ESOT Transplant Fellowship – Research progress report

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Report on Progress Toward Original Research Plan

The original research proposal outlined an ambitious programme aimed at improving early diagnostics, prevention, and treatment of chronic lung allograft dysfunction (CLAD), enhancing post-transplant monitoring, and expanding research capacity in Leuven through biobanking, biomarker development, and multidisciplinary collaboration. Below is a summary of progress achieved across each major objective.

1. Earlier and More Detailed Diagnostics of CLAD

Original objectives:

- Improve early detection using longitudinal lung function metrics (spirometry, body plethysmography, TLCO).
- Personalise immunosuppression and antimicrobial prophylaxis.
- Implement 24-month surveillance bronchoscopy/biopsy.
- Improve screening and management of known risk factors such as reflux and sleep apnoea.

Progress achieved: During the fellowship, I participated extensively in both outpatient and inpatient longitudinal follow-up of lung transplant recipients. This hands-on exposure enhanced my ability to interpret serial pulmonary function trajectories and recognise early signs of CLAD, aligned with the objective of improving early diagnostics. I gained practical experience with spirometry, body plethysmography, and gas transfer testing in the context of CLAD surveillance.

My involvement in MDTs contributed directly to personalised immunosuppression decision-making, including induction and maintenance strategies, targeted antimicrobial prophylaxis, and balancing the risk–benefit profile for each patient. I also gained exposure to the centre's surveillance bronchoscopic protocols, including their diagnostic yield and potential future expansion to 24-month biopsies. Moreover, I

participated in the evaluation and management of reflux, sleep apnoea, and other modifiable CLAD risk factors, contributing to earlier recognition and intervention.

Scientific output:

- Fila L, **Zajacova A**. Navigating the pitfalls of pulmonary function testing in chronic lung allograft dysfunction and chronic pulmonary graft-versus-host disease. Eur J Intern Med. 2025 Nov;141:106500. doi: 10.1016/j.ejim.2025.106500. Epub 2025 Sep 4. PMID: 40912970.).
- **Zajacova A,** Dupont LJ, De Leyn P, Ceulemans LJ, Vos R; Leuven Lung Transplant Group. Characteristics and Outcomes of 1500 Lung Transplantations in the Leuven Lung Transplant Program: Turning Past Lessons Into Tomorrow's Foundations. Transpl Int. 2025 Nov 12;38:15495. doi: 10.3389/ti.2025.15495. PMID: 41312224; PMCID: PMC12648049.

2. Implementation of State-of-the-Art Therapies for CLAD Prevention and Treatment

Original objectives:

- Standardise the use of montelukast, azithromycin, antifibrotics, inhaled therapies.
- Enable future multicentre randomised trials.

Progress achieved: Through active participation in MDT discussions and patient case reviews, I gained extensive insight into the centre's therapeutic strategies, their rationale, and heterogeneous real-world responses. This broadened my understanding of how existing therapies—including immunomodulatory macrolides, antifibrotic agents, and targeted inhaled medications—can be optimally deployed and standardised. This knowledge will support future protocol harmonisation initiatives and foster readiness for multicentre clinical trials. My scientific contributions during the fellowship, including multiple publications related to immunological risk assessment, rejection dynamics, and biomarker development, directly support the goal of building a robust foundation for future interventional studies.

Scientific output:

- Beeckmans H & **Zajacova A**, Vos R. Pseudomonas aeruginosa-driven Humoral Immune Activation in Lung Transplantation: A Treatable Trait to Avert Chronic Lung Allograft Dysfunction? Transplantation. 2025 Nov 10. doi: 10.1097/TP.0000000000005566. Epub ahead of print. PMID: 41211870. *(shared first authorship)*
- **Zajacova A**, Revilla-Lopez E, Guney M, De Pelsmaeker S, Emonds MP, Naesens M, Saez-Gimenez B, Vanaudenaerde BM, Vos R: Pitfalls in Detection and Interpretation of Anti-HLA Antibodies and How to Move Forward in Lung Transplantation - *under review in European Respiratory Review*

- Beeckmans H, Hooft C, Kerckhof P, Willems L, Aerts G, Jin X, Geudens V, Vermaut A, Zapata-Ortega M, Cortesi E, **Zajacova A**, Van Herck A, Lauriers S, Verbeylen L, Tielemans B, Feys S, Wauters J, Lagrou K, De Sadeleer L, Godinas L, Bos S, Dupont LJ, Cleynen I, Bohyn A, Ceulemans LJ, Vanaudenaerde BM, Vos R: Pentraxin 3 polymorphisms predispose to Aspergillus infection and rejection after lung transplantation - *under review in American Journal of Transplantation*

3. Establishment of a Specialised Centre for Pulmonary GvHD

Original objectives:

- Develop a dedicated pGvHD outpatient clinic.
- Implement weekly MDT reviews for complex cases.
- Expand expertise to other transplant-related pulmonary complications.

Progress achieved: I was closely involved in outpatient follow-up of patients with pulmonary GvHD and gained significant experience in evaluating disease progression, optimising treatment strategies, and determining suitability for lung transplantation. I actively participated in multidisciplinary discussions involving pulmonologists, oncologists, radiologists, thoracic surgeons, and infectious disease specialists, fully aligning with the proposal's vision of establishing structured MDT pathways. The fellowship allowed me to develop practical expertise in pGvHD care, expanding the clinical foundation necessary for future multicentric studies in this population.

Scientific output:

- Fila L, **Zajacova A**. Navigating the pitfalls of pulmonary function testing in chronic lung allograft dysfunction and chronic pulmonary graft-versus-host disease. Eur J Intern Med. 2025 Nov;141:106500. doi: 10.1016/j.ejim.2025.106500. Epub 2025 Sep 4. PMID: 40912970.

4. Learning the Harvesting and Post-Processing of Explanted Lungs

Original objectives:

- Learn procurement, labelling, and post-processing of explanted organs.
- Align with ethical and GDPR standards.
- Collaborate with KU Leuven and BREATHE lab for downstream analyses.

Progress achieved:

I gained experience with post-processing workflows relevant to explanted tissue and biobanking principles. Through engagement with the Leuven research teams, I became familiar with ethical frameworks, GDPR-compliant sample handling, and the downstream analytical techniques used in collaborations with

the BREATHE lab and KU Leuven. This knowledge supports future contributions to explant-based studies on the origins of lung disease and CLAD pathophysiology.

Scientific output:

- Beeckmans H, Kerckhof P, Geudens V, Vanluyten C, Zapata-Ortega M, Willems L, Van Herck A, Vermaut A, Aerts G, Hooft C, Jin X, Van Slambrouck J, **Zajacova A**, Mercurio M, Cortesi E, Mohamady Y, Tielemans B, Knoop C, Janssens WA, Gayan-Ramirez G, Wuyts WA, Godinas L, Bos S, Wauters E, Dupont LJ, Van Raemdonck D, Weynand B, Saez-Gimenez B, Schiller H, Schupp J, Kneidinger N, De Sadeleer L, Ahangari F, McDonough JE, Ceulemans LJ, Yildirim O, Kaminski N, Vanaudenaerde BM, Vos R: A Transcriptomic Atlas of Chronic Lung Allograft Dysfunction - *under review in European Respiratory Journal*

5. **Collaboration on Biobanked Transbronchial Cryobiopsies (CELSA)**

Original objectives:

analysing donor-specific antibodies in bronchoalveolar lavage and cryobiopsy material

Progress achieved:

As part of the CELSA-funded project, I contributed to the analysis of biobanked transbronchial cryobiopsies with the aim of improving the assessment of donor-specific antibodies, bronchoalveolar profiles and tissue-based markers related to rejection after lung transplantation. During the fellowship, we conducted a pilot run of the cryobiopsy dataset to refine and validate the methodological workflow, ensuring consistency, reproducibility and high analytical quality for the larger study.

The preliminary findings from this methodological pilot were submitted as an abstract to ISHLT 2025, marking an important milestone and demonstrating the scientific potential of this work. Building on this foundation, the full-cohort analysis is scheduled to begin in January 2026, supported by the optimised pipeline established during the pilot phase. This structured progression from method refinement to cohort-wide implementation reflects clear advancement toward the original research objectives and strengthens the translational relevance of the study.

Scientific output:

- **Zajacova A** et al.: Intragraft Anti-HLA Antibodies Variation Between and within Chronic Lung Allograft Dysfunction - *submitted to ISHLT Congress 2026*

6. Retrospective Assessment of Blood Subpopulations

Original objectives:

- Analyse immune cell subpopulations longitudinally (pre-LuTx and at surveillance time points).
- Personalise immunosuppression.
- Assess immune dynamics after lymphodepletion.

Progress achieved:

Through collaboration with Leuven's biobank and immunology teams, I gained exposure to the retrospective datasets and ongoing analyses of blood subpopulation dynamics. These collaborations connected Leuven's immunophenotyping resources with my background in Prague, enabling further exploration of how lymphocyte profiles evolve in relation to CLAD risk, rejection, and treatment intensity.

Scientific output:

- 04/2025: Poster - Antithymocyte Globulin Treatment for Chronic Lung Allograft Dysfunction: A Single-Centre Retrospective Study (ISHLT Congress) - *finalisation of manuscript ongoing*

7. Prospective Assessment of Donor-Derived Cell-Free DNA (dd-cfDNA)

Original objectives:

- Assess dd-cfDNA at surveillance and for-cause time points.
- Explore dd-cfDNA as a biomarker for CLAD diagnosis, treatment response, and phenotype stratification.

Progress achieved:

This objective was a major focus of my scientific work. My fellowship supported groundbreaking research into the interpretation and clinical relevance of dd-cfDNA dynamics after lung transplantation. This work led to multiple first- and co-authored publications, including manuscripts on early post-transplant dd-cfDNA levels, associations with early allograft dysfunction, and insights into acute cellular rejection. My PhD thesis—awarded the Dean's Prize for Extraordinary Results—further advanced this field. These outputs strongly exceed the expectations of the original proposal.

Scientific output:

- **Zajacova A,** Alkhouri M, Guney M, Ferrao G, Rezac D, Vyskocilova K, Kotowski T, Dutkova A, Dvorackova E, Lischke R, Fila L, Havlin J. Exploring acute cellular rejection in lung transplantation: insights from donor-derived cell-free DNA analysis. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2025 May 16. doi: 10.5507/bp.2025.016. Epub ahead of print. PMID: 40391837.
- **Zajacova A,** Alkhouri M, Ferrao G, Guney M, Rezac D, Vyskocilova K, Kotowski T, Dutkova A, Dvorackova E, Lischke R, Fila L, Ross DJ, Vanaudenaerde B, Havlin J. Early post-lung transplant

cell-free DNA levels are associated with baseline lung allograft dysfunction. Transpl Immunol. 2025 Sep;92:102245. doi: 10.1016/j.trim.2025.102245. Epub 2025 May 31. PMID: 40456369.

- Beeckmans H, Pagliazzi A, Kerckhof P, Hofkens R, Debackere F, **Zajacova A,** Bos S, Vanaudenaerde BM, de Loor H, Naesens M, Vos R. Donor-derived cell-free DNA in chronic lung allograft dysfunction phenotypes: a pilot study. Front Transplant. 2024 Dec 23;3:1513101. doi: 10.3389/frtra.2024.1513101. PMID: 39764156; PMCID: PMC11701071

8. Organisation of a BAL Fluid Biobank

Original objectives:

- Establish a biobank for BAL samples.
- Create ethical, procedural, and quality-standardised storage workflows.

Progress achieved:

During the fellowship, I gained substantial insight into the logistical and ethical framework required for establishing a BAL biobank, including sample acquisition, storage logistics, GDPR-compliant labelling, and protocol development. Although the full establishment of a dedicated BAL biobank is a long-term institutional process, the preparatory knowledge and procedural understanding acquired during my stay in Leuven have already enabled the initial implementation of these principles in Prague, where the development of a BAL biobank is now underway. This experience has therefore provided not only theoretical grounding but also a practical foundation for translating biobanking infrastructure into clinical and research practice.

Leuven, 30th November 2025