Research Focus 1 – Regulation-related Research and Innovative Product Testing to Ensure High Quality, Safe, and Effective Allergen Products for Allergy Diagnostics and Therapy:

- **Investigations on the occurrence and significance of Api m 10 and other allergen components in therapy allergen products (Vera Mahler)**

Api m 10 (Icarapin), a major allergen in honeybee venom, has been under discussion as a putative predictor of treatment failure in the context of presumed potential underrepresentation in marketable venom immunotherapy products. Api m 10 is known to be of instable nature with rapid degradation and of low abundance in honeybee venom. Previous qualitative analysis by mass spectrometry performed in our Division was able to detect Api m 10 in all investigated venom immunotherapy products. Whether the amount of Api m 10 contained in AIT-products is sufficient to induce a specific protective immune response is of utmost importance concerning safety and efficacy of venom immunotherapy products. Aim of this project, using a model with BALB/c mice, is to obtain supportive information in vivo on the amount of Api m 10 which is sufficient to elicit a protective immune response. Furthermore, Api m 10-specific monoclonal antibodies (mAK) are to be obtained for the development of immunochemical in vitro assay systems for the analysis of Api m 10 in complex bee venom mixtures.

- **Quality control of the diagnostics for occupational type I allergies**

As a consequence of pharmaceutical companies´ decision to withdraw marketing authorizations, the availability of commercial test allergens (e.g. prick test solutions, SPTs) for diagnosis of occupational allergies has been decreasing for years. This results in a diagnostic gap with considerable limitations of patient care.

In cooperation with the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum (IPA), in this feasibility study we aim to establish standard operating procedures (SOPs) for protein extraction from 20 occupational allergen sources and explore compounding of test allergen preparations under pharmacy conditions taking into account practical aspects and legal requirements. These include compliance with the German Medicinal Products Act (Arzneimittelgesetz (AMG)), the monographs of the European Pharmacopoeia on allergen products and their starting materials as well as compliance with the Pharmacy Operating Ordinance (ApBetrO). According to AMG Section 13 (2) no. 1 in combination with Section 13 (2a) sentence 2 no. 3 compounding of medicinal products is an essential part of the working routine in German pharmacies. However, is not yet implemented for test allergens.

In this feasibility study we want to find out whether suitable extraction and standardization procedures can be established and documented in the form of SOPs, which can be used in public pharmacies as working instructions for the production of qualitatively acceptable and stable test allergens on a pharmacy scale which may contribute to reduce the existing diagnostic gap in the field of occupational allergies.

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- **Establishment of replacement batches for the recombinant major allergens Bet v 1 and Phl p 5a Ph. Eur. reference standards**

In 2012, the first European allergen reference standards have been established by the European Directorate for the Quality of Medicines and HealthCare (EDQM). The two Chemical Reference Standards (CRS) contain specific amounts of recombinant Bet v 1, the major allergen from birch pollen, and Phl p 5a, a major allergen from Timothy grass (1, 2). An upcoming change of the Ph. Eur. General Monograph on Allergen Products will make the use of these CRS in combination with the respective standard ELISA systems for allergen quantification mandatory (3). Thus, it was decided to initiate the project BSP163 to generate replacement batches of the two CRS to meet the anticipated increased demand. Bulk preparations of both recombinant allergens had been provided to the EDQM in 2014. Suitability of the bulks had to be demonstrated before new CRS batches could be produced from them. The necessary experiments were performed at the University of Salzburg and at the Paul-Ehrlich-Institut and included a.o. analyses via allergen-specific ELISAs and β-hexosaminidase release from humanized rat basophil leukaemia cells as well as amino acid analysis and control of protein identity by mass spectrometry. Results confirmed that both allergen bulks were of satisfactory quality to proceed to the next step of BSP163, the production of the replacement CRS batches, to ensure the availability of the Ph. Eur. allergen reference standards. The establishment of the new CRS batches will be based on a ring trial, which is scheduled for 2023.

For the role of project leader in BSP163, the Paul-Ehrlich-Institut receives financial support from the EDQM as agreed in a scientific advisor contract.

References

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- **Review of the allergen content of birch pollen over time (10 years).**

Birch pollen are the natural source material used for manufacturing of all marketed products for allergen-specific immunotherapy (AIT) of birch pollen allergy. In previous studies, we have shown that differences in allergen content between batches of AIT products can be linked to the use of different pollen batches in production (1). Based on these findings we now investigate the degree of biodiversity of birch pollen in more detail. The main question is whether clear trends can be observed over time or are associated with the locations of the trees. For example, climate change might alter the allergen content of birch pollen over the years. This would pose a challenge in production as well as regulation of the respective AIT products, especially if the major birch pollen allergen Bet v 1 is subject to change.

In the project we are testing a large number of commercially available birch pollen batches, which have been harvested between 2008 and 2021 in four different European countries (France, Sweden, Czech Republic and Slovakia). From each of the 30 pollen batches, three

independent aqueous extractions were prepared and are being analysed using allergen-specific ELISAs to quantify four birch pollen allergens (Bet v 1, Bet v 4, Bet v 6 and Bet v 7).

References

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