18FLT PET depicts heterogeneous proliferation pathology in IPAH patient lung: a potential biomarker for PAH

Ashek & Spruijt et al.; Heterogeneous lung 18FLT uptake in IPAH

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Commentary

With the focus now on developing drugs that tackle pulmonary vascular remodelling (as opposed to vasoconstriction), we explored the use of dynamic $^{18}$FLT PET imaging with kinetic analysis in idiopathic PAH patients (IPAH) as a non-invasive tool to report on pulmonary vascular pathology. We show that IPAH patients demonstrate increased lung $^{18}$FLT uptake, reflected in $^{18}$FLT phosphorylation k3, with both inter- and intra-variability in the signal. Using animal models and cells from IPAH patients we demonstrate that lung $^{18}$FLT uptake reflects pulmonary vasculature cellular proliferation and responds to drugs that reduce pulmonary vascular remodelling. We suggest that $^{18}$FLT PET might be a useful tool for identifying patients who might benefit most from anti-remodelling therapy and so inform precision medicine.
Abstract

Background - Pulmonary vascular cell hyperproliferation is characteristic of pulmonary vascular remodelling in pulmonary arterial hypertension (PAH). A non-invasive imaging biomarker is needed to track the pathology and assess the response to novel treatments targeted at resolving the structural changes. Here we evaluated the application of radioligand 3'-deoxy-3'[18F]-fluorothymidine (18FLT) using positron emission tomography (PET).

Methods and Results - We performed dynamic 18FLT PET scanning in 8 idiopathic PAH patients (IPAH) and applied in-depth kinetic analysis with a reversible 2-compartment 4k model. Our results show significantly increased lung 18FLT phosphorylation (k3) in IPAH patients compared with non-PAH controls (0.086±0.034 min^-1 vs 0.054±0.009 min^-1; P<0.05). There was heterogeneity in the lung 18FLT signal both between IPAH patients and within the lungs of each patient, compatible with histopathological reports of lungs from IPAH patients. Consistent with 18FLT PET data, thymidine kinase TK1 expression was evident in the remodelled vessels in IPAH patient lung. In addition, hyperproliferative pulmonary vascular fibroblasts isolated from IPAH patients exhibited upregulated expression of TK1 and the thymidine transporter, ENT1. In the monocrotaline and Sugen hypoxia rat PAH models, increased lung 18FLT uptake was strongly associated with peripheral pulmonary vascular muscularization and the proliferation marker, Ki-67 score, together with prominent thymidine kinase TK1 expression in remodelled vessels. Importantly, lung 18FLT uptake was attenuated by two anti-proliferative treatments, dichloroacetate and the tyrosine kinase inhibitor, imatinib.

Conclusion – Dynamic 18FLT PET imaging can be used to report hyperproliferation in pulmonary hypertension and merits further study to evaluate response to treatment in IPAH patients.

Keywords: 18FLT, PET, pulmonary arterial hypertension, remodelling, proliferation

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**Introduction**

Pulmonary arterial hypertension (PAH) is characterized by structural remodelling of the pulmonary vasculature leading to increased pulmonary artery pressure and progressive right heart dysfunction\(^1\)\(^-\)\(^3\). Histopathology of lungs from PAH patients at the end stage of their disease show a complex pulmonary pathology including aberrant proliferation of pulmonary vascular cells, inflammatory cell infiltration and deposition of extra-cellular matrix\(^4\)\(^-\)\(^6\). The licensed treatments for PAH are based on restoration of an imbalance in vasoactive factors favoring vasoconstriction. While these drugs improve symptoms, they fail to halt the disease and have a limited effect on survival\(^7\).

Innovative treatment strategies that inhibit the excessive proliferation of lung vascular cells, e.g. with the receptor tyrosine kinase inhibitor imatinib\(^8\) and the metabolic modulator dichloroacetate (DCA)\(^9\), show great promise in the preclinical setting\(^10\). Inter-individual variation in response to treatment is well recognized and was evident in the imatinib (IMPRESS) trial as well as the recent DCA study\(^11\),\(^12\). A possible explanation is heterogeneity in pulmonary vascular remodelling in PAH, with some patients exhibiting profound active vascular proliferation but others a more quiescent state of end-stage fixed pulmonary vascular pathology. A new tool for assessing PAH pathology *in vivo* would greatly improve the potential to translate therapeutic strategies targeted at resolving pulmonary vascular remodelling into the clinic. Ideally, such a tool would allow a strategy of precision medicine with therapies tailored to individual patients.

Positron emission tomography (PET) with appropriate ligands allows for spatial detection and localization of pathophysiological processes *in vivo*. Radioactive ligands such as \(^{18}\)F-fluorodeoxyglucose (\(^{18}\)FDG)\(^13\) and \(^{18}\)F-3'-fluoro-3'-deoxythymidine (\(^{18}\)FLT)\(^14\),\(^15\) have been used in oncology to stratify patients and assess disease progression and response to treatment, and predict clinical outcomes. \(^{18}\)FDG tracks general cellular glucose metabolism, which may correlate with proliferation in some tumors, but does not provide a direct measure of cell growth\(^16\). \(^{18}\)FLT is a marker for cell proliferation\(^14\),\(^15\) that correlates closely with histological markers, such as proliferating cell nuclear antigen (PCNA), Ki-67 and S phase fraction\(^17\)-\(^20\). \(^{18}\)FLT is a fluorine-modified thymidine analogue that is transported into the cell via passive diffusion and by nucleoside transporters, such as the equilibrative nucleoside transporter...
(ENT1), in a manner similar to thymidine\textsuperscript{21}. Thereafter, \textsuperscript{18}FLT is phosphorylated by thymidine kinase 1 (TK1) and trapped intracellularly at a rate proportional to TK1 activity, which is elevated during the S phase of the cell proliferation cycle but decreased or absent in quiescent cells\textsuperscript{22-25}. This mechanism has been used to accurately measure DNA synthesis in cell cultures using 3H-thymidine, and supports the view that \textsuperscript{18}FLT more accurately reports on proliferation. Importantly, \textsuperscript{18}FLT PET is recognised for evaluating whole tumour proliferation heterogeneity\textsuperscript{26}.

Few studies have explored the application of PET imaging in PAH. Previously we have described \textsuperscript{18}FDG-PET as a tool for following response to treatment in animal models and the signal from patients with PAH\textsuperscript{9,12}, but it reports both proliferation and inflammation. Here we evaluate the potential of \textsuperscript{18}FLT PET as an imaging biomarker for assessing the distinctive hyper-proliferative pulmonary vascular pathology seen in PAH. First, we examined \textsuperscript{18}FLT lung uptake in idiopathic PAH (IPAH) patients in comparison to control subjects. Second, we assessed whether \textsuperscript{18}FLT PET could track pulmonary pathology in animal PAH models and the response to targeted therapies. Third, we examined ENT1 and TK1 expression in lung tissues and pulmonary vascular fibroblasts isolated from IPAH patients to understand the determinants of lung \textsuperscript{18}FLT uptake in PAH.

**Methods**

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

**Patient population**

8 patients (Table 1) diagnosed with IPAH according to ERS guidelines\textsuperscript{27} were recruited from the VU University Medical Center; both treatment naive and treated patients were included in this study. We made use of \textsuperscript{18}FLT PET data sets from an oncology study of non-small cell lung cancer patients\textsuperscript{28} from the VU University Medical Center. Six subjects with one-sided lung tumor were identified and \textsuperscript{18}FLT uptake from the tumor-free side of the lung was used as control. The study was approved by the
Medical Ethical Review Committee of the VU University Medical Center (Toetsingonline: NL49166.029.14) and all subjects gave informed consent.

Right heart catheterizations and cardiac magnetic resonance imaging

All patients underwent right heart catheterization and cardiac magnetic resonance imaging (CMR) within one month of their PET/CT scan, and in this period all patients were stable on pulmonary hypertension targeted therapy. A balloon-tipped, flow-directed 7.5F, triple-lumen Swan-Ganz catheter (Edwards Life sciences LLC, Irvine, CA) was inserted in the pulmonary artery via the jugular vein or femoral vein under local anesthesia and constant ECG monitoring, and measurements were performed according to guidelines\textsuperscript{27}. Catheter ports were placed in the right atrium, right ventricle and pulmonary artery and the zero reference level for the pressure transducer was placed at mid-thoracic level in supine position.

CMR scans were performed on a Siemens 1.5T Sonato or Avanto scanner (Siemens Medical Solutions, Erlangen, Germany). Acquisition and post-processing were performed as described previously\textsuperscript{29}. Summarized, short-axis images of the right and left ventricle were analysed using MASS software (MEDIS Medical Imaging Systems, Leiden, The Netherlands). Endocardial borders of the right ventricle were manually traced on end-diastolic images and on end-systolic images. Right and left ventricular end-diastolic and end-systolic volumes were assessed using the Simpson rule\textsuperscript{30}.

IPAH Patients\textsuperscript{18}FLT PET

\textsuperscript{18}FLT was synthesized as described previously\textsuperscript{31}. Minimum 4 hours fasting was instructed prior to the PET scan, IPAH patients were scanned on a Philips Ingenuity PET/CT (Philips Healthcare, Best, The Netherlands), whilst control data sets were from a Philips Gemini TF-64 PET/CT (Philips Healthcare, Best, The Netherlands) using an identical scanning protocol. First, following a scout-CT scan, IPAH patients were positioned with lungs centered in the field of view of the PET scanner. Then, a dynamic 60-min emission scan was started simultaneously with automated bolus intravenous injection of 370 MBq of \textsuperscript{18}FLT (0.8 mL/s). After completion of the emission scan, a low-dose CT scan was
performed to correct for photon-attenuation and scatter. Finally, emission data were reconstructed in a 36 frame (1x10, 8x5, 4x10, 3x20, 5x30, 5x60, 4x150, 4x300 and 2x600 s) using the 3-Dimensional Row-Action Maximum Likelihood Algorithm (3D-RAMLA), applying all appropriate corrections for dead time, decay, scatter, attenuation and normalization. During the scan, venous blood samples were withdrawn at 5, 10, 20, 30, 40 and 60 minutes post injection and measured for the presence of radiolabeled metabolites (\(^{18}\)F-glucuronide) and the ratio of plasma and whole-blood activity concentrations as described\(^{32}\).

Kinetic Model for Data Analysis

\(^{18}\)FLT PET data were analysed using the plasma kinetic model as described previously\(^{31}\). The model consists of three or four rate constants, of which \(k_3\) represents phosphorylation of \(^{18}\)FLT by thymidine kinase and this rate constant was assumed to most closely represent proliferation rate, thus used to represent the lung \(^{18}\)FLT uptake. The model can be described using:

\[
C_{PET}(t) = C_T(t) + V_A \cdot C_A(t) + V_V \cdot C_A(t)
\]  

(1), in which \(C_{PET}(t)\) represents the measured pulmonary activity concentrations, \(C_T(t)\) the activity according to the plasma kinetic model, \(C_A(t)\) and \(C_V(t)\) are arterial and venous blood concentrations, respectively. To correct for contamination of the signal by blood-pool activity, \(V_A\) (arterial blood volume fraction) and \(V_V\) (venous blood volume fraction) were added to the model. For \(C_T(t)\), both an irreversible 2-compartment 3k model (2T3k) and a reversible 2-compartment 4k model (2T4k) were tested and the best fit was obtained. The use of an irreversible or reversible two-tissue model was evaluated using Akaike Information Criterion (AIC)\(^{33}\). For all patients and controls, Akaike Information Criterion (AIC) showed a significantly improved fit using a reversible 2T4k model (Supplementary Figure 1) and consequently, all results below are obtained using this model. In this model, \(K_1\) represents the rate constant for transport of \(^{18}\)FLT from blood into tissue, \(k_2\) represents the rate constant of transfer from tissue back to blood, \(k_3\) reflects the rate constant of phosphorylation of \(^{18}\)FLT, and \(k_4\) describes the rate of dephosphorylation\(^{34}\).

Input Functions and model selection
The lung has a dual circulation with perfusion coming from both the pulmonary and systemic (bronchial) circulation, recent literature suggests that systemic perfusion may become more important in PAH, e.g. increased from 1% to 18%-30% (total systemic cardiac output to bronchial output).\textsuperscript{35-37}. Definition of the appropriate model and input function was performed in two steps. First, for all controls, the input functions were obtained in the right-ventricular cavity (RV), pulmonary artery (PA) or ascending aorta (AA). Spill-over from the blood was included in all analyses and an additional correction for spill-over of activity from the second blood compartment (i.e. bronchial or pulmonary) was performed by drawing a region of interest in either the pulmonary artery or left atrium (LA). We tested the following combinations of input functions for the controls: RV with and without spillover from the LA or AA (RV, RVPA and RVLA), PA with and without spillover from the LA or AA (PA or PALA) and AA with and without spillover from the PA (AA, AALA and AAPA). These combinations were tested for all possible compartmental modeling including both the irreversible two-tissue (2T3k) model and for the reversible two-tissue (2T4k) model. For control and patient data, adding spillover corrections for input function resulted in reduced Akaike Information Criterion (AIC), indicating a significantly improved fit. Based on AIC, the reversible two-tissue model was used for all PET data analysis. Optimal fits were obtained for PALA in 50% of the lungs, AAPA in 20% and RVLA in 30%, showing that an input from the pulmonary circulation with pulmonary-venous spillover correction yielded optimal fits in a large majority of regions. Since we were looking for an input function model that could be applied to other tracers, we chose to discard the RV input function to avoid potential issues of spillover from the RV wall in tracers that show myocardial uptake.

After this initial analysis, the presence or absence of a dual circulation was tested by modifying the kinetic model, allowing for an input from both the arterial (obtained from the ascending aorta) and pulmonary (PA) circulation. When this method was applied to the data of our PAH patients, $K_{1,bronchial}$ was consistently smaller than 0.0001 whilst $K_{1,pulmonary}$ was on average 0.056 mL*g\(^{-1}\)*min\(^{-1}\) (range 0.044-0.079). In addition, using two different input function (Pulmonary artery/Ascending aorta) yield virtually identical values with no significant differences between both measurements. This indicated an absence of significant bronchial input or at least an inability to measure this input. These results led to
the conclusion that a single input from the pulmonary artery with spill-over correction from the pulmonary artery and the left-atrium yielded optimal results and used as input function.

Because the best model fit was obtained using a pulmonary input function, this was used for further calculations. Five 1-cm diameter regions of interest (ROI) were drawn in the pulmonary artery at the level of the pulmonary trunk on early frames showing the first-pass of the bolus through the pulmonary circulation. Average activity through time $C_{PA}(t)$ was obtained from this region and assumed to represent the total supply of radioactivity to the lungs $C_A(t)$ in eq. (1). To obtain the arterial input function $C_{PL}(t)$, $C_{PA}(t)$ was multiplied with a sigmoid function fitted through the ratio of plasma and whole-blood activity concentrations and a second sigmoid function fitted through the fraction of non-metabolized tracer. This correction was performed as described previously$^{31}$. A second set of ROIs was drawn in left atrial cavity (LA) in at least three slices. Average activity concentrations $C_{LA}(t)$ were obtained and used as correction factor for venous blood concentrations in the lungs $C_V(t)$ in eq. (1).

**Lung Segmentation**

Pulmonary activity concentrations were obtained using the low-dose CT scan. Using automated region-growing methods, a region of low tissue density between 0.05 and 0.6 g/mL was obtained for each lung. A layer of 2 voxels (8mm) was eroded from the borders of each lung region to reduce the effects of respiratory motion, spill-in of activity from the liver and other partial volume effects. To avoid any effects of a gradient signal in the lungs and of differences in the field of view of the scanner and patient positioning, data from a 2 cm region at the level of the bifurcation of the pulmonary artery were used for analysis using the ROIs drawn in the pulmonary artery. (Supplementary Figure 2)

**Parametric Mapping**

To assess the spatial distribution of $^{18}$FLT uptake within the lungs, volume of distribution was estimated from the slope of the linear regression on the Logan plot$^{38}$. A parametric 3-dimensional map was generated by using volume of distribution values measured per voxel basis, typically 200000 voxels ($2\text{mm}^2$ per voxel) for a human subject.
Animals and Experimental Design

Adult Sprague-Dawley (SD) rats (body weight 200-250g, Charles River, UK) were used, littermates age matched animals were assigned to study groups randomly. Only male rats were used to establish pulmonary hypertension models according to our previous experience. All experiments were conducted in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 (London, UK).

Monocrotaline (MCT) model: Pulmonary hypertension was induced by subcutaneous injection of monocrotaline (MCT, 60 mg/kg; Sigma-Aldrich) in rat. In our experience, pulmonary arterial pressure is marginally elevated in rats 1 week after MCT injection but significantly increased after 4 weeks. Histological examination of the MCT lung shows progressive vascular remodelling (proliferation) 1 and 4 weeks post MCT injection. Experimental design: a) $^{18}$FLT PET – following MCT induced PH progression; $^{18}$FLT PET scans were performed in control and MCT PAH rats one week and four weeks after injection. b) $^{18}$FLT PET – to assess the effects of treatments in MCT PH model; both imatinib and DCA have demonstrated efficacy in reducing pulmonary vascular proliferation the MCT induced PAH rat in our previous study. Rats were divided into 4 groups (n=6 per group) (i) control (C), (ii) MCT 4weeks (4W) and MCT rats treated with (iii) Dichloroacetate acid 70mg/kg/day in drinking water (DCA, Sigma-Aldrich, UK), (iv) imatinib 100mg/kg/day (IMA, LC Laboratories, USA) by oral gavage. Each treatment was started 2 weeks post MCT injection and continued for the following 2 weeks. At the end of the treatment, $^{18}$FLT PET scans were performed and tissues were collected for biochemical and histological examination.

Sugen hypoxia model (SuHx): Pulmonary hypertension was induced by a single subcutaneous injection of SU5416 (25mg/kg, Tocris Bioscience, Bristol, UK) followed by 4 weeks in normobaric hypoxic chamber (FIO$_2$ = 10%, maintained by nitrogen supply). Control rats were injected with the
vehicle alone and kept in normoxic condition. After 4 weeks hypoxia exposure, all the SuHx rats were returned to normoxia for an additional 6 weeks before performing $^{18}$FLT PET imaging.

In vivo $^{18}$FLT PET in rat

In vivo imaging was performed using a Siemens Inveon small-animal multimodality PET/CT system (Siemens Healthcare Molecular Imaging) using a protocol described previously. Briefly, rats were anaesthetized with isoflurane (2-4%), ventilation was adjusted to maintain pO2, pCO2 and pH within normal range. After the completion of the CT scan, $^{18}$FLT (approximately 35 MBq, <0.5ml) was injected through tail vein catheter. Dynamic emission scans were acquired in list-mode format for 60 minutes using conventional full-ring, whole-body PET. During PET scanning, serial blood samples were taken via a femoral artery line (20 µl each) 8 samples at 1st minute, and then at 2, 3, 5, 10, 15, 30, 45 and 60 minutes. At the end, animals were sacrificed and tissue samples (lung, RV, kidney, liver) were collected for gamma-counting, as well as snap frozen for biochemical measurement. The ratio of RV to left ventricle plus the septum mass (RV/ LV + septum) was used as an index of RV hypertrophy. Left lungs were inflated and fixed with 10% formalin for histology examination.

Data Analysis

Following the PET scanning of each animal, the data was saved using an anonymous coding system in the Biological Imaging Centre at Imperial College. PET image data were sorted into 0.5mm sinogram bins and 33 time frames and images were reconstructed using filtered back projection with CT-based attenuation correction. The frame durations used for the study were 12×5s, 4×15s, 6×30s, 11×300s. The reconstructed CT and PET images were analysed using the 3-dimensional visualization toolbox in the Inveon Research Workplace software. (Siemens Healthcare Molecular Imaging).

The whole lung tissue time-activity curves (TACs) were calculated from lung PET images co-registered with ROI on CT lung images, covering the lung volume with clearly visible boundaries.
adjusted by CT density thresholding. Cumulative images over 0 to 60 min were used for kinetic analysis of tracer uptake. The image derived input functions (IDIFs) were determined individually by sampled arterial plasma time activity curves or the PET images derived activity curve from the LV blood pool.

Decay corrected whole lung TACs were normalized for injected activity (kBq) and body weight to obtain standardized uptake value (SUV). The dynamic image data was fitted to a 2-compartment 4k model using the Matlab-based in house kinetic analysis software package (CLICKFIT). In this model, $K_1$ represents the rate constant for the transport of $^{18}$FLT from blood into tissue, $k_2$ represents the rate constant of the transport of $^{18}$FLT from tissue to blood, $k_3$ reflects the rate constant of phosphorylation of $^{18}$FLT, and $k_4$ describes the rate of $^{18}$FLT dephosphorylation.

**Bio-distribution**

The plasma and tissue samples (lung, RV, LV, septum, liver, and kidney) harvested following PET scanning were weighed and measured for radioactivity in a gamma counter. All data were corrected for radioactivity decay. The amount of radioactivity was expressed as percentage of injected dose per gram of tissue or blood (%ID/g).

**Histology Examination**

Rat transverse lung sections were processed for elastic Van Gieson (EVG) and hematoxylin and eosin (H&E) staining. Peripheral vessels less than 100 µm in lung were counted blindly under microscope (40X) and pulmonary vascular remodelling was expressed as the proportion of vessels with double elastic lamina to total vessels counted. Lung sections were also processed for Ki-67 (1:50, Thermo Scientific), CD68 (1:50; Serotec), TK1 (1:50; Abcam) and ENT1 (1:100; Abcam) immunostaining. Ki-67 score was calculated on a minimum of 20 randomly selected 40X high-power fields per slide and Ki-67 positive cells around the vessels (<100µm) were counted (approximately 40-50 vessels per slide). Number of Ki-67 positive cells per vessels was calculated for comparison between groups. CD68-positive cells were counted in whole-lung tissue sections and expressed as per per mm².
Human IPAH lung sections were obtained from the Imperial College Pulmonary Hypertension biorepository (ethics reference numbers: 01-210 & 2001/6003) and processed with TK1 (1:50; Abcam) antibody. For immunohistochemistry, horseradish-peroxidase-conjugated secondary anti-rabbit antibody (1:200) was used. Double immunofluorescence with anti-αSMA (1:100 Sigma) was performed using fluorescence secondary antibodies, anti-mouse Alexa 488 and anti-rabbit Alexa 568 (1:2,000, Invitrogen). Images (green for TK1 and red for αSMA) were detected under Leica fluorescence microscope (DM2500).

**Western Blotting**

Rat lung samples were homogenized in RIPA buffer (150 mmol/L sodium chloride, 1.0% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mmol/L Tris, pH 8.0, supplemented with protease inhibitor; Roche). Western blotting was performed according to the manufacturer’s suggestions (rabbit polyclonal anti-TK1 and anti-ENT1 1:1000; Abcam). Proteins were detected by Novex ECL chemiluminescence (Invitrogen). Optical densities of individual bands were measured, and protein expression was standardized with GAPDH.

**Cell Culture**

Pulmonary artery adventitial fibroblasts isolated from the lungs of IPAH patients and healthy donors were obtained from the University of Giessen and Marburg Lung centre tissue bank\(^9\). These cells has been used in our previous study\(^9\), the IPAH pulmonary arterial fibroblasts exhibited greater proliferation capacity and increased FDG uptake. We extracted RNA by the Trizol method from pulmonary artery adventitial fibroblasts and donor cells cultured in 6-well plates. Total RNA was transcribed with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), followed by real-time polymerase chain reaction analysis of ENT1 and thymidine metabolism enzyme genes (TK1 and thymidine phosphorylase TP) using primers described in Table 2.
**Statistical Analysis**

Data are presented as mean±SEM. Normal distribution was verified with the Kolmogorov-Smirnov test, and variance of homogeneity was tested by the Levene test. Differences between groups were assessed by either the Student *t* test or an appropriate ANOVA followed by the Bonferroni post hoc test for multigroup comparisons. Correlations between $^{18}$FLT uptake ($k_3$) and clinical variables were determined by simple linear regression analyses. A *P* value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows version 20 (IBM Corp., Armonk, NY) and GraphPad Prism for Windows version 5 (GraphPad Software, La Jolla, CA).

**Results**

**Increased Rate of $^{18}$FLT Phosphorylation $k_3$ in IPAH Patient lungs**

The clinical characteristics of the IPAH patients studied and details of the controls included in this study are summarized in Table 1. We calculated the rates of $^{18}$FLT transportation by $2T4k$ modelling based on sixty-minute PET imaging dynamic data acquisition. Compared with $^{18}$FLT PET uptake in control subjects, $^{18}$FLT phosphorylation, expressed as $k_3$ (Figure 1A), was increased significantly in IPAH patient lungs (0.086±0.034 vs 0.054±0.009; *P*<0.05). There was variation in $k_3$ within the IPAH group, such that some patients exhibited uptake more than 2-fold above the control group whereas others were in the range of controls. Further analysis by 3-dimensional parametric mapping of computed per-voxel $^{18}$FLT uptake from IPAH patients demonstrated that focal areas of relatively high uptake were distributed unevenly throughout the lung parenchyma (Figure 1B, Supplementary Figure 3A). Two representative IPAH patients with lowest and highest mPAP (Patient A 25mmHg vs Patient B 92mmHg) and PVR (Patient A 134 dyn.s/cm$^5$ vs Patient B 1005dyn.s/cm$^5$) demonstrated this variation (Supplementary Figure 4A); computed per-voxel $^{18}$FLT uptake from Patient B showed a distinctive uneven regional pattern of $^{18}$FLT signal compared to Patient A.
We next looked at the relationship between lung $^{18}$FLT uptake and cardiopulmonary haemodynamics in the IPAH group. There was no statistic significant correlation between lung $^{18}$FLT uptake (phosphorylation rates $k_3$) and mean pulmonary artery pressure (mPAP, $r=0.606$, $p=0.111$) or pulmonary vascular resistance (PVR $r=0.0205$, $p=0.625$) (Figure 1C). Interestingly, $k_3$ correlated significantly with right ventricular end-diastolic volume index ($r=0.736$, $p=0.037$), right ventricular end-systolic volume ($r=0.809$, $p=0.015$), right ventricular ejection fraction ($r=0.711$, $p=0.048$) and the load on the right ventricle (arterial elastance $r=0.711$, $p=0.049$) statistically. The correlation between $k_3$ and cardiac index was not significant statistically ($r=0.23$, $p=0.604$) (Supplementary Figure 4B).

Interestingly, prominent TK1 immunostaining was observed in the remodelled pulmonary arteries of IPAH patient lungs (Figure 2A, Supplementary Figure 5).

**Increased TK1, TP and ENT1 Expression in IPAH Fibroblast**

The vascular remodelling in PAH involves all cellular elements of the vessel wall, including fibroblasts. We have shown previously that these pulmonary fibroblasts from IPAH patients exhibit greater proliferation *in vitro*\(^9\). Here, we examined the expression of the thymidine metabolism enzymes TK1 and TP, as well as the thymidine transporter ENT1, in pulmonary fibroblasts isolated from 9 IPAH patients and 12 donor lungs in culture. IPAH pulmonary fibroblasts demonstrated increased expression of ENT1, TP and TK1 in comparison to control donor cells (Figure 2B).

**Increased Lung $^{18}$FLT Uptake in a Monocrotaline and Sugen hypoxia PAH Model**

To interpret the relationship between lung $^{18}$FLT uptake and the underlying pulmonary vascular pathology, further studies were conducted in the MCT and SuHx PAH rat. Static images obtained from 60 min dynamic PET acquisitions demonstrated significantly higher accumulation of $^{18}$FLT in both MCT and SuHx rat lung (Figure 3A); $^{18}$FLT SUV in the 4 week MCT group was >50% greater than in the control group (Supplementary Figure 6). Dynamic 60 min PET data acquisition was used for kinetic modeling using a 2T4K compartmental model. There was a progressive increase in $k_3$ (Figure 3B) in 1 week and 4 week MCT rat lungs, supported by direct tissue $^{18}$FLT measurements (Figure 3C). Similar
to MCT PAH rat model compartmental modeling identified a significant increase in $k_3$ in SuHx rats (Figure 3D) compare to controls and this was further confirmed by direct measurement of lung tissue $^{18}$FLT uptake by gamma counter (Figure 3E).

Histological examination of the MCT lung showed progressive vascular remodelling over 1 and 4 weeks post MCT injection, as previously demonstrated\(^9\). The remodelled vessels in both MCT and SuHx rat lung demonstrated Ki67 expression as well as prominent TK1 expression (Figure 4A). A significant increase in Ki67 expression was observed as early 1 week post MCT injection (Supplementary Figure 6). The increased $k_3$ in both MCT and SuHx rats were in proportion to the vascular pathology and closely correlated ($r^2$=0.78, $P$>.0001, MCT; $r^2$=0.81, $P$>.0001, SuHx) with the Ki-67 score (Figure 4.B and C).

**Attenuated Lung $^{18}$FLT Uptake with Imatinib and DCA Treatment in the MCT Model**

We investigated the response of $^{18}$FLT PET measurements to treatments with proven anti-proliferative efficacy in the MCT rat model. Treatment with DCA or imatinib during weeks 2 to 4 post MCT injection attenuated pulmonary vascular remodelling, as demonstrated by significant reductions in the proportion of remodelled vessels with double elastic lamina (Figure 5A), Ki67 score (5B), and right ventricular hypertrophy (RV/LV+sep ratio, DCA: 0.48±.03 imatinib: 0.32±.02; $P$<0.001 in comparison to placebo group: 0.61±.02, Figure 5C), consistent with previous data\(^9\). This was accompanied by a marked reduction in $k_3$ (Fig 5D) and further supported by the direct measurement of lung tissue $^{18}$FLT uptake (Figure 5E).

Western blot analysis of lung homogenates demonstrated a significant increase in ENT1 and TK1 expression in the 4 week monocrotaline rat lung, which was attenuated by both imatinib and DCA treatment, respectively (Figure 5F).

**Discussion**
This is the first study to explore dynamic $^{18}$FLT PET imaging in IPAH patients and a rodent PAH models to report on pulmonary vascular pathology. We found that lung $^{18}$FLT uptake was increased in IPAH patients compared with control subjects, and that there is heterogeneity in the lung $^{18}$FLT signal in IPAH, both between patients and within the lungs of each patient. The MCT and SuHx rat models allowed us to investigate this signal in more depth. Increased lung $^{18}$FLT uptake in MCT rats responded to drugs that reduce pulmonary vascular remodelling in animal models. Further in depth kinetic 2T4k modelling of the lung $^{18}$FLT signal showed an increased rate of $^{18}$FLT phosphorylation, $k_3$, together with prominent TK1 immunostaining in the remodelled pulmonary arteries of IPAH patient lungs and increased expression of thymidine metabolism genes in pulmonary arterial fibroblasts isolated from IPAH patients. Our data support the contention that the lung $^{18}$FLT PET signal reflects pulmonary vasculature cellular proliferation in PAH.

We have previously shown that $^{18}$FDG uptake is increased in the lungs of IPAH patients. A limitation of $^{18}$FDG is that it tracks general cellular metabolism and we are challenged in distinguishing inflammation from proliferation by this approach. In the field of oncology, the $^{18}$FLT ligand has been used for imaging tumor cell proliferation, and valued for improving the accuracy of anti-proliferative target delineation. $^{18}$FLT has been shown to correlate better than $^{18}$FDG with cell proliferation markers in excised tumors and has potential to better differentiate growing tumors from inflammation. Importantly, background activity of $^{18}$FLT is low in the thorax as shown in studies of lung cancer. The potential clinical superiority of $^{18}$FLT over $^{18}$FDG PET is evident in our data, where there is very low noise in the $^{18}$FLT signal at 95% threshold (Voxels with $R^2 > 0.95$); in comparison, $^{18}$FDG lung uptake is more patchy at 60% threshold, indicating a relatively higher noise threshold (Supplementary Figure 7). In the current study, dynamic PET scanning data from IPAH patients allowed us to perform the in-depth kinetic analysis of the rates of $^{18}$FLT transportation in the lung by 2T4k modelling. We found a significantly higher rate of $^{18}$FLT phosphorylation in a proportion of IPAH patient lungs.

The $^{18}$FLT phosphorylation reaction is the TK1 rate-limiting step during S-phase of the cell cycle, determining the uptake and retention of $^{18}$FLT in tissue. TK1 overexpression has been described
in various tumour tissues including brain, breast and lung tumors and TK1 expression is correlated closely with Ki67 expression as well as $^{18}$FLT uptake\textsuperscript{22-24}. The measurement of intracellular levels of phosphorylated thymidine (as a surrogate of TK1 activation level) is regarded as an accurate method for estimating cellular growth\textsuperscript{16}. Barthel et al\textsuperscript{45}, using tumour implants with variant TK1 expression (denoted $+/-$ and $-/-$) in mice, have shown that $+/-$ tumours produced 48\% more TK1 enzyme on average and also grew faster compared with $-/-$ tumours. Importantly, uptake of $^{18}$FLT was higher in $+/-$ tumours compared with $-/-$ tumours, while a converse pattern was found with $^{18}$FDG imaging. These findings support the conclusion that imaging DNA synthesis is more accurate for the assessment of the proliferative potential of tumors, compared with radiotracer measurements of glucose consumption.

Direct correlation of $^{18}$FLT uptake with vascular remodelling in the same human study subjects is not possible. We examined lung sections obtained from the Imperial College Pulmonary Hypertension Biorepository and observed prominent TK1 immunostaining in remodelled pulmonary arteries in IPAH patient lungs. We also used pulmonary fibroblasts, an active contributor to vascular remodelling in IPAH, isolated from IPAH patients to explore the biology in vitro. Consistent with increased lung uptake of $^{18}$FLT PET and phosphorylation of $^{18}$FLT in IPAH patients, we observed significantly increased expression of thymidine metabolism enzymes, TK1 and TP, as well as the thymidine transporter, ENT1, in these hyper-proliferative pulmonary vascular cells. These data are consistent with a lung $^{18}$FLT signal that depicts the hyper-proliferative vascular pathology documented in the pulmonary hypertensive lung.

There was considerable variation in the distribution of lung $^{18}$FLT uptake within the small cohort of IPAH patients. Some patients exhibited a more than 2-fold increase in $k_3$ above the control group, whereas others were in the range of controls. This is not surprising as vascular proliferation is unlikely to be constant throughout the course of the disease. Little is known about the natural history of the vascular pathology of IPAH; for example, it could be episodic with periods of vascular proliferation interspersed with periods of relative quiescence, or vascular remodelling could be an early event with little cellular activity during the later stages of the disease. A cross sectional study of IPAH patients
would be expected to reflect this variation. Furthermore, we analysed data by 3-dimensional parametric mapping of computed per-voxel (2mm² per voxel) ¹⁸FLT uptake. There was an uneven pattern in the ¹⁸FLT signal distribution within the lungs of patients with severe disease, which may reflect the patchy distribution of the vascular pathology within the lung seen on histological examination⁴,⁶.

This temporal and spacial variability in pulmonary vascular remodelling in the lungs of PAH patients is likely to explain the absence of an association between overall lung ¹⁸FLT phosphorylation and mean pulmonary artery pressure or pulmonary vascular resistance in this small cohort of IPAH patients. The lack of association does not diminish the utility of ¹⁸FLT PET as a clinical tool, but offers new possibilities for drug development and personalized medicine. With the focus now on developing drugs that tackle pulmonary vascular remodelling (as opposed to vasoconstriction) and many potential drug targets, it is important to have an efficient method for filtering out agents that have therapeutic potential by this mechanism of action from those that are not likely to work. ¹⁸FLT PET could offer a bridging biomarker; a method for evaluating the action of a drug in an animal model that can also be used to provide mechanistic data in patients and provide early dose-response information before any change in vascular resistance.

To that end, the changes in ¹⁸FLT PET signal in response to treatment in the experimental MCT and SuHx models are significant. The remodelled vessels in the MCT and SuHx rat lungs demonstrated increased Ki-67 expression as well as prominent thymidine kinase TK1 expression. k₃ was also increased in proportion to the proliferation Ki-67 score. An increase in ³FLT signal was seen at 1 week, before significant changes in hemodynamic measurements are observed in this model⁹. Treatment with DCA and imatinib attenuated pulmonary hypertension and vascular remodelling and reduced the disease-driven increase in lung TK1 and ENT1 expression. ¹⁸FLT PET lung uptake was also reduced in the treated animal groups, consistent with decreased peripheral vascular muscularization.

These data support the concept that ¹⁸FLT PET may be a useful tool to evaluate novel anti-proliferative therapies in patients with PAH. It can be a critical part of clinical trials where the objective
is to gain evidence that a new treatment is targeting the proliferative component that drives the structural changes in the pulmonary vasculature, and further investigation is urgently needed. We suggest that, given the variability in uptake between patients, it might also be a useful tool for identifying patients who might benefit most from anti-proliferative therapy and so support precision medicine.

**Sources of Funding**

This research is supported by project grant from the British Heart Foundation (PG/14/88/31183). For the clinical studies in PAH patients, we received support from the Netherlands CardioVascular Research Initiative; the Dutch Heart Foundation, Dutch Federation of University Medical Centres, the Netherlands Organisation for Health Research and Development and the Royal Netherlands Academy of Sciences.

**Disclosures**

None.
References


Table 1: Clinical Characterization of Patients with Idiopathic Pulmonary Arterial Hypertension (IPAH) and 6 controls with unilateral lung cancer

<table>
<thead>
<tr>
<th></th>
<th>IPAH (n=8)</th>
<th>Controls (n=6)</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>46 ± 12</td>
<td>68 ± 9</td>
</tr>
<tr>
<td>Sex (M:F) (n)</td>
<td>4 : 4</td>
<td>3 : 3</td>
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<tr>
<td>NYHA functional class I/II/III/IV (n)</td>
<td>2/6/0/0</td>
<td>-</td>
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<tr>
<td>6MWD (m)</td>
<td>518 ± 125</td>
<td>-</td>
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<tr>
<td>NT-ProBNP (median IQR)</td>
<td>190 (30-1280)</td>
<td>-</td>
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</table>

**Treatment**

- Treatment naive: 1
- Monotherapy (ERA or PDE5I): 1
- Dual combination therapy (ERA+PDE5I): 3
- Triple combination therapy (ERA + PDE5I + prostacyclin): 3

**Hemodynamic characteristics**

- Heart rate (beats/min): 79 ± 8
- Mean pulmonary artery pressure (mmHg): 52 ± 20
- Pulmonary arterial wedge pressure (mmHg): 8 ± 3
- Mean right atrial pressure (mmHg): 7 ± 4
- Pulmonary vascular resistance (dyn.s/cm^5): 686 ± 482
- Cardiac output (l/min): 5.6 ± 1.7
- Mixed venous O2 saturation (%): 68 ± 10

**Cardiac magnetic resonance imaging**

- RVEDVI (ml/m^2): 93 ± 21
- RVESVI (ml/m^2): 59 ± 23
- RVEF (%): 39 ± 11
- LVEF (%): 63 ± 8

Subject characteristics. M = males; F = females; NYHA = New York Heart Association functional class; 6MWD = six minute walk distance; ERA = Endothelin Receptor Antagonists; PDE5I = Phosphodiesterase-5 Inhibitors. RVEDI = right ventricular end diastolic volume index; RVESV = right ventricular end systolic volume index; RVEF = right ventricular ejection fraction; LVEF = left ventricular ejection fraction.
Table 2. Real-Time Polymerase Chain Reaction Primers for the Thymidine Kinase 1 (TK1), Equilibrative Nucleotide Transporter 1 (ENT1) and Thymidine Phosphorylase (TP).

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>TK1</td>
<td>CCGTGGTTAAAGCGGTCG</td>
<td>GGACGCCTCTCATCAACTCT</td>
</tr>
<tr>
<td>ENT1</td>
<td>ACTCCAAAGTCTCAGCAGCAGG</td>
<td>AGAGTTCCGCTCAGGCAAG</td>
</tr>
<tr>
<td>TP</td>
<td>AGGAGGCACCTTGGATAAGC</td>
<td>GCCCCTCCACGAGTTTCTTA</td>
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Figure legends:

Figure 1. A. Lung $^{18}$FLT uptake (rate of $^{18}$FLT phosphorylation, $k_3$) was significantly increased in IPAH patient group (n=8, 0.086±0.034) compared with controls (n=6, 0.054±0.009; *$p<0.05$). Patients with prostacyclin treatment are marked in green and the treatment-naive patient is marked in black. B. Upper panel: Parametric map of lung $^{18}$FLT uptake calculated from Logan analysis from two IPAH patients having lowest (Patient A) and highest (Patient B) among the patient group (mPAP presented in Supplementary Figure 4A; CMR data in Supplementary Figure 3B). Lower panel: 3D parametric map (axial view) generated from computed per-voxel $^{18}$FLT lung uptake from IPAH patients A and B, and a control subject (coronal view in Supplement Figure 4A). Considerably higher uneven regional $^{18}$FLT uptake was observed in patient B. C. There was no significant correlation between lung $^{18}$FLT uptake ($k_3$) with mean pulmonary artery pressure (mPAP) or pulmonary vascular resistance index (PVRI). But lung $^{18}$FLT uptake ($k_3$) was closely correlated to right ventricular dimensions (RVEDVI and RVESVI) and right ventricular function (RVEF) as well as to the load on the right ventricle (Ea). (RVEDVI = right ventricular end-diastolic volume; RVESVI = right ventricular end-systolic volume; RVEF = right ventricular ejection fraction; Ea = arterial elastance).

Figure 2. A. Thymidine kinase, TK1, expression in lungs from a patient with idiopathic pulmonary arterial hypertension (IPAH). Double immunofluorescence and immunostaining demonstrate co-localization of TK1 and smooth muscle actin in the remodelled vessels from the patient with IPAH. The representative pictures for fluorescence immunostaining and light microscopy immunostaining are not from serial sections. The focus of the fluorescence stain section was set on the vessel medial layer, confirm our observation using immunostaining (See also Supplementary Figure 5). B. Pulmonary artery adventitial fibroblasts isolated from lungs of patients with idiopathic pulmonary arterial hypertension (IPAH) exhibited increased expressions of ENT1, TK1 and TP in transcription level compared with cells from donor lungs. Data are expressed as mean±SEM; n≥9 in each group. **$P<0.01$, *$P<0.05$. 
Figure 3. A. Representative static images from dynamically acquired positron emission tomography images over 60 minutes after $^{18}$FLT injection. B. Compartmental analysis demonstrating a significantly increased lung $^{18}$FLT uptake (phosphorylation rate $k_3$) in rats 1 week (1W) and 4 weeks (4W) post monocrotaline (MCT) injection. C. Measurement of $^{18}$FLT uptake by gamma counter confirms significantly increased $^{18}$FLT uptake in monocrotaline rats. %ID/gm indicates percentage of injected dose per gram of tissue. Data are expressed as mean±SEM; n≥5 in each group. **P<0.01, *P<0.05 vs control (C) group. D. and E. Significantly increased $^{18}$FLT uptake was observed in 10 week SuHx rat lungs compared to control measured by both compartmental analysis and gamma counter respectively. Data are expressed as mean±SEM; per group. *P<0.01 , *P<0.05 compared to control (C) group.

Figure 4. A. Immunostaining with EVG, Ki67 and TK1 in both monocrotaline and SuHx rat lung sections demonstrated profound pulmonary vascular remodelling (muscularization) with increased expression of Ki-67 positive nuclei and prominent TK1 expression respectively. B. and C. Relationship between $^{18}$FLT phosphorylation rate and proliferation index Ki-67 score in monocrotaline ($r^2=0.77, P<0.0001$) and SuHx ($r^2=0.81, P<0.0001$) rat lung respectively.

Figure 5. A. Treatment with dichloroacetate (DCA) and imatinib (IMA) attenuated pulmonary vascular muscularization. B. Effect of treatments on Ki-67–positive nuclei surrounding remodelled peripheral pulmonary vessels. C. Effect of treatments on attenuation of right ventricular hypertrophy in the monocrotaline (MCT) rat. D. and E. Treatment with DCA and imatinib attenuated lung $^{18}$FLT uptake in monocrotaline rats. %ID/gm indicates percentage of injected dose per gram of tissue. F. Summary data and representative western blot for TK1 and ENT1 expression in whole lung homogenate. Both TK1 and ENT1 expression, normalized to GAPDH, were increased in the monocrotaline rat and attenuated by DCA and imatinib treatment. Data are expressed as mean±SEM; n≥ 5 per group. ***P<0.001, **P<0.01, *P<0.05.
Figure 1.

A

![Graph showing lung \(^{18}F\)-FLT uptake](image)

- Control (n=6)
- IPAH (n=8)

> Lung \(^{18}F\)-FLT uptake (phosphorylation rates)

> **P=0.031**

---

B

![Images of Patient A, Patient B, and Control Subject](image)

---

C

![Graphs showing correlation between variables](image)

- mPAP (mmHg)
- PVR
- RVDDV1 (mL/m²)
- RVESV1 (mL/m²)
- RVEF (%)
- Ees (mmHg/ml)

> r=0.606 (p=0.111)
> r=0.205 (p=0.625)
> r=0.756 (p=0.037)
> r=0.809 (p=0.015)
> r=0.731 (p=0.048)
> r=0.709 (p=0.049)
Figure 2.

A

IPAH

IPAH

B

Relative mRNA Expression ENT1

Relative mRNA Expression TK1

Relative mRNA Expression TP

Donor  IPAH

Donor  IPAH

Donor  IPAH

20µm
Figure 3.
Figure 4.

A. EVG, Ki67, TK1

Control

MCT

SuHx

B. MCT rat Lung $^{18}$FLT uptake ($k_3$) and proliferation (Ki-67)

C. SuHx rat Lung $^{18}$FLT uptake ($k_3$) and proliferation (Ki-67)
Figure 5.

A. Muscularization

B. Proliferation Ki-67 score

C. RV Hypertrophy

D. Lung $^{18}$FLT uptake (phosphorylation rate $k_p$)

E. $^{18}$FLT uptake (Tissue count)

F. TK1 expression

GAPDH

TK1 MCT IMA DCA

ENT1 MCT IMA DCA
SUPPLEMENTAL MATERIAL

\(^{18}\text{FLT PET depicts heterogeneous proliferation pathology in IPAH patient lung: a potential biomarker for PAH}\)

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Supplementary Figures

Supplementary Figure 1.

Supplementary Figure 2.
Supplementary Figure 3.

A

Patient A  Patient B  Control subject

B

Patient A  Patient B  Cardiac parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient A</th>
<th>Patient B</th>
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<tbody>
<tr>
<td>RVEDVI (mL/m²)</td>
<td>69</td>
<td>126</td>
</tr>
<tr>
<td>RVEF (%)</td>
<td>38</td>
<td>17</td>
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<tr>
<td>mPAP (mmHg)</td>
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<td>69</td>
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<td>RAP (mmHg)</td>
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<td>PVRI (dyn.s/cm²/m²)</td>
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<td>SvO2 (%)</td>
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<td>73</td>
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<tr>
<td>6MWD (m)</td>
<td>528</td>
<td>466</td>
</tr>
<tr>
<td>NT proBNP</td>
<td>27</td>
<td>1593</td>
</tr>
</tbody>
</table>

Supplementary Figure 4.

A.

B.

$r = 0.23$  ($p = 0.604$)
Supplementary Figure 5.

Supplementary Figure 6.

A. \(^{18}\text{FLT uptake (SUV}_{60})\)

B. Ki67 score

\[ \begin{align*}
\text{Control} & \quad 1\text{W}_\text{MCT} & \quad 4\text{W}_\text{MCT} \\
\text{Positive nuclei per vessel} & \quad 15 & \quad 10 & \quad 5 \\
\end{align*} \]
Supplementary Figure 7.

FLT (95% threshold)  
FDG (60% threshold)
Supplementary Figure Legends

**Supplementary Figure 1.** A. Representative graph for model comparison. The 9 models employed are the 2k, 3k and 4k, each of which without Vb, with fixed Vb, and with Vb estimated. AIC (Akaike Information Criterion) in its small sample formulation was computed based on the Weighted-Residual-Sum-of-Squares WRSS obtained from each fit. According to this parsimony criterion, the model with the lowest AIC (4k model in this example) was selected as best model. B. Representative fitting generated by 4k-compartmental model using PALA as input function from one of the patient (red dot indicates measured lung tissue activity and blue line indicates estimated lung tissue activity).

**Supplementary Figure 2.** The region of interest used in all cases corresponds with the lung parenchyma visible on five consecutive slides at the level of the pulmonary artery trunk from which the image derived input function is derived. A: the red dotted rectangle illustrates the region of interest on a sagittal CT scout image of the thorax. The blue line corresponds to the level of image B and C.; B: The transverse low-dose CT image corresponds to the blue line in A at the level of the pulmonary artery trunk; C: The image derived input function was drawn (red circle) in the pulmonary artery at the level of the pulmonary artery trunk on five consecutive slides.

**Supplementary Figure 3.** Coronal view of parametric map of lung $^{18}$FLT uptake calculated from Logan analysis from two different IPAH patients having lowest (Patient A) and highest (Patient B) mPAP among the patient group and a healthy control subject. Similar to axial view, relative uneven $^{18}$FLT lung uptake was observed in patient B. B. Upper panel: CMR short axis view showing increased RV volume and D-shaped interventricular septum in patient B. Lower panel: CMR four chamber view showing increased right ventricular and right atrial volume as well as tricuspid regurgitation in patient B. RVEDVI = right ventricular end-diastolic volume; RVEF = right ventricular ejection fraction; mPAP = mean pulmonary artery pressure; RAP = right atrial pressure; PVRI = pulmonary vascular resistance index; SvO2 = mixed venous oxygen saturation; 6MWD = six minute walking distance.
Supplementary Figure 4. A. Mean pulmonary arterial pressure from all patients. Lowest and highest mPAP is marked with A and B respectively. B. There was no significant correlation between lung $^{18}$FLT uptake ($k_3$) and cardiac index (CI).

Supplementary Figure 5. A. Representative images (4x and 20x objective) of thymidine kinase (TK1) expression in lungs section from a patient with idiopathic pulmonary arterial hypertension (IPAH) (upper panel) and a healthy control subject (lower panel). Prominent TK1 expression was observed in the remodelled vessels in the IPAH lung.

Supplementary Figure 6. A. Standard uptake value of Lung $^{18}$FLT uptake in monocrotaline (MCT) rat was increased after 4 weeks (4W_MCT) compare to controls. B. Immunostaining with Ki67 in monocrotaline lung sections demonstrated increased expression of Ki-67 positive nuclei around remodelled vessels within 1 week following MCT injection.

Supplementary Figure 7. Comparison of signal to noise ratio per-voxel lung FLT (left) and FDG (right) signal. Representative figures showing per-voxel uptake. On the left per-voxel uptake at 95% threshold (voxels with $R^2 > 0.95$) for the same slice in the scan of patient B demonstrated that every lung voxel signal is within this tolerance, indicating a very low noise in FLT images. In comparison, when applying the same method for FDG lung uptakes (on the right) the coverage is spotty at a threshold of 60%, indicating the relative higher noise threshold.