Multi-Modality Imaging (PET, CLI) of a PSMA-Targeted Cys-Diabody in Tumor-Bearing Mice

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Introduction

Prostate Specific Membrane Antigen (PSMA) is a clinically validated biomarker of prostate cancer that is being investigated as a target for antibody-based imaging and therapy. The aims of this study were to:

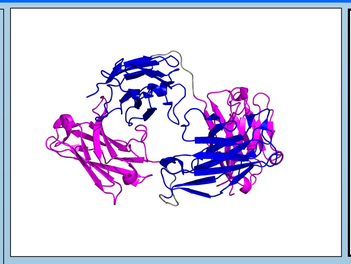
1. **Determine the ease of radio-labeling a NODAGA-conjugated anti-PSMA cys-diabody (NODAGA-Cys-Db)**
2. **Determine the feasibility of using that radiotracer to image PSMA-positive human tumor xenografts in a mouse model of prostate cancer using both Positron Emission Tomography (PET) and Cerenkov Luminescence Imaging (CLI)**

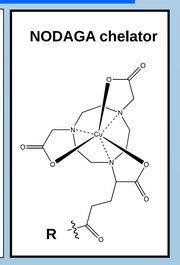
Methods

NODAGA-Cys-Db was radiolabeled with $^{64}CuCl_2$ to a specific activity of ~0.5 MBq/µg and its binding activity was verified in live-cell binding assays. Nu/Nu mice harboring PSMA-positive (22Rv1) and –negative (PC3) xenografts were then injected with 20 µg of radiolabelled NODAGA-Cys-Db. Serial images were acquired on both PET (2, 4, 16, and 26 hrs) and CLI (1, 4, 16 and 24 hrs) at various time points after i.v. injection of the radiotracer. The PET imaging was performed using a clinical PET/CT scanner (Biograph 16 Truepoint) with 10 minute PET acquisition. The CLI imaging was performed on an IVIS Spectrum. Images were made with a 300 second exposure, medium binning and a 19.6 cm field of view. Imaging results obtained at 24 hrs were compared to tissue uptake as quantified by biodistribution.

Radiotracer Structure

The anti-PSMA radiotracer is a non-covalent single-chain Fv dimer (diabody, Db) conjugated to the NODAGA chelate through maleimide chemistry.

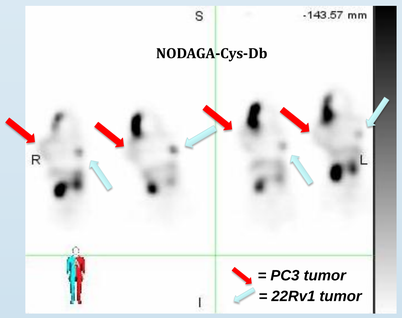


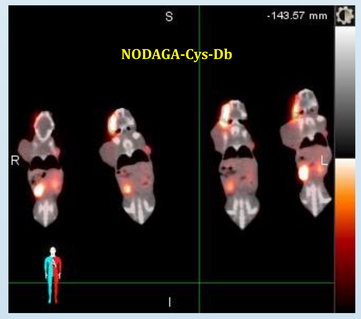


Left: Crystal structure of representative diabody (PDB # 1MOE). Vh domains in magenta, Vl domains in blue, linker in gray.
Right: NODAGA chelator, R = site of conjugation to diabody.

Results

PET/CT Imaging of NODAGA-Cys-Db 24 hours post-injection

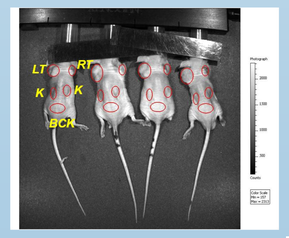


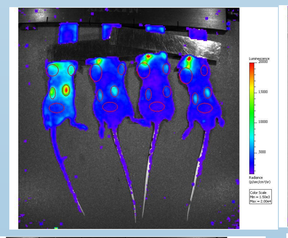


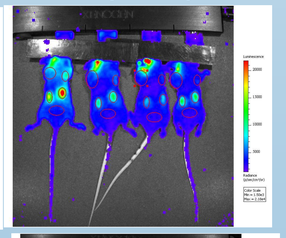
PET (left/white background) and fused PET/CT (right, black background) images showing an approximate 5-fold selective uptake of NODAGA-Cys-Db into 22Rv1 tumors vs PC3. The decay-corrected tumor uptake determined from PET imaging increased to its peak value at about 4 hrs after injection and remained nearly steady until the end of the 24 hr study period. The activity in blood declined during this period, while kidney and liver uptakes were steady.

Cerenkov Imaging of NODAGA-Cys-Db

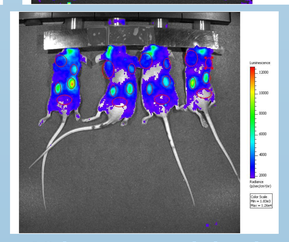
1 hour post injection *4 hours post injection*

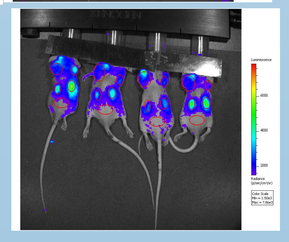






LT – left tumor
RT – right tumor
K – kidney
BCK -background

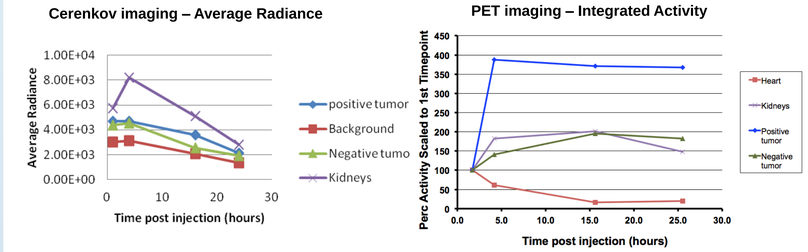




16 hours post injection *24 hours post injection*

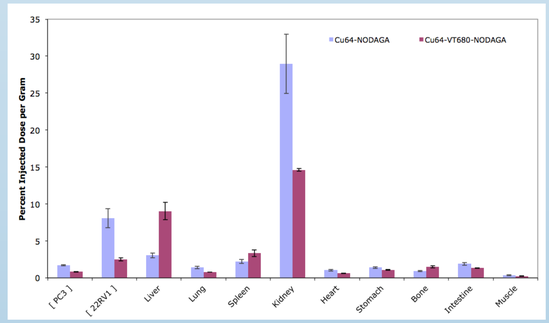
In Cerenkov images signal in PSMA-positive tumors and kidneys peaked at 4 hrs, with the maximum difference in average radiance between the PSMA-positive and –negative tumors occurring at 16 hrs post injection.

Image Quantification



LEFT: Average radiance of the regions of interest from the CL images for one mouse (all images showed a similar pattern). The peak difference in signal between the different tumor types occurs at 16 hours.
RIGHT: Integrated PET activity of different organs (average of all mice).

Biodistribution of Probes



Biodistribution results obtained immediately after the 24 hr imaging timepoint demonstrated NODAGA-Cys-Db reached levels of 5-fold selective uptake in 22Rv1 (8.1 %ID/g) vs PC3 (1.7 %ID/g). Kidneys, as seen both by imaging and biodistribution, represent the organs with highest level of uptake, consistent with the predicted first-pass renal clearance of this radiotracer

Conclusion

When radiolabeled with ^{64}Cu the anti-PSMA targeted NODAGA-Cys-Db can selectively image PSMA-positive tumors in a nu/nu mouse model of prostate cancer via both PET and CLI, with the tumor uptake increasing and blood pool activity decreasing with time during the 24 hr period of the study. CLI permits a rapid and simple estimate of distribution of the imaging agent using widely available optical imaging systems, allowing one to determine optimal imaging times with precision. Additional studies are warranted to more fully evaluate the imaging capabilities of this radiotracer.