68Ga-Pentixafor PET/CT imaging for in-vivo CXCR4 receptor mapping in different lung cancer histologic sub-types: Correlation with quantitative receptors’ density by immunochemistry techniques

Ankit Watts
PGIMER: Post Graduate Institute of Medical Education and Research

Baljinder Singh (drbsingh5144@yahoo.com)
PGIMER: Post Graduate Institute of Medical Education and Research  https://orcid.org/0000-0001-8464-7765

Harmandeep Singh
PGIMER: Post Graduate Institute of Medical Education and Research

Amanjit Bal
PGIMER: Post Graduate Institute of Medical Education and Research

Hameet Kaur
PGIMER: Post Graduate Institute of Medical Education and Research

Ninjit Dhanota
PGIMER: Post Graduate Institute of Medical Education and Research

Sunil K Arora
PGIMER: Post Graduate Institute of Medical Education and Research

Bhagwant R Mittal
PGIMER: Post Graduate Institute of Medical Education and Research

Digambar Behera
PGIMER: Post Graduate Institute of Medical Education and Research

Research Article

Keywords:

Posted Date: August 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1948325/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Purpose

In-vivo CXCR4 receptor quantification in different lung cancer (LC) sub-types using $^{68}$Ga-Pentixafor PET/CT and correlation with quantitative CXCR4-receptors’ tissue density by immunochemistry analyses.

Methods

$^{68}$Ga-Pentixafor PET/CT imaging was performed prospectively in 94 (77M: 17F, mean age 60.15 ± 10.12 yrs) LC patients. CXCR4 receptors’ expression was estimated in all the patients on lung tissue by immunohistochemistry (IHC) and FACS analyses. SUV$_{\text{max}}$ on PET, Intensity score on IHC and Mean fluorescence Index (MFI) on FACS analyses were measured.

Results

75/94 (79.8%) cases had NSCLC, 14 (14.9%) had SCLC and 5 (5.3%) had lung NETs. All LC types showed increased CXCR4 expression on PET (SUV$_{\text{max}}$) and FACS (MFI). However, both these parameters (mean SUV$_{\text{max}}$ = 10.30 ± 5.0; mean MFI = 349.0 ± 99.0) were significantly (p = 0.005) higher in SCLC as compared to NSCLC and lung NETs. PET SUV$_{\text{max}}$ in adenocarcinoma (n = 16) were 8.00 ± 1.9 which was significantly (p = 0.003) higher than in squamous cell carcinoma (n = 54; 6.2 ± 2.15) and NOS (n = 5; 5.8 ± 1.5) subtypes of NSCLC. A significant correlation (r = 0.697; p = 0.001) was seen between SUV$_{\text{max}}$ and MFI values in squamous cell NSCLC as well as in NSCLC-adenocarcinoma (r = 0.538, p = 0.031) which supports the specific uptake of $^{68}$Ga-Pentixafor by CXCR4 receptors. However, this correlation was not significant in SCLC (r = 0.435, p = 0.121) and NET (r = 0.747, p = 0.147) which may be due to the small sample size. $^{68}$Ga-Pentixafor PET/CT provided good sensitivity (85.7%) and specificity (78.1%) for differentiating SCLC from NSCLC (ROC cut-off SUV$_{\text{max}}$ = 7.24). Almost similar sensitivity (87.5%) and specificity (71.4%) were observed (ROC cut-off SUV$_{\text{max}}$ = 6.67) for differentiating adenocarcinoma and squamous cell variants of NSCLC.

Conclusion

Higher CXCR4 expression was seen in SCLC as compared to NSCLC and NETs on $^{68}$Ga-Pentixafor PET imaging. The findings may potentially supplement the existing data for inclusion and expanding CXCR4-based radioligand therapies in LC beyond haematological malignancies.

Introduction

18-Fluorine Fluorodeoxyglucose-Positron Emission Tomography ($^{18}$F-FDG-PET) integrated with computed tomography (CT) as hybrid PET/CT imaging remains the mainstay for staging and diagnostic work-up in lung cancer (LC) patients [1, 2]. Nevertheless, $^{18}$F-FDG-PET has limitations such as inability to differentiate inflammatory/infectious pathologies from tumour/recurrence and has limited clinical utility for detecting brain metastasis due to high physiological tracer uptake in the normal brain cortex [3, 4]. In view of these drawbacks of $^{18}$F-FDG PET/CT imaging, development of more specific newer PET radiopharmaceuticals is a focus research area [5].

Advances in molecular cancer biology have demonstrated that many of these promising tumor targets are receptors and have been reported as earliest targets for cancer diagnosis as well as precision therapy, with notable success in the effective treatment in few cancers [6]. An important class of targets is CXCR4 - a chemokine receptor that is widely expressed in 30 different human cancers including lung carcinoma [7–9]. Recently, a CXCR4 targeting $^{68}$Ga-Pentixafor PET tracer has gained attention in PET oncology [10–12]. This PET probe has exhibited promising results in 'first proof of concept' study in various solid tumors including lung carcinoma [13]. In the present study, $^{68}$Ga-Pentixafor PET imaging was performed to image the CXCR4 over expression in various lung cancer subtypes and findings were validated with simultaneous tissue characterisation and quantification of CXCR4 receptors by histopathological, IHC and FACS analyses.

Materials And Methods

This study was approved by the Institute ethic committee (IEC) as a Ph.D thesis protocol of the first author (AW). A written informed consent was obtained from all the patients enrolled in this study.
Patients

A total of 94 patients with biopsy proven lung carcinoma were enrolled prospectively from July 2016 to March 2019. All the patients were subjected to either bronchoscopic or image guided biopsy from the lung lesions. The tissue diagnosis was made on the basis of routine histopathological analysis of fixed lung tissue cores.

CXCR4 receptors’ expression

In-vitro tissue analysis was done to document the CXCR4 receptor expression using Immunohistochemistry and Fluorescence-activated cell sorting (FACS) techniques.

Immunohistochemistry (IHC) CXCR4 staining

Immunohistochemistry analysis was done on paraffin embedded tumor sections to assess CXCR4 expression. The dewaxed slides after rehydration were incubated with primary anti-CXCR4 monoclonal antibody-UMB2 (AB124824, Abcam, Waltham, MA, USA) at room temperature in moist chamber for 1.5h. After PBS wash, it was incubated with secondary antibody (Ab209101, Abcam, Waltham, MA, USA) conjugated with signal amplifier, horse radish per-oxidase (HPR) for 45-min. Finally, dehydrated slides were used for visual scoring based on intensity of CXCR4 stained cells (1+,2+ or 3+) and percentage of CXCR4 positive tumor cells (5-10%=1, 10-50%=2, >50%=3) in the whole population of cells as seen under the microscope by an experienced pathologist. Final scoring was computed by considering both the criteria and maximum score that could be attained was 9.0. The IHC analysis could be performed only in 60/94 (64.0%) of the study subjects. This included 31/54 (NSCLC-squamous cell), 11/16 (NSCLC-adenocarcinoma), 5/5 (NSCLC-NOS), 8/14 (SCLC) and 2/5 (lung NETs) respectively.

Fluorescence-activated cell sorting (FACS)

Fresh lung tissue biopsy samples obtained in normal saline (NS) were processed to make single cell suspension. The cell suspension was divided equally into two falcon round bottom tubes. To label the CXCR4 cells in the single cell suspension, 5.0μl of Phycoerythrin (PE) labelled CD184 (BD Pharmingen Inc, San Diego, USA) antibody was added to one of the tubes & the other tube was marked as unstained. The final stained and unstained tubes were subjected to FACS analysis (FACS Calibur, BD Pharmingen Inc, San Diego, USA). The data acquired for unstained cells population was used to set the gate for CXCR4 positive cells analysis. Mean fluorescence Index (MFI) and Percentage of stained CXCR4 cells were obtained as quantitative parameters.

68Ga-Pentixafor radiolabelling

The labelling of 68Ga with Pentixafor was done under Good manufacturing practice (GMP) condition in a fully automated synthesizer (Scintomics, Munich, Germany) procured under the DST-FIST grant (Government of India). The radiolabelling was done using the standard procedure as has been reported previously [14].

68Ga-Pentixafor PET/CT scan

68Ga-Pentixafor PET/CT imaging was performed in all (n=94) the patients at 60-min after intravenous administration of 111.0MBq-185.0MBq of the radiopharmaceutical. The whole-body PET/CT (Discovery STE16/Discovery; 710/Discovery MIDR, GE Healthcare, Milwaukee, USA) acquisition was started at 1.0-h. Scanogram (120kVp & 10mAs) was done first to define the scan range for CT & PET whole body scans. Whole body contrast enhanced CT was done with the following acquisition parameters: voltage of 120KeV, current of 150-250mA (smart modulated mA), slice thickness of 3.75mm, tube rotation time of 0.5 sec, pitch of 0.98:1 and matrix size 512x512. PET acquisition was done with 3 min/bed position for a total of 6 to 9 bed positions from skull to proximal thighs in caudo-cranial direction. Semi-quantitative analysis was done on reconstructed fused PET and CT images by computing SUV_{max} values of the primary lung tumor.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences (IBM, USA, SPSS statistics 20). Pearson's correlation analysis was applied between CXCR4 expression (MFI) and SUV_{max} values. A receiver operating characteristic (ROC) curve analysis was done to derive cut-off values of SUV_{max}. All statistical tests were two-sided and were performed at a significance level of p<0.05.
Results

Ninety-four patients (77M: 17F, mean age 60.15 ± 10.12 years; range 36–82 years) were recruited. Histopathological diagnosis confirmed that 75 patients had non-small cell lung carcinoma (NSCLC) with 54 as Squamous cell variant, 16 as Adenocarcinoma, 5 as NOS and 14 had small cell lung carcinoma (SCLC), 5 had lung neuroendocrine tumors (NETs) (Table-1). All subtypes of lung cancer showed increased tracer uptake in the primary lesions on $^{68}$Ga-Pentixafor-PET scan that was indicative of high CXCR4 tumour positivity. Representative $^{68}$Ga-Pentixafor MIP and axial-fused PET/CT images are presented comprehensively in Figure-1 in one patient each of SCLC (A, A1), NSCLC-adenocarcinoma (B, B1), NSCLC-squamous (C,C1) and lung NET (D,D1) respectively. The corresponding FACS histograms depicting the quantitative CXCR4 receptors expression (MFI) and percent-stained cells in these patients are also given in Figure - 1 as A2; A3, B2; B3, C2; C3 and D2; D3 respectively. Typically, the immunohistochemistry stained sections of the lung using anti-CXCR4 antibody showing tumor positivity score of 3+ and > 50.0% of the stained cells population in a patient of NSCLC (squamous cell) is presented in Figure-2.

SCLC patients (n = 14) showed higher mean SUV$_{\text{max}}$ value of 10.30 ± 5.0 (range 6.55–26.64; median = 8.92) as compared to all other types of lung cancer and the correspondingly higher mean MFI value of 349.00 ± 98.5 was noted in SCLC. The percentage of CXCR4 stained cells was found to be 45.58 ± 22.35%. IHC analysis could be performed in 8/14 patients. CXCR4 tumor positivity on the stained slides was observed only in 6/8 patients. The mean visual score was found to be 4.50 ± 4.0. No significant correlation was found in SCLC group between SUV$_{\text{max}}$ and MFI values ($r=0.435$, $p=0.121$), between SUV$_{\text{max}}$ and percentage stained cells ($r=-0.036$, $p=0.902$) and between SUV$_{\text{max}}$ and IHC visual score ($r=0.482$, $p=0.226$).

Among the subtypes of NSCLC patients, patients of adenocarcinoma (n = 16) had higher mean SUV$_{\text{max}}$ value of 8.00 ± 1.90 (range 4.7–12.2; median = 7.73) and the corresponding mean MFI value of 288.3 ± 121.5 and mean percentage of CXCR4 stained cells was 47.75 ± 22.70%. A significant positive correlation ($r=0.538$, $p=0.031$) was found between SUV$_{\text{max}}$ and MFI values. However, no significant correlation was found between SUV$_{\text{max}}$ and the percentage-stained cells ($r=0.129$, $p=0.634$).

In patients of NSCLC- squamous cell variant (n = 54), the mean SUV$_{\text{max}}$ value was estimated to be 6.16 ± 2.14 (range 3.17–14.98; median = 5.63) which was lower than the SCLC and adenocarcinoma patients. A similar trend was seen in the mean MFI values (135.72 ± 80.11) and the mean percentage of CXCR4 positive cells (40.60 ± 21.4%). The IHC analysis revealed CXCR4 tumor positivity on the stained slides in 29/31 patients and the mean visual scoring was estimated to be 5.1 ± 2.7. A highly significant ($r=0.690$, $p=0.0001$) positive correlation was observed between SUV$_{\text{max}}$ and MFI values. Similarly, a significant correlation ($p<0.05$) was seen between SUV$_{\text{max}}$ and the percentage-stained cells ($r=0.296$; $p=0.030$) in NSCLC (squamous cell) only. No significant correlation between SUV$_{\text{max}}$ and MFI was noted in any other group of patients.

In the small group (n = 5) of 'not otherwise specified' (NOS) variant of NSCLC, the mean SUV$_{\text{max}}$ value was found to be 5.8 ± 1.5 (median = 5.5). The corresponding mean MFI value was found to be 159.8 ± 37.90 and the mean percentage of stained CXCR4 expressing cells was estimated to be 39.40 ± 20.60%. IHC analysis in this group did not reveal any histochemical evidence of the CXCR4 tumor positivity. No significant correlation between SUV$_{\text{max}}$ and MFI values only in the primary lung tumor in all the 94 (100.0%) patients. The mean SUV$_{\text{max}}$ value was found to be 5.23 ± 1.23 (median = 5.12) and the mean MFI and the percentage stained cells were estimated to be 60.64 ± 25.00 & 26.00 ± 16.3% respectively. The IHC analysis was performed in all the 5 patients of this group. CXCR4 tumor positivity on the stained slides was observed only in 2/5 patients. There was no significant correlation between SUV$_{\text{max}}$ and MFI values ($r=0.747$, $p=0.147$). Likewise, no significant correlation was seen between SUV$_{\text{max}}$ and the percent stained cells ($r=0.747$; $p=0.147$).

In the nutshell, $^{68}$Ga-Pentixafor PET/CT findings showed increased tracer uptake (SUV$_{\text{max}}$) in the primary lung tumor in all the 94 (100.0%) patients. And the tracer uptake varied as a function of the quantitative CXCR4 receptors' density and both decreased in the order viz. SCLC, NSCLC-adenocarcinoma, NSCLC-squamous, NOS and lung NETs respectively. On the other hand, the IHC results were inconsistent and the CXCR4 tumor positivity rate was observed to be 62% (37/60) only and did not show any correlation with the SUV$_{\text{max}}$ values in any of the LC sub-groups. However, for the percentage stained cells, a positive correlation ($p=0.05$) was observed with SUV$_{\text{max}}$ values only in the NSCLC-squamous cell group of patients.

Box and whisker plots analysis (Figure- 3A) demonstrated that the mean SUV$_{\text{max}}$ value was significantly ($p = 0.005$) higher in SCLC as compared to NSCLC group (including all the variants). Similarly, the mean SUV$_{\text{max}}$ value in SCLC was significantly higher than in the
squamous cell lung cancer patients. However, the mean SUV_{max} value in adenocarcinoma patients did not differ significantly from that observed in SCLC patients.

The ROC curve analysis estimated the cut-off SUV_{max} value of \(^{68}\text{Ga}-\text{Pentixafor}\) uptake as 7.24 in differentiating SCLC from NSCLC. The observed cut off value of 7.24 provided sensitivity and specificity of 87.5% and 72.0% respectively (Figure-3B). While a cut-off SUV_{max} value of 6.67 was estimated to differentiate the two most common NSCLC sub-types i.e. adenocarcinoma and squamous cell carcinoma with a sensitivity and specificity of 87.5% and 71.4% respectively (Figure-3C).

**Discussion**

The CXCR4/CXCL12 ‘receptor-ligand pair’ plays a prominent role in cell proliferation and metastasis in at-least 30 different human cancers [15, 16]. \(^{68}\text{Ga}-\text{Pentixafor}\) - a CXCR4 targeting radioligand allows in vivo visualization non-invasively of tumors expressing these receptors. The use of \(^{68}\text{Ga}-\text{Pentixafor}\) PET/CT imaging has proven the potential of this tracer in evaluating the whole-body disease burden of CXCR4 receptors in many haematological and solid human malignancies [17]. Further, high contrast PET images demonstrated by this tracer have led to the development of beta emitting \(^{90}\text{Y}/^{177}\text{Lu}\) Pentixather as a powerful \(^{68}\text{Ga}\) and \(^{90}\text{Y}/^{177}\text{Lu}\) theranostic pair [18–20]. This theranostic pair has been introduced successfully for the treatment of advanced stage multiple myeloma, lymphoma and leukaemia [21–23].

CXCR4 stromal cell derived 1-α factor is critical in cancer growth and metastasis. Typically, the rising activity of this factor in lymph nodes, bone, bone marrow, lung and liver has been reported to trigger the metastasis of CXCR4 expressing tumor cells [24–25]. CXCR4 receptors’ over-expression thus has been recognized as an adverse prognostic factor in various malignancies including lung cancer [26–28]. Therefore, \(^{68}\text{Ga}-\text{Pentixafor}\) PET/CT based in vivo whole-body quantification of CXCR4 receptors is viewed as a very promising diagnostic or therapeutic imaging biomarker in a variety of cancer patients [29–30].

In this study, we present \(^{68}\text{Ga}-\text{Pentixafor}\) PET/CT imaging results in 94 lung cancer patients and validation of the quantitative PET parameters with simultaneous tissue characterization and quantification of CXCR4 receptors’ density. To the best of our knowledge, this is the first study reporting the tracer uptake as a function of CXCR4 receptors’ density identified by IHC and FACS in primary lung cancer tissue of different histologic types. We observed that all sub-types of lung cancer showed increased tracer uptake in the primary lung lesions on \(^{68}\text{Ga}-\text{Pentixafor}\) PET, which was indicative of tumor CXCR4 over-expression. The highest CXCR4 expression was seen in SCLC, which is the most aggressive lung cancer sub-type characterized by rapid doubling time, high growth fraction and early development of metastatic spread [31]. CXCR4 activation is also linked to metastatic behaviour of cancer cells metastasizing to organs by invasive and migratory responses and adhesion to narrow stromal cells in SCLC [32, 33]. SCLC swiftly metastasizes to other organs and much more rapidly than NSCLC types. Hence, the finding of \(^{68}\text{Ga}-\text{Pentixafor}\) SUV_{max} and MFI values highlight higher CXCR4 expression in SCLC than that in NSCLC variants which in turn validates the specificity of in-vivo CXCR4 PET imaging by \(^{68}\text{Ga}-\text{Pentixafor}\).

Despite, the higher CXCR4 expression (MFI) and the tracer uptake (SUV_{max}) in SCLC, we did not find a significant correlation between these two parameters which is probably due to the small number of patients (n = 14) in this group. In an extensive meta-analysis of 24 studies and 2037 lung cancer patients, CXCR4 was not significantly related to the prognosis factors such as age, gender, tumor size, smoking, etc. [26]. However, these authors reported that CXCR4 expression correlated with some prognosis factors such as N-stage (N1, N2 vs. N0), M-stage (M1 vs. M0), tumor-stage, etc. It has been reported that \(^{68}\text{Ga}-\text{Pentixafor}\) PET/CT showed a higher CXCR4 receptors’ density (MFI = 142.0; SUV_{max} = 13.2) in a SCLC patient than in a patient (MFI = 120.0; SUV_{max} = 8.8) with NSCLC variant [28]. It was also observed that in the SCLC patient, \(^{18}\text{F}-\text{FDG}\) PET/CT showed SUV_{max} value of 8.0 as against the SUV_{max} value of 13.2 on \(^{68}\text{Ga}-\text{Pentixafor}\) PET/CT. And \(^{68}\text{Ga}-\text{Pentixafor}\) PET/CT picked up additional brain metastatic lesions in the NSCLC patient.

It is thus highlighted that \(^{68}\text{Ga}-\text{Pentixafor}\) PET/CT demonstrating higher tracer uptake (SUV_{max}) is supported by higher receptors’ density (MFI) in SCLC. The Receiver Operating Characteristic (ROC) curve analysis of \(^{68}\text{Ga}-\text{Pentixafor}\) SUV_{max} values provided a cut-off value of 7.24 to differentiate SCLC from NSCLC (sensitivity 87.5% and specificity 72.0%). However, no definitive trend for sensitivity and specificity with \(^{18}\text{F}-\text{FDG}\) PET/CT has been reported for this differentiation [34–35].

In a recent study, Buck et al. reported that a very high tracer (\(^{68}\text{Ga}-\text{Pentixafor}\)) uptake (SUV_{max}>12.0) was found in multiple myeloma (n = 113) followed by adrenocortical carcinoma (n = 30), mantle cell lymphoma (MCL, n = 20), adrenocortical adenoma (n = 6) and SCLC (n = 12) [17]. They concluded that these results may provided a roadmap to detect patients who may benefit from CXCR4 targeted therapies. The suitability of \(^{68}\text{Ga}-\text{Pentixafor}\) for non-invasive high contrast imaging of CXCR4 over-expressing cancers has been demonstrated
initially for haematological malignancies [36–40]. With the subsequent development of $^{177}$Lu-Pentixather as a therapeutic companion, the first CXCR4 targeted radiotheranostic concept has been translated into the clinic [21, 41]. An encouraging therapeutic response of $^{177}$Lu/$^{90}$Y-Pentixather for radioligand therapy (RLT) in advance stage multiple myeloma and other lymphoproliferative diseases have been reported [18–20]. The other potential therapeutic applications of this theranostic pair are being explored in prospective clinical trials.

In NSCLC and lung NETs, the mean $SUV_{max}$ and MFI values were lower than SCLC patients. Though, we did not find a significant correlation between these two parameters in SCLC, but the same exhibited a significant correlation in NSCLC variants (adenocarcinoma; n = 16; r = 0.538; p = 0.031; squamous cell; n = 54; r = 0.690; p = 0.0001). Similarly, a significant correlation ($p < 0.05$) was seen between $SUV_{max}$ values and the percentage-stained cells ($r = 0.296; p = 0.030$) in NSCLC-squamous cell patients. Therefore, NSCLC variants with the evidence of significant CXCR4 over-expression may also be considered for RLT using alpha and beta labelled CXCR4 targeting radionuclide theranostics. In a recent study, Watts et al. reported that $^{68}$Ga-Pentixafor PET/CT allows non-invasive assessment of CXCR4 expression in rare lung cancers i.e. haemangioendothelioma, sarcomatoid carcinoma and hemangiopericytoma and in lung metastasis cases [42]. The highest $SUV_{max}$ of 13.0 was noted in the case of haemangioendothelioma. Therefore, the lung cancer cases other than SCLC and NSCLC which express significant quantity of CXCR4 expression also holds great potential both for imaging and treatment using $^{68}$Ga-Pentixafor/$^{177}$Lu-Pentixather theranostic pair. The precision radiomolecular oncology using such targeted radiotheranostic approach challenging the classical statistical evidence based medicine has been reported [43]. $^{68}$Ga-Pentixafor PET/CT could be of special clinical significance in response assessment to CXCR4 based radiotherapeutics. And in a recent study, the varied physiological distribution of $^{68}$Ga-Pentixafor in spleen has been reported to be of great prognostic significance [44].

$^{68}$Ga-Pentixafor PET/CT scan findings indicated an increased tracer uptake ($SUV_{max}$= 5.23 ± 1.23) in all the 5 lung- NET patients. No significant correlation was seen between $SUV_{max}$ and the percent stained cells ($r=-0.293; p = 0.663$). There is only a single study in the literature by Werner et al., who have investigated the role of $^{68}$Ga-Pentixafor in imaging GEP NET tumors [45]. These authors compared the diagnostic performance of three tracers i.e. $^{18}$F-FDG, $^{68}$Ga-DOTATATE and $^{68}$Ga-Pentixafor in 12 GEP NET patients and found concordant (positive) findings between $^{68}$Ga-Pentixafor and $^{68}$Ga-DOTATATE in 4/5 poorly differentiated NETs. However, $^{68}$Ga-Pentixafor demonstrated superiority and picked up more number (n = 66) of metastatic lesions as compared to $^{68}$Ga-DOTATATE which detected only 12 lesions. In this regard, these authors reported that an increasing number of CXCR4 (+)/SSTR (-) metastasis were identified in patients with increasing tumor aggressiveness. The usefulness of $^{18}$F-FDG-PET/CT in poorly dedifferentiated NETs has been described previously. These NET variants pose a serious therapeutic challenge with the currently available $^{177}$Lu-DOTATATE therapy and thus, $^{177}$Lu-Pentixather RLT targeting CXCR4 receptors may be useful in NETs having poor SSTR expression.

Therefore, the demonstration of variable quantitative CXCR4 receptors' expression supported by the matching pattern of $^{68}$Ga-Pentixafor tissue uptake in different LC sub-types provides a convincing data for using this imaging modality for radio-theranostic applications. This may potentially supplement the existing data for inclusion and expanding CXCR4 -based radioligand therapies in LC beyond haematological malignancies.

**Declarations**

**Funding**

The research work was carried out using the grant by the Department of Science and Technology (DST), Government of India under the DST-FIST program (grant SR/FST/LSI-548/2012) for setting up the infrastructure (Automated chemistry module and other accessories and chemicals) required for the present work.

**Competing interests**

The authors have no other potential competing or conflict of interest relevant to this article.

**Authors contributions**

*All authors contributed to the study conception and design.* **Ankit Watts:** Radiopharmaceutical synthesis, quality control testing, PET/CT imaging, image reconstruction, image quantification, IHC/FACS analysis, sample preparation, data curation, manuscript writing and statistical analysis. **Baljinder Singh:** Study conceptualization, manuscript editing, grant/funding to carry out the research work, Supervision, Project administration **Harmandeep Singh:** Data Interpretation, PET biopsy, manuscript editing. **Bhagwant R Mittal:** Study Designing, Data Interpretation, formal analysis, PET biopsy, manuscript editing, Supervision. **Amanjit Bal:** Histopathological
Immunohistochemical analyses of the lung tissues, manuscript editing. **Harneet Kaur**: Manuscript writing/editing and statistical analysis. **Ninjit Dhanota**: Sample preparation, FACS analysis and data interpretation. **Sunil K Arora**: Sample preparation, FACS analysis and data interpretation, formal analysis, Supervision. **Digambar Behera**: Study Designing, Patients’ enrolment, clinical examination, bronchoscopy /biopsy, Supervision. *The first draft of the manuscript was written by [AW, BS] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.*

**Ethics approval:**

*This study was performed in line with the principles of the Declaration of Helsinki. Approval was (vide letter No. INT/IEC/2017/194 dated 23.08.2017) granted to the study as PhD project of the first author by the Institute Ethics Committee (IEC) of the Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India.*

**References**


Page 8/13


Tables

Table 1: Patients’ details and the quantitative results of \(68^{\text{Ga}}\)-Pentixafor PET/CT (SUV\(_{\text{max}}\)), FACS (MFI & Percent stained cells) and IHC analysis in all study subjects.
<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Sub-Type (number of patients)</th>
<th>Sex (Male: Female)</th>
<th>Mean age (years)</th>
<th>$^{68}$Ga-Pentixafor PET/CT Imaging</th>
<th>Quantitative parameters of FACS analysis (mean± S.D)</th>
<th>Immuno-histochemistry (IHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Non-small cell lung carcinoma (NSCLC)</em></td>
<td>Squamous cell (n=54)</td>
<td>49M: 5F</td>
<td>62.6± 9.5 (range=39-82)</td>
<td>6.2± 2.14</td>
<td>135.7± 80.1</td>
<td>5.1± 2.71 (n=29)</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma (n=16)</td>
<td>7M: 9F</td>
<td>56.6± 8.7 (range=47-70)</td>
<td>8.0± 1.90</td>
<td>288.3± 121.50</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>NOS (n=5)</td>
<td>4M: 1F</td>
<td>61.2± 7.0 (range=52-59)</td>
<td>5.8± 1.5</td>
<td>159.80± 37.90</td>
<td>39.40± 20.60 (n=5)</td>
</tr>
<tr>
<td><em>Small cell lung carcinoma (SCLC)</em></td>
<td>SCLC (n=14)</td>
<td>13M: 1F</td>
<td>62.5± 7.5 (range=50-75)</td>
<td>10.30± 5.0</td>
<td>349.0± 98.5</td>
<td>45.60± 22.35 (n=8)</td>
</tr>
<tr>
<td><em>Neuroendocrine tumors (NETs)</em></td>
<td>NET-primary lung (n=5)</td>
<td>5M: 0F</td>
<td>50.0± 8.5 (range=36-57)</td>
<td>5.23± 1.23</td>
<td>60.64± 25.0</td>
<td>26.0± 16.3 (n=2)</td>
</tr>
</tbody>
</table>

**Figures**
Figure 1

$^{68}$Ga-Pentixafor MIP and axial-fused PET/CT images are presented comprehensively in Figure-1 in one patient each of SCLC (a, b) with $\text{SUV}_{\text{max}} = 13.2$, NSCLC-adenocarcinoma (e,f) with $\text{SUV}_{\text{max}} = 12.2$, NSCLC-squamous (i,j) with $\text{SUV}_{\text{max}} = 7.2$ and lung NET (m, n) with $\text{SUV}_{\text{max}} = 5.2$ respectively. The corresponding FACS histograms depicting the quantitative CXCR4 receptors expression (MFI) and percent-unstained and stained population of CXCR4 expressing cells in these patients are also given in Figure -1 as c,d (MFI=414.0), g,h (MFI=289.0) , k,l (MFI=99.0) and o,p (MFI=100.0) respectively. The unstained population of cells was used for gating to calculate the percentage of stained cells and intensity (MFI).
**Figure 2**

Immunohistochemistry in paraffin embedded lung tissue in a patient of NSCLC - squamous cell carcinoma using anti-CXCR4 antibody showing 3+ CXCR4 intensity and >50% stained tumor cells.
Figure 3

The comparative Box and whisker plots showing differing $SUV_{max}$ values in different histological types of NSCLC and SCLC (a). The ROC-curve analysis (at $SUV_{max}$ cut off =7.24) provided sensitivity (x-axis) and specificity (y-axis) of 85.7% and 78.1% for differentiating SCLC versus NSCLC (b). Similar ROC analysis provided sensitivity and specificity of 87.5% and 71.4% (at $SUV_{max}$ cut off =6.67) for differentiating NSCLC- adenocarcinoma and squamous cell variants (c).