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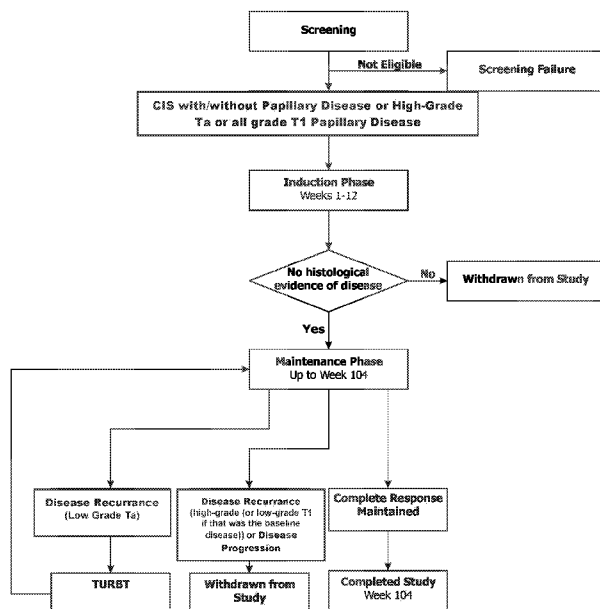
(54) **Title:** DOSING STRATEGIES FOR TARGETING EPCAM POSITIVE BLADDER CANCER

Figure 1

(57) **Abstract:** The present disclosure provides methods for treatment of bladder cancer. The methods include administering an immunoconjugate to a subject in a dosing regimen that includes an induction phase with twice weekly followed by once weekly dosing, and optionally a maintenance phase. The immunoconjugates useful in the methods provided include immunoconjugates having a binding protein that specifically binds to Ep-CAM and a toxin molecule.



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DOSING STRATEGIES FOR TARGETING EPCAM POSITIVE BLADDER CANCER**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Provisional Application No. 62/132,251 filed March 12, 2015 and U.S. Provisional Application No. 62/267,386 filed December 15, 2015, the contents of each of which is hereby incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The content of the text file submitted electronically herewith is incorporated herein by reference in its entirety: A computer readable format copy of the Sequence Listing (filename: VIVE_029_00US_SeqList_ST25.txt); date recorded: March 6, 2015; file size 25 KB).

BACKGROUND

[0003] Bladder cancer is the 6th most common cancer in the United States; it is the 3rd most common cancer in men and the 11th most common in women. Approximately 75% of bladder cancers are of the non-muscle invasive type (Babjuk *et al.*, 2013). Non-muscle invasive bladder cancers (NMIBCs) are categorized as Ta (non-invasive papillary carcinoma), T1 (tumor invades lamina propria or subepithelial connective tissue), and Tis (carcinoma *in situ*). Ta tumors are the most common, representing about 70% of NMIBCs, but only about 7% of these are categorized as high-grade (Sylvester *et al.*, 2005). About 20% of NMIBCs are T1 tumors (Anastasiadis *et al.*, 2012). T1 tumors are more aggressive than Ta tumors, and considered high-risk (Babjuk *et al.*, 2013; American Urological Association, 2014). Flat, high-grade tumors confined to the mucosa (non-invasive) are characterized as carcinoma *in situ* (CIS), ((Babjuk *et al.*, 2013) and these represent approximately 10% of the NMIBCs (Anastasiadis *et al.*, 2012).

[0004] The usual first treatment for NMIBC (high grade Ta, T1, and CIS) is transurethral resection of the bladder tumors (TURBT), followed by intravesical immunotherapy, most commonly with bacillus Calmette-Guérin (BCG) (Babjuk *et al.*, 2013, American Urological Association 2014). In patients with T1 tumors, a second TURBT is recommended (Babjuk *et al.*, 2013, American Urological Association 2014). Local and systemic side effects are common with intravesical BCG therapy, causing discontinuation of treatment in approximately 20% of patients (Sylvester *et al.*, 2011). Approximately 75% of patients experience local side effects (including cystitis, irritative voiding symptoms, and hematuria), while 40% report systemic side effects,

including general malaise and fever (Sylvester *et al.*, 2011). Intravesical BCG failure occurs in up to 40% of patients (Sylvester *et al.*, 2011). Because of the high risk for development of muscle invasive disease, cystectomy is recommended for CIS and high-grade Ta and T1 patients who experience disease recurrence following intravesical therapy. For patients unable or unwilling to undergo cystectomy, treatment options are limited. Thus, there is a need in the art for safe and effective therapies and optimal dosing regimens of safe and effective therapies for bladder cancer.

SUMMARY OF THE INVENTION

[0005] In one aspect, the present disclosure provides methods for treating bladder cancer in a subject in need thereof, wherein the methods comprise administering to the subject an immunoconjugate for a first dosing period followed by a second dosing period, wherein the first dosing period comprises administration of the immunoconjugate twice per week, and wherein the second dosing period comprises administration of the immunoconjugate once per week. In some embodiments, the first and second dosing periods are an induction phase of treatment. In some embodiments, the first and second dosing periods are each independently selected from about 4, about 5, about 6, about 7 or about 8 weeks in length. In some embodiments, the first dosing period is a period of at least about 6 consecutive weeks and the second dosing period is a period of at least about 6 consecutive weeks.

[0006] In one aspect, the methods further comprise administering the immunoconjugate to the subject for a third dosing period, wherein the immunoconjugate is administered once per week every other week. In some embodiments, the third dosing period is a maintenance phase of treatment. In some aspects, the third dosing period is started in a subject having no histological evidence of high-grade disease following the second dosing period. In other embodiments, the third dosing period is started in a subject that entered the study with low-grade T1 disease and having no evidence of low-grade T1 disease or any high grade disease at the end of the second dosing period. Thus, in some embodiments, a third dosing period or maintenance phase is initiated in a subject in which an event as defined herein has not occurred; and/or in a subject in which complete response as defined herein is achieved following the induction phase of treatment. In some embodiments, the third dosing period begins at least one week or at least two weeks following the end of the second dosing period. In some embodiments, the third dosing period begins no more than 3 weeks following the end of the second dosing period. In some

embodiments, the maintenance period is continued for a period of at least about 6 weeks, at least about 8 weeks, at least about 10 weeks, at least about 12 weeks, at least about 14 weeks, at least about 16 weeks, at least about 6 months, at least about 12 months, at least about 18 months, or at least about 24 months. In other embodiments, the maintenance phase is continued until disease recurrence or disease progression.

[0007] In one aspect, the methods further comprise removing a bladder tumor prior to the first administration of the immunoconjugate. In some embodiments, the bladder tumor is removed by transurethral resection or fulguration.

[0008] In some embodiments, the immunoconjugate comprises a binding protein and a toxin. In further embodiments, the binding protein binds to the extracellular domain of human Ep-CAM. In some embodiments, the immunoconjugate comprises a binding protein that binds to the extracellular binding domain of Ep-CAM, wherein the binding protein is an immunoglobulin binding protein or a non-immunoglobulin polypeptide ligand.

[0009] In some embodiments, the immunoglobulin binding protein is an antibody or an antigen-binding fragment thereof. In further embodiments, the binding protein is a monoclonal antibody, Fab, Fab', (Fab')₂, single chain fragment (scFv), disulfide-stabilized fragment (dsFv), or a single domain antibody (sdAb). In some embodiments, the antibody or antigen-binding fragment thereof is a murine, chimeric, or humanized antibody or antigen-binding fragment thereof. In particular embodiments, the antibody or antigen-binding fragment thereof is humanized.

[0010] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to the amino acid sequence set forth in SEQ ID NO: 7; a heavy chain CDR2 region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to the amino acid sequence set forth in SEQ ID NO: 8; and a heavy chain CDR3 region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to the amino acid sequence set forth in SEQ ID NO: 9. In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain CDR1 region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to the amino acid sequence set forth in SEQ ID NO: 4; a light chain CDR2 region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to the amino acid

sequence set forth in SEQ ID NO: 5; and a light chain CDR3 region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to the amino acid sequence set forth in SEQ ID NO: 6. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 7, 8, and 9, respectively; and a light chain CDR1, CDR2, and CDR3 consisting of SEQ ID NOs: 4, 5, and 6, respectively.

[0011] In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to an amino acid sequence as set forth in SEQ ID NO: 12. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to an amino acid sequence as set forth in SEQ ID NO: 13. In some embodiments, the antibody or antigen-binding fragment thereof comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to an amino acid sequence as set forth in SEQ ID NO: 3. Thus, in some embodiments, the antibody or antigen-binding fragment thereof is an scFv that binds to human Ep-CAM, wherein the amino acid sequence of the scFv corresponds to SEQ ID NO: 3. In some embodiments, the antibody or fragment thereof comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or 100% homology to SEQ ID NO: 3 and comprises SEQ ID Nos: 4, 5, 6 and 7, 8, and 9.

[0012] In certain embodiments, the immunoconjugates useful in the methods of the present disclosure comprise a toxin. In some embodiments, the toxin is selected from *Pseudomonas* exotoxin A (ETA) or variants thereof, gelonin, bouganin, saporin, ricin, ricin A chain, bryodin, diphtheria toxin, and restrictocin. In some embodiments, the toxin is *Pseudomonas* exotoxin A (ETA) or a variant thereof. In further embodiments, the toxin is ETA(242-608). In some embodiments, the toxin comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to SEQ ID NO: 11.

[0013] In some embodiments, the immunoconjugates useful in the methods of the present disclosure comprise a binding protein that is attached to a toxin by a peptide linker. Peptide linkers may be cleavable linkers or non-cleavable linkers. In some embodiments, the linker is a

cleavable linker selected from the group consisting of a furin sensitive linker, a cathepsin sensitive linker, a metalloproteinase linker, or a lysosomal hydrolase sensitive linker. In some embodiments, the linker is a flexible linker. In some embodiments, the linker is between about 5 and about 50 amino acids in length. In further embodiments, the linker is between about 10 and about 30 amino acids in length. In further embodiments, the linker is about 20 amino acids in length. In some embodiments, the linker has at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to SEQ ID NO: 10.

[0014] In some embodiments, the immunoconjugates useful in the methods provided herein comprise an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to SEQ ID NO: 2. In some embodiments, the immunoconjugates consist of an amino acid sequence as set forth in SEQ ID NO: 2. In some embodiments, the immunoconjugates useful in the methods provided herein comprise an amino acid sequence having at least at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to amino acids 23-665 of SEQ ID NO: 2. In some embodiments, the immunoconjugates useful in the methods provided herein consist of an amino acid sequence as set forth as amino acids 23-665 of SEQ ID NO: 2. In some embodiments, the immunoconjugates useful in the methods provided herein comprise an amino acid sequence having at least at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% homology to amino acids 23-669 of SEQ ID NO: 2. In some embodiments, the immunoconjugates useful in the methods provided herein consist of an amino acid sequence as set forth as amino acids 23-669 of SEQ ID NO: 2.

[0015] In some embodiments, the immunoconjugate is administered to the subject at the same dose level in the first and second dosing periods, such that the subject receives a particular dose of the immunoconjugate twice per week in the first dosing period and the same dose of the immunoconjugate once per week in the second dosing period. In other embodiments, the immunoconjugate is administered to the subject at a higher or lower dose level in the second dosing period relative to the first dosing period. In other embodiments, the immunoconjugate is administered to the subject at the same dose level in the first, second, and third dosing periods, such that the subject receives the same dose of the immunoconjugate twice per week in the first dosing period, once per week in the second dosing period, and once every other week in the third dosing period. In still further embodiments, the immunoconjugate is administered to the subject

at different dose levels in the first, second, and /or third dosing periods. In some embodiments, the immunoconjugate is administered to the subject at a dose level of about 5 mg to about 75 mg per dose. In further embodiments, the immunoconjugate is administered to the subject at a dose level of about 10 mg to about 60 mg per dose. In further embodiments, the immunoconjugate is administered to the subject at a dose level of about 20 mg to about 40 mg per dose. In further embodiments, the immunoconjugate is administered to the subject at a dose level of about 30 mg. In some embodiments, the immunoconjugate is administered to the subject at a dose level of 30 mg in the first and second dosing periods. In further embodiments, the immunoconjugate is administered to the subject at a dose level of 30 mg in the first, second, and third dosing periods. In some embodiments, the methods further comprise additional dosing periods in which the dosing level and/or frequency of dosing is further modified.

[0016] In some embodiments, the doses of immunoconjugate are administered to the subject at least 48 hours apart. In some embodiments, no more than 2 doses of immunoconjugate are administered to the subject within any 7 day period.

[0017] In some embodiments, the immunoconjugate is administered by instillation into the bladder. In further embodiments, the immunoconjugate is administered via a catheter. In some embodiments, the bladder is emptied prior to administration of the immunoconjugate. For example, in some embodiments, the bladder is emptied by the subject by voiding the bladder and/or the bladder is emptied via the catheter prior to administration of the immunoconjugate. In some embodiments, the immunoconjugate is held in the bladder for at least about 15 minutes, at least about 30 minutes, at least about 45 minutes, at least about 1 hour, at least about 1.5 hours, at least about 2 hours, at least about 2.5 hours, at least about 3 hours, or longer. In some embodiments, the subject is positioned in one or more of the following positions during the time the immunoconjugate is in the bladder: upright, prone, supine, and in the left and right lateral decubitus positions. In further embodiments, the subject is position in each of the upright, prone, supine, and left and right lateral decubitus positions during the time the immunoconjugate is in the bladder.

[0018] In one aspect, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the NMIBC is classified as carcinoma in situ (CIS), Ta, or T1. In some embodiments, the subject has BCG refractive or BCG resistant bladder cancer. In some embodiments, the subject has been previously treated with BCG, interferon, or a combination

thereof. In some embodiments, the subject has previously been treated additional intravesicular therapy such as mitomycin, gemcitabine, a taxane, interferon, valrubicin or a combination thereof. In some embodiments, the subject failed to respond to previous BCG with or without interferon therapy. In other embodiments, the subject responded to previous BCG with or without interferon therapy but disease subsequently recurred. In some embodiments, the subject has been treated mitomycin, gemcitabine, a taxane, interferon, valrubicin or a combination thereof after failure to respond to BCG with or without interferon therapy. In other embodiments, the subject has been treated mitomycin, gemcitabine, a taxane, interferon, valrubicin or a combination thereof after a relapse following BCG with or without interferon therapy. In some embodiments, the subject received at least 6, at least 7, at least 8, or at least 9 instillations of BCG. In further embodiments, the at least 6, at least 7, at least 8, or at least 9 instillations of BCG were over 2 cycles of BCG.

[0019] In some embodiments, the methods for treating bladder cancer provided herein further comprise administering to the subject at least one additional therapeutic agent. In further embodiments, the additional therapeutic agent is a chemotherapeutic drug or a radiotherapeutic drug. In some embodiments, the chemotherapeutic drugs include, but are not limited to, cisplatin, carboplatin, paclitaxel, docetaxel, 5-fluorouracil, bleomycin, methotrexate, ifosamide, oxaliplatin, cyclophosphamide, dacarbazine, temozolomide, gemcitabine, capecitabine, cladribine, clofarabine, cytarabine, floxuridine, fludarabine, hydroxyurea, pemetrexed, pentostatin, thioguanadine, daunorubicin, doxorubicin, epirubicin, idarubicin, topotecan, irinotecan, etoposide, eniposide, colchicine, vincristine, vinblastine, and vinorelbine. Exemplary cancer specific agents and antibodies include, but are not limited to, Afatinib, Aldesleukin, Alemtuzumab, Axitinib, Belimumab, Bevacizumab, Bortezomib, Bosutinib, Brentuximab vedotin, Cabozantinib, Canakinumab, Carfilzomib, Cetuximab, Crizotinib, Dabrafenib, Dasatinib, Denosumab, Erlotinib, Everolimus, Gefitinib, Ibritumomab tiuxetan, Ibrutinib, Imatinib, Ipilimumab, Lapatinib, Nilotinib, Obinutuzumab, Ofatumumab, Panitumumab, Pazopanib, Pertuzumab, Ponatinib, Regorafenib, Rituximab, Romidepsin, Ruxolitinib, Sipuleucel-T, Sorafenib, Temsirolimus, Tocilizumab, Tofacitinib, Tositumomab, Trametinib, Trastuzumab, Vandetanib, Vemurafenib, Vismodegib, Vorinostat, Ziv-aflibercept, and any combination thereof. In some embodiments, the additional therapeutic agent is administered to the subject prior to, concurrently with, or subsequent to administration of the immunoconjugate. In some

embodiments, the additional therapeutic agent is administered systemically. For example, in some embodiments, the additional therapeutic agent is administered by intravenous injection.

[0020] Features discussed in relation to the disclosed methods of treating bladder cancer comprising administration of an immunoconjugate are also applicable to the medical use of the immunoconjugate in treating cancer.

[0021] In some embodiments, an immunoconjugate for use in treating bladder cancer is provided. In some embodiments, the immunoconjugate comprises binding protein that binds to the extracellular domain of human Ep-Cam and a toxin. In certain embodiments, the use comprises administration of the immunoconjugate at least twice per week for a first dosing period of at least six consecutive weeks, followed by a second dosing period wherein the immunoconjugate is administered once per week for a period of at least six consecutive weeks. In certain embodiments, the use comprises a third dosing period wherein the immunoconjugate is administered once per week every other week. In certain embodiments, the third dosing period is up to 24 months in length or until disease recurrence or disease progression. In certain embodiments, the immunoconjugate is administered to a subject who has undergone transurethral resection to remove a NMIBC tumor from their bladder prior to the first dosing period.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] **Figure 1** is a schematic flow diagram showing steps in a clinical protocol testing a dosing scheme of Vicinium comprising an induction dosing phase and a maintenance dosing phase.

DETAILED DESCRIPTION

Bladder cancer and EpCAM

[0023] The present disclosure provides methods for treatment of bladder cancer comprising administering to a subject in need thereof an immunoconjugate comprising a binding protein specific for Ep-CAM and a toxin, wherein the immunoconjugate is administered in a dosing regimen comprising an induction phase and, optionally, a maintenance phase. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). Other types of bladder cancer include early stage bladder cancer, non-metastatic bladder cancer, primary bladder cancer, advanced bladder cancer, locally advanced bladder cancer (such as unresectable

locally advanced bladder cancer), metastatic bladder cancer, bladder cancer in remission, progressive bladder cancer, or recurrent bladder cancer. In some embodiments, the subject has been diagnosed with bladder cancer or is at risk of developing bladder cancer. In some embodiments, the bladder cancer is NMIBC that is refractory to or intolerant of other treatment options, such as bacillus Calmette-Guerin (BCG) treatment or interferon treatment.

[0024] Ep-CAM (for Epithelial Cell Adhesion Molecule, which also known as 17-1A, KSA, EGP-2 and GA733-2). Ep-CAM is a transmembrane protein that is highly expressed in many solid tumors, including carcinomas of the bladder, lung, breast, ovary, colorectum, and squamous cell carcinoma of the head and neck, but weakly expressed in most normal epithelial tissues. The role of Ep-CAM in cancer formation remains unclear; however, its expression correlates with the rate of cellular proliferation. Ep-CAM-specific antibodies have been used to image and detect primary tumors and metastases in patients with small cell lung cancer and non-small cell lung cancer. Among anti-Ep-CAM MAbs, PANOREX®, which is a murine monoclonal antibody also known as edrecolomab, had been approved for the treatment of colon cancer in Germany, and is in clinical trials in the United States. Of note, however, PANOREX® treatment has been associated with undesirable side effects, including abdominal cramps, nausea, transient diarrhea and cutaneous urticarial lesions. Clinical trials with other Ep-CAM-targeted antibodies have been less successful; antibody BIS-1 was associated with peripheral vasoconstriction, dyspnea and fever, and antibody 3622W94 was associated with acute necrotizing pancreatitis. The search for an effective, low-toxicity, anti-Ep-CAM antibody continues: a fully humanized anti-Ep-CAM antibody, MT201, purported to act via Antibody-Dependent Cellular Cytotoxicity (“ADCC”), has been reported. A humanized, stabilized, single-chain, anti-Ep-CAM antibody, 4D5MOC-B, which is derived from murine monoclonal antibody MOC31, has also been developed, and is described in International Patent Application No. PCT/EP00/03176, Publication No. WO 00/61635, filed Apr. 10, 2000 and published Oct. 19, 2000, and in Willuda *et al.* (Cancer Research 59, 5758-5767, 1999). A Phase I dose escalating trial of the Ep-CAM immunoconjugate Vicinium was reported in Kowalski *et al.* (Drug Des Devel Ther. 2010;4:313-20 (2012)); and a Phase II study of once-weekly administration of Vicinium was reported in Kowalski *et al.* (J Urol. 188(5):1712-82012 (2012)).

Ep-CAM Binding proteins

[0025] In one aspect, the present disclosure provides an immunoconjugate comprising a binding protein that binds to Ep-CAM and a toxin. In one embodiment, the binding protein is an antibody or a fragment thereof. Antibodies and fragments thereof are selected from polyclonal antibodies, monoclonal antibodies, Fab, Fab', (Fab')₂, single chain fragments (scFv), disulfide-stabilized fragments (dsFv), single domain antibodies (sdAb), diabodies, and cys-diabodies (cysteine-modified diabodies). In some embodiments, diabodies include bivalent, bispecific antibodies or antibody fragments. In some embodiments, diabodies include bivalent antibodies that are made up of the same or different antibody or antibody fragment polypeptides; thus, in some embodiments, the diabodies are homo-multimers or hetero-multimers. Antibodies and fragments thereof may comprise an immunoglobulin constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM. The antibody or fragment thereof may be from any species including mice, rats, rabbits, hamsters, and humans. In one embodiment, the antibody or fragment is chimeric. Chimeric antibodies or fragments thereof are antibody molecules that combine a non-human animal variable region and a constant region or portion of a constant region that is human.

[0026] In some embodiments, the antibody or fragment thereof is humanized. As used herein, humanized antibody or humanized antibody fragment means that the antibody or fragment comprises CDRs that are derived from a non-human antibody and framework regions that are derived from a human antibody. The humanization of antibodies from non-human species has been well described in the literature. See for example EP-B1 0 239400 and Carter & Merchant 1997 (Curr Opin Biotechnol 8, 449-454, 1997).

[0027] The antigen-binding fragments of any of the above-described monoclonal antibodies, chimeric antibodies or humanized antibodies are prepared using means known in the art, for example, by preparing nested deletions using enzymatic degradation or convenient restriction enzymes. In some embodiments, the humanized antibodies, chimeric antibodies or immunoreactive fragments thereof are screened to ensure that antigen binding has not been disrupted by the humanization, chimerization, or fragmentation of the parent monoclonal antibody. This may be accomplished by any of a variety of means known in the art, including, for example, use of a phage display library.

[0028] In one aspect, the binding protein is a non-immunoglobulin polypeptide ligand, such as a protein scaffold. Protein scaffolds are known in the art and comprise proteins or polypeptides

that bind to one or more antigens. Such molecules can be designed to bind to a target tumor antigen such as EpCAM, and can be engineered to a desired affinity or avidity or can be designed to tolerate a variety of physical conditions such as extreme pH ranges and high temperatures. Protein scaffolds include, for example, affibodies, adnectins, anticalins, kunitz domain proteins, and other non-immunoglobulin binding proteins known in the art such as, for example, those disclosed in Gebauer and Kerra, *Curr. Opin. Chem. Biol.* 13; 245 (2009).

Toxins

[0029] In one aspect, the present disclosure provides an immunoconjugate comprising an antibody fragment that binds to the extracellular domain of human Ep-CAM and a toxin. In some embodiments, the immunotoxin comprises more than one toxin. For example, in some embodiments, the immunoconjugate comprises a diabody comprising more than one toxin.

[0030] In some embodiments, the toxin is a molecule that blocks protein synthesis in a target cell, therein leading to cell death. Thus, in some embodiments, the toxin is a cytotoxin. Cytotoxins are known in the art and include, for example, *Pseudomonas* exotoxin A (ETA) or variants thereof; gelonin, bouganin, saporin, ricin, ricin A chain, bryodin, diphtheria toxin, and restrictocin.

[0031] In some embodiments, the toxin is a toxic portion of *Pseudomonas* exotoxin A (ETA), or a variant thereof. The toxic portion may comprise one or more *Pseudomonas* exotoxins known in the art. For example, variants of *Pseudomonas* exotoxin, as well as methods of making and using constructs comprising *Pseudomonas* exotoxin and variants, are described in Kreitman, 1995, "Targeting pseudomonas exotoxin to hematologic malignancies," *Seminars in Cancer Biology* 6: 297-306; Pastan, 2003, "Immunotoxins containing pseudomonas exotoxin A: a short history," *Cancer Immunol. Immunother.* 52: 338-341), U.S. Patent Application No. US2003054012; U.S. Pat. No. 6,531,133; U.S. Pat. No. 6,426,075; U.S. Pat. No. 6,423,513; U.S. Pat. No. 6,074,644; U.S. Pat. No. 5,980,895; U.S. Pat. No. 5,912,322; U.S. Pat. No. 5,854,044; U.S. Pat. No. 5,821,238; U.S. Pat. No. 5,705,163; U.S. Pat. No. 5,705,156; U.S. Pat. No. 5,621,078; U.S. Pat. No. 5,602,095; U.S. Pat. No. 5,512,658; U.S. Pat. No. 5,458,878; U.S. Pat. No. 5,082,927; U.S. Pat. No. 4,933,288; U.S. Pat. No. 4,892,827; U.S. Pat. No. 4,677,070; U.S. Pat. No. 4,545,985; International Publication Nos. WO98/20135, WO93/25690, WO91/18100, WO91/18099, WO91/09949, and WO88/02401; Kondo et al., 1988, "Activity of immunotoxins constructed

with modified pseudomonas exotoxin a lacking the cell recognition domain.” J Biol Chem. 263:9470-9475; Batra et al., 1989, “Antitumor activity in mice of an immunotoxin made with anti-transferring receptor and a recombinant form of pseudomonas exotoxin.” Proc Natl. Acad. Sci. USA 86:8545-8549; Puri et al., 1991, “Expression of high-affinity interleukin 4 receptors on murine sarcoma cells and receptor-mediated cytotoxicity of tumor cells to chimeric protein between interleukin 4 and Pseudomonas exotoxin.” Cancer Res 51:3011-3017; Siegall et al., 1992, “Cytotoxicity of chimeric (human murine) monoclonal antibody BR96 IgG, F(ab')₂, and Fab' conjugated to Pseudomonas exotoxin.” Bioconjug-Chem 3:302-307; Hall et al., 1994, “In vivo efficacy of intrathecal transferrin-Pseudomonas exotoxin A immunotoxin against LOX melanoma.” Neurosurgery 34:649-655; Kuan and Pai, 1995, “Immunotoxins containing pseudomonas exotoxin that target Le y damage human endothelial cells in an antibody-specific mode: relevance to vascular leak syndrome.” Clin Cancer Res 1:1589-1594; Kreitman, 1995, “Targeting pseudomonas exotoxin to hematologic malignancies.” Sem Cancer Biol 6:297-306; Kawooya et al., “The expression, affinity purification and characterization of recombinant pseudomonas exotoxin 40 (PE40) secreted from Escherichia coli.” J Biotechnol 42:9-22; Kaun and Pai, 1995, “Immunotoxins containing pseudomonas exotoxin that target LeY damage human endothelial cells in an antibody-specific mode: Relevance to vascular leak syndrome.” Clin Cancer Res 1:1589-1594; Puri et al., 1996, “Preclinical development of a recombinant toxin containing circularly permuted interleukin 4 and truncated Pseudomonas exotoxin for therapy of malignant astrocytoma.” Cancer Res 56:5631-5637; Pai et al., 1996, “Treatment of advanced solid tumors with immunotoxin LMB-1: An antibody linked to Pseudomonas exotoxin.” Nature Med. 3:350-353; Pai et al., 1998, “Clinical Trials with pseudomonas exotoxin immunotoxins.” Curr Top. Microbiol. Immunol. 234: 83-96; Klimka et al., 1999, “An anti-CD30 single chain Fv selected by phage display and fused to pseudomonas exotoxin A (Ki-4(scFv)-ETA') is a potent immunotoxin against a Hodgkin-derived cell line.” British J Cancer 80:1214-1222; Rand et al., 2000, “Intratumoral administration of recombinant circularly permuted interleukin-4-Pseudomonas exotoxin in patients with high-grade glioma.” Clin Cancer Res 6:2157-2165; Leland et al., 2000, “Human breast carcinoma cells express type II IL-4 receptors and are sensitive to antitumor activity of chimeric IL-4-pseudomonas exotoxin fusion protein in vitro and in vivo.” Molecular Medicine Today 6:165-178; Tur et al., 2001, “An anti-GD2 single chain Fv selected by phage display and fused to Pseudomonas exotoxin A develops specific cytotoxic

activity against neuroblastoma derived cell lines.” *Int J Mol. Med* 8:579-584; Onda et al., 2001, “Cytotoxicity of antiosteosarcoma recombinant immunotoxins composed of TP-3 Fv fragments and a truncated pseudomonas exotoxin A.” *J Immunother* 24:144-150; 18. “Synergistic interaction between an anti-p185her-2 pseudomonas exotoxin fusion protein [scfv(frp5)-eta] and ionizing radiation for inhibiting growth of ovarian cancer cells that overexpress HER-2.” Schmidt et al., 2001, “Synergistic interaction between an anti-p185HER-2 pseudomonas exotoxin fusion protein [scFv(FRP5)-ETA] and ionizing radiation for inhibiting growth of ovarian cancer cells that overexpress HER-2.” *Gynecol Oncol* 80:145-155; Pastan, 2003, “Immunotoxins containing pseudomonas exotoxin A: a short history.” *Cancer Immunol Immunother* 52:338-341; Li et al., 1996, “Crystal structure of the catalytic domain of Pseudomonas exotoxin A complexed with a nicotinamide adenine dinucleotide analog: implications for the activation process and for ADP ribosylation.” *Proc Natl. Acad Sci USA*. 9:6902-6906; Kreitman and Pastan, 2003, “Immunobiological treatments of hairy-cell leukaemia.” *Best Pract Res Clin Haematol*. 16:117-33.

[0032] In some embodiments, the toxin comprises an ETA variant that, when administered alone, is substantially unable to bind to cells. In some embodiments, the cytotoxic portion comprises ETA(252-608). ETA(252-608), which is a truncated form of ETA that lacks the cell binding domain, is a single polypeptide fusion protein produced by continuous translation of a single construct. In some embodiments, ETA(252-608) comprises an amino acid sequence according to SEQ ID NO: 11.

[0033] In other nonlimiting embodiments, the immunoconjugate comprises a binding protein that specifically binds to human EpCAM and a toxin, wherein the toxin comprises an agent that acts to disrupt DNA. Thus, toxins may comprise, without limitation, enediynes (e.g., calicheamicin and esperamicin) and non-enediyne small molecule agents (e.g., bleomycin, methidiumpropyl-EDTA-Fe(II)). Other toxins useful in accordance with the invention include, without limitation, daunorubicin, doxorubicin, distamycin A, cisplatin, mitomycin C, ecteinascidins, duocarmycin/CC-1065, and bleomycin/pepleomycin. In other nonlimiting embodiments, the toxin comprises an agent that acts to disrupt tubulin. Such toxins may comprise, without limitation, rhizoxin/maytansine, paclitaxel, vincristine and vinblastine, colchicine, auristatin dolastatin 10 MMAE, and peloruside A. In other nonlimiting embodiments, the toxin portion of an immunotoxin of the invention may comprise an alkylating agent

including, without limitation, Asaley NSC 167780, AZQ NSC 182986, BCNU NSC 409962, Busulfan NSC 750, carboxyphthalatoplatinum NSC 271674, CBDCA NSC 241240, CCNU NSC 79037, CHIP NSC 256927, chlorambucil NSC 3088, chlorozotocin NSC 178248, cis-platinum NSC 119875, clomesone NSC 338947, cyanomorpholinodoxorubicin NSC 357704, cyclodisone NSC 348948, dianhydrogalactitol NSC 132313, fluorodopan NSC 73754, hepsulfam NSC 329680, hycanthone NSC 142982, melphalan NSC 8806, methyl CCNU NSC 95441, mitomycin C NSC 26980, mitozolamide NSC 353451, nitrogen mustard NSC 762, PCNU NSC 95466, piperazine NSC 344007, piperazinedione NSC 135758, pipobroman NSC 25154, porfiromycin NSC 56410, spirohydantoin mustard NSC 172112, teroxirone NSC 296934, tetraplatin NSC 363812, thio-tepa NSC 6396, triethylenemelamine NSC 9706, uracil nitrogen mustard NSC 34462, and Yoshi-864 NSC 102627. In other nonlimiting embodiments, the toxin portion of an immunotoxin of the invention may comprise an antimitotic agent including, without limitation, alcolcolchicine NSC 406042, Halichondrin B NSC 609395, colchicine NSC 757, colchicine derivative NSC 33410, dolastatin 10 NSC 376128 (NG—auristatin derived), maytansine NSC 153858, rhizoxin NSC 332598, taxol NSC 125973, taxol derivative NSC 608832, thiocolchicine NSC 361792, trityl cysteine NSC 83265, vinblastine sulfate NSC 49842, and vincristine sulfate NSC 67574

[0034] In other nonlimiting embodiments, the toxin portion of an immunotoxin of the invention may comprise an topoisomerase I inhibitor including, without limitation, camptothecin NSC 94600, camptothecin, Na salt NSC 100880, aminocamptothecin NSC 603071, camptothecin derivative NSC 95382, camptothecin derivative NSC 107124, camptothecin derivative NSC 643833, camptothecin derivative NSC 629971, camptothecin derivative NSC 295500, camptothecin derivative NSC 249910, camptothecin derivative NSC 606985, camptothecin derivative NSC 374028, camptothecin derivative NSC 176323, camptothecin derivative NSC 295501, camptothecin derivative NSC 606172, camptothecin derivative NSC 606173, camptothecin derivative NSC 610458, camptothecin derivative NSC 618939, camptothecin derivative NSC 610457, camptothecin derivative NSC 610459, camptothecin derivative NSC 606499, camptothecin derivative NSC 610456, camptothecin derivative NSC 364830, camptothecin derivative NSC 606497, and morpholinodoxorubicin NSC 354646

[0035] In other nonlimiting embodiments, the toxin portion of an immunotoxin of the invention may comprise an topoisomerase II inhibitor including, without limitation, doxorubicin NSC

123127, amonafide NSC 308847, m-AMSA NSC 249992, anthrapyrazole derivative NSC 355644, pyrazoloacridine NSC 366140, bisantrene HCL NSC 337766, daunorubicin NSC 82151, deoxydoxorubicin NSC 267469, mitoxantrone NSC 301739, menogaril NSC 269148, N,N-dibenzyl daunomycin NSC 268242, oxanthrazole NSC 349174, rubidazole NSC 164011, VM-26 NSC 122819, and VP-16 NSC 141540.

[0036] In other nonlimiting embodiments, the toxin portion of an immunotoxin of the invention may comprise an RNA or DNA antimetabolite including, without limitation, L-alanosine NSC 153353, 5-azacytidine NSC 102816, 5-fluorouracil NSC 19893, acivicin NSC 163501, aminopterin derivative NSC 132483, aminopterin derivative NSC 184692, aminopterin derivative NSC 134033, an antifol NSC 633713, an antifol NSC 623017, Baker's soluble antifol NSC 139105, dichlorallyl lawsone NSC 126771, brequinar NSC 368390, fltorafur (pro-drug) NSC 148958, 5,6-dihydro-5-azacytidine NSC 264880, methotrexate NSC 740, methotrexate derivative NSC 174121, N-(phosphonoacetyl)-L-aspartate (PALA) NSC 224131, pyrazofurin NSC 143095, trimetrexate NSC 352122, 3-HP NSC 95678, 2'-deoxy-5-fluorouridine NSC 27640, 5-HP NSC 107392, alpha-TGDR NSC 71851, aphidicolin glycinate NSC 303812, ara-C NSC 63878, 5-aza-2'-deoxycytidine NSC 127716, beta-TGDR NSC 71261, cyclocytidine NSC 145668, guanazole NSC 1895, hydroxyurea NSC 32065, inosine glycidialdehyde NSC 118994, machecin II NSC 330500, pyrazoloimidazole NSC 51143, thioguanine NSC 752, and thiopurine NSC 755.

[0037] Furthermore, a toxin may be altered to decrease or inhibit binding outside of the context of the immunoconjugate, or to reduce specific types of toxicity. For example, the toxin may be altered to adjust the isoelectric point to approximately 7.0 such that liver toxicity is reduced.

Linkers

[0038] The immunoconjugates useful in the present invention may comprise linkers, such as peptide linkers. For example, in some embodiments, the immunoconjugate comprises an Ep-CAM binding protein linked to a toxin molecule via a peptide linker. Peptide linkers may be cleavable linkers or non-cleavable linkers. Cleavable peptide linkers are known in the art and include, but are not limited to, furin sensitive linkers, cathepsin sensitive linkers, metalloproteinase linkers, and lysosomal hydrolase sensitive linkers. In some embodiments, the

linker is a flexible linker. Peptide linkers may be about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acids long. In certain embodiments, the present disclosure provides methods for treating bladder cancer comprising administering to a subject in need thereof an immunoconjugate comprising a binding protein that specifically binds to Ep-CAM, a toxin molecule, and a peptide linker linking the binding protein to the toxin molecule.

VB4-845

[0039] In one aspect of the present disclosure, the immunoconjugate is Vicinium. Vicinium contains the active pharmaceutical ingredient VB4-845, which is a recombinant fusion protein produced in *Escherichia coli* (*E. coli*) that expresses a humanized single-chain antibody fragment (scFv) specific for the epithelial cell adhesion molecule (EpCAM) antigen linked to ETA(252-608). VB4-845 is disclosed in U.S. Patent No. 8,545,840, which is incorporated herein by reference in its entirety. Sequences for VB4-845 are provided below in Table 1.

Table 1. VB4-845 sequences

Description	Sequence	SEQ ID NO
VB4-845 immuno-conjugate	MKYLLPTAAAGLLLLLAAQPAMAHHHHHHDIQMTQSPSSLSASVGD RVTITCRSTKSLLSNGITYLYWYQQKPGKAPKLLIYQMSNLSAG VPSRFSSSGSGTDFTLTISLQPEDFATYYCAQNLEIPRTFGQGT KVELKRATPSHNSHQVPSAGGPTANS GTSGSEVQLVQSGPGLVQP GGSVRISCAASGYTFTNYGMNWVKQAPGKGLEWMGWINTYTGEST YADSFKGRFTFSLDTSASAAYLQINSLRAEDTAVYYCARFAIKGD YWGQGTLLTVSSEFGGAPEFPKPSTPPGSSGLEGGSLAALTAHQA CHLPLETFTRHRQPRGWEQLEQCGYPVQRLVALYLAARLSWNQVD QVIRNALASPGSGGDLGEAIREQPEQARLALTAAAESERFVRQG TGNDEAGAASADVSLTCPVAAGECAGPADSGDALLERNYPTGAE FLGDGGDVSEFSTRGTQNWTVERRLLQAHRLQLEERGYVVFVGYHGTFL EAAQSIVFGGVRARSQDLDAIWRGFYIAGDPALAYGYAQDQEPDA RGRIRNGALLRVYVPRSSLPGFYRTGLTLAAPEAAGEVERLIGHP LPLRLDAITGPEEEGGRLETILGWPLAERTVVI PSAIPTDPRNVG GDLDPSSI PDKEQAI SALPDYASQPGKPPHHHHHHKDEL	2

Ep-CAM scFv	DIQMTQSPSSLSASVGDRVITTCRSTKSLLSNGITYLYWYQQKPGKAPKLLIYQMSNLAGVPSRFSSSGSGTDFTLTISSLQPEDFATYYCAQNLEIPRTFGQGTKVELKRATPSHNSHQVPSAGGPTANSGETSGSEVQLVQSGPGLVQPGGSVRISCAASGYTFTNYGMNWVKQAPGKGLEWMGWINTYTGESTYADSFKGRFTFSLDTSASAAYLQINSLRAEDTAVYYCARFAIKGDYWGQGTLTSS	3
VB4-845 LCDR1	RSTKSLLSNGITYLY	4
VB4-845 LCDR2	QMSNLAG	5
VB4-845 LCDR3	AQNLEIPRT	6
VB4-845 HCDR1	NYGMN	7
VB4-845 HCDR2	WINTYTGESTYADSFKG	8
VB4-845 HCDR3	FAIKGDY	9
VB4-845 scFv-toxin Linker	EFGGAPEFPPKSTPPGSSGL	10
ETA(252- 608)	EGGSLAALTAHQACHLPLETFTTRHRQPRGWEQLEQCGYPVQRLVALYLAARLSWNQVDQVIRNALASPGSGGDLGEAIREQPEQARLALTAAAESERFVRQGTGNDEAGAASADVSLTCPVAAGECAGPADSGDALLERNYPTGAFLGDGGDVSFSTRGTQNWTVRLLQAHRLQEE RGYVFGYHGTFLAAQSIIVFGGVRARSQDLDAIWRGFYIAGDPALAYGYAQDQEPDARGRIRNGALLRVYVPRSSLPGFYRTGLTLAAP EAAGEVERLIGHPLPLRLDAITGPEEEGGRLETILGWPLAERTVVI PSAIPTDPRNVGGDLDPSSIPDKEQAISALPDYASQPGKPP	11
VB4-845 light chain variable region	DIQMTQSPSSLSASVGDRVITTCRSTKSLLSNGITYLYWYQQKPGKAPKLLIYQMSNLAGVPSRFSSSGSGTDFTLTISSLQPEDFATYYCAQNLEIPRTFGQGTKVELKR	12
VB4-845 heavy chain variable region	EVQLVQSGPGLVQPGGSVRISCAASGYTFTNYGMNWVKQAPGKGL EWMGWINTYTGESTYADSFKGRFTFSLDTSASAAYLQINSLRAEDTAVYYCARFAIKGDYWGQGTLTSS	13
VB4-845 light-heavy chain linker	ATPSHNSHQVPSAGGPTANSGETSGS	14

[0040] In one aspect, the mechanism of action of Vicinium is dependent upon the 2 components of VB4-845. Once bound to the EpCAM antigen on the surface of carcinoma cells, VB4-845 is internalized through an endocytic pathway. Furin contained within the endosomal compartment

cleaves a proteolytic site on the surface of ETA(252-608), releasing ETA(252-608). The ETA(252-608) induces cell death by irreversibly blocking protein synthesis through adenosine diphosphate (ADP)-ribosylation of a post-translationally modified histidine residue of elongation factor-2 (EF-2), called diphthamide (Oppenheimer *et al.*, 1981). The truncated version of ETA, ETA(252-608), has been engineered to retain the active domains necessary to induce cell death, but the cell binding domain has been eliminated thereby preventing the ETA(252-608) moiety from entering the cell in the absence of some alternate vehicle, such as *via* antibody-mediated internalization. Binding to EpCAM must occur to result in ETA(252-608)-mediated effects.

[0041] The ETA(252-608) component of the Vicinium fusion protein can cause an immunogenic response when administered systemically to humans. In one aspect, Vicinium is administered in a locally targeted manner in order to limit its systemic exposure and to maximize the concentration of the drug in its target cells. For example, in some embodiments, Vicinium is administered via bladder instillation. In further embodiments, Vicinium is administered into an empty bladder via catheterization. In some embodiments, by administration via instillation, the probability of systemic exposure and subsequent generation of neutralizing antibodies is decreased. Furthermore, in some embodiments, the high local concentrations of Vicinium maximize the likelihood of achieving a therapeutic benefit.

[0042] Preclinical study data have shown that Vicinium exhibits potent activity [inhibitory concentration 50% (IC₅₀) = 0.001 - 10 pM] against numerous EpCAM-positive cell lines, with selectivity for EpCAM-expressing tumors. *In vivo* pharmacology demonstrated that Vicinium effectively inhibits tumor growth in several human xenograft animal models. Studies in rats found that the toxicological effects of Vicinium occur at doses 1,000-fold greater than the IC₅₀ for activity on tumor cells, with a safety margin of at least 5- to 100-fold.

[0043] The present disclosure, in one aspect, provides an improved dosing regimen for Vicinium in non muscle invasive bladder cancer subjects. The dosing regimen comprises an induction phase period comprising 6 weeks of twice-per-week dosing followed by 6 weeks of once per week dosing with Vicinium. In one embodiment, following the induction phase is a maintenance phase. In some embodiments, the maintenance phase comprises at least 1, at least 2, at least 3, at least 6, at least 8, at least 10, at least 12, at least 18, at least 24, at least 36, at least 48, at least 60, at least 72, at least 84, or at least 90 weeks of dosing with Vicinium once every other week. In some embodiments, the maintenance phase comprises up to 91 weeks of dosing with Vicinium.

In one aspect, the dosing regimen disclosed herein provides an improved therapeutic result, for example, by preventing disease recurrence.

Definitions

[0044] As used herein, the term “animal” includes all members of the animal kingdom, including humans. As used herein, the term “subject” or “patient” refers to any member of the subphylum chordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species. The terms “mammals” and “animals” are included in this definition. In particular embodiments, the term “subject” as used herein refers to a human that has been diagnosed with bladder cancer, e.g., non muscle-invasive bladder cancer.

[0045] As used herein, the phrase “effective amount” is used interchangeably with “therapeutically effective amount” or “therapeutically effective dose” and the like, and means an amount of a therapeutic agent that is effective for treating cancer. As used herein, the phrase “treating cancer” refers to inhibition of cancer cell proliferation, inhibition of cancer spread (metastasis), inhibition of tumor growth, reduction of cancer cell number or tumor growth, decrease in the malignant grade of a cancer (e.g., increased differentiation), or improved cancer-related symptoms. Effective amounts of an immunotoxin as provided herein may vary according to factors such as the disease state, age, sex, weight of the animal.

[0046] As used herein, the term “induction phase” refers to an initial course of therapy with the immunoconjugates provided herein. The induction phase for the immunoconjugates provided herein comprises a first dosing period of a twice per week dosing regimen and a second dosing period of a once per week dosing regimen.

[0047] As used herein, the term “maintenance phase” refers to a dosing period that follows an induction phase of therapy. Thus, as described herein, the maintenance phase is initiated in a subject following the second dosing period of the induction phase. The maintenance phase for the immunoconjugates provided herein comprises a dosing regimen of once every other week. In some embodiments, the maintenance phase is initiated in subjects who exhibit a complete response to the induction phase of therapy. As used herein, the term “complete response” refers to the response to a therapy in a subject. For example, a subject who exhibits no histological evidence of high-grade disease following the induction phase has exhibited a complete response to the induction phase. In some embodiments, a subject who has exhibited a complete response

to the induction phase enters the maintenance phase. Subjects with histologically confirmed evidence of high-grade disease following the induction phase will not enter the maintenance phase. Subjects that enter the study with low grade T1 disease and do not experience a recurrence of T1 disease or have evidence of high-grade disease following the induction phase enter the maintenance phase. Maintenance therapy continues until disease recurrence (i.e., the subject recurs with histologically-confirmed CIS or high-grade papillary Ta or T1 disease, or low-grade T1 if that was the baseline disease) or disease progression (histologically-confirmed disease stage \geq T2), cystectomy, 24 months from start of study, or death, whichever comes first. A subject with disease recurrence or progression will be withdrawn from the study. A subject who relapses with low-grade papillary disease will be allowed to continue maintenance therapy following tumor removal unless the subject had low-grade T1 disease and relapses with low-grade T1 disease.

[0048] In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC), and subjects subjected to the methods provided herein exhibit event-free survival following the first dose of the immunoconjugate. "Event-free survival" as used herein refers to the interval from the date of first dose of an immunoconjugate provided herein to an event. An "event" herein is defined as high-grade disease, high-grade disease tumor recurrence, tumor progression to muscle invasive bladder cancer, cystectomy for any reason, low grade T1 disease that occurs in a subject that entered the study with low-grade T1 disease, or death.

[0049] In some embodiments, the bladder cancer is NMIBC that is classified as carcinoma in situ (CIS), and subjects subjected to the methods provided herein exhibit a complete response following administration of the immunoconjugate. As used herein, "complete response" is defined as one of the following a) normal cystoscopy and non-positive or non-suspicious urine cytology or an abnormal cystoscopy but negative biopsy(ies) (or biopsy(ies) revealing only low-grade disease) and a non-positive or non-suspicious urine; b) negative biopsy (or biopsy revealing only low-grade disease) and non-positive repeat urine cytology; or c) cystoscopy or biopsy revealing only low-grade papillary disease that may be managed by transurethral resection of bladder tumor (TURBT), and not requiring cystectomy or other chemotherapy.

[0050] The term "no evidence of high-grade disease," as used herein, includes at least one of the following: a) normal cystoscopy and non-positive or non-suspicious urine cytology, or a negative biopsy or a biopsy revealing only low-grade papillary disease; b) negative biopsy (or biopsy

revealing only low-grade papillary disease) and non-positive repeat urine cytology; or c) cystoscopy or biopsy revealing only low-grade papillary disease that may be managed by transurethral resection of the bladder tumor (TURBT), and not requiring cystectomy. Thus, “high grade disease” may be identified by, for example, a biopsy revealing something greater in severity than low-grade papillary disease.

[0051] As used herein, the terms “disease recurrence” or “tumor recurrence” refer to the return of histologically-confirmed CIS or high-grade papillary TA or T1 disease. As used herein, “time to disease recurrence” is defined as the interval from the date of the first documented no histological evidence of high-grade disease to the date of first recurrence of histologically-confirmed high-grade disease. In some embodiments, the time to disease recurrence in a subject that received or is receiving the immunoconjugate provided herein is delayed relative to the time to disease recurrence that would occur in a subject that received other available intravesicular therapy.

[0052] As used herein, the term “disease progression” refers to histologically-confirmed disease at a stage at or above T2. As used herein, “time to progression” is defined as the time from the date of first dose of the immunoconjugate to the date of histologically confirmed, invasive disease of stage \geq T2. In some embodiments, the time to disease progression is delayed relative to the time to disease progression that would occur in a subject that received other available intravesicular therapy.

[0053] “Progression-free survival” is defined herein as the time from the date of first dose of the immunoconjugate to the earlier of (i) the date of histologically-confirmed, stage \geq T2 invasive disease being determined, or (ii) the date of death. “Overall survival” is defined herein as the time from the date of the first dose of immunoconjugate to death due to any cause.

[0054] The terms “VB4-845” and “Vicinium” are used interchangeably herein and refer to an immunotoxin that comprises a) the scFv humanized antibody 4D5MOC-B that is fused to b) a truncated form of *Pseudomonas* exotoxin A that consists of amino acids 252-608. In some embodiments, Vicinium is referred to herein as oportuzumab monatox.

[0055] The terms “pharmaceutically acceptable excipient” and “pharmaceutically acceptable carrier” are used interchangeably herein and include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in

the art. Supplementary active ingredients also can be incorporated into the compositions. The antibodies and antigen-binding fragments thereof provided herein may be administered together with other biologically active agents. See, for example, Remington: The Science and Practice of Pharmacy, 1995, Gennaro ed. Pharmaceutically acceptable carriers are in general clinical use and/or have approval by a regulatory agency of the Federal or state government, listing in the United States Pharmacopoeia, or general acceptance by those skilled in the relevant art.

[0056] “Failure of BCG therapy,” as used herein, is defined as treatment with BCG therapy that does not result in an improved outcome in a subject that was treated with BCG therapy for bladder cancer. In some embodiments, a failure of BCG therapy means having had an inadequate response to at least 9 instillations of BCG over two cycles (1 induction course + 1 maintenance cycle of at least 3 doses, or 2 full induction courses), or have developed recurrent disease following at least 9 instillations of BCG. In other embodiments, failure of BCG therapy means having had an inadequate response to at least 7 instillations of BCG over 2 cycles (1 induction course of at least 5 doses + 1 maintenance cycle of at least 2 doses, or 2 induction courses). A subject with non muscle invasive bladder cancer whose disease is no longer responding to BCG therapy may be either refractory or early relapsing. In a subject whose high-grade disease is refractory to BCG therapy, evaluable high-grade disease remains after adequate BCG treatment. In a subject whose high-grade disease is early-relapsing following BCG therapy, the subject underwent a complete response after adequate BCG treatment but has had an emergence of high-grade disease subsequent to the complete response within 6 months of the last dose of BCG. A subject with low grade T1 disease in which recurrence of T1 disease occurs is also considered early-relapsing.

[0057] As used herein, the term “variant” refers to any pharmaceutically acceptable derivative, analogue, or fragment of an immunotoxin, an antibody or antibody fragment, a toxin (e.g., *Pseudomonas* toxin), or cancer therapeutic described herein. A variant also encompasses one or more components of a multimer, multimers comprising an individual component, multimers comprising multiples of an individual component (e.g., multimers of a reference molecule), a chemical breakdown product, and a biological breakdown product. In particular, non-limiting embodiments, an immunotoxin may be a “variant” relative to a reference immunotoxin by virtue of alteration(s) in the Ep-CAM-binding portion and/or the toxin portion of the reference immunotoxin. For example, a variant immunotoxin may contain multimers of

the antibody portion and/or the toxin portion. A variant of the toxin portion of the molecule retains toxicity of at least 10 percent and preferably at least 30 percent in a standard assay used to measure toxicity of a preparation of the reference toxin.

[0058] As used herein, the phrase “cancer therapeutic” and the like refers to compounds or treatments that are effective in treating or preventing cancer including, without limitation, chemical agents, other immunotherapeutics, cancer vaccines, anti-angiogenic compounds, certain cytokines, certain hormones, gene therapy, radiotherapy, surgery, and dietary therapy. One or more additional cancer therapeutic agents, including chemotherapeutic drugs and cancer specific antibodies, can be administered in addition to the immunoconjugates provided herein, before, during, or after the immunoconjugate administration. Exemplary therapeutic agents include, but are not limited to, 2,2,2 trichlorotriethylamine, 3-HP, 5,6-dihydro-5-azacytidine, 5-aza-2'-deoxycytidine, 5-azacytidine, 5-fluorouracil, 5-HP, 5-propagermanium, 6-azauridine, 6-diazo-5-oxo-L-norleucine, 6-mercaptopurine, 6-thioguanine, abrin, Aceglarone, acivicin, Aclacinomycin, actinomycin, actinomycin D, aldesleukin, Alemtuzumab, allocolchicine, allutamine, alpha-fetoprotein, alpha-TGDR, Altretamine, aminocamptothecin, Aminoglutethimide, aminopterin derivative, amonafide, amsacrine, an antifol, anastrozole, ancitabine, angiogenin antisense oligonucleotide, angiostatin, anthramycin, anthrapyrazole derivative, anti-thrombin, aphidicolin glycinate, ara-C, asparaginase, auristatin, autologous cells or tissues, Avastin, azacitidine, azaserine, aziridine, Axitinib, Belimumab, Bevacizumab, Bortezomib, Bosutinib, Brentuximab vedotin, Cabozantinib, Canakinumab, Carfilzomib, Cetuximab, Crizotinib, Dabrafenib, AZQ, *Bacillus*, Baker's soluble antifol, batimastat, BCG live vaccine, bcl-2 antisense oligonucleotide, BCNU, benzodepa, betamethasone, beta-TGDR, biaomycin, bicalutamide, bisantrene, bleomycin, brequinar, buserelin, Busulfan, cactinomycin, calicheamicin, calusterone, campath-1, camptothecin, camptothecin Na salt, capecitabine, carboplatin, Carboplatin, carboquone, carboxyphthalatoplatinum, carcinoembryonic antigen, carmofur, carmustine, camptothecin derivatives, carubicin, carzinophilin, CBDCA, CCNU, CHIP, Chlorabutin, Chlorambucil, chlormadinone acetate, chlornaphazine, chlorozotocin, chromomycins, cisplatin, cisplatinum, cladribine, clomesone, colchicine, colchicine derivative, collagen 14-amino acid peptide, cortisol, cortisone, cyanomorpholinodoxorubicin, cycloarabine, cyclocytidine, cyclodisone, cyclophosphamide, cyclophosphamide, cytarabine, cytochalasin B, cytosine arabinoside, dacarbazine, daclinomycin, dactinomycin, Dasatinib, daunorubicin, defosfamide,

dehydrotestosterone, demecolcine, denopterin, deoxydoxorubicin, dexamethasone, dianhydrogalactitol, diaziquone, dichlorallyl lawsone, diphtheria toxin, distamycin A, Docetaxel, dolastatin 10, doxifluridine, doxorubicin, droloxifene, dromostanolone, Duocarmycin/CC-1065, ecteinascidins, edatrexate, eflomithine, elliptinium acetate, emetine, emitefur, endostatin, enocitabine, epipodophyllotoxin, epirubicin, epitiostanol, erbitux, Erlotinib, esperamicin, estramustine, estrogen, ethidium bromide, etoglucid, etoposide, Fadrozole, Fenretinide, fibronectin 29 kDa N-terminal proteolytic fragment, Fibronectin 40 kDa C-terminal N-terminal proteolytic fragment, florafbr (pro-drug), floxuridhe, floxuridine, fludarabine, fluorodopan, flutamide, folinic acid, formestane, fosfestrol, fotemustine, gallium nitrate, Gefitinib, gemcitabine, gemcitabine, gemtuzumab, glucocorticoid, goserelin, gramicidin D, granulocyte monocyte colony stimulating factor, guanazole NSC 1895, Guerin, Halichondrin B, hepsulfam, hexamethylmelamine, hexestrol, human chorionic gonadotropin, hycanthone, hydroxyurea, idarubicin, Ifosamide, Imatinib, improsulfan, inosine glycodialdehyde, interferon, interferon-alpha, interferon-beta, interferon-gamma, interleukin-12, interleukin-15, interleukin-18, interleukin-1, interleukin-2, interleukin-2, interleukin-6, interleukins, Irinotecan, iubidazone, kringle 5, L-alanosine, Lapatinib, L-asparaginase, lauprolide acetate, lentinan, letrozole, leuprolide, leuprolide acetate (LUPRON), levamisole, lidocaine, liposomal dihydroxyanthracindione, lomusline, lomustine, lonidamine, lymphokines, lymphotoxin, LYSODREN, macbecin, macrophage inflammatory protein, m-AMSA, mannomustine, maytansine, mechlorethamine, mechlorethamine oxide hydrochloride, medroxyprogesterone, megestrol acetate, melanocyte lineage proteins, melengestrol, melphalan, menogaril, mepitiostane, mercaptopurine, mesna, methidiumpropyl-EDTA-Fe(II)), methotrexate, methotrexate derivative, meturedepa, miboplatin, miltefosine, mineral corticoid, mithramycin, mitobronitol, mitoguazone, mitolactol, mitolanc, mitomycin C, mitotane, mitoxantrone, mitozolamide, mopidamol, morpholinodoxorubicin, mutated tumor-specific antigens, mycophenolic acid, N-(phosphonoacetyl)-L-aspartate (PALA), N,N-dibenzyl daunomycin, nerve growth factor, Nilotinib, nilutamide, nimustine, nitracine, nitrogen mustard, nogalamycin, nonautologous cells or tissues, novembichin, olivomycins, ontak, Onyx-015, oxaliplatin, oxanthrazole, paclitaxel, PCNU, pegaspargase, pelomside A, pentostatin, peplomycin, perfosfamide, phenamet, phenesterine, picamycin, piperazine, piperazinedione, pipobroman, pipo sulfan, pirarubicin, piritrexim, platelet derived growth factor, platelet factor-4 7.8 kDa

proteolytic fragment, platelet factor-4 13 amino acid peptide, plicamycin, podophyllinic acid 2-ethyl-hydrazide, podophyllotoxin, polyestradiol phosphate, porfimir, porfiromycin, prednimustine, prednisone, procabazine, procaine, progestine, prolactin 16 kDa proteolytic fragment, propranolol, *Pseudomonas* exotoxin, PSK, pteropterin, puromycin, pyrazofurin, pyrazoloacridine, pyrazoloimidazole, Ranimustine, razoxane, retinoid, rhizoxin, rhizoxinlmaytansine, ricin A, rituxan, rituximab, riuxlmab, Roquinimex, Serpin (Serine Protease Inhibitor), Sizofican, sobuzoxane, Sorafenib, SPARC, 20-amino acid peptide, Spirogermanium, spirohydantoin mustard, straplozocin, streptonigrin, streptozocin, Sunitinib, Tamoxifen, Taxol, Taxol derivative, tegafur, temozoamide, teniposide, tenuazonic acid, teroxirone, testolactone, tetracaine, tetraplatin, thalidomide, Thiamiprine, thiocolchicine, thioepa, thiopurine, thio-tepa, Thrombospondin I 19 amino acid peptide, tissue plasminogen activator, Tomudex, topotecan, toremifene, trastuzutnaban, tretinoin, triaziquone, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, trilostane, trimetrexate, triptorelin, trityl cysteine, trofosfamide, Trontecan, tubercidin, tumor necrosis factor-like cytokine, tumor necrosis factors, Ubenimex, uracil mustard, uracil nitrogen mustard, uredepa, urethan, Vandetanib (ZD6474), VEGF antisense oligonucleotide, vinblastine, vinblastine sulfate, vincristine, vincristine sulfate, vindesine, vinorelbine, VM-26, VP-16, Yoshi-864, Zinostatin, zorubicin, Afatinib, Denosumab, Everolimus, Ibritumomab tiuxetan, Ibrutinib, Imatinib, Ipilimumab, Lapatinib, Nilotinib, Obinutuzumab, Ofatumumab, Panitumumab, Pazopanib, Pertuzumab, Ponatinib, Regorafenib, Romidepsin, Ruxolitinib, Sipuleucel-T, Sorafenib, Temsirolimus, Tocilizumab, Tofacitinib, Tositumomab, Trametinib, Trastuzumab, Vandetanib, Vemurafenib, Vismodegib, Vorinostat, Ziv-aflibercept, and any combination thereof.

[0059] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Unless otherwise stated, the practice of the present invention employs conventional molecular biology, cell biology, biochemistry, and immunology techniques that are well known in the art and described, for example, in *Methods in Molecular Biology*, Humana Press; *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook et al., 1989), *Current Protocols in Immunology* (J. E. Coligan et al., eds., 1991); *Immunobiology* (C. A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: a practical approach* (D. Catty, ed.,

IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Phage display: a laboratory manual (C. Barbas III et al, Cold Spring Harbor Laboratory Press, 2001); and Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999). The skilled person will recognize that any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

[0060] All publications referenced herein are incorporated by reference in their entireties for all purposes. Compositions and methods encompassed in the present disclosure will be further described with respect to the following examples; however, the scope of the invention is not to be limited thereby.

Examples

EXAMPLE 1. Clinical Study of the treatment of non muscle invasive bladder cancer with twice weekly dosing followed by once weekly dosing of Vicinium.

[0061] A study is conducted to assess the efficacy and tolerability of Vicinium therapy for the treatment of non muscle-invasive CIS and/or high-grade papillary disease of the bladder in subjects who have previously failed treatment with BCG.

Endpoints

[0062] In one aspect, the primary endpoint of the study is event-free survival following 18 months of Vicinium therapy. Event-free survival is defined as the interval from the date of the first dose of the immunoconjugate (Vicinium) to an event. An event is defined as persistent high-grade disease, high-grade tumor recurrence, tumor progression to muscle invasive bladder cancer, cystectomy for any reason, or death. Tumor recurrence or progression is histologically confirmed by bladder biopsy.

[0063] In another aspect, the primary endpoint of the study is complete response rate in patients with CIS with or without resected papillary disease following the initiation of Vicinium therapy. A complete response is defined as one of the following: a) normal cystoscopy and non-positive or non-suspicious urine cytology; b) negative biopsy (or biopsy revealing only low-grade disease) and non-positive repeat urine cytology; or c) cystoscopy or biopsy revealing low-grade (Ta) papillary disease that may be managed by transurethral resection of bladder tumor (TURBT), and not requiring cystectomy or other chemotherapy. In subjects who have a complete

response, the duration of response will be estimated (Kaplan-Meier Estimate). In this aspect, the secondary endpoints include event-free survival.

[0064] The secondary endpoints of the study include i) time to cystectomy (defined as the time from the date of the first dose of the immunoconjugate (Vicinium) to physical removal of the bladder); ii) no evidence of high-grade disease (high grade diseases is determined by direct examination or by histology) after 3, 6, 9, 12, 15, 18, 21, and 24 months of Vicinium therapy; time to disease recurrence; time to progression; progression-free survival rate; overall survival; and safety and tolerability of Vicinium.

[0065] No evidence of high-grade disease is defined as one of the following: a) normal cystoscopy and non-positive or non-suspicious urine cytology, or a negative biopsy or a biopsy revealing only low-grade papillary disease; b) negative biopsy (or biopsy revealing only low-grade papillary disease) and non-positive repeat urine cytology; c) negative biopsy (or biopsy revealing only low-grade papillary disease) and positive repeat urine cytology and a finding of transitional cell carcinoma (TCC) in the upper urinary tract; or d) cystoscopy or biopsy revealing only low-grade papillary disease that may be managed by transurethral resection of the bladder tumor (TURBT), and not requiring cystectomy. Time to disease recurrence is defined as the number of weeks from the date of the first documented no histological evidence of high-grade disease to the date of first recurrence of histologically-confirmed high-grade disease. Time to progression is defined as the time from the date of first dose of the immunoconjugate (Vicinium) to the date of histologically confirmed, invasive disease of stage $\geq T2$. Progression-free survival is defined as the time from the date of first dose of the immunoconjugate (Vicinium) to the date of histologically-confirmed, stage $\geq T2$ invasive disease being determined, or to the date of death. Overall survival is defined as the time from the date of the first dose of the immunoconjugate (Vicinium) to death due to any cause.

Study Design

[0066] The study is an open-label, non-randomized, multicenter, multiple-dose study of Vicinium in subjects with histologically-confirmed non muscle-invasive bladder cancer - carcinoma in situ (CIS), high-grade Ta or any grade T1 papillary disease or CIS plus papillary disease - who failed previous treatment with BCG. The subjects will include subjects who have received at least 7 instillations over 2 cycles (1 induction course of at least 5 doses + 1 maintenance cycle of at least 2 doses, or 2 induction courses) of BCG (with or without

interferon) and have failed to respond (BCG refractory subjects), or subjects who have recurred after achieving a disease-free status following at least 9 instillations of BCG therapy, as described above (BCG relapsing subjects).

[0067] The study design comprises an Induction Phase that is 12 weeks in duration and a Maintenance Phase. The total amount of treatment may be up to 104 weeks including induction and maintenance phase. . The study is an outpatient study, though intravesicular dosing is performed in the study clinic. Vicinium will be administered as monotherapy, and efficacy and tolerability/safety will be assessed.

[0068] Subjects will receive intravesicular Vicinium instillations twice weekly (BIW) for 6 weeks followed by once weekly for 6 weeks for a total of 18 doses in the Induction Phase. The twice weekly doses are to be administered at least 48 hours apart (and no more than 2 doses may be administered within any 7-day period.) After completion of the Induction Phase, subjects will undergo testing to determine their eligibility to continue in the trial. If there is no histological evidence of high-grade disease, the subject will enter the Maintenance Phase. Subjects who enter the study with low grade T1 disease and have a recurrence of low grade T1 disease will not enter the Maintenance Phase. During the Maintenance Phase, Vicinium is administered once every other week for up to 21 months. Subjects with histologically-confirmed evidence of high-grade disease will be withdrawn from the study as a treatment failure.

[0069] A schematic flow diagram of the study is provided in **Figure 1**.

[0070] The subject will be asked to empty his/her bladder prior to administration of Vicinium. Appropriate study personnel will then insert a catheter, and the catheter will be drained. Vicinium is then instilled into the bladder through the catheter. Subjects will be instructed to hold Vicinium in the bladder for 2 hours. They will also be asked to refrain from fluid intake to reduce urine flow during this period. Subjects will be required to position themselves upright (sitting or standing), supine, prone, and in the left and right lateral decubitus positions, for at least 15 minutes each, in any order. At the end of 2-hour period, the subject will void to empty the bladder or the bladder will be emptied by catheter drainage.

[0071] An appropriate local anesthetic and sterile lubricant may be used at the time of catheterization. In addition, a single dose of antibiotic may be given prophylactically at the time of catheterization, at the discretion of the Investigator.

[0072] The administration procedures for each dose of Vicinium throughout the study are the same as described above.

[0073] Vicinium is administered twice weekly via bladder instillation as described above in Weeks 1-6. Each treatment separated by at least 48 hours (and no more than 2 doses may be administered within any 7-day period.) As an example, while a Monday/Thursday or Tuesday/Friday would be optimal, a Monday/Wednesday or a Tuesday/Thursday dosing schedule could be used. Concomitant medications are recorded prior to dosing. Vital signs and collection of AEs, including SAEs, are conducted prior to dosing and prior to clinic discharge. Following the last dose in Week 6, prior to discharge from the clinic, clinical laboratory assessments are to be performed: blood samples collected for hematology and chemistry, urine sample for urinalysis.

[0074] During Weeks 7-11, Vicinium is administered once weekly via bladder instillation, as described above. Concomitant medications are recorded prior to dosing. Vital signs and collection of AEs, including SAEs, are conducted prior to dosing and prior to clinic discharge.

[0075] The following assessments are to be performed at Week 12. Urine cytology, concomitant medications, vital signs, and AEs, including SAEs, are to be collected prior to dosing. Following the dose in Week 12 and prior to discharge, the following clinical laboratory assessments are to be performed: vital signs, AEs including SAEs, physical exam, hematology and chemistry, urinalysis, and triplicate ECG (spaced one minute apart) for QTc analysis.

[0076] During Weeks 13-16, cystoscopy and biopsies will be performed. Biopsies may be random or direct biopsies. Samples will be collected and graded by a central pathologist to determine a subject's eligibility to continue in the trial. To enter the Maintenance Phase, a subject must have no histological evidence of high-grade disease. Any subject with histological evidence of high-grade disease will be discontinued from the trial as a treatment failure.

[0077] Week 14 is the earliest a subject may start Maintenance therapy dosing. Maintenance therapy cannot be initiated until all testing procedures are completed and confirmation of no histological evidence of high-grade disease has been provided by the central pathologist. Week 17 is the latest a subject may start Maintenance therapy.

[0078] Subjects who have no histological evidence of high-grade disease after the Induction Phase are eligible to enter the Maintenance Phase. Subjects in the Maintenance Phase will be dosed once every other week. The first Maintenance dose will begin no earlier than Week 14

(i.e., at least 1 week must separate the end of Induction and the start of Maintenance therapy) and no later than Week 17 (i.e., 4 weeks following completion of the Induction Phase).

[0079] Maintenance therapy continues until disease recurrence (subject recurs with histologically-confirmed CIS or high-grade papillary Ta or T1 disease) or disease progression (histologically-confirmed disease stage \geq T2) or up to 24 months from start of study. A subject with disease recurrence or progression will be withdrawn from the study. A subject who relapses with low-grade papillary disease will be allowed to continue Maintenance therapy following tumor removal. A period of at least 2 weeks, but no more than 3 weeks, must separate tumor removal and dosing of Vicinium.

[0080] A positive cytology without cystoscopic evidence of malignant disease will be evaluated (complete evaluation of the upper urinary tract by an appropriate imaging technique; bilateral ureteral cytologies; blind bladder biopsies; urethral evaluations and biopsy) and followed as clinically appropriate, at discretion of the Investigator. The subject may remain in the study at the discretion of the Investigator. A subject with a second consecutive positive cytology will be considered as having had an event at the time of the second positive or suspicious but non-diagnostic cytology if subsequent follow-up discovers recurrence of high-grade disease, but that subject may remain in the study at the Investigator's discretion prior to that determination of high-grade disease.

[0081] The primary endpoint will be evaluated at Month 17 by directed biopsy. EpCAM determination by immunohistochemistry will be obtained on all positive biopsies. In addition, the following procedures will be completed within 2 weeks of the final study visit: physical exam (including height and weight); triplicate ECG (spaced one minute apart) for QTc analysis; vital signs; concomitant medications; AEs, including SAEs; clinical laboratory sample collection (blood samples for hematology and chemistry and urine samples for urinalysis); cystoscopy; and urine cytology.

[0082] The schedule of visits and assessments during the induction phase is provided below in **Table 2**. The schedule of visits and assessments during the maintenance phase is provided below in **Table 3**. The term "study drug" refers to Vicinium.

Table 2. Screening/baseline and induction phase

[0083] Assessment	Screening and Baseline		Induction Phase			Weeks 13 - 16
	-8 to 0	-1 to 0	1	2-6	7-11	12
Week						

Informed consent	X						
Inclusion/Exclusion Criteria	X						
TURBT	X ¹						X ¹¹
Cystoscopy	X ²						X
Biopsy (directed)	X ^{2,3}						X
Cytology	X		X ⁷			X ⁹	
Demographics	X						
Medical history (including previous anti-tumor therapy)	X						
Physical examination	X		X ⁷			X ¹⁰	
Weight and height			X ⁷				
Prostatic urethral biopsy (males only)	X						
Upper tract radiological imaging	X ⁴						
Triplicate ECG	X ⁵					X ¹⁰	
Vital signs	X		X ⁸	X ⁸	X ⁸	X ⁸	
Clinical Labs (hematology, chemistry, urinalysis)		X	X ⁷	X ¹⁰		X ¹⁰	
Anti-Vicinium antibody test		X					
Serum Vicinium Levels ¹³		X	X	X	X		
Urine pregnancy test		X ⁶					
Concomitant medication	X	X	X ⁹	X ⁹	X ⁹	X ⁹	
Adverse events (AEs / SAEs)	X	X	X ⁸	X ⁸	X ⁸	X ⁸	
Drug administration (BIW) ¹²			X	X			
Drug administration (once/week)					X	X	

¹ Must be performed at least 2 weeks prior to study drug administration; all papillary disease must be completely resected. In subjects with T1 disease, a second TURBT must have been performed in order to qualify for study enrollment.

² Must have documented non-muscle invasive urothelial bladder cancer that is histologically confirmed by the protocol-specified, independent central pathology reviewer prior to first dose of study drug.

³ Random biopsies not required prior to study drug administration.

⁴ Not required if performed within 6 months before start of dosing.

⁵ Single tracing only at Screening; thereafter, triplicate ECGs are to be performed.

⁶ Females of childbearing potential only (defined as meeting at least one of the following criteria: a) has not undergone bilateral salpingo-oophorectomy, tubal ligation and/or hysterectomy; b) is greater than age 50 years and has had at least one menstrual period over the last 24 months).

⁷ Evaluation performed pre-dose before first dose in Week 1. Note: Week 1 clinical laboratory assessments only need to be performed if the Baseline laboratory assessments were performed >7 days before the first dose of study medication.

⁸ Evaluation performed pre-dose and prior to leaving the clinic.

⁹ Evaluation performed pre-dose.

¹⁰ Evaluation performed prior to leaving the clinic after the last dose in Week 6 (for clinical labs only) and Week 12 (clinical labs, physical exam, and ECG).

¹¹ Performed before the start of Maintenance only in subjects with low-grade papillary disease. A period of at least 2 weeks, but no more than 3 weeks, must separate tumor removal and the start of Vicinium Maintenance therapy.

¹² Twice weekly (BIW) dosing with each dose separated by at least 48 hours (and no more than 2 doses within any 7-day period). As an example, while a Monday/Thursday or Tuesday/Friday would be optimal, a Monday/Wednesday or Tuesday/Thursday dosing schedule could be used.

¹³ Levels are to be taken at baseline (pre dose of first instillation) and one hour following the initial instillation, the final of the twice-weekly instillations (scheduled 12th instillation), and the final of the once-weekly instillations (scheduled 18th).

Table 3. Maintenance Phase

Assessment	Maintenance Phase (Week 17-104)							Final Visit
	Following Induction Phase Treatment	Weeks 14, 17, 24	Monthly	Every 3 Months	Every 6 Months	Month 12	Month 18	Month 24 (End-of-Study)
Earliest week to start		Week						

Maintenance dosing		14						
Last week to start Maintenance dosing		Week 17						
Cytology				X ³				X ⁷
Cystoscopy	X ¹			X ³				X ⁷
Biopsy (directed)	X ^{1,2}						X ²	X ²
Physical examination					X ⁴			X ⁷
Triplicate ECG								X ⁷
Vital signs			X ³					X ⁷
Weight and height						X ⁴		X ⁷
Anti-Vicinium antibody test ⁴		Week 24						
Clinical Labs (hematology, chemistry, urinalysis)				X ⁵				X ⁷
TURBT ⁶	X			X				
Concomitant medication			X ⁴					X ⁷
Adverse events (AEs / SAEs)			X ³					X ⁷
Drug administration (once every other week)			X					

¹ Performed following completion of the Induction Phase to determine qualification for the Maintenance Phase.

² Evaluation performed as soon as possible, but no more than 2 weeks, following dosing.

³ Evaluation performed pre-dose and prior to leaving the clinic.

⁴ Evaluation performed pre-dose.

⁵ Evaluation performed 1 week and no more than 2 weeks following dosing.

⁶ TURBT only for subjects with low-grade papillary disease. A period of at least 2 weeks, but no more than 3 weeks, must separate tumor removal and the start of Vicinium Maintenance therapy following completion of Induction, or the re-start of Vicinium Maintenance therapy in those subjects who relapse with low-grade papillary disease during Maintenance therapy.

⁷ Evaluation performed within 2 weeks of end of-study.

Vicinium and Administration of Vicinium

[0084] Subjects will receive Vicinium twice weekly (BIW) for 6 weeks and once weekly for 6 weeks during the Induction Phase, and then enter the Maintenance Phase, if eligible. Only subjects who have no histological evidence of high-grade disease after the Induction Phase are eligible to enter the Maintenance Phase. The treatment regimens for the induction and maintenance phases are provided below in **Table 4**.

Table 4. Treatment regimens

Study Phase	Treatment Regimen
Induction Phase (Weeks 1-12)	<p>One intravesical dose of Vicinium 30 mg in 50 mL of saline instilled twice weekly (BIW) for 6 weeks followed by once weekly for 6 weeks, for a total of 12 weeks. The twice weekly doses are to be administered at least 48 hours apart (and no more than 2 doses may be administered within any 7-day period.) As an example, while a Monday/Thursday or Tuesday/Friday would be optimal, a Monday/Wednesday or a Tuesday/Thursday dosing schedule could be used. There will be a total of 18 doses over the 12-week period of the Induction Phase.</p>
Maintenance Phase (up to Week 104)	<p>One intravesical dose of Vicinium 30 mg in 50 mL saline instilled once every other week, for up to 24 months (Week 104) from the start of the Induction Phase.</p> <p>Only subjects who have no histological evidence of high-grade disease after the Induction Phase are eligible to enter the Maintenance Phase.</p> <p>Dosing for the first maintenance cycle will begin as soon as practical following completion of the Induction Phase, within the following timeframe: no earlier than Week 14 (i.e., at least 1 week, must separate the end of Induction and the start of Maintenance therapy) and no later than Week 17.</p>

[0085] To reduce urine flow during the instillation and dwell of Vicinium in the bladder, subjects will be asked to refrain from drinking any liquids beginning 2 hours prior to Vicinium instillation through completion of the 2-hour time period during which drug is held in the bladder. Immediately prior to bladder catheter insertion, subjects will empty their bladder. Appropriate study personnel will then insert a catheter into the bladder, and the catheter will be drained. An appropriate local anesthetic and sterile lubricant may be used at the time of catheterization. In addition, a single dose of an oral antibiotic may be given prophylactically at the time of catheterization, at the discretion of the Investigator. The use of any medications must be recorded on the subject case report form.

[0086] Vicinium 30 mg in 50 mL of saline will be instilled into the catheter. Subjects will be instructed to hold Vicinium in the bladder for 2 hours, and asked to refrain from fluid intake to reduce urine flow during this period. Subjects will be required to be upright (sitting or standing), prone, supine, and in the left and right lateral decubitus positions, for at least 15 minutes each, in any order. If physically unable, this should be recorded in the CRF. At the end of 2-hour period, the catheter will be removed and the subject will void to empty the bladder.

[0087] In this clinical study, Vicinium will be administered as a monotherapy. Beginning 2 weeks prior to the initial dose of Vicinium and throughout the 24-month study period, no anti-

cancer therapies, including hormone therapy, radiation therapy, immunotherapy, vaccine therapy, biological therapy, or gene-based therapies will be permitted. Additionally, no other investigational agent is permitted within 4 weeks before the start of Vicinium and throughout the 24-month study period. Any other medication that is considered necessary for the well being of the subject, and that is not expected to interfere with the evaluation of Vicinium, may be given at the discretion of the Investigator.

[0088] In subjects who develop low-grade papillary disease during the Maintenance Phase, Vicinium must be interrupted for a minimum of 2 weeks, but for no more than 3 weeks, following tumor removal before restarting Maintenance therapy. Vicinium administration may be postponed for up to 2 weeks if a subject develops any local Grade 3 Adverse Event as defined using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03. Vicinium administration may also be postponed for up to 2 weeks at the discretion of the Investigator for any systemic Grade 3 AE. If during these 2 weeks the symptoms improve to Grade 1 or less, dosing may resume. If after 2 weeks the symptoms do not improve, treatment should not be resumed. However, if the AE is assessed by the Investigator as not related to Vicinium and continued treatment is expected to result in a clinical benefit for the subject, treatment may resume as determined in consultation with the Medical Monitor on a case-by-case basis.

[0089] Vicinium™ (oportuzumab monatox) is a clear, colorless solution for intravesical administration. It is provided as a frozen product in a glass vial, and then thawed at room temperature prior to preparation for administration. Cloudiness, a change in color and/or the presence of particulate matter may indicate the product has deteriorated. Vicinium 30 mg in 50 mL of saline will be administered intravesically.

[0090] For each dose, 1 vial of Vicinium containing 7 mL of Vicinium at a concentration of 5 mg/mL is to be removed from the freezer and allowed to thaw at room temperature (20-25°C with excursions to 15-30°C permitted) for 1 hour ± 15 minutes in an upright position. If large particles are observed or the solution is cloudy in appearance, the vial will be allowed to stand for a maximum additional time of 30 minutes or until a clear solution is observed, whichever comes first. If particulates are still observed after 30 minutes, a fresh vial of Vicinium will be obtained from the freezer. Vials will not be inverted or shaken or placed on their sides during the thawing process. Vials should not be exposed to direct light or heat sources.

Study Population

[0091] To qualify for participation, a subject must meet all of the following Inclusion Criteria at the screening/baseline visit:

1. Histologically-confirmed high-grade non muscle-invasive bladder cancer - CIS, Ta or T1 papillary disease or both. This diagnosis is confirmed by the protocol-specified, independent central pathology reviewer prior to subject enrollment in the trial and the start of study treatment.
2. Documented receipt and failure (i.e., not intolerance) of BCG therapy (with or without interferon) for CIS or high-grade Ta or any grade T1 of the bladder, or BCG-refractory or BCG-resistant disease. Male or non-pregnant, non-lactating female, age 18 years or older at date of consent.
3. Females of childbearing potential and all males with partners of childbearing age are eligible only if they agree to use appropriate contraceptive techniques or abstinence during the 24-month study period. A female of childbearing potential is defined as meeting at least one of the following criteria: a) has not undergone bilateral salpingo-oophorectomy, tubal ligation and/or hysterectomy; b) is greater than age 50 years and has had at least one menstrual period within the last 24 months.
4. Bladder biopsy mapping the location of the tumors and quantifying the affected area of bladder within 8 weeks before Vicinium administration.
5. Life expectancy of at least 4 years.
6. Adequate organ function, as defined by the following criteria:
 - a. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 3.0 \times$ upper limit of normal (ULN);
 - b. Total serum bilirubin $\leq 1.5 \times$ ULN (CTCAE Grade ≤ 1);
 - c. Serum creatinine $\leq 2.0 \times$ ULN; subjects with serum creatinine $> 1 \times$ ULN must also have creatinine clearance ≥ 50 mL/min;
 - d. Hemoglobin ≥ 8.0 g/dL; subjects receiving therapeutic erythropoietin preparations (i.e., epoetin alfa, darbepoetin alfa) are eligible to enroll;
 - e. Absolute neutrophil count $\geq 1500 \times 10^9/L$;
 - f. Platelets $\geq 75,000 \times 10^9/L$.
7. Ability to understand and sign an Independent Ethics Committee- or Institutional Review Board-approved informed consent document indicating that the subject (or legally acceptable representative) has been informed of all pertinent aspects of the trial prior to enrollment and is willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures.

[0092] A subject will not be eligible for participation if any one or more of the following criteria apply.

8. The subject is pregnant or breastfeeding.
9. Evidence of urethral or upper tract transitional cell carcinoma (TCC) by biopsy or upper tract radiological imaging (e.g., intravenous pyelogram, computed tomography urogram, or retrograde pyelogram) or evidence of higher stage disease by pelvic imaging within the past 2 years.
10. Subjects with hydronephrosis, except for those subjects where hydronephrosis has been longstanding (i.e., predates the diagnosis of the CIS, Ta or T1 by more than 2 years) and diagnostic evaluation at Screening shows no evidence of tumor.
11. Any intravesicular or other chemotherapy treatment within 2 weeks or any investigational agent within 4 weeks prior to the initial dose of Vicinium.
12. Current severe urinary tract infection or history of recurrent severe bacterial cystitis.
13. Active, uncontrolled impairment of the urogenital, renal, hepatobiliary, cardiovascular, gastrointestinal, neurologic or hematopoietic systems which, in the opinion of the Investigator, would predispose the subject to the development of complications from the administration of intravesical therapy and/or general anesthesia.
14. History of other primary malignancy (other than squamous or basal cell skin cancers) that will require concomitant cancer therapy during the 24 months of the study.
15. A QTc interval of >450 msec for males or > 470 msec for females at the Screening ECG. If the finding is due to a correctable cause (e.g., hypokalemia, hypomagnesemia), a repeat ECG upon correction may be performed to determine subject eligibility.
16. Subjects who, in the opinion of the Investigator, cannot tolerate intravesical administration or intravesical surgical manipulation (cystoscopy, biopsy) due to the presence of serious comorbid condition(s) (e.g., uncontrolled cardiac or respiratory disorders).

Assessment of Efficacy

[0093] Standard cystoscopy is to be performed. Any subject who underwent fluorescence-guided biopsy for the diagnostic samples used for qualification into the study must continue using this modality for cystoscopy throughout the study. During each cystoscopic assessment, bladder mapping must be performed, including documentation of each tumor location and quantification of the overall area of the bladder affected. Cystoscopies will be performed at the end the Induction Phase and every 3 months during the Maintenance Phase. Cystoscopy should be done at least 1 week, but no more than 2 weeks, after the last dose at each of the above time points. Cystoscopy is also performed at Final Visit, within 2 weeks following last dose of Vicinium administration.

[0094] Induction Phase cytology samples will be collected at Week 1 prior to the initial (first) dose of Vicinium and pre-dose of Week 12. Cytology samples are also collected every 3 months

during the Maintenance Phase (just prior to the first dose of the month) and at Final Visit (within 2 weeks following last dose of Vicinium administration).

[0095] Directed biopsies are required for all subjects after completion of the Induction Phase, at Month 18 during the Maintenance Phase, and at Final Visit. No random biopsies are required but may be obtained during workups of suspicious or positive cytologies without obvious anatomical abnormalities. Biopsies are to be obtained and graded as soon as possible, but no more than 2 weeks, after the last dose at each time point. Biopsies at all other time points will only be obtained if evident or suspicious lesions are seen during cystoscopy or when positive or suspicious cytology is observed in the case of negative cystoscopy.

[0096] Directed biopsies should be taken from all areas suspicious for disease, including any area of apparent persistent disease and/or any area of inflammation. A minimum of 2 biopsies should be obtained for each subject at each biopsy time point.

Assessment of Safety/Tolerability

[0097] An adverse event (AE) is defined as any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (that may include an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product. Any worsening of the subject's disease under study or other medical conditions will also be considered an AE, unless it is within the normal range of disease fluctuation for that subject. A laboratory test abnormality constitutes an AE only if it is clinically significant such that it induces clinical signs or symptoms, leads to a new diagnosis that was not present at baseline or worsened during active treatment to Grade 3 or Grade 4 toxicity, or requires therapy. An abnormal laboratory value that constitutes a serious adverse event (SAE) or leads to Vicinium discontinuation must be reported and recorded as an AE. All AEs should be treated appropriately. All AEs regardless of suspected causality, occurring from the time of signed Informed Consent and until 30 days after study discontinuation must be recorded on the Adverse Events CRF (AE CRF) with the following minimum information: type of event, duration (start and end dates), severity, seriousness, causality to Vicinium, action taken, and outcome. All AEs will be assessed using the NCI-CTCAE (version 4.03); where applicable, pre-study conditions will be graded using the same scale. For each episode, the highest severity grade attained should

be recorded. Adverse events not included in the NCI-CTCAE are to be recorded. Intensity (mild, moderate, or severe) and relationship to Vicinium (not related, unlikely related, possibly related, probably related, or definitely related) will be graded by the Investigator.

[0098] A serious adverse event (SAE) or serious suspected adverse reaction is considered serious if, in the view of either the Investigator or sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening AE, defined as an AE that, in the view of either the Investigator or the Sponsor, places the patient or subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death;
- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions;
- A congenital anomaly/birth defect;
- Inpatient hospitalization or prolongation of existing hospitalization. The following will not be considered as a hospitalization:
 - Elective or pre-planned hospital stays for conditions that existed prior to informed consent and have not worsened or hospital stays for social reasons.
 - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of an SAE given above and not resulting in hospital admission. “23-hour” hospitalizations for observation should be discussed with the Medical Monitor to determine appropriateness for SAE reporting.
 - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based on appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

[0099] Laboratory assessments will be performed at Baseline, and at the following times in the Induction Phase: before the first dose in Week 1 (if Baseline assessments were performed >7 days prior to the first dose of Vicinium at Week 1), after the last dose in Week 6, and after the last dose in Week 12. During the Maintenance Phase, laboratory assessments will be performed every 3 months (collected 1 week, but no more than 2 weeks following last dose) and at Final Visit (collected no more than 2 weeks following last dose). The following laboratory assessments will be performed:

- Serum chemistry, including total bilirubin, ALT, AST, alkaline phosphatase, albumin, lactate dehydrogenase, BUN, creatinine, glucose, sodium, potassium, calcium, phosphorus
- Hematology, including Complete Blood Count (CBC) with differential, hemoglobin, hematocrit, and platelets
- Urinalysis with red blood cell (RBC) and white blood cell (WBC) counts

[00100] All female subjects of childbearing potential (defined as any female who meets at least one of the following criteria: a) has not undergone bilateral salpingo-oophorectomy, tubal ligation and/or hysterectomy; b) is greater than 50 years and has had at least one menstrual period over the last 24 months) will have a urine pregnancy test at Baseline.

[00101] Blood will also be collected for determination of plasma anti-Vicinium at Baseline and 6 months of therapy.

[00102] A physical examination must be performed by the Investigator or qualified member of the clinic study staff at Screening and at Week 1 prior to the initial dose of Vicinium in the Induction Phase. Physical examinations are also to be performed after last dose in Induction Phase, every 6 months during the Maintenance Phase, and at EOS (or early withdrawal).

[00103] Weight and height will be measured at Week 1 prior to the initial dose of Vicinium in the Induction Phase, at Month 12 prior to first dose of Month 12, and at EOS (or early withdrawal).

[00104] Complete physical examinations will be performed at Screening and at EOS. All other examinations will be abbreviated and focused, at the discretion of the Investigator, to assess the subject's clinical status, any changes since the previous visit, and any possible AEs.

[00105] A single 12-lead ECG will be performed at Screening to determine subject eligibility. Triplicate ECGs (consisting of three individual ECGs spaced one minute apart) for QTc analysis will be performed after the last dose in Induction Phase prior to discharge from the clinic, and at EOS or early withdrawal (within 2 weeks following last dose of Vicinium administration). The ECG should be initiated after at least 10 minutes of quiet rest in a supine position.

[00106] Vital signs (blood pressure, pulse, respirations and temperature) will be measured at the following times: Screening, each Induction Phase dosing visit (before dosing and prior to discharge from the clinic), each Maintenance Phase dosing visit (before dosing and prior to

discharge from the clinic), and EOS (or early withdrawal). Each vital sign assessment will consist of 2 measurements. Prior to recording the first measurement, the subject should lie in a supine position for 5 minutes. Prior to recording the second measurement, the subject should stand for 3 minutes.

Statistics

[00107] The type I (alpha error) for all hypothesis testing will be set at 0.05, and all statistical tests will be two-sided. All confidence intervals will be two-sided 95% confidence intervals. Data will be summarized by reporting the frequency and percentage of subjects in each category for categorical and ordinal measures, and means, standard deviations (SD), medians, minimum and maximum for continuous measures.

[00108] Modified intent-to-treat (mITT) Population: The mITT population includes any subject who receives at least one dose of study medication and has an evaluable baseline assessment of disease (biopsy, cystoscopy, and cytology). The mITT population will be the primary efficacy analysis population.

[00109] Safety Population: The safety population includes any subject who receives at least one dose of study medication.

[00110] The number and percentage of subjects with CIS with or without papillary disease with a complete response will be summarized separately at each assessment. Ninety-five percent (95%) confidence intervals around the complete response rate will be calculated using the Clopper-Pearson method. For subjects with CIS with or without papillary disease who achieve complete response, the duration of response, defined as the start of the complete response to the first documented case of progression or death, will be estimated using the method of Kaplan-Meier. Any patient who does not experience progression or death will be censored at the last non-missing assessment.

[00111] Event-free survival will be estimated using the method of Kaplan-Meier. Subjects who do not experience an event will be censored at the last non-missing assessment. Subjects with persistent disease during the Induction Phase will be treated as having an event at Day 1. The ninety-five percent (95%) confidence interval around the event-free survival rate at 18 months will be presented. The study will be deemed successful if the lower bound of the 95% confidence interval excludes 20%. Analyses of other time-to-event efficacy endpoints (time to

cystectomy, time to disease recurrence, time to progression, progression-free survival, and overall survival) will be analyzed in a similar manner.

[00112] The number and percentage of subjects with no evidence of high-grade disease will be summarized separately at each assessment. Ninety-five percent (95%) confidence intervals around the no evidence of high-grade disease rate will be calculated using the Clopper-Pearson method.

[00113] Subset analyses by pathology at study entry will be performed as determined by final distribution.

[00114] Adverse events will be coded using the latest available version of the Medical Dictionary for Regulatory Activities (MedDRA). The NCI-CTCAE 4.03 terms are grouped by MedDRA Primary SOCs. Separate tables and/or listings for will be provided for AEs by maximum intensity, treatment-emergent AEs, relationship to study medication, discontinuation of study medication, and death. Tabulations and listings of values for vital signs and clinical laboratory tests will be presented. The tabulation of clinical laboratory tests will be classified as falling above, below, or within normal range. Follow-up ECG and physical examination data will be summarized in tabular format.

[00115] Assuming a nonparametric distribution for event-free survival time and the length of follow-up is 24 months, a sample size of 134 subjects will provide at least 80% power to test the null hypothesis that the event-free survival rate at 18 months is 20% versus the alternative hypothesis that the event-free survival rate at 18 months is $\geq 30\%$. (SWOG One Sample Non-Parametric Survival Calculator
https://www.swogstat.org/stat/public/one_nonparametric_survival.htm)

References

1. American Urological Association. Guideline for the management of noninvasive bladder cancer: (stages Ta, T1, Tis): 2007 update. Updated February 2014. Available at: <https://www.auanet.org/education/guidelines/bladder-cancer.cfm>. Last accessed April 25, 2014.

2. Anastasiadis A, de Reijke TM. Best practice in the treatment of nonmuscle invasive bladder cancer. *Ther Adv Urol*. 2012;4(1):13-32.
3. Babjuk M, Burger M, Zigeuner R, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. *Eur Urol*. 2013;64(4):639-53.
4. Bladder cancer treatment (PDQ®). General information about bladder cancer. Available at: <http://www.cancer.gov/cancertopics/pdq/treatment/bladder/HealthProfessional/page1>. Last accessed April 25, 2014.
5. Kowalski M, Entwistle J, Cizeau J, et al. A phase I study of an intravesically administered immunotoxin targeting EpCAM for the treatment of nonmuscle-invasive bladder cancer in BCG-refractory and BCG-intolerant patients. *Drug Des Devel Ther*. 2010;4:313-20.
6. Kowalski M, Guindon J, Brazas L, et al. A phase II study of oportuzumab monatox: an immunotoxin therapy for patients with noninvasive urothelial carcinoma in situ previously treated with bacillus Calmette-Guérin. *J Urol*. 2012;188(5):1712-8.
7. Oppenheimier NJ, Bodley JW. Diphtheria toxin. Site and configuration of ADP-ribosylation diphthamide in elongation factor 2. *J Biol Chem*. 1981;256(16):8579-81.
8. Sylvester RJ, van der Meijden A, Witjes JA, et al. High-grade Ta urothelial carcinoma and carcinoma in situ of the bladder. *Urology*. 2005;66(6 Suppl 1):90-107.
9. Sylvester RJ. Bacillus Calmette-Guérin treatment of non-muscle invasive bladder cancer. *Int J Urol*. 2011;18(2):113-20.

CLAIMS

1. A method of treating bladder cancer in a subject in need thereof comprising administering to the subject an immunoconjugate, wherein the immunoconjugate comprises (i) a binding protein that binds to the extracellular domain of human Ep-CAM and (ii) a toxin;

wherein the immunoconjugate is administered to the subject twice per week for a first dosing period of at least about six consecutive weeks, followed by a second dosing period wherein the immunoconjugate is administered once per week for a period of at least about six consecutive weeks.

2. The method of claim 1, wherein the binding protein is an antibody or fragment thereof.

3. The method of claim 2, wherein the antibody or fragment thereof is a monoclonal antibody, Fab, Fab', (Fab')₂, single chain fragment (scFv), disulfide-stabilized fragment (dsFv), or a single domain antibody (sdAb).

4. The method of claim 2, wherein the antibody or fragment thereof comprises a heavy chain CDR1 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 7; a heavy chain CDR2 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 8; a heavy chain CDR3 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 9; a light chain CDR1 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 4; a light chain CDR2 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 5; and a light chain CDR3 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 6.

5. The method of claim 4, wherein the antibody or fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 consisting of an amino acid sequence as set forth in SEQ ID NOS: 7, 8, and 9, respectively; and a light chain CDR1, CDR2, and CDR3 consisting of an amino acid sequence as set forth in SEQ ID NOS: 4, 5, and 6, respectively.

6. The method of claim 2, wherein the antibody or fragment thereof is humanized.

7. The method of claim 6, wherein the antibody fragment is a humanized scFv.
8. The method of claim 6 wherein the humanized scFv comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 3.
9. The method of claim 6, wherein the humanized scFv comprises an amino acid sequence according to SEQ ID NO: 3.
10. The method of claim 1, wherein the toxin is ETA(252-608).
11. The method of claim 10, wherein the ETA(252-608) comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 11.
12. The method of claim 10, wherein the ETA(252-608) comprises an amino acid sequence according to SEQ ID NO: 11.
13. The method of claim 1, wherein the binding protein is attached to the toxin by a peptide linker.
14. The method of claim 13, wherein the peptide linker is a cleavable linker or a non-cleavable linker.
15. The method of claim 14 wherein the cleavable linker is a furin sensitive linker, a cathepsin sensitive linker, a metalloproteinase linker, or a lysosomal hydrolase sensitive linker.
16. The method of claim 14, wherein the non-cleavable linker is a flexible linker.
17. The method of claim 16, wherein the flexible linker is between 10 and 30 amino acids in length.
18. The method of claim 17, wherein the flexible linker is 20 amino acids in length.

19. The method of claim 16, wherein the flexible linker comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 10.
20. The method of claim 16, wherein the flexible linker comprises an amino acid sequence according to SEQ ID NO: 10.
21. The method of claim 1, wherein the immunoconjugate comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 2.
22. The method of claim 1, wherein the immunoconjugate comprises an amino acid sequence according to SEQ ID NO: 2.
23. The method of claim 1, wherein the immunoconjugate is administered to the subject at a dose level of about 10mg to about 60mg per dose.
24. The method of claim 23, wherein the immunoconjugate is administered to the subject at a dose level of about 30 mg per dose.
25. The method of claim 1, wherein the doses of the first dosing period are administered at least 48 hours apart.
26. The method of claim 1, wherein no more than 2 doses are administered within any 7 day period in the first dosing period.
27. The method of claim 1, wherein the immunoconjugate is administered by instillation into the bladder.
28. The method of claim 27, wherein the immunoconjugate is administered via a catheter.

29. The method of claim 28, wherein the bladder is emptied via the catheter prior to administration of the immunoconjugate.
30. The method of claim 27, wherein the immunoconjugate is held in the bladder for 2 hours.
31. The method of claim 27, wherein the subject is positioned upright, prone, supine, and in the left and right lateral decubitus positions during the time that the immunoconjugate is in the bladder.
32. The method of claim 31, wherein the subject is positioned upright, prone, supine, and in the left and right lateral decubitus positions for at least 15 minutes per position.
33. The method of claim 1, wherein the bladder cancer is BCG refractive or BCG resistant.
34. The method of claim 1, wherein the subject has previously been treated with BCG and/or interferon.
35. The method of claim 34, wherein the subject failed to respond to BCG and/or interferon therapy or wherein the subject achieved a disease-free status following BCG and/or interferon therapy but disease subsequently recurred.
36. The method of claim 34, wherein the subject received at least 9 instillations over 2 cycles of BCG.
37. The method of claim 1, wherein the bladder cancer is non-muscle invasive bladder cancer (NMIBC).
38. The method of claim 37, wherein the NMIBC is classified as carcinoma in situ (CIS), Ta, or T1.

39. The method of claim 1, wherein the dosing schedule further comprises administering the immunoconjugate to the subject for a third dosing period, wherein the immunoconjugate is administered once per week every other week during the third dosing period.
40. The method of claim 37, wherein the third dosing period is started in subjects having no histological evidence of high-grade disease following the second dosing period.
41. The method of claim 39, wherein the third dosing period begins at least one week following the end of the second dosing period.
42. The method of claim 39, wherein the third dosing period begins no more than 3 weeks following the end of the second dosing period.
43. The method of claim 39, wherein the immunoconjugate is administered at a dose of about 10 to about 60 mg per dose during the third dosing period.
44. The method of claim 43, wherein the immunoconjugate is administered at a dose of about 30 mg per dose.
45. The method of claim 1, further comprising removing a bladder tumor prior to administration of the immunoconjugate.
46. The method of claim 45, wherein the bladder tumor is removed via transurethral resection.
47. The method of claim 1, further comprising administering to the subject at least one additional therapeutic agent.
48. The method of claim 47, wherein the additional therapeutic agent is a chemotherapeutic drug or a radiotherapeutic drug.

49. The method of claim 48, wherein the additional therapeutic agent is selected from the group consisting of cisplatin, carboplatin, paclitaxel, docetaxel, 5-fluorouracil, bleomycin, methotrexate, ifosamide, or any combination thereof.

50. The method of claim 49, wherein the additional therapeutic agent is administered to the subject concurrently with the immunoconjugate.

51. A method of treating bladder cancer in a subject in need thereof, the method comprising:
removing a NMIBC tumor from the bladder of the subject via transurethral resection, and
administering to the subject an immunoconjugate, wherein the immunoconjugate comprises (i) a binding protein that binds to the extracellular domain of human Ep-CAM and (ii) a toxin;

wherein the immunoconjugate is administered to the subject twice per week for a first dosing period of at least about six consecutive weeks, followed by a second dosing period wherein the immunoconjugate is administered to the subject once per week for a period of at least about six consecutive weeks, followed by a third dosing period wherein the subject is administered the immunoconjugate once per week every other week.

52. The method of claim 51, wherein the binding protein is an antibody or fragment thereof.

53. The method of claim 52, wherein the antibody or fragment thereof comprises a heavy chain CDR1 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 7; a heavy chain CDR2 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 8; a heavy chain CDR3 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 9; a light chain CDR1 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 4; a light chain CDR2 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 5; and a light chain CDR3 having at least 80% homology to the amino acid sequence set forth in SEQ ID NO: 6

54. The method of claim 53, wherein the antibody or fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 consisting of an amino acid sequence as set forth in SEQ ID NOs: 7, 8,

and 9, respectively; and a light chain CDR1, CDR2, and CDR3 consisting of an amino acid sequence as set forth in SEQ ID NOS: 4, 5, and 6, respectively.

55. The method of claim 52, wherein the antibody or fragment thereof is a monoclonal antibody, Fab, Fab', (Fab')₂, single chain fragment (scFv), disulfide-stabilized fragment (dsFv), or a single domain antibody (sdAb).

56. The method of claim 55, wherein the antibody or fragment thereof is humanized.

57. The method of claim 56, wherein the antibody fragment is a humanized scFv.

58. The method of claim 57 wherein the humanized scFv comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 3.

59. The method of claim 57, wherein the humanized scFv comprises an amino acid sequence according to SEQ ID NO: 3.

60. The method of claim 51, wherein the toxin is ETA(252-608).

61. The method of claim 60, wherein the ETA(252-608) comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 11.

62. The method of claim 60, wherein the ETA(252-608) comprises an amino acid sequence according to SEQ ID NO: 11.

63. The method of claim 51, wherein the binding protein is attached to the toxin by a peptide linker.

64. The method of claim 63, wherein the peptide linker is a cleavable linker or a non-cleavable linker.

65. The method of claim 64 wherein the cleavable linker is a furin sensitive linker, a cathepsin sensitive linker, a metalloproteinase linker, or a lysosomal hydrolase sensitive linker.
66. The method of claim 64, wherein the non-cleavable linker is a flexible linker.
67. The method of claim 66, wherein the flexible linker is between 10 and 30 amino acids in length.
68. The method of claim 67, wherein the flexible linker is 20 amino acids in length.
69. The method of claim 66, wherein the flexible linker comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 10
70. The method of claim 66, wherein the flexible linker comprises an amino acid sequence according to SEQ ID NO: 10
71. The method of claim 51, wherein the immunoconjugate comprises an amino acid sequence having at least 80% homology to SEQ ID NO: 2.
72. The method of claim 51, wherein the immunoconjugate comprises an amino acid sequence according to SEQ ID NO: 2.
73. The method of claim 51, wherein the immunoconjugate is administered to the subject at a dose level of about 10mg to about 60mg per dose.
74. The method of claim 73, wherein the immunoconjugate is administered to the subject at a dose level of about 30 mg per dose.
75. The method of claim 51, wherein the doses are administered at least 48 hours apart.

76. The method of claim 51, wherein no more than 2 doses are administered within any 7 day period.

77. The method of claim 51, wherein the immunoconjugate is administered by instillation into the bladder.

78. The method of claim 77, wherein the immunoconjugate is administered via a catheter.

79. The method of claim 78, wherein the bladder is emptied via the catheter prior to administration of the immunoconjugate.

80. The method of claim 77, wherein the immunoconjugate is held in the bladder for 2 hours.

81. The method of claim 77, wherein the subject is positioned upright, prone, supine, and in the left and right lateral decubitus positions during the time that the immunoconjugate is in the bladder.

82. The method of claim 81, wherein the subject is positioned upright, prone, supine, and in the left and right lateral decubitus positions for at least 15 minutes per position.

83. The method of claim 51, wherein the bladder cancer is BCG refractive or BCG resistant.

84. The method of claim 51, wherein the subject has previously been treated with BCG and/or interferon.

85. The method of claim 84, wherein the subject failed to respond to BCG and/or interferon therapy or wherein the subject achieved a disease-free status following BCG and/or interferon therapy but disease subsequently recurred.

86. The method of claim 84, wherein the subject received at least 9 instillations over 2 cycles of BCG.

87. The method of claim 1, wherein the bladder cancer is non-muscle invasive bladder cancer (NMIBC).
88. The method of claim 87, wherein the NMIBC is classified as carcinoma in situ (CIS), Ta, or T1.
89. The method of claim 51, wherein the third dosing period begins at least one week following the end of the second dosing period.
90. The method of claim 51, wherein the third dosing period begins no more than 3 weeks following the end of the second dosing period.
91. The method of claim 51, further comprising administering to the subject at least one additional therapeutic agent.
92. The method of claim 91, wherein the additional therapeutic agent is a chemotherapeutic drug or a radiotherapeutic drug.
93. The method of claim 92, wherein the additional therapeutic agent is selected from the group consisting of cisplatin, carboplatin, paclitaxel, docetaxel, 5-fluorouracil, bleomycin, methotrexate, ifosamide, or any combination thereof.
94. The method of any one of the preceding claims, wherein the immunoconjugate is VB4-845.
95. An immunoconjugate for use in treating bladder cancer, wherein the immunoconjugate comprises (i) a binding protein that binds to the extracellular domain of human Ep-CAM and (ii) a toxin; wherein the immunoconjugate is administered twice per week for a first dosing period of at least six consecutive weeks, followed by a second dosing period wherein the immunoconjugate is administered once per week for a period of at least six consecutive weeks.

96. An immunoconjugate for use in treating bladder cancer, wherein the immunoconjugate comprises (i) a binding protein that binds to the extracellular domain of human Ep-CAM and (ii) a toxin; wherein the immunoconjugate is administered twice per week for a first dosing period of at least six consecutive weeks, followed by a second dosing period wherein the immunoconjugate is administered once per week for a period of at least six consecutive weeks, followed by a third dosing period wherein the immunoconjugate is administered once per week every other week, and wherein the immunoconjugate is administered to a subject who has undergone transurethral resection to remove a NMBIC tumor from their bladder prior to the first dosing period.

97. The method of claim 96 wherein the third dosing period is up to 24 months in length or until disease recurrence or disease progression.

98. The method of claim 8, 9, 58 or 59 wherein SEQ ID NO: 3 comprises SEQ ID Nos: 4, 5, 6 and 7, 8 and 9.

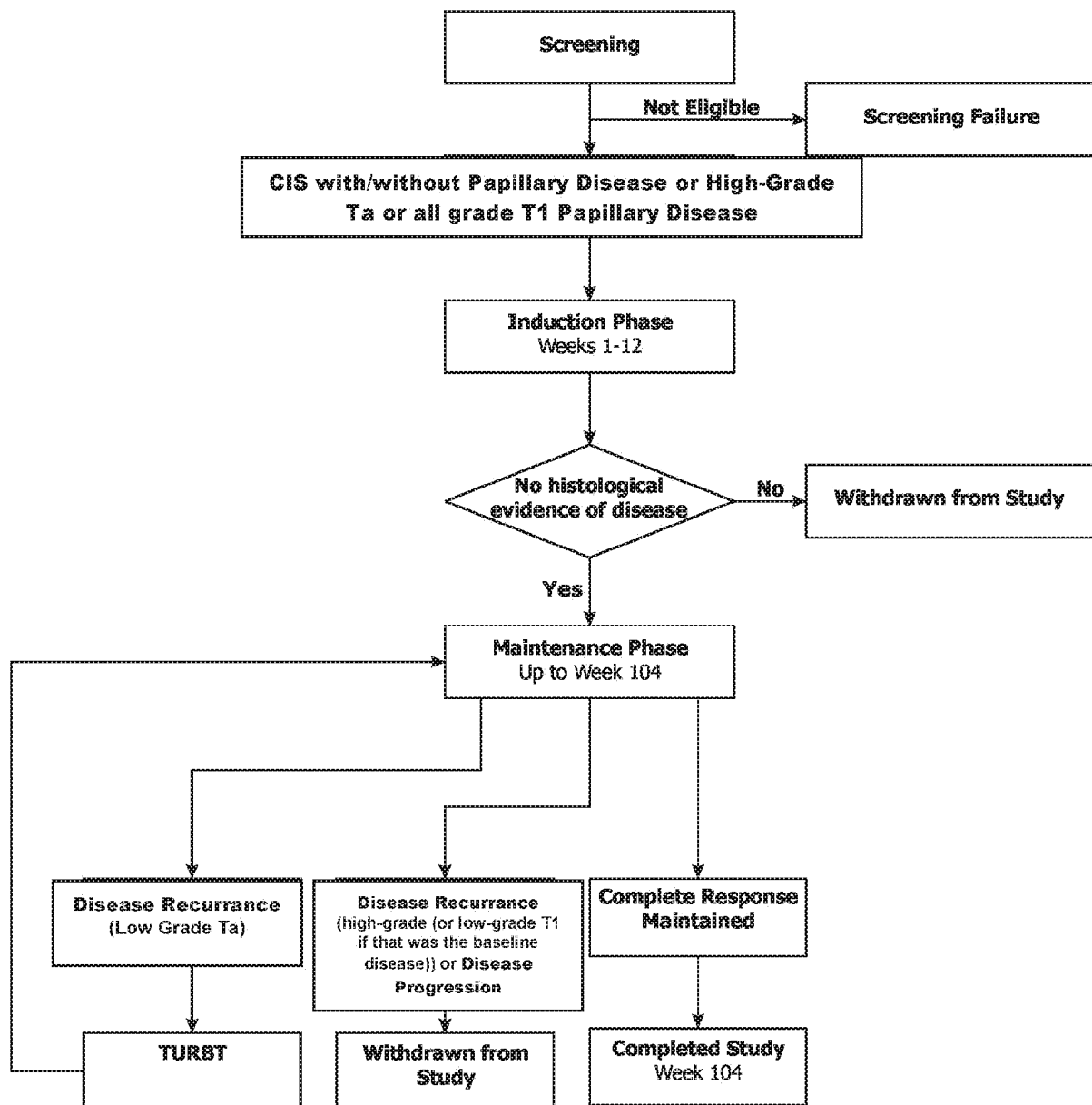


Figure 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/022085

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; A61K 47/48; A61K 49/16; C07K 14/21 (2016.01)

CPC - A61K 47/48569; A61K 47/48715; C07K 16/30; C07K 2317/622 (2016.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 39/395; A61K 47/48; A61K 49/16; C07K 14/21 (2016.01)

CPC - A61K 47/48569; A61K 47/48715; C07K 16/30; C07K 2317/622 (2016.05)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/178.1; 435/7.23; 530/391.7 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar, Google, PubMed.

Search terms used: EPCAM, TACSTD1; bladder; cancer, non-muscle invasive bladder cancer, NMIBC; conjugate, immunoconjugate; dosing regimen; supine, prone, upright, decubitus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/166002 A1 (VIVENTIA BIO INC.) 16 October 2014 (16.10.2014) entire document	95
Y		1-30, 33-80, 83-94, 96-98
Y	US 2014/0193436 A1 (PRUDENT et al) 10 July 2014 (10.07.2014) entire document	1-30, 33-50, 63-70, 87, 88, 94, 98
Y	US 2010/0215670 A1 (CIZEAU et al) 26 August 2010 (26.08.2010) entire document	15
Y	MCKIERNAN et al. "Phase I Trial of Intravesical Docetaxel in the Management of Superficial Bladder Cancer Refractory to Standard Intravesical Therapy," 01 July 2006 (01.07.2006), J Clin Oncol, Vol. 24, Pgs. 3075-3080. entire document	29, 79
Y	KOWALSKI et al. "A Phase I study of an intravesically administered immunotoxin targeting EpCAM for the treatment of nonmuscle-invasive bladder cancer in BCG-refractory and BCG-intolerant patients," Drug Design, Development and Therapy, 13 November 2010 (13.11.2010), Vol. 4, Pgs. 313-320, entire document	30, 33-36, 46, 51-80, 83-93, 96, 97
Y	KOWALSKI et al. "A Phase II Study of Oportuzumab Monatox: An Immunotoxin Therapy for Patients with Noninvasive Urothelial Carcinoma In Situ Previously Treated with Bacillus Calmette-Guérin," J Urol. 19 September 2012 (19.09.2012), Vol. 188, Pgs. 1712-1718. entire document	37, 38, 40
A	US 2004/0022726 A1 (GOLDENBERG et al) 05 February 2004 (05.02.2004) entire document	81, 82

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 May 2016

Date of mailing of the international search report

10 JUN 2016

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/022085

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-14 were searched.