Preclinical Modeling of Laser Interstitial Thermal Therapy in Murine Brain Tumors for Translational Immuno-oncology

Duke Neurosurgery

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INTRODUCTION

- Cytoreduction is a cornerstone of the management of intracranial tumors.
- Following laser interstitial thermal therapy (LITT), ablated tumor tissue remains *in situ*, allowing for an anti-tumor immune response.
- The post-LITT immune response has not been well characterized previously.

Objective: To develop a murine model of stereotactic laser ablation for identification of combinatorial treatment approaches.

METHODS

Our murine LITT system is depicted in **Figure 1**. Following creation of a craniotomy, a 1064 nm diode-based Nd:YAG laser (Neurolase, Monteris Medical) was used to thermally ablate normal brain tissue or CT-2A tumors in C57BL/6 mice**.** Ablations were performed at + 2 ML, + 1 AP, -3 DV using a stereotactically inserted 400 µm core fiber (ThorLabs); simultaneous temperature recording was performed 2 mm posterior to the ablation site using a needle thermocouple (MT-29, Physitemp). T2 sequences with TE of 54 ms and TR 3000 ms were acquired using a 7T Bruker BioSpec 70/20 small animal MRI using Bruker Paravision 6 software (Center for In Vivo Microscopy, Duke University School of Medicine). Formalin-fixed, paraffin-embedded mouse brains were stained and imaged using a Keyence BZ-X800.

RESULTS

To identify a well-tolerated thermal dose, we ablated brains from non-tumor bearing mice at 1, 1.5, or 2 W for 30 – 90 seconds each, finding that 1 W for 60 s did not cause post-LITT mortality (**Fig. 2A**, red points = 24h mortality). Notably, temperature measurements at lower power settings appeared more consistent overall (**Fig. 2B - 2D**). On H&E staining at 24h, ablations at lower powers and times produced a controlled ablation that did not char tissue (**Fig. 2E**). These data suggest higher, mortality-associated doses (1.5 W for 90s and beyond) produced large cavitary lesions associated with tissue vaporization and charring, causing inaccuracy in temperature measurement **(Fig. 2F**).

Although real-time MR thermometry is not feasible for high-throughput animal studies, we used serial T2-weighted MRI imaging to accurately track CT-2A tumor growth (**Fig. 3A**,n = 5 mice) and identify a safe lesion size for ablation, selecting 10 days post-implantation for subsequent experiments **(Fig. 3B).** Based on tumor diameter at these timepoints, we selected 2 mm posterior to the implantation site to monitor temperature at the lesion edge to mitigate damage to surrounding tissue. (**Fig. 3C**).

RESULTS

Figure 1. Murine LITT System
A needle thermocouple and fiber optic were threaded into a 1 mL syringe held in a stereotactic probe holder (left panel) and inserted into a burr-hole craniotomy (middle and right panels) to a depth of 3 mm at +2 ML, +1 AP.

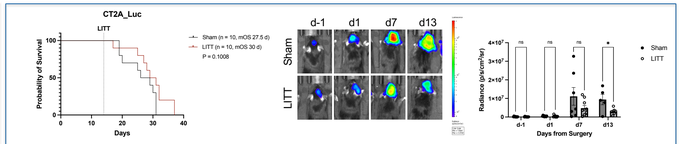

Figure 5. Semi-quantitative Assessment of Cytoreduction
A luciferase-expressing subclone of CT-2A (CT2A_Luc) was implanted into mice for serial imaging pre- and post-LITT. **A.** There was a numerical difference in overall survival in LITT-treated mice. **B.** Representative images and **C.** comparison of radiance at each imaging timepoint. * = P < 0.05, Student's t-test.

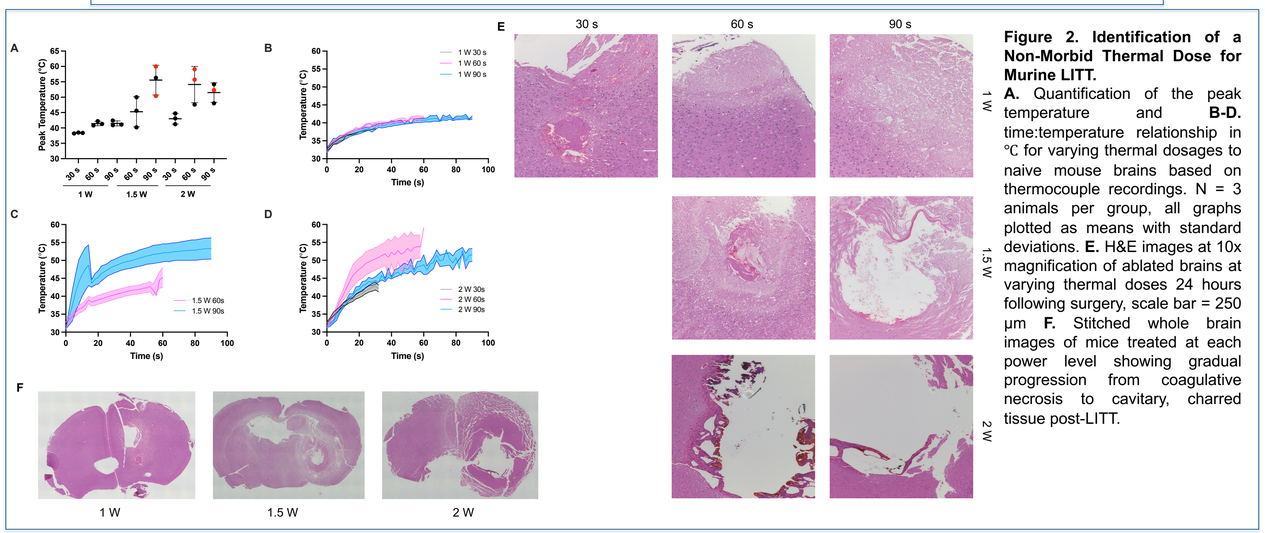

Figure 2. Identification of a Non-Morbid Thermal Dose for Murine LITT.
A. Quantification of the peak temperature and **B-D.** time:temperature relationship in ℃ for varying thermal dosages to naive mouse brains based on thermocouple recordings. N = 3 animals per group, all graphs plotted as means with standard deviations. **E.** H&E images at 10x magnification of ablated brains at varying thermal doses 24 hours following surgery, scale bar = 250 µm **F.** Stitched whole brain images of mice treated at each power level showing gradual progression from coagulative necrosis to cavitary, charred tissue post-LITT.

We next implanted CT-2A tumors for ablation with the selected dose, following mice for survival between the LITT and sham-ablated groups. A subset of mice underwent imaging at 1, 3, and 7 days post-LITT. The peak temperature at the lesion edge achieved with intra-tumoral ablations was 43.79 ± 2.99 ℃ **(Fig. 4A-B**), slightly higher than that of naïve brain tissue at the same thermal dose (41.43 ± 0.71 ℃). Median survival for sham-operated mice was 25.5 days vs 28 days for LITT-treated mice, P = 0.0172, **(Fig. 4C**). T2-weighted MRI scans revealed hyperintense lesions consistent with coagulated, hemorrhagic portions of treated tumor at post-LITT days 1 and 3 **(Fig. 4D**). Interestingly, tumor did not appear to regrow in these spaces at post-LITT day 7, with flow voids remaining in their place. H&E staining of mice at sacrificed at clinical endpoints revealed persistent hemorrhagic and fibrotic lesions along the fiber tract (**Fig. 4E**). These findings were also seen on studies employing bioluminescence imaging (**Fig. 5A– 5C**).

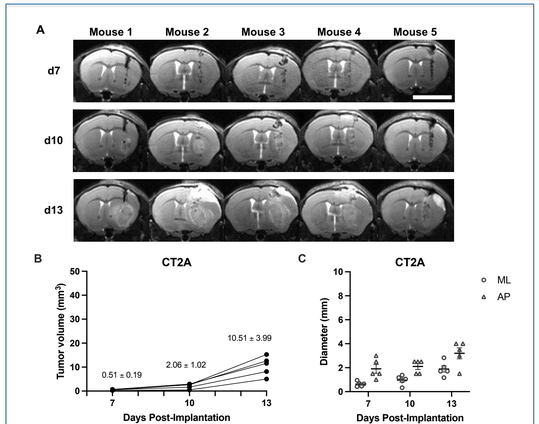

Figure 3. Segmentation of Murine Brain Tumors Yields Parameters for LITT Targeting without Real-Time MR Thermometry
A. Serial T2-weighted MRI of CT2A brain tumors at day 7, 10, and 13 post-implantation. N = 5 mice, scale bar = 5 mm. **B-C.** Segmented tumor volumes and perpendicular diameters at each time point. All values are means and standard deviations.

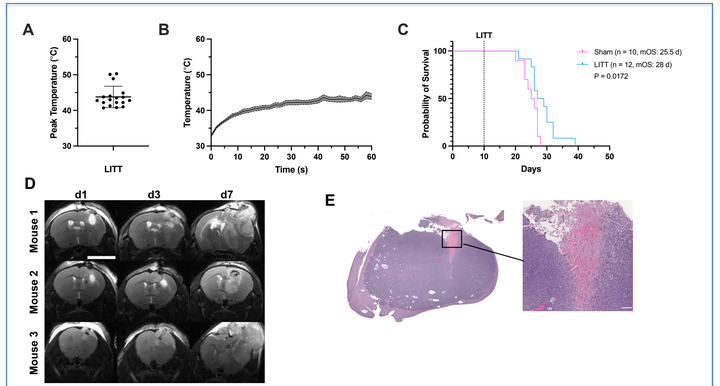

Figure 4. Murine LITT Increases Survival and Recapitulates Essential Clinicopathological Findings
A. Peak temperature and **B.** time:temperature curves for mice treated with LITT. N = 18 mice, error bars = mean and standard deviation. **C.** Kaplan-Meier curve of survival for sham surgery (burr-hole craniotomy and dural puncture) versus LITT-treated mice. Median overall survival = 25.5 vs 28 days, log-rank test, P = 0.0172. **D.** Serial T2-weighted MRI of mice treated with LITT at post-LITT day 1, 3, and 7. Scale bar = 5 mm. **E.** Representative H&E staining of a LITT-treated mouse at clinical endpoint. Left panel = stitched whole brain image, inset = 10x magnification, scale bar = 250 µm.

CONCLUSIONS

- Our model successfully recapitulates the essential clinical features of LITT: a cytoreductive survival benefit, lesion expansion on MRI, and focal necrosis with hemorrhagic vasculature on H&E.
- Our model can be used in a modular fashion to investigate the effects of laser-induced hyperthermia on syngeneic glioma or brain metastasis cell lines, as well as in patient-derived xenografts or murine epilepsy paradigms.

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