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Letters to the Editor

Aflibercept Does Not Suppress Angiopoietin-2 in Patients With nAMD or DME

We read with interest the article titled "Aflibercept Suppression of Angiopoietin-2 in a Rabbit Retinal Vascular Hyperpermeability Model" by Lange and colleagues[1] who reported that antivascular endothelial growth factor-A (VEGF) agents, including aflibercept, suppressed angiopoietin-2 (Ang-2) protein, and retinal/choroidal Ang-2 gene expression in rabbits. After reviewing the article, we have several questions about the model and the conclusions drawn:

- Translatability of this rabbit model to human diseases

Our group recently reported data from 4 large, randomized phase III trials enrolling more than 3000 patients with neovascular age-related macular degeneration (nAMD) or diabetic macular edema (DME), where we found that aflibercept had no effect on ocular Ang-2 concentrations (Cheung G., et al., conference presentation, Macular Society Annual Meeting, February 15–18, 2023, Miami, FL). In contrast, patients treated with the dual Ang-2 and VEGF-A inhibitor, faricimab, demonstrated rapid and sustained suppression of ocular Ang-2 concentrations (Bogman K., et al., conference poster, Association for Research in Vision and Ophthalmology, April 23–27, 2023, New Orleans, LA).

Aqueous humor (AH) samples evaluated in our analysis were optionally collected per protocol at multiple timepoints in the randomized, controlled, phase III trials in patients with nAMD (TENAYA/LUCERNE [NCT03823287/NCT03823300]) or DME (YOSEMITE/RHINE [NCT03622580/NCT03622593]).[2–5] AH data were collected from 157 faricimab-treated patients, corresponding to 669 free Ang-2 specimens and 127 aflibercept-treated patients corresponding to 531 free Ang-2 specimens. Free Ang-2 concentrations were assessed using a validated bead-based immunoassay on the Single Molecule Array immunoassay platform. The assay was developed in accordance with industry recommendations and the performance parameters were within industry and health authority recommendations (European Medicines Agency [EMA] 2019 and US Food and Drug Administration [FDA] 2019). The lower limit of quantification was 4.04 pg/mL and the upper limit of quantification was 1750 pg/mL, defining the quantification range of the assay in 100% human AH.

The Figure shows AH Ang-2 concentrations versus relative time (predose on day 1, day 7, week 4, and week 8) after administration of faricimab 6.0 mg or aflibercept 2.0 mg. Profiles are shown up to 8 weeks after dosing to match the observation window for both treatments. We found that AH Ang-2 concentrations were rapidly suppressed after faricimab administration and remained suppressed throughout the observation window and through 16 weeks postdose, consistent with the extended durability of faricimab seen in clinical trials. In contrast, there were no relevant changes from baseline in AH Ang-2 concentrations in patients treated with aflibercept. Similar results were observed in patients treated with ranibizumab in the faricimab phase II trials (Csaky KG., et al., conference presentation, AAO Retina Subspecialty Day, October 11–12, 2019, San Francisco, CA). Hence, the results of this rabbit model do not seem to translate to what was observed in patients with nAMD and DME.

Lange et al. show data to suggest that this rabbit VEGF challenge model leads to an increase in Ang-2 protein and mRNA, which is reduced by aflibercept, and to a lesser degree other anti-VEGF agents. However, the inability of anti-VEGF agents, including aflibercept, to reduce intraocular Ang-2 levels in patients leads us to question the clinical translatability of the rabbit model findings. Further to the issue of translatability, we note that rabbit retinal vasculature is very different to human retinal vasculature, with the rabbit retina being largely avascular, and that rabbits do not have a fovea.[6] However, a more plausible explanation for this disparity may be differences in the underlying cause(s) of Ang-2 elevation between the rabbit model and humans with nAMD or DME. This study demonstrates that excessive VEGF can induce Ang-2 levels. However, as the authors note in their discussion of the model limitations, anti-VEGF treatment was administered before the VEGF challenge, which is misaligned with how retinal diseases are managed in clinical practice. Such anti-VEGF treatment could

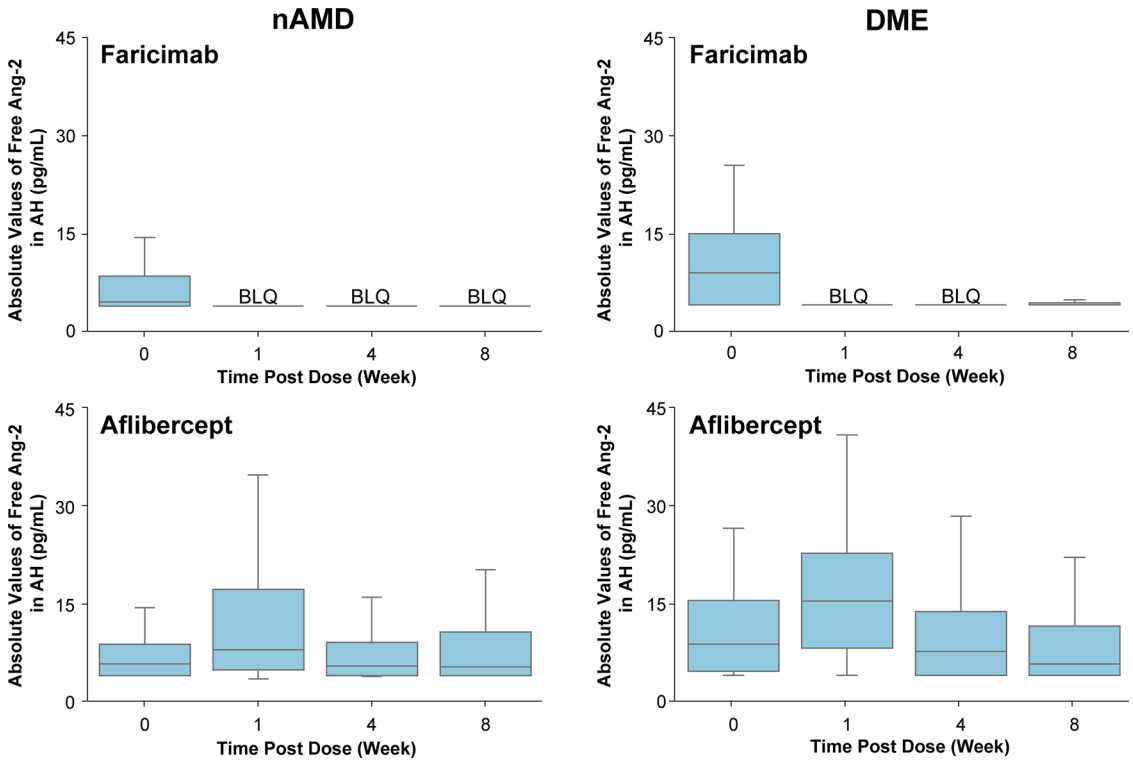

Figure. Aqueous humor free Ang-2 concentrations over time (predose, 7 days, 4 weeks, and 8 weeks) after faricimab 6.0 mg or aflibercept 2.0 mg dosing in patients with nAMD or DME. AH, aqueous humor; BLQ, below the limit of quantification; DME, diabetic macular edema; nAMD, neovascular age-related macular degeneration.

easily block VEGF and the downstream effect of Ang-2 elevation. On the other hand, in clinical disease, we know that other factors, such as hypoxia, can upregulate Ang-2. Given that aflibercept does not bind Ang-2 and that the reasons underlying Ang-2 elevations in patients with retinal disease are multifactorial (and not due to an artificial bolus of VEGF), it is unsurprising that the clinical trial data do not show any reduction in Ang-2 levels with aflibercept.

- Inadequate representation of the faricimab phase III trial population

We found it perplexing that a preclinical paper went into such a long discussion about enrollment and patient population for a phase III trial. Baseline characteristics, such as best-corrected visual acuity letters and lesion type, in the faricimab TENAYA and LUCERNE trials were similar to those in the more recent anti-VEGF trials (Lanzetta PL, conference presentation, AAO Annual Meeting, September 30–October 3, 2022, Chicago, IL),[7] reflecting the evolution in the field toward treating patients earlier in the disease course.

In summary, our findings from patients with nAMD or DME demonstrate that faricimab, a dual Ang-2 and VEGF-A inhibitor, suppresses ocular Ang-2 concentrations. In contrast, no such suppression was observed in patients who received the VEGF inhibitor, aflibercept. Lange et al. demonstrate the downstream effects of VEGF on a host of angiogenic factors, including Ang-2, in the rabbit VEGF challenge model. However, the extrapolation of a component of these findings, specifically the effects of aflibercept on Ang-2, to the clinical realm is problematic when confronted with data from clinical trials. Hence, the conclusion "that direct inhibition of these secondary targets (ANG-2) may not be clinically relevant" may be true in the rabbit VEGF challenge model, but appear unsupported in humans with nAMD or DME.

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