A Cell-targeted Photodynamic Nanomedicine Strategy for Head & Neck Cancers

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Statement of Purpose: Surgical resection and radiotherapy are the current clinical mainstays for the treatment of head and neck (H&N) cancers. These can cause significant trauma and functional/cosmetic debilitation of tissue, especially if repetitive treatment becomes necessary due to aggressive tumor recurrence. Hence there is significant clinical interest in alternate treatment modalities which can effectively and selectively eradicate H&N tumor and can be safely repeated if needed. To this end, Photodynamic Therapy (PDT) is a promising modality, where activation of a photosensitizer (PS) by appropriate light ultimately leads to generation of cytotoxic reactive oxygen species (ROS). In the absence of the activating light the PS itself confers minimal toxicity (a significant advantage over chemotherapy) and the activating light itself is non-ionizing (a significant advantage over radiotherapy). Therefore PDT can provide dual selectivity in tumor treatment if the PS can be specifically targeted to the cancer cell followed by spatio-temporally selective photoirradiation of the cancer tissue. Building on this rationale, we are investigating a cell-selective nanomedicine approach for targeted PDT of H&N cancers. We have previously shown that the NIR-activable PS Pc 4 can be formulated within polymeric micelles [1] and the micelles can be specifically targeted to cancer cells over-expressing Epidermal Growth Factor Receptors (EGFRs) using GE-11 peptide ligands to enhance Pc 4 internalization [2]. Building on this work and rationalizing from the established EGFR over-expression in H&N Squamous Cell Carcinomas (SCCs), here we have used fluorescence spectroscopy and confocal microscopy to demonstrate *in vitro* the EGFR-targeting capability and EGFR-mediated internalization of GE-11-decorated Pc 4 nanoformulation in H&N SCC 15 cells. Building on this *in vitro* data, we have further demonstrated that the EGFR-targeted formulation results in enhanced intra-tumoral drug uptake and enhanced PDT response, *in vivo*, in SCC-15 xenografts in mice.

Methods: The EGFR-targeting GE-11 peptide with an additional cysteine residue on the tyrosine N-terminus (Cys-GE-11) was conjugated to the maleimide (Mal) terminus of Mal-polyethyleneglycol-co-polycaprolactone (Mal-PEG-PCL) through thioether reaction to form GE11-PEG-PCL. This was incorporated at 10 mol% along with just PEG-PCL to form EGFR-targeted micelles loaded with Pc 4 in the hydrophobic core. Targeted and nontargeted Pc 4-nanoformulations were incubated with SCC 15 cells and Pc 4 fluorescence was monitored to determine intracellular uptake. Confocal microscopy was used to determine spatio-temporal distribution of cellular uptake of the nanoformulation. Non-targeted formulation and low EGFR MCF7 cells were used as two negative controls. Cells were stained with Hoeschst 3342 (stains nucleus blue) and Lysotracker Green (stains lysosomes green). The EGFR-targeted Pc 4-loaded nanoformulation was further tested for *in vitro* PDT efficacy on EGFR-overexpressing SCC-15 cells using MTT and clonogenic assays. Subsequently, an SCC 15 xenograft tumor model in mice was used to study *in vivo* drug delivery and PDT effect of the Pc 4-nanoformulations. For this, targeted nanoformulation, non-targeted nanoformulation or a control group of Pc 4 formulated in 10% Intralipid, were administered via tail vein and at appropriate time-points the tumoral versus skin concentration of Pc 4 was monitored. Subsequently, tunors were photoirradiated (fluence rate: 132 mW/cm^2) and tumor regression over time was determined.

Results: EGFR-targeted nanoformulation resulted in faster and higher uptake of Pc 4 in EGFR-overexpressing SCC 15 cells and subsequent PDT in vitro rendered higher cell-killing with drastic reduction in clonogenicity. For subsequent in vivo studies, as shown in **Figure 1**, following systemic administration of the different Pc 4 formulations at Day 0 and tumor photoirradiation at Day 2, tumors treated with targeted and nontargeted Pc 4 nanoformulations, as well as, those treated with the 10% Intralipid-based formulation all showed initial regression whereas, the control tumors without photoirradiation continued to grow. Over time, the EGFR-targeted nanoformulation group maintained significantly regressed tumors compared to non-targeted group.

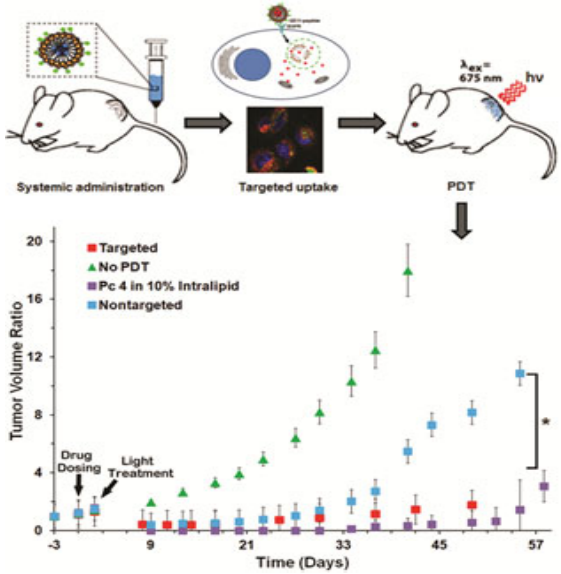

Figure 1. Tumor response to EGFR-targeted Pc 4-PDT

Conclusions: Our results show that EGFR-targeting can be utilized for enhanced delivery of a photosensitizer nanoformulation to EGFR-overexpressing H&N cancers, for cell-targeted photodynamic treatment of these cancers.

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References: [1] Master et al *J Pharm Sci. 2010.*
[2] Master et al *Nanomedicine: NBM 2011.*