Nanoparticle mediated photodynamic tumor ablation as a therapeutic strategy for oral cancers in biological and clinically relevant animal models

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Introduction

The positive margin remains a challenge for the head and neck surgeon. A positive margin leads to a 40% locoregional recurrence rate and the need for postoperative adjuvant treatments with their associated toxicity and side effects. Therefore, there is a need for development of alternative adjuvant treatment strategies. Photodynamic therapy (PDT) maximizes tissue preservation compared with oncological margin ablation, and favors re-mucosalization with less fibrosis as interstitial collagenous fibers are spared. Lack of specific accumulation of photosensitizers within the tumor resulting in suboptimal tumor ablation and skin photosensitivity have been some of the limitations of PDT. Our research group developed a novel multifunctional theranostic porophysome (PS) nanoparticle for oral cancer surgical guidance and therapy[1,2,3,4]. This molecule is capable of providing intra-operative fluorescence for surgical guidance and simultaneously acting as a photosensitizer for PDT treatment. We explored the efficacy of PS-mediated PDT as an adjuvant strategy in biological and clinically relevant SCC animal models.

Methods and Materials

Athymic mice injected with Human SCC (Cal33) cells in the right lower limb were used as our biologically relevant model. After growing to a size less than 10 mm, a first phase-proof of concept analysis was done, and animals were randomized into a PDT, laser control (LC) and particle control (PC) group. PDT was performed 24hs after the injection of PS, using an external beam laser (100 mW, 1 cm diameter). Three timed endpoints were established at 72hs, 7 and 14 days after treatment. Tumor size progression, hematoxylin-eosin (HE), TUNEL and Caspase-3 staining were utilized to evaluate the treatment response. A second-phase with long term follow-up with a 14 days endpoint was done including a natural progression (NP) group.

New Zealand rabbits were used as our clinically relevant model, in which rabbit SCC-VX2 cells were injected in the tongue and grown to a size of 10-15 mm. PDT was utilized as adjuvant treatment to the surgical bed after incomplete resections were performed leaving a 2-5mm positive margin. The control group were animals with positive margins without PDT. Tumor regrowth in the rabbits was monitored by CT, MRI and histology.

Results

- Thirty-five Cal33 tumor bearing mice were used for the first phase, with 100% of the animals developing tumors. Mean tumor size was 9 mm in maximum diameter. The 18 mice in the PDT group had a significant reduction in size (p=0.0004) before and after treatment **(Fig 1)**, compared with 9 PC mice (p=0.56) and 8 LC mice, which exhibited significant growth (p=0.003) **(Fig 2)**. Tumors in PDT group that had not regressed by 7 days post treatment showed complete TUNEL and Caspase-3 staining, compared with no/minimal staining in PC or LC **(Fig 3)**.
- Forty mice were used for the second phase with long term follow-up. The PDT group exhibited an 80% complete clinical response (12/15). PC (0/7), LC (0/10) and NP (0/8) had no clinical response 14 days after PDT. This reached statistically difference as well (p=0.007).
- Four rabbits had surgical resection with positive margins and adjuvant PDT (treatment) and there were three control rabbits. Residual tumor margin was demonstrated with real time intra-operative fluorescence **(Fig 4)**. None of the rabbits in the treatment group experienced tumor regrowth 4 months after treatment, whereas 100% of the rabbits in the control group had tumor regrowth **(Fig 5)**. These findings were confirmed longitudinally by CT/MRI and by histology at endpoint. All tumours were visible by PS mediated fluorescence at 24 hs post-nanoparticle injection, with at least a two-fold SBR.

Fig 1: Clinical response of PDT, Laser Control and Particle Control groups at 72hs, 7 and 14 days follow-up.

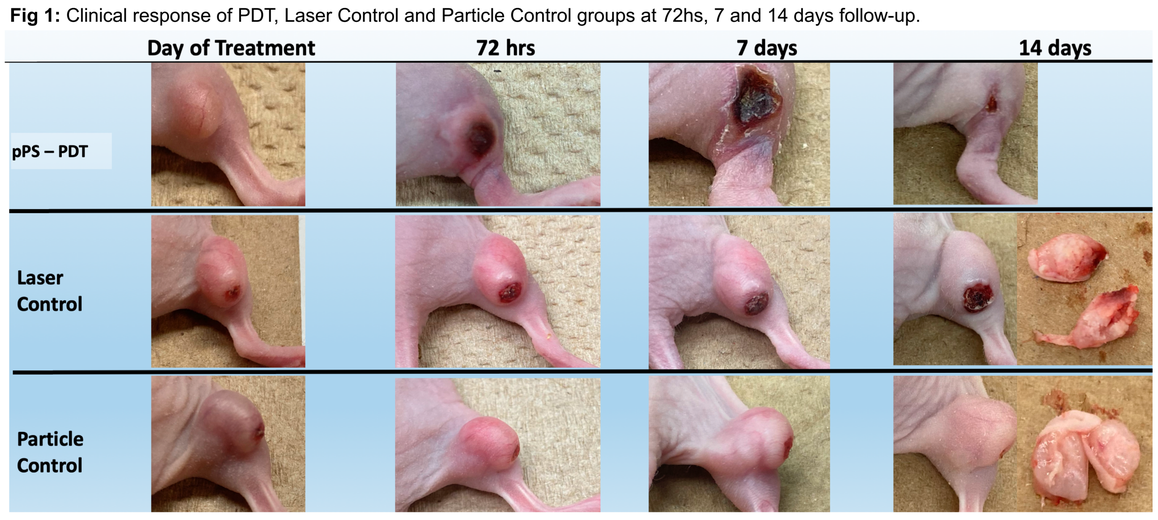


Fig 2: Pre and post treatment tumor size comparison between PDT, particle control (PC), and Laser Control (LC) groups, in the first phase-proof of concept.

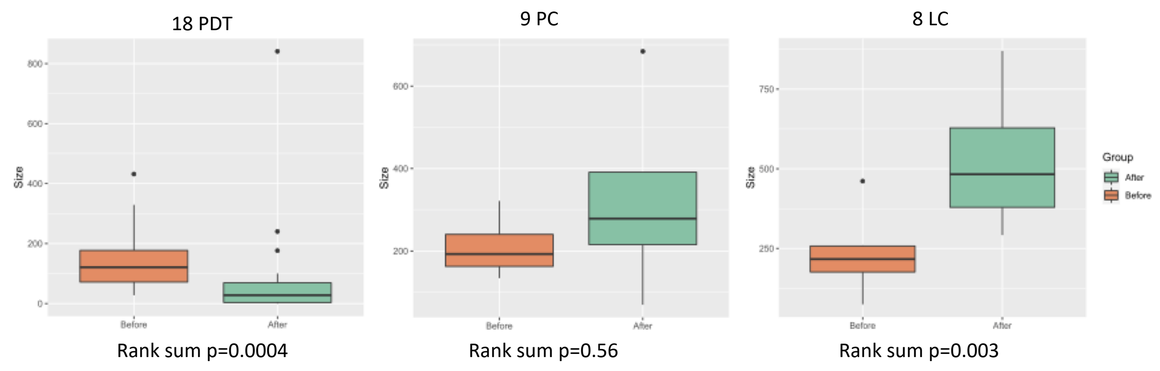


Fig 3: H-E, TUNEL and Caspase 3 of the different groups 72 hs after the intervention. TUNEL and Caspase 3 show higher degree of staining in the PDT group.

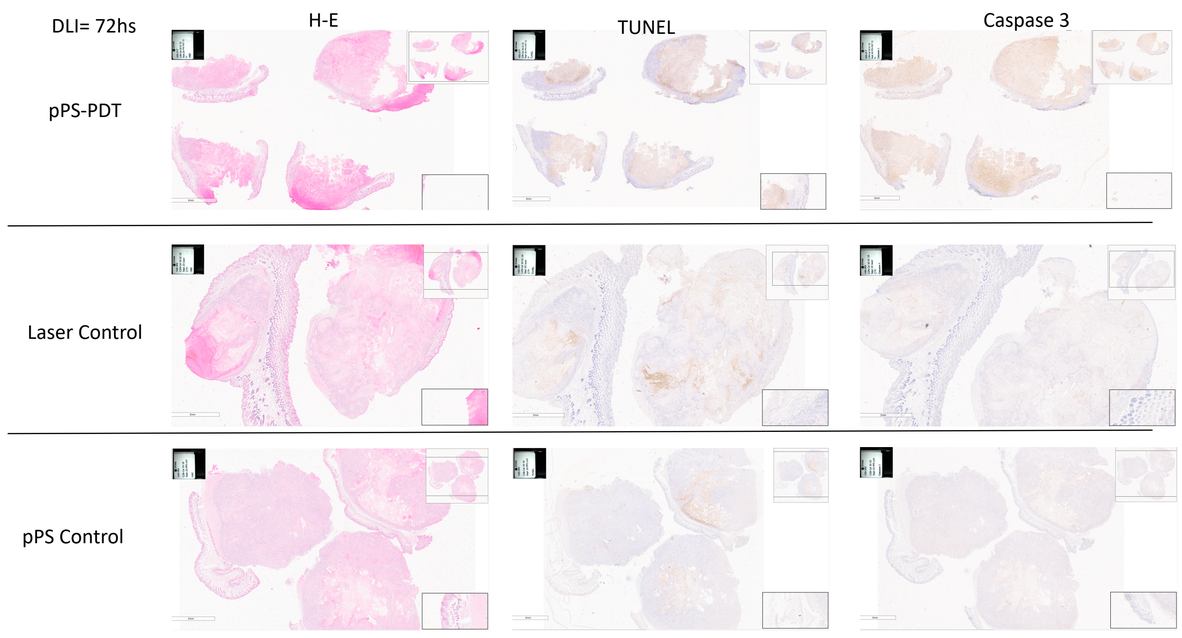


Fig 4: Intraoperative Fluorescence showing the positive margin.

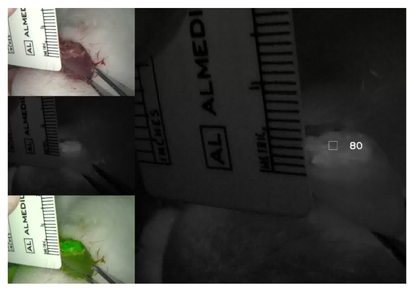


Fig 5: Rabbits with adjuvant therapy with PDT after positive margins and control group showing growth of the latter one.



Discussion and Conclusions

This preclinical study demonstrates the potential feasibility of using PS mediated PDT as an "on-the table" adjuvant treatment for oral SCC with close or positive margins. Subsequent steps will be to increase the cohort of animal models and continuing method optimization in order to translate the approach to human clinical settings.

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