Alymsys®
(bevacizumab-maly)

Proven Similarity with Avastin®[1,2]

amneal

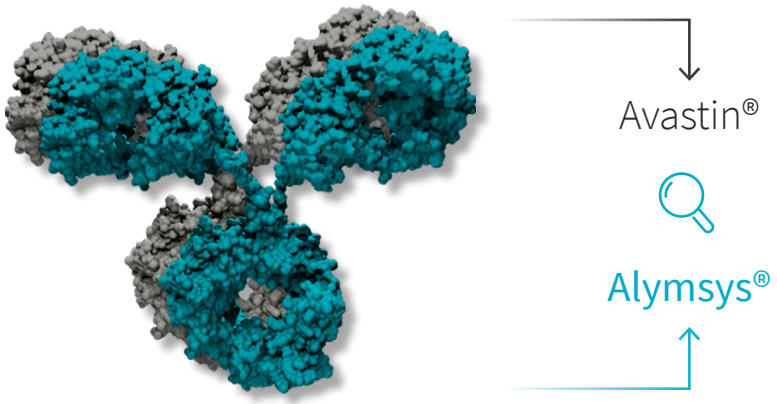
Proven Analytical Similarity

A biosimilar medicine is a biological medicine which is highly similar to an existing approved biological product.[3] Similarity studies are needed to generate evidence substantiating the similar nature, in terms of quality, safety and efficacy, of the biosimilar and the chosen Reference Product (RP).[3,4,5,6]

According to regulatory guidelines, all quality attributes of the biosimilar product are not expected to be identical to the RP, but the analytical data submitted should be such that firm conclusions on the physicochemical and biological similarity between the RP and the biosimilar can be made.[3,4,5,6]

Alymsys® (bevacizumab-maly) has been developed in comparison with Avastin® (bevacizumab) as the RP. High similarity is demonstrated by using state-of-the-art orthogonal analytical methods and any difference observed in quality attributes are justified not to be clinically relevant.[1,2,3,4,5,6]

Over 50 analytical methods were applied to evaluate > 90 quality attributes and **confirm high analytical similarity between Alymsys® and Avastin®:**[1,2]



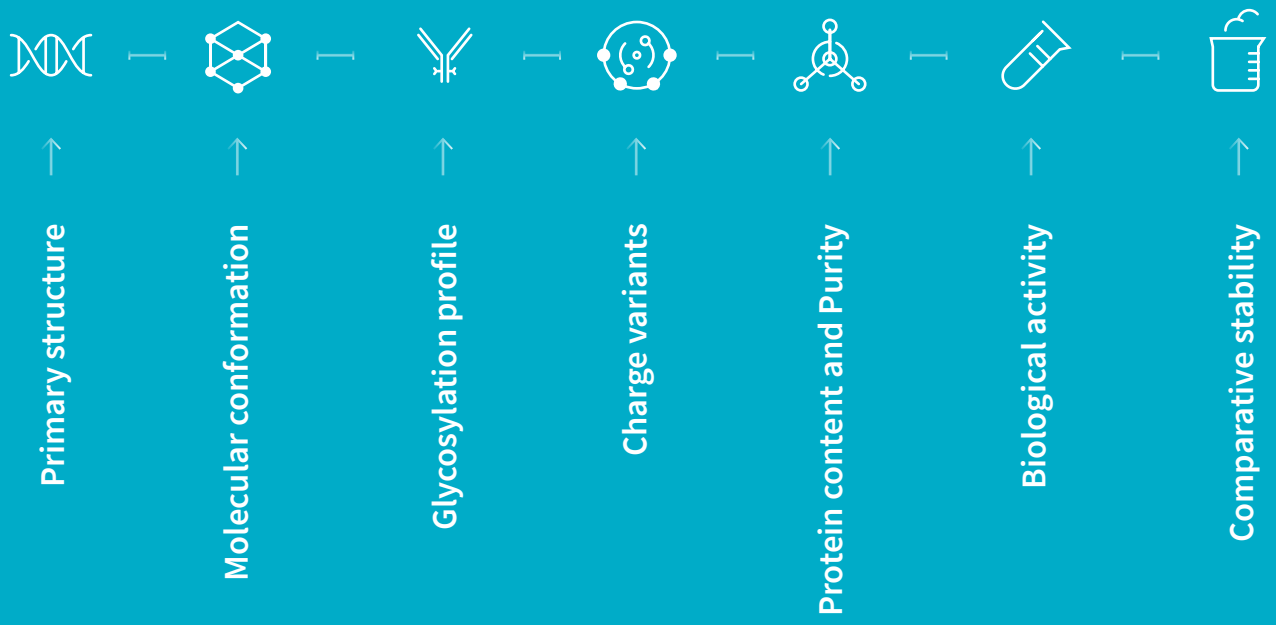
Click here for full Prescribing Information

Alymsys (bevacizumab-maly)

amneal

Proven Analytical Similarity

As per regulatory guidelines recommendations, an exhaustive analytical similarity study was carried out.[4,6] RP and Alymsys® lots were analyzed for primary structure, molecular conformation, glycosylation, charge variants, protein content, purity and biological activity.[1,2] In addition, comparative stability studies were carried out and process-related impurities were compared.[2] From the in-depth comparative characterization of Alymsys® and the RP, the following conclusions were reached:[1,2]



Alymsys
(bevacizumab-maly)

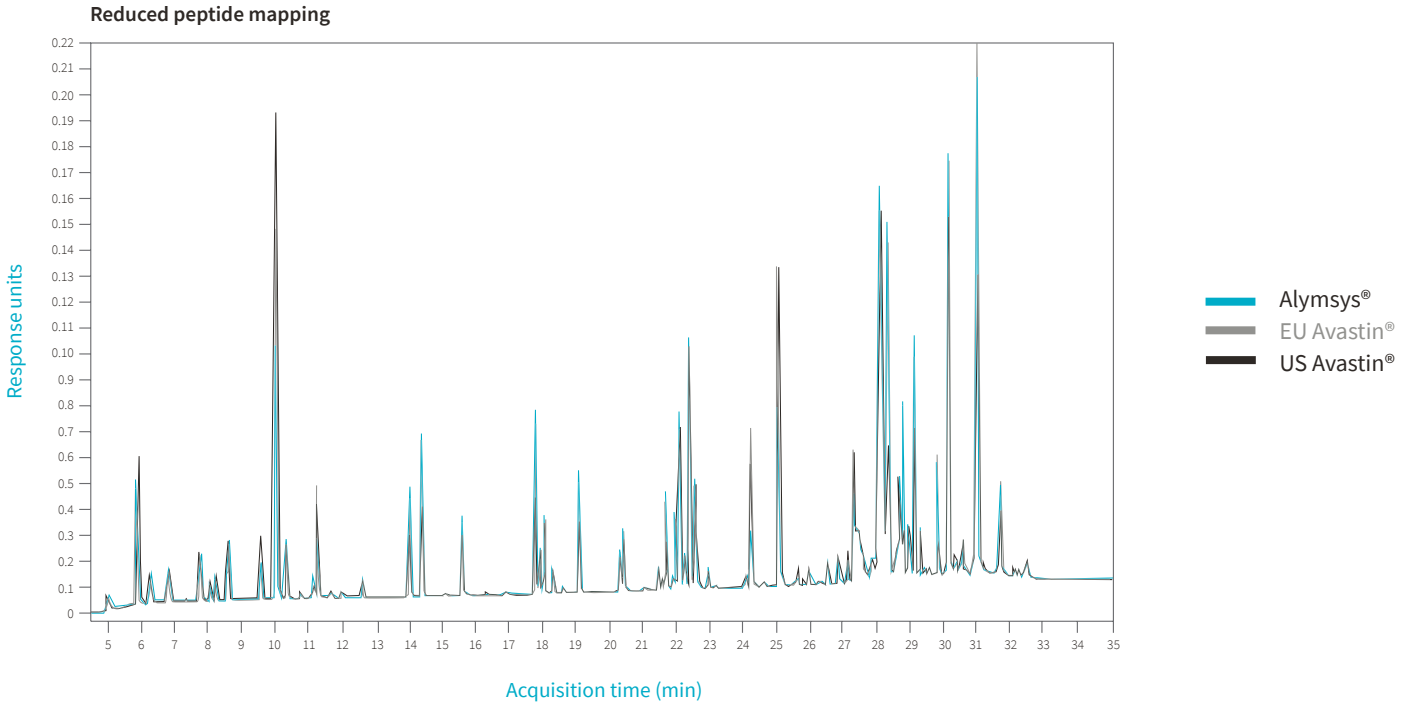
amneal

Primary Structure

Same amino acid sequence confirmed for Alymsys® and Avastin®[1,2]

The target amino acid (AA) sequence of the biosimilar should be confirmed and is expected to be the same as for the RP.[4,6]

Each peak observed corresponds to a part of the monoclonal antibody (peptide) after its digestion by trypsin. The detection of the same peptides by mass spectrometry confirms **the identical AA sequence between Alymsys® and Avastin®.** Orthogonal mass spectrometry methods confirm the **same primary structure for Alymsys® and Avastin®.**[1,2]



Alymsys® (bevacizumab-maly)

amneal

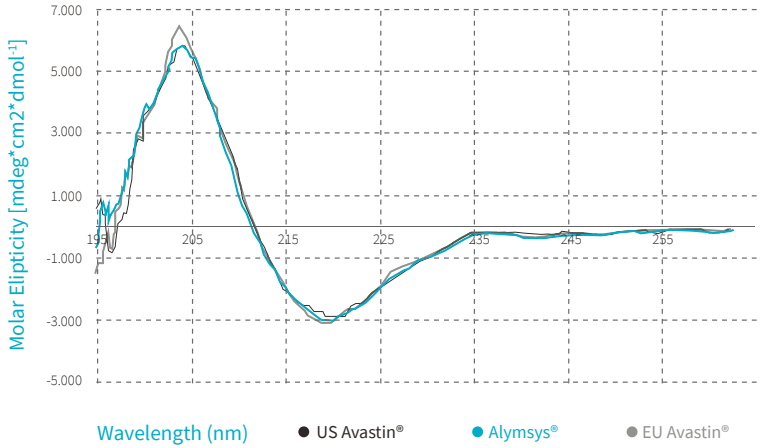
Molecular Conformation

Highly similar molecular conformation between Alymsys® and Avastin®[1,2]

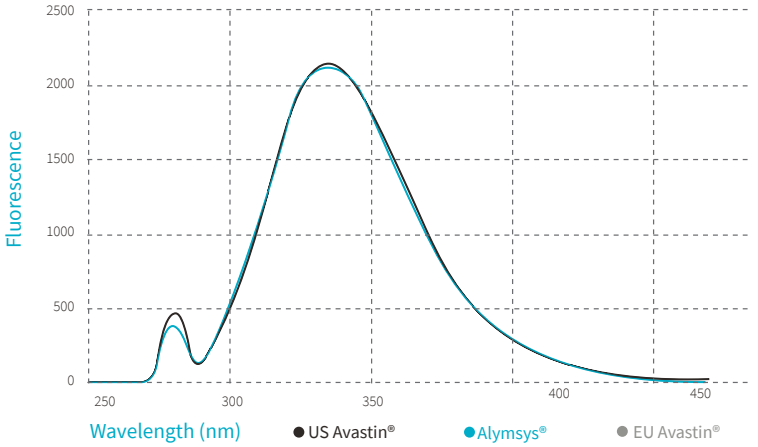
Multiple structural characterization assays have confirmed similar molecular conformation between Alymsys® and Avastin®.[1,2]

Superimposed circular dichroism (CD) and fluorescence spectra confirm **similar secondary and tertiary structure between Alymsys® and Avastin®.**[1,2]

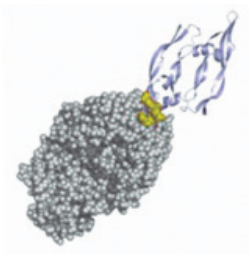
Circular dichroism



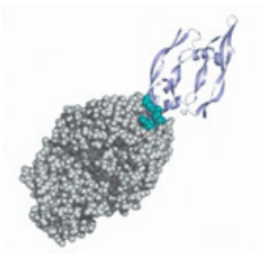
Fluorescence



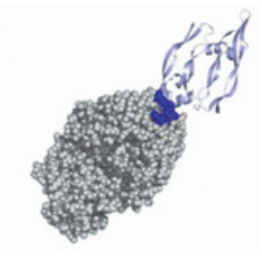
Epitope mapping confirms **same interaction with VEGF 165.**[1,2]



VEGF 165 EU Avastin®



VEGF 165 Alymsys®



VEGF 165 US Avastin®

Alymsys® (bevacizumab-maly)

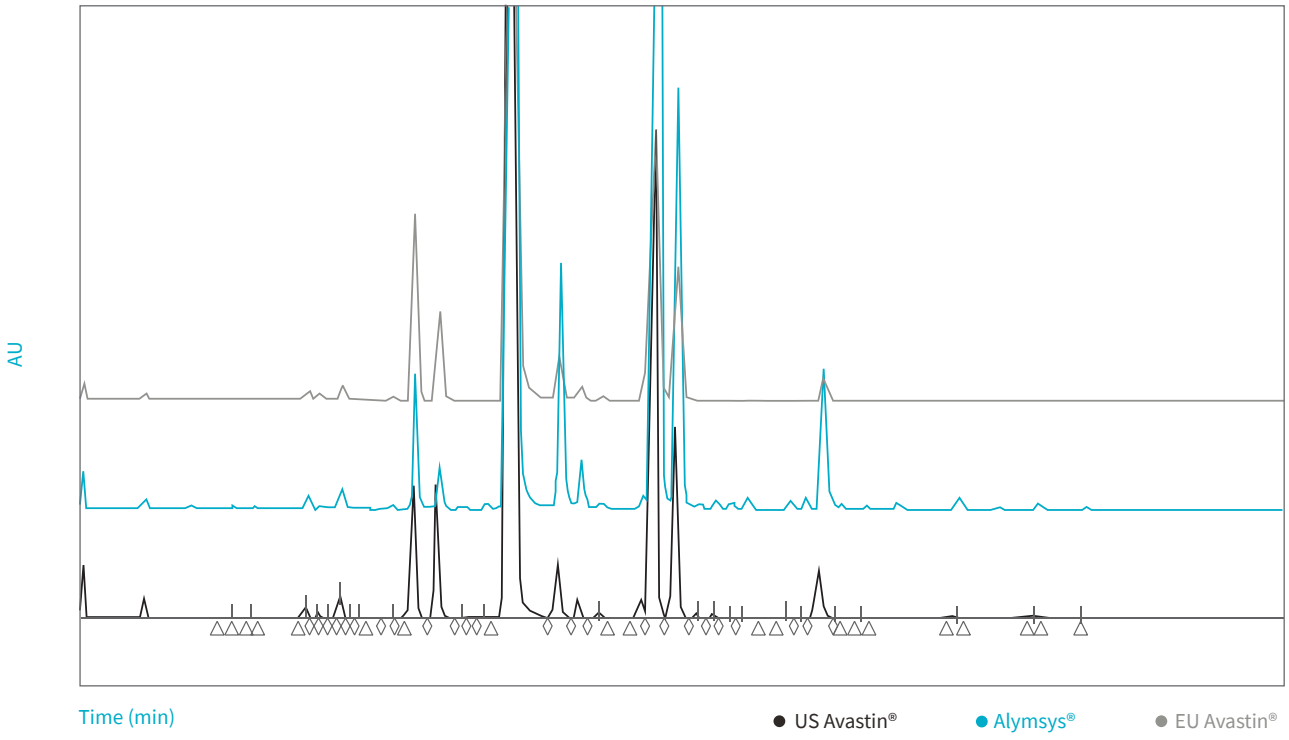
amneal

Glycosylation Profile

Similar N-glycans identity and distribution[1,2]

Protein glycosylation is an important quality attribute which may impact the product's immunogenicity, PK, safety and efficacy. Alymsys® and Avastin® glycosylation profiles have been characterized by multiple assays including chromatography and mass spectrometry methods which have confirmed minor differences which are not clinically meaningful. Variability in glycosylation depends on the cell line and manufacturing process which are specific to each product.[7] The Alymsys® manufacturing process was designed to achieve a quality profile close to the RP and the small differences observed are not clinically meaningful based on bevacizumab mechanism of action (MoA).[1,2,8]

Glycosylation profile by HILIC



HILIC = Hydrophilic Interaction Liquid Chromatography

Alymsys® (bevacizumab-maly)

amneal

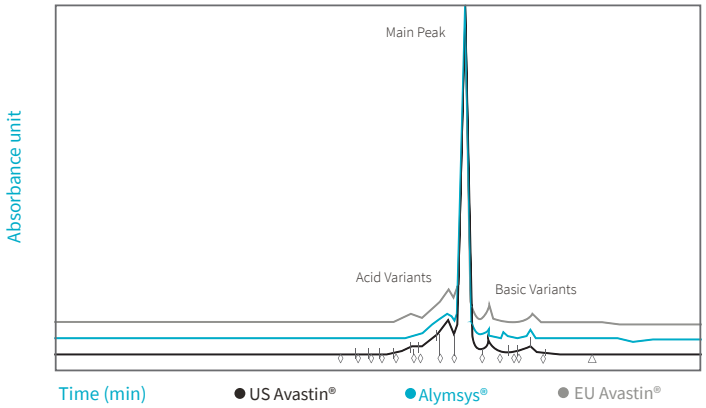
Charge Variants

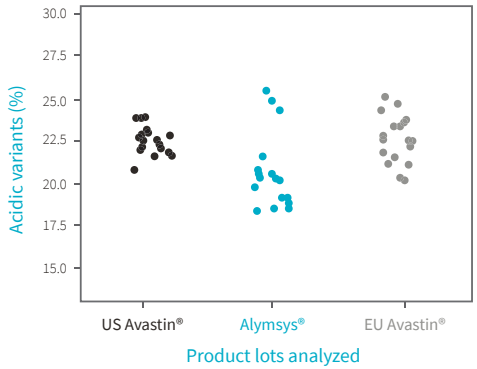
Similar post-translational modifications[1,2]

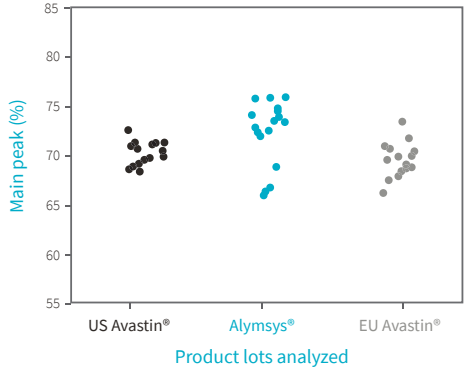
Large proteins exist as multiple charged species, with presence of positively and negatively charged AA and negatively charged glycans (sialic acids). Protein degradation during their shelf life can also create new charges e.g. by oxidation or deamidation of the AA forming the primary sequence. Charge variants must be controlled since they may affect the efficacy and safety of the molecule.[9]

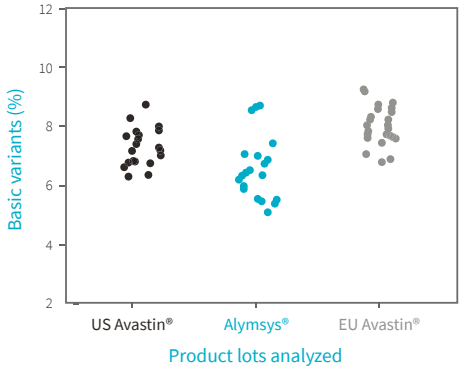
The distribution of charge variants is similar in Alymsys® and Avastin® when considering the age of the samples, as demonstrated by such techniques as cation exchange chromatography and capillary isoelectric focusing.[1,2]

Cation exchange chromatography









Alymsys® (bevacizumab-maly)

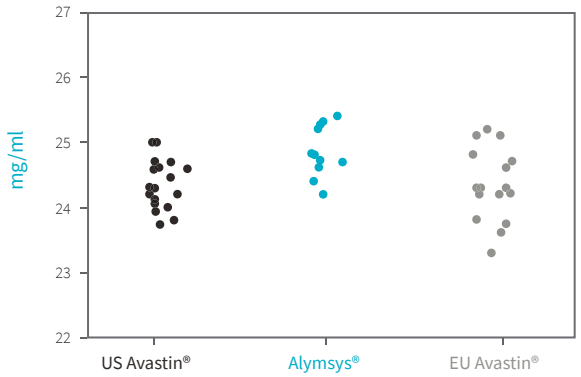
amneal

Protein Content and Purity

Alymsys® and RP have similar protein concentration and purity profile.[1,2]

Similar protein concentration was measured for Alymsys® and Avastin®.[1,2]

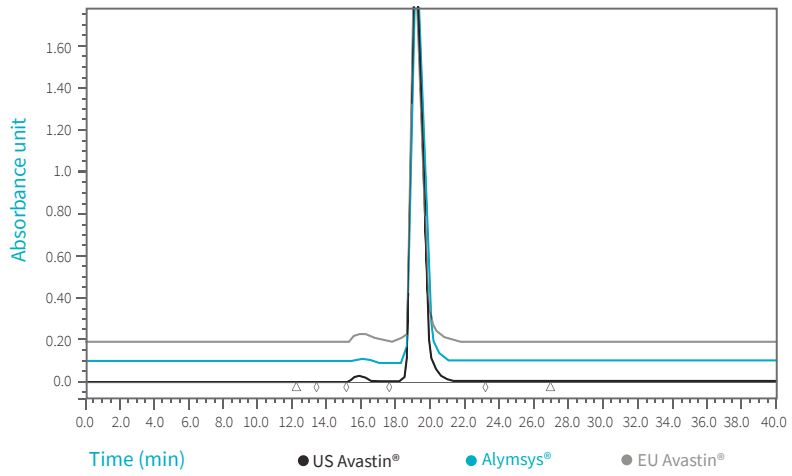
Protein concentration by Ultraviolet



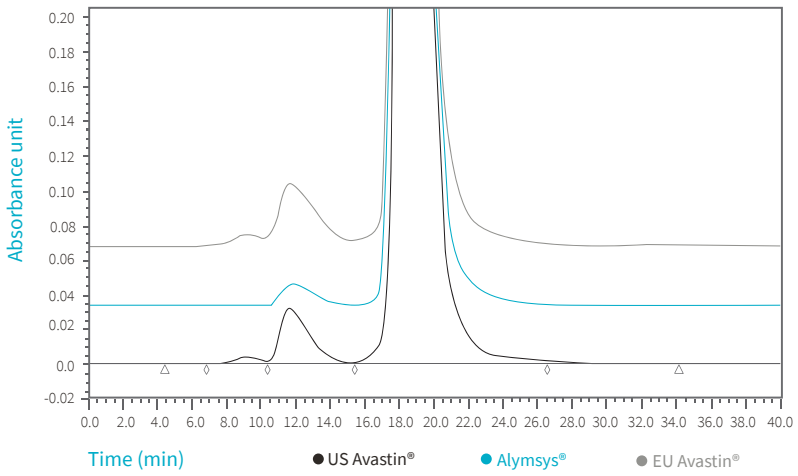
Biotherapeutic monoclonal antibodies are highly pure molecules, but aggregates and fragments may affect the biological activity and safety.[9]

Both Alymsys® and Avastin® are highly pure products with > 95% purity measured by size exclusion chromatography. Low levels of aggregates are present in both products with slightly lower amounts in Alymsys® (higher purity) as confirmed by multiple orthogonal methods.[1,2]

Size exclusion chromatography



Size exclusion chromatography (zoom)



Alymsys® and the RP have been tested for process-related impurities showing similar residual levels in both products.[2]

Alymsys® (bevacizumab-maly)

amneal

Biological Activity

Biological activity in vitro reflects the mechanisms of action in vivo.[1,2]

Bevacizumab, the active ingredient, is a recombinant humanized monoclonal antibody that selectively binds to all human vascular endothelial growth factor A (VEGF A) isoforms with high affinity. Its neutralizes VEGF A by sterically disrupting the ability of VEGF to bind its receptors on the surface of endothelial cells to promote angiogenesis.[10]

Similar binding to VEGF A 165 (figure 1), the main VEGF A variant as well as other variants (121, 189, 206) was demonstrated for Alymsys® and Avastin®.[1,2]

A cells antiproliferation bioassay using human umbilical vein endothelial cells (HUVEC) as a model representing the MoA showed similar potency for Alymsys® and the RP (figure 2).[1,2]

Binding to FcRn*, responsible for the long half life of IgGs, was similar for Alymsys® and the RP (figure 3).[1,2]

Figure 1

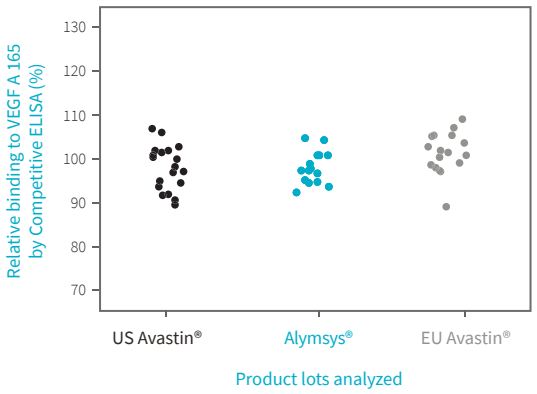


Figure 2

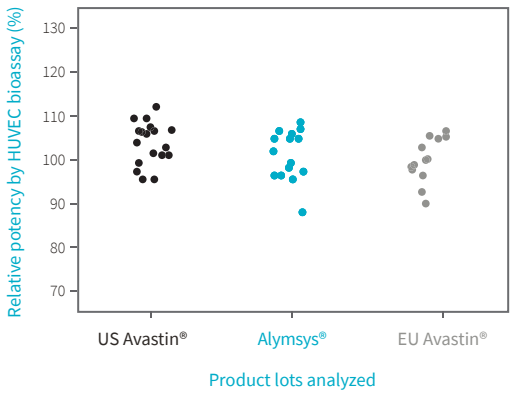
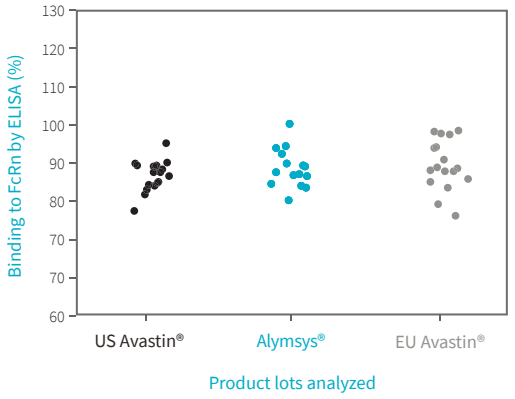


Figure 3



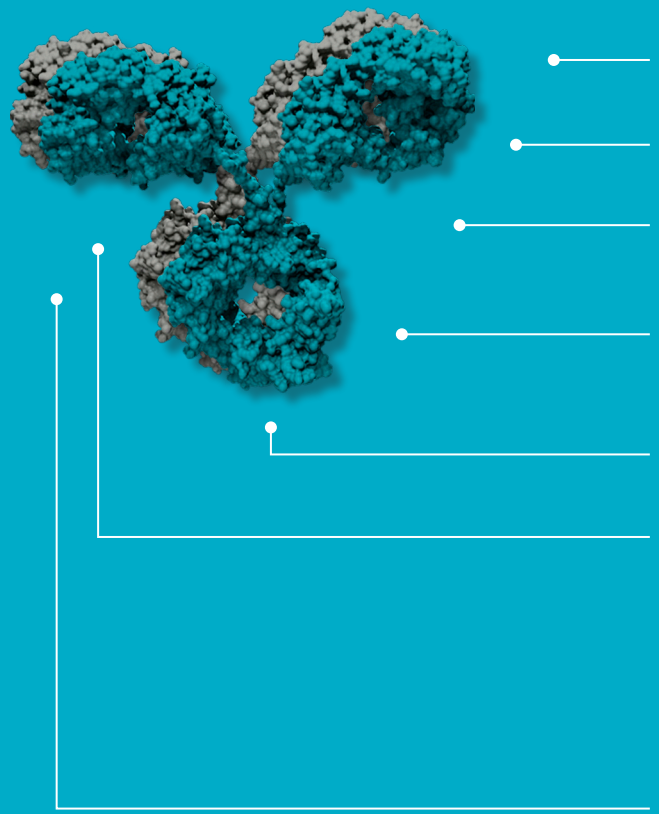
Comparative Stability

Alymsys® and Avastin® stability was compared in studies under accelerated conditions at 25°C and forced degradation conditions such as high temperature (45°C), agitation, oxidative stress, high and low pH and photodegradation. The two products showed similar degradation pathways and kinetics.[2]

Alymsys® (bevacizumab-maly)

amneal

Alymsys® and Avastin® were extensively characterized by orthogonal state-of-the-art assays which demonstrate analytical similarity:[1,2]



Primary structure | The same AA composition is confirmed in Alymsys® and Avastin®.[1,2]

Molecular conformation | Similarity between Alymsys® and Avastin® in terms of molecular conformation was demonstrated.[1,2]

Glycosylation profile | Similar glycoforms profile with the same main glycoforms. Few minor quantitative differences were found which are not clinically meaningful based on bevacizumab mechanism of action which doesn't involve Fc-related activity.[1,2]

Charge variants | The post-translational modifications analysis demonstrated similar relative content of acidic and basic forms between both proteins when considering the age of the sample.[1,2]

Protein content and Purity | Similar protein content and high purity of Alynsys® and Avastin® with slightly less aggregates in Alymsys®.[1,2]

Biological activity | Multiple state-of-the-art orthogonal methods demonstrate the high similarity of Alymsys® to its RP regarding biological activity:
1) Highly similar binding to VEGF 165 and other main VEGF A variants is shown between Alymsys® and Avastin®.[1,2]
2) Three separate bioassays confirm similar VEGF neutralization activity of Alymsys® and Avastin®.[1,2]
3) Similar binding to FcRn, responsible for IgGs long half-life, is found between Alymsys® and Avastin®.[1,2]

Comparative stability: Comparative accelerated and forced degradation studies have shown a similar behavior and degradation profile of Alymsys® and Avastin®.[2]

References:
1. Sacristan D. et al. Analytical Similarity Assessment of Bevacizumab Biosimilar MB02 Using Multiple State-of-the-art Assays. DIA Europe 2021. DOI: https://doi.org/10.26226/morressier.60281ebbc1114ae23b81412f. 2. (a) FDA Summary Basis of Approval of Alymsys (SBoA). April 2022. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/761231Orig1s000TOC.cfm 2. (b) EMA Alymsys® European Public Assessment Report (EPAR). April 2021. https://www.ema.europa.eu/en/documents/assessment-report/alymsys-epar-public-assessment-report_en.pdf. 3. (a) Food and Drug Administration, FDA. Scientific Considerations in Demonstrating Biosimilarity to a Reference Product. Guidance for Industry, 2015. 3. (b) Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues. EMA 2014. 4. Guideline on similar biological medicinal products containing biotechnology derived proteins as active substances: quality issues. (Rev.1). (EMA/CHMP/BWP/247713/2012). 5. CDER/CBER, April 2015. Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product; Guidance for Industry. 6. CDER/CBER, May 2019. Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations; Draft Guidance for Industry. 7. Liu, L. Antibody glycosylation and its impact on the pharmacokinetics and pharmacodynamics of monoclonal antibodies and Fc-fusion proteins. 2015. 8. Trukhin D, Poddubskaya E, Andric Z, et al. STELLA Investigators. Efficacy, Safety and Immunogenicity of MB02 (Bevacizumab Biosimilar) versus Reference Bevacizumab in Advanced Non-Small Cell Lung Cancer: A Randomized, Double-Blind, Phase III Study (STELLA). BioDrugs. 2021 Jul;35(4):429-444. doi: 9. 1007/s40259-021-00483-w. 9. Ambrogelly, A., et al. (2018). "Analytical comparability study of recombinant monoclonal antibody therapeutics." MAbs 10(4): 513-538. 10. (a) FDA Summary Basis of Approval of Avastin (SBoA). August 2004 https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/STN-125085_Avastin.cfm 10. (b) EMA Avastin® EPAR. 2006. https://www.ema.europa.eu/en/documents/scientific-discussion/avastin-epar-scientific-discussion_en.pdf

*Click here for full Prescribing Information for Alymsys®

Alymsys®
(bevacizumab-maly)

Alymsys.us | amneal.com

In partnership with:
mAbxience
From lab to life

amneal