

Deciphering the clinical efficacy mechanism of buloxibutid (C21) in idiopathic pulmonary fibrosis



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Aims

Buloxibutid (also known as C21) is a potent and selective angiotensin II type 2 receptor (AT2R) agonist under development for oral treatment of idiopathic pulmonary fibrosis (IPF). Buloxibutid has been dosed in >300 subjects and found to be well tolerated.

The function of the renin-angiotensin system (RAS) and the protective functions of AT2R are illustrated in Figure 1.

The AT2R is highly expressed on alveolar epithelial cells type 2 (AEC2) in both healthy subjects and IPF patients (Figure 2).

The AEC2 have important maintenance functions in the alveoli (Figure 3), and when dysfunctional, they are believed to play an important role in IPF pathophysiology (Figure 4).

The role of matrix metalloproteinases (MMPs) in IPF is complex and incompletely understood. However, MMP-13, a collagenase with fibrolytic anti-fibrotic capacity, has been suggested to play a protective role in IPF¹.

In a recently completed open-label phase 2a IPF trial (AIR), buloxibutid (100 mg BID) stabilized and improved lung function as measured by FVC over a 36-week period (Figure 5)².

Here we aim to shed light on the potential modes of action of buloxibutid in IPF.

Methods & Results

Receptor autoradiography in human lung tissue (Figure 6): The AT2R is highly expressed in the human lung, while the expression of AT1R is comparatively very low.

Precision cut IPF lung slices - TGFβ1 and collagen (Figure 7): Buloxibutid dose-dependently inhibited TGFβ1 and collagen.

Primary human AEC2 (Figure 8): Buloxibutid significantly downregulated genes involved in epithelial-to-mesenchymal transition (EMT).

Primary human airway epithelial cell + myofibroblast co-culture (Figure 9): Buloxibutid dose-dependently inhibited myofibroblast α-smooth muscle actin (αSMA) and N-cadherin protein expression. This was not seen in a myofibroblast monoculture system, suggesting paracrine signaling between the two cell types.

Precision cut IPF lung slices – Surfactant proteins (Figure 10): Buloxibutid greatly increased surfactant protein A, B and C gene expression. Similar results were seen with two buloxibutid analogues.

Clinical phase 2a IPF trial – plasma MMP-13 (Figure 11): Plasma MMP-13 increased in IPF patients treated with buloxibutid 100 mg BID for up to 36 weeks. There was no significant increase in plasma levels of the pro-fibrotic MMP-7, as measured at week 24 in 18 of the patients in a previous interim analysis.

Acknowledgment: We thank Prof Donna Davies, Dr Franco Conforti and Dr Joseph Bell at University of Southampton for the work with primary human AEC2.

Figure 2. AT2R – Lung single cell gene expression

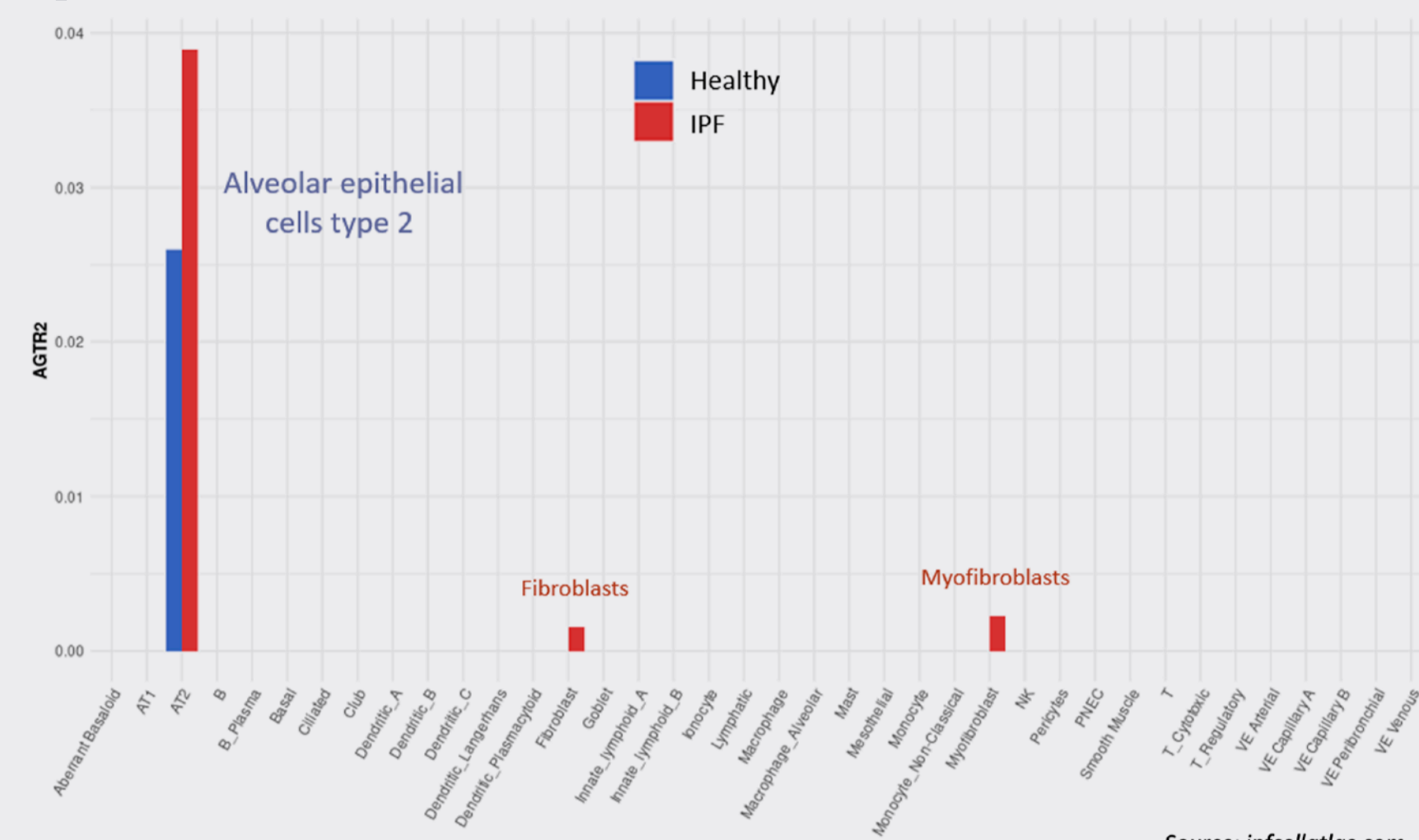


Figure 3. Alveolar epithelial cells type 1 (AEC1) and 2 (AEC2)

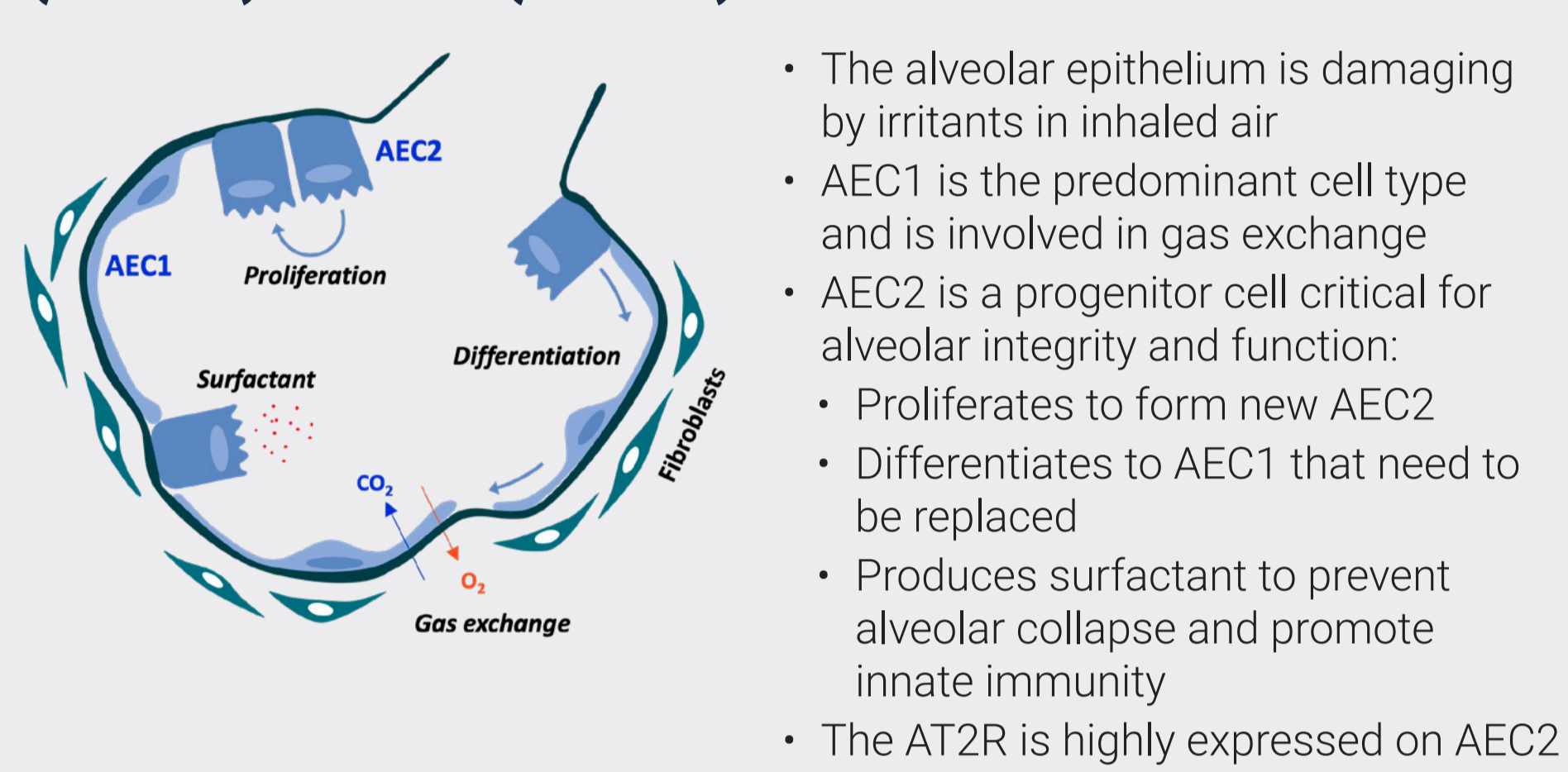


Figure 4. Role of AEC2 in IPF progression

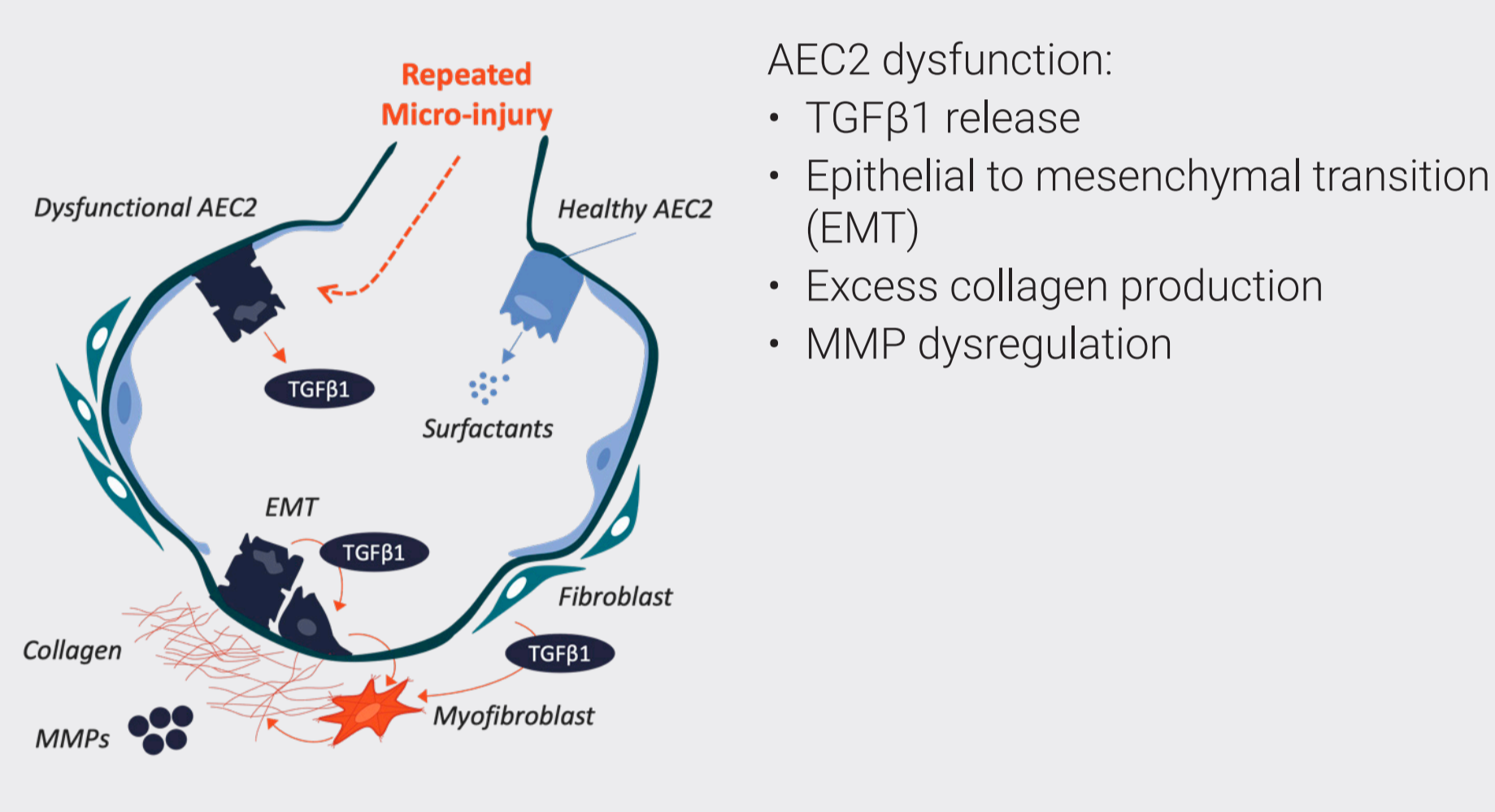
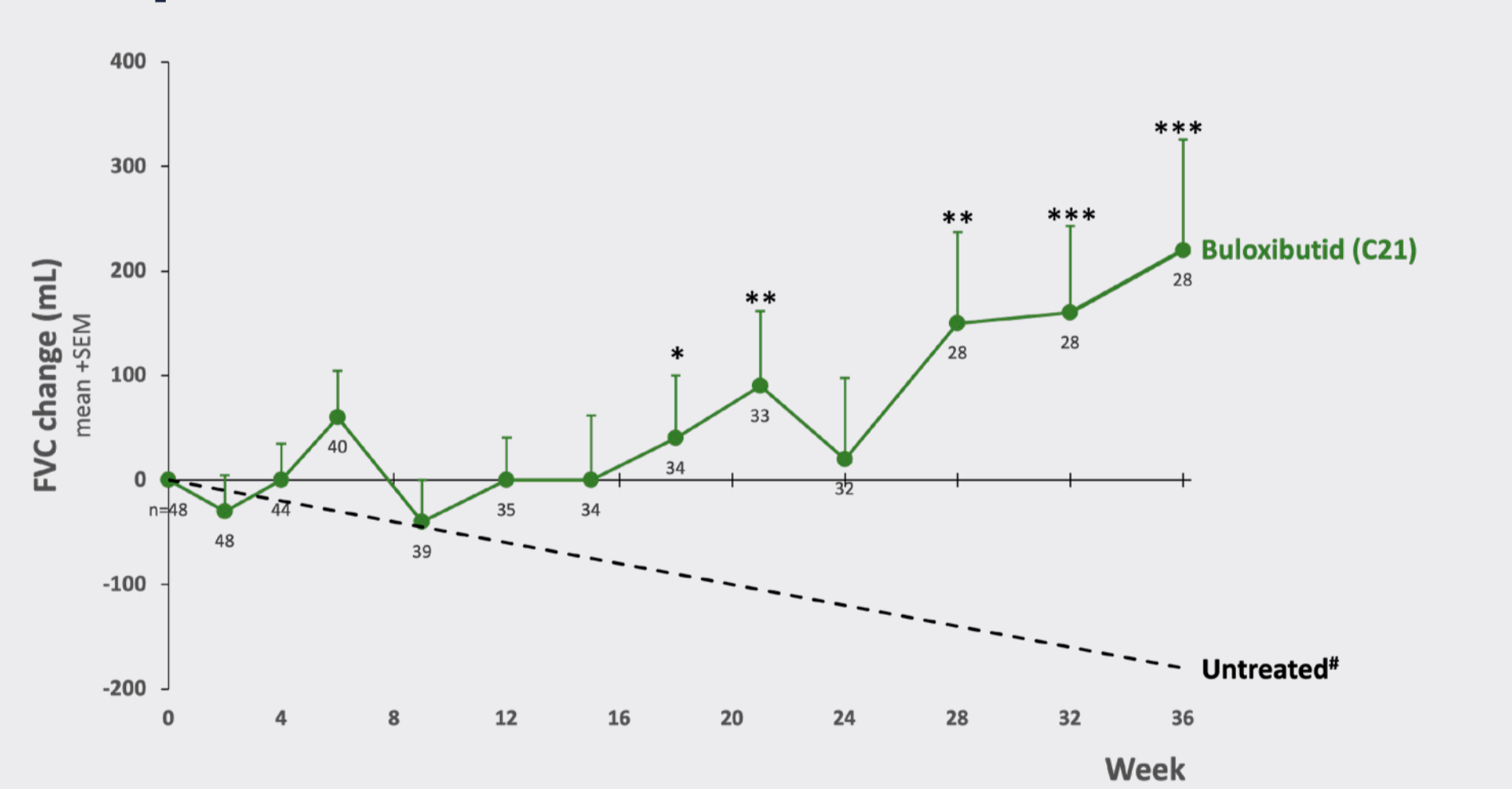


Figure 5. Effect of buloxibutid on FVC in IPF patients

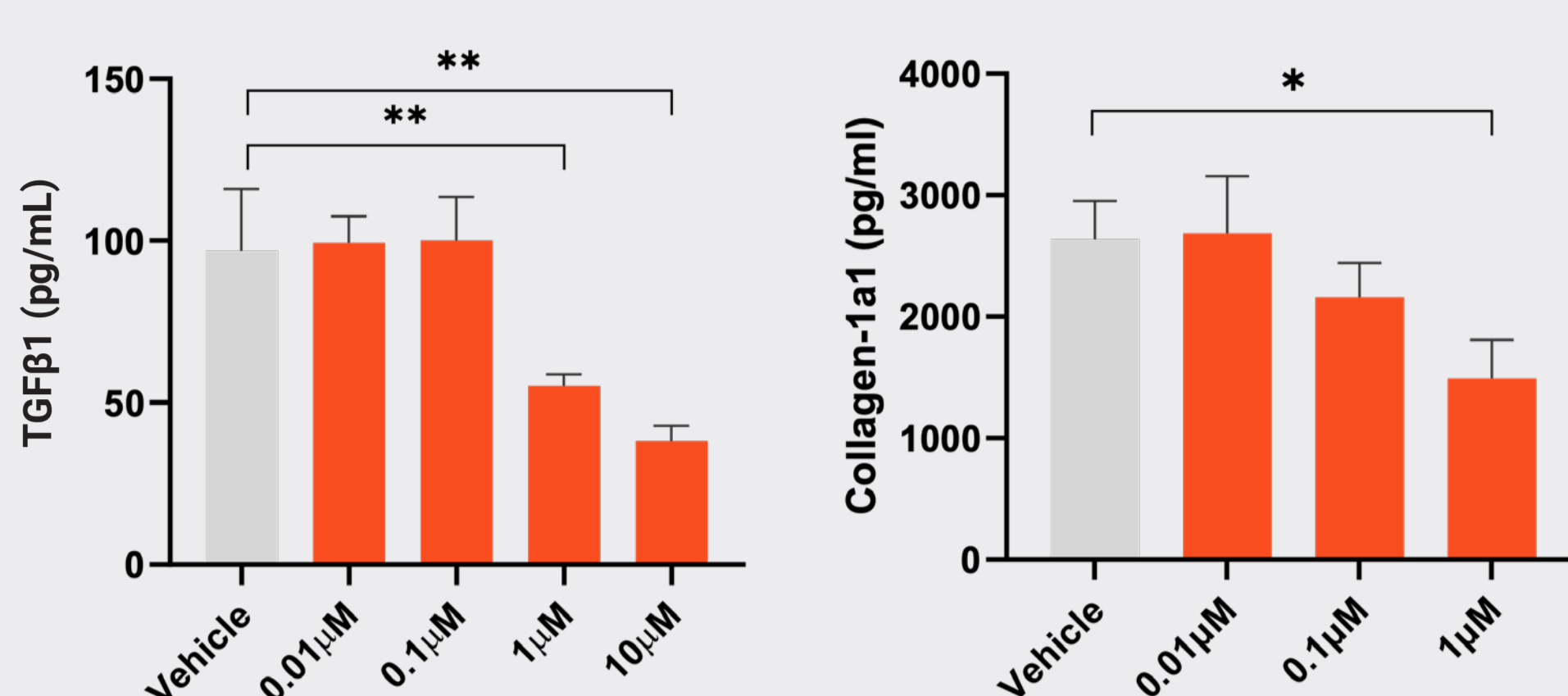


Note: n=48 patients with 2-week FVC data. Observed values, no imputation.
Untreated: Expected average decline for untreated patients based on published placebo data^{1,2}.
*p<0.05, **p<0.01, ***p<0.001; t-test versus expected untreated decline corresponding to -120 mL/24 weeks

¹Noble et al. Eur Respir J. 2016 Jan; 47(1): 243–253

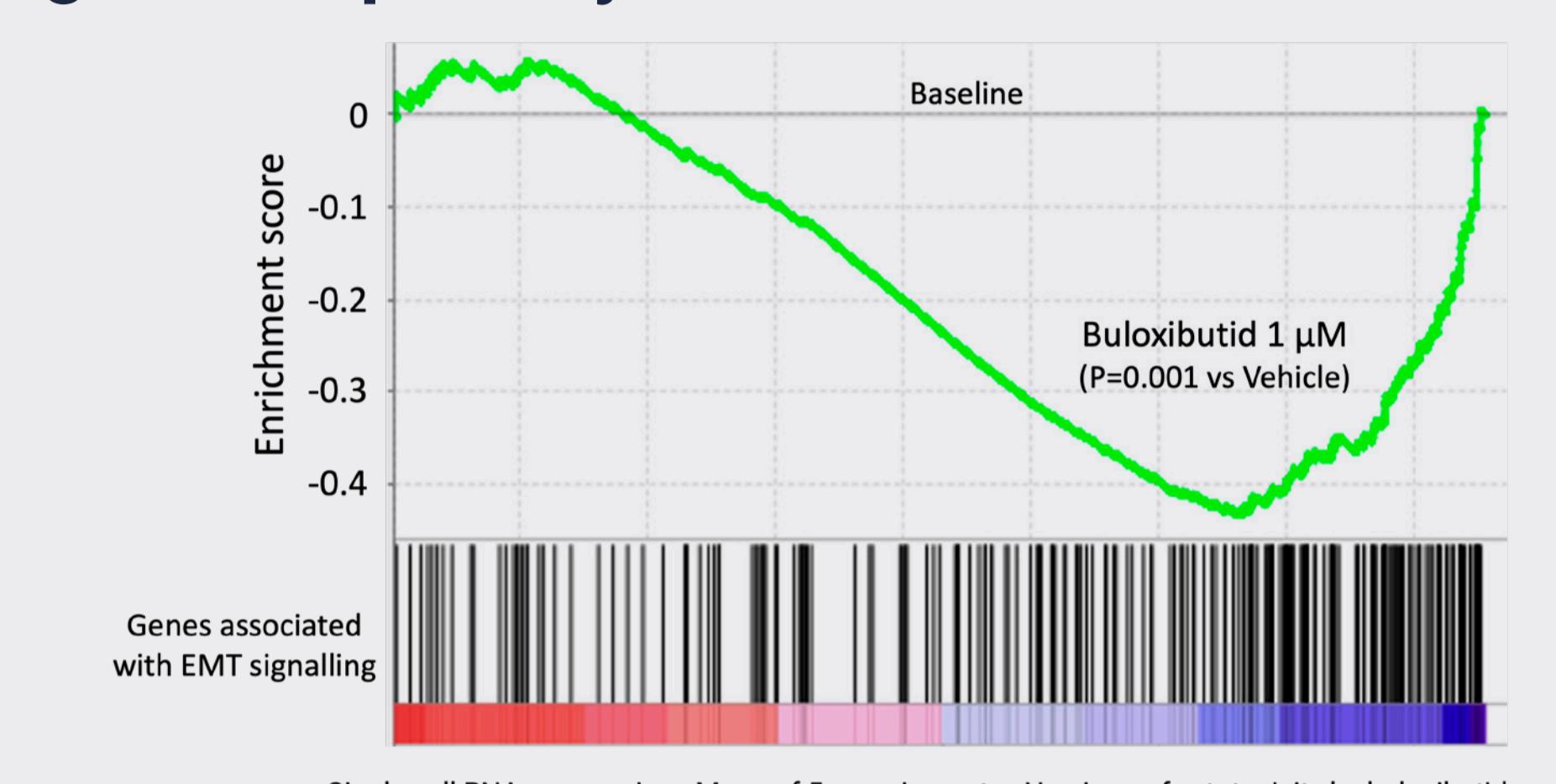
²Richeldi et al. Engl J Med 2014; 370:2071-2082

Figure 7. Buloxibutid reduces TGFβ1 and collagen in human IPF lung slices



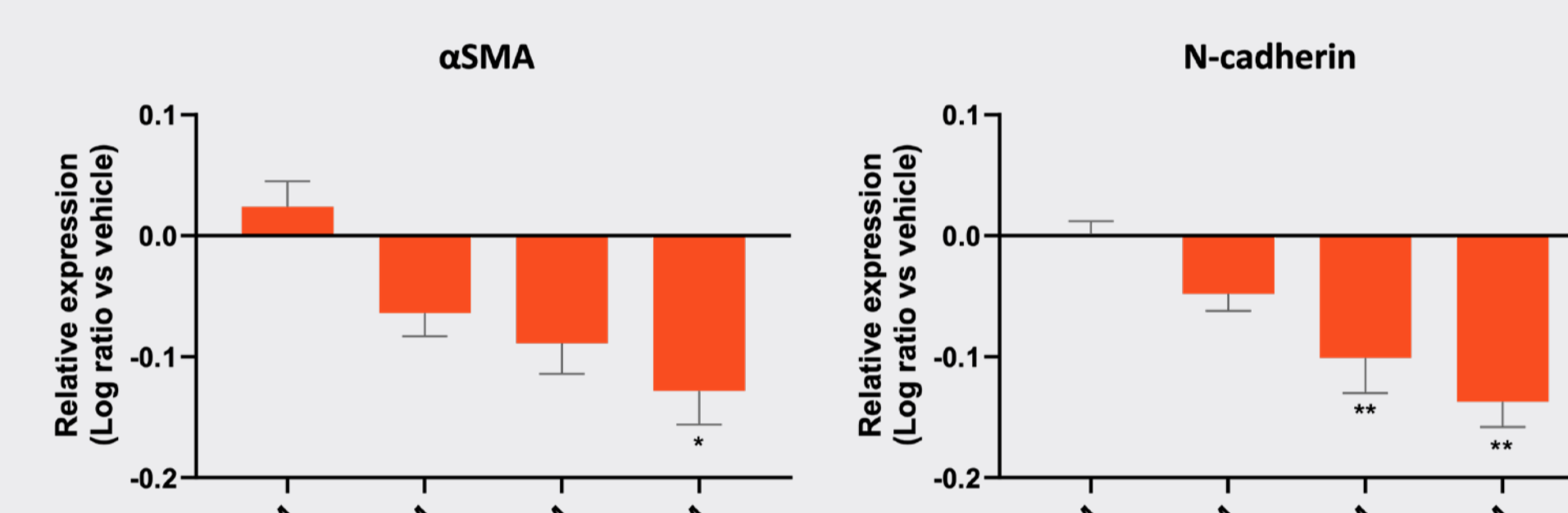
Data represent mean ± SEM of 5 separate tissue slices at each concentration, sampled after 96h exposure to buloxibutid or vehicle.

Figure 8. Buloxibutid downregulates EMT genes in primary human AEC2



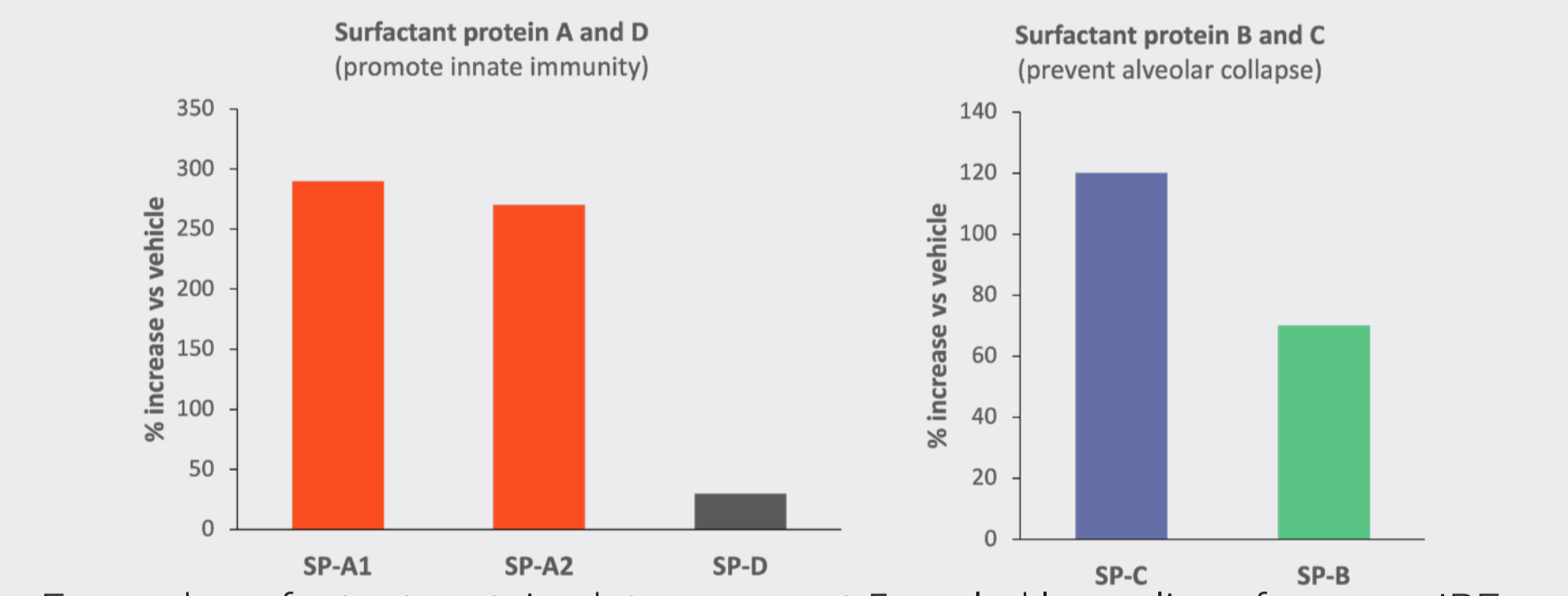
Single cell RNA sequencing. Mean of 5 experiments. No signs of cytotoxicity by buloxibutid
Source: Unpublished (collaboration with Prof D Davies, Univ of Southampton)

Figure 9. Buloxibutid inhibits αSMA and N-cadherin expression in primary human airway epithelial cell + myofibroblast co-cultures



BioMAP platform (fibrosis panel): Stimulation with TGFβ1 and TNF; proteins analyzed with immunoassay

Figure 10. Buloxibutid increased surfactant protein gene expression in human IPF lung slices



For each surfactant protein, data represent 5 pooled lung slices from one IPF patient incubated with 1 μM buloxibutid for 96 hours (no increase was seen with 0.1 μM)

Figure 11. Increased plasma MMP-13 in IPF patients treated with buloxibutid

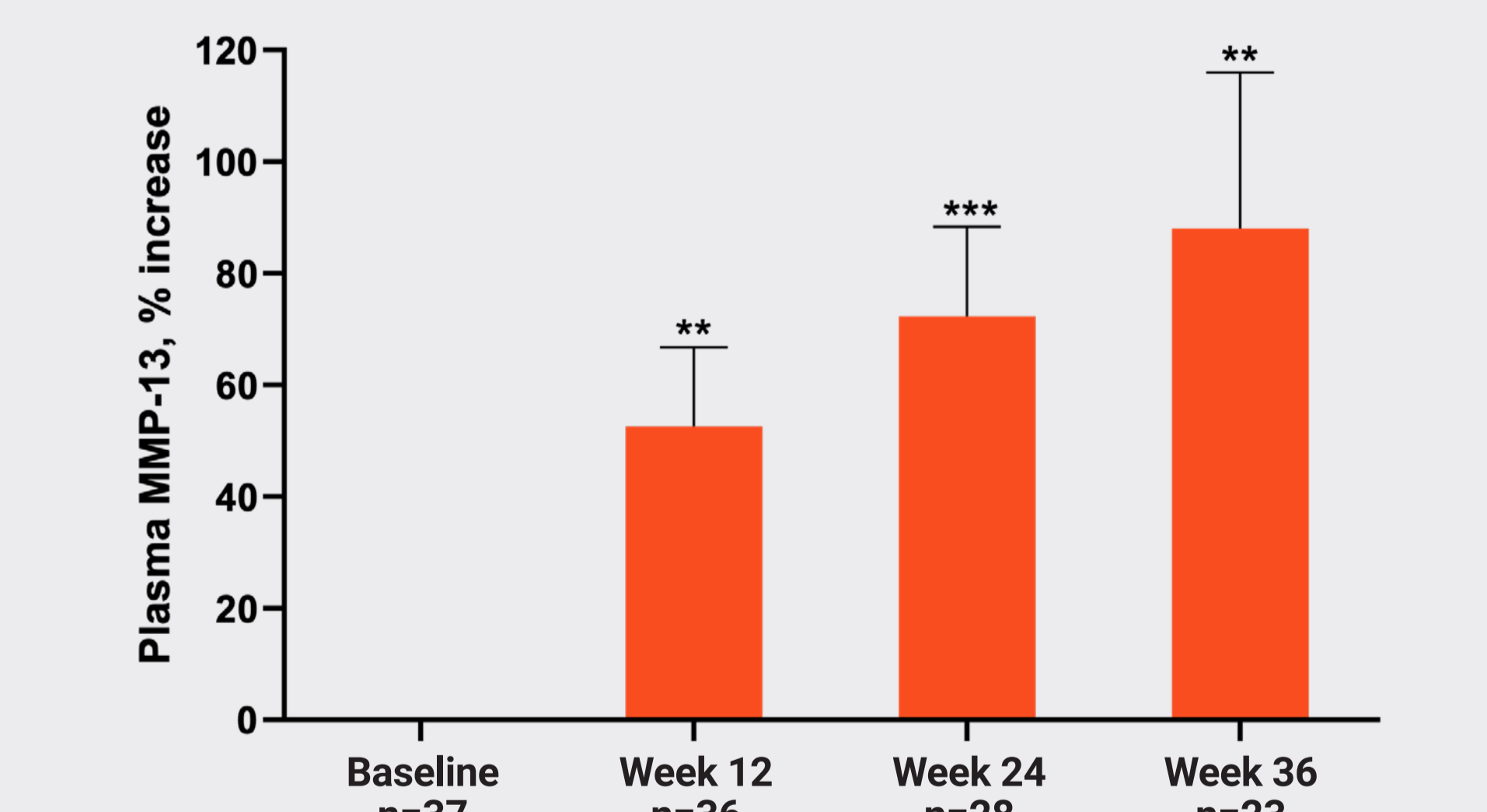
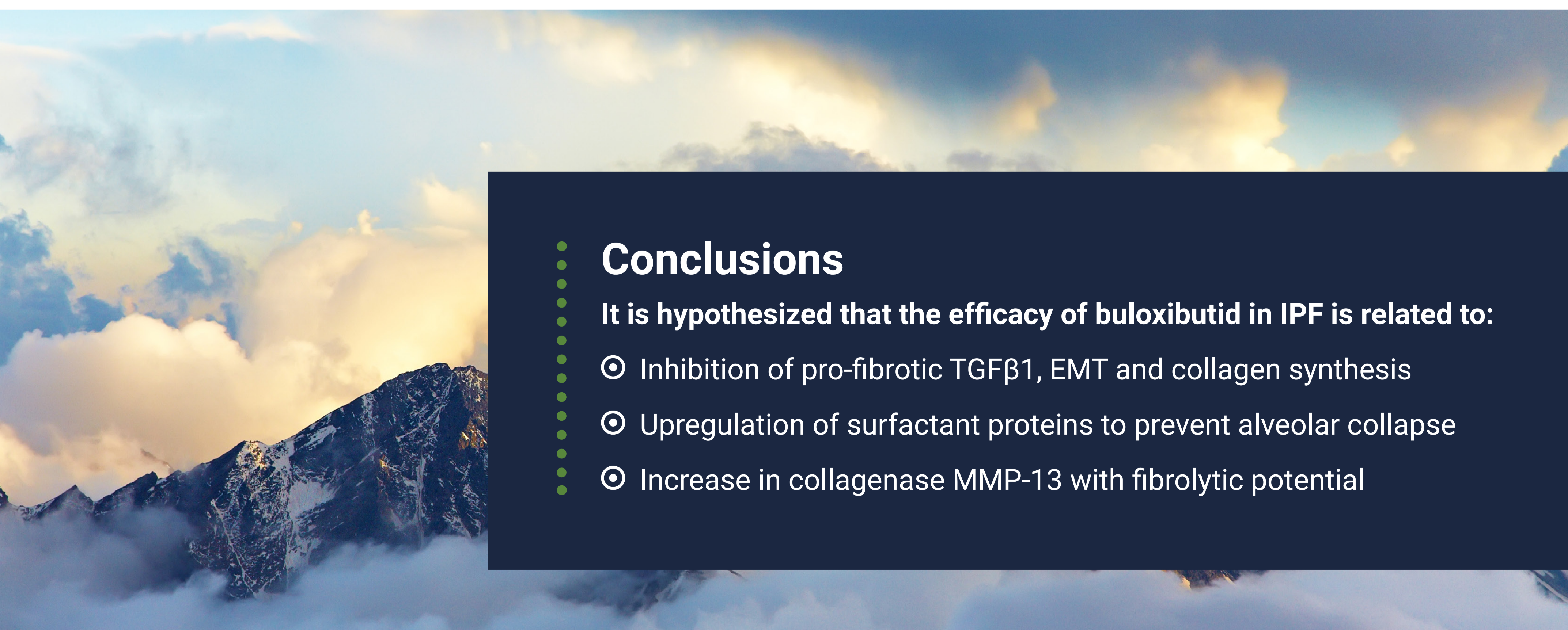
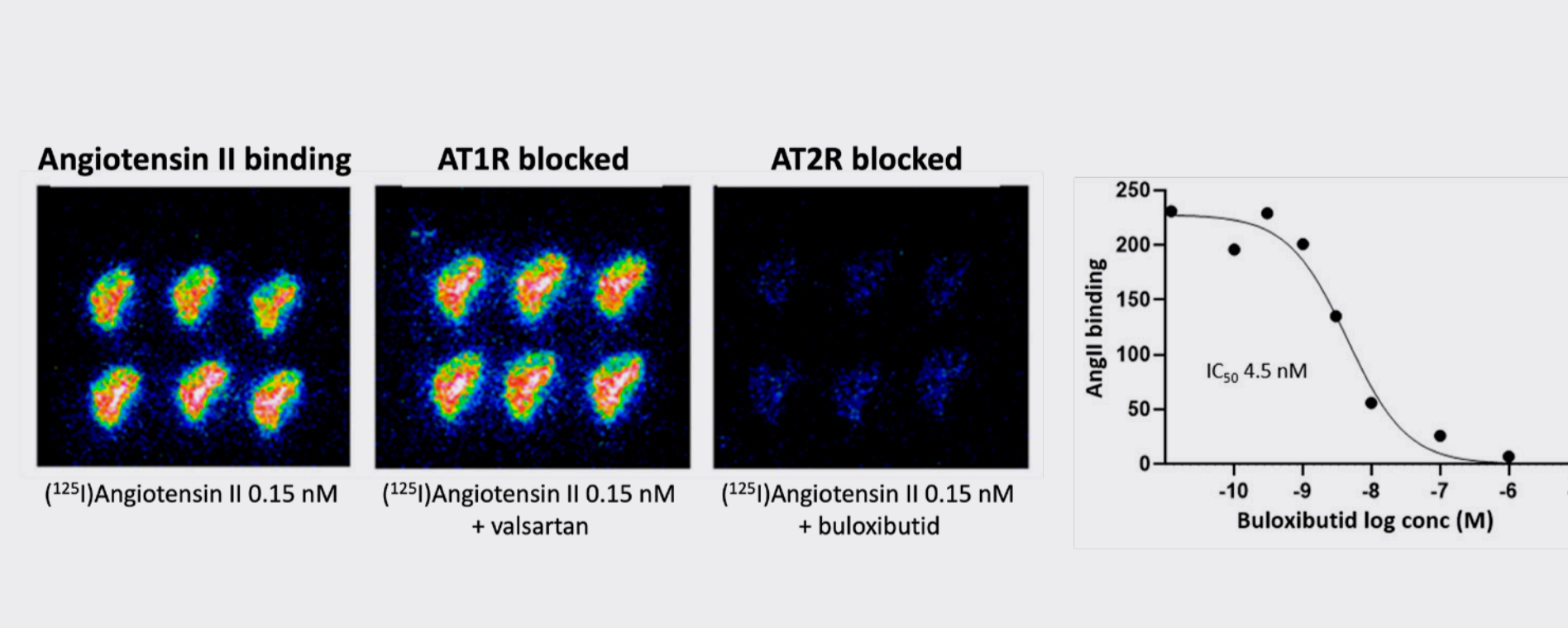


Figure 6. AT1R and AT2R expression in the human lung



Conclusions

It is hypothesized that the efficacy of buloxibutid in IPF is related to:

- Inhibition of pro-fibrotic TGFβ1, EMT and collagen synthesis
- Upregulation of surfactant proteins to prevent alveolar collapse
- Increase in collagenase MMP-13 with fibrolytic potential