Supplemental Materials

Comparing the Diagnostic Potential of ^{68}Ga-Alfatide II and ^{18}F-FDG in Differentiating Between Non-Small Cell Lung Cancer (NSCLC) and Tuberculosis Patients

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SUPPLEMENTAL METHODS

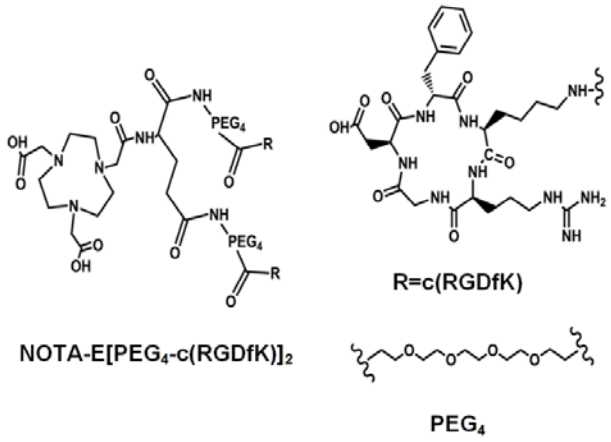
^{68}Ga-Alfatide II Preparation

^{68}Ga-Alfatide II was synthesized with the following protocol: 20 μg Alfatide Ⅱ was added to a mixture of 50 μL of sodium acetate solution (1.25 M) and 1 mL of $^{68}GaCl_3$ eluent (370–666 MBq, pH 1.3-1.5) was obtained from a ^{68}Ge/^{68}Ga generator (ITG Isotope Technologies Garching GmbH, Garching, Germany). The mixture was then heated at 100℃ for 10 min. Following addition of 10 mL water, the mixture is loaded on a pre-activated C-18 Sep-pak Plus cartridge (500 mg, Waters). The cartridge is then washed with additional 6 mL water. The loaded peptide is eluted using 1 mL 10 mMHCl/ethanol. The resulting solution was analyzed by instant thin-layer chromatography (TLC, Bioscan, Washington, DC, USA) and high performance liquid chromatography (HPLC, Agilent Technologies Inc., CA, USA). Instant thin-layer chromatography (ITLC) was performed with silica-gel paper strips (VARIAN) in a 1:1

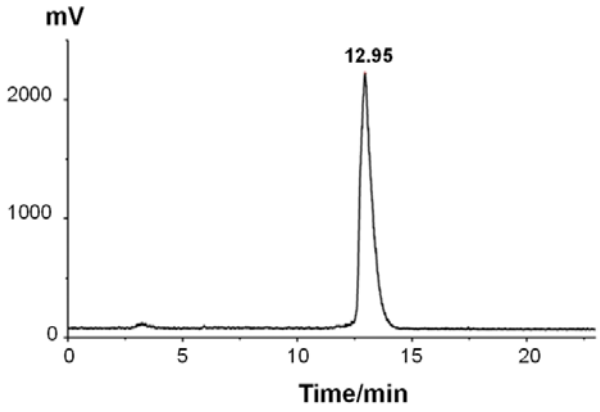
mixture of methanol and sodium acetate developing solution. Analytical HPLC was performed with an RPC-18 column (ZORBAX, 5 μm, 4.6 x 250 mm). The HPLC method is described below: solvent Aconsists of 0.05% trifluoroacetic acid (TFA) in water, and solvent B consists of 0.05% TFA in acetonitrile with the flow rate of 1 mL/min; the gradient is 0-3 min: 5-5% Solvent B; 3-20min: 5-65% Solvent B. The final ^{68}Ga-Alfatide Ⅱ solution was filtered with a 0.22 μm Millex-GP filter (EMD Millipore) that was demonstrated to be bacterium/endotoxin-free.

Immunohistochemistry

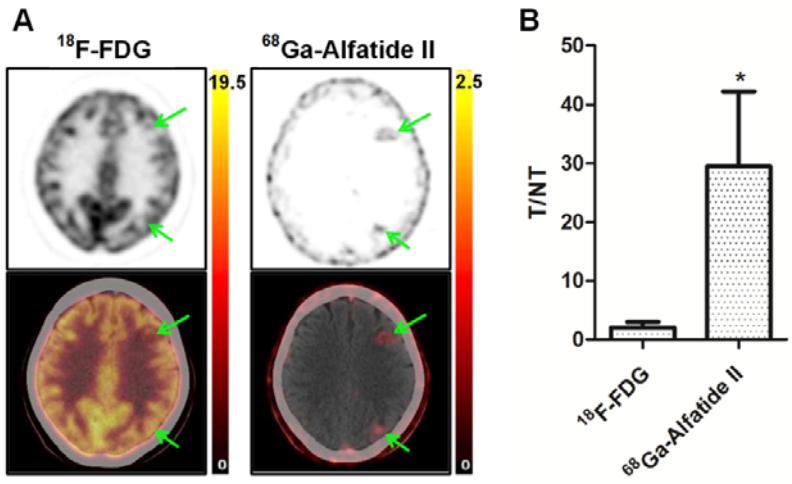
Tissue samples of the lung cancer patients were procured by surgery within 1 wk following PET/CT scans. Reference TB samples were taken from TB patients who received bronchoscopic biopsy. Tissue samples were sectioned and stained using the biotinylated monoclonal anti-integrin αvβ3 antibody. Sections were processed by peroxidase staining.



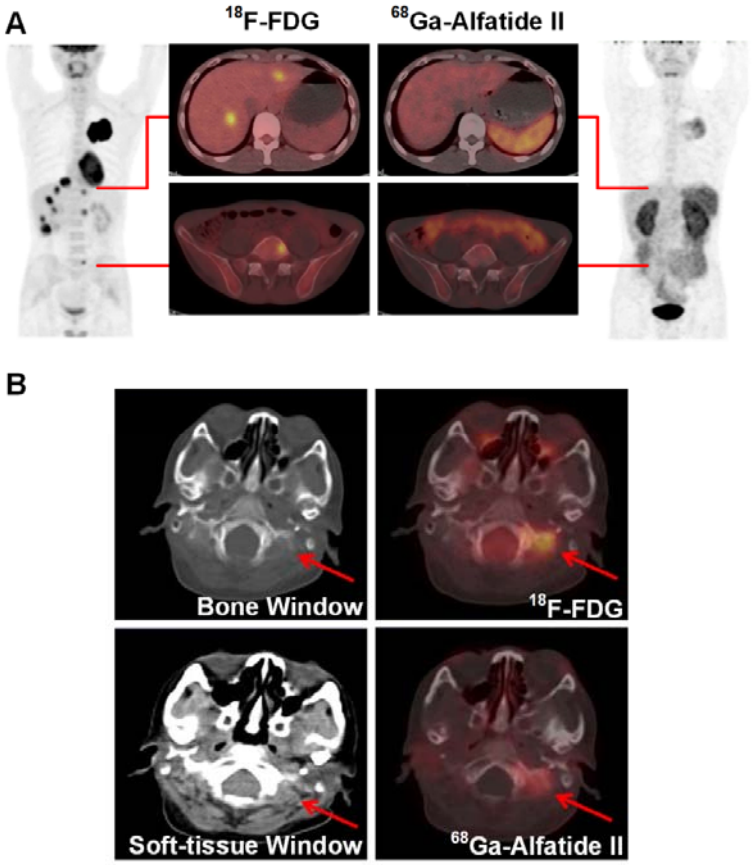
SUPPLEMENTAL FIGURE 1 Chemical Structure of Alfatide II.



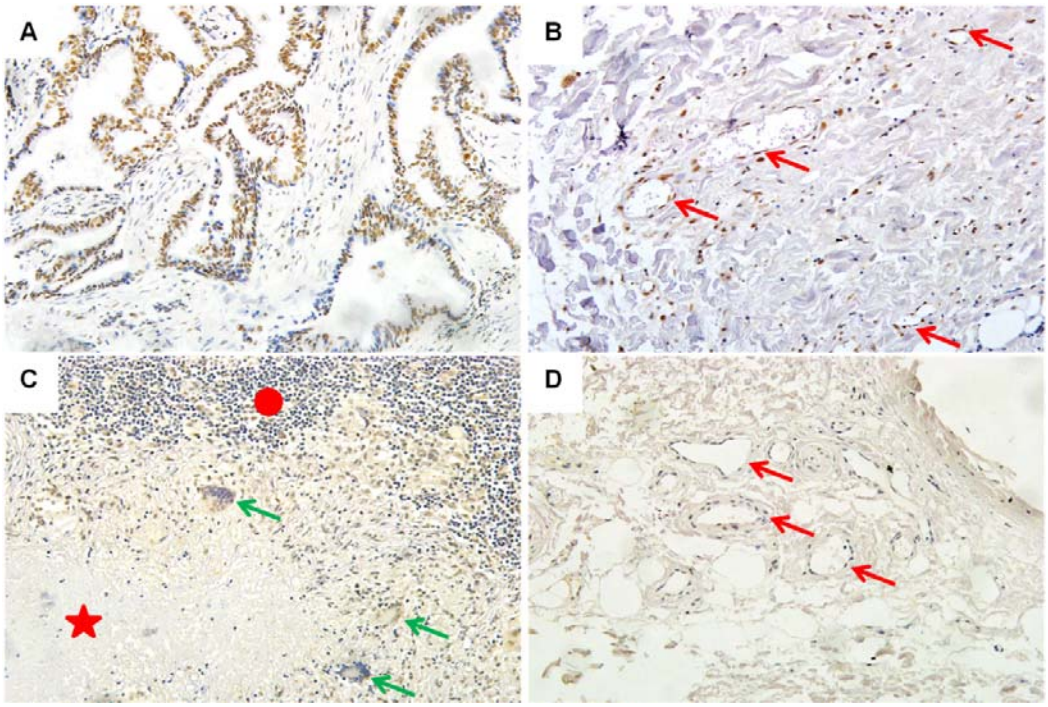
SUPPLEMENTAL FIGURE 2 Radioactive high performance liquid chromatography result showing the radiochemical purity of ^{68}Ga-Alfatide II tracer.



SUPPLEMENTAL FIGURE 3 (A) Representative transaxial ^{18}F-FDG and ^{68}Ga-Alfatide II PET and PET/CT images ofan adenocarcinoma patient with brain metastasis. Brain metastasis lesions are indicated by green arrows. (B) Quantitation of the target/non-target ratio (T/NT) of all brain metastasis lesions in the ^{18}F-FDG and ^{68}Ga-Alfatide II images. Contralateral normal brain region was selected as the non-tumor tissue. *P< 0.05.



SUPPLEMENTAL FIGURE 4 (A) ^{18}F-FDG and ^{68}Ga-Alfatide II coronal PET projection images and representative transaxial PET/CT images of an adenocarcinoma patient with multiple liver and bone metastases. Transaxial PET/CT images of the two tracers showing liver or bone metastasis in the same slice. (B) Representative transaxial CT images (upper left: bone window, bottom left: soft tissue window) and PET/CT images in another adenocarcinoma patient with a large bone metastasis. Bone metastasis is indicated by red arrows. Soft tissue exists in the osteolytic region and both tracers show positive uptakes.



SUPPLEMENTAL FIGURE 5 Clinical immunohistochemical staining qualitatively shows the expression of αvβ3 in the NSCLC cells (A), NSCLC neovasculature (B), tuberculosis granuloma (C), and the vasculature in tuberculosis lesions (D). Red arrowsindicate the vasculature epithelium. Green arrows indicate the multinucleate giant cells. Red star indicatescaseous necrosis. Red circle indicates lymphocytes (Magnification, × 400).