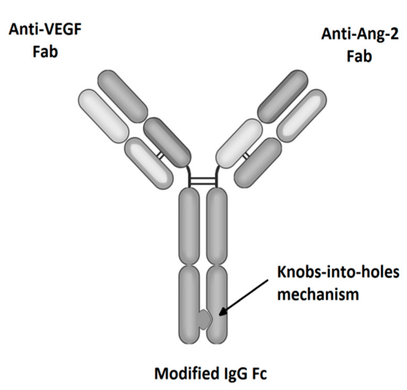
Storage of faricimab and high-dose aflibercept in polypropylene syringes does not impair antibody integrity after 28 days

Alexander Taschauer[1,2], Arthur Sedivy[3], Daniel Egger[2,4], Sebastian M. Waldstein[2,4], Claudia Wunder[1,2]

[1] Hospital Pharmacy, University Hospital St. Pölten, Dunant-Platz 1, 3100 St. Pölten, Austria
[2] Karl Landsteiner University of Health Sciences, Dr. Karl-Dorrek-Strasse 30, 3500 Krems, Austria
[3] Protein Technologies, Vienna Biocenter Core Facilities GmbH, Dr. Bohr-Gasse 3, 1030 Vienna, Austria
[4] Department of Ophthalmology, Landesklinikum Mistelbach-Gänserndorf, Liechtensteinstrasse 67, 2130 Mistelbach, Austria

Background and Aims

The IgG1-derived antibody faricimab and the recently approved high-dose formulation of the fusion protein aflibercept (114.3mg/mL) represent promising therapeutic approaches in the treatment of neovascular age-related macular degeneration (nAMD).



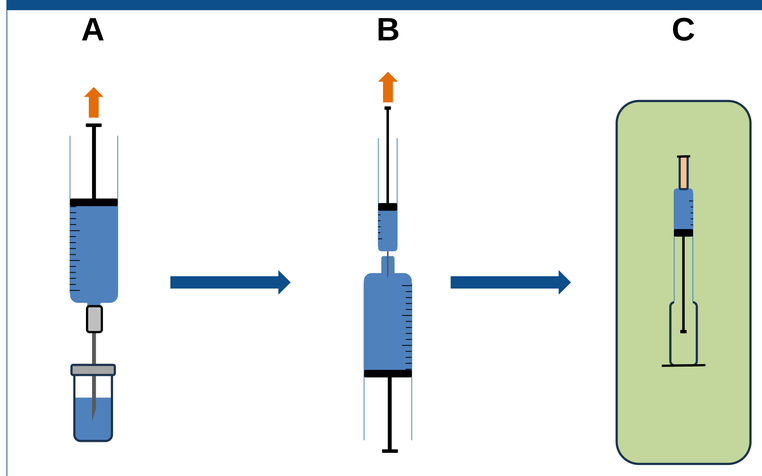
Faricimab is characterized by a heterodimeric IgG1 structure with an anti-VEGF-A- and an anti-Ang-2-Fab region.[1] The Fc-region is modified to reduce systemic exposure and immunogenic side effects. Aflibercept is a fusion protein, which binds both VEGF-A and -B. The IgG-derived Fc-region is unmodified.

Both for faricimab and aflibercept reliable stability data to enable compounding and storage in syringes are scarce. We therefore evaluated the long-term stability of faricimab and aflibercept after compounding in polypropylene syringes (BD Microfine® / Sample Type 1 and ZeroResidual® from SJJ Solutions / Sample Type 2) over a storage time of 28 days.

Materials and Methods

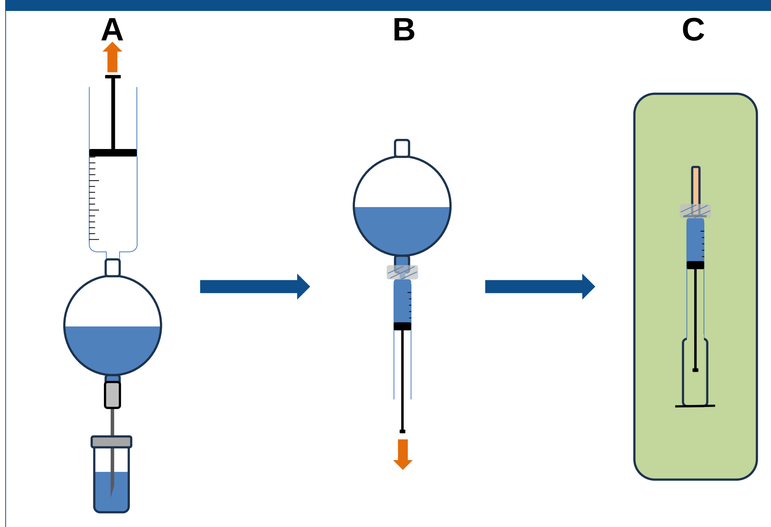
Sample Type 1 and 2 were prepared under clean room A (GMP) conditions. Samples from freshly punctured vials were used as day 0 control. Product characteristics were analyzed using size exclusion chromatography, nano differential scanning fluorimetry (nanoDSF; thermal stability), UV-Vis (3D-structure and aggregation index), dynamic light scattering (DLS; particle size distribution), pH measurement, sterility tests and endotoxin quantification. For quantification of binding affinity, we established a new method based on grating-coupled interferometry (GCI). In the case of faricimab, this method enabled the evaluation of the simultaneous binding mechanism of both VEGF and angiopoietin 2 (Ang-2) in solution.

Sample Type 1: BD Microfine®



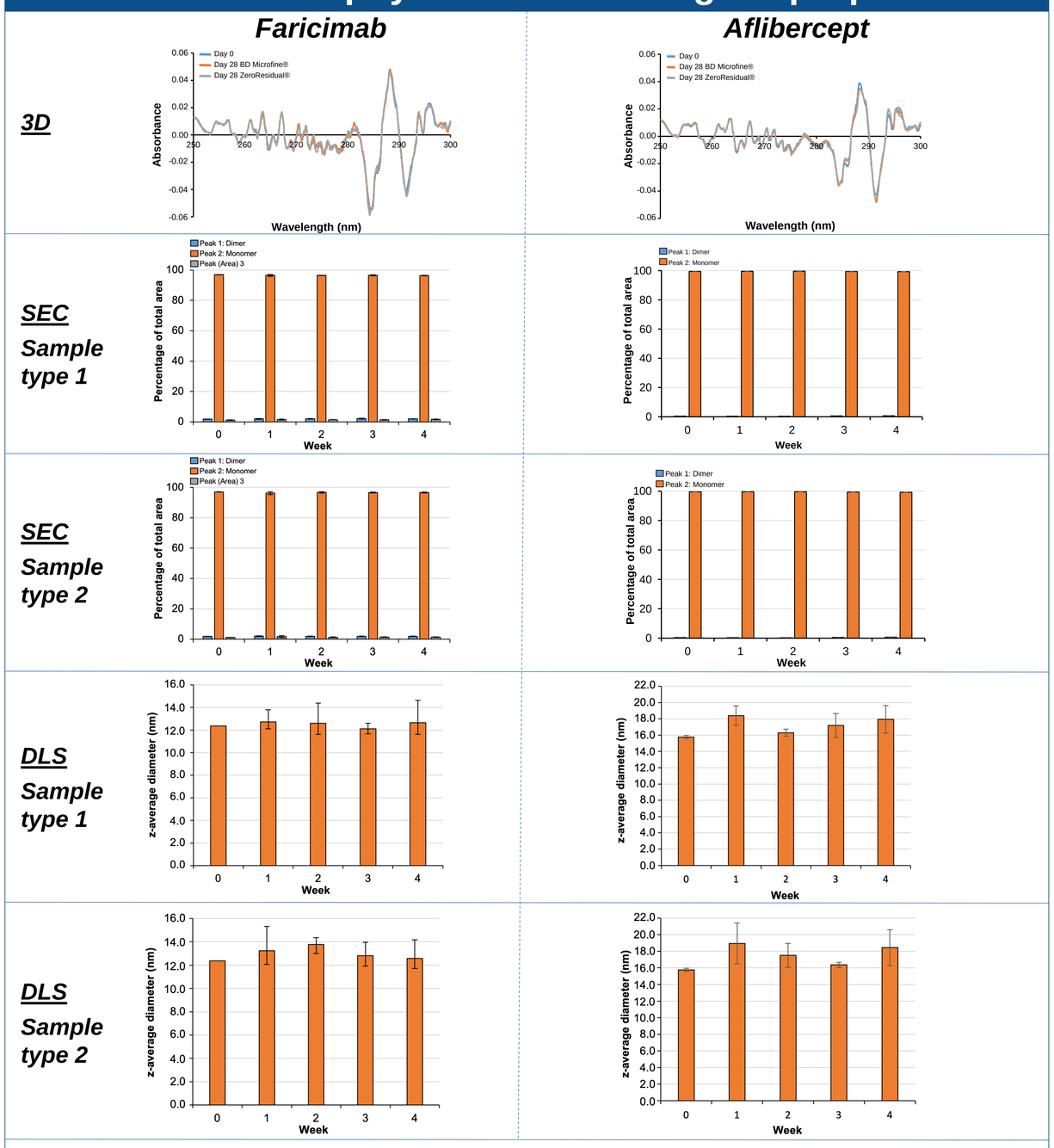
(A) The content of >1 vial of either faricimab or aflibercept was drawn up into a reservoir syringe through a filter canula.
(B) The content of the reservoir syringe was compounded into BD Microfine® syringes through the syringe cone.
(C) Samples were stored at 2-8°C (light protection) with capped canula and transport protection of the plunger in sealed sterile bags.

Sample Type 2: ZeroResidual® SJJ



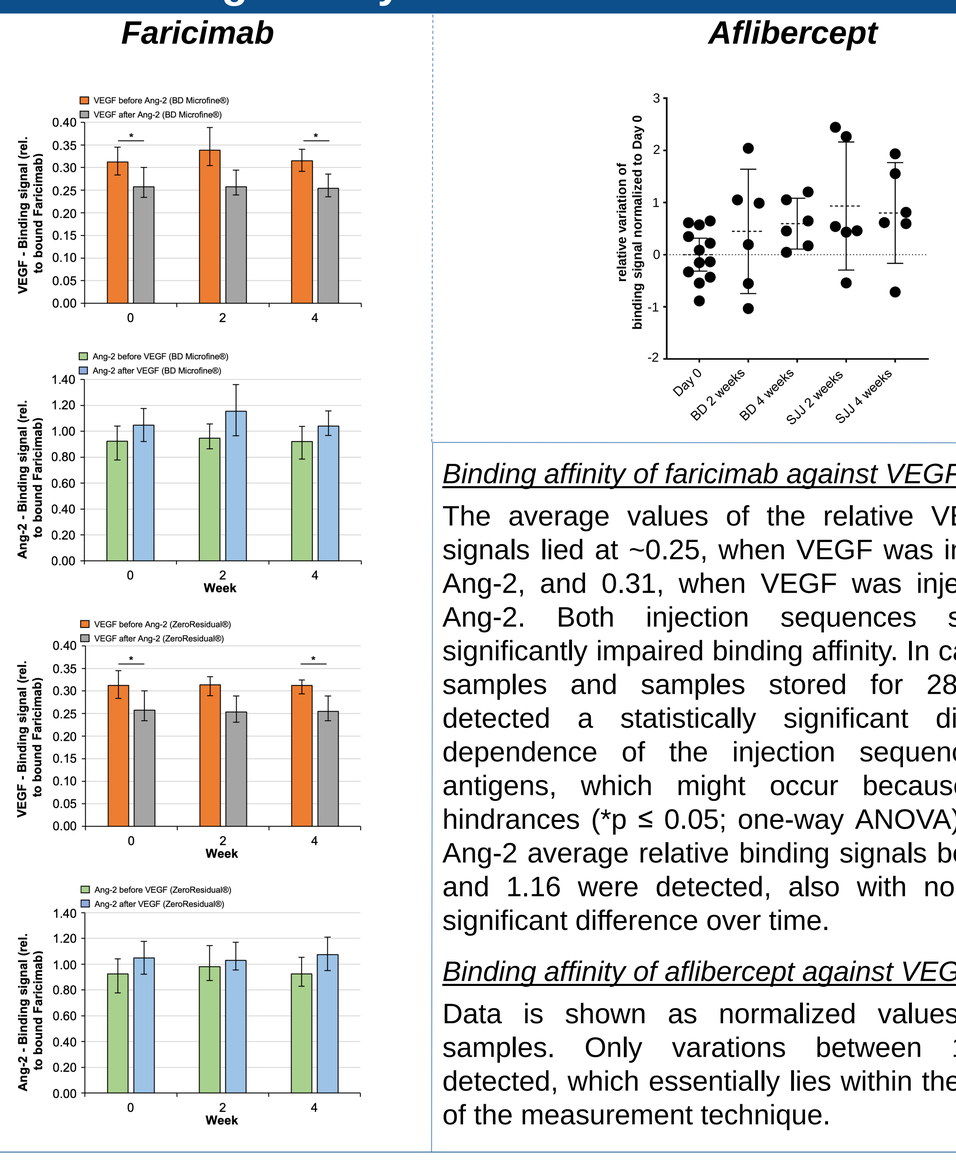
(A) The content of >1 vial of either faricimab or aflibercept was drawn up into a reservoir bubble-adapter through a filter canula.
(B) The content of the reservoir syringe was compounded into ZeroResidual® SJJ syringes.
(C) Samples were stored at 2-8°C (light protection) with capped canula and transport protection of the plunger in sealed sterile bags.

Results: Chemical/physical/microbiological properties



Evaluation of compound stability of faricimab (left column) and aflibercept (right column).
Both faricimab and aflibercept showed no significant alterations in the 3D-structure based on the second-derivative UV-Vis spectrum between 250nm and 300nm over 4 weeks. In case of faricimab, the molecular weight distribution (measurement by OmniSEC) showed 2% dimerization and around 97% monomer at day 0. Aflibercept had an oligomerization degree of 0.5% (as dimer) and 99.5% monomer. Both compounds didn´t show significant changes over 4 weeks.
Both faricimab and aflibercept had a z-average hydrodynamic diameter between 10 and 20nm without significant changes over time.
Data not graphically shown: Thermal stability was measured by nanoDSF. Both faricimab and aflibercept showed two unfolding transitions and onset temperatures of 57°C (faricimab) and 47°C (aflibercept) without alterations in the temperature curve profile over 4 weeks. Also the pH value of undiluted faricimab (5.5) and aflibercept (5.8) as well as the aggregation index stayed stable. All samples were free of aerobic and anaerobic bacteria and showed an endotoxin concentration below the detection limit of 2.5 EU/mL.

Results: Binding affinity



Binding affinity of faricimab against VEGF and Ang-2:

The average values of the relative VEGF-binding signals lied at ~0.25, when VEGF was injected after Ang-2, and 0.31, when VEGF was injected before Ang-2. Both injection sequences showed no significantly impaired binding affinity. In case of day 0 samples and samples stored for 28 days, we detected a statistically significant difference in dependence of the injection sequence of both antigens, which might occur because of steric hindrances (*p ≤ 0.05; one-way ANOVA). In case of Ang-2 average relative binding signals between 0.92 and 1.16 were detected, also with no statistically significant difference over time.

Binding affinity of aflibercept against VEGF-A :

Data is shown as normalized values to day 0 samples. Only varations between 1-2% were detected, which essentially lies within the uncertainty of the measurement technique.

Conclusion and Relevance

- Faricimab[2] and high-dose aflibercept sustained their integrity after compounding into polypropylene syringes (BD Microfine® and ZeroResidual®) for up to 4 weeks, when stored under light protection at 2°C to 8°C.
- An endotoxin concentration below the detection threshold and no signs of bacterial growth in both sample types (BD Microfine® and ZeroResidual®) further proof the quality of the compounding process.
- The compounding procedure was done in accordance with the EU guidelines for good manufacturing practice of medicinal products under controlled clean room conditions by appropriately trained personnel. Deviating from this process could result in the introduction of bacterial contaminants and an increased risk of infection after administration.
- The findings facilitate batch compounding rather than individual compounding for each patient while increasing patient safety and maintaining cost-effectiveness for intravitreal injections in real-world settings.

References:
1) Liberski et al: Int. J. Mol. Sci. 2022 (DOI: 10.3390/ijms23169424)
2) Taschauer et al: Eye (Lond). 2024 (DOI: 10.1038/s41433-024-03511-5)

Abstract Number:
3PC-001