferation (PI > 2)), compared with moDCs or PBMCs (<20% for both). Surprisingly, sensitivity in CLV allergic patients was always lower (25%), independently of the APCs used. No proliferation was observed in tolerant subjects with any of the APCs used.

Conclusion: The APCs used in the LTT influence the evaluation of immediate allergic reactions to AX. The employment of mDCs as APCs improve the results obtained with LTT in those patients. However, no differences were observed in CLV immediate allergic reactions. This could be due to the non-inclusion of the specific determinants that induced the reaction and not to the APCs used.

0894 | Evaluation of immediate reactions and cross-reactivity due to metronidazole and ornidazole as rare antibiotic elicitors

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Background: Little is known about the value of diagnostic approaches for immediate hypersensitivity reactions (IHRs) due to 5-Nitroimidazole antibiotics such as metronidazole and ornidazole. The aim was to evaluate the usefulness of skin tests, drug provocation tests and basophil activation test (BAT) for the diagnosis of IHRs due to 5-nitroimidazole antibiotics and to determine possible cross reactivity among these drugs.

Method: Forty-nine patients with a history of IHRs due to 5-nitroimidazole antibiotics were included to this study. Ten nitroimidazole tolerable healthy subjects formed the control group. After detailed clinical history, skin tests (STs), and single-blind placebo-controlled drug provocation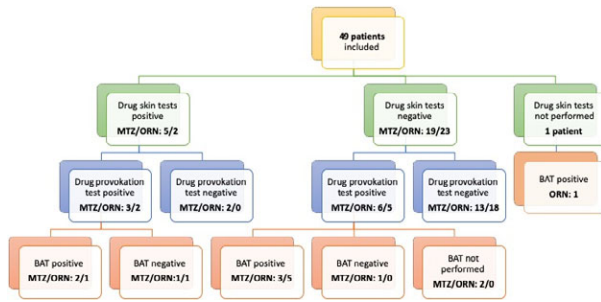 tests (SBPCDPTs) were performed with both 5-Nitroimidazole antibiotics whereas BAT was applied only with the culprit drug.

Results: The mean age of the patients was 44.14 ± 11.71 years and 91.8% of them were female. The most and the least common reaction types were urticaria/angioedema (34.7%) and anaphylaxis (14.3%) respectively. Although SBPCDPTs were positive in 16 patients, only 7 of them had positive STs. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of STs were 31.25%, 93.94%, 71.43% and 73.81% respectively. BAT was performed in 14 patients with positive DPT results, in one patient with a history of anaphylaxis and in 10 control subjects. BAT was positive in 12 patients and the sensitivity of the test was 80%. The test was negative in all control subjects. The optimal concentration of both drugs for BAT was determined as 5 mg/mL. The diagnostic workup used in the study was shown as a flow chart in Figure 1. The clinical findings, *in vivo* test and BAT results of the patients with positive drug provocation test results were shown in Table 1. No cross-reactivity among two drugs was observed according to *in vivo* test results.

Conclusion: Our study showed that both provocation tests and BAT are useful diagnostic tools for IHRs due to 5-nitroimidazole antibiotics and may be interchangeably used.

Patient	Culprit drug	Reaction type	Metronidazole			Ornidazole		
			STs	DPT	BAT(%)	STs	DPT	BAT(%)
			STs	DPT	BAT(%)	STs	DPT	BAT(%)
1	ORN	Urt/AE	Neg	Neg	NP	Neg	Poz	22.25
2	ORN	Urt	Neg	Neg	NP	Neg	Poz	43.53
3	MTZ	Anaphy	Neg	Poz	55.08	Neg	Neg	NP
4	ORN	Anaphy	Poz	Neg	NP	Neg	NP	11.53
5	ORN	Anaphy	Neg	NP	-	Neg	NP*	6.21
6	ORN	Urt/AE	Neg	Neg	NP	Neg	Poz	8.75
7	ORN	AE	Neg	Neg	NP	Poz	Poz	21.67
8	ORN/MTZ	Urt	Neg	Neg	-	Neg	Poz	23.10
9	MTZ	AE	Neg	Poz	14.23	Neg	Neg	NP
10	MTZ	Urt	Poz	Poz	41.66	Neg	Neg	NP
11	MTZ	Urt	Poz	Poz	32.70	Neg	Neg	NP
12	MTZ	Anaphy	Neg	Poz	-	Neg	Neg	NP
13	ORN	Urt/AE	Neg	Neg	NP	Neg	Poz	-
14	MTZ	AE	Poz	Poz	-	Neg	Neg	NP
15	MTZ	Urt/AE	Neg	Poz	NP	Neg	Neg	NP
16	MTZ	Urt/AE	Neg	Poz	NP	Neg	Neg	NP
17	MTZ	Urt/AE	Neg	Poz	19.17	Neg	Neg	NP

Urt, urticaria; AE, Angioedema; Anaphy, Anaphylaxis; NP, not performed; Neg, negative; Poz, positive; ORN, ornidazole; MTZ, metronidazole. Only the positive BAT results were demonstrated and BAT results below the level of 5% and SI < 2 were showed as (–).*Provocation was not performed due to anaphylaxis observed by the study staff.

LTT sensitivity after ROC curves was up to 19% with CLV itself, and up to 36% when cells from patients were cultured with the different synthetic AD. Interestingly, it was found that some patients selectively recognised AD-I or AD-II, so, analysing all analogs together, the sensitivity of the test increased up to 63% and 72% in CD4^{+} and CD4^{+}Th2 cells respectively. The specificity was 100% in all cases.

Conclusion: The inclusion of synthetic AD of CLV increases LTT sensitivity compared with CLV itself, without affecting specificity. The use of these synthetic structures in different *in vitro* assays could improve the current diagnosis of CLV allergy.

0999 | Recognition of synthetic antigenic determinants of clavulanic acid in patients with immediate allergic reactions to betalactams using lymphocyte transformation test

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Background: *In vitro* tests are useful to improve the diagnosis of immediate allergic reactions to drugs (IR). Nevertheless, those carried out with clavulanic acid (CLV) are not good enough due to their low sensitivity, impairing their inclusion in clinical routine. This low sensitivity could be explained because, after the intake of CLV, the drug is degraded, and only the different resulting metabolites are able to modify proteins and form the haptenic structures that are specifically recognised by the immune system. To evaluate this hypothesis, we used the Lymphocyte Transformation Test (LTT) to evaluate specific-proliferation of different cell populations after culturing them with synthetic antigenic determinants (AD) of CLV.

Method: Two AD were generated based on two different degradation pathways of CLV. Three different analogs CLV1-3 and CLV4-6 were synthetised from AD-I and AD-II respectively, with different stability and ability to modify proteins. Monocytes were isolated from 15 allergic patient with IR to CLV and from 15 healthy controls (HC). Monocytes were differentiated into dendritic cells (moDCs) by culturing with IL-4 and GM-CSF. moDCs were cultured with CLV itself and with each synthetic analog for 5 days. Then, moDCs were cultured with lymphocytes that were previously labeled with Carboxyfluorescein succinimidyl ester (CFSE). Proliferation of CD3^{+}, CD8^{+}, CD4^{+}, CD4^{+}Th2, and Treg cells was assessed by flow cytometry, analysing the CFSE dilution.

Results: Higher proliferation was found when CLV-1, CLV-2, CLV-5, and CLV-6 were cultured with cells from allergic patients compared with HC, mainly in CD4^{+} with aTh2 cytokine pattern ($P < .005$). Moreover, higher proliferation was observed in Treg cells from patients compared with HC ($P < .05$). No difference was observed when cells from patients were cultured with CLV itself compared with HC.

1047 | Anaphylaxis-derived extracellular vesicles induce vascular permeability

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Background: Anaphylaxis is the most serious manifestation of allergic disorders. It is a reaction of hypersensitivity, with rapid development and several organs are affected among which those belonging to the circulatory system stand out. The analysis of the endothelial niche due to the increased vascular permeability associated to anaphylaxis, allows to improve the knowledge of the underlying molecular bases. Moreover, our previous studies identify a differential protein extracellular vesicle (EV) pattern in anaphylaxis. Therefore, because of its role in cellular communication, the objective of our study is to analyze the role of the human anaphylaxis-derived EVs in endothelial permeability.

Method: EVs were purified by centrifugation and ultracentrifugation from plasma collected from 16 patients during the acute phase of anaphylaxis (food, drug or others) which were compared with baseline samples of plasma obtained from the same patients 15 days after recover. The sampling was carried out following criteria based on the grading system of clinical symptoms; moderate (2) and severe (3). An *in vitro* system of human microvascular endothelial cells- lung (HMVEC-L) was carried out and cells were incubated with 100ug of EVs (acute and basal) over different periods of time (10 min, 1 h, 2 h, 5 h). Vascular permeability was determined by measuring the resistance of the cell monolayer through an EndOhm device. Confocal microscopy confirmed the status of the monolayer by staining with Texas Red and the interaction between EVs and HMVEC-L by PKH67 co-staining.

Results: Vascular permeability assays based on endothelial cells monolayers demonstrated that EVs obtained from the acute phase of anaphylaxis significantly increase the leakage measured by decreased resistance values. This effect was observed when compared with baseline EVs stimulated for 2 and 5 hours. Moreover, the interaction between EVs and the endothelial cell monolayer was

confirmed by combined staining of Texas Red and PKH67 in both cases.

Conclusion: Our study demonstrates that anaphylaxis-derived EVs from the acute phase increase vascular permeability in HMVEC-L. In addition, it confirms differences in the EVs′ functional behavior between both acute and basal phases. Their interaction with endothelial cells was also demonstrated. Our study reveals a possible clinical relevance of EVs in anaphylaxis and helps to understand the pathophysiology and the molecular bases underlying to the reactions.

1194 | Basophil activation test in anaphylaxis to non-steroidal anti-inflammatory drugs – is it useful?

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Background: Drug provocation tests (DPT) are the gold standard diagnosis method in non-steroidal anti-inflammatory (NSAID) hypersensitivity (HS). Although basophil activation test (BAT) has promising results in immediate HS reactions with beta-lactams, platinum compounds or neuromuscular blockers, its utility in the reactions with NSAIDs (except pyrazolones) is not well established.

Method: From the 401 patients referred to the drug outpatient clinic with history of HS to NSAIDs, from January 2006 to December 2018, we've selected those with confirmed diagnosis of NSAIDs-induced urticaria/ angioedema (NIUA) or Single-NSAID-induced urticaria/ angioedema or anaphylaxis (SNIUAA), based on a positive provocation challenge with acetylsalicylic acid (ASA). From these patients, we included those with immediate reaction of anaphylaxis, who had performed a diagnostic DPT with the culprit drug and ASA. We also included a control group of non-allergic patients. In NIUA and SNIUAA groups, two BATs were performed in each patient, both with the culprit drug (confirmed in DPT) and ASA and in the control group, with ASA and NSAID, according with clinical history of patients from NIUA/ SNIUAA groups, in 3 different concentrations for each drug. We considered a positive BAT if the stimulation index was > 2%, in one drug concentration.

Results: 24 patients, average age 49.8 ± 16.2 years old, 15 (63%) female. A total of 48 BAT were performed in this population. Each group (SNIUAA, NIUA and control) had 8 patients, for a total of 16 BAT. In the SNIUAA group (6 female), BAT performed with culprit drug (1 diclofenac, 2 clonidine, 5 dipyrone) was positive in all patients; only one BAT with ASA was positive, which was considered as a false positive; BAT sensibility in this group was 87,5%. In the NIUA group (5 female), BAT performed with culprit drug was positive in 4 patients (3 diclofenac, 1 dipyrone) and negative in the others (3 diclofenac, 1 dipyrone); BAT for ASA was positive in 6 patients and

negative in 2; there were 4 false negatives with culprit drug and 2 with ASA, so that BAT sensibility in this group was 37,5%. In the control group (4 female), all the BATs were negative, so that specificity was 100%.

Conclusion: BAT was more predictive in patients with selective HS reactions to NSAIDs (sensibility of 87.5% in SNIUAA), which is in line with literature. It can be an additional tool in the diagnosis of NSAIDs HS, especially in case of severe reactions; however, more studies are needed to confirm its reliability.

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Clinical Perspectives in Allergen Immunotherapy: Where are We Now?

0089 | Long-term effects of allergen immunotherapy to grass pollen

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Background: Allergen-specific immunotherapy (AIT) is a crucial therapy for allergic rhinitis (AR). However, the long-term effectiveness of AIT remains to be explored. The objective of this study was to evaluate the clinical long-term effect of allergen injection immunotherapy to grass in patients with allergic rhinitis when immunotherapy was discontinued.

Method: It was a prospective follow-up observation of patients with AR who completed preseasonal AIT for grass pollen. After receiving 3 years of AIT (ClinicalTrials.gov - Protocol Record MC56871/12), 31 patients were compared to a placebo group after an additional 3 years of observation. Combined symptom medication score (CSMS), quality of life (RQLQ) and concentration of allergen-specific IgE and IgG_4 for *Phleum pratense* were monitored during observation. The local grass pollen counts during May-August were determined by volumetric pollen trap every year of observation.

Results: Three years after AIT was discontinued, a significant clinical effect based on CSMS was still observed compared with the baseline (before AIT), just after immunotherapy and 3 years later as follows: 7.85 (range: 3.67-8.98) *vs* 4.63 (range: 3.76-7.80) *vs* 4.03 (range: 3.12-7.81). Significant differences in CSMS were observed every year between the study groups and the placebo group ($P < .05$). Serum-specific IgE against *Phleum pratense* decreased during the AIT trial and remained at the same levels 3 years after immunotherapy compared to the placebo group. There were no significant changes between specific IgG_4 levels compared to results just after AIT.

RQLQ stayed at a better level *vs* placebo throughout the observation.

Conclusion: The positive effect obtained after allergen immunotherapy to grass pollen was sustained after a long period after therapy. However, further controlled studies are required to assess this result.

0239 | Efficacy and safety of ragweed SLIT-tablet from a large trial in children with allergic rhinoconjunctivitis

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Background: Ragweed sublingual immunotherapy (SLIT)-tablet improves symptoms and decreases symptom-relieving medication use in adults with allergic rhinitis with or without conjunctivitis (AR/C) but has not been evaluated in children. This international, double-blind, placebo-controlled trial evaluated the efficacy and safety of ragweed SLIT-tablet in children with AR/C.

Method: Polysensized children (N = 1025) aged 5 to 17 years with ragweed AR/C with or without asthma ($FEV_1 \geq 80\%$ predicted) were randomized 1:1 to daily ragweed SLIT-tablet (12 SQ-Amb) or placebo for up to 28 weeks (NCT02478398). 42.7% had a history of asthma. Symptom-relieving medication was provided to both treatment arms. The primary endpoint was the average total combined score (TCS; sum of rhinoconjunctivitis daily symptom score [DSS] and daily medication score [DMS]) over the peak ragweed pollen season (RPS). Key secondary endpoints were average TCS during entire RPS, and DSS and DMS during peak RPS. Asthma outcomes included number of daily inhalations of as-needed short-acting beta$_2$-agonist (SABA) and number of weekly nocturnal awakenings due to asthma symptoms requiring SABA in subjects with asthma at baseline (n = 406) during peak RPS.

Results: Relative improvements in TCS with ragweed SLIT-tablet compared with placebo were −38.3% (95% CI, −46.0%, −29.7%; least square [LS] mean difference = 2.73; $P < .001$) during peak RPS and −32.4% (95% CI, −40.7%, −23.3%; LS mean difference = 1.86; $P < .001$) during the entire RPS. DSS and DMS were improved with ragweed SLIT-tablet compared with placebo by −35.4% (95% CI, −43.2%, −26.1%; LS mean difference = 1.40; $P < .001$) and −47.7% (95% CI, −59.8, −32.5%; LS mean difference = 1.84; $P < .001$), respectively, during peak RPS. SABA use and nocturnal awakenings were improved with ragweed SLIT-tablet compared with placebo by −68.1% (95% CI, −87.6%, −39.0%; LS mean difference = 0.14; $P = .002$) and −75.1% (95% CI, −99.3, −35.2%; LS mean difference = 0.08; $P = .017$), respectively. There were no reported severe or serious asthma events, events of anaphylaxis, airway compromise, or severe treatment-related systemic allergic reactions.

Conclusion: This is the largest allergy immunotherapy trial in children with ragweed AR/C demonstrating that ragweed SLIT-tablet significantly improves AR/C symptoms and asthma control outcomes and decreases symptom-relieving medication use for AR/C and asthma. Treatment was well tolerated.

0350 | Disease modifying effect of sublingual immunotherapy tablets in patients with Japanese cedar pollinosis. A double-blind, randomised, parallel-group, placebo-controlled study

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Background: Japanese Cedar (JC) pollen sublingual immunotherapy (SLIT) tablets are licensed for the treatment of JC pollinosis in Japan. We conducted a clinical trial to investigate disease-modifying effect of the SLIT-tablets after 3-year treatment and 2-year follow-up period covering five JC pollen seasons.

Method: A total of 1042 patients with JC pollinosis (5-64 years) were equally randomized to receive tablets containing placebo (P), 2000, 5000, or 10,000 Japanese allergy units (JAU) of JC pollen for 15 months to identify an optimal dose. Patients receiving P (n = 240) and the optimal active treatment dose (5000 JAU; A, n = 236) were re-randomized to receive P or A for an additional 18 months (i.e. four groups: placebo to placebo (PP; n = 159), 5000 JAU to placebo (AP; n = 78), placebo to 5000 JAU (PA; n = 81), and 5000 JAU to 5000 JAU (AA; n = 158), allocation ratio 2:1:1:2), comprising total three years of treatment and two years of follow-up. Clinical efficacy was evaluated by the total nasal symptom and medication score (TNSMS) during the peak symptom period of each JC pollen season.

Results: TNSMS was significantly lower in AA, AP and PA groups (vs PP, $P < .001$ in all groups) during all 3 years treatment. The largest relative reduction in TNSMS during treatment was seen in the AA group in the third year of treatment (vs PP, 46.3%, $P < .001$). The AA group had significantly lower TNSMS during 2 years of blinded follow-up after completion of 3 years of treatment (vs PP, 45.3% in the first follow-up season, $P < .001$, 34.0% in the second follow-up season, $P < .001$). The proportion of participants who did not use rescue medication was significantly higher in the AA group than in the PP group during the peak symptom period of each JC pollen seasons (vs PP, $P < .001$, respectively). Most common adverse drug reactions were mild local reactions related to the administration site.

Conclusion: The study confirms the sustained clinical efficacy of JC pollen SLIT-tablets during 3 years of treatment, and disease modifying effect for at least 2 years after completion of the treatment.

0506 | Effects of combined treatment with allergen immunotherapy and dupilumab on nasal allergen challenge and tolerability of immunotherapy

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Background: Interleukin (IL)-4 and IL-13 play key roles in the pathogenesis of allergic responses, and antagonism of these cytokines may contribute to the beneficial effects of allergy immunotherapy. We hypothesized that the addition of dupilumab, a monoclonal antibody that inhibits IL-4 and IL-13 signaling, to cluster subcutaneous immunotherapy (SCIT) would enhance efficacy in blocking allergic responses and improve tolerability during SCIT updosing as compared with SCIT alone.

Method: We conducted a multicenter, randomized, double-blind, 4-arm study (NCT03558997) in 103 adults with Timothy grass (TG) allergic rhinitis, comparing 16 weeks of SCIT (cluster protocol with 8-week build-up to 4,000 bioequivalent allergy units and 8-week maintenance), subcutaneous dupilumab (300 mg every 2 weeks), SCIT+dupilumab, and placebo. Patients underwent a nasal allergen challenge with TG extract at baseline and at Week 17. The primary endpoint in the study was total nasal symptom score (TNSS) area under the curve for the first hour (AUC 0–1) comparing SCIT+dupilumab with SCIT.

Results: More patients receiving SCIT+dupilumab tolerated SCIT updosing than those receiving SCIT alone. In the SCIT group, 31% of patients terminated treatment due to SCIT-related allergic reactions vs 4% in the SCIT+dupilumab group (*P* = .0238). At Week 17, the pre-specified primary analysis of TNSS AUC 0–1 treatment effect did not demonstrate a differential benefit comparing SCIT+dupilumab vs SCIT. The pre-specified sensitivity analysis demonstrated a placebo-corrected reduction of 24.6% (*P* = .0474) in TNSS AUC 0–1 with SCIT+dupilumab compared with 16.3% reduction with SCIT (*P* = .1871). Assessment of efficacy was limited by the unanticipated number of dropouts in the SCIT group. The SCIT+dupilumab group demonstrated a reduction in SCIT-associated rise in TG-specific (s) IgE compared with the SCIT group (56% median reduction vs 81% median increase; *P* < .0001) and an increase in sIgG4/sIgE (1.72 vs 0.75; *P* < .0001) and sIgG/sIgE (1.11 vs 0.18; *P* < .0001) log ratios.

Conclusion: Dupilumab significantly improved the tolerability of cluster SCIT and demonstrated complete blockade of the rise in sIgE seen with SCIT updosing, resulting in increased sIgG4/sIgE and sIgG/sIgE log ratios. Both groups had improvement in TNSS at Week 17. Future studies are needed to further evaluate whether dupilumab improves upon the efficacy of SCIT.

0612 | Assessment of efficacy of allergen immunotherapy in patients with allergic rhinitis and atopic asthma

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Background: Allergen immunotherapy is one of the main methods of pathogenetic therapy of allergic rhinitis (AR) and atopic asthma (AA), leading to the modification of the disease. Goal was to assess the efficacy of sublingual allergen immunotherapy (SLIT) in patients with AR and AA, and the dynamics of secretion IL4, IL13, IFNγ, IL12, IL10 and TGFβ with SLIT.

Method: Were examined 693 patients with AR and combination of AR and AA from the age of 6 to 43 years. AR was confirmed in 126(49%) children and 161(37%) in adults, combination of AR and AA in 131(51%) children and 275(63%) in adults. The efficacy of SLIT was evaluated using standardized questionnaires and scales recommended by EAACI. Patients with AR used combined symptom and medication score (CSMS), patients with AA and AR used the Control of Allergic Rhinitis and Asthma Test scale (CARAT10). Plasma levels of IL4, IL13, IFNγ, IL12, IL10 and TGFβ (in pg/mL) were measured in 80 patients using ELISA. Statistical analysis was performed using Statistica 10.0. Study supported grant International Scientific Council of Young Scientists KSMU.

Results: The initial CSMS score in children with AR was 4.33[3.8; 4.7; *P* < .01]. After 1 year SLIT, the score was 2 [1; 3.2; *P* < .01]; after 5 years-0[0; 0; *P* < .01]. In adults this indicator initially amounted 2 [1; 3; *P* < .01]. The score decreased to 1[0; 1; *P* < .01] after 1 year SLIT, after 5 years-0 [0; 0; *P* < .01]. The initial score CARAT10 was 20.5[17; 24; *P* < .01] in children with AA and AR, after 1 year SLIT-28[24; 30; *P* < .01], after 5 year-29[29; 30; *P* < .01]. The initial score was 24[21; 26; *P* < .01] in adults with AA and AR, after 1 year-27[24; 28.5; *P* < .01], after 5 years SLIT-30[29; 30; *P* < .01]. Initial levels of serum cytokines were IL4 (0.9 ± 0.4),IL13(8 ± 1.7), IFNγ (0.35 ± 0.1), IL12(8.9 ± 3.5),IL10(3.5 ± 1.8) and TGFβ (89.2 ± 7.3). After the 1st year of SLIT was noted increase in TGFβ to 91.2 ± 18.4 (*P* = .07), which indirectly indicates the activation of Treg and Breg. The IL10 stayed at the same level 4 ± 1.6. We watched a tendency to increase IFNγ to 2.3 ± 0.9 and IL12 - 13 ± 4.5, but significant differences weren't revealed. The levels of IL4 and IL13 (0.5 ± 0.3 and 2.7 ± 1.3, respectively) did not significantly change.

Conclusion: SLIT is highly effective method for treatment of AR and AA in children and adults, too makes to achieve control of disease control and to decrease the volume of therapy. The obtained dynamics of level cytokines indicates an increase functional activity of Treg, Tr1 and Breg, which indicates the reconstruction of immune response.

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Pollution, Aeroallergens and Allergy Risk

0276 | The expression of inflammatory mediators in macrophages, epithelium and dendritic cells after urban particulate matter stimulation in triple co-culture scheme

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Background: Environmental pollution is an important element affecting the public health. Numerous studies have shown the carcinogenic, mutagenic, and genotoxic potential of particulate matter (PM) after metabolic activation. The cross-talk between the external and internal matrix in respiratory tract can occur due to macrophages/dendritic cells (DCs) transepithelial network. The aim of this work was to evaluate the expression of selected mediators of inflammation in epithelial, DCs and macrophages using triple co-culture model after urban particulate matter (UPM) stimulation in control group.

Method: A triple co-culture model containing 1) primary nasal epithelial cells cultured in the air–liquid interface (ALI); 2) macrophages (moMφs monocyte derived macrophages) located on top of the epithelium; 3) the subepithelial moDCs (monocyte derived DCs) was used in the study. *In vitro* models from 6 healthy subjects was stimulated with 100 µg/mL UPM (from Gliwice, Poland) for 24 hours in various culture scheme (mono-, di- and triple-co-culture, the models consist normal as well as previously UPM (200 µg/mL) treated moMφs). The mRNA expression of IL-1β, IL-6, IL-8, MMP7, MMP9 IL-33 and TSLP was measured in epithelial cells as well as in moMφs by real time PCR. The expression of CD83 and CCR3 on DCs (CD45+HLA-DR+CD11c+ cells) was evaluated by flow cytometry.

Results: IL-6 and IL-8 mRNA expression after UPM stimulation was increased in triple co-cultures and epithelial/moDCs co-cultures compared to epithelial cells alone and in triple co-cultures compared to epithelial/moMφs co-cultures. We found that only IL-1β mRNA expression was elevated in moMφs after UPM 24 h stimulation. An increased expression of CD83 (marker of DCs maturation) was observed on moDCs from UPM treated "double stressed" triple co-culture (contained UPM pre-treated moMφs) compared to moDCs monoculture and moDCs from UPM treated "normal" triple co-culture (contained unstimulated moMφs). Interestingly, the expression of CCR3 (receptor associated with eosinophilic and allergic response) on moDCs from UPM stimulated triple co-culture with UPM pre-treated moMφs was higher than in moDCs co-cultivated with ECs (without UPM stimulation).

Conclusion: The inflammatory response of nasal epithelial cells and moDCs to UPM stimulation is affected by macrophages/epithelial/DCs interactions in healthy people.

0400 | Indoor air quality in primary schools is associated with atopic sensitization, airway inflammation and FEV1 reversibility in schoolchildren

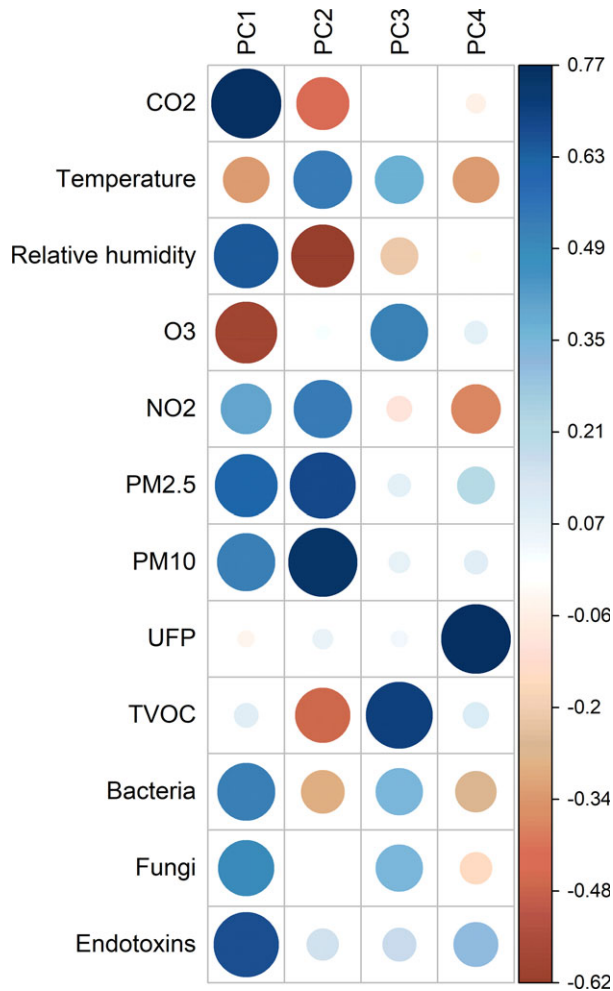Cavaleiro Rufo J[1,2]; Paciência I[1,2]; Farraia M[1,2]; Delgado L[2]; Annesi-Maesano I[3]; Moreira A[1,2]

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Background: Apart from home, primary school classrooms represent environments of relevant and prolonged exposure to indoor air quality (IAQ) in schoolchildren. The aim of this study was to understand how the synergetic interactions of IAQ parameters in primary schools are associated with the prevalence of allergic diseases and asthma in schoolchildren.

Method: IAQ parameters, including temperature, relative humidity, CO_2, ozone, nitric dioxide, PM10, PM2.5, ultrafine particles, volatile organic compounds, and airborne bacteria, fungi and endotoxins, were measured in 71 classrooms distributed through 20 primary schools in the city of Porto, Portugal. A total of 845 children (mean age 9.0 ± 0.8, 429 males) attending the classrooms were included and performed spirometry with bronchodilation, skin-prick tests, pupillometry, exhaled nitric oxide, and had their parents filling a questionnaire about allergy and asthma symptoms, as well as demographic and socioeconomic data. Adjusted mixed models were then performed with school and classroom specified as random-effect variables. To understand the synergistic effect of the IAQ parameters, principal component analysis was used.

Results: Classrooms with higher temperature and humidity were significantly attended by children with a higher exhaled nitric oxide (β [95%CI]= 3.04 [0.90 : 5.18] and 3.52 [0.66 : 6.37], respectively). PM2.5 was associated with significantly higher average and maximum pupil constriction velocity (β [95%CI]= 0.40 [0.14 : 0.67] and 0.40 [0.02 : 0.79], respectively), as well as a significantly higher reversibility in FEV1 (β [95%CI]= 1.53 [0.18 : 2.87]). Total bacteria concentrations in classrooms were inversely associated with FEV1 reversibility (β [95%CI]= -0.74 [-1.34 : -0.15]). There were no significant associations between IAQ parameters and the prevalence of asthma, although a higher risk was observed for endotoxins (OR [95%CI]= 1.37 [0.99 : 1.91]). Regarding the synergistic effect of the parameters, the main component (characterized by high CO_2, relative humidity, endotoxins, bacteria, PM10 and PM2.5) was significantly associated with allergic sensitization (OR [95%CI]= 4.55 [1.39 : 14.85]).

Conclusion: Classrooms with an IAQ profile characterized by lower ventilation profiles and with higher concentrations of particles and gram-negative bacteria were associated with a higher prevalence of allergy in schoolchildren.

phenological observations. Protein composition was analysed by SDS-PAGE and densitometric analyses, while the concentration of Bet v1 was evaluated by fluoroenzymeimmunoassay. The obtained results were estimated at the background of the PM10 level and the birch pollen seasons in Krakow.

Results: SDS-PAGE showed a similar electrophoretic patterns for most of the analyzed samples; however, differences in staining intensities of the individual subunits were observed in all years of the studies. Densitometric analyses showed the great variability among eleven major protein bands, especially in HMW proteins (MW > 70 kDa) (V% around 134.0%). Significant differences in the Bet v1 concentrations were found among the study years (Friedman test; $P = .032$) and in environments of different pollution level (Kołmogorov-Smirnov test; $P < .05$). The highest Bet v1 concentrations were observed in 2018, in spite of that the pollen season intensity was not as high as in 2019, but twice higher as in 2017. Pollen collected in locations with a higher PM10 level contained higher Bet v1 concentration than in the less polluted areas.

Conclusion: The profile and concentration of birch pollen proteins (especially Bet v1 the major birch allergen) depend on year of vegetation and pollution level (PM10). The pollen allergenic potential is determined by the level of pollen exposure and the content of the main allergenic components. Strong variation in protein composition in relation to major allergenic proteins can affect the level of clinical symptoms of allergic people.

0765 | Occasional sources and level of indoor pollution in primary schools of relative low polluted region of Eastern Europe

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Background: Airborne fine particles produce detrimental effects on human health. The growing public health concern caused by respiratory allergies and other chronic non communicable diseases can't be solved without appropriate control of risk factors.

The aim of the study was the evaluation of level and main sources of the indoor aerosol pollution in eleven schools in Vilnius as model of middle size Eastern European city during the period from September 2017 to May 2018.

0756 | Can the birch pollen be more aggressive in the polluted environments?

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Background: Chemical components of air pollution can influence plant pollen in two different ways (i) directly, causing some changes in the surface morphology of pollen, facilitating the penetration of inorganic particles and the release of the natural allergens; (ii) indirectly through the modification of secondary structure of birch pollen allergenic proteins. The aim of the study was to estimate the variability in the protein content and their subunits composition in pollen collected from the birch trees growing in environments differentiated regarding the air pollution levels.

Method: The study was performed in southern Poland in 2017-2019. Male inflorescences were collected from the 20 selected sites (3 trees per each) before pollen release estimated based on the

Method: A Condensation Particle Counter (CPC, TSI model 3007, particle number concentration (PNC) over the full range of sizes (from 0.01 to > 1 μm) and an Optical Particle Sizer (OPS, TSI model 3330, 16 channels of particle number (PNC) and mass concentrations (PMC) in the size range of 0.3-10.0 μm) were used. Measurements were focused on the situation in the classrooms and corridors of the primary schools.

Results: Only in two suburban schools the situation may be considered satisfactory due to minimal indoor aerosol particle concentration. In the other schools, in different seasons, maximum values of the aerosol number and mass concentrations in classrooms varied in the range of 2800-31000 part/cm^3 and of 70-590 μg/m^3, respectively. Canteens were recognised as a main source of indoor air pollution. However, in some cases, occasional sources: construction works, scraping of the exterior walls of buildings near schools and a use of petrol-powered trimmers for cutting the green plantings during lessons may induce enhanced aerosol mass and number concentrations indoors in the range of 1000 - 1600 μg/m3 (OPS) and up to 66400 part/cm3 (CPC), respectively. Our results confirmed that outdoor school sport yard during the games may be important source of indoor air pollution of 0.3-3 μm size range particles.

Conclusion: Even in relatively low polluted region of Eastern Europe there are big differences in the sources and levels of occasional aerosol pollution within one middle size city. Peculiarities of occasional and seasonal pollution should be taken in to account for the elaboration of prophylaxis as well as daily management of chronic non communicable diseases in children.

1058 | Air pollution exposure and allergic rhinitis exacerbation: The role of nasal microbiota and extracellular vesicle communication

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Background: Allergic Rhinitis (AR) is a systemic airway disease involving the entire respiratory tract. Lifestyles and environmental factors (e.g. particulate matter, PM) have a role in disease pathogenesis and recurrence.

The aim of the study is to assess whether the exposure to PM$_{10}$ and PM$_{2,5}$, chosen as paradigmatic environmental stressors, could modify the homeostasis and composition of the nasal microbiota (NM) and extracellular vesicle (EV9 signalling network, showing a role in allergic AR exacerbation.

Method: The microbial community was examined through metabarcoding analysis of the V3-V4 16S rRNA gene regions amplified from upper-airway tracts of 25 AR cases and 25 healthy individual controls to perform α-diversity and taxonomic studies. EV size, concentration and cellular origin for each subject were assessed by nanoparticle tracking analysis (NTA) and flow-cytometry (FC). Information on daily PM$_{10}$ and PM$_{2,5}$ concentrations at the municipality of residence in the 7 days preceding nasal sampling (i.e. Day -1 to Day -7) was assigned to each subject by ArcGIS software. Multivariable and logistic analyses were applied on NM, NTA and FC outcomes.

Results: When relative abundances of each phylum were considered, in controls Actinobacteria (50.8%) was the most represented, followed by Firmicutes (34.7%) and Proteobacteria (12.8%) while in cases Proteobacteria were 38.8%, Actinobacteria were 37.1% and Firmicutes were 23.4%.

Cases showed a higher concentration of all the investigated EV types, derived from platelets (CD61+), activated endothelium (CD62e+), monocytes (CD14+), eosinophils (CD294+), neutrophils (CD177+), mastocytes (CD203c+), epithelial cells (EPCAM+), GRAM+ bacteria (Lipoteichoic Acid+), GRAM- bacteria (LPS+). The effect was greatest in the case of mastocytes EVs which were increased 2.5-fold in cases *vs* controls (*P* < .001). EVs were modified by PM exposure at several time lags. In particular, a negative association between PM10 and eosinophil EVs was observed (beta = −0.016; *P* -value = .017).

As we clustered subjects according to their NM, we observed this variable was a strong effect modifier of the association between PM exposure and EV release.

Conclusion: Our findings start to provide an insight on the effect of air pollution on EVs, taking into account the effect of NM, in patients with AR. Further research is necessary to disentangle the mechanism exerted by inhaled pollutants in modulating EVs and NM, and therefore AR exacerbation.

1120 | Air pollution-induced augmented allergenicity of Platanus hybrida pollen in urban environments

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Background: Air pollution aggravates asthma and respiratory allergies evoking higher incidence and/or symptoms worsening in heavily polluted areas. The relative contribution of an additive pollution-pollen effect and of pollen modifications exerted by the pollutants to the increased prevalence of respiratory allergic diseases remains unclear. The objective of this work was to evaluate the effect of atmospheric pollution in the *P. hybrida* pollen allergenicity.

Method: Pollen was harvested (in 2018 and 2019) from trees growing in the urban area of Évora (38.575099, -7.905096) and a rural area 12 km outside the city, the *Herdade da Mitra* (38.531037, -8.014918). NO_2 was monitored by the ESA Sentinel-5P satellite using the TROPOMI instrument. For pollen production evaluation, in 2019, inflorescences were collected in phase II (5/tree) from 5 trees in each site. Anthers (3) from each inflorescence were homogenized in 70% ethanol; Microscopy slides with the suspensions were prepared and pollen was counted following the standard methodology. Micro BCA Protein Assay Kit was used to determine the protein in pollen extracts. Pla a1 expression was determined by a specific ELISA. Immunoreactive bands were identified by western blot, using sera from sensitized individuals.

Results: Tropospheric NO_2 reached 2.40×10^{-05} $mol.m^{-2}$ in Évora city and 2.35×10^{-5} $mol.m^{-2}$ in Mitra (fig. 1). The average pollen production was similar in both sites, but the dispersion of the values was higher in Évora (Évora: 10,977-39,273 pollen/anther; Mitra: 21,023-25,290 pollen/anther). Pollen harvested in Évora showed 2- to 4-fold lower protein content (see table 1) and ~20% higher Pla a1 content (see table 1). Six IgE-reactive proteins have shown higher intensity in pollen harvested in Évora (MW 73.7 ± 4.6; 47.8 ± 1.5; 29.8 ± 1.1; 26.7 ± 0.4; 24.4 ± 0.9; 17.4 ± 0.6 kDa), three of those corresponding to known allergens Pla a1 (~48 kDa confirmed by Western blotting), Pla TLP (~24 kDa) and PLa a8 (~17 kDa).

Conclusion: These results show that, despite the geographical proximity, a higher concentration of air pollutants is found in urban environments and this is affecting plane pollen production and biochemistry, including the augmented expression of several allergens. It suggests that higher allergenic pollen in urban environments is one cause of the highest prevalence of respiratory allergic diseases.

	Protein content, µg/mg pollen		Pla a 1 content, ng/mg pollen	
	Évora	Mitra	Évora	Mitra
	Évora	Mitra	Évora	Mitra
2018	96	490	103.7 ± 4.2	92.2 ± 2.7
2019	330	702	56.2 ± 5.1	43.5 ± 1.0
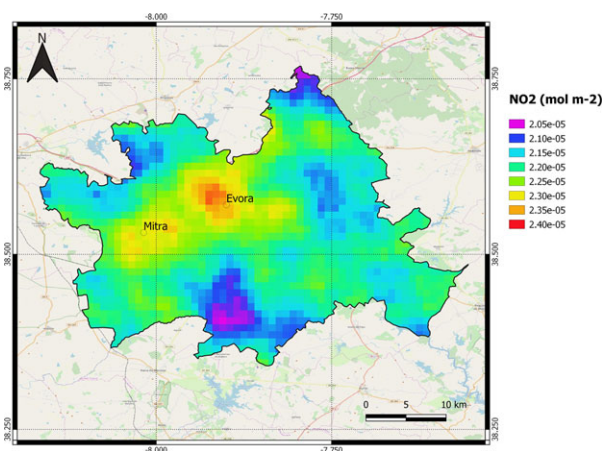
OAS 29
What is New in Urticaria and Angioedema?

0186 | Diagnosis and treatment of urticarial vasculitis: An international survey

Kolkhir P[1,2]; Bonnekoh H[1]; Kocatürk E[3]; Hide M[4]; Metz M[1]; Sanchez Borges M[5]; Krause K[1]; Maurer M[1]

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Background: Urticarial vasculitis (UV) is characterized by long lasting urticarial skin lesions and histological findings of leukocytoclastic vasculitis. We conducted an online survey to examine how UV patients are diagnosed and treated by international specialists and to reveal the challenges in managing UV patients worldwide.

Method: A web-based questionnaire was sent to the members of the World Allergy Organization, the American Academy of Allergy, Asthma & Immunology, the GA^2LEN network of Urticaria Centers of Reference and Excellence (UCARE) and the Russian, Turkish and Japanese societies of Allergology and Immunology and/or Dermatology.

Results: In total, 883 physicians from 92 countries completed the survey. UV was reported to be rare in clinical practice, with an average of five patients per physician per year. More than two thirds of physicians reported wheals, burning of the skin and residual hyperpigmentation in 60-100% of UV patients. The most frequently reported reason for receiving referrals of patients with UV was to establish the diagnosis. The most important features for establishing the diagnosis of UV were wheals of longer than 24 h duration (72%), the results of skin biopsy (63%), and post-inflammatory hyperpigmentation (46%). The most common tests ordered in UV patients were complete blood count, erythrocyte sedimentation rate, C-reactive protein, complement components, antinuclear antibodies and skin biopsy. Physicians considered UV to be of unknown cause in most patients, and drugs and systemic lupus erythematosus to be the most common identifiable causes. Two of three physicians reported to use second-generation antihistamines in standard dose as the first line therapy in UV patients. The greatest perceived challenges in the management of UV were the limited efficacy of drugs and the absence of clinical guidelines and treatment algorithms.

Conclusion: UV is a challenging disease. Skin biopsy, a gold standard for UV diagnosis, is not performed regularly. This may lead to misdiagnosis of UV, e.g. as chronic spontaneous urticaria, and to inadequate treatment. International consensus-based recommendations for the classification of UV and the diagnostic workup and treatment as well as prospective studies evaluating potentially safe and effective drugs for the treatment of UV are necessary.

0610 | Emergency room visits of children and adults with Urticaria - prevalence, characterization and outcome

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Background: Acute and chronic Urticaria with or without angioedema are common medical problems in all ages.

Objectives: To define the prevalence, characterization, treatment and outcome of patients who visit the emergency room (ER) due to Urticaria.

Method: A retrospective study of all ER visits (pediatrics and adults; 123,014 patients) at Kaplan Medical Center between 01/0/7/2015-31/03/2017. Urticaria ER visit was defined when it was the main medical complain/problem. All patients were followed for at least 1 year (mean 2.4 ± 0.6 years) following their ER visit.

Results: 1525 adults (> 16 years of age, 64% females, mean age 44 ± 20 years) and 934 children (45% females, mean age 5.6 ± 4.6 years) were admitted to the ER due to Urticaria during the time of the study (2.5% of all ER visits for adults as well as for children). Co-angioedema was more common in adults with Urticaria (27%) then in children (13%). 87% of the adults and 95% of children had acute (< 6 weeks) Urticaria. In most patients (children-53%, adults- 67%) the cause for the acute Urticaria was not defined. Viral infections (31% in children) and drug hypersensitivity (21% in adults) were the most common defined causes for the Urticaria. ER readmissions for Urticaria were more common in patients with chronic (*vs* acute) Urticaria (54% Vs 13% in adults *P* < .001; 34% Vs 10% in children *P* < .001). More adults, compared with children, were treated in the ER with 2nd generation(non-sedating) H1 antihistamines (68% Vs 0%; *P* < .005), corticosteroids (68% Vs 3.5%; *P* < .005) and H2 blockers (48% Vs 0.5%; *P* < .005). 5.3% of all ER visits for Urticaria resulted in hospital admission (6.5% and 3.5% for adults and children respectively; *P* = .0001) Only 43% of adults and 7% of the children attend allergy clinics within the follow up period. At the end of the study most patients with chronic Urticaria were in complete remission without any treatment (76% in adults; 86% in children)

Conclusion: Acute and chronic Urticaria are major clinical problems leading to ER visits. Better programs are mandatory to improve the treatment and follow-up of those patients.

0635 | Changes in disease activity and treatment patterns in patients with chronic urticaria during pregnancy: Results of the PREG-CU study, a UCARE project
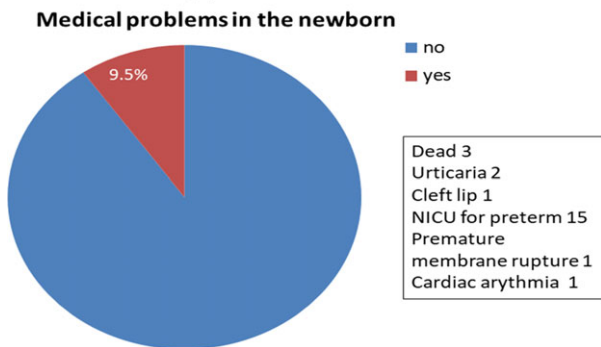
Kocatürk E[1]; Al-Ahmad M[2]; Krause K[3]; Gimenez-Arnau A[4]; Thomsen SF[5]; Conlon N[6]; Marsland A[7]; savk E[8]; Criado R[9]; Danilycheva I[10]; Fomina D[11]; Godse K[12]; Khoshkhui M[13]; Gelincik A[14]; Degirmentepe EN[15]; Ertan S[16]; Ensina LF[17]; Kasperska-Zajac A[18]; Rudenko M[19]; Valle S[20]; Medina I[21]; Bauer A[22]; Zhao Z[23]; Staubach P[24]; Boulliet L[25]; Maurer M[3]

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Background: Chronic urticaria (CU) predominantly affects women, and sex hormones can modulate disease activity in female CU patients. As of now, however, the impact of pregnancy on disease activity and the choice and outcome of treatment in pregnant CU patients are largely unknown. To analyse the impact of pregnancy on the course of CU, and to investigate the use of treatments and their outcomes in pregnant patients with CU.

Method: PREG-CU was a prospective, international, multicenter, observational study of the Urticaria Centers of Reference and Excellence (UCARE) network. Data were collected via a 47-item-questionnaire completed by patients, who became pregnant and had CU, within the last 3 years. We included patients with chronic spontaneous urticaria (CSU), chronic inducible urticaria (CIndU), or both.

Results: A total of 296 pregnancies of 296 CU patients from 13 countries were analysed (mean age: 33.8 ± 6.06 years, duration of CU: 84.7 ± 76.33 months; CSU: n = 192, CSU+CIndU: n = 56, CIndU: n = 37). One hundred ninety-nine (67.2%) and 35 (11.8%) patients were on antihistamine and omalizumab treatment, respectively, before pregnancy. During pregnancy, 64% continued their treatment, while 14% stopped, and others took only on demand. Exacerbations during pregnancy occurred in 43.3% of the patients mostly at the

first (17.4%) and the third (19.7%) trimester. During pregnancy, 172 (60.0%) patients received treatment including; standard dose non-sedating antihistamines (97), high dose antihistamines (15), sedating antihistamines (22), omalizumab (17) and other treatments (21). The frequency of angioedema decreased from 39.2% to 17.1% during pregnancy compared to before pregnancy. Overall, 28.4% of the patients described their urticaria got worsened and 50.8% reported their urticaria got better during pregnancy. From 282 patients, 218 (77.3%) defined their pregnancy as non-problematic while 64(22.7%) defined it as being problematic. Preterm delivery counted for 11.1% of the pregnancies and 9.5% of the newborns had medical problems.

Conclusion: The severity of urticaria seems to diminish during pregnancy and outcomes of the pregnancy is similar to those females in the normal population.

Medical problems in the newborn

9.5%

- no
- yes

Dead 3
Urticaria 2
Cleft lip 1
NICU for preterm 15
Premature membrane rupture 1
Cardiac arythmia 1

0716 | **Ligelizumab achieves high rate of complete response in patients with moderate to severe chronic spontaneous urticaria**

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Background: Ligelizumab, a next generation high-affinity humanised monoclonal anti-IgE antibody, has demonstrated greater control of symptoms of hives, itch and angioedema *vs* omalizumab and placebo in adult patients (pts) with chronic spontaneous urticaria (CSU) up to 20 weeks (Wks) in the core Phase 2b study. Here, we present the efficacy of ligelizumab 72 and 240 mg *vs* omalizumab at Wks 4 and 12 in pts with moderate and severe CSU at baseline (BL).

Method: Data from the Phase 2b trial (NCT02477332), a randomised, double-blind study of ligelizumab (24, 72 or 240 mg every 4 weeks [q4w] or 120 mg single dose) *vs* omalizumab 300 mg q4w or placebo in adult pts with moderate to severe CSU (weekly urticaria activity score [UAS7]≥16), was analysed. UAS7 values were assigned to five score ranges (bands): UAS7 = 28–42, severe activity CSU; UAS7 = 16–27, moderate activity CSU; UAS7 = 7–15, mild activity CSU; UAS7 = 1–6, low activity CSU; UAS7 = 0, urticaria-free. The percentage of pts with BL moderate to severe CSU activity achieving complete control of their symptoms (UAS7 = 0) or low-activity/well-controlled (UAS7 ≤ 6) at Wks 4 and 12 in the ligelizumab (72 mg and 240 mg) and omalizumab 300 mg arms are reported here.

Results: At BL, the distribution of pts in moderate to severe CSU activity was balanced across treatment arms (moderate: 23.8%–37.6%; severe: 58.8%–75%). At Wk 4, 70.0% and 48.1% of pts with moderate CSU at BL and treated with ligelizumab 72 and 240 mg achieved UAS7 ≤ 6, respectively, *vs* 34.4% with omalizumab (Table). Among pts with severe CSU activity at BL, 42.9% and 41.0% achieved UAS7 ≤ 6 with ligelizumab 72 and 240 mg, respectively, *vs* 28.0% with omalizumab.

At Wk 4, 35.0% and 25.9% of pts with moderate CSU at BL and treated with ligelizumab 72 and 240 mg achieved UAS7 = 0, respectively, *vs* 12.5% with omalizumab (Table). Among pts with severe CSU activity at BL, 28.6% and 32.1% achieved UAS7 = 0 with ligelizumab 72 and 240 mg, respectively, *vs* 22.0% with omalizumab.

Conclusion: From Wk 4 onwards, a markedly higher percentage of patients who had moderate disease activity at BL or severe disease activity at BL had a complete response with ligelizumab (72 mg or 120 mg) treatment vs omalizumab (300 mg).

Table. Disease activity at Weeks 4 and 12 in patients with moderate to severe disease at baseline

	Visit	Treatment group	Number of patients at baseline	Disease activity			
				Overall responders (UAS7 ≤ 6)	Free (UAS7 = 0)	Low activity/ Well-controlled (UAS7 = 1-6)	Mild (UAS7 = 7-15)
Patients with moderate disease (UAS7 ≥ 16 to < 28) at baseline	Week 4	Ligelizumab 72 mg q4w (N = 84)	20	70.0%	35.0%	35.0%	20.0%
		Ligelizumab 240 mg q4w (N = 85)	27	48.1%	25.9%	22.2%	25.9%
		Omalizumab 300 mg q4w (N = 85)	32	34.4%	12.5%	21.9%	40.6%
	Week 12	Ligelizumab 72 mg q4w (N = 84)	20	80.0%	60.0%	20.0%	5.0%
		Ligelizumab 240 mg q4w (N = 85)	27	55.5%	40.7%	14.8%	22.2%
		Omalizumab 300 mg q4w (N = 85)	32	59.4%	34.4%	25.0%	12.5%
Patients with severe disease (UAS7 ≥ 28 to 42) at baseline	Week 4	Ligelizumab 72 mg q4w (N = 84)	63	42.9%	28.6%	14.3%	11.1%
		Ligelizumab 240 mg q4w (N = 85)	56	41.0%	32.1%	8.9%	8.9%
		Omalizumab 300 mg q4w (N = 85)	50	28.0%	22.0%	6.0%	16.0%
	Week 12	Ligelizumab 72 mg q4w (N = 84)	63	50.8%	38.1%	12.7%	14.3%
		Ligelizumab 240 mg q4w (N = 85)	56	46.5%	41.1%	5.4%	25.0%
		Omalizumab 300 mg q4w (N = 85)	50	40.0%	20.0%	20.0%	16.0%

q4w, every 4 weeks; UAS7, weekly urticaria activity score.

0758 | A randomized open labeled trial to compare the efficacy of antihistamine dosing-up and add-on treatment with H2-receptor antagonist in patients with chronic urticaria

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Background: The guidelines recommend to increase the dose of H1-antihistmaine up to four-fold when standard dose is not effective in chronic urticarial (CU) patients. However, this decision is not based on concrete evidence. We aimed to compare the efficacy and safety of antihistamine dosing-up, combined 4 H1RAs, change 2 different H1RAs and add-on treatment with H2-receptor antagonist in patients with CU.

Method: A 4-week randomized open labeled trial was conducted in 109 CU patients (age 39.8 ± 12.7 years and 61 (56%) females) whose urticaria were not controlled with standard dose of H1-antihistamines. Urticaria control status, urticaria activity score (UAS), urticaria control test (UCT), CU-specific quality of life (CU-QOL), visual analogue scale (VAS) by patients were compared after 4 weeks of treatment.

Results: The proportion of well-controlled CU was 57.7% in the dose-up group, 46.4% in the combined 4H1RAs, 37.0% in the adding H2RA, 32.1% in the changing 2 different H1RAs (P = .007, linear-by-linear association test). In comparison between groups of 2 H1RAs (adding H2RA and changing H1RAs) and 4H1RAs (combined 4H1RAs and four-fold H1RA), urticaria control status was different (well controlled 34.5% vs 51.9%, uncontrolled 27.3% vs 13.0%, P = .031). Changes from the baselines in UCT, CU-QOL, patients' VAS were not significantly different among 4 study groups, whereas UAS reduced the most in dosing-up group (−5.2 ± 4.9) y adding H2RA (−4.0 ± 4.0), combined 4H1RAs (−2.14 ± 3.9), and changing

2 different H1RAs (2.1 ± 3.7, P = .018). The most common adverse reactions were drowsiness and dry mouth, but no difference among the 4 study groups was observed.

Conclusion: The efficacy of 4-week treatment with dosing-up H1RA in CU patients was the greatest compared with the combined 4H1RA, adding H2RA, and changing 2 different H1RA groups on urticaria control and UAS. Four-fold H1RA in patients with CU was well tolerable as much as standard dose of H1-antihistamines.
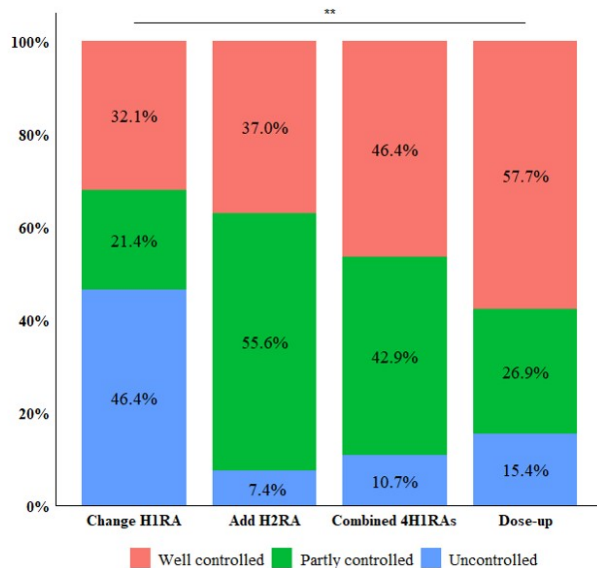
0907 | **Treatment with ligelizumab achieves over forty percent higher complete response rate in chronic spontaneous urticaria patients originally treated with omalizumab**

Maurer M[1]; Giménez-Arnau AM[2]; Soong W[3]; Bernstein JA[4]; Sussman G[5]; Metz M[1]; Lanier B[6]; Hide M[7]; Sitz K[8]; Hua E[9]; Barve A[10]; Severin T[11]; Janocha R[11]

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Background: Ligelizumab, a humanized monoclonal anti-IgE antibody, binds to IgE with stronger affinity than omalizumab as previously demonstrated. Here, we report efficacy and safety of ligelizumab 240 mg up to 1 year in an open-label, single-arm extension study (NCT02649218) in patients originally treated with omalizumab in the core study (NCT02477332) and presented with active disease (7-day Urticaria Activity Score [UAS7] ≥12) in the 16 weeks after omalizumab cessation.

Method: In the 20 weeks core Phase 2b trial, adult patients with moderate to severe CSU (defined by a 7-day Urticaria Activity Score [UAS7] ≥16) were randomised to receive ligelizumab 24, 72 or 240 mg, omalizumab 300 mg, ligelizumab 120 mg (single dose) or placebo every 4 weeks (q4w) for five injections. Following a 16 weeks wash out after last dose, eligible patients (UAS7 ≥ 12) entered a 1-year open-label, single-arm (ligelizumab 240 mg q4w) extension study. The mean absolute change in UAS7 response at Week 12 from baseline as well as the proportions of patients achieving completely-controlled disease UAS7 = 0 for patients treated with omalizumab in the core study were calculated.

Results: From the core study population, 70.6% (226/320) of patients were eligible based on their disease activity score (UAS7 ≥ 12) and willing to enter the extension study, after the washout period (Week 32). Out of all the patients eligible for the extension study, 88.9% (201/226) of these completed 1-year open-label treatment (T_x). The mean absolute change in UAS7 from baseline to Week 12 in patients treated with omalizumab 300 mg in the core study was -17.65 (n = 53) whereas retreatment with ligelizumab 240 mg, showed a -20.88 change (n = 53). The percentage of patients achieving a complete response (UAS7 = 0) at week 12 with omalizumab T_x in the core study was 30.2%, increasing to 43.4% upon retreatment with ligelizumab when assessed again after 12 weeks of Tx. The percentage of patients achieving a complete response (UAS7 = 0) at week 20 with omalizumab T_x in the core study was 32.1%, and 56.6% upon retreatment with ligelizumab when assessed again after 52 weeks of Tx (Figure 1).

Conclusion: CSU patients initially treated with omalizumab experienced upon retreatment with 240 mg ligelizumab over forty percent higher rate of complete responses after 12 weeks of treatment. This improved response was sustained throughout the treatment period.
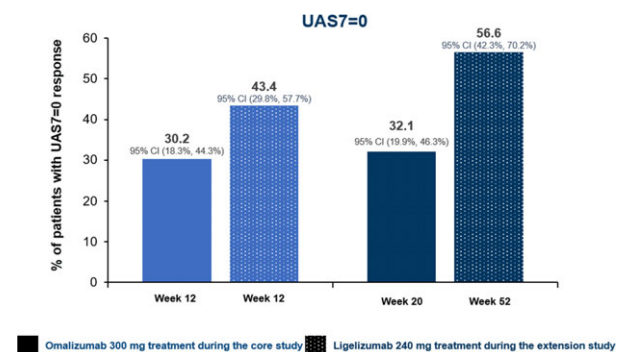
OAS 30
Allergy Management in the 21st Century: Optimisation, Knowledge and Education

1054 | Impact of food allergy on household expenditures among Manitoba families

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Background: As it currently stands, the impact of food allergy on household expenditures is poorly understood. Consequently, the present study aimed to address this gap by estimating the percentage of after-tax dollars spent on food by families with a child with food allergy.

Method: We recruited families with a child attending a follow-up visit at a paediatric allergy clinic in Winnipeg, Canada. Families completed the EcoQ questionnaire, designed to establish food allergy-related family costs, including food (groceries, eating away from home, take away) and medication (over-the-counter [OTC] and prescription). Low-income was defined based on Statistics Canada's Low-Income Measure Threshold, on after tax-dollars. Food cost comparisons were made with Manitoba averages, using data from Statistics Canada.

Results: At present, we have recruited 31 families. Children (10/31; 32.3% female) were, on average, 6.8 ± 4.9 years old. A total of 8 (25.8%) families were low-income. Most children (21/31; 67.7%) had one food allergy, although 4 (12.9%) had 3 or more. The most common food allergy was peanuts/nuts (25/31; 80.6%), followed by egg (9/31; 29.9%) and fish (8/31; 25.8%). In our study population, the average after-tax annual family income was 80,316 Canadian dollars (CAD). Of this, 17.5% was spent on food, amounting to a total annual expenditure of 14,058 CAD. In contrast, Statistics Canada data show the average Manitoban family spends 12% of their income or 8,466 CAD annually on food. Among the study families, food costs did not differ based on the number of food allergies (1 *vs* 1+). The proportion of income spent on groceries was double for low-income compared to middle-income families, but was similar for eating away from home and take away meals. Both OTC and prescription medication costs were comparable, regardless of the number of food allergies. In contrast, low-income families spent less on OTC (275 *vs* 5 CAD) and prescription (525 *vs* 285 CAD) medications, compared to middle-income families.

Conclusion: Manitoba families with a food allergic child spend an additional 5.5% of their annual income on food related expenditures relative to the provincial average. Low-income families with a food allergic child spend disproportionately more on groceries, but much less on medications, compared to middle-income families.

1134 | The importance of education on nasal spray and eye drop technique: Results from two community-pharmacy based studies

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Background: To obtain maximum therapeutic effect while minimizing side effects, it's imperative to adopt correct nasal spray or eye drop technique. In contrast to numerous reports on inhaler technique, information on nasal spray and eye drop technique is limited. Therefore, the aim of this research was to assess nasal spray technique and eye drop technique on a primary care level.

Method: Combined results of two cross-sectional observational studies in community pharmacies in Belgium. Patient inclusion criteria were: being ≥ 18 years, using a nasal spray for persistent rhinitis according to ARIA guidelines (for nasal spray technique) or using eye drops for ≥ 1 month (for eye drop technique). Participants demonstrated their administration technique and completed a self-administered questionnaire. Technique was scored based on a standardized checklist.

Results: The nasal spray technique (NST) was evaluated for 1276 patients (mean age of 49.1 ± 16.7 years) and eye drop technique (EDT) for 678 patients (mean age of 68.9 ± 12.4 years). Although patients perceived the use of a nasal spray or eye drops as not difficult (median difficulty scores 0.4 (on a 0-10 VAS, IQR 1) and 1.0 (0-10 VAS, IQR 3), respectively), only 16.1% of the sample exhibited perfect NST and 3% perfect EDT. For NST, we observed that 25.0% of nasal spray suspensions were not shaken before use, 61.1% of patients did not bend the head forward, and 63.7% aimed the nasal spray towards the nasal septum. Most common deviations in EDT were: touching the bottle to the eye or eyelid (40.7% of patients), and failing to close the eye (67.8%) and perform nasolacrimal occlusion for at least one minute (94.7%) after drop instillation. Notably, 20% of ophthalmic suspension were not shaken before use. About 60% of the sample of nasal spray users recalled having had education in NST compared to 51% of eye drop users recalling education on EDT. Eye drop users mainly received the education from an ophthalmologist (82.0%), while for nasal spray users the education was

most frequently provided by their community pharmacist (50.5%). Both for NST and EDT, education was in more than half of the cases limited to verbal instructions only.

Conclusion: These primary care-based studies showed both suboptimal nasal spray and eye drop technique. A proactive role of community pharmacists in detecting and resolving these problems could be helpful.

1364 | Optimization of allergen immunotherapy care using an integrated eHealth environment

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Background: Allergen immunotherapy (AIT) is an effective therapy for allergic airway disease. However, the treatment period is long and time-consuming. Despite the fact that most patients want their care nearby, general practitioners (GP's) in the Netherlands are reluctant to provide AIT in a primary care setting. Common reasons are lack of experience, fear of anaphylaxis and insufficient reimbursement. Our aim was to improve management of AIT (subcutaneous and sublingual) for allergy patients by developing a care path between GP's and specialists.

Method: This is an observational study looking at feasibility, patient-satisfaction and safety. Seven allergy specialists working in 5 hospitals and 3 GP's formed a taskforce and developed an integrated care path supported by an online platform for data transition between patients, GP's and specialists (www.allergie-netwerk.nl). Assumptions were that the initial phase of AIT was performed in the hospital and the maintenance phase continued in primary care after 3 months. All patients eligible for AIT (according to EAACI guideline: Roberts G, Allergy 2018), who consented with the study protocol and had acceptable language/computer skills were included in the study. Patients were followed for 1 year and received combined allergic rhinitis asthma test (CARAT), rhinitis quality of life questionnaire (RQLQ) and asthma quality of life questionnaire(AQLQ) every 3 months during the study period. Data are expressed as medians (range).

Results: The study started in Sept 2017. So far, 163 patients are included (female 53,4%): 113 patients had subcutaneous immunotherapy (SCIT), 49 sublingual immunotherapy (SLIT) and 1 both. During the initial phase (Q1) 33 patients were lost to follow-up. On

the moment of data-extraction (Jan 2020), 37 patients were actively treated in primary care (SCIT 24, SLIT 13: 28% of per protocol population). Although 106 patients completed 1-year follow-up, only 55 patients (52%) responded to the Q4 questionnaires. The CARAT improved from 19 (Q0) to 25 (Q4); 40% had MCID of > 4 points. The RQLQ went from 2,0 (Q0) to 1,14 (Q4); AQLQ from 5,9 (Q0) to 6,2 (Q4). Efficacy outcomes and side-effects were comparable between primary and secondary care.

Conclusion: Transition of AIT care from hospital to primary care setting is feasible and safe if precautions have been met. However, it is difficult to get GP's involved and to keep patients committed using an online platform.

1376 | Quality of life in mastocytosis scale (QLMS) - the construction of the disease-specific questionnaire

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Background: The quality of life questionnaires are available in Poland but they are designed for general population and don't encompass the specificity of difficulties experienced by people suffering from mastocytosis. The aim of the presented study was to develop a questionnaire measuring the quality of life in patients with mastocytosis, and including the issues and symptoms typical for this group.

Method: The study involved 85 patients (57 women and 28 men) with mastocytosis treated at the Department of Allergology, University Clinical Centre in Gdańsk (Poland). The study was approved by the ethical committee at the Medical University of Gdańsk (approval no. NKBBN/550/2015). Participation in the study was anonymous and voluntary. All patients signed informed consent to participate in the project. The study procedures started in 2015 and were completed in 2019. The following questionnaires were used: HADS-M, EORTC QLQ-C30, STAI, Cantril Ladder, SWLS and an initial version of Quality of Life in Mastocytosis Scale (QLMS).

Results: The initial exploratory factor analysis allocated 45 questions to 11 subscales. Further on, the authors conducted item reduction based on psychometric criterion and qualitative item analysis. Then, the final EFA was performed on 24 questions. The results of KMO sampling adequacy measure and Bartlett's sphericity test were adequate. The analysis revealed that QLMS includes 4 inherently coherent subscales (leisure time, life limitations, professional life, and protective behaviors). The total variance explained by a 4-factor scale is 66.92%.

All the Cronbach's alpha values were very high (from 0.802 to 0.898). The MQLS scores demonstrate a good correlation with the results of HADS, Cantril Ladder, STAI, and SWLS, what confirms the high external construct validity of the questionnaire. The macrocytosis type (systemic and cutaneous) did not differentiate the scores of the scale.

Conclusion: The analyses revealed that QLMS is a reliable and valid tool. It takes into account the specific difficulties experienced by

patients with mastocytosis. QLMS offers a deeper insight in the quality of patient's life, including the difficulties in professional life, everyday life, leisure time, or those associated with protective behaviors. Including psychological and physical difficulties in the diagnostic and therapeutic process may be beneficial for the treatment, and contribute to increasing patients' quality of life.

OAS 31
Epidemiological and Interventional Studies of Food Allergy

0052 | Lifestyle and helminth infection associated with taxonomic and functional shifts in gut microbiota in children with food allergy: The europrevall-INCO study

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Background: The hygiene hypothesis, initially proposed to explain the rising prevalence of atopic disorders, has been related to reduced exposure to farm/rural lifestyle and helminth infection. Recent evidence suggested lifestyle and helminth infection can alter the normal gut microbiota. To establish associations between alternation of gut microbiota and lifestyle as well as helminth infection in children with food allergy (FA) in southern China.

Method: We collected data from 11,473 children, aged 7-12 years, enrolled in the EuroPrevall-INCO study from urban Guangzhou and rural Shaoguan, China. Clinical data, including atopy, total IgE and blood eosinophil, as well as stool and blood samples were collected in a sub-cohort of 751 subjects. Gut microbiota of 130 children with FA and 96 healthy children were analyzed by 16S sequencing, 96 of them were followed up by shotgun metagenomics. We further measured IgG antibodies to five helminths, including *Schistosoma japonicum*, *Toxocara canis*, *Ascaris lumbricoides*, *Trichinella spiralis* and Strongyloides. Multivariate association with linear models (MaAsLin2) was used to compare taxonomic and functional shifts adjusting for age, gender and BMI.

Results: We observed that rural/urban lifestyle, but not FA, could be separated by diversity, including core microbiota and Prevotella-to-Bacteroides ratio, which was confirmed by enterotype analysis showing urban subjects dominated with Bacteroides enterotype (94.0%). Prevotella_9 enterotype enterotype was higher in rural subjects (43.2%, 6.0% in urban) and was found a risk factor for FA in a multivariate logistic model adjusted for lifestyle and helminth

infection. Mollicutes was found increased in subjects with FA after controlling for lifestyle, parasites, total IgE and eosinophil. MaAsLin2 analysis showed functional shift in FA was related to six microbial pathways, i.e. D-fructuronate (PWY-7242) and Butanoate (PWY-5022), which were driven by abundance of *E. coli* and *Megamonas*.

Conclusion: Our study provides the first comprehensive association study of gut microbiota with lifestyle and helminth infection, suggesting diet- and helminth-based treatment may be therapeutically relevant for food allergy through altering gut microbiota.

0991 | Prevalence of food sensitisation and food allergy in children across Europe

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Background: Prevalence estimates of food sensitisation (FS) and food allergy (FA) based on standardised data collection, are essential for international and inter-generational comparisons. The EuroPrevall project has made it possible to establish such estimates

for adults across Europe, but equivalent figures are not yet available for children.

Method: The objective in this study was to determine prevalence of self-reported FA, FS, probable FA (symptoms plus sIgE-sensitisation), and challenge-confirmed FA, and their causative foods, in European school-age children, using data collected during the well-standardised EuroPrevall project. In this multi-centre cross-sectional study, children were recruited from the general population of eight cities across Europe. A total of 16.935 randomly sampled school-age children answered the phase I screening questionnaire, through which self-reported FA was determined. All children with, and a random sample of children without self-reported FA to 24 commonly implicated foods, were invited to phase II, which involved a more extensive questionnaire and serology testing, to establish FS and probable FA. Food challenge was performed in phase III.

Results: Prevalence of self-reported FA was lowest in Athens (6.5%), and highest in Lodz (24.6%). Prevalence of FS ranged from 11.0% in Reykjavik to 28.7% in Zurich. Primary (non-cross-reactive) FS was most prevalent in all centres, but FS due to PR-10 cross-reactivity was also common in Central-Northern Europe. Symptoms and sensitisation coincided as probable FA in 1.9-5.6% of the population in Reykjavik, Athens, Zurich, Utrecht, Vilnius, Madrid and Lodz, in ascending order. Cow's milk, hen's egg, hazelnut, walnut, peanut, lentil, apple, peach, kiwi, banana, carrot, and celery most often caused probable FA in the participating centres. Upon comparison to previously published adult data, prevalence of FS was found to be higher in the current cohort of children, whereas probable FA was equally or more common in the adult cohort.

Conclusion: In school-age children across Europe, prevalence estimates of food sensitisation (FS) and food allergy (FA) demonstrate considerable geographical variation. Animal source FA, plant source FA through primary (non-cross-reactive) FS, and birch pollen-related FA, all occur frequently. Prevalence differences between children and adults suggest a rise in prevalence of FS over time, which may lead to an increase in prevalence of FA in the future.

1002 | Development of IgE-sensitization and symptoms to peanut over time: A 24 year follow up

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Background: IgE-sensitization, presence of Immunoglobulin E antibodies (IgE-ab), to peanut is associated with increased risk for allergic peanut reactions. Longitudinal studies on peanut sensitization and symptoms up to adulthood are sparse. Our aim was to study how IgE sensitization to peanut extracts and peanut allergen molecules develops over time and how this relates to self-reported peanut allergy in adulthood.

Method: BAMSE is a Swedish population-based birth cohort of 4089 children. Questionnaire data covering background factors and peanut allergy symptoms (at baseline, 4, 8, 16 and 24 years) was collected. ImmunoCAP was used to measure IgE to peanut extract in 1237 individuals with blood samples available at 4, 8, 16 and 24 years and IgE to peanut allergen molecules (Ara h 1-3, 8 & 9) in 1605 individuals at 8 and 24 years of age.

Results: The prevalence of peanut extract sensitization, defined as IgE ≥ 0.35 kU_A/L, was 5.4%, 8.0%, 7.5% and 6.2% at 4, 8, 16 and 24 years of age, respectively. Peanut extract IgE-ab levels remained stable over time, but IgE-ab levels to peanut allergen components tended to decrease. Thirty-three participants (20%) developed peanut extract sensitization between 8 and 24 years, although low levels (median 1,4, range 0.7-2.6 kU_A/L), most of them (85%) had a co-existing birch sensitization. Five individuals (7%) developed sensitization to Ara h 2 (≥ 0.1 kU_A/L) between 8 and 24 years of age, of whom three had an IgE-ab level of ≤ 0.12 kU_A/L. Twelve participants (18%) lost their Ara h 2 IgE-sensitization between 8 and 24 years of age, ten of them had initially low Ara h 2 IgE-ab levels (≤2 kU_A/L). Ara h 2 IgE-level

at 8 and 24 years was related to reported symptoms at 24 years: An Ara h 2-IgE of 9.53 and 1.54 kU_A/L at 8 and 24 years, respectively, corresponded to a 95% likelihood of peanut symptoms at 24 years.

Conclusion: Sensitization to peanut extract plateaus after 8 years of age. Development of Ara h2 sensitization rarely emerges after eight years of age. Ara h 2 sensitization is a strong predictor of reported peanut allergy in adulthood.

1497 | Early introduction of peanut, egg, and milk among black and white food-allergic children in the forward study

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Background: Early dietary introduction of certain allergenic foods before 6 months of age may decrease food allergy (FA) incidence. Considering racial differences in US FA prevalence, this study characterizes timing of peanut, egg, and milk introduction among food-allergic Black and White children.

Method: Black and White children (0-12 years old) with a diagnosed FA were enrolled into a prospective, multi-site, cohort study. In the intake survey, parents of children with peanut (n = 182; 68 Black/114 White), egg (n = 136; 99 White/37 Black), and milk (n = 82; 27 Black/55 White) allergies reported timing of dietary introduction of each allergenic food. Age of introduction was categorized into ≤ 6 months, 7-10 months, and ≥ 11 months. Pearson X^2 tests were used to compare timing of food introduction by race.

Results: Only 2.9% of Black children with peanut allergy were introduced to peanut at ≤ 6 months, 8.8% between 7-10 months, and 88.2% at ≥ 11 months, compared to White children (21.9%, 25.4%, and 52.6% respectively) (X^2 = 4.66; P < .001). For milk, 25.9% of Black children were introduced at ≤ 6 months, 18.5% between 7-10 months, and 55.6% at ≥ 11 months, compared to White children (49.1%, 23.6%, and 27.3%) (X^2 = 6.52; P = .04). Finally, 10.8% of Black children were introduced egg at ≤ 6 months, 27.0% between 7-10 months, and 62.2% at ≥ 11 months, compared to White children (25.3%, 32.3%, and 42.4%) (X^2 = 5.07; P = .08).

Conclusion: Peanut, milk, and egg are introduced earlier to White children compared to Black children, which may contribute to racial differences in FA prevalence. Additional education on the NIAID's 2017 Prevention of Peanut Allergy Guidelines may be needed.

1531 | Prenatal egg consumption and infant sensitization to egg and peanut in the CHILD cohort

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Background: Egg and peanut are common food allergens in young children. We examined associations between maternal egg consumption during pregnancy and infant sensitization to egg and peanut at ages 1 and 3 years. We also investigated if the timing of infant introduction to egg and peanut modified these associations.

Method: CHILD participants were recruited from the general population before birth. Number of days per week on which egg was consumed during pregnancy was reported prenatally. Infant diet was reported at birth and every 3-6 months. At ages 1 and 3 years, sensitization to egg and peanut were measured by skin prick testing and atopic dermatitis was diagnosed clinically. Multivariable logistic regression was used to examine associations among frequency of maternal egg consumption during pregnancy, timing of infant dietary egg and peanut introduction, and infant sensitization to egg and peanut.

Results: Among 2912 CHILD participants at 1 year, 7.4% were sensitized to egg and 5.0% were sensitized to peanut; at 3 years, 2.2% were sensitized to egg and 3.8% were sensitized to peanut. Infant sensitization to egg and peanut at 1 and 3 years did not vary depending on frequency of prenatal egg consumption as long as it was less than daily. After evaluating for potential confounding by moderate-severe atopic dermatitis in the first year, timing of breastfeeding, parental food allergy and other atopic conditions, older siblings, sibling food allergies, race, sex, study centre and socioeconomic status, infants of mothers who ate egg at least daily while pregnant (3.8%) were over twice as likely to be sensitized to egg at ages 1 year (adjusted odds ratio [OR] 2.63; 95% confidence interval [CI]: 1.46-4.72) and 3 years (OR 4.19; 95% CI: 1.80-9.76). The associations persisted regardless of timing of infant dietary egg introduction before or after 6, 9 or 12 months. Furthermore, infants of mothers who ate egg at least daily while pregnant were over twice as likely to be sensitized to peanut at ages 1 year (OR 2.48; 95% CI: 1.24-4.99) and 3 years

(OR 2.68; 95% CI: 1.19-6.01). The associations persisted regardless of timing of infant dietary peanut introduction.

Conclusion: Infant sensitization to egg and peanut was more likely with daily maternal consumption of egg prenatally, even after accounting for ages of infant dietary egg and peanut introduction, respectively. Investigations of the mechanisms of these associations are ongoing.

1563 | No cashew allergy in children introduced to cashew by age 1 year

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Background: Evidence that introduction of peanut and egg before 12 months of age reduces the risk of food allergy has led to a paradigm shift in infant feeding advice, with guidelines recommending introduction of allergenic foods such as egg and peanut in the first year of life. However, due to lack of evidence, there was no specific recommendation for tree nut introduction. We aimed to determine whether the introduction of cashew in the first year of life is associated with cashew allergy risk at age 6 years.

Method: A population-based sample of 5276 children was recruited into the HealthNuts study at age 1 year and followed up to age 6 years. At recruitment, parents completed a questionnaire including data on the timing of food introduction in the infant's diet; skin prick testing (SPT) was performed to 4 foods (not cashew) and infants with evidence of sensitisation were invited for oral food challenge (OFC). At age 6, participants completed a comprehensive health assessment which included SPT to 8 foods including cashew. Those with SPT wheal ≥ 1 mm were offered OFC. Cashew allergy at age 6 years was informed by OFC, SPT and questionnaire responses.

Results: Complete data on cashew introduction, relevant confounders were available for 2925 with a cashew allergy outcome and 2539 for cashew sensitisation. By 12 months of age 4.8% (N = 140/2925, 95%CI 4.0%-5.6%) of infants had consumed cashew. The prevalence of cashew sensitisation at age 6 years was 4.8% (95%CI 4.0-5.7) and cashew allergy was 3.4% (95%CI 2.8%-4.1%). No child who ate cashew ≤ 12 months of age developed cashew allergy at age 6 (0%; 95%CI 0%-2.6%), compared to 3.6% (95%CI 2.9%-4.4%) of children who had not consumed cashew by age 12 months. After adjustment for confounding variables, there was weak evidence that introduction of cashew by age 12 months was associated with reduced odds of cashew allergy (aOR 0.19, 95% CI 0.00-1.09; *P* = .07); this association was independent of age of peanut introduction. The magnitude of association was similar for cashew sensitisation but confidence intervals were wider (aOR 0.22; 95% CI, 0.03-1.61; *P* = .14).

Conclusion: We present the first evidence that introduction of cashew in the first year of life is associated with a reduced risk of cashew allergy in a population-based cohort, although confidence intervals were wide and marginally crossed the null. Clinical trials are required to assess how cashew, can be safely and effectively introduced into the infant's diet and its impact on cashew allergy.

OAS 32
Diagnosis and Management of Drug Allergy

0514 | Title: Treating Through in Drug Allergy: What do the allergists think about?

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Background: The standard approach to drug hypersensitivity reactions (HSR) is to replace the agent that causes the reaction with a safe alternative. After a risk-benefit analysis, the treatment regime with a presumed culprit drug could be maintained despite the possibility of a HSR and this approach is termed as 'treating through (TT)'. We aimed to observe the perspective of allergists on this approach.

Method: A 20-question survey, specially prepared for the study, was sent to adult and pediatric allergy fellows and consultants via e-mail.

Results: 180 allergists (81 adult, 69 pediatric) completed the survey. The majority (57%) were seeing at least one or even more patients with the suspicion of drug allergy daily and considering themselves (61%) as sufficiently knowledgeable in regards to drug allergy. TT method hasn't been heard by 39% of pediatric allergists and 22% of adult allergists (*P* = .031). A total of 56 physicians (38%) reported having no experience in TT before, 83% have described it as a risky procedure. The ones who had performed this approach have opted in drug HSR presented with urticaria (57.5%), maculopapular erythema (37.8%), and flushing (27.2%) and they haven't experienced any serious side effects. The majority of physicians prefer not to use this method for cases with nephropathy, high liver enzyme values, diffuse pustulosis and anaphylaxis. Physicians stated that they would never perform a TT with agents such as allopurinol, aspirin and taxanes.

Conclusion: Mild drug HS reactions seem to encourage allergists to perform TT. Awareness about this approach and careful determination of risk-benefit analyze may increase the use of this practical method by allergists in selected cases.

Question	Replies (Percentage)
Main diagnosis and presentation of the case(s) considered for the treating through approach (more than one option could be marked)	Chronic urticaria and NSAID (41%)
	Chronic urticaria and antibiotic (35%)
	Bacterial infection-maculopapular eruption with antibiotic (33%)
	Bacterial infection-angioedema with antibiotic (12%)
	Pneumoniae paracetamol or NSAID (7%)
	Viral infection, paracetamol or NSAID (12%)
	Gastroenteritis (4%)
	Urticaria with COX2 inhibitor (15%)
	Maculopapular eruption with chemotherapeutics (29%)
The outcome of TT approach	Mostly successful (46%)
	Mild adverse effects but successful (37%)
	Moderate adverse effects but successful (16%)
	Severe adverse effects, the treatment has been stopped (0%)
	Severe adverse effects ended up with a desensitization (0%)

NSAID: Nonsteroidal anti-inflammatory drugs.

0623 | Teicoplanin hypersensitivity in peri-operative anaphylaxis

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Background: Teicoplanin is a first generation glycopeptide antibiotic effective against Gram positive bacterial infections including multi-resistant staphylococci and Clostridium difficile infection-associated colitis. Teicoplanin is also often used as second line therapeutic treatment in patients labelled penicillin allergic and first line prophylactic treatment within the peri-operative setting during surgical procedures. Teicoplanin use in the United Kingdom has increased considerably.

Method: We sought to evaluate the clinical characteristics, investigation outcomes and predictors of allergy in a cohort of patients with completed work-up for suspected peri-operative teicoplanin anaphylaxis and immediate hypersensitivity reactions.

Medical records of all patients referred to Guy's and St Thomas' NHS Foundation Trust, over a 5-year period, for peri-operative anaphylaxis where teicoplanin was implicated as one of the index drugs were reviewed.

Results: 73% (30 of 41) of patients investigated were confirmed to have acute-onset teicoplanin hypersensitivity: 6 by DPT and 24 by skin testing.

Patients with reaction onset of ≤ 10 minutes of receiving teicoplanin ($P < .01$) and acute MCT of greater than 14 µg/L or greater than the threshold predicted by the international consensus equation ($P = .014$) were more likely to be confirmed to have immediate teicoplanin hypersensitivity.

There was a NPV for combined SPT and IDT of 65%. 1 in 3 (6 of 17) patients with negative teicoplanin skin tests (SPT and IDT) were positive on teicoplanin DPT.

Of patients confirmed to have acute-onset teicoplanin hypersensitivity, 53% (16 of 30) had a documented penicillin allergy label at the time of teicoplanin administration.

Conclusion: Time to reaction and acute and baseline MCT levels are useful predictors of teicoplanin hypersensitivity. Teicoplanin skin testing has a low NPV. Therefore, DPT is recommended in all SPT and IDT negative patients. Graded teicoplanin DPT was not associated with severe reactions.

0846 | Evaluation of platelet to lymphocyte ratio (PLR) in various types of hypersensitivity to NSAIDs

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Background: Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) is divided into five subtypes with different clinical picture and different mechanisms. Platelet to lymphocyte ratio (PLR) is used in assessment of inflammatory process and a tendency to the microthrombosis. The purpose of the study was to find out if PLR may be useful in discriminating of various types of hypersensitivity to NSAIDs.

Method: The study was based on a retrospective analysis of the data of all patients hospitalized in 2011-2018 and diagnosed with NSAIDs hypersensitivity. Appropriate types of hypersensitivity to NSAIDs were diagnosed based on detailed medical history and drug provocation tests. Exclusion criteria were accompanying disease (acute and chronic inflammatory disease) not related to the primary diagnosis of drug hypersensitivity, that could affect the results of laboratory test. The index was calculated based on absolute number of platelet divided by absolute number of lymphocytes of peripheral blood. The results are presented as median and mid-range and were compared using the Kruskal-Wallis test.

Results: The final analysis covered 98 patients (72 women, 73%), mean age 40.2 years. NERD (NSAIDs exacerbated respiratory disease) was diagnosed in 20 (20.4%) patients, NECD (NSAIDs exacerbated cutaneous disease) in 20 (20.4%), NIUA (NSAIDs inducted urticaria/angioedema) in 38 (38.7%) and SNIUAA (Single NSAIDs inducted urticaria/angioedema or anaphylaxis) in 19 (19.4%) patients.

The groups were comparable in age and gender distribution. The absolute numbers of lymphocytes and platelets were comparable among all studied groups but the significantly lower PLR values were found in NECD group ($P = .0013$) than in the NERD, NIUA and SNIUAA group. In the NECD group PLR was 65.30 (25.5-140.0); NERD 131.58 77.4-250.8; NIUA 129.10 (55.6-234.2) and SNIUAA 122.40 (68.0-237.6).

Conclusion: Lower values of PLR in NECD may indicate the lower tendency to inflammation in this type of NSAIDs hypersensitivity as compared to others. Earlier studies showed significantly higher eosinophil to lymphocyte ratio (ELR) in NERD group and comparable values of neutrophils to lymphocytes ratios (NLR) in all types of hypersensitivity to NSAIDs. Thus these unexpensive, simply markers may be useful in diagnostic work-up of patients sensitive to NSAIDs.

1139 | A cohort study into intravenous drug provocation tests in perioperative allergy

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Background: Perioperative allergic reactions are rare, yet dangerous complications of anesthesia. Currently, skin-testing (ST) is the most prevailing diagnostic in perioperative hypersensitivity reactions. However, STs are known to give false positive and false negative results. Therefore, intravenous (IV) drug provocation-tests (DPTs) may deserve a more prominent place in perioperative allergy diagnostics. The goal of this study was to investigate the value of IV DPT testing in perioperative allergy diagnostics.

Method: We conducted a cohort study of 27 patients, referred to our Dutch Perioperative Allergy Center (DPAC) for assessing the culprit of perioperative allergic reactions from 2016 to 2019. All patients were subjected to a full allergological investigation including STs and DPTs.

The primary outcome measures was the culprit agent and discrepancies between STs and DPTs.

Results: With the aid of DPT, a culprit was identified in 12 cases (44%). In 11 cases (41%) no perioperative used agent could be determined as the culprit or an alternative perioperative diagnosis was deemed more likely, and 4 patients (14%) pulled out from the diagnostic process.

Discrepancies between the outcomes of STs and DPTs are displayed in table 1. In 8/27 (30%) patients, negative STs were followed by a positive DPT, whereas in 4/27 (15%) positive STs were followed by negative DPTs. Hence, over 40% of our patients would have received a false diagnosis based on STs alone.

Conclusion: In our cohort, intravenous DPT proved to be of added value in perioperative drug allergy diagnostics. Solely performing STs for suspected perioperative allergy may lead to patients being re-exposed to the culprit drug or false allergy labels. Intravenous DPTs tests for perioperative allergy are safe, provided that they are performed in a monitored setting with appropriate supervision from an anaesthesiologist.

Table 1: Discrepancies between STs and DPTs

Patient	Per-operative reaction	Agent	SPT*	IDT**	Provocation
Negative skin test, positive/ambiguous provocation test					
Patient 4	Hypersensitivity grade III	Metamizole	Negative	Negative	Positive
Patient 6	Hypersensitivity grade I	Cefazolin	Negative	Negative	Positive
Patient 7	Hypersensitivity grade III	Cefazolin	Negative	Negative	Positive
Patient 18	Other	Rocuronium	Negative	Negative	Ambiguous
Patient 22	Hypersensitivity grade I	Morphine	Negative	Negative	Positive
Patient 25	Hypersensitivity grade III	Metamizole	Negative	Negative	Positive
Patient 26	Anaphylaxis grade III	Morphine	Negative	Negative	Positive
Patient 27	Hypersensitivity grade I	Morphine	Negative	Negative	Positive
Positive/ambiguous skin-test, negative drug-provocation test:					
Patient 9	Anaphylaxis grade III	Sufentanil	Negative	Positive	Negative
Patient 10	Hypersensitivity grade II	Midazolam	Negative	Ambiguous	Negative
Patient 11	Hypersensitivity grade I	Metamizole	Negative	Ambiguous	Negative
Patient 12	Other	Midazolam	Negative	Positive	Negative

*Skin prick tests
**Intradermal tests

1214 | Hypersensitivity reactions to iodinated contrast media: Still a matter of debate

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Background: Iodinated contrast media (ICM) are widely used in medical settings for diagnostic and therapeutic purpose and adverse reactions (ADR) are reported in 1%–3% of diagnostic procedures. Because of their frequent daily use, adverse reactions to represent a relevant issue. Premedication with antihistamines and corticosteroids is still widely used, but its efficacy is still controversial while the possibility for an under-estimation of the risk is a certainty.

Method: We retrospectively included 98 patients who consecutively referred to our Allergology Unit from 2015 to 2018 for adverse reactions to ICM.

An allergologic workup was performed including skin tests and drug provocation tests (DPT) with several ICM. Severity of the reaction was graded using Ring and Messmer scale for immediate reactions (IHR) while delayed one (DHR) were classified in mild, moderate and severe. Drug provocation test (DPT) was performed in single or double sessions, depending on the type of the reaction, using 95 mL of the chosen compound without premedication.

Results: The skin tests resulted positive in 10 out of 98 patients. DPT was performed with culprit or alternative ICM. Eighty-eight patients could tolerate the first DPT; overall, a safe alternative ICM was found in 94 patients out of 98 patients. While it was non possible to identify a safe alternative in 4 patients. No severe ADR were recorded.

Subsequently a telephone interview was conducted to assess if they had been re-exposed to ICM for radiologic procedure. Thirty-nine patients had been re-exposed, without any premedication in 13 cases: 12 of them had tolerated the ICM, while one reacted again despite a negative DPT with the same ICM. Overall, the negative predictive value (NPV) of this protocol was elevated (92.3%) for patients undergoing DPT and subsequent exposure to the same ICM in a real-life setting.

Conclusion: Our proposed diagnostic protocol has proven to be safe and its NPV is high compared to real life re-exposure.

1329 | **Drug provocation test in non-immediate beta lactam hypersensitivity workup: One-day vs prolonged protocol in pediatric patients**

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Background: Drug provocation tests (DPT) are the gold standard to establish a beta lactam (BL) hypersensitivity (HS) diagnosis in non-serious, non-immediate skin reactions. There is no consensus about the DPT protocol to be used.

With our study, we aim to compare outcomes in patients submitted to a single day or a prolonged DPT.

Method: Retrospective analysis of data from children under evaluation for a suspected BL HS reporting non-severe, non-immediate skin reactions.

All performed a DPT with the implicated drug using a single day or a prolonged protocol.

We collected demographic data, clinical history concerning the suspected reaction and the DPT results.

Results: A total of 248 patients were included, 158 (63,7%) did a prolonged scheme of DPT and 90 (36,3%) a 1 day protocol reaching a daily therapeutic dose. Overall only 14 (5,6%) DPT were positive.

From the 90 patients submitted to a single day DPT, 55 were male (61,1%) and the median age at the time of reaction was 3,5 years. 72 (80,9%) reported a maculopapular rash and 14 (15,7%) urticaria. The implicated drug was amoxicillin in 50 (55,6%) cases.

6 (6,7%) DPT were positive: 4 (66,7%) reacted to amoxicillin and 2 (33,3%) to amoxicillin-clavulanic acid. 4 (66,7%) had a maculopapular rash and 2 (33,3%) reacted with urticaria in de the same day as DPT. 158 patients did a prolonged DPT, 86 male (54,4%) and the median age at the time of the reaction was 2,9 years. 126 (79,7%) reported a maculopapular rash and 21 (13,3%) urticaria. The implicated drug was amoxicillin-clavulanic acid in 77 (48,7%) cases.

8 (5%) DPT were positive: The median duration of DPT was 4.7 days (minimum 3 days, maximum 7 days). 5 (62,5%) reacted to amoxicillin and 3 to amoxicillin-clavulanic acid. 7 (87,5%) had a maculopapular rash and 1(12,5%) reacted with urticaria.

There was no significant statistical difference between both groups in gender, age or manifestations of reaction ($P < .05$). The implicated drug was different in both groups ($P = .11$), amoxicillin being the most implicated drug in single day DPT and amoxicillin-clavulanic acid in prolonged DPT. Although there was a superior rate of positive DPT in the single day DPT, there was no statistical difference between protocols ($P < .05$).

Conclusion: Only 5,6% of the patients were diagnosed as having HS. The rate of confirmed reactions was not statistically different between the two used protocols.

A single day protocol seems to have a similar diagnostic sensitivity and avoids unnecessary antibiotic intake.

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Environment, Aeroallergen and Health

0766 | **Phenological phases of pollination and climate change**

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Background: Rising CO2 levels and climate change may be resulting in some shift in the geographical range of certain plant species, as well as in increased rate of photosynthesis. Many plants respond accordingly with increased growth and reproduction and possibly greater pollen yields, that could affect allergic diseases among other things.

The aim of this study is the evolution of aerobiological measurements in France for 25-30 years. This allows to follow the main phenological parameters in connection with the pollination and the ensuing allergy risk.

Method: The RNSA (French Aerobiology Network) has pollen background-traps located in more than 60 towns throughout France. These traps are volumetric Hirst models. The main taxa studied here are birch, grasses and ragweed for more than 25 years over some cities of France.

Results: Concerning birch but also other catkins or buds' trees pollinating in late winter or spring, it can be seen an overall advance of the pollen season start date until 2004 and then a progressive delay, the current date being nearly the same as it was 20 years ago, and an increasing trend in the quantities of pollen emitted.

For grasses and ragweed, we only found a few minor changes in the start date but a longer duration of the pollen season.

As regards the trees, the start date of the new production of catkins or buds is never the 1st of January but depends on the species. For example, it is early July for birch. For breaking dormancy, flowering, and pollinating, the trees and other perennial species need a period of accumulation of cold degrees (Chilling) and later an accumulation of warm degrees (Forcing). With climate change these periods may be shorter or longer depending of the autumn and winter temperature. Therefore, a change in the annual temperature may have a direct effect on the vegetal physiology and hence on pollen release. It may also explain why the quantities of pollen produced are increasing.

The Poaceae reserve very contrasted patterns which make it impossible to identify a general tendency. This is probably due to the great diversity of taxa grouped under the generic term Poaceae, which are clearly not equally sensitive to climate change.

Conclusion: Trees with allergenic pollen blowing late winter or early spring pollinate since 2004 later and produce amounts of pollen constantly increasing. Grasses and ragweed have longer periods

of pollination with either slightly higher or most often lower pollen production.

1079 | Dynamic and Behavior of plane tree pollen and its relationship with Pla a 1 aeroallergen concentration in Évora (Portugal)

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Background: *Platanus* pollen is an important cause of allergy in many cities of Western Europe, where this pollen comes from plane tree, *Platanus orientalis* L. var. *acerifolia* Dyand in Aiton, widely used as an ornamental species in parks, gardens and other urban green areas. The major allergen of *Platanus* pollen is Pla a 1; allergic patients to this pollen type present 60% of IgE and a prevalence of 83-87%. In this paper, we studied this pollen type and the allergen Pla a 1 along the year 2018 (March 20th - April 23rd) in the atmosphere of the city of Évora (Portugal). The aims of this work were to analyze the aerobiological characteristics of the *Platanus* pollen and to study the relationship between the airborne concentration of *Platanus* pollen and the major allergen Pla a 1. Furthermore, we studied closely the influence of meteorological variables on the airborne concentration of this pollen type.

Method: Pollen sampling was carried out using a Hirst volumetric spore trap and for allergen sampling, a high-volume cascade impactor ChemVol was used. In the analysis of the aerobiological samples the procedure established by the International Association for Aerobiology (IAA) was followed and the quantification of the allergen was carried out using the ELISA technique using anti-Pla a 1 antibody. The Main Pollen Season (MPS) was calculated as 95% of total annual pollen, obtained after removing 2.5% of the start and end of total production.

Results: The results obtained in Évora during 2018 indicate that the main pollen season of the *Platanus* pollen took place from March 28th to April 20th. The day of maximum concentration was recorded on April 1st with 619 pollen grains/m^3. There were 16 days of risk of allergy (> 50 pollen grains/m^3) for allergic people of which seven are considered as high-risk level (> 200 pollen grains/m^3). Regarding the relationship between the concentration of airborne pollen and allergen, the results indicate that there is a clear relationship between both (R = 0.632, *P* < .01). Temperature and precipitation are the meteorological variables that most influence the airborne *Platanus* pollen in Évora (year 2018).

Conclusion: The allergenic load (Pla a 1) coincides with the presence and magnitude of the concentration of pollen in the atmosphere. Pollen counts are good indicators and useful to alert the allergic

population because pollen constitute the main allergen carrier although other environmental factors are involved on the dispersion of allergens.

1234 | Allergic symptoms are highly associated between environmental exposure unit exposures and a natural seasonal exposure to birch pollen but the magnitude of the maximum change is different

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Background: Environmental exposure units (EEU)s deliver controlled exposures of inhaled allergens compared with the variable allergen exposure patients experience when participating in field studies. EEU and field studies may be combined into a single "hybrid" trial, where the same anti-allergic therapy is investigated in patients exposed in the EEU as well as in a field for direct comparability in the same subject population.

Method: Objectives: To date, there have been limited data generated from hybrid trials with no consensus regarding optimal trial design. This single-center prospective observational study was conducted to inform study design for future potential hybrid trials evaluating new anti-allergic therapies.

Results: Birch allergic participants (N = 76) were exposed to birch pollen across 3 allergen exposures: (1) EEU priming: out-of-season EEU challenge (2 back-to-back 3 hour EEU sessions on consecutive days), (2) During a natural seasonal exposure, and (3) Natural priming: in-season EEU challenge (one 3 hour exposure, 2 weeks after the start of the birch pollen season). Total nasal symptom score (TNSS), total ocular symptom score (TOSS), total symptom score (TSS=TNSS+TOSS), including other measures, were assessed every 30 minutes during EEU exposures and daily during the natural season. TSS scores were highly associated between EEU priming and natural priming in the EEU (maximal TSS: *r* = 0.79, *P* < .001; area under the curve over 3 hours TSS (AUC): *r* = 0.78, *P* < .001). There was also a good association between maximal TSS during natural season and the natural priming EEU exposure (*r* = 0.73, *P* < .001) and during natural season and the EEU priming (*r* = 0.54, *P* < .001). Subjects had a higher maximum change from baseline TSS during EEU priming (second 3 hour EEU session), *vs* during the first 3 hour EEU session (unprimed) (*P* < .001), *vs* during a natural priming EEU (*P* < .001), *vs* during a natural seasonal exposure (*P* < .001), respectively [TSS max change mean (SD): 12.4 (5.4) *vs* 9.8 (4.5) *vs* 8.5 (4.4) *vs* 7.6 (4.5).

Conclusion: Allergic symptoms are highly associated in patients exposed to birch pollen in an EEU and in the field; however, the magnitude of change in symptom scores is highest with EEU priming in an

EEU. A hybrid trial design may demonstrate clinical efficacy for novel therapies with fewer subjects and with shorter timelines, expediting the availability of new therapies to patients.

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Allergen Immunotherapy: New Decade, New Approaches

0223 | House dust mite allergen-specific immunotherapy using an adjuvant- and allergoid-based approach in a murine model of allergic asthma

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Background: House dust mites (HDM) represent a major perennial allergen source that is linked to allergic asthma and other allergic diseases. Allergen-specific immunotherapy (AIT) is the only disease-modifying and potentially curative approach to treat HDM allergy. Hence, the design of HDM AIT with superior efficacy and minimal side effects is of major interest. In this study we established a murine model of HDM allergy and addressed the effects of the adjuvants microcrystalline tyrosine (MCT) and monophosphoryl lipid A (MPL), both of which are thought to trigger a protective Th1-like immune response, during AIT carried out with HDM allergoids.

Method: C57BL/6 mice were intranasally sensitized with native HDM extract. Next, a physiological HDM AIT mouse model based on HDM allergoids and adjuvants was designed and compared with crude HDM extract-based AIT. Subsequently, allergen-specific immune responses of the differently treated mice were analyzed focusing on lung function, immune cell infiltration, T cell response, cytokine profile, immunoglobulin secretion and lung transcriptome levels.

Results: HDM allergic mice that were intranasally sensitized and challenged with native HDM extract showed a strong Th2 immune response that could be strongly resolved in mice that received AIT with allergoids combined with MCT and MPL as well as with native HDM extract. Anti-CD3/CD28 re-stimulated cells from lungs, nose-draining lymph nodes and spleens demonstrated a significant reduction of Th2/Th17 cytokines in mice treated with HDM allergoids in presence of MCT or MCT + MPL. Further, the Th1- and IgG-inducing potential of the allergoids was enhanced by the addition of both adjuvants to the formulation. As clinical parameter, lung function measurements were performed which showed significant improvements by AIT in comparison to HDM allergic mice. Results of the HDM-specific immunotherapy model, including lung transcriptome

analysis, indicated immunomodulatory properties of the allergoids combined with adjuvants, with a reduction of the HDM allergy driven Th2 immune response and airway inflammation.

Conclusion: In this study we were able to establish a murine model of HDM allergy that uses physiological nasal sensitization and subsequent AIT. MCT and MPL are adjuvants that lower the Th2-inducing properties of HDM extract *in vivo*. Combined with allergoids, these substances have the potential to address unmet needs such as efficacy of HDM AIT.

0595 | Successful CpG/Fel d 1-based immunotherapy reduces Th2 effector and memory cell compartments in a mouse model of allergic asthma

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Background: Adjuvants are useful tools to improve allergen-specific immunotherapy (AIT). Recent evidence shows that CpG oligodeoxynucleotides (CpG-ODN), used at a higher dose than the ones applied in previous clinical AIT trials, can induce immune tolerance. In previous experiments, we confirmed that high dose-CpG-AIT reduced allergic hallmarks such as airway eosinophilia and hyper-responsiveness, as well as Fel d 1-specific IgE in a mouse model of allergic asthma. However, the type 2 T helper (Th2) response was not investigated in detail. It has been shown that the Th2 response can affect the prognosis of allergic disease and relapse after AIT, especially through the Th2 memory compartment. Here we sought to investigate the impact of CpG-AIT on the Th2 response and its implication on the success of CpG-AIT

Method: BALB/c mice were sensitized by three i.p. injections containing a mixture of Fel d 1 and alum. Subsequently, the mice received three courses of CpG-AIT i.p. using a solution of Fel d 1 and CpG-ODN (1 mg CpG/kg body mass) and a final nasal instillation challenge with Fel d 1. Lungs (effector organ) and draining mediastinal lymph nodes (MLN) were analyzed by flow cytometry 18 h after the final challenge. Three groups of animals were analyzed: i) allergic, without CpG-AIT; ii) allergic, CpG-AIT-treated; and iii) non-allergic, untreated control

Results: A clear improvement of airway allergic symptoms was observed upon CpG-AIT with Fel d 1. Among others, the eosinophil ratio in lungs was reduced by three fold. Although MLN T cell and CD4 T cell ratios remained unchanged, the proportion of Th2 cells was lowered in the MLN of CpG-AIT-treated mice by two fold, to a level of non-allergic controls. Moreover, Th2 effector cell (CD62L^{low} CD44^{low}) and Th2 effector memory cell (CD62L^{low} CD44^{hi}) ratios were reduced in the AIT group by three fold

Conclusion: Analysis of the lungs and MLN of CpG-AIT treated mice showed a reduction of the allergic effector cells as well as of the Th2 cells, key players of the allergic immune response. In-depth characterization of the Th2 lineage revealed a reduction in

the Th2 effector and effector memory responses in Fel d 1/CpG-AIT-treated mice, indicating that CpG-AIT not only reduces the allergic effector response, but also weakens the ability of Th2 cells to maintain a long-term response. These results will shed light in further understanding how high dose CpG-AIT modulates the immune system towards immune tolerance by lowering the reservoir of Th2 cells.

0813 | Glycan modification of rPhl p5a leads to superior suppression of allergic inflammation in SCIT mouse model of allergic asthma

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Background: Allergen-specific immunotherapy (AIT) is a treatment for allergic airway disease that depends on regulatory T cell (Tregs) induction by dendritic cells (DCs) to achieve long-term tolerance. We established that linkage of specific sialic acid residues to antigens (sialylation) enhances their binding to immune inhibitory Siglec receptors on DCs. Sialylated (Sia) proteins show increased internalization, processing and loading onto MHC molecules, while instructing a tolerogenic DC phenotype. Here, we test whether AIT using sialylated recombinant Phleum pratense 5a (Sia-rPhl p5a), or sialylated Phl p5a- and Phl p1-derived peptides enhance efficacy of subcutaneous immunotherapy (SCIT) in a grass pollen (GP)-driven mouse model of allergic asthma.

Method: T-cell activation by DCs loaded with unmodified or Sia-Phl p5a peptides was measured *in vitro*. Suppression of allergic inflammation by Sia-rPhl p5a, and peptides derived from Phl p1 and Phl p5a, was compared to that of the unmodified counterparts. To this end, BALB/cByJ mice were GP sensitized, treated with s.c. injections of saline, sialylated or control rPhl p5a, Phl p1 or Phl p5a peptides, or GP extract, followed by intranasal GP challenges. We measured ear swelling responses (ESR), inflammatory cells in bronchoalveolar lavage (BAL) and lung tissue and airway hyperresponsiveness (AHR) to methacholine.

Results: CFSE labeled CD4^{+} T-cells isolated from GP-sensitized mice showed increased proliferation and higher production of TGF-β1 in response to sia-peptide-loaded DCs *in vitro*, as compared to their unsialylated controls. *In vivo*, SCIT using GP and rPhl p5a effectively suppressed AHR and airway inflammation. Remarkably, Sia-rPhl p5a SCIT significantly enhanced suppression of ESR and AHR as well as IL-5 and IL-13 levels and eosinophilia in BALF compared to unmodified rPhl p5a. In addition, sialylation of Phl p5a derived peptides, but not of Phl p1 derived peptides, enhanced suppression of ESR and AHR.

Conclusion: Sialylation of rPhl p5a protein or peptides leads to superior suppression of allergic inflammation while Phl p1 derived peptides were ineffective irrespective of sialylation. AIT with sialylated rPhl p5a and or peptides is safe and inclusion of additional epitopes into an allergen vaccine for clinical use might well result in a further increase of efficacy. Therefore, glycan-allergen conjugates have the potential to be developed as novel improved allergen-specific IT treatment.

1129 | Targeting IL-10-producing regulatory B cells with a novel hypoallergenic depigmented and polymerized phleum pratense candidate for allergen immunotherapy in seasonal allergic rhinitis

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Background: Allergen extracts that have undergone chemical modification (allergoids) are commonly used in allergen immunotherapy (AIT). Tolerogenic B cell responses following AIT are essential in promoting immune tolerance. Immunogenicity models for evaluating IL-10-producing regulatory B cells (Bregs) response are not well established. We hypothesized that depigmented and polymerized allergen extract induces IL-10-producing regulatory B cells (Bregs) with tolerogenic properties.

Method: Peripheral blood mononuclear cells (PBMCs) were collected from 14 grass pollen allergic patients (GPA) and 6 healthy controls (NAC). Cells were stimulated with varying concentrations of native *P. pratense* extract (Phl p), depigmented Phl p and depigmented-polymerized Phl p extract (DPG-POL) for 72 hours. Induction of IL-10 producing Bregs was evaluated by multiparametric flow cytometry. Moreover, an unbiased analysis was performed using viSNE and FlowSOM to generate clusters based on cell surface expression and their abundance (phenotype) profile and identify IL-10^{+} Bregs subsets.

Results: PBMCs stimulated with DPG-POL (1 μg/mL) resulted in increased induction of IL-10^{+}CD19^{+}CD5^{hi} and IL-10^{+}CD19^{+}CD5^{hi}CD38^{hi}CD24^{hi} Bregs subsets (both, $P < .05$) compared to native and depigmented Phl p extracts. This induction was observed in a DPG-POL dose-dependent manner. Moreover, DPG-POL also showed a trend towards induction of IL-10^{+}CD27^{+}CD19^{+} Bregs. The induction of IL-10-producing Bregs by DPG-POL was validated using an unbiased algorithmic clustering analysis using FlowSOM and identified three relevant meta clusters out of ten with increased abundance of IL-10 producing Bregs: Meta cluster 4 (CD19^{+}CD5^{hi}CD24^{+}CD38^{hi}); Meta cluster 7 (CD19^{+}CD5^{hi}CD38^{+}), and Meta cluster 10

(CD19^{+}CD5^{hi}CD24^{+}CD38^{+}). Moreover, the unbiased analyses revealed increased proportions of IL-10^{+}Bregs in NAC compared to GPA in response to CPG (synthetic oligonucleotides containing unmethylated CpG dinucleotides in specific sequence contexts, acting as a Toll-like receptor 9 agonist) stimulation (positive control).

Conclusion: For the first time, we report the advantages of unbiased clustering analysis in conjunction with flow cytometry to identify specific subsets of IL-10 producing Bregs and to evaluate the immunogenicity of DPG-POL Phl p extract.

1148 | Effects of toll-like receptor 7 on dendritic cells and B cells in inducing tolerogenic responses during allergic inflammation: A proof of concept study

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Background: Previous investigations into the actions of Toll-like Receptor 7 (TLR7) agonist GSK2974932 revealed suppression of grass pollen induced allergic responses *in vitro*. The mechanism of action remains unclear, and we hypothesised that TLR7 stimulation of dendritic cells (DCs) alone, can alter the allergic response from memory T_H2 cells, and promote B regulatory cells.

Method: Peripheral Blood Mononuclear Cells were collected from 25 patients with seasonal allergic rhinitis. Pan-DCs or CD19^{+} B cells were enriched and stimulated with dose-ranging concentrations of GSK297 in the presence of 10 μg/mL *Phleum pratense* allergen for 24 or 72 hours respectively. Primed DCs were co-cultured with memory CD4^{+} cells for 7 days. Cytokine production from cultures was determined by ELISAs for IL-10 or IL-5 and IFN-α/IP-10 production through a Luminex MagPix assay. Flow cytometry was used to determine the proportion of IL-4^{+} T_H2 cells and B regulatory cell subsets, while ^{3}H-Thymidine incorporation was used to assess proliferation.

Results: GSK297 significantly induced both IFN-α and IP-10 ($P < .001$) from isolated pan-DCs after 24 hours, with a maximal response obtained at 1 μM, while IL-10 was not induced (n = 9). GSK297 stimulation provided a near two-fold reduction in IL-5 production and proliferation from memory T cells after 7 days, with the highest suppression observed at the maximum IFN-α/IP-10 response. Flow cytometry revealed a significant reduction in IL-4 expression ($P < .05$) within the memory T_H2 compartment (CD4^{+}, CD45RO^{+}, CD27^{-}, CRTH2^{+}). Culture of isolated CD19^{+} B cells with GSK297 revealed a dose-dependent increase in IL-10 production, with a maximum response at 10 μM ($P < .01$) (n = 18). Increases in IL-10 production were also mirrored in the expression of IL-10 within B10 regulatory cells (CD19^{+}, CD24^{hi}, CD27^{+}) ($P < .001$).

Conclusion: For the first time, we have shown the actions of TLR7 activation on DCs alone is sufficient to suppress the allergic response to grass pollen in memory T cells, potentially mediated via IFN-α production from stimulated DCs. Moreover, TLR7 activation promoted IL-10 B regulatory cells that are important in tolerance induction. This study highlights that the role of TLR7 needs to be evaluated in large clinical study in conjunction with AIT.