Background Enhancing anti-tumor immunity is a foundational therapeutic strategy against cancer.1 The Aliya Pulsed Electric Fields (PEF) proprietary system has been shown to promote local and systemic anti-tumor immunity activation in pre-clinical murine models through the activation of immunogenic cell death (ICD) and the release of damage associated molecular patterns (DAMPs) and tumor-specific antigens.2 3 Unlike apoptosis, often immunosuppressive, ICD mechanisms generate a potent immune response by releasing DAMPs that are recognized by and attract immune cells,4 ICD mechanisms can suppress cancer cell proliferation and migration.5 6 In this study we evaluated PEF’s capability for modulating anti-tumor immunity in patients with NSCLC.

Methods The treat-and-resect INCITE ES study enrolled patients with suspected or confirmed NSCLC stage IA2-IB and no cancer treatment history within two years. This two-arm study included 34 patients in the treatment group and 8 patients in the control group. Treatment group subjects received AliyaTM PEF (GTI-00018 investigational device; Galvanize Therapeutics) after the diagnostic biopsy, whereas the control group only had the biopsy. Blood, serum, and tissue samples were collected pre- and post-PEF at specific time-points (figure 1).

Results Ingenuity Pathway Analysis (IPA) of serum cytokines predicts that host innate immunity pathways that drive immune cell trafficking, differentiation and activation are activated in PEF-treated samples, including the Acute Phase Response, IL-6, JAK-STAT and Th17/IL-17 signaling (figure 2A).7–9 scRNA-Seq from this initial cohort of patients additionally shows that PEF-treated tumors have a significant increase in the proportion of plasma B cells, T cells and neutrophils, with neutrophils expressing genes indicative of functional activation (figure 2B).7 In samples from PEF-treated patients, IPA further predicts activation of ICD mechanisms, such as pyroptosis and HMGB1 signaling (figure 3A,B).4 10 After this initial innate immune response, IPA predicts activation of Th2 signaling, responsible for dampening acute inflammation and orchestrating adaptive anti-tumor immunity (figure 4A).11 Flow cytometry analysis further suggests activation of adaptive immunity, as PEF samples have higher levels of circulating B cells and effector memory T cells, and lower Tregs (figure 4B). Within the tumor microenvironment, PEF tumors show increased proportion of cytotoxic CD8+ T cells, plasma B cells and tumor leukocytes overexpressing antigen-presenta-
Abstract 697 Figure 1 Sample collection and time points used for examining local and systemic immune effects of PEF treatment. (A) Enrolled patients underwent a standard of care diagnostic biopsy to confirm their tumor was malignant. After malignancy was confirmed, patients in the treatment group received PEF. Tumor tissue was collected on the day of the diagnostic biopsy (Pre-PEF, labeled as ‘biopsy’ in the diagram) and on the day of tumor resection (22 ± 7 days [average ± SD] after PEF; labeled as ‘resected tissue’ in the diagram). Peripheral blood and serum samples were collected prior to PEF (day 0), and approximately on days 3, 10, 21, and on the day of tumor resection, as indicated by the blue (serum samples) and purple (blood samples) arrows. A CT-scan was performed 0 and prior to resection (gray arrows). Subjects in the control group received a biopsy on day 0 but no PEF, and all other samples were collected as in the treatment group. (B) Single-cell RNA-seq (scRNA-Seq) from pre-PEF and post-PEF tumor samples (n=8 pre-PEF, n=9 post-PEF) was performed to examine PEF-induced changes in the relative cell frequency and gene expression of tumor immune cells. Flow cytometry and serum cytokine profiling were used to evaluate systemic changes in the immune cell populations and levels of 71 cytokines/chemokines at each timepoint relative to Day 0.

Abstract 697 Figure 2 PEF energy transiently activates the innate immune response systemically and within the tumor microenvironment (TME). (A) Heatmap of serum cytokine Ingenuity Pathway Analysis showing the most significantly affected pathways predicted to be activated or inhibited in response to PEF energy delivery. Serum cytokines were analyzed using a 71-analyte Luminex multiplex assay (n=30 treated, n=6 control). Heatmap values correspond to Z-score activity predictions, based on the log2 ratio (PEF-treated samples/control samples) of the cytokine values at each timepoint with respect to the baseline values (Day 0). Orange and blue represent predicted activation and inhibition, respectively. A manually curated list of innate immune signaling pathways with absolute Z-score values >2 (significant) is shown, with early activation at days 3 or 10 that subsides by resection. (B) Signaling pathways enriched in genes upregulated in neutrophils from PEF-treated tumors compared to non-treated control tumors. Top signaling pathways based on adjusted p-value significance from Enrichr MSigDB Hallmark 2020 are shown [12].

Abstract 697 Figure 3 PEF energy induces immunogenic cell death mechanisms. (A) Heatmap of serum cytokine Ingenuity Pathway Analysis showing the predicted pattern of activation of immunogenic cell death signaling pathways, pyroptosis, and HMGB1 signaling. Heatmap values correspond to Z-score activity predictions, based on the log2 ratio (PEF-treated samples/Control samples) of the cytokine values at each timepoint with respect to the baseline values (Day 0). Orange and blue represent predicted activation and inhibition, respectively. (B) Heatmap showing the relative expression of the TXNIP gene in the indicated populations obtained from scRNA-Seq. Values correspond to the normalized average expression of TXNIP in cells from biopsy samples (n=8, pre-PEF) and resected PEF-treated tumors (n=9, post-PEF). TXNIP encodes for thioredoxin-interacting protein, a major regulator of cellular redox signaling that binds the NLRP3 inflammasome and has essential functions in pyroptosis and as a tumor suppressor gene [13].
Abstract 697 Figure 4  Adaptive immune mechanisms induced by PEF. (A) Heatmap of serum cytokine Ingenuity Pathway Analysis showing the predicted activation pattern of the Th2 signaling pathway. Heatmap values correspond to Z-score activity predictions, based on the log2 ratio (PEF-treated samples/Control samples) of the cytokine values at each timepoint with respect to the baseline values (Day 0). Orange and blue represent predicted activation and inhibition, respectively. (B) Flow cytometry analysis of peripheral blood B cells, effector memory T cells, and Tregs in PEF-treated samples and control samples (n=3 treated, n=3 control) approximately 10 days after PEF energy delivery. (C) Cell populations showing significantly increased expression of the indicated antigen-presenting genes (HLA-DQA2, HLA-DQB1, HLA-DRB5, HLA-DRA) via scRNA-Seq in tumors treated with PEF (n=9, post-PEF) compared to biopsy samples (n=8, pre-PEF).

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