MATERIALS AND METHODS

Patients

Three patients with recurrent metastatic breast cancer and known lesions identified by CT or ^{18}F-FDG PET/CT underwent diagnostic imaging with radiolabeled ABY-002. Patients received a less than 100 μg of ABY-002 (~80-90 μg) labeled with indium-111 or gallium-68 at the GMP certified radiopharmacy of the Zentral Klinik Bad Berka, Germany. The exploratory research was approved by the local ethics committee in Bad Berka, Germany. Patients were imaged between November 2005 and April 2006 in accordance with the German regulations concerning administration of radiolabeled substances to humans. Each patient's written informed consent was obtained prior to inclusion.

Radiopharmaceutical

ABY-002. The peptide containing 58 amino acids was made by Fmoc peptide synthesis in a single chemical process by Bachem. DOTA-(t-bu)ester (Bachem) was used for N-terminal modification of the peptide. After sterile filtration, 100 μg of $DOTA^0$-$Z_{HER2:342\text{-}pep2}$ (ABY-002) was packaged in sterile and pyrogen-free vials and delivered as lyophilized white powder.

Labeling of ABY-002 with ^{111}In. Pharmaceutical grade ^{111}In-$InCl_3$ (185 MBq stock solution in 0.05 N HCl) was purchased from Mallinckrodt Medical B.V. 100 μg ABY-002 dissolved in 200 μL 0.2 M ammonium acetate buffer pH 5.0

was mixed with 165 MBq (~200 µL) ^{111}In-$InCl_3$. The mixture was incubated for 30 min at 80°C. RP-HPLC analysis showed a radiochemical purity of >97%. ^{111}InABY-002 was diluted to 10 mL with sterile 0.9% NaCl followed by sterile filtration using a Milex GV 0.22 µm 13 mm filter unit. The injected activity was determined by measuring the activity in the syringe before and after administration using a dose calibrator.

Labeling of ABY-002 with ^{68}Ga

A 1110 MBq ^{68}Ge/^{68}Ga generator based on TiO_2 solid phase (Cyclotron Co Ltd) was eluted with 10 mL 0.1 N HCl and the eluate was purified and pre-concentrated on a AG 50W-X8 200-400 mesh cation exchange column (BioRad). The column was eluted with 0.4 mL 4N HCl, and after addition of 0.4 mL 7N HCl the generated tetrachloro-galate complex was absorbed onto a SAX anion exchange column. The resin was dried in a helium stream for one minute and ^{68}Ga was eluted with 0.2 mL water. 300 MBq ^{68}Ga was added to 100 µg ABY-002 dissolved in 500 µL 1.5 M HEPES buffer pH 3.5 and incubated for 10 min at 90°C. The radiochemical yield was >97%. ^{68}Ga-labeled ABY-002 was further purified on a small C-18 cartridge. After washing with water, ^{68}Ga-ABY-002 was eluted with 0.4 mL ethanol and diluted and sterilized as described above. Analysis of the eluate revealed a radiochemical purity of >99.5%.

Gamma Camera imaging.

^{111}In-ABY-002 scintigraphies were performed on MEDISO X-Ring and Spirit DH-V gamma camera; MEGP collimator; peak at 172 and 246 keV; 20% energy window.

PET/CT imaging.

Patients were examined on a dual-modality PET/CT tomograph (Biograph Duo; Siemens). The CT component of the biograph BGO duo corresponds to a Somatom Emotion Duo (Siemens). After 100 mL of intravenous contrast administration, contrast enhanced CT followed by two-dimensional PET emission scanning was performed as described earlier (*1*).

Image analysis.

The CT transmission images were used for attenuation correction of the PET emission data. After scatter and attenuation correction, PET emission data were reconstructed using an attenuation-weighted ordered-subsets maximization expectation approach with 2 iterations and 8 subsets on 128 x 128 matrices and with a 5 mm Gaussian post-reconstruction filtering. Image analysis of the CT was performed on a syngo viewing station (Siemens). The PET/CT images were assessed using the esoft workstation (Siemens). Any abnormal uptake of ^{111}In-ABY-002 above the background not corresponding to the physiological distribution visible in salivary glands, thyroid, liver, kidney, blood pool activity, urinary bladder and small intestine was considered to be pathological.

Blood kinetics

The blood kinetics of intravenously injected radiolabeled ABY-002 was assessed from samples collected from 2 min up to 94 hours after injection for ^{111}In-ABY-002 and from 2 min up to 4 hours after injection for ^{68}Ga-ABY-002. The radioactivity was measured in a gamma counter (Isomed 2000, Nuklear Medizintechnik Dresden) and the values obtained were used for kinetic analysis using Origin Pro 7G (OriginLab) and exponential curve fit ExpDec ½.

RESULTS

Patient 1. Figure 1 shows the distribution of ^{111}In-ABY-002 during a 30 minutes dynamic scan. The subclavian vein and the blood pool radioactivity in the heart were visible on the one minute image but not on later images. The left kidney and the liver could be identified after two minutes. Image intensity increased up to 5 minutes and remained constant for the remaining 25 minutes.

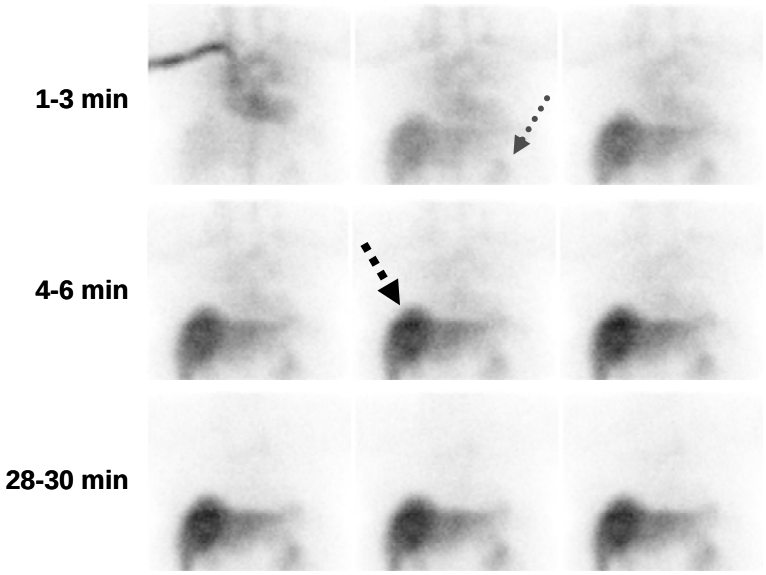

Figure 1. Gamma camera and PET/CT imaging of patient 1. Gamma camera imaging was performed after intravenous injection (i.v.) of 123 MBq ^{111}In-ABY-002 with peptide mass dose of < 100 µg, and PET imaging after i.v. administration of 293 MBq ^{18}F-FDG. The arrows indicate the localization of the kidney (grey dotted arrows) and the liver (black hatched arrows). Gamma camera dynamic scan (anterior view) from one to 30 min after injection of ^{111}In-ABY-002. Images for 1-6 and 28-30 min are shown. The subclavian vein and the heart pool can be seen on the one minute image.

Patient 2 was a 39 year old woman, with HER2-positive (HercepTest™ score 3+) mammary carcinoma diagnosed 18 month earlier. The patient had not been treated with trastuzumab prior to the ^{111}In-ABY-002 SPECT investigation. One metastasis had been identified by sonography and ^{18}F-FDG PET in the left lobe of the liver segment 2 (2.5 cm, SUV 9.0) one month earlier. In addition ^{18}F-FDG uptake suggestive of a metastasis was observed in a mediastinal lymph node (ventral to the carina, SUV 4.2). Images from PET/CT examination using ^{68}Ga-ABY-002 could not provide unambiguous evidence for HER2 receptor positive liver metastasis due to high background uptake of the labeled ABY-002 molecule in the liver (**Fig. 2**). The SUV in the CT-morphologically hypodense liver lesion was 18.2, while normal liver tissue showed a SUV of 15.6 - 18.2. The liver tumor was removed by surgery and the HER2 status was determined with a HercepTest score 3+. Tumor infiltration was detected by histopathology in three additional extrahepatic lymph nodes removed during surgery. None of these was identified as metastatic lesion, neither by CT, ^{18}F-FDG PET nor ^{68}Ga-ABY-002 PET. The mediastinal lymph node showed no ^{68}Ga-ABY-002 uptake.

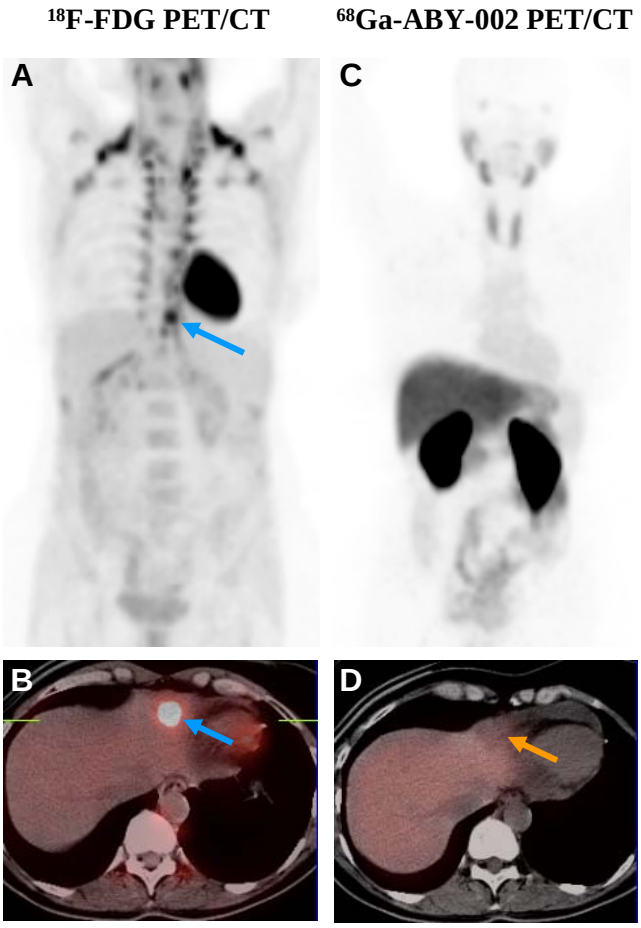

Figure 2. PET/CT imaging of patient 2. PET imaging was performed after intravenous injection of (A,B) 284 MBq ^{18}F-FDG or (C,D) 187 MBq ^{68}Ga-ABY-002 with peptide mass dose of < 100 µg. (A,B) MIP images are (B,D) PET/CT fusion images. The arrows indicate the localization of the liver metastasis. All PET/CT images were taken 95 min after injection.

Patient 3. Analysis with ^{111}In-ABY-002 two weeks after a ^{18}F-FDG PET scan. SPECT images 20 hours after injection clearly visualized all five known lesions in the pelvic area (**Fig. 3**).

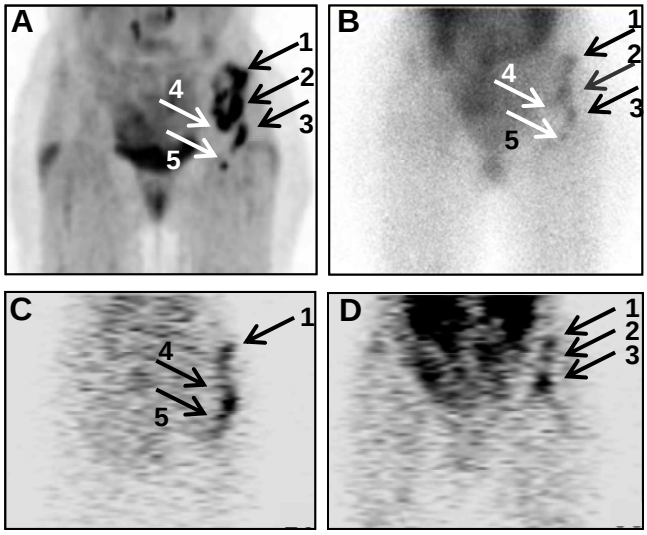

Figure 3. Gamma camera, SPECT and PET imaging of patient 3. Imaging was performed after intravenous injection of 278 MBq ^{18}F-FDG (A) or 101.3 MBq ^{111}In-ABY-002 with peptide mass dose of < 100 µg (B-D). (A) ^{18}F-FDG PET MIP image of the pelvic area. (B) Planar ^{111}In-ABY-002 scan of the hip region 20 hours after injection. (C,D) ^{111}In-ABY-002 SPECT imaging 20 hours after injection. The arrows indicate the localization of identified ABY-002 positive metastases in the iliac spine (1), the soft tissue close to the iliac bone (2), acetabulum and femur head (3), musculus sartorius (4) and musculus quadriceps with potential lymphangiosis carcinomatosa (5). Biopsies taken from the quadriceps muscle four months later confirmed solid carcinoma metastasis. Histologically, the growth pattern was compatible with mammary carcinoma with 80-90% expressing the proliferation associated antigen MIB-1.

More than 50% of the tumor cells showed a strong and mostly complete membrane staining for HER2 (mean HercepTest™ score 2+, minimal value 0, maximal value 3+).

REFERENCES

1. Prasad V, Ambrosini V, Hommann M, Hoersch D, Fanti S, Baum RP. Detection of unknown primary neuroendocrine tumours (CUP-NET) using ^{68}Ga-DOTA-NOC receptor PET/CT. *Eur J Nucl Med Mol Imaging*. 2010;37:67-77.