Performance characteristics of ^{18}F-fluciclovine positron emission tomography/computed tomography prior to retroperitoneal lymph node dissection

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Abstract

Introduction: We aimed to determine whether anti-1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid (^{18}F-fluciclovine) positron emission tomography/computed tomography (PET/CT) can accurately detect residual non-seminomatous germ cell tumor (NSGCT) prior to retroperitoneal lymph node dissection (RPLND). There is no reliable way to differentiate between fibrosis/necrosis, teratoma, and viable germ cell tumor in patients receiving post-chemotherapy RPLND. Functional imaging, including ^{18}F-fludeoxyglucose (18F-FDG) PET/CT, has been disappointing. Due to the need for better imaging modalities, our prospective, pilot study aims to investigate the accuracy of ^{18}F-fluciclovine PET/CT in detecting residual tumor prior to RPLND.

Methods: From March 2018 to May 2019, 10 eligible patients underwent preoperative ^{18}F-fluciclovine PET/CT prior to undergoing bilateral, full-template RPLND or excision of mass (for one re-do RPLND) in a prospective, phase 2 study. Correlation between ^{18}F-fluciclovine PET/CT and RPLND pathology were evaluated on a per-patient level.

Results: A total of 10 patients (mean age 29±7.6 years) underwent ^{18}F-fluciclovine PET/CT prior to surgery. Nine of 10 patients received chemotherapy prior to RPLND. Correlation between ^{18}F-fluciclovine PET/CT and RPLND pathology was seen in 3/10 (30%) patients. Five of 10 patients (50%) with negative ^{18}F-fluciclovine PET/CT were found to have residual disease/teratoma on RPLND. Compared to the reference standard of RPLND, ^{18}F-fluciclovine PET/CT demonstrated 29% sensitivity and 33% specificity. No patients experienced any adverse events due to ^{18}F-fluciclovine PET/CT.
Conclusions: Despite a different mechanism of action from ^{18}F-FDG, ^{18}F-fluciclovine has low sensitivity and specificity for residual teratoma in the retroperitoneum.

Introduction

Testicular germ cell tumors (TGCT) represent the most common solid malignancy in young men and account for about 1% of cancers in men(1). Many of the controversies regarding management of non-seminomatous germ cell tumors (NSGCT) stem from discrepancy between clinical staging and pathologic staging and the fact that current imaging modalities are unable to accurately identify extra-testicular disease or distinguish between post-chemotherapy necrosis/fibrosis versus viable germ cell tumor and/or teratoma. Contrast-enhanced axial imaging CT is the standard imaging modality to identify retroperitoneal lymph nodes. 'Positive' or 'suspicious' retroperitoneal lymph nodes are defined by size cutoffs, for which there is no standard definition. A 1-cm cut-point is commonly used but lacks sensitivity(3–5). From series reporting on 'failure' of active surveillance, we can surmise that CT scans of the abdomen are under-staging patients in about 20-30% of cases(6). To improve upon staging, metabolic imaging with the use of 18F fludeoxyglucose (18F FDG) Positron Emission Tomography/Computed Tomography (PET/CT) has been investigated for NSGCT but has not been shown to be effective in this setting as it is unable to distinguish between mature teratoma and necrosis/fibrosis due to little or no uptake of FDG with either histology(7–10). Due to these limitations, the National Comprehensive Cancer Network guidelines do not recommend the use of 18F FDG PET/CT either in the primary or the post-chemotherapy setting for NSGCT.

Given the significant need for new imaging modalities to improve management of NSGCT, both in the primary setting and in the post-chemotherapy setting to determine response to therapy, we designed a prospective study using anti-1-amino-3-18F fluorocyclobutane-1-carboxylic acid, a synthetic radiolabeled leucine amino acid analog which is commonly referred to as 18F fluciclovine (Axumin™, Blue Earth Diagnostics, Ltd. Oxford, UK). This agent allows for cancer detection utilizing mechanisms governing amino acid uptake, a process which is elevated in malignant tumors. In May 2016, 18F fluciclovine was FDA-approved for use with PET/CT in men with suspected prostate cancer recurrence(11,12). Given its different mechanism

of action compared to FDG, we hypothesize that it may improve diagnostic accuracy in NSGCT, in particular its ability to distinguish teratoma from benign necrosis. Our prospective study aims to investigate the accuracy of 18F fluciclovine PET/CT in detecting residual tumor and how this correlates with histopathologic outcomes in patients with NSGCT undergoing primary or post-chemo RPLND.

Methods

Study design and population

From March 2018 to May 2019, 16 patients were screened for enrollment in this prospective phase II study after approval by the local institutional review board with informed consent and Health Insurance Portability and Accountability Act compliance. Study inclusion criteria included patients with histologically confirmed NSGCT after orchiectomy who were scheduled to undergo either primary RPLND or NSGCT that was previously treated with cisplatin-based chemotherapy who were scheduled to undergo post-chemotherapy RPLND (ClinicalTrials.gov Identifier: NCT03426865). Patients must have had measurable disease within their retroperitoneum. A total of 10 eligible patients were enrolled and underwent pre-operative 18F fluciclovine PET/CT prior to undergoing retroperitoneal surgery (Figure 1). Patients were excluded if they were under 18 years of age, had less than 6 months of life expectancy, had poor performance status, liver failure or known allergic or hypersensitivity reactions to previously administered radiopharmaceuticals of similar composition.

The experimental 18F fluciclovine PET/CT was required to be completed within 30 days prior to RPLND. A total of 10 patients underwent 18F fluciclovine PET/CT with a mean (standard deviation) of 7.3 ± 4.7 days prior to retroperitoneal surgery. Nine of ten patients underwent RPLND after completion of chemotherapy, while one patient underwent primary RPLND. Of the nine patients who underwent PC-RPLND, 7 had residual tumor after completion of chemotherapy and underwent 18F fluciclovine PET a mean 55 days after chemotherapy. Two patients had late recurrence and underwent 18F fluciclovine PET at 172 and 872 days after completion of chemotherapy. All patients had normal serum HCG and AFP following completion of chemotherapy and/or prior to PC-RPLND.

Labs and correlative radiology, as directed per clinical care, were required within 60 days prior to 18F fluciclovine PET/CT. 18F fluciclovine was administered with a 1-2 minute infusion for a mean dose of 10.7 ± 0.63 mCi. After approximately 3-5 minutes of uptake time, the patient was positioned supine in the PET/CT scanner for standard whole-body PET/CT scan from mid-thigh to skull base.

Surgeons were blinded to the results of 18F fluciclovine PET/CT scan prior to surgery to ensure no deviations from standard of care based on PET/CT results. Nine patients underwent bilateral full template RPLND with nerve sparing when possible. In one case, re-operative

retroperitoneal mass excision was performed following a prior RPLND at an outside institution. All radiographically visible extra-retroperitoneal sites of disease were resected when >1 cm.

Data analysis

18F fluciclovine PET/CT images were interpreted as positive or negative by multiple board-certified radiologist and nuclear medicine physicians during the study. At the conclusion of the study, a single board-certified nuclear medicine physician with expertise in urologic nuclear medicine who was blinded to the pathologic results re-review all 10 studies. In the absence of established interpretation criteria, for qualitative baseline assessment, focal 18F fluciclovine uptake less than blood pool was considered negative and focal uptake more than bone marrow was considered as positive. Repeat analysis of the data was also undertaken by considering regions of focal uptake more than blood pool (rather than bone marrow) to be consider sufficient for positivity.

For quantitative analysis purposes, the SUVmax (representing the single voxel with the highest activity concentration) was determined using MimVista (version 6, Cleveland, OH) analysis workstation for the 18F fluciclovine PET/CT. The specimens were evaluated by four pathologists with sub-specialty training in urologic pathology. Correlation between 18F fluciclovine PET/CT and RPLND pathology were evaluated on a per patient level. In order to evaluate on a 'per packet' level, we aimed to send each lymph node packet separately to pathology, however the post-chemotherapy tissue changes and residual masses made defining strict borders between lymph node packets impractical.

Results

The mean (interquartile range 25-75%) for retroperitoneal lesions SUVmax was 4.2 (3.0 – 3.7), blood pool SUVmax was 1.4 (1.2 – 1.6) and lumbar spine bone marrow SUVmax was 4.8 (4.2 – 5.8). Baseline patient characteristics are shown in Table 1. Nine out of ten patients had undergone prior chemotherapy with one patient proceeding directly to surgery. The mean size of the largest retroperitoneal mass prior to RPLND was 3.6cm (range 0.8-12.3cm). RPLND histology was negative for residual GCT or teratoma in 3 cases (30%), positive for teratoma in 5 cases (50%), and positive for GCT (seminoma) in 1 cases (10%), and positive for embryonal rhabodomyosarcoma in 1 case (10%).

Diagnostic performance of 18F-fluciclovine PET/CT

Correlation between 18F fluciclovine PET/CT and RPLND pathology was seen in 3/10 (30%) of patients. Patients with correlation of retroperitoneal histology and 18F fluciclovine consisted of one patient with positive 18F fluciclovine PET/CT and embryonal rhabdomyosarcoma detected on resection of the recurrent retroperitoneal mass, one patient with positive retroperitoneal and pelvic nodes with teratoma on final pathology, and one patient with residual retroperitoneal masses on cross sectional imaging with negative 18F fluciclovine PET/CT and fibrosis in the

final specimen with no evidence of germ cell tumor or teratoma (Table 2). Five patients (50%) with negative 18F fluciclovine PET/CT were found to have residual disease on RPLND, with 1 patient with seminoma and 4 patients with teratoma. Two patients (20%) had positive 18F fluciclovine PET/CT but only benign findings on final pathology. Compared to the reference standard of RPLND, 18F fluciclovine PET/CT has 29% sensitivity, 33% specificity, positive predictive value of 50% and negative predictive value of 17% in these 10 patients.
Repeat analysis of the 18F fluciclovine PET/CT results using a wider criteria for positive uptake wherein regions of focal uptake more than blood pool (rather than bone marrow) are considered sufficient for positivity improves correlation between retroperitoneal histology and 18F fluciclovine (Figure 2). Using this cutoff, there was an increase from four to eight positive 18F fluciclovine PET/CT scans with an additional patient with seminoma and three patients with teratoma detected, demonstrating a correlation of 7/10 (70%). Compared to the reference standard of RPLND, 18F fluciclovine PET/CT with the wider cut-off demonstrates 86% sensitivity, 33% specificity, positive predictive value of 75% and negative predictive value of 50% in these 10 patients.

Adverse events
No patients experience any adverse events as a direct result of undergoing an 18F fluciclovine PET/CT.

Discussion
18F Fluciclovine is a novel radioactive diagnostic agent that is FDA-approved for use with PET/CT with promising findings in men with suspected prostate cancer recurrence(11,12). Given the different mechanism of action compared to standard 18F FDG PET/CT, we were hopeful that it may be able to distinguish teratoma from benign necrosis. However, in our prospective study utilizing 18F fluciclovine in patients with residual masses after chemotherapy prior to RPLND, there was poor sensitivity and specificity for detection of teratoma from fibrosis/necrosis in the retroperitoneum. While cure rates are generally high with chemotherapy alone, a significant portion of patients will have viable germ cell tumor left in their retroperitoneum and/or may have teratoma histology in the retroperitoneum that is chemotherapy-resistant(13). Similar to existing literature on the histology of residual mass following chemotherapy(14,15), our study found necrosis/fibrosis in 30%, residual GCT in 20% and teratoma in 50% of the patients.

In contrast to FDG-PET, 18F fluciclovine PET uses a synthetic radiolabeled leucine amino acid analog to identify cells that have increased uptake of amino acids, a process generally seen in malignancy, given increased need for amino acids for protein synthesis and cell division(16). Therefore radiolabeling of amino acids is a potentially useful imaging technique that may be more tumor specific(17). In addition to the FDA-approved usage in men with suspected prostate cancer recurrence, it is currently being investigated in a number of different malignancies. Ulaner et al demonstrated that 18F fluciclovine PET/CT had excellent sensitivity

by detecting locally advanced breast cancers and axillary nodal metastases(18). 18F fluciclovine had similar overall detection of disease as FDG but demonstrated different performance characteristics, suggesting that different properties were measured within the tumor. In a small phase IIa trial, Kondo et al examined the use of 18F fluciclovine in patients with malignant glioma prior to brain tumor resection, a situation where FDG-PET/CT has limited sensitivity(19,20). The authors found that 18F fluciclovine identified tumors in all five patients and delineated wider regions of tumor extent with two histopathologically confirmed tumors located in regions that were positive on 18F fluciclovine PET/CT but not on contrast-enhanced MRI(19).

In our prospective study of 10 patients, 18F fluciclovine PET/CT only had 29% sensitivity and 33% specificity, missing 4 patients with teratoma and 1 patient with residual GCT and was falsely positive in 2 patients with only necrosis/fibrosis on final pathology using our study criteria for positive retroperitoneal pathology on imaging. However, 4/10 (40%) patients in the study with retroperitoneal pathology had mild uptake above blood pool levels but less than bone marrow levels. If these patients are considered to be positive on 18F fluciclovine PET/CT, the sensitivity improves to 86% without affecting specificity. As the existing threshold criteria for positivity were established using prostate cancer metastasis, adjusting the thresholds for testicular cancer may lead to improved ability to identify residual GCT and teratoma. This post-hoc analysis would need to be verified in a prospective manner. It should also be noted that one patient with positive 18F fluciclovine PET/CT after prior RPLND demonstrated embryonal rhabdomyosarcoma on resection of the recurrent retroperitoneal mass. Whether this is an isolated finding or a potential role for 18F fluciclovine PET/CT in recurrent disease needs further exploration. We were also able to demonstrate that 18F fluciclovine PET/CT was well tolerated with no adverse events attributable to the agents. Furthermore, no patients were delayed from undergoing RPLND as a result of undergoing 18F fluciclovine PET/CT.

The strength of this study include the prospective clinical trial design which decreases study biases. All patients were already scheduled for RPLND and the surgeons and pathologists were blinded to the results of the 18F fluciclovine PET/CT. The 18F fluciclovine PET/CT were re-reviewed by a single radiologist and nuclear medicine physician who was blinded to the pathology findings. The study's obvious limitation is the small population which affected our ability to adequately compare the characteristics of the two groups with positive and negative 18F fluciclovine PET/CTs.

Conclusions

Despite a different mechanism of action compared to 18F FDG PET/CT, 18F fluciclovine PET/CT also demonstrates poor sensitivity and specificity for teratoma. While further experience with this imaging modality for testis cancer may help improve performance characteristics, currently, it does not appear to be useful at helping guide further management of NSGCT in the post-chemotherapy setting.

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Figures and Tables

Fig. 1. Study flowchart. BEP: bleomycin, etoposide, platinum; CT: computed tomography; NSGCT: non-seminomatous germ cell tumor; PET: positron emission tomography; RPLND: retroperitoneal lymph node dissection; VDC/IE/VAC: vincristine/doxorubicin/cyclophosphamide

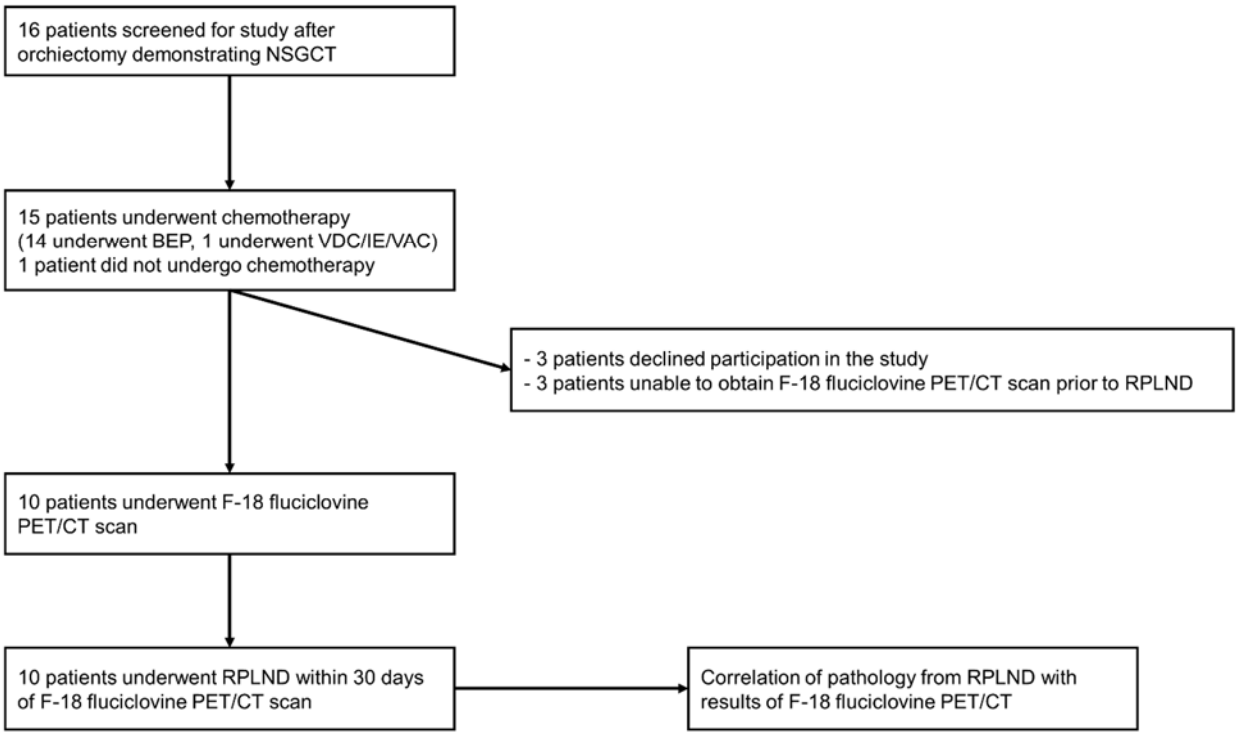

Fig. 2.

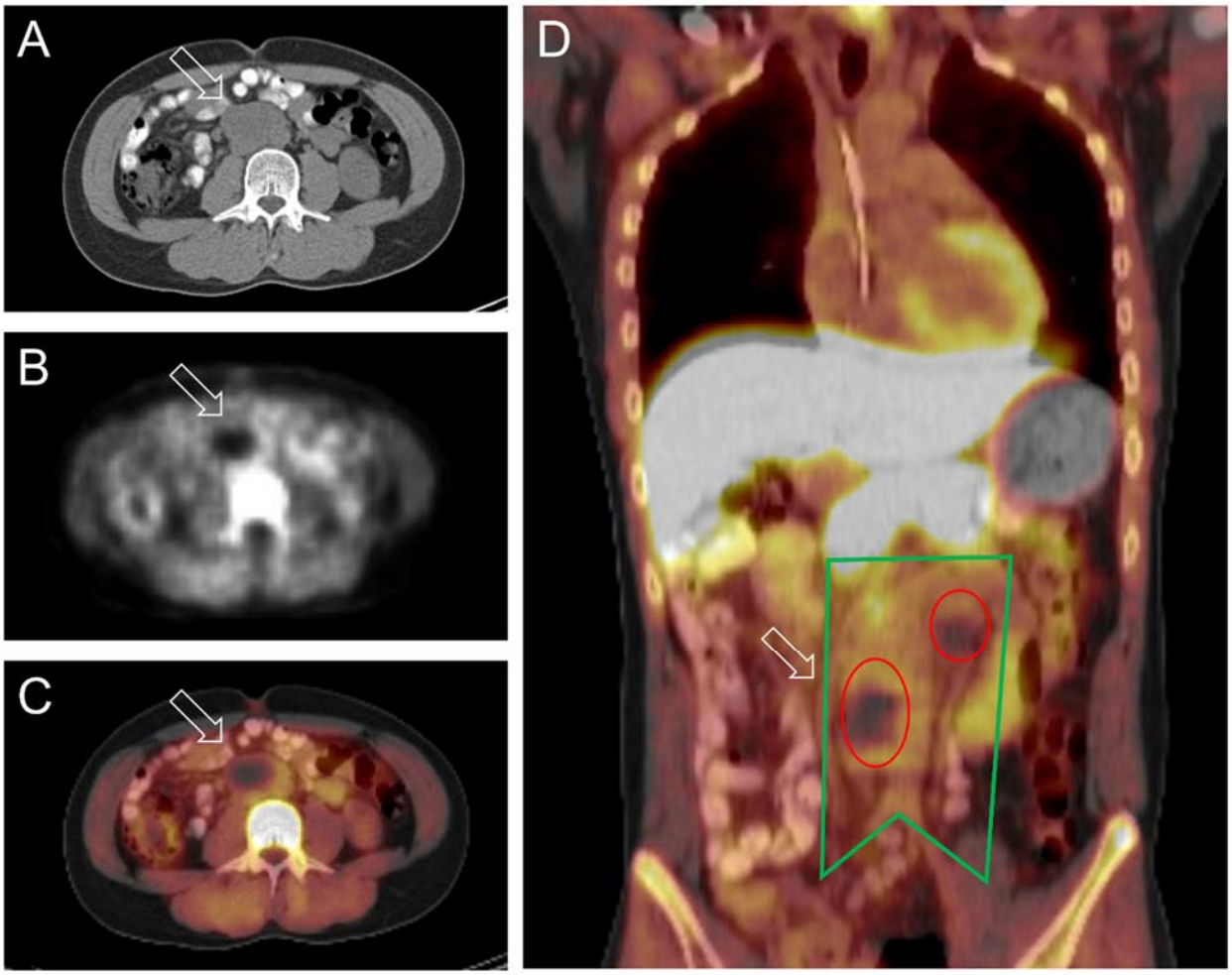

Table 1. Patient characteristics	
Characteristic	**N=10**
Age (yrs), mean (SD)	29 (7.6)
Tumor markers, median (IQR)	
Pre-orchiectomy	
LDH (u/L)	300 (257–672)
AFP (μg/L)	34.5 (16.1–298.3)
HCG (u/L)	332.0 (22.5–2137.3)
Post-orchiectomy	
LDH (u/L)	275 (166–474)
AFP (μg/L)	4.0 (2.4–5812.5)
HCG (u/L)	40 (6.5–1252.0)
Post-chemotherapy	
LDH (u/L)	167 (197–307)
AFP (μg/L)	4.4 (2.9–8.3)
HCG (u/L)	2.0 (2.0–2.0)
Post-RPLND	
LDH (u/L)	169 (160–195)
AFP (μg/L)	3.2 (1.3–4.1)
HCG (u/L)	2.0 (2.0–2.0)
Orchiectomy histology	
Patient #1	60% Embryonal sarcinoma, 35% Choriocarcinoma, 5% Yolk sac
Patient #2	95% Embryonal rhabdomyosarcoma and PNET 5% seminoma
Patient #3	50% Embryonal carcinoma, 50% Teratoma
Patient #4	95% Seminoma, 5% Embryonal carcinoma
Patient #5	100% Intratubular seminoma
Patient #6	79% Embryonal carcinoma, 20% Yolk Sac, 1% Choriocarcinoma
Patient #7	60% Seminoma, 30% Embryonal carcinoma,

	9% Teratoma, 1% Yolk sac
Patient #8	60% Teratoma, 40% Yolk sac
Patient #9	75% Embryonal carcinoma, 20% Choriocarcinoma, 5% Yolk sac
Patient #10	40% Embryonal carcinoma, 30% Yolk Sac, 1 % Choriocarcinoma, 29% Teratoma
Clinical stage group, n (%)	
Stage I	2 (20)
Stage II	2 (20)
Stage III	6 (60)
IGCCCG risk group, n (%)	
Good	6 (60)
Intermediate	3 (30)
Poor	1 (10)
Chemotherapy regimen, n (%)	
None	1 (10)
BEP	8 (80)
VDC	1 (10)
Positive pre-op ^{18}F-fluciclovine-PET/CT, n (%)	
No	7 (70)
Yes	3 (10)
Residual lesion histology, n (%)	
Necrosis and fibrosis	3 (30)
Teratoma	5 (50)
Seminoma	1 (10)
Embryonal rhabdomyosarcoma	1 (10)

AFP: alpha-fetoprotein; BEP: bleomycin, etoposide, platinum; HCG: beta human chorionic gonadotropin; IGCCCG: International Germ Cell Cancer Collaborative Group;
IQR: interquartile range; LDH: lactic acid dehydrogenase; PET/CT: positron emission tomography/computed tomography; RPLND: retroperitoneal lymph node dissection;
VDC/IE/VAC: vincristine/doxorubicin/cyclophosphamide.

Table 2. Correlation between ^{18}F-fluciclovine PET/CT and pathology				
Patient	**Size of largest post-chemotherapy LN**	**18F- fluciclovine PET/CT**	**Pathology**	**Correlation between PET/CT and pathology**
Patient #1	4.3 cm para-aortic LN	Negative	2/5 LNs from left common iliac positive for teratoma	No
Patient #2*	12.3 cm right retroperitoneal mass	Positive – Right retroperitoneal mass with peripheral tracer uptake	Right retroperitoneal mass positive for embryonal rhabdomyosarcoma	Yes
Patient #3	2.9 cm left para-aortic LN	Positive – multiple left sided retroperitoneal nodes and bilateral external iliac nodes	4/7 para-aortic LN positive for teratoma 4/13 interaortocaval LN positive for teratoma 1 spermatic cord node positive for teratoma	Yes
Patient #4	2.5 cm para-aortic LN	Positive – left para-aortic LN	All LN benign	No
Patient #5	2.1 cm aortocaval LN	Negative	All LN benign	Yes
Patient #6	1.6 cm left para-aortic LN	Negative	7/19 para- and pre-aortic LN positive for teratoma	No
Patient #7	8 mm aortocaval LN	Negative	1/16 Aortocaval LN positive for seminoma	No
Patient #8	3.4 cm para-aortic LN	Positive – right external iliac node	All LN benign	No
Patient #9	4.5 cm left para-aortic LN	Negative	4/9 Interaortocaval LN positive for teratoma	No

			4/5 para-aortic LN positive for teratoma 1/1 right hilar LN positive for teratoma	
Patient #10	2.3 cm aortocaval LN	Negative	1/8 Interaortocaval LN positive for teratoma	No

*This patient underwent RPLND two years prior with newly diagnosed recurrent right retroperitoneal mass. LN: lymph node; PET/CT: positron emission tomography/computed tomography; RPLND: retroperitoneal lymph node dissection.