Photodynamic therapy for Non-Muscle Invasive Bladder Cancer (NMIBC) mediated by instilled photosensitizer TLD1433 and green light activation

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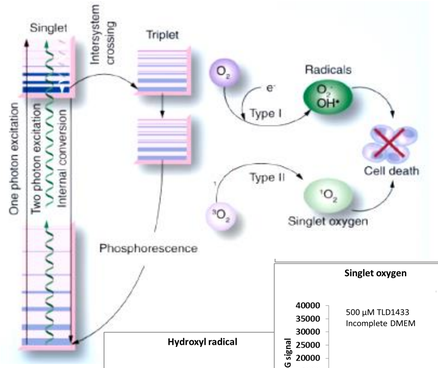
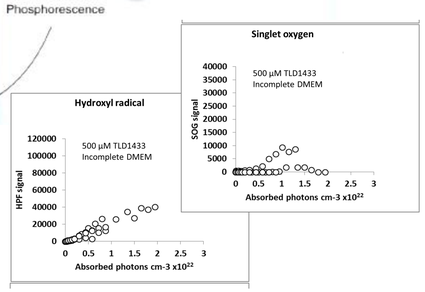
Introduction

Bladder cancer is the most common malignant tumor in the urinary tract. An estimated 72,570 new bladder cancer cases occurred in the US in 2013 and resulted in approximately 15,210 deaths. Transurethral resection of a bladder tumor is the standard first line treatment. Although effective at treating the tumor, the recurrence rate ranges from 60% to 70%.

Photodynamic therapy (PDT), where a photosensitizer (PS) converts light into cytotoxic radical oxygen species to cause cell death, was approved for bladder cancer in 1993. It failed clinically due to morbidity affecting the muscle layers, resulting in reduced bladder volume and incontinence.





Here we present a new approach to PDT treatment of bladder cancer using a novel Ruthenium coordination complex based PS (TLD1433) and a short (525 nm) activation wavelength, both providing for a steep PDT dose gradient in the bladder wall.
The AY-27 rat orthotopic cancer model was used to test PDT efficacy.

Method

Efficacy of TLD1433 mediated PDT was tested in vitro in AY-27 and HT1376 bladder cancer cells. Light activation of the photosensitizer was provided at 525 to 532 nm to evaluate PDT efficacy.

Rat bladders were seeded with 1.5×10^6 AY-27 tumor cell following an acid/base wash. 90% of bladders had visible tumors at 2 to 3 weeks. TLD1433 was instilled into the bladder for one hour at 0.6 (N=18) or 6.0 (N=9) mg mL^{-1}. Immediately afterwards, the bladder was rinsed and irradiated with 525 nm to a target radiant exposure of 90 J cm^{-2}. Bladder wall and tumors were collected two days later and histologically analyzed with H&E staining.

Results

TLD1433-only or light-only treatment showed no effect on tumor growth, heathy urothelium, or bladder musculature. At 48 hrs post PDT treatment with TLD1433, full depth tumor necrosis was observed at both 0.6 and 6.0 mg mL^{-1} concentrations in the vast majority of tumors including deeper growing tumors reaching the muscle layer. At the same time point bladder muscle tissue appeared normal and the urothelium showed only local inflammation near the tumor.

From the estimated tissue uptake selectivity between the AY-27 tumors and normal bladder wall a potential therapeutic ratio ~ 200 was determined. TLD1433 mediated PDT sensitivity of the tumour and normal tissue as estimated in the worst case scenario reduced the therapeutic ratio by a factor of ~16. However, light propagation simulations indicated that careful light dosimetry is required for treatment of NMIBC in humans to mitigate muscle damage, due to the unknown optical properties of the bladder wall.

PDT and Bladder Cancer: Current Status

Health Canada approval 1993: Whole-bladder PDT (with hematoporphyrin derivative [Photofrin®] I.V.) for patients with recurring superficial papillary bladder cancer who have failed standard intravesical therapy.

Warnings included transient increased micturition frequency (60%), hematuria (56%), dysuria (36%), urgency (32%) suprapubic pain (20%).
Additionally, strangury (32%), genital edema (24%), urinary incontinence (20%) urinary tract infection (12%), but also transient reduction in bladder capacity and **even irreversible bladder contracture occurred in 20% of patients.**
However, one Photofrin mediated PDT can be as effective as multiple BCG treatments.

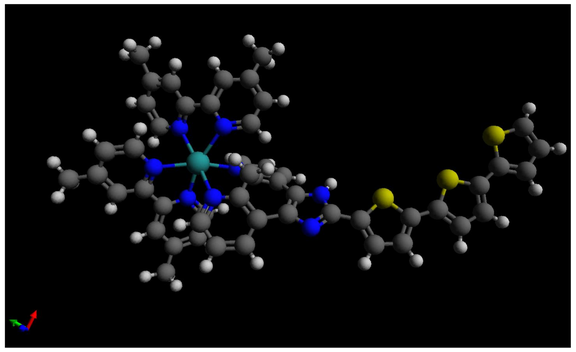
Early response rates
At 2-3 month 50-80% had complete response and at 1-2 years complete response was observed in 20-60% of patients.

Dosimetry
With strict dosimetry, long term response up to 60% were reported, and for intravesical ALA induced PPIX long term response of 52 -60% .

Adjuvant or combination therapies
PDT + BCG or intravesical immunotherapy mitomycin C and PDT may have enhanced response.

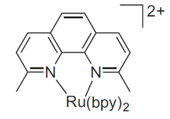
The Photosensitizer TLD1433:

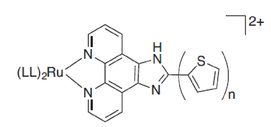
[Ru(II)(4,4′-dimethyl-2,2′-bipyridine(dmb))$_2$-(2-(2′,2″:5″,2‴-terthiophene)-imidazo[4,5-f][1,10]phenanthroline)]$^{2+}$

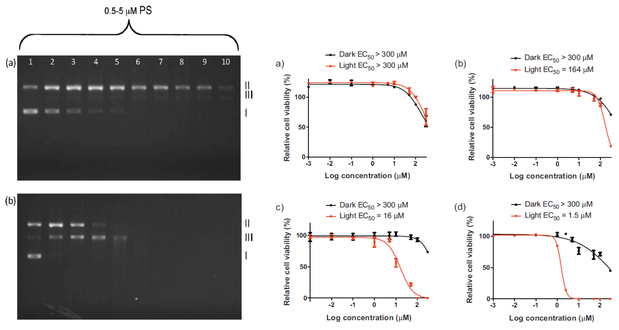


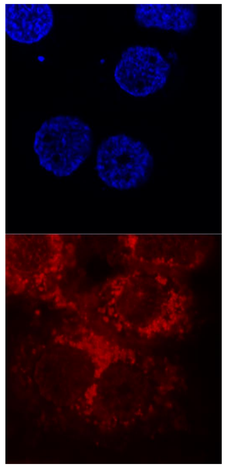
TLD 1433 (originally synthesized by S. MacFarland, Acadia University, N.S. Canada)
It comprises of two identical dimethylated bipryidine ligands and one terthiophene.
Triplet state energy: 2.22 eV with trans (3T*) conformation energy 1.72 eV (->1.57 eV) for longer chains.
Energy of intra-ligand triplet excited state (3IL) lower than metal-to-ligand charge transfer state (3MLCT) increasing T- life time ~250 nsec.

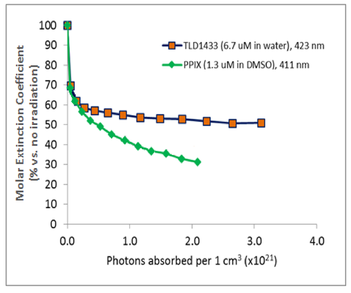
(1) Type II $^1O_2$ generating
(2) Type I compounds, charge transfer
(3) Photo-caging complexes releasing bioactive molecules
(4) Photo-adduct forming with DNA





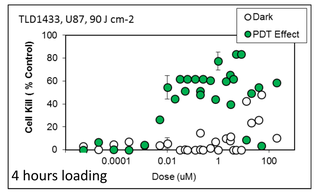


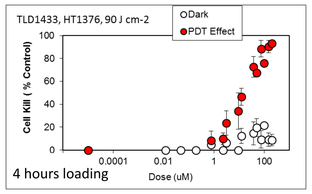


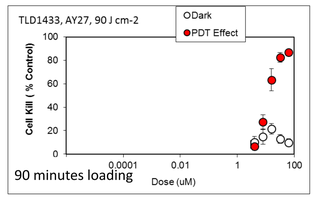


Photonsensitizer preparation includes generating a TLD-1433 stock solution [6mM] in sterile water requiring 1-2 minutes of vortexing and 1-2 minutes sonication. This results in a yellow-red solution. It remains stable for 24 hours at a pH > 5.62
For in vivo and clinical use vials containing 240 mg are available.

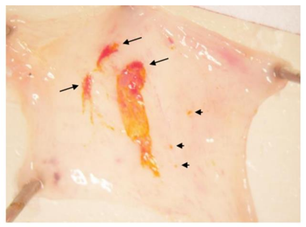
In vitro efficacy: Tumour cell response







Bladder model: Tissue uptake



Strong staining of tumors. Large tumors (long arrows) where utilized for photonsensitizer extractions as well as unstained normal tissue.

	Normal	Tumor
AVG	0.4± 0.09 mg/kg	77±18 mg/kg

Up to 192 fold therapeutic index based on durg selectivity .

To estimate tissue responsivity the Photodynamic Threshold model was applied. The threshold establishes the number of photons absorbed per unit volume to cause tissue destruction.

$$Dose_{PDT} = 2.3\varepsilon[TLD1433]\phi(d)$$

$$\frac{T_{tumour}}{[TLD1433]_{tumour}}\frac{\phi(d)}{\phi(0)} < \frac{T_{urothelium}}{[TLD1433]_{urothelium}}$$

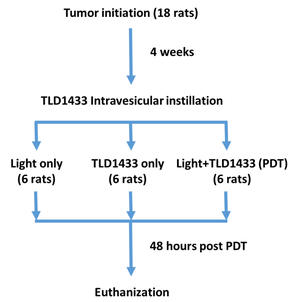
$$T_{tumour} < 2.12\ 10^{18}\ h\upsilon\ cm^{-3}$$

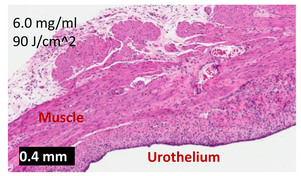
$$T_{urothelium} > 0.16\ 10^{18}\ h\upsilon\ cm^{-3}$$

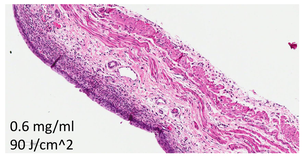
$$T_{muscle} > 0.128\ 10^{18}\ h\upsilon\ cm^{-3}$$

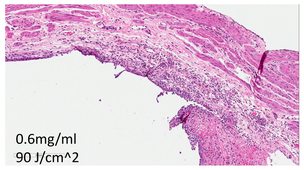
Therapeutic index could be reduced to as low as 11.6 indicating the need to control the radiant exposure in the bladder

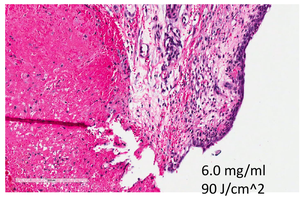
In vivo bladder cancer : Histology

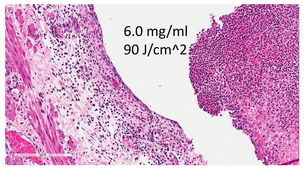












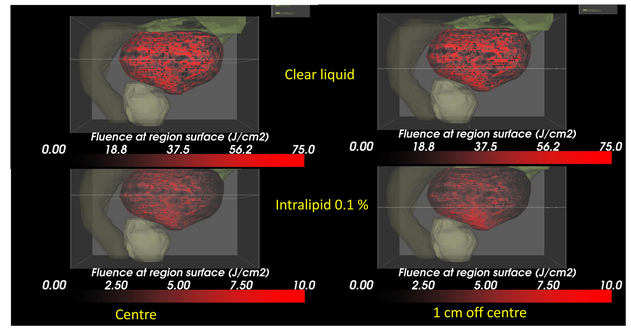
Observations

In the absence of light, TLD1433 instillation for 60 minutes at a concentration of 6 mg ml^{-1} does not lead to tumor, urothelium and muscle cell death 48 hrs later.

A radiant exposure of 90 J cm^{-2} results in mild submucosal inflammation, no observable urothelium damage but tumor necrosis up to 1 mm in depth.

These observations are a strong indicator that the selectivity of the photosensitizer accumulation during instillation in combination with the light activation still provides a PDT therapeutic ratio > 10 or at least comparable to 2 effective penetration depth for 525 nm light, whereby an effective penetration depth reduced the incident photon density to 37% of its original.

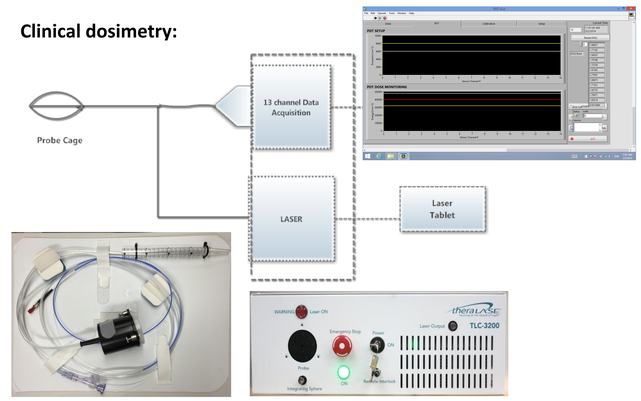
Homogeneity of bladder irradiation



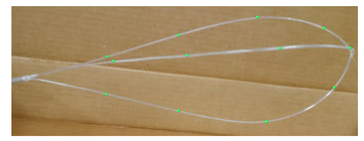
A transparent liquid in the bladder provides at equal power [W] delivers a higher irradiance [Wcm^{-2}] and hence radiant exposure [Jcm^{-2}] on the bladder surface.

Additionally, a transparent liquid void makes the local radiant exposure across the bladder wall less sensitive to positioning uncertainties of the isotropic emitter.

Clinical dosimetry:



The dosimetry setup comprises the laser source emitting at 525 nm with up to 3 W, an isotropic emitter contained in the centre of the dosimetry cage. The dosimetry cage carries 3 time 4 irradiance [W cm^{-2}] sensors viewing the inside of the bladder, see below, position of sensors are indicated on one cage arm, which are connected to the data acquisition system. The controller of the data acquisition system provide the Urologist with a real time feedback of the irradiance and radiant exposure [W cm^{-2}], whereby the former allows the Urologist to improve source position for more homogenous irradiation and the latter to determined when a sufficient photon density was achieve at the surface.



Conclusions

The Ru (II) coordination complex TLD1433 shows very high selectivity towards NMIBC after 1 hour of instillation in a pre-clinical model at a concentration which is far below the NOEAL systemic dose equivalent. Green light activation at 523 nm does not cause histological identifiable damage to the urothelium and the muscle layer. Tumour necrosis was observed up to a depth of approximately 1mm.
This suggests that personalized TLD1433 mediated PDT is a viable option for NMIBC when an a priori defined photon and photosensitizer dose is delivered.

Acknowledgement:
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Relevant publication
A ruthenium(II) based photosensitizer and transferrin complexes enhance photo-physical properties, cell uptake, and photodynamic therapy safety and efficacy
Pavel Kaspler, Savo Lazic, Sarah Forward, et. al.
PHOTOCHEMICAL & PHOTOBIOLOGICAL SCIENCES **15** (4) : 481-495 2016
A novel class of ruthenium-based photosensitizers effectively kills in vitro cancer cells and in vivo tumors
Fong, Jamie; Kasimova, Kamola; Arenas, Yaxal; et al.
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Ru(II) dyads derived from alpha-oligothiophenes: A new class of potent and versatile photosensitizers for PDT
Shi, Ge; Monro, Susan; Hennigar, Robie; et al.
COORDINATION CHEMISTRY REVIEWS **282** (SI) : 127-138 2015
Photodynamic inactivation of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus with Ru(II)-based type I/type II photosensitizers
Arenas, Yaxal; Monro, Susan; Shi, Ge; et al.
PHOTODIAGNOSIS AND PHOTODYNAMIC THERAPY **10** (4) : 615-625 2013,