PHOTOTHERMAL THERAPY GENERATES A THERMAL WINDOW OF IMMUNOGENIC CELL DEATH IN NEUROBLASTOMA

Rohan Fernandes, George Washington University, Washington, DC USA
rfernandes@gwu.edu
Elizabeth E. Sweeney, George Washington University, Washington, DC, USA
Juliana Cano-Mejia, University of Maryland, College Park, MD, USA

Key Words: Photothermal therapy, immunogenic cell death, thermal dose window, Prussian blue nanoparticles, antitumor immune response

Nanoparticle-based photothermal therapy (PTT) has been widely investigated in cancer therapy as a rapid and minimally invasive tumor ablation technique. Over the past two decades, several reports utilizing nanoparticles for PTT of diverse tumor types *in vitro* and *in vivo* have been described. An emerging area of interest is the effect of PTT on the immune system during tumor therapy, since PTT not only causes tumor cell death, but can also release tumor antigens and endogenous adjuvants (e.g. heat shock proteins, damage-associated molecular patterns (DAMPs)) under certain conditions. These effects have the potential to increase tumor immunogenicity, which can trigger improved therapeutic responses. Engaging the immune system during PTT is important as it offers the potential for persistent treatment responses and immunological memory.
Here, we describe a thermal "window" of immunogenic cell death (ICD) elicited by nanoparticle-based photothermal therapy (PTT) in an animal model of neuroblastoma. ICD is a highly favorable cell death phenotype that initiates an adaptive immune response and is associated with improved therapeutic outcomes in cancer.[1] In studies using Prussian blue nanoparticles to administer photothermal therapy (PBNP-PTT) to established localized tumors in the neuroblastoma model, we observed that PBNP-PTT conformed to the "more is better" paradigm, wherein higher doses of PBNP-PTT generated higher cell/local heating and thereby more cell death, and consequently improved animal survival. However, *in vitro* analysis of the biochemical correlates of ICD elicited by PBNP-PTT, namely ATP and high motility group box 1 protein (HMGB1) release and increased surface calreticulin expression, demonstrated that PBNP-PTT triggered a thermal window of ICD. Specifically, the aforementioned markers of ICD were highly expressed within an optimal temperature (thermal dose) window of PBNP-PTT (63.3-66.4 °C) as compared with higher (83.0-83.5 °C) and lower PBNP-PTT (50.7-52.7 °C) temperatures, which both yielded lower expression of ICD markers (Fig. 1A). Subsequent vaccination studies in the neuroblastoma model confirmed our *in vitro* findings wherein PBNP-PTT administered within the optimal temperature window (63.3-66.4 °C) resulted in long-term survival (33.3% at 100 days) compared with PBNP-PTT administered within the higher (0%) and lower (20%) temperature ranges, and controls (0%) (Fig. 1B). Our findings demonstrate a tunable immune response to heat generated by PBNP-PTT, which should be critically engaged in the administration of PTT, both alone and when PTT is administered in combination with immune adjuvants (e.g. TLR agonists) and/or immunotherapies (e.g. immune checkpoint inhibitors) for maximizing its therapeutic benefits.

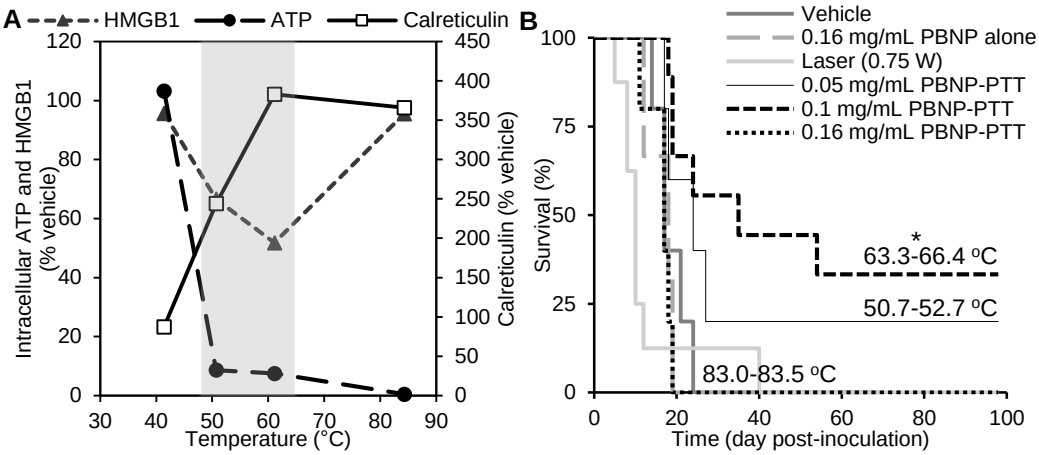

Figure 1. PBNP-PTT exhibits a thermal window of immunogenic cell death. A) Expression of ICD markers (HMGB1, ATP, and Calreticulin) showing increased expression between 48-65 °C (gray band) in vitro. B) Long-term survival in mice vaccinated with Neuro2a cells treated with different thermal doses of PBNP-PTT and subsequently challenged with Neuro2a cells exhibiting maximum long-term survival for animals vaccinated with PBNP-PTT-treated cells at ~65 °C compared with animals in the lower (~51 °C) and higher (~83 °C) temperature groups; * indicates $p<0.05$ as determined by a log-rank test.

REFERENCE: 1. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol.* Feb 2017;17(2):97-111.