Antibody imaging as biomarker in early cancer drug development and treatment
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CHAPTER 6

ImmunoPET with naked mesothelin antibody in pancreatic and ovarian cancer patients before anti-mesothelin antibody-drug conjugate treatment

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Abstract

Purpose: Mesothelin (MSLN) is frequently overexpressed in pancreatic and ovarian cancers, making it a potential drug target. We performed an $^{89}$Zr-PET imaging study with MMOT0530A, a MSLN antibody, in conjunction with a phase I study with the antibody-drug conjugate DMOT4039A, containing MMOT0530A bound to MMAE. The aim was to study antibody tumor uptake, whole-body distribution and relation between uptake, response to treatment and MSLN expression.

Experimental design: Before DMOT4039A treatment, patients received 37 MBq $^{89}$Zr-MMOT0530A followed by PET/CT imaging 2, 4, and 7 days postinjection. Tracer uptake was expressed as standardized uptake value (SUV). MSLN expression was determined with immunohistochemistry (IHC) on archival tumor tissue.

Results: Eleven patients were included, 7 with pancreatic and 4 with ovarian cancer. IHC MSLN expression varied from absent to strong. Suitable tracer antibody dose was 10 mg MMOT0530A and optimal imaging time was 4 and 7 days postinjection. Tumor tracer uptake occurred in 37 lesions with mean SUV max of 13.1 ($\pm$ 7.5) on PET 4 days pi, with 11.5 ($\pm$ 7.5) in ($n$=17) pancreatic and 14.5 ($\pm$ 8.7) in ($n$=20) ovarian cancer lesions. Within patients, a mean 2.4-fold ($\pm$ 1.10) difference in uptake between tumor lesions existed. Uptake in blood, liver, kidneys, spleen, and intestine reflected normal antibody distribution. Tracer tumor uptake was correlated to IHC. Best response to DMOT4039A was partial response in one patient.

Conclusions: With $^{89}$Zr-MMOT0530A-PET, pancreatic and ovarian cancer lesions as well as antibody biodistribution could be visualized. This technique can potentially guide individualized antibody-based treatment.

Translational Relevance

Mesothelin (MSLN) has been recognized as an interesting target for immunotoxins and antibody-drug conjugates (ADC). DMOT4039A, composed of anti-MSLN antibody MMOT0530A and cytotoxic agent MMAE, is evaluated in patients with pancreatic and ovarian cancer. In early drug development, information regarding target presence, organ distribution at the whole-body level, as well as binding of the antibody to the target could be extremely helpful to guide and individualize drug dosing. This study shows that immunoPET with the $^{89}$Zr-labeled MMOT0530A is able to visualize whole-body distribution and quantify uptake in pancreatic and ovarian tumor lesions. Whole-body organ-level uptake of the tracer was highest in liver. Tumor lesion uptake between patients and within patients varied. These data support further development of more immunoPET tracers consisting of the 'naked' antibody of an ADC to determine whole-body target expression and organs at risk for toxicity, to ultimately guide dosing and confirm delivery of the ADC.
Introduction

Recognition of tumor-specific molecular characteristics involved in all hallmarks of cancer has led to the development of many targeted cancer drugs. Despite the success of targeted cancer drugs, regretfully, for several tumor types, such as pancreatic and ovarian cancer, no important 'drugable' targets are available. A promising approach is to use tumor-specific membrane proteins (even those with no known role in tumorigenesis) as targets for toxin delivery by several innovative drug types such as immunotoxins and antibody-drug conjugates (ADC). An interesting target molecule in this respect is the membrane-bound surface glycoprotein mesothelin (MSLN). The biological function of MSLN is still largely unknown. It is expressed minimally by normal mesothelial cells lining pleural, pericardial, and peritoneal surfaces. Interestingly, besides in mesotheliomas, MSLN is also highly overexpressed in 80 to 100% of pancreatic and ovarian cancers and to a lesser extent in several other human cancers.

MSLN has been recognized as a potential drug target for more than 10 years. Several approaches to target MSLN have been investigated in clinical trials, such as inducing antibody-dependent cellular toxicity and applying adoptive T-cell immunotherapy. An immunotoxin was also developed, with antitumor activity shown in phase I/II studies. Moreover, a preliminary report about MSLN for drug delivery by an ADC with a maytansinoid cytotoxin showed a partial response (PR) in one and stable disease (SD) in two mesothelioma patients.

Currently, ADCs hold a lot of interest in oncology. Adotrastuzumab emtansine (T-DM1), recently registered by FDA and the European Medicines Agency (EMA), prolongs progression-free survival (PFS) and overall survival with less toxicity in human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer patients compared with the combination of lapatinib with capecitabine. Moreover, a dozen ADCs for different antigens are in different phases of development. It would be helpful to safely and accurately predict the behavior of the ADCs in early drug development.

Noninvasive antibody imaging using single photon emission computed tomography (SPECT) and positron emission tomography (PET), that is, immunoPET, can serve this goal. In general, PET provides better spatial and temporal resolution than SPECT based on its physical principle of detecting coincident gamma pairs instead of single gamma rays. ImmunoPET can be used to determine target antigen expression at whole-body level and to provide information about antibody biodistribution and organ pharmacokinetics, information that is usually lacking from phase I study designs. Zirconium-89 (Zr) is the preferred radioisotope for PET imaging of internalizing targets such as MSLN, as it residualizes in the target tissue after cellular internalization, causing increasing tumor-to-normal tissue ratios over time. Several studies with 89Zr-immunoPET in cancer patients have shown that after administration of 37 MBq (1 mCi) 89Zr-labeled antibody, quantitative assessment of tumor uptake and whole-body biodistribution is feasible. Labeling a complete ADC with a radioisotope could lead to instability of the molecule.
'naked' antibody for PET imaging of the target will, however, also provide insight into drug distribution, because the process that drives tracer uptake (i.e., tissue exposure and penetration, and also expression of the target and internalization of the antibody) is similar and therefore PET with the 'naked' antibody of an ADC seems rational.

In human MSLN-expressing tumor-bearing mice, $^{89}$Zr-labeled anti-MSLN antibody MMOT0530A showed progressive and antigen-specific tumor uptake on microPET at 1, 3, and 6 days after tracer injection.$^{31}$ Therefore, we performed a clinical PET imaging study with the 'naked' $^{89}$Zr-labeled MMOT0530A in conjunction with the phase I study of ADC DMOT4039A, composed of humanized IgG1 monoclonal antibody MMOT0530A and the potent mitotic agent monomethyl auristatin MMAE. The aim was to determine and quantify tumor antibody uptake, whole-body distribution, and organ pharmacokinetics in patients with unresectable pancreatic or platinum-resistant ovarian cancer. In addition, the relation between tracer uptake and MSLN expression and response to DMOT4039A treatment was explored.

Patients and methods

Patient population
Patients with histologically confirmed, unresectable and/or metastatic pancreatic or platinum-resistant ovarian cancer and measurable disease according to RECIST 1.1, who were included in the phase I study with DMOT4039A (ClinicalTrials.gov identifier NCT01469793) in the University Medical Center Groningen (UMCG, Groningen, the Netherlands) or the VU University Medical Center (VUMC, Amsterdam, the Netherlands), were eligible for this imaging study (ClinicalTrials.gov identifier NCT01832116). Other inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance score 0 or 1, adequate bone marrow (absolute neutrophil count ≥ 1.5 x 10$^9$/L, hemoglobin ≥ 9 g/dL, and platelet count ≥ 100 x 10$^9$/L), liver (total bilirubin ≤ 1.5 x upper limit of normal (ULN) and aspartate aminotransferase and alanine aminotransferase ≤ 2.5 x ULN) and renal function (serum creatinine ≤ 1.5 x ULN). Major exclusion criteria were history of severe allergic reactions to antibody therapies and prior treatment with MSLN-targeted therapy. This trial was approved by the Medical Ethical Committee of the UMCG and the Central Committee on Research Involving Human Subjects, a competent authority in the Netherlands. All patients provided written informed consent.

Study design
Patients received 37 MBq (1 mCi) $^{89}$Zr-MMOT0530A (effective radiation dose of approximately 18-22 mSv, based on radiation dosimetry studies of other $^{89}$Zr-labeled antibodies with comparable characteristics$^{32,33}$) intravenously and were observed for 1 hour to detect any infusion-related adverse events. To determine the suitable tracer dose, the first cohort of two patients received $^{89}$Zr-labeled MMOT5030A (~1 mg) without any additional
unlabeled antibody. In the second cohort, the radiolabeled antibody was complemented with unlabeled antibody to a total amount of 10 mg MMOT0530A. The unlabeled antibody was co-infused with the $^{89}$Zr-labeled antibody. To determine the optimal antibody tracer dose, the distribution of the tracer in the body as a whole was analyzed. From other antibody tracers, we know that an additional dose of unlabeled antibody is often needed for imaging. When the amount of tracer still present at day 7 postinjection is high enough to visualize the circulation clearly, we consider this to be the consequence of an adequate protein dose in the tracer. To determine the optimal day for PET scanning, we analyzed all lesions and all organs at all 3 PET scans for each patient. The PET moment on which an adequate tracer amount was present in the circulation and most tumor lesions in most patients had maximum tracer uptake was considered to be the best PET scan.

Clinical grade $^{89}$Zr-MMOT0530A was produced in the UMCG essentially as was described previously. $^{31,34}$ PET scans were acquired from the top of the skull to mid-thigh with a 64-slice PET/CT camera (Biograph mCT, Siemens in the UMCG, and Gemini TF or Ingenuity TF, Philips in the VUMC), for 5 minutes per bed position at day 2 and 4, and 10 minutes per bed position at day 7 after tracer injection. For attenuation and scatter correction, immediately after the PET scan, a low-dose CT scan was acquired with the same PET/CT camera, as part of the same procedure.

Diagnostic CT scans were performed within 21 days before tracer injection and after every 2 cycles of DMOT4039A. CT scans were evaluated centrally at UMCG for measurable lesions according to RECIST 1.1. $^{35}$ After the last PET scan (either on the same day or within a week thereafter), patients continued in the phase I study and received treatment with DMOT4039A. $^{36}$ Archival tumor tissue (both primary and metastatic tissue, if available) was tested for MSLN expression with an immunohistochemical (IHC) assay using 19C3 mouse anti-human antibody. $^{37}$ IHC scoring was based on at least 10% of tumor cells staining positive, scoring 3+ for strong, 2+ for moderate, 1+ for weak, and 0 for <10% cells staining.

$^{89}$Zr-MMOT0530A PET analysis
All PET scans were reconstructed similarly (256 matrix, 3 iterations, 21 subsets, and 8 mm filters) and visually analyzed by an experienced nuclear medicine physician. All regions with high tracer uptake, compared to normal organs, were further analyzed using diagnostic CT and the number and locations of visible tumor lesions on the PET scan were determined. Quantification of radioactivity concentration in tumor lesions and normal organs was performed using A Medical Image Data Examiner (AMIDE) software (version 0.9.3, Stanford University, Stanford, CA). $^{38}$ In addition to the amount of injected activity and bodyweight, the amount of radioactivity within a lesion or organ served to determine the standardized uptake values (SUV). To assess the present radioactivity, three-dimensional spherical volumes of interest (VOI) were manually drawn around tumor lesions. To assess the biodistribution of the tracer background, VOIs were drawn in the circulation (measured in the left ventricle), liver, spleen, kidney, intestine, lung, brain, bone marrow, femur head, and thigh muscle. To assess the radioactivity concentration in tumor lesions and organs,
SUV<sub>max</sub> (the maximum voxel intensity in the VOI) was calculated. Tumor-to-blood ratios (TBR) were determined using SUV<sub>max</sub> in tumor lesions and SUV<sub>max</sub> in blood pool. In addition, to calculate the percentage of the tracer in the liver, in all PET scans of all patients, the liver was three-dimensionally delineated and the volume of the liver with the corresponding radioactivity concentration present in the liver at that PET moment was calculated. Subsequently, the total liver tracer uptake was expressed as percentage of radioactivity still present in the whole-body at that PET scan (hereby correcting for 89Zr-decay and excretion both).

**Pharmacokinetic assessments**

Blood samples for pharmacokinetic analyses of 89Zr were collected at 5 time points: before and 15 minutes postinjection, as well as at day 2, 4, and 7 postinjection (same days as the PET scans). Radioactivity was measured in 1 mL whole blood samples per time point by use of a calibrated well-type gamma-counter (LKB Instruments). Thereafter, radioactivity (determined in activity per mL) was converted to SUV equivalent values, using weight, injected amount of radioactivity, and moment of blood sampling (thereby correcting for 89Zr decay). The SUV uptake in the circulation determined by PET was correlated to the calculated SUV value in the blood samples at the corresponding days.

In addition, the apparent clearance (Cl), volume of distribution (V<sub>d</sub>), and elimination half-life (t<sub>1/2</sub>) of 89Zr-labeled MMOT0530A were calculated using a noncompartmental pharmacokinetic model in the “KINFIT module” of the software package MWPharm v 3.81 (Mediware).

**Statistical analyses**

Data are presented as mean ± SD. Associations between parameters were calculated using the Pearson correlation test (CP) for two continuous variables and the Spearman correlation test (CS) for one continuous and one categorical variable. An independent t test was performed to compare tumor uptake between pancreatic and ovarian cancer lesions. P values < 0.05 were considered significant.

**Results**

**Patient characteristics**

Between March 2013 and February 2014, a total of 11 patients (7 patients with pancreatic and 4 patients with ovarian cancer) eligible for participation in the phase I study with DMOT4039A, were consecutively enrolled in this study; 2 men and 9 women with a median age of 62 years (range 44-70) (Table 1). Three primary pancreatic tumors and two primary ovarian tumors were still in situ at the moment of trial participation.

**89Zr-MMOT0530A PET**

The first two patients received only 89Zr-labeled MMOT0530A (~1 mg protein dose)
Table 1. Patient characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients, n=11</th>
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<tbody>
<tr>
<td>Pancreatic cancer (n)</td>
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</tr>
<tr>
<td>Ovarian cancer (n)</td>
<td>4</td>
</tr>
<tr>
<td>Gender, male/female (n)</td>
<td>2/9</td>
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<tr>
<td>Age (median in years, range)</td>
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<tr>
<td>Primary tumor in situ</td>
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<tr>
<td>Pancreatic cancer (n)</td>
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</tr>
<tr>
<td>Ovarian cancer (n)</td>
<td>2</td>
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<tr>
<td>Tumor lesions on PET scan</td>
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<tr>
<td>IHC MSLN expression on primary tumor</td>
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<td>n (disease type)</td>
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</tr>
<tr>
<td>1+</td>
<td>1 (pancreatic cancer)</td>
</tr>
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<td>2+</td>
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<tr>
<td>3+</td>
<td>0</td>
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<tr>
<td>Unknown</td>
<td>4 (3 ovarian cancer, 1 pancreatic cancer)</td>
</tr>
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</table>

IHC, immunohistochemistry; MSLN, mesothelin

Figure 1. $^{89}$Zr-MMOT0530A tracer uptake in normal organs of all patients on PET on 2, 4 and 7 days pi in respectively blue, green and yellow bars (n = 11). Error bars display SD. Abd cav, abdominal cavity.
without additional unlabeled antibody, the 9 patients thereafter were administered $^{89}$Zr-labeled MMOT0530A and unlabeled MMOT0530A antibody in a total amount of 10 mg (range 9.4 - 10.4). The mean radioactivity at time of injection was 36.78 MBq (± 1.26). No infusion-related reactions or adverse events were observed in this imaging study.

**Tracer dose and organ distribution.** In the first 2 patients, who received approximately 1 mg $^{89}$Zr-MMOT0530A, mean SUV$_{\text{max}}$ in the circulation (measured in the left ventricle on PET scans) decreased fast from 9.2 (± 1.6) on day 2 to 6.0 (± 2.0) on day 4 to 3.6 (± 0.2) on day 7 postinjection. In the next 9 patients, in whom approximately 10 mg additional cold MMOT0530A was administered, more labeled antibody remained in the circulation with a mean SUV$_{\text{max}}$ of 10.2 (± 3.2), 8.3 (± 2.1) and 6.8 (± 2.9) on day 2, 4, and 7 postinjection.

![Figure 2. $^{89}$Zr-MMOT0530A PET in a pancreatic cancer patient day 2 (A), day 4 (B), and day 7 (C) after tracer injection, showing whole-body distribution with highest uptake in circulation (heart), liver, kidneys, and primary tumor (red circle). A fusion with diagnostic CT shows the primary pancreatic tumor (red circle) and the high liver uptake in healthy liver (D).](image-url)

**Figure 2.** $^{89}$Zr-MMOT0530A PET in a pancreatic cancer patient day 2 (A), day 4 (B), and day 7 (C) after tracer injection, showing whole-body distribution with highest uptake in circulation (heart), liver, kidneys, and primary tumor (red circle). A fusion with diagnostic CT shows the primary pancreatic tumor (red circle) and the high liver uptake in healthy liver (D).
respectively (Supplementary Fig. S1A). With a mean SUV<sub>max</sub> of 6.8 (± 2.9) at day 7 postinjection, sufficient tracer was available for ongoing tumor uptake until day 7 postinjection. Moreover, visibility of tracer present in the circulation improved using 10 mg of unlabeled antibody added to the labeled MMOT530A (Fig. 2A-C). Therefore, a suitable tracer dose was determined to be a total of 10 mg MMOT0530A (of which ~1 mg was <sup>89</sup>Zr-labeled).

The <sup>89</sup>Zr-MMOT0530A organ distribution showed (based on the PET scan 4 days postinjection) a high SUV<sub>max</sub> in the circulation (7.9 ± 2.2), as well as in the liver (13.2 ± 4.1), kidneys (9.1 ± 4.5), and the spleen (8.0 ± 3.4). The high intestinal mean SUV<sub>max</sub> at day 4 of 8.0 (± 2.7) reflected excretion with highest uptake in patients with habitual constipation. Low uptake was observed in muscle (2.4 ± 1.3), lung (2.4 ± 1.0), bone (1.5 ± 0.6), and brain tissue (1.1 ± 0.3).

The tracer distribution on the 3 consecutive PET scans was comparable between patients. The widest ranges were observed for the liver (Supplementary Fig. S1B) and kidneys (Supplementary Fig. S1C) and to a lesser extent also for the blood pool, spleen, and intestine. Mean SUV<sub>max</sub> in the liver was 11.1 (±3.3), 13.2 (±4.1), and 14.7 (±5.8) and in the kidney 8.3 (± 2.6), 9.1 (±4.5), and 9.0 (±3.7) on PET 2, 4, and 7 days postinjection respectively. Mean SUV on PET scan series showed a decline in tracer in the blood pool as expected. The other organs showed stable tracer uptake over time. Fig. 1 shows the organ distribution for the PET scans 2, 4, and 7 days pi for all patients.

On the PET scans, we determined a mean liver volume of 1.60 L (± 0.26 L). The absolute liver uptake in was decreasing over time with a radioactivity level of 3.68 MBq (± 7.38), 2.58 MBq (± 3.57), and 1.39 MBq (± 2.76) on PET at days 2, 4, and 7 respectively. However, the percentage injected dose of radioactivity per gram of liver tissue (assuming a tissue density of 1 g/ mL; %ID/g) was increasing over time, with a mean %ID/g of 0.82 (±0.30), 1.00 (±0.29), and 1.07 (±0.31) on PET at 2, 4, and 7 days postinjection respectively. The mean liver uptake at PET 4 days postinjection was in the first cohort 22.5% (22.6% and 22.47%) and in the second cohort 18.2% (± 2.4).

The pharmacokinetic variables shown in Table 2 are presented for both cohorts. The <sup>89</sup>Zr-MMOT0530A clearance measured in whole blood in cohort 1 was 2-fold faster compared to cohort 2; with 0.066 L/hour in cohort 1 compared with 0.033 L/hour in cohort 2. Consequently, t<sub>1/2</sub> was also shorter in cohort 1 (70 hours) than in cohort 2 (105 hours).

**Tracer uptake in tumor lesions.** <sup>89</sup>Zr-MMOT0530A uptake was observed in at least one tumor lesion in all patients (range 1-8 per patient). Representative PET/CT scans from one pancreatic cancer patient are shown in Fig. 2. We used the PET scan of day 4 to present the tumor uptake analyses, as on this PET scan most tumor lesions had maximum uptake and an adequate amount of tracer was available in the circulation (Fig. 3). Because of the decay of <sup>89</sup>Zr, the PET scans at day 7 were more difficult to analyze visually and at day 2 tumor uptake did not yet reach it maximum. Mean SUV<sub>max</sub> of all lesions was 13.1 (± 7.5) on PET 4 days postinjection. A total of 37 quantifiable tumor lesions were detected, of which 36 were also visible on the diagnostic CT scan. One lesion, a lymph node in the neck, was
positioned outside the field of view of the CT scan. Eleven of the 37 lesions were not measurable according to RECIST 1.1 due to being cystic (n=3), peritoneal localization (n=4), or a diameter of <15 mm on the short axis in case of lymph nodes (n=4).

Heterogeneity was present between and within patients as \(^{89}\)Zr-MMOT0530A tumor uptake varied greatly. No clear pattern was found to explain the heterogeneity in tumor uptake. Mean SUV\(_{\text{max}}\) on PET 4 days postinjection on patient-based analysis was 13.4 (± 6.9) with a 5.3-fold difference in mean tumor uptake between patients. The lowest mean SUV\(_{\text{max}}\) was 5.1 in a patient with pancreatic cancer and the highest was 27.2 in a patient with ovarian cancer. Also, a large intrapatient variation of the tumor SUV\(_{\text{max}}\) values was found within 8 patients with more than one lesion, with a mean difference of 2.4 fold (± 1.1). Mean SUV\(_{\text{max}}\) on day 4 postinjection was 14.4 (± 13.7) for primary tumor lesions (n=5), and 12.9 (± 6.4) for metastatic lesions. Figure 4 shows tumor uptake for all tumor lesions per patient and per disease.

### Table 2. \(^{89}\)Zr pharmacokinetics in whole blood samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (±SD) Cohort 1 (n = 2)</th>
<th>Mean (±SD) Cohort 2 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl (L/h)</td>
<td>0.066 (0.014)</td>
<td>0.033 (0.004)</td>
</tr>
<tr>
<td>V(_d) (L)</td>
<td>6.66 (1.433)</td>
<td>4.90 (0.876)</td>
</tr>
<tr>
<td>(t_1/2) (h)</td>
<td>70.36 (0.297)</td>
<td>105.17 (22.131)</td>
</tr>
</tbody>
</table>

Cl, clearance; L, liter; h, hour; V\(_d\), volume of distribution; \(t_1/2\), elimination half-life

Cohort 1: ~1 mg \(^{89}\)Zr-labeled MMOT0530A. Cohort 2: ~1 mg \(^{89}\)Zr-labeled MMOT0530A supplemented with unlabeled MMOT0530A to a total of 10 mg MMOT0530A.

**Figure 3.** \(^{89}\)Zr-MMOT0530A PET uptake expressed in SUV\(_{\text{max}}\) (on y-axis) on 2, 4, and 7 days respectively, on x-axis for all 37 tumor lesions.
Tracer uptake differed between lesions of patients with pancreatic and ovarian cancer (Fig. 5). Mean $SUV_{\text{max}}$ on day 4 postinjection in lesions of pancreatic cancer ($n=17$) was $11.5 \pm 5.6$, while in those of ovarian cancer ($n=20$) this was $14.5 \pm 8.7$, $P=0.221$. Five patients had primary tumors in situ. The two primary ovarian cancers had a $SUV_{\text{max}}$ of 8.4 and 38.9, respectively. In the primary pancreatic tumors ($n=3$) mean $SUV_{\text{max}}$ was $8.2 \pm 0.9$. Metastatic tumor lesions of pancreatic origin ($n=14$) had a mean $SUV_{\text{max}}$ of $12.1 \pm 6.0$, whereas for metastatic lesions of ovarian cancer origin ($n=18$) this was $13.5 \pm 6.9$. TBR was rising over time for all except one patient; mean TBR for all patients was $0.9 \pm 0.4$ at PET 2 days.
postinjection, 1.7 (± 0.8) and 2.3 (±1.2) at PET 4 and 7 days postinjection, respectively. In patients with pancreatic cancer, the TBR [0.9 (± 0.4), 1.5 (± 0.8) and 1.9 (± 1.1)] was lower than in patients with ovarian cancer [1.0 (± 0.4), 1.9 (± 0.8) and 2.6 (± 1.3)]. Primary pancreatic lesions had a TBR of 0.70, 1.1, and 1.28 at PET 2, 4, and 7 days postinjection, respectively.

Six measurable lesions on diagnostic CT, according to RECIST 1.1, were not visible on PET. This was the case for one abdominal tumor mass (maximum diameter 30 mm) in a patient with ovarian cancer, for a retroperitoneal lymph node (15x16 mm) in a patient with pancreatic cancer and for 2 lung lesions (~10 mm) in a patient with pancreatic cancer, and 2 liver lesions (maximum diameters 16 and 11 mm) in a pancreatic cancer patient in whom other liver metastases were visible (uptake did not correspond with metastases on CT). Furthermore, cystic lesions, some peritoneal lesions, and 9 small lymph nodes (< 15 mm short axis on diagnostic CT) were not visualized on PET. Interestingly, in one patient with pancreatic cancer, high uptake was observed in both adrenal glands (SUVmax on day 4 postinjection were 21.6 in the right and 19.9 in the left adrenal gland), while on diagnostic CT, the adrenal glands were classified as fatty adenoma.

Correlation ⁹⁹Zr-MMOT0530A blood pool activity on PET versus ⁹⁹Zr-activity in blood samples

In 8 patients, whole blood samples for ⁹⁹Zr activity measurements were available. The SUV equivalents from ex vivo measurements of blood samples at 2, 4, and 7 days postinjection correlated well with image-derived SUV values of the blood pool measured by PET (Pearson correlation 0.765, P = 0.000; Fig. 6).

**Figure 6.** Correlation between SUV in 1 mL whole blood samples at day 2, 4, and 7 after ⁹⁹Zr-MMOT0530A injection and SUVmax of blood pool as measured in the left ventricle on corresponding PET scans (n = 8 patients). Blue, day 2 postinjection; green, day 4 postinjection; grey: day 7 postinjection. Pearson correlation 0.765, P = 0.000.
MSLN IHC expression versus PET uptake
MSLN expression levels determined in primary \( (n=7) \) and metastatic \( (n=7) \) archival tumor samples varied from 0 to 3+ in 10 patients (Table 1). Primary tissue was available in 3 of the 4 patients with ovarian cancer, and metastatic tumor tissue was available in only 1 patient. Archival tissue was available in 6 of 7 patients with pancreatic cancer, only metastatic tissue in 2 patients, and both primary and metastatic tissue in 4 patients. IHC score was 0 in both primary and metastatic tissue in one patient, while in one patient, the IHC score was higher in primary compared with metastatic tissue (IHC score 3+ vs. 2+). In all others, the IHC scores were consistent.
On a patient-based analyses, the IHC score correlated with the mean SUV\text{max} per patient on PET day 4 (Spearman's correlation 0.689, P = 0.027). However, no correlation was found when the two tumor types were analyzed separately. In ovarian cancer the correlation coefficient was 0.775 (P = 0.225). For pancreatic cancer tissue, the correlation coefficient was 0.676 (P = 0.14).

**Response to DMOT4039A and PET uptake**

Five patients received the weekly schedule (dose 0.8 - 1.2 mg/kg) and 6 patients the every-3-week schedule (dose 2.4 - 2.8 mg/kg) of DMOT4039A. In 9 of 11 patients best response was SD, one patient experienced immediate progressive disease (PD), and one patient had a confirmed PR ongoing for 311+ days. Nine patients with stable disease and one with PR had a mean PFS of 121 days (range 28 – 311+, from start of treatment to PD).\textsuperscript{35} PET uptake on a per-patient basis (mean PET uptake over all lesions in one patient) did not correlate with PFS (CP -0.101, P = 0.768). On a per-lesion analysis of 26 lesions that were measurable according to RECIST 1.1, there was no correlation between PET uptake and best response on CT in percentage compared with baseline (CP -0.06, P = 0.786). The patient with ongoing PR showed PET tracer uptake in 2 liver metastases and in the primary pancreatic tumor (Fig. 7), with SUV\text{max} values on PET 4 days postinjectiob of 21.0, 12.0, and 7.4, respectively.

**Discussion**

This is the first-in-human study evaluating anti-MSLN antibody tumor uptake and whole-body distribution, using the naked antibody of an ADC with \textsuperscript{89}Zr-MMOT0530A PET for whole-body antibody distribution. In addition to primary pancreatic and ovarian cancers, metastatic lesions were also visualized.

A relatively small amount of 10 mg MMOT0530A was found to be a suitable protein dose for PET imaging. With a lower protein dose, presence of the tracer in the circulation was too low at day 7 to be optimally visualized and consequently would likely prohibit optimal tumor uptake. The optimal moment for PET scanning was 4 days after tracer injection, because most tumor lesions had maximum uptake at that moment. Although tumor-to-background ratios were lower at day 4 than day 7 they were easier to analyze given de ongoing decay of \textsuperscript{89}Zr.

Invasive determination of \textsuperscript{89}Zr in whole blood was completely in line with the PET findings. In patients who received 1 mg antibody (cohort 1), the \textsuperscript{89}Zr-labeled MMOT0530A half-life was shorter than in the patients receiving 10 mg antibody (cohort 2). This is most likely due to faster antibody clearance in the first cohort with the lower antibody dose. For certain antibody-based tracers with dose-dependent antibody kinetics, higher doses of unlabeled antibody are needed to counteract the rapid clearance at lower doses.\textsuperscript{39,40} \textsuperscript{89}Zr-MMOT0530A uptake in liver is relatively high and rises over time in contrast to other uptake in other organs. This might be due to hepatic catabolism of MMOT0530A as
opposed to target antigen expressed, as MSLN is not normally expressed in normal liver. Hepatic catabolism might be promoted by the antibody complexing with MSLN antigen shed into the circulation. Shedding of antigen into the tumor interstitium is a well-known process for cell-surface proteins including MSLN, which can also influence tumor uptake of MSLN targeting agents in preclinical models.\textsuperscript{41,42} However, liver uptake levels of this antibody are comparable to that of other antibodies, such as trastuzumab and huJ591.\textsuperscript{26,43} The high uptake in the liver suggests that it is appropriate to monitor the liver as a potential site of toxicity with the ADC DMOT4039A. In the phase I study, the dose-limiting toxicities were hypophosphatemia and hyperglycemia and clinically significant liver toxicity, expressed as liver function abnormalities, occurred in less than 10\% of the patients.\textsuperscript{36}

\textsuperscript{89}Zr-MMOT0530A tumor uptake was heterogeneous between and within patients. We observed a mean 5.3-fold difference between, and 2.4-fold difference within patients. Inter- and intra-patient heterogeneity is a widely acknowledged phenomenon in oncology, especially since the multi-region sequencing of tumor samples from primary renal carcinomas and their metastatic sites showed in 4 patients that target heterogeneity was not only present between different lesions within one patient, but even within one lesion.\textsuperscript{44} By recognizing the existence and extent of heterogeneity, PET imaging of a tumor-specific target adds valuable information for individualized treatment decisions.

MSLN-specific tracers have been developed (from different antibodies) for SPECT, as well as PET. A Copper-64 (\textsuperscript{64}Cu)-anti MSLN Fab fragment visualized MSLN-expressing xenografted tumors, as did several Indium-111(\textsuperscript{111}In)-labeled anti-MSLN antibodies.\textsuperscript{45-48} Moreover, an antibody targeting MSLN was recently conjugated to quantum dots encapsulated in micelles to detect human tumor xenografts in mice.\textsuperscript{49}

In the preclinical study preceding this clinical trial, \textsuperscript{89}Zr-MMOT0530A was used for PET imaging of MSLN-expressing human pancreatic tumor xenografts.\textsuperscript{31} Antigen-specific tracer uptake occurred with increasing uptake over time; mean TBR increased from 0.5 via 1.3 to 2.4 at 24, 72, and 144 hours postinjection, respectively.

In general, radionuclide labeled antibody uptake is higher in tumor lesions of patients than in xenografted animal models. However, in the current clinical study TBRs in primary pancreatic cancer lesions were relatively low and lower than in the subcutaneously implanted human pancreatic tumors in the preclinical study. This difference may be explained by the higher injected dose of about 1 MBq with 0.5 mg/kg MMOT0530A per mice than the dose administered in human. In pancreatic cancer, there is a known discrepancy between results in preclinical assays and clinical findings of new drugs. A possible cause might be the influence of the microenvironment in human pancreatic cancer. It has been suggested that the pancreatic stromal tissue diminishes tumor perfusion and thereby tumor penetration and delivery of therapeutics in adequate doses.\textsuperscript{50} In the preceding preclinical assessment of this tracer, the pancreatic tumors in mice did not contain the same relative amount of stromal tissue as human pancreatic tissue, which may explain the difference in TBR between the preclinical and the current clinical study. Interestingly, primary tumor lesions in pancreatic cancer patients could be visualized, indicating that the antibody did reach these
lesions. This was also the case in a recent small study, in 4 patients with mesothelioma and 2 patients with pancreatic cancer. Here, 4 mCi $^{111}$In-labeled MSLN antibody amatuximab was administered and subsequent SPECT showed uptake in pancreatic cancer lesions but a higher uptake in mesothelioma lesions.\textsuperscript{51}

Although not statistically significant, we saw in our study a similar pattern as $^{89}$Zr-MMOT0530A PET showed a higher uptake in ovarian cancer lesions compared to pancreatic cancer lesions (mean SUV$\text{max}$ of ovarian versus pancreatic lesions were 14.5 versus 11.5, respectively). This might indicate that pancreatic tumor tissue is more difficult to be reached by antibodies than mesothelioma or ovarian cancer lesions, possibly due to extensive stromal tissue in pancreatic cancer.

Moreover, in two patients with pancreatic cancer, the metastatic lesions showed higher tracer uptake than the primary lesions (1.9 and 2.9 fold, respectively). For one of these two patients both primary and metastatic tissue (a biopsy from lymph nodes) were available for MSLN expression analysis: the primary tumor showed a higher expression (3+) than the metastatic tissue (2+). This again suggests heterogeneity between primary and metastatic lesions, which might explain the differential antibody PET uptake. Overall, IHC score correlated well to the mean PET uptake in all lesions in a patient.

MSLN expression determined by IHC did not correlate with PET uptake in this study. IHC was performed on archival tumor tissue obtained during surgery or from biopsies. MSLN expression may have changed over time, and intrapatient heterogeneity will likely play a role as well. For a better understanding about the relation between target expression based on IHC and target expression based on PET uptake, fresh tumor biopsies would be most informative. However, this will still provide information about a small part of a tumor lesion, whereas with PET, the whole lesion is being assessed. In addition, other factors such as differences in perfusion can also affect antibody uptake by tumor lesions.

Recently, two ADCs in development for prostate cancer, STEAP1 and TEN2B, have been radiolabeled with $^{89}$Zr for PET imaging in mice.\textsuperscript{52} Tumor tracer uptake was rising in parallel to efficacy of the ADC treatment, suggesting resembling mechanisms for uptake. Since the stability of the ADC is uncertain when both the cytoxin and the radionuclide are attached to the antibody, we chose to label the naked antibody for PET imaging.

Apart from providing information for early drug development on tracer-antibody organ distribution (and potential organs at risk of toxicity) and tracer-antibody accumulation in the different tumor lesions, this imaging approach might also be of interest in later stages of drug development to select patients that are most likely to benefit from the treatment. As an example, the ZEPHIR study (ClinicalTrials.gov identifier NCT01565200) assesses the predictive value of pretreatment $^{89}$Zr-trastuzumab PET in metastatic breast cancer patients before treatment with the ADC T-DM1. In an exploratory patient-based analysis, the combination of $^{89}$Zr-trastuzumab PET and an early 18-Fluorine ($^{18}$F) fluorodeoxyglucose (FDG) PET showed a negative predictive value for RECIST response of 100%, indicating the combined techniques to be promising in identifying patients unlikely to respond to
T-DM1. Interestingly, this imaging study also showed highly heterogeneous $^{89}$Zr-trastuzumab uptake between and within patients.\textsuperscript{53}

Given our findings, $^{89}$Zr-MMOT0530A PET may be of interest to be used in future trials with DMOT4039A as a complementary tool to select patients with the highest chance of benefit from treatment with DMOT4039A.

Supplementary figure 1. A. PET uptake in the circulation per cohort, as determined by region of interest drawn in left ventricle; cohort 1 (in blue) contained 2 patients (0 mg additional MMOT0530A), cohort 2 (in green) contained 9 patients (10 mg additional MMOT0530A). On X axis the different PET moments: 2, 4 and 7 days postinjection. On the Y axis the mean SUVmax per cohort. B. PET uptake in the liver per patient. On X axis the different PET moments: 2, 4 and 7 days postinjection. On the Y axis the SUVmax per patient. C. PET uptake in one kidney per patient. On X axis the different PET moments: 2, 4 and 7 days postinjection. On the Y axis the SUVmax per patient.
CHAPTER 6

References


