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TREATMENT EFFECTS OF WST11 VASCULAR TARGETED PHOTODYNAMIC THERAPY IN UROTHELIAL CELL CARCINOMA AND FEASIBILITY, SAFETY, AND LONG TERM OUTCOMES IN THE UPPER URINARY TRACT OF SWINE

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Abstract

Purpose—Surgical management of upper tract urothelial carcinoma requires removal of kidney and ureter, compromising renal function. Non-surgical alternatives have potentially prohibitive safety concerns. We examine the feasibility and safety of ablation of the ureter and renal pelvis using endoluminal vascular-targeted photodynamic therapy in a porcine model and report efficacy of WST11 vascular-targeted photodynamic therapy in a murine model.

Materials and Methods—Following approval, we performed 28 endoluminal ablations in the ureters and renal pelvis of 18 swine. Intravenous infusion of WST11 (4mg/kg) followed by laser illumination (10 minutes) was performed via percutaneous access or retrograde ureteroscopic approach. Animals were followed clinically with laboratory testing, imaging and histology was evaluated at several post-ablation time points. A murine xenograft was created with the 5637 human urothelial cell carcinoma line to determine sensitivity to this therapy.

Results—At 24 hours, 50 mW/cm laser fluence produced superficial necrosis of the ureter and deeper necrosis (penetrating the muscularis propria or adventitia) was produced by treatment with 200 mW/cm in the ureter and renal pelvis. At 4 weeks, superficial urothelium had regenerated over the treatment site. No symptomatic obstruction, clinically relevant hydronephrosis, or abnormality of lab testing was noted up to 4 weeks. In mice, 80% had no evidence of tumor at 19 days after WST11 vascular-targeted photodynamic therapy.

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Conclusions—Urothelial cell carcinoma appears to be sensitive to WST11 vascular-targeted photodynamic therapy. Depth of WST11 vascular-targeted photodynamic therapy treatment effects can be modulated in a dose-dependent manner by titration of light intensity. Moreover, this treatment modality, applied to the porcine upper urinary tract, is feasible via antegrade and retrograde access.

Keywords

WST11 compound; photochemotherapy; kidney neoplasms; ureteral neoplasms

Introduction

UTUC is a rare and clinically challenging disease that can be associated with exposure to chemical industrial agents, tobacco, and heritable genetic factors. In 2014, roughly 5% of bladder cancer patients in the US were found to harbor UTUC, including 3,700 renal pelvis and 3,000 ureteral cancers.¹

Surgical management of UTUC commonly requires radical nephroureterectomy, resulting in the compromise of overall kidney function.² Therefore, UTUC poses a complex management scenario when functional preservation is desired. Conservative therapies for UTUC, largely unchanged over the last several decades, are offered in specialized centers for highly selected cases. Modest oncologic success has been reported, with recurrence rates of 48% to 60% and ultimate failure rates (defined as upstaging or progressive disease) as high as 24%.³ Commonly used thermal ablation modalities, including electrosurgical resection, cryoablation, and laser ablation, are plagued by risk of inconsistent treatment effects and risks such as ureteral stricture (11%) and/or perforation.³ Studies of radiation and instillation therapies suggest potentially prohibitive safety concerns. Treatments to better address the largely unmet needs in this vulnerable patient population are lacking.

WST11 (TOOKAD[®] Soluble, Steba Biotech, France), is a water-soluble near-infrared-activated compound.⁴⁻⁶ Following IV administration, WST11 non-covalently binds to albumin and is sequestered within the circulation, with a half-life <60 minutes in humans,⁵ thus avoiding long-term skin toxicity. Preclinical studies,^{7,8} show that when WST11 is activated by light, free radicals produced in the blood mediate events resembling profound, transient, and localized ischemia-reperfusion injury.^{7,9} This causes arrest of tumor blood supply by rapid occlusion which leads to tumor necrosis and eradication within 48 hours.¹⁰

In non-tumor bearing large animals, WST11-VTP in normal tissues of hepatic and renal parenchyma, has demonstrated titratable degrees of tissue necrosis with sparing of larger vessels and tissue-scaffolding.¹¹⁻¹³ WST11-VTP of tumor bearing solid organs was found to be safe and effective in prostate cancer clinical trials.^{14,15} WST11-VTP may be uniquely suited for use in structurally complex endoluminal organs such as the upper urinary tract that might otherwise require challenging endoscopic fulguration or extirpative surgery. For development of clinical trials, preclinical modeling of tissue effects under appropriate endoluminal conditions in normal tissues are needed to serve as a reference of safety. Notably, tumor bearing models of comparable organ systems in large animals do not exist.

With the hypothesis that soft tissue ablation of superficial urothelium can be safely achieved and depth of ablation effect titrated by light dosing parameters, we undertook a series of studies using porcine and murine models to assess acute and chronic WST11-VTP ablation effects in the upper urinary tract using clinically relevant delivery mechanisms.

Materials and Methods

Porcine Experimental Plan

The Animal Use Protocol for this study was approved by the Institutional Animal Care and Use Committee. Tissue and radiographic effects of WST11-VTP of the ureter at 24 hours, 1, 2, and 4 weeks posttreatment and of the renal pelvis at 24 hours and 4 weeks were evaluated in a healthy non-tumor bearing porcine model. Based on prior studies, applied light fluencies ranged from 50 mW/cm to 200 mW/cm,^{14,16} and the time of illumination was fixed at 10 minutes.

To characterize treatment effects at escalating light doses, euthanasia and organ harvest were performed on 10 animals at 24 hours following VTP, 1 animal at 1 week, 1 animal at 2 weeks, and 6 animals at 4 weeks.

Eighteen female Yorkshire pigs weighing ~35 kg (Archer Farms, Darlington, MD) underwent acclimation, observation, and evaluation of baseline laboratory values two days prior to treatment. Accordance with the Guide for the Care and Use of Laboratory Animals was ensured. After a 12 hour fast animals were sedated with tiletamine/zolazepam (4.4 mg/kg) and given glycopyrrolate (0.007 mg/kg). Each pig was intubated and maintained on inhaled isoflurane anesthetic (1.5% to 2.5%) during the procedure. A 16-gauge angiocatheter was placed in an ear vein for blood collection and WST11 administration.

Surgical Procedures and WST11 Delivery in the Porcine Model

Percutaneous Antegrade Approach—Under anesthetic, an experienced radiologist (HT) established percutaneous access to the renal pelvis and ureter under ultrasound guidance, with antegrade nephrostogram confirmation (fig. 1, *a*). Clear polyethylene terephthalate balloon catheters (Venton Medical, Salem, NH) were passed antegrade and positioned in the mid to distal ureter and confirmed by radiographic markings (fig. 1, *b*). The 600- μ m laser fibers with 2-cm cylindrical, light-diffusing tips (MedLight S.A., Switzerland) were passed through the catheters and positioned with the diffusing tip at the level of the balloon and the site of treatment was recorded. Animals intended for 24-hour posttreatment euthanasia underwent bilateral ablations. To avoid potential complication from ureteral obstruction, only unilateral ablations were performed in animals undergoing survival longer than 24 hours.

Retrograde Ureteroscopic Approach—Following anesthesia, the genitalia were prepped and draped in the standard surgical fashion and a flexible ureteroscope (Olympus URF-V, Olympus American, Center Valley, PA) was used to navigate the urogenital sinus to the ureteral orifice into which a 5 French ureteral catheter was advanced. Retrograde pyelogram with Omnipaque 350 contrast medium (GE Healthcare, Milwaukee, WI) and fluoroscopy were used to obtain evaluation of anatomy. Ureteroscopy was performed by

introducing the endoscope into the ureter over a guide wire. The laser fibers (600 μ m) with 2 cm cylindrical-diffusing tips (MedLight S.A., Switzerland) were positioned within a major calyx to provide illumination to the related polar moiety. At least 1 ablation was performed through the ureteroscope on each animal. When 2 simultaneous sites of ablation were performed, one additional laser fiber was guided into the upper pole of the kidney through a ureteral access catheter and visual confirmation was achieved with ureteroscopy (fig. 1, c–e).

Illumination Source

Once all laser fibers were placed solubilized WST11 (Steba Biotech, France) was administered intravenously at 4 mg/kg over 15 minutes. Target tissues were illuminated for 10 minutes by a 753-nm medical diode laser (V-Gen, Tel Aviv, Israel or Biolitec, East Longmeadow, MA) at fluence rates of 50 mW/cm, 100 mW/cm, or 200 mW/cm.

Clinical Follow-Up With Laboratory and Imaging Studies

The animals were evaluated per the institutional guidelines for discernible signs of distress. Laboratory studies, including a complete blood count and comprehensive serum chemistry panels, were obtained weekly up to 4 weeks. For hematology, blood was collected into EDTA tubes and automated analysis performed on an IDEXX Procyte DX hematology analyzer (IDEXX Laboratories, Westbrook, ME). For chemistry, blood was collected into serum separator tubes and analyzed on a Beckman Coulter AU680 (Beckman Coulter, Brea, CA).

CT images were obtained by a multidetector-row CT scanner (LightSpeed 16, GE Healthcare, Milwaukee, WI) 24 hours post-ablation and weekly until the endpoint. Three-phase contrast-enhanced CT images were obtained. Fifty mL contrast medium (1.5 mL/kg Iohexol 300; GE Healthcare, Milwaukee, WI) was injected at a rate of 2.5 mL/s. Arterial-, nephrographic-, and excretory-phase images were obtained at 30, 60, and 900 seconds after contrast injection. A slice collimation of 16 mm $\times$ 1.5 mm was used for image acquisition, and 3-mm thickness axial and coronal images were reconstructed. Images were reviewed by an experienced radiologist (HT).

Necropsy and Histologic Evaluation

Animals were euthanized under anesthesia at 24 hours (n=10), 1 week (n=1), 2 weeks (n=1), and 4 weeks (n=6) following WST11-VTP treatment. Euthanasia was performed with intravenous injection of pentobarbital sodium (87 mg/kg) and phenytoin sodium (11 mg/kg), (Euthasol; Vibrac AH, Fort Worth, TX).

Treatment sites were excised en bloc at necropsy by removing the treated kidney and entire ureter to bladder, and photographed. The kidneys and ureters were bisected longitudinally and subdivided along their proximal-distal axis. Untreated contralateral organs were obtained for control samples. All specimens were fixed in 10% neutral buffered formalin, processed in alcohol and xylene, paraffin embedded, sectioned at 5 micron thickness, and stained with H&E. Sections from selected specimens were also stained with Masson's trichrome stain for collagen and TUNEL for cell death.¹⁷

H&E stained sections were assessed for the presence of necrosis, hemorrhage, edema, inflammation, and fibrosis. Measuring treatment depth based on gross inspection was inaccurate and therefore not utilized. A board-certified comparative veterinary pathologist (SM) performed all histological assessments.

Mouse Model

To study the primary in vivo effects of WST11-VTP treatment, we utilized the human **high grade** urothelial carcinoma cell line, 5637 (ATCC, Manassas, VA) in a heterotopic murine model. Cells were cultured in Roswell Park Memorial Institute medium media supplemented with 10% fetal bovine serum (Life Technologies, Grand Island, NY). Ten million cells mixed with matrigel (1:1) were subcutaneously injected into bilateral flanks of 7–8 week old male athymic nude mice (n=18) (National Cancer Institute, Frederick, MD). When tumors reached approximately 100 mm³, one flank was treated with WST11-VTP while the contralateral side was used for a control. An anesthetic cocktail of 150 mg/kg ketamine and 10 mg/kg xylazine was administered intraperitoneally prior to treatment and was supplemented with inhaled isoflurane. A single dose of carprofen (5 mg/kg) and 1 mL of subcutaneous warm saline was administered. WST11 was infused via tail vein over 5 minutes at a dose of 9 mg/kg and a 1 cm fiber was inserted into the tumor. After WST11 infusion, illumination at a light fluence of 150 mW/cm using a 753 nm medical diode laser (V-gen, Israel) for a total of 10 minutes was administered. Following VTP, tumors were monitored for response and growth kinetics biweekly for the first 2 weeks and then weekly. Tumors were measured by the same individual using calipers in 3 dimensions to calculate tumor volume. Animals were sacrificed at day 19 post-WST11-VTP treatment using inhaled carbon dioxide gas.

Results

Percutaneous Access Ureteral Ablations

24 Hours After VTP—Ureters at the time of necropsy appeared grossly normal, as did surrounding tissues (bowels and blood vessels). Histologic analysis of treated sections **showed** the deepest anatomic level of coagulative necrosis (Table 1). A light fluence dose-dependent effect was observed indicating deeper treatment effect at higher fluence, characterized as a homogenous region of coagulative necrosis from the luminal urothelium to the deep treatment margin.

Treated tissues were characterized by well demarcated areas of necrosis and positive TUNEL staining with variable degrees of hemorrhage. Data is shown in Table 1 and Figure 2.

1–4 Weeks After VTP—Pigs were euthanized 1 week (n=1), 2 weeks (n=1), or 4 weeks (n=1) after percutaneous unilateral VTP at 200mW/cm. Four week data was obtained in one animal treated at 50 mW/cm and 1 animal at 100 mW/cm. No perforation or urinoma formation was identified from serial CT imaging in any animal. Three animals developed hydronephrosis (1 from each light fluence setting), each of which resolved without intervention by 3 weeks (fig. 3). No animals exhibited discernible signs of distress indicative

of symptomatic obstruction. Representative images of microscopic effects of those at 200 mW/cm are provided in Figure 4.

Retrograde Approach to Renal Pelvis Ablations

A total of 9 renal pelvis ablations at 200 mW/cm were performed using a retrograde ureteroscopic approach with 5 ablation sites (n=3) evaluated at 24 hours and 4 (n=3) ablation sites evaluated at 4 weeks. No visible changes to the urothelium were detectable immediately following VTP. Light hematuria resolved by 24 hours post-ablation in all animals. All animals functioned normally and gained or maintained weight over the course of the study.

Laboratory Tests—All serum creatinine concentration increased at 24 to 48 hours following unilateral renal pelvis ablation (Table 2). For animals surviving 4 weeks, creatinine continued to gradually increase as shown in Table 2. Hemoglobin concentration for the animals at baseline ranged from 10.6 to 12.0 g/dL. At 1 week post-ablation, hemoglobin decreased and remained stable until week 4 (Table 2). At 48 hours after VTP, the white blood cell count increased in all but one of the surviving animals and was due to an increase in absolute neutrophil count (data not shown). Table 2 demonstrates a downward trend by 2 weeks with continued decrease out to 4 weeks.

Imaging Follow-Up—CT urography showed no hydronephrosis within the kidney at 24 hours post-ablation and no evidence of urinary extravasation or infection was noted during the 1–4 weeks following VTP ablation.

Histological Response: 24 Hours Post-Ablation—The urothelium was completely sloughed at all ablation sites. H&E demonstrated significant necrosis into the muscularis propria with hemorrhage within the lamina propria and hyperemia of the muscularis. The necrosis was confirmed with TUNEL. Figure 5 shows normal urothelium versus ablated site on H&E and TUNEL staining.

Renal parenchyma surrounding the renal pelvis ablation sites had areas of necrosis and loss of cellular detail in the renal tubules and glomeruli. The deepest level of damage to the renal pelvis and surrounding renal parenchyma at each site is shown in Table 3.

Histological Response: 4 Weeks Post-Ablation—On gross inspection of the renal pelvis and calyces, there were no visible lesions, but the surrounding parenchyma in 1 kidney showed a wedge-shaped scar adjacent to the ablation site (fig. 6). On microscopic examination, urothelium was present at all ablation sites, although appeared thinner than non-ablated portions of renal pelvis urothelium. The areas where ablation extended into the renal cortex also showed regeneration of renal tubules with intervening stroma and fibrosis. The changes to adjacent renal cortex are shown with H&E and confirmation of fibrosis using Masson's trichrome (fig. 7). Of note, renal parenchyma not in direct contact with the ablation zone had no evidence of lesions. Table 3 shows the deepest level of damage for each ablation at 4 weeks post ablation.

Mouse Model

At 22 days after tumor grafting 18 mice were randomized and subjected to WST11-VTP (10 tumors for ablation, 8 tumors for control). On day 4 after WST11-VTP, all 10 treated tumors had partial (3) or complete (7) eschar formation and no palpable or measurable tumor indicating treatment effect. By the endpoint of the study, animals in the untreated control group were sacrificed due to excessive tumor growth (19 days post-VTP), and only 2 animals in the treatment group had measurable recurrence at the treatment site. Those that recurred had a partial eschar noted at 4 days after the treatment. The contralateral control tumors continued to grow while 80% of the treated tumors remained clinically undetectable (non-palpable or measurable) up to 19 days after WST11-VTP (fig. 8).

Discussion

Similar to its counterpart in the bladder, UTUC often manifests as multifocal metachronous tumors. This is frequently described as a pan-urothelial process prone to implantation of tumor cells with genetic clonality.¹⁸ A modality for safe and effective endoluminal soft tissue ablation in the upper urinary tract is needed in this challenging disease.

Our study demonstrates that 2 clinically relevant approaches utilizing WST11-VTP of the ureter and renal pelvis can be titrated to produce superficial or deeper ablation. Both approaches are well tolerated and without major complications.

A gradual increase in serum creatinine concentration was noted from 24-48 hours until 4 weeks after surgery. The gradual increase remained well within the established reference range for swine (1.0-2.7 mg/dL)¹⁹ and likely represents physiological changes with increased muscle mass (30% increase in body weight from baseline, data not shown). Histological examination of the renal parenchyma distant from the ablations showed no injury. The decreased hemoglobin and increased WBC count are likely due to tissue necrosis and inflammation and these both resolved by 4 weeks post treatment.

In porcine models, WST11-VTP was shown to induce non-thermal tissue ablation in liver and kidney while preserving critical organ structures.¹² Moreover, cellular necrosis was complete in contrast to residual cellular viability seen following thermal based ablation influenced by heat sink effects.¹² WST11-VTP differs from classical PDT²⁰⁻²⁶ and offers major advantages, including absence of prolonged skin photosensitivity, which has been a major barrier to widespread utilization of traditional PDT.²²⁻²⁴ WST11-VTP has been successfully and safely applied in phase II clinical trials for tissue ablation in cancer bearing organs.^{14,15} Results from studies in prostate cancer have shown well-preserved quality of life, indicating the potential to overcome the disadvantages of other ablation approaches. A phase II prospective trial in prostate cancer confirmed the drug concentration (4 mg/kg) and light dose (200 J/cm) necessary to achieve interstitial tumor ablation and demonstrated safety up to 6 months after treatment.¹⁴ Data from phase III studies in over 500 patients using these optimal parameters will be available this year. Our current study reports on the results of similar treatment parameters adapted to endoluminal WST11-VTP.

We have also shown that human urothelial cancer is sensitive to WST11-VTP under relevant and similar treatment conditions in a murine model with demonstration of complete primary tumor ablation. These findings provide basis, safety, feasibility, and conceptual rationale for prospective clinical trials using WST11-VTP in UTUC.

Limitations to our preclinical studies include the necessary use of a non-tumor bearing large animal model to mimic the anatomy in humans. We expect that treatment effects in tumor bearing tissues may respond differently due to tumor thickness and pathophysiology of the tumor microenvironment. The use of ureteral stents was not evaluated but would likely be recommended in clinical studies. The challenges of treating several sites in the urinary tract either synchronously or sequentially also bears consideration, but were not tested. Our murine model with human tumor xenografts have shown high sensitivity to WST11-VTP, although small numbers with limited follow-up. The cell line 5637 is a high grade tumor and therefore we are unable to describe the effects that WST11-VTP may have to a low grade UTUC. It is reassuring that treatment effects were consistent and recovery uneventful in small and large animal studies.

Our study demonstrates that WST11-VTP appears to be safe and feasible using both percutaneous and ureteroscopic access to the porcine upper urinary tract and provides data for initial dosing parameters for upcoming prospective clinical trials. We also documented WST11-VTP sensitivity in an in vivo murine model. Future studies are necessary to support this conclusion and to create the foundation for future clinical trials.

Conclusion

WST11-VTP can be successfully applied to the porcine upper urinary tract safely. Human xenograft lines of UTUC in a murine model are responsive to WST11-VTP. Light fluence administered for WST11-VTP can be adjusted to obtain differing levels of tissue ablation with stability of organ functionality under treatment conditions.

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Figure 1.

WST11 vascular-targeted photodynamic therapy of ureter and renal pelvis. A: Pretreatment antegrade nephrostogram showing ureteral course into pelvis. B: Ureteral balloon placement with radiographic markers (arrow) in the distal ureteral segment. Laser fiber placement through the ureteral catheter (arrow marks opening of ureteral catheter) was confirmed in the renal pelvis via fluoroscopy as noted in C. The placement of the fiber through the ureteral catheter was confirmed to be in correct position by direct vision with the flexible ureteroscope as shown in D and the position of the fiber placed through the ureteroscope was confirmed directly as shown in E (arrow represents 2 cm diffusing tip of laser).

Figure 2.

Ureteral necrosis 24 hours after WST11 VTP with different light fluencies. A, D: Hematoxylin and eosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining of urothelium at 50 mW/cm with only multifocal superficial urothelial necrosis and loss. B, E: H&E and TUNEL staining at 100 mW/cm with diffuse urothelial loss and necrosis and hemorrhage through the lamina propria, with marked hemorrhage (arrow). C, F: H&E and TUNEL staining at 200 mW/cm with diffuse urothelial loss and necrosis and hemorrhage noted through lamina propria to muscularis (arrow). This necrosis is evident with the TUNEL staining.

Figure 3.

Computed tomography urography illustrating transient hydronephrosis and subsequent resolution with WST11 vascular-targeted photodynamic therapy (VTP) of the ureter at 200 mW/cm. Hydronephrosis after percutaneous ureteral WST11 VTP is noted on treatment side (arrows) within 24 hours after VTP (A), with improvement at 1 wk (B) and complete resolution at 4 wk (C).

Figure 4.

Ablation and regeneration of urothelium after ureteral with WST11 VTP at 200 mW/cm. A, D: Normal appearing, intact untreated ureter on hematoxylin and eosin (H&E) and trichrome stains. B, E: H&E shows completely lost urothelium with diffuse replacement of the muscularis smooth muscle with collagen (blue) present on trichrome stain. C, F: 4 weeks after VTP, urothelium regeneration is evident on H&E (although urothelium appears thinner than in the untreated sample) and viable muscularis (red) is observed.

Figure 5.

Ablations at 24 hours. A: Untreated renal pelvis urothelium (arrow) on hematoxylin and eosin (H&E) stain; C: terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) stain of the urothelium (arrow). B: 24 hours posttreatment H&E reveals completely absent urothelium with hemorrhage and edema in lamina propria layer around the renal pelvis urothelium with sharp demarcation between ablated and normal tissue (dotted line) on TUNEL stain in D.

Figure 6.

Gross examination of kidney: 4 weeks after WST11 VTP to renal pelvis. Gross inspection of bi-valved kidney shows no changes to renal collecting system. Region of renal parenchyma surrounding the ablation site (shown by circle) shows wedged shaped scar overlying the area of treatment near a renal papilla.

Figure 7.

Ablations after 4 weeks. 7A shows untreated renal cortex on hematoxylin and eosin (H&E) and negative stain with Masson trichrome (C) of the renal tubules and glomerulus (shown by arrow in all figures). 7B shows renal cortex surrounding area of renal pelvis ablation with normal tubules and glomerulus with decreased number of tubules with intervening fibrosis. This stroma intervening between tubules is shown with a positive Masson trichrome stain (blue) for collagen deposition as shown in 7D.

Figure 8.

Xenograft urothelial cell carcinoma mouse model and vascular-targeted photodynamic therapy (VTP) response. Tumor growth kinetics of treated and untreated primary human urothelial carcinoma xenograft tumors demonstrating sensitivity to WST11-VTP. Of the animals that underwent VTP (n=10) for subcutaneous implanted tumors, 2 were noted to have measurable tumors at day 19 after ablation. All tumors in untreated control animals (n=8) continued to grow over time prompting animal euthanasia by post-ablation day 19.

Table 1

Depth of ureteral ablation of swine identified on staining 24 hours post-ablation^{*}

Light fluence (n=numbers of animals treated)	Deepest Layer of Necrosis per Ablation (number of animals)		
	Urothelium/Lamina Propria	Muscularis	Adventitia
50 mW/cm (n=2)	2	0	0
100 mW/cm (n=6)	1	4	1
200 mW/cm (n=5)	1	3	1

For ureteral ablations, depth of ablation was established on microscopy as the deepest layer of ureteral wall necrosis identified on hematoxylin and eosin and terminal deoxynucleotidyl transferase dUTP nick-end labeling staining according to defined anatomic boundaries: urothelium/lamina propria, muscularis, and adventitia.

^{*} This treatment occurred immediately after the completion of the WST11 infusion.

Table 2

Laboratory changes after WST11 vascular-targeted photodynamic therapy to unilateral renal pelvis of pigs

	Animal #1	Animal #2	Animal #3	Animal #4	Animal #5	Animal #6
Creatinine (mg/dL)						
Baseline	1.31	1.13	1.21	0.9	1.12	1.19
24–48 h	1.41	1.51	1.59	1.1	1.26	1.36
1 wk	X	X	X	1.09	1.36	1.34
2 wk	X	X	X	1.12	1.43	1.27
3 wk	X	X	X	1.26	1.47	1.29
4 wk	X	X	X	1.53	1.51	1.45
Hemoglobin (g/dL)						
Baseline	11.9	10.6	10.6	12	11.8	11.7
24–48 h	X	X	X	11.5	10.1	9.9
1 wk	X	X	X	9.7	8.8	8.5
2 wk	X	X	X	9.3	8.7	7.2
3 wk	X	X	X	9	8.9	8.5
4 wk	X	X	X	9.7	9.1	8.6
White Blood Cell (K/uL)						
Baseline	12.64	13.62	15.43	16.62	20.05	24.38
24–48 h	X	X	X	21.49	26.15	20.67
1 wk	X	X	X	20.53	26.84	24.45
2 wk	X	X	X	18.23	21.28	21.33
3 wk	X	X	X	17.61	19.36	18.43
4 wk	X	X	X	15.36	18.2	15.05

Table 3

Degree of histological damage after WST11 VTP to unilateral renal pelvis^{*}

Depth of Ablation	Number of Ablations at 24 hours (n=5)	Number of Ablations at 4 weeks (n=4)
Urothelium	0	0
Lamina Propria	0	2
First 1/3 of Renal Parenchyma	3	1
First 2/3 of Renal Parenchyma	1	1
Through Entire Parenchyma	1	0

For renal pelvis ablations, depth of ablation was identified according to defined anatomic boundaries: urothelium, lamina propria, first 1/3 of renal parenchyma, first 2/3 of renal parenchyma, and through the entire renal parenchyma extending to the renal capsule.

^{*} All ablations to renal pelvis were at maximal light fluence of 200 mW/cm