THE CHARACTERIZATION OF OSTEOLYTIC AND OSTEOBLASTIC LESIONS IN A PROSTATE CANCER MOUSE MODEL WITH THE USE OF 18F-FDG AND 18F-FLUORIDE PET/CT SCANS

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INTRODUCTION
Prostate adenocarcinoma affects millions of males annually and is associated with both osteoblastic and osteolytic metastases in bone. Murine models that grow human prostate cancer cell lines have been developed to study tumor behavior and treatment strategies. Multiple limitations exist in the use of these models to evaluate the osteolytic and osteoblastic characteristics of these tumor lines. Multiple radiographic grading systems have been published in the literature that assess osteolytic and osteoblastic activity. However, there is significant inter- and intraobserver variability when these lesions are measured. Furthermore, despite the inconsistency in the soft tissue measurements of tumor burden, this is often used as an endpoint for treatments. For this reason, the development of an imaging method that can quantify the characteristics of a prostate cancer lesion in vivo would be advantageous in providing an objective assessment in the evaluation of treatment outcomes. In addition, since histologic specimens would not be required, animals could be followed longitudinally throughout the study, obviating the need for animals at multiple timepoints. The aim of our study was to evaluate the use of positron emission tomography (PET) scanning in the characterization of osteolytic and osteoblastic prostate cancer lines in a mouse model.

MATERIALS AND METHODS
Cell Lines: Human prostate cancer cell lines PC-3 (purely osteolytic lesion) and LAPC-9 (purely osteoblastic lesion) were used in this study. Cells were cultured in Iscove’s medium supplemented with 15% fetal bovine serum and 1% glutamine and maintained at 37°C in a humidified atmosphere with 5% CO2.

Surgical technique: An equal amount of Matrigel was added to single-cell suspensions of LAPC-9 or PC-3 to form a 10 uL mixture containing 1 x 105 cells and injected into the left tibia of 8-week old male SCID mice via a previously published protocol. Three animals were used for each different timepoints for each tracer for both PC-3 and LAPC-9 cells. Each animal was then radiographed, sacrificed, and tibia harvested for tumor measurement and histomorphometric analysis.

PET Scanning procedure: Both 18F-FDG and 18F-Fluoride ion tracers were used in this study. Animals were anesthetized via isoflurane induction chamber via an ARC-approved protocol. Tail-vein injections using 250 uci of tracer were then performed under a high-flow hood. After one-hour uptake, mouse bladders were expressed for residual urine, and mice were then positioned securely with fixation of all four extremities to plastic pegs. Animals were scanned for 10 minutes using a microPET Focus machine and for 30 minutes using microCAT. PET/CT images were then merged using a previously published protocol.

Data Analysis: All PET/CT images were analyzed using Amide®, Version 0.7.154. For 18F-Fluoride ion and 18F-FDG scans, regions of interest (ROIs) were drawn over the injected left tibia and contralateral control tibia using a standardized isocountur value for both sides. ROIs were then 3-dimensionally reconstructed and size calculations were then made. Lesion size was calculated by subtracting the size of right tibial ROI from that of the left. For PC-3 tumors, microCT images were used to identify bone loss in comparison to the contralateral control tibia. ROIs were drawn over bilateral tibias using a standardized isocontour value (~2,20). 3-dimensionally reconstructions and size calculations were done using Amide®. Lesion size was identified by subtracting the size of left tibial ROI from that of the right.

RESULTS
Tumor burden measurements using 18F-FDG PET/CT scans had strong correlations with those using soft tissue measurements using linear regression calculations (Figure 1) with an R-squared value of 0.91. Results of 18F-Fluoride ion PET/CT scans are shown in Figure 2. A progressively larger osteoblastic lesion was seen longitudinally at multiple timepoints. In addition, a small lesion (5.0 mm3) was measured using PET scan at the 4-wk timepoint that was undetectable using plain radiographs. Using microCT imaging, osteolytic lesion sizes at 2, 4, and 6-week timepoints, were measured at 1.5, 12.6, 24.2 mm3 respectively. Lesions measured by PET Scan also correlated with histomorphometric analysis of harvested specimens and plain radiographs (data not shown). Merged PET/CT images allowed for excellent visualization of soft tissue and bony involvement of prostate cancer lesions (Figures 3).

DISCUSSION
18F-FDG and 18F-Fluoride ion PET/CT scans can be invaluable tools in characterizing and quantifying the osteolytic and osteoblastic lesions of human prostate cancer cell lines. Our results indicate that using novel ROI drawing protocols and software analysis, osteolytic, osteoblastic lesion sizes, and tumor burden can be quantified at various timepoints. By using novel in vivo imaging methods, the animals required for statistical power in further studies could be reduced. Furthermore, 18F-Fluoride ion PET scans are able to quantify a small osteoblastic lesion before it is discernable on plain radiographs. Novel imaging and quantitation techniques may become important tools in detecting differences in treatment strategies in animal models of metastatic disease. For this reason, 18F-FDG and 18F-Fluoride ion PET/CT scans deserve further study to properly elucidate their role in prostate cancer animal models.