



To the EAACI Vice President Science - EAACI Headquarters,

Project title: Systemic and Mucosal Immune Signatures of Grass Pollen Immunotherapy With and Without Dupilumab in Seasonal Allergic Rhinitis

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Type, duration, and location of Fellowship: EAACI Research Fellowship from 01.09.2025 to 02.03.2026 (6 months), London, United Kingdom.

Host Institution and Supervisor name: Immunomodulation and Tolerance Group (ITG), Allergy & Clinical Immunology, National Heart & Lung Institute, Imperial College London; Professor Mohamed H. Shamji.

The following questions will be addressed:

- What questions were addressed and why?
- What was the nature of the research?
- What was the result?
- How will the findings impact future research?

Introduction

Allergen immunotherapy (AIT) is the only disease-modifying treatment for allergic rhinitis and can produce clinical benefits that persist beyond the treatment period. These effects are associated with changes in allergen-specific humoral immunity, including the induction of blocking antibodies that interfere with IgE-dependent allergen recognition. While IgG4 has been extensively studied, sublingual immunotherapy (SLIT) is also associated with allergen-specific IgA responses, including IgA1 and IgA2, which may contribute to mucosal immune regulation and inhibition of IgE-allergen interactions.

The fellowship project was developed within the GRADUATE clinical trial, which provides longitudinal serum and nasal samples from adults with moderate-to-severe seasonal allergic rhinitis treated with grass pollen SLIT with or without Dupilumab, together with a placebo comparator. Samples collected across treatment and post-treatment timepoints provide an opportunity to investigate the evolution of systemic and mucosal antibody responses and their relationship with clinical tolerance.

The principal research question was therefore how grass pollen SLIT, with or without Dupilumab, shapes systemic and mucosal functional antibody responses and whether these immunological signatures are associated with clinical reactivity and sustained tolerance. The planned project focused on allergen-specific IgA1 and IgA2 in serum and nasal fluid and on functional inhibition of IgE-allergen interactions using the IgE-Facilitated Allergen Binding (IgE-FAB) assay (Figure 1).

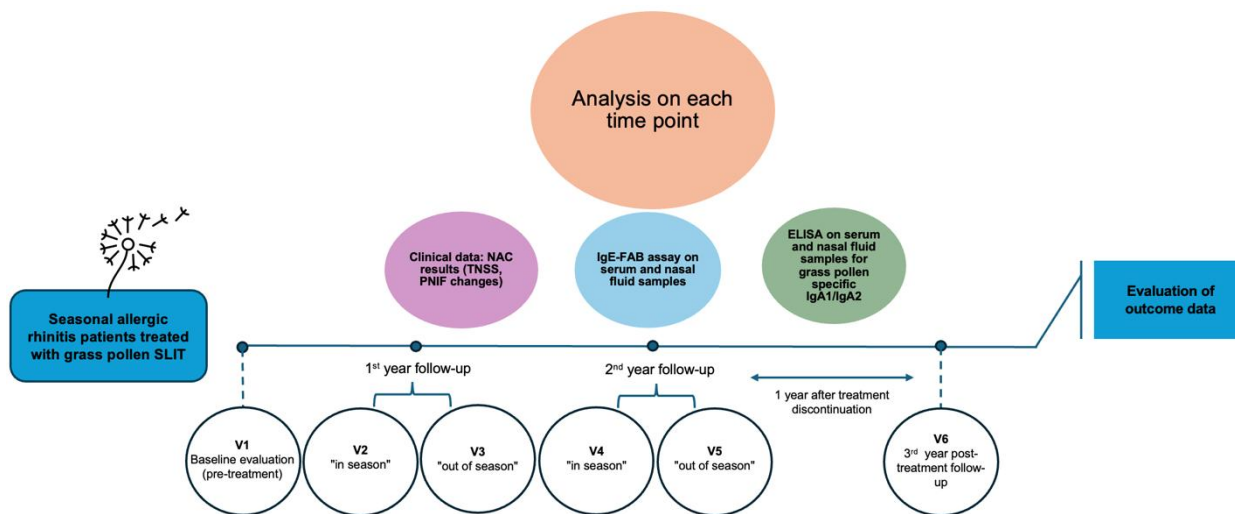


Figure 1. Schematic overview of the study sampling and analysis timeline

Nature of the Research and Planned Experimental Approach

This was a laboratory-based translational and mechanistic research project using pre-existing biological samples from the GRADUATE trial. The fellowship project required no new patient recruitment or clinical intervention.

1. Allergen-specific IgA1 and IgA2 ELISA

The ELISA component was designed to quantify grass pollen-specific IgA1 and IgA2 in serum and nasal fluid. The intended workflow included allergen coating, blocking, incubation with diluted biological samples, subclass-specific detection, enzymatic development, optical density measurement, and assay quality assessment. The objective was to characterise longitudinal changes in IgA1 and IgA2 across treatment groups and timepoints and subsequently compare systemic and mucosal responses.

2. IgE-Facilitated Allergen Binding (IgE-FAB)

The functional component of the project involved IgE-FAB, a flow-cytometry-based assay used to assess facilitated allergen binding to CD23-expressing B cells and the capacity of treatment-induced antibodies to inhibit IgE-allergen complex binding. The planned analysis was intended to complement quantitative antibody measurements by providing a functional measure of inhibitory antibody activity.

3. Integration with clinical outcomes

After completing the laboratory analyses, the immunological data will be integrated with clinical outcomes from the GRADUATE trial, particularly nasal allergen challenge measures such as total nasal symptom score and nasal airflow parameters. This will allow assessment of whether systemic and mucosal antibody responses are associated with reduced clinical reactivity during treatment and with sustained tolerance after treatment.

Activities and Training During the Fellowship

During my fellowship in the Immunomodulation and Tolerance Group at Imperial College London, I gained practical experience in translational allergy and immunology research and participated in laboratory activities, human biological sample management, experimental data analysis, and laboratory operations.

My hands-on laboratory training included peripheral blood mononuclear cell (PBMC) isolation, basophil activation testing (BAT) with diamine oxidase (DAO), IgE-Facilitated Allergen Binding (IgE-FAB), and enzyme-linked immunosorbent assays (ELISA) using different aeroallergens and food allergens. I also

gained experience analysing and interpreting flow cytometry data using FlowJo, and in data visualisation and statistical plotting using GraphPad Prism.

I completed the mandatory training requirements of Imperial College London and worked in accordance with institutional research governance procedures. This included training related to the Human Tissue Act and the appropriate handling, processing, storage, and management of human biological samples. I participated in receiving incoming samples, cross-checking sample information and documentation, creating and maintaining sample indexes, and ensuring that samples were appropriately catalogued and stored to maintain traceability.

I also contributed to routine laboratory activities and gained experience in laboratory organisation, instrument and equipment maintenance, and troubleshooting flow cytometers and other laboratory equipment. Working within a multidisciplinary research group provided valuable exposure to the practical organisation of translational immunology research and to collaborative experimental work.

Experimental Work Performed for the Fellowship Project

As part of my own fellowship project, I performed preliminary optimisation and validation of allergen-specific IgA1 and IgA2 ELISAs. I used archived serum samples from individuals who had previously received grass pollen SLIT for this preliminary work before applying the assay to the longitudinal GRADUATE samples.

The optimisation process provided practical experience with the complete ELISA workflow, including preparation of plates and reagents, sample dilution, detection procedures, evaluation of assay performance, and interpretation of experimental readouts. The purpose of these experiments was to establish workable assay conditions for IgA1 and IgA2 measurement and to provide a methodological basis for the subsequent analysis of GRADUATE samples.

In parallel, training in the IgE-FAB methodology allowed me to understand the experimental workflow and the role of this functional assay in assessing inhibitory antibody activity. This was particularly relevant to the overall project because combining quantitative IgA measurements with functional IgE-FAB data is intended to provide a more integrated assessment of treatment-induced humoral immunity.

Adaptation of the Original Research Plan

The original fellowship plan anticipated progression from assay optimisation to batch analysis of the longitudinal GRADUATE serum and nasal samples, followed by IgE-FAB experiments and integration of the laboratory findings with clinical outcomes. Due to unforeseen health circumstances, I was unable to personally complete all subsequent experimental phases within the fellowship period.

The scientific aims of the project have not changed. The remaining experimental work is continuing within the Immunomodulation and Tolerance Group. My role in the project continues through the analytical and scientific stages, including data analysis, interpretation of the findings, integration of laboratory and clinical data, preparation of figures and tables, and manuscript development. This adaptation enables the original objectives of the fellowship project to be pursued despite the change in the planned experimental timeline.

Results and Current Status

The full experimental dataset from the GRADUATE substudy is not yet complete. The remaining ELISA and IgE-FAB analyses are ongoing within the host research group. Therefore, final longitudinal comparisons between treatment groups and time points, as well as correlations between antibody responses and clinical outcomes, are not yet available. To avoid selective reporting while the experimental work is ongoing, this final report does not present preliminary biological comparisons from the incomplete GRADUATE dataset.

Once the experimental dataset is complete, the planned analysis will evaluate longitudinal changes in serum and nasal IgA1/IgA2, functional IgE-FAB activity, and their relationships with clinical measures of nasal allergen reactivity.

Impact of Findings on Future Research

Although the complete GRADUATE laboratory analysis could not be finalised within the fellowship period, the work performed provides a methodological foundation for the ongoing study and has several implications for future research.

Integration of systemic and mucosal immunity

The completed and ongoing work will enable paired assessment of serum and nasal antibody responses. This may improve understanding of whether local mucosal immunity and systemic humoral immunity provide complementary information about tolerance development during SLIT.

Functional assessment of treatment-induced antibodies

Combining IgA1/IgA2 measurements with IgE-FAB will allow interpretation of quantitative antibody responses alongside functional inhibitory activity. This approach may help identify immune signatures that are more closely related to clinical response than antibody concentration alone.

Mechanistic evaluation of SLIT with Dupilumab

The GRADUATE design provides an opportunity to examine how Dupilumab co-administration influences the humoral response to grass pollen SLIT. The completed dataset may therefore help explain interactions between biologic therapy and allergen-specific immune tolerance.

Future translational research

The laboratory and analytical skills acquired during the fellowship will support future mechanistic allergy studies and the development of translational research combining clinical phenotyping with immunological assays at my home institution.

Publications and Presentations

No publication or scientific presentation has yet been generated from this fellowship project because the experimental work and final analyses are still ongoing. The aim is to prepare the completed findings for scientific presentation and peer-reviewed publication. Future outputs resulting from this exchange project will acknowledge that I was a recipient of an EAACI Research Fellowship, and EAACI will be informed of resulting publications.

Conclusion and Personal Reflection

This EAACI Research Fellowship was a highly valuable experience for my academic and scientific development. It provided hands-on exposure to laboratory techniques that are central to translational allergy research, including ELISA, IgE-FAB, BAT, PBMC isolation, and flow cytometry analysis, while also strengthening my understanding of biological sample management, research governance, experimental optimisation, and data interpretation.

An important aspect of the fellowship was learning how mechanistic laboratory studies can be linked to carefully phenotyped clinical trial data to address clinically relevant questions. The experience also highlighted areas in which I would like to develop further, particularly advanced analysis of longitudinal immunological data and integration of functional immune assays with clinical outcomes.

The scientific collaboration established during the fellowship is continuing through the ongoing GRADUATE project. I expect the technical skills, research perspective, and collaborative experience gained during the fellowship to contribute directly to my future work in pediatric allergy and immunology and to the development of future translational research projects.

Acknowledgments

I would like to express my sincere gratitude to the European Academy of Allergy and Clinical Immunology (EAACI) for supporting this Research Fellowship. I am especially grateful to my host supervisor, Professor Mohamed H. Shamji, for welcoming me into the Immunomodulation and Tolerance Group and for his scientific guidance, mentorship, and support. I would also like to thank Dr Janice A. Layhadi and all members of the ITG team for generously sharing their knowledge and expertise and for their support and friendship throughout my fellowship.

Sincerely,
Damla Baysal Bakır, MD