Evaluation of Prostate Cancer with Radiolabeled Amino Acid Analogs

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Conventional imaging of prostate cancer has limitations related to the frequently indolent biology of the disease. PET is a functional imaging method that can exploit various aspects of tumor biology to enable greater detection of prostate cancer than can be provided by morphologic imaging alone. Radiotracers that are in use or under investigation for targeting salient features of prostate cancer include those directed to glucose, choline, acetate, prostate-specific membrane antigen, bombesin, and amino acids. The tumor imaging features of this last class of radiotracers mirror the upregulation of transmembrane amino acid transport that is necessary in carcinomas because of increased amino acid use for energy requirements and protein synthesis. Natural and synthetic amino acids radiolabeled for PET imaging have been investigated in prostate cancer patients. Early work with naturally occurring amino acid–derived radiotracers, such as L-^{11}C-methionine and L-1-^{11}C-5-hydroxytryptophan, demonstrated promising results, including greater sensitivity than ^{18}F-FDG for intraprostatic and extraprostatic cancer detection. However, limitations with naturally occurring amino acid–derived compounds, including metabolism of the radiotracer itself, led to the development of synthetic amino acid radiotracers, which are not metabolized and therefore more accurately reflect transmembrane amino acid transport. Of the synthetic amino acid–derived PET radiotracers, anti-1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid (^{18}F-FACBC or ^{18}F-fluciclovine) has undergone the most promising translation to human use, including the availability of simplified radiosynthesis. Several studies have indicated advantageous biodistribution in the abdomen and pelvis with little renal excretion and bladder activity—characteristics beneficial for prostate cancer imaging. Studies have demonstrated improved lesion detection and diagnostic performance of ^{18}F-fluciclovine in comparison with conventional imaging, especially for recurrent prostate cancer, although issues with nonspecific uptake limit the potential role of ^{18}F-fluciclovine in the diagnosis of primary prostate cancer. Although work is ongoing, recently published intrapatient comparisons of ^{18}F-fluciclovine with ^{11}C-choline reported higher overall diagnostic performance of the former, especially for the detection of disease relapse. This review is aimed at providing a detailed overview of amino acid–derived PET compounds that have been studied for use in prostate cancer imaging.

Amino acids serve many physiologic roles, including acting as the basic building blocks of protein, as components in energy metabolism, and as precursors of bioactive molecules (*1,2*). Amino acid transport is upregulated in carcinomas because of increased amino acid use for energy requirements and protein synthesis (*2–4*). Therefore, the molecular imaging of amino acid activity is an attractive target, especially in situations in which ^{18}F-FDG PET has limitations, such as in the detection of prostate cancer (*5–7*). Amino acid–based radiotracer cancer imaging with both naturally occurring and synthetic radiolabeled amino acids chiefly reflects substrate transport across the tumor cell membrane (*1,2,8*).

Amino acids are transported across plasma membranes by proteins that are encoded by members of the solute carrier family of genes (*1*). Specific amino acid transporters that are overexpressed in prostate cancer include system L amino acid transporters (LAT1, LAT3, and LAT4), system alanine-serine-cysteine transporters (ASCT1 and ASCT2), the system A sodium-coupled neutral amino acid transporter, system X^-c, system $B^{0,+}$, and proton-assisted transporters (PAT1 and PAT4) (*9–18*). Of these amino acid transporters, ASCT2 and LAT1 appear to be the most important in cancer metabolism and have been described as "partners in crime" (*3,10*). LAT1 expression in prostate cancer specimens has been correlated with poor survival and an elevated Gleason score (*13,14*). ASCT2 expression has also been associated with more aggressive behavior of prostate cancer, and the expression of both ASCT2 and LAT3 has been shown to be stimulated by androgen signaling in androgen-dependent prostate cancer (*19–21*).

In many cancers, including prostate cancer, a metabolic shift occurs when glutamine is used as an alternative energy source, instead of glucose (*4,22*). Targeting ASCT2 transport of glutamine inhibits prostate cancer growth in vitro and in PC-3 xenografts in nude mice (*22*). Changes in glutamine metabolic activity in prostate cancer appear to be related to v-*myc* avian myelocytomatosis viral oncogene homolog (*MYC*) protein overexpression, and both leucine and glutamine are important factors in mammalian target of rapamycin feedback signaling in cancer (*1,10,20,22–24*). Interestingly, preclinical data have suggested that metformin-induced suppression of glucose oxidation in tandem with glutamine inhibition reduces cellular proliferation in prostate cancer (*25*).

INVESTIGATIONS WITH RADIOLABELED NATURALLY OCCURRING AMINO ACIDS

Radiolabeled naturally occurring amino acids, such as L-^{11}C-methionine (^{11}C-MET) and L-1-^{11}C-5-hydroxytryptophan, were the first radiotracers used in amino acid–based molecular imaging of prostate cancer (26–29).

A pharmacokinetic study was performed on 10 patients who had castration-resistant cancer and progressive or new lesions on bone scans and who underwent PET imaging with ^{11}C-MET and ^{18}F-FDG (26). Higher uptake in index skeletal lesions with ^{11}C-MET than with ^{18}F-FDG, more rapid blood clearance of ^{11}C-MET, and lower (potentially interfering) urinary excretion of ^{11}C-MET were observed. In a subsequent study of 12 patients with rising prostate-specific antigen (PSA) levels and progressive disease, ^{11}C-MET was noted to be more sensitive than ^{18}F-FDG: ^{11}C-MET and ^{18}F-FDG detected 72.1% (251/348) and 48% (167/348) of bone and soft-tissue lesions, respectively (27). Figure 1 shows an example of ^{11}C-MET PET imaging of prostate cancer.

In a study of primary prostate cancer, a cohort of 20 subjects with mean PSA levels of 9.36 ng/mL and negative repeat biopsies underwent ^{11}C-MET PET. Suspect ^{11}C-MET uptake was noted in 75% of subjects (15/20), 46.7% of whom (7/15) had prostate cancer confirmed by ^{11}C-MET–guided biopsy (28). However, the fact that 8 subjects with focal ^{11}C-MET uptake and negative guided biopsies had either benign prostatic hyperplasia (6/8) or chronic prostatitis (2/8) demonstrates a limitation in the evaluation of primary prostate cancer. In a more recent study, 20 patients with suspected primary prostate cancer underwent both ^{11}C-MET and dual-phase (60- and 120-min) ^{18}F-FDG PET/CT (30). Although for a Gleason score of 8 or greater there was no significant difference between ^{11}C-MET PET and ^{18}F-FDG PET, ^{11}C-MET had greater sensitivity than ^{18}F-FDG for the detection of cancers with Gleason scores of 6 and 7.

The neurotransmitter amine precursor 5-hydroxytryptophan has also been studied because neuroendocrine differentiation in prostatic adenocarcinoma is linked to high-grade and refractory disease (31,32). A report on L-1-^{11}C-5-hydroxytryptophan in a cohort of 10 subjects with castration-resistant prostate cancer showed that L-1-^{11}C-5-hydroxytryptophan PET may be useful in the diagnosis and monitoring of treatment of metastatic castration-resistant prostatic adenocarcinoma (29).

SYNTHETIC OR NON–NATURALLY OCCURRING PET RADIOTRACERS

Early work with radiolabeled naturally derived amino acid PET radiotracers set the stage for the investigation of synthetic or non–naturally occurring amino acid PET radiotracers for the imaging of prostate cancer. The advantages of these radiotracers include the potential for radiolabeling with ^{18}F, simplified radiosynthesis, and more straightforward kinetics because of the lack of accumulation of metabolites (1).

The most comprehensive work to date with a synthetic amino acid PET radiotracer for prostate cancer imaging has been conducted with *anti*-1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid (^{18}F-FACBC or ^{18}F-fluciclovine) (33). (The standard international nomenclature for this radiotracer is ^{18}F-fluciclovine; therefore, ^{18}F-fluciclovine is used in this review.) ^{18}F-labeled 1-amino-cycloalkane-1-carboxylic acids such as ^{18}F-fluciclovine are a class of α,α-dialkyl amino acids that have a fluorine atom on the side chain, that are covalently bonded to each other to form a cyclic ring, and that are neither metabolized nor incorporated into proteins (3,12,34–36).

Initial preclinical experiments with ^{18}F-fluciclovine reported in vitro uptake in DU145 prostate carcinoma cells and that radiotracer transport was most like that of leucine via system L, especially LAT1 (34,37). Subsequent studies demonstrated that system alanine-serine-cysteine transporters—specifically, ASCT2—play the largest role in ^{18}F-fluciclovine transport; however, in a more acidic intratumoral environment, LAT1 transport is relatively elevated (12,35,38). Ono postulated that as a tumor progresses into castration resistance, LAT1 transport of ^{18}F-fluciclovine becomes more active (38). The predominance of ASCT2-mediated cellular influx and efflux suggests that ^{18}F-fluciclovine transport is more similar to that of glutamine than to that of leucine (39). In vitro studies demonstrated that ^{18}F-fluciclovine uptake is enhanced in LNCaP (androgen receptor–positive) but not DU145 (androgen receptor–negative) cell lines in response to androgen stimulation and that, in both prostate cancer cell lines, relative ^{18}F-fluciclovine uptake is greater than that of methionine, choline, and acetate (21). Work with a rat orthotopic prostate cancer model showed that, compared with ^{18}F-FDG, ^{18}F-fluciclovine better visualized the tumor with a significantly lower level of accumulation in the bladder (34).

Human dosimetry studies with ^{18}F-fluciclovine described a commonality of biodistribution with other amino acid radiotracers, such as ^{11}C-MET (40–42). Physiologic ^{18}F-fluciclovine activity shows the highest intensity in the pancreas and liver; moderate, heterogeneous intensity in the marrow, salivary glands, lymphoid tissue, and pituitary; and variable intensity in the bowel (Fig. 2). Brain parenchyma activity is minimal, and in most patients, bladder activity is absent or mild (43). Compared with ^{11}C-MET, ^{18}F-fluciclovine seems to have more rapid pancreatic clearance and comparatively lower levels of renal and bladder activity.

The initial visualization of metastatic prostate cancer nodal disease with ^{18}F-fluciclovine took place fortuitously during a study of renal lesions (44). A subsequent pilot study in 9 patients with primary prostate carcinoma and 6 patients with suspected recurrence reported promising results, including the visualization of the tumor despite negative ^{111}In-capromab-pendetide findings (45). Time–activity curves typically peak at 5–20 min, with subsequent
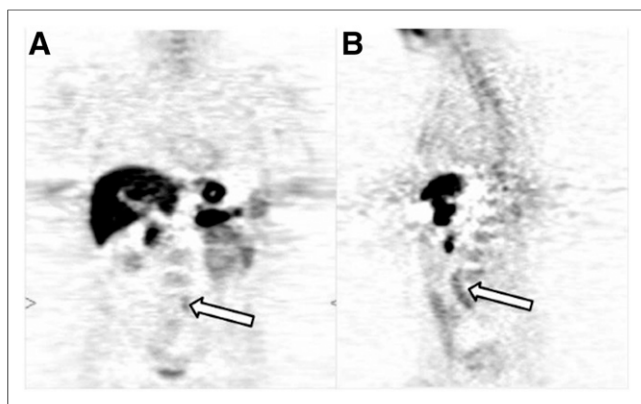

FIGURE 1. Coronal (A) and sagittal (B) PET images demonstrating ^{11}C-MET uptake in prostate cancer retroperitoneal nodal metastasis (arrows). Note intense physiologic hepatic and pancreatic activities. (Images courtesy of Steve Larson, Memorial Sloan Kettering Cancer Center, and Homer Macapinlac, M.D. Anderson Cancer Center.)
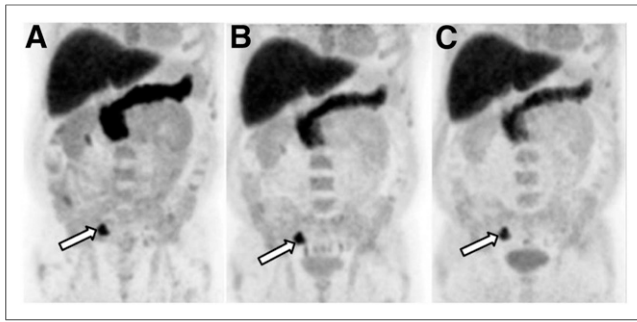
FIGURE 2. Multiple-time-point anterior projection images of typical ^{18}F-fluciclovine biodistribution at 5–16 min (A), 17–28 min (B), and 29–40 min (C). Note activity in metastatic right pelvic node (arrows) and typical absent to minimal bladder activity.

washout; therefore, early imaging is recommended. Although most work to date has involved the use of ^{18}F-fluciclovine PET for recurrent disease, investigators have also reported the results of studies for primary disease and initial staging.

PRIMARY PROSTATE CANCER DIAGNOSIS AND INITIAL STAGING

For primary prostate cancer, the challenges of using ^{18}F-fluciclovine PET are similar to those reported with ^{11}C-MET PET. In a study of 10 patients with primary prostate carcinoma, although the SUV$_{max}$ was significantly higher in malignant sextants than in nonmalignant sextants and the Gleason score generally correlated with the SUV$_{max}$, overlap of the SUV$_{max}$ in the benign and malignant sextants limited the utility of ^{18}F-fluciclovine PET for radiotherapy planning (46). In a prospective study of 21 patients with primary prostate cancer and with whole-mount step-section analysis as a reference standard, Turkbey et al. reported that although the activity of ^{18}F-fluciclovine was significantly higher in prostate cancer than in normal prostate, ^{18}F-fluciclovine did not discriminate malignancy from benign prostatic hyperplasia (47). Interestingly, although a sector-based analysis of ^{18}F-fluciclovine demonstrated a sensitivity of 67% and a specificity of 66%—values inferior to the 73% sensitivity and 79% specificity of T2-weighted MRI—the highest positive predictive value (PPV), 82%, was achieved with a combination of ^{18}F-fluciclovine and MRI for tumor localization.

In a report of a multicenter phase 2b clinical trial for the staging of primary prostate cancer, 68 patients with a mean PSA level of 88.6 ng/mL underwent both whole-body ^{18}F-fluciclovine PET/CT and conventional imaging with CT and bone scanning (48). Overall similar accuracies—85.5% and 87.3%—were reported for ^{18}F-fluciclovine PET/CT and conventional imaging, respectively. However, in 13 patients (23 regions), ^{18}F-fluciclovine PET was positive for nodes 5–9 mm in short-axis diameter that were not detected by CT, and in 7 patients, ^{18}F-fluciclovine PET was positive for skeletal lesions that were not detected by conventional imaging, although these results were not confirmed with the study reference standard. Of note, neither modality detected nodal metastases 5 mm or smaller in 7 patients who underwent prostatectomy and extended lymph node dissection. Other groups have also studied the kinetics of ^{18}F-fluciclovine in primary prostate cancer and the detection of untreated metastatic prostate cancer in an early-phase study, with encouraging results (49,50).

RECURRENT PROSTATE CANCER DETECTION AND STAGING

^{18}F-fluciclovine PET seems to be most useful in patients with biochemical failure and suspected recurrent disease. In a single-center trial at Emory University of patients with biochemical failure and negative bone scan results, 53 patients underwent ^{18}F-fluciclovine PET/CT and routine clinical CT (30 with and 23 without intravenous contrast material) within a 90-d interval. The scan positivity rates for recurrent disease were 77.4% (41/53) for ^{18}F-fluciclovine and 18.9% (10/53) for CT (51). The positivity rates varied with absolute PSA levels: 37.5% at PSA levels of less than 1 ng/mL, 77.8% at PSA levels of 1–2 ng/mL, 91.7% at PSA levels of greater than 2–5 ng/mL, and 83.3% at PSA levels of greater than 5 ng/mL. The respective CT positivity rates were 12.5%, 0.0%, 41.7%, and 16.7%. In addition, the positivity rate was greater with a shorter doubling time and with a higher original Gleason score. Significantly higher sensitivity was also reported for prostate or prostate bed disease and extraprostatic disease in a subset analysis of patients who underwent CT with intravenous contrast material as a comparator. In a retrospective study at a different center of 30 scans in 26 patients with suspected or proven recurrence, 53.3% of ^{18}F-fluciclovine studies were positive, and the detection of disease was positively correlated with absolute PSA levels and negatively correlated with PSA doubling times (52). Schuster et al. had reported earlier that, on the basis of logistic regression, a patient with a PSA level of 1 ng/mL had a 71.8% probability of positive ^{18}F-fluciclovine scan results, but optimal PSA trigger values have yet to be defined (53).

In published reports from the Emory University trial mentioned earlier, ^{18}F-fluciclovine imaging demonstrated superior diagnostic performance compared with ^{111}In-capromab-pendetide in a comprehensive analysis of 93 patients for whom both studies were obtained within 90 d (53,54). A strength of that trial was that histologic proof of the presence of disease on a per-patient basis was established for 96.1% of index lesions. For the 91 of 93 patients for whom a reference standard was established for the prostate or prostate bed, ^{18}F-fluciclovine PET had 90.2% sensitivity, 40.0% specificity, 73.6% accuracy, 75.3% PPV, and 66.7% negative predictive value. The respective values for ^{111}In-capromab pendetide were 67.2%, 56.7%, 63.7%, 75.9%, and 45.9%. For the 70 of 93 patients for whom a reference standard was established for the presence or absence of extraprostatic disease, ^{18}F-fluciclovine PET had 55.0% sensitivity, 96.7% specificity, 72.9% accuracy, 95.7% PPV, and 61.7% negative predictive value. The respective values for ^{111}In-capromab pendetide were 10.0%, 86.7%, 42.9%, 50.0%, and 41.9%. Overall, ^{18}F-fluciclovine PET detected 14 more patients with prostate or prostate bed recurrence (55 vs. 41) and 18 more patients with extraprostatic involvement (22 vs. 4), resulting in upstaging for 25.7% of patients. Malignant lymph nodes ranging from 0.5 × 0.5 to 2.3 × 2 cm were detected.

Figure 3 shows an example of local recurrence detection with ^{18}F-fluciclovine. Detection of retroperitoneal nodal disease and utility with skeletal lesions are shown in Figure 4 and Figure 5, respectively.

COMPARISON WITH CHOLINE FOR DETECTION AND RESTAGING OF RECURRENT DISEASE

Choline is a substrate for the synthesis of phosphatidylcholine, which is the major phospholipid in the cell membrane (55). Choline kinase activity is substantially upregulated in tumor cells (56), especially in prostate cancer. Consequently, ^{11}C-choline PET and ^{18}F-choline PET have been widely used for prostate cancer imaging,
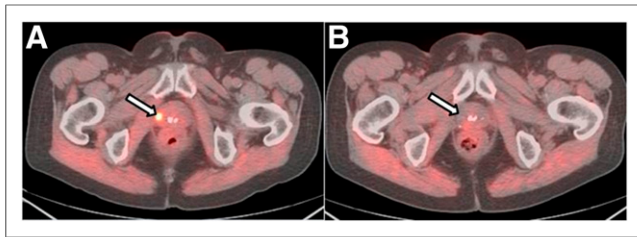
FIGURE 3. Axial coregistered ¹⁸F-fluciclovine PET/CT images demonstrating biopsy-proven prostate cancer recurrence (arrow) after radiotherapy (A) and absence of activity at site of lesion (arrow) after cryotherapy (B).

particularly for prostate cancer relapse detection. Therefore, comparing the performance of promising radiolabeled amino acid analogs, such as ¹⁸F-fluciclovine, with that of choline for the detection of prostate cancer is of interest, especially for patients with relapse. Because of variance in patient inclusion criteria and reference standards, making this determination from the literature alone is problematic; therefore, a trial directly comparing radiotracers in the same patient is preferable. In a recently completed study at the University of Bologna, 100 patients with biochemical failure after radical treatment underwent ¹¹C-choline PET and ¹⁸F-fluciclovine PET within 1 wk. The results of preliminary, interim, and final analyses from this trial have been published (57–59).

In the preliminary analysis of 15 patients, ¹⁸F-fluciclovine had a better detection rate than ¹¹C-choline in patient-based and lesion-based analyses (57). Findings from this early report were confirmed through an interim analysis (58). A final report from this trial for 89 patients for whom the reference standard of clinical follow-up for at least 1 y was available provided a complete description of diagnostic performance (59). In this analysis, choline and ¹⁸F-fluciclovine had sensitivities of 32% and 37%, specificities of 40% and 67%, accuracies of 32% and 38%, PPVs of 90% and 97%, and negative predictive values of 3% and 4%, respectively; these results confirmed the overall superior performance of ¹⁸F-fluciclovine. When the patients were divided into groups with different PSA levels, the true positivity rates for ¹⁸F-fluciclovine and choline at PSA levels of less than 1 ng/mL were 21% and 14%, respectively; those at PSA levels of 1 to less than 2 ng/mL were 29% and 29%, respectively; those at PSA levels of 2 to less than 3 ng/mL were 45% and 36%, respectively; and those at PSA levels of greater than or equal to 3 ng/mL were 59% and 50%, respectively. In general, ¹⁸F-fluciclovine had a higher PPV than ¹¹C-choline for local relapse, lymph node metastases, and bone lesions (Fig. 6). In addition, the authors also reported practical advantages of ¹⁸F-

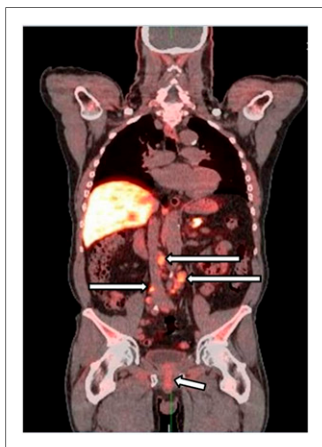**FIGURE 4.** Coronal coregistered ¹⁸F-fluciclovine PET/CT image demonstrating extensive retroperitoneal nodal prostate cancer metastasis (long arrows) and mild background activity in prostate after therapy (short arrow). (Image courtesy of Trond Bogsrud, Oslo University Hospital, Oslo, Norway.)

fluciclovine over ¹¹C-choline; these included more favorable biodistribution, with lower background activity in the abdomen and pelvis, and—of course—a longer half-life. No similar comparison of ¹⁸F-fluciclovine with ¹⁸F-choline has been reported, but the overall accuracies of ¹¹C-choline and ¹⁸F-choline have been noted to be equivalent (60).

A New Drug Application filing for ¹⁸F-fluciclovine for the detection of recurrent prostate carcinoma was accepted by the U.S. Food and Drug Administration for priority review on the basis of data from more than 700 prostate cancer patients imaged in the United States, Norway, and Italy. Subsequently, in May 2016, approval was granted for the indication of suspected recurrent prostate cancer in patients with elevated PSA after prior therapy (61). Industry-sponsored phase 3b trials in the United States and the United Kingdom are ongoing. In our experience, ¹⁸F-fluciclovine PET may be most useful for the detection of small and borderline nodes in the range of 5–15 mm and for occult skeletal lesions not detected by conventional imaging, with a lower sensitivity for blastic skeletal lesions than for mixed and lytic or silent lesions (43).

EMERGING AMINO ACID–BASED PET RADIOTRACERS

Other PET radiotracers derived from naturally occurring and synthetic amino acids are in various stages of preclinical and early clinical investigations. The development of fluorinated methionine and tryptophan derivatives has been reported (62–64). In addition, ¹¹C and ¹⁸F system A transport radiotracers have been tested in preclinical prostate cancer animal models and are in the early stages of human translation (3,65,66). A homolog of ¹⁸F-fluciclovine, anti-1-amino-2-¹⁸F-fluorocyclopentane-1-carboxylic acid (¹⁸F-FACPC), had been evaluated as a prostate cancer imaging agent in humans. However, despite promising preclinical data, the results for 5 patients who underwent both ¹⁸F-FACPC PET and ¹⁸F-fluciclovine PET demonstrated suboptimal disease conspicuity with ¹⁸F-FACPC relative to that with ¹⁸F-fluciclovine; these findings served as an example of the challenge of translation from the bench to the bedside (67,68). Cationic transport with O-2((2-¹⁸F-fluoroethyl) methyl-amino)ethyltyrosine has been studied as a probe for system $B^{0,+}$ transport within prostate cancer PC3 cells and mice xenografts, with good results (18).

Glutamate, glutamine, and glutamic acid PET radiotracers have been developed (69). Progress has been made with a system X^-c glutamate derivative PET radiotracer, (4S)-4-(3-¹⁸F-fluoropropyl)-L-glutamate (¹⁸F-FSPG), with which primary prostate cancer in 5 of 10 patients and recurrent or metastatic prostate cancer in 7 of 10
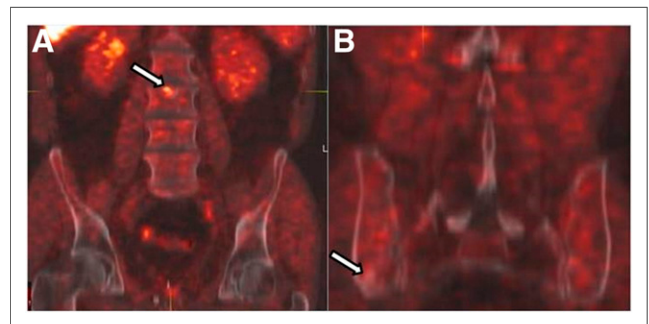

FIGURE 5. (A) Coronal coregistered ¹⁸F-fluciclovine PET/CT images demonstrating focal activity located in mixed lytic–sclerotic lesion near vertebral end plate and proven to represent metastasis (arrow). (B) No activity was present in purely blastic metastasis (arrow).
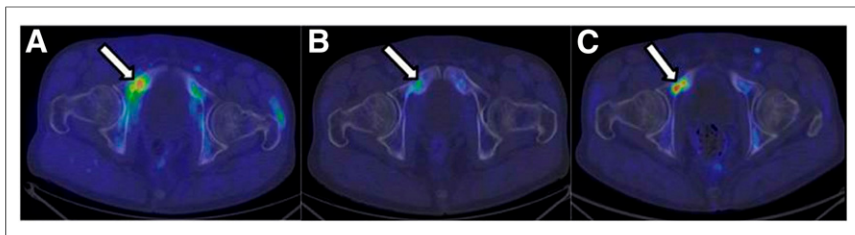
FIGURE 6. (A and B) Coregistered ^{18}F-fluciclovine axial PET/CT image (A) with definitively positive findings in right pubic ramus (arrow) and ^{11}C-choline PET/CT image (B) with mild non-specific asymmetry at same location (arrow). (C) ^{11}C-choline PET/CT findings became positive 1 y later (arrow).

patients were successfully visualized (*70*). Finally, although not specifically yet studied with prostate cancer, immuno-PET imaging with radiotracers such as ^{89}Zr-DFO-Ab2, targeting the extracellular domain of LAT1, enable direct interrogation of the amino acid transport complex itself (*8*).

CONCLUSION

The upregulation of amino acid transport in prostate cancer and the interrelationship of the intracellular amino acid pool with tumor signaling provide the biologic underpinnings of the molecular imaging of prostate cancer with amino acid–based radiotracers. Although initial work had been completed with PET radiotracers derived from naturally occurring amino acids, most progress has been made with radiotracers based on non–naturally occurring amino acids, such as ^{18}F-fluciclovine. On the basis of clinical data to date, the detection of recurrent locoregional disease and the distant spread of disease, rather than the detection of primary prostate cancer, seem to be the most promising applications for this class of radiotracers. Further study to determine utility in various clinical situations beyond disease detection, such as the response to therapy and imaging-guided treatment modulation, is ongoing.

DISCLOSURE

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