VEGF–ANG-2 axis in angiogenesis: Implications of anti-VEGF therapies on endothelial cell permeability

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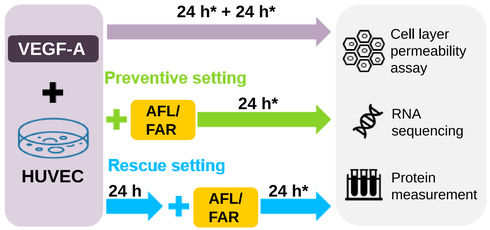
Background

- Anti-vascular endothelial growth factor (VEGF) drugs used for treating retinal vascular diseases work by inhibiting VEGF-A binding to its receptors, expressed primarily by endothelial cells[1]
- Response to anti-VEGF treatment often varies and is driven by multiple factors[2]
- Anti-VEGF agents differ in design, binding affinity for VEGF-A, and binding to additional factors potentially involved in disease progression (e.g. angiopoietin-2 [ANG2] or placental growth factor)[1]
- The clinical benefit of inhibiting ANG2 in addition to VEGF-A remains unclear[3]
- In a rabbit model, inhibition of VEGF-A by aflibercept (AFL) was found to indirectly affect many angiogenesis proteins, including ANG2[4]
- This study investigates the effect of VEGF-A inhibition and additional inhibition of ANG2 on several readouts, by directly comparing AFL (VEGF-A inhibition) and faricimab (FAR, VEGF-A, and ANG2 co-inhibition) in a human umbilical vein endothelial cell (HUVEC)-based in vitro model

Objectives & study design

1. **How does VEGF-A affect vascular permeability?**
2. **What is the effect of AFL and FAR on this process?**



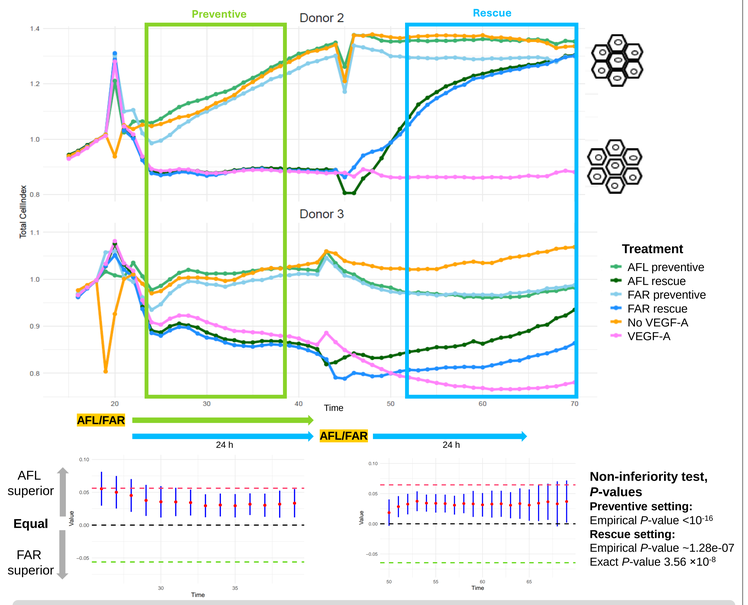
*Denotes the time at which the cells or culture supernatants were harvested for analysis.

Methods

- VEGF-A_{165} (10 ng/mL) was added to confluent (HUVECs, n=3 donors), followed by AFL or FAR at 10-fold molar excess, either simultaneously (**preventive setting**) or after 24 h of VEGF-A exposure (**rescue setting**)
- Cell-layer permeability was evaluated on the xCELLigence platform. Non-inferiority of the effect of AFL in both settings across time points was assessed by Tukey's range test (**Figure 1**)
- For HUVEC transcriptomics by RNA-Seq, an average 35.2 million reads per sample were mapped to Gencode-annotated transcripts using RSEM and STAR. Differential gene expression was assessed using the R-package DESeq2 with the model including donor as a fixed effect. Differential expression *P*-values were adjusted for multiple testing using the false discovery rate method (**Figure 2**)
- Supernatant ANG2 and TEK tyrosine kinase (Tie2) proteins were measured by Meso Scale Discovery (MSD) immunoassay. Differential protein abundance was analyzed using the R-Package limma, with log-transformed concentrations as input. *P*-values were adjusted for multiple testing using Holm's method (**Figures 3 & 4**)
- All experiments were run with 3 replicates per donor

Results

FIGURE 1: FAR is non-superior to AFL in reversing vascular permeability

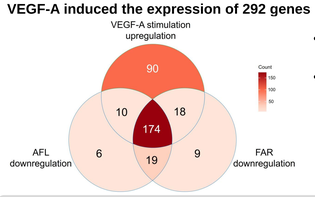


- **In this model, ANG2 co-blockade does not have an additional effect on permeability**

AFL, aflibercept; FAR, faricimab; VEGF-A, vascular endothelial growth facto- A. Donor 1 was excluded from analysis due to irresponsiveness to controls (forskolin, thrombin).

FIGURE 2: AFL and FAR comparably reverse the VEGF-A-induced global transcriptional changes in HUVECs

VEGF-A induced the expression of 292 genes

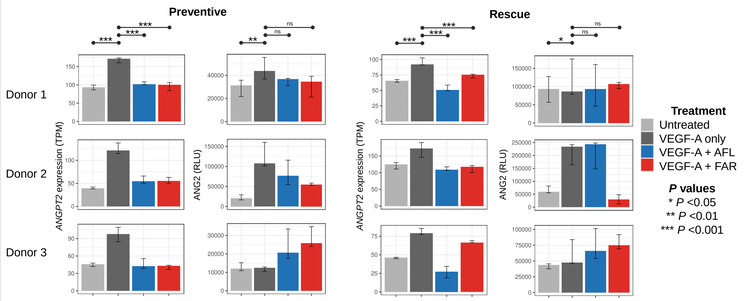


- Enrichment was observed for the angiogenesis and "negative regulation of cell adhesion" pathways
- In the combined analysis (preventive/rescue settings):
  - 174 (60%) of upregulated genes are downregulated by AFL and FAR
  - Only 10 (3%) and 18 (6%) of downregulated genes are specific to one of the treatments

- **Within this system, blocking ANG2 does not appear to have any additional effect on the prevention or rescue of ANG2-induced genes**

AFL, aflibercept; ANG2, angiopoietin-2; FAR, faricimab; HUVEC, human umbilical vein endothelial cells; VEGF-A, vascular endothelial growth factor-A.

FIGURE 3: AFL and FAR reduce VEGF-A-induced *ANGPT2* expression and ANG2 secretion

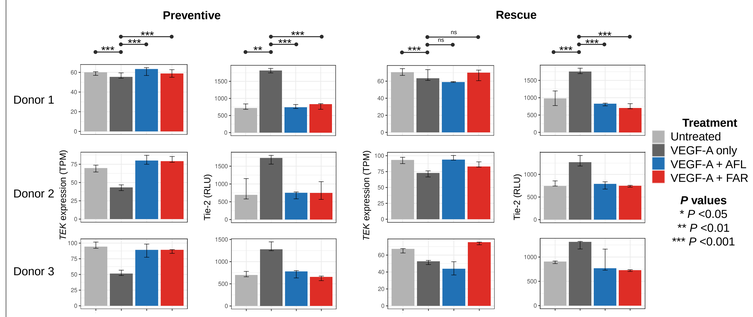


- Consistent with the rabbit study,[4] VEGF-A significantly upregulates the expression of *ANGPT2* in a human-derived, cell-based model. ***AFL and FAR significantly prevent or rescue this effect***
- VEGF-A-induced *ANGPT2* expression results in significantly elevated ANG2 secretion (with high inter-donor variability for the latter)

- **VEGF-A further drives *ANGPT2* gene expression and ANG2 protein secretion**
- **AFL and FAR comparably and significantly reverse this effect at the RNA level**

Error bars denote SD. AFL, aflibercept; ANG2, angiopoietin-2 protein; *ANGPT2*, ANG2-encoding gene; FAR, faricimab; HUVEC, human umbilical vein endothelial cells; ns, no significance; RLU, relative light unit(s); TPM, transcripts per million; VEGF-A, vascular endothelial growth factor-A.

FIGURE 4: AFL and FAR reverse VEGF-A-induced *TEK* expression and soluble Tie2 release



- *TEK* expression is suppressed or downregulated by VEGF-A and rescued by AFL as well as FAR
- Soluble Tie2 levels increase with VEGF-A exposure and are reversed with AFL as well as FAR

- **VEGF-A decreases *TEK* gene expression and increases Tie2 (the receptor for ANG2) shedding**

Error bars denote SD. AFL, aflibercept; FAR, faricimab; ns, no significance; RLU, relative light unit(s); *TEK*, Tie2 receptor-encoding gene; Tie2, angiopoietin receptor 2; TPM, transcripts per million; VEGF-A, vascular endothelial growth factor-A.

Conclusions

- VEGF-A increased endothelial permeability and activated the angiogenesis transcriptional program in HUVECs. Accordingly, *ANGPT2* and *TEK* expression was upregulated and downregulated, respectively, while secreted ANG2 and shed Tie2 receptor proteins were strongly increased
- AFL and FAR comparably reversed these VEGF-A-induced biological effects, with no clear differences between the two agents
- VEGF-A/ANG2 co-inhibition did not confer additional benefits to VEGF-A inhibition alone in this human endothelial cell-based model
- Our results point to **VEGF-A as the primary regulator of the endothelial barrier and vascular integrity**

Lead author disclosures

Tobias Strunz is an employee of Bayer and has disclosed personal financial interest in Bayer.

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