

TERTIARY LYMPHOID STRUCTURES (TLS) OBSERVED IN NON-SMALL CELL LUNG CANCER (NSCLC) TUMORS TREATED WITH PULSED ELECTRIC FIELDS

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Background Tertiary lymphoid structures (TLS) may develop in non-lymphoid tissues in response to a variety of different stimuli and can serve as foci for generating anti-tumor immunity.¹ TLS formation is emerging as a strong prognostic and predictive biomarker² associated with patient survival benefits in NSCLC.^{3,4} Pulsed Electric Fields (PEF) have been reported to induce an immunogenic form of cell death and thus may enhance adaptive immunity in the setting of cancer. The treat-and-resect INCITE ES study enrolled adults with suspected or confirmed NSCLC stage IA2-IB (>1 to ≤4 cm) and without a history of treatment for cancer within the previous two years. **Methods** The INCITE ES study design includes both control and treatment groups with 8 enrolled control group subjects and 30 enrolled treatment group subjects. Treatment group subjects received PEF (Aliya™ System, GTI-00018 investigational device; Galvanize Therapeutics, San Carlos, CA) either percutaneously or endoscopically at time of biopsy prior to surgical resection. Blood, bronchoalveolar lavage (BAL) when applicable, and tissue samples were collected over the course of the study for appropriate pre- and post-PEF comparison.

Serial histologic sections were obtained from an initial cohort of 12 patients (n=1 control, n=11 treatment group) on the day of surgery 17-21 days post-PEF delivery, stained for standard H&E as well as duplex stained for pan-cytokeratin (panCK) and CD20, and reviewed by an independent pathologist.

Results TLS were identified and characterized according to their maturity and localization within or adjacent to the tumor (see criteria in Table 1). Intratumor TLS were observed admixed among tumor cells or within the invasive margin (figures 1 to 5), including within the cellular depletion zone induced by PEF (figures 6 and 7). Independent of tumor morphology, a significant quantity of 49.8 ± 55.8 TLS per tumor was observed post-PEF (n=11, average \pm S.D.). TLS across treated tumors showed varying proportions of mature vs. immature TLS, using the criteria in Table 1. No TLS were identified in the available pre-PEF biopsy specimens (figures 2 and 8). TLS density was greater in PEF specimens compared to the non-treated control, where only three immature TLS were observed (figure 9).

Conclusions This initial cohort suggests that PEF may induce the formation of TLS within the tumor, including proximal to the PEF delivery zone. The observed density and detection of mature TLS may suggest ongoing immune activity. As such, PEF has the potential to induce or enhance an immune response irrespective of tumor morphology.

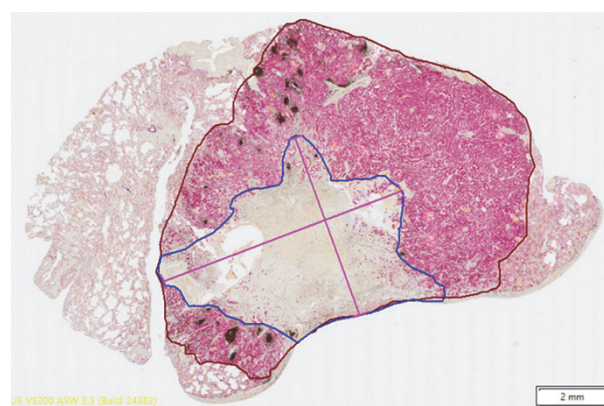
Trial Registration The study is registered on clinicaltrials.gov (NCT04732520).

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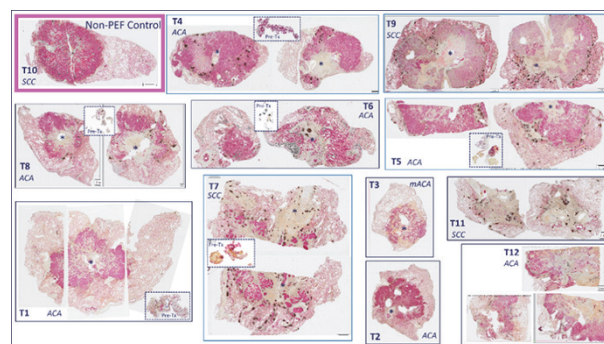
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Ethics Approval This abstract discusses the INCITE ES clinical study. Participants gave informed consent before taking part in the study. The study obtained ethics approval from the Ethics Committee for Research with Drugs (CEIm) of the Salamanca Health Area (Salamanca, Spain, reference 20/1615 (E.C.P.S.)), Committee on Research Involving Human Subjects (CMO) of Radboud University Medical Center (Nijmegen, the Netherlands, NL76406.091.21), and the Joint Chinese University of Hong Kong – New Territories East Cluster Research Ethics Committee (Hong Kong SAR, reference 2021.294-T).

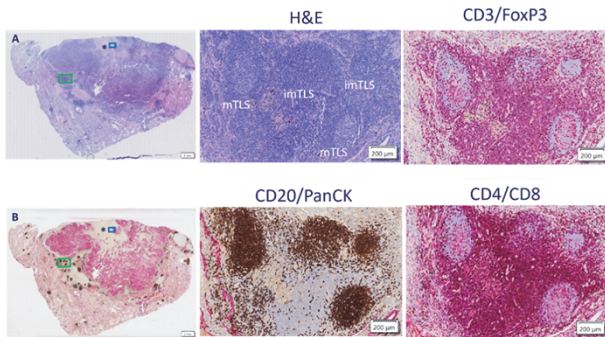


Abstract 702 Figure 1 Example of outlined resected tumor region and CDZ post-PEF. Tumors were resected post PEF delivery and underwent immunostaining. The duplex staining with PanCK (pink) identifies epithelial cells and CD20+ B cells (brown). Areas of dense pink coloration are tumor and the remaining tissue is non-cancerous lung parenchyma. The residual tumor area is outlined in maroon to the leading tumor edge adjacent to non-cancerous lung parenchyma. The PEF-induced cellular depletion zone (CDZ) is outlined in blue. On average, the CDZ measures 0.9 ± 0.3 cm (longest dimension) by 0.6 ± 0.2 cm (longest perpendicular dimension) after a single PEF delivery (n=9).

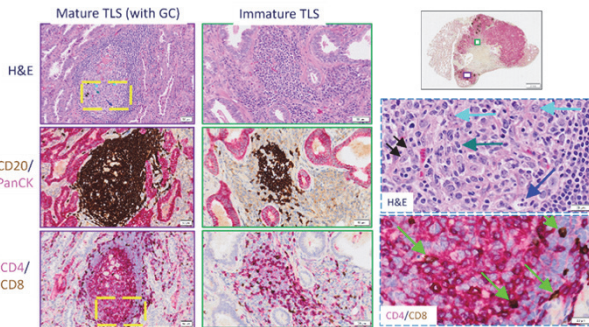


Abstract 702 Figure 2 Compilation of resected NSCLC tumors from INCITE ES study. Tumors underwent duplex PanCK and CD20+ staining. One non-treatment control specimen resected 35 days post biopsy is included (T10). Tumors after a single delivery of PEF energy were resected 17 to 21 days after PEF. Inset images are of pre-PEF biopsy specimens with duplex stain, when available. Colored asterisk (*) in each image denotes the estimated location of the cellular depletion

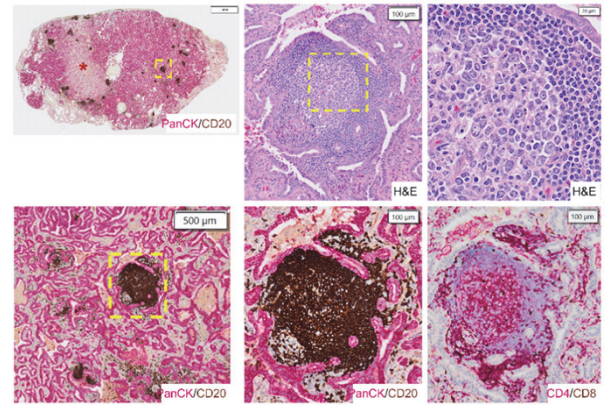
zone. Acronyms ACA, mACA, and SCC indicate tumor histopathology adenocarcinoma, mucinous adenocarcinoma, and squamous cell carcinoma, respectively.



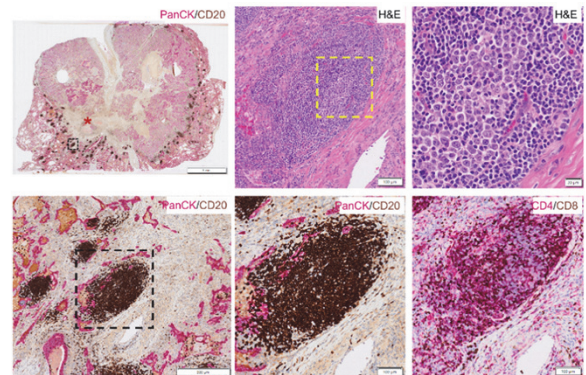
Abstract 702 Figure 3 TLS in tumor periphery 19 days after a single PEF delivery. Representative H&E and IHC images of a right upper lobe tumor. Characterization of the TLS indicates presence of CD20+ B cells (brown), CD3+ T-cells (red), FoxP3+ regulatory T-cells (Tregs, brown), CD4+ helper T-cells (red) and cytotoxic CD8+ T-cells (brown). Higher magnification images of the area denoted by the green box in A and B show two mTLS and two immature imTLS. Asterisks (*) in images shown in A and B denote the estimated location of the cellular depletion zone.



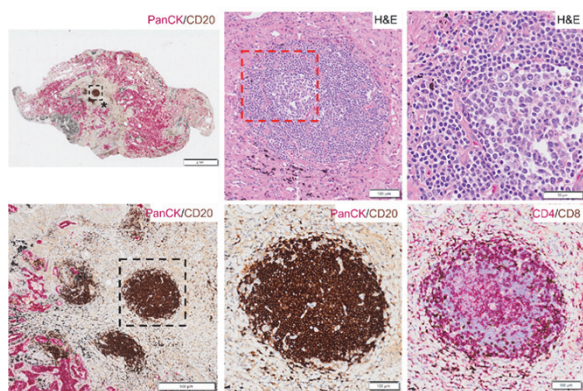
Abstract 702 Figure 4 Mature and immature TLS in a tumor post-PEF energy delivery. Representative H&E and IHC images of a left lower lobe tumor. Purple-outlined (left column) and green-outlined (middle column) boxes representing mature TLS (mTLS) and immature TLS (imTLS), respectively, are higher power magnification of the specimen sampled at approximately 7 o'clock and 12 o'clock, respectively, in the wide field view (upper right). Both mTLS and imTLS are identified by a collection of dense, small CD20+ B cells (brown, middle row), generally juxtaposed with T cell aggregate (CD4+ helper T-cells (red) and cytotoxic CD8+ T-cells (brown), bottom row). The mTLS contains a germinal center (GC) discernable via H&E. The center of the GC is shown in the right column (yellow-dashed outline represents area of higher magnification) to identify dendritic cells (black arrows) and CD8+ T-cells (bright green arrows). Mature CD20+ B cells within the GC exhibit the characteristic morphology of proliferating centroblasts (cyan arrows) and centrocytes (dark green arrow). A GC may also contain tingible body macrophages (dark blue arrow) licensed for phagocytosis by follicular dendritic cells within the germinal center.



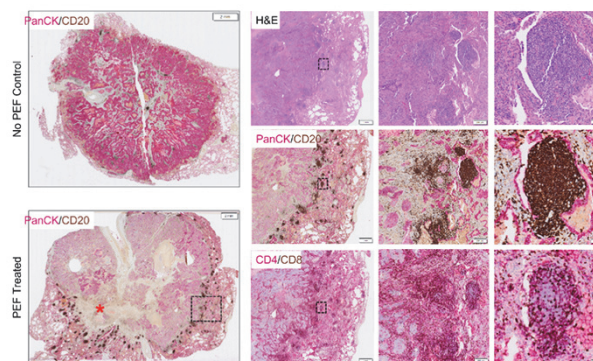
Abstract 702 Figure 5 Representative mature TLS located intratumorally post-PEF. Images were captured from duplex PanCK and CD20, duplex CD4 and CD8, and H&E-stained tissue sections of tumor T4. The whole tissue from the resection sample is shown with red asterisk (*) denoting the estimated location of the cellular depletion zone and the yellow dashed box indicating the mTLS within the tumor bed that is shown at higher magnification as indicated in subsequent image panels. The mTLS contains a germinal center (GC) discernable via H&E. The high magnification image of the region denoted by the dashed yellow box in the H&E image demonstrates mature CD20+ B cells within the GC exhibiting the characteristic morphology of proliferating centroblasts and centrocytes. Accumulation of CD4+ and CD8+ T cells can be seen within and surrounding the mTLS.



Abstract 702 Figure 6 A mature TLS located at the CDZ-lung parenchyma interface. Images were captured from duplex PanCK and CD20, duplex CD4 and CD8, and H&E-stained tissue sections of tumor T9. The whole tissue from the resection sample is shown with the red asterisk (*) denoting the estimated location of the CDZ and the black dashed box indicating the mTLS that is shown at higher magnification as indicated in subsequent image panels. The mTLS contains a germinal center (GC) discernable via H&E. The high magnification image of the region denoted by the dashed yellow box in the H&E image demonstrates mature CD20+ B cells within the GC exhibiting the characteristic morphology of proliferating centroblasts and centrocytes. Accumulation of CD4+ and CD8+ T cells can be seen within and surrounding the mTLS.

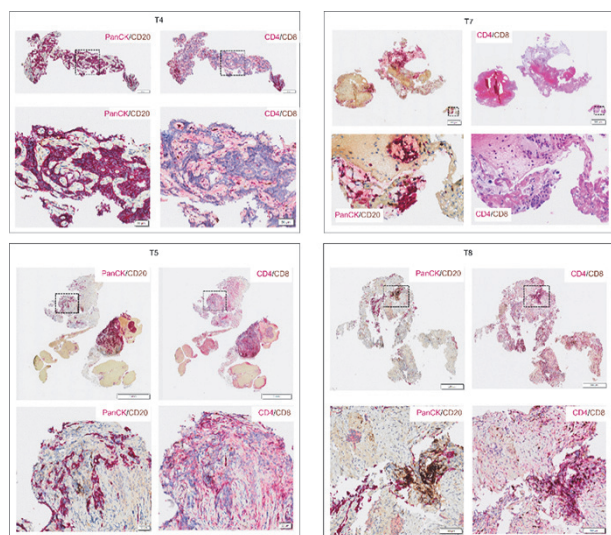


Abstract 702 Figure 7 A mature TLS located within the cellular depletion zone (CDZ). Images were captured from duplex PanCK and CD20, duplex CD4 and CD8, and H&E-stained tissue sections of tumor T6. The whole tissue from the resection sample is shown with the black asterisk (*) denoting the estimated location of the CDZ and the black dashed box indicating the mTLS within the CDZ that is shown at higher magnification as indicated in subsequent image panels. The mTLS contains a germinal center (GC) discernable via H&E. The high magnification image of the region denoted by the dashed red box in the H&E image demonstrates mature CD20+ B cells within the GC exhibiting the characteristic morphology of proliferating centroblasts and centrocytes. Accumulation of CD4+ and CD8+ T cells can be seen within and surrounding the mTLS.



Abstract 702 Figure 9 TLS accumulation in PEF-treated tumor and control tumor. Non-PEF control (T10) and a PEF-treated (T9) demonstrating the accumulation of TLS at the tumor periphery in the PEF-treated sample. Images were captured from duplex PanCK and CD20, duplex CD4 and CD8, and H&E-stained tissue sections. The whole tissue from the resection sample is shown with the red asterisk (*) denoting the estimated location of the CDZ in the PEF-treated sample, and the black dashed box indicating an imTLS that is shown at the invasive tumor margin at higher magnification as indicated in subsequent image panels (right). Accumulation of CD4+ and CD8+ T cells can be seen within and surrounding the imTLS.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0702>



Abstract 702 Figure 8 Representative pre-PEF tumor biopsy specimens. Images were captured from duplex PanCK and CD20 and duplex CD4 and CD8 stained tissue sections of tumors T4, T5, T7, and T8. The black dashed box indicates the region of the tissue at shown at higher magnification in the lower panels for each sample. While some CD20+ staining is evident in the pre-treatment biopsy from sample T8, this did not meet the criteria for being considered a TLS.