Anti-EphrinB2 neutralizing antibody suppresses fibrosis in preclinical in vitro and in vivo models

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Rationale

Fibrosis is complex disease characterized by persistent inflammation, aberrant synthesis and accumulation of extracellular matrix (ECM), and ultimately loss of organ function as healthy tissue is replaced by scar tissue.

Dysregulated Eph-Ephrin signaling has been implicated in a wide array of fibrotic diseases including systemic sclerosis and idiopathic pulmonary fibrosis (IPF). Although the exact mechanism is not well understood, dysregulation of the EphrinB2-EPHB signaling axis in particular is thought to play a role in fibrotic disease progression.

To test this hypothesis, we generated neutralizing antibodies to EphrinB2 and investigated their anti-fibrotic efficacy both in vitro and in preclinical mouse models of skin and lung fibrosis.

Methods

In-vitro assays: Inhibition of EphB4 Receptor phosphorylation was evaluated by treating Human Umbilical Vein Endothelial Cells (HUVECs) with EphrinB2-Fc +/- anti-EphrinB2 antibody or isotype control for 1h and quantifying pEphB4 via ELISA (RayBiotech). HUVEC transwell migration was evaluated by coating the membrane fibronectin and seeding 1k cells/well. Anti-EphrinB2 or isotype control were added to the top well and 10% FBS in the bottom well. Wells were imaged using an Incucyte (Sartorius).

In-vivo: Bleomycin mouse models of skin and lung fibrosis were conducted at Aragen under IACUC approved protocols. Study designs are shown in the Results. Inflammatory cytokines were evaluated via MSD 10-plex. Histological analysis was conducted at Dallas Tissue and evaluated by a board-certified pathologist.

Conclusions

Anti-EphrinB2 antibody inhibited EphB4 phosphorylation and migration in HUVECs.

Anti-EphrinB2 antibody significantly reduced fibrosis in preclinical mouse models of skin and lung fibrosis.

Based on these data, anti-EphrinB2 warrants further assessment in clinical trials.

Acknowledgments

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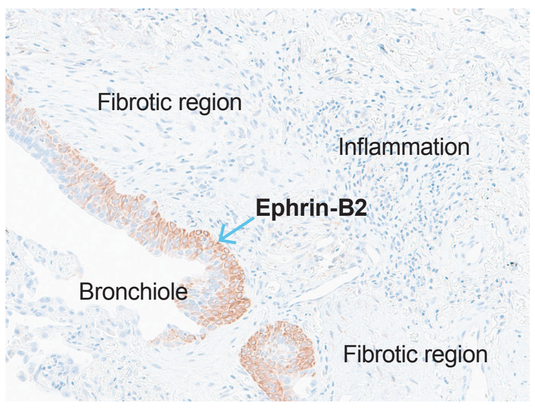
PhenoVista: Stephen Gilmore, Matt Stoltz

Dallas Tissue: Jessica Durrant, Alex Van Engelenburg

Results

EphrinB2 co-localizes with fibrotic foci in human IPF lung tissue (FFPE)

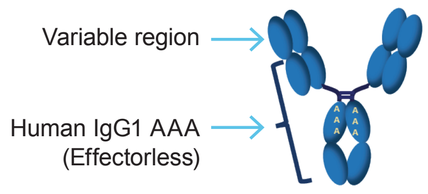


FFPE: formalin fixed, paraffin-embedded

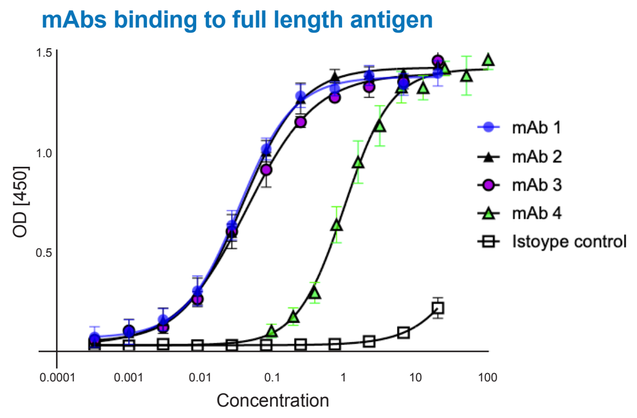
EphB2 expression is dysregulated in aberrant basaloid cells in human IPF

SOURCE: IPFCellAtlas.com; Modified from Kaminski/Rosas (2020)

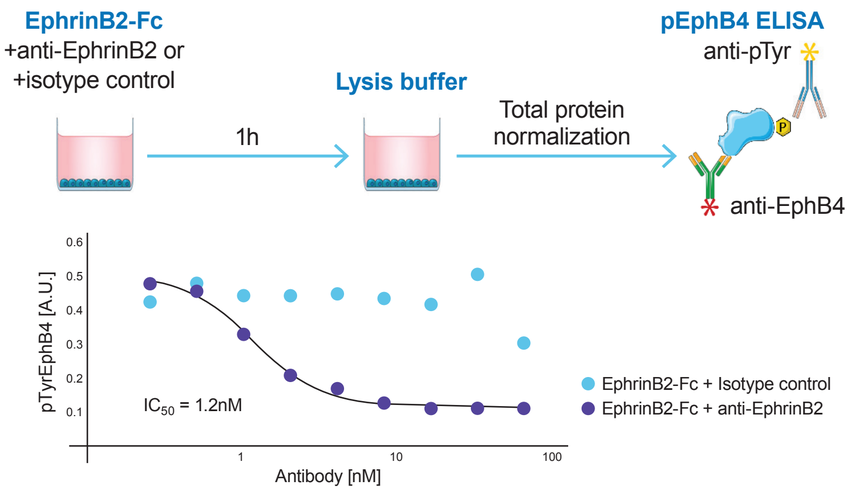
Anti-EphrinB2 human IgG1 monoclonal antibody binds EphrinB2 with high specificity and high affinity



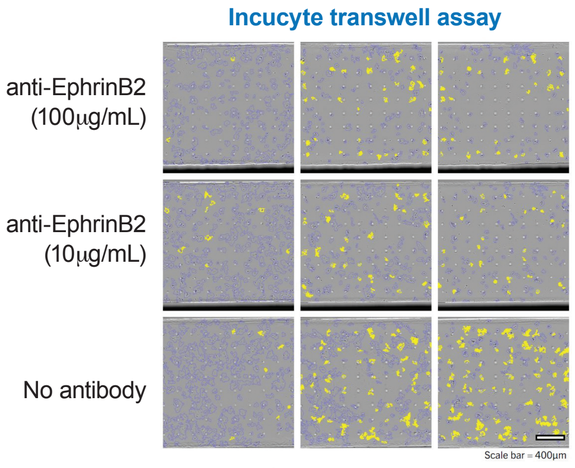
mAb ID	EC_{50} [nM]
mAb1	0.034
mAb 2	0.038
mAb 3	0.044
mAb 4	1.00
Isotype control	NA



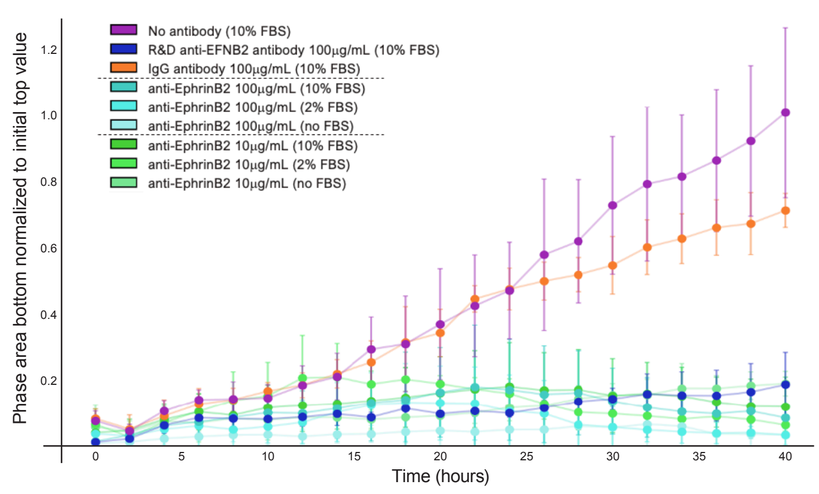
Anti-EphrinB2 inhibits EphB4 phosphorylation in HUVECs



Anti-EphrinB2 inhibits HUVEC migration in transwell assay

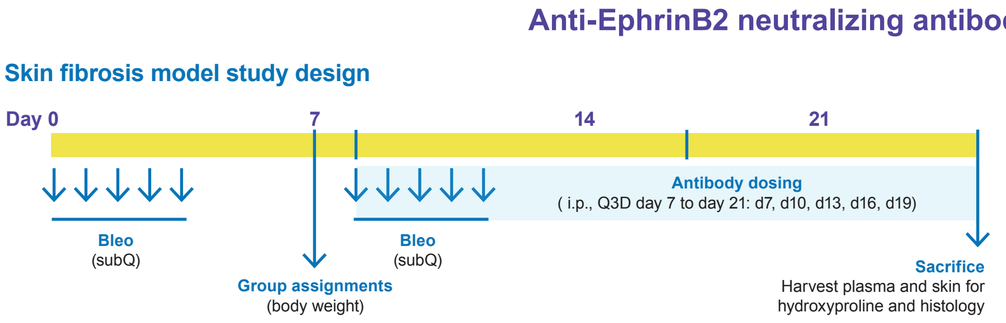


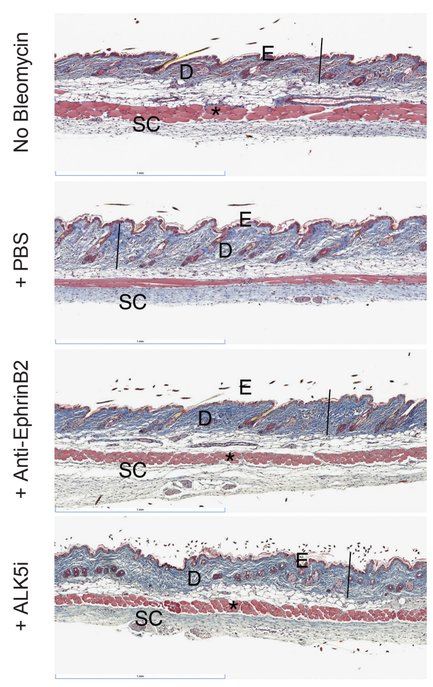
Yellow = HUVECs which have migrated to lower chamber



Anti-EphrinB2 neutralizing antibody suppresses skin and lung fibrosis in bleomycin mouse models

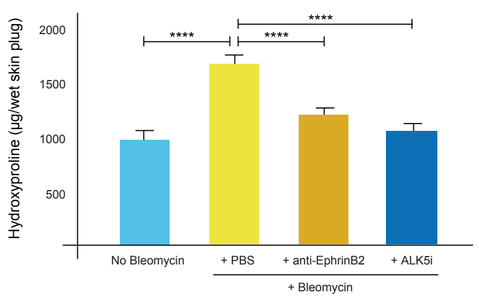
Skin fibrosis model study design



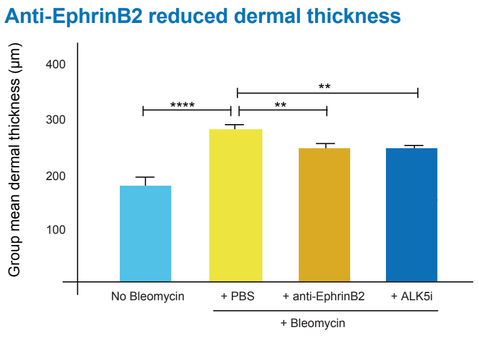


E – epidermis; D – dermis; SC – subcutaneous adipose tissue

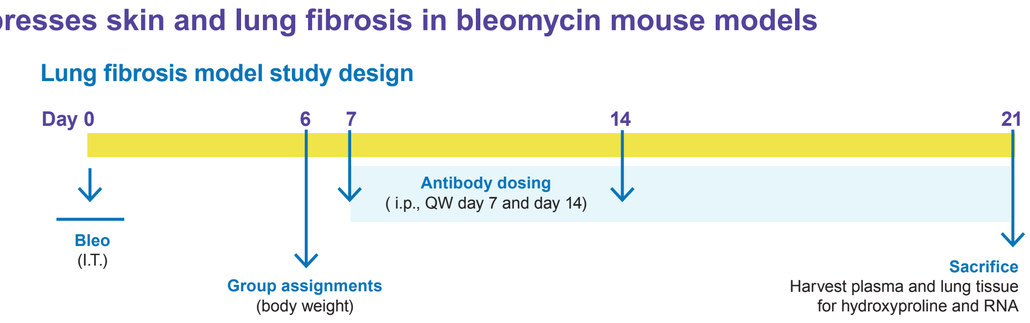
Anti-EphrinB2 suppressed collagen deposition in skin



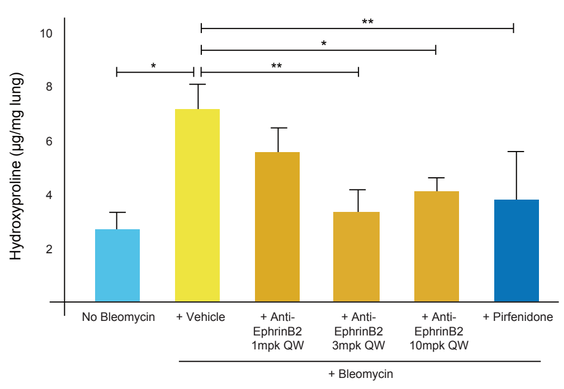
Anti-EphrinB2 reduced dermal thickness



Lung fibrosis model study design

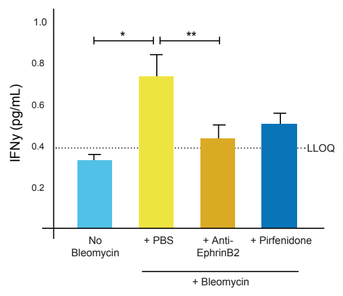


μg Hydroxyproline per mg of lung (collagen deposition)

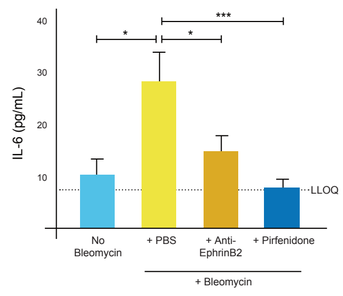


	+ anti-EphrinB2 1mpk	+ anti-EphrinB2 3mpk	+ anti-EphrinB2 10mpk	+ Pirfenidone
Reduction in hydroxyproline	**35.7%**	**85.8%**	**68.7%**	**75.7%**
SD	78.40	69.94	44.34	39.92
SEM	20.24	18.69	11.45	10.31

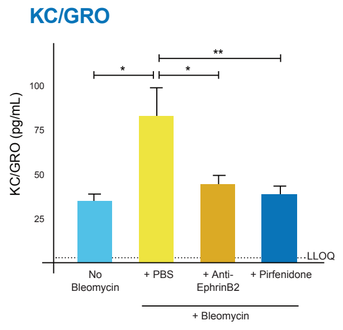
IFNγ



IL-6



KC/GRO



TNFα