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ORIGINAL ARTICLE

Treatment of carcinoma in situ of the urinary bladder with an alpha-emitter immunoconjugate targeting the epidermal growth factor receptor: a pilot study

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Abstract

Purpose Patients with carcinoma in situ (CIS) of the bladder refractory to bacillus Calmette-Guérin (BCG) treatment are usually treated with cystectomy. Therefore, new treatment options with preservation of the urinary bladder are needed. The objective of the study was to investigate the feasibility, safety and efficacy of a novel targeted alpha-emitter immunotherapy for CIS after BCG treatment failure.

Methods A pilot study was conducted in 12 patients (age range 64–86 years, ten men, two women) with biopsy-proven CIS of the bladder refractory to BCG treatment. The patients were treated intravesically with a single instillation (one patient was treated twice) of the alpha-emitter ^{213}Bi coupled to an anti-EGFR antibody (366–821 MBq). The primary aims of the study were to determine the feasibility of treatment with the ^{213}Bi-immunoconjugate and evaluation of adverse effects. Therapeutic efficacy was monitored by histological mapping of the urinary bladder 8 weeks after treatment and at different time points thereafter.

Results The study proved that intravesical instillation of the ^{213}Bi-immunoconjugate targeting EGFR is feasible. No adverse effects were observed and all blood and urine parameters determined remained in their normal ranges. Therapeutic efficacy was considered satisfactory, in that three of the 12 patients showed no signs of CIS 44, 30 and 3 months after treatment.

Conclusion Intravesical instillation of ^{213}Bi-anti-EGFR monoclonal antibody was well tolerated and showed therapeutic efficacy. Repeated instillation and/or instillation of higher activities of the ^{213}Bi-immunoconjugate might lead to better therapeutic outcomes. A phase I clinical trial is planned.

Keywords Alpha-emitter ^{213}Bi · ^{225}Ac/^{213}Bi generator · Targeted therapy · Bladder cancer · Feasibility · Adverse effects · Therapeutic efficacy

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Introduction

Worldwide bladder cancer is the seventh most frequent malignancy in men and the seventeenth most frequent malignancy in women [1]. At the time of diagnosis, approximately 70% of all bladder cancers are non-muscle-invasive (NMIBC) [2]. NMIBC comprise stage Ta tumors confined to the epithelium and stage T1 tumors that have penetrated the neighboring lamina propria, as well as high-grade carcinoma in situ (CIS/Tis), i.e. flat tumors restricted to the urothelium [3]. NMIBC are classified into low, intermediate and high-risk disease according to the risk of relapse and of developing muscle-invasive cancer. Among high-risk NMIBC, CIS deserves closer attention due to its propensity to invade neighboring tissues [4].

The standard therapy for CIS involves intravesical instillation of bacillus Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis*. BCG induces a local immune response in the bladder that finally supports eradication of tumor cells. In contrast to the treatment of other NMIBC tumors, extensive surgical removal of tumor tissue by transurethral resection is generally not applicable in CIS due to extensive spread in the bladder urothelium. The therapeutic efficacy of BCG treatment is monitored by regular posttreatment biopsies. Approximately 40% of patients showing a complete response to BCG induction therapy relapse at a median of 18 months [5]. Following BCG failure, BCG immunotherapy can be repeated. Repeated BCG treatment has been reported to result in freedom from disease at 2 years in 30–41% of patients [6]. Nevertheless, 60–70% of these patients relapse and are consequently classified as BCG-unresponsive. The preferred treatment option for BCG nonresponders is currently radical cystectomy [7]. However, the benefit of radical cystectomy is compromised by a drastically reduced quality of life. Therefore, several salvage bladder-preserving therapies are used that have shown therapeutic efficacy in some patients. These options include other immunotherapies, chemotherapy, device-assisted therapy (e.g. thermochemotherapy) and inhibition of immunological checkpoints [8].

Nevertheless, new effective bladder-preserving therapies that are free from side effects are urgently needed. In the long run, new therapeutic options might replace first-line BCG treatment that has been reported to induce serious side effects in some patients [9]. Because epidermal growth factor receptor (EGFR) is overexpressed in up to 75% of bladder cancers, it is a promising structure for use in targeted therapy [10, 11].

Radioimmunotherapy with alpha-particle-emitting radionuclides has been shown to be a promising concept in targeted cancer therapy [12]. Furthermore, intravesical instillation of radioimmunoconjugates composed of the alpha-emitter ^{213}Bi and a monoclonal antibody (MAb) targeting EGFR has demonstrated therapeutic efficacy in a nude mouse model of human bladder cancer [13, 14]. Therefore, we initiated a pilot study enrolling 12 patients with BCG-refractory CIS of the bladder to investigate safety, toxicity and therapeutic efficacy of intravesical instillation of ^{213}Bi-anti-EGFR MAb as a single administration in 11 patients and two administrations in one patient.

Materials and methods

A pilot study was designed for patients suffering from CIS of the bladder, unresponsive to BCG treatment. The aims of the study were to determine the feasibility and outcome of intravesical instillation of the alpha-emitter ^{213}Bi coupled to a MAb targeting EGFR. The study was performed as a collaboration between the Department of Urology and the Department of Nuclear Medicine of the Technische Universität München (TUM) as well as the Directorate for Nuclear Safety and Security of the European Commission JRC in Karlsruhe, Germany. Approval for the study was granted by the research ethics board of the Klinikum r.d. Isar of TUM. Signed informed consent was provided by all study participants. Twelve patients (ten men, two women) aged between 64 and 86 years with biopsy-proven CIS were enrolled between October 2013 and March 2017.

Preparation, quality control and administration of the ^{213}Bi-radioimmunoconjugate

The anti-EGFR MAb cetuximab (IgG1, GMP grade) was purchased from Merck KGaA (Darmstadt, Germany). Covalent coupling of the bifunctional chelator p-SCN-Bn-CHX-A - DTPA (Macrocyclics, Plano, TX, USA) to the anti-EGFR antibody was performed using a standard method [15]. Labeling of the chelated anti-EGFR antibody with the alpha-emitter ^{213}Bi ($t_{½}$ = 45.6 min) and quality control were done as described previously [13] with minor modifications. Briefly, ^{213}Bi eluted from a ^{225}Ac/^{213}Bi generator (approximately 1.11–1.48 GBq) was incubated with 100 μg of the chelate-conjugated anti-EGFR antibody for 5 min at room temperature. Subsequently, 900 μg of native cetuximab was added to prevent radiation-mediated decomposition of the radioimmunoconjugate. The ^{213}Bi-radioimmunoconjugate was separated from free ^{213}Bi by gel filtration using a 10DG desalting column (Bio-Rad, Munich, Germany). Finally, the ^{213}Bi-radioimmunoconjugate (3 ml) was stabilized by addition of 2.2 ml 20% ascorbic acid (pH 6.0), sterilized via filtration (Millex-LG, 0.20 μm; Merck-Millipore, Darmstadt, Germany) and injected into the bladder of the patient via a catheter.

Assessment of EGFR-targeting by the ^{213}Bi-radioimmunoconjugate

Targeting of the ^{213}Bi-radioimmunoconjugate was confirmed in vitro 1 day before instillation into patients using EJ28-luc bladder carcinoma cells that overexpress EGFR as a tumor surrogate, and the binding assay was carried out as described previously [13]. Binding to EJ28-luc cells of ≥10% of ^{213}Bi-immunoconjugates was considered adequate whereas binding of ≤3% was considered insufficient.

Analysis of sterility and bacterial endotoxins of the ^{213}Bi-radioimmunoconjugate sample

Following sterile filtration an aliquot of ^{213}Bi-anti-EGFR MAb (0.5 ml) was retained. The sample was sent for testing to a company providing comprehensive pharmaceutical testing services (Eurofins BioPharma GmbH, Munich, Germany).

Sterility assay was performed according to the European Pharmacopoeia (EP) 8.0. Quantitative bacterial endotoxin testing was carried out using the LAL assay according to EP 8.8.

Treatment of patients with the ^{213}Bi-radioimmunoconjugate

The ^{213}Bi-immunoconjugate (5.7 ml, 366–821 MBq) was injected using a syringe into the emptied bladder of the patient via a transurethral catheter. The syringe was then washed with 30 ml phosphate-buffered saline which was also injected into the bladder. Patients were asked to turn over repeatedly to ensure equal distribution of the radioimmunoconjugate in the bladder. The localization of the radioimmunoconjugate was monitored via SPECT imaging. The radioimmunoconjugate was retained in the bladder for 120 min and was then drained via the catheter. Patients were kept under observation as inpatients overnight. The efficacy of treatment with ^{213}Bi-anti-EGFR MAb was monitored via bladder mapping 6–8 weeks after treatment. Blood and urine (U-Stix, Roche, Germany) parameters were analyzed at several time points before and after treatment.

Estimation of absorbed dose delivered to the bladder wall

We used OLINDA/EXM software to estimate the absorbed dose (in Gy) in the bladder wall from the urinary content [16]. Based on three SPECT studies, calculation was performed using an average activity of ^{213}Bi-anti-EGFR MAb of 540 MBq, a bladder volume of 200 ml, and a time interval of 120 min. To include the biological effectiveness (RBE) of the alpha-radiation, we also estimated the biological effective dose (BED in Sv) using a RBE of 5 for alpha-particles. During the branched decay of ^{213}Bi to stable ^{209}Bi both alpha- and beta-particles and gamma-radiation are emitted [12]. We normalized the values obtained for the alpha, beta and gamma radiation to the surface area of the bladder wall.

Results

^{213}Bi labeling of the anti-EGFR antibody and bladder instillation

The ^{213}Bi-radioimmunoconjugate was prepared immediately before intravesical instillation due to the short half-life of ^{213}Bi. For this, ^{213}Bi eluted from the generator and the CHX-A -DTPA chelated anti-EGFR antibody were incubated and processed as described previously. Depending on the loading of the ^{225}Ac/^{213}Bi generator with ^{225}Ac, the preparation resulted in specific activities in the range 0.37–0.82 GBq ^{213}Bi/mg anti-EGFR antibody with >99% of the added ^{213}Bi bound to the antibody. Accordingly, between 366 and 821 MBq of the ^{213}Bi-anti-EGFR immunoconjugate was injected into the patient's bladder (Fig. 1). We concluded that both preparation and intravesical instillation of the ^{213}Bi-anti-EGFR conjugate could be managed safely. Sterility testing was positive in all cases and bacterial endotoxin levels were submarginal.

Binding of ^{213}Bi-anti-EGFR MAb to EJ28 bladder cancer cells

Incubation of the ^{213}Bi-radioimmunoconjugate at specific activities in the range 0.37–0.82 GBq/mg with EJ28 bladder cancer cells resulted in 66% (±6%) binding, demonstrating that the ^{213}Bi-radioimmunoconjugate was undamaged. In contrast, binding of a ^{213}Bi-immunoconjugate with a tenfold higher specific activity (3.7–8.2 GBq/mg) decreased to about 3% (data not shown). This might have been due to destruction of the anti-EGFR antibody via alpha particle irradiation. Therefore, addition of nonchelated anti-EGFR antibody (900 μg) to the ^{213}Bi-anti-EGFR MAb is required to ensure functionality of the ^{213}Bi-immunoconjugate.

Tolerance of ^{213}Bi immunoconjugate instillation and blood/urine parameters

All 12 patients treated with the ^{213}Bi-immunoconjugate showed excellent tolerance without any signs of adverse

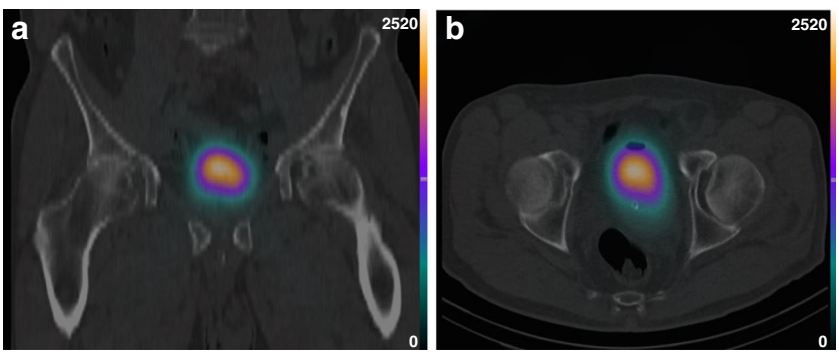

Fig. 1 Distribution of ^{213}Bi-anti-EGFR MAb after intravesical instillation visualized via SPECT/CT imaging: **a** coronal view, **b** axial view. ^{213}Bi activity remains in the bladder; systemic uptake and reflux into the ureters were not observed. The *colored bars* represent total counts (0 no signal, 2,520 maximum signal)

effects. Moreover, ^{213}Bi was not detected in blood samples taken from patients during and 1–2 h after treatment. Excellent tolerance of the treatment was supported by analysis of blood and urine parameters at different time points before and after intravesical instillation of ^{213}Bi-anti-EGFR MAb. Blood parameters did not suggest any adverse effects (Table 1). Only in patient 6, was blood coagulation reduced both before and after treatment, due to concomitant treatment with blood coagulation inhibitors. Likewise, routine laboratory testing of urine parameters did not suggest any adverse effects of intravesical instillation of ^{213}Bi-anti-EGFR MAb (data not shown).

Absorbed dose delivered to the bladder wall

The dosimetric calculation carried out using OLINDA/EXM software gave an average dose to the complete bladder wall from the urinary content of 2,538 mGy, resulting from

Table 1 Blood parameters of patients before and after intravesical instillation of ^{213}Bi-anti-EGFR MAb

Patient	Sex	Time of evaluation[b]	Hb (g/dl)	Hct (%)	RBC ($\times 10^{12}$/l)	WBC ($\times 10^{9}$/l)	Plt ($\times 10^{9}$/l)	INR (ratio)	aPTT (s)	Quick (%)[e]	Crea (mg/dl)
1	F	Before treatment	10.7[c]	33.6[c]	4.2	4.8	406	1.0	26	106	1.2[c]
		After treatment	11.1[c]	35.0	4.6	5.4	400	1.0	26	103	1.3[c]
2	M	Before treatment	15.1	42.7	4.8	4.8	155	1.1	31	91	1.4[c]
		After treatment	15.2	43.6	4.9	5.5	144	1.1	30	85	1.3
3	M	Before treatment	15.2	44.7	5.2	9.2	192	1.0	28	96	1.0
		After treatment	15.2	45.3	5.2	8.2	204	n.a.	n.a.	n.a.	1.2
4	M	Before treatment	14.1	41.4	4.4	4.75	221	1.1	31	91	1.2
		After treatment	14.5	42.4	4.5	4.8	199	1.0	30	93	1.2
5	M	Before treatment	12.5[c]	37.3[c]	4.1[c]	7.75	225	1.0	34	98	0.8
		After treatment	13.8	41.0	4.5	4.75	245	1.1	40	93	0.9
6[a]	M	Before treatment	14.5	41.7	4.7	9.75	423	2.2[d]	38	33[d]	0.9
		After treatment	15.6	44.8	5.1	8.0	336	1.4[d]	40	57[d]	0.9
7	M	Before treatment	13.7	42.5	4.5	6.9	266	1.0	33	105	1.7[d]
		After treatment	12.2[c]	38.3[c]	4.1	6.0	241	1.0	36	103	n.a.
8	M	Before treatment	15.1	42.8	5.2	7.6	226	1.0	30	99	0.9
		After treatment	14.9	43.9	5.0	9.1	244	1.0	30	94	1.0
9	M	Before treatment	13.8	39.8	4.5	6.9	232	1.0	27	95	1.0
		After treatment	14.5	41.7	4.7	9.6	231	1.0	26	99	1.1
10	F	Before treatment	14.7	45.0	4.8	5.2	200	0.9	24	111	0.7
		After treatment	15.2	45.8	4.9	5.9	207	0.9	24	109	0.6
11	M	Before treatment	10.9[c]	32.5[c]	3.7[c]	8.5	316	1.1	29	90	1.7[d]
		After treatment	11.7[c]	35.4[c]	4.0[c]	9.0	297	1.0	29	90	1.6[d]
12 (1)	M	Before treatment	15.1	43.2	4.7	4.7	121[c]	1.0	35	93	1.4[c]
		After treatment	15.6	44.7	4.9	6.5	124[c]	1.0	34	94	1.3
12 (2)	M	Before treatment	15.7	45.6	5.0	5.0	116[c]	1.0	36	105	1.5[c]
		After treatment	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Normal ranges: hemoglobin 12.0–16.0 g/dl in women, 13.0–17.5 g/dl in men; hematocrit 35–48% in women, 39–62% in men; red blood cell count 3.5–5.5 × 10^{12} /l in women, 4.2–6.9 × 10^{12} /l in men; white blood cell count 3.5–11.0 × 10^{9} /l in women and men; thrombocyte/platelet count 140–450 × 10^{9} / l in women and men; International Normalized Ratio 0.9–1.2 in women and men; activated partial thromboplastin time 18–45 s in women and men; Quick value (prothrombin time) 70–120% in women and men; creatinine 0.6–1.1 mg/dl in women, 0.7–1.3 mg/dl in men. Values are within their respective normal range except as indicated.

Hb hemoglobin, *Hct* hematocrit, *RBC* red blood cell count, *WBC* white blood cell count, *Plt* thrombocyte/platelet count, *INR* International Normalized Ratio (standardized prothrombin time), *aPTT* activated partial thromboplastin time, *Quick* Quick value, *Crea* creatinine, *n.a.* not analyzed

[a] Patient was treated with coagulation inhibitors; treatment explains strong deviation from INR and Quick value normal ranges

[b] Blood parameters were analyzed between days −25 and 0 before treatment and between days +1 and +47 after treatment

[c] Tolerable deviation from normal range

[d] Strong deviation from normal range

[e] After treatment of patients with coagulation inhibitors such as 4-hydroxycoumarins, the Quick value decreases to 15–36%

540 mGy (alpha) plus 1,944 mGy (beta) plus 54 mGy (gamma). Normalized to a surface area of 170 cm^2, the estimated dose to the bladder wall was therefore 15 mGy/cm^2. The calculation takes into account the fact that the beta-particles emitted during the branched decay of ^{213}Bi from unbound ^{213}Bi-anti-EGFR MAb within the bladder volume of 200 ml have a distinctly longer range than the alpha-particles emitted. Assuming a RBE of 5 for alpha particles, the BED to the bladder wall was 4,698 mSv and 28 mSv/cm^2. From the absorbed dose per injected activity of 4.7 mGy/MBq, the range of absorbed doses to the bladder wall in all patients treated with ^{213}Bi activities from 366 to 821 MBq was in the range 1.72–3.86 Gy.

Therapeutic efficacy of intravesical instillation of ^{213}Bi-anti-EGFR MAb

CIS patients refractory to BCG therapy and referred for cystectomy were offered the possibility to participate in a pilot study for evaluation of the tolerability and therapeutic efficacy of ^{213}Bi-anti-EGFR MAb instillation. Eleven patients received a single intravesical instillation and one patient received two intravesical instillations of 366–821 MBq of ^{213}Bi-anti-EGFR MAb, and the therapeutic efficacy of the treatment, i.e. CIS status, was monitored 6–8 weeks after treatment.

Patient characteristics and the results of the evaluation of therapeutic efficacy of ^{213}Bi-anti-EGFR MAb treatment are summarized in Table 2. Three patients (patients 1, 3, and 6) showed a clear benefit from a single intravesical instillation of ^{213}Bi-anti-EGFR MAb: bladder mapping 6–8 weeks after treatment showed no CIS in these patients. Of these patients, patients 1 and 6 showed no signs of CIS 44 and 30 months, respectively, after treatment. Patient 3 relapsed 15 months after treatment and cystectomy was performed 9 months later. In patient 12 instillation of ^{213}Bi-anti-EGFR MAb resulted in partial elimination of the tumor load and therefore a second intravesical treatment with ^{213}Bi-anti-EGFR MAb (650 MBq) was performed 4 months after the first treatment. Evaluation 3 months later showed complete eradication of CIS. The remaining patients did not benefit from intravesical treatment except for the increase in time to cystectomy in some patients.

Discussion

Intravesical treatment of CIS of the bladder may allow cystectomy to be avoided. Among intravesical therapies available, BCG is used most commonly and has a high success rate. Nevertheless, BCG treatment is associated with side effects that may outweigh its potential benefit [17]. Therefore, other intravesical therapies with fewer adverse side effects are desirable and are under investigation. The chemotherapeutic compounds used for intravesical treatment, including mitomycin C, doxorubicin, valrubicin, epirubicin and interferon alpha 2a, are associated with fewer local and systemic adverse effects than BCG; however, they are associated with a higher risk of tumor recurrence [17]. A phase II study of intravesical adenovirus-mediated interferon-α2b gene therapy (rAd-IFNα) in patients with relapse after BCG therapy has shown promising response rates and acceptable toxicity [18]. Immunotherapy targeting checkpoint inhibitors PD-L1 and PD-1 is another new concept for eradication of tumor cells [19]. The therapeutic efficacy of atezolizumab and pembrolizumab inhibiting PD-L1 and PD-1 is currently under evaluation in BCG-refractory bladder cancer in several phase I/II trials [20].

Treatment of CIS of the bladder with ^{213}Bi-anti-EGFR MAb

Another promising concept is the use of highly cytotoxic alpha-emitters coupled to antibodies specifically targeting bladder cancer cells. Intravesical instillation of the alpha-emitter ^{213}Bi coupled to an anti-EGFR antibody has shown promising results in a mouse model [13]. Moreover, targeted alpha-emitter therapies have been shown to be effective in the treatment of glioma [21], ovarian cancer [22, 23] castration-resistant prostate cancer [24], neuroendocrine tumors [25] and acute myeloid leukemia [26]. Therefore, a pilot study was initiated to evaluate the feasibility, tolerability and efficacy of ^{213}Bi-anti-EGFR MAb treatment in patients with BCG-refractory CIS. A single intravesical instillation (or two instillations) of 366–821 MBq of ^{213}Bi-anti-EGFR immunoconjugate (with specific activities in the range 0.37–0.82 GBq/mg) was tolerated without any adverse effects. Four patients showed a complete response, i.e. no visible CIS, 8 weeks after the first or second treatment. Three of these four patients were still tumor-free at the time of this report, i.e. 3 months after the second treatment and 30 and 44 months after the first treatment. To improve the therapeutic outcome, several modifications of the current procedure are discussed.

Dosimetric analyses

Since the patients treated with ^{213}Bi-anti-EGFR MAb clearly differed with regard to tumor burden and EGFR expression and since we did not quantify binding of ^{213}Bi-anti-EGFR MAb to the bladder tumor lesions prior to therapy, e.g. via imaging, dosimetric calculations of ^{213}Bi doses to the tumor lesions are not practicable. Therefore, extensive dosimetry will be the subject of a separate study. Nevertheless, we performed a dosimetric calculation to estimate the energy dose delivered to the bladder wall using OLINDA/EXM software. The absorbed doses to the bladder wall in all treated patients varied in the range 1.72–3.86 Gy. According to Meredith et al.

Table 2 Patient characteristics and outcomes of ^{213}Bi-anti-EGFR MAb treatment as at June 2017

Patient	Sex	Age (years)	CIS diagnosis	Pretreatment	CIS verification	EGFR status	^{213}Bi-anti-EGFR Therapy	^{213}Bi activity (MBq)	8-week follow-up	Next steps	Status/months since treatment
1	F	81	Mar 2013	BCG: May–Jul 2013	Aug 2013	n.a.	30 Oct 2013	366	CR – no malignancy	Repeated cytoscopic monitoring	CR/44
2	M	78	May 2008	Mitomycin: 2007 BCG: 2008 + May–Jul 2013	Jul 2013	1+	30 Oct 2013	444	SD – CIS	Cystectomy (Feb 2014)	44
3	M	76	Nov 2012	Mitomycin: 5× BCG: Jun 2013 to Mar 2014	Mar 2014	1+	30 Apr 2014	821	CR – no malignancy	CIS relapse (Jul 2015); cystectomy (Apr 2016)	38
4	M	72	Oct 2011	BCG: Nov 2011 to Jan 2012 + Dec 2012 to Apr 2013	Feb 2014	1+	30 Apr 2014	722	SD – CIS	BCG; CIS relapse (Nov 2016); cystectomy recommended	38
5	M	74	Apr 2009	BCG: Apr 2009 to Sep 2009	Oct 2014	1+	29 Oct 2014	426	SD – CIS	Cystectomy (May 2016)	32
6	M	68	Jul 2014	BCG: Jul 2014 to Sep 2014	Oct 2014	n.a.	22 Dec 2014	529	CR – no malignancy	Repeated cytoscopic monitoring	CR/30
7	M	74	Aug 2012	BCG: Jan 2013 to Mar 2014	Apr 2015	2+	3 Mar 2015	584	SD – CIS	Cystectomy (Apr 2015)	28
8	M	72	Mar 2015	Mitomycin: 2013 BCG: Mar to Jul 2015	Jul 2015	1+	29 Sep 2015	514	SD – CIS	Cystectomy (Feb 2016)	21
9	M	86	Jul 2013	BCG: Sep to Oct 2014 Gemcitabine: Oct 2014	May 2015	1+	23 Nov 2015	599	SD – CIS	Cystectomy recommended	19
10	F	77	Jan 2014	BCG: Apr 2014 to Apr 2016	Jun 2016	1+/2+	22 Sep 2016	596	SD – CIS	Cystectomy recommended	9
11	M	80	Feb 2016	BCG: Apr to Jul 2016	Oct 2016	2+	24 Nov 2016	821	SD – CIS	Cystectomy recommended	7
12 (1)	M	64	Jan 2015	BCG: Jun 2015 to Aug 2015 + Jan 2016 to Jun 2016	Sep 2015 + Sep 2016	1+	24 Nov 2016	685	PR – CIS	Second ^{213}Bi therapy Mar 2017	[see 12 (2)]
12 (2)	M	64	–	–	Feb 2017	1+	24 Mar 2017	650	CR – no malignancy	Repeated cytoscopic monitoring	CR/3

CR complete response, *PR* partial response, *SD* stable disease, *CIS* carcinoma in situ

[27], the maximum tolerated absorbed dose to the bladder wall is approximately 40 Gy. Therefore, administration of higher doses of ^{213}Bi-anti-EGFR MAb seems feasible. Moreover, to further improve the therapeutic outcomes of CIS treatment using ^{213}Bi-anti-EGFR MAb, quantification of the tumor burden using the PET tracer ^{68}Ga coupled to anti-EGFR MAb seems promising.

Improvement of therapeutic efficacy of ^{213}Bi-anti-EGFR MAb

One option is to increase the dose of ^{213}Bi. As discussed above, the ^{213}Bi doses used in this study (366–821 MBq) resulted in absorbed doses to the bladder wall far below the maximum tolerated doses and thus did not cause any adverse effects. Therefore, the highest ^{213}Bi-activity tolerated without side effects should be ascertained in a dose-escalation study. The use of this activity could then improve therapeutic efficacy. Moreover, repeated intravesical instillation of ^{213}Bi-anti-EGFR MAb might improve the therapeutic outcome, as has been demonstrated in a nude mouse model [14]. Because alpha-emitter immunoconjugates target the outer cell layers of tumor nodules, repeated instillation could contribute to a gradual decrease in the tumor volume. Indeed, the beneficial effect of a second instillation of ^{213}Bi-anti-EGFR MAb was seen in patient 12.

In addition, therapeutic efficacy could possibly be enhanced with a combined administration of an alpha-emitter and a beta-emitter anti-EGFR immunoconjugate. Beta-emitters such as ^{90}Y, ^{131}I, and ^{177}Lu are commonly used in targeted cancer therapy. Because of the different characteristics of alpha-emitters and beta-emitters, i.e. short path length combined with high linear energy transfer (LET) and long path length combined with low LET, respectively, the effects of each could be complementary to successfully eradicate tumors of various sizes.

The use of the alpha-emitter ^{225}Ac ($t_{½}$ = 9.92 days) instead of ^{213}Bi coupled to an EGFR ligand that is internalized upon binding to the receptor is another promising option. The feasibility and remarkable therapeutic efficacy of intravenous ^{225}Ac-PSMA-617 has recently been demonstrated in patients with metastatic castration-resistant prostate cancer [24]. Furthermore, adverse effects observed after intravenous administration should not occur following intravesical instillation.

In the study described here, only patients with no remaining treatment options were enrolled, i.e. only patients in whom all established standard therapies had failed were treated with ^{213}Bi-anti-EGFR MAb. Therefore, improvement in therapeutic efficacy could be achieved if targeted alpha-emitter immunotherapy is used together with BCG as a co-first-line therapy. Moreover, the use of other antibodies (coupled to the alpha-emitter ^{213}Bi) that target different receptors overexpressed on the surface of bladder cancer cells could extend the therapeutic options. In this respect, antibodies targeting MUC1 and MUC2 [10], FGFR1 [28], FGFR3 [29], HER2 (ERBB2) [30], and integrin $\alpha 3\beta 1$ [31] are promising candidates.

As mentioned above, MAbs targeting PD-L1 on cancer cells trigger T-cell-mediated killing of tumor cells. Coupling of ^{213}Bi to such antibodies (atezolizumab, durvalumab, avelumab) [20, 32] generates radioimmunoconjugates that can also be used to directly kill bladder cancer cells.

Furthermore, immunotherapy targeting PD-L1 combined with radiation therapy has been shown to result in prolongation of long-term survival in mouse cancer models [33]. This might be due to radiation-induced stimulation of the immune system. Accordingly, treatment of murine adenocarcinoma cells with the alpha-emitter ^{213}Bi triggers an immunogenic response that finally inhibited tumor growth in immunocompetent mice [34]. Therefore, bladder cancer immunotherapy, i.e. conventional BCG or experimental anti-PD-L1, combined with irradiation via targeted ^{213}Bi-anti-EGFR MAb could possibly improve the therapeutic response. Likewise, the antitumor efficiency of adoptive T-cell transfer (ACT) can be boosted by irradiation. In immunocompetent mice bearing multiple myeloma cells, combined treatment with ^{213}Bi-anti-CD138 MAb and OVA-specific CD8$^+$ T cells resulted in prolongation of survival [35]. Again, activation of the immune system via alpha radiation could conceivably be complemented by direct eradication of tumor cells via alpha-emitter conjugates.

Conclusion

Treatment of CIS of the bladder with the alpha-emitter ^{213}Bi coupled to a MAb targeting EGFR was shown to be a safe treatment option without any adverse effects. There are a number of strategies available to improve the therapeutic efficacy. These strategies will be addressed and evaluated in a clinical study.

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Compliance with ethical standards

Conflicts of interest None.

Ethical approval The study was approved by the local Ethics Committee.

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