ORIGINAL RESEARCH

Evaluation of the Structural, Physicochemical, and Biological Characteristics of SB11, as Lucentis® (Ranibizumab) Biosimilar

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ABSTRACT

Introduction: SB11 was recently approved as a ranibizumab biosimilar by the US Food and Drug Administration (FDA) and the European Commission (EC) as a therapy for retinal vascular disorders under the brand name Byooviz™. This study was performed to assess the analytical similarity between SB11 and reference products from the European Union (EU-ranibizumab) and United States (US-ranibizumab).

Methods: A comprehensive structural, physicochemical, and biological characterization was performed utilizing state-of-the-art analytical methods. Comparisons included the following: primary structure related to amino acid sequence and post-translational modifications; higher order structure; product-related substances and purity/impurity including size and charge variants. In addition, biological characterization included a series of mechanism of action (MoA)-related bioassays such as vascular endothelial growth factor (VEGF)-A binding assay (VEGF-A 165 and its isoforms), cell-based VEGF-A 165 neutralization assay, and anti-proliferation assay using human umbilical vein endothelial cells (HUVEC).

Results: The amino acid sequence of SB11 was identical to that of reference products, and post-translational modification profiles and higher order structures of SB11 were shown to be indistinguishable from the reference products. Product-related size and charge variants and aggregates were also similar. Using a broad range of VEGF-related functional assays, we demonstrated that SB11 has similar biological properties to reference products in VEGF-A binding activities (VEGF-A 165 and isoforms (VEGF-A 110, VEGF-A 121, and VEGF-A 189)), VEGF-A 165 neutralization, and HUVEC anti-proliferation. Overall, SB11 exhibits high similarity compared to EU/US-ranibizumab.

Conclusion: Based on the comprehensive analytical similarity assessment, SB11 is highly similar to the EU/US-ranibizumab with respect to structural, physicochemical, and biological properties.

Keywords: Biosimilar; Byooviz™; Lucentis®; Ranibizumab; SB11; Samsung bioepis

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Key Summary Points

SB11 has been developed as a ranibizumab biosimilar and approved by the US Food and Drug Administration (FDA) and European Commission (EC).

SB11 demonstrated high similarity with reference products (EU/US-ranibizumab) in terms of structural, physicochemical, and biological properties.

INTRODUCTION

The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PLGF) controls angiogenesis and vascular permeability. In particular, VEGF-A, which is overexpressed in retinal cells, is a critical mediator of neovascular ocular diseases due to ocular angiogenesis and vascular permeability. Therefore, anti-angiogenic therapy by VEGF-A neutralization is the standard of care for treating pathological neovascularization diseases [1–5]. Ranibizumab inhibits the interaction between VEGF-A and VEGF-A receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells to prevent VEGF-A signaling, thereby inhibiting cell functions such as proliferation, permeabilization, migration, and angiogenesis [6].

Lucentis® is a humanized anti-VEGF antibody fragment (Fab) approved by the US FDA and EC and other countries for the treatment of retinal vascular diseases that cause visual loss or blindness due to angiogenesis promotion in the eyes. The indications of retinal vascular diseases include neovascular age-related macular degeneration (nAMD), proliferative diabetic retinopathy (PDR), diabetic macular edema (DME), myopic choroidal neovascularization (CNV), and macular edema following retinal vein occlusion (RVO) [7, 8]. SB11 has been developed as a biosimilar of Lucentis® and obtained marketing authorization in the EU and US in September 2021 as the first biosimilar of Lucentis® [nAMD, PDR, DME, CNV, and RVO (branch RVO or central RVO) for EU and nAMD, CNV, and RVO for US] [9, 10].

Biosimilar development requires a stepwise approach to demonstrate similarity between a proposed biosimilar and an authorized original biological medicine (reference product). The foundation of the biosimilarity assessment is a comprehensive structural and functional similarity evaluation using state-of-the-art analytical techniques [11–14]. To determine the analytical similarity of SB11 and EU/US-ranibizumab, the products were characterized in terms of the primary structure, higher order structures, and physicochemical and functional properties. In this study, a pre-defined quality range approach with EU/US-ranibizumab or side-by-side comparison was applied according to the quality attribute characteristics. The data presented in this journal demonstrate that the comprehensive characteristics of SB11 and EU/US-ranibizumab are very similar.

METHODS

This article does not contain any studies with human participants or animals performed by any of the authors. All SB11 samples were prepared and experiments conducted aseptically in a biological safety cabinet.

Reference Products

EU/US-ranibizumab, which expired from December 2016 to August 2021, was purchased from local distributors and then stored according to the manufacturer's instructions.

Peptide Mapping

Peptide mapping was performed to analyze structural integrity, including post-translational modifications and disulfide bond by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI–MS/MS). To achieve denaturation and reduction, ranibizumab was mixed with 8 M urea and 1 M dithiothreitol; 1 M iodoacetamide was added. The sample was

digested with trypsin (11047841001, Roche, Basel, Switzerland), Lys-C (90051, Thermo scientific, Waltham, MA, USA), and Asp-N (11058541103, Roche). For the disulfide linkage analysis, the reducing step was not performed, and the non-reduced samples were digested with trypsin. The digestion samples underwent reverse-phase ultra performance liquid chromatography-mass spectrometry (RP-UPLC-MS) using a BEH300 C18 column (186003687, Waters, Milford, MA, USA) at 60 °C. Peptides were eluted by a linear gradient at a flow rate of 0.3 ml/min for 100 min. Data were collected and processed by MassLynx v4.1 (Waters) and/ or BiopharmaLynx v1.2 (Waters).

Circular Dichroism Spectroscopy

For far-UV studies, ranibizumab was dialyzed with 10 mM sodium citrate buffer and diluted with 10 mM sodium citrate. For near-UV studies, the samples were not dialyzed but instead diluted with SB11 formulation buffer. Circular dichroism spectroscopy (CD) measurements were performed using a Chirascan Q100 (Applied Photophysics, Leatherhead, UK) with 0.1-mm pathlength cells for far-UV and 10-mm pathlength cuvette for near-UV. The observed CD data in ellipticity for each sample were blank-subtracted and the average of the triplicate scans was used to make the CD plot. The CDNN algorithm was used to fit the far-UV CD data for prediction of secondary structure.

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) spectra were recorded from a Nicolet iS50 FT-IR spectrophotometer (Thermo Scientific), equipped with a Smart Orbit diamond ATR accessory and the OMNIC software package for spectrometer control and data analysis. All spectra were acquired at 4 cm^{-1} resolution between 4000 and 525 cm^{-1}, and a background scan with the matching SB11 formulation buffer was acquired prior to a scan of each sample. Ranibizumab spectra were partitioned into peak areas according to structural contribution and

the results averaged over three replicates per sample.

Differential Scanning Calorimetry

Nano-differential scanning calorimetry (DSC) (TA Instruments, New Castle, DE, USA) was used to analyze the melting temperature of samples. The ranibizumab and SB11 formulation buffer were scanned from 15 to 100 °C using a scan rate of 1.5 °C/min. Ranibizumab was diluted with the SB11 formulation buffer prior to the run. To determine the baseline value, the SB11 formulation buffer was loaded into a sample channel and scanned to verify a reproducible baseline for subtraction from sample data. Data were analyzed by TA Instruments NanoAnalyze software.

Hydrogen/Deuterium Exchange-Mass Spectrometry

Hydrogen/deuterium exchange-mass spectrometry (H/DX-MS) was adapted to compare higher order structures between ranibizumab samples. H/DX-MS was initiated by a 1:8 dilution of sample in D_2O buffer at intervals of 10 s, 1 min, 10 min, 1 h, and 4 h before quenching and injecting into the mass spectrometer. Peptides were digested on an immobilized pepsin column, and the trapped peptide fragments were eluted by a gradient of 8% to 92% acetonitrile in 15 min. Mass spectra were collected in MS^E mode, and data were analyzed by ProteinLynX Global Server^{TM} (Waters) to identify peptides and by DynamX software (Waters) to calculate deuterium uptake and generate butterfly and difference plots.

Size Exclusion-High Performance Liquid Chromatography

Ranibizumab was injected onto a TSK-Gel Super SW2000 SW$_{XL}$ analytical column (0018674, Tosoh, Tokyo, Japan), which was connected to a Waters HPLC system; monitoring was done by UV detection at 280 nm. The flow rate was 0.2 ml/min. Data were acquired and processed by Empower®3 software (Waters).

Capillary Electrophoresis-Sodium Dodecyl Sulfate

Reduced and non-reduced capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) analyses were conducted with a high-performance capillary electrophoresis system (PA800 plus Pharmaceutical Analysis System; SCIEX, Framingham, MA, USA). For the reduced condition, ranibizumab was mixed with a 10-kDa internal standard, SDS-MW sample buffer (A10663, Beckman Coulter, Brea, CA, USA), and 2-mercaptoethanol and then boiled at 70 °C for 5 min. For the non-reduced condition, 2-mercaptoethanol was replaced with iodoacetamide. The sample was electrokinetically introduced onto a capillary (base fused-silica capillary; Beckman Coulter) by applying voltage at -5 kV for 20 s and separated in the capillary cartridge. Electrophoresis was performed at a constant voltage with an applied field strength of -497 V. Data were acquired and processed by 32 Karat software (SCIEX) with integration capabilities.

Imaged Capillary Isoelectric Focusing

Ranibizumab was mixed with pharmalyte 3–10, pharmalyte 8–10.5, 1% methyl cellulose, distilled water, pI 6.61 marker, and pI 9.5 marker. The mixture was loaded onto an iCE3 icIEF instrument (ProteinSimple, San Jose, CA, USA) using a capillary cartridge at 4 °C. Data were acquired by CFR software (ProteinSimple) and processed by Empower®3 software (Waters).

VEGF-A 165/189/121/110 Binding Assay

Relative binding activities of ranibizumab to VEGF-A 165/189/121/110 were measured using an enzyme-linked immunosorbent assay (ELISA). A recombinant human vascular endothelial growth factor (rhVEGF), VEGF-A 165 (293-VE/CF, R&D Systems, Minneapolis, MN, USA), VEGF-A 189 (8147-VE/CF, R&D Systems), VEGF-A 121 (4644-VS/CF, R&D Systems), and VEGF-A 110 (5336-VE-010/CF, R&D Systems), was absorbed onto a 96-well plate. Serial dilutions of ranibizumab bound to rhVEGF followed by the sequential addition of anti-human IgG (Fab-specific)-peroxidase antibody, tetramethylbenzidine (TMB, T0440, Sigma-Aldrich, St. Louis, MO, USA) substrate, and 1 N sulfuric acid. The measurement of the absorbance at a wavelength of 450 nm quantitated the relative binding activity of ranibizumab to rhVEGF. The VEGF binding activity of ranibizumab sample was calculated relative to a reference standard using a four-parameter logistic model [PLA (Parallel Line Analysis); Stegmann Systems GmbH, Rodgau, Germany].

HUVEC Anti-Proliferation Assay (VEGF-A 165)

HUVEC (C2519A, Lonza, Basel, Switzerland) in endothelial cell growth medium (EBM™-2, CC-3156, Lonza) was treated on microplates where VEGF A-165 (293-VE/CF, R&D systems) and serially diluted ranibizumab were pre-incubated and proliferated for 4 days at 37 °C, 5% CO_2 incubator. The relative cell proliferation was measured by CellTiter-Blue® cell viability assay kit (G8082, Promega, Madison, WI, USA) using a SpectraMax (M3; Molecular Devices, San Jose, CA, USA). Anti-proliferation activity was calculated relative to a reference standard four-parameter logistic model (PLA; Stegmann Systems GmbH).

VEGF-A 165 Neutralization Assay

Engineered cell lines for VEGF signaling (NFAT-RE-*Luc2P*/KDR 293 cells, E8510, Promega) in assay media (DMEM, 11995, Gibco, Grand Island, NY, USA) were treated on microplates where VEGF A-165 (293-VE/CF, R&D systems) and serially diluted ranibizumab had been pre-incubated. After cell treatment, the microplate was incubated at 37 °C, 5% CO_2 incubator for 3.5–6 h to induce luciferase gene expression by VEGF signaling. The relative VEGF neutralizing activity was measured by a luminescent detection system (Steady-glo®, E2520, Promega) via microplate reader (EnVision, 2104, Perkin Elmer, Waltham, MA, USA). VEGF neutralization activity was calculated relative to a reference standard four-parameter logistic model (PLA; Stegmann Systems GmbH).

RESULTS

To demonstrate the similarity between SB11 and the EU/US-ranibizumab, the analytical characterization was performed using state-of-the-art methods, the primary structure, higher order structure, and physicochemical and functional activities.

The analytical methods utilized for the similarity assessment are summarized in Table 1.

Primary Structure

The peptide chromatograms of trypsin or Lys-C digested SB11 and EU/US-ranibizumab showed a highly similar peak profile without missing or additional new peaks with comparative retention time (Fig. 1A, B). In addition, the data confirmed that the amino acid sequences of SB11 and EU/US-ranibizumab were identical and that the sequence coverage by MS/MS was 100% (data not shown). Based on the results of intact mass and peptide mapping analysis, the

Table 1 Summarized quality attributes

Category	Product quality attributes		Analytical methods
Primary Structure	Molecular weight		LC-MS
	Amino acid sequence		LC-ESI-MS/MS
	Peptide mapping		
	Oxidation		
	Deamidation		
	Disulfide bond		
Higher order Structure	Secondary and tertiary structure—CD spectra		CD spectrometry (far-UV, near-UV)
	Secondary structure		FT-IR
	Thermal stability/heat-induced protein denaturation pattern		DSC
	Aggregates characteristics/size and shape of macromolecules		SV-AUC, SEC-MALS
	Protein tertiary structure/conformation and conformational dynamics		H/DX/MS
Purity and impurities	%Monomer and %HMW		SE-HPLC
	%Main species		CE-SDS (non-reducing/reducing)
Charge variants	Acidic and basic variants		CEX-HPLC, icIEF
Biological activity	MoA-related biological activities	Anti-proliferation activity	Cell-based assay
		VEGF-A 165 binding	ELISA
		VEGF-A 165 neutralization	Cell-based assay
	Additional biological activities	VEGF-A 121 binding	ELISA
		VEGF-A 110 binding	ELISA
		VEGF-A 189 binding	ELISA
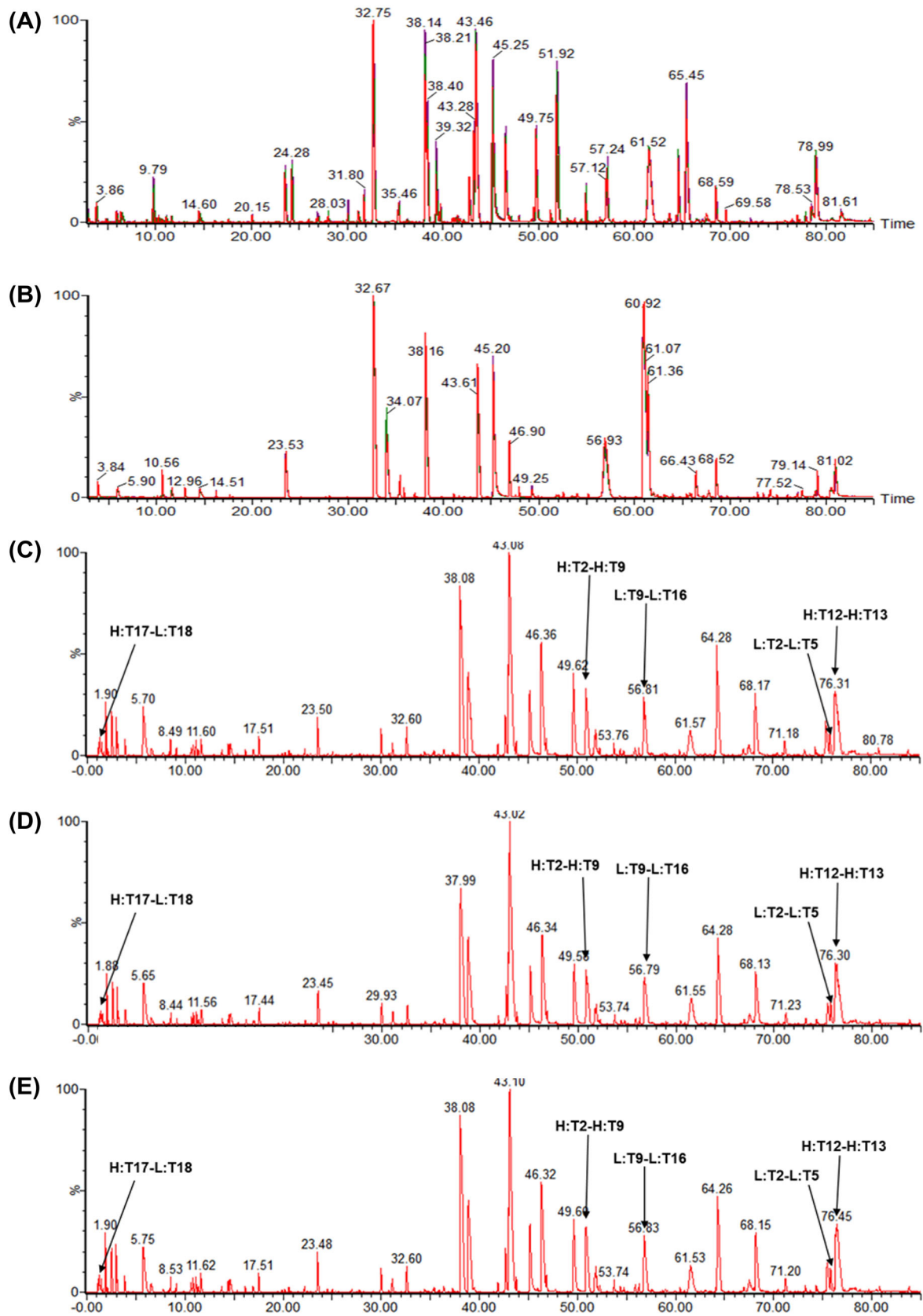
◄**Fig. 1 A, B** Overlay of the peptide mapping profiles of SB11 (red), EU-ranibizumab (purple), and US-ranibizumab (green). Trypsin-digested condition (**A**), Lyc-C-digested condition (**B**). **C–E** Comparison of disulfide linked peptides of SB11 (**C**), EU-ranibizumab (**D**), and US-ranibizumab (**E**)

molecular weights of SB11 and EU/US-ranibizumab were identical (considering assay variability; 0.01% of molecular weight and 30 ppm for intact protein and peptide, data not shown). Sequence variants generated by post-translational modifications such as oxidation and deamidation were identified. The CDR region of Fab contains methionine (Met) residues that are susceptible to oxidation and asparagine (Asn) residues that are susceptible to deamidation [15]. The relative levels of Met oxidation and Asn deamidation were similarly very low (< 0.4%) in all samples (data not shown). Disulfide bond analysis of SB11 and EU/US-ranibizumab under non-reducing condition after trypsin digestion showed that all ten cysteine residues are properly involved in the formation of the five disulfide bonds (Fig. 1C–E, Table 2).

Higher Order Structures

The higher order structures of SB11 and EU/US-ranibizumab were compared using CD, FT-IR

spectroscopy, and DSC. The far UV CD spectrum of SB11 and EU/US-ranibizumab showed comparable profiles throughout much of the far-UV region, especially at the local minimum at around 218 nm, which strongly corresponds to the secondary structure of protein folding (Fig. 2A). However, as the curves reach the far-UV region lower than about 210 nm, there was some divergence within samples. In this region, the buffer absorbance and concentration differences can play a role; this non-overlap does not necessarily correlate with a difference in protein folding. In addition, the samples showed comparable near-UV CD profile maxima at 258 nm, 265 nm, 285 nm, and 291 nm, respectively (Fig. 2B). No significant differences between the samples were observed, suggesting the samples share common tertiary structure characteristics. Secondary structure was further identified by FT-IR spectroscopy. The second derivative spectra of SB11 and EU/US-ranibizumab in the amide I band (1600–1700 cm^{-1}) and their distribution into the amide regions support high similarity in secondary structures (Fig. 2C). The DSC thermograms of samples showed highly similar thermal profiles and thermal transition midpoint temperatures, indicating that the thermal stability and conformation of SB11 are highly similar to those of EU/US-ranibizumab (Fig. 2D). Tertiary structure was also determined using H/DX-MS. The

Table 2 Detected disulfide-linked peptides and experimental masses for representative batches of SB11 and EU/US-ranibizumab

Type of disulfide bond		Cysteine sites	Theoretical mass (Da)	Theoretical m/z (charge state)	Experimentally detected m/z		
					SB11	EU-ranibizumab	US-ranibizumab
Heavy chain	H[a]:T[b]2–H:T9	Cys_{22}–Cys_{96}	3407.47	682.50	682.50	682.50	682.50
	H:T12–H:T13	Cys_{150}–Cys_{206}	7916.92	1132.00	1132.00	1132.00	1132.00
Light chain	L[c]:T2–L:T5	Cys_{23}–Cys_{88}	7343.40	1469.69	1469.69	1469.69	1469.69
	L:T9–L:T16	Cys_{134}–Cys_{194}	3555.75	712.16	712.16	712.16	712.16
Interchain	H:T17–L:T18	Cys_{226}–Cys_{214}	756.24	757.25	757.25	757.25	757.25

[a]Heavy chain
[b]Tryptic peptide
[c]Light chain

dynamics of deuterium uptake over time (10 s to 4 h) revealed high symmetry between SB11 and EU/US-ranibizumab (Fig. 2E, F).

Purity and Impurity

The purity of the products was analyzed via product monomer content and amount of intact Fab, heavy chain, and light chain determined by SE-HPLC and CE-SDS, respectively. In SE-HPLC analysis, all samples showed prominent monomer peaks (Fig. 3A, B). The level of high molecular weight (%HMW) species of SB11 and EU/US-ranibizumab was comparably low (Table 3). The results were confirmed by orthogonal analyses using size exclusion chromatography-coupled multi-angle light scattering (SEC-MALS) and sedimentation velocity analytical ultracentrifugation (SV-AUC) (data not shown). In CE-SDS analysis, the results showed that the electrophoretic profiles of SB11 and EU/US-ranibizumab were similar (Fig. 3C, D). The level of intact Fab species (%Main for non-reduced CE-SDS), heavy chain (%Main 2 for reduced CE-SDS), and light chain (%Main 1 for reduced CE-SDS) in SB11 were similar to those of EU/US-ranibizumab (Table 3).

Charge Heterogeneity

To assess charge variants of SB11 and EU/US-ranibizumab, icIEF analysis was performed. The results of icIEF showed that the isoelectric point (pI) values of all samples were identical and their electropherograms were similar (Fig. 3E, F). The results for %Main, %Acidic, and %Basic of all samples were comparable (Table 3). These results have been confirmed by orthogonal analytical method which is cation exchange-high performance liquid chromatography (CEX-HPLC) (data not shown).

Biological Activity

As MoA-related biological activities of ranibizumab reflect physiological and pathological conditions, the VEGF-A 165 binding, VEGF-A 165 neutralization assay and anti-proliferation assay were performed. Additionally, binding

activities for VEGF-A isoforms, excluding the VEGF-A 165, were evaluated to demonstrate similarity between SB11 and EU/US-ranibizumab.

MoA-Related Biological Activities

Reference product lots with different expiry dates within the approved shelf life were used for establishing similarity ranges (mean ± 3 SD). SB11 drug products manufactured at commercial scale were compared with EU-ranibizumab and US-ranibizumab for the biological activities. Evaluation of the VEGF-A 165 binding activity by ELISA showed the mean %relative binding activity of SB11, EU-ranibizumab, and US-ranibizumab were 98%, 100%, and 100%, respectively. No meaningful differences between SB11, and EU/US-ranibizumab were observed and VEGF-A 165 binding activities of SB11 were within the similarity range (Fig. 4A). The relative VEGF-A 165 neutralization potency of SB11 was similar to that of EU/US-ranibizumab. Mean %relative potencies of SB11, EU-ranibizumab, and US-ranibizumab were 99%, 100%, and 101%, respectively. In addition, VEGF-A 165 neutralization potencies of SB11 were within predetermined mean ± 3 SD of similarity range (Fig. 4A). For the HUVEC anti-proliferation assay, the mean values of %relative anti-proliferation potencies of SB11, EU-ranibizumab, and US-ranibizumab were 100%, 101%, and 102%, respectively. All SB11 batches also showed relative potencies within the similarity range, demonstrating promising similarity in HUVEC anti-proliferation potency (Fig. 4A). These results support that SB11 is similar to EU/US-ranibizumab in terms of MoA-related biological activities.

Additional Biological Activities

Additional biological activities and VEGF-A 110, VEGF-A 121, and VEGF-A 189 binding activities were assessed of SB11 in a side-by-side comparison. Mean values of %relative VEGF-A 110 binding of SB11, EU-ranibizumab, and US-ranibizumab were 102%, 98%, and 101%, respectively, and those of %relative VEGF-A 121
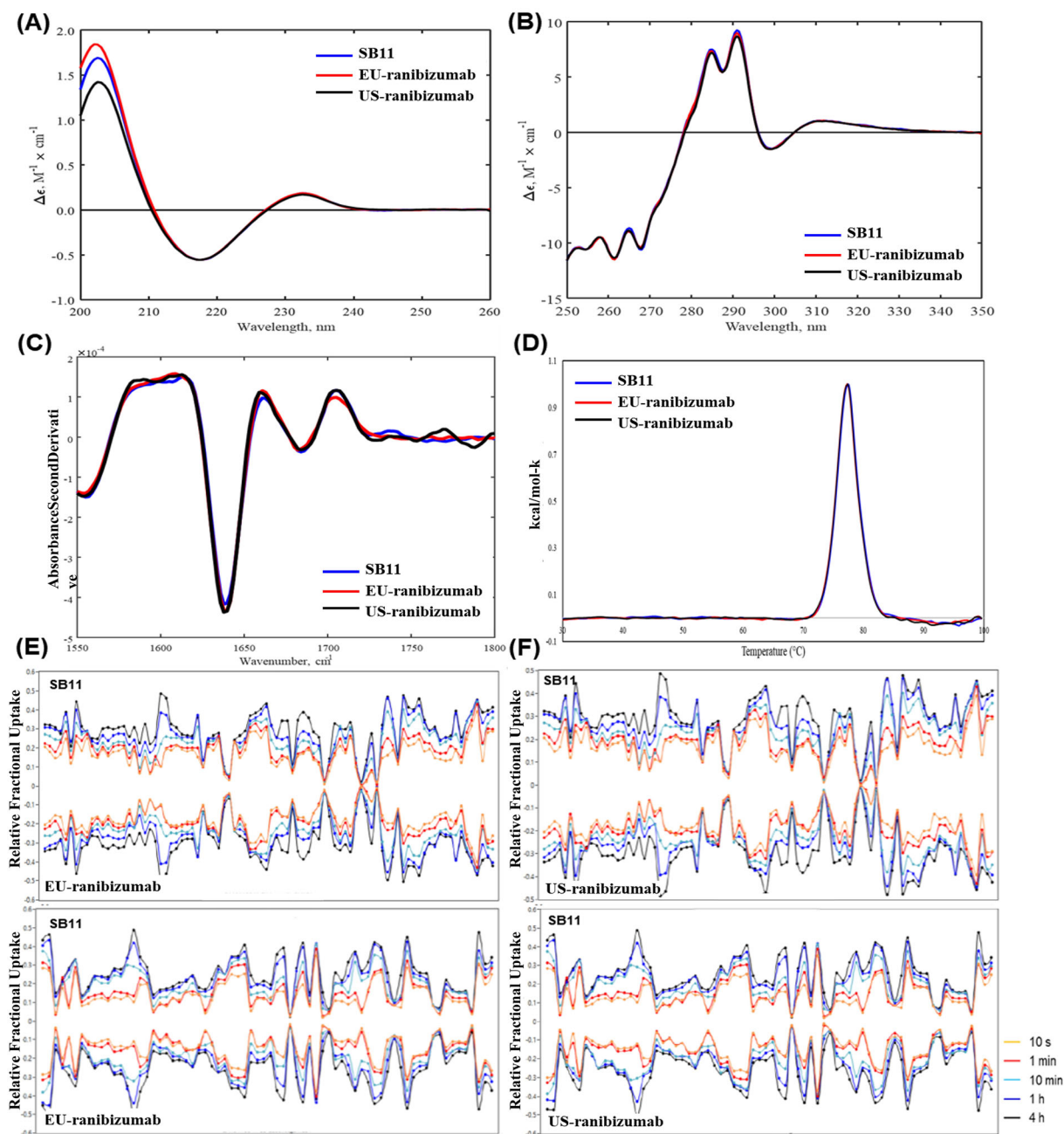
Fig. 2 A–D Comparison for higher order profiles of SB11 and EU/US-ranibizumab. **A** Far-UV CD spectra, **B** near-UV CD spectra, **C** second derivative FT-IR spectra, **D** DSC thermograms. **E,F** Comparison deuterium uptake profiles of SB11 and EU/US-ranibizumab. **E** Butterfly plot of SB11 and EU-ranibizumab for heavy chain (top) and light chain (bottom). **F** Butterfly plot of SB11 and US-ranibizumab for heavy chain (top) and light chain (bottom)

binding activities were 101%, 103%, and 98%, respectively. In addition, mean values of %relative VEGF-A 189 binding activities of SB11, EU-ranibizumab, and US-ranibizumab were 99%, 97%, and 96%, respectively. No differences were observed between SB11 and EU/US-ranibizumab
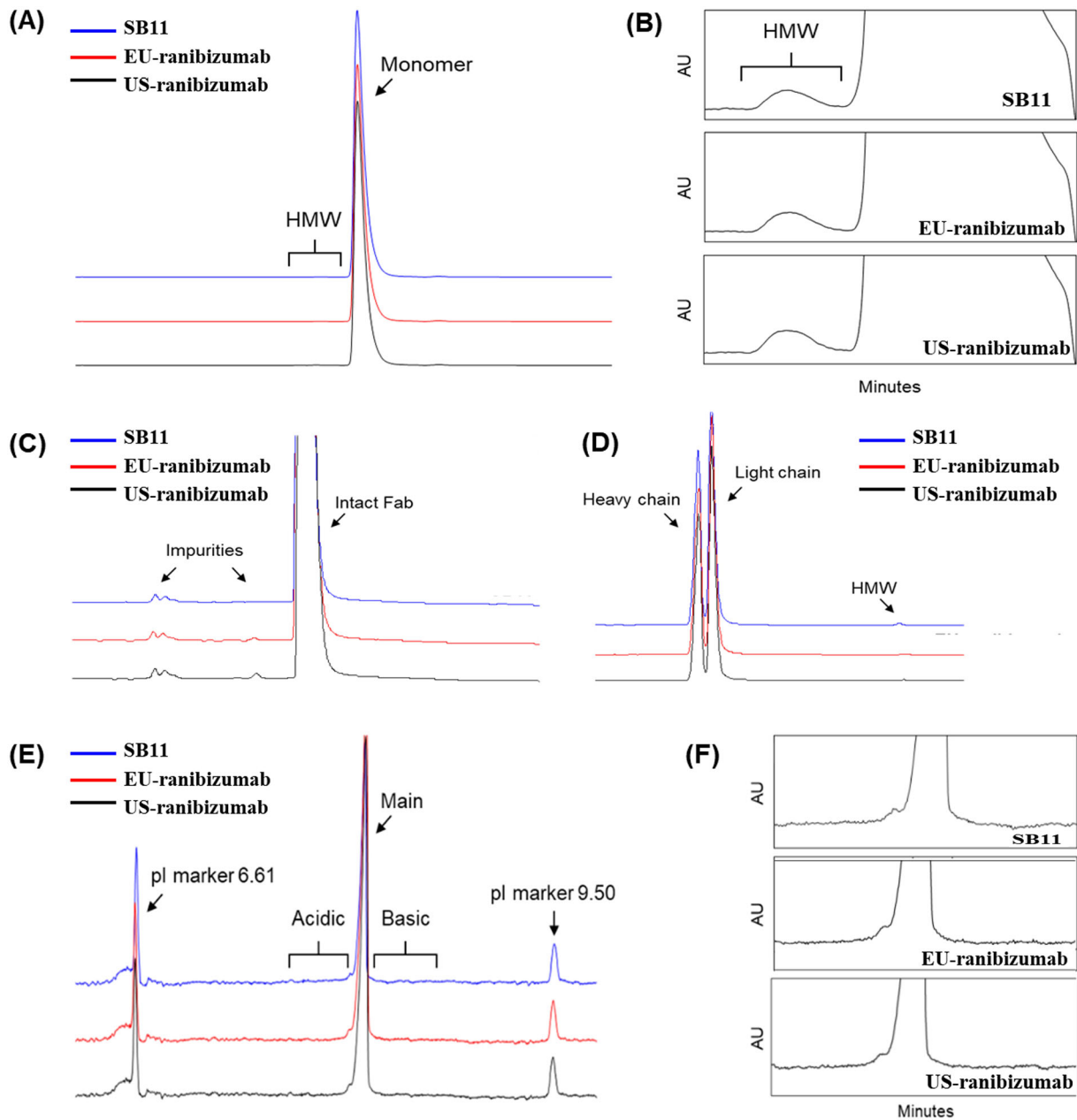
Fig. 3 A, B Comparison of SE-HPLC profiles of SB11 and EU/US-ranibizumab. A Overlay of SE-HPLC profiles, B enlarged view of SE-HPLC profiles, C,D comparison for CE-SDS profiles of SB11 and EU/US-ranibizumab. C Overlay of CE-SDS profiles under non-reduced conditions and D reduced conditions. E,F Comparison of charge variants of SB11 and EU/US-ranibizumab. E Overlay of icIEF profiles, F enlarged view of icIEF profiles

in the side-by-side comparison; relative VEGF-A 110, VEGF-A 121, and VEGF-A 189 binding activities of SB11 were similar to those of EU/US-ranibizumab (Fig. 4B).

DISCUSSION

A biosimilar needs to demonstrate a high level of similarity with the reference products in

Table 3 Summary of the physicochemical analysis results for SB11 and EU/US-ranibizumab

Analysis	Attributes	SB11	EU-ranibizumab	US-ranibizumab
SE-HPLC	%Monomer (%)	99.9–100.0	99.9–100.0	99.8–99.9
	%HMW (%)	0.0–0.1	0.0–0.1	0.1–0.2
CE-SDS (non-reduced)	%Main (%)	99.3–99.4	99.0–99.1	98.8–98.9
CE-SDS (reduced)	%Main1 (%)	42.7–43.0	43.0–43.1	43.0–43.1
	%Main2 (%)	56.5–56.8	56.8–56.9	56.7–56.8
icIEF	pI	8.2	8.2	8.2
	%Acidic (%)	3.1–4.1	3.2–3.9	2.9–3.6
	%Main (%)	93.9–95.3	94.2–95.4	94.5–95.5
	%Basic (%)	1.6–1.9	1.4–1.9	1.4–1.8

terms of structural and functional properties as well as clinical outcomes. The totality of the evidence generated from analytical, functional, non-clinical, and clinical studies is conducted in a stepwise manner. Comparative analytical characterization is the foundation for the biosimilar development. Since our study focuses on the biosimilarity demonstration in respect to structural and functional characterization, other clinical aspects (efficacy, safety,
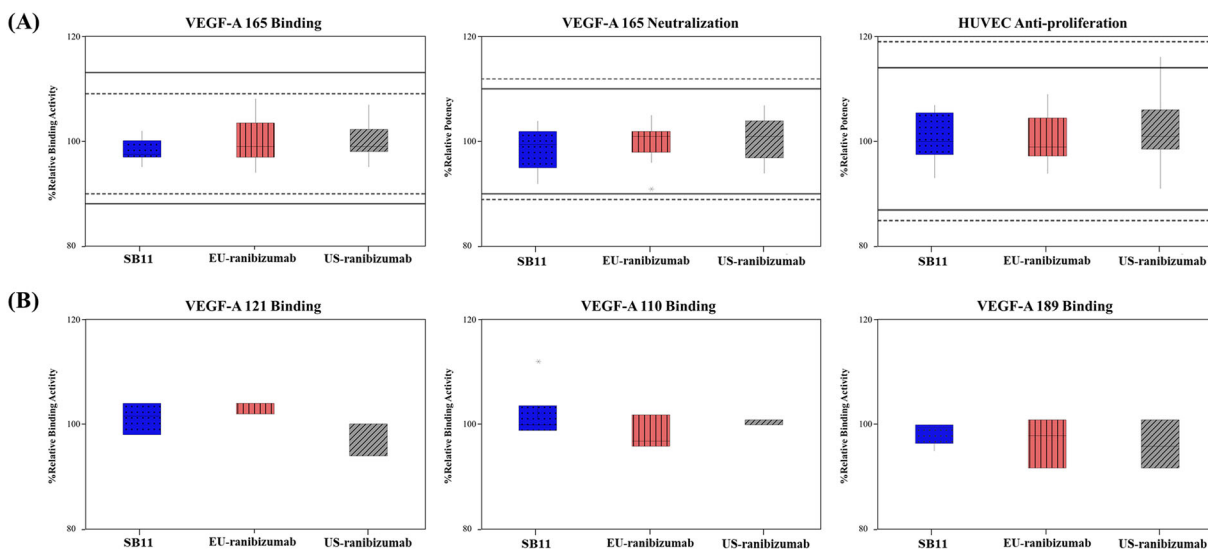
Fig. 4 Comparison of the biological activities of SB11 and EU/US-ranibizumab. Dotted line shows the similarity range (mean ± 3 SD) of EU-ranibizumab. Solid line shows the similarity range (mean ± 3 SD) of US-ranibizumab®. **A** MoA-related biological activities; VEGF-A 165 binding (left), VEGF-A neutralization (middle), and HUVEC anti-proliferation (right). **B** Additional biological activities; VEGF-A 121 binding (left), VEGF-A 110 binding (middle), and VEGF-A 189 binding (right)

pharmacokinetics, and immunogenicity) of SB11 in Phase 3 study have not been reported and discussed in the study. However, the comprehensive analytical characterization package presented here complements the clinical similarity of SB11 and reference product observed in the comparative clinical trial [16, 17].

For the broad analytical characterization of SB11 as biosimilar of Lucentis®, the quality attributes used for the similarity assessment were ranked ('tiered') according their potential impact on PK/PD, safety, efficacy, and immunogenicity [13]. According to quality risk assessment, structural, physicochemical, and functional studies were conducted in line with FDA/EMA standards [12, 13]. For each quality attribute, pre-defined quality ranges were established on the basis of analysis of batches of EU/US-ranibizumab and side-by-side comparisons were performed for similarity demonstration. To establish a quality range, a sufficient number of reference product lots were secured and analyzed over a long period, and the similarity range was statistically established through their quality monitoring.

EU-ranibizumab and US-ranibizumab show comparability in quality aspects (data not shown), and similarity of SB11 to EU/US-ranibizumab was demonstrated in terms of the structural, physicochemical, and biological activities using state-of-the-art methods. For structural integrity, there was no apparent difference between SB11 and EU/US-ranibizumab. Especially, the levels of deamidation and oxidation were important in that these post-translational modifications have potential to affect VEGF-A binding affinity [18]. The results showed the similarity not only for the modification level but also for the VEGF-A binding affinity. No differences were observed in the tertiary structures between SB11 and EU/US-ranibizumab, showing high similarity. Moreover, no relevant differences were observed in terms of purity/impurity and charge variants, strongly supporting the similarity between SB11 and EU/US-ranibizumab. Therefore, the results can support the similarity in structural and physicochemical properties between SB11 and EU/US-ranibizumab.

VEGF-A 165 is the key determinant of MoA of ranibizumab and is most extensively investigated in respect to its function, signaling, expression, and pathological roles. As a potent stimulator of angiogenesis, VEGF-A 165 is considered the prototypical pro-angiogenic VEGF-A isoform [19]. In terms of biological activities, VEGF-A 165 neutralization and HUVEC antiproliferation were similar for SB11 and EU/US-ranibizumab, and there were no differences in binding activities for VEGF-A 165 and its isoforms (VEGF-A 110, VEGF-A 121, and VEGF-A 189). Taken together, the observed structural, physicochemical, and biological similarities between SB11 and EU/US-ranibizumab are part of the totality of the evidence supporting biosimilarity of SB11 and EU/US-ranibizumab.

CONCLUSION

SB11 was developed as a ranibizumab biosimilar in accordance with guidelines of international regulatory organizations. Extensive analytical characterization between SB11 and EU/US-ranibizumab was conducted on structural, physicochemical, and biological properties to support biosimilarity of SB11. Analytical characterization results showed that SB11 is highly similar to EU/US-ranibizumab with respect to overall critical and non-critical quality attributes performed.

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article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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Disclosures. Eunji Kim, Jihyeon Han, Yunjung Chae, Hyerim Park, Saerom Kim, Seokkyun Kim, Jungmin Lee, and Beom Chan Kim are employees of Samsung Bioepis Co., Ltd.

Compliance with Ethics Guidelines. This article does not contain any studies with human participants or animals performed by any of the authors. All SB11 samples were prepared and experiments conducted aseptically in a biological safety cabinet.

Data Availability. The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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