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Early View

Invited review

ERS international Congress 2023: highlights from the Basic and Translational Sciences Assembly

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ERS international Congress 2023: highlights from the Basic and Translational Sciences Assembly

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Take home message

ERJOR: In case you missed the #ERS2023, this article highlights key messages of the @EuroRespSoc @3Assembly: Basic and Translational Sciences sessions that were discussed in Milan! @SaraOcana1 @EarlyCareerERS @ERSpublications

Abstract

In this article, early career members of the Assembly 3: Basic and Translational Sciences of the European Respiratory Society summarise the key messages discussed during six selected sessions that took place at the ERS congress 2023 in Milan, Italy. Aligned with the theme of the congress, the first topic covered is 'micro- and macro-environments and respiratory health', followed by a summary of the 'Scientific year in review' session. Next, recent advances in experimental methodologies and new technologies were highlighted within the 'tissue modelling and remodelling' session, as well as summary of the translational science session, 'what did you always want to know about omics analyses for clinical practice?', organised as part of the ERS Translational Science Working group's aims. Details on how the next-generation sequencing can be integrated with laboratory methods were provided in the 'lost in translation: new insights into cell-to-cell crosstalk in lung disease' session and a final summary of studies presented in the 'from the transcriptome landscape to innovative preclinical models in lung diseases' session linking the transcriptome landscape with innovative preclinical models was included in this review. The wide range of topics covered in the selected sessions and the high quality of the research discussed highlight the strength of the basic and translational science being presented at the international respiratory conference organised by the ERS.

Abbreviations

Alveolar type 2 (AT2)
Alveolar type 1 (AT1)
Aryl hydrocarbon receptor (AHR)
Birt-Hogg-Dubé syndrome (BHD)
Black carbon (BC)
Bronchoalveolar lavage (BAL)
Charcot-Leyden crystals (CLC)
Chronic obstructive pulmonary disease (COPD)
Cystic fibrosis (CF)
Cystic fibrosis transmembrane conductance regulator (CFTR)

Cytometry by time-of-flight (CyTOF)
Digital spatial profiling (DSP)
European Respiratory Society (ERS)
Fibrotic hypersensitivity pneumonitis (fHP)
Forced expiratory volume in 1 second (FEV_1)
Forced vital capacity (FVC)
Fractional exhaled nitric oxide (FeNO)
Human antigen R (HuR)
Human induced pluripotent stem cell (hiPSC)
Human precision-cut lung slices (hPCLS)
Idiopathic pulmonary fibrosis (IPF)
Interstitial lung diseases (ILDs)
IPSC-derived AT2 (iAT2)
Isthmin-1 (ISM1)
Keratin 17 (KRT17)
Matrix metallopeptidase (MMP)
Multiple Iterative Labeling by Antibody Neodeposition (MILAN)
Mycobacterium tuberculosis (Mtb)
Neutrophil trap (NET)
Particulate matter ≤2.5 µm ($PM_{2.5}$)
Particulate matter ≤10 µm (PM_{10})
Phosphatase and tensin homolog (PTEN)
Post-COVID pulmonary fibrosis (PCPF)
RNA sequencing (RNAseq)
Single-cell RNA sequencing (scRNAseq)
Single-nuclei RNA sequencing (snRNAseq)
Surfactant protein C (SP-C)
Surfactant protein C (SP-C) gene (*SFTPC*)
Terminal airway-enriched secretory cells (TASCs)
Tissue inhibitor of metalloproteinase (TIMP)
Transient receptor potential vanilloid 4 (TRPV4)
Type 2 helper T (Th2) cell
Volatile organic compounds (VOCs)

The European Respiratory Society (ERS) International Congress 2023 took place in a hybrid form, hosting more than 20,000 participants, attending either in person (17,309 registrations) in Milan, Italy or participating online (3,215 registrations). The congress focused on tackling key areas of respiratory medicine: pollution, climate change and sustainable developments. As in previous years [1-3], there were numerous types of sessions including oral presentations, symposia, hot topics, poster presentations and year in review [4]. In this article, early career members of the ERS Assembly 3 [5] summarise some of the most relevant sessions describing the latest state of the art technologies, and sessions giving insights into the future direction of basic and translational respiratory science. Additional content can be accessed on the virtual platform (https://live.ersnet.org/home/ers/ers2023/en-GB).

Micro- and macro-environments and respiratory health – Oral presentation session

The respiratory system is closely linked with the environment. Various elements in the surroundings impact lung function, ranging from endotypes, the microbiome, and the microenvironment, to the macroenvironment, including indoor and outdoor air pollution and green spaces. This also encompasses the utilization of inhaler treatments. These factors interact with one another within a complex network, influencing respiratory health as assessed by spirometry measurements such as forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1), fractional exhaled nitric oxide (FeNO), symptom burden, allergic rhinitis, chronic obstructive pulmonary disease (COPD), and the incidence of asthma (Fig. 1).

In this session, Dr Helena Backman (Luleå, Sweden) showed that COPD endotypes can be identified through different lung function trajectories with unique biomarker profiles. Three trajectories (T1: mean age 65 years, ever-smokers 72%, T2: mean age 58 years, ever-smokers 100%, T3: mean age 71 years, ever-smokers 78%) were described exhibiting combinations of different FEV_1, mean high sensitive C-reactive protein, matrix metallopeptidase (MMP)-9, and MMP-9/tissue inhibitor of metalloproteinase (TIMP)-1 ratio values [6, 7]. Miss Beatrice Cornu Hewitt (Utrecht, Netherlands) presented a study assessing the association between livestock-related emissions, (e.g., bacteria, antimicrobial resistance genes), and the oropharyngeal-acquired resistome (defined as an inherited set of genes used to resist infections) structure in COPD individuals versus healthy individuals. This study showed that the individuals' with COPD airway exhibited a higher resistome diversity, while *E.coli* was associated with significant differences in the oropharyngeal resistome of all individuals in the study [8-11]. Dr Randi Bertelsen (Bergen, Norway) explained the link between the exposure to indoor bacteria and lung function and inflammation in children. More specifically, higher microbial diversity is associated with better lung function (measured by FVC and FEV_1 z-scores) in males and increased inflammation (measured by FeNO) and lower lung function in females [12].

Dr Zhebin Yu (Stockholm, Sweden) next showed an association between air pollution exposure and long COVID symptoms. This study combined an estimation of particulate matter ≤2.5 µm ($PM_{2.5}$), ≤10 µm (PM_{10}), black carbon (BC), and nitrogen oxide levels with the evaluation of long COVID symptoms acquired via questionnaires from 753 participants. Exposure to $PM_{2.5}$ was linked to long COVID, dyspnea, and altered smell/taste [13-15]. Dr Carlos Valencia-Hernandez (London, United Kingdom) presented the association of urban green spaces with lung function in ages from six to sixteen years in three European birth cohorts. Despite the high heterogeneity between the studies, the presence of green environment was linked to a small increase in FEV_1 and FVC values, although analysis of further cohorts is ongoing [16]. Dr Inês Paciência (Oulu, Finland) explained the role of exposure to air pollution as a modifier on the association between access and exposure to green spaces and development of allergic rhinitis. A study including 2568 participants demonstrated the beneficial role of green spaces, which is more important in cases of high air pollution exposure [17]. Miss Rina So (London, United Kingdom) explained the risk of COPD and asthma in relation to air pollution. This study investigated the Danish population and the annual mean levels of $PM_{2.5}$, NO_2, and BC. Higher exposure was related to higher asthma and COPD incidence defined by hospital contact [18-21].

Finally, Dr Joachim Heinrich (Munich, Germany) presented a study on long-term exposure to ambient ozone in 3014 adults from 17 centers in nine countries. Higher exposure was associated with faster lung functional decline estimated by spirometry [22, 23]. Dr Henning Kothe (Hamburg, Germany)

explained the impact of cooking methods on indoor air pollution and lung function in rural Rwanda. Indeed, replacing traditional cooking with improved cookstoves resulted in a reduction in IAP and an improvement in lung function [24]. Finally, Dr Joan B Soriano (Madrid, Spain) showed the estimated economic burden and carbon footprint in metric tons of CO_2 equivalent of the change of inhalers for non-clinical reasons and the consequent lack of adherence to treatment in Spain, producing a great economic and environmental cost [25].

Scientific year in review – Year in Review

In this Scientific year in review session, the speakers summarised the latest advances in translational respiratory science made by labs from across the world over the last year. Dr Rosa Faner (Barcelona, Spain) demonstrated the importance of gene-environment interactions in the pathogenesis of COPD. Being born severely pre-term (<28 weeks gestation age) was associated with a 7-fold increased risk of developing COPD by age 30-50 [26]. This links to another study of preterm children who underwent COPD polygenic risk scoring. Those who had the highest risk scores developed reduced FEV_1 at the age of 5, which shows that COPD-associated genes may play a role in preterm children developing obstructive airways disease [27].

Dr Faner also discussed the role of epigenetics in COPD. A study focussing on ethnically-diverse children living in low-income areas identified a genetic variant that was partly mediated by DNA methylation changes associated with smoking history – this variant was associated with reduced FEV_1 [28]. In addition, studies continue to show the importance of telomere shortening in the development of COPD [29]. This led to an insightful conversation amongst the panellists about the potential to screen for individuals at risk of COPD using telomere length and polygenic risk scores.

Dr Maor Sauler (Connecticut, United States) presented the latest research into alveolar defects in obstructive lung disease. He discussed that pro-inflammatory macrophages are associated with ferroptosis of alveolar type 2 (AT2) epithelial cells in lungs exposed to cigarette smoke [30]. In addition, recent data show that the loss of zinc transporter ZIP8 results in impaired AT2 cell function and subsequent lung fibrosis. Exogenous zinc then renewed the activity of AT2 cells, raising the potential of zinc as a therapeutic target in idiopathic pulmonary fibrosis (IPF) [31]. Previous studies have shown that transfection with specific miR-200 family members (including miR-200c-3p) restored trans-differentiation of AT2 cells obtained from people with IPF to alveolar type 1 (AT1) cells [32]. More recent research showed that this is through down-regulation of the endothelial Flt1 receptor [33]. Flt1 knock-out mice were protected from lung fibrosis upon exposure to bleomycin, and fibrosis was even reversed in these mice [33].

Dr Sauler showed research focusing on small airway disease in COPD. Single-cell RNA sequencing (RNAseq) (scRNAseq) identified a new cell type found in distal airways, termed 'terminal airway-enriched secretory cells' (TASCs), which secretes surfactant. There is loss of TASCs in the distal airways of end-stage COPD individuals, which may contribute to the loss of distal airways seen in COPD [34].

Dr Melanie Königshoff (Pennsylvania, United States) focused on anti-ageing targets in IPF (Fig. 2). Airway basal cells in IPF are reprogrammed to a keratin 17 $(KRT17)^{high}$ and phosphatase and tensin homolog $(PTEN)^{low}$ cell type. These cells contributed to fibrosis development when implanted into mouse lungs, changes that were attenuated by the Src kinase inhibitor saracatinib [35]. Saracatinib,

initially developed as an oncological treatment, reverses several fibrotic pathways – a trial of saracatinib in IPF is ongoing [36, 37].

Dysfunction of the endothelial transcription factor ERG occurs during aging, and was associated with increased systemic inflammation, vascular remodelling and impaired lung fibrosis recovery following bleomycin administration [38]. Lower levels of another endothelial transcription factor, FOXF1, were observed in endothelial cells obtained from people with IPF. FOXF1-deficient endothelial cells were associated with accelerated lung fibrosis and inflammation, and lung delivery of FOXF1 cDNA via nanoparticles attenuated lung fibrosis development in mice treated with bleomycin, showing the potential of this finding as a treatment strategy in IPF [39]. These results emphasise the importance of the lung endothelium in ageing and the pathogenesis of IPF.

Dr Wolfgang Kübler (Berlin, Germany) presented advances in the understanding of tissue barrier dysfunction in pathogen-associated respiratory failure. He described that the matrikine endostatin is increased in the lungs of ARDS individuals, including COVID-19-related ARDS, and this promoted thrombin-induced epithelial barrier dysfunction, and platelet and neutrophil activation [40]. Loss of the endothelial aryl hydrocarbon receptor (AHR) also increased tissue barrier dysfunction and subsequent movement of inflammatory cells into alveoli following influenza infection. A diet rich in AHR ligands (indoles) protected against tissue barrier dysfunction, demonstrating the importance of the gut-lung axis in viral infections [41].

Dr Kübler furthermore described novel targets for pneumonia-related acute lung injury. Cystic fibrosis (CF) transmembrane conductance regulator (CFTR), the membrane channel involved in the pathogenesis of CF, was down-regulated following *Streptococcus pneumoniae* infection. This led to endothelial barrier dysfunction through various mechanisms, including the activation of voltage-gated calcium channels and transient receptor potential vanilloid 4 (TRPV4). The CFTR potentiator ivacaftor reduced endothelial permeability following *Streptococcus pneumoniae* infection [42]. Vasculotide, agonist of the angiopoietin receptor Tie2, reduced lung permeability and acute lung injury when used with ampicillin in mice infected with *Streptococcus pneumoniae* and were mechanically ventilated [43].

This session highlighted the breadth and quality of translational respiratory research over the last year, covering many causes of impaired tissue regeneration and lung function in lung disease (Fig. 2).

Tissue modelling and remodelling – Oral presentation session

Tissue remodeling is a process occurring due to aberrant repair responses to tissue damage, leading to the loss of tissue integrity, disrupted extracellular matrix homeostasis, and replacement with disorganized structural cells [44]. Alongside fibrosis, tissue remodeling is a common feature in many respiratory diseases, such as asthma, COPD, and IPF [45]. In this session, the speakers used various experimental methodologies including murine models, human *ex vivo*/*in vitro* cell culture models, and single cell-omics technologies to model diseased tissues and tease out the mechanisms underlying tissue remodelling.

Murine models: Mutations in surfactant protein C (SP-C) gene (*SFTPC*) in AT2 epithelial cells have been linked to sporadic and familiar IPF and a fibrotic lung phenotype [46, 47]. Using a murine model of lung fibrosis where mutant *Sftpc*^{I73T} (I^{ER}-SP-C^{I73T}) was inducibly expressed, Dr Luis Rodriguez (Philadelphia, United States) showed a role for epithelial metabolic dysfunction in IPF mediated by AT2 glycolytic

reprogramming, mitochondrial dysfunction and altered AMPK signals which could be rescued by Metformin (indirect AMPK agonist). Next, Dr Sabina Janciauskiene Wallmark (Hannover, Germany) showed the beneficial effects of plasma-purified alpha1-antitrypsin therapy in preventing the development of Obliterative bronchiolitis, and attenuating acute rejection in an orthotopic model (Balb/C mice as donors and C57BL/6 as recipients) for lung transplantation [48].

Human *ex vivo*/*in vitro* culture models: fibroblast-derived MMPs have been postulated to be drivers of extensive lung tissue destruction and remodelling during *Mycobacterium tuberculosis* (Mtb) infection [49]. Using primary human lung fibroblasts treated with control or Mtb-infected monocytes, Miss Ramla Cusman (London, United Kingdom) showed that MMP-1 and MMP-3 were elevated in fibroblasts treated with Mtb-infected monocytes, and that inhibiting glycolysis with 2-Deoxy-D-glucose resulted in dose-dependent reduction in MMP-1 and reduction in *TIMP-1* gene expression. These results propose that fibroblast MMP and TIMP-1 secretion are monocyte-dependent and suggest host-directed strategies targeting metabolic pathways may decrease lung fibrosis in tuberculosis. Using nasal epithelial cells obtained from people with severe asthma, Dr Marianne Baastrup Soendergaard (Copenhagen, Denmark) explained that people who are unable to down-titrate anti-IL5 tended to have impaired wound healing (determined by a wound/scratch test), suggesting that epithelial dysfunction could be a marker of incomplete remission on treatment. In this study, complete responders to anti-IL-5 had better results on lung function tests and improved symptoms compared to non-complete responders [50].

This session also included work on human induced pluripotent stem cell (hiPSC)-derived lung cells, such as a study on Birt-Hogg-Dubé syndrome (BHD), a rare autosomal dominant disorder caused by germline mutations in the tumour suppressor gene, *FLCN*, encoding for the protein folliculin [51]. Dr Alejandro Rodriguez Ruiz (Leiden, Netherlands) generated a BHD *in vitro* model by deleting *FLCN* in hiPSCs using CRISPR-Cas9 and differentiating those cells into iPSC-derived AT2 (iAT2) epithelial cells. Together with primary AT2 cells obtained from people with BHD, which were used to validate the *in vitro* model, Dr Rodriguez Ruiz utilized a lung-on-chip model to expose these cells to breathing related-stresses [52]. Additionally, hiPSCs were used by Miss Anja Schweikert (Dublin, Ireland) to generate iAT2 cells to investigate whether estradiol affects development of pulmonary fibrosis in non-diseased organoids. Epidemiological data on disease onset of IPF, as well as data in a bleomycin mouse model, suggest a role for sex hormones in disease pathogenesis [53]. Even though no significant differences were found in AT2 markers or selected proinflammatory or fibrotic genes in response to estradiol, it would be interesting to further investigate the effect of sex hormones in diseased iAT2 and AT2 cells to understand potential sex-specific differences in the disease.

Single cell-omics: Mr Niklas Jonathan Lang (Munich, Germany) explained how he could observe an induction of multi-lineage conserved fibrogenic cell states by 1) coupling *ex vivo* cytokine and drug perturbations of human precision-cut lung slices (hPCLS) with scRNAseq to study early lung fibrogenesis directly in human tissue and 2) comparing the data against an *in vivo* multi-cohort single cell atlas from pulmonary fibrosis individuals. Using micro-CT staged human tissues, he characterized the appearance and interaction of *CTHRC1*$^{+}$ myofibroblasts, *KRT17*$^{+}$/*KRT5*$^{-}$ basaloid epithelial cells, and an ectopic *PLVAP*$^{+}$/*VWA1*$^{+}$ endothelial cell state in the thickened alveolar septum of early-stage pulmonary fibrosis. This supports the use of hPCLS for drug testing and provides a framework for in-tissue perturbational single cell genomics [54]. Utilising a Multiple Iterative Labeling by Antibody

Neodeposition (MILAN) methodology on tissue sections of COPD and IPF explanted lungs, Dr Emanuela Elsa Cortesi (Leuven, Belgium) found five distinct cell clusters (basal, AT1, AT2, intermediate AT2-to-AT1, macrophages) based on 9 phenotypic markers. They also demonstrated increased levels of LGR6 in basal, AT2 cells and intermediate alveolar progenitor populations located in fibrotic regions, and in areas of inflammatory infiltration in COPD and IPF lungs that is associated with increased levels of p21 senescence marker [55]. Next, Mr Quazi Islam (Montreal, Canada) demonstrated a role for human antigen R (HuR) in lung fibroblast differentiation during IPF [56] by analysing TGF-β-treated HuR siRNA knockdown and vector-control-treated normal fibroblasts and IPF-fibroblasts using concomitant RNAseq and mass spectrometry-based proteomics techniques. Lastly, Dr Puja Mehta (London, United Kingdom) provided late-breaking data from single cell transcriptomic and T cell receptor profiles of bronchoalveolar lavage (BAL) cells obtained from people with post-COVID-19 (>3 months from acute disease) who have residual lung abnormalities with predominant 1) inflammatory or 2) fibrotic radiological appearances from a CT scan. Dr Mehta showed that the two participant groups were transcriptionally similar and exhibited clonal expansion and high TCR clustering without enrichment for SARS-CoV-2 reactive sequences, indicating that the purported radiological sub-phenotypes in such groups may well be a different manifestation of the same disease. Therefore, T cell directed therapies might be beneficial for these people regardless of radiological appearance.

What did you always want to know about omics analyses for clinical practice? – Hot topic

Rapid advances in omics technologies have provided us with the tools to dissect biological processes at single cell resolution. Integration of omics data (multi-omics) can reveal clinically important endotypes and phenotypes, with the potential to identify new therapeutic targets.

Dr Martijn Nawijn (Groningen, the Netherlands) explained that use of transcriptomics is key to understanding how cellular activity is related to its genetic information. He focused on the transition from bulk to scRNAseq, which has revolutionised pathogenesis studies by providing in-depth analysis of differences in cell-type composition, activity and (sub)phenotype within complex samples, and information on cell-cell interactions and transitions in cell state [57]. The first study presented using scRNAseq in asthma identified a novel mucous ciliated cell state, and dominance of type 2 helper T (Th2) cell signalling [58]. Further work utilising scRNAseq showed heterogeneity within Th2 cell populations and identified a subset of pathogenic IL-9 expressing Th2 cells that was increased in allergic asthmatic individuals compared to allergic individuals without asthma [59]. Strikingly, post-allergen challenge, Th2 cells were only present in the airways in asthma, and airway epithelial cells demonstrated a dramatically altered transcriptional response in subjects with asthma but not in those with allergy alone [60]. The online resource Human Lung Cell Atlas integrates multiple scRNAseq respiratory system datasets, facilitating disease comparisons at the single cell level with the potential to identify novel targets for intervention [61].

Dr Ian Adcock (London, United Kingdom) discussed the increasing sophistication of proteomic techniques, enabling selective quantification of proteins within a complex sample. Mass cytometry by time-of-flight (CyTOF) utilises heavy-metal isotope-labelled antibodies to detect and quantify multiple proteins in single cells [62]. Thus, CyTOF can identify distinct cell populations, e.g. lung adenocarcinoma-associated immune cells [63] and various immune cell populations in interstitial lung diseases [64]. Proteomic signatures can also be used to identify clinical phenotypes: sputum proteome clusters in asthma represented discrete molecular sub-phenotypes and identified candidate protein

biomarkers [65]. It was emphasized that identification of protein "hits" requires validation over time, and it remains challenging to relate cell subtype to functionality and to demonstrate disease relevance. Using machine learning, nasal fluid protein signatures were mapped to transcriptomic datasets, identifying subsets of severe asthmatics [66]. Further, differentially expressed gene/protein pathway analysis in this study revealed potential novel therapeutic targets.

Digital spatial profiling (DSP) is a complementary technique that adds a crucial layer of information, linking transcriptomics and proteomics to imaging. Dr Francesca Polverino (Houston, United States) described how spatial omics is a cutting-edge tool that allows structural navigation of the lung by digitally selecting regions of interest [67]. Identification of gene and protein enrichments within a specific spatial context using the same input material can predict pathologies associated with specific lung regions. The first DSP study in COPD demonstrated that the immune checkpoint, PD-L1, was spatially clustered with protein markers of activated T cells, as well as genes involved in cancer progression. In bronchioles, PD-L1 expression was associated with functionally active alveolar macrophages and directly correlated with lung function [68].

To fully understand heterogeneity in disease, it is vital to combine multiple omics platforms. Dr Rosa Faner Canet (Barcelona, Spain) illustrated different multi-omic integration approaches to inform clinical medicine. One approach is to use clinical phenotypes to identify underlying biological mechanisms (endotypes): COPD clusters, identified by spirometry and imaging, revealed differential protein and gene expression associated with distinct clinical outcomes [69]. Alternatively, multi-omics can expose mechanistic links that identify clinical phenotypes: integration of the sputum transcriptome, proteome and metabolome with the serum proteome demonstrated that airway microbiota metabolites may mediate COPD pathophysiology [70]. Ultimately, the approach used for multi-level integration depends on the research question, and the selection of platforms may influence the endotype uncovered.

In conclusion, this session highlighted the power of omics to reveal novel disease mechanisms and lead us towards precision medicine. Collaboration is vital, both for robustness and validation by increasing cohort size, and for multi-disciplinary interpretation of outcomes. The challenge is to integrate clinical and multi-omic data longitudinally for therapeutic translation.

Lost in translation: new insights into cell-to-cell crosstalk in lung disease – Oral presentation session

This session showcased how integration of next-generation sequencing with laboratory methods could be used to investigate cell-to-cell crosstalk (Fig. 3). First, Dr Laurens De Sadeleer (Munich, Germany) introduced epithelial-mesenchymal crosstalk. This process is vital in lung regeneration and repair after injury, and of particular interest in IPF [71, 72]. Using single-nuclei RNA sequencing (snRNAseq), laser capture microdissection, spatial transcriptomics and multiplexed immunofluorescence, novel injury-associated profibrotic cell states were successfully identified. Importantly, further analysis revealed niche ligand-specific cell-to-cell interactions distinct between normal and early stages of IPF.

Next, Dr Nahal Mansouri (Lausanne, Switzerland) focussed on the role of basophils in regulating tumours, combining multi-level analyses of scRNAseq with complex laboratory models enabled the exploration of the role of understudied basophils and their interaction with regulatory T cells. Clinically relevant interventions (antihistamines) disturbed the interactions between basophils/Tregs,

promoting tumour progression in mice. This work highlighted a surprising role of cell-to-cell crosstalk that directly impacted the risk of metastasis in humans.

Dr Amanda Oliver (Cambridge, United Kingdom) shared a recent integrated cell atlas of healthy and diseased lungs [73]. The value of this database was demonstrated by combining scRNAseq and spatial transcriptomics to reveal novel circuits of cell communication between epithelial cells and $CD4^+$ T cells. They highlighted increased abundance and activation of resident memory T cells in asthmatics, an important cell type in the lung [74]. This integrated multi-omics approach identified increased interactions of goblet cells with other epithelial cells, and with CD4 T cells, which is mediated via the major histocompatibility complex in people with persistent asthma. This work provides valuable insights into targetable mechanisms behind regulatory networks of T cell activation in asthma.

Dr Jong Huat Tee (Singapore, Singapore) presented valuable insight into the anti-inflammatory role of Isthmin-1 (ISM1) in allergic asthma, whose function has been described for other conditions [75, 76]. A knockout mouse model showed that ISM1 reduces eosinophil number in BAL fluid and reduces adiponectin secretion from AT2 epithelial cells. The multicellular effect of ISM1 deficiency directly correlated with intensified inflammation, necroptosis and airway hyperresponsiveness. This presents ISM1 as a mediator of cellular interaction and a potential therapeutic tool in allergic asthma [77].

Next, Dr Dóra Paróczai (Szeged, Hungary) presented findings on extracellular neutrophil traps (NET) in airway inflammation. Charcot-Leyden crystals (CLC), known to induce neutrophil recruitment and NET formation [78], were demonstrated to have diminished effects in complement protein-depleted mice. Granulocyte-macrophage colony-stimulating factor increased uptake of CLC, increasing NET formation and complement proteins C3 and C5aR1. These findings reveal a novel therapeutic target in people with unresponsive asthma via NET-based anti-inflammatory pathways.

Dr Ken Bracke (Ghent, Belgium) utilised RNAseq to explore cellular crosstalk in COPD using B cells co-cultured with fibroblasts. Together with immunohistochemical co-staining for B cells and stromal cell markers, the localisation of B cells was highlighted to impact COPD's inflammatory and remodelling pathways, building upon previous findings [79]. As such, B cells, lymphoid follicles, and fibroblasts have a dynamics role as critical regulators of COPD [80, 81]. Miss Heloisa Zimermam (Ribeirao Preto, Brazil) explored the complex immune cell communication networks. Dendritic cells have a protective role in tumour microenvironments [82, 83]. The findings of this work show ineffective dendritic cells function in the tumoral front area. This was specific to adenocarcinoma as opposed to squamous cell carcinoma. Therefore, cell interactions are disease subtype-specific, informing therapeutic interventions.

Returning to COPD, Dr Harriet Owles (London, United Kingdom) focussed on IL36γ and its effects on lung macrophages [84]. Supernatants from IL36γ-stimulated small airway fibroblasts were exposed to monocyte-derived macrophages. Here, they show that increased levels of IL36γ impair macrophage phagocytosis in COPD. Notably, IL36γ expression/release is increased by viral infection [85], making this novel cell-cell crosstalk relevant for acute COPD exacerbations [86].

Finally, Dr Caroline Lindo (Lund, Sweden) used surgical lung tissue samples to reveal the relationship between eosinophils, microbes, and immune cell patterns [87]. Combining *in situ* hybridisation, multiplexed immunohistochemistry and spatial analysis enabled an investigation of immune infiltration patterns. No spatial correlation of infiltration with bacteria, viruses or fungi was found. However, spatially distinct cell niches were revealed. Eosinophils and a type 2 inflammatory features

were linked with basophils, indicating a spatial correlation [85]. This patchy pattern of immune cell niches results in a complex mix of inflammatory signatures, which impacts treatment effectiveness [88].

From the transcriptome landscape to innovative preclinical models in lung diseases – Oral presentation session

This session highlighted a variety of state-of-the-art, innovative approaches to explore complex aspects of lung diseases, providing valuable insights into chronic airway diseases, pulmonary fibrosis, post-infection complications, and conditions like COPD that result in skeletal muscle wasting.

Dr Arnaud Bourdin (Montpellier, France) presented a novel model combining the bronchial epithelium and submucosa, both playing an important role in many chronic airway diseases including asthma. The model consists of human bronchial fibroblasts seeded on a collagen-chitosan matrix, iPSC-derived bronchial epithelial cells (forming basal, goblet, club and neuroendocrine cells) [89] and iPSC-derived neurons. To facilitate axonal integration into the existing airway epithelium, Schwann cells were added (previously described to improve nerve regeneration [90]), resulting in an improved innervation of the airway epithelium that can be used to model chronic airway diseases.

Ultra-strong exercise induces physiological responses in the human body. Dr Agnieszeka Smolinska (Maastricht, the Netherlands) described that volatile organic compounds (VOCs), which can be measured in exhaled breath using high-resolution TD-GC-MS, change after running an ultra-marathon. Breath was collected pre- and post-ultra-marathon from 24 healthy participants. Here, 811 VOCs were differentially regulated, with 12 being significantly decreased and 51 significantly increased post-ultra-marathon. Seven of the significantly upregulated compounds after the ultra-marathon suggest physiological responses like fatty acid oxidation, inflammation and altered gut microbiome activity.

Lung explants mostly recapitulate the end stage of IPF, limiting the outcome of these models. Additionally, scRNAseq studies of lung explants are lacking spatial information [91-94]. Dr Aurélien Justet (Caen, France) applied high-resolution spatial transcriptomics to earliest clinical-grade IPF samples to recapitulate the architecture of the human airways. Early disease was characterized by change in cell type proportions (decreased AT1, AT2 cells and general capillaries) and increased COL15 venous and ectopic airway cells, suggesting respiratory unit loss. Thus, spatial transcriptomic analysis allows the investigation of cellular changes of the alveolar niches.

Dr Ana Lilia Serna Valverde (Nottingham, United Kingdom) used a hiPSC-derived model with a *SFTPC* mutation generated from an individual with IPF and CRISPR gene-edited wild-type control [95, 96] to investigate the impact of the *SFTPC* mutation on the iAT2 cell response to infection with Influenza A virus subtype H1N1 [97, 98]. Interestingly, bulk RNAseq revealed, in addition to top genes involved in IPF and infection, that wild-type cells mainly show GO terms associated to the anti-bacterial defence response, whilst the mutant cell line mainly displayed GO terms associated with the cells' reaction to its environment. This model demonstrates the potential of gene-edited iAT2s as *in vitro* platforms for human respiratory infection modelling.

Dr Yuki Yamamoto (Kyoto, Japan) presented an IPF model composed of healthy iPSC-derived alveolar organoids [99] co-cultured with lung fibroblasts. scRNAseq showed that this model after treatment with bleomycin recapitulated key mechanisms of fibrosis sharing 76.3% of upregulated pathways with

IPF human-derived lung samples. Treating these fibrotic organoids with HL001, a LPA1 antagonist [100], showed a restorative effect with decrease of fibrosis, and increase of AT2 cell marker. Consistent with a previous report [101], murine and human organoid models proved the effectiveness of HL001 in IPF.

By combining hPCLS generated from lung tissue from IPF donors with snRNAseq Dr Martin Decaris (San Francisco, United States) investigated bexotegrast, a dual αVβ6/ αVβ1 integrin inhibitor in the fibrotic lung models. He showed that bexotegrast reduces ECM-related gene expression in fibroblasts, attenuates CTHRC1$^+$ pro-fibrotic fibroblast subpopulation and reduces fibrogenic gene expression pathways in aberrant basaloid cells [102].

Lung tissue biopsies may aid the diagnosis of fibrotic interstitial lung diseases (ILDs); however, less invasive alternatives are needed. Dr Avraham Unterman (Tel Aviv, Israel) used scRNAseq to investigate novel biomarkers in BAL [103, 104] by characterizing differences in BAL composition between fibrotic hypersensitivity pneumonitis (fHP) and IPF. They found that the proportions of non-FABP4$^+$ macrophages, regulatory T cells and CLEC9A$^+$ dendritic cells are significantly increased in fHP vs IPF. In addition, fHP macrophages showed a pro-inflammatory activation pattern. These findings may help to differentiate IPF from fHP without the need of invasive techniques.

Mr Mohammad Shadab Ali (New Delhi, India), delved into the molecular underpinnings of the severe post-infection complication known as post-COVID pulmonary fibrosis (PCPF) [105]. This was achieved by comparing BAL samples obtained from people with PCPF with those obtained from non-ILD individuals. Analyses of KEGG and IPA pathways unveiled the involvement of pathways associated with the nervous system in PCPF, and identified key regulators play a crucial role in the cytoskeleton organization. The insights gained from this molecular investigation enhance our comprehension of PCPF and present potential therapeutic targets.

Next, Dr Pauline Henrot (Pessac, France) explored the involvement of CXCR4$^+$ cells [106] in skeletal muscle wasting among people with COPD [107]. Using an early COPD mouse model with a CXCR4 deletion, the study found that this deletion prevented a decrease in muscle endurance and the loss of oxidative myofibers. Dr Henrot intends to employ snRNAseq to further analyze the inflammatory infiltrate and dysregulated pathways.

Collectively, this session showcased innovative approaches in utilising transcriptomics (Table 1) to advance our understanding of disease mechanisms and identify potential drug targets. This emphasizes the significance of continued research in this field.

Concluding remarks

The selected sessions summarised in this review article showcased the diversity in basic and translational respiratory science and the remarkable progress presented at this year ERS congress. The studies delved into the intricate interplay of micro- and macro-environmental factors impacting respiratory health, emphasizing the urgency for comprehensive strategies addressing both environmental influences and individual behaviours. They illuminated the transformative potential of omics technologies, revealing cellular states and interactions that were previously unseen and paving the way for precision medicine. The exploration of cell-to-cell crosstalk provided deep insights into the complex networks underlying lung diseases, offering promising avenues for targeted interventions.

Additionally, innovative preclinical models and advanced molecular analyses unveiled novel aspects of various lung conditions, laying the groundwork for future research and therapeutic development. The topics discussed at the ERS congress 2023 collectively underscored the collaborative efforts and interdisciplinary approaches driving the advancements in respiratory science, offering hope for improved treatments and a healthier respiratory future for people worldwide.

Table 1: Summary of the presented innovative approaches to model lung diseases presented as part of the 'From the transcriptome landscape to innovative preclinical models in lung diseases' oral presentation session.

Models used	**Transbronchial cryobiopsy**	**hPCLS**	**BAL**	**Exhaled breath**	**iPSC-derived models**	**Mouse model**
Diseases/conditions investigated	IPF	IPF	IPF, fHP, hPCLS	Ultra-strong exercise	Asthma, IPF	COPD
Purpose	Investigate cellular changes of the alveolar niche in IPF [108]	Test drug bexotegrast [109]	Investigate biomarkers to characterize differences between fHP and IPF [110], Investigate molecular basis of PCPF [111]	Investigate physiological response [112]	Create novel innervated airway epithelium model [113], model respiratory infection with H1N1 [98], test drug HL001 [114]	Investigate role of CXCR4 in skeletal muscle wasting [115]
Read-out	High resolution spatial transcriptomics	snRNAseq	scRNAseq	High resolution TD-GC-MS	Bulk RNAseq and scRNAseq	Functional tests and whole tissue proteomics

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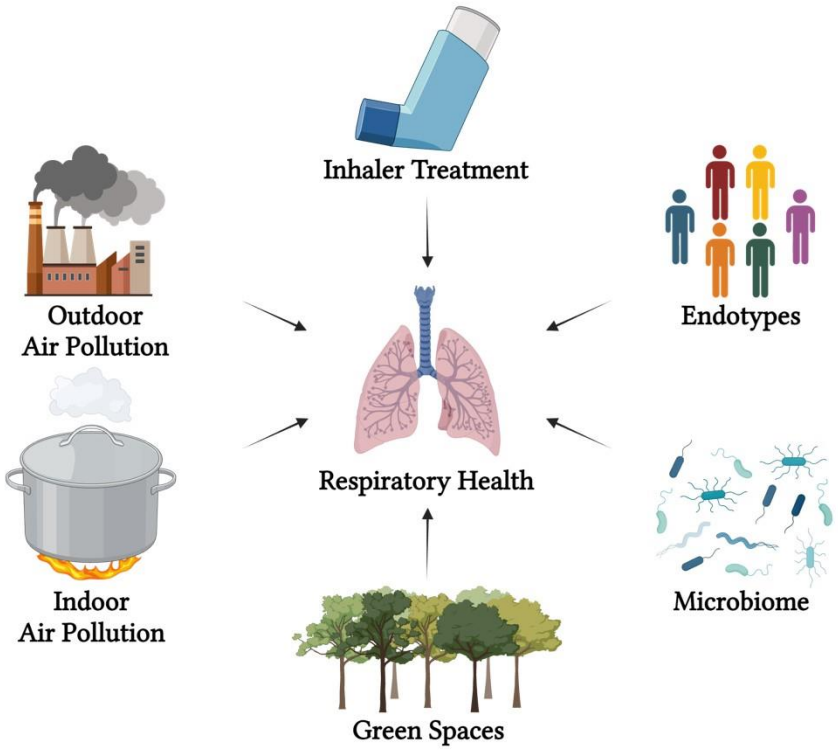

Figure 1. Schematic of the discussed environmental factors that affect lung function, including the microenvironment level of pathophysiological changes (endotypes), and microbiome, the macroenvironment level of indoor and outdoor air pollution and green spaces, as well as the use of inhaler treatment. Created with BioRender.com.

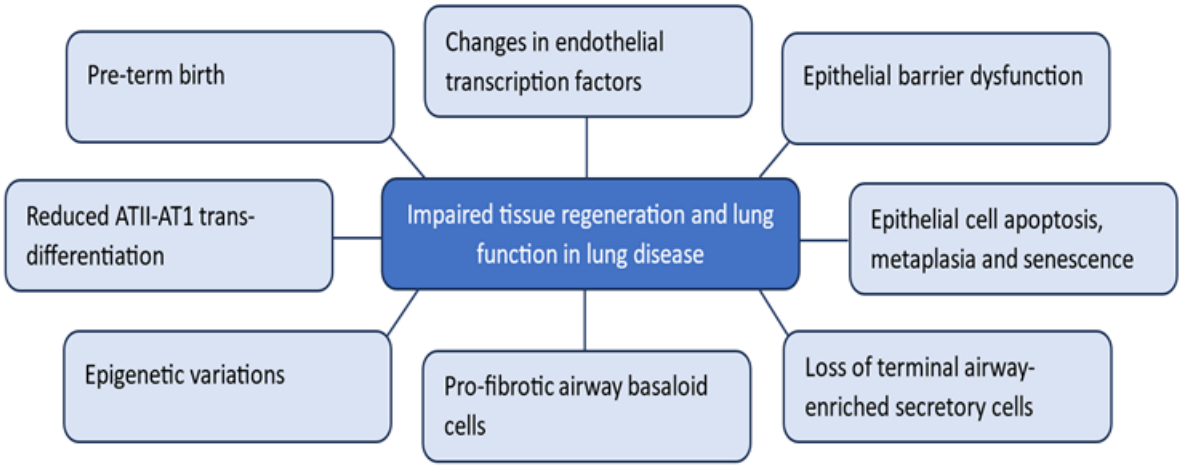

Figure 2. Causes of impaired tissue regeneration and lung function in lung disease.

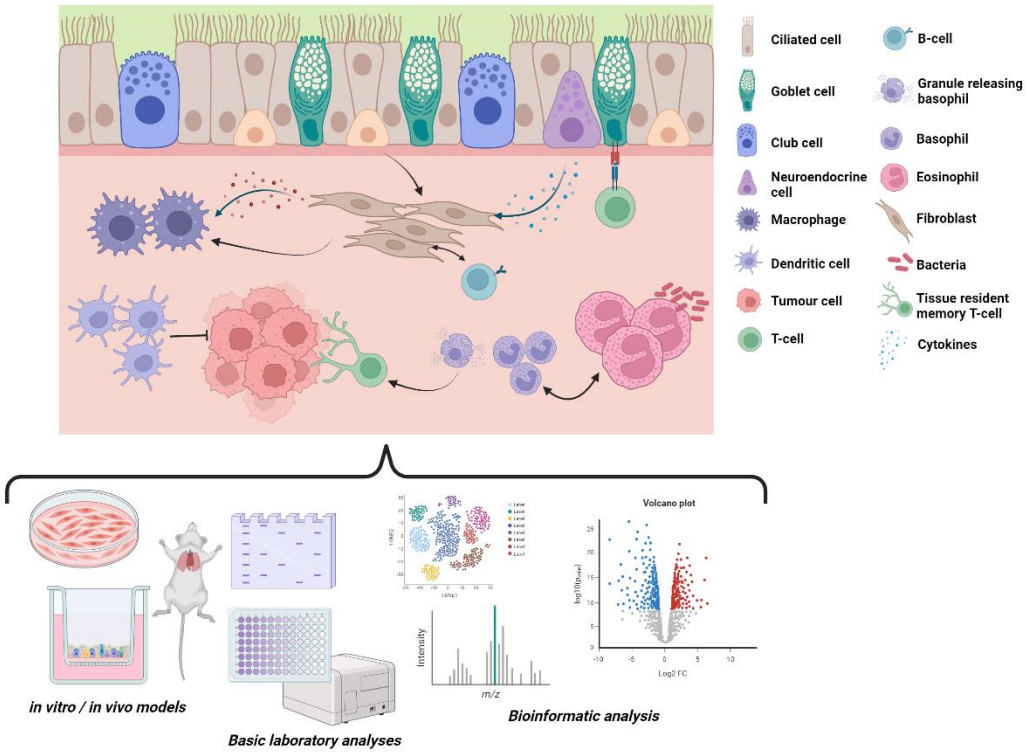

Figure 3. Cell crosstalk in the airways is highly complex, thus, there is a need to integrate basic laboratory techniques, multi-omics and bioinformatic analyses to holistically understand these interactions. Created with BioRender.com.