

Labelling chemistry and characterization of [⁹⁰Y/¹⁷⁷Lu]-DOTA-Z_{HER2:342}-3 Affibody molecule, a candidate agent for locoregional treatment of urinary bladder carcinoma

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Abstract. The direct instillation of radiolabelled conjugates in the urinary bladder is a promising path for the treatment of bladder carcinoma. The targeting of HER2/neu receptors expressed on the surface of many bladder carcinoma cells shows potential to be developed as a therapeutic strategy, and patients identified with a high risk of progression may benefit from adjuvant targeted radionuclide therapy. A phage-display selected Affibody molecule (Z_{HER2:342}) which binds to HER2/neu with picomolar affinity, can be used for targeting HER2/neu-expressing bladder carcinomas. A DOTA-derivative of Z_{HER2:342}, designated as DOTA-Z_{HER2:342}-3, is considered as a suitable targeting agent for therapy. The DOTA chelator provides stable labelling with radiometals, and the low molecular weight (7.2 kDa) of the DOTA-Z_{HER2:342}-3 compound is expected to enable efficient tumor penetration. DOTA-Z_{HER2:342}-3 was radiolabelled with ⁹⁰Y and ¹⁷⁷Lu in 1 M ammonium acetate buffer, at pH 5.5, and in the presence of ascorbic acid. Nearly quantitative labelling yields were achieved for both nuclides after 15 min of incubation at 60°C. After chelation, the conjugates retained their capacity to specifically bind to HER2/neu-expressing SKOV-3 cells. The radiolabelled affibody conjugate (DOTA-Z_{HER2:342}-3) demonstrated high antigen-binding capacity and good cellular retention. Bio-distribution in normal mice demonstrated low uptake in all organs and tissues except for kidneys.

Introduction

Urinary bladder carcinoma is a common malignancy in the developed countries, and the incidence of this disease is

increasing. Most urinary bladder carcinomas are diagnosed using computed tomography (CT) and cystoscopic procedures; in a majority of cases the procedure is followed by endoscopic surgery. Transurethral resection (TUR) is the primary therapy for superficial bladder carcinoma, but recurrence rates from 15 to 30% are expected after 50 months (1,2). The disease can be treated more efficiently if TUR is followed by intravesical bacille Calmette-Guérin (BCG) therapy (3-6). This adjuvant does not specifically target the disease. It has also been shown that intravesical chemotherapy could be effective in decreasing the short-term incidence of tumour recurrence (1,7). No evidence was found however that such treatment could decrease the long-term incidence of tumor recurrence, according to 60- to 77-month follow-up study (1).

The direct instillation of radioactive targeting agents in the bladder can offer a promising path to the treatment of bladder carcinoma. Radioactive targeting conjugates can be injected into the bladder and left for several hours to allow tissue penetration. The fluid is then removed from the patient. During incubation, the radioactive targeting molecules diffuse into the bladder tissue and bind to molecular targets expressed by the cancer cells. Most of the disadvantages related to invasive surgery are avoided through this procedure. In comparison to external radiation therapy, the patient is treated with a radiation therapy technique that leaves limited damage to the surrounding healthy tissue.

Systemic radionuclide therapy of disseminated cancer is often limited by the risk of exceeding the dose burden on critical organs such as red bone marrow, the liver, and the kidneys, which is often the case when using small targeting proteins and peptides. This issue can be avoided by using the technique of intravesical instillation, in which direct instillation of radioconjugates in the bladder allows the delivery of cytotoxic substances directly to the tumour cells. New radio-immunotherapeutical conjugates have been developed in the last 15 years with the prospect of treating bladder cancer through such procedures. Most of these involved antibodies radiolabelled with ¹¹¹In (8,9), ¹³¹I (10), ⁶⁷Cu (11,12), ¹⁸⁸Re (13). However, the large size of monoclonal antibodies (~150 kD) is a general limitation to the efficiency of intravesical treatments due to slow diffusion and limited penetration. Therefore,

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smaller antibody fragments (14-18) and single domain antibodies (19-21) have been considered for application.

Affibody molecules ('affibody' is used to denote 'Affibody®'), a class of affinity ligands, have been described in previous reports and studies (22-25). The affibody protein is a low molecular weight molecule (7.2 kDa), characterized by a robust structure. Affibody molecules showing high affinity to HER2/neu-expressing cells have been developed (26,27) recently. The receptor HER2/neu, also called ErbB2, is a transmembrane protein belonging to the human epidermal growth factor tyrosine kinase receptor family (28). In urinary bladder carcinoma, it can be expressed in up to 80% of primary cancers, especially in metastatic cases (29,30). It is either absent or only slightly expressed in the majority of normal adult tissues (31,32). An increase in HER2/neu activity is associated with both increased proliferation of cells, and resistance to apoptosis. Therefore, the HER2/neu receptor can be used to target bladder cancer. Pre-clinical investigation has confirmed that anti-HER2/neu affibody ligands can selectively target HER2/neu-expressing tumor xenografts *in vivo* (27,33-36).

We have selected the molecule DOTA-Z_{HER2:342}-3 for further *in vitro* and *in vivo* characterization, aiming at developing a molecular targeting radiotherapeutic that would be an alternative to existing bladder carcinoma treatments. This targeting agent is based on the affibody ligand Z_{HER2:342}, which binds to HER2 with an affinity of 22 pM (35). To enable stable binding of therapeutic radiometals, a DOTA-chelator was coupled to affibody ligand Z_{HER2:342} using succinimide ester-based chemistry. An extended characterization study of the binding properties of DOTA-Z_{HER2:342}-3, comprising real-time biospecific interaction analysis (biosensor binding studies, BIAcore) and LS-MS will be reported elsewhere. The aims of the present study were: i) to establish the labelling chemistry of the affibody compound DOTA-Z_{HER2:342}-3 with the radioisotopes ⁹⁰Y and ¹⁷⁷Lu; ii) to evaluate *in vitro* the stability and the tumour-binding capacity of the compound (to HER2/neu expressing cells); and iii) to study *in vivo* the biodistribution of the radioactive conjugate in normal mice. For this study, we have selected the two nuclides ⁹⁰Y and ¹⁷⁷Lu. The first radioisotope is a pure β -emitter with a radiation spectrum well-suited for the treatment of relatively bulky tumours (3.9 mm mean range in soft tissues), whereas ¹⁷⁷Lu is selected in the case of millimetric and submillimetric metastasis (0.67 mm mean range in soft tissues).

Materials and methods

Materials. ⁹⁰Y was purchased from Schering, Berlin (yttrium chloride in 0.05 M HCl, radiochemical purity >99%, 2 GBq/ml at calibration time) and ¹⁷⁷Lu from IDB Holland, Petten (lutetium chloride in 0.05 M HCl, radiochemical purity >99%, 3 GBq/ml at calibration time). High-quality Milli-Q water was used for the preparation of all solutions, and all commercially available chemicals were of analytical grade or better. Ammonium acetate buffer (1 M) at pH 5.6 and containing 5 mg/ml of ascorbic acid was used for the radiolabelling. The buffer was purified from metal contamination using Chelex 100 resin (Bio-Rad Laboratories, Richmond, CA, USA). Sodium citrate was used during ITLC procedures (instant thin

layer chromatography) and EDTA was used for challenging the chelation of the radioisotopes by DOTA. DOTA-Z_{HER2:342}-3 affibody molecules were kindly provided by Affibody AB (Bromma, Sweden).

Instrumentation. During cell studies and organ distribution experiments, the radioactive samples were measured by means of an automated gamma-counter [3-in. NaI (Tl) well detector, 1480 WIZARD; Wallac Oy, Turku, Finland]. For the measurement of ⁹⁰Y (bremstrahlung) and ¹⁷⁷Lu (primary gammas and bremstrahlung), the collection window was set to 0-1000 keV and 0-600 keV respectively. The distribution of radioactivity along the ITLC strips was measured with a Cyclone storage phosphor system (Perkin-Elmer) and analyzed using OptiQuant image analysis software. Cells were counted using an electronic cell counter (Z2; Beckman-Coulter, Fullerton, CA, USA).

Radiolabelling. The experiments were performed in 50 μ l of 1 M acetate buffer, pH 5.5, using 1.5 ml microcentrifugation tubes (Elkay, Costelloe, Ireland) each one containing 50 μ g of DOTA-Z_{HER2:342}-3. ⁹⁰Y was dissolved in acetate buffer to a concentration of 1 MBq/ μ l, and the affibody conjugate (50 μ g) was mixed with 25 μ l of this radioisotopic solution. In the studies involving ¹⁷⁷Lu, the affibody ligand conjugate (50 μ g, 50 μ l, acetate buffer) was radiolabelled with 22.2 μ l of ¹⁷⁷Lu (0.034 μ g/ μ l). The ratio of ¹⁷⁷Lu:DOTA-Z_{HER2:342}-3 was kept to 1:3. All experiments were carried out at least in duplicate. Heating was performed in a temperature-controlled heating block. ITLC was performed with each compound to assess the labelling yield (silica gel impregnated glass fibre sheets from Pall Corporation, Ann Arbor, Michigan).

Stability tests. DOTA-Z_{HER2:342}-3 (50 μ g) was radiolabelled with ⁹⁰Y (at 50°C) or with ¹⁷⁷Lu (at 60°C) for 30 min, in accordance with the optimal conditions established in the first step of this study. ITLC was performed shortly after radiolabelling. Samples were diluted with PBS and kept at room temperature. Further ITLC tests were performed after 1, 3 and 24 h. In parallel to the PBS stability tests, the quality of DOTA chelation was challenged by submitting the radiolabelled compounds to a PBS solution containing EDTA in 50-fold molar excess over Affibody molecules. Samples were incubated at room temperature, and ITLC was performed on the compounds after 1, 3 and 24 h. All tests were performed in duplicate.

Binding of DOTA-Z_{HER2:342}-3 to SKOV3 cells. The tumour-binding capacity of the molecule was evaluated by adding the radiolabelled compound to SKOV-3 cells, a human carcinoma cell-line expressing a high level of HER2/neu (1-2x10⁶ per cell). Cells (ATCC, Rockville, MD, USA) were cultured in Ham's F-10 medium, supplemented with 10% fetal calf serum (Sigma, USA), 2 mM L-glutamine and PEST (100 IU/ml penicillin, 100 μ g/ml streptomycin), all obtained from Biochrom (Berlin, Germany). This medium is referred to as complete medium in the text below. An *in vitro* specificity test was performed by using cells cultured in Petri dishes (3.5-cm diameter) maintained in a humidified incubator (37°C, 5% CO₂). Cell monolayers were grown to a density of approximately 0.4x10⁶ cells per dish. Prior to the experiments,

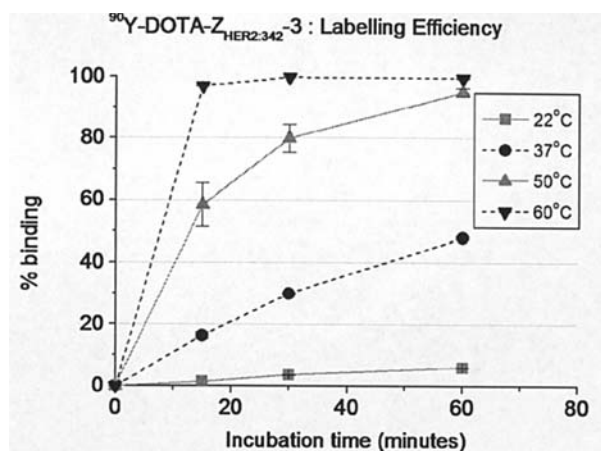


Figure 1. Labelling efficiency of DOTA- $Z_{HER2:342}$ -3 with ^{90}Y in 1 M ammonium acetate buffer (pH 5.6) as a function of time and at different temperatures. Data are presented as mean values of the results of two experiments \pm maximum errors. Error bars may not be observable if they are smaller than point symbols.

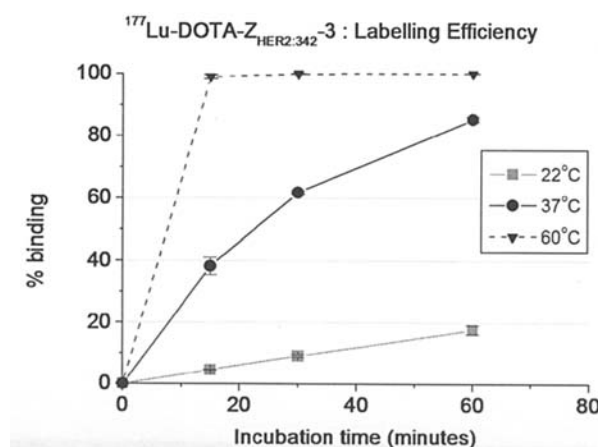


Figure 2. Labelling efficiency of DOTA- $Z_{HER2:342}$ -3 with ^{177}Lu in 1 M ammonium acetate buffer (pH 5.6) as a function of time and at different temperatures. Data are presented as mean values of the results of two experiments \pm maximum errors. Error bars may not be observable if they are smaller than point symbols.

the cell layers were rinsed with 1 ml of complete medium. In half of the dishes, high concentrations of non-radioactive $Z_{HER2:342}$ affibody molecule were added with 0.5 ml of complete medium for blocking the HER2/neu receptors (approximately 100 molar excess). The other set of dishes received 0.5 ml of complete medium only. Medium containing the radiolabelled conjugate was then added to both series of dishes in a ratio of approximately one affibody molecule per receptor (7 ng per dish in 0.5 ml of medium). After 1-h incubation, the cells were washed 6 times with cold serum-free medium. The cells were then trypsinized with 0.5 ml of trypsin-EDTA solution (0.05% trypsin, 0.02% EDTA in buffer, Flow Irvine, UK) and maintained at 37°C for 15 min. Finally, 1 ml of standard medium was added to each dish. The cell suspension (1 ml) was transferred to scintillation vials and the cell-associated radioactivity was measured by an automated gamma counter. The rest of the suspension (0.5 ml) was used to evaluate the number of cells in the Petri dish.

For cell retention studies, similar Petri dishes were washed with complete medium, and then pre-incubated with 1 ml of radiolabelled conjugate (ratio of 1:1, $Z_{HER2:342}$ -3:receptor) for 2 h at 37°C. The incubation was interrupted by washing the cells 6 times with cold, serum-free culture medium. Complete medium (1 ml) was added to the cells, and incubated again for up to 75 h at 37°C. At pre-determined time points, incubation media were collected and counted for radioactivity. Finally, the cells were washed 6 times with cold, serum-free medium, then trypsinized and resuspended in 0.5 ml of complete medium for radioactivity measurement.

The antigen binding capacity (ABC), which is analog to the immunoreactivity for labelled antibodies, was evaluated in the following way. In order to preserve the receptor expression, SKOV-3 cells were scraped from the surface of cell culture bottles. After re-suspension in medium, cells were counted in an automated cell counter and cell pellets containing 6×10^6 cells were formed in Eppendorf tubes by gentle centrifugation (2500 \times g). This procedure was performed in triplicate. The supernatant was then completely removed from the centrifugation tubes and replaced by 1 ml of medium

containing labelled conjugate, in a ratio of ~ 1 affibody molecule per 100 HER2/neu receptors. Cells were gently re-suspended and incubated for 4 h at 4°C under slight shaking. After incubation the cells were centrifuged (7500 \times g) and 0.5 ml of supernatant medium was taken from the centrifugation tubes and placed in separate Eppendorf tubes. Radioactivity was measured on both samples and antigen binding capacity (ABC) was calculated as $ABC = (A-B)/(A+B) \times 100\%$, where A is the radioactivity of the Eppendorf tube containing both cell pellet and 0.5 ml of supernatant and B is the radioactivity of the Eppendorf tube containing 0.5 ml of supernatant only.

Biodistribution of subcutaneously injected [^{177}Lu]-DOTA- $Z_{HER2:342}$ -3 in normal mice. To evaluate the *in vivo* behavior of the radioactive conjugate, biodistribution studies were performed with normal mice (NMRI, adult females). The animals were handled according to guidelines by the Swedish Animal Welfare Agency, and the experiments were approved by the local ethics committee. The animals were injected subcutaneously with 100 μ l of [^{177}Lu]-DOTA- $Z_{HER2:342}$ -3 (100 kBq, 3.3 μ g in PBS) each, and were sacrificed at 4 and 24 h post injection. They were anesthetized by a mixture of Rompun (1 mg/ml) and Ketalar (10 mg/ml), 0.1 ml per 10 g of animal weight, and sacrificed by heart puncture. Blood was collected, and the heart, bladder (emptied), pancreas, spleen, stomach, liver, kidneys, lungs, large intestine, skin, muscle, bone and brain were dissected, weighed, and measured for radioactive content with an automated gamma counter. Organ values were calculated as a percent of injected activity per g of organ (% I.A./g).

Results

Radiolabelling. Reaction kinetics with ^{90}Y and ^{177}Lu were investigated at room temperature, 37°C, 50°C (^{90}Y only) and 60°C. The reaction kinetics is represented in Figs. 1 and 2. Addition of ^{90}Y and ^{177}Lu to the solution of DOTA- $Z_{HER2:342}$ -3 molecules in 1 M ammonium acetate at pH 5.6 gives high

Table I. *In vitro* stability of ^{177}Lu and ^{90}Y -labelled DOTA- $\text{Z}_{\text{HER2}:342}$ -3. Data are presented as a percent of affibody molecule-associated radioactivity \pm maximum error.

Incubation time (h)	^{90}Y -DOTA- $\text{Z}_{\text{HER2}:342}$ -3		^{177}Lu -DOTA- $\text{Z}_{\text{HER2}:342}$ -3	
	EDTA (50-fold excess)	PBS	EDTA (50-fold excess)	PBS
1	99.2 \pm 0.2	99.5 \pm 0.2	99.65 \pm 0.05	99.65 \pm 0.05
3	99.25 \pm 0.25	99.65 \pm 0.05	99.65 \pm 0.05	99.75 \pm 0.15
24	96.75 \pm 2.25	99.0 \pm 0.0	99.3 \pm 0.0	92.3 \pm 1.2

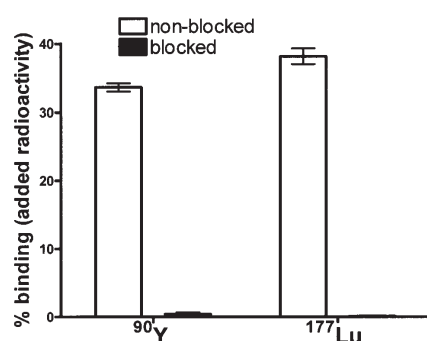


Figure 3. Cell-binding specificity of ^{90}Y - and ^{177}Lu -DOTA- $\text{Z}_{\text{HER2}:342}$ -3 labelled at 60°C during 15 min. The test was performed with ovarian carcinoma cell line SKOV-3, expressing a high level of HER2 receptors. In the blocking experiment, binding sites were saturated by adding to the cells an excess of non-labelled $\text{Z}_{\text{HER2}:342}$ -3. Cells were incubated with ^{177}Lu -DOTA- $\text{Z}_{\text{HER2}:342}$ -3. Data are presented as mean values for three samples \pm maximum errors.

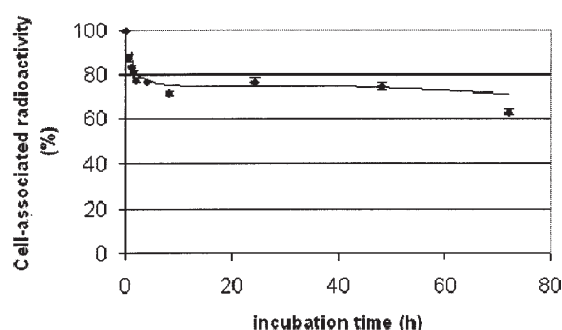


Figure 4. Cellular retention of ^{177}Lu -DOTA- $\text{Z}_{\text{HER2}:342}$ -3 radioactivity in SKOV-3 cells. Data are normalized to the cell-associated radioactivity value at $t=0$, which corresponds to the time at which incubation with the radioactive conjugate was interrupted. All data points represent mean values from three gamma-counting measurements \pm maximum errors.

reaction yields (>95%) after only 15 min at 60°C. The reaction kinetics is faster with ^{177}Lu than with ^{90}Y . Our results demonstrated that the rapid labelling of the molecule DOTA- $\text{Z}_{\text{HER2}:342}$ -3 can be achieved at a moderate temperature and within a reasonable time for both radioisotopes. The stability of the label was tested *in vitro* after 1, 3 and 24 h post-incubation, and the results of the ITLC tests are shown in Table I, for samples submitted to PBS and EDTA solution (50-fold excess).

Cellular binding and retention. Data obtained from the cell-binding specificity tests with ^{90}Y - and ^{177}Lu -DOTA- $\text{Z}_{\text{HER2}:342}$ -3

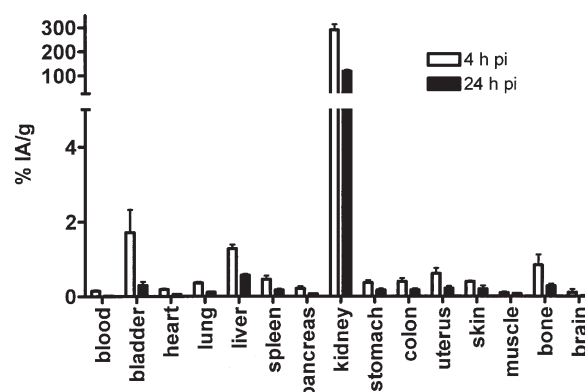


Figure 5. Biodistribution of ^{177}Lu -DOTA- $\text{Z}_{\text{HER2}:342}$ -3 in NRMI mice. Data represents the mean values of four animals \pm standard deviation.

are given in Fig. 3. Without blocking of the HER2/neu receptors, the cellular association was 34 \pm 1% and 38 \pm 2% for ^{90}Y and ^{177}Lu respectively. Large excesses of affibody ligands added to the cell dishes reduced cell-associated radioactivity to 0.4% and 0.5%. Previous studies demonstrated that the $\text{Z}_{\text{HER2}:342}$ molecule binds specifically to HER2/neu receptors. The results reported in this study confirm the presence of a receptor-mediated type of binding after coupling of chelator and labelling (DOTA; ^{90}Y and ^{177}Lu) to the core molecule ($\text{Z}_{\text{HER2}:342}$). The antigen binding capacity (ABC) was determined to be 89.1 \pm 0.5%. The cellular retention of ^{177}Lu was studied as a function of time, and the results are shown in Fig. 4. The cellular retention was high and stable (to ~75%) between 2 and 48 h.

Biodistribution in normal nude mice. Normal nude mice injected with ^{177}Lu -DOTA- $\text{Z}_{\text{HER2}:342}$ -3 were euthanized after 4 and 24 h post-injection. Blood concentration and organ distribution of ^{177}Lu were determined. Biodistribution results (Fig. 5) indicate rapid clearance of the radioisotope from blood and very low uptake in most organs. Virtually no uptake was detected in the brain. Limited uptake was noticed in bone, and after 24 h the initial concentration showed a 2/3 decrease. Therefore, we can assume that there is probably a very limited permanent retention of the radioisotope in bone. For all organs, we observed a general tendency towards a decrease in the radioactivity accumulation over time. Despite generally low uptake values, most of the radiotracer was found in the kidneys after 4 h (>280% I.A./g), and after 24 h (117% I.A./g). This

result indicates that the affibody complex is eliminated via the kidneys.

Discussion

The improvement of local intravesical treatments involving radiolabelled conjugates could help treat or at least control the progression of primary bladder carcinoma. Patients suffering from bladder cancer are an ageing population, for whom treatments such as external beam therapy or invasive surgery should be substituted by non-invasive procedures. At later stages of the disease, external beam therapy can be an effective method for treating bladder carcinoma, but there are risks of over-irradiating healthy tissues surrounding the bladder. Targeted radionuclide therapy is therefore one of the most promising approaches to deliver a therapeutical dose to disseminated tumour cells with minimal damage to the surrounding tissue (37,38), and at various stages of the disease. The urinary bladder provides a natural and simple access to fluid instillation treatments. Many drawbacks related to the systemic injection of radiolabelled small protein conjugates can be avoided, such as high uptake of the radionuclide in the kidneys.

Previous studies involving the use of monoclonal antibodies have shown the potential of radioimmunotargeting for the diagnostics and therapy of bladder cancer. Monoclonal antibodies have been radiolabelled with ^{111}In and $^{99\text{m}}\text{Tc}$ (8,9,39-42) for SPECT and scintigraphy imaging. Both ^{67}Cu and ^{188}Re have been suggested (11-13) as nuclides to be used for the therapy of bladder cancer. However, reported cystological data obtained from patients treated through direct instillation with targeting immunoagents generally indicate low uptake of radioactivity. The large size of intact immunoglobulins is the most probable reason explaining the poor tissue penetration of the compound given the limited duration of the urinary bladder instillation treatment. In contrast to antibodies, affibody molecules are small. Thus a molecule such as $\text{Z}_{\text{HER2:342}}$, which has a high affinity to HER2/neu receptors, can diffuse more efficiently into the interstitial space of the affected tissue and bind to HER2/neu receptors expressed on the surface of non-superficial cancer cells. However, such a strategy requires the development of a labelling chemistry adapted to this type of affinity ligands.

The affibody molecules are known to be very stable in harsh labelling conditions (high temperature and non-physiological pH), which is a major advantage in radiochemistry. However, long incubation times during labelling can affect the molecule by provoking radiolysis, and higher temperatures increase the risk of denaturation. The results of this study show that DOTA-affibody molecules can be radiolabelled with ^{90}Y and ^{177}Lu at high chelation yields (>95%), and under reasonable conditions of time and temperature. An almost quantitative labelling was achieved within 15 min at 60°C, without affecting the binding capacity of the targeting agent. Results for the *in vitro* stability tests suggest that the coupling of the radionuclide to DOTA- $\text{Z}_{\text{HER2:342}}$ -3 is chelator-mediated. No evidence was found for the presence of interfering side chains of amino acids that can provide occasional low-affinity binding sites for the radiometals. Purified buffer solutions must be used in order to avoid the presence of competing metal ions. It is

worth mentioning that despite the availability in the NIST database (43) of DOTA chelation stability constants for various nuclides, information about reaction kinetics is limited to a small number of studies. Therefore, the present work offers a complement to available data reported on the labelling of DOTA with ^{177}Lu and ^{90}Y .

SKOV-3 cells are known to express a high level of HER2/neu receptors, and therefore have been used to demonstrate the capacity of radiolabelled affibody ligand to bind living HER2/neu-expressing cells. The high cellular retention (>70% from 2-48 h) found in our experiments suggests that the use of DOTA- $\text{Z}_{\text{HER2:342}}$ -3 radiolabelled with metals can be a promising approach to minimize rapid leakage of radioactivity from the tumour cells.

Neither the development of a bladder cancer xenograft model on mice, nor the design of equipment for reproducing direct bladder instillation on such small animals are within the scope of this study. However, biodistribution studies with normal mice can provide crucial information about the general behaviour of the radiolabelled conjugate. Injected subcutaneously, the radioactive compound is drained to the blood through the lymphatic system of the animal. Information extracted from such an animal model and procedure is helpful to anticipate the physiological behavior of the same radioactive compound after extraction from the bladder tissue through the lymphatic system, assuming good penetration of the molecule DOTA- $\text{Z}_{\text{HER2:342}}$ -3 following direct instillation procedures. ^{177}Lu -DOTA- $\text{Z}_{\text{HER2:342}}$ -3 was subcutaneously injected in normal nude mice, and the animals were euthanized and dissected after 4 and 24 h post-injection. Comparing the results obtained for 4 and 24 h post-injection (Fig. 5), the affibody molecule appears to be efficiently cleared from the blood. Very limited uptake is noted in the liver. Most of the activity is excreted through the kidneys to the urinary tract. Despite an appreciable radioactivity clearance from the kidneys (3-fold decrease, comparing 4 h to 24 h post injection), the uptake in this organ remains very high. This could represent a serious local overdose risk to the patient should systemic injections be considered for the treatment of bladder carcinoma and metastases. The conjugate is however being developed for local direct instillation procedures aimed at treating primary cancers, which would drastically lower the dose to the kidneys.

This study provides crucial pre-clinical information necessary to establish the path of detailed *ex vivo* and *in vivo* studies involving human bladder cancers. Further pre-clinical trials could focus on the instillation of radioactive compounds in *ex vivo* human bladders, removed from patients during cystectomy procedures. Immunoscintigraphy or SPECT imaging studies could follow this type of *ex vivo* treatment, complemented with biopsies taken at different places on the *ex vivo* treated organs.

In conclusion, DOTA-affibody molecules can be radiolabelled with ^{90}Y and ^{177}Lu rapidly and at high yields, at a temperature of 60°C. The reactions were complete after 15 min. The DOTA- $\text{Z}_{\text{HER2:342}}$ -3 compound binds very specifically to HER2/neu receptors expressed in bladder carcinomas. Due to its stability, good affinity and intracellular retention, the affibody molecule is a promising targeting conjugate for the treatment of bladder cancer through direct instillation procedures.

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