The Canonical Wnt Pathway in the Pathogenesis of Cardiac Allograft Vasculopathy

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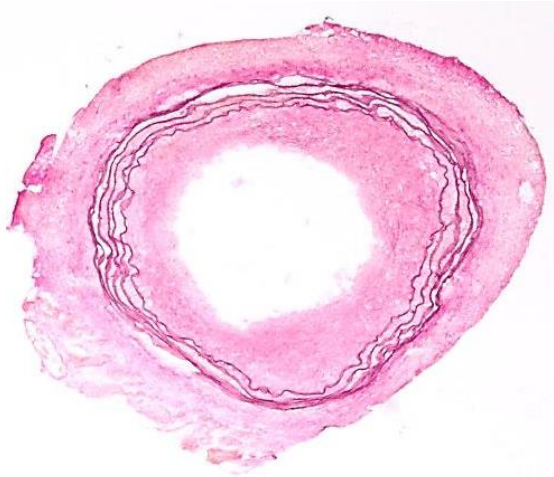
Cardiac allograft vasculopathy (CAV)

- Major limiting factor for long-term survival after heart transplantation
  - Morbidity about 50 % within 10 years after transplantation *(Khush 2019)*
  - After the 3rd year one of the main causes for death *(Khush 2019)*
- Pathogenesis
  - Ongoing immune reaction results in accumulation and proliferation of smooth muscle cells (SMC), leukocytes and mononuclear cells in the vessel wall (especially in the intima)

    (Waller et al. 2003; Labarrere et al. 2016)
  - Cell accumulation leads to diffuse concentric narrowing of the vascular lumen by forming the so-called neointima → ultimately vessel occlusion and organ failure

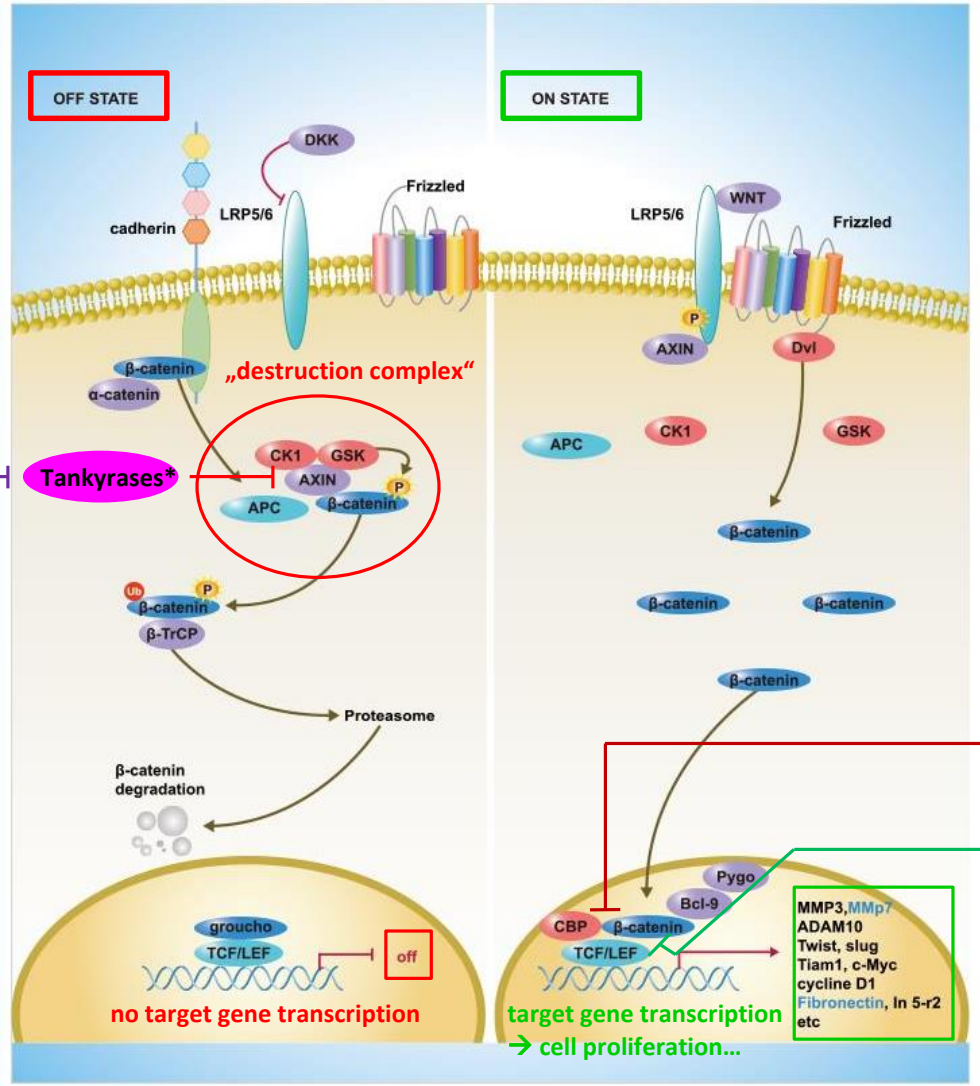
    (Rahmani et al. 2006; Labarrere et al. 2016)



Mouse aortic allograft with obvious neointima formation

The canonical wnt signaling pathway

In the absence of wnt ligands, intracellular β-catenin is degraded by a cytoplasmic "destruction complex" (left side).
If the wnt pathway is activated by binding of a wnt ligand to its membrane bound receptor (Frizzled) and coreceptor (LRP5/6), the destruction complex is inactivated and β-catenin reaches the cell nucleus, where it induces the transcription of wnt target genes, which promote cell survival and proliferation (right side).
XAV-939, ICG-001 and PKF118-310 interrupt the wnt pathway at different steps.



XAV-939

ICG-001

PKF118-310

* Tankyrases promote the degradation of axin, thereby inducing the disintegration of the „destruction complex"

https://www.sinobiological.com/Canonical-beta-Catenin-Dependent-Wnt-Signaling-a-1396.html

Links between the wnt signaling pathway and CAV

The wnt pathway modulates processes related to the pathogenesis of CAV:

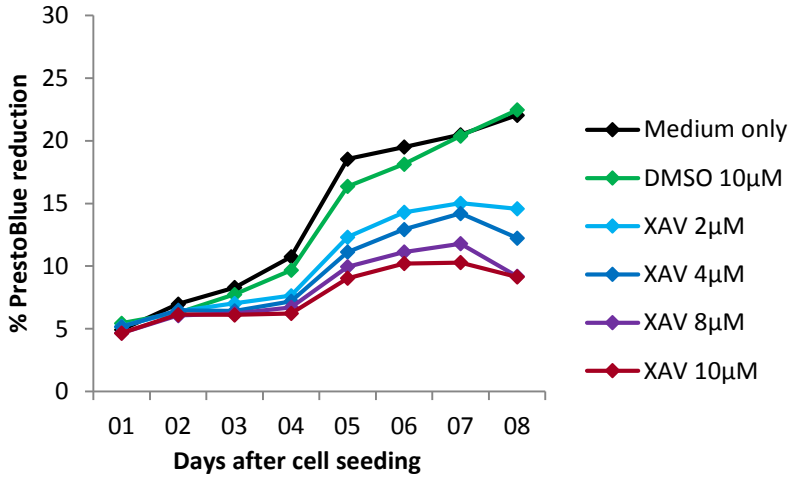
- β-catenin promotes proliferation of vascular smooth muscle cells (VSMCs)
  (Quasnichka et al. 2006)
- β-catenin is necessary for the development of neointima after vessel injury
  (Riascos-Bernal et al. 2017)

- XAV-939 inhibits migration and proliferation of VSMCs *in vitro*
  (Chen et al. 2016; Yang et al. 2015)
- XAV-939 attenuates neointima formation after carotis ligation in mice
  (Chen et al. 2016)

- ICG-001 and PKF118-310 inhibit VSMC proliferation *in vitro*
  (Riascos-Bernal et al. 2017)

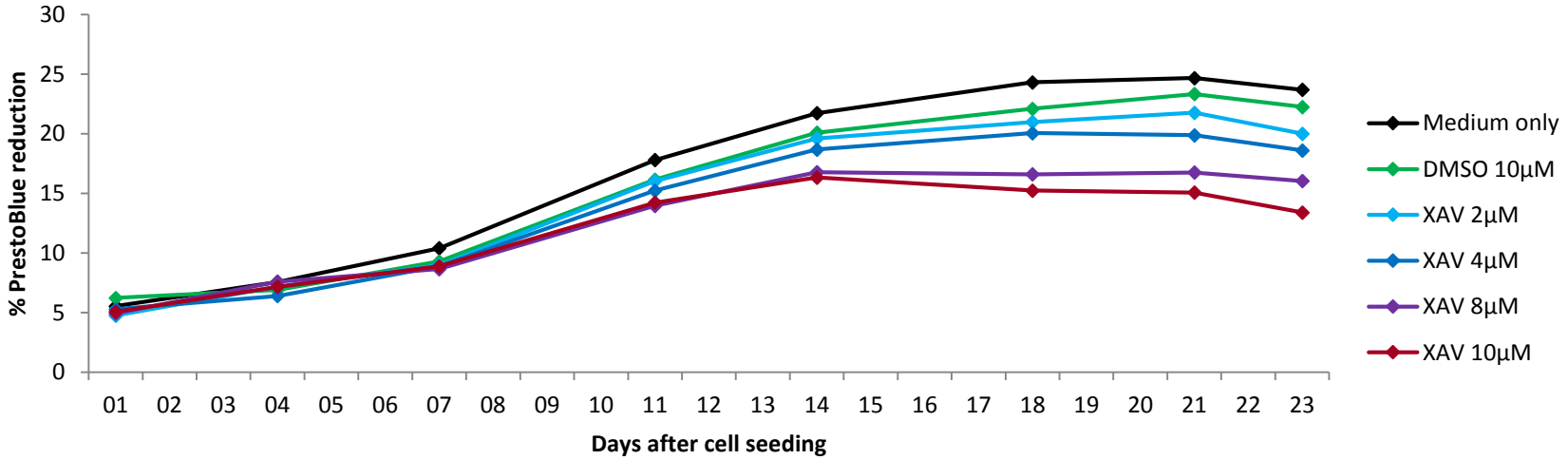
In vitro experiments with XAV-939

huSMCs



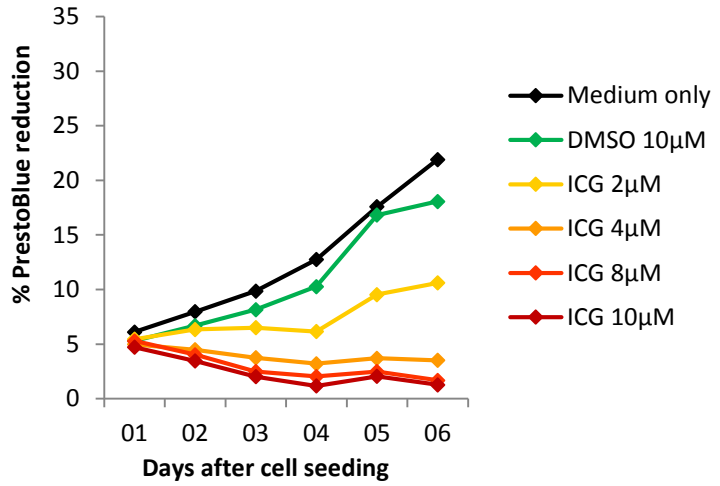
XAV-939 dose-dependently inhibited the proliferation of cultured human and murine VSMCs measured as metabolized PrestoBlue reagent.

muSMCs



In vitro experiments with ICG-001

huSMCs



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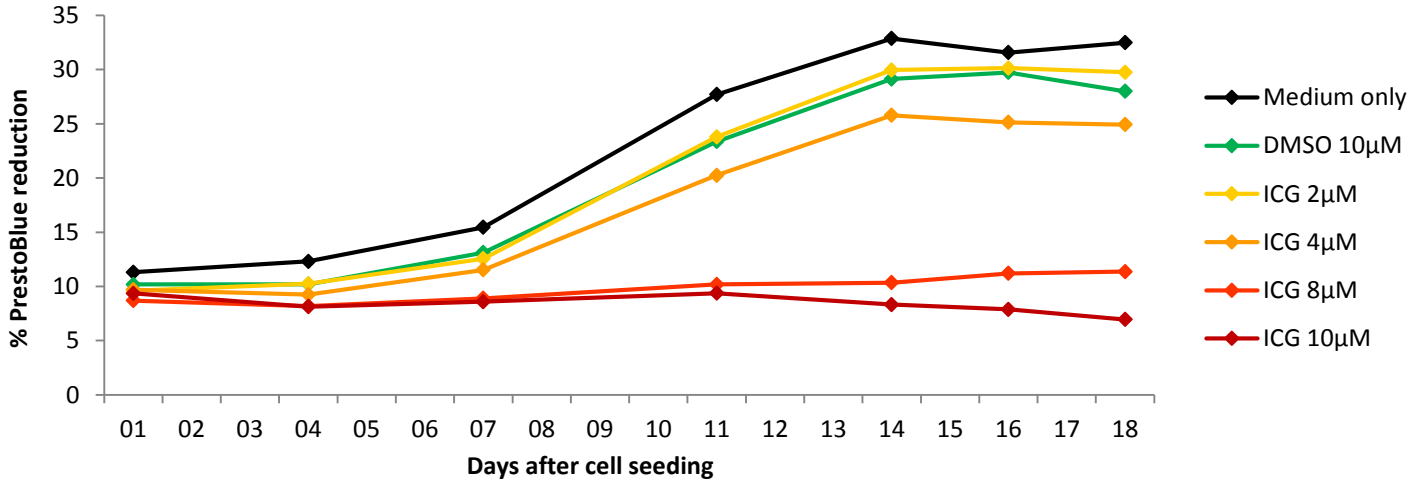
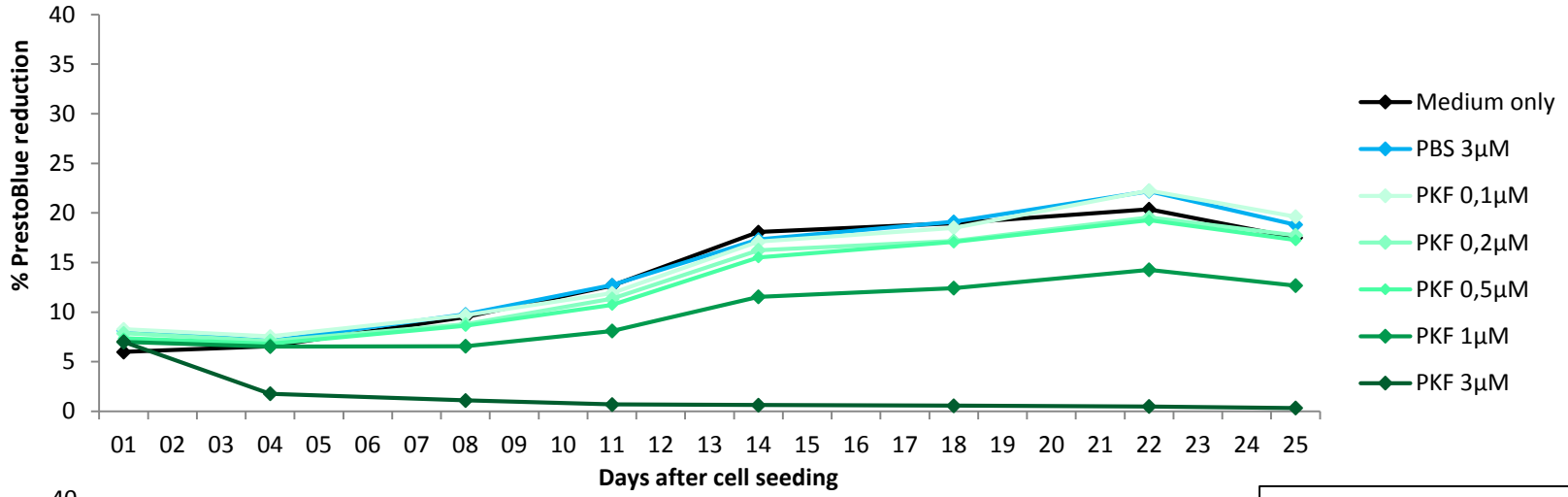
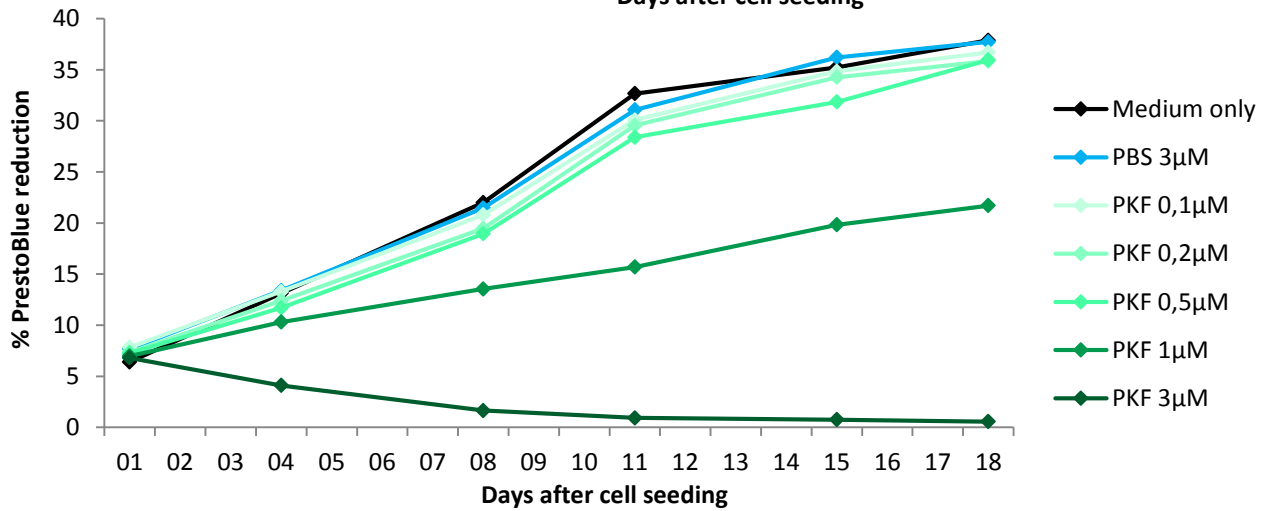


In vitro experiments with PKF118-310

huSMCs



muSMCs



PKF118-310 dose-dependently inhibited the proliferation of cultured human and murine VSMCs measured as metabolized PrestoBlue reagent. In the highest concentration it even caused cell death.

Wnt target gene expression after wnt inhibition *in vitro*

To verify that the observed proliferation inhibition of VSMCs after incubation with the wnt inhibitors is indeed based on a blockade of the wnt pathway, we analyzed the expression of typical wnt target genes in VSMCs. Red arrows indicate changes in target gene expression after treatment of cells with wnt inhibitors compared to untreated controls.

Expected changes after treatment with wnt inhibitors:

Cyclin D1 ↓

p21 ↑

Axin2 ↓

Observed changes after treatment with wnt inhibitors for two weeks:

Cyclin D1 → (XAV-939) and ↑ (ICG-001 and PKF118-310)

p21 → (XAV-939) and ↑ (ICG-001 and PKF118-310)

Axin2 ↓ (XAV-939, ICG-001 and PKF118-310)

Target gene expression only partially matched the expectations. Downregulation of Cyclin D1 could not be found.

Wnt target gene expression after wnt inhibition *in vitro*

We repeated the experiment with a shorter incubation time of the cells with the wnt inhibitors (24 hours instead of two weeks as before) because of respective descriptions in the literature.

Expected changes after treatment with wnt inhibitors:

Cyclin D1 ↓

p21 ↑

Axin2 ↓

Observed changes after treatment with wnt inhibitors for 24 hours:

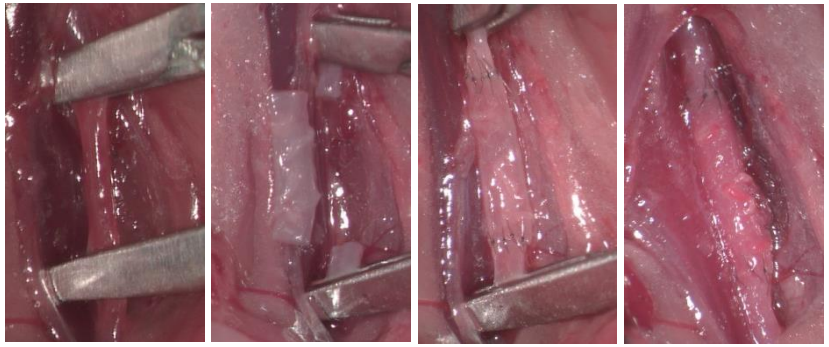
Cyclin D1 → (XAV-939, ICG-001) and ↓ (PKF118-310)

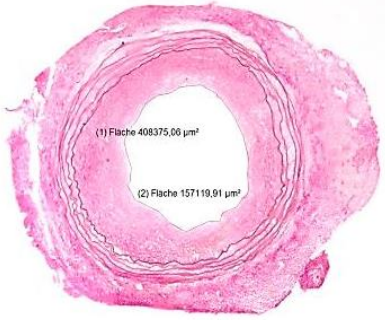
p21 → (XAV-939) and ↑ (ICG-001, PKF118-310)

Axin2 ↓ (XAV-939, ICG-001 and PKF118-310)

Target gene expression still only partially matched the expectations for XAV-939 and ICG-001, but was satisfactory for PKF118-310.

Animal experiment - design

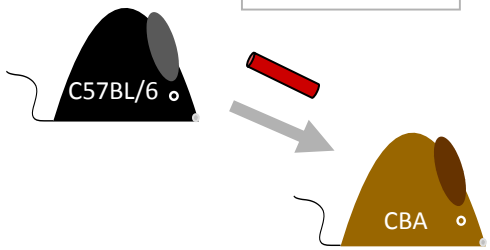


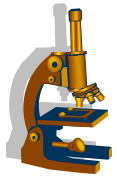


Abdominal Aortic Transplantation

PCR

Histology, Immunofluorescence





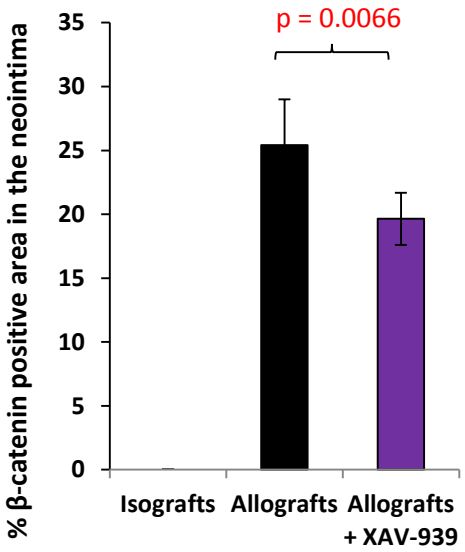
Day 0 — 14 — 30

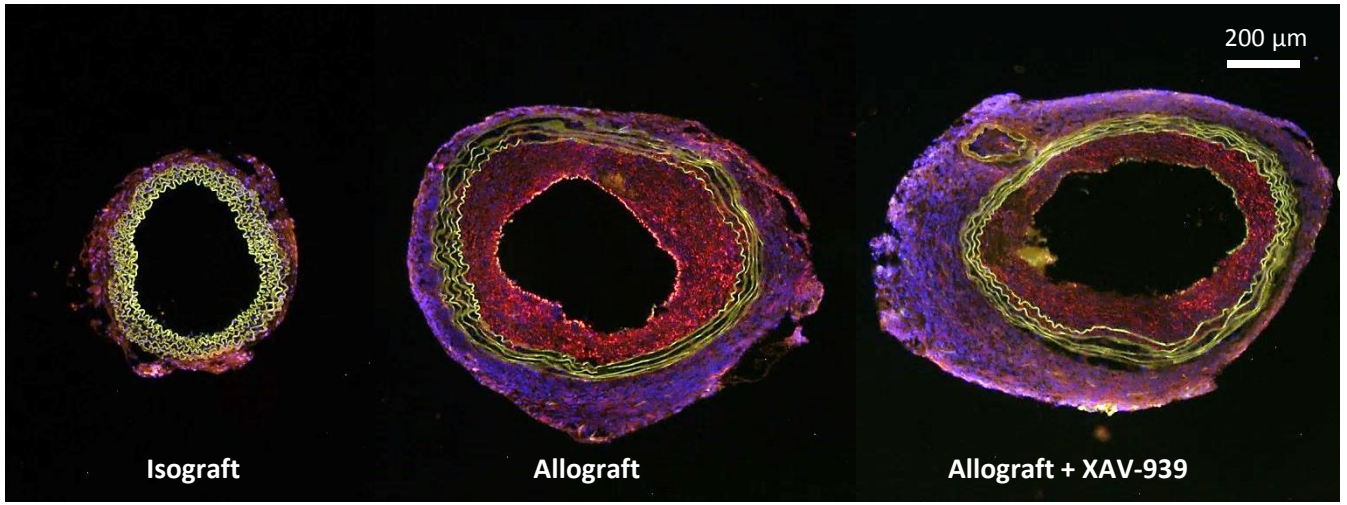
Treatment with Wnt inhibitors:

XAV-939 (5 mg/kg/d i.p.) or
ICG-001 (4 mg/kg/d i.p.) or
PKF118-310 (0.85mg/kg/d i.p.)

(dosages adapted from published reports)

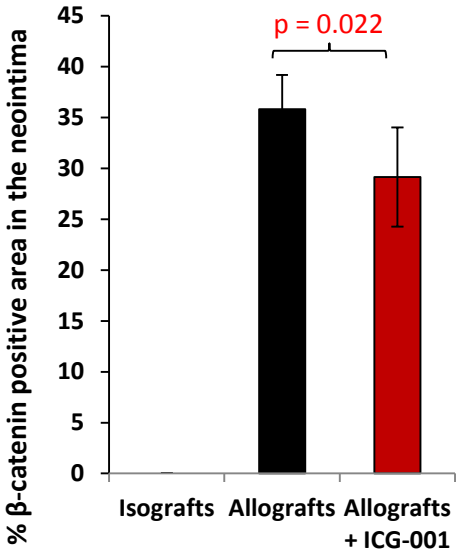
Neointimal expression of central wnt signaling molecule β-catenin 30 days after treatment with XAV-939

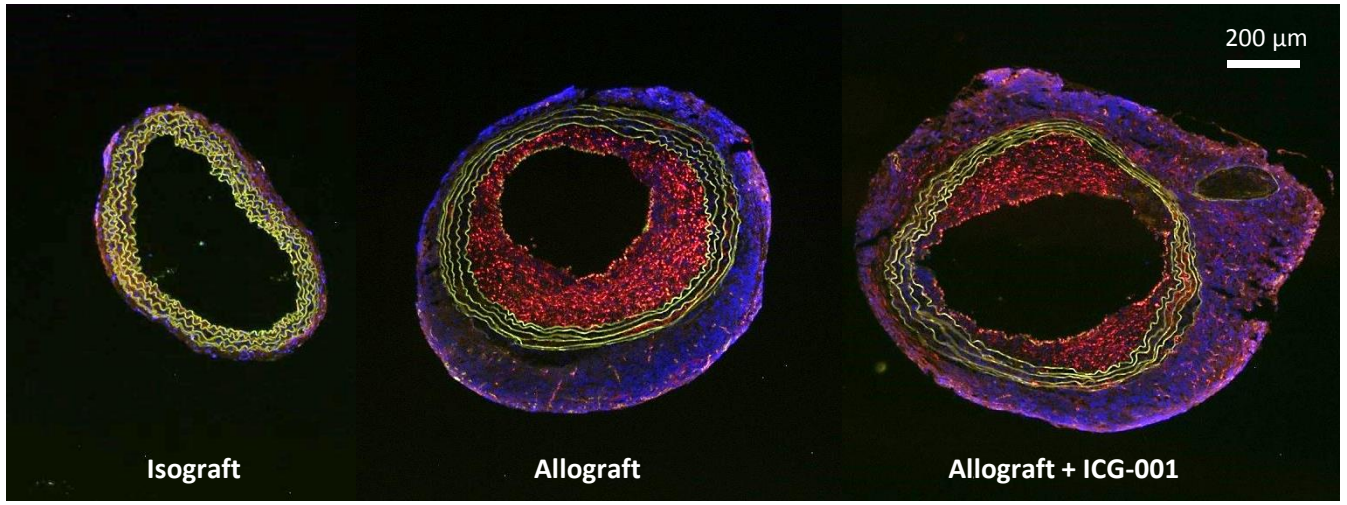




Red fluorescence marks β-catenin expression. Treatment with XAV-939 significantly reduced β-catenin expression in the aortic neointima compared to untreated allografts.

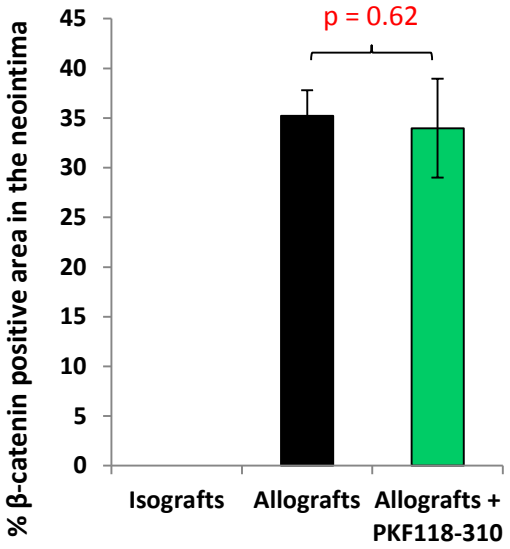
Neointimal expression of central wnt signaling molecule β-catenin 30 days after treatment with ICG-001





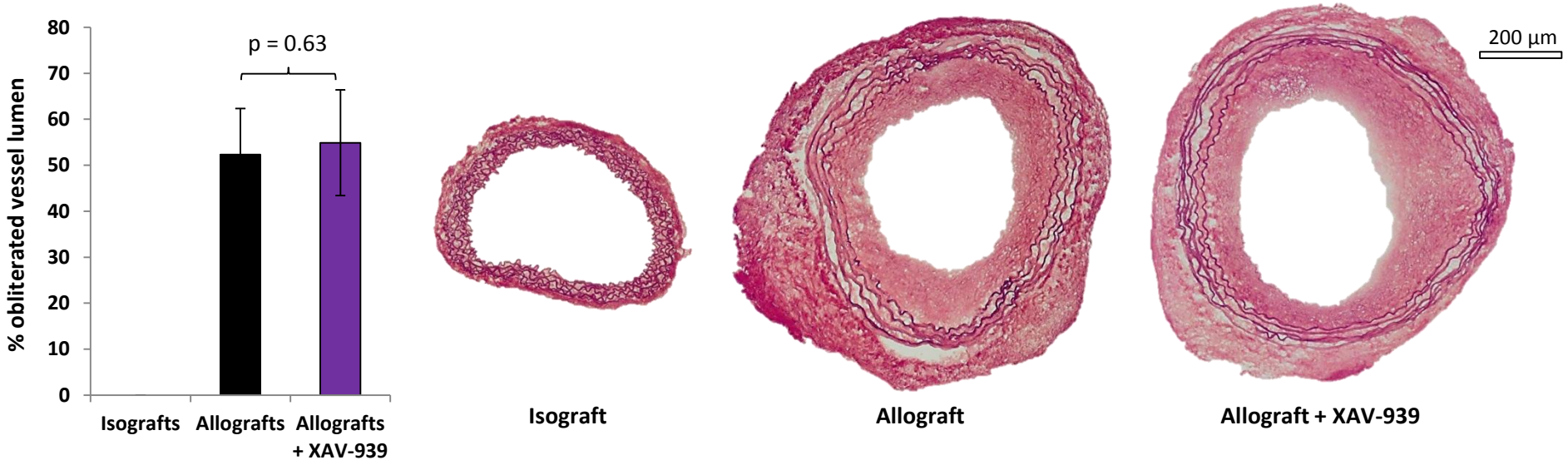
Red fluorescence marks β-catenin expression. Treatment with ICG-001 significantly reduced β-catenin expression in the aortic neointima compared to untreated allografts.

Neointimal expression of central wnt signaling molecule β-catenin 30 days after treatment with PKF118-310



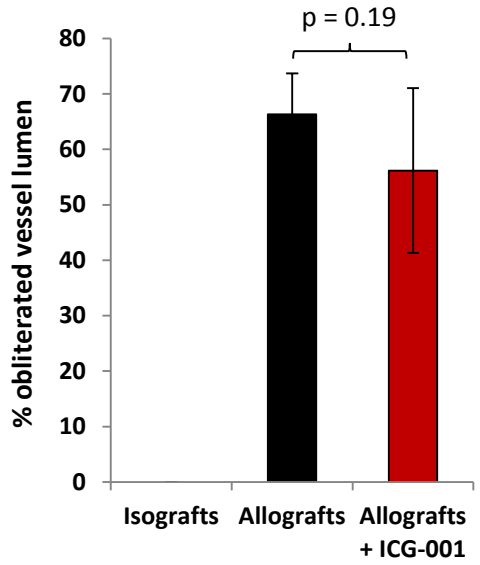
Red fluorescence marks β-catenin expression. Treatment with PKF118-310 had no influence on the expression of β-catenin in the aortic neointima compared to untreated allografts.

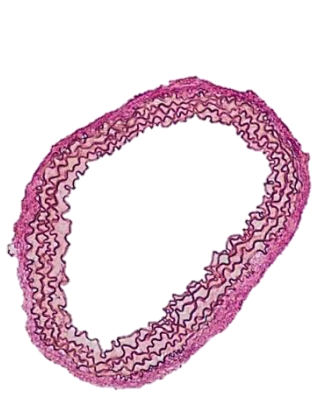
Neointimal proliferation 30 days after treatment with XAV-939



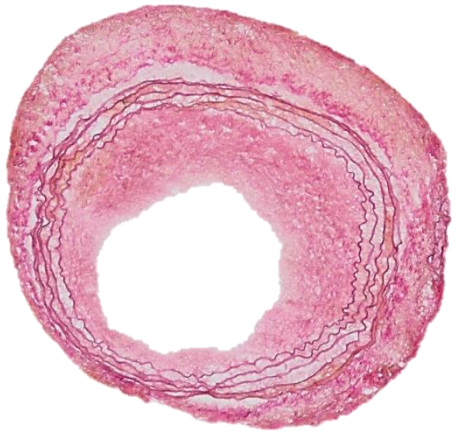
Neointimal area was measured and the percentage of obliterated vessel lumen calculated. Treatment with XAV-939 did not influence neointimal proliferation.

Neointimal proliferation 30 days after treatment with ICG-001

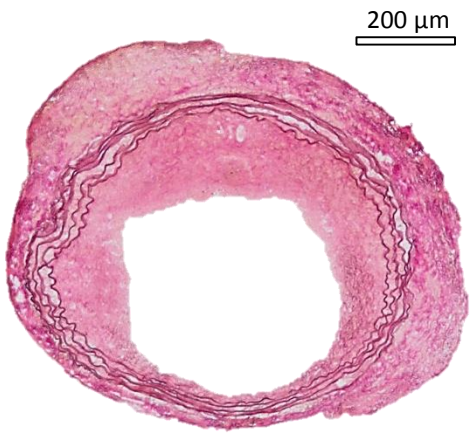




Isograft



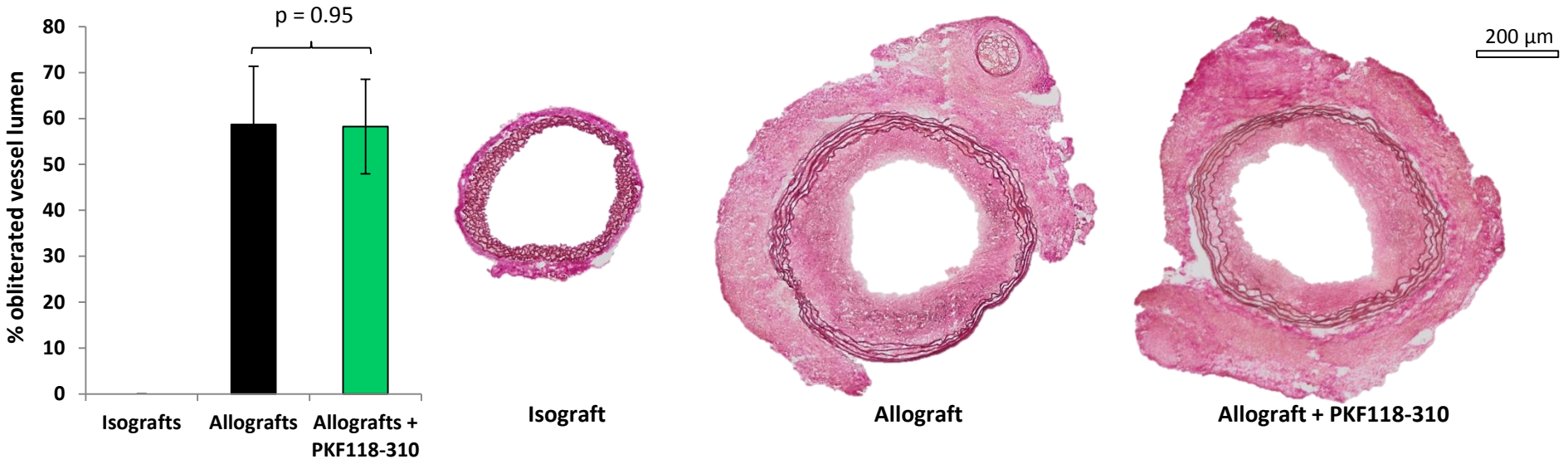
Allograft



Allograft + ICG-001

Neointimal area was measured and the percentage of obliterated vessel lumen calculated. Treatment with ICG-001 did not influence neointimal proliferation.

Neointimal proliferation 30 days after treatment with PKF118-310



Neointimal area was measured and the percentage of obliterated vessel lumen calculated. Treatment with PKF118-310 did not influence neointimal proliferation.

Wnt target gene expression in aortic transplants after 14 days of treatment with wnt inhibitors

mRNA expression of wnt target genes was measured in aortic transplants. Red arrows indicate changes after treatment with wnt inhibitors compared to untreated control allografts.

Expected changes after treatment with wnt inhibitors:

Cyclin D1 ↓

p21 ↑

Axin2 ↓

Observed changes after treatment with wnt inhibitors:

Cyclin D1 ↓ (XAV-939) → (ICG-001) ↑ (PKF118-310)

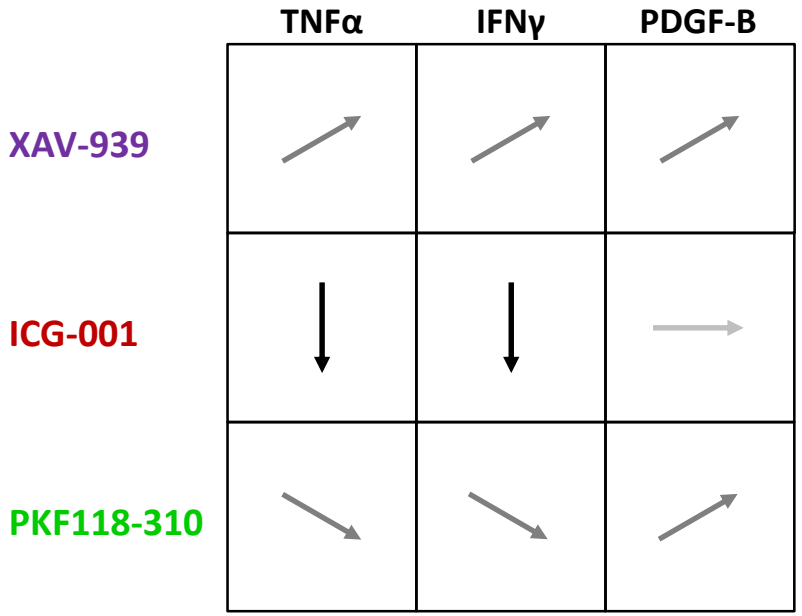
p21 ↓ (XAV-939, ICG-001) → (PKF118-310)

Axin2 ↑ (XAV-939, ICG-001) → (PKF118-310)

Target gene expression did not show sufficient wnt inhibition in aortic transplants.

Cytokine expression in aortic transplants

mRNA expression of classic pro-inflammatory cytokines TNFα, IFN γ and growth factor PDGF-B was measured in aortic transplants after 14 days of treatment with wnt inhibitors. Arrows indicate changes compared to untreated control allografts.



Decreased expression of inflammatory markers could be found for PKF118-310 and even stronger for ICG-001, but not for XAV-939.

Summary

- Dose-dependent growth inhibition of human and murine VSMCs *in vitro* after treatment with XAV-939, ICG-001 and PKF118-310
- ICG-001 and PKF118-310 induce regulation of Wnt target genes *in vitro*
- XAV-939 and ICG-001 significantly reduced expression of β-catenin, the central signaling molecule of the wnt pathway, in aortic neointima
- No relevant inhibition of luminal obliteration by any wnt inhibitor
- No sufficient influence on wnt target gene expression in aortic transplants by any wnt inhibitor
- Decreased expression of inflammatory cytokines after treatment with ICG-001 and PKF118-310, but not XAV-939

Conclusion

- No clinically relevant reduction in CAV development in spite of strong anti-proliferative effects of all wnt inhibitors on VSMCs *in vitro*
- Anti-proliferative effect *in vivo* is not strong enough to prevent neointima formation/CAV development
- Underdosage of inhibitors in the animal model cannot be ruled out
- Suspected reason for lacking *in vivo* effects of wnt inhibitors: Complexity of the wnt pathway combined with multifactorial pathogenesis of CAV → compensation of anti-proliferative effects of wnt inhibitors by other mechanisms (e.g. growth factor release from activated platelets or other cells)

Thank you for your interest!

For questions, please send me a message to: annika.kuckhahn@uk-erlangen.de