

ImmunoPET Imaging of Prostate Stem Cell Antigen (PSCA) in Pancreatic Xenografts: Comparison of Intact IgG with Engineered Antibody Fragments

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Background: Early diagnosis and efficient therapeutic intervention remains a major challenge in the clinical management of pancreatic cancer patients. Radiolabeled antibodies and engineered antibody (Ab) fragments such as the minibody (scFv-C_H3; ~80 kDa) and Cys-diabody (scFv dimer; ~50 kDa) provide the desired specificity for recognizing cell surface antigens. These platforms retain similar binding affinity and specificity to antibodies with the added advantage of improved pharmacokinetics and clearance optimized for targeted diagnostic imaging. The humanized parental 2B3 mAb (1), specific for prostate stem cell antigen (PSCA), was affinity matured and reformatted into a minibody (A11 Mb) that was validated previously in prostate and pancreatic cancer models (2). In this study the A11 diabody with a terminal cysteine (Cys-Db) was generated and compared with the A11 Mb and parental 2B3 *in vivo* to determine their suitability as imaging agents in pancreatic cancer.

Methods: 2B3, A11 Mb and Cys-Db were radioiodinated with I-124 ($t_{1/2}$ 4.2 days) using the Iodogen method. Groups of 4-5 nude, female mice harboring Capan-1 (low PSCA expresser) and MIA-PaCa-2 (negative control) tumors were injected with 140 μ Ci (Cys-Db) or 100 μ Ci (Mb and 2B3) and serially imaged by PET/CT. One mouse from each group was subjected to a 0-2 hour dynamic image and one representative mouse was imaged with FDG to confirm viability of the tumors. After the last scan, mice were sacrificed and tumor, blood and organs were harvested and the percent injected dose per gram (%ID/g) was determined. Regions of interest (ROIs) were drawn over the heart and tumors to calculate blood clearances and tumor uptakes at the different scan times.

Results: The radiochemical purities after radioiodination were ~90%, the specific activities were ~5 μ Ci/ μ g and cell-based immunoreactivities ranged from 34-75%. FDG scans confirmed that the tumors were viable. Specific targeting to Capan-1 xenografts was observed with all three constructs. Clear delineation of the tumors was seen as early as 2 hours with the A11 Cys-Db, and at 24 hours with the A11 Mb and 2B3. ROI analyses revealed rapid clearance of the A11 Cys-Db with most of the blood activity gone by 8 hrs. The A11 Mb exhibited intermediate blood clearance with most of the activity gone by 24 hrs while 2B3 showed a slow clearance profile consistent for intact antibodies. The elimination phase ($t_{1/2\beta}$) was determined to be 3.63, 8.66, and 80.45 hrs for the A11 Cys-Db, A11 Mb and 2B3, respectively. ROI analyses revealed a positive tumor to blood ratio of 9.5 for the A11 Cys-Db at 24 hrs which was 7-fold over that of the A11 Mb and 15-fold over that of the 2B3. Biodistribution analyses revealed a positive tumor to negative tumor ratio of 5.7 for A11 Cys-Db at 24 hrs. The A11 Mb had similar tumor to negative tumor ratio (5.8) at 48 hrs.

Conclusion: Specific targeting and high contrast images were obtained with the ¹²⁴I-labeled A11 Cys-Db and Mb in a pancreatic xenograft with low expression of PSCA. The A11 Cys-Db exhibited improved properties over the A11 Mb and 2B3 as an imaging agent and yielded high contrast images at an earlier time point. When considered together these data suggest that A11 Cys-Db may be a promising agent for early imaging of pancreatic cancer. We are currently investigating the properties of A11 Cys-Db and Mb in a high expressing PSCA pancreatic tumor model.

1. Olafsen, T *et al.* J Immunother. 2007;30(4):396-405
2. Lepin, EJ *et al.* Eur J Nucl Med Mol Imaging. 2010;37(8):1529-38