Staying in the flow: preferential PDE4B inhibitor nerandomilast (BI 1015550) improves features of vascular dysfunction in lung fibrosis *in vitro* and *in vivo*

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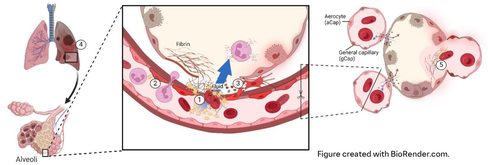
INTRODUCTION

- Nerandomilast (BI 1015550) is an oral preferential inhibitor of PDE4B and a potential treatment for IPF and PPF.[1–4]
- Preclinical studies have shown that nerandomilast has anti-inflammatory and anti-fibrotic properties.[1]
- There is increasing evidence that vascular dysfunction is an important contributor to disease progression in pulmonary fibrosis.[5]
- Pathologic endothelial-associated features of pulmonary fibrosis include: 1) increased vascular permeability and coagulation, 2) increased immune cell infiltration, 3) endothelial-to-mesenchymal transition, 4) deregulated angiogenesis, and 5) loss of endothelial cell type specialization.[5–7]



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AIM

- To investigate the activity and mode of action of nerandomilast in a rat model of Bleo-induced lung fibrosis *in vivo* and in disease-relevant 3D models of endothelial injury *in vitro*.

METHODS

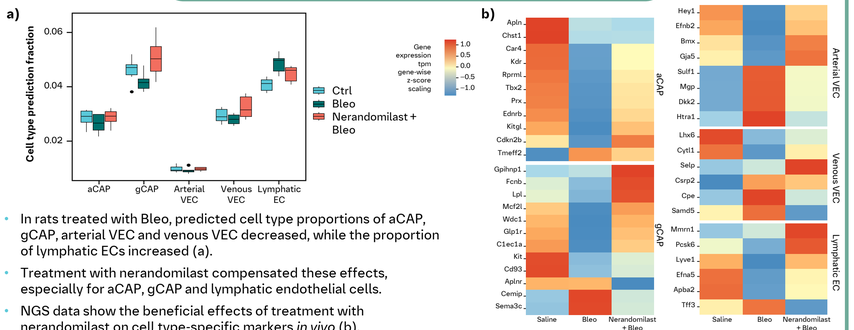
- Bleo-induced lung fibrosis in rats was treated with nerandomilast (2.5 mg/kg, twice daily from Day 10–20) and lung tissue was analyzed using RNA-Seq.
- HPMECs were cultured in microfluidic chips (MIMETAS) to form functional tubes; fibrotic changes were induced by stimulation with an IPFrc.[8]
- IPFrc-induced vascular permeability was measured in a 3D *in vitro* culture of HPMECs under bi-directional flow by the leakage of FITC-dextran from the perfusion to the ECM gel channel.
- Immune cell infiltration was measured by the adhesion of calcein-labeled monocytes.
- Protein expression of CD31 (PECAM-1) and VCAM-1 was measured using Simple Western capillary gel electrophoresis and immunostaining.

CONCLUSIONS

- In both *in vitro* and *in vivo* models of pulmonary fibrosis, nerandomilast had beneficial effects on features of vascular dysfunction.
- Nerandomilast normalized endothelial cell type specialization, inhibited immune cell infiltration and reduced microvascular permeability by stabilizing endothelial barrier integrity.
- Preclinical and clinical studies to date indicate that nerandomilast is a potential treatment for IPF and PPF.

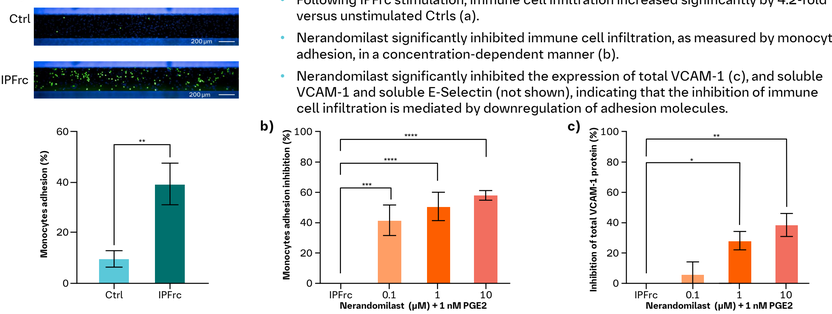
RESULTS

Nerandomilast normalizes endothelial cell type specialization in Bleo rat



- In rats treated with Bleo, predicted cell type proportions of aCAP, gCAP, arterial VEC and venous VEC decreased, while the proportion of lymphatic ECs increased (a).
- Treatment with nerandomilast compensated these effects, especially for aCAP, gCAP and lymphatic endothelial cells.
- NGS data show the beneficial effects of treatment with nerandomilast on cell type-specific markers *in vivo* (b).

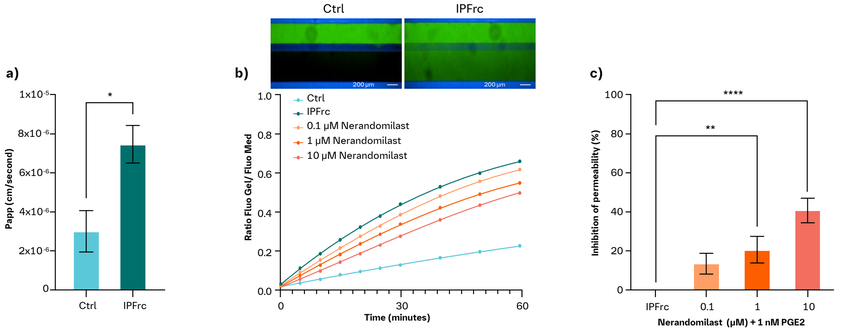
Nerandomilast inhibits immune cell infiltration



- Following IPFrc stimulation, immune cell infiltration increased significantly by 4.2-fold versus unstimulated Ctrls (a).
- Nerandomilast significantly inhibited immune cell infiltration, as measured by monocyte adhesion, in a concentration-dependent manner (b).
- Nerandomilast significantly inhibited the expression of total VCAM-1 (c), and soluble VCAM-1 and soluble E-Selectin (not shown), indicating that the inhibition of immune cell infiltration is mediated by downregulation of adhesion molecules.

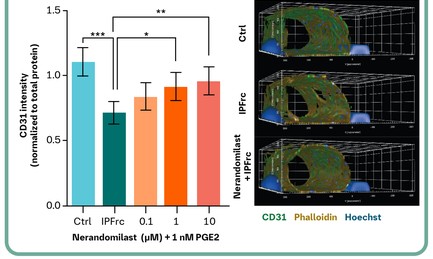
Nerandomilast stabilizes endothelial barrier integrity by reducing microvascular permeability

- Stimulation of healthy HPMECs with an IPFrc significantly increased vascular permeability by 2.5-fold (a).
- Treatment with nerandomilast significantly inhibited microvascular permeability in a concentration-dependent manner by up to 41% (b, c).



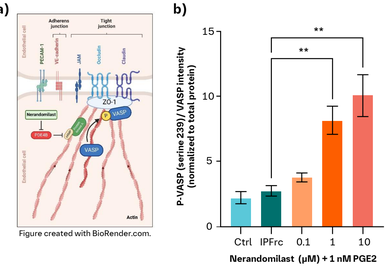
Nerandomilast stabilizes endothelial barrier integrity by enhancing cell–cell junctions

- Following IPFrc stimulation, human microvascular endothelial CD31protein expression significantly decreased by 0.4-fold compared with unstimulated Ctrls.
- Treatment with nerandomilast significantly increased CD31 protein expression in a concentration-dependent manner (a).
- The effects of nerandomilast were confirmed by immunostaining (b).



Nerandomilast stabilizes endothelial barrier integrity by enhancing tight junctions

- In tight junctions, P-VASP is located in a complex with ZO-1 protein (a).
- Nerandomilast increases VASP phosphorylation at serine 239 in a concentration-dependent manner (b), indicating that nerandomilast increases subsequent translocation of P-VASP and downstream stabilization of tight junctions in a complex with ZO-1.[9]



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*p<0.05; **p<0.005; ***p< 0.0005; ****p< 0.0001.

ABBREVIATIONS

aCAP, aerocyte capillary cells; gCAP, general capillary cells; Bleo, bleomycin; cAMP, cyclic adenosine monophosphate; CD31, cluster of differentiation 31; Ctrl, control; EC, endothelial cells; ECM, extracellular matrix; FITC-dextran, fluorescein isothiocyanate-dextran; Fluo Gel, fluorescence intensity ECM gel channel; Fluo Med, fluorescence intensity perfusion channel; HPMEC, human pulmonary microvascular endothelial cells; IPF, idiopathic pulmonary fibrosis; IPFrc, IPF-relevant cytokine cocktail; JAM, junctional adhesion molecules; NGS, next-generation sequencing; P, phosphorylated; Papp, apparent permeability coefficient; PECAM-1, platelet endothelial cell adhesion molecule-1; PGE2, prostaglandin E2; PPF, progressive pulmonary fibrosis; tpm, transcripts per million; VASP, vasodilator-stimulated phosphoprotein; VCAM-1, vascular cell adhesion molecule 1; VEC, vascular endothelial cells; VE, vascular endothelial; ZO-1, zonula occludens-1.

DISCLOSURES

DR, FW, CHM, PN and FEH are employees of Boehringer Ingelheim. PN and FEH have commercial interests in active patents at Boehringer Ingelheim.

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