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 13,210 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 cytostatic radical oxygen species to cause cell death, was approved for bladder cancer in 1993. It failed clinically due to morbidity affecting the muscle layer, resulting in reduced bladder volume and incompleteness.

Here we present a new approach to PDT treatment of bladder cancer using a novel Rhodamine coordinate complex based PS (TLD1433) and a short (252 nm) activation wavelength, both providing for a steep PDT dose gradient in the bladder wall. The 252-nm nanophotonic cancer model was used to test PDT efficacy.

**Method**

Efficacy of TLD1433 mediated PDT was tested in vitro in AI-27 and HT1376 bladder cancer cells. Light activation of the photosensitizer was provided at 252 to 532 nm to evaluate PDT efficacy.

The PDT efficacy was observed to be maximally 90% of viable bladder tumor cells at 2 to 3 weeks. TLD1433 was instilled into the bladder for one hour at 0.5 (N=9) or 1.0 (N=9) mg mL-1. Immediately afterwards, the bladder was rinsed and irradiated with 252 nm to a target radiant exposure of 50 J cm-2. Bladder wall and tumor tissues were collected two days later and histologically analyzed with H&E staining.

**Results**

TLD1433- and light-only treatment showed no effect on tumor growth, healthy urothelium, or bladder musculature. At 48 hrs post PDT treatment with TLD1433, full depth tumor necrosis was observed at both 0.5 and 6.0 mg mL-1 concentrations in the vast majority of tumors including deep 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 AI-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 1.5. 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 properties of the bladder wall.

**PDT and Bladder Cancer: Current Status**

Health Canada approval 1990. Whole-bladder PDT (with hematoporphyrin derivative Photofrin™) is used for patients with recurring superficial papillary bladder cancer who have failed standard interventional therapy.

Worries included transient increased infection frequency (10%), hematoma (6%), dysuria (6%), urgency (12%) suprapubic pain (20%). Additionally, strongyloides (12%), genital eczema (2%), 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 effective as multiple BCG treatments.

**Early response rates**

At 2-3 month 50-60% complete response and at 1-2 years complete response was observed in 10-60% of patients.

**Dosimetry**

With in vivo dosimetry, long-term response up to 60% were reported, and for intravesical AIA induced PRXK long-term response of 52-60%.

**Additional photodynamic therapy**

PDT + BCG or intravesical immunotherapy mitomycin C and PDT may have enhanced responses.

**The Photonsensitizer TLD1433:**

![TLD1433](image)

TLD1433 (originally synthesized by S. MacFarlane, Acadia University, N.S. Canada) is comprised of two identical chromophore(s) porphyrine ligands and one terminal thiol radical.

Triplet state energy: 3.22 eV with transition (3P7) conformation energy 1.72 eV (1.55 eV) for longer chains.

Energy of intra-ligand triplet excited state (1E2) lower than metal-to-ligand charge transfer state (1MLCT) increasing 1.81 eV time ~350-nsec.

1. Type II O2 generating,
2. Type II compounds, charge transfer
3. Photo-caging complexes releasing bioactive molecules
4. Photo-oxidation forming with DNA

**In vitro efficacy: Tumour cell response**

Photoinactivation dose: 1 2.14 (TLD1433) (dL)

**In vivo bladder cancer: Histology**

![Histology](image)

Strong staining of tumor. Large tumors (long arrows) where utilized for photostimulation extractions as well as obtained normal tissue.

**Bladder model: Tissue uptake**

![Tissue Uptake](image)

**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 60 min later.

- A radiative exposure of 90 J cm-2 results in mild submucosal inflammation, no observable urothelial damage but tumor necrosis up to 1 mm in depth.

These observations are a strong indicator that the selectivity of the photostimulans accumulation during instillation in combination with the light activation still provides PDT therapeutic ratio of 10 or at least comparable to 2 effective penetration depth for 532 nm light, whereas 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 (W cm²) than the bladder surface.

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

**Clinical dosimetry:**

![Dosimetry](image)

The dosimetry setup employs the laser beam shaping at 532 nm with 5 x 5 cm in the bladder wall, centered to the vicinity of the dosimetry setup. The dosimetry setup uses 3 cm 3 irradiance [5 cm] ensuring moving for the border of the bladder, for inner, circumferential, surface around which is covered by the dose equivalent column. Thermocouples in the bladder wall, which are connected to the data acquisition system. Thermocouples in the bladder wall, which are connected to the data acquisition system. Thermocouples in the bladder wall, which are connected to the data acquisition system. Thermocouples in the bladder wall, which are connected to the data acquisition system.

**Conclusions**

The Ru [III] 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 NOEL systemic dose equivalent. Green light activation at 532 nm does not cause histological identifiable damage to the urothelium and the muscle layer. Tissue necrosis was observed up to a depth of approximately 1 mm.

This suggests that personalized TLD1433 mediated PDT is a viable option for NMIBC when a prior defined photosensitizer and photosensitizer dose is delivered.

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**References**

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