Simultaneous Ang-2/VEGF-A Inhibition Prevents Subretinal Fibrosis Progression in Preclinical Mouse Models of Choroidal Neovascularization (CNV)

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Disclosures

Financial Disclosures
- RF, SU, FR, ML, JC, MGG, PW: Employee: F. Hoffmann-La Roche Ltd.

Study Disclosures
- This study includes research conducted on human subjects
- Institutional Review Board approval was obtained prior to study initiation
- Animal experiments were approved and conducted in strict adherence to the Swiss federal ordinance on animal protection and welfare (reference BS-2734), as well as according to the rules of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research guidelines, European Directive 86/609/EEC, and the Roche Ethics Committee on Animal Welfare
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The Angiopoietin Pathway Maintains Vascular Stability and Homeostasis Under Physiological Conditions\textsuperscript{1-3}

1. Ang-1 from platelets and pericytes binds to and activates Tie2 on endothelial cells…

2. …tightening endothelial cell junctions…

3. …and recruiting pericytes, which wrap around mature vessels…

4. …resulting in maintenance of vascular stability and homeostasis

Ang-1, angiopoietin-1; Tie2, tyrosine kinase with immunoglobulin-like and epidermal growth factor homology domains-2; VEGF-A, vascular endothelial growth factor-A; VEGFR2, vascular endothelial growth factor receptor-2.
Ang-2 Promotes Vascular Instability in Disease by Blocking Ang-1–Tie2 Signaling

1. Endothelial tight junctions weaken, leading to vascular leakage...

2. ...and increased inflammation due to leukocyte migration

3. Pericyte dropout and loss further destabilizes vessels

4. Vascular sprouting and neovascularization occur

5. Immature, leaky vessels drive vascular instability

Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; Tie2, tyrosine kinase with immunoglobulin-like and epidermal growth factor homology domains-2; VEGFR2, vascular endothelial growth factor receptor-2.
Faricimab Is the First Bispecific Antibody Designed for Intraocular Use: 1 Molecule, 2 Targets

Anti–Ang-2 Fab
Enhances vascular stability
Reduces inflammation and vascular leakage

Anti–VEGF-A Fab
Inhibits vascular leakage and neovascularization

Modified Fc
Reduces systemic exposure
Reduces inflammatory potential

Ang-2, angiopoietin-2; Fab, fragment antigen binding; Fc, fragment crystallizable; VEGF-A, vascular endothelial growth factor-A.
**Dual Ang-2/VEGF-A Inhibition With Faricimab Demonstrated ≥ Q12W Dosing Intervals in ~80% of Patients in the First Year of Phase 3 Trials in nAMD**

- **TENAYA (NCT03823287) and LUCERNE (NCT03823300)** met primary endpoint

- **nAMD disease control with faricimab**
  - Noninferiority in mean change from baseline in BCVA with faricimab dosed up to Q16W to aflibercept Q8W in patients with nAMD at week 48
  - Durability up to Q16W at week 48 with faricimab
  - Meaningful reductions in CST with faricimab up to Q16W comparable with aflibercept Q8W through week 48

- **Safety**
  - Faricimab was well tolerated. There were no reported cases of vasculitis or occlusive retinitis

- **Long-term data**
  - TENAYA and LUCERNE are 2-year studies. The long-term extension study, AVONELLE-X, will generate 4-year long-term data

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Ang-2, angiopoietin-2; BCVA, best-corrected visual acuity; CST, central subfield thickness; nAMD, neovascular age-related macular degeneration; Q8W, every 8 weeks; Q12W, every 12 weeks; Q16W, every 16 weeks; VEGF-A, vascular endothelial growth factor-A.
How Can the Sustained Efficacy of Faricimab Seen in Clinical Trials Be Explained?

- Sustained inhibition of both Ang-2 and VEGF-A
- Vessel-stabilizing effects of dual Ang-2 and VEGF-A inhibition

In these analyses, we utilized 2 CNV mouse models (laser-induced CNV and spontaneous CNV) to investigate (using fibronectin and active collagen staining) if dual Ang-2/VEGF-A inhibition–dependent vessel stabilization also prevents fibrosis, which is often linked to vision loss in patients with nAMD.
Fibrosis is attributed to excess deposition of extracellular matrix components, such as collagen.

Collagen has a unique triple helical structure that is unfolded in tissues during diseases, development, or mechanical injury.

CHPs (developed by 3Helix Inc.):

- Bind to unfolded/remodeling collagen based on the structural recognition of the individual alpha-chains, not a defined epitope.
- Allow us to distinguish active lesions from stable scars and healthy collagen-rich tissues.
- Are fluorophore labeled and bind independent of species and collagen types; can be used together with antibodies on the same tissue section.


CHP, collagen hybridizing peptide.
CHPs Detect Active Fibrotic Lesions Without Binding to Healthy Collagen-Rich Tissue

CHPs bind specifically to remodeling collagen/active fibrotic lesions, but not to healthy collagen in blood vessels.

CHP, collagen hybridizing peptide; CNV, choroidal neovascularization; DAPI, 4′,6-diamidino-2-phenylindole; NASH, nonalcoholic steatohepatitis.
Assessment of Ang-2/VEGF-A Inhibition on Fibrosis Development in a Laser-Induced CNV Mouse Model

Mouse cross-reactive tool antibody:
- IgG control
- Anti–VEGF-A
- Anti–Ang-2
- Bispecific anti–VEGF-A/anti–Ang-2 (VA2) and
- Untreated control

n = 4 mice (male, C57BL/6, age = 10–12 weeks);
7–8 RPE/choroid flat mounts

Ang-2, angiopoietin-2; CNV, choroidal neovascularization; IgG, immunoglobulin G; IP, intraperitoneal; RPE, retinal pigment epithelium; VEGF-A, vascular endothelial growth factor-A.
Ang-2/VEGF-A Inhibition Prevented Fibrosis Progression
3 Weeks After Laser-Induced CNV in Mice

* $P < 0.05$; ** $P < 0.01$ versus IgG control.

n = 4 mice (male, C57BL/6, age = 10–12 weeks); 7–8 RPE/choroid flat mounts.

Ang-2, angiopoietin-2; CNV, choroidal neovascularization; CHP, collagen hybridizing peptide; IgG, immunoglobulin G; VEGF-A, vascular endothelial growth factor-A.
JR5558 Mice Develop Spontaneous Bilateral CNV

Spontaneous CNV in a Novel Mutant Mouse Is Associated With Early VEGF-A–Driven Angiogenesis and Late-Stage Focal Edema, Neural Cell Loss, and Dysfunction

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- The sCNV mouse model (JR5558) can be used to study both early and late events associated with CNV
- The sCNV mouse model is validated for drug discovery and is used for investigating therapeutic targets in nAMD
- Preclinical experiments in JR5558 mice suggest the involvement of the Ang/Tie pathway in vascular stability and subretinal macrophage infiltration
- The mouse model develops CNV lesions with age that continue to grow without regressing, developing fibrotic changes
CHPs Colocalize With Fibrosis and EMT Markers in RPE/Choroid Flat Mounts From JR5558 Mice

<table>
<thead>
<tr>
<th>Fibrosis-Associated Proteins</th>
<th>EMT-Associated Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>merged</td>
<td>isolectin B4</td>
</tr>
<tr>
<td>Collagen I Propeptide</td>
<td>Collagen I</td>
</tr>
<tr>
<td>CHP</td>
<td>isolectin B4</td>
</tr>
<tr>
<td>CHP</td>
<td>Collagen III</td>
</tr>
<tr>
<td>merged</td>
<td>isolectin B4</td>
</tr>
<tr>
<td>Collagen III</td>
<td>Collagen III</td>
</tr>
<tr>
<td>CHP</td>
<td>isolectin B4</td>
</tr>
<tr>
<td>CHP</td>
<td>fibronectin</td>
</tr>
</tbody>
</table>

CHPs colocalize with fibrosis markers (collagen I, collagen III, and fibronectin) and EMT markers (vimentin and Loxl2) in active fibrotic lesions.

RPE/choroid flat mount staining, male JR5558 mice, age = 3 months. Scale bar = 50 µm.
CHP, collagen hybridizing peptide; EMT, endothelial-to-mesenchymal transition; Loxl2, lysyl oxidase like 2; RPE, retinal pigment epithelium.
Assessment of Sustained Ang-2 Inhibition on Subretinal Fibrosis in JR5558 Mice

Animal arrival in the facility at ~30 days old

Mouse cross-reactive tool antibody:
- IgG control
- Anti–VEGF-A
- Anti–Ang-2
- Bispecific anti–Ang-2/anti–VEGF-A (VA2); and
- Untreated control

Antibody injection (IP)
- P30
- P44
- P45
- P52
- P59
- P75
- P88

Tissue collection
- PT1
- PT2
- PT3

Baseline

Animal arrival in the facility at ~30 days old

n = 6–13 eyes per group
Ang-2/VEGF-A Inhibition Prevented Fibronectin Deposition Better Than Either Treatment Alone Relative to IgG Control in JR5558 Mice

### Post-treatment 1
1 week after last antibody dose (P60)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Area of Fibronectin Signal, µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100,000</td>
</tr>
<tr>
<td>IgG Control</td>
<td>200,000</td>
</tr>
<tr>
<td>Anti–Ang-2</td>
<td>250,000</td>
</tr>
<tr>
<td>Anti–Ang-2/VEGF-A</td>
<td>350,000</td>
</tr>
</tbody>
</table>

**Δ = 41%**  
***Δ = 38%**  
Δ = 21%

**n = 8–10 RPE/choroid flat mounts**

### Post-treatment 2
3 weeks after last antibody dose (P75)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Area of Fibronectin Signal, µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100,000</td>
</tr>
<tr>
<td>IgG Control</td>
<td>200,000</td>
</tr>
<tr>
<td>Anti–VEGF-A</td>
<td>250,000</td>
</tr>
<tr>
<td>Anti–Ang-2</td>
<td>350,000</td>
</tr>
<tr>
<td>Anti–Ang-2/VEGF-A</td>
<td>500,000</td>
</tr>
</tbody>
</table>

**Δ = 47%**  
**Δ = 29%**  
Δ = 19%

**n = 7–8 RPE/choroid flat mounts**

### Post-treatment 3
5 weeks after last antibody dose (P88)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Area of Fibronectin Signal, µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100,000</td>
</tr>
<tr>
<td>IgG Control</td>
<td>200,000</td>
</tr>
<tr>
<td>Anti–VEGF-A</td>
<td>250,000</td>
</tr>
<tr>
<td>Anti–Ang-2</td>
<td>350,000</td>
</tr>
<tr>
<td>Anti–Ang-2/VEGF-A</td>
<td>500,000</td>
</tr>
</tbody>
</table>

**Δ = 54%**  
*Δ = 36%**  
Δ = 21%

**n = 12–13 RPE/choroid flat mounts**

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*P < 0.05; **P < 0.01; ***P < 0.001.

Foxton RF et al. Data on file (F. Hoffmann-La Roche Ltd.).

Ang-2, angiopoietin-2; IgG, immunoglobulin G; P, postnatal day; RPE, retinal pigment epithelium; VEGF-A, vascular endothelial growth factor-A.
Ang-2/VEGF-A Inhibition Prevented CHP Binding Better Than Either Treatment Alone Relative to IgG Control in JR5558 Mice

Quantification (ImageJ)

3 weeks post treatment (P75)

** ns
Δ = 66%

ns Δ = 45%

ns Δ = 20%

n = 4 mice; 6–7 RPE/choroid flat mounts
## Conclusions

**Faricimab dual mechanism and sustained efficacy**
- Faricimab is a bispecific antibody, which independently binds and neutralizes both Ang-2 and VEGF-A.
- Faricimab demonstrated improved durability up to Q16W at week 48 in the phase 3 clinical trials for nAMD and DME.

**Dual Ang-2/VEGF-A inhibition**
- We present data from 2 independent CNV mouse models (laser-induced and spontaneous CNV) to assess the role of dual Ang-2/VEGF-A inhibition on subretinal fibrosis using fibronectin and CHP staining.

**Prevention of fibrosis and vascular stability**
- Dual inhibition of Ang-2 and VEGF-A led to sustained prevention of fibrosis based on fibronectin deposition and CHP staining, suggesting that Ang-2 and VEGF-A may drive subretinal fibrosis and contribute to vascular instability.

**Outlook**
- Future studies are underway to determine how dual Ang-2/VEGF-A inhibition limits subretinal fibrosis.

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Ang-2, angiopoietin-2; CHP, collagen hybridizing peptide; CNV, choroidal neovascularization; DME, diabetic macular edema; nAMD, neovascular age-related macular degeneration; Q16W, every 16 weeks; VEGF-A, vascular endothelial growth factor-A.