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## TSANZ Oral Abstracts

TO 001

### CLUSTER ANALYSIS OF INFLAMMATORY BIOMARKER EXPRESSION IN THE INTERNATIONAL SEVERE ASTHMA REGISTRY (ISAR)

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**Introduction/Aim:** Allergy, eosinophilic inflammation, and epithelial dysregulation are implicated in severe asthma pathogenesis. We characterized biomarker expression in adults with severe asthma.

**Methods:** Within the International Severe Asthma Registry (ISAR), we analyzed data from 10 countries in North America, Europe and Asia, with pre-specified thresholds for biomarker positivity (serum IgE ≥75kU/L, blood eosinophils ≥300 cells/uL, and FeNO ≥25 ppb), and with hierarchical cluster analysis using biomarkers as continuous variables.

**Results:** Of 1175 patients; 64% were female, age (mean ±SD) 53±15 years, body mass index (BMI) 30±8, post-bronchodilator $FEV_1$ predicted 74±20%. By pre-specified thresholds, 59% were IgE positive, 57% eosinophil positive, and 58% FeNO positive. There was substantial overlap; 59% were positive for either two or three biomarkers. Five distinct clusters were identified: Cluster 1 (61%, low-to-medium biomarkers) comprised highly symptomatic, older females with elevated BMI and frequent exacerbations; Cluster 2 (18%, elevated eosinophils and FeNO) older females with lower BMI and frequent exacerbations; Cluster 3 (14%, extremely high FeNO) older, highly symptomatic, lower BMI and preserved lung function; Cluster 4 (6%, extremely high IgE) younger, long duration of asthma, elevated BMI, and poor lung function; Cluster 5 (1.2%, extremely high eosinophils) younger males with low BMI, poor lung function, and high burden of sino-nasal disease and polyposis.

**Conclusion:** There is significant overlap of biomarker positivity in severe asthma. Distinct clusters according to biomarker expression exhibit unique clinical characteristics, suggesting the occurrence of discrete patterns of underlying inflammatory pathway activation and providing pathogenic insights relevant to the era of monoclonal biologics.

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TO 002

### INHIBITION OF LC3-ASSOCIATED PHAGOCYTOSIS (LAP) IN COPD AND IN RESPONSE TO CIGARETTE SMOKE

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**Introduction/Aim:** In COPD, we have shown defective macrophage clearance of apoptotic cells by efferocytosis that may lead to secondary necrosis of the uncleared cells and contribute to chronic airway inflammation. The precise mechanisms for this phenomenon remain unknown. LC3-associated phagocytosis (LAP) is a recently described pathway that has been shown to be indispensable for effective efferocytosis. We hypothesized that LAP and its regulators are inhibited in COPD and by exposure to cigarette smoke, potentially contributing to the chronic airways inflammation associated with COPD.

**Methods:** BAL-derived alveolar macrophages, lung tissue macrophages obtained from lung resection surgery, and monocyte-derived macrophages (MDM) were prepared from COPD patients and controls. Lung/airway samples from mice chronically exposed to cigarette smoke were also investigated. Differentiated THP-1 cells were exposed to cigarette smoke extract (CSE). The LAP pathway including Rubicon, as an essential positive regulator of LAP, Atg5, LC3, NOX2, TIM4 and associations with efferocytosis and inflammation (TNF-α) were examined using western blot, RT-PCR, ELISA, flow cytometry and/or immunofluorescence, and the effects of azithromycin and dexamethasone; anti-inflammatory therapies used in COPD, were also assessed. Half-life and possible degradation/ubiquitination of Rubicon upon cigarette smoke exposure was assessed using cyclohexamide chase assay and proteasome inhibitors. Ubiquitination/ immunoprecipitation experiment was used to evaluate the interaction between ubiquitin and Rubicon protein.

**Results:** Rubicon was significantly depleted in alveolar macrophages of COPD patients compared with non-COPD control subjects ($p=0.0286$, $n=4$). Rubicon protein abundance in alveolar macrophages of cigarette smoke-exposed mice ($p=0.0022$, $n=6$) and cigarette smoke-exposed MDM and THP-1 was decreased ($p=0.0022$, $n=6$; $p<0.0001$, $n=6$ respectively), with a concomitant impairment of efferocytosis ($r=0.9633$, $p=0.0020$, $n=6$). When Rubicon protein synthesis was inhibited in THP-1 macrophages using cyclohexamide, our preliminary data showed enhanced degradation in cigarette smoke exposed compared to control and cyclohexamide only treated macrophages. We also noted increased expression of LC3 which is critical for LAP pathway in COPD (*$p=0.0118$, $n=3$) and THP-1 macrophages (**$p=0.0022$; $n=6$). Further, THP-1 macrophages exposed to cigarette smoke extract exhibited higher levels of other key components of LAP pathway including Atg5 (*$p=0.0159$; $n=6$) and TIM-4 (*$p=0.0158$, $n=3$). There was a strong positive correlation between Rubicon protein expression and efferocytosis ($p=0.002$, $r^2=0.9279$, $n=6$). Conversely Rubicon protein expression inversely correlated with levels of TNF-α ($p=0.0142$, $r= -0.9009$, $n=6$). Interestingly,