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(54) Title: COMBINATION THERAPY FOR TREATING BLADDER CANCER

(57) Abstract: The present specification provides compositions comprising a photosensitizing agent and an indoloquinone compound, kits comprising these compositions, and methods of treating bladder cancer using such compositions and kits.



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Combination Therapy for Treating Bladder Cancer
Guru Reddy

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Patent Application No. 61/641,421 filed May 2, 2012. The contents of this application is incorporated by reference herein in its entirety.

INTRODUCTION

[002] Bladder cancer is the seventh most common cancer worldwide. In 2006, there were an estimated 280,000 cases of bladder cancer in Europe and more than 60,000 new cases occurred in the United States.

[003] The most common type of bladder cancer (about 90%) is transitional cell carcinoma (TCC) which derives from the urothelium, the cellular lining of the urethral system (ureters, bladder and urethra). Transitional cell carcinoma (TCC) can be classified as either superficial (pTa and pT1), meaning that tumor involvement is limited to the mucosal or submucosal layer of the urothelium, or muscle invasive ($\geq$ pT2). About 75% of newly detected bladder cancers are superficial at initial presentation, i.e., without muscle invasion. More specifically, superficial transitional cell carcinomas consist of papillary tumors that are confined to the mucosa (Ta), papillary or sessile tumors extending into the lamina propria (T1) and carcinoma in situ (CIS).

[004] Superficial bladder cancers can be stratified into prognostic risk classes according to tumor stage, grade, size, number, and recurrence pattern. Low-stage, low-grade primary tumors (stage Ta, grades G1-G2) have a 30% recurrence rate over 2 years and do not usually progress to muscle invasion, while at the other extreme, multiple, highly recurrent or large T1 G3 tumors have up to a 70%-80% recurrence rate and a 10%-30% progression rate to a muscle-invasive stage. CIS presents the highest risk of tumor progression.

[005] Management of superficial bladder cancer may be achieved by transurethral resection, an endoscopic surgical removal of all visible lesions. Transurethral resection of bladder tumor (TUR-BT) is often followed by a course of adjuvant intravesical chemotherapy or immunotherapy with the aim of both eradicating remaining tumor cells and preventing tumor recurrence. See, e.g., Herr, H.W., Intravesical therapy—a critical review, Urol. Clin. N. Am. 14:

399-404 (1987). The validity of this treatment is supported by the significant reduction in superficial tumor recurrence observed following adjuvant chemotherapy, when compared to TUR-BT alone. Although anti-neoplastics (Mitomycin C [MMC], epirubicin and thioTEPA) and immunotherapy (Bacillus-Calmette-Guerin; "BCG") administered intravesically are effective at reducing tumor recurrence rates, it is unclear whether disease progression to muscle invasive tumors is prevented. See, e.g., Newling, D., Intravesical therapy in the management of superficial transitional cell carcinoma of the bladder: the experience of the EORTC GU group, *Br. J. Cancer* 61: 497-499 (1990); Oosterlink, et al., A prospective European Organization for Research and Treatment of Cancer Genitourinary Group randomized trial comparing transurethral resection followed by a single instillation of epirubicin or water in single stage Ta, T1 papillary carcinoma of the bladder, *J. Urol.* 149: 749-752 (1993). This observation in conjunction with the fact that mortality from bladder cancer is still high underscores the need to develop more effective therapeutic agents (Oosterlink et al. 1993). As such, there is a need to develop either more potent and/or less toxic agents against TCC or to use current therapeutics better in terms of targeting treatment to individuals (or pathological subgroups) that are likely to benefit.

[006] Apaziquone (also known as EO9 or EOQUIN[®], Spectrum Pharmaceuticals, Inc.) is a chemotherapeutic agent that it is enzymatically reduced to active metabolites that damage DNA and cause cell death. Apaziquone has been clinically evaluated but despite reports of three partial remissions in phase I clinical trials, no activity was seen against non-small cell lung carcinoma (NSCLC), gastric, breast, pancreatic and colon cancers in subsequent phase II trials. See, e.g., Schellens, J. H. M., et al., Phase I and pharmacologic study of the novel indoloquinone bioreductive alkylating cytotoxic drug EO9, *J. Natl. Cancer Inst.* 86: 906-912 (1994); Dirix, L. Y., et al., EO9 phase II study in advanced breast, gastric, pancreatic and colorectal carcinoma by the early clinical studies group, *Eur. J. Cancer* 32A: 2019-2022 (1996). These findings are particularly disappointing in view of the preclinical studies together with reports that several tumor types have elevated NAD(P)H quinine oxidoreductase (NQO1) levels. Hendriks, H. R., et al., EO9: A novel bioreductive alkylating indoloquinone with preferential solid tumor activity and lack of bone marrow toxicity in preclinical models, *Eur. J. Cancer* 29A: 897-906 (1993); Malkinson, A. M., et al., Elevated NQO1 activity and messenger RNA content in human non small cell lung carcinoma - Relationship to the response of lung tumor xenografts to MMC, *Cancer Res.* 52: 4752-4757 (1992); Smitskamp-Wilms, E., et al., NQO1 activity in normal and neoplastic human tissues: An indicator of sensitivity to bioreductive agents?, *Br. J. Cancer*

72: 917-921 (1995); Siegel, D., et al., Immunohistochemical detection of NAD(P)H:Quinone oxidoreductase in human lung and lung tumors. Clin. Cancer Res. 4: 2065-2070 (1998). Several possible explanations have been proposed to explain apaziquone's lack of clinical efficacy. Recent studies have demonstrated that the failure of apaziquone in the clinic may not be due to poor pharmacodynamic interactions but may be the result of poor drug delivery to tumors. See, e.g., Phillips, R. M., et al., Evaluation of a novel in vitro assay for assessing drug penetration into avascular regions of tumors, Br. J. Cancer 77: 2112-2119 (1998). The rapid plasma elimination of apaziquone ($t_{1/2}$ = 10 min in humans) in conjunction with poor penetration through multicell layers suggests that apaziquone will not penetrate more than a few microns from a blood vessel within its pharmacokinetic lifespan (Schellens et al, 1994, Phillips et al, 1998). Intratumoural administration of apaziquone to NQO1 rich and deficient tumors produced significant growth delays (although a distinction between damage to the aerobic or hypoxic fraction was not determined) suggesting that if apaziquone can be delivered to tumors, therapeutic effects may be achieved. See, e.g., Cummings, J., et al., Pharmacological and biochemical determinants of the antitumour activity of the indoloquinone EO9, Biochem. Pharmacol. 55: 253-260 (1998). While these undesirable characteristics are a serious setback for the treatment of systemic disease, paradoxically they may be advantageous for treating cancers which arise in a third compartment such as superficial bladder cancer. In this scenario, drug delivery is not problematical via the intravesical route and the penetration of apaziquone into avascular tissue can be increased by maintenance of therapeutically relevant drug concentrations within the bladder (using a one hour instillation period for example).

[007] Hexyl aminolevulinate (such as, e.g., CYSVIEW[®]/HEXVIX[®], PhotoCure, ASA) is a photosensitizing agent used to enhance detection of non-muscle invasive urinary bladder cancer (NMIBC). While inclusion of hexyl aminolevulinate results in superior florescent cytосcopy and detection, whether residual hexyl aminolevulinate adversely affects anticancer activity of subsequent chemotherapy treatment is unclear. To better understand possible negative effects of hexyl aminolevulinate on apaziquone activity, anticancer activity of apaziquone was evaluated in a panel of human transitional cell bladder carcinoma cell lines alone and following pretreatment with hexyl aminolevulinate. Unexpectedly, these studies revealed that hexyl aminolevulinate has antitumor activity and there appears to be synergistic interaction between hexyl aminolevulinate and apaziquone.

[008] Thus, the present specification is directed to compositions and methods for treating bladder cancer.

SUMMARY

[009] Aspects of the present specification disclose methods of treating a bladder cancer in an individual in need thereof, the method comprising: administering to the individual a pharmaceutical preparation including a photosensitizing agent and a pharmaceutical preparation including an indoloquinone compound. In some aspects of the disclosed methods, a pharmaceutical preparation including a photosensitizing agent may be administered systemically or locally. In some aspects of the disclosed methods, a pharmaceutical preparation including an indoloquinone compound may be administered systemically or locally. In some aspects of the disclosed methods, a photosensitizing agent is a hexyl aminolevulinate. In some aspects of the disclosed methods, an indoloquinone compound is an apaziquone. In some aspects of the disclosed methods, administration is for a time sufficient to treat the bladder cancer. In some aspects of the disclosed methods, administration reduces a symptom associated with the bladder cancer and is indicative of treating the bladder cancer. In some aspects of the disclosed methods, administration of a pharmaceutical preparation including a photosensitizing agent and a pharmaceutical preparation including an indoloquinone compound is simultaneous. In some aspects of the disclosed methods, administration of a pharmaceutical preparation including a photosensitizing agent and a pharmaceutical preparation including an indoloquinone compound is sequential. In embodiments “sequential” or “sequentially” can mean immediately following or following within a certain time period, such as within a time interval of 1 minute, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, or the like.

[010] Other aspects of the present specification disclose an indoloquinone compound and a photosensitizing agent in combination for uses in the treatment of a bladder cancer. In some aspects of the disclosed combinations, an indoloquinone compound and a photosensitizing agent are separate pharmaceutical preparations. In some aspects of the disclosed combinations, an indoloquinone compound is simultaneously or sequentially administered with a photosensitizing agent. In some aspects of the disclosed combinations, a photosensitizing agent is a hexyl aminolevulinate. In some aspects of the disclosed combinations, an indoloquinone compound is an apaziquone.

[011] Yet other aspects of the present specification disclose uses of an indoloquinone compound in the manufacture of a medicament for the treatment of a bladder cancer by simultaneous and/or sequential administration with a pharmaceutical preparation including a photosensitizing agent. In some aspects of the disclosed uses, a photosensitizing agent is a hexyl aminolevulinate. In some aspects of the disclosed uses, an indoloquinone compound is an apaziquone.

[012] Still other aspects of the present specification disclose kits comprising a composition comprising an indoloquinone compound, a composition comprising a photosensitizing agent, and a container. In some aspects of the disclosed kits, an indoloquinone compound and a photosensitizing agent are separate pharmaceutical preparations. In some aspects of the disclosed kits, a photosensitizing agent is a hexyl aminolevulinate. In some aspects of the disclosed kits, an indoloquinone compound is an apaziquone.

DETAILED DESCRIPTION

[013] Photodynamic therapy (PDT) is used clinically to treat a wide range of medical conditions, including wet age-related macular degeneration and malignant cancers. PDT applications typically involve three key components: a photosensitizing agent, a light source, and tissue oxygen. The basis of PDT involves the application of light of a certain wavelength that excites a photosensitizing agent which generates an energy transfer and production of reactive oxygen species. The combination of these three components leads to the chemical destruction of any tissues having both taken up the photosensitizing agent and been exposed to light energy of the appropriate wavelength.

[014] Of the many photosensitizing agents that have been used in PDT, each has its own unique excitation properties. Usually, the photosensitizing agent is excited from a ground singlet state to an excited singlet state. It then undergoes intersystem crossing to a longer-lived excited triplet state. However, an important factor in the successful use of PDT is that light is needed to activate a photosensitizing agent. This factor, more than any other, limited the development of PDT because most wavelengths of light can not penetrate through more than 1 cm of tissue using standard laser technology and low powered LED technology.

[015] In order to achieve the selective destruction of the target area using PDT while leaving normal tissues untouched, either the photosensitizing agent can be applied locally to the target area or photosensitive targets can be locally excited with light. For instance, in the treatment of skin conditions, including acne, psoriasis, and skin cancers, the photosensitizing agent can be applied topically and locally excited by a light source. In the local treatment of internal tissues and cancers, after a photosensitizing agent has been administered intravenously, light can be delivered to the target area using a medical device, such as, e.g., an endoscope or fiber optic catheter.

[016] Compared to normal tissues, most types of cancers are especially active in both the uptake and accumulation of a photosensitizing agent, which makes cancers especially vulnerable to PDT. Since a photosensitizing agent can also have a high affinity for vascular endothelial cells, PDT can be targeted to the blood carrying vasculature that supplies nutrients to tumors, increasing further the destruction of tumors.

[017] Although a wide array of photosensitizing agents for PDT exist, they all aim to achieve certain characteristics including, without limitation, high absorption at long wavelengths (about 700 nm to about 850 nm) to enable deeper tissue penetration and treatment of larger tumors, high singlet oxygen quantum yield, high chemical stability and/or low photobleaching to enable longer treatment duration, low dark toxicity and/or preferential targeting and/or uptake in target tissue to reduce unwanted side effects or harm to non-targeted tissues. The major difference between different types of photosensitizing agents can be in the parts of the cell that each targets. Unlike radiation therapy, where tumor cell damage is done by targeting cell DNA, most photosensitizing agents target other cell structures, such as, e.g., the nuclear envelope (talaporfin), mitochondria (ALA), or lysosomes (methylene blue).

[018] Photosensitizing agents may be divided into ones based from porphyrin structure, chlorophyll structure or light absorbing dyes. Non-limiting examples of photosensitizing agents include violanthrone, isoviolanthrone, fluoresceine, rubrene, 9,10-diphenylanthracene, tetracene, 13,13'-dibenzantronile, and levulinic acid like an aminolevulinic acid (ALA) like hexyl aminolevulinate (5-ALA hexylester), an azadipyrromethene, methoxsalen (9-methoxy-7H-furo[3,2-g]chromen-7-one), porfimer sodium, psoralen, silicon phthalocyanine Pc 4, talaporfin (N-[(2S,3S)-7-carboxy-3-(2-carboxyethyl)-12-ethyl-2,8,13,18-tetramethyl-17-vinyl-2,3-dihydroporphyrin-5-yl]acetyl}-L-aspartic acid), temoporfin (3,3',3'',3'''-(2,3-dihydroporphyrin-

5,10,15,20-tetrayl)tetraphenol), and verteporfin (3-[(23S,24R)-14-ethenyl-5-(3-methoxy-3-oxopropyl)-22,23-bis(methoxycarbonyl)-4,10,15,24-tetramethyl-25,26,27,28-tetraazahexacyclo[16.6.1.13,6.18,11.113,16.019,24]octacosa-1,3,5,7,9, 11(27),12,14,16,18(25),19,21-dodecaen-9-yl]propanoic acid). Several photosensitizers are commercially available for clinical use, such as, e.g., ALLUMERA® (PhotoCure, ASA), PHOTOFRIN®, (Pinnacle Biologics, Inc.), VISUDYNE® (QLT Ophthalmics, Inc.), LEVULAN® (DUSA Pharmaceuticals, Inc.), FOSCAN® (Scotia Pharmaceuticals), METVIX® (methyl aminolevulinate; PhotoCure, ASA), CYSVIEW®/HEXVIX® (PhotoCure, ASA), and APTOCINE®/LASERPHYRIN® (Light Sciences Oncology), while others are currently in development, such as, e.g., AMPHINEX® (PCI Biotech), ANTRIN® (Pharmacyclics, Inc.), AMELUZ® (Biofrontera, AG), CEVIRA® (PhotoCure, ASA), LUMACAN® (PhotoCure, ASA), PHOTOCHLOR® (2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-a; Photodynamic Therapy Center), PHOTSENS, PHOTREX® (Miravant Medical Tech), and VISONAC® (PhotoCure, ASA).

[019] Aspects of the present specification disclose a pharmaceutical preparation comprising a photosensitizing agent disclosed herein. In certain aspects, a pharmaceutical preparation disclosed herein further comprises a coating agent. The coating agents disclosed herein provide better adhesion of the composition to the bladder wall. Consequently, the preparation and, in particular, the photosensitizing agent contacts and may be able to penetrate the avascular tissue that comprises for a time sufficient to treat the bladder cancer. In one embodiment, the coating agent is propylene glycol. In other embodiments, the coating agent can be hydroxypropylcellulose, carboxymethylcellulose, chitosan hydrochloride, lectin, or polycarbophil.

[020] Aspects of the present specification disclose a pharmaceutical preparation that is a lyophilized preparation of a photosensitizing agent disclosed herein. As those skilled in the art will appreciate, the compositions can be lyophilized by those methods known or developed in the art. Dosage amounts may vary due to several factors including, but not limited to, characteristics of the individual, type and/or stage of cancer, and/or the specific therapeutic composition administered.

[021] In one embodiment, a pharmaceutical preparation disclosed herein comprises a photosensitizing agent disclosed herein. In aspects of this embodiment, a pharmaceutical preparation disclosed herein comprises about 30 mg, about 40 mg, about 50 mg, about 60 mg,

about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, or about 150 mg of a photosensitizing agent. In other aspects of this embodiment, a pharmaceutical preparation disclosed herein comprises at least 30 mg, at least 40 mg, at least 50 mg, at least 60 mg, at least 70 mg, at least 80 mg, at least 90 mg, at least 100 mg, at least 110 mg, at least 120 mg, at least 130 mg, at least 140 mg, or at least 150 mg of a photosensitizing agent. In yet other aspects of these embodiment, a pharmaceutical preparation disclosed herein comprises about 10 mg to about 150 mg, about 30 mg to about 150 mg, about 50 mg to about 150 mg, about 70 mg to about 150 mg, about 30 mg to about 130 mg, about 30 mg to about 110 mg, about 30 mg to about 90 mg, about 50 mg to about 110 mg, or about 60 mg to about 100 mg of a photosensitizing agent.

[022] In other aspects of this embodiment, a pharmaceutical preparation disclosed herein comprises about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, or about 150 mg of a hexyl aminolevulinate. In yet other aspects of this embodiment, a pharmaceutical preparation disclosed herein comprises at least 30 mg, at least 40 mg, at least 50 mg, at least 60 mg, at least 70 mg, at least 80 mg, at least 90 mg, at least 100 mg, at least 110 mg, at least 120 mg, at least 130 mg, at least 140 mg, or at least 150 mg of a hexyl aminolevulinate. In still other aspects of these embodiment, a pharmaceutical preparation disclosed herein comprises about 10 mg to about 150 mg, about 30 mg to about 150 mg, about 50 mg to about 150 mg, about 70 mg to about 150 mg, about 30 mg to about 130 mg, about 30 mg to about 110 mg, about 30 mg to about 90 mg, about 50 mg to about 110 mg, or about 60 mg to about 100 mg of a hexyl aminolevulinate.

[023] In another embodiment, a pharmaceutical preparation disclosed herein is a liquid useful for injection into an individual. In aspects of this embodiment, a pharmaceutical preparation comprises a photosensitizing agent disclosed herein and water. In another embodiment, a pharmaceutical preparation disclosed herein comprises a photosensitizing agent disclosed herein and a buffered solution. In aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, or about 15 mM of a photosensitizing agent. In other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises at least 1 mM, at least 2 mM, at least 3 mM, at least 4 mM, at least 5 mM, at least 6

mM, at least 7 mM, at least 8 mM, at least 9 mM, at least 10 mM, at least 11 mM, at least 12 mM, at least 13 mM, at least 14 mM, or at least 15 mM of a photosensitizing agent. In yet other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 1 mM to about 15 mM, about 3 mM to about 15 mM, about 5 mM to about 15 mM, about 7 mM to about 15 mM, about 3 mM to about 13 mM, about 3 mM to about 11 mM, about 3 mM to about 9 mM, about 5 mM to about 11 mM, or about 6 mM to about 10 mM of a photosensitizing agent.

[024] In other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, or about 15 mM of a hexyl aminolevulinate. In yet other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises at least 1 mM, at least 2 mM, at least 3 mM, at least 4 mM, at least 5 mM, at least 6 mM, at least 7 mM, at least 8 mM, at least 9 mM, at least 10 mM, at least 11 mM, at least 12 mM, at least 13 mM, at least 14 mM, or at least 15 mM of a hexyl aminolevulinate. In still other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 1 mM to about 15 mM, about 3 mM to about 15 mM, about 5 mM to about 15 mM, about 7 mM to about 15 mM, about 3 mM to about 13 mM, about 3 mM to about 11 mM, about 3 mM to about 9 mM, about 5 mM to about 11 mM, or about 6 mM to about 10 mM of a hexyl aminolevulinate.

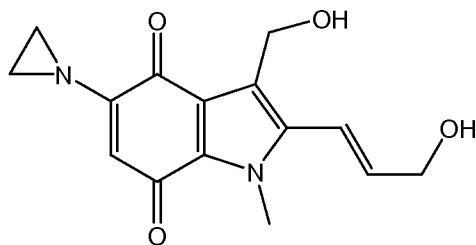
[025] In another embodiment, a pharmaceutical preparation disclosed herein is a liquid useful for intravesical instillation into the bladder of an individual. In aspects of this embodiment, pharmaceutical preparation comprises a photosensitizing agent disclosed herein, propylene glycol, and water or a buffered solution. In aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 110 mM, about 120 mM, about 130 mM, about 140 mM, or about 150 mM of a photosensitizing agent. In aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, at least 90 mM, at least 100 mM, at least 110 mM, at least 120 mM, at least 130 mM, at least 140 mM, or at least 150 mM of a photosensitizing agent. In yet other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 10 mM to about 150 mM, about

30 mM to about 150 mM, about 50 mM to about 150 mM, about 70 mM to about 150 mM, about 30 mM to about 130 mM, about 30 mM to about 110 mM, about 30 mM to about 90 mM, about 50 mM to about 110 mM, or about 60 mM to about 100 mM of a photosensitizing agent.

[026] In other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 110 mM, about 120 mM, about 130 mM, about 140 mM, or about 150 mM of a hexyl aminolevulinate. In yet aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, at least 90 mM, at least 100 mM, at least 110 mM, at least 120 mM, at least 130 mM, at least 140 mM, or at least 150 mM of a hexyl aminolevulinate. In still other aspects of these embodiment, a liquid pharmaceutical preparation disclosed herein comprises about 10 mM to about 150 mM, about 30 mM to about 150 mM, about 50 mM to about 150 mM, about 70 mM to about 150 mM, about 30 mM to about 130 mM, about 30 mM to about 110 mM, about 30 mM to about 90 mM, about 50 mM to about 110 mM, or about 60 mM to about 100 mM of a hexyl aminolevulinate.

[027] Indoloquinone compounds, include, without limitation, apaziquone. Apaziquone, also known as EO9 or NSC-382459, is a fully synthetic bioreductive alkylating indoloquinone. It is a pro-drug that generates cytotoxic species after enzymatic activation. The enzyme DTD (DT-diaphorase, also called NAD(P)H:quinone oxidoreductase-1, or NQO1) plays a prominent role in the activation of apaziquone under aerobic conditions. Apaziquone is also cytotoxic under hypoxic conditions, such as in cells with low DTD activity. The basic mechanism of activation of apaziquone is believed to be similar to that of other indoloquinones, involving reduction by cellular enzymes that transfer one or two electrons, forming semiquinone and hydroquinone, respectively. Oxidation of the semiquinone under aerobic conditions results in a redox cycle that can cause cell death by forming reactive oxygen species (ROS), resulting in DNA strand breaks. The semiquinone/hydroquinone can, particularly under hypoxic conditions, alkylate and crosslink DNA and other macromolecules, causing cell death.

[028] The chemical name for apaziquone is 5-(aziridin-1-yl)-3-(hydroxymethyl)-2-[(1E)-3-hydroxyprop-1-enyl]-1-methyl-1H-indole-4,7-dione, and this compound has the following structural formula:



[029] Aspects of the present specification disclose a pharmaceutical preparation comprising an indoloquinone composition disclosed herein. In certain aspects, a pharmaceutical preparation disclosed herein further comprises a coating agent disclosed herein. The coating agents disclosed herein provide better adhesion of the composition to the bladder wall. Consequently, the preparation and, in particular, the indoloquinone compound contacts and may be able to penetrate the avascular tissue for a time sufficient to treat the bladder cancer. In one embodiment, the coating agent is propylene glycol. In other embodiments, the coating agent can be hydroxypropylcellulose, carboxymethylcellulose, chitosan hydrochloride, lectin, or polycarbophil.

[030] In one embodiment, a liquid pharmaceutical preparation disclosed herein comprises apaziquone, propylene glycol, and water. Apaziquone concentrations can be present in a range from about 300 μ M to about 400 μ M. Propylene glycol concentrations can be present in a range from about 6% (v/v) to about 34% (v/v). In another embodiment, a pharmaceutical preparation comprises apaziquone and propylene glycol, wherein the concentration of propylene glycol is a range of about 6% (v/v) to about 14% (v/v), about 16% (v/v) to about 24% (v/v), or about 26% (v/v) to about 34% (v/v). In yet another embodiment, a pharmaceutical preparation comprises apaziquone and propylene glycol, wherein the concentration of propylene glycol is about 30% (v/v), about 20% (v/v), or about 10% (v/v). In still another embodiment, the preparation comprises about 347 μ M apaziquone. In another embodiment, a preparation comprises about 0.025 mg/mL to about 0.25 mg/mL apaziquone. In yet another embodiment, a preparation comprises about 0.1 mg/mL apaziquone.

[031] A pharmaceutical preparation comprising an apaziquone may further comprise sodium bicarbonate (NaHCO_3), disodium edetate (EDTA), and/or mannitol. Sodium bicarbonate can be present in a range from about 0 mg/mL to about 60 mg/mL. Mannitol can be present in a range from about 0 mg/mL to about 3.0 mg/mL. In one embodiment, a preparation comprises from about 1 mg/mL to about 20 mg/mL sodium bicarbonate. In one embodiment, a preparation

comprises from about 2.5 mg/mL to about 10 mg/mL sodium bicarbonate. In another embodiment, a preparation comprises about 5.125 mg/mL sodium bicarbonate. In another embodiment, a preparation comprises about 0.35 mg/mL to about 3 mg/mL mannitol. In another embodiment the preparation comprises 1.25 mg/mL mannitol. In another embodiment, a preparation comprises about 0.625 mg/mL mannitol. In another embodiment, the preparation comprises about 5.125 mg/mL sodium bicarbonate, about 1.25 mg/mL mannitol and about 0.1 mg/mL apaziquone in a solution comprising EDTA, propylene glycol, and water.

[032] In another embodiment, a pharmaceutical preparation comprises an apaziquone, sodium bicarbonate and mannitol in a solution comprising propylene glycol, EDTA and water wherein the propylene glycol is present in a concentration range of about 6% (v/v) to about 14% (v/v), about 16% (v/v) to about 24% (v/v), or about 26% (v/v) to about 34% (v/v). In another embodiment, the propylene glycol is present in a concentration of about 10% (v/v), about 20% (v/v), or about 30% (v/v). In another embodiment, the preparation comprises about 300 μ M to about 400 μ M apaziquone and about a 10% (v/v) propylene glycol. In yet another embodiment, the preparation comprises about 300 μ M to about 400 μ M apaziquone and about 20% (v/v) propylene glycol. In a further embodiment, the preparation comprises about 300 μ M to about 400 μ M apaziquone and about a 30% (v/v) propylene glycol. In yet another embodiment, the preparation comprises about 347 μ M apaziquone and about 30% (v/v) propylene glycol. These described embodiments can comprise about 0 mg/mL to about 60 mg/mL sodium bicarbonate and in particular embodiments will comprise about 1 mg/mL to about 20 mg/mL sodium bicarbonate, about 2.5 mg/mL to about 10 mg/mL sodium bicarbonate, or about 5.125 mg/mL sodium bicarbonate. These described embodiments can also comprise about 0.5 mg/mL to about 3.0 mg/mL mannitol and in particular embodiments will comprise about 0.625 mg/mL mannitol or about 1.25 mg/mL mannitol.

[033] In one embodiment, a pharmaceutical preparation comprises about 347 μ M apaziquone, about 30% (v/v) propylene glycol, about 5.125 mg/mL sodium bicarbonate, about 1.25 mg/mL mannitol, about 0.1 mg/mL sodium edentate, and water. In another embodiment, a pharmaceutical preparation comprises about 347 μ M apaziquone, about 20% (v/v) propylene glycol, about 5.125 mg/mL sodium bicarbonate, about 1.25 mg/mL mannitol, about 0.1 mg/mL sodium edentate, and water. In another embodiment, a pharmaceutical preparation comprises about 347 μ M apaziquone, about 10% (v/v) propylene glycol, about 5.125 mg/mL sodium bicarbonate, about 1.25 mg/mL mannitol, about 0.1 mg/mL sodium edentate, and water.

[034] Aspects of the present specification disclose a pharmaceutical preparation that is a lyophilized preparation of an indoloquinone compound disclosed herein. As those skilled in the art will appreciate, the compositions can be lyophilized by those methods known or developed in the art. In one embodiment, a lyophilized formulation comprises about 1 mg/mL to about 8 mg of apaziquone, about 2 mg to about 30 mg sodium bicarbonate, and about 10 to about 60 mg mannitol. In one embodiment, a lyophilized preparation comprises about 2 mg/mL to about 6 mg of apaziquone, about 5 mg to about 15 mg sodium bicarbonate, and about 20 to about 40 mg mannitol. In another embodiment, a lyophilized preparation comprises about 4 mg of apaziquone, about 5 mg sodium bicarbonate, and about 50 mg mannitol. Dosage amounts may vary due to several factors including, but not limited to, characteristics of the individual, type and/or stage of cancer, and/or the specific therapeutic composition administered.

[035] A lyophilized preparation described herein may be reconstituted with any pharmaceutically acceptable diluent to produce a pharmaceutical preparation as disclosed herein. A reconstitution vehicle may comprise propylene glycol and water. A reconstitution vehicle disclosed herein dissolves the lyophilized disclosed herein and produces a stable solution for administration for up to 24 hours. Propylene glycol concentrations can be present in a range from about 0% (v/v) to about 60% (v/v). A reconstitution vehicle disclosed herein may further comprise sodium bicarbonate and disodium edetate. Sodium bicarbonate can be present in a range from about 0 mg/mL to about 60 mg/mL. EDTA concentrations can be present in a range from about 0 mg/mL to about 5 mg/mL. In one embodiment, a reconstitution vehicle disclosed herein comprises about 20% (v/v) to about 40% (v/v) propylene glycol, about 1 mg/mL to about 5 mg/mL sodium bicarbonate, about 0.01 mg/mL to about 1 mg/mL EDTA, and water. In one embodiment, a reconstitution vehicle comprises about 60% (v/v) propylene glycol, about 5 mg/mL sodium bicarbonate, about 0.2 mg/mL disodium edentate and water. In another embodiment, a reconstitution vehicle comprises about 40% (v/v) propylene glycol, about 5 mg/mL sodium bicarbonate, about 0.2 mg/mL disodium edentate and water. In yet another embodiment, a reconstitution vehicle comprises about 20% (v/v) propylene glycol, about 5 mg/mL sodium bicarbonate, about 0.2 mg/mL disodium edentate and water.

[036] One type of pharmaceutical preparation is a reconstituted lyophilized preparation. In one embodiment, a reconstituted lyophilized preparation comprises a photosensitizing agent disclosed herein. In another embodiment, a reconstituted lyophilized preparation comprises an

indoloquinone compound disclosed herein. Such a preparation is formed upon reconstitution of the lyophilized preparation disclosed herein with a reconstitution vehicle disclosed herein.

[037] A reconstituted lyophilized preparation disclosed herein can then be optionally diluted to a desired concentration and administered to an individual. In one embodiment, the final concentration of indoloquinone compound is in a range of about 300 μ M to about 400 μ M and the final concentration of propylene glycol is in a range from about 6% (v/v) to about 34% (v/v). In one embodiment, a reconstituted lyophilized preparation disclosed herein comprises about 347 μ M apaziquone, about 30% (v/v) propylene glycol, about 5.125 mg/mL sodium bicarbonate, about 1.25 mg/mL mannitol, about 0.1 mg/mL sodium edentate, and water.

[038] Aspects of the present specification provide, in part, a pharmaceutical kit. A pharmaceutical kit disclosed herein comprises at least one of the compositions disclosed herein. Additionally such kits contain instructions providing guidance on the administration of the disclosed compositions as disclosed herein. The components of the kit may be contained in a single container.

[039] In one embodiment, a pharmaceutical kit is provided comprising a) a pharmaceutical preparation comprising an indoloquinone composition disclosed herein; and b) instructions for administration the pharmaceutical preparation. In an aspect of this embodiment, a pharmaceutical kit is provided comprising a) a pharmaceutical preparation comprising an indoloquinone composition disclosed herein; b) a pharmaceutical preparation comprising a photosensitizing agent disclosed herein; and c) instructions for administration the pharmaceutical preparations.

[040] In another embodiment, a pharmaceutical kit is provided comprising a) a pharmaceutical preparation comprising an apaziquone composition disclosed herein; and b) instructions for administration the pharmaceutical preparation. In an aspect of this embodiment, a pharmaceutical kit is provided comprising a) a pharmaceutical preparation comprising an apaziquone composition disclosed herein; b) a pharmaceutical preparation comprising a hexyl aminolevulinate disclosed herein; and c) instructions for administration the pharmaceutical preparations.

[041] Aspects of the present specification disclose methods of treating a bladder cancer. In one embodiment, the method comprises the step of administering a pharmaceutical preparation comprising a photosensitizing agent disclosed herein to an individual, and administering a pharmaceutical preparation comprising an indoloquinone compound disclosed herein to the individual. In an aspect of this embodiment, the administration of the pharmaceutical preparations is for a time sufficient to treat a bladder cancer. In another aspect of this embodiment, the administration of the pharmaceutical preparations reduces a symptom associated with the bladder cancer. In certain aspects the symptom reduced is at least one of hematuria, dysuria, urinary tract infections, pain, weight loss, anemia, frequent urination, or the like.

[042] In yet another aspect of this embodiment, the administration of the pharmaceutical preparations occurs after transurethral resection of bladder tumor (TUR-BT). In other aspects of this embodiment, administration of the pharmaceutical preparation comprising a photosensitizing agent disclosed herein may be intravenously or by intravesical instillation to the bladder of the individual. In other aspects, of this embodiment, administration of the pharmaceutical preparation comprising an indoloquinone compound disclosed herein may be by intravesical instillation to the bladder of the individual.

[043] In another embodiment, the method comprises the step of administering a pharmaceutical preparation comprising a photosensitizing agent disclosed herein intravenously to an individual and administering a pharmaceutical preparation comprising an indoloquinone compound disclosed herein by intravesical instillation to the bladder of the individual. In an aspect of this embodiment, the administration of the pharmaceutical preparations is for a time sufficient to treat a bladder cancer. In another aspect of this embodiment, the administration of the pharmaceutical preparations reduces a symptom associated with the bladder cancer. In yet another aspect of this embodiment, the administration of the pharmaceutical preparations occurs after TUR-BT.

[044] In another embodiment, a method of treating a bladder cancer comprising the steps of performing TUR-BT in an individual in need thereof and administering a pharmaceutical preparation comprising a photosensitizing agent disclosed herein to an individual, and administering a pharmaceutical preparation comprising an indoloquinone compound disclosed herein to the individual. In aspects of this embodiment, administration of the pharmaceutical preparation comprising a photosensitizing agent disclosed herein may be intravenously or by

intravesical instillation to the bladder of the individual. In other aspects of this embodiment, administration of the pharmaceutical preparation comprising an indoloquinone compound disclosed herein may be by intravesical instillation to the bladder of the individual. In aspects of this embodiment, a pharmaceutical preparation is administered within about 6 hours following TUR-BT, within about 5 hours following TUR-BT, within about 4 hours following TUR-BT, within about 3 hours following TUR-BT, within about 2 hours following TUR-BT, or within about 1 hour following TUR-BT.

[045] In yet another embodiment, a method of treating a bladder cancer disclosed herein is performed in conjunction with a photodynamic therapy.

[046] In still another embodiment, a method of treating a bladder cancer disclosed herein is not performed in conjunction with a photodynamic therapy. As such, an individual being treated for a bladder cancer using a method disclosed herein may not also be treated using a photodynamic therapy.

[047] Aspects of the present specification disclose, in part, treating an individual suffering from a bladder cancer. As used herein, the term “treating,” refers to reducing or eliminating in an individual a clinical symptom of a bladder cancer; or delaying or preventing in an individual the onset of a clinical symptom of a bladder cancer. For example, the term “treating” can mean reducing a symptom of a condition characterized by a bladder cancer by, e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. The actual symptoms associated with a bladder cancer are well known and can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the location of the bladder cancer, the cause of the bladder cancer, and/or the severity of the bladder cancer. Those of skill in the art will know the appropriate symptoms or indicators associated with a specific bladder cancer and will know how to determine if an individual is a candidate for treatment as disclosed herein.

[048] In one embodiment, the present methods are used for the treatment of a non-invasive bladder cancer (SBCs). In another embodiment, the present methods are used for the treatment of a transitional-cell carcinoma of the bladder. In another embodiment, the present methods are used for the treatment of a bladder cancer that is a TNM stage Ta or T1 and/or a histologic grade G1 or G2.

[049] A pharmaceutical preparation is administered to an individual. An individual comprises all mammals including a human being. Typically, any individual who is a candidate for a conventional bladder cancer treatment is a candidate for a bladder cancer treatment disclosed herein. Pre-operative evaluation typically includes routine history and physical examination in addition to thorough informed consent disclosing all relevant risks and benefits of the procedure.

[050] With reference to a therapy comprising a pharmaceutical preparation disclosed herein, the amount of a photosensitizing agent disclosed herein and the amount of an indoloquinone compound disclosed herein used with the methods of treatment disclosed herein will typically be an effective amount. As used herein, the term “effective amount” is synonymous with “therapeutically effective amount”, “effective dose”, or “therapeutically effective dose” and when used in reference to treating a bladder cancer means the minimum dose of a photosensitizing agent and/or an indoloquinone compound necessary to achieve the desired therapeutic effect and includes a dose sufficient to reduce a symptom associated with a bladder cancer. An effective amount refers to the total amount of a photosensitizing agent and/or an indoloquinone compound administered to an individual in one setting. The effectiveness of a photosensitizing agent and/or an indoloquinone compound in treating a bladder cancer can be determined by observing an improvement in an individual based upon one or more clinical symptoms, and/or physiological indicators associated with the condition. An improvement in a bladder cancer can also be indicated by the lack of recurrence or relapse or a prolonging the rate of recurrence or relapse.

[051] The appropriate effective amount of a photosensitizing agent and/or an indoloquinone compound to be administered to an individual for a particular bladder cancer can be determined by a person of ordinary skill in the art by taking into account factors, including without limitation the type of bladder cancer, the location of the bladder cancer, the cause of bladder cancer, the severity of the bladder cancer, the degree of relief desired, the duration of relief desired, the particular a photosensitizing agent and/or an indoloquinone compound used, the rate of excretion of the a photosensitizing agent and/or an indoloquinone compound used, the pharmacodynamics of the photosensitizing agent and/or an indoloquinone compound used, the nature of the other compounds to be included in the composition, the particular route of administration, the particular characteristics, history and risk factors of the individual, such as, e.g., age, weight, general health and the like, or any combination thereof. Additionally, where

repeated administration of a composition comprising disclosed herein is used, an effective amount of a photosensitizing agent and/or an indoloquinone compound will further depend upon factors including, without limitation, the frequency of administration, the half-life of a photosensitizing agent and/or an indoloquinone compound used, or any combination thereof. It is known by a person of ordinary skill in the art that an effective amount of a photosensitizing agent and/or an indoloquinone compound can be extrapolated from *in vitro* assays and *in vivo* administration studies using animal models prior to administration to humans.

[052] A pharmaceutical preparation disclosed herein can be administered to an individual using a variety of routes. Routes of administration suitable for a method of treating a bladder cancer as disclosed herein include both local and systemic administration. Local administration results in significantly more delivery of a composition to a specific location as compared to the entire body of the individual, whereas, systemic administration results in delivery of a composition to essentially the entire body of the individual.

[053] The pharmaceutical preparations disclosed herein may be administered to an individual in need of treatment for bladder cancer following TUR-BT. In one embodiment, a pharmaceutical preparation may be administered to an individual via intravesical instillation. In aspects of this embodiment, a pharmaceutical preparation may be administered in a single instillation or a plurality of installations. In another aspect of this embodiment, a pharmaceutical preparation may be administered in a single instillation given within six hours after a TUR-BT. In another aspect, the pharmaceutical preparation may be administered in a single instillation given less than six hours after a TUR-BT. In another embodiment, a pharmaceutical preparation disclosed herein may be administered to an individual intravenously.

[054] In one embodiment, a method of treating a bladder cancer includes administering a volume of a pharmaceutical preparation disclosed herein of between about 2 mL and about 80 mL. In another embodiment, a method of treating a bladder cancer includes administering a volume of reconstituted lyophilized pharmaceutical preparation of between about 30 mL and about 60 mL. In another embodiment, a method of treating a bladder cancer includes administering a volume of reconstituted lyophilized pharmaceutical preparation of about 40 mL. Dosage volumes may vary due to several factors including, but not limited to, characteristics of the individual, type and/or stage of cancer, and/or the specific pharmaceutical preparation administered.

[055] In yet another embodiment, a pharmaceutical preparation disclosed herein can be delivered to the bladder wall by a liposome. According to one embodiment, the liposomes used are unilamellar or multilamellar and contain at least one cationic phospholipid such as stearylamine, 1,2-diacyl-3-trimethylammonium-propane (TAP) or 1,2-triacyl-3-dimethylammonium-propane (DAP). In another embodiment, the surface liposomes may be coated with polyethylene glycol to prolong the circulating half-life of the liposomes. In yet another embodiment, neutrally charged liposomes such as, but not limited to, phosphatidylcholine and cholesterol can also be used for liposomal entrapment of the compositions disclosed herein. In another embodiment, a pharmaceutical preparation can be delivered to the bladder wall by a microsphere such as those known or developed in the art.

[056] "Start of instillation" is the time instillation of a pharmaceutical preparation begins, following TUR-BT, while the "end of retention" is the time the administered pharmaceutical preparation and other bladder contents are drained or voided, i.e. the time at which retention of the drug in the bladder is terminated.

EXAMPLES

[057] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of representative embodiments now contemplated. These examples should not be construed to limit any of the embodiments described in the present specification, including those pertaining to the compounds, pharmaceutical compositions, pharmaceutical kits, or methods of treating bladder cancer.

Example 1

Apaziquone and Hexyl Aminolevulinate Single Agent and Combination Experiments

[058] To evaluate the anticancer activity of hexyl aminolevulinate as well as apaziquone alone and following pretreatment with hexyl aminolevulinate, a series of experiments were performed using a panel of human transitional cell bladder carcinoma cell lines.

[059] Cells from human transitional cell bladder carcinoma cell lines were seeded in 96-well plates at approximately 2×10^4 cells/well and cultured in appropriate growth media supplemented

with FBS for 24 hours before initiating a drug treatment. The cell lines used were 1) RT4, a cell line originally established using cells from a transitional cell bladder carcinoma from a 63 year old male; 2) T24, a cell line originally established using cells from a transitional cell bladder carcinoma from an 81 year old female; and 3) RT112, a cell line originally established using cells from a transitional cell bladder carcinoma from a female of unknown age.

[060] For hexyl aminolevulinate single agent studies, serum-containing media was removed and serum-free media including either 0.5 μ M, 1 μ M, 2 μ M, 4 μ M, 8 μ M or 16 μ M hexyl aminolevulinate was added to cells and incubated for three hours. Control experiments were conducted where cells were exposed only to a vehicle. Following treatment, drugged media was removed and cells washed with serum-containing media or left unwashed and incubated with serum-containing media for 24 hours.

[061] For single agent studies with apaziquone, serum-containing media was replaced with serum-free media and the cells were incubated for three hours. After incubation, the serum was removed and growth media including either 0.0001 μ M, 0.001 μ M, 0.01 μ M, 0.05 μ M, 0.1 μ M, 0.5 μ M, 1 μ M, 5 μ M, or 10 μ M apaziquone was added to cells and incubated for 60 minutes. Control experiments were conducted where cells were exposed only to a vehicle. Following treatment, drugged media was removed and cells washed with serum-containing media and incubated with serum-containing media for 23 hours.

[062] For combination studies, cells were treated with hexyl aminolevulinate as above. Following treatment, drugged media was removed and cells washed with serum-containing media or left unwashed and added with 5 μ M apaziquone for 60 minutes. Control experiments were conducted where cells were exposed only to a vehicle. Following treatment, drugged media was removed and cells washed with serum-containing media and incubated with serum-containing media for 23 hours.

[063] To assess a growth inhibition cell viability was determined using the MTS assay. The MTS assay was performed at the 27 hour post-treatment timepoint in all experiments. This colorimetric procedure measures conversion of the MTS reagent (a tetrazoleum salt) to formazan by living cells. Formazan production is quantified by spectrophotometric measurement at 490 nm and is proportional to viable cell number. Following treatment, 100 μ L of growth medium was removed and cells incubated with 20 μ L CellTiter 96® AQueous One

Solution Reagent (1.9 mg/mL in PBS, pH 6.0) for 1-3 hours at 37°C. Absorbance (OD) values were measured using a μ Quant microplate reader at a single wavelength of 490 nm. Data from each experiment was collected and expressed as a normalized %Maximum Growth Inhibition (Effect) using the following calculation: %Maximum Growth Inhibition = $(1 - (OD_{\text{test}} / OD_{\text{vehicle}})) \times 100$, where OD_{test} is the optical density of the tested sample, and OD_{vehicle} is the optical density of the vehicle in which the drug is dissolved. An IC_{50} value (drug concentration which results in a half-maximal response) was calculated from % Maximum Growth Inhibition values using the formula: $y = \min + (\max - \min) / (1 + 10^{(\log IC_{50} - x) \text{Hillslope}})$, where (x) is the logarithm of agonist concentration, (y) is the response, and (min) and (max) are the variable lower and upper plateaus, respectively. The variable Hillslope characterizes the slope of the curve at its midpoint. The IC_{50} is the drug concentration for y halfway between min and max.

[064] Overall apaziquone and hexyl aminolevulinate reported growth inhibitory activity towards the three evaluated bladder lines with RT4 the most sensitive and RT112 the most resistant to both agents (Table 1). For example, one hour apaziquone treatment was sufficient to inhibit cell growth in the three evaluated lines with IC_{50} value ranges of 1.8-6.7 μ M reported in T24, 1.0-2.8 μ M in RT112 and 0.7-7.6 μ M in RT4 (Table 1). Thus, most IC_{50} values were below 5 μ M, the threshold for translatable activity potential in humans. Maximum percentage inhibition or effect ranged from 47% in RT112 to 86% in RT4 (Table 1). Hexyl aminolevulinate alone for 1 or 3 hours resulted in IC_{50} values ranging from 0.3 μ M in RT4 to 7.7 μ M in the T24 line (Table 1). Thus, single agent hexyl aminolevulinate treatment resulted in IC_{50} values less than 5 μ M in all evaluated lines. Maximum percentage inhibition or effect ranged from 84% in RT112 to 99% in RT4.

Table 1. Apaziquone and Hexyl Aminolevulinate Single Agent and Combination Experiments

Cell Line	Apaziquone		Hexyl Aminolevulinate		Combination	
	Max Effect	IC_{50} Value	Max Effect	IC_{50} Value	Max Effect	IC_{50} Value ¹
T24						
1 hr (Wash)	50%	6.7 μ M	89%	2.5 μ M	100%	0.6 μ M
1 hr (No wash)	62%	1.8 μ M	90%	3.9 μ M	100%	0.3 μ M
3 hr (Wash)	63%	2.0 μ M	90%	1.0 μ M	99%	0.2 μ M
3 hr (No wash)	70%	2.7 μ M	87%	7.7 μ M	99%	0.03 μ M
RT112						
1 hr (Wash)	55%	2.8 μ M	86%	2.8 μ M	96%	2.8 μ M
1 hr (No wash)	47%	1.8 μ M	85%	2.5 μ M	89%	2.5 μ M

3 hr (Wash)	60%	1.8 μ M	86%	1.9 μ M	95%	1.9 μ M
3 hr (No wash)	65%	1.0 μ M	84%	2.6 μ M	84%	2.0 μ M
RT4						
1 hr (Wash)	78%	5.0 μ M	97%	2.8 μ M	100%	0.07 μ M
1 hr (No wash)	73%	7.6 μ M	98%	0.3 μ M	100%	0.05 μ M
3 hr (Wash)	78%	2.9 μ M	99%	2.6 μ M	98%	0.01 μ M
3 hr (No wash)	86%	0.7 μ M	96%	2.8 μ M	96%	0.01 μ M
¹ The IC ₅₀ value is for hexyl aminolevulinate.						

[065] Combination of apaziquone and hexyl aminolevulinate resulted in enhanced growth inhibitory activity and maximum percentage inhibition in each tested line. For example, combination of these agents resulted in IC₅₀ values ranging from 0.01 μ M in RT4 to 2.8 μ M in the Rt112 line (Table 1). Maximum percentage inhibition or effect ranged from 84% in RT112 to 100% in both T24 and RT4 lines. Thus, combination of apaziquone and hexyl aminolevulinate resulted in a synergistic increase in growth inhibition and maximum percentage inhibition. No notable differences were reported with extended hexyl aminolevulinate pretreatment or cell washing between drug treatments.

[066] In closing, it is to be understood that although aspects of the present specification are highlighted by referring to specific embodiments, one skilled in the art will readily appreciate that these disclosed embodiments are only illustrative of the principles of the subject matter disclosed herein. Therefore, it should be understood that the disclosed subject matter is in no way limited to a particular methodology, protocol, and/or reagent, etc., described herein. As such, various modifications or changes to or alternative configurations of the disclosed subject matter can be made in accordance with the teachings herein without departing from the spirit of the present specification. Lastly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Accordingly, the present invention is not limited to that precisely as shown and described.

[067] Certain embodiments of the present invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the present invention to be practiced otherwise than

specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described embodiments in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[068] Groupings of alternative embodiments, elements, or steps of the present invention are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other group members disclosed herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[069] Unless otherwise indicated, all numbers expressing a characteristic, item, quantity, parameter, property, term, and so forth used in the present specification and claims are to be understood as being modified in all instances by the term “about.” As used herein, the term “about” means that the characteristic, item, quantity, parameter, property, or term so qualified encompasses a range of plus or minus ten percent above and below the value of the stated characteristic, item, quantity, parameter, property, or term. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical indication should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and values setting forth the broad scope of the invention are approximations, the numerical ranges and values set forth in the specific examples are reported as precisely as possible. Any numerical range or value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Recitation of numerical ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate numerical value falling within the range. Unless otherwise indicated herein, each individual value of a numerical range is incorporated into the present specification as if it were individually recited herein.

[070] The terms “a,” “an,” “the” and similar referents used in the context of describing the present invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the present invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the present specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[071] Specific embodiments disclosed herein may be further limited in the claims using consisting of or consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term “consisting of” excludes any element, step, or ingredient not specified in the claims. The transition term “consisting essentially of” limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the present invention so claimed are inherently or expressly described and enabled herein.

[072] All patents, patent publications, and other publications referenced and identified in the present specification are individually and expressly incorporated herein by reference in their entirety for the purpose of describing and disclosing, for example, the compositions and methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

CLAIMS

1. A method of treating a bladder cancer in an individual in need thereof, the method comprising: administering by intravesical instillation into the bladder of the individual a photosensitizing agent and an indoloquinone compound.
2. The method of claim 1 wherein the administration is for a time sufficient to treat the bladder cancer.
3. The method of claim 2 wherein the indoloquinone compound comprises an apaziquone.
4. The method of claim 1 wherein reducing a symptom associated with the bladder cancer is indicative of treating the bladder cancer.
5. The method of claim 3 wherein the photosensitizing agent comprises a hexyl aminolevulinate.
6. The method of claim 1 wherein the bladder cancer comprises at least one of non-invasive bladder cancer or transitional-cell carcinoma of the bladder.
7. An indoloquinone compound and a photosensitizing agent in combination for use in the treatment of a bladder cancer.
8. The use of claim 7 wherein the photosensitizing agent comprises a hexyl aminolevulinate.
9. The use of claim 8 wherein the indoloquinone compound comprises an apaziquone.
10. The use of claim 9 wherein the treatment of bladder cancer comprises intravesical instillation into the bladder.
11. An indoloquinone compound for use in the treatment of a bladder cancer by simultaneous or sequential administration with a photosensitizing agent.
12. The use of claim 11 wherein the photosensitizing agent comprises a hexyl aminolevulinate.

13. The use of claim 12 wherein the indoloquinone compound comprises an apaziquone.
14. The use of claim 13 wherein the treatment of bladder cancer comprises intravesical instillation.
15. Use of an indoloquinone in the manufacture of a medicament for the treatment of a bladder cancer by simultaneous and/or sequential administration with a photosensitizing agent.
16. The use of claim 15 wherein the indoloquinone compound comprises an apaziquone.
17. The use of claim 16 wherein the photosensitizing agent comprises a hexyl aminolevulinate.
18. A kit comprising a composition comprising an indoloquinone compound, a composition comprising a photosensitizing agent, and a container.
19. The kit of claim 18 wherein the indoloquinone compound comprises an apaziquone.
20. The kit of claim 19 wherein the photosensitizing agent comprises a hexyl aminolevulinate.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/039313

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K41/00 A61P35/00 A61K45/06
ADD.

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)

A61K

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

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

EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. REDDY ET AL: "660 Effect of hexyl aminolevulinate on antitumor activity of apaziquone in human bladder cell lines", EUROPEAN UROLOGY SUPPLEMENTS, vol. 11, no. 1, 1 February 2012 (2012-02-01), pages e660-e660a, XP055070462, ISSN: 1569-9056, DOI: 10.1016/S1569-9056(12)60657-5	1-20
Y	the whole document ----- -/--	1-20



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

10 July 2013

Date of mailing of the international search report

19/07/2013

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/039313

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/132234 A1 (PHOTOCURE ASA [NO]; GORDON KIRSTEEN [GB]; GODAL ASLAK [NO]; KLAVENESS) 22 November 2007 (2007-11-22)	7,8,11, 12,15,18
Y	page 1, lines 4-9 page 3, lines 4-8 page 6, lines 30-32 page 9, lines 10-12 page 12, lines 22-23 example 3	1-20
X	----- SKYRME ROB J ET AL: "A phase-1 study of sequential mitomycin C and 5-aminolaevulinic acid-mediated photodynamic therapy in recurrent superficial bladder carcinoma", BJU INTERNATIONAL, BLACKWELL SCIENCE, OXFORD, GB, vol. 95, no. 9, 1 June 2005 (2005-06-01), pages 1206-1210, XP002452414, ISSN: 1464-4096	1,2,4,6, 7,11,15, 18
Y	the whole document -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/039313

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
W0 2007132234	A1	22-11-2007	NONE
