



COMMENT

Aflibercept vs ranibizumab biosimilars: manufacturing differences that might matter in the long run

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Eye (2026) 40:168–169; <https://doi.org/10.1038/s41433-025-04167-5>

The emergence of biosimilars has revolutionised the treatment landscape for retinal diseases, with ranibizumab biosimilars leading the way over the past few years across various global markets [1–3]. Recently, aflibercept 2 mg biosimilars have gained regulatory approval from prominent authorities such as the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA), paving the way for their clinical application. With numerous aflibercept 2 mg biosimilar molecules currently in development, impending approvals are anticipated [4–6].

This article aims to elucidate the key differences between ranibizumab and aflibercept 2 mg biosimilars, focusing on their manufacturing processes. Understanding these differences is crucial, as they may have significant implications for the long-term uptake and adoption of these biosimilar molecules in clinical practice. By examining the nuances of their production, we can better predict their potential impact on market dynamics and patient access to these therapies.

Aflibercept is a recombinant human fusion glycoprotein consisting of two identical polypeptide chains, each comprising the VEGFR1 and VEGFR2 domains fused to the hinge region of the Fc domain of human IgG1. It acts as a decoy receptor that traps circulating VEGF-A, VEGF-B, and placenta growth factor (PlGF) with high affinity. In contrast, ranibizumab was generated by inserting murine anti-VEGF-A complementary determining regions (CDRs) into a consensus human IgG1 framework, which lacks the Fc region. It contains only the Fab fragment of a humanised IgG1 monoclonal antibody, wherein the light chain remains linked to the N-terminal portion of the heavy chain by a disulfide bond.

Escherichia coli (*E. coli*) is a commonly used host cell for protein expression due to its cost-effectiveness and rapid growth rate. However, expressing active protein in *E. coli* is technically challenging, particularly for complex proteins like ranibizumab, an antibody fragment. Ranibizumab consists of two protein chains (Fab fragment of light and heavy chains) that require proper folding and assembly. The two chains are assembled with various protein-binding forces, including intra-chain and inter-chain disulfide bond formation, to adopt the active biological conformation. Unfortunately, *E. coli* often struggles to correctly fold these chains, leading to the formation of inclusion bodies – insoluble, inactive protein aggregates.

To obtain an active, properly folded protein, unique molecular biology techniques have been applied with appropriate plasmid

vector construction. For example, the innovator ranibizumab is produced by *E. coli* using recombinant technology, wherein both the light-chain and heavy-chain genes linked in tandem are cloned in a plasmid vector. The two subunits are expressed by the cells in a soluble form and secreted into the periplasmic space, where they are folded into their native conformation and joined by a covalent disulfide bond. In another instance (by a different manufacturer), ranibizumab is produced in the form of inclusion bodies in *E. coli*, wherein the two chains are expressed independently by separate *E. coli* cell culture processes, followed by isolation, solubilisation, oxidative refolding of the two chains, purification, and membrane ultrafiltration or diafiltration to obtain the desired protein. This process can be lengthy, cumbersome, and labour-intensive. Both processes may result in a low yield of protein [7, 8].

Aflibercept is produced using Chinese Hamster Ovary (CHO) cells, a widely used mammalian host-cell system for biopharmaceutical manufacturing. CHO cells offer several advantages, including proper protein folding, glycosylation, and secretion, which are crucial for producing complex recombinant proteins like aflibercept. The CHO cell line used for aflibercept production is engineered to express the recombinant fusion protein, which consists of VEGFR1 and VEGFR2 receptor domains fused to the Fc region of human IgG1. The protein is expressed in its active, folded form and secreted into the culture medium by the cells, isolated, and purified by conventional chromatography techniques, including affinity and interaction column chromatography in combination with membrane ultrafiltration or diafiltration. The use of mammalian CHO host cells ensures that aflibercept meets the required quality and regulatory standards for human use [9]. This process eliminates the need for refolding, reducing the complexity and potential variability associated with the production process.

The yields of aflibercept and ranibizumab during manufacturing can differ significantly due to the distinct expression systems and processes employed. Aflibercept, produced in CHO cells, typically involves a more controlled cell culture process to obtain the desired yield and Quality Target Product Profile (QTPP). Aflibercept expression levels in CHO cells have been found to be fairly high. Additionally, the downstream purification process is straightforward, as the aflibercept protein is secreted by the cells in a soluble, folded form, and an affinity column chromatography step is involved in the process. In contrast, ranibizumab, when

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Received: 23 July 2025 Revised: 11 November 2025 Accepted: 3 December 2025

Published online: 10 December 2025

expressed in inclusion bodies in *E. coli*, often faces challenges in terms of extracting the protein through cell lysis and proper refolding, followed by purification, which can affect the overall yield and quality of ranibizumab. When expressed in its soluble, folded form in the periplasmic space of *E. coli* cells, the yield of ranibizumab is not expected to be high. However, in both cases, the downstream process is critical to obtaining ranibizumab protein with the desired level of purity and target product quality.

In conclusion, the distinct manufacturing processes of aflibercept 2 mg biosimilars and ranibizumab biosimilars may influence their cost-effectiveness. Given aflibercept's widespread adoption and popularity among retina specialists, with a maximum market share [10], the biosimilar version of it is likely to follow a similar trajectory, potentially driving cost savings through increased competition. While innovator molecules have unique pricing strategies, the manufacturing process of biosimilars may play a crucial role in determining the accessibility and affordability of these products for patients. If aflibercept 2 mg biosimilars achieve a high adoption rate, manufacturers may offer a cost-effective alternative to other available anti-VEGF therapies, including innovators and ranibizumab biosimilars, ultimately benefiting patients and healthcare systems.

REFERENCES

- Sharma A, Kumar N, Parachuri N, Bandello F, Kuppermann BD, Loewenstein A. Biosimilars for Retinal Diseases: An Update. *Am J Ophthalmol*. 2020;224:36–42.
- Sharma A, Reddy P, Kuppermann BD, Bandello F, Lowenstein A. Biosimilars in ophthalmology: "Is there a big change on the horizon?". *Clin Ophthalmol*. 2018;12:2137–43.
- Sharma A, Kondo M, Iwahashi C, Parachuri N, Kumar N, Bandello F, et al. Approved biosimilar ranibizumab—a global update. *Eye*. 2022. <https://doi.org/10.1038/s41433-022-02246-5>.
- Lee H, Sharma A, Woo SJ. Clinical Trials of Aflibercept Biosimilars: A Review. *J Ocul Pharmacol Ther*. 2025. <https://doi.org/10.1089/jop.2025.0040>.
- Sharma A, Loewenstein A, Kumar N, Parachuri N, Bandello F, Kuppermann BD. Aflibercept biosimilars - update on the development progress. *Eye*. 2023. <https://doi.org/10.1038/s41433-023-02813-4>.
- Sharma A, Woo SJ, Lee CS, Kumar N, Parachuri N, Bandello F, et al. Aflibercept 2 mg biosimilars-will they lead the Anti-VEGF biosimilar world globally. *Eye (Lond)*. 2025;39:1026–7.
- Tungekar AA, Fulewar P, Kumthekar R, Bhambure R. Understanding in-vivo refolding of antibody fragments (Fab): Biosimilar Ranibizumab a case study. *Process Biochem*. 2024;146:484–97.
- Cloning, expression and purification method for the preparation of Ranibizumab. US Patent application no.: US 2016/0289314A1.
- European Medicines Agency. Eylea (aflibercept) solution for injection: EU Summary of Product Characteristics. 2022. https://www.ema.europa.eu/en/documents/product-information/eylea-epar-product-information_en.pdf. Accessed on July 08, 2025.
- <https://www.coherentmarketinsights.com/industry-reports/eylea-market#:~:text=Eylea%20Market%20Insights%2C%20By%20Dosage,by%20the%20%20mg%20dosage>. Accessed on July 08, 2025.

AUTHOR CONTRIBUTIONS

AS: conception, analysis, drafting, integrity check, final approval. FB, SB, NK, NP; drafting, revision, analysis, integrity check.

COMPETING INTERESTS

Ashish Sharma: CONSULTANT: for Novartis, Allergan, Bayer, Lupin, Sun and Intas, Speaker fee MS Pharma. Francesco Bandello: CONSULTANT: Allergan, Bayer, Boehringer- Ingelheim, FidiaSooft, Hofmann La Roche, Novartis, NTC Pharma, Sifi, Thrombogenics, Zeiss. Sanjay Bandyopadhyay: Employee of Zydus Lifesciences. Nikulaa Parachuri: None. Nilesh Kumar: None.

ADDITIONAL INFORMATION

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