

# In Vivo Testing of $^{177}\text{Lu}$ -Labelled Anti-PSMA Antibody as a New Radioimmunotherapeutic Agent Against Prostate Cancer

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**Abstract.** Aim: The goal of the present study was to test the  $^{177}\text{Lu}$ -labelled anti-PSMA monoclonal antibody 3/F11 ( $^{177}\text{Lu}$ -DOTA-3/F11) as a new radioimmunotherapeutic agent in a prostate cancer SCID mouse xenograft model. Materials and Methods: The mAb 3/F11 was  $^{177}\text{Lu}$  labelled using 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) as chelating agent. DOTA-3/F11 was tested for cell binding and serum immunoreactivity by flow cytometry. The biodistribution and the therapeutic efficacy of  $^{177}\text{Lu}$ -DOTA-3/F11 in mice bearing PSMA-positive C4-2 prostate cancer xenografts were evaluated. Results: 3/F11 and DOTA-3/F11 showed high and specific cell binding and similar serum half-lives of approximately seven days. Biodistribution studies revealed an increasing tumour uptake of  $^{177}\text{Lu}$  DOTA-3/F11 over time with maximum tumour-to-muscle and tumour-to-blood ratios after 72 h. A single dose of 1 MBq  $^{177}\text{Lu}$ -DOTA-3/F11 inhibited tumour growth and prolonged survival. Conclusion: This study indicated that  $^{177}\text{Lu}$ -DOTA-3/F11 may be a suitable radioimmunotherapeutic agent for the treatment of prostate cancer.

Prostate cancer is the most common malignancy, with about 25% of all diagnosed cases among men in industrialised countries, and represents the second leading cause of cancer-related deaths (1). Early stages of the disease can be cured by surgical or radiation therapy, but no curative treatment currently exists for advanced stages. Therefore, there is a growing demand for new targeted therapies for the management of this cancer.

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Radioimmunotherapy combines the specific binding of an antibody to a tumour antigen with therapeutic radionuclides that induce tumour cell death based on their high linear energy transfer (LET) (2). Additionally, radioimmunotherapy uses the crossfire effect, which, depending on the emitted particle range of the chosen radioisotope, may kill surrounding antigen-negative bystander cells without direct binding of the antibody (3).

Advanced prostate cancer represents an eligible candidate for radioimmunotherapeutic intervention, because it is often localised as small-volume metastases in lymph nodes or in bones that receive high levels of antibodies (4). Additionally, serum-specific prostate-specific antigen (PSA) is a useful marker for treatment outcome.

Recently, three different monoclonal antibodies (mAbs) were generated, 3/A12, 3/E7 and 3/F11, which show a high and specific binding to the prostate-specific membrane antigen (PSMA) (5, 6). PSMA is considered as the most suitable target antigen in prostate cancer, since it is highly restricted to the surface of prostate epithelial cells, overexpressed in prostate cancer, present at all tumour stages, internalised after antibody binding, and is not secreted into the blood circulation (7). In the present study, the anti-PSMA mAb 3/F11 was labelled with the beta-particle-emitting radionuclide lutetium-177 ( $^{177}\text{Lu}$ ) and tested for its *in vivo* activity in a prostate cancer SCID mouse xenograft model.

## Materials and Methods

**Cell lines and hybridoma 3/F11.** The PSMA-positive, androgen-independent prostate cancer cell line C4-2 and the PSMA-negative prostate cancer cell line DU 145 were obtained from ATCC (Rockville, MD, USA) and grown in RPMI-1640 medium with 10% foetal calf serum (FCS). The anti-PSMA hybridoma 3/F11 (mIgG2a) was generated as previously described (5) and propagated in Dulbecco's modified Eagle's medium (DMEM) with HAT supplement, 5% low-immunoglobulin FCS (Invitrogen, Karlsruhe, Germany), and 5% hybridoma-cloning supplement (PAA Laboratories

GmbH, Pasching, Austria). All cells were propagated in the presence of penicillin (100 U/ml) and streptomycin (100 mg/ml) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>.

**Purification and DOTA-labelling of the anti-PSMA mAb 3/F11.** The anti-PSMA mAb 3/F11 was purified from the hybridoma supernatant by Protein G-Sepharose chromatography (GE Healthcare Biosciences AB, Uppsala, Sweden). After dialysis against PBS, the protein content was measured with the BCA Protein Reagent Kit (Pierce Technology, Rockford, IL, USA). 3/F11 was labelled with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelating agent according to a modified method originally published by Lewis *et al.* (8). In brief, 8 mg of mAb 3/F11 in 1 ml 0.1M Na<sub>2</sub>HPO<sub>4</sub> buffer, pH 7.5 containing 1.2 g/l Chelex 100 were mixed with 100 µl of a freshly prepared solution of 10 mg/ml DOTA-NHS (Macrocyclics, Dallas, TX, USA) in 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.5. Then the reaction mixture was incubated at 4°C for 24 h with continuous end-over-mixing, after which it was dialysed against 0.25 M ammonium acetate (pH 5.4) for subsequent radiolabelling.

**Radiolabelling of DOTA-3/F11.** Initially, 100 µl of a solution of DOTA-conjugated 3/F11 (4 mg/ml in 0.25M NH<sub>4</sub>OAc, pH 5.4) were incubated with approximately 30 µl of <sup>177</sup>Lu (180-280 MBq; ITG GmbH, Garching, Germany) in 0.01M HCl at 40°C for 40 min. After this, 20 µl of 10 mM DTPA and 100 µl of 1% BSA were added. Quality was controlled by HPLC (Biosil SEC 125-5; Bio-Rad Laboratories, München, Germany; 300×7.8 mm; PBS; 1 ml/min; 220 nm). The antibody was purified over a Sephadex G-25 column (GE Healthcare, Freiburg, Germany), if necessary (radiolabelling <90%). For adjustment of the specific activity, 20 µg of DOTA-conjugated 3/F11 was always injected into the mice in the following *in vivo* experiments.

**Flow cytometry.** C4-2 or DU145 target cells were freshly harvested from tissue culture flasks and a single-cell suspension was prepared in PBS with 3% FCS and 0.1% NaN<sub>3</sub>. Then, 1×10<sup>5</sup> cells per well in a 96-well plate were incubated with 25 µl of unlabelled or DOTA-labelled 3/F11 at concentrations between 0.025 and 25 µg/ml for 1 h on ice. After three rounds of washing with PBS, the cells were incubated with 25 µl of goat anti-mouse IgG-R-PE (10 µg/ml, Becton Dickinson, Mountain View, CA, USA) for 40 min on ice. The cells were then washed repeatedly and resuspended in 200 µl of PBS containing 1 mg/ml propidium iodide, 3% FCS and 0.1% NaN<sub>3</sub>. The relative fluorescence of stained cells was measured using a FACScan flow cytometer and CellQuestPro software (both from Becton Dickinson, Franklin Lakes, NJ, USA). Mean fluorescence intensity (MFI) values were considered after background subtraction of the goat anti-mouse IgG-R-PE signal.

**In vivo experiments.** Five to six-week-old SCID-mice (weight: 20-25 g) were obtained from Charles River Laboratories (Sulzfeld, Germany). The animals were kept under sterile and standardised environmental conditions and received autoclaved food, water and bedding. All experiments were carried out according to the German animal protection law with permission from the responsible local authorities.

For the measurement of serum half-lives, 25 µg single doses of 3/F11 or DOTA-3/F11 were injected into the tail vein of the SCID mice (each n=3). Serum samples were collected at 0 h, 1, 5, 8 and 12 days after injection. Afterwards, serum immunoreactivities of 3/F11 and DOTA-3/F11 were determined by flow cytometry on C4-2 cells, as described above.

For tumour inoculation, 3×10<sup>6</sup> C4-2 cells in 100 µl PBS were mixed with 100 µl Matrigel (Collaborative Biomedical Products, Chicago, IL, USA) at 4°C and administered subcutaneously into the right flank of each animal. Growing tumours were palpated; diameters were measured by a calliper and recorded twice a week. Tumour volumes were calculated using the formula  $V = \frac{4}{3} \times \pi r^3$ , where r is the tumour radius.

For testing the biodistribution of <sup>177</sup>Lu-DOTA-3/F11, mice bearing C4-2 tumours with approximately 2-mm diameter (which corresponded to a volume of approximately 4-5 mm<sup>3</sup>) were randomised and intravenously injected with 20 µg <sup>177</sup>Lu-DOTA-3/F11 each, corresponding to an activity of 300 kBq. After 4 h (n=4), 24 h (n=4), 48 h (n=11), 72 h (n=5), and 168 h (n=4), the mice were sacrificed by CO<sub>2</sub> gas overdose and tumours and organs were removed and weighed. The concentration of <sup>177</sup>Lu-DOTA-3/F11 for each tissue was then measured in a gamma counter and the percentage of injected activity per gram tissue (% i.a./g) was calculated.

For testing the *in vivo* activity with regard to survival and tumour growth, groups of five mice, each bearing C4-2 xenografts of approximately 4-5 mm<sup>3</sup> in volume were randomised and intravenously injected with single doses of 20 µg <sup>177</sup>Lu-DOTA-3/F11 with activities of 0.5, 1, or 2 MBq. Ten control mice were injected with PBS.

During the experiment, mice were daily checked for signs of toxicity and survival. The tumour size was measured twice a week until natural death of the animals or sacrifice, if the tumour size was >12-14 mm diameter or if mice showed signs of cachexia. After death of the animals, an autopsy was performed and livers and kidneys were resected, paraffin-embedded, and stained with haematoxylin and eosin for further histological examination. *In vivo* data are expressed as mean±standard error of the mean.

## Results

**PSMA-specific cell binding of 3/F11 and DOTA-3/F11.** Approximately 10 to 15 mg mAb 3/F11 with >95% purity were routinely gained after purification of 1 l hybridoma supernatant. After DOTA-labelling, specific binding of 3/F11 and DOTA 3/F11 was tested by flow cytometry on PSMA-positive C4 2 and PSMA-negative DU 145 prostate cancer cells. Similar half-maximal saturation concentrations (HMSC) of about 1 µg/ml for both molecules were examined on C4-2 cells (Figure 1), whereas cell binding was absent for PSMA-negative DU 145 cells (data not shown).

**Serum half-lives of 3/F11 and DOTA-3/F11.** For the determination of the serum half-lives of 3/F11 and DOTA-3/F11, single doses of 25 µg each were intravenously injected into SCID mice without tumour xenografts. Then, serum samples were collected at several time points after injection and measured for serum immunoreactivity by flow cytometric analyses on C4-2 cells. As shown in Figure 2, DOTA-3/F11 and 3/F11 showed serum half-lives of approximately seven days.

**In vivo activity of <sup>177</sup>Lu-DOTA-3/F11.** A radiochemical purity of more than 90% was determined for <sup>177</sup>Lu-DOTA-3/F11

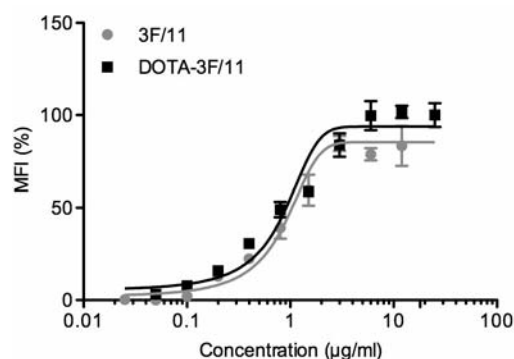


Figure 1. Binding of 3/F11 and DOTA-3/F11 to PSMA-positive C4-2 cells. Cells were incubated with different concentrations of 3/F11 or DOTA-3/F11, washed to eliminate unbound material, stained with goat anti-mouse Ig-R-PE as secondary antibody, and analysed by flow cytometry. Data are expressed as mean  $\pm$  standard deviation of three independent experiments.

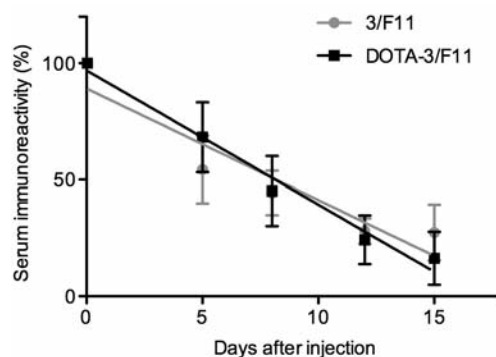


Figure 2. Immunoreactivity of 3/F11 and DOTA-3/F11 in the serum of SCID mice. Mice ( $n=3$  each) were intravenously injected with 25  $\mu$ g of 3/F11 or DOTA-3/F11, respectively. Then immunoreactivity was measured at different times after injection by flow cytometry. Data are expressed as mean  $\pm$  standard deviation.

after radiolabelling. After intravenous application into mice bearing C4-2 tumours, the *in vivo* biodistribution of  $^{177}\text{Lu}$  DOTA-3/F11 was assessed quantitatively by gamma counting of selected organs and tissues at different time points. The mean activities remaining in the blood and in the liver decreased from  $27.7 \pm 0.5\%$  i.A./g after 4 h to  $10.7 \pm 1.2\%$  i.A./g after 72 h and from  $17.3 \pm 1.3\%$  i.A./g after 4 h to  $10.0 \pm 0.8\%$  i.A./g after 72 h, respectively. At the same time, the mean activity in the C4-2 tumours increased from  $14.6 \pm 2.6\%$  i.A./g to  $44.8 \pm 11.7\%$  i.A./g. All other organs showed a low constant or slightly decreasing activity during the period of measurement (Figure 3A).

From the biodistribution data, an increasing tumour-to-blood ratio from  $0.5 \pm 0.1$  after 4 hours to  $4.6 \pm 1.3$  after 72 hours (Figure 3B) and a rising tumour-to-muscle ratio from  $15.0 \pm 2.5$  after 4 h to  $71.9 \pm 25.6$  after 72 h (Figure 3C) was calculated.

Mice of the groups treated with 0.5 MBq  $^{177}\text{Lu}$ -DOTA-3/F11 or PBS had to be sacrificed between days 9 and 20 after treatment because of fast tumour growth. Mean survival time was 16.3 days for mice treated with 0.5 MBq  $^{177}\text{Lu}$ -DOTA-3/F11, while it was 15.0 days for mice treated with PBS. All mice of the 2 MBq group died or had to be sacrificed due to toxic side effects leading to cachexia between days 13 and 18 after treatment and therefore showed a mean survival time of only 14.4 days (Figure 4). Toxicity was based on extensive haemorrhage of the subcutaneous tissue around the tumour site. Additionally, a strong deficiency of femoral bone marrow was observed during autopsy. Histological examination of the kidneys and livers showed no changes compared to organs of PBS control mice (data not shown).

This is in contrast to the mice treated with 1 MBq  $^{177}\text{Lu}$ -DOTA-3/F11, which had a mean survival time of 33.6 days.

While two mice of the group had to be sacrificed at day ten of treatment due to fast growing tumours, another two mice showed prolonged survival until day 39 and one mouse even survived until day 70, before it had to be sacrificed due to signs of cachexia (Figure 4). Compared to the PBS control mice, prolonged survival of these mice was associated with an inhibition of tumour growth (Figure 5).

## Discussion

There is continuing interest in utilising antibodies to deliver radionuclides or cytotoxic agents to prostate cancer metastases for an increased therapeutic efficacy directly at the tumour target and for a decreased toxicity at normal tissues (7). Radiolabelled antibodies are used with success for the therapy of lymphoma and other haematological malignancies (9-11), but for solid tumours radioimmunotherapy is still under investigation. There have been several attempts to apply it for different tumour types such as colorectal, ovarian, breast and renal carcinomas (12). In the present study, the mAb 3/F11 was directed against prostate cancer for the first radiotherapeutic experiments. Like the mAbs 3/A12 and 3/E7, the mAb 3/F11 is highly specific to cell-adherent PSMA (5), but binds to another conformational epitope and shows the highest antigen binding (6, 13).

For the radiolabelling of 3/F11, DOTA was used, which is known to be a highly stable chelator. 3/F11 and DOTA-3/F11 specifically bound to PSMA-expressing C4-2 cells with similar HMSC values, whereas no binding was detected to PSMA-negative DU 145 control cells. These data were comparable to those acquired in positron emission tomography (PET) imaging studies with 3/F11 (14) and indicated that the DOTA-labelling of 3/F11 neither affects the PSMA specificity nor inhibits the binding capacity of the mAb.

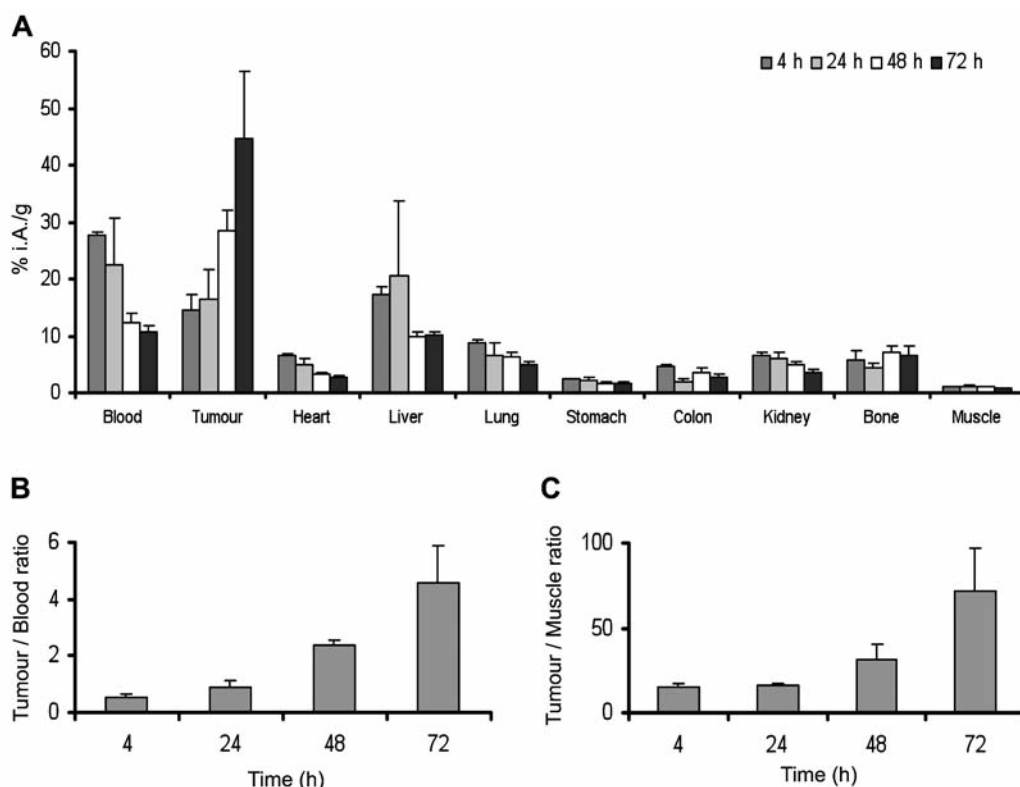


Figure 3. A: Biodistribution by gamma counting of  $^{177}\text{Lu}$ -DOTA-3/F11 in SCID mice bearing PSMA-positive C4-2 xenografts. Data are expressed as mean % injected activity per g tissue (% i.a./g)  $\pm$  standard error of the mean. Tumour-to-blood (B) and tumour-to-muscle (C) ratios of  $^{177}\text{Lu}$ -DOTA-3/F11 as calculated from the biodistribution data.

The biodistribution data showed that the tumour uptake of  $^{177}\text{Lu}$ -DOTA-3/F11 gradually increased with time. A more than 70-fold activity compared to the muscle and a more than 4.5-fold activity compared to the blood was measured 72 h after injection. It is suggested that the high tumour uptake and retention may be based on the rapid internalisation of the  $^{177}\text{Lu}$  DOTA-3/F11-PSMA complex into the target cells after binding (13), followed by metabolism and trapping of the radioactivity at the tumour site (15).

Remarkably, the treatment of SCID mice bearing small prostate cancer xenografts with a single dose of 1 Mbq  $^{177}\text{Lu}$ -DOTA-3/F11 showed a more than two-fold enhanced mean survival and a delay in tumour growth. However, this therapeutic window is small since mice treated with 2 MBq  $^{177}\text{Lu}$ -DOTA-3/F11 apparently died of myelotoxicity, which is the predominant dose-limiting factor in radioimmunotherapeutic approaches with  $^{177}\text{Lu}$  (16-18).

In the present study, the C4-2 prostate cancer SCID mouse xenograft model was used, because PET imaging studies have shown that the  $^{64}\text{Cu}$ -labelled 3/F11 already has excellent biodistribution and tumour uptake in this model (14). Yet, since it is known that SCID mice are relatively radiosensitive to ionising radiation compared to other mouse

strains (19, 20), the use of mice with reduced radiosensitivity (e.g. athymic nude mice) may broaden the therapeutic window of the developed method. Additionally, a fractionated-dose regimen with multiple administrations of  $^{177}\text{Lu}$  DOTA-3/F11 may be more advantageous and less toxic compared to a single-dose treatment (21, 16).

In conclusion, the *in vivo* experiments with  $^{177}\text{Lu}$ -DOTA-3/F11 in the C4-2 SCID mouse xenograft model supported its potential for further preclinical evaluation and application as a radioimmunotherapeutic agent for the treatment of prostate cancer.

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## References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ: Cancer statistics, 2009. *CA Cancer J Clin* 59(4): 225-249, 2009.
- Eriksson D and Stigbrand T: Radiation-induced cell death mechanisms. *Tumor Biol* 31(4): 363-372, 2010.



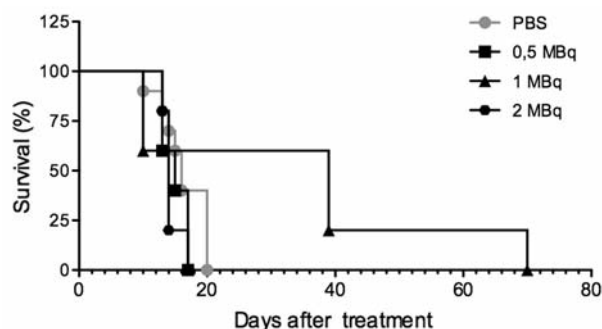


Figure 4. Survival of SCID mice bearing C4-2 xenografts after treatment with a single dose of  $^{177}\text{Lu}$ -DOTA-3/F11.

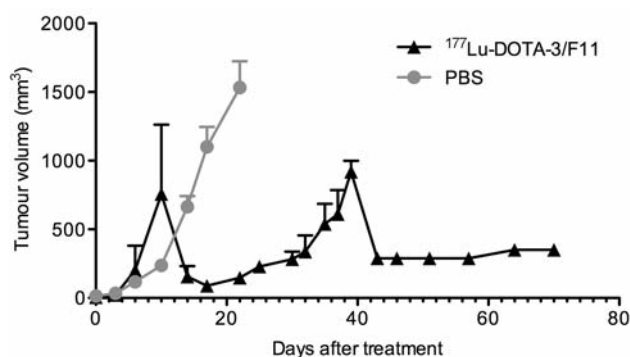


Figure 5. Effect of a single-dose of 1 MBq  $^{177}\text{Lu}$ -DOTA-3/F11 treatment on tumour growth of SCID mice bearing C4-2 xenografts. Control mice were injected with PBS. Data are expressed as mean  $\pm$  standard error of the mean. Drops of the curve of mice treated with 1 MBq  $^{177}\text{Lu}$ -DOTA-3/F11 at days 10 and 39 are due to the sacrifice of animals due to fast growing tumours at these days.

- 3 Bethge WA and Sandmaier BM: Targeted cancer therapy using radiolabeled monoclonal antibodies. *Technol Cancer Res Treat* 4(4): 393-405, 2005.
- 4 Arya M, Bott SR, Shergill IS, Ahmed HU, Williamson M and Patel HR: The metastatic cascade in prostate cancer. *Surg Oncol* 15(3): 117-128, 2006.
- 5 Elsaesser-Beile U, Wolf P, Gierschner D, Buehler P, Schultze-Seemann W and Wetterauer U: A new generation of monoclonal and recombinant antibodies against cell-adherent prostate-specific membrane antigen for diagnostic and therapeutic targeting of prostate cancer. *Prostate* 66(13): 1359-1370, 2006.
- 6 Wolf P, Freudenberg N, Buehler P, Alt K, Schultze-Seemann W, Wetterauer U and Elsaesser-Beile U: Three conformational antibodies specific for different PSMA epitopes are promising diagnostic and therapeutic tools for prostate cancer. *Prostate* 70(5): 562-569, 2010.
- 7 Elsaesser-Beile U, Buehler P and Wolf P: Targeted therapies for prostate cancer against the prostate-specific membrane antigen. *Curr Drug Targets* 10(2): 118-125, 2009.
- 8 Lewis MR, Kao JY, Anderson AL, Shively JE and Raubitschek A: An improved method for conjugating monoclonal antibodies

with *N*-hydroxysulfosuccinimidyl DOTA. *Bioconjug Chem* 12(2): 320-324, 2001.

- 9 DeNardo G and DeNardo S: Dose intensified molecular targeted radiotherapy for cancer-lymphoma as a paradigm. *Semin Nucl Med* 40(2): 136-144,
- 10 Goldsmith SJ: Radioimmunotherapy of lymphoma: Bexxar and Zevalin. *Semin Nucl Med* 40(2): 122-135,
- 11 Sharkey RM and Goldenberg DM: Novel radioimmunopharmaceuticals for cancer imaging and therapy. *Curr Opin Investig Drugs* 9(12): 1302-1316, 2008.
- 12 Boerman OC, Koppe MJ, Postema EJ, Corstens FH and Oyen WJ: Radionuclide therapy of cancer with radiolabeled antibodies. *Anticancer Agents Med Chem* 7(3): 335-343, 2007.
- 13 Liu J, Kopeckova P, Buehler P, Wolf P, Pan H, Bauer H, Elsaesser-Beile U and Kopecek J: Biorecognition and Subcellular Trafficking of HPMA Copolymer-Anti-PSMA Antibody Conjugates by Prostate Cancer Cells. *Mol Pharm* 6(3): 959-970, 2009.
- 14 Alt K, Wiehr S, Ehrlichmann W, Reischl G, Wolf P, Pichler BJ, Elsaesser-Beile U and Buehler P: High-resolution animal PET imaging of prostate cancer xenografts with three different (64)Cu-labeled antibodies against native cell-adherent PSMA. *Prostate* 70(13): 1413-1421, 2010.
- 15 Wu AM and Senter PD: Arming antibodies: prospects and challenges for immunoconjugates. *Nat Biotechnol* 23(9): 1137-1146, 2005.
- 16 Schlom J, Siler K, Milenic DE, Eggensperger D, Colcher D, Miller LS, Houchens D, Cheng R, Kaplan D and Goeckeler W: Monoclonal antibody-based therapy of a human tumor xenograft with a  $^{177}\text{Lu}$ -labeled immunoconjugate. *Cancer Res* 51(11): 2889-2896, 1991.
- 17 Vallabhajosula S, Goldsmith SJ, Kostakoglu L, Milowsky MI, Nanus DM and Bander NH: Radioimmunotherapy of prostate cancer using 90Y- and  $^{177}\text{Lu}$ -labeled J591 monoclonal antibodies: effect of multiple treatments on myelotoxicity. *Clin Cancer Res* 11(19 Pt 2): 7195s-7200s, 2005.
- 18 Vallabhajosula S, Goldsmith SJ, Hamacher KA, Kostakoglu L, Konishi S, Milowski MI, Nanus DM and Bander NH: Prediction of myelotoxicity based on bone marrow radiation-absorbed dose: radioimmunotherapy studies using 90Y- and  $^{177}\text{Lu}$ -labeled J591 antibodies specific for prostate-specific membrane antigen. *J Nucl Med* 46(5): 850-858, 2005.
- 19 Urushibara A, Kodama S, Suzuki K, Desa MB, Suzuki F, Tsutsui T and Watanabe M: Involvement of telomere dysfunction in the induction of genomic instability by radiation in SCID mouse cells. *Biochem Biophys Res Commun* 313(4): 1037-1043, 2004.
- 20 Biedermann KA, Sun JR, Giaccia AJ, Tosto LM and Brown JM: SCID mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. *Proc Natl Acad Sci USA* 88(4): 1394-1397, 1991.
- 21 Vallabhajosula S, Smith-Jones PM, Navarro V, Goldsmith SJ and Bander NH: Radioimmunotherapy of prostate cancer in human xenografts using monoclonal antibodies specific to prostate-specific membrane antigen (PSMA): studies in nude mice. *Prostate* 58(2): 145-155, 2004.

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