Manuscripts during doctoral studies (First author manuscripts highlighted in red):

Included in thesis:

Paper I: Wagner DE, **Alsafadi HN,** Lehmann M, Korfei M, Mutze K, Stein M, Costa R, Skronska-Wasek W, Forty E, Eley E, Klee S, Ota C, Schiller H, Günther A, Chambers A, Kaminski N, Königshoff M. **A Deranged Hippo-YAP/TAZ-LOX axis in distal epithelial cells modifies the ECM niche in IPF.** (In preparation)

Summary:

Idiopathic pulmonary fibrosis (IPF) is a fatal chronic lung disease effecting around 25 in 100,000 persons and is predicted to rise. IPF is characterized by excessive extracellular matrix (ECM) deposition in the lung accompanied by progressive loss of lung function. At present, there is no cure and median survival following diagnosis is between 2-3 years, worse than many malignancies. The cause of IPF remains unknown, but epithelial injury, reprogramming and subsequent deranged repair has recently gained traction as playing a prominent pathological role. In particular, deranged activity of developmental pathways is emerging as a potential mechanism driving the development of lung fibrosis. Recently, the nuclear effectors of the Hippo pathway and known mechano-transducers, YAP/TAZ have been shown to be aberrantly activated in IPF. However, the cause and effect of their dysregulation as well as the therapeutic potential of targeting YAP/TAZ remains unexplored. Uncovering the role of YAP/TAZ in the onset and progression of IPF and the identification of mechanisms which regulate their activity may open new possibilities for designing new therapies for IPF. In this study, we determine the mechanisms of epithelial Yap/Taz derangement in fibrosis and their effect on the deposition of the extracellular matrix through activation of lysyl oxidase, an enzyme known to crosslink various collagens and other ECM components.

Role in the paper:

- Bioinformatics analysis of microarray data.
- In vitro experiments on primary alveolar type II experiments.
- Developed single cell RNAseq analysis pipelines.
- Developed an in vitro assay to measure cross-linking of collagen I in order to identify the role of epithelial lysyl oxidase (Lox) in the various set ups.
- Contributed to writing and Figure preparation.

Paper II: **Alsafadi HN,** Margareta Mittendorfer, Deniz Bolukbas, John Stegmayr, Iran Silva, Martina De Santis, Victoria Ptasinski, Lynne Murray, Wagner DE. **Simultaneous isolation of murine proximal and distal lung epithelial cells.** In preparation. (Manuscript attached as **APPENDEX A**).

Summary: The lung epithelium consists of several cell-types with functions varying based on their location and is the major site of injury for several chronic lung disease. Proximal (tracheal/bronchial) cells populate air passage and modulate inflammation while distal (alveolar) cells facilitate gas exchange. Cell isolation methods are heavily utilized to study these cell populations in vitro. However, Cell-type-specific surface markers that distinguish epithelial progenitors are lacking making sorting methods obsolete. Thus, a robust isolation method is needed to distinguish between these cells. We developed a 3D-printed lobe divider (3DLD) to simultaneously isolate proximal and distal epithelial cells from the same murine lung. We evaluated isolated cells by their capacity to differentiate. Both proximal and distal cells gave rise to differentiated airway and alveolar epithelia respectively as evident by organoid formation assays and air-liquid-interface (ALI) cultures with colony formation efficiency consistent with literature. Interestingly, this method yielded

distal alveolar organoids with smaller size distribution than previous methods and increased colony formation efficiency. We provide an inexpensive reproducible method for isolating proximal and distal progenitors from individual mice. This allows for direct examination of disease specific effects on the different epithelial compartments while reducing experimental animal numbers.

Role in the paper: Lead author of the project. Design of experiments, performing experiments, data collection, data analysis, data interpretation, manuscript writing.

Paper III: Gercken M, **Alsafadi HN,** Wagner DE, Lindner M, Burgstaller G, Königshoff M. **Generation of human lung tissue slices for disease modeling**. JoVE (2019). DOI: doi:10.3791/58437

Summary: Translation of novel discoveries to human disease is limited by the availability of human tissue-based models of disease. Precision-cut lung slices (PCLS) used as 3D lung tissue cultures (3D-LTCs) represent an elegant and biologically highly relevant 3D cell culture model, which highly resemble in situ tissue due to their complexity, biomechanics and molecular composition. Tissue slicing is widely applied in various animal models. 3D-LTCs derived from human PCLS can be used to analyze responses to novel drugs, which might further help to better understand the mechanisms and functional effects of drugs in human tissue. The preparation of PCLS from surgically resected lung tissue samples of patients, who experienced lung lobectomy, increases the accessibility of diseased and peritumoral tissue. Here, we describe a detailed protocol for the generation of human PCLS from surgically resected soft-elastic patient lung tissue. Agarose was introduced into the bronchoalveolar space of the resectates, thus preserving lung structure and increasing the tissue's stiffness, which is crucial for subsequent slicing. 500 μm thick slices were prepared from the tissue block with a vibratome. Biopsy punches taken from PCLS ensure comparable tissue sample sizes and further increase the amount of tissue samples. The generated lung tissue cultures can be applied in a variety of studies in human lung biology, including the pathophysiology and mechanisms of different diseases, such as fibrotic processes at its best at (sub-)cellular levels. The highest benefit of the 3D-LTC ex vivo model is its close representation of the in situ human lung in respect of 3D tissue architecture, cell type diversity and lung anatomy as well as the potential for assessment of tissue from individual patients, which is relevant to further develop novel strategies for precision medicine.

Role in the paper:

- Optimization of PCLS protocol.
- Wrote parts of the manuscript
- Prepared figures for the manuscript

Paper IV: Stegmayr J, **Alsafadi HN** Langwiński W, Niroomand A, Lindstedt S, Leigh ND, Wagner DE. **Isolation of high yield and quality RNA from human precision-cut lung slices for RNA-sequencing and computational integration with larger patient cohorts.** AJP Lung (2020). In revision at AJP Lung, minor revision submitted on 14th Oct 2020 (Attached as **APPENDEX B**)

Summary: Precision-cut lung slices (PCLS) have gained increasing interest as a model to study lung biology and disease, as well as for screening novel therapeutics. In particular, PCLS derived from human tissue can better recapitulate some aspects of lung biology and disease as compared to PCLS derived from animals (e.g. clinical heterogeneity), but access to human tissue is limited. A number of different experimental readouts have been established for use with PCLS, but obtaining high yield and quality RNA for downstream gene expression analysis has remained challenging. This is particularly problematic for utilizing the power of next-generation sequencing techniques, such as RNA-sequencing

(RNA-seq), for non-biased and high through-put analysis of PCLS human cohorts. In the current study, we present a novel approach for isolating high quality RNA from a small amount of tissue, including diseased human tissue, such as idiopathic pulmonary fibrosis (IPF). We show that the RNA isolated using this method is of sufficient quality for both RT-qPCR and RNA-seq analysis. Furthermore, the RNA-seq data from human PCLS was comparable to data generated from native tissue and could be used in several established computational pipelines, including deconvolution of bulk RNA-seq data using publicly available single-cell RNA-seq data sets. Deconvolution using Bisque revealed a diversity of cell populations in human PCLS derived from distal lung tissue, including several immune cell populations, which correlated with cell populations known to be present and aberrant in human disease, such as IPF.

Role in the paper: RNAseq data analysis, established piplelines for deconvultion of bulkRNAseq using scRNAseq reference datasets.

Paper V: **Alsafadi HN,** … , Cantu C, Wagner DE. Identification of Yap/Taz co-transcriptional partners in the fibrotic lung epithelium.

Not included in the PhD thesis (Potential to include):

1. **Alsafadi HN,** Uhl FE, Pineda R., Bailey K, Rojas M, Wagner DE, Königshoff M. Applications and Approaches for Three-Dimensional Precision-Cut Lung Slices. Disease Modeling and Drug Discovery. (2020) *AJRCMB.* DOI: 10.1165/rcmb.2019-0276TR. REVIEW PAPER
   Type: Review Paper
   Role: Lead author
   Future work-load: NONE.

2. Conlon T, John-Schuster G, Heide D, Lehmann M, Costa R, Prokosch S, Hetzer J, Verleden S, Lopez M, **Alsafadi HN,** Günes G, Zabeh M, Lindner M, Burgstaller G, Becker L, Irmler M, Stoeger T, Beckers J, Wagner DE, Hrabe de Angelis M, O'Conner T, Dejardin E, Eickelberg O, Konigshoff M, Heikenwalder M, Yildirim ÖA. Inhibiting LTβR-signaling reverses COPD by blocking epithelial apoptosis and activating WNT-induced regeneration. Accepted for Publication in Nature.
   Type: Primary Article
   Role: Performed Experiments with 3D model (human PCLS) and accompanied data processing/Analysis.
   Future work-load: NONE.

3. Martina M De Santis, **Alsafadi HN**, Sinem Tas, Deniz A Bölükbas, Sujeethkumar Prithiviraj, Iran A. N. Da Silva, Margareta Mittendorfer, Chiharu Ota, John Stegmayr, Melanie Königshoff, Jeffery A Wood, Manlio Tassieri, Paul E Bourgine, Sandra Lindstedt, Sofie Mohlin, Darcy E Wagner. Extracellular Matrix Reinforced Bioinks for 3D Bioprinting Human Tissue. In revision Advanced Materials.
   Type: Primary article
   Role: Established protocols for human bronchial epithelial cell (HBEC) isolations and cultivation, 3D Bioprinting of airways, imaging.
   Future work-load: Minimal. Experiments for revision already performed.

4. Uhl FE., Burgess J, Schweitzer K., Zvarova B., Pouliot RA., De Santis MM., Bölükbas D., **Alsafadi HN**, Mohlin S., Lindstedt S., Petrache I., Deng B., Lam Y., Weiss D J, Wagner DE. Extracellular matrix from COPD patients impairs angiogenesis. In preparation
   Type: Primary Article
   Role: Characterization of Collagen IV in native tissue and decellularized tissue.
   Future work-load: NONE.

5. Tas S, Bölükbas DA, **Alsafadi HN**, Wagner DE. Use of decellularized porcine lung derived extracellular matrix hydrogel for lung organoid culture
   Type: Primary Article
   Role: HBEC isolation / maintenance. Design of experiments using HBECs/ALI/Organoid formation.
   Future work-load: Minimal; section in methods, proofreading, potential involvement in revisions (consulting)

6. Bölükbas DA, De Santis MM, **Alsafadi HN,** Doryab A, Wagner DE. The Preparation of Decellularised Mouse Lung Matrix Scaffolds for Analysis of Lung Regenerative Cell Potential. *In: Bertoncello I. (eds) Mouse Cell Culture. Methods in Molecular Biology, vol 1940. Humana Press, New York, NY* (2019)
   Type: Book chapter
   Role: Wrote parts on RNA isolation from lung tissue.
   Future work-load: NONE.

7. Wagner DE, Ikonomou L, Gilpin SE, Magin CM, Cruz F, Greaney A, Magnusson M, Chen Y, Davis B, Vanuytsel K, Enes SR, Krasnodembskaya A, Lehmann M, Westergren-Thorsson G, Stegmayr J, **Alsafadi HN,** Hoffman ET, Weiss DJ, Ryan AL. Stem Cells, Cell Therapies, and Bioengineering in Lung Biology and Disease 2019. Accepted for publication at ERJ Open.
   Type: Conference Report
   Role: Co-Wrote final section and produced statistics figures.
   Future work-load: NONE.

Authorship has been secured on other manuscripts that are in production. Future work related to these projects is minimal.