Diphtheria Toxin–Epidermal Growth Factor Fusion Protein DAB389EGF for the Treatment of Bladder Cancer

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Abstract

Purpose: The novel fusion protein, DAB389EGF, is composed of both the catalytic and the translocation domains of diphtheria toxin that are fused to the human EGF, providing a targeting and a toxicity component. We tested DAB389EGF for antitumor activity in both in vitro and in vivo urinary bladder cancer models.

Experimental Design: Human bladder cancer lines were treated with DAB389EGF and assessed for growth inhibition and clonogenic suppression. Using 6- to 8-week-old female athymic nude mice implanted orthotopically with HTB9 cells, DAB389EGF was administered intravesically twice weekly for 2 weeks. The response of the luciferase-expressing HTB9 cells was monitored via bioluminescence as the primary endpoint.

Results: Treatment response with DAB389EGF was specific and robust, with an IC50 ranging from 0.5 to 15 ng/mL in eight tested bladder cancer cell lines, but greater than 50 ng/mL in the EGF receptor (EGFR)-negative H520 control cell line. Simulating short-duration intravesical therapy used clinically, a 2-hour treatment exposure of DAB389EGF (10 ng/mL) produced clonogenic suppression in three selected bladder cancer cell lines. In vivo, luciferase activity was suppressed in five of six mice treated with DAB389EGF [70 mL (1 ng/mL) per mouse], as compared with only one of six mice treated with a control diphtheria toxin (DT) fusion protein. Histologic assessment of tumor clearance correlated with the bioluminescent changes observed with DAB389EGF treatment. Immunocompetent mice treated with intravesical DAB389EGF did not show any nonspecific systemic toxicity.

Conclusions: The intravesical delivery of targeted toxin fusion proteins is a novel treatment approach for non–muscle-invasive urinary bladder cancer. With appropriate targeting, the treatments are effective and well-tolerated in vivo. Clin Cancer Res; 19(1): 148–57. ©2012 AACR.

Introduction

Urinary bladder cancer is common, with nearly 70,000 new cases and 15,000 deaths expected in 2011 in the United States (1). The majority of these new cases are *superficial* or non–muscle-invasive and treated with cystoscopic resec-

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-12-1258

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148 Clin Cancer Res; 19(1) January 1, 2013
EGFR protein overexpression in bladder cancer is not limited to muscle-invasive tumors but is also seen in low-grade, non–muscle-invasive cases (15). Taken together and based on these features, EGFR is a rational target for intravesical therapy with an EGF-targeted toxic fusion protein.

We hypothesize that an EGFR-targeting conjugated toxin protein will effectively treat bladder cancer in vivo without significant systemic toxicity. The aim of this present study is to examine the effect of DAB\textsubscript{388}EGF in both cell culture and orthotopic mouse models of bladder cancer.

Materials and Methods

Cell culture and reagents

Human bladder cancer cells J82, RT4, CRL1749 (CRL), T24, TCCSUP (SUP), HTB9 (American Type Culture Collection) and lung cancer cell lines H520 and A549 were grown in OptiMEM (Gibco) with 3.75% FBS (Gemini) and 100 μg/mL streptomycin–100 IU/mL penicillin sulfate (Life Technologies). All cell lines were incubated at 37°C with 5% CO\textsubscript{2}. EGFR antibody and related secondary antibody were purchased from Cell Signaling Technology. The DAB\textsubscript{389}EGF (DT-EGF) and DAB\textsubscript{388}GM-CSF [granulocyte-macrophage colony-stimulating factor (GM-CSF)] fused to DT\textsubscript{m} were prepared as previously described (8, 16).

Western blot analysis

For Western blot assessment, the cells were plated in culture dishes until confluent and harvested by scraping and washing with PBS. Cells were collected by centrifugation at 1,100 rpm and pellets were resuspended in lysis solution [10 mmol/L Tris, 150 mmol/L NaCl, 1% Triton X-100, 1 mmol/L EDTA, 1 mmol/L EGTA, 0.2 mmol/L sodium orthovanadate, 0.5% NP-40, 0.3 mmol/L phenylmethylsulfonylfluoride (PMSF), 10 μg/mL aprotinin]. Protein estimation was conducted with Pierce protein dye, using a DU 800 Beckman Coulter spectrophotometer. Fifty micrograms of protein was electrophoresed using NuPAGE 4%–12% Bis-Tris Gel (Invitrogen). The protein was transferred to a nitrocellulose membrane (Invitrogen) using a wet method at 100 volts for 1 hour. The membrane was then blocked with 5% (w/v) milk and placed in a rotator for 1 hour at room temperature. The primary antibody was added in milk (2.5% w/v) and allowed to incubate overnight at 4°C after which it was washed with PBS/0.05% Tween-20 3 times (15 minutes each) before the appropriate horseradish peroxidase (HRP)-linked secondary antibody was added and incubated for an additional hour at room temperature. The membrane was again washed 3 times for 15 minutes before the addition of Pierce SuperSignal chemiluminescent substrate and exposed. The films were scanned using EPSON PERFECTION V500 PHOTO and quantified by ImageJ (NIH, Bethesda, MD).

Cell proliferation assay

Cell proliferation was assessed using a tetrazolium-based assay (CellTiter 96 AQueous One Solution, Promega Corporation). Three thousand cells in 50 μL of media per well
were plated in 96-well plates in triplicate using the bladder cell lines. Twenty-four hours after plating, 50 μL cell culture medium containing different concentrations of DAB389EGF were added to give a total volume of 100 μL in each well. Fifty microliter cell culture media was used in the control condition. Seventy-two hours after the treatment, 20 μL of the AQueous One Solution was added to each well. Colorimetric analysis using a 96-well plate reader (Vmax Kinetic Microplate Reader, Molecular Devices) was conducted between 2 and 4 hours (wavelength of 490 nm), depending on cell type and cell density. Cell proliferation assays were conducted in triplicate.

**Clonogenic assays**

Clonogenic survival was defined as the ability of the cells to maintain their clonogenic capacity and to form colonies. Depending on cell type, 200 to 500 cells were seeded into 6-well dishes in 2 mL of medium. The next day, cells were treated with DAB389EGF, which were then maintained for 7 to 10 days at 37°C in a 5% CO2 incubator. The cells were fixed with 12.5% acetic acid in 30% methanol and then stained with Brilliant Blue R. The plate was photographed or the colonies containing 50 or more cells were counted. The effect of short-duration DAB389EGF treatments was also examined. J82, SUP, and T24 cells were seeded (300 cells per well) in a 6-well plate in 2 mL of medium as above. Twenty-four hours later, 2 mL of medium containing DAB389EGF was used to replace the normal medium for 2 to 24 hours, following which the treatment medium was removed and quickly replaced with fresh medium. The clonogenic survival was examined according to the clonogenic assay method described above.

**DAB389EGF stability test**

A total of $4 \times 10^5$ J82 cells were seeded in 6-well plates (an empty well served as a no-cell control) for 24 hours. The normal medium was replaced with 2 mL, 20 ng/mL DAB389 EGF in medium for 2 hours in a 37°C incubator. The treatment medium containing DAB389EGF was then harvested and tested for cell killing and used to treat T24, J82, and SUP for 3 days, giving a final DAB389EGF treatment concentration of 10 ng/mL, as described.

**Preparation of HTB9-Luc cells**

For imaging purposes, the human bladder cancer cell line HTB9 was infected with a lentivirus containing the firefly luciferase gene. Briefly, the full-length luciferase-coding DNA sequence was amplified by PCR reaction from the pGL-3 vector (Promega) and inserted at BamHI and NotI sites of the lentiviral vector pLEX. In addition, Kozak sequence GCCACC was added between BamHI and start codon ATG of luciferase to ensure the luciferase expressed correctly. To generate luciferase-expressing lentivirus (Lenti-luc), this vector was co-transfected using calcium phosphate precipitation method with packaging plasmid psPAX2 and envelope plasmid pMD2.G into HEK-293T cells. Supernatant was collected and filtered with 0.45-μm microfilter for infection. HTB9 cells were then infected with Lenti-luc virus (25 μL viral supernatant/ml medium) and mixed with polybrene (4 μg/mL medium). A single clone with strong luminescent intensity was selected with puromycin and subcultured. Approximately passages 3 to 4 HTB9-Luc cells were used for further work.

**Animals**

Female nu/nu and C57BL/6 mice were purchased from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD). The animals were housed 4 per cage in a specific pathogen-free animal facility and fed with regular chow diet with water ad libitum. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee.

**Orthotopic implantation**

HTB9-Luc cells were harvested from 70% to 80% confluent cultures by exposure to trypsin. Proteinolysis was stopped with medium containing 10% FBS, and the cells were washed once in serum-free medium and resuspended in PBS. Female athymic nude mice 6 to 8 weeks of age had their bladders catheterized with a 30-gauge plastic catheter, under sterile conditions and using lubricant, by separating the animal legs and exposing the external urethral orifice for catheterization. Anesthesia was accomplished with pentobarbital sodium 60 mg/kg. The adequacy of anesthesia was monitored every 2 to 3 minutes by response to toe/skin pinch. The bladder was washed with PBS and scratched with the catheter tip before instilling 100 μL of 1.5 × 10⁶ HTB9-Luc cells. The mild physical irritation caused by the catheter scratching is believed to increase the tumor development rate and degree of scratching contributes to the tumor stage (i.e., superficial). The urethra was temporarily closed with a single, sterile suture at the distal portion of the urethra thus retaining the cells in the bladder for 3 hours. This single suture was placed in the distal portion of the urethra under sterile conditions and accomplished through a single-needle pass. The suture was removed by cutting the suture with a scissors and pulling through the non-knotted end, without further invasive animal manipulation. Mice with xenograft tumor implantation confirmed by positive luciferase activity 7 days after human cancer cell implantation were used for subsequent DAB389EGF treatment.

**Bioluminescence measurement**

The primary endpoint of the in vivo study was bioluminescent activity after 4 intravesical treatments. Each mouse received 150 mg luciferin/kg body weight (e.g., for a 20 g mouse, we injected 200 μL to deliver 1.5 mg of luciferin). The luciferin was injected intraperitoneally (i.p.) 12 minutes before imaging. Luciferin-injected mice were anesthetized and monitored using the XG-8 isofluorane device. The mice were first placed into an induction chamber and anesthetized with a high initial dose of isofluorane (8%) and then maintained with a lower dose of isofluorane (2.5%) through a manifold located on the imaging stage. Imaging of the mice was then conducted under
continuously anesthetized conditions. The mice were imaged with In Vivo Imaging System (IVIS) 200 Imaging System (Xenogen Corp.). Finally, 4 weeks after tumor cell implantation, the mice were sacrificed and bladders were taken for hematoxylin and eosin (H&E) staining and immunohistochemistry stain using whole-mount bladder step sections.

**In vivo treatment**

One week after implantation, the degree of bioluminescence was measured and used to equally distribute the mice into balanced treatment and control groups. Drugs were stored at −80°C and thawed on ice. Seventy microliter of 1 ng/μL drug in PBS per mouse was instilled into the animal bladder following a similar procedure as cancer cell implantation procedure. This intravesical treatment was maintained for 2 hours each time, roughly simulating the short duration of intravesical therapy used in current clinical practice. The mice were treated twice per week for 2 weeks. Tumor growth was monitored weekly by the IVIS Imaging System. Following sacrifice, the bladders were harvested, and formalin-fixed and paraffin-embedded for immunohistochemistry and fluorescent in situ histologic (FISH) analysis.

**Histologic analysis**

Tissue processing and H&E staining of 5-μm tissue sections were conducted either by the Diabetes and Endocrinology Research Center Histology Core Facility at the Barbara Davis Center for Childhood Diabetes or by the Prostate Diagnostic Laboratory, University of Colorado Anschutz Medical Campus (Aurora, CO). The slides were reviewed by a pathologist, F.G. La Rosa. The pathology evaluation of urinary bladder specimens was done to confirm the presence or absence of tumor as observed in the bioluminescence measurement. Examination of bladder, liver, and kidney specimens was also done to evaluate for toxicity-related changes in these tissues.

**FISH analysis**

Formalin-fixed, paraffin-embedded mouse tissue sections were subjected to a dual-color FISH assay using a human/mouse probe mix to allow for unambiguous identification of the human xenograft in murine bladder when required. Human and mouse Cot-1 DNA (Invitrogen) were labeled with SpectrumRed- and SpectrumGreen-conjugated dUTPs, respectively (Abbott Molecular) using the Nick Translation Reagen Kit (Abbott Molecular Catalog#32-801300), according to manufacturer’s instructions. FISH assays were conducted according to standard protocol (17). Initially, the slides were incubated for 2 hours at 56°C, deparaffinized in Citri-Solv (Fisher) and washed in 100% ethanol. The slides were sequentially incubated in 2× SSC at 75°C for 15 minutes, digested in 0.50 mg/ml Proteinase K/2× SSC at 45°C for 15 minutes, washed in 2× SSC for 5 minutes, and dehydrated in ethanol. Probe mix was applied to the selected hybridization area, which was covered with glass coverslip and sealed with rubber cement. DNA denaturation was conducted for 15 minutes at 85°C and hybridization was allowed to occur at 37°C for 15 hours. Post-hybridization washes were conducted sequentially with 2× SSC/0.3%NP-40 (pH 7.0–7.5) at 72°C for 2 minutes and 2× SSC at room temperature for 2 minutes, followed by dehydration in ethanol. Chromatin was counterstained with DAPI (0.3 μg/mL in Vectashield mounting medium, Vector Laboratories). The work was conducted by University of Colorado Cancer Center Cytogenetics Core Program directed by M. Varella-Garcia.

**Toxicology studies**

C57BL/6 mice were treated intravesically with 70 μL at concentrations of 1 and 43 ng/μL (yielding a total dose administered for 70 ng and 3 μg) of DAB389EGF per mouse every other day for 4 doses. In addition, systemic tolerance was assessed by giving 70 μL (43 ng/μL) DAB389EGF per mouse intravenously every other day for 2 doses, based on the use of this dose in previous in vivo work with this agent (8). The mice were euthanized at the completion of the experiments and their tissues of liver, kidney, and bladder were collected and formalin-fixed. H&E stains were conducted for these paraffin-embedded mouse tissue sections.

**Statistical analysis**

The Student t-test was used for comparisons of cell proliferation results of 2 groups (A540 vs. H520), and Fisher statistical test was conducted between in vivo results of DAB389EGF versus DAB388GM-CSF treatment groups. A one-way ANOVA was used to determine whether the means were significantly different overall for the remainder of the statistical analysis. If the overall means were significantly different, we carried out a pairwise comparison. Tukey method for multiple comparison was used to compare differences between groups. SEM was indicated for each value by a bar. All analyses were conducted using GraphPad Prism version 5.0c for Windows (GraphPad Software) and SAS statistical software (SAS).

**Results**

**DAB389EGF treatment inhibited the growth of the human bladder cells and is correlated with EGFR expression**

As shown in Fig. 1A, DAB389EGF significantly inhibited cellular growth in all tested human bladder cancer cells. To account for differences in the observed level of IC50, we quantified the EGFR protein expression level of the tested cell lines via Western blotting, as shown in Fig. 1B. HTB9 and T24 cells exhibited higher EGFR expression than in J82 and CRL cells, which is consistent with their sensitivity to DAB389EGF (Fig. 1A).

To further investigate the relationship between cell killing efficacy and EGFR expression, we conducted cell proliferation (3-day treatment) and clonogenic assay (10-day treatment) using 2 well-characterized lung cancer cell lines with (A549) and without (H520) EGFR expression. Cell proliferation results show that the A549 cell line has much higher
sensitivity to DAB389EGF than H520 (Supplementary Fig. S1A). Furthermore, clonogenic assay results show that at the concentration of 25 ng/mL, DAB389EGF almost completely inhibits the colony formation in A549 cells with little influence on H520 cell growth (Supplementary Fig. S1B).

After 10 days of treatment, clonogenic assay for selected 4 human bladder cancer cell lines was conducted (Fig. 1C), showing that a higher concentration of DAB389EGF had greater inhibition of colony formation, indicating that this suppression is dose-dependent.

Short-duration DAB389EGF treatment suppressed clonogenicity

To prepare for in vivo intravesical testing, short-duration treatments with DAB389EGF treatment were used in vitro for the clonogenic assay. As shown in Fig. 2A, a 2-hour exposure to DAB389EGF treatment showed growth inhibition in 3 selected bladder cancer cell lines (J82, SUP, and T24). Meaningful reductions in clonogenic formation were seen with 2 hours of exposure and large or even complete suppression was seen with a 24-hour treatment. To further explore the stability of DAB389EGF, considering that in a clinical application that the dwell time would be less than 3 hours, we preincubated this agent with J82 cells for 1 to 3 hours and then applied the collected medium containing DAB389EGF to 3 selected cell lines (T24, J82, and SUP). As shown in Fig. 2B–D, the media with and without cells exhibits similar cell killing efficiency in a manner comparable with the nonincubated (control) treatment. The efficacy of DAB389EGF did not vary despite pretreatment incubation with J82 cells for various time course (1–3 hours) before the treatment phase when we examined the cell killing effects of 3 cell lines T24, J82, and SUP. All together, these results reveal that DAB389EGF exhibits significant cell killing effect with a 2-hour exposure and is very stable in cell culture, providing a strong rationale for studying clinically applicable intravesical treatment in vivo.

Establishment of orthotopical xenograft bladder cancer model

Using human bladder cancer HTB9 cells, we have successfully established an orthotopic xenograft bladder cancer model confirmed by bioluminescent imaging, H&E staining, and FISH. First, HTB9 cells were transfected so that they...
expressed luciferase and single luciferase-bearing cell clones were selected on the basis of bioluminescent expression after luciferin exposure (Fig. 3A). Their luciferase activity was further confirmed after 3 to 4 passages in culture (Fig. 3B). Bioluminescent imaging provides for in vivo determination of bladder cancer cell implantation and tumor formation in the nude mice. Using the technique described, an implantation rate of approximately 75% was obtained, which was assessed at 1 week after implantation (Fig. 3C). Once tumor implantation was confirmed at day 7, we selected and followed a cohort of 4 mice with positive luciferase activity for 2 months to establish the stability of long-term, orthotopic implantation with this method and found that these mice have positive but relatively decreased luciferase activity (Fig. 3D). On the basis of this information, we selected mice for intravesical DAB389EGF treatment on the basis of luciferase activity 7 days after the cells were administered and established the relative stability of this model over several months, with a low spontaneous tumor loss rate. Unlike a syngeneic tumor model, these mice bearing the positive luciferase activity do not necessarily develop palpable tumors and the tumor implants do not appear to be fatal with longer term follow-up. On the basis of serial FISH and histologic assessments with this model, bioluminescent findings appear to be the most reliable marker of tumor presence and this was the primary endpoint of this study.

**Treatment of DAB389EGF**

Mice were selected for DAB389EGF treatment on the basis of confirmation of tumor implantation, via the presence of luminescent activity 7 days after the cell administration procedure, with the treatment schema shown in Fig. 4A. As a control treatment, the related agent DAB388GM-CSF was used and seemed suitable, as the urothelium is not known to express GM-CSF receptors (18). Six mice were
treated with intravesical DAB389EGF and 6 with control (DAB388GM-CSF). After 1 week, a subjective decrease in luminescent activity was observed in the DAB389EGF-treated mice, with nearly uniform loss of luminescent activity after 2 weeks treatment (Fig. 4B). In contrast, there was little decrease of luminescent activity with DAB388GM-CSF treatment in all but one of the mice after the 2-week treatment phase (Fig. 4B). At the conclusion of the treatments, luciferase activity was lost in 5 of the 6 mice treated with DAB389EGF compared with 1 of the 6 mice treated with control (Fig. 4B and Table 1). Fisher statistical test confirmed that the difference between DAB389EGF and DAB388GM-CSF treatment groups is significant ($P < 0.05$).

The bladders were then examined histologically, and the findings were in agreement with the luciferase results, with loss of bladder cancer tissue observed in 5 of the DAB389EGF-treated mice as compared with 1 of the DAB388GM-CSF–treated mice. The presence of tumor xenograft was additionally confirmed in selected mice by using species-specific dual-color FISH assay (human versus murine) as noted in Fig. 4C. This assay is highly sensitive and specific and is able to detect a very small number of xenograft (human) cells in the murine background.

**Preliminary toxicity results**

Previous work has showed that DAB389EGF at doses of $\geq 5 \mu g$ given intravenously in BALB/c mice leads to listlessness, lethargy, dehydration, and reduced activity (19). In the current experiments, we used the immunocompetent C57BL/6 mice to examine the toxic effect of DAB389EGF when given intravesically compared with intravenous treatment. In these mice, there was no obvious behavioral toxicity or reduction in activity when given 4 treatments intravesically, twice per week for 2 weeks, with either 70 ng or 3 $\mu g$ DAB389EGF. Histologic examination of organ tissue from the mice given intravesical treatment showed no changes or toxicity as evidenced in Fig. 5. In contrast, in the intravenously treated group, histologic examinations of kidney, liver, and bladder reveal the presence of severe renal tubular necrosis and rare apoptotic cells in the kidney (Fig. 5A) and liver (Fig. 5B) with 2 treatments of 3 $\mu g$ DAB389EGF every other day, although the toxicity in the urothelium (Fig. 5C) was not evident. As a result of subjective changes in the intravenously treated mice, the intravenous treatment was stopped in all mice after 2 treatments, with the observation of reduced activity and general weakness in the mice, consistent with systemic toxicity.

**Discussion**

In this work, we present a novel use of an EGFR-directed fusion protein with a modified DT. Bladder cancer frequently overexpresses EGFR on the luminal surface, whereas such expression is uncommon on the normal urothelium. Using an orthotopic, xenograft
model of bladder cancer, we showed a difference in the elimination of the implanted tumor after 2 weeks of intravesical treatment with DAB389EGF, as compared with the control DT chimeric protein (DAB388GM-CSF). Importantly, in immunocompetent mice, there was clear, generalized toxicity with the intravenous DAB389EGF treatment, but there was no evidence of histologic or generalized toxicity in the intravesically treated mice at equal doses.

While targeted agents have made significant inroads into the clinical management of most common cancer types, there is no integration of such agents into the routine treatment of urothelial carcinoma. Importantly, in most cases, the use of inhibiting a particular molecular pathway is dependent on "pathway addiction," frequently denoted by an activation mutation, such as the EGFR mutations in non–small cell lung cancer (20), which predicts clinical benefit with the systemic use of EGFR inhibitors. Another weakness of selective pathway inhibition via a systemic agent is the common "cross talk" that occurs, in which the neoplastic cells use a secondary growth pathway in response to the inhibition of one pathway by a targeted therapy (21). In contrast to other targeted agents, currently in clinical practice and given systemically toxic fusion proteins do not require pathway addiction for activity, they simply require differential expression of the receptor in question between the targeted tumor cells and the normal background; the receptor merely serves as a doorway, allowing entry of the chimeric, therapeutic protein component. EGFR in bladder cancer serves as a very favorable "targeting" receptor or portal in this light, in that is commonly and

Table 1. Luciferase activity results of DAB389EGF treatment

<table>
<thead>
<tr>
<th>Total mice</th>
<th>Luciferase activity loss</th>
<th>Luciferase activity presence</th>
</tr>
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<tbody>
<tr>
<td>DAB389EGF</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>DAB388GM-CSF</td>
<td>6</td>
<td>1</td>
</tr>
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NOTE: P < 0.05 DAB389EGF vs. DAB388GM-CSF.
differentially expressed. Importantly, bladder cancer also offers a direct and unique method of delivery, via simple catheterization and intravesical administration. There are also available clinical assays to assess EGFR expression, allowing for the selection of patients for DAB389EGF therapy, based on EGFR expression levels in tumor biopsies. BCG and other medical therapies for bladder cancer are currently given in this manner and the use of DAB389EGF in this way would not represent a change in general practice.

Of note, denileukin diftitox (ONTAK), a chimeric protein of DT and IL-2, is currently in clinical use, in addition to other analogous agents currently in clinical evaluation (22), establishing the realistic translational potential of this approach in the novel intravesical setting.

Another advantage of intravesical therapy is the protective effect of the physiologic space provided by the bladder itself. As noted in our experiments, the intravesical delivery of DAB389EGF at more than 40 times the effective therapeutic levels (70 ng compared with 3000 ng) was very well tolerated, whereas similar high doses of DAB389EGF given intravenously led to toxicity. Simply, the bladder physically contains the fusion protein when delivered intravesically, limiting systemic exposure. In a clinical application, this will improve the clinical tolerability and safety of such an approach in the novel intravesical setting.
approach. Of note, we also did not see any toxicity in the mice with orthotopic bladder xenograft in pace, and therefore an altered bladder wall from tumor growth, when treated with 70 ng of intravesical DAB389EGF during the therapeutic treatment experiments.

In summary, non–muscle-invasive bladder cancer is common, frequently recurs after therapy, with little change in the medical therapy over 2 decades. The intravesical administration of DAB389EGF yielded a high rate of tumor clearance after 2 weeks of therapy using an orthotopic, xenograft model of bladder cancer, in contrast to control-treated mice. In addition, intravesical treatment limits systemic exposure of the therapy, reducing nonspecific toxicities. The use of targeted toxic fusion proteins represents a novel therapeutic advantage, building on currently principles of intravesical treatment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: X. Yang, L.-J. Su, A. Thorburn, M. Glode, T.W. Flaig
Writing, review, and/or revision of the manuscript: X. Yang, E. Kessler, A. Thorburn, A.E. Frankel, M. Varella-Garcia, M. Glode, T.W. Flaig
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Yang, E. Kessler, Y. Li, T.W. Flaig

Grant Support
This work was supported by a Paul Calabresi K12 clinical scholar grant awarded to the University of Colorado Denver (K12CA086913 to T.W. Flaig). This effort was also supported in part by a State of Colorado/University of Colorado Technology Transfer grant (CUH19561/H-State: 10HCF-37) via Bioscience Discovery Evaluation Grant Program through the State of Colorado (ODET) and also the Cancer League of Colorado. FISH was carried out by University of Colorado Cancer Center Cytogenetics Core Program directed by Dr. Marelle Varella-Garcia supported by grant P30-CA046594.

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Received April 21, 2012; revised October 10, 2012; accepted October 11, 2012; published OnlineFirst November 21, 2012.

References


www.aacnjournals.org
Cancer Clin Res; 19(1) January 1, 2013

Published OnlineFirst November 21, 2012; DOI: 10.1158/1078-0432.CCR-12-1258

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