Clinical quantification of the integrin αvβ6 by [18F]FB-A20FMDV2 positron emission tomography in healthy and fibrotic human lung (PETAL Study)

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Abstract

Purpose The RGD-integrin, αvβ6, plays a role in the pathogenesis of pulmonary fibrosis through activation of transforming growth factor beta (TGFβ). This study sought to quantify expression of αvβ6 in the lungs of healthy humans and subjects with pulmonary fibrosis using the αvβ6-selective [18F]FB-A20FMDV2 PET ligand.

Methods [18F]FB-A20FMDV2 PET/CT scans were performed in healthy subjects and those with fibrotic lung disease. Standard uptake values (SUV) and volume of distribution (VT) were used to quantify αvβ6 expression. In subjects with fibrotic lung disease, qualitative assessment of the relationship between αvβ6 expression and the distribution of fibrosis on high resolution computed tomography was conducted.

Results A total of 15 participants (6 healthy, 7 with idiopathic pulmonary fibrosis (IPF) and 2 with connective tissue disease (CTD) associated PF) were enrolled. VT and SUV of [18F]FB-A20FMDV2 were increased in the lungs of subjects with pulmonary fibrosis (PF) compared with healthy subjects. Geometric mean VT (95% CI) was 0.88 (0.60, 1.29) mL/cm³ for healthy subjects, and 1.40 (1.22, 1.61) mL/cm³ for subjects with IPF; and SUV was 0.54 (0.36, 0.81) g/mL for healthy subjects and 1.03 (0.86, 1.22) g/mL for subjects with IPF. The IPF/healthy VT ratio (geometric mean, (95% CI of ratio)) was 1.59 (1.09, 2.32) (probability ratio > 1 = 0.996). Increased uptake of [18F]FB-A20FMDV2 in PF was predominantly confined to fibrotic areas. [18F]FB-A20FMDV2 measurements were reproducible at an interval of 2 weeks. [18F]FB-A20FMDV2 was safe and well tolerated.

Conclusions Lung uptake of [18F]FB-A20FMDV2, a measure of expression of the integrin αvβ6, was markedly increased in subjects with PF compared with healthy subjects.

Keywords Idiopathic pulmonary fibrosis · [18F]FB-A20FMDV2 PET/CT · αvβ6 integrin · Biomarker · Pulmonary fibrosis · PET · Clinical study
**Introduction**

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive scarring of the lungs of unknown aetiology. It results in respiratory failure and has a median untreated survival of 3 years from diagnosis [1]. Diagnosis is made by a multidisciplinary team, primarily after high-resolution computed tomography (HRCT) of the lungs. The observation of subpleural, basal honeycombing is pathognomonic for IPF and reflects the histological feature of usual interstitial pneumonia (UIP) [2]. HRCT imaging has been considered to stage IPF (Walsh 2018). Visual quantification of disease on HRCT in IPF provides prognostic information. However, this approach is hampered by its subjective nature, heterogeneity and variability. Computer-based quantification of disease on HRCT may provide more objective and reproducible data. However, this field suffers from the lack of large, well-annotated imaging datasets which hinder the development and testing of new computer-based tools, and lack of prospective trials for imaging biomarker validation. HRCT is not generally considered sensitive enough to be used as an efficacy endpoint in clinical studies, requiring years of treatment to observe a response.

There is no cure for IPF and pharmacological treatments are limited to drugs (pirfenidone and nintedanib) that slow disease progression but have significant side effects [3]. Unmet need remains high and new treatments are required. In some individuals with connective tissue disease (CTD) such as rheumatoid arthritis or systemic sclerosis, interstitial lung disease (ILD) including pulmonary fibrosis may develop with features and prognosis similar to those for IPF [4, 5].

The αvβ6 integrin is a cell surface adhesion receptor that is induced on damaged epithelium [6]. In its activated form, the αvβ6 integrin interacts with extra-cellular matrix (ECM) via ligands bearing the arginine-glycine-aspartic acid (RGD) tripeptide sequence [7]. Binding of ECM ligands to αvβ6 promotes cell adhesion, activation of intra-cellular signalling pathways and local release of activated TGFβ from latent complexes in the matrix [7–9]. Active TGFβ mediates multiple pro-fibrotic processes resulting in progressive lung scarring, ultimately resulting in organ failure and death [10]. αvβ6 integrins have been demonstrated to play a role in the aetiology and progression of several fibrotic diseases including IPF, renal and hepatic fibrosis, as well as some cancers [11, 12]. A number of drugs targeting αvβ6 integrin across fibrotic diseases are currently in development including an inhaled small molecule inhibitor of αvβ6 under clinical development by GlaxoSmithKline R&D and an intravenous monoclonal antibody under development by Biogen (see studies registered on clinical trials.gov: NCT01371305, NCT02612051 and NCT03069989) [13]. Semiquantitative analysis of αvβ6 integrin expression in lung biopsy specimens from individuals with IPF has been shown to have potential prognostic significance, with higher levels predicting more rapid progression, and mortality [14]. Non-invasive tracking of αvβ6 integrin expression could therefore provide a mechanism to assess drug action, disease activity and prognosis.

The envelope protein of foot and mouth disease virus (FMDV) contains a 20 amino acid peptide sequence, NAVPNLRGDLQVLAQKVART (A20FMDV2) that binds the αvβ6 integrin with high affinity and selectivity [15–18]. Automated GMP-compatible radiochemistry non-clinical radiosiodimetry of the A20FMDV2 peptide is described in the accompanying article [19]. [18F]FB-labelled A20FMDV2 has an acceptable toxicology profile, showed no adverse effects in healthy subjects and has an effective dose (0.0217 mSv/MBq) that permits multiple scans in a single subject [20]. In the murine bleomycin model, the evolution of pulmonary fibrosis is accompanied by an increased expression of αvβ6 integrins that is reproducibly quantifiable using SPECT assessment of A20FMDV2 binding [21].

In this study, we investigated the potential for [18F]FB-A20FMDV2 to safely and reproducibly quantify αvβ6 integrins in the lungs of healthy subjects as well as those with pulmonary fibrosis due to IPF or CTD-ILD.

**Materials and methods**

**Study design**

Study objectives and endpoints are presented in Table 1. Healthy participants underwent one [18F]FB-A20FMDV2 PET/CT scan each. Subjects with pulmonary fibrosis underwent an HRCT and two [18F]FB-A20FMDV2 PET/CT scans, up to 2 weeks apart, to assess test/retest variability. Healthy participant data was used to optimise scanning procedures prior to recruitment of participants with fibrotic lung disease.

Subjects were screened and recruited at Hammersmith Medicines Research (HMR), London, UK, and imaging assessments were conducted at Invicro, London, UK. The study was approved by the London—Brent Research Ethics Committee, UK (reference 13/LO/1792), and permission to administer radioisotopes was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC) of the UK (Ref: 630/3925/30809). The study is registered in clinical trials.gov (NCT02052297; RES116235).

**Participants**

Main inclusion criteria were as follows: male subjects ≥ 45 years and female subjects ≥ 55 years at the time of signing the informed consent; a positive Allen’s test in at least one arm for arterial blood sampling; body weight ≥ 50 kg and body mass index (BMI) within the range 19.0–35.0 kg/m². Additionally,
self-reported healthy subjects or individuals with IPF [1] systemic sclerosis (SSc) [22] or rheumatoid arthritis (RA) [23] who had confirmed severe or progressive interstitial lung disease (ILD) on HRCT [24] were enrolled. Exclusion criteria included the following: a current history of liver disease, claustrophobia, radiation exposure in research studies greater than 10 mSv over the preceding 3 years or greater than 10 mSv in any single year, human immunodeficiency virus (HIV) or Hepatitis B/C virus positive status, anaemia (< 11 g/dL), abnormal blood coagulation profile and previous or current exposure to animals that may harbour FMDV2.

**Sample size**

The sample size was estimated to provide a precision of < 50% for the parameters of interest (volume of distribution \((V_T)\) and/or standardised uptake values \([SUV]\)). Preclinical data was available to conduct a preliminary estimate of the number of subjects that may be required to achieve this precision. Coefficients of variation \((CV)\) for standardised uptake values \([SUV]\) in rat tissues at various timepoints \((n = 3\) per timepoint) following administration of \([^{18}\text{F}]\text{FB-A20FMDV2}\) ranged between 3 and 150% for different tissues and incubation timepoints [19]. The mean CVs across all rodent tissues were 39–58%; for the lung, the mean CV ranged from 3 to 26%. Using these estimates for SUV variability from the rodent, the precision of the mean SUV calculated as half the width of a 95% confidence interval for the mean and expressed as a percentage of the estimated mean, for 6, 8 and 20 humans, was calculated for this study (Table 2).

Thus, a sample size of 6 was likely to achieve the required precision, depending on the observed CV (%) in humans. Thus, a sample size re-estimation was planned to confirm the required sample size.

**Immunogenicity assay**

A direct immunoassay for A20FMV2 was developed and validated [20]. Serum samples were stored at \(-70 ^\circ C\) until assayed.

**Imaging**

\(A20FMDV2\) was labelled with fluorine-18 by conjugation of the resin bound precursor \((A20FMDV2)\) to the prosthetic group \([^{18}\text{F}]\)-fluorobenzoic acid \([^{18}\text{F}]\text{FB}\), followed by acidic cleavage from the resin, purification by semi-preparative HPLC and reformulation in saline. The synthetic procedure of \([^{18}\text{F}]\text{FB-A20FMDV2}\) was adapted from a previously described method [19, 25]. An automated procedure was developed in-house using an Eckert and Ziegler Modular Lab system coupled with a semi-preparative HPLC system.

A venous cannula was inserted into a cubital or forearm vein and an arterial cannula was inserted into the radial artery under local anaesthesia. GMP grade \([^{18}\text{F}]\text{FB-A20FMDV2}\) [19] was injected via the venous cannula and emission data acquired for up to 240 min. The arterial cannula was used to collect blood samples throughout the scan to enable the quantification of the total and unmetabolised \([^{18}\text{F}]\text{FB-}

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**Table 1** Objectives and endpoints

| Objectives | Endpoints |
|------------|-----------|
| **Primary** | **Uptake and distribution of \([^{18}\text{F}]\text{FB-A20FMDV2}\) in organs of interest (volume of distribution \((V_T)\), and/or standardised uptake values \([SUV]\)).** |
| To determine the utility of \([^{18}\text{F}]\text{FB-A20FMDV2}\) to quantify \(\alpha\varepsilon\beta_6\) availability in healthy, IPF and CTD-ILD participants | |
| **Secondary** | **Qualitative assessment of the relationship between the distribution of fibrosis in the lungs from IPF and CTD-ILD participants and the uptake of \([^{18}\text{F}]\text{FB-A20FMDV2}\).** |
| To compare \([^{18}\text{F}]\text{FB-A20FMDV2}\) uptake in areas of the lungs with varying degrees of fibrosis as determined by high resolution computed tomography (HRCT). | Volume of distribution \((V_T)\), and/or standardised uptake values \([SUV]\) in IPF and ILD vs healthy lungs. |
| To compare \([^{18}\text{F}]\text{FB-A20FMDV2}\) uptake in IPF and CTD-ILD lungs versus healthy lungs. | |
| To determine the reproducibility of \([^{18}\text{F}]\text{FB-A20FMDV2}\) uptake in IPF and CTD-ILD participant lungs. | |

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**Table 2** Estimated precision for mean SUV

| CV (%) | \(N = 6\) | \(N = 8\) | \(N = 20\) |
|-------|---------|---------|----------|
| 39    | 41%     | 33%     | 18%      |
| 58    | 61%     | 48%     | 27%      |
| 100   | 105%    | 84%     | 47%      |

\(^1\) Calculated as half the width of a 95% confidence interval for the mean and expressed as a percentage of the estimated mean.
A20FMDV2-related radioactivity in whole blood and plasma throughout the duration of the scan. The data provided by the arterial blood samples collected was used to derive an input function for the analysis of emission PET data.

Whole blood arterial activity data was obtained from a continuous sampling system (ALLOG AB, Mariefred, Sweden), measured at a frequency of 1 Hz for the first 15 min of each scan (900 data points per scan). Manual (discrete) blood samples were withdrawn at the following timepoints: 5, 10, 15, 20, 25, 30, 40 and 50 min after scan start. Discrete samples were analysed for radioactivity in whole blood and plasma components using a Perkin Elmer (Cambridge, UK) Wizard 1470 gamma counter. A subset of the discrete plasma samples (5, 10, 15, 20, 30 and 50 min) were analysed to determine the fraction of radioactivity corresponding to the intact radiotracer compound, as opposed to radioactive metabolites.

The total effective radiation dose received was not expected to exceed 7.2 mSv for the healthy subjects who had a single PET/CT scan and 23.0 mSv for the pulmonary fibrosis participants who had two PET/CT scans as well as a high-resolution CT scan. This initial dosimetry estimate was based on a conservative approach using a dose conversion factor of 33.5 μSv/MBq injected activity based on preclinical data for the novel radioligand and a maximum administered activity of 180 MBq.

Following assessment of human dosimetry [20], the dose conversion factor was later revised downwards to 21.7 μSv/MBq, with correspondingly lower dose estimates of 5.1 mSv for the healthy and 18.8 mSv for the IPF patient group. Furthermore, following review of the quality of the images from healthy and IPF participants, the maximum administered activity for the CTD-ILD participants, was reduced from 180 to 150 MBq, thus lowering the maximum estimated radiation dose to 17.6 mSv.

**Image acquisition**

Dynamic [18F]-FB-A20FMDV2 PET scans were acquired in list mode on a Siemens PET/CT system Biograph 6 TruePoint with TrueV (Siemens Healthcare, Erlangen, Germany). For the first scan, a high-resolution CT (HRCT) was achieved prior to the PET/CT. A low-dose CT scan was performed immediately before each PET study in order to estimate tissue attenuation (CT-AC). Following intravenous bolus injection of the radiotracer, dynamic PET emission data were acquired for at least 90 min (frame durations 8 × 15 s, 3 × 60 s, 5 × 120 s, 3 × 300 s, 6 × 600 s). If scans were longer than 90 min, additional 600-s frames were used. The dynamic images were reconstructed using OSEM (2 iterations and 8 subsets) for visualisation purposes. Corrections were applied for attenuation, randoms and scatter.

**Image analysis**

Attenuation correction CT (AC-CT) was used to semi-automatically delineate the left and right lungs. Then, a manual QC of each lung mask was performed: starting from one axial extremity, masks were displayed overlaid with the AC-CT image and non-lung labelled regions were manually removed (e.g. trachea). Morphological operations were applied to both lungs to remove 5 mm of the border to minimise any spillover of radioactivity from adjacent tissues. Finally, a correction was performed to remove potential breathing artefacts at the base of the lung and to avoid any radioactivity spillover from the liver.

Tissue fraction (TF) correction uses the low-dose attenuation correction CT scan to calculate the fraction of the lung volume that is air rather than tissue/blood [26]. As air cannot contain any radioligand, correcting for the tissue fraction yields outcome parameters that better reflect the tissue-specific uptake. The TF represents the amount of tissue remaining after the volume of air has been removed.

Time activity curves (TACs) were extracted from the dynamic [18F]FB-A20FMDV2 PET images for the left lung, right lung and total lungs (left + right lung). Static (SUV) and dynamic (VT) outcome parameters (described below) were derived from these TACs and the input function. Subsequently, SUV and VT were corrected for tissue fraction by dividing the outcome parameter by the TF.

**Volume of distribution and standard uptake values**

PET emission data and blood radioactivity data were collected for each scan and fitted to a variety of kinetic models to determine the optimal model to quantify the data. A physiological parameter of interest (VT; volume of distribution) was calculated for the tissue of interest.

VT is a measure of the total (i.e. both non-displaceable and specific binding) distribution of the radioligand into the tissue [27] and is equivalent to an equilibrium partition coefficient. The one-tissue compartment (TC) model was selected via Akaike Information Criterion (AIC) as the most appropriate model [28]. MIKAT fits the one TC model to the data using non-linear optimisation techniques to estimate the individual rate constants (K1, k2), then calculates VT from these rate constants as,

\[
VT = \frac{K_1}{k_2}
\]
In addition to $V_T$, the model also estimates the volume of blood ($V_B$) in the region of interest.

**Statistical analyses**

This was an exploratory study and no formal statistical hypotheses were tested. Instead, estimation approaches were used to obtain mean values (including 95% confidence intervals) for the output parameters (i.e. $V_T$ and SUV), following administration of $[^{18}F]$FB-A20FMDV2 to healthy, IPF or CTD-ILD participants, and test-retest variability was assessed. SUV values reported are averaged over 10 to 30 min post injection of the tracer.

Statistical analysis of $V_T$ and SUV using data from healthy participants and those with IPF was performed to estimate the difference in $\alpha v \beta 6$ binding in both the combined lungs of IPF participants compared with healthy participants.

A linear mixed model with a subject random effect was fitted to the data with $V_T$ or SUV as the response and visit (scan 1 and scan 2) and group (IPF and healthy) as the independent variables with and without tissue fraction correction. The probability ratio > 1 and probability ratio > 2 were also computed using the calculated $t$ statistic and Kenward-Roger degrees of freedom [29] from the model.

When test-retest was performed, individual test-retest variability of the binding output parameters (difference scan 2 – scan 1 divided by the mean of scan 1 and scan 2, expressed as a percentage) in IPF patients was calculated for each tissue of interest. Using these data, a Bland-Altman plot of difference scan 2 – scan 1 against (mean of scan 1 and scan 2) was produced for each tissue of interest, and limits of agreement were computed. Estimates (95% CI) for within and between-subject variability in IPF patients were obtained from a mixed model fitted separately for each tissue of interest, including scan occasion as a fixed effect and subject as a random effect.

No statistical analysis was performed to compare data from healthy participants to subjects with CTD-ILD due to the small number of participants with this diagnosis recruited in the study.

Safety data was listed and summarised descriptively. No formal statistical analysis of safety data was conducted.

### Results

#### Participants

A total of 15 participants (6 healthy, 7 with a diagnosis of IPF and 2 with a diagnosis of CTD-ILD) were enrolled and completed the study (Table 3). Of the two participants with CTD-ILD, one had RA and the other SSc. None of the subjects was withdrawn from the study. Seven participants with IPF were sufficient to achieve a precision of < 50% for the parameters of interest $V_T$ and/or SUV and therefore recruitment was stopped. The small number of participants with CTD-ILD was due to recruitment being stopped after the study had achieved its primary endpoint in IPF subjects.

Use of prior and concomitant medications was reported in all 7 subjects with IPF and 2 subjects with CTD-ILD. Use of prior and concomitant medications was as expected in these disease populations. Four subjects with IPF were taking pirfenidone and two subjects were taking glucocorticoids (one subject with IPF for comorbid shingles and one subject for CTD).

#### Clinical safety and tolerability

A total of 10 subjects out of 15 (75%) experienced AEs during the study (Table 4); none of which led to withdrawal. The most frequently reported AE was pain in extremities, which was reported in 4 subjects (27%), probably related to the arterial cannulation procedure. Each of the remaining AEs was reported in a single subject. All AEs were mild or moderate in intensity and had resolved by the follow-up visit. None of the AEs was considered by the investigator to be related to the test material (PET radioligand) administration. There were no deaths or SAEs.

#### Immunogenicity

Immunogenicity was assessed by the presence of antibodies to A20FMDV2. Antibodies were observed in 3 subjects. One healthy participant had detectable antibodies prior to scanning and at follow-up. Two IPF participants had detectable

| Demographics | Healthy (N = 6) | IPF (N = 7) | CTD-ILD (N = 2) |
|--------------|----------------|------------|----------------|
| Age in years (mean (SD)) | 61.2 (9.22) | 70.6 (5.65) | 63.5 (3.54) |
| Sex (%): Female/Male | 3 (50)/3 (50) | 1 (14)/6 (86) | 1 (50)/1 (50) |
| BMI (kg/m²) (mean (SD)) | 25.35 (2.91) | 24.81 (3.26) | 30.70 (3.39) |
| Height (cm) (mean (SD)) | 171.5 (8.38) | 175.7 (7.48) | 168.0 (15.56) |
| Weight (kg) (mean (SD)) | 74.7 (10.7) | 76.6 (10.6) | 87.9 (25.6) |
antibodies prior to any scanning procedures, between the two scans and at follow-up. These data may imply previous exposure to FMDV2 and the presence of a cross-reacting antibody in subjects with IPF. All results were negative or very low (<2 titre) on re-testing.

Since all results were observed prior to scanning as well as at follow-up, and all results remained as low titres during the study, none of these results was considered clinically significant. Antibodies were not detected in any other subject.

**PET imaging**

Human whole body dosimetry and pharmacokinetics of \([^{18}F]FB-A20FMDV2\) are reported elsewhere [20].

Dynamic \([^{18}F]FB-A20FMDV2\) PET images were successfully acquired for six healthy participants (scan duration 56–240 min), seven IPF participants (dynamic test-retest scans of 90 min in duration) and two CTD-ILD participants (one had a single dynamic scan of 90 min in duration and the other had dynamic test-retest scans of 90 min each in duration). Injected dose (MBq), molar activity at injection time (GBq/μmol), injected mass (μg) and type of blood sampling (arterial, venous or both) are tabulated for each participant (Table 5). Due to variations in radiochemical yield, a lower mass of radioligand and/or lower radioactive dose was used for some scans. However, the administered mass of radiotracer was always sufficiently small to ensure it was at tracer concentrations, and good quality image, blood and metabolite data were acquired for all acquisitions. A qualitative assessment showed that uptake of \([^{18}F]FB-A20FMDV2\) is low in lung tissue with a normal appearance on CT and uptake is increased in fibrotic regions as observed on CT image. Images from a representative participant with IPF are presented (Fig. 1).

Following intravenous administration, \([^{18}F]FB-A20FMDV2\) was rapidly metabolised with 5% of parent radioactivity remaining in plasma approximately 30 min post injection.

**Time activity curves**

Dynamic PET images were used to obtain TAC for the whole lung (Fig. 2). Subjects with IPF had higher uptake than healthy subjects using both uncorrected data (Fig. 2a) and tissue fraction–corrected data (Fig. 2b).

**Volume of distribution**

Geometric mean values (95% CI of geomean) for volume of distribution \((V_T)\) without tissue fraction correction were 0.88 (0.60, 1.29) mL/cm\(^3\) for healthy participants, and 1.40 (1.22, 1.61) mL/cm\(^3\) for subjects with IPF (Fig. 3a). Geometric mean values for \(V_T\) with tissue fraction correction were 2.98 (2.17, 4.09) mL/cm\(^3\) for healthy participants and 3.49 (3.11, 3.91) mL/cm\(^3\) for participants with IPF (Fig. 3b). \(V_T\) was higher in subjects with IPF compared with healthy subjects, regardless of tissue fraction correction (Fig. 3a and b).

**Standardised uptake value**

Geometric mean values for SUV without tissue fraction correction were 0.54 (0.36, 0.81) g/mL for healthy subjects and 1.03 (0.86, 1.22) g/mL for subjects with IPF (Fig. 3c). Geometric mean values for SUV with tissue fraction correction were 1.82 (1.40, 2.36) g/mL for healthy subjects and 2.56 (2.22, 2.97) g/mL for subjects with IPF (Fig. 3d).
**Analysis of left and right lung separately**

VT and SUV were higher in participants with IPF when measured in the whole lung as well as in the individual left and right lungs (Fig. 3).

**VT and SUV changes in participants with IPF vs. healthy participants**

Geometric means and adjusted IPF/healthy ratio of VT and SUV of [18F]FB-A20FMDV2 in lungs of healthy and IPF participants without (Fig. 4a and c respectively) and with (Fig. 4b and d respectively) tissue fraction correction are illustrated.

Without tissue fraction correction, the adjusted geometric mean VT of the IPF/healthy ratio (95% CI of ratio) estimated from the model was reported as 1.59 (1.09, 2.32) and was considered statistically significant at the 5% level. The probability ratio > 1 = 0.988 and probability ratio > 2 = 0.096 was also computed.

Without tissue fraction correction, the adjusted geometric mean SUV of the IPF/healthy ratio (95% CI of ratio) was reported as 1.91 (1.27, 2.87) and was considered statistically significant at the 5% level (probability ratio > 1 = 0.996, probability ratio > 2 = 0.398). With tissue fraction correction, the adjusted geometric mean SUV of the IPF/healthy ratio (95% CI of ratio) was reported as 1.17 (0.85, 1.61) and was not considered statistically significant at the 5% level. The probability ratio > 1 = 0.988 and probability ratio > 2 = 0.096 were also computed.

One of the healthy participants had VT and SUV uptake values that were higher than the other healthy subjects and within the observed range for IPF participants. Whilst we did not acquire diagnostic quality HRCT images for the healthy participants, further examination of this participant’s low dose AC-CT showed diffuse reticular shadowing consistent with interstitial lung disease. The participant was referred for appropriate clinical assessment and follow-up.

### Table 5 PET scan acquisition data including injected dose (MBq), molar activity (Aₘ) at injection time (GBq/μmol), injected mass (μg) and type of blood sampling (arterial, venous or both)

| Sample   | Injected dose (MBq) | Aₘ @ injection (GBq/μmol) | Injected mass (μg) | Blood samples |
|----------|---------------------|---------------------------|--------------------|---------------|
| Healthy  |                     |                           |                    |               |
| 201      | 130.8               | 31.6                      | 9.45               | Arterial      |
| 202      | 160.9               | 16.9                      | 21.7               | Arterial      |
| 203      | 156.7               | 25.2                      | 14.2               | Arterial      |
| 204      | 146.4               | 20.7                      | 16.1               | Arterial      |
| 205      | 77.70               | 24.0                      | 7.40               | Arterial      |
| 206      | 90.50               | 12.0                      | 17.2               | Arterial      |
| IPF      |                     |                           |                    |               |
| 301      | 121.0               | 23.2                      | 11.9               | Arterial      |
| 301      | 33.94               | 60.1                      | 1.29               | Arterial      |
| 302      | 142.8               | 27.4                      | 11.9               | Venous        |
| 302      | 135.7               | 26.4                      | 11.8               | Venous        |
| 303      | 130.3               | 26.5                      | 11.2               | Venous        |
| 303      | 134.9               | 16.2                      | 19.1               | Venous        |
| 304      | 78.51               | 78.5                      | 2.29               | Arterial      |
| 304      | 87.27               | 31.0                      | 6.43               | Arterial and venous |
| 305      | 146.5               | 21.0                      | 15.9               | Arterial      |
| 305      | 94.38               | 18.4                      | 11.7               | Arterial and venous |
| 306      | 83.41               | 29.9                      | 6.38               | Arterial and venous |
| 306      | 125.4               | 116                       | 2.47               | Arterial      |
| 307      | 96.87               | 16.0                      | 13.8               | Venous        |
| 307      | 157.7               | 25.8                      | 14.0               | Venous        |
| CTD-ILD  |                     |                           |                    |               |
| 401      | 113.9               | 24.5                      | 10.7               | Venous        |
| 401      | 124.8               | 20.0                      | 14.3               | Venous        |
| 402      | 129.0               | 14.1                      | 20.9               | Venous        |
V_T and SUV test/re-test variability within participants with IPF

Figure 5 presents Bland-Altman plots of V_T and SUV to assess reproducibility of scans in both lungs combined for [18F]FB-A20FMDV2 in participants with IPF. The mean difference of the V_T and SUV (scan 2 – scan 1) was in the range of 0 to 0.3 and 0 to 0.1 respectively, with and without tissue fraction correction. The geometric mean ratio of the V_T and SUV (scan 2/scan 1) with and without tissue fraction correction were not statistically significantly different from 1 at the 5% level.

Uptake in lungs of CTD-ILD

Only 2 participants with a diagnosis of CTD-ILD were recruited. The participant with RA-ILD had only 1 scan, and the participant with SSc-ILD had 2 scans. V_T and SUV for SSc-ILD were at the lower end of the range for both scans (SUV scan 1 = 0.42 g/mL and SUV scan 2 = 0.65 g/mL; V_T scan 1 = 0.56 and V_T scan 2 = 1.06). It may be significant that the HRCT of this participant with SSc-ILD had mild fibrotic changes in the lung bases posteriorly and very mild changes anteriorly. For RA-ILD, V_T and SUV were at the higher end of the range for scan 1 (SUV scan 1 = 1.40 mL/cm^3 and V_T scan 1 = 1.51 mL/cm^3). This participant with RA-ILD had severe fibrotic changes in all lobes on HRCT.

Discussion

[18F]FB-A20FMDV2 is a potent, selective ligand for integrin αvβ6, as shown by in vitro cell-free and cellular binding assays [18, 30, 31] and by in vivo animal models of cancer [25, 32] and pulmonary fibrosis [33, 21]. In addition, it has been tested in a first dose in human study of the safety, tolerability, biodistribution, radio-dosimetry and immunogenicity of [18F]FB-A20FMDV2 [20]. Visual inspection of these PET images from healthy humans suggested that uptake of radioactivity was observed in the thyroid, salivary glands, liver, stomach wall, spleen, ureters and bladder [20]. Immunohistochemistry and SPECT imaging of healthy rodent tissues revealed that αvβ6 is
expressed constitutively in the epithelium of the gall bladder, stomach wall, duodenum, ileum, colon, and at lower levels in the lungs and skin [34, 35]; largely supporting the observed expression in healthy humans [20].

In the current clinical study, [18F]FB-A20FMDV2 PET/CT was used to selectively image and quantify \( \alpha_v \beta_6 \) integrin in the lungs of healthy individuals and those with established fibrotic lung disease. As anticipated based on published immunohistochemistry of lung biopsies from subjects with IPF [14], we demonstrate increased levels of \( \alpha_v \beta_6 \) integrin expression in the lungs of subjects with IPF compared with lungs of healthy subjects. This increased expression of \( \alpha_v \beta_6 \) integrin was generally observed in fibrotic regions of the lung. The \([18F]FB-A20FMDV2\) signal was shown to be reproducible over a 2-week period, indicating its potential as a measure of target engagement in early phase drug development studies.

Although \( \alpha_v \beta_6 \) integrins are induced on epithelial cells in response to damage, detectable background levels of expression were observed in healthy human lungs, as previously described in mice [21]. In one healthy, clinically asymptomatic participant however, an unexpectedly high PET signal was observed that, together with the observation of possible reticular shadowing on the AC-CT, suggested further investigation and assessment of possible lung pathology. The
identification of asymptomatic interstitial lung abnormalities (ILAs) in screened healthy populations is an emerging problem in clinical trials and a proportion of these cases may progress towards IPF [36]. It is possible that identification of pathogenic pathways such as increased levels of the αvβ6 integrin may help determine the natural history of this condition and although no overt disease was observed in this volunteer, the identification of radiological and molecular abnormalities would suggest continued careful follow-up is indicated. No further information on this participant is currently available.

Comparison of lung-based PET signals between healthy and pulmonary fibrosis subjects is complicated by regional differences in air and tissue fraction due to the fibrosis. Tissue fraction (TF) correction is used to define the volume of air (which cannot contain any radioligand) in the lung using CT scan and removes this contribution from the analysis. The lungs of IPF subjects have a smaller air fraction than healthy subjects especially in the fibrotic regions; hence, an increased PET signal could be observed due to the density change even in the absence of altered tissue uptake [37]. In this study, even after TF correction, a smaller, more variable, but still significant increase in the SUV was observed in pulmonary fibrosis. The kinetic modelling required to derive $V_T$ also increases the variability, and these two sources of increased variability together are likely to explain why the difference in tissue-fraction-corrected $V_T$ between fibrotic and healthy lungs did not meet the criterion for significance. On the other hand, the test-retest reproducibility would suggest that TF correction is not required in short-term studies using within-subject comparisons, because tissue density is unlikely to change over a few weeks.

Individuals with usual interstitial pneumonia (UIP) pattern on HRCT and an underlying diagnosis of connective tissue disease do not have IPF. However, due to the similar HRCT pattern and underlying histopathology of UIP, there may be similarities in the underlying mechanisms of fibrosis. We show here that αvβ6 integrin expression can be measured by PET scan in these subjects and is generally localised to the fibrotic regions. As only two subjects with CTD-ILD were included in the study, it is not possible to robustly compare PET signals between CTD-ILD and IPF. Of the two CTD-ILD subjects, the one with SSc-ILD had relatively low levels of

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**Fig. 4** Geometric means and adjusted IPF/healthy ratio of $V_T$ and SUV of $[^{18}F]$FB-A20FMDV2 in lungs of healthy and IPF participants. Data from individual participants is presented (scan 1 from healthy participants numbers 201–206, scans 1 and 2 from IPF participants numbers 301–307) in black and CTD-ILD participants numbers 401 and 402 in red). a, b Geometric mean $V_T$ and 95% CI are illustrated using the left-hand axes. c, d Geometric mean SUV and 95% CI are illustrated using the left-hand axes. Adjusted IPF/healthy ratio and 95% CI are shown on the right-hand axes in blue. a, c Not corrected for tissue fraction. b, d Corrected for tissue fraction. Note that the data from CTD-ILD participants did not contribute to the calculation of the geometric means or the adjusted IPF/Healthy ratio.
αvβ6 expression whilst the subject with RA-ILD had relatively high levels. These differences may be due to sampling bias in such a small sample, may be related to the observed extent of fibrotic disease in the lungs on HRCT, or to differing mechanisms of fibrosis between SSc and RA.

Other PET ligands are under development to quantify expression levels of the integrin αvβ6. Cystine knot peptides have been conjugated with 18F-fluorobenzoate, injected into mice xenografted with αvβ6-positive or αvβ6-negative pancreatic tumours and PET scans conducted [38]. Target specificity was confirmed by low tumour uptake in αvβ6-negative tumours compared with high uptake in αvβ6-positive tumours. These cystine knot peptide tracers show translational promise for molecular imaging of integrin αvβ6 expression in pancreatic and other cancers [38]. In fact, [18F]FP-R01-MG-F2, an αvβ6-specific cysteine knot peptide is under development in a clinical study in 10 subjects with IPF (NCT03183570). It is currently premature to determine the relative benefit of each of the αvβ6 integrin PET ligands that are under development, but emerging data may provide insight into the optimal use of these tools.

In conclusion, we have developed a non-invasive imaging technique using [18F]FB-A20FMDV2 to quantify expression of αvβ6 integrin in the lungs of subjects with fibrotic interstitial lung disease. It is likely that PET approaches such as this will have far-reaching implications in the development of novel integrin targeting therapies for fibrotic diseases.

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Data sharing Anonymised individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.
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Compliance with ethical standards

Conflict of interest  This study was sponsored by GlaxoSmithKline R&D (study numbers 200262/NCT02612051). GlaxoSmithKline reviewed the manuscript for factual accuracy.

FJW is an employee and shareholder of GSK. He was previously a consultant to ECNP R&S, GlaxoSmithKline, IPPEC, King’s College London, Lundbeck A/S, Mentis Cura ehh and Pfizer Inc. and has received travel expenses as a guest speaker from Orion Pharma Ltd.

PTL was a GlaxoSmithKline employee at the time of the study and is currently a shareholder. She now works or has worked as an independent consultant to GlaxoSmithKline R&D, the Francis Crick Institute, Galecto, Mereo BioPharma, Peptinnovate, BerGenBio, LifT BioSciences, Afortiori, Morphic Therapeutics.

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ARK is an employee of GlaxoSmithKline. WAF, CP, NG, SK are employees of GlaxoSmithKline and hold shares. MC and RPM were employees of GlaxoSmithKline at the time of the study.

AL, CC, JR and MO have no conflicts of interest to declare.

RG, JD, SM, AS, JP are employees of Invicro and received funding from GSK for the conduct of this study. RG has also received consultant fees from AbbVie, Biogen and Cervical. AS holds GSK shares.

Ethical approval  All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent  Informed consent was obtained from all individual participants included in the study.

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