Buloxibutid (C21) activates the angiotensin II type 2 receptor in alveolar epithelial type II cells, increasing MMP-13, a marker associated with fibrosis resolution in idiopathic pulmonary fibrosis

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Background

IPF is a progressive, fibrosing lung disease with few effective treatment options.

In the 36-week Phase 2a AIR open-label trial, oral buloxibutid, a selective angiotensin II type 2 receptor (AT2R) agonist, was associated with stabilized and improved lung function with an observed mean FVC change at week 36 of +216 mL in completers (Figure 1), and +9 mL FVC change using conservative imputation[1].

The AT2R is constitutively expressed on alveolar epithelial cells type II (AEC2) and represents a tissue-protective mechanism that counteracts injury through anti-inflammatory, anti-fibrotic and vasodilatory signalling (Figure 2); in IPF it is further upregulated on AEC2, fibroblasts and myofibroblasts (Figure 3).

AEC2 produce surfactant and maintain alveolar homeostasis by proliferating and differentiating into alveolar epithelial cells type 1 (AEC1). When dysfunctional, as in IPF, AEC2 fail to regenerate new AEC1 and drive fibrosis via e.g. TGF-β signaling (Figure 4).

Preclinical data suggest that buloxibutid supports AEC2 viability[2] and to stimulate surfactant production[3], consistent with AT2R-mediated preservation of alveolar epithelial integrity.

Matrix metalloproteinase-13 (MMP-13) has been suggested to promote fibrosis resolution in IPF[4], and in the AIR IPF trial, plasma levels were significantly elevated during buloxibutid treatment[1] (Figure 5). For comparison, plasma levels of MMP-7 did not increase.

Here, we investigated MMP-13 localization in the rat lung.

Methods

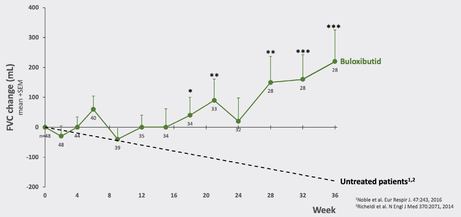
Rat bleomycin model:
Bleomycin was administered intratracheally to Sprague Dawley rats (1000 IU in 200µL 0.9% NaCl/animal), and the experiment was terminated on day 28. Lungs were perfused with cold PBS and inflated with 4% PFA and placed in 4% PFA and paraffin embedded. Blocks were sectioned at 4 µm thickness for further analysis using RNAscope.

RNAScope®:
RNAScope in situ hybridization assay was used to detect and localize surfactant protein C (SpC, a specific marker of alveolar epithelial type II cells, AEC2) and matrix metallopeptidase 13 (MMP13) RNA in the tissue sections. Briefly, samples were fixed, pretreated and hybridized with target-specific probes, followed by signal amplification and fluorescent detection using the ECHO revolution microscope.

Results

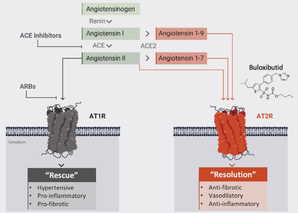
MMP-13 commonly co-localized with SpC-positive AEC2 cells in the rat lung (Figure 6).

Figure 1. Buloxibutid stabilized and improved lung function in IPF



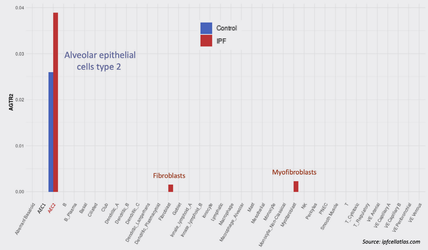
Change of FVC over time in IPF patients treated with 100 mg buloxibutid twice daily for 36 weeks (Phase 2a AIR trial). Observed values, no imputation.
*p<0.05, **p<0.01, ***p<0.001; t-test versus expected untreated decline corresponding to -120 mL/24 weeks.

Figure 2. Renin-Angiotensin System and AT2R agonist buloxibutid



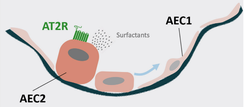
- Angiotensin II (formed mainly in the lung) activates AT1 and AT2 receptors with similar potency
- AT1R is widely expressed, while AT2R is mainly expressed on alveolar epithelial cells, but is upregulated at sites of disease/tissue injury
- AT1R effects include increase in blood pressure, the basis for ACEi and ARB development
- AT2R is a tissue protective mechanism
- Buloxibuted is a potent (IC50 5.7 nM) and selective (2900x vs AT1R) AT2R agonist

Figure 3. AT2R expression in healthy and IPF lung



AT2R expression in human lung based on single-cell RNA sequencing (gene name: AGTR2). Source: ipfcellatlas.com

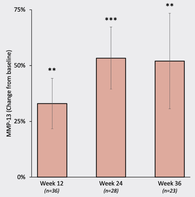
Figure 4. Alveolar Epithelial Cells Type I (AEC1) and Type II (AEC2)



AEC2 - progenitor cells critical for alveolar integrity and function:

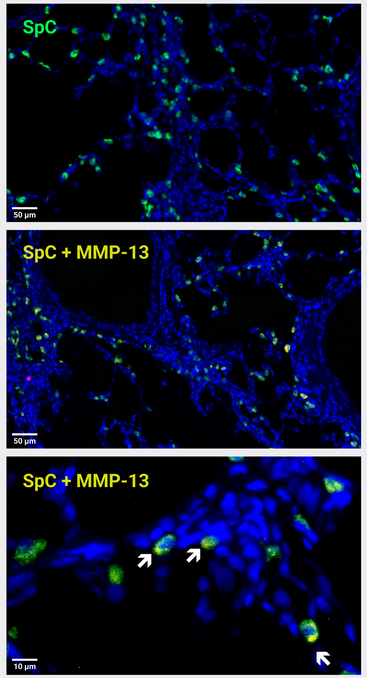
- AEC2 have highest AT2R expression (>6 times higher than any other cell)
- Continuously replace damaged AEC1 and AEC2
- Produce surfactant to prevent alveolar collapse
- Dysfunctional AEC2 drive IPF pathology

Figure 5. Plasma MMP-13 in IPF patients treated with buloxibutid



Plasma levels of MMP-13 in IPF patients treated with 100 mg buloxibutid twice daily for 36 weeks (Phase 2a AIR trial). Bars represent geometric mean post baseline to baseline ratios; error bars indicate standard errors derived from log transformed data.
** p < 0.01; *** p < 0.001
(paired Wilcoxon signed rank tests).

Figure 6. Surfactant protein C and MMP-13 in rat lung



RNAscope-based RNA detection in rat lung. Green cellular areas represent surfactant protein C (SpC) and arrows in bottom panel indicate yellow cellular areas representing co-localization of SpC and MMP-13.

Conclusions

- 36 weeks treatment with buloxibutid stabilized and improved lung function in IPF patients.
- This clinical benefit was accompanied by a sustained elevation of plasma MMP-13, consistent with an AT2R-driven fibrosis resolution mechanism.
- AEC2 were identified as a source of the collagenase MMP-13 in rat lung, suggesting that AT2R activation may drive local fibrolytic activity in alveoli.
- Plasma MMP-13 may represent a promising pharmacodynamic biomarker for monitoring AT2R engagement and treatment response in IPF.

References

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[3] Raud et al., Am J Respir Crit Care Med 2024; 209:A32464.
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