Update on BSP090 – Recombinant allergens as reference materials

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Biological Standardisation Programme 090 (BSP090) is an initiative of the European Directorate for Quality of Medicines and healthcare (EDQM) that developed from the EU CREATE project, which established the feasibility of using recombinant allergens as biological reference materials for standardization purposes. Proteomics and IgE binding data from CREATE established that rBet v 1 from birch pollen and rPhl p 5 from timothy pollen were ideal candidates for which high quality reference materials could be developed. The aim of BSP090 was to establish Reference Substances suitable for the European Pharmacopoeia (Ph. Eur.) for Bet v 1 and Phl p 5a, as well as to validate reference ELISA methods for allergen quantification in mass units.

Both recombinant allergens were manufactured under GMP conditions and showed excellent IgE binding using the stripped basophil histamine release assay. The rBet v1 showed excellent performance in two ELISA systems manufactured by ALK-ABELLO and by Stallergenes. As a result of the BSP090 studies, the recombinant major pollen allergens were adopted by the European Pharmacopoeia Commission (Oct 2012) to serve as chemical reference substances (CRS) in ELISA to quantify Bet v 1 and Phl p 5 in allergenic products. These standards are available from the EDQM: Cat No. Y0001565 (10.25µg rBet v 1/vial) and Cat No. Y0001566 (8.56µg rPhl p 5/vial). See: http://www.edqm.eu/site/News-EDQM-standards-1572.html.

The two ELISA systems performed well and could not be distinguished in Phase 2 studies of reproducibility, sensitivity, accuracy and robustness. However, the Stallergenes ELISA showed better performance in Phase 3 studies of natural Bet v 1 measurements in allergen extracts and was thus selected by the Ph. Eur. Allergens Working Group for inclusion into the Ph. Eur. as official method to quantify Bet v 1 (June 2014). An ELISA for rPhl p 5 (Allergopharma) is currently being evaluated by the working group for inclusion into the Ph. Eur.

Mass spectrometry (MS) is increasingly being used as an analytical tool to define the purity and composition of purified allergens as an adjunct to allergen manufacturing and standardization and as a precise measuring tool. Our group has used LC/MS/MS to analyse the purity of manufactured allergens and to assess relative abundance using Normalized Spectral Abundance Factors. Using this analysis, NSAF values of 99.60% and 100.00% have been obtained for nDer p 2 and rBet v 1, respectively. For peanut allergens, values of 96.2% and 95.6% were obtained for nAra h 2 and rAra h 8. The MS analysis allows contaminating proteins/allergens or host expression system proteins to be identified and precisely measured, thereby facilitating improvements in purification and enhancing standardization.

Future priorities of the Ph. Eur. Allergens Working Group are to develop Ph. Eur reference substances for dust mite allergens (Der p 1 and Der p 2), together with associated ELISA for
allergen measurement. Such standards are urgently needed for dust mite immunotherapy trials and to harmonize environmental exposure assessments. Involvement of regulatory authorities in the EU and in the US is critical to develop allergen standards for diagnostic and therapeutic purposes, especially with the widespread application of molecular diagnostics in the allergy field. European Medicines Agency (EMEA) guidelines recommend measurement of individual allergens of clinical significance by antibody-based techniques or MS, which form part of the quality assessment for marketing authorization (see Guideline on Allergen Products: Production and Quality Issues, 2009, available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC50003333.pdf). While there has been substantial progress in allergen standardization over the past decade, production of purified allergen standards needs to be accelerated within the industry to develop standardized allergen products for diagnosis and treatment of allergic patients.

References


