5-16-2022

Interstitial photothermal therapy generates durable treatment responses in neuroblastoma

Debbie Ledezma

Preethi B. Balakrishnan

Jacob Medina

Allan Bowen

Anshi Shukla

See next page for additional authors

Authors

Debbie Ledezma, Preethi B. Balakrishnan, Jacob Medina, Allan Bowen, Anshi Shukla, Emily Oakley, Gal Shafirstein, Mario Miscuglio, and Rohan Fernandes

INTERSTITIAL PHOTOTHERMAL THERAPY GENERATES DURABLE TREATMENT RESPONSES IN NEUROBLASTOMA

Debbie Ledezma, The George Washington University, USA
dkledezma@gwu.edu
Preethi B. Balakrishnan, The George Washington University, USA
Anshi Shukla, The George Washington University, USA
Jacob Medina, The George Washington University, USA
Allan Bowen, The George Washington University, USA
Emily Oakley, Roswell Park Cancer Institute, USA
Gal Shafirstein, Roswell Park Cancer Institute, USA
Mario Miscuglio, The George Washington University, USA
Rohan Fernandes, The George Washington University, USA

Key Words: Photothermal therapy, Prussian blue nanoparticle, interstitial laser, thermal dose, neuroblastoma

High-risk neuroblastoma is a pediatric cancer accounting for 15% of childhood cancer-related mortality due to the limited success of standards-of-care. A promising approach to improve survival rates of high-risk neuroblastoma is photothermal therapy (PTT), a thermal therapy that we and others have shown to generate an *in situ* vaccine effect and elicit a potent antitumor response in combination with immunotherapy.[1-3] While the majority of studies involving PTT have primarily utilized external beam lasers, we propose to interstitially administer PTT (I-PTT), a more clinically relevant approach for neuroblastoma, which is a deep-seated tumor not amenable to the use of external lasers. In this study, we compare I-PTT with external laser PTT (S-PTT) in terms of: 1) their efficiency and distribution of heat in a target area, 2) PTT-induced immunogenicity, and 3) overall therapeutic benefit. I-PTT was administered using Prussian blue nanoparticles that our research group uses as PTT agents and an interstitial laser. For interstitial delivery of the laser into a target area such as a tumor, we used a fiber coupler to couple the external beam laser to an optical fiber fitted with a terminal diffuser. We conducted studies comparing the immune and therapeutic effects generated by both I-PTT and S-PTT *in vitro* and *in vivo*. We determined that I-PTT generates more efficient heating than S-PTT, attaining temperatures at least 10.5°C higher for the same output laser power (Fig 1A). The heating efficiency with I-PTT enhanced the N2A and 9464D neuroblastoma cell killing *in vitro* compared to S-PTT, when assessed at a low thermal dose of 2.0 logCEM43 (Fig 1B-C). I-PTT also induced similar levels of immunogenic cell death as S-PTT, as measured by its biochemical correlates: ATP, calreticulin, HMGB1 (data not shown). In animal studies using syngeneic neuroblastoma tumor-bearing mice, I-PTT and S-PTT were administered at comparable thermal doses in both the N2A and 9464D model to assess the efficacy of each PTT strategy. We observed that I-PTT efficiently ablated tumors at a greater rate than S-PTT, improving the overall survival from 60% to 90% in the N2A tumor model and from 0% to 80% in the harder to treat 9464D tumor model (data not shown). Upon tumor rechallenge of long-term surviving mice in the N2A model, I-PTT-treated mice exhibited improved tumor rejection compared to S-PTT-treated mice, as evidenced by the 33% versus 16% tumor-free survival (Fig 1D) observed in the rechallenged I-PTT group compared to S-PTT, respectively. Taken together, these results suggest that besides providing access to deeper tumors, I-PTT is a vastly improved approach for administering PTT compared to S-PTT in terms of heating efficiency, immunogenicity, and therapeutic benefit. Consequently, it is ideally positioned for combination with immunotherapy to provide novel treatment regimens for neuroblastoma and other solid tumors.

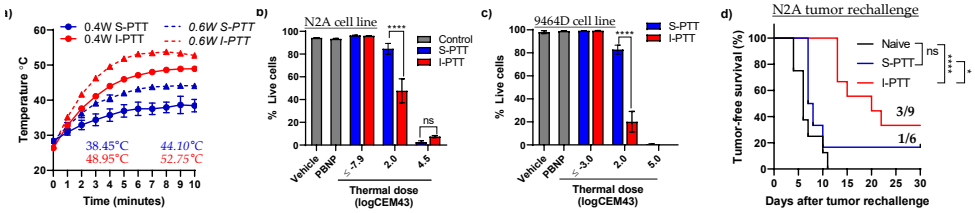

Figure 1 – Interstitial PTT heats efficiently to ablate neuroblastoma in vitro and improve rejection of tumor in vivo. ***a****) At similar laser powers, I-PTT heated to higher temperatures than S-PTT. At similar thermal doses, I-PTT was more effective than S-PTT in reducing* ***b****) N2A and* ***c****) 9464D cell viability in vitro.* ***d****) I-PTT treated mice were better at rejecting tumors upon rechallenge compared to S-PTT, as shown in the tumor-free survival plot.*

References: 1) Bear, et al. PLoS One, 2013, 8 (7), e69073. 2) Chen, et al. Nat. Commun. 2016, 7, 13193. 3) Cano-Mejia, et al. Transl Oncol 2020, 13 (10), 100823.