A phase II study of tocilizumab in group 1 pulmonary arterial hypertension

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Abstract

Background Inflammation and autoimmunity are important causes of pulmonary arterial hypertension (PAH). Compelling preclinical data from patients and animal models supports the therapeutic blockade of IL-6 signalling. In the first monoclonal therapeutic study to report immunomodulation in PAH we used an open label phase 2a study design to test the safety and efficacy of tocilizumab, a repurposed IL-6 receptor antagonist, in PAH.

Methods We undertook an exploratory open-label, 6-month study of IV tocilizumab (8mg/kg) over 6 months in group 1 PAH. The co-primary endpoints were safety, as defined by the incidence and severity of adverse events, and pulmonary vascular resistance (PVR) measured during right heart catheterization (RHC). Exploratory secondary endpoints included 6-minute walk test (6MWT), NT-proBNP, WHO class and CAMPHOR questionnaire. Additional exploratory analyses of serum cytokines, flow cytometric immunophenotyping and whole blood RNAseq were undertaken.

Findings Twenty-nine patients (M/F 10/19; mean age 54.9 (SD11.4)) were recruited between January 2016 to April 2017. Nineteen patients had idiopathic or heritable PAH (H/IPAH) and ten connective tissue disease associated PAH (CTD-PAH). Six patients were withdrawn prior to drug administration; one chest infection, one exacerbation of co-morbid disease, 4 at baseline RHC. Twenty-three patients received at least one dose of the study drug (mITT). Drug was discontinued in 4 patients due to serious adverse events. Vomiting occurred in one patient and was classified as a suspected unexpected serious adverse reaction. There were no deaths. In both ITT and mITT analyses PVR was unchanged. WHO class, 6MWT, NT-proBNP and CAMPHOR were additionally not suggestive of a treatment effect. Four of 6 (67%) of connective tissue disease patients improved their PVR >15% vs 3/13 (23%) of idiopathic/heritable PAH. Tocilizumab reduced CRP and increased IL-6 as expected but did not alter leucocyte subsets. RNAseq pathway analyses demonstrated altered B cell activation. No inflammatory markers predicted treatment response.

Interpretation Immunosuppression with tocilizumab is feasible in PAH patients with adverse events consistent with the known literature. Tocilizumab is not effective in prevalent populations of unstratified patients with PAH. Our data suggests future stratified medicine approaches to immunomodulation should, at present, focus on endotyping by autoimmune aetiology rather than inflammatory biomarkers.

Introduction

A strong association of PAH with dysregulated immunity and inflammation has been established (1, 2). Auto-immune diseases are causative in PAH, most prominently scleroderma, but also notably rheumatoid arthritis, SLE, mixed connective tissue disease, HIV and lymphoproliferative disorders (3). There is also an association of the idiopathic and heritable forms of PAH with auto-immune thyroid disease, links to HLA subtypes (4) and the presence of auto-antibodies in up to 93% of patients (5, 6). Idiopathic PAH has previously been speculated to be an auto-immune disease (2). More locally, within the pulmonary vascular lesions, there is accumulation of inflammatory cells including T and B lymphocytes (7) with altered T regulatory cell function (8, 9) and changes in B cell gene expression (10). It is clear therefore, that inflammation and dysregulated immunity play a significant role in a spectrum of causes of PAH. From the perspective of identifying pathways that are targetable, IL-6 has emerged as a strong candidate. IL-6 has been well-characterised as raised in peripheral blood and within the lung in PAH (7, 11). Interleukin-6 is an independent marker of prognosis outperforming traditional markers such as PVR and NT-proBNP (12). Over-expression of IL-6 in animal models using transgenic mice leads to pulmonary hypertension (13) and in hypoxia, IL-6 deficient mice are protected (14). Administration of recombinant IL-6 to rats also recapitulates a PAH phenotype (15). Tocilizumab is an IL-6 receptor antagonist established as safe, well tolerated and effective, primarily in rheumatoid arthritis (16), and has shown promise in scleroderma (17). IL-6 receptor antagonism attenuates murine PAH and recent exciting data has suggested that additionally ectopic IL-6 signalling directly drives vascular changes in animal models (18). The rationale for blocking Il-6 therefore extends beyond attenuating the contribution of inflammation to disease, to the direct effects of IL-6 on vascular remodeling. In uncommon cases, where the underlying cause of PAH is an established inflammatory process such SLE, mixed connective tissue disease and Castleman's disease, there have been case reports of regression of PAH with tocilizumab (19-21). We therefore proposed a phase II open-label single-arm proof of concept study of tocilizumab in PAH.

Methods

Study design and participants

In this multi-centre open label single-arm study, patients were recruited across 8 centres in the UK (appendix). The study was approved by the local Research Ethics Committee (Leicester Central- 15/EM/0401).

Patients on stable therapy aged 18-70 were enrolled with a diagnosis of group 1 PAH: Idiopathic or Heritable PAH, PAH associated with connective tissue disease excluding SLE, RA and mixed CTD. Selected exclusion criteria included subjects on IV or SC infusions, active infection, peripheral blood platelets <100x109/L neutrophil count <2x109/L, concomitant treatment with biologics, evidence of coronary artery and left heart disease, TLC ≥60% of predicted normal and FEV1 ≥60% of predicted normal and a 6MWT of <100m (full inclusion/exclusion criteria in appendix). All participants provided written informed consent.

Procedures

Participants were given tocilizumab IV monthly (8mg/kg; Roche Pharmaceuticals) for 6 months on day 1, weeks 4, 8, 12, 16 and 20 (6 doses). Patients attended monthly for infusions and safety data collection. Worsening of pulmonary arterial hypertension was defined by the occurrence of three of the following: A decrease in the 6MWT distance of at least 15% from baseline; the need for additional treatment for PAH; and the worsening of symptoms of PAH including at least one of: a change from baseline to a higher WHO functional class and the appearance or worsening of signs of right heart failure unresponsive to oral diuretic therapy. Peripheral blood sampling was undertaken at trial baseline and end of study (EOS). Blood was collected for flow cytometric evaluation of leucocyte subsets, RNAseq and serum mediators of inflammation IL-1β, IL-6, IL-8 and TNFα (appendix). Blood was collected in citrate phosphate dextrose adenine (CPDA) and processed within 30 minutes of sampling after transfer to a lymphocyte separation tube (EZ Lympho-Sep), which were centrifuged as per manufacturer instructions. After a red cell lysis step (reagent) PBMNCs were separated, resuspended in PBS+2mM EDTA and after overnight -80C, transferred to liquid nitrogen (appendix). Immunophenotyping was undertaken on a Fortessa flow cytometer (BD Biosciences) (appendix). Optimised combinations of primary antibodies were developed based upon those proposed by the Human Immunophenotyping Consortium (22). A minimum of $1x10^6$ events were collected for each analyte. Blood was also processed for assay of cytokines in serum SST tubes and Tempus™ Blood RNA tubes (3ml blood per tube, Thermo Fisher Scientific). Samples were stored at -80^{o}c until completion of the trial. Cytokines were measures from serum samples using a Mesoscale Discovery VPLEX multispot assay platform (Gaithersburg, MD, USA) on the MesoScale Discovery Sector S600 analyser. Samples were prepared and analysed as per manufacturer instructions (MSD Human Proinflammatory Panel 1- K15049D). RNA extraction was performed using the Tempus™ Spin RNA Isolation kit (Thermo Fisher Scientific) and included the optional DNase treatment step using AbsoluteRNA Wash Solution (Thermo Fisher Scientific) in accordance with manufacturer's protocol. Extracted RNA was then concentrated using RNeasy Minelute columns (Qiagen) and eluted in a final volume of 14μl before depletion of globin mRNA using GLOBINclear™ (Thermo Fisher Scientific). Final elution of globin depleted RNA was in 30μl RNase free water. The yield and RNA integrity score (RIN) of the samples was determined using the Eukaryote Total RNA nano chip kit (Agilent Technologies) and run on an Agilent 2100 bioanalyser (Agilent Technologies), RIN numbers were calculated using the 2100 Expert Software (Agilent Technologies). Libraries for cDNA were prepared with TruSeq Stranded mRNA Library preparation kit (Illumina) which generates Poly-A enriched strand-specific libraries. 1μg high quality RNA was inputed and all protocols were performed following the manufacturer's instructions. Completed libraries were assessed by DNA 1000 chip (Agilent Biotechnologies) on an Agilent 2100 Bioanalyser (Agilent Biotechnologies) before normalisation and pooling. Pooled indexed libraries were submitted at 10nM and sequencing was performed on a HiSeq4000 instrument (Illumina) using a single end 50bp run. Geneset analysis were run through cpdb.molgen.mpg.de geneset enrichment database.

Outcomes

The co-primary end-points were of safety and efficacy, adjudged respectively on the occurrence of adverse events and serious adverse events as classified by use of the Medical Dictionary for Regulatory Activities, and pulmonary vascular resistance (PVR) delta as measured by invasive haemodynamic assessment via fluid-filled right heart catheter using cardiac output measured by thermodilution technique. Secondary safety and exploratory efficacy endpoints included 6 MWT distance, Borg Dyspnea Index, N-Terminal pro-BNP, WHO Functional Class assessment, CAMPHOR assessment, analysis of flow cytometric peripheral blood leucocyte immunophenotyping and serum and plasma measurements of circulating cytokines IL-1β, IL-6, IL-8 and TNFα. A full description of the study schedule is included in the appendix. Post-hoc descriptive statistics of disease subtype were prespecified in the Statistical Analysis Plan.

Statistical analysis

The sample size (n) was decided to detect a 30% reduction in PVR after 6 months of treatment with 90% power and 5% statistical significance was n=17, accounting for a conservative drop-out rate, the minimum target for recruitment was n=21 as published (23). The main analysis reported here are specified on the modified intention to treat (mITT) set. This is defined as the set of patients who have had at least one dose of Tocilizumab and at least one post-baseline result. An additional ITT analysis as a sensitivity analysis, i.e. to assess the existence of potential bias did not differ from the mITT. Estimates of treatment response rate and associated confidence intervals (or credible intervals and posterior probabilities) are reported. The exception to this is the Wilcoxon signed rank test which is carried out on the PVR fold change as defined in the protocol paper (23). An additional Bayesian analysis, considered to be more informative of the treatment response of Tocilizumab on PVR was prespecified using an expert elicited prior. Any predictor effect on secondary outcomes are reported with confidence intervals. All analysis reported here were performed using R (version 3.3.3).

Results

Twenty-nine patients (M/F 10/19; mean age 54.9 (SD11.4) were recruited in total between January 2016 to April 2017. Fifteen patients had idiopathic PAH, ten connective tissue disease associated PAH (CTD-PAH), and four heritable/ BMPR2 associated PAH (figure 1). Six patients were withdrawn prior to drug administration; one chest infection, one exacerbation of co-morbid disease, four at baseline RHC. Twenty-three patients received study drug.

Figure 1 Consort diagram

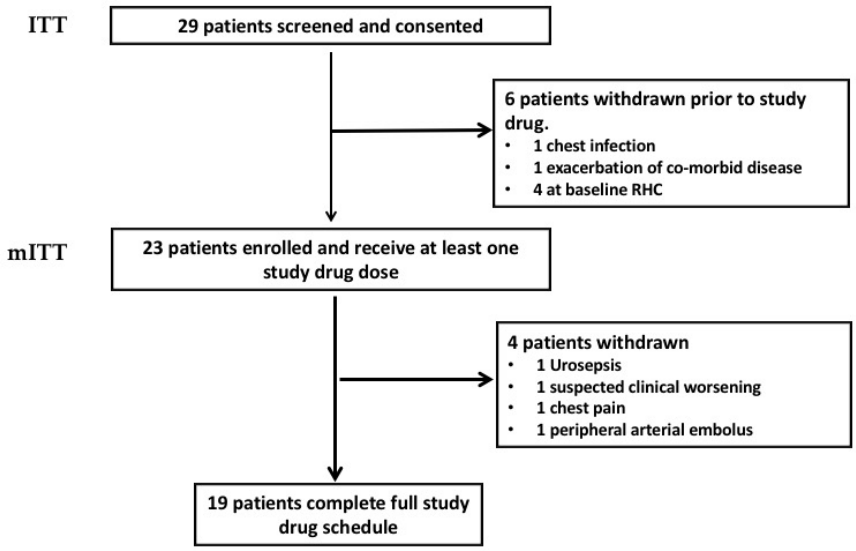
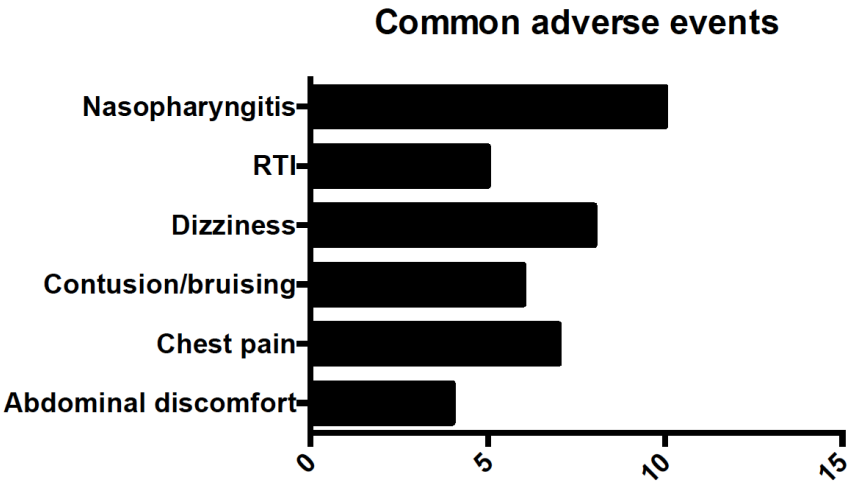


Table 1 Baseline demographics

Sex M/F	10/19
Age mean (SD)	54.9 (11.4)
IPAH/FPAH/CTD	16/3/10
PVR dyne.sec/cm^{-5} mean (SD)	612.30 (317.63)
mPAP mmHg mean (SD)	43.57 (11.07)
NT-proBNP pg/ml median (IQR)	329.50 (62.0, 611.0)
Cardiac Output L/min median (IQR)	5.14 (3.49, 5.67)

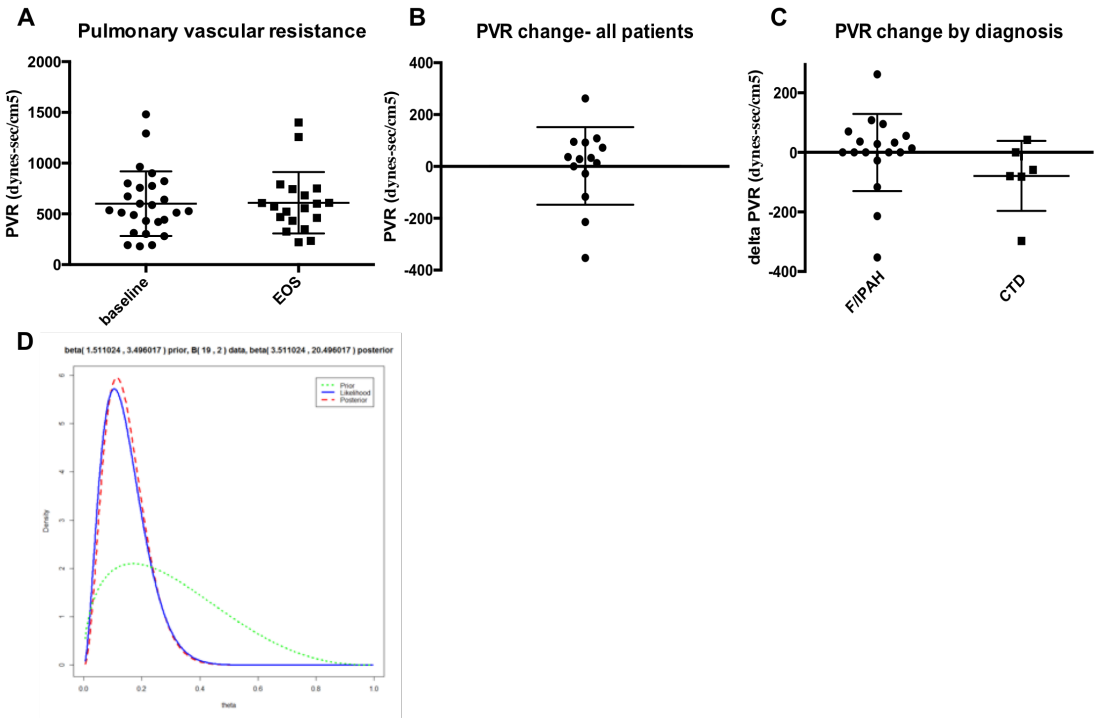
Drug was discontinued in 4 patients due to serious adverse events. These were urosepsis, chest pain, peripheral arterial embolus and one suspected clinical deterioration that was subsequently adjudged on independent blinded review not to fulfil criteria. Vomiting occurred in one patient and was classified as a suspected unexpected serious adverse reaction. There were no deaths. The most common adverse event was nasopharyngitis (figure 2). Adverse events were graded mild (80.0%), moderate (16.6%) and severe (3.4%).

Figure 2 Adverse events



There was no significant difference in delta PVR at 6 months (figure 3A,B). In *a-priori* analyses of PVR stratified by aetiology, 4 of the 6 CTD-PAH patients receiving study drug with an EOS RHC had a greater than 15% reduction in PVR vs 3 of 13 F/IPAH (figure 3C).

Figure 3 Co-primary endpoint- change in PVR



A Bayesian analysis led to the same conclusions as the frequentist analysis (figure 3d). The expert elicited prior was combined with the observed data to produce a posterior density for the probability of the study drug achieving a PVR fold-change

of at least -30%. (Figure 3D). The posterior probability of the study drug being successful in the above sense with at least a 30% chance was less than 1%.

Exploratory secondary endpoint analyses were consistent with the primary endpoint with no strong data to support a treatment effect (table2). 6MWT increased by mean 19.1m (SD 60.8), NT-proBNP increased by median 22.5pg/mL (IQR 275) and CAMPHOR decreased by median 2 (IQR 10). WHO functional class changed in 6 patients; improving in 4 patients and deteriorating in 2.

Table 2 Secondary endpoints

6MWT metres Δ m (mean/SD)	19.1 (60.8)
NT-proBNP Δ pg/mL (median/IQR)	22.5 (275)
BORG score Δ (mean/SD)	0.05 (0.05)
CAMPHOR Δ (median/IQR)	-2 (10)
WHO class: unchanged/improved/deteriorated	17/4/2

In RNAseq analyses there were no significant single gene expression changes after FDR correction. Network analyses, however, of gene-set over-representation and ontological analyses are dominated by immune mediated processes (appendix). Enriched protein complex-based sets are also dominated by protein complexes associated with T and B cell mediated immunity, notable the B cell receptor (BCR) (appendix). Principal component analyses of RNAseq gene expression and expression changes did not discriminate either CTD-PAH or the responders in the cohort (appendix). Immunophenotyping did not show any significant changes in either B or T cell populations (appendix). IL-1β, IL-8 and TNFα did not change however Il-6 increased by median 9.5pg/mL (IQR 7.3) and CRP decreased by 1.2mg/L (IQR 2.2) (appendix). None of our extensive immunophenotyping data correlated with treatment response or predicted responders vs non-responders (data not shown).

Discussion

Despite a wealth of preclinical data, targeting the IL-6 receptor PAH is unlikely to be a therapy applicable to an unstratified, stable population of PAH patients already established on targeted vasodilator therapy. This proof of concept trial was powered to pick up a large treatment effect of over 30%. Given the extensive new potential side effects we would be exposing patients to by immunosuppressing them, we feel it is advisable to set a high bar for treatment effect and that this strategy should only be pursued if the effect is clinically meaningful.

In considering why IL-6 blockade did not improve patients with PAH despite robust preclinical evidence, it is important to understand our current model of PAH pathophysiology. In PAH the traditional description of disease is progressive and classically is not conceptualized in terms of inflammatory activity, flaring of

disease activity or "relapsing/remitting". It is notable however that in most of the published work on inflammation in PAH the positive signals are driven by non-normal distribution, i.e. a tail of positive patients rather than a normally distributed homogeneous increase in markers of inflammation in the whole population. This raises the possibility that either 1) only a subset of patients are truly being driven by inflammation or autoimmunity, or 2) alternatively- in PAH, inflammatory activity flares. A useful analogy is multiple sclerosis where therapy is still uniformly ineffective in progressive disease when compared to relapsing remitting disease (https://www.aan.com/Guidelines). To clarify this will require longitudinal assessment on large carefully endotyped cohorts. At present we do not have a clear evidence basis on how to stratify patients more likely to respond based on markers of inflammation. In our study there was no correlation between clinical response and any markers of inflammation, in particular the most obvious to target; IL-6. Our extensive inflammatory interrogation demonstrates the difficulties developing biomarkers for trial stratification in the context of rare diseases where numbers in phase 2 studies will be limited. Even in larger cohorts such rheumatoid arthritis, meta-analyses trials stratified by IL-6 have demonstrated disappointing correlations with clinical response (24). This strategy is currently not in practice in any of the current indications for IL-6 receptor antagonism.

A possible alternative explanation for the negative trial is that tocilizumab is not having a profound enough effect on the immune system. It is notable that leucocyte populations did not change and RNAseq did not show significant changes in gene expression in whole blood when adjusted for multiple testing. It is reassuring, however that IL-6 increased, CRP decreased and B and T cell activation pathways were the top ontology hits in the RNAseq, suggesting that there is target engagement on B and T cell activity. It is possible that gene expression changes would have been more significant had we performed analyses on subsets of B and T cells rather than whole blood.

We excluded several patients at trial entry because of changes in haemodynamics, and it could be argued that these patients are more likely to be active from an inflammatory perspective, and reflecting this 50% of the excluded subjects had a CRP>10mg/L. It would not have been ethical to continue with the trial in these cases as there are licensed, effective third line therapies to be considered. It could be argued that our stable patients are less likely to have active inflammation (though the IL-6 and CRP levels are in line with previous reports). It is likely that any further work in IPAH will need to careful consider how to endotype and immunophenotype patients to try to enrich for patients with active inflammation. Our extensive work on a small population demonstrates that this will not be a simple task. With appropriate safety data, it is reasonable to consider whether incident populations would be one way of not biasing studies towards patients who are more likely to be stable and lack ongoing active inflammation. From a practical perspective, now that upfront dual therapy is established, it will be difficult to consider treating patients who are deteriorating on oral therapy. The gold standard for deteriorating dual therapy treated patients is IV therapy and this carries obvious additional risk in the context of immunomodulation.

Our entry criteria for CTD-PAH excluded the patient groups with anecdotal evidence for large effect sizes, specifically SLE and mixed connective tissue disease. This is because these patients are uniformly already immunosuppressed and for a first proof of concept it was not felt appropriate or safe to have a subset on dual immunosuppression or a switch over trial design. We additionally did not want to skew results towards rarer sub-populations. Despite this, there was still a response in 4 of 6 patients CTD-PAH subgroup. Immunosuppression in CTD outside of the context of PAH is not controversial, but at present the effects on the pulmonary hypertensive phenotype are not known. We feel it is now time to consider how trials of immunosuppression can be performed in CTD-PAH. These will have some new complexity as patients are usually diagnosed with PAH after the diagnosis of CTD is made and are established on a heterogeneous mix of immunosuppression. Further complicating trial design, some patients, in particular those with scleroderma, are frequently not immunosuppressed and therefore will be treatment naive. Any trials will therefore have to consider the mixed disease population and mixed background immunosuppression regimes.

A potential criticism of our study is the open label nature, though perhaps not for the classical reason. Our previous meta-analysis data shows that PVR is not a placebo responsive endpoint (23). The normal distribution of our primary endpoint data and additional negative exploratory secondary endpoints are strongly in line with this. In previous studies of PVR over 4-6 months of treatment, it is more likely to deteriorate than improve in placebo arms and it is therefore possible that we have missed a signal by masking any deterioration effect. Our counter-argument to this is that we would not wish to pursue immunosuppression as a therapeutic strategy, with all its attendant risks and problems, if it was not able to significantly improve patient haemodynamics and function. This trial has also made a significant effort in trying to address existing challenges of conventional trial design in PAH by including and reporting a secondary analysis based on Bayesian analysis using an expert elicited prior. Classical frequentist trial models are increasingly infeasible as trial populations are becoming more stratified and targeted. Bayesian statistical methods are being increasingly used, led by cancer research, as a solution to this difficulty (25-27). They are currently being recognised as an option for trial in rare diseases by the EMA and the FDA, who are drafting specific regulatory documents for these designs. The Bayesian analysis in this case should reassure the skeptics (28) that rare disease trial design can learn from oncology.

In summary, treatment with tocilizumab is feasible in PAH but demonstrated no significant effects on haemodynamics or exploratory secondary endpoints in familial or idiopathic PAH. A potential improvement was noted in the small subgroup of patients with CTD-PAH and this needs further consideration. Any future trials in immunomodulation in PAH need to consider whether endotyping and stratification of patients earlier in the disease process can be undertaken.

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