Nanopulse Stimulation (NPS) is a non-thermal tumor therapy that delivers ultrashort electrical pulses (100-600ns) to tumor cells. NPS opens nanopores in the membrane of the ER, allowing the efflux of Ca²⁺ into the cytoplasm, causing ER stress and the production of ROS. These effects induce an immunogenic cell death (ICD) that both eliminates a primary tumor and inhibits the growth of a secondary re-challenge tumor in preclinical models1. To date, the primary mechanisms of action of most known ICD inducers is ER stress and ROS production leading to intrinsic mitochondrial apoptosis, and the release and translocation of damaged associated molecular patterns (DAMPs)² that bind to pattern recognition receptors (PRRs) to prime the adaptive immune response. Here we sought to profile the pathways involved in ER stress, apoptotic cell death and the immune response after NPS treatment, using the NanoString PanCancer Immune Panel with an additional 30 spike-in genes designed to investigate apoptotic cell death pathways.

Methods
C57/B6 albino mice (N=12) were injected intradermally with 1million syngeneic B16-F10 melanoma cells into the left flank. When tumors reached ~5mm in diameter they were treated with NPS (N=5, 500 pulses, 200 ns in duration applied at 25 kV/cm at 5 pps) or were surgically resected and harvested as untreated tumor controls (G1; N=3). Tumors treated with NPS were harvested 2hrs (G2; N=3), 4hrs (G3; N=3) and 24hrs (G4; N=3) after treatment and placed into formalin for fixation followed by embedding in paraffin. mRNA was extracted and hybridized to bar-coded probes that correspond to 800 gene transcripts (770 PanCancer Immune Panel + 30 spike-in). Transcripts were read using the NanoString nCounter® and analyzed with nSolver software (see below).

NanoString Technology Overview

References
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Conclusions
NanoString profiling revealed that transcripts coding for components previously identified as important for the mechanism of ICD, such as ER stress-induced intrinsic apoptotic pathways, key DAMPs and PRRs, as well a number of immune mediators and cells involved in priming the adaptive immune response were upregulated in tumor tissues 24hrs after NPS treatment. We plan to continue to utilize the NanoString platform in future studies to help us to further understand the mechanisms involved in NPS-treatment of malignant tumors.