Preliminary efficacy of [90Y] DOTA-biotin-avidin radiotherapy against non-muscle invasive bladder cancer

Alessandra Alì  
Columbia University Irving Medical Center

Dev Leibowitz  
Columbia University Irving Medical Center

Nikunj Bhatt  
Columbia University Irving Medical Center

Mikhail Doubrovin  
Columbia University Irving Medical Center

Gleneara E. Bates-Pappas  
Memorial Sloan-Kettering Cancer Center Department of Psychiatry and Behavioral Science

Robert N. Taub  
Columbia University Irving Medical Center

James M McKiernan  
Columbia University Irving Medical Center

Akiva Mintz  
Columbia University Irving Medical Center

Andrei Molotkov  
am3355@cumc.columbia.edu  
Columbia University Irving Medical Center

Research Article

Keywords: bladder cancer, [90Y]DOTA-biotin-avidin, intravesical radionuclide therapy, avidin

Posted Date: April 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1559077/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Title: Preliminary efficacy of [90Y]DOTA-biotin-avidin radiotherapy against non-muscle invasive bladder cancer

Authors: Alessandra Ali¹, Dev Leibowitz¹, Nikunj Bhatt¹, Mikhail Doubrovin¹, Gleneara E. Bates-Pappas², Robert N. Taub³, James M McKiernan⁴, Akiva Mintz*,¹, Andrei Molotkov¹

Affiliations:
¹ Department of Radiology, Columbia University Irving Medical Center, New York, NY 10032, USA.
² Department of Psychiatry and Behavioral Sciences, Memorial Sloan Kettering Cancer Center, New York, NY 10021, USA.
³ Department of Medicine, Columbia University Irving Medical Center, New York, NY 10032, USA (retired).
⁴ Department of Urology, Columbia University Irving Medical Center, New York, NY 10032, USA.

Correspondence:
* Akiva Mintz, Vice Chair of Translational Imaging; Director, Columbia University PET Center; Chief, Division of Nuclear Medicine; 722 West 168th Street, Floor R1 New York, NY 10032 USA;
Tel: +1-212-305-5521, am4754@cumc.columbia.edu

First author:
Alessandra Ali, Post-Doctoral Research Scientist, Department of Radiology, Columbia University Irving Medical Center, 722 West 168th Street, Floor R1 New York, NY 10032 USA;
Tel: +1-212-342-0555, aa4560@cumc.columbia.edu, ORCID ID:0000-0001-7065-6353

Acknowledgments
The Columbia University image-guided therapeutics development pipeline is supported by the National Center for Advancing Translational Sciences (NCATS) UL1TR001873 (Reilly) through the ACCELERATE Resource.
Abstract

Purpose: Bladder cancer represents 3% of all new cancer diagnoses per year. We propose intravesical radionuclide therapy using the β-emitter $^{90}$Y linked to DOTA-biotin-avidin ([$^{90}$Y]DBA) to deliver short-range radiation against non-muscle invasive bladder cancer (NMIBC).

Material and methods: Image-guided biodistribution of intravesical DBA was investigated in an animal model by radiolabeling DBA with the $^{68}$Ga and dynamic microPET imaging following intravesical infusion of [$^{68}$Ga]DBA for up to 4 hours and post-necropsy γ-counting of organs. The antitumor activity of [$^{90}$Y]DBA was investigated using an orthotopic MB49 murine bladder cancer model. Mice were injected with luciferase-expressing MB49 cells and treated via intravesical administration with 9.2 MBq of [$^{90}$Y]DBA or unlabeled DBA 3 days after the tumor implantation. Bioluminescence imaging was conducted after tumor implantation to monitor the bladder tumor growth. In addition, we investigated the effects of [$^{90}$Y]DBA radiation on urothelial histology with immunohistochemistry analysis of bladder morphology.

Results: Our results demonstrated that DBA is contained in the bladder for up to 4 hours after intravesical infusion. A single dose of [$^{90}$Y]DBA radiation treatment significantly reduced growth of MB49 bladder carcinoma. Attaching $^{90}$Y to the DOTA-avidin prevents its re-absorption into the blood and distribution throughout the rest of the body. Furthermore, immunohistochemistry demonstrated that [$^{90}$Y]DBA radiation treatment did not irreversibly damage the bladder urothelium, which appeared similar to the normal urothelium of healthy mice.

Conclusion: Our data demonstrates the potential of intravesical [$^{90}$Y]DBA as a treatment for non-muscle invasive bladder cancer.

Keywords: bladder cancer, [$^{90}$Y]DOTA-biotin-avidin, intravesical radionuclide therapy, avidin
Introduction

Bladder cancer is the 10th most commonly diagnosed cancer worldwide; approximately 573,000 new cases were diagnosed in 2020 and 213,000 deaths were estimated [1, 2]. Approximately 75% of bladder cancers are diagnosed as non-muscle invasive bladder cancer (NMIBC) [3] and 10-20% of the recurrent cases of NMIBC progress to muscle invasive bladder cancer (MIBC) [4]. The standard treatment for NMIBC is the transurethral resection followed by an adjuvant therapy to prevent recurrence and/or progression according to the tumor grade classification [5]. Intravesical bacillus Calmette-Guérin (BCG) is the gold standard therapy for adjuvant treatment in patients with intermediate and high-risk urothelial NMIBC, while a single chemotherapy instillation is recommended for tumors classified as low risk [6, 7]. However, these adjuvant treatments are accompanied by significant adverse events and 30%-50% of patients do not respond or relapse within 5 years after appropriate BCG therapy [8]. Given the high recurrence rates after BCG therapy, safe and effective adjuvant locoregional therapies are needed in order to limit the recurrence and/or progression of NMIBC.

In recent years, there has been a much greater emphasis on radionuclide therapy, which demonstrates increased selectively in delivering radiation doses to the desired molecular targets expressed by cancer cells and reduced cytotoxicity to the surrounding normal tissue [9, 10]. We hypothesize that infusion of radioactive agents directly into the bladder that can reach the surface tumor cells as well as those below the most superficial layer may offer a promising path for treating relapsed NMIBC [11], avoiding the disadvantages related to radical cystectomy, external beam radiation or systemic treatments.

Avidin is a 67 KDa tetrameric glycoprotein that binds to biotin with high affinity. Avidin is positively charged with isoelectric point of 10.5 and contains terminal N-acetylglucosamine and mannose residues able to bind lectins [12, 13]. Lectins are carbohydrate-binding proteins expressed on the surface of tumor cells that act to internalize their ligands [12]. Therefore, glycoproteins like avidin recognized by lectins could be used as carriers of therapeutic drugs for different types of tumors including urinary bladder cancer [13-15]. Furthermore, cationized avidin binds rapidly to the cell surface and enters into cells by endocytotic uptake [16]. Previous study demonstrated that the intravesical administration of radiolabeled avidin resulted in a preferential accumulation in tumor tissue compared to normal urothelium [17]. Our goal is to develop an effective minimally invasive therapy that can reach below the urothelium of the bladder to selectively target cancer cells at or below the surface.

We propose an intravesical therapeutic application of [90Y]-DOTA-biotin-avidin ([90Y]DBA) complex in order to deliver short range radiation up to 12 mm deep [10] to reach the surface and throughout the bladder wall, which is 3-5 mm thick in humans when distended. We therefore sought to test the distribution and efficacy of DBA-based radiotherapy in rodents using real-time PET imaging and an orthotopic bladder cancer model.

Material and methods

Cell culture
MB49 mouse urothelial carcinoma cells (ATCC, VA, USA) were cultured as recommended by the manufacturer and stably transfected with a firefly luciferase- and GFP-expressing plasmid pBMN-CMV-copGFP-Luc2-Puro (Addgene, MA, USA) using Lipofectamine 3000 Transfection Reagent (Invitrogen, MA, USA). Transfected MB49 cells were selected using puromycin and sorted on Influx flow sorter (BD Biosciences, CA, USA).

**Avidin-Cy7 cellular uptake**

Avidin was labeled with Cy7 near infrared fluorescent dye according to manufacturer instructions (Lumiprobe, MD). MB49 cells (5x10^4) were seeded overnight to a µ-slide 8 well cell culture chamber (IBIDI GmbH, Germany). The cells were incubated with 1 or 5 µg/mL of avidin-Cy7 for 24 h. Post-incubation, each well was washed 2x with PBST for 5 min and fixed with 4% paraformaldehyde (PFA) for 30 min. The cells were washed 3x with PBST for 10 min each and the nuclei were counterstained with DAPI (Sigma-Aldrich, USA). Cellular uptake of avidin-Cy7 was observed by RVL-100G Echo Revolve microscope (Echo, San Diego, CA, USA).

**Animals**

Female C57BL/6NTac mice (B6) and NTac:SD rats (SD) were purchased from Taconic (NY, USA) and maintained on a normal diet. All animal experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee of Columbia University Irving Medical Center.

**68Ga labeling of DOTA-biotin-avidin**

68Ga was eluted from a 68Ge/68Ga generator. 181 MBq (4.9 mCi) of 68Ga was equilibrated to pH=4.0 with 1 M sodium acetate buffer followed by addition of 15 µg DOTA-biotin. Reaction was incubated at 90°C for 15 min in a thermomixer. Formation of [68Ga]DOTA-biotin was monitored by radio-TLC (ITLC-SG, 1 M NH4OAc/methanol (1:1). Retention factor (Rf) of free 68Ga = 0.0-0.2, Rf of [68Ga]DOTA-biotin = 0.8-1.0). The [68Ga]DOTA-biotin was further confirmed by reverse-phase HPLC using a Phenomenex Luna C18(2) column (5µm, 250 x 4.6 mm) eluted with a mobile phase consisting of 0.1% trifluoroacetic acid (TFA)/H2O (solvent A) and 0.1% TFA/acetonitrile (solvent B), and a gradient consisting of 5% B for 2 min followed by 90% B in 8 min (total 10 min) at a flow rate of 1 mL/min. Formed [68Ga]DOTA-biotin (>97% pure) was used as such for avidin labelling. [68Ga]DOTA-biotin (98.79 MBq, 500 µL) pH was adjusted to pH=5.0 with 1 M NaHCO3 followed by the addition of 750 µg of avidin (75 µL in PBS). The reaction was incubated at 30°C for 15 min in a thermomixer. Radiochemical purity of [68Ga]DOTA-biotin-avidin ([68Ga]DBA) was confirmed by size exclusion HPLC using a Superdex 200 Increase 10/300 GL eluted with an isocratic mobile phase consisting of PBS at a flow rate of 0.75 mL/min. Formed [68Ga]DOTA-biotin-avidin (>97% pure) was used for experiments.
For free unbound $^{68}$Ga infusion $^{68}$Ga was eluted from a $^{68}$Ge/$^{68}$Ga generator, equilibrated to pH=4.0 with 1 M sodium acetate buffer, and injected into the rat’s bladder as described in the next session.

**Biodistribution of $^{68}$Ga[DBA**

An INSYTE.w.22G catheter (BD Biosciences, CA, USA) was inserted into bladder of SD rats as previously described [18]. 44-52 MBq (1.2-1.4 mCi) of $^{68}$GaDBA or free unbound $^{68}$Ga in 0.5 mL of PBS was injected into bladder of SD rats using the catheter. Immediately after $^{68}$Ga injection, rats were placed on a microPET scanner (Inveon, Siemens, Germany), and a 4-hour dynamic scan was acquired. After the PET scan, radioactivity was removed from the rat’s bladder and the bladder was washed 3 times with 1 mL of PBS each wash. Rats were dissected and blood, liver, bladder, kidneys and muscle samples were collected and counted on HIDEX gamma counter (HIDEX, Finland).

**MB49 cell implantation**

B6 mice were anesthetized with isoflurane and placed in supine position. An INSYTE.w.24G catheter (BD Biosciences, CA, USA) was inserted into the bladder through the urethra [18], and the bladder was emptied by draining out the urine. To damage the urothelium and enable tumor cell implantation, 100 μL of 0.1 μg/mL poly-L-lysine solution (PLL, MW = 70,000 to 150,000) (Sigma, MO, USA) was injected into the bladder. After 15 min incubation, PLL solution was removed, 50,000 of MB49 cells in 50 μL of DPBS were injected into the bladder and mice were kept for 45 min under anesthesia in a supine position to allow for MB49 cell implantation. After the incubation with MB49 cells, the catheter/syringe were removed and mice were allowed to recover from anesthesia.

**$^{90}$Y labeling of DOTA-biotin-avidin**

$^{90}$YL3 was obtained from Eckert & Ziegler Nuclitec GmbH (Germany). 213 MBq (5.77 mCi) of $^{90}$Y was equilibrated to pH=7 with 2 M ammonium acetate buffer followed by the addition of 250 μg of DOTA-biotin and incubated for 30 min at 90°C in a thermomixer. Formation of $[^{90}Y]DOTA$-biotin was monitored by radio-TLC (ITLC-SG, 50 mM EDTA pH 5, Rf of free $^{90}$Y= 0.9-1.0, Rf of $[^{90}Y]$$-$$DOTA$-biotin = 0.0-0.2). Formed $[^{90}Y]$$DOTA$-biotin (>99% pure) was used for avidin labelling. 9 mg of avidin in 30 μL PBS was added to $[^{90}Y]$$DOTA$-biotin, and the reaction mixture was incubated at 25°C for 15 min in a thermomixer. Radiochemical purity of $[^{90}Y]$$DOTA$-biotin-avidin ($[^{90}Y]$$DBA$) was confirmed by Radio-TLC (ITLC-SG, 1 M NH$_2$OAc/methanol 1:1, Rf of free $[^{90}Y]$ = 0.9-1.0, Rf of $[^{90}Y]$$DOTA$-biotin = 0.7-0.8, Rf of $[^{90}Y]$$DOTA$-biotin-avidin = 0.0-0.2). Purity of $[^{90}Y]$$DBA$ was further confirmed by HPLC using size excusing Superdex 200 Increase 10/300 GL column eluted with an isocratic mobile phase (PBS) at a flow rate of 0.75 mL/min. $[^{90}Y]$$DBA$ in PBS (>97% pure) was used for mice treatment.

$[^{90}Y]$$DBA$ treatment
MB49 tumor-bearing mice were randomly assigned into two groups: (1) control group – received intra-bladder injection of unlabeled DBA, and (2) treated group – received intra-bladder injection of [\textsuperscript{90}Y]DBA. Tumor bearing mice were catheterized and received a single intra-bladder injection with either DBA or 9.2 MBq (250 µCi) of [\textsuperscript{90}Y]DBA 72 hours after MB49 cell implantation. Mice were treated for 2 hours. At the end of the treatment, bladders were washed 3 times with PBS and catheters were removed.

**Luciferase imaging**

Mice were injected intraperitoneally with 0.25 mL of luciferin (Research Products International Corp, IL, USA) solution (30 mg/mL in PBS). 15 min after luciferin injection, mice were imaged for 20 min using a 3D optical/CT scanner (MILabs, Netherlands).

**Histology and immunohistochemistry**

Bladders were dissected, fixed in 4% PFA for 24 hours, incubated overnight in 30% sucrose, embedded in OCT (Fisher scientific, MA, USA), and sectioned. Next, primary antibodies were used: goat anti-GFP polyclonal antibody (Bio-techne, MN, USA, catalog AF4240, 1:200), chicken anti-keratin-5 antibody (Biolegend, CA, USA, catalog 905901, 1:500), mouse anti-uroplakin-3 antibody (Fitzgerald, MA, USA, catalog 10R-U103, 1:50), rabbit anti-Ki67 antibody (Abcam, MA, USA, catalog ab15580, 1:100) with corresponding secondary antibodies (1:1000). Nuclei were visualized by DAPI (Sigma-Aldrich, USA) staining. All imaged were obtained using RVL-100G Echo Revolve microscope (Echo, San Diego, CA, USA).

**Quantification and Statistical Analysis**

Statistical analysis was performed using Prism 8.0 (San Diego, CA, USA). Statistical $p$-values were calculated using Mann-Whitney tests.

**Results**

**Uptake of avidin-Cy7 by MB49 tumor cells**

To demonstrate the feasibility of our approach, we studied the ability of MB49 cells to uptake avidin from cell medium by using the avidin labeled with Cy7. We found a significant dose dependent internalization of avidin-Cy7 by MB49 cells 24 hours after incubation (Fig. 1). This confirms prior work demonstrating bladder cancer uptake of avidin based $[^{99m}Tc]N_2$-Lys-Biotin [17].

$[^{68}Ga]DBA$ is contained in the bladder and does not significantly enter the systemic circulation

To study the distribution of the DBA complex using microPET, we radiolabeled DBA with $^{68}$Ga, which binds DOTA similar to $^{90}$Y. We hypothesized that linking $^{68}$Ga to avidin through the DOTA-biotin bond will prevent its leakage through the urothelium and getting into
the circulation to expose systemic organs and compared it to the free $^{68}$Ga. To examine the distribution of $^{68}$GaDBA after intravesical delivery, we infused rats (n=4) with $^{68}$GaDBA, and scanned animals for 4 hours using microPET. Following microPET and 3 washes with PBS, delayed microPET images were obtained and animals were sacrificed and systemic organs were counted for the presence of $^{68}$Ga (Fig. 2 and Table 1). We found that the >99% of the injected $^{68}$GaDBA was contained within the bladder without detectable activity in systemic organs (Fig. 2a, b). Furthermore, >99% of $^{68}$GaDBA was washed out after 3 rinses of PBS (Fig. 2c). We confirmed the $^{68}$GaDBA microPET distribution by post-necropsy $\gamma$ counting (Table 1) and did not detect any significant accumulation of $^{68}$GaDBA in systemic organs outside the bladder (<0.05 % of injected dose per gram of body weight (%ID/g)) and only 0.1 %ID/g in the bladder. These data demonstrate that the radiolabeled DBA complex was not significantly absorbed into circulation after intravesical administration and was nearly completely washed out after 3 washes with PBS, making this strategy viable for therapeutic applications using a $^{90}$Y payload. In comparison, we found a 29-fold increase in blood $^{68}$Ga activity when rats received intravesical infusion of free unbound $^{68}$Ga (Table 1).

**Table 1** Tissue uptake of $^{68}$Ga and $^{68}$GaDBA 4h after intravesical injection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Free $^{68}$Ga, Bq</th>
<th>$^{68}$GaDBA, Bq</th>
<th>Uptake increase, times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.09 ± 0.04</td>
<td>0.003 ± 0.001</td>
<td>29, p=0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>0.04 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>4</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>1.3</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.3 ± 0.07</td>
<td>0.1 ± 0.06</td>
<td>3</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.01 ± 0.004</td>
<td>0.002 ± 0.0004</td>
<td>5.5</td>
</tr>
</tbody>
</table>

**Intravesical $^{90}$Y DBA therapy is safe and effective against orthotopic MB49 tumors**

To study effects of intravesical $^{90}$YDBA therapy on bladder cancer, we treated mice bearing orthotopic syngeneic MB49 bladder cancers (Fig. 3a). We found that tumors grew below the urothelial surface 72 hours post implantation (Fig. 3b). To test the efficacy of our approach, we infused the bladders of tumor bearing mice with $^{90}$YDBA or with unlabeled DBA using urethral catheters to deliver $^{90}$YDBA directly into the bladder. $^{90}$YDBA was retained in the bladder for 2 hours, removed via the catheter and subsequently washed 3 times with PBS. We evaluated tumor growth in the $^{90}$YDBA and control groups using bioluminescence 10-days post tumor implantation. We found that tumors developed in 100% of the control mice in the bladder. In contrast, 50% of the treated mice had no tumor (p=0.02). Moreover, $^{90}$YDBA treated mice (n=8) had significantly less (p=0.01) tumor burden compared to mice treated with unlabeled DBA (n=8), demonstrated by significantly lower bioluminescence signal (Fig. 3c, d).
Intravesical $^{90}$YDBA treatment does not demonstrate any adverse effects on treated mice

We examined animal weight and urothelium histology after treatment. We did not observe any significant difference in weights between control and $^{90}$Y treated groups (Fig. 4). To study the effect of the $^{90}$YDBA treatment on the bladder urothelium, we dissected bladders from $^{90}$YDBA treated mice and compared them to normal (no tumor) and control (untreated) mice. H&E staining revealed typical urothelium morphology in normal mice (Fig. 5a). Large tumors (asterisks on Fig. 5a) were found in the bladders of control mice. In contrast, mice implanted with orthotopic tumors and subsequently treated with $^{90}$YDBA demonstrated normal bladder morphology and had no tumors in the treatment area, indicating successful locoregional treatment (Fig. 5a). We further analyzed the urothelial integrity by staining sections for keratin 5 (Krt5) and uroplakin 3 (Urp3). Normal bladder urothelium is comprised of 3 cell layers: (1) basal cells expressing Krt5+/p63+/Urp-, (2) intermediate cells expressing Krt5-/Urp+/p63+, and (3) terminally differentiated Urp+/p63-/Krt5- umbrella cells. As expected, intact bladder urothelium of normal mice contained three distinct layers of cells: Krt5+/Urp3- basal cells, Krt5-/Urp3+ intermediate cells, and morphologically distinct Krt5-/Urp3+ giant umbrella cells (Fig. 5b). As previously reported [19, 20], we did not find Urp3+ cells in the bladder urothelium of tumor bearing untreated mice (Fig. 5b). Instead, urothelium of these mice consisted of multiple layers of Krt5+ cells. In contrast, the bladder urothelium of $^{90}$YDBA treated mice had a normal basal layer of Krt5+ cells and intermediate/top layers of Urp3+ cells that closely resemble urothelium of normal untreated mice (Fig. 5b).

Adult undamaged urothelium is quiescent epithelium that contains very few proliferating cells. Damage induces cell proliferation and regeneration of the urothelium [21]. We stained bladder sections with anti-Ki67 antibody and found that both the bladder urothelium of the normal mice as well as tumor bearing mice treated with $^{90}$YDBA (10 days after treatment) did not have Ki67+ cells, indicating a quiescent urothelium (Fig. 5c). This suggests that $^{90}$YDBA did not irreversibly damage bladder urothelium as the urothelium is not substantially different from the normal untreated mice. In contrast, we detected multiple Ki67+ cells in urothelium of untreated tumor bearing mice (Fig. 5c).

Discussion

NMIBC patients who have not responded to BCG have an urgent unmet need for improved therapeutic options. The standard of care (SOC) consists of transurethral resection with post-operative intravesical infection with BCG “immunotherapy” [22, 23]. However, an estimated 40% of patients fail to respond to this SOC. Of patients who do initially respond 30-50% relapse within 5 years [24, 25]. Following BCG failure, there is no clear SOC. $^{90}$Y is routinely used in clinical practice for locoregional radioablation of liver cancer with $^{90}$Y labeled microspheres [26]. This approach has proven to be safe, even if up to 10% of radioactivity leaks into circulation. In contrast, local radioablation of refractory NMIBC using an intravesical approach can potentially near completely sequester radioactivity in the bladder and treat the entire urothelial surface as well as the entirety of the distended bladder wall. Previously, $\alpha$-emitter $^{213}$Bi coupled to a monoclonal antibody (mAb) targeting EGFR has demonstrated therapeutic efficacy in BCG-refractory carcinoma of the
However, antigenic and vascular heterogeneity of tumors combined with the extremely short range of the $\alpha$-emitters (~0.1 mm) might limit the efficacy if the biomarker is not expressed or if there is nascent tumor cells below the surface layer. These limitations might be overcome with intravesical infusion of $\beta$-emitters that have a range of up to 12 mm. This range permits the so called “crossfire” effect, where $\beta$ particles can access several cell layers adjacent to the targeted cell in the event not all cancer cells are successfully targeted. Furthermore, $\beta$ particles can traverse the entire distended bladder wall, depriving tumor cells from seeking refuge below the surface layer. Crossfire is critical to $\beta$ particles emitter therapy to improve tumor dose homogeneity and to ensure sufficient dose to each cell [28]. Therefore, in our study, we tested the efficacy of $[^{90}Y]$DBA intravesical therapy using an orthotopic MB49 murine bladder tumor model. We hypothesized that the range of the $\beta$-emitter $^{90}Y$ will be effective at killing tumor cells both at the surface and below the urothelium. To minimize $^{90}Y$ leakage through the bladder urothelium, we attached it to avidin. Avidin has a positive charge that facilitates cell uptake and has been shown to bind lectins expressed on cancer cells [29]. Our labeling approach using $[^{90}Y]$biotin coupled with avidin takes advantage of the high biotin-avidin affinity ($K_d$~$10^{-15}$ M) and can be easily converted into a kit format for clinical use. Our real-time PET study of the DBA complex demonstrated that it is indeed retained in the bladder for up to 4 hours and can be nearly completely washed out with 3 saline washes. These characteristics are critical to successfully translate our approach to clinical use, as they indicate that the DBA treatment will not significantly distribute and damage normal organs and can be removed in a controlled manner that will not cause any radiation safety concerns when the therapy is over.

To test the preliminary feasibility, safety and efficacy of $[^{90}Y]$DBA, we used a syngeneic MB49 model implanted below the urothelial surface to test our hypothesis that $[^{90}Y]$DBA can effectively reach beyond the surface urothelium of the bladder. Our results demonstrated significant efficacy after a 2-hour intravesical treatment with $[^{90}Y]$DBA measured by bioluminescent signal from the luciferase expressing tumor cells. A shortcoming of the MB49 model is that it is highly aggressive and metastatic, which cannot be treated by a locoregional approach. Thus, we designated a 10-day end point in this preliminary study to evaluate the safety of our approach and to obtain an initial efficacy readout. We found that 50% of $[^{90}Y]$DBA treated animals did not have any tumor after treatment (compared to 100% having tumors in the control group). Importantly, the 50% of treated animals in which tumors were present, demonstrated disease in the urethra, liver and lungs, which were outside the field of treatment. Thus, while the MB49 model is good for preliminary efficacy studies, we are currently performing longer term survival studies using carcinogen models, which better represent NMIBC as they do not typically metastasize but are less consistent and take many months to establish.

Damage to the bladder urothelium initiates a regenerative response which repairs most of the urothelium within 72 h [21]. Our study demonstrated that $[^{90}Y]$DBA treatment did not irreversibly damage urothelium and we observed a completely restored urothelium 10 days after treatment. While we did not observe any indication of acute radiation cystitis in treated mice, shorter-term and longer-term follow-up studies are being performed to evaluate for more acute and delayed adverse events.

Conclusion
We demonstrated that radiotherapy using a single administrated dose of intravesical $^{90}$YDBA (9.8 MBq) significantly treated bladder tumors in an orthotopic model and did not cause irreversible damage to the urothelium. $^{90}$YDBA was contained within the bladder, preventing radiation toxicity to the rest of the body. These preliminary results support the further study of $^{90}$YDBA as a promising treatment for refractory NMIBC that can potentially spare patients from the morbidity related to bladder resection, external beam radiation or systemic therapies.
References


Statements & Declarations

Funding

The Columbia University image-guided therapeutics development pipeline is supported by the National Center for Advancing Translational Sciences (NCATS) UL1TR001873 (Reilly) through the ACCELERATE Resource.

Conflict of interest

RNT and GEB are listed inventors on the patents related to [90Y]GBA held by Columbia University. RNT and GEB are founders of Biobina, LLC, which is developing [90Y]GBA as a therapeutic for bladder cancer.

Author Contributions


Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All animal experiments were approved by the Institutional Animal Care and Use Committee of Columbia University Irving Medical Center (AC-AABL2550)

Consent to participate

Not applicable

Consent to publish

Not applicable
Figure legends

Fig. 1 MB49 bladder cancer cell uptake of avidin-Cy7. MB49 cells were incubated 24 h with 1 or 5 µg/mL of Cy7-avidin. (a) Cy7-avidin (cyan) in MB49 cells. (b) DAPI (blue) is used to counterstain nuclei. (c) Overlay of A and B. (d) MB49 cells are positive for GFP

Fig. 2 Biodistribution of $^{68}$GaDBA injected into bladder of healthy rats. a Whole body microPET images 4 hours after intravesical infusion of $^{68}$GaDBA demonstrating fully contained activity within the bladder (asterisk). %ID/g= percentage injected dose per gram. b Quantification of percent of activity in bladder compared to the remainder of the body demonstrating >99% of activity isolated to the bladder. c Effectiveness of saline washes after a 4 hour bladder exposure to $^{68}$GaDBA showing the amount of activity recovered after removing the initial activity (wash 1) and subsequent bladder drainings after infusions of saline. Recovered activity is the sum of all washes and accounts for nearly 100% of injected activity.

Fig. 3 $^{90}$YDBA treatment effect on MB49 bladder tumor growth. a Timeline of the study in which mice bearing orthotopic MB49 carcinoma were treated with $^{90}$YDBA or unlabeled DBA (CTR) via an intravesical route for 2 hours on day 3 after MB49 cells implantation followed by luciferase imaging. b Immunofluorescence images of representative MB49 bladder cancer 3 days after implantation. GFP+ MB49 cells are stained with anti-GFP antibodies (green); antibodies to Krt5 (red) are used to detect basal urothelial cells. Scale bar: 130 µm. c Representative bioluminescence images of mice treated with $^{90}$YDBA and control unlabeled DBA. d Luciferase signal quantification of treated and control groups demonstrated significantly reduced tumor burden in $^{90}$Y-treated mice 10 days after treatment compared to the control treated mice (n=8 per group).

Fig. 4 Weight changes in the control (blue line) and $^{90}$YDBA (red line) treated mice demonstrate no significant difference. Data are shown as mean ± standard error.

Fig. 5 H&E and immunofluorescent staining of bladder sections from healthy mice, mice implanted with orthotopic MB49 tumors (untreated), and mice implanted with orthotopic MB49 tumors and subsequently treated with $^{90}$YDBA (MB49 + $^{90}$YDBA). (a) Representative H&E staining of normal, untreated MB49 and $^{90}$YDBA treated bladder sections. Scale bars: 890 µm; *, MB49 tumor. (b) Immunofluorescent staining with antibodies to Krt5 (green) and Urp3 (red) of bladder urothelium from normal, MB49 and MB49+$^{90}$YDBA treated mice. DAPI (blue) is used to counterstain nuclei. (c) Immunofluorescent staining with antibodies to Ki67 (red)
of bladder urothelium from normal (left panel), MB49 (middle panel) and MB49+[90Y]DBA treated (right panel) mice. DAPI (blue) is used to counterstain nuclei. The yellow dashed lines outline urothelium. Scale bars: 60 \mu m
Figures

Figure 1

MB49 bladder cancer cell uptake of avidin-Cy7. MB49 cells were incubated 24 h with 1 or 5 µg/mL of Cy7-avidin. (a) Cy7-avidin (cyan) in MB49 cells. (b) DAPI (blue) is used to counterstain nuclei. (c) Overlay of A and B. (d) MB49 cells are positive for GFP

Figure 2

Biodistribution of [ 68Ga]DBA injected into bladder of healthy rats. a Whole body microPET images 4 hours after intravesical infusion of [ 68Ga]DBA demonstrating fully contained activity within the bladder (asterisk). %ID/g= percentage injected dose per gram. b Quantification of percent of activity in bladder compared to the remainder of the body demonstrating >99% of activity isolated to the bladder. c Effectiveness of saline washes after a 4 hour bladder exposure to [ 68Ga]DBA showing the amount of activity recovered after removing the initial activity (wash 1) and subsequent bladder drainings after infusions of saline. Recovered activity is the sum of all washes and accounts for nearly 100% of injected activity

Figure 3

[ 90Y]DBA treatment effect on MB49 bladder tumor growth. a Timeline of the study in which mice bearing orthotopic MB49 carcinoma were treated with [ 90Y]DBA or unlabeled DBA (CTR) via an intravesical route for 2 hours on day 3 after MB49 cells implantation followed by luciferase imaging. b Immunofluorescence images of representative MB49 bladder cancer 3 days after implantation. GFP+ MB49 cells are stained with anti-GFP antibodies (green); antibodies to Krt5 (red) are used to detect basal urothelial cells. Scale bar: 130 µm. c Representative bioluminescence images of mice treated with [ 90Y]DBA and control unlabeled DBA. d Luciferase signal quantification of treated and control groups demonstrated significantly reduced tumor burden in 90Y-treated mice 10 days after treatment compared to the control treated mice (n=8 per group)
Figure 4

Weight changes in the control (blue line) and [90Y]DBA (red line) treated mice demonstrate no significant difference. Data are shown as mean ± standard error.

Figure 5

H&E and immunofluorescent staining of bladder sections from healthy mice, mice implanted with orthotopic MB49 tumors (untreated), and mice implanted with orthotopic MB49 tumors and subsequently treated with [90Y]DBA (MB49 + [90Y]DBA). (a) Representative H&E staining of normal, untreated MB49 and [90Y]DBA treated bladder sections. Scale bars: 890 μm; *, MB49 tumor. (b) Immunofluorescent staining with antibodies to Krt5 (green) and Urp3 (red) of bladder urothelium from normal, MB49 and MB49+[90Y]DBA treated mice. DAPI (blue) is used to counterstain nuclei. (c) Immunofluorescent staining with antibodies to Ki67 (red) 16 of bladder urothelium from normal (left panel), MB49 (middle panel) and MB49+[90Y]DBA treated (right panel) mice. DAPI (blue) is used to counterstain nuclei. The yellow dashed lines outline urothelium. Scale bars: 60μm.