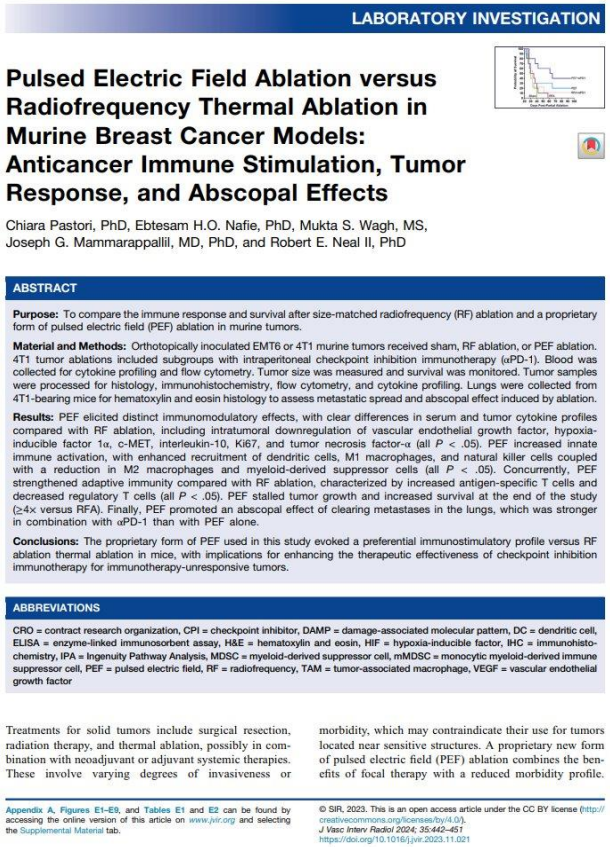
Disclosure for Galvanize Website That References Scientific Information on Unapproved Uses (SIUU)

- These references include publications from studies sponsored by Galvanize Therapeutics.
- Two references summarize pre-clinical studies that investigated Aliya PEF effects on orthotopic murine breast cancer tumors.
- Two references summarize data reported from INCITE-ES (NCT04732520), a safety and feasibility study focused on early-stage, resectable NSCLC.
- While sound scientific principles were used to identify the components of the host immune response (both in the murine animal model and the human patient) as reported in each reference, each study has significant limitations that prevent the definitive assignment of Aliya PEF as having demonstrated clinical benefit in any human cancer patient population.
- At a minimum, additional clinical assessment is required to address these limitations.
- "The Aliya System" is cleared by the FDA for the surgical ablation of soft tissue but has not been cleared by FDA for any therapeutic indication, and the safety and effectiveness of the Aliya System for treatment of human cancer of any type has not been established. FDA clearance is restrictive, and we cannot promote the uses or properties of the Aliya System that fall outside the cleared intended use statement. FDA's guidance documents, however, allow us to provide clinically relevant scientific information such as these references.

Pastori et al. "Pulsed Electric Field Ablation versus Radiofrequency Thermal Ablation in Murine Breast Cancer Models: Anticancer Immune Stimulation, Tumor Response, and Abscopal Effects". Journal of Vascular and Interventional Radiology35, no. 3 (March 1, 2024): 442-451.e7.

- Results from an animal study comparing the local and metastatic tumor response and immunostimulatory effects from using the Aliya System with a PEF dose titrated to ablate ~80% of mouse tumor volumes compared to similarly dosed radiofrequency ablation were reported. The authors present data showing preferential immunogenicity for PEF, reduced tumor growth, and better survival. They report PEF results in reduced pro-oncologic factors within the tumor, promoted immunostimulatory cytokines, increased local innate immunity, recruited dendritic cells, natural killer cells, and M1 macrophages, while decreasing M2 macrophages and myeloid-derived suppressor cells compared with RF ablation. The authors show PEF causes increased systemic adaptive immunity, including activated and antigen-specific T cells, and decreased regulatory T cells compared with RF ablation. The authors report that PEF improved metastatic clearance compared with RF ablation, which was further enhanced when combined with anti-PD-1 therapy.

- The limitations of the study include that it was performed in an animal model. While the analysis is an indication of potential Aliya System performance in an animal model of breast cancer, clinical assessment is required to address these limitations and applicability in human cancer patients.



LABORATORY INVESTIGATION

Pulsed Electric Field Ablation versus Radiofrequency Thermal Ablation in Murine Breast Cancer Models: Anticancer Immune Stimulation, Tumor Response, and Abscopal Effects

Chiara Pastori, PhD, Ebtesam H.O. Nafie, PhD, Mukta S. Wagh, MS, Joseph G. Mammarappallil, MD, PhD, and Robert E. Neal II, PhD

ABSTRACT

Purpose: To compare the immune response and survival after size-matched radiofrequency (RF) ablation and a proprietary form of pulsed electric field (PEF) ablation in murine tumors.

Material and Methods: Orthotopically inoculated EMT6 or 4T1 murine tumors received sham, RF ablation, or PEF ablation. 4T1 tumor ablations included subgroups with intraperitoneal checkpoint inhibition immunotherapy (αPD-1). Blood was collected for cytokine profiling and flow cytometry. Tumor size was measured and survival was monitored. Tumor samples were processed for histology, immunohistochemistry, flow cytometry, and cytokine profiling. Lungs were collected from 4T1-bearing mice for hematoxylin and eosin histology to assess metastatic spread and abscopal effect induced by ablation.

Results: PEF elicited distinct immunomodulatory effects, with clear differences in serum and tumor cytokine profiles compared with RF ablation, including intratumoral downregulation of vascular endothelial growth factor, hypoxia-inducible factor 1α, c-MET, interleukin-10, Ki67, and tumor necrosis factor-α (all $P < .05$). PEF increased innate immune activation, with enhanced recruitment of dendritic cells, M1 macrophages, and natural killer cells coupled with a reduction in M2 macrophages and myeloid-derived suppressor cells (all $P < .05$). Concurrently, PEF strengthened adaptive immunity compared with RF ablation, characterized by increased antigen-specific T cells and decreased regulatory T cells (all $P < .05$). PEF stalled tumor growth and increased survival at the end of the study (≥4× versus RFA). Finally, PEF promoted an abscopal effect of clearing metastases in the lungs, which was stronger in combination with αPD-1 than with PEF alone.

Conclusions: The proprietary form of PEF used in this study evoked a preferential immunostimulatory profile versus RF ablation thermal ablation in mice, with implications for enhancing the therapeutic effectiveness of checkpoint inhibition immunotherapy for immunotherapy-unresponsive tumors.

ABBREVIATIONS

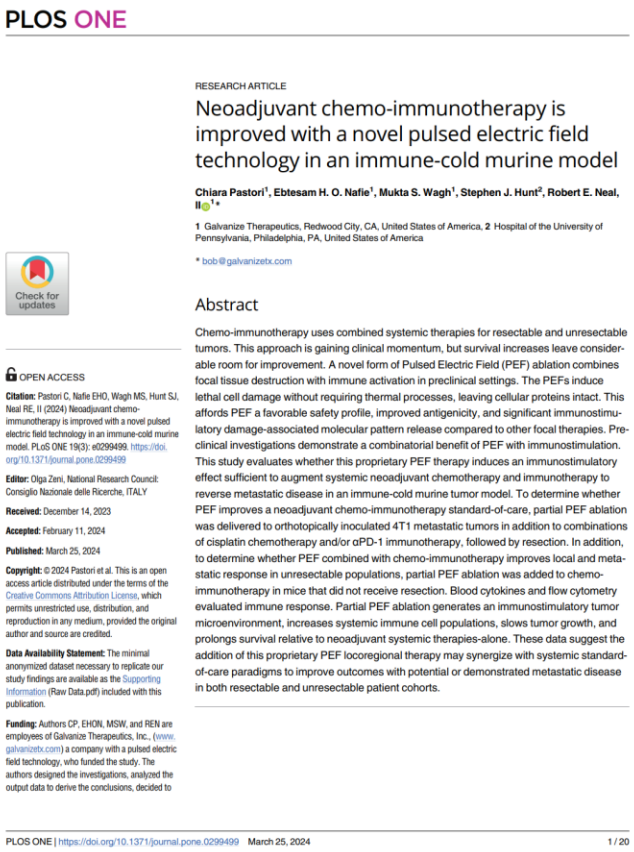
CRO = contract research organization, CPI = checkpoint inhibitor, DAMP = damage-associated molecular pattern, DC = dendritic cell, ELISA = enzyme-linked immunosorbent assay, H&E = hematoxylin and eosin, HIF = hypoxia-inducible factor, IHC = immunohistochemistry, IPA = Ingenuity Pathway Analysis, MDSC = myeloid-derived suppressor cell, mMDSC = monocytic myeloid-derived immune suppressor cell, PEF = pulsed electric field, RF = radiofrequency, TAM = tumor-associated macrophage, VEGF = vascular endothelial growth factor

Treatments for solid tumors include surgical resection, radiation therapy, and thermal ablation, possibly in combination with neoadjuvant or adjuvant systemic therapies. These involve varying degrees of invasiveness or morbidity, which may contraindicate their use for tumors located near sensitive structures. A proprietary new form of pulsed electric field (PEF) ablation combines the benefits of focal therapy with a reduced morbidity profile.

Pastori et al. “Neoadjuvant Chemo-Immunotherapy Is Improved with a Novel Pulsed Electric Field Technology in an Immune-Cold Murine Model.”PLOSONE19, no. 3 (March 25, 2024): e0299499

- This study evaluates whether a proprietary PEF therapy induces an immunostimulatory effect sufficient to augment systemic neoadjuvant chemotherapy and immunotherapy to reverse metastatic disease in an immune-cold murine tumor model. Partial PEF ablation was delivered to orthotopically inoculated 4T1 metastatic tumors in addition to combinations of cisplatin chemotherapy and/or αPD-1 immunotherapy, followed by resection. In addition, to determine whether PEF combined with chemo-immunotherapy improves local and metastatic response in unresectable populations, partial PEF ablation was added to chemo-immunotherapy in mice that did not receive resection. Blood cytokines and flow cytometry evaluated immune response. Partial PEF ablation was found to generate an immunostimulatory tumor microenvironment, increase systemic immune cell populations, slow tumor growth, and prolong survival relative to neoadjuvant systemic therapies-alone. The authors conclude that the addition of this proprietary PEF locoregional therapy may synergize with systemic standard-of-care paradigms to improve outcomes with potential or demonstrated metastatic disease in both resectable and unresectable patient cohorts.

- The limitations of the study include that it was performed in an animal model. While the analysis is an indication of potential Aliya System performance in an animal model of breast cancer, clinical assessment is required to address these limitations and applicability in human cancer patients.



PLOS ONE

RESEARCH ARTICLE

Neoadjuvant chemo-immunotherapy is improved with a novel pulsed electric field technology in an immune-cold murine model

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Abstract

Chemo-immunotherapy uses combined systemic therapies for resectable and unresectable tumors. This approach is gaining clinical momentum, but survival increases leave considerable room for improvement. A novel form of Pulsed Electric Field (PEF) ablation combines focal tissue destruction with immune activation in preclinical settings. The PEFs induce lethal cell damage without requiring thermal processes, leaving cellular proteins intact. This affords PEF a favorable safety profile, improved antigenicity, and significant immunostimulatory damage-associated molecular pattern release compared to other focal therapies. Preclinical investigations demonstrate a combinatorial benefit of PEF with immunostimulation. This study evaluates whether this proprietary PEF therapy induces an immunostimulatory effect sufficient to augment systemic neoadjuvant chemotherapy and immunotherapy to reverse metastatic disease in an immune-cold murine tumor model. To determine whether PEF improves a neoadjuvant chemo-immunotherapy standard-of-care, partial PEF ablation was delivered to orthotopically inoculated 4T1 metastatic tumors in addition to combinations of cisplatin chemotherapy and/or αPD-1 immunotherapy, followed by resection. In addition, to determine whether PEF combined with chemo-immunotherapy improves local and metastatic response in unresectable populations, partial PEF ablation was added to chemo-immunotherapy in mice that did not receive resection. Blood cytokines and flow cytometry evaluated immune response. Partial PEF ablation generates an immunostimulatory tumor microenvironment, increases systemic immune cell populations, slows tumor growth, and prolongs survival relative to neoadjuvant systemic therapies-alone. These data suggest the addition of this proprietary PEF locoregional therapy may synergize with systemic standard-of-care paradigms to improve outcomes with potential or demonstrated metastatic disease in both resectable and unresectable patient cohorts.

OPEN ACCESS

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Data Availability Statement: The minimal anonymized dataset necessary to replicate our study findings are available as the Supporting Information (Raw Data.pdf) included with this publication.

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J. Iding, et al. “Tertiary lymphoid structures (TLS) observed in non-small cell lung cancer (NSCLC) tumors treated with Pulsed Electric Fields”. Journal for Immunotherapy of Cancer 2022;10(Suppl 2):A1–A1595.

- The enclosed poster reprint contains a discussion of the authors’ findings from a histopathologic examination of early-stage NSCLC tumors resected after being treated with the Aliya System after patients enrolled in the INCITE-ES clinical study NCT04732520. 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, reviewed by an independent pathologist, and showed a significant quantity of TLS per tumor in post-PEF samples compared to control and that TLS maturity varied across treated tumors. The authors conclude that ablation with the Aliya System may induce the formation of TLS within the tumor, including proximal to the Aliya PEF delivery zone and that the observed accumulation and detection of mature TLS may suggest ongoing immune activity.

- The limitations of the poster include data presented on only a small cohort of initial patients that were available at the time of presentation (total patients enrolled N=36 treated and N=8 control) and comparisons made to a single control patient. In addition, INCITE-ES was designed as a safety and feasibility study and not powered to identify any effects of the Aliya System on the formation of TLS in treated tumors. Finally, while the analysis is an indication of potential Aliya System performance, additional clinical assessment is required to address these limitations.

Abstracts

702 **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 biomarker[2] 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-000118 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 ± 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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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

M. Jimenez, et al. “Pulsed Electric Fields Induce a Stepwise Activation of Host Anti-Tumor Immunity in Patients with Early-Stage Non-Small Cell Lung Cancer”. Journal for Immunotherapy of Cancer 2023;11(Suppl 1):A1–A1686.)

- The INCITE-ES study (NCT04732520) enrolled adults with suspected or confirmed NSCLC stage IA2-IB (>1 to ≤4 cm) and no cancer treatment history within two years. The two-arm study included 8 control and 34 treatment group patients. Treatment group patients received PEF from the Aliya System at the time of standard of care diagnostic biopsy prior to subsequent surgical resection. Their analysis revealed that Aliya PEF energy delivery shows the potential to trigger the activation of host innate immunity pathways that drive immune cell trafficking, differentiation and activation, including the Acute Phase Response, IL-6, JAK-STAT and Th17/IL-17 signaling. The authors report that Aliya PEF may induce multiple aspects of host adaptive immunity within treated tumors including increased proportion of plasma B cells and tumor leukocytes overexpressing antigen-presentation and antibody production genes, Th2 signaling (which is responsible for dampening acute inflammation and orchestrating adaptive anti-tumor immunity), as well as increasing levels of circulating B cells and effector memory T cells. The authors indicate that Aliya PEF may activate immunogenic cell death (ICD) mechanisms, such as pyroptosis and MAVS/STING signaling, which are able to induce host immune activation. The authors conclude that their findings from this initial cohort suggest Aliya PEF may be capable of inducing a stepwise activation of host anti-tumor immune mechanisms.

- While sound scientific principles were used to identify the components of the host immune response as reported in the poster, the study is limited by the lack of adequate control specimens for comparison. Further, INCITE-ES was designed as a safety and feasibility study and was not powered appropriately to identify any effects of the Aliya System on the host immune response. Finally, while the analysis is an indication of potential Aliya System performance, additional clinical assessment is required to address these limitations.

Abstracts

697 **PULSED ELECTRIC FIELDS INDUCES A STEPWISE ACTIVATION OF HOST ANTI-TUMOR IMMUNITY IN PATIENTS WITH EARLY-STAGE NON-SMALL CELL LUNG CANCER (NSCLC)**

[1,2]Marcelo Jimenez, [1]José Fernández García-Hierro, [3]Javier Flandes Aldeyturriaga, [4]Erik HFM van der Heijden, [5]Calvin Ng, [5]Rainbow Lau, [6]Borja Recalde, [2,6]Carlos Prieto, [2]Alberto Orfao, [4]Roel Verhoeven, [7]Alicia Moreno-Gonzalez, [7]Beryl Hatton, [7]Ebtesam Nafie, [7]Gloria Mas Martin*, [8]William Krimsky. *[1]Hospital Universitario de Salamanca, Salamanca, Castilla y Leon, Spain; [2]Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Castilla y Leon, Spain; [3]Hospital Universitario Fundacion Jimenez Diaz, Madrid, Madrid, Spain; [4]Radboudumc, Nijmegen, Gelderland, Netherlands; [5]The Chinese University of Hong Kong, Hong Kong, China; [6]Bioinformatics Service Servicio de Apoyo a la Investigación de la Universidad de Salamanca (NUCLEUS), Salamanca, Castilla y Leon, Spain; [7]Galvanize Therapeutics, Inc., Redwood City, CA, USA

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 immune 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 timepoints (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-presentation genes (figure 4C).

Conclusions Data from this initial cohort show the first clinical evidence that PEF may have the potential to induce inflammatory changes in the tumor microenvironment capable of engaging host innate and adaptive immune responses, which may elicit anti-tumor activity.

Acknowledgements The authors wish to acknowledge Dr Cristina Teodosio and Julio Pozo for their assistance with the flow cytometry analysis.

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

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Ethics Approval This study was approved by C.E.I.M. reference 20/1615 (E.C.P.S.) (Spain); CUHK-NTEC CREC 2021.294-T (Hong Kong); CMO Arnhem-Nijmegen Region NL-number: NL76406.091.21 (Netherlands).

Consent The patient was identified as an appropriate candidate and consented for study, standard of care procedures, and publication of the data for the Ethics Committee-approved INCITE ES trial (NCT04732520)