

## Biochemical and Biophysical Characterization of Synthetic anti-VEGF Antigen Binding Fragment for Treating Wet-AMD and Diabetic Retinopathy

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Wet Age-related macular degeneration (wet-AMD) is a progressive neurodegenerative disease of the retina, affecting the central vision. Wet-AMD and Diabetic Retinopathy (DR) is a leading cause of irreversible vision loss, worldwide affecting the population older than 50 years. Products that significantly addresses market access representing a global ocular market of >\$25B and >200M patients globally.

The vision behind FAB201 development is to provide an efficacious and cost-effective treatment for wetAMD patients. Currently approved anti-VEGF drugs like Lucentis and Eylea in the market are expensive and not widely accessible in developing countries. Keeping this in mind to meet the unmet medical needs and to provide affordable treatment to developing countries.

We have developed an anti-VEGF Fab molecule (FAB201 and FAB293) for wet-AMD and DR, filed US patent, successfully finished the discovery, proof of concept, development, pre-clinical toxicity studies and cGMP manufacturing for our proprietary molecule. FAB201, that is close to going to Phase-1 trails in US and Australia for wet AMD and DR.

FAB201 and FAB293 are 48kDa synthetic human anti-VEGF Fab (Fragment Antigen Binding). It is a novel biologic (has unique CDRs in light chain & heavy chain) expressed in microbial system (E. coli BL21).

FAB201 and FAB293 has been developed using Phage display library with several rounds of affinity maturation for selection of clones with highest binding affinity, followed by site directed mutagenesis in CDRs for improved antigen binding towards hVEGF (Vascular Endothelial Growth Factor).

The Fab molecules were engineered and fully characterized using advanced Biochemical & Biophysical characterization techniques.

- Quantitation assayo ELISA (Synergy H1-M multimode plate reader from Biotek)
- In-vitro binding affinityo Quantikine Kit and Surface Plasmon Resonance (SPR) by- Biacore 3000 (GE Healthcare); CM5 Chip, research grade (Catalog No. BR- 1003-99, Biacore-GE Healthcare)
- In-vitro efficacy studyo VEGF induced hTERT-RPE1 proliferation assay by BioTek Multiscan reader
- o HUVEC by Fluorescence plate reader
- Protein/product characterizationo PI by Biorad Protean i12 IEF System
- o Intact mass by ESI-Q-TOF instrument (Waters QTOF SYNAPT G2 Mass Spectrometer)
- o Disulfide bond analysis by LC-MS/MS using a Waters QTOF SYNAPT G2 Mass Spectrometer
- o Peptide mass fingerprinting (PMF) by LC-MS/MS using a Waters QTOF SYNAPT G2 Mass Spectrometer
- o AAA sequencing by LC-MS/MS using a Waters QTOF SYNAPT G2 Mass Spectrometer
- o CD spectra by Jasco J-815 Spectropolarimeter
- o CE-SDS Page by PA 800 Plus Protein Characterization System; Sciex with PDA detector
- o Capillary Iso electric focusing (cIEF) by Rotein Simple Maurice C; GE Ettan IPGPhor3
- o Protein crystallography by NMR-JEOL- 600 MHz (14.1T) NMR
- o SVP analysis by Beckman HIAC 9703+ Liquid Particle Counter
- o Epitope mapping by HDX-MS; LTQ-FTICR mass spectrometer (Thermo Fisher, Waltham, MA)
- o Hydrodynamic size by DLS, Malvern Zetasizer
- In-process related impurity testingo Host Cell Protein (HCP) by Micro plate reader Thermo Fisher, Model No.-51119300
- o Host Cell (Hc)-DNA by Quantitative PCR system Roche, Light Cyclor 480 II
- o Endotoxin by Endotoxin Kit KTA2 Charles River Laboratories; Synergy H1-M multimode plate reader from Biotek
- o Protein L leachate by Medicago Protein L Ligand Leakage ELISA kit (Cat. No.: 10-0027/10-0028); Synergy H1-M multimode plate reader from Biotek
- Product related impurities testingo SDS-PAGE by Gel electrophoresis system, BioRad

- o SEC-UPLC by Agilent 1260 Infinity II HPLC system with auto vial sampler  
Column: TOSOH TSK gel UP-SW3000, 4.6 x 300 mm (I.D. x L); Particle size: 2  $\mu\text{m}$
- o RP-HPLC by Agilent 1260 Infinity II HPLC system with auto vial sampler and Shimadzu LC2010C HT  
Column: Agilent, ZORBAX SB-300, C8, 2.1 x 100 mm, 3.5  $\mu\text{m}$  (861775-906)  
Guard column: ZORBAX 300SB-C8 4-Pack, Narrow bore (2.1 x 12.5mm, 5  $\mu\text{m}$ ) (821125-918)
- o SVP by Beckman HIAC 9703+ Liquid Particle Counter

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