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Research Article

Fluorothymidine Positron Emission Tomography (FLT-PET) Repeatability and Response Evaluation in Advanced Pancreatic Cancer Patients Treated with Gemcitabine-Based Chemotherapy

Angela Lamarca[1,2,3], Prakash Manoharan[4], Peter J Julyan[5], Mahbubunnabi Tamal[6], Madhu Rao[7], Andrew Cotterill[7], Ioannis Trigonis[2], Mairéad G McNamara[1,3], Richard A Hubner[1,3], Zarni Win[8], Juan W Valle[1,3#] and Azeem Saleem[9,10#*]

[1]Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, United Kingdom
[2]The University of Manchester, Division of Informatics, Imaging and Data Sciences, Manchester, United Kingdom
[3]Division of Cancer Sciences, Manchester Academic Health Science Centre, University of Manchester, United Kingdom
[4]Department of Radiology, The Christie NHS Foundation Trust, Manchester, United Kingdom
[5]Christie Medical Physics and Engineering, The Christie NHS Foundation Trust, Manchester, United Kingdom
[6]Department of Biomedical Engineering, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
[7]Department of Cellular Pathology, The Pennine Acute Hospitals NHS Trust, Manchester, United Kingdom
[8]Department of Radiology, Imperial College Healthcare NHS Trust, London, United Kingdom
[9]Invicro, A Konica Minolta Company, Burlington Danes Building, Hammersmith Hospital, Du Cane Road, London, United Kingdom
[10]Department of Surgery and Cancer, Imperial College London, United Kingdom
[#]Contributed equally to the work

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ABSTRACT

Purpose: This study aimed to evaluate the feasibility and repeatability of [^{18}F]-fluorothymidine positron emission tomography (FLT-PET) and its utility as a proliferative imaging marker to evaluate response in patients with advanced pancreatic adenocarcinoma (PDAC) receiving gemcitabine-based chemotherapy.

Methods: PDAC patients due to commence gemcitabine-based chemotherapy underwent FLT-PET over 60 minutes, before (baseline) and after 28 days of chemotherapy. Repeatability was assessed by a second FLT-PET scan within 7 days of baseline scan and before starting chemotherapy. Scans were assessed by two independent physician's to determine inter-reporter concordance. FLT-PET uptake over 45-60 minutes was estimated as maximum and mean standardised uptake values (SUVmax and SUVmean). Exploratory analysis of tissue biomarkers was performed from archival tissue samples.

Results: All 18 of the 21 patients consented who were imaged had primary tumour in-situ and 83% had metastases with 60% in liver. 17 patients received gemcitabine-based treatment. Thirty-five FLT-PET scans were acquired (89% evaluable) and 26 lesions delineated (17 primary tumours, 9 liver metastases). At baseline, liver metastases showed higher uptake compared with primary tumour with mean (SD) SUVmax [7.2 (1.1) vs 4.5 (1.3); $p < 0.001$] and SUVmean [4.7 (0.6) vs 2.1 (0.6); $p < 0.001$)]. There was good intra-patient repeatability and inter-reporter concordance with mean (SD) test-retest difference and inter-reporter Lin's concordance coefficient being 4.9% (17.6) and 0.703 for SUVmax and -5.4% (SD 9.8) and 0.710 for SUVmean, respectively. However, gemcitabine-capecitabine combination therapy resulted in a higher FLT uptake compared to gemcitabine alone, although this did not translate to clinical benefit. No relationship was observed between tissue markers and FLT in half of the subjects imaged whose tissue was available.

Conclusions: FLT-PET is a feasible and reproducible imaging technique in patients with PDAC to evaluate proliferation-targeting therapy, using a simplified imaging protocol in well-designed clinical trials.

[*]*Correspondence to: Dr. Azeem Saleem, Invicro, Burlington Danes Building, Imperial College, Hammersmith Hospital, Du Cane Road, London; Tel: +442080086124; E-mail: azeem.saleem @invicro.co.uk*

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in men and women [1]. Five-year survival rate is around 5% for all stages: 20% for patients diagnosed with early stage disease and less than 1% for advanced stages [2, 3]. Most patients (up to 80%) are diagnosed at an advanced stage and palliative chemotherapy is an option in fit patients, which aims to prolong overall survival (OS) and to improve quality of life. Since gemcitabine was established as the primary chemotherapeutic agent in the treatment of advanced PDAC in 1997, multiple studies with combination therapies such as gemcitabine/erlotinib, gemcitabine/capecitabine, 5-fluorouracil, oxaliplatin and irinotecan (FOLFIRINOX) and gemcitabine/nab-paclitaxel have shown improvement in OS compared to single agent gemcitabine alone [4-8]. However, none of these regimens have extended the median OS beyond 1 year. There is therefore a clear need to improve the management of patients with pancreatic cancer and considerable effort has been invested in the development of novel therapies, albeit with limited success.

Radiological follow-up (computerised tomography (CT) or magnetic resonance (MR)) is the cornerstone of assessment of response to treatment, based on Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 [9]. The evaluation of changes in tumour size using RECIST as an endpoint, and as a surrogate for OS in clinical therapeutic studies creates a unique challenge in poorly-responding tumours such as PDAC, where increments in morphological response to novel and cytostatic therapies may be minimal. Therefore, lack of early signals of response to therapy can be easily missed if a 30% decrease in the diameter of target lesion(s) is required for definition of an "active compound", which may result in some active cytostatic compounds being inadvertently rejected. Functional imaging techniques such as positron emission tomography (PET) may therefore have the unique potential to provide information on early response signals that may not only prevent inadvertent rejection of active compounds, but may also allow for the development of novel combination therapies. The potential to image tumour proliferation, a hallmark of cancer, by assessing the turnover of the deoxyribonucleic acid (DNA) nucleoside thymidine (that is exclusively incorporated in to the cellular DNA) has been evaluated non-invasively with [^{11}C]thymidine- PET [10]. However, the short half-life of [^{11}C] thymidine and the presence of radiolabelled metabolites has limited its use and led to evaluation of its analogues such as [^{18}F]-fluoro-3′-deoxy-3′-L-fluorothymidine (FLT) that may overcome these limitations [11-14]. [^{18}F]-Fluoro-3′-deoxy-3′-L-fluorothymidine is actively taken up by cells by nucleoside transporters and, like thymidine, is phosphorylated to mono-/bi-/tri-phosphate by the cytosolic enzyme thymidine kinase-1 (TK1). However, unlike thymidine, FLT is not incorporated into the DNA and the phosphorylated FLT is not de-phosphorylated and therefore is trapped in the cells. The imaging of FLT trapping and accumulation in cells using FLT-PET represents TK1 activity, which is thought to be proportional to cellular proliferation and DNA synthesis by the salvage pathway [11, 15, 16]. The physiologic uptake of FLT by the liver, kidneys, and bladder, leading to a high background signal, has limited the use of FLT in some indications [17-19]. As FLT and gemcitabine, one of the primary chemotherapeutic agents for the treatment of PDAC, are both transported into the cell via the human equilibrative nucleoside transporter-1 (hENT1), FLT-PET has been postulated as a potential biomarker of gemcitabine uptake [19-22].

Preclinical models have explored changes on FLT uptake following gemcitabine administration and confirmed direct competition of gemcitabine with the radiotracer for cellular uptake; correlation with TK1 expression has also been confirmed [23]. In this study, the aim was to assess the feasibility of imaging proliferation using FLT-PET in patients with advanced PDAC. Test-retest variability of FLT-PET imaging and changes in tumour FLT uptake with gemcitabine-based chemotherapy were also assessed. Finally, any correlations between FLT uptake and pathological markers, including hENT1 expression were explored.

Material and Methods

I Study population and regulatory approval

This study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice (GCP). The study conduct was approved by the Greater Manchester West Research Ethics Committee (REC; REC no. 12/NW/0243), the Administration of Radioactive Substances Advisory Committee (ARSAC; Research certificate No. RPC: 595/3742/2860), UK and The Christie NHS Foundation Trust (recruiting centre). All participants provided written informed consent prior to participation in the study.

Study eligibility criteria included patients with locally advanced or metastatic PDAC, with Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0-2 who were due to start first-line gemcitabine-based palliative chemotherapy. Eligible patients were also required to have at least one potentially PET-evaluable lesion (defined as a lesion of 2 cm or larger on CT or MR). Female patients who were pregnant or breast feeding, patients known to have allergy to intravenous iodine contrast or patients with other uncontrolled inter-current illnesses were excluded from the study.

II Study design

Once subjects passed screening, they had a pre-chemotherapy baseline FLT PET scan (BS) and a post-treatment response scan (RS) performed after the last day of cycle 1 of chemotherapy and prior to the start of cycle 2 of chemotherapy (i.e. between days 22-28 of the 28-day cycle). A cohort of patients who had consented for repeatability assessment, had a second pre-chemotherapy FLT-PET repeatability scan (RPS) within 7 days of the first baseline scan (Figure 1). The scans were scheduled to fit in with the chemotherapy schedule, and their treatment schedule was not modified by taking part in the study. This pilot study aimed to recruit ten patients with evaluable BS and RS and four patients with evaluable BS and RPS. The number of subjects required was based on pragmatic considerations for pilot studies in such a cohort of patients. Since patients who withdrew consent before any study scan, or after a single scan, would not be evaluable, ethics permission was sought to recruit a maximum of 20 patients to allow replacement of non-evaluable patients.

III PET imaging procedure

All PET scans were performed in the outpatient (ambulatory) setting at the University of Manchester Wolfson Molecular Imaging Centre (WMIC, Manchester, UK) on a Biograph TruePoint TrueV 6 (Siemens) PET-CT scanner [24]. An x-ray topogram was initially performed to localise the area to be scanned, followed by a low dose CT scan for attenuation correction. This was followed by intra-venous administration of FLT (target dose 330 MBq) and simultaneous commencement of the PET scan. PET data was acquired in 3-dimensional mode for up to 60 minutes. An additional contrast-enhanced CT (ceCT) was performed after the PET scan, to aid localisation of the lesions in patients who had not had a recent (within 1-2 weeks) ceCT. Patients were discharged home on completion of the scans.

IV Chemotherapy administration

Chemotherapy administration followed institutional practice at The Christie NHS Foundation Trust (Manchester, UK). All recruited patients were scheduled to receive gemcitabine-based chemotherapy as follows: 1) gemcitabine monotherapy: gemcitabine 1000 mg/m^2 intravenously (iv) administered at days 1, 8 and 15 of a 28-day cycle; 2) gemcitabine and capecitabine combination: gemcitabine 1000 mg/m^2 iv on days 1, 8 and 15 together with capecitabine 825 mg/m^2 orally twice daily on days 1 to 21 of a 28-day cycle; 3) gemcitabine and nab-paclitaxel combination: gemcitabine 1000 mg/m^2 iv on days 1, 8 and 15 together with nab-paclitaxel 125 mg/m^2 iv on days 1, 8 and 15 of a 28-day cycle. Patients taking part in other clinical trials exploring novel gemcitabine-based combinations were also recruited to the study.

V Assessment of response to chemotherapy

Response to chemotherapy was determined clinically and radiologically, as per standard of care with 3-monthly radiological imaging following initiation of chemotherapy. Response was assessed as per RECIST v1.1 [9]. Based on our institutional data from 374 advanced PDAC patients treated with standard of care chemotherapy outside the setting of clinical trials (real-world data), wherein the median overall survival (OS) was 4.9 months, we termed patients who achieved a partial response or stable disease as best response in any of the radiological reassessments or those who had an OS of at least 5 months as having attained 'clinical benefit' from the treatment in this study. Patients who had progressive disease, or who died before any radiological assessment was performed, were classified as having "no benefit" [25].

VI Pathological analysis of tumour samples

Archival paraffin-embedded tumour tissue was retrieved and immunohistochemical (IHC) analysis was performed on completion of all the PET scans in order to assess other biomarkers of interest involved in PDAC aggressiveness and response to gemcitabine. Cytology samples were excluded from such analysis. Analysis was performed by the pathology team (MR, AC) at the Pennine Acute NHS Foundation Trust (Manchester, UK) employing the following antibodies: mouse monoclonal anti-human Ki67 (Clone Mib-1; Dako, Denmark), rabbit polyclonal anti-Thymidine Kinase 1 (Thermo Scientific, USA), rabbit polyclonal anti-ribonucleotide reductase subunit M (RRM1) (Thermo Scientific, USA), mouse monoclonal anti-human human antigen R (HuR or ELAVL1) (Clone 3A2; Thermo Scientific, USA) and rabbit monoclonal anti-human hENT1 (Clone SP120; Spring Bioscience, USA). Percentage of cells showing expression (%) and intensity (graded from 0-3) was stated for the following: RRM, TK1, hENT1 and HuR. Expression of Ki67 was stated as a percentage. The relationship between hENT expression and FLT uptake (standardised uptake value (SUVmax and SUVmean)) was explored.

VII PET data analysis

Positron emission tomography data acquired 45-60 minutes post-injection were reconstructed as a static image using 3D ordered subset expectation maximisation (OSEM) into a 256 x 256 x 109 matrix, which corresponds to a voxel size of 2.67 x 2.67 x 2.02 mm^3. The reconstructed PET images were then smoothed using a 3D 4 mm Gaussian filter. Regions of interest (ROIs) were manually defined, outlining the tumour on the static PET image with the aid of the ceCT scan, and attenuation CT of the PET-CT scan by two dually accredited radiologists and nuclear medicine physicians (PM, ZW) at separate sites (The Christie NHS Foundation Trust, Manchester and Imperial College Healthcare NHS Trust, London), respectively to assess inter-reporter concordance. All normal tissue regions were manually delineated by a clinical oncologist with research expertise in translational molecular imaging (AS). Seven regions were delineated: primary pancreatic tumour, liver metastases and normal tissues, including liver, right and left kidneys, vertebrae (at least 3 vertebral bodies in the scanner field of view) and spleen. Standardised uptake value was defined as the uptake in the defined region normalised for the administered activity and body weight and maximal (SUVmax) and mean (SUVmean) in the regions of interest (ROIs) were calculated.

VIII Statistical analysis

Frequency tables were used for descriptive analysis of the data. Repeatability was explored using Bland-Altman plots, and by describing test-retest differences, with mean and standard deviation (SD) and 95%-confidence intervals (95%-CI), as appropriate. For the repeatability analysis, mean test-retest difference was calculated as follows: ((Scan2-scan1)/((Scan1+Scan2)/2)) x100. Concordance between continuous variables was analysed with Lin's Concordance rho coefficient. Linear regression and logistic regression were applied when appropriate. Patients included in the reassessment cohort had treatment-related changes (%) calculated as follows: ((after treatment-baseline)/baseline) x 100; t-test and non-parametrical methods were employed for comparisons of means, where appropriate.

Results

I Patients, feasibility and safety

Twenty-one of 58 patients (36%) approached for the study consented (Figure 2). A total of 18 patients had at least one baseline FLT-PET performed, with 13 of the 18 subjects having repeat scans. Of the 5 subjects unable to have a second scan, three had worsening of their performance status and in 1 patient, chemotherapy was not initiated due to rapidly progressive disease. Eighty-three percent of patients recruited had distant metastases at time of study entry. The median size of primary

pancreatic tumours was 4.95 cm (range 2.3-7). Of the 18 patients scanned, 17 patients started palliative chemotherapy with gemcitabine-based chemotherapy, with 2 patients (12%) achieving a partial response by RECIST v.1.1 as best response. Patient recruitment, treatment characteristics and best response achieved are summarised in (Table 1). FLT-PET imaging was shown to be feasible in patients with advanced PDAC with 31 evaluable scans of the 35 (89%) (Table 2). Four scans were not evaluable due to patient movement during scan (n=2), protocol violation (n=1) and tissue extravasation of FLT at scan start (n=1). As a result, only 4 of the 5 (80%) paired scans were suitable for repeatability comparison and 10 of the 12 (83%) baseline-post-chemotherapy pairs were suitable for response evaluation. The protocol violation was due to a patient receiving cycle 2 day 1 of chemotherapy before the response scan, rather than after the scan (this case is presented as a case study in this manuscript; see Supplementary Material A). All subjects tolerated the scanning procedure well. The patient who had extravasation of FLT did not develop any significant adverse clinical sequelae. There were no other study-procedure related adverse events.

Table 1: Summary of patients' characteristics, chemotherapy administered and best-response achieved.

Patient characteristics		n	%
Patients with baseline PET scans (n=18)			
Gender	Female	10	56
	Male	8	44
Age (years)	Median (range)	63.8 (53.1-77.9)	
Location of primary tumour	Head	8	44
	Neck	3	17
	Body	4	22
	Tail	2	11
	Not identifiable	1	6
Size of primary tumour (cm)	Median (range)	4.95 (2.3-7)	
Stage	Locally advanced	3	17
	Metastatic	15	83
Sites of metastases	Liver	9	60
ECOG Performance Status	0	4	22
	1	11	61
	2	3	17
Patients treated with chemotherapy (n =17)			
Chemotherapy regimen	Gemcitabine single agent	7	41
	Gemcitabine + capecitabine	4	24
	Gemcitabine + *nab*-paclitaxel	4	24
	Gemcitabine + other drugs (TH-302 or vandetanib; clinical trials)	2	11
Best radiological response to chemotherapy	Partial response	2	12
	Stable disease	7	41
	Progressive disease	3	18
	Not assessed	5	29

Eighteen patients were consented for the study, of which 17 started palliative chemotherapy. Of these, 7 had stable disease and 2 had partial response as assessed by RECIST v1.1.

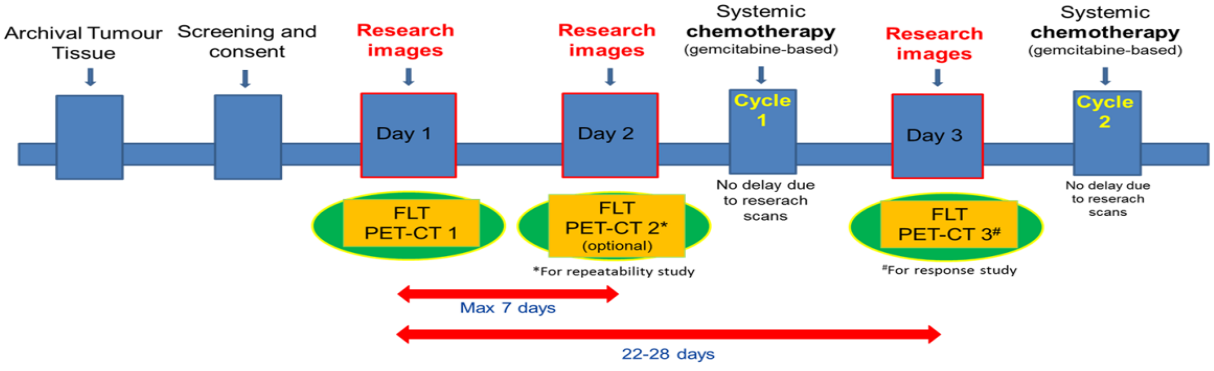

Figure 1: Study design
Patients recruited on to the study, who passed screening, underwent a baseline FLT scan before start of treatment. A second scan prior to start of chemotherapy was performed (n=5) to assess repeatability, and after chemotherapy to assess response (n=11).

Table 2: Feasibility of FLT-PET imaging in patients with PDAC.

Total of scans acquired (N=35)		Total	Evaluable	Evaluable (%)
Evaluable scans	Total	35	31*	89
	Baseline san	18	17	94
	Repeatability scan	5	4	80
	Response scan	12	10	83
Patients	Total	18	12[#]	67

A total of 35 scans were performed, of which *4 scans (11%) were not evaluable due to protocol violation (n=1), FLT tissue extravasation at scan start (n=1), patient movement during scan (n=2); [#]Of the 18 patients who had a baseline scan, 6 (33%) patients did not have a repeat scan, due to inability to have a second scan (n=3, due to poor PS at time of repeatability/response scan), chemotherapy never started (n=1), repeatability scan not evaluable due to patient' movement (n=1), and response scan not evaluable due to protocol violation (n=1; administration of cycle 2 day 1 of chemotherapy before response scan).

II Visualisation of PET images and tissue uptake

Visualisation of the fused static PET-CT image showed higher FLT uptake in the liver consistent with its role as an organ for FLT metabolism (Figure 3). The higher background uptake in the abdomen required the assistance of a diagnostic CT scan for identification and delineation of intra-abdominal disease. Of the 26 lesions (17 primary pancreatic tumours and 9 liver metastases) delineated on the evaluable baseline scans, a significantly mean (SD) lower uptake was seen within the primary tumour compared to the liver metastases [SUVmax (4.5 (1.3) vs 7.2 (1.1); $p < 0.001$) and SUVmean (2.1 (0.6) vs 4.7 (0.6); $p < 0.001$ (Table 3). For normal tissue, mean (SD) uptake was the highest in vertebral body, consistent with the role of vertebral marrow as a myeloproliferative tissue followed by liver and kidneys, the eliminatory organs and spleen (Table 3).

III Repeatability of imaging

III.I Intra-patient repeatability of tumour uptake

Intra-patient tumour uptake repeatability was assessed in 8 scans (from 4 patients with paired baseline and repeatability scans). Eight pairs of tumour lesions (BS and RS) were delineated (4 primary tumours and 4 liver metastases). There was good intra-patient repeatability in tumoural uptake for both the primary pancreatic tumours and liver metastases (Table 3). For all tumours, the mean (SD) percentage test-retest difference and Lin's concordance coefficient (ρ) of 4.9 (17.6) and 0.792, respectively for SUVmax. For SUVmean the mean (SD) percentage test-retest difference and Lin's concordance coefficient (ρ) were -5.4 (9.8) and 0.947 for SUVmean, respectively for all tumours (Figure 4A). Good intra-patient repeatability was replicated in tumoural uptake (total of 6 pairs of lesions delineated: 4 primary tumours and 2 liver metastases) when images were reviewed by a second independent radiologist. The mean (SD) percentage test-retest difference and Lin's concordance coefficient (ρ) were -1.5 % (11.1) and 0.893, respectively for SUVmax and mean (SD) percentage test-retest difference and Lin's concordance coefficient (ρ) were -10.1 % (12.9) and 0.599, respectively for SUVmean (Figure 4B).

III.II Inter-reporter variability on tumour uptake

Inter-reporter concordance in tumoural uptake between the two radiologists reporting independently was also good with mean (SD) percentage test-retest difference and Lin's concordance coefficient (ρ) of -5.1 % (17.4) and 0.703, respectively for SUVmax and mean (SD) percentage test-retest difference and Lin's concordance coefficient (ρ) of -1.2 % (18.7) and 0.710, respectively for SUVmean (Figure 4C)

III.III Intra-patient repeatability in normal tissue uptake

The mean test-retest difference for the various normal tissues is also given in Table 3. Amongst normal tissues, the variability (test-retest difference) in vertebral uptake parameters SUVmax was 6.1% (SD 5.3) and SUVmean was 8.2% (SD 9.8). Similar variability was observed in the spleen [mean test-retest differences were 6.0% (SD 12.1) for SUVmax and 8.1% (SD 18.6) for SUVmean] and liver [mean SUVmax test-retest difference -4.9% (SD 7.9), mean SUVmean test-retest difference -6.6% (SD 11.3].

In contrast, the mean test-retest difference for the left kidney was 16.8% (SD 21.6) for SUVmax and 14.5% (SD 17.0) for SUVmean; which varied from the right kidney [5.3% (SD 16) for SUVmax and 17.2% (SD 9.7) for SUVmean]. These differences could be attributed to slower renal elimination of the radiotracer from the renal pelvis visually observed in some of the patient's kidneys as dilated renal pelvis.

IV Changes within normal tissue following chemotherapy

An increase in uptake in the vertebral body uptake was observed after chemotherapy [SUVmax 22.5% (95% CI: 13, 33); SUVmean 21.8% (95% CI: 14, 30) (Table 3). Similarly, an increase in FLT splenic uptake was found [SUVmax response 27.4% (95% CI: 6, 48); SUVmean 30.6% (95% CI: 11, 50)] after chemotherapy, with some subjects exceeding the mean test-retest difference. Both kidneys showed a decrease in the FLT uptake after therapy, with left kidney [SUVmax -20.6% (95% CI: -32, -9); SUVmean response -22.2% (95% CI: -29, -16)] having a larger decrease than the right kidney [SUVmax response -15.5% (95% CI: -33, 1); SUVmean response -17.7% (95% CI: -32, -4)]. Changes within healthy liver were minimal [SUVmax response 2.2% (95% CI: -14, 18); SUVmean response 1.5% (95% CI: -12, 15)].

Table 3: Mean (SD) for tumours and normal tissues during the baseline scan mean (SD) percentage (%) change in uptake between baseline scan and repeatability scans, both of which were performed prior to administration of chemotherapy.

Tissue	Mean (SD) uptake at baseline scan		Mean (SD) percentage change in uptake in repeatability scans	
	SUVmean	SUVmax	SUVmean	SUVmax
Pancreatic tumour	2.1 (0.6)	4.5 (1.3)	-3.1 (12.8)	8.0 (24.3)
Liver metastases	4.7 (0.6)	7.2 (1.1)	-7.7 (7.3)	1.9 (10.5)
Liver	6.6 (1.1)	8.0 (1.3)	-6.6 (11.3)	-4.9 (7.9)
Kidney (Right)	3.4 (0.7)	5.1 (1.3)	17.2 (9.7)	5.3 (16.0)
Kidney (Left)	3.3 (0.8)	4.8 (0.9)	14.5 (17.0)	16.8 21.6
Vertebra	8.7 (0.7)	12.6 (2.0)	8.2 (9.8)	6.1 (5.3)
Spleen	2.3 (0.7)	3.1 (0.9)	8.1 (18.6)	6.0 (12.1)

V Changes following gemcitabine-based chemotherapy in tumours

Of the 10 patients who had PET scans and whose disease was evaluable for response by RECIST, 6 had benefitted from chemotherpy (2 achieved a partial response and 4 achieved stable disease and had overall survival of more than 5 months). In the 16 tumour lesions evaluated from 10 subjects for response after chemotherapy, no changes in the tumoural SUV (SUVmean and SUVmax) were observed following administration of FLT, with the mean (SD) percentage change in SUVmax and SUVmean in primary pancreatic tumours being 1.6 (26) and -8.1% (15), respectively. For liver metastases, the mean (SD) percentage changes in SUVmax and SUVmean after therapy were 5.4 (13) and -3.7 (14) (Table 4A). There were no differences in FLT uptake between subjects who clinically benefited from chemotherapy and those who did not (Table 4B). In addition, we did not observe that either baseline SUV or changes in SUV after therapy that were predictive of benefit from gemcitabine-based chemotherapy.When FLT uptake was evaluated for the various treatment regimens, we found the largest decrease in tumoural FLT uptake after gemcitabine-capecitabine combination chemotherapy with mean percentage (SD) decrease in SUVmax and SUVmean of -11.5 (13.7) and -16.1 (5.6), respectively (Table 4C). On comparison of uptake between various chemotherapy regimens, a significant decrease in SUVmax ($p< 0.05$) and SUVmean ($p < 0.01$) was observed gemcitabine-capecitabine combination when compared with therapy with single agent gemcitabine (Table 4C)

Table 4: Changes between baseline and response scans and factors predictive of benefit from gemcitabine-based chemotherapy

A: Overall changes in uptake in tumour lesions after therapy			
	All lesions (n=16)	Primary tumour (n=10)	Liver metastases (n=6)
Percentage change in SUVmax after therapy. Mean (SD)	3.0 (21)	1.6 (26)	5.4 (13)
Percentage change in SUVmean after therapy. Mean (SD)	-6.4 (14)	-8.1 (15)	-3.7 (14)
B: Changes in tumour uptake in patients with and without clinical benefit from gemcitabine chemotherapy			
	Patients with Clinical benefit (n=6)	Patients with no Clinical benefit (n=4)	p-value (t-test)
Mean (SD) percentage reduction in SUVmax after therapy.	2.7 (25.4)	3.3 (17.8)	0.27
Mean (SD) percentage reduction in SUVmean after therapy.	-10.5 (13.8)	- 2.4 (16.7)	0.78

C: Changes in uptake in tumour lesions with chemotherapy regimen				
	Gemcitabine alone (n=5)	Gemcitbine- all combintaion (n=11)	Gemcitabine –Capecitabine (n=5)	Gem+Nab-paclitaxel (n=3)
Mean (SD) percentage reduction in SUVmax after therapy	16.7 (20.1) *	3.0 (21.2)*	-11.5 (13.7)	7.0 (15.1)
Mean (SD) percentage reduction in SUVmean after therapy	3.0 (9.1) *	- 6.4 (14.3)*	-16.1 (5.6)	-11.3 (18.4)

*Significant ($p < 0.05$) difference in FLT uptake was observed when gemcitabine was compared with gemcitabine-capecitabine but not with any of the other comparisons.

Table 5: Immunohistochemistry analysis of biopsy samples (n=9).hENT1: Human equilibrative nucleoside transporter 1; RRM: Ribonucleotide reductase subunit M; TK1: Thymidine kinase 1; HUR: human antigen R; Ki67: protein Ki-67.

	No Expression	Mild Expression	Moderate Expression	High Expression	Benefit prediction of gemcitabine-based chemotherapy (logistic regression) p-value)
hENT1	1 (11.1%)	3 (33.3%)	4 (44.4%)	1 (11.1%)	0.438
RRM	1 (11.1%)	6 (66.7%)	2 (22.2%)	0 (0%)	0.399
TK1	0 (0%)	3 (33.3%)	2 (22.2%)	4 (44.4%)	0.181
HUR	0 (0%)	1 (11.1%)	3 (33.3%)	5 (55.6%)	-
Ki67 Median (95%CI) (range)	15 (10-20) (5-70)	-	-	-	-

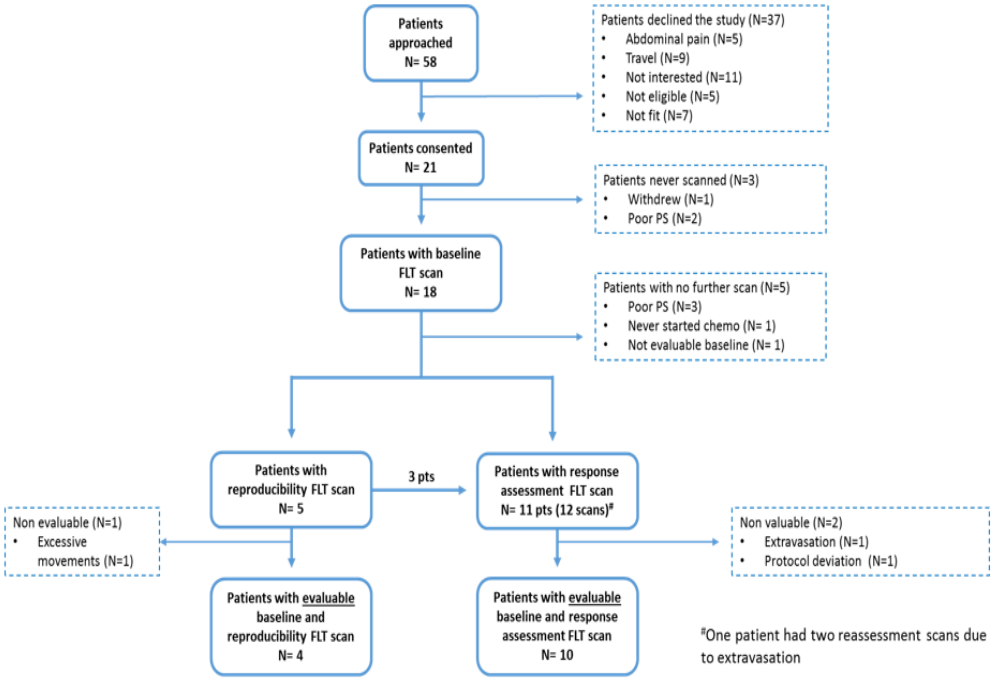

Figure 2: Consort Diagram
Diagram showing the subjects approached, recruited and scanned in this study

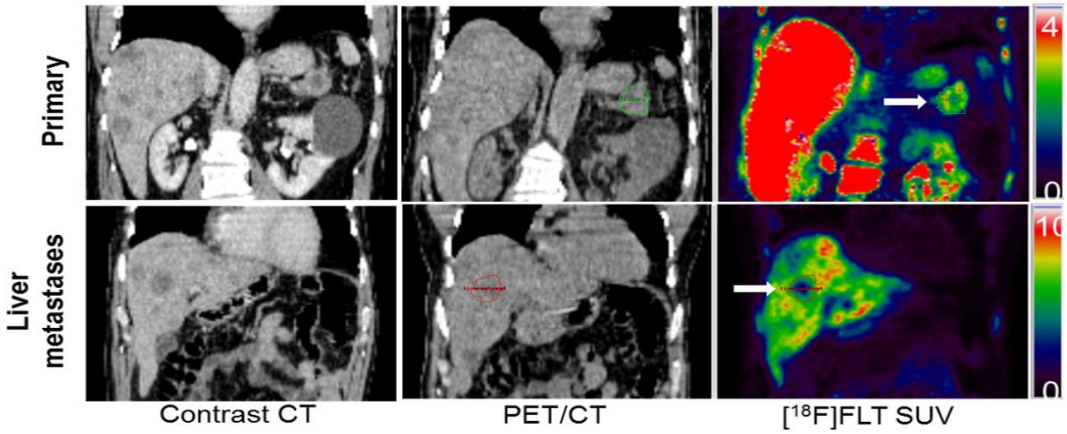

Figure 3: Delineation of primary tumours and liver metastases on the static baseline FLT-PET image on which the primary tumour and metastatic lesions were delineated. FLT uptake was higher in liver metastases compared to primary pancreatic tumour.

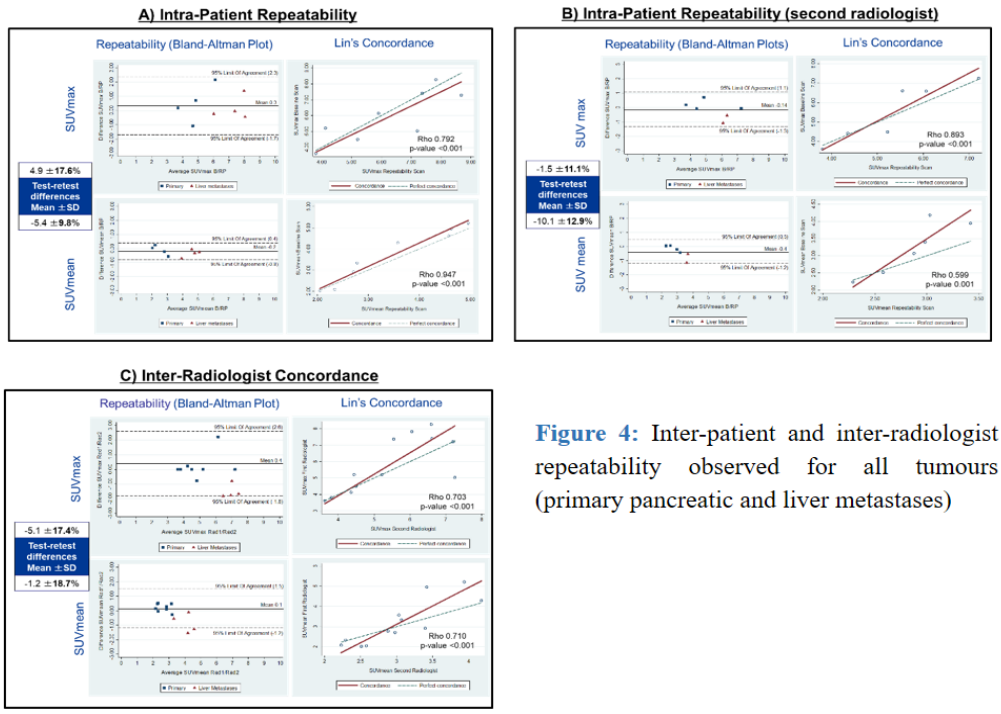

Figure 4: Inter-patient and inter-radiologist repeatability observed for all tumours (primary pancreatic and liver metastases)

VI Pathological biomarkers and relationship with PET and clinical parameters

Nine of 18 patients imaged (50%), had biopsy sample available for pathological IHC analysis. Results are summarised in Table 5. The median Ki67 was 15%. Expression of hENT1 was mild in 33.3%, moderate in 44.4% and high in 11.1% of patients; 11.1% did not show any hENT1 expression. None of these biomarkers' expression was able to predict benefit from chemotherapy from the limited samples available. Expression of hENT1 did not correlate with benefit from gemcitabine-based chemotherapy (Table 5). In addition, hENT1 presence (measured as a continuous variable, %) did not correlate with SUVmax within the primary pancreatic cancer. The relationship between baseline primary tumoural uptake of FLT and clinical and pathological expression was evaluated. Linear regression analysis identified that gender (female; Odds Ratio (OR) 4.04 (95%-CI 1.2, 13.58)), TK1 (continuous variable (%); OR 1.03 (95%-CI 1.01, 1.06)) and HUR (continuous variable (%); OR 1.14 (95%-CI 1.03, 1.25)) expression were related with higher primary tumour SUVmax at baseline (univariate analysis). However, none of these factors-maintained significance in the multivariable analysis (Supplementary material B).

Discussion

These results confirm the feasibility of performing FLT imaging in patients diagnosed with PDAC treated with palliative chemotherapy. However, there were several challenges in patient recruitment, and it is likely that such challenges are related to the targeted population. Patients diagnosed with advanced PDAC have significant ongoing symptoms and impaired performance status; thus, the prolonged scan acquisition time required for scanning may have resulted in moderate acceptance by patients. Although, it challenging to speculate on the causes of a similarly poor recruitment rate for another study comparing FDG and FLT PET scanning in PDAC (18), it is likely that imaging method development studies, without a potential therapeutic benefit, in a cohort of subjects with limited overall survival, is likely to result in poor study uptake. It is therefore essential that the molecular imaging community devises innovative methods or study designs that provide most information on a simplified and/or an opportunistic protocol, and thereafter expand to a more detailed methods study that would ideally be incorporated or added on to studies providing potential benefit to subjects. A limited number of previous studies using FLT-PET have been performed in patients with pancreatic cancer mainly as a diagnostic tool. Quon et al compared FLT with FDG imaging in five patients and concluded that FDG was superior for identification of the primary lesion due to lower SUV with FLT compared to FDG [26-30]. Similarly, in a larger prospective study in 33 patients with pancreatic cancer due to undergo surgery, Herrmann et al confirmed that FDG-PET had a higher sensitivity and overall accuracy in detecting pancreatic cancer compared to FLT-PET [28]. Nakajo et al observed that FLT and FDG were similar in detecting primary lesions in a study of 15 newly-diagnosed patients with pancreatic cancer, but FDG was superior to FLT for metastatic disease [18]. FLT-PET however had a higher specificity compared to FDG-PET in pancreatic cancer and was exclusively taken up by malignant lesions (100% specificity in detecting malignancy), compared to benign lesions, in a study of 31 subjects with undefined pancreatic lesions [28, 29].

This study did not aim to evaluate FLT as a diagnostic tool, nor seek to compare FDG with FLT. This study aimed to evaluate the feasibility and repeatability if FLT PET imaging in patients with PDAC as a potential readout of the proliferative pathway, a key hallmark of cancer [31]. The FLT uptake values observed in pancreatic cancer in this study match that in the published studies [18, 26, 28]. A higher uptake of FLT was observed in the liver metastases compared to uptake in the primary lesion. Despite a higher amount of background activity in the liver, due

to its role in metabolising FLT, hepatic metastases were identified in all the subjects. However, PET visualisation of disease in this study was likely aided by the availability of the ceCT scan for the patients evaluated. Amongst normal tissues, the highest uptake of FLT was observed in vertebral body, consistent with its role as a proliferative organ [32].

On evaluating the repeatability of FLT-PET in patients with PDAC, good inter-reporter concordance was confirmed in tumour uptake, as reported previously and intra-subject repeatability of the semi-quantitative uptake parameters (SUV) [18]. In normal tissue, better repeatability scores were observed within high uptake organs (bone marrow and liver), compared to tumour or other low uptake normal tissue (spleen, kidneys) and likely influenced by the higher noise in low uptake tissues. Tumour repeatability data obtained was similar to that obtained from FDG- and FLT- PET studies in other malignancies [33, 34].

The effects of gemcitabine-based chemotherapy on uptake of FLT was also evaluated. Changes observed in FLT uptake after chemotherapy remained within expected variability observed in repeatability scans for most observations. This study did not confirm the findings of Challapalli and colleagues who found that changes in FLT uptake after chemotherapy were predictive of response [26]. Neither was a differential in FLT uptake observed in subjects who clinically benefited from chemotherapy, versus those who did not. Although a significant decrease in FLT tumour uptake (SUV) was observed in subjects receiving gemcitabine capecitabine combination therapy compared to gemcitabine alone, this did not translate to clinical benefit. However, it is likely that despite the tendency to decrease the proliferative potential of tumours in combination therapy, this is perhaps not efficacious enough to lead to cell death and thereby improved objective response.

An increased uptake in vertebral body uptake 3 to 4 weeks after chemotherapy was indicative of increased proliferative activity consequent to marrow recovery after chemotherapy, as was also observed in other studies [35, 36]. An increase in the splenic FLT uptake paralleled that in the vertebral body; this is more likely to reflect the FLT activity in the blood pool, although a potential role in haematopoiesis cannot be completely ruled out without corroborative tissue data.

In one patient (subject 4), who inadvertently had the post-treatment scan a few hours after gemcitabine administration, a significant decrease in vertebral body uptake was observed, likely due to the rapid decrease in proliferative activity in the marrow immediately after chemotherapy (Supplementary Material A). Normal tissue uptake also decreased after chemotherapy in this subject. Gemcitabine, like fluoropyrimidines, inhibits TS, leading to an increase in TK activity, by triggering the salvage pathway, and therefore should result in an increase in FLT uptake immediately after chemotherapy. However, the absence of tumoural FLT flare with gemcitabine unlike fluoropyrimdines, in pre-clinical models has been attributed to the competition in tumoural uptake between FLT and gemcitabine, both of which use the same nucleoside transporter, hENT1. It is difficult to delineate if the decreased uptake in tumour and normal tissues in this subject is a true representation of decreased proliferative activity, or as a result of competition between gemcitabine and FLT for transport in to tissue [23, 37]. No relationship between the imaging and pathologic parameters were identified. Although *in vitro* studies have shown that FLT uptake was predictive of gemcitabine transport, by virtue of sharing of hENT1 as the transporter and toxicity in cancer cell line an increased response was not found in patients who had a higher FLT uptake at baseline [38]. However, it is possible that such subjects did indeed have greater gemcitabine uptake, but this did not translate into clinical benefit, as the drug levels do not necessarily translate to a response in all tumours. Moreover, the limited pathological samples from the small number of the subjects studied precludes making a definitive conclusion on the relationship between hENT1, FLT uptake and the pharmacodynamic effect of gemcitabine in this study.

In conclusion, these findings confirm feasibility and repeatability for performing FLT-PET in patients with PDAC. Importantly, FLT PET, due to its high specificity in pancreatic tumours and the ability to confirm changes in tissue proliferation, may serve as a useful tool to detect proof of mechanism of drug action with a limited and patient-friendly imaging protocol, specifically if a 'No-go' decision is required in early drug development. Although changes in normal tissue uptake provide valuable information on the drug, given the action and biology that would be important for drug development studies, the high background uptake in liver and vertebral body, common sites of metastatic disease, preclude using FLT-PET as a diagnostic tool. Importantly, unless the efficacy (in terms of cell death and objective response) of chemotherapy increases significantly, the changes in response obtained with FLT-PET will likely fall within the limits of repeatability and is therefore unlikely to serve as a useful biomarker of response, or drug development, currently in this patient population.

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Conflicts of interest

None of the authors have any conflict of interest pertaining to this work.

Ethical approval

All procedures performed were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration and its later amendments.

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