Use of Collagen-Hybridizing Peptides to Assess Active Fibrosis in 2 Mouse Models of Choroidal Neovascularization

Peter Westenskow, PhD
Lucas Bennink, PhD; Richard Foxton, PhD; Mike Kirkness, PhD; and Markus Linder, PhD

1 Roche Pharma Research and Early Development, Roche Innovation Center, F. Hoffmann-La Roche Ltd., Basel, Switzerland
2 3Helix, Salt Lake City, UT

Presented at the Association for Research in Vision and Ophthalmology
Denver, CO | May 1–4, 2022
Virtual | May 11–12, 2022
Disclosures

Financial Disclosures
- PW, RF, ML: Employee: F. Hoffmann-La Roche Ltd.
- LB, MK: Employee: 3Helix.com

Study and Product Disclosures
- Faricimab has been approved for the treatment of neovascular age-related macular degeneration and diabetic macular edema in the United States and Japan, and is currently being studied for the treatment of macular edema due to retinal vein occlusion. Please note that faricimab has not been approved for use outside these countries, or for use outside its approved indications.
- 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.
- Funding was provided by F. Hoffmann-La Roche Ltd. for the study and third-party writing assistance, which was provided by Ellen M. Ross, PhD, of Envision Pharma Group.
Fibrosis and Collagen-Hybridizing Peptides (CHPs)

**Fibrosis**

Excessive deposition of ECM components, such as collagen and fibronectin, within and around damaged tissue.

RPE cells can undergo EMT contributing to fibrosis in the retina.

**Subretinal Fibrosis Causes Irreversible Vision Loss in Patients With nAMD**

Current ophthalmic imaging techniques detect fibrosis with limited efficiency.

There is a clinical need for novel tools to monitor subretinal fibrosis-related changes.

**Collagen Hybridizing Peptides**

CHPs bind to single α-chain collagen structures, allowing identification of unfolded/remodeling collagen in situ and in vivo (a hallmark of active fibrosis).

**Intact/Stable Collagen**

**Unfolded/Remodeling Collagen**

Fluorophore labeled

Bind independently of species and collagen type

CHPs have shown diagnostic potential in various organ systems, but their use in the eye is currently unexplored.

---


CHP, collagen-hybridizing peptide; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; nAMD, neovascular age-related macular degeneration; RPE, retinal pigment epithelium.
In Situ Imaging of Subretinal Fibrosis With R-Labeled (sCy3) CHPs

JR5558 Mice – Spontaneous Choroidal Neovascularization (CNV)
In JR5558 Eyes, R-CHP (sCy3) Binding Was Associated With Fibrosis and EMT-Related Biomarkers

In RPE/choroid, R-CHP binding was associated with:

- **Fibrosis markers** (fibronectin, collagen I propeptide, collagen I and III)
- **EMT-related markers** (vimentin, Loxl2)
In JR5558 Eyes, CHPs Bound to Remodeling, But Not Intact, Collagen, and Detected Increased Collagen Remodeling as Mice Aged

Active Fibrotic Lesion (Collagen Remodeling)

Healthy Tissue (Intact Collagen)

R-CHPs are specific for denatured collagen upregulated in active fibrotic lesions and have minimal affinity for intact collagen surrounding quiescent vasculature

R-CHPs can detect increased collagen remodeling associated with increased fibrosis

Left: representative immunohistochemistry images of RPE/choroid flatmounts from JR5558 mice; center flat mount scale bar = 1 mm; all other panels scale bar = 50 µm. Right: mean ± SEM of R-CHP–positive area in RPE/choroid flatmounts from JR5558 mice.

CHP, collagen-hybridizing peptide; R-CHP, sCy3-labeled collagen-hybridizing peptide; RPE, retinal pigment epithelium; SEM, standard error of the mean.
Can CHPs Be Used In Vivo to Image Fibrosis in the Subretinal Space?

JR5558 and Laser-Induced CNV (LCNV)
sCy7.5-CHPs Bound Directly to, and Enabled In Vivo Imaging of, Collagen in Subretinal Fibrosis in JR5558 Mice

Ex vivo staining with isolectin B4, fibronectin, and R-CHP confirmed presence of fibrosis and damaged collagen in both control and targeted JR5558 mice

Control (nontargeted) sCy7.5-CHPs were not detected

Left: representative images of IR, FA, and cSLO of JR5558 retinas; MFI of sCy7.5-CHP (normalized to control); ** P < 0.01 versus control (nontargeted) sCy7.5-CHPs.

Right: representative immunohistochemistry images of RPE/choroid flatmounts from JR5558 mice. Scale bars = 50 µm.

CHP, collagen-hybridizing peptide; cSLO, scanning laser ophthalmoscope; FA, fluorescein angiography; IR, infrared reflectance; MFI, mean fluorescence intensity; R-CHP, sCy3-labeled collagen-hybridizing peptide; sCy7.5-CHP, sCy7.5-labeled collagen-hybridizing peptide.
In LCNV, sCy7.5-CHP Integration Correlated With Fibrosis Severity

sCy7.5-CHP binding increased as laser intensity/fibrosis severity increased

Fibrosis severity was confirmed ex vivo with R-CHP and fibronectin staining

sCy7.5-CHP binding increased with increasing R-CHP and fibronectin expression

Left: representative images of IR and cSLO of JR5558 retinas; MFI of sCy7.5-CHP (normalized to 150 mW); ** P < 0.01; *** P < 0.001 versus 150 mW using ordinary 1-way ANOVA.

Middle: representative immunohistochemistry images of RPE/choroid flatmounts from JR5558 mice. Scale bars = 50 µm.

Right: Correlation of positive area (µm²) for the indicated parameter normalized to the mean. r and P values calculated using 2-tailed correlation with Pearson correlation coefficients.

Merged R-CHP Fibronectin

sCy7.5-CHP–Positive Area, µm² (In Vivo)

Fibronectin-Positive Area, µm² (Ex Vivo)

Fibronectin-Positive Area, µm² (Ex Vivo)

R-CHP–Positive Area, µm² (Ex Vivo)

r = 0.76

P = 0.03

r = 0.69

P = 0.058

ANCOVA, analysis of variance; cSLO, scanning laser ophthalmoscope; IR, infrared reflectance; LCNV, laser-induced choroidal neovascularization; MFI, mean fluorescence intensity; R-CHP, sCy3-labeled collagen-hybridizing peptide; sCy7.5-CHP, sCy7.5-labeled collagen-hybridizing peptide.
LCNV Lesions Show Decreased Collagen Remodeling in Stabilized vs Fresh Wounds

**Middle:** representative images of IR and cSLO of JR5558 retinas. Right, top: MFI of sCy7.5-CHP (normalized to 1 week); **** \( P < 0.0001 \) versus 1 week.
Right, bottom: mean ± SEM of R-CHP—positive area (normalized to 1 week); ** \( P < 0.01 \) versus 1 week.

**sCy7.5-CHP** binding was decreased at 8 weeks versus 1 week after injury.

**sCy7.5-CHP** can detect decreased collagen remodeling in stabilized versus fresh wounds.

**Legend:**
- Laser injury injection
- CHP injection
- Imaging
- Time (days)
- 0 2 7 51 56

**Fresh Wound**
- 1 Week After Injury
- IR
- sCy7.5-CHP (cSLO)

**Stabilized Scar**
- 8 Weeks After Injury
- IR
- sCy7.5-CHP (cSLO)

**Graphs:**
- **sCy7.5-CHP (In Vivo)**
  - 1 wk vs 8 wks
  - **** \( P < 0.0001 \)
- **R-CHP (Ex Vivo)**
  - 1 wk vs 8 wks
  - ** \( P < 0.01 \)

**LCNV Lesions** Lesions Show Decreased Collagen Remodeling in Stabilized vs Fresh Wounds

- LCNV, laser-induced choroidal neovascularization
- MFI, mean fluorescence intensity
- R-CHP, sCy3-labeled collagen-hybridizing peptide
- sCy7.5-CHP, sCy7.5-labeled collagen-hybridizing peptide
- SEM, standard error of the mean
sCy7.5-CHPs Enabled Monitoring of Reduced Fibrosis Following Treatment With a Bispecific Anti-VEGF/Anti–Ang-2 (VA2) Antibody in JR5558 Mice

Left: representative images of IR and cSLO of JR5558 retinas; MFI of sCy7.5-CHP (normalized to IgG); ** P < 0.01 versus IgG.

Middle: representative immunohistochemistry images of RPE/choroid flatmounts from JR5558 mice; scale bars for 2 images on left = 1 mm; scale bars for 2 images on right = 50 µm.

Right, top: mean ± SEM of fibronectin and R-CHP–positive area (normalized to IgG); ** P < 0.01; *** P < 0.001 versus IgG using unpaired Student’s t test.

Right, bottom: Correlation of positive area (µm²) for the indicated parameter normalized to the mean. r and P values calculated using 2-tailed correlation with Pearson correlation coefficients.

Ang-2, angiopoietin-2; CHP, collagen-hybridizing peptide; cSLO, scanning laser ophthalmoscope; IgG, immunoglobulin G; IR, infrared reflectance; MFI, mean fluorescence intensity; R-CHP, sCy3-labeled collagen-hybridizing peptide; RPE, retinal pigment epithelium; sCy7.5-CHP, sCy7.5-labeled collagen-hybridizing peptide; SEM, standard error of the mean; VA2, bispecific anti-VEGF/anti–Ang-2 antibody; VEGF, vascular endothelial growth factor.
Fluorophore-Labeled CHPs: The First Direct Imaging of Collagen in Animal Models of Subretinal Fibrosis in nAMD

In JR5558 mouse eyes, ex vivo staining with R-CHPs demonstrated that CHPs:

1. Bind specifically to denatured collagen upregulated in active fibrosis
2. Allowed detection of increased collagen remodeling and fibrosis in mouse models of nAMD

**sCy7.5-labeled CHPs** can image fibrosis in vivo with cSLO

**LCNV Mouse Model**
- CHP binding increased with increasing fibrosis severity (laser intensity)
- CHPs detected increased collagen remodeling in stabilized scars versus fresh wounds

**JR5558 Mice**
- Enabled monitoring of antifibrotic effects of a bispecific anti-VEGF/Ang-2 antibody

**R-CHPs and sCy7.5-CHPs** have the potential to:

1. Support clinical development of nAMD treatments and antifibrotic therapeutics
2. Become a key diagnostic tool for detection of active fibrosis in the retina

**Ongoing work:**
- Primary human RPE cell models
- Nonhuman primate models

Ang-2, angiopoietin-2; CHP, collagen-hybridizing peptide; cSLO, scanning laser ophthalmoscope; IgG, immunoglobulin G; IR, infrared reflectance; LCNV, laser-induced choroidal neovascularization; MFI, mean fluorescence intensity; nAMD, neovascular age-related macular degeneration; R-CHP, sCy3-labeled collagen-hybridizing peptide; RPE, retinal pigment epithelium; sCy7.5-CHP, sCy7.5-labeled collagen-hybridizing peptide; VA2, bispecific anti-VEGF/anti-Ang-2 antibody; VEGF, vascular endothelial growth factor.