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Development of One-Step High Sensitive BEVACIZUMAB ELISA

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HISTORY

- Bevacizumab is a recombinant humanized monoclonal antibody and in 2004 it became the first clinically used angiogenesis inhibitor. Its development was based on the discovery of human vascular endothelial growth factor (VEGF), a protein that stimulated blood vessel growth, in the laboratory of Genentech scientist Napoleone Ferrara. Ferrara later demonstrated that antibodies against VEGF inhibit tumor growth in mice. His work validated the hypothesis of Judah Folkman, proposed in 1971, that stopping angiogenesis might be useful in controlling cancer growth..

CHEMISTRY

- Bevacizumab was originally derived from a mouse monoclonal antibody generated from mice immunized with the 165-residue- form of recombinant human vascular endothelial growth factor. It was humanized by retaining the binding region and replacing the rest with a human full light chain and a human truncated IgG1 heavy chain with some other substitutions. The resulting plasmid was transfected into Chinese Hamster Ovary cells which are grown in industrial fermentation systems

MECHANISM OF ACTION

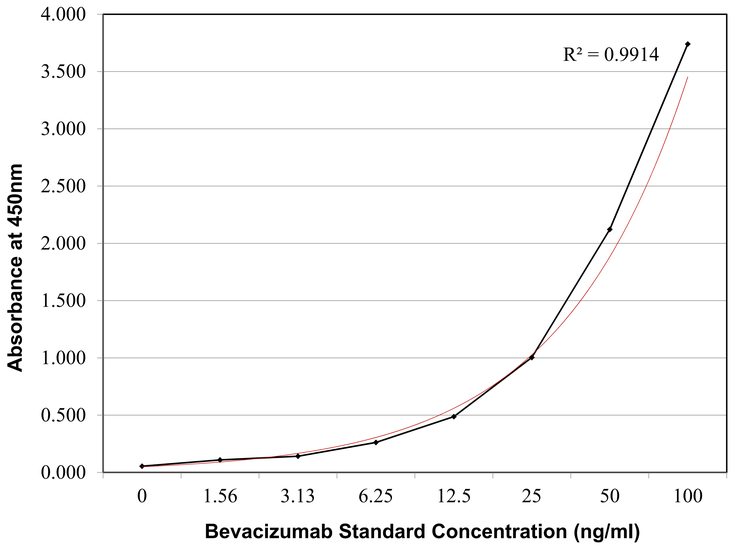
- Bevacizumab is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A). VEGF-A is a growth factor protein that stimulates angiogenesis in a variety of diseases, especially in cancer Bevacizumab was the first available angiogenesis inhibitor in the United States.

APPROVAL

- It received its first approval in the United States in 2004, for combination use with standard chemotherapy for metastatic colon cancer It has since been approved for use in certain lung cancers, renal cancers, ovarian cancers, and glioblastoma multiforme of the brain.
- In July 2014, two pharming companies, PlantForm and PharmaPraxis, announced plans to commercialize a biosimilar version of bevacizumab made using a tobacco expression system in collaboration with the Fraunhofer Center for Molecular Biology.
- In Sept 2017 the US FDA approved Amgen's biosimilar (generic name bevacizumab-awwb, product name Mvasi) for six cancer indications.

PRINCIPLE

Bevacizumab ELISA is a one-step enzyme immunoassay on the basis of humanized monoclonal antibodies specific to Bevacizumab. Assay Diluent, horseradish peroxidase (HRP) labelled humanized monoclonal antibodies specific to Bevacizumab and Standard or test specimens are dispensed simultaneously into the wells of a microtitre plate coated with humanized monoclonal antibodies specific to Bevacizumab. After an incubation time of 2.0hr at 37°C unbound components are removed by a washing step. HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 30 min reaction time into a blue product. The enzyme reaction is terminated by stop solution dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 is directly proportional to the specifically bound amount of Bevacizumab.



Standard curve generated after incubating anti-bevacizumab antibody on the precoated plate. Reading was taken after adding the substrate at 450nm and reaction was stopped by an acid.

Specificity

No cross reaction was observed with sera spiked with the other therapeutic antibodies including Human IgG1, kappa from human myeloma plasma, Infliximab, Adalimumab, Rituximab, Ustekinumab and Alemtuzumab

Sensitivity

- Limit of Detection (LOD) - 1ng/ml i.e., The lowest detectable level that can be distinguished from the zero standard is less than 1 ng/mL.
- Limit of Quantitation : (LOQ) – 1.53ng/ml
- Assay Range : 1.53ng/ml – 100 ng/ml

Precision

- Intra-assay: Less than 5% for bevacizumab range 1.53-100ng/mL.
- Inter-assay: Less than 10 % for bevacizumab range 1.53-100ng/mL.

Calculation & Interpretation Of Results

- Using the diluted standards (12.5, 6.25; 3.13; 1.56; 0 ng/mL) disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each of 4 standards on the vertical (Y-axis) axis versus the corresponding Bevacizumab concentration on the horizontal (X-axis) axis, thus creating a standard curve by 4 points obtained.
- The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of bevacizumab from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the bevacizumab concentration for the unknown sample.

CONCLUSION

The kit developed at Krishgen gives rapid, accurate, reproducible, results and may be useful for pharmacokinetic and pharmacokinetic-pharmacodynamic studies as well in therapeutic drug monitoring of bevacizumab.