Validation of an Enzyme-Linked Immunosorbent Assay (ELISA) Method for the Determination of Bevacizumab Reference Product and Biosimilar in Human Serum

C. Becker 1, Y. He 1, R. Weaver 1, M. Calvarese 1, C. Xia 1, L. Calliste 1, X. Wang 1, C-H. Cai 2, J. Kolman 1
1 QPS, LLC, 2 Pfizer Inc.

Purpose
Bevacizumab (Avastin®) is a humanized monoclonal antibody (mAb) specific for human VEGF. The goal of the validation is to demonstrate a method capable of quantifying the Bevacizumab reference product and biosimilar within the assay variability limits in human serum from normal and targeted populations.

Methods
The Bevacizumab ELISA was constructed as a sandwich assay where Bevacizumab was captured by VEGF and detected by anti-human IgG HRP conjugate. Following the AAPS focus group recommendation, the analytical similarity of calibration curves from both drugs was compared during method development while the QCs’ were assessed during the validation. The reference product and biosimilar drugs were also evaluated for matrix selectivity in normal, solid tumor, hemolyzed and lipemic matrices, as well as for dilution linearity, ADA and VEGF interference, and stability in human serum.

Results
During the method development, calibration curves were constructed for the US/EU sourced reference products and the biosimilar. A total of 3 curves for each of the compounds were analyzed using a statistical approach. An acceptable degree of parallelism was observed. The reference product was selected as the calibration curve for validation, where 6 levels of QCs were found to be analytically similar. The inter/intra- batch precision and accuracy were acceptable for each compound with inter-batch %CV between 3.4% and 13.5% and %RE between 12.7% and 6.0%. Dilution linearity was demonstrated up to 1.25 mg/mL. Matrix specificity and selectivity were successfully evaluated in normal, solid tumor and hemolyzed human serum lots. However, 0/6 lipemic lots recovered in range indicating hyperlipidemia may affect the assay results. Interference was not observed with up to 3000 pg/mL of soluble VEGF or 2000 ng/mL of ADA. Stabilities of both Bevacizumab reference product and biosimilar in human serum after freeze/thaw cycles, bench-top storage and long-term frozen storage were also established.

Conclusion
One ELISA assay was validated for the analysis of the Bevacizumab reference product and biosimilar. The assay exhibited acceptable precision, accuracy, specificity from 250 to 3000 ng/mL and is acceptable to quantify Bevacizumab in human serum to support clinical trials.