Histotripsy, a novel image-guided, robotically assisted sonic therapy platform, is a non-invasive and non-thermal tumor ablation modality. We have previously shown that histotripsy potentiates profound innate and adaptive anti-tumor responses in addition to direct tumor destruction. In this study, we sought to characterize the biomarkers of tumor cell death pathways immediately after histotripsy and after the induction of adaptive anti-tumor immune responses in preclinical settings.

Methods Immunocompetent C57BL/6 mice were inoculated with bilateral subcutaneous flank injections of Hepa1-6 hepatocellular carcinoma to generate 8–10 mm tumors within 8–11 days. Unilateral subcutaneous histotripsy was then performed. Mice were euthanized at 6h, and 1, 3 and 10–12 days post-treatment (dpt). Tumors were measured, harvested, fixed, sectioned and studied using multicolor immunohistochemistry.

Results Histotripsy decreased treated tumor growth by 50% and abscopal tumor growth by 30–40% compared to untreated tumors at 12dpt, evidencing a systemic anti-tumor immune response that inhibited growth of distant untreated tumor. Treated tumors showed immediate tissue liquefaction in the ablation zone with marked extranuclear translocation of the damage associated molecular pattern HMGB1. At 1dpt, 100% of tumor cells within the ablation zone showed HMGB1 translocation, and 70% of tumor cells at the periphery of the ablation zone showed HMGB1 translocation. Caspase 3 cleavage was not observed in the direct ablation zone, but at the junction of the ablated and non-ablated tissue ~40% cells that released HMGB1 showed cleaved Caspase 3. Caspase 9 cleavage was observed in ~50% cells that had cleaved Caspase 3, suggesting early programed cell death with mitochondrial damage and cytochrome C release 1 dpt; the presence of inflammasome integration/activation suggested pyroptosis induction. Areas of tumor well outside the zone of ablation and within untreated tumors contralateral to ablated tumors did not show early DAMP release or apoptotic cell death compared to the control tumors. However, a robust immune cell infiltration was observed in these locations at 10–12dpt, involving CD8 T-cell infiltration and areas of tumoral HMGB1 release in the vicinity of the infiltrating CD8 T cells - indicating the induction of immune rejection of treated and untreated tumors by histotripsy.

Conclusions Our results indicate that histotripsy ablation promotes tumor cell destruction through both immediate mechanical disruption, as well as possible adjacent apoptotic and pyroptotic death. Systemic CD8 T-cell mobilization and immunological cell death in the treated and the contralateral tumors is a novel long term therapeutic benefit.

REFERENCE