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PII: S0360-3016(20)30184-X

DOI: <https://doi.org/10.1016/j.ijrobp.2020.02.014>

Reference: ROB 26189

To appear in: *International Journal of Radiation Oncology • Biology • Physics*

Received Date: 4 October 2019

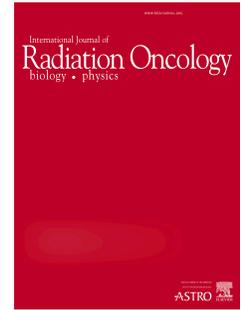
Revised Date: 28 January 2020

Accepted Date: 2 February 2020

Please cite this article as: Saleem A, Helo Y, Win Z, Dale R, Cook J, Searle GE, Wells P, Integrin  $\alpha\beta 6$  positron emission tomography imaging in lung cancer patients treated with pulmonary radiotherapy, *International Journal of Radiation Oncology • Biology • Physics* (2020), doi: <https://doi.org/10.1016/j.ijrobp.2020.02.014>.

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## Integrin $\alpha\beta 6$ positron emission tomography imaging in lung cancer patients treated with pulmonary radiotherapy

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Running title: Integrin imaging in lung cancer

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**Conflict of interest:** None of the authors have any conflict of interest pertaining to this study. GES is an employee and AS, YH are ex-employees of Invicro which funded the study. AS currently holds a consultancy contract with Invicro. AS and GES are ex-employees of GlaxoSmithKline, which sponsored and provided healthy volunteer study data.

**Funding:** This study was funded and sponsored by Invicro, A Konica Minolta Company.

**Acknowledgements:** The authors would like to acknowledge and thank the subjects who took part in the study. We would also like to thank the Rohini Akosa, clinical study co-ordinator, James Davies, PET technologist, Piran Sucindran, Head of Quality assurance and other imaging, clinical, quality assurance staff at Invicro and Oscar Riches and Fatjon Dekaj clinical co-coordinators at St Bartholomew's hospital for their help in conduct of the study. We acknowledge with gratitude GlaxoSmithKline for provision of healthy volunteer imaging data used for analysis in this study.

## Running title: Integrin imaging in lung cancer

### Abstract

**Purpose:** Post radiotherapy (RT) lung fibrosis is a major barrier to improved cure rate in lung cancer. Integrin  $\alpha\beta6$  plays a key role in fibrogenesis by activating transforming growth factor- $\beta$ . Positron emission tomography (PET) studies with a fluorine-18 radiolabelled  $\alpha\beta6$  radioligand, [ $^{18}\text{F}$ ]-FBA-A20FMDV2 were performed to assess uptake and relationship to RT dose parameters explored.

**Methods:** Recently treated non-small cell lung cancer (NSCLC) patients (< 6 months after radiotherapy) had [ $^{18}\text{F}$ ]-FBA-A20FMDV2-PET scans, co-registered with the RT planning CT and segmented to RT doses of > 40 Gy (excluding tumour), 25-40 Gy, 15-25 Gy, 8-15 Gy and < 8 Gy. PET uptake (standardised uptake value; SUV) corrected for tissue density between 10 and 60 minutes ( $\text{SUV}_{10-60}$ ) was calculated and compared with RT dose, dose per fraction, biological effective dose (BED). PET uptake was also evaluated in healthy volunteers (HV).

**Results:** 6 NSCLC (3M; 3 F) subjects scanned between 6 -22 weeks after RT and 6 HVs (3M; 3 F) were evaluated. Higher mean PET uptake ( $\text{SUV}_{10-60}$ ) was observed in the irradiated lung compared to the healthy lung (2.98 vs. 1.99;  $p < 0.05$ ). A significant and positive pharmacodynamic (PD) relationship was observed between radioligand uptake ( $\text{SUV}_{10-60}$ ) and dose per radiotherapy fraction ( $r^2$ : 0.63;  $p < 0.001$ ) and with BED for fibrosis ( $r^2= 0.38$ ;  $p < 0.001$  for  $\alpha/\beta$  3 Gy and  $r^2= 0.33$ ;  $p < 0.001$  for  $\alpha/\beta$  5 Gy).

**Conclusions:** Higher uptake in the irradiated lung and a PD relationship between  $\alpha\beta6$  radioligand uptake versus radiotherapy dose per fraction and BED for lung fibrosis is consistent with RT induced activation of  $\alpha\beta6$  integrin and supports a role for  $\alpha\beta6$  in the induction of lung fibrosis after pulmonary RT.  $\alpha\beta6$ -PET imaging may potentially aid in the assessment and management of radiation induced pulmonary fibrosis.

**Key words:**

$\alpha\beta6$  integrin, Transforming Growth Factor- $\beta$ , PET imaging, Radiation induced lung fibrosis, pulmonary radiotherapy,  $\alpha\beta6$  radioligand

**Summary:**

Radiotherapy (RT) induced lung fibrosis is a major cause of morbidity in cancer survivors and its management an unmet clinical need. Integrin  $\alpha\beta6$  plays a key role in initiation of fibrosis. In this study, we observed PET uptake of the  $\alpha\beta6$  radio-ligand is up regulated after RT and is related to radiation dose per fraction and biological effective dose for fibrosis.  $\alpha\beta6$ -PET imaging could therefore potentially be used as a tool in therapy development and management for RT induced lung fibrosis.

## Introduction

Radiotherapy (RT) induced lung fibrosis is a chronic side effect and an important cause of morbidity (1). However, diagnosis remains a challenge, with post-RT lung fibrosis diagnosed by a combination of symptoms, lung function tests and visual assessment of density changes on CT scans and once definitive CT changes are observed, treatment options are limited. There is therefore an unmet need for early detection and treatment of RT induced lung fibrosis.

Transforming growth factor (TGF)- $\beta$  plays a key role in the initiation and maintenance of fibrosis (2) and highly expressed in lungs of patients with idiopathic pulmonary fibrosis (IPF) (3). TGF- $\beta$  is released in an inactive form and activated by tissue-restricted activators such as the membrane protein, integrin  $\alpha\beta 6$ , which is constitutively bound to TGF- $\beta$  (4, 5).  $\alpha\beta 6$  plays an integral role in the fibrogenic pathway, as mice with impaired TGF- $\beta$  signalling and  $\alpha\beta 6$  null mice do not develop bleomycin-induced lung fibrosis (5-7). In normal healthy lung, there is very low level of  $\alpha\beta 6$  expression and there is up regulation of  $\alpha\beta 6$  after lung injury and RT (8, 9).

A 20 amino acid peptide sequence contained in the envelope protein of foot and mouth disease virus that binds to  $\alpha\beta 6$  integrin with high affinity and selectivity has been radio-labelled with fluorine-18 ( $[^{18}\text{F}]$ -FBA-A20FMDV2 (Cancer Research Technology UK patented);  $[^{18}\text{F}]$ IMAFIB) and positron emission tomography (PET) clinical studies performed to quantify radioligand uptake. Higher uptake of  $\alpha\beta 6$  integrin radioligand was observed in patients with IPF compared to healthy volunteers (HVs) (10) and radioligand uptake as a measure of target engagement evaluated in another study with an IPF-targeting drug asset (NCT03069989).

In this pilot study, we evaluated  $\alpha\beta 6$  expression in the irradiated and healthy lung by assessing the uptake of  $[^{18}\text{F}]$ -FBA-A20FMDV2 with PET in HVs, Non-small cell cancer (NSCLC) patients treated with pulmonary RT and explored the potential relationship between RT parameters and radioligand uptake.

## Methods

The study was approved by a UK National Research Ethics Committee. All participants provided written informed consent prior to participate. Six NSCLC subjects underwent PET-CT scans between 6 and 22 weeks after completion of RT. We were unable to recruit the 6 RT-naïve NSCLC subjects initially planned due to the limited time available to schedule the PET scan before start of their RT. We therefore compared post-RT PET data with anonymised PET data from 6 HVs from another study with the same radioligand (10) kindly provided to us by that study sponsor.

Detailed information on methods is provided in Supplementary material. Briefly, a low-dose CT scan of the chest was performed to estimate tissue attenuation (AC-CT) followed by PET after the intravenous administration of [<sup>18</sup>F]-FBA-A20FMDV2, radiosynthesised to good manufacturing practice, as described previously (11). All but 2 NSCLC subjects were unable to tolerate to the full duration of the scan were scanned for 60 minutes. Although HV data was available for 90 minutes, PET data for 60 minutes was only used for analysis.

PET-CT and RT planning (RTP) images were converted to NIfTI (Neuroimaging Informatics Technology Initiative) format and re-sliced to match the PET image resolution (Figure 1). The lung was segmented using known Hounsfield unit (HU) values and regions of interest (ROIs) outlined on the images for RT dose windows > 40 Gy (excluding tumour), 25-40 Gy, 15-25 Gy, 8-15 Gy and < 8 Gy. In HVs, the whole lung was considered as single ROI. Time versus radioactivity uptake curves (TACs) were generated and the mean standardised uptake value of the radioligand between 10 and 60 minutes normalised for the administered radioactivity and corrected for subject body weight ( $SUV_{10-60}$ ; g/ml) calculated. Uptake ( $SUV_{10-60}$ ) was also calculated after tissue density correction was made to exclude air within the ROI, as described previously (12, 13). Biological effective doses (BED) for lung fibrosis using the linear quadratic (LQ) cell survival radiobiology model typically used for conventional radiotherapy fractionation were calculated (14, 15) using  $\alpha/\beta$  values of both 5 Gy and 3 Gy (14, 16, 17). Scatter plot and regression co-efficient ( $r^2$ ) and significance values calculated. The

extent and location of fibrotic changes on the 6 month post-RT CT scans was visually evaluated by a radiologist.

## Results

In contrast to similar TAC profiles observed in non-tumorous irradiated lung (Figure 2a) and HV lung (Figure 2b), TACs for tumours visualised in 4 of the 6 subjects' post-RT (supplementary figure S1) were different demonstrating variable uptake. Higher (mean  $SUV_{10-60}$  (SD)) uptake was observed in irradiated lung (exclusive of tumour) compared to the healthy lung both with tissue density correction (2.97 (0.86) versus 1.99 (0.45);  $p < 0.05$ ) and without tissue correction (0.93 (0.6) versus 0.56 (0.21);  $p < 0.05$ ).

Evaluation of uptake in irradiated tumour bearing lung (ipsilateral) exclusive of tumour and non-tumour bearing lung (contralateral) and did not reveal any difference in uptake between the lungs receiving the same radiation dose (Figure 3), ruling out potential local paracrine effects that may result in higher expression of integrin in ipsilateral lung or in close proximity to the tumour. PET uptake in healthy lung was the least confirming a RT dose versus uptake gradient (Figure 3), suggesting up-regulation in  $\alpha\beta6$  expression with radiation dose. We also ruled out an irradiated volume effect by not finding any relationship between the PTV and radioligand uptake.

Although a pharmacodynamic (PD) relationship between PET uptake and the RT dose was observed in most subjects (Figure 4), this correlative trend did not reach statistical significance when (figures 5a) when group data was evaluated (Figure 5 a). By contrast, we observed a statistically significant relationship between the dose per fraction and radioligand uptake in irradiated lung ( $r^2 = 0.63$ ;  $p < 0.001$ ; figure 5b); this relationship was not as a result of the single SBRT subject as the significant relationship was maintained (Supplementary figure 2) even after exclusion of the SBRT subject. A moderate but significant correlation ( $p < 0.001$ ) was also observed between PET radioligand uptake and the BED indicative of fibrosis calculated using  $\alpha/\beta$  values of 3 Gy and 5 Gy (Figures 5 and 5d).

Overall the correlation between radiation parameters and PET uptake in lung was not altered irrespective of lung tissue density correction (figure 5 and supplementary figure S3).

On visual evaluation of the 6 month post-RT CT scan, the area with the most prominent fibrotic changes was in close proximity to the tumours in the region that received the highest RT dose. We did not observe CT fibrotic changes in regions away from the tumour regions and unlike PET, a dose-dependent gradient of fibrotic changes was not observed on CT (supplementary figure S4).

## Discussion

We evaluated [<sup>18</sup>F]-FBA-A20FMDV2-PET to assess the expression of  $\alpha\beta6$  and observed higher uptake of the  $\alpha\beta6$  radioligand in the irradiated lung compared to the un-irradiated lung, consistent with tissue immunohistochemistry data that shows up regulation of  $\alpha\beta6$  in irradiated lung (9). Increased radioligand uptake observed in the irradiated lung, irrespective of RT dose implies the absence of radiation dose threshold for  $\alpha\beta6$  up regulation and therefore RT is likely to trigger a profibrogenic cascade by activation of TGF- $\beta$ . However, lack of generalised CT fibrotic changes at 6 months may reflect the inability of CT to detect subtle changes, variability in the time course or reversibility of the fibrogenic processes perhaps due to intrinsic and extrinsic factors etc that are beyond the scope of this study.

We have also for the first time shown a linear PD relationship between  $\alpha\beta6$  radioligand uptake versus dose per fraction and BED for pulmonary fibrosis in the irradiated lung and was not confounded by the single SBRT subject in our study cohort. We however did not observe a PD relationship between total dose and radioligand uptake, which suggests that the dose per fraction may play a significant role in the up regulation of  $\alpha\beta6$ . The greater weight apportioned to dose per fraction in the quadratic component of the LQ cell survival model is also reflected as a PD relationship between radioligand uptake and BED (3Gy and 5Gy) for fibrosis calculated in this study (18).

Although a majority of tumours showed higher uptake in keeping with higher  $\alpha\beta6$  expression in epithelial tumours (19) and its role in oncogenesis, uptake was variable and was not observed in a third of tumours after RT; this may either be due to lack of  $\alpha\beta6$  expression in those tumours or a post-RT decrease in tumour integrin expression. However, these deductions are speculative and our inability to image RT-naive NSCLC subjects and the absence of tissue data was a limitation.

We acknowledge other study limitations including limited subject number, heterogeneity in RT dose and post-RT scan time. However, the numbers included were pragmatic and based on experience from previous such PET studies where reasonable conclusions have been made (10, 20). In our view, it is unethical to put many more subjects through procedures that do not result in any benefit for them in such hypothesis generating pilot studies. Any further PET studies with the radioligand as a read out of target engagement should be performed within an interventional study and any further methodological issues addressed then, if necessary (10).

In conclusion, the ability to image  $\alpha\beta6$  expression using PET and the observation of a RT-PET uptake PD relationship has the potential to aid development of therapy targeting TGF- $\beta$   $\alpha\beta6$  fibrogenic cascade.

## Figure legends:

**Figure 1:** Flow chart (figure 1a) and images (figure 1b) depicting the data analysis methodology that was followed to evaluate PET uptake and relate it to the radiotherapy doses received by the lung obtained from the radiotherapy planning scans. In subjects who received radiotherapy regions of interest in the lungs of each subject were defined based on the radiotherapy doses received. PET uptake values for all of lung were considered as a single region of interest in healthy volunteers for each subject.

**Figure 2:** Time versus radioactivity uptake curves for healthy subjects (a) and irradiated lung exclusive of tumour (b) showed similar profiles. On visualisation, this is suggestive of a higher uptake in the irradiated lung.

**Figure 3:** PET uptake in lung versus radiation doses in subjects who were treated with radiotherapy and in un-irradiated lung (dose is given here as 0 Gy) shows a dose-uptake gradient with healthy volunteers demonstrating least radioligand uptake. The plot also shows no difference in PET radioligand uptake in the ipsilateral lung (tumour bearing lung) exclusive of tumour and the contralateral lung (non-tumour bearing lung).

**Figure 4:** Individual subject plots showing the relationship between PET uptake versus radiotherapy dose without (a) and with (b) tissue density correction. In 5 out of 6 subjects a significant relationship was observed with tissue density correction.

**Figure 5:** PET uptake in irradiated lung exclusive of tumour corrected for tissue density versus radiotherapy dose (a), dose per fraction (b), biologically effective doses for fibrosis using  $\alpha/\beta$  of 3Gy (c) and 5Gy (d). A significant relationship between PET uptake and dose per fraction and BED for fibrosis but not with total radiation dose was observed.

**Supplementary figure S1:** Tumours visualised in the PET-CT scans in 4 of the 6 subjects after completion of their RT are illustrated in the top panel and defined as ROIs in red and the non-tumour containing lung ROIs defined in blue. The corresponding TACs illustrated in the lower panel are illustrated in red and blue respectively and both different profiles.

**Supplementary figure S2:** Relationship between PET uptake and radiotherapy dose per fraction showed a significant correlation was maintained when the subject who received SBRT (subject 11) was excluded. The left plot was without tissue density correction and the right plot with tissue density correction.

**Supplementary figure S3:** PET uptake in lung (without issue density correction) versus radiation doses in the subjects who were treated with radiotherapy and in un-irradiated lung shows a dose-uptake gradient with healthy volunteers demonstrating least radioligand uptake. The plot also shows no difference in PET radioligand uptake in the lung in which the tumour was located (ipsilateral lung exclusive of tumour) and the contralateral lung in which did not bear the tumour. Corresponding figures with tissue density correction are shown in figure 5.

**Supplementary figure S4:** Diagnostic CT scan for a subject (subject 17) performed 6 months after completion of RT demonstrating fibrotic changes (shown as green in right panel) in close proximity to the tumours and in the region that received the highest radiotherapy dose.

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## Tables

Table 1:

| Subj. No                        | Age, Gender | Tumour location  | RT Dose, Treatment regimen | Dose per fraction (Gy) | Interval between RT completion and PET (weeks) | Activity injected (MBq) |
|---------------------------------|-------------|------------------|----------------------------|------------------------|--|-------------------------|
| <b>POST-RADIOTHERAPY COHORT</b> |             |                  |                            |                        |  |                         |
| 11                              | 66, F       | Right lower lobe | 55 Gy/5 #, SBRT*           | 11                     | 8  | 40.48                   |
| 12                              | 56, F       | Left lower lobe  | 64 Gy/32 #, EBRT*          | 2                      | 22   | 8.86                    |
| 16                              | 51, F       | Right Upper lobe | 45 Gy/15#, EBRT (CRT*)     | 3                      | 13   | 15.97                   |
| 17                              | 75, M       | Right Upper lobe | 55 Gy/20 #, EBRT           | 2.75                   | 6  | 62.51                   |
| 20                              | 70, M       | Left Upper lobe  | 64 Gy/32 #EBRT             | 2                      | 7  | 22.83                   |
| 21                              | 60, M       | Right Upper lobe | 64 Gy/32 #, EBRT (CRT)     | 2                      | 14   | 122                     |
| <b>HEALTHY VOLUNTEER COHORT</b> |             |                  |                            |                        |  |                         |
| 1                               | 48, M       | NA               | NA                         | NA                     | NA   | 130.8                   |
| 2                               | 75, M       | NA               | NA                         | NA                     | NA   | 160.9                   |
| 3                               | 57, F       | NA               | NA                         | NA                     | NA   | 156.7                   |
| 4                               | 65, F       | NA               | NA                         | NA                     | NA   | 146.4                   |
| 5                               | 67, M       | NA               | NA                         | NA                     | NA   | 77.70                   |
| 6                               | 61, F       | NA               | NA                         | NA                     | NA   | 90.50                   |

Table 1: Subject demographics, radiation fractionation details and administered radioactivity. EBRT: External beam radiotherapy. SBRT: Stereotactic radiotherapy. CRT: Chemo-radiotherapy

Figure 1

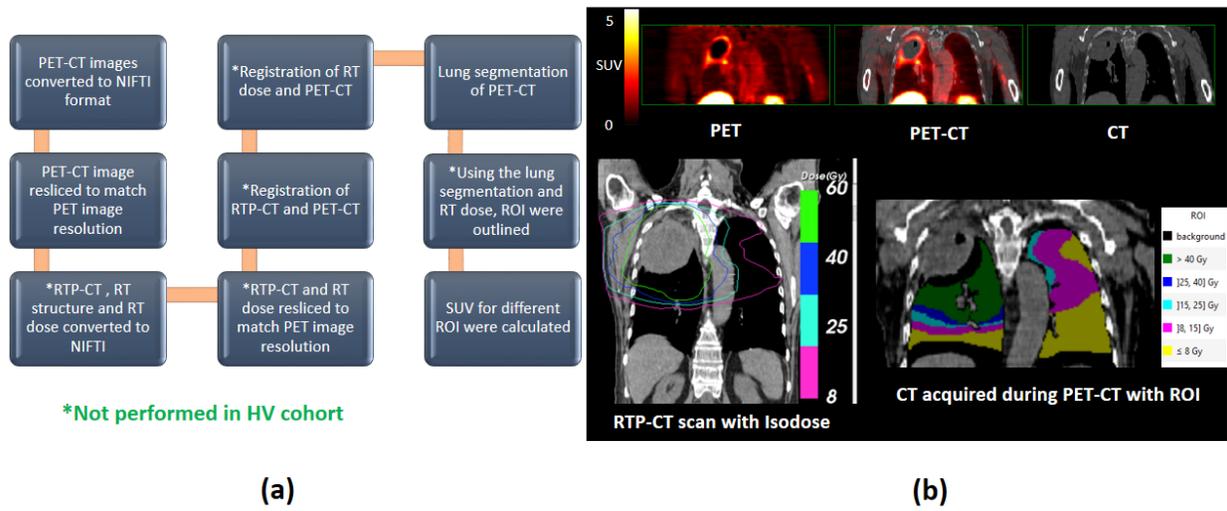


Figure 2

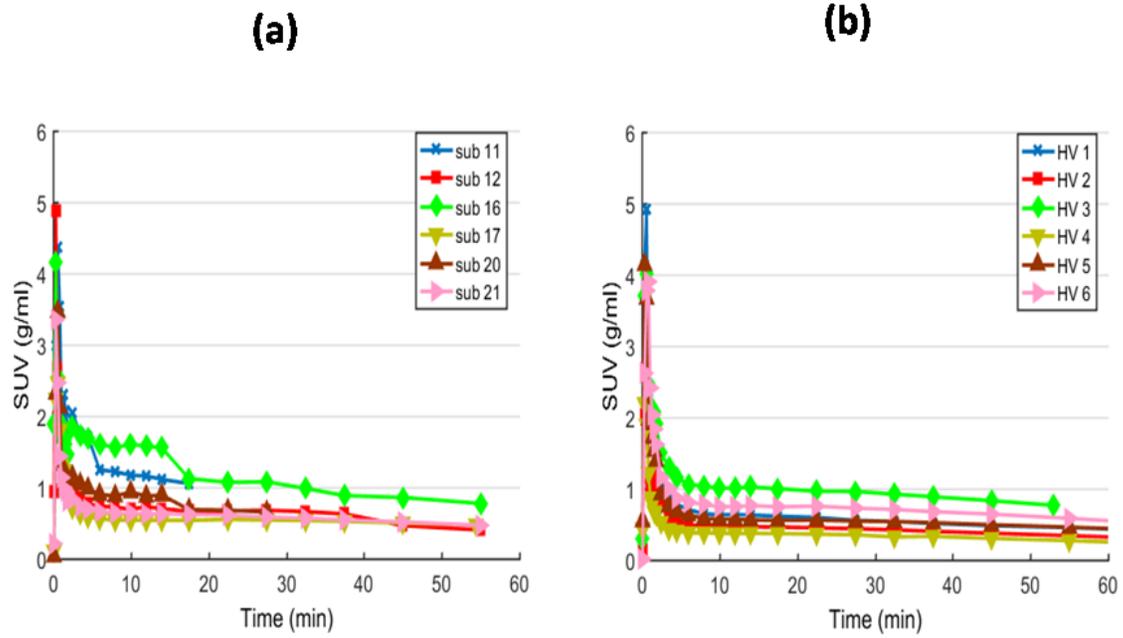


Figure 3

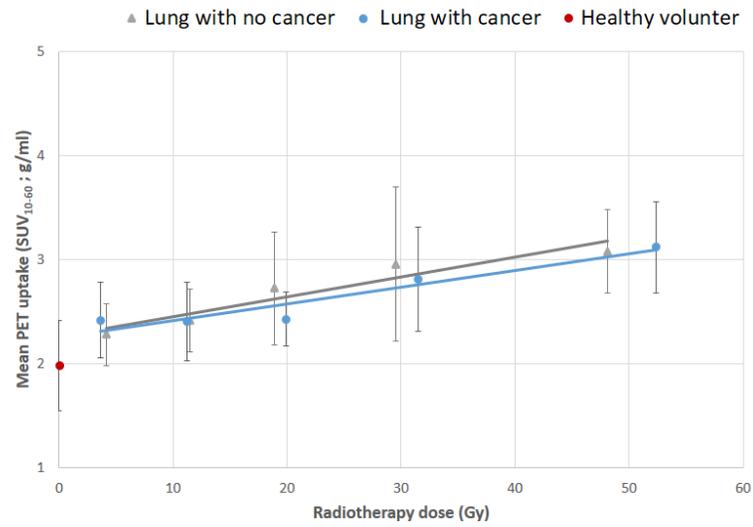
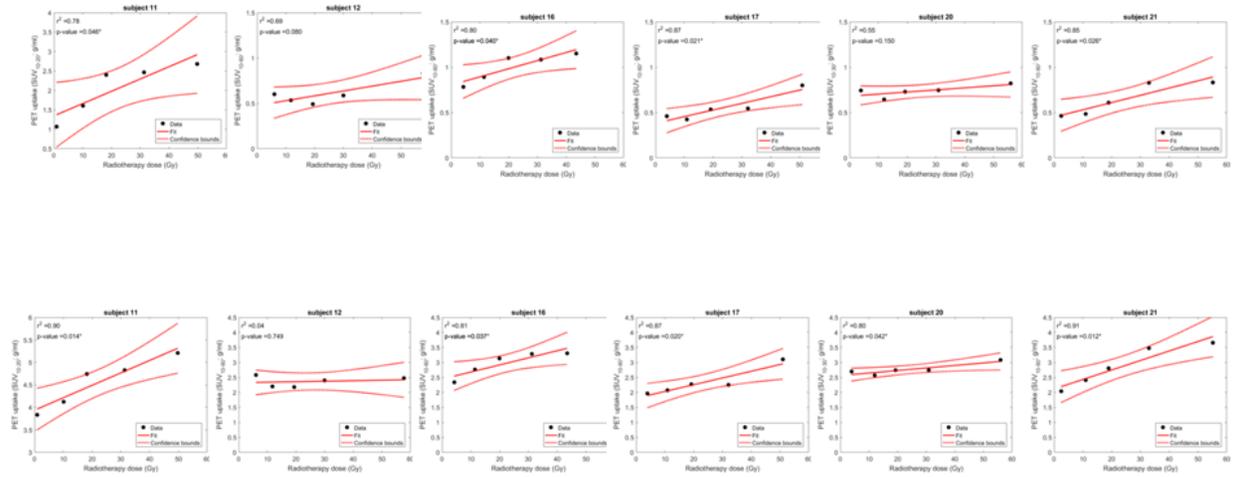


Figure 4

(a)



(b)

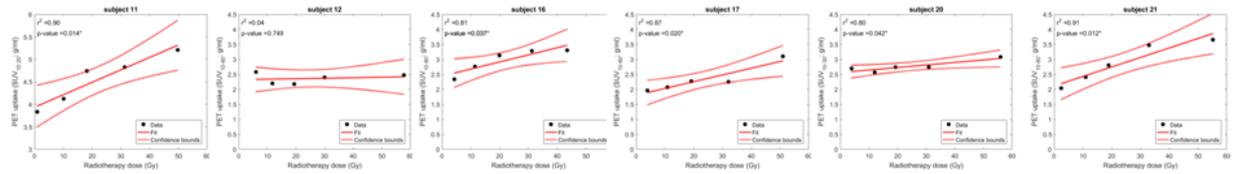


Figure 5

