Expression of the Human Thromboxane Receptor in Pulmonary Hypertension & Related Cardiopulmonary Diseases

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BACKGROUND

- ***NTP42***, a novel antagonist of the thromboxane (TX)A_2 receptor (TP), is in clinical development for the treatment of pulmonary arterial hypertension (PAH), and other pulmonary hypertension (PH) and cardiopulmonary diseases.
- Clinical Phase I studies in 92 healthy volunteers showed that ***NTP42*** is safe, well-tolerated, displaying favourable pharmacokinetic and pharmacodynamic properties, with direct evidence of specific TP target engagement.[1]
- In preclinical models, ***NTP42*** improves pulmonary haemodynamics and vascular remodelling, inflammation, and fibrosis. ***NTP42*** also promotes beneficial right ventricular (RV) adaptation, decreasing cellular hypertrophy, and increasing vascularization, leading to improved RV geometries, contractility, and function.[2,3,4]
- These preclinical findings suggest both pulmonary-specific and direct cardioprotective benefits for ***NTP42***, highlighting its potential as a multimodal therapy in PAH, PH, and related cardiopulmonary diseases. To further explore this potential, TP expression was examined herein in lung and RV tissue from patients with PAH and other cardiopulmonary diseases.
- TP expression was also examined in tissues from corresponding preclinical models, including the monocrotaline (MCT), Sugen-hypoxia (SuHx) and *BMPR2* Δ71 models, the pulmonary artery banding (PAB) and Zucker fatty/spontaneously hypertensive heart failure F1 hybrid (ZSF-1) models, as well as the bleomycin-induced model of pulmonary fibrosis (BLM-PF).
- Finally, publicly-available single-cell, single-nucleus, or bulk RNA sequencing datasets were also interrogated to investigate TP gene (*TBXA2R*) expression in relevant healthy and cardiopulmonary disease cohorts.

METHODS

- Formalin-fixed and paraffin-embedded (FFPE) PAH and control lung tissue (n = 12 each) was obtained from the Royal Papworth Hospital NHS Foundation Trust Tissue Bank (Cambridge, UK). FFPE lung tissue from Group 3 PH (associated with idiopathic pulmonary fibrosis; PH-iPF; n = 3) and control subjects (n = 4) was obtained from the Hospices Civils de Lyon Biobank (Lyon, France). Control tissue was taken from pneumonectomy/biopsy specimens and confirmed malignancy-free.
- FFPE and fresh-frozen RV tissue from healthy donors (n = 5), or PAH (n = 5) or dilated cardiomyopathy (DCM; n = 3) patients, following autopsy, were obtained from the Institute of Cardiometabolism and Nutrition BioCollection (Paris, France).
- All protocols to obtain human biomaterials conformed with the recommendations of the Declaration of Helsinki, where the respective institutions' review boards approved these protocols and informed written consent was obtained from patients or their surrogates for the use of biomaterials for research purposes.
- TP immunohistochemical (IHC) staining and quantitation methods, and the MCT-PAH, SuHx-PAH, PAB, *BMPR2* Δ71 and ZSF-1 preclinical (rat) models have been detailed previously.[4] With respect to the BLM-PF model (*unpublished data*), rats received a single instillation of BLM (2.5 mg/kg) or saline (Control), and lung tissue was harvested 28 days later.
- The respective sources of the publicly-available single-cell, single-nucleus, or bulk RNA sequencing datasets used in the bioinformatic assessments are referenced in-line in the figures below.
- Where appropriate, unpaired t-tests were used to compare data, where */**/***/**** denote $P < 0.05/0.01/0.001/0.0001$.

RESULTS

Expression of the TP in Healthy and Diseased Lung Tissue

- In the Control lungs (**Figure 1A – C & Figure 2A – C**), TP expression was observed in bronchiolar epithelial and smooth muscle (SM) tissue, in alveolar pneumocytes and macrophages. In the pulmonary arteries, TP is expressed within the vascular endothelium and SM.
- In PAH lung tissue (**Figure 1D – F**), marked expression of the TP occurs in the remodelled pulmonary vasculature. Abundant endothelial and SM expression was noted in large obliterative and plexiform-like lesions (**Figure 1D & E**), a characteristic morphological hallmark of advanced PAH. In smaller remodelled vessels (**Figure 1F**), the TP is expressed in the endothelium and SM, as well as in perivascular tissues and in the associated-inflammatory cell infiltrates.
- In PH-iPF lung tissue (**Figure 2D – F**), abundant TP expression was also evident in the endothelium and SM in the remodelled vessels and surrounding vascular lesions. In addition, marked expression was also observed in histopathological features of iPF, including in regions of fibrosis and immune cell infiltration. Notably, the TP was also found in epithelioid cells associated with vascular and fibrotic lesions (*e.g.*, **Figure 2E**), reminiscent of the recently-described aberrant basaloid-type epithelial cells that are implicated in driving pro-fibrotic mechanisms in iPF.[5]

Figure 1. Representative images of TP IHC in Control and PAH lungs.

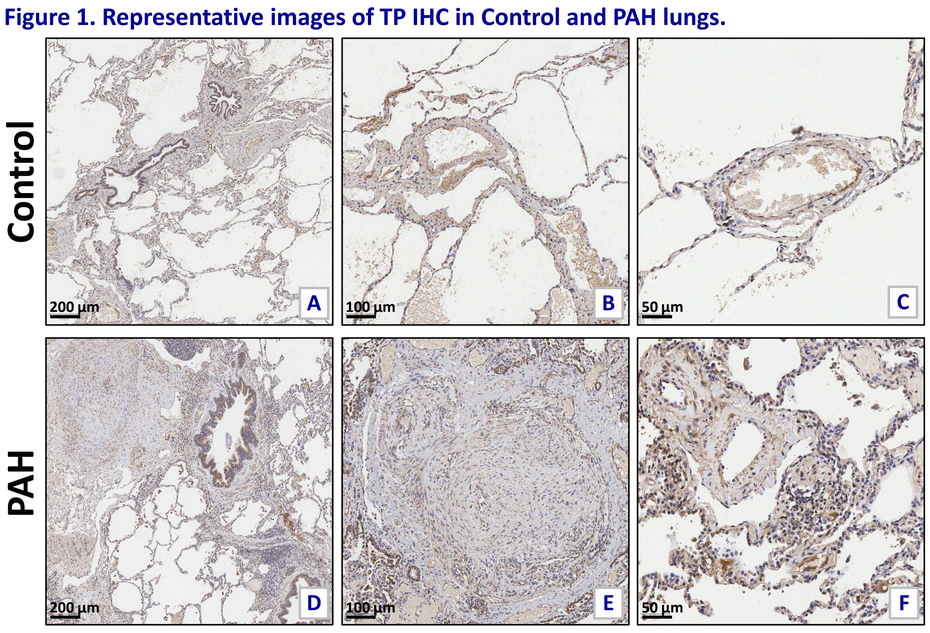
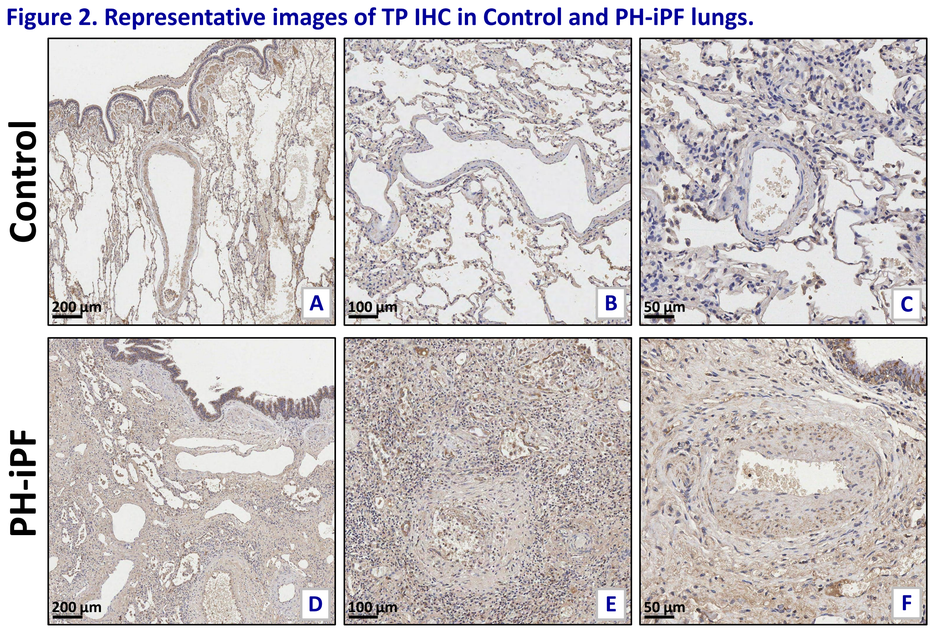
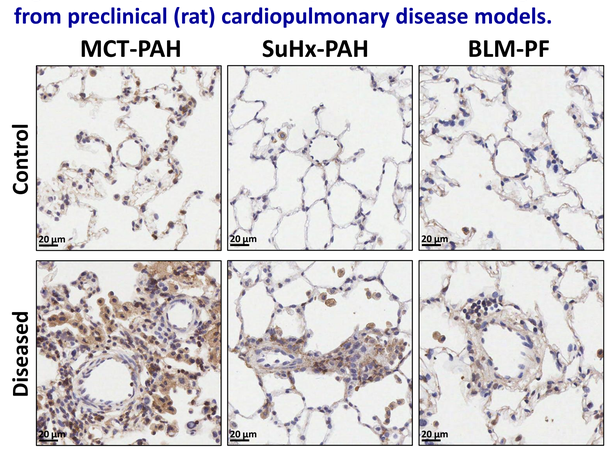


Figure 2. Representative images of TP IHC in Control and PH-iPF lungs.



- Abundant TP expression was also evident in remodelled pulmonary vasculature in rat lungs from preclinical MCT-PAH, SuHx-PAH, and BLM-PF models (**Figure 3**).

Figure 3. Representative images of TP IHC in lung tissue from preclinical (rat) cardiopulmonary disease models.



Expression of the TP in Healthy and Diseased RV Tissue

- In Control RV tissue (**Figure 4A – C**), TP expression was observed at a low level throughout the myocardium, with greater expression evident in nuclear and perinuclear regions.
- In PAH tissue (**Figure 4D – F**), TP expression was particularly evident in the enlarged RV cardiomyocytes and was also found in distinct cell types in fibrotic regions (*e.g.*, **Figure 4D**).
- Elevated TP expression was also observed in RV samples from DCM (**Figure 4G – I**), a primary cardiomyopathy which results in ventricular dilation and functional impairment that can drive the development of Group 2 PH-associated with left heart disease.
- Increased expression of the TP was also observed in RV tissue from a range of relevant preclinical (rat) disease models (**Figure 5**), including the MCT- and SuHx-induced PAH models, the PAB model of RV overload, spontaneously in rats harbouring the *BMPR2* Δ71 mutation, a genetic locus of heritable PAH, and in ZSF1-obese rats, a model of metabolic syndrome, Type 2 diabetes, hypertension, and heart failure with preserved ejection fraction (HFpEF) which also manifests PH and RV dysfunction.
- Quantitative PCR analysis in matching fresh-frozen tissue samples confirmed elevated TP expression levels in PAH & DCM RV, relative to Control tissues (**Figure 6A**). Furthermore, IHC quantitation confirmed significant increases in TP expression (signal intensity) across all preclinical models used in the current studies (**Figure 6B**).
- Notably, the increased TP expression observed in RV tissue from the clinical specimens and preclinical models did not occur in matching left ventricular tissue (*data not shown*).

Figure 4. Representative images of TP IHC in Control, PAH, & DCM RV.

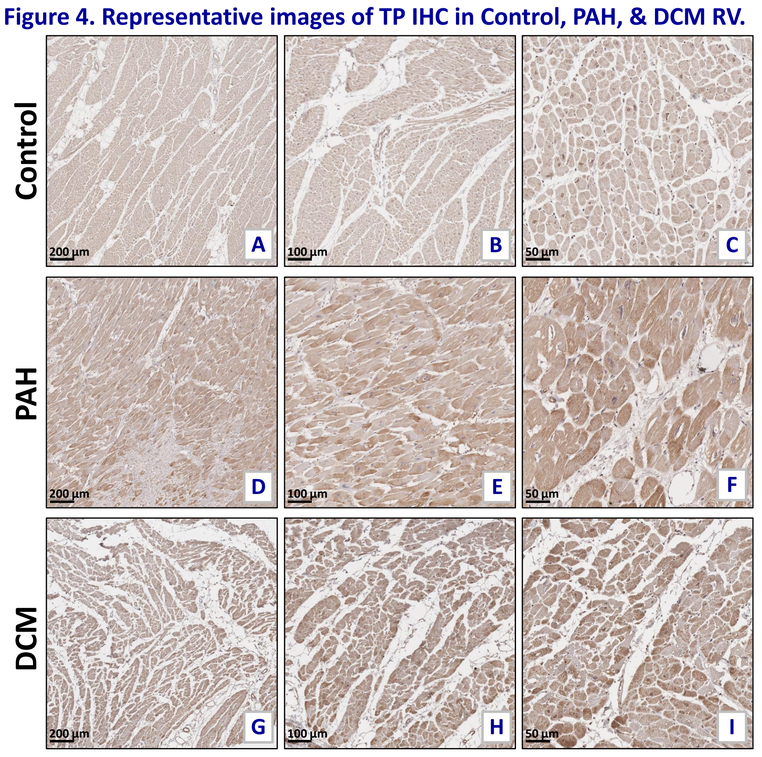


Figure 5. Representative images of TP IHC in in RV tissue from preclinical (rat) cardiopulmonary disease models.

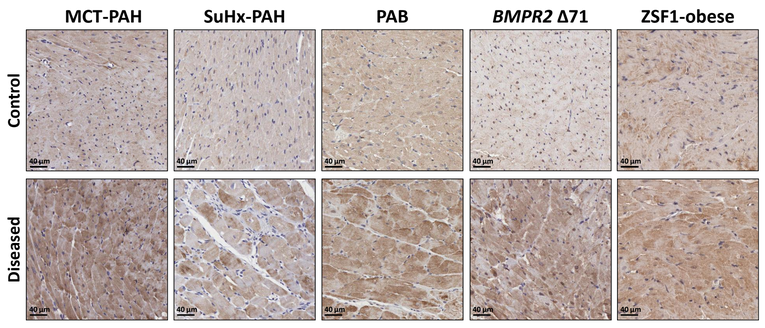
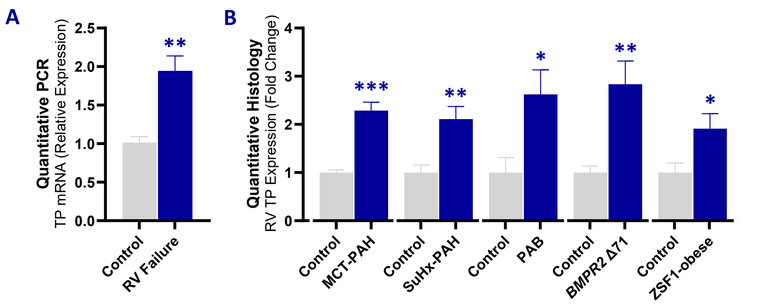


Figure 6. Quantitative assessments of TP expression in clinical & preclinical RV tissues.



Bioinformatic Assessments of TP Gene (*TBXA2R*) Expression

- Analysis of publicly-available single-cell/nucleus RNA sequencing datasets from healthy lung[6] and heart[7] tissues confirmed widespread expression of the *TBXA2R* in vascular cells (endothelial and SM), fibroblasts, and inflammatory cells in both tissues, with specific expression also noted in epithelial cell and cardiomyocyte populations in the lung and heart, respectively.
- Compared with control data, marked elevations in *TBXA2R* expression was noted in key pulmonary vascular cell types in PAH (**Figure 7**; Data[8]), specifically in the RV in heart failure (HF) with both reduced/preserved ejection fraction (HFrEF/HFpEF; **Figure 8**; Data[9]), and in multiple lung cell types implicated in iPF and PH-iPF development and progression (**Figure 9**; Data[5, 10-12]).

Figure 7. *TBXA2R* Expression in PAH Lung.

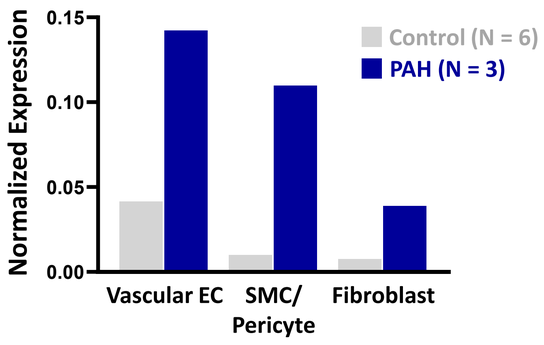


Figure 8. *TBXA2R* Expression in HF RV.

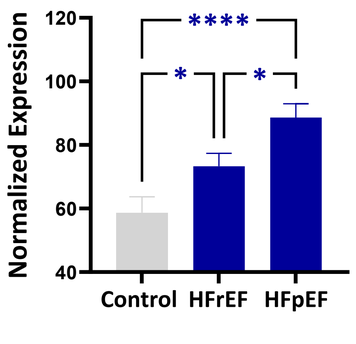


Figure 9. *TBXA2R* Expression in iPF Lung.

Lung Cell Types Implicated in iPF Development & Progression	
Vascular Endothelial Cells	Elevated **2.6-fold**
Smooth Muscle Cells/Pericytes	Elevated **8.8-fold**
Fibroblasts & Myofibroblasts	Elevated **5.2-fold**
Macrophages	Elevated **2.7-fold**

SUMMARY & CONCLUSIONS

- ***NTP42*** is a novel TP antagonist in clinical development for the treatment of PAH, as well as for other PH and cardiopulmonary diseases.
- Widespread expression of the TP-drug target was observed in remodelled pulmonary vascular cells and other disease-associated cell types in lung tissue from Groups 1 (PAH) & 3 (PH-iPF) patients, and related preclinical models.
- TP expression was also elevated in RV tissue in the PAH and DCM/HF heart, and in diverse preclinical models of PAH, HF and RV failure.
- These findings strengthen the evidence that the TP is a *bone-fide* target for diverse cardiopulmonary diseases and highlight the therapeutic potential of ***NTP42*** in treatment of both the underlying pulmonary and cardiac dysfunctions.

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