Pretargeted Dual-Modality Immuno-SPECT and Near-Infrared Fluorescence Imaging for Image-Guided Surgery of Prostate Cancer

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Abstract
Radical removal of malignant lesions may be improved using tumor-targeted dual-modality probes that contain both a radiotracer and a fluorescent label to allow for enhanced intraoperative delineation of tumor resection margins. Because pretargeting strategies yield high signal-to-background ratios, we evaluated the feasibility of a pretargeting strategy for intraoperative imaging in prostate cancer using an anti–TROP-2 x anti-HSG bispecific antibody (TF12) in conjunction with the dual-labeled diHSG peptide (RDC018) equipped with both a DOTA chelate for radiolabeling purposes and a fluorophore (IRDye800CW) to allow near-infrared optical imaging. Nude mice implanted s.c. with TROP-2–expressing PC3 human prostate tumor cells or with PC3 metastases in the scapular and suprarenal region were injected i.v. with 1 mg of TF12 and, after 16 hours of tumor accumulation and blood clearance, were subsequently injected with 10 MBq, 0.2 nmol/mouse of either 111In-RDC018 or 111In-IMP288 as a control. Two hours after injection, both microSPECT/CT and fluorescence images were acquired, both before and after resection of the tumor nodules. After image acquisition, the biodistribution of 111In-RDC018 and 111In-IMP288 was determined and tumors were analyzed immunohistochemically. The biodistribution of the dual-label RDC018 showed specific accumulation in the TROP-2–expressing PC3 tumors (12.4 ± 3.7% ID/g at 2 hours postinjection), comparable with 111In-IMP288 (9.1 ± 2.8% ID/g at 2 hours postinjection). MicroSPECT/CT and near-infrared fluorescence (NIRF) imaging confirmed this TROP-2–specific uptake of the dual-label 111In-RDC018 in both the s.c. and metastatic growing tumor model. In addition, PC3 metastases could be visualized preoperatively with SPECT/CT and could subsequently be resected by image-guided surgery using intraoperative NIRF imaging, showing the preclinical feasibility of pretargeted dual-modality imaging approach in prostate cancer. Cancer Res; 74(21); 6216–23. ©2014 AACR.

Introduction
Despite advances in diagnostic procedures and clinical management, prostate cancer remains associated with significant morbidity and is the second leading cause of cancer-related deaths in men in the Western world.

At present, extensive research is focused on the development of new molecular imaging techniques to improve detection and staging of this disease. In this regard, radiolabeled antibodies that target the prostate cancer–associated cell surface antigens seem to be particularly promising. This is supported by the clinical application of the PSMA-directed antibody capromab pendetide, also known as Prostascint (7E11-C53) or the antibody J591 (1). However, antibodies typically show slow clearance from the circulation (t1/2 = 2–3 days), which requires a relatively long interval between time of injection of the radiolabeled antibody and subsequent image acquisition to achieve adequate contrast. In addition, the slow blood clearance results in limited target-to-background ratios. To overcome these limitations and to improve targeting of tumors, various pretargeting techniques have been developed. Previously, we reported a study in which we evaluated a pretargeting approach that targets TROP-2–expressing prostate cancer (2). TROP-2, also known as EGP-1 (epithelial glycoprotein-1), is a 46-kDa transmembrane glycoprotein expressed on diverse carcinomas, such as of the lung, bladder, breast, cervix, ovary, stomach, and prostate (3). We previously applied the trivalent bispecific Abs (bsAbs) TF12, which consists of two anti-TROP-2 Fab fragments and one anti-HSG (histamine–succinyl–glycine) Fab fragment. In this strategy, an unlabeled bsAb with affinity for both the tumor cell and a radiolabeled diHSG peptide was injected. After TF12 had accumulated in the tumor and was cleared from the blood,
the $^{111}$In-labeled diHSG peptide IMP288 was administered, which was rapidly and selectively trapped at the tumor. This method allowed for imaging within 1 to 2 hours after injection of the radiolabeled peptide and higher target-to-background ratios than with the directly labeled anti-TROP2 antibody hRS7 (2, 4).

At present, surgical removal of prostate cancer lesions is the treatment of choice for patients with low- and intermediate-risk localized disease, and in selected patients with high-risk localized disease (5). The use of intraoperative imaging might guide the surgeon in the detection of malignant tissue, which might improve the outcome, and reduce morbidity and treatment-related side effects. However, the application of nuclear imaging–based pretargeting strategies seems to be limited for intraoperative imaging purposes as tumor tissue cannot be delineated precisely by radionuclide detection in the intraoperative setting.

Near-infrared fluorescence (NIRF) imaging with fluorophores conjugated to tumor-targeting agents is rapidly emerging as a new sensitive intraoperative imaging technique for improved and real-time detection of malignant lesions during surgery (6–9). However, the penetration depth of both excitation and emission light in tissues is usually limited to several millimeters (6). One strategy to deal with this limited penetration depth is combining NIRF and radionuclide imaging techniques. With this dual-modality approach, the burden of the disease could be assessed preoperatively, followed by localization of the tumor lesions intraoperatively using a gamma probe, and subsequent intraoperative guidance for surgical removal of the tumor lesions based on the fluorescent component of the dual-label agent.

Here, we evaluated a bispecific antibody-based pretargeting strategy for prostate cancer preclinically, using a novel hapten, designated RDC018, a peptide that is based on the extensively characterized tetrapeptide IMP288 with two HSG haptons and a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelate. RDC018 is an IMP288 analog that is C-terminally conjugated with the fluorophore IRDye800CW (Fig. 1). The DOTA chelate of RDC018 was labeled with $^{111}$In. The pretargeting characteristics of the TF12/RDC018 approach were evaluated in a mouse tumor model bearing TROP-2–expressing prostate cancer metastases. In addition, the feasibility of this dual-label pretargeting strategy for image-guided surgery based on microSPECT/CT and NIRF imaging was investigated.

Materials and Methods

Pretargeting reagents TF12 and RDC018

BsAb TF12, having both TROP-2- and HSG-binding specificities, was produced using the dock-and-lock technology as described previously by Rossi and colleagues (10). The NIR fluorescent peptide RDC018 is a peptide-hapten derived from IMP288, a DOTA-conjugated $^{3}$-Tyr-$^{3}$-Lys-$^{3}$-Glu-$^{3}$-Lys tetrapeptide, in which both lysine residues are substituted with an HSG moiety via their $\varepsilon$-amino group. In addition to the DOTA chelator for radiolabeling, RDC018 also contains a NIR fluorescent IRDye800CW moiety (Fig. 1).

Cell culture

The TROP-2–expressing human prostate cancer cell line PC3, originally derived from a PC bone metastasis was obtained from the American Type Culture Collection (CRL 1435). Cells were cultured in RPMI-1640 medium, supplemented with 10% fetal calf serum (Life Technologies) and 2 mmol/L glutamine.

Mouse models

Male BALB/c nude mice (Janvier), 8- to 9-week-old, housed in individual ventilated cages (5 mice per cage) under nonsterile standard conditions with free access to standard animal chow and water, were adapted to laboratory conditions for 1 week before experimental use. Mice were subcutaneously inoculated with $3 \times 10^{5}$ PC3 cells (right flank) suspended in 200 $\mu$L of 67% complete RPMI-1640 medium with 33% Matrigel (BD Biosciences). PC3 xenografts grew to approximately 0.1 g in 7 days after tumor cell inoculation. To induce metastatic prostate cancer growth, mice were anesthetized with isoflurane and $5 \times 10^{5}$ PC3 cells in 50 $\mu$L of PBS were inoculated with a 30-gauge needle through the diaphragm into the left cardiac ventricle after midline incision (11). PC3 metastases were allowed to grow for 10 to 12 weeks after tumor cell inoculation.

All experiments have been approved by the Institutional Animal Welfare Committee of the Radboud University Medical Center and were conducted in accordance to the guidelines of the Revised Dutch Act on Animal Experimentation.

Radiolabeling of IMP288 and RDC018

IMP288 was labeled with $^{111}$In (Covidien) at a specific activity of 60.5 MBq/nmol under strict metal-free conditions. Briefly, 60 MBq of $^{111}$In was added to 2.4 $\mu$L of IMP288 in 0.1 mol/L MES buffer (pH 5.4; three times the volume of $^{111}$In-chloride) during 20 minutes of incubation at 95°C. Following incubation, 50 mmol/L ethylenediaminetetraacetic acid (EDTA) was added to the labeling reaction to a final concentration of 1 mmol/L EDTA to chelate unincorporated $^{111}$In. The labeling efficiency, determined by instant thin-layer chromatography on silicagel strips (ITLC-SG; Gelman Sciences) using 0.1 mol/L ammonium acetate (NH$_4$Ac) buffer with 0.1 mol/L EDTA (pH 5.5) as the mobile phase, reached 97%.

RDC018 was radiolabeled under metal-free conditions at a specific activity of 25.5 MBq/nmol. For this reaction, 127 MBq of $^{111}$In was added to 7.2 $\mu$L of RDC018 in 0.1 mol/L MES buffer pH 5.4 (three times the volume of $^{111}$In-chloride) for 20 minutes of incubation at 95°C. Following incubation, 50 mmol/L ethylenediaminetetraacetic acid (EDTA) was added to the labeling reaction to a final concentration of 1 mmol/L EDTA to chelate unincorporated $^{111}$In. The labeling efficiency, determined by instant thin-layer chromatography on silicagel strips (ITLC-SG; Gelman Sciences) using 0.1 mol/L ammonium acetate (NH$_4$Ac) buffer with 0.1 mol/L EDTA (pH 5.5) as the mobile phase, reached 97%.

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Immunoreactivity

To demonstrate bispecific immunoreactivity of TF12, a serial dilution of PC3 cells in RPMI medium containing 0.5% BSA ($1.6 \times 10^{7}$ to $2.6 \times 10^{9}$ cells in 0.5 mL) was incubated with TF12 (50 $\mu$g/mL) for 30 minutes at 37°C. Cells were washed...
twice in RPMI medium containing 0.5% BSA followed by incubation with $^{111}$In-RDC018 (250 Bq). To determine non-specific binding, a duplicate of the lowest cell concentration was incubated without TF12. After incubation, cells were centrifuged and washed with 500 μL RPMI medium containing 0.5% BSA. The activity in the vials and in the cell pellet was determined in the gamma counter (Wizard 3$^{\text{TM}}$ 1480; LKB-Wallac).

Immunohistochemical analysis of TROP-2–expressing tumors

Expression of TROP-2 in subcutaneous PC3 tumors was determined immunohistochemically on 5-μm frozen tissue sections. The tissue sections were fixed with acetone (100%) for 10 minutes at −20°C. After overnight drying, the sections were blocked with 20% normal goat serum (Bodincot) for 30 minutes and stained with the anti–TROP-2 humanized monoclonal antibody hRS7 (4 μg/mL antibody in PBS 1% BSA) for 1 hour at room temperature under light-protected conditions. Subsequently, the tissue sections were washed in PBS and stained with a secondary goat-anti-human HRP (Abcam) at a dilution of 1:100 in PBS, 1% BSA, for 30 minutes at room temperature. After incubation, the tissue sections were washed with PBS three times. Finally, tumor sections were incubated with 0.019 g of 3,3-diaminobenzidine tetrahydro-chloride (DAB) substrate (Bright DAB; Immunologic) in 25 mL of PBS and 62.5 μL of H$_2$O$_2$ for 10 minutes for development, followed by a hematoxylin counterstaining. Immunohistochemical analysis of the resected s.c. PC3 tumors revealed TROP-2 expression in all tumors (not shown).

Pretargeted immuno-SPECT/CT and NIRF imaging

In the first experiment, male BALB/c nude mice ($n = 4$) with subcutaneous PC3 xenografts in the right flank were intravenously injected into the tail vein with 1 mg of TF12 (6.4 nmol) in 200 μL of PBS followed by i.v. injections of either $^{111}$In-RDC018 [10 MBq, 0.6 μg (0.2 nmol)] or $^{111}$In-IMP288 [10 MBq, 0.4 μg (0.2 nmol)] 16 hours later. Two additional groups of mice ($n = 2$) did not receive TF12 before injection of the radiolabeled peptide to determine the non-TF12-mediated localization of the peptide. One of these groups received only $^{111}$In-RDC018 (10 MBq, 0.6 μg/mouse) and one group received only $^{111}$In-IMP288 (10 MBq, 0.4 μg/mouse).

Two hours after injection of $^{111}$In-RDC018, mice were anesthetized with isoflurane and imaged on a small-animal micro-SPECT/CT scanner (U-SPECT II; MILabs) with a 1.0-mm diameter pinhole collimator tube (acquisition time, 30 minutes) in prone position. After microSPECT/CT imaging, the mice were euthanized by O$_2$/CO$_2$ asphyxiation and NIRF images were acquired on the IVIS Imaging System (acquisition time, 5 minutes; binning, medium; Fstop, 2; excitation, 745 nm; excitation autofluorescence, 675 nm; emission, ICG; lamp level, high; FOV, D). MicroSPECT/CT scans were reconstructed with MILabs reconstruction software, which uses an ordered-subset expectation maximalization algorithm.

Biodistribution of pretargeted $^{111}$In-RDC018 and $^{111}$In-IMP288

Subsequently, the biodistribution in mice ($n = 4$) with s.c. PC3 tumors of $^{111}$In-RDC018 was compared with that of $^{111}$In-IMP288. Tissues of interest (tumor, muscle, lung, spleen,
kidney, liver, pancreas, stomach, duodenum, and prostate) were dissected, weighed, and the radioactivity was measured in a γ-counter to determine the biodistribution of $^{111}$In-IMP288 and $^{111}$In-RDC018. Blood samples were obtained by heart puncture. For calculation of the uptake of radioactivity in each tissue as a fraction of the injected dose, an aliquot of the injection dose was counted simultaneously.

**Dual-modality microSPECT/CT and NIRF image-guided surgery**

In the second experiment, male BALB/c nude mice ($n = 20$) with PC3 metastases in the submandibular, shoulder, and adrenal gland region that developed after injection of PC3 cells into the right cardiac ventricle were injected intravenously with the bsAb TF12 (1 mg/mouse), followed by i.v. injection of $^{111}$In-RDC018 (0.6 μg/mouse, 20 MBq/μg) at 16 hours after injection of TF12. Mice were imaged 2 hours later on both the IVIS Imaging System and a small-animal microSPECT/CT scanner with the same settings as in the first experiment. MicroSPECT/CT scans were performed preoperatively, followed by NIRF imaging of the mice in the supine position after surgical removal of skin, abdominal muscle layers, and peritoneum. After NIRF image acquisition, the visualized tumor lesions were resected, followed by NIRF imaging to check whether residual tumor tissue was left in situ. In addition, microSPECT/CT scans were performed after resection of the tumor lesions, using the same scanning parameters that were used for preoperative evaluation.

**Statistical analyses**

Statistical analyses were performed with GraphPad Prism, version 5.03 (GraphPad). Results are presented as mean ± standard deviation (SD). To determine statistical differences, unpaired t tests were used.

**Results**

**Tumor growth and development of metastases**

Subcutaneous PC3 tumors in the right flank reached a tumor size of approximately 0.1 g at 7 days after tumor cell inoculation. To induce metastases, mice ($n = 20$) received injections of PC3 cells into the left cardiac ventricle. The mice were dissected after 10 to 12 weeks and soft-tissue PC3 metastases were most frequently found in the submandibular, shoulder, and adrenal gland region. In addition, several PC3 metastases were found in the rectovesical pouch. Besides the formation of soft-tissue PC3 metastases at different anatomic sites, microCT imaging showed the development of PC3 bone metastases in the vertebral column ($n = 3$) and in the ribs ($n = 2$) of the mice.

**Biodistribution of $^{111}$In-RDC018 and $^{111}$In-IMP288**

Analysis of the bispecific immunoreactivity of TF12 revealed that in the in vitro assay up to 82% of the added peptide specifically bound to the PC3 cells (results not shown). $^{111}$In-RDC018 and $^{111}$In-IMP288 specifically accumulated in the s.c. TF12-pretargeted TROP-2–expressing PC3 tumors (right flank). Uptake of $^{111}$In-RDC018 and $^{111}$In-IMP288 in the PC3 tumor was comparable at 2 hours after injection [12.4 ± 3.7% ID/g ($n = 4$) and 9.1 ± 2.8% ID/g ($n = 4$), respectively], Fig. 2A. Tumor uptake of $^{111}$In-RDC018 and $^{111}$In-IMP288 in nonpretargeted tumors was significantly lower, reaching 2.1 ± 0.3% ID/g ($P = 0.020$) and 2.2 ± 0.0% ID/g ($P = 0.029$) for $^{111}$In-RDC018 and $^{111}$In-IMP288, respectively. Overall, the biodistribution of $^{111}$In-RDC018 and $^{111}$In-IMP288 in BALB/c nude mice with TF12-pretargeted s.c. PC3 xenografts (right flank) measured at 2 hours after injection of the radiolabeled haptens ($n = 4$). Two groups of mice did not receive TF12 before injection of the radiolabeled haptens to determine nonspecific binding. Tumor uptake for both $^{111}$In-RDC018 and $^{111}$In-IMP288 was significantly higher in the TF12-pretargeted tumors compared with nonpretargeted tumors. B, representative SPECT/CT (panel 1) and NIRF image (panel 2) of a BALB/c nude mouse with a s.c. PC3 xenograft (right flank) pretargeted with TF12 and 2 hours after injection of $^{111}$In-RDC018. In addition to tumor uptake, on the SPECT image also, rest activity originating from renal excretion of the hapten can be observed.
biodistribution profiles of $^{111}$In-RDC018 and $^{111}$In-IMP288 show similar patterns (Fig. 2A). One essential difference in the biodistribution profiles of $^{111}$In-RDC018 and $^{111}$In-IMP288 after pretargeting with TF12 is renal accumulation. Whereas the accumulation of $^{111}$In-IMP288 in the kidneys was relatively low ($2.4 \pm 0.6\% \text{ ID/g}$), renal uptake of $^{111}$In-RDC018 was significantly higher, reaching $7.6 \pm 0.9\% \text{ ID/g}$ at 2 hours after injection ($P < 0.0001$). In addition, blood levels of $^{111}$In-RDC018 were significantly higher compared with $^{111}$In-IMP288 ($4.7 \pm 0.6\% \text{ ID/g}$ and $1.7 \pm 0.9\% \text{ ID/g}$ ($P = 0.0016$), respectively), indicating slower blood clearance. Moreover, liver uptake of $^{111}$In-RDC018 and $^{111}$In-IMP288 differed significantly, reaching $2.8 \pm 0.3\% \text{ ID/g}$ for $^{111}$In-RDC018 and $1.1 \pm 0.4\% \text{ ID/g}$ for $^{111}$In-IMP288 ($P = 0.0006$).

Tumor-to-blood ratios of $^{111}$In-RDC018 and $^{111}$In-IMP288 in the TF12-pretargeted tumors were $2.6 \pm 0.7$ and $5.9 \pm 2.0$ ($P = 0.021$), respectively.

**Pretargeted immuno-SPECT/CT and NIRF imaging**

Five BALB/c nude mice with s.c. TROP-2–expressing PC3 tumors (right flank) were imaged by both fluorescence imaging and microSPECT/CT at 2 hours after injection of $^{111}$In-RDC018 (18 hours after pretargeting with TF12). A typical set of microSPECT/CT images with corresponding fluorescence images of mice in prone and left lateral positions is shown in Fig. 2B. PC3 xenografts were clearly and specifically visualized with both imaging modalities.

**Dual-modality microSPECT/CT and NIRF image-guided surgery**

BALB/c nude mice with PC3 metastases were imaged by both fluorescence imaging and microSPECT/CT at 2 hours after injection of radiolabeled RCD018. A typical example of a microSPECT/CT image with corresponding NIRF image of a mouse with two bone metastases in the vertebral column is shown in Fig. 3. Another example of dual-modality micro-SPECT/CT and NIRF imaging of a mouse with TF12-pretargeted bone metastases originating from the ribs is depicted in Fig. 4.

After preoperative evaluation of tumor lesions by micro-SPECT/CT, image-guided surgery of the tumor lesions was performed. First, the exact tumor location was identified by NIRF imaging. Subsequently, these tumor lesions were resected and NIRF and microSPECT/CT imaging was repeated to ensure radical surgical resection. In Fig. 5, the feasibility of the dual-modality probe for intraoperative image-guided surgical resection of the metastatic tumor nodules is shown.

**Discussion**

In the present preclinical study, the in vivo tumor-targeting characteristics were evaluated of the dual-modality fluorescent $^{111}$In-labeled hapten RDC018 in TROP-2–expressing prostate cancer pretargeted with the trivalent bsAb TF12. The feasibility of this approach for image-guided surgery was demonstrated in mice bearing TROP-2–expressing metastases.

In a previous study, the potential for imaging pretargeted prostate cancer with the bsAb TF12 and the radiolabeled hapten IMP288 was evaluated (2). In mice with s.c. PC3 tumors, it was shown that this pretargeting approach allows for rapid and sensitive imaging of TROP-2–expressing prostate cancer. For intraoperative imaging purposes, radionuclide detection can be used to localize tumor nodules with a gamma probe; however, precise delineation of tumor lesions or assessment of tumor cell–containing surgical margins remains challenging. NIRF imaging is a sensitive technique that may be exploited to

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*Figure 3.* Sequentially acquired dual-modality microSPECT/CT and NIRF image with $^{111}$In-RDC018 in a mouse with two TF12-pretargeted tumors. PC3 bone metastases located in two vertebrae with soft tissue outgrowth were visualized 2 hours after injection of $^{111}$In-RDC018. A, CT scan illustrating the alterations in bone structure in the two vertebrae. B, microSPECT/CT scan showing uptake in the bone metastases with soft tissue outgrowth, as well as accumulation in kidneys and bladder due to renal excretion of $^{111}$In-RDC018. C, corresponding NIRF image showing specific uptake in both PC3 metastases and kidneys.
improve visualization of small to microscopic malignant lesions and potentially of positive resection margins during surgery. So far, several preclinical studies have evaluated methods to intraoperatively visualize tumor tissue and resection margins in prostate cancer (12–14). Targeted approaches using antibodies or peptides are particularly promising to apply dual-modality imaging. Very recently, the first clinical trial using the anti-VEGF antibody bevacizumab tagged with the NIRF dye IRDye800CW (clinicaltrials.gov, NCT01508572) has begun, with the purpose to show the feasibility of tumor-targeted intraoperative fluorescence imaging in patients with breast cancer.

Because the penetration depth of emitted light in tissue is limited to several millimeters, the combination with a radio-label would allow intraoperative probe-guided tumor detection and image-guided surgery.

In the present study, analysis of biodistribution of $^{111}$In-RDC018 and $^{111}$In-IMP288 showed that the addition of the IRDye800CW fluorophore to the IMP288 diHSG peptide hapten only slightly affected the in vivo behavior of the molecule. The overall biodistribution profile of both peptides was similar; however, RDC018 showed enhanced hepatic uptake. Previously, this has also been observed for IRDye800CW-conjugated antibodies (15) and may be due to the increased lipophilicity of RDC018 compared with IMP288.

Biodistribution studies demonstrated high and specific PC3 tumor targeting for both $^{111}$In-RDC018 as well as $^{111}$In-IMP288. Using this subcutaneous tumor model, we provide proof-of-principle that $^{111}$In-RDC018 can be used for rapid dual-modality imaging of TROP-2–expressing tumors. As early as 2 hours after injection, high tumor-to-background ratios were achieved. With both imaging modalities, the fluorescent $^{111}$In-labeled hapten-peptide RDC018 accumulated specifically in the TROP-2–expressing PC3 tumors, whereas no specific tumor targeting with $^{111}$In-RDC018 was observed in mice with subcutaneous tumors that were not pretargeted with TF12.

Accordingly, the nonpretargeted PC3 tumors were not visualized by NIRF imaging. These results clearly demonstrate that the accumulation of $^{111}$In-RDC018 is TF12 mediated. While the uptake of $^{111}$In-RDC018 in other tissues remained low, intermediate accumulation in the kidneys was observed, which is most likely caused by renal clearance of the peptide. The enhanced renal retention of RDC018 compared with that of IMP288 suggests enhanced tubular reabsorption of the dual-labeled peptide in the kidneys, which may be due to the presence of the IRDye800CW moiety in RDC018. Similarly, blood levels of RDC018 are higher than those obtained with IMP288 in the same model, most likely because the IRDye800CW moiety of the RDC018 peptide caused the enhanced residence time in the circulation. A clearing agent...
may not help in this case, as it is unlikely that the enhanced blood levels are due to complex formation of the peptide with TF12 in the circulation, because the affinity of TF12 for the HSG hapten is the same for IMP288 and RDC018.

High accumulation of small-sized fluorescent tracers has been observed previously. Banerjee and colleagues synthesized a dual-label PSMA ligand (based on glutamate urea), which was tagged with both $^{111}$In and IRDye800CW for targeting PSMA-expressing prostate cancer in mice with subcutaneous xenografts (16). In this study, high tumor uptake in the PSMA-expressing PC3-PIP xenografts was observed, whereas uptake in the PSMA-negative PC3-FLU tumors remained low. Both tumor cell lines were derived from PC3 human prostate cancer cells and only differ in their expression of PSMA (PIP: PSMA$^+$, FLU: PSMA$^-$). PSMA-expressing tumors could be visualized specifically on both SPECT/CT and NIRF imaging. However, intense radiotracer uptake was observed in the kidneys, which were attributed to the tracer’s route of excretion and specific uptake by PSMA expressed in the kidneys.

In addition to biodistribution and NIRF imaging experiments, we also performed sequential dual-modality microSPECT/CT and NIRF imaging to demonstrate the feasibility of this pretargeting approach using the bsAb TF12 and the dual-label fluorescent hapten-peptide $^{111}$In-RDC018. Preferential accumulation of the dual-label hapten-peptide $^{111}$In-RDC018 was confirmed with both NIRF and microSPECT/CT imaging in TROP-2–expressing soft-tissue and bone metastases. PC3 metastases could be visualized clearly with both microSPECT/CT and NIRF imaging, showing accurate conformity of single tumor lesions by both imaging modalities. In addition, image-guided surgery based on microSPECT/CT and NIRF images was performed to provide proof-of-principle for feasibility of using this pretargeting approach for intraoperative detection of tumor lesions. It was shown that metastases imaged by microSPECT/CT and NIRF before surgery could be resected with image guidance using this pretargeting approach. Moreover, NIRF imaging could be performed intraoperatively to ensure that the tumor tissue was resected completely without leaving visible tumor-cell–containing resection margins in situ. MicroSPECT/CT imaging, which was performed after surgery to exclude false-negative observations from intraoperatively conducted NIRF imaging, confirmed complete resection of all metastatic tumor lesions.

For clinical translation of the current approach, toxicity tests will have to be carried out. The toxicity of IMP288 was investigated in mice: high doses were administered without any signs of toxicity. We have applied the IMP288 peptide labeled with In-111 in patients (100 $\mu$g/patient), without any toxicity (17). The toxicity of IRDye800CW has been studied extensively in rats (18). Also, the first clinical studies with IRDye800CW-conjugated antibodies are ongoing and so far no toxicity has been reported.

In conclusion, compared with targeting approaches using dual-labeled antibodies, pretargeting strategies may yield better signal-to-background ratios. Here, we show feasibility of the $^{111}$In-labeled near-infrared fluorescent hapten-peptide RDC018 for dual-modality detection of prostate cancer metastases in a mouse model of TROP-2–expressing soft-tissue and bony prostate cancer metastases pretargeted with the bsAb TF12. Both soft-tissue as well as bony prostate cancer lesions were specifically and sensitively detected in vivo. Whereas radionuclide imaging may allow preoperative detection and
intraoperative localization of tumor lesions, NIRS imaging enables subsequent accurate delineation of tumors and real-time assessment of resection margins. In addition, proof-of-principle for image-guided resection of metastatic lesions using this dual-modality pretargeting approach was provided.

Disclosure of Potential Conflicts of Interest
D.M. Goldenberg and W.J. McBride have ownership interest (including patents) in Immunomedics, Inc. W.J.G. Oyen received other commercial research support. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Lütje, M. Rijpkema, C. Frielink, W.J.G. Oyen, O.C. Boerman

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