

UC Berkeley

UC Berkeley Previously Published Works

Title

Pulsed field ablation in medicine: irreversible electroporation and electropermeabilization theory and applications.

Permalink

<https://escholarship.org/uc/item/8f78c5bs>

Journal

Radiology and Oncology, 59(1)

Authors

Jacobs, Edward

Rubinsky, Boris

Davalos, Rafael

Publication Date

2025-03-01

DOI

10.2478/raon-2025-0011

Peer reviewed

review

Pulsed field ablation in medicine: irreversible electroporation and electropermeabilization theory and applications

Edward J Jacobs¹, Boris Rubinsky², Rafael V Davalos¹

¹ Wallace H Coulter School of Biomedical Engineering, Georgia Institute of Technology & Emory Medical School, Atlanta, Georgia, USA

² Department of Bioengineering and Department of Mechanical Engineering, University of California, Berkeley, Berkeley, California, USA

Radiol Oncol 2025; 59(1): 1-22.

Received 21 November 2024

Accepted 7 December 2024

Correspondence: Rafael Davalos, Ph.D., Wallace H. Coulter School of Biomedical Engineering, Georgia Institute of Technology & Emory Medical School, Atlanta, Georgia, USA. E-mail: Rafael.Davalos@bme.gatech.edu

Disclosure: R.D. and B.R. have patents in the field of bioelectrics.

This is an open access article distributed under the terms of the CC-BY license (<https://creativecommons.org/licenses/by/4.0/>).

Background. Focal ablation techniques are integral in the surgical intervention of diseased tissue, where it is necessary to minimize damage to the surrounding parenchyma and critical structures. Irreversible electroporation (IRE) and high-frequency IRE (H-FIRE), colloquially called pulsed-field ablation (PFA), utilize high-amplitude, low-energy pulsed electric fields (PEFs) to nonthermally ablate soft tissue. PEFs induce cell death through permeabilization of the cellular membrane, leading to loss of homeostasis. The unique nonthermal nature of PFA allows for selective cell death while minimally affecting surrounding proteinaceous structures, permitting treatment near sensitive anatomy where thermal ablation or surgical resection is contraindicated. Further, PFA is being used to treat tissue when tumor margins are not expected after surgical resection, termed margin accentuation. This review explores both the theoretical foundations of PFA, detailing how PEFs induce cell membrane destabilization and selective tissue ablation, the outcomes following treatment, and its clinical implications across oncology and cardiology.

Conclusions. Clinical experience is still progressing, but reports have demonstrated that PFA reduces complications often seen with thermal ablation techniques. Mounting oncology data also support that PFA produces a robust immune response that may prevent local recurrences and attenuate metastatic disease. Despite promising outcomes, challenges such as optimizing field delivery and addressing variations in tissue response require further investigation. Future directions include refining PFA protocols and expanding its application to other therapeutic areas like benign tissue hyperplasia and chronic bronchitis.

Key words: pulsed-field ablation; irreversible electroporation; pulsed electric fields; margin accentuation; oncology; atrial fibrillation

Electropermeabilization theory

Electropermeabilization is a biophysical phenomenon in which exogenous electric fields (EFs) increase the permeability of the cellular membrane (Figure 1). The application of an electric potential across tissue generates an EF whose shape and magnitude depend on the local electrical tissue

properties. The EF induces ion movement (i.e., current) within the tissue (Figure 2), and the subsequent charge concentration around cells generates an electric potential across the cellular membrane. This transmembrane potential (TMP) permeabilizes the cellular membrane through phospholipid oxidation¹⁻⁶, modulation of electrically-induced proteins⁷, and the generation of nano-scale pores

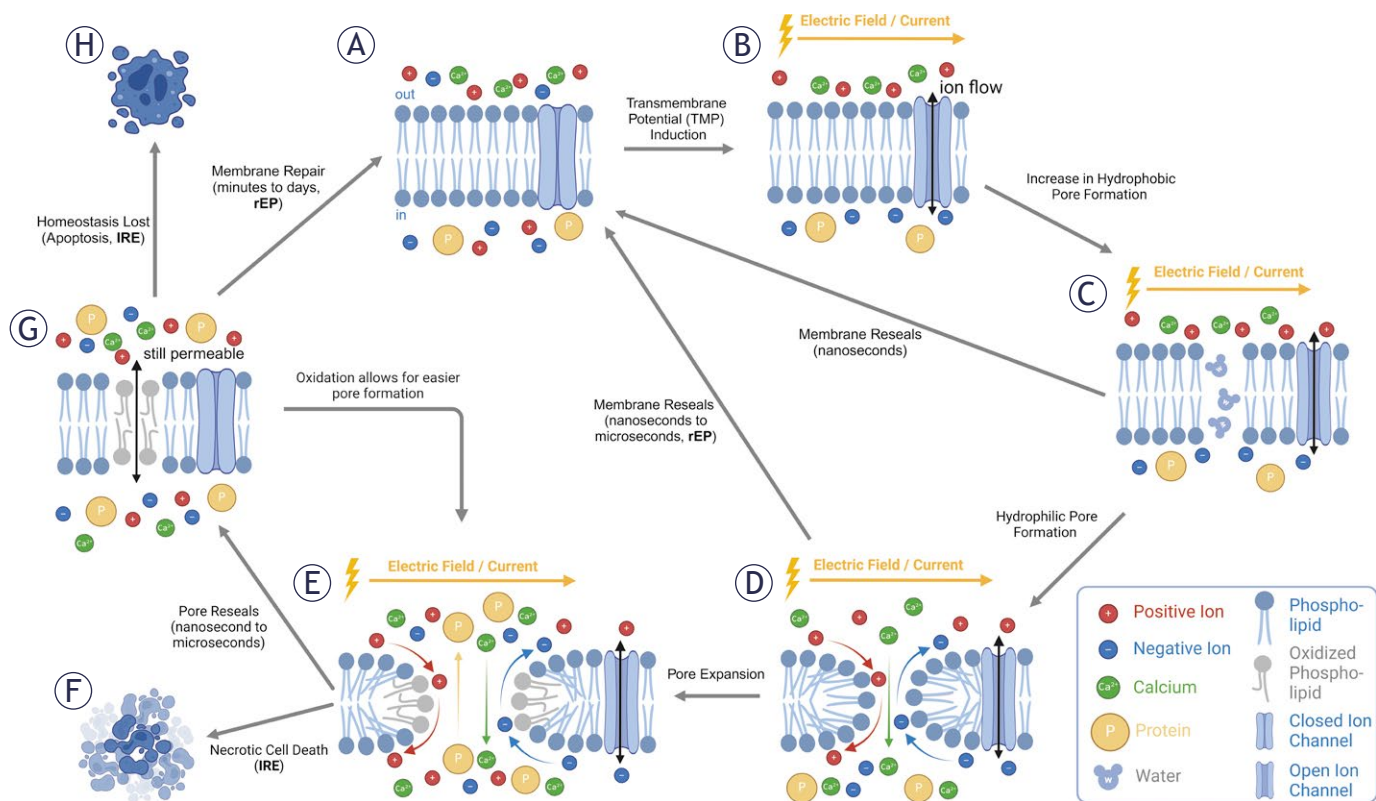


FIGURE 1. Conceptual schematic of the molecular mechanisms of electroporation. (A) An intact cell membrane (B) in an exogenous electric field experiences an induced transmembrane potential. (C) Hydrophobic pores become energetically favorable as water infiltrates the bilayer. With the removal of the applied electric field, the hydrophobic pore reseals within nanoseconds. (D) If higher and longer external electric fields are applied, phospholipids invert to form small hydrophilic pores that allow the passage of ions and small molecules. Elastic forces within the membrane allow for these pores to reseal within nanoseconds to microseconds after the removal of the electric field. (E) With higher magnitude and longer duration electric fields, pores number may increase, and nucleated pores may expand or combine, allowing the transport of larger molecules and higher quantities across the membrane. Significant lipid oxidation is indicated to occur at high electric fields. (F) If excessive, the lipid bilayer may hemorrhage leading to lytic (necrotic) cell death. (G) After cessation of the applied electric field, the cell membrane may remain permeable due to the presence of lipid oxidation, which, in return, also allows for easier pore formation upon the introduction of another electric field. (H) As significant mass transport occurs over the cell membrane, the cell may lose homeostasis and die through regulated cell death, or (G) the cell may repair the permeable and damaged cell membrane to regain homeostasis.

(electroporation).⁸ Standard electroporation theory and experiments suggest that pores are the dominant factor in mass transport across the membrane following electroporation⁹ and that pore formation occurs when the induced TMP exceeds a critical threshold (~ 0.258 V).¹⁰ The magnitude of the induced TMP is dependent on the local geometry of the membrane and directly related to cell size and shape.^{11,12} Once the exogenous EF is removed, the hydrophobic interactions, Van der Waals forces, and electrostatic interactions within the phospholipid bilayer may cause the pores to reseal within seconds to hours.¹³⁻¹⁵ The transitory formation of pores is called reversible electroporation (rEP) and has been used for decades to deliver chemotherapeutics (electrochemotherapy; ECT)¹⁶⁻¹⁸, calcium (calcium electroporation; CaEP)¹⁹⁻²⁵, ge-

netic material (gene electrotherapy, GET)^{26,27}, and otherwise impermeable substances²⁸ into cells. With the application of higher magnitude and protracted pulses, pore nucleation increases within the cellular membrane, and existing pores expand, allowing for increased mass transport, consequently with the increased likelihood of losing homeostasis or causing cellular membrane hemorrhage.^{7,29,30}

Concomitant to pore formation, the applied EF generates reactive oxygen species (ROS) that can induce lipid oxidation within the membrane.¹⁻⁶ Lipid oxidation increases the spacing between lipids and decreases membrane thickness, leading to increases in membrane permeability and electrical conductivity.^{5,6,31} Since oxidative agents are slowly removed from the membrane³², these effects

also persist after pores reseal.^{4,5} Further, subsequent pore formation and increased oxidation may occur more easily at locations of previous oxidation³³, and oxidative lipids may diffuse throughout the membrane between applied pulses.³⁴ Excessive oxidation can occur using higher magnitude EFs, longer pulses, and more pulses²⁻⁴, leading to complete bilayer disruption and cell death.³¹

Further, PEFs can destabilize and fragment cytoskeletal elements³⁵, including actin filaments³⁶⁻³⁹, microtubules^{40,41}, and intermediate filaments⁴¹⁻⁴³, which collectively maintain cell shape, enable intracellular transport, and support membrane stability.⁴⁴ The membrane and cytoskeleton are functionally and structurally linked, so disruption can exacerbate membrane deformation and impair cellular mechanical properties, increasing the susceptibility of the membrane to subsequent pore formation and enhancing ion and molecule transport.^{39,45} Cytoskeletal disruption may also interfere with cellular signaling pathways reliant on cytoskeletal integrity, affecting processes such as cell adhesion, motility, and division^{36,42,45}, with implications in blood vessel permeabilization.⁴⁶⁻⁴⁸ As with membrane oxidation, cytoskeletal damage can persist even after the EF is removed, leading to prolonged changes in cell structure and negatively impacting cell viability and function.^{49,50}

Pulsed field ablation techniques in medicine

Irreversible electroporation (IRE) was initially considered the upper limit of rEP and, as such, something to be avoided when post-treatment viability is desired.¹¹ With their seminal paper, R. Davalos, L. Mir, and B. Rubinsky mathematically described that EFs necessary to induce clinically relevant volumes of IRE did not simultaneously generate significant Joule heating and subsequent thermal damage.⁵¹ Edd *et al.* supported this hypothesis by generating contiguous ablations in rat livers at EFs indicated to not cause thermal damage.⁵² Following, Al-Sakere *et al.* reported the first successful use of IRE in oncology, achieving complete regression in 92% (12/13) of treated cutaneous mouse tumors using an optimized waveform (80 monophasic pulses of 100 μs at 0.3 Hz and 2500 V/cm), with a maximum measured temperature of 37.5°C.⁵³ The results from these studies demonstrated the feasibility of increasing the number of pulses from conventional ECT (8 pulses) without inducing thermal damage and provided the founda-

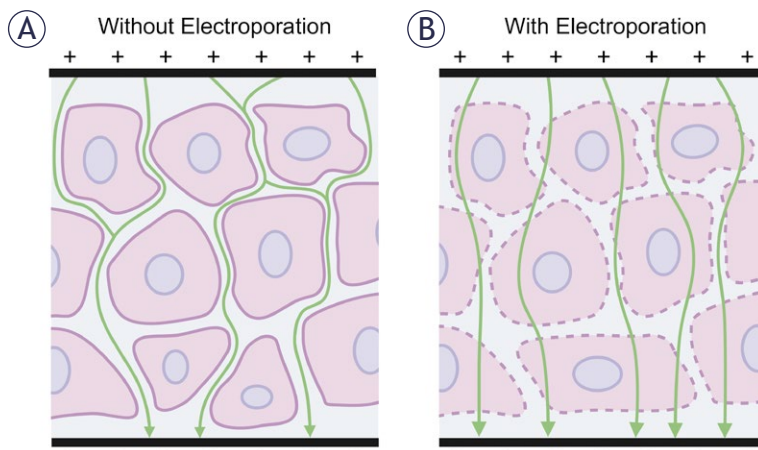


FIGURE 2. Electric field and current through heterogeneous tissue. (A) Without electroporation, current (green arrows) passes around the cells (pink) through the extracellular space (blue). (B) Electroporation allows for current to pass through the cells, but it is still influenced by tissue heterogeneity.

ation for parameters used in current IRE protocols.

Shortly after, Bertacchini *et al.* developed the first IRE generator approved for clinical use.⁵⁴ Since the introduction of IRE in the clinic in 2010, over 100 clinical trials have been registered worldwide (Figure 3), with hundreds of research articles published demonstrating safe and effective treatment of prostate⁵⁵⁻⁶⁴, pancreas⁶⁵⁻⁷⁴, liver⁷⁵⁻⁸⁴, and kidney⁸⁵⁻⁹⁷ tumors, but feasibility in many other solid tumors like lung⁹⁸⁻⁹⁹ and brain¹⁰⁰ has been demonstrated.

IRE as a clinical technique is described as a non-thermal focal ablation modality that employs high-magnitude (1–3 kV) and short (70–100 μs) monophasic pulses (Figure 4A) generated between conductive electrodes placed into or around the targeted tissue. In clinical practice, conventional

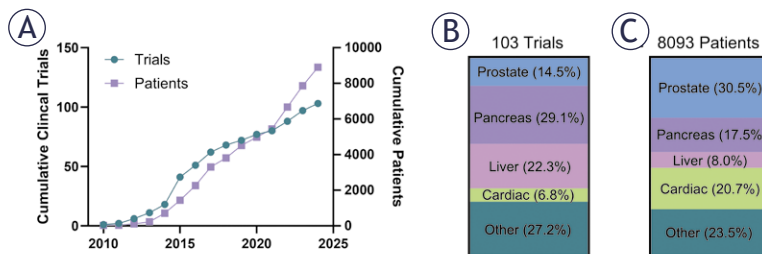


FIGURE 3. (A) cumulative registered patient and trial numbers for IRE and PFA on ClinicalTrials.gov. (B) Breakdown of trials and (C) patient populations by tissue type. Other contains renal, lung, stomach, esophageal, gallbladder, hilus pulmonis, extremity, lymph node, intestinal, rectal, laryngeal, head and neck, and breast cancers; benign prostate hyperplasia; chronic bronchitis; tonsillar hypertrophy.

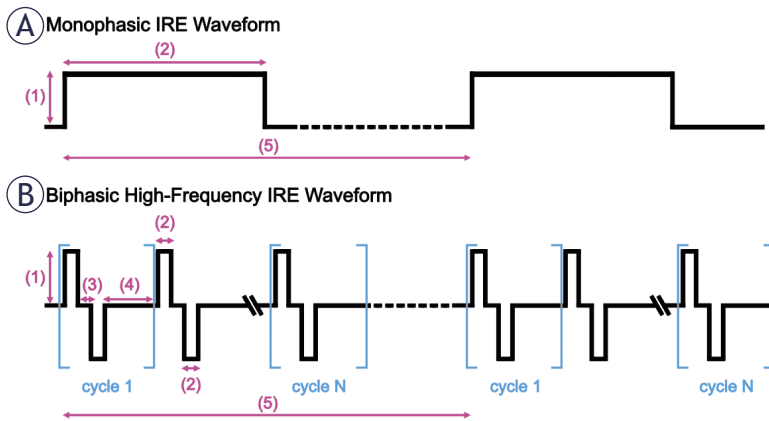


FIGURE 4. (A) Monophasic IRE waveform and (B) Biphasic H-FIRE waveform. (1) Magnitude (voltage or current), (2) pulse width, (3) interphase delay, (4) interpulse / intercycle delay, (5) burst repetition interval.

monophasic IRE pulses must be delivered using general anesthesia and prophylactic neuromuscular blockers to reduce muscle contractions.^{53,101-103} Induced muscle contractions are undesirable in debilitated patients and can cause an involuntary shift in the electrode locations, leading to incomplete ablation of the target region or puncture of neighboring critical structures (e.g., blood vessels, nerves). Early experience with IRE was also associated with incidence of cardiac dysrhythmia, so pulse delivery is now synchronized to the R-wave on electrocardiogram (ECG) recording with a 0.05 s delay to avoid interference with normal cardiac rhythm.¹⁰⁴ IRE is still contraindicated in patients with cardiac arrhythmia, as pulses cannot be con-

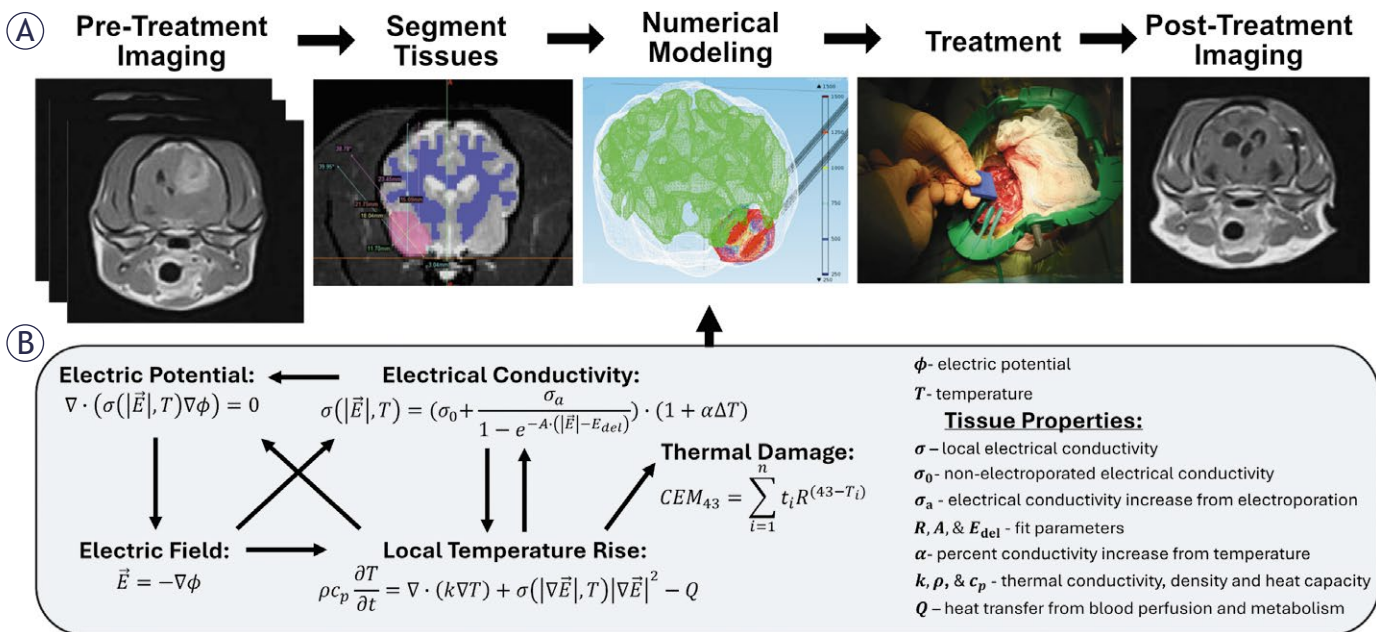


FIGURE 5. Pulsed field ablation treatment planning pipeline. (A) Images of the region of interest are taken through CT, MRI, US, or other modalities. Typically, segmentation is performed to define individual tissue regions before computational modeling, as the dynamic electric field, temperature, and conductivity distributions are tissue dependent. Numerical modeling is performed with the intent to maximize targeted tissue coverage with a critical electric field while minimizing deleterious effects on nearby structures. Following, the protocol is implemented for the treatment of the target tissue. While computational modeling can inform treatments, the exact application of PFA can often differ from *a priori* computational modeling. Post-treatment imaging is frequently used to assess acute and long-term ablation success. (B) Tissue electroporation modeling is multifaceted and requires knowledge about multiple electroporation-dependent and electroporation-independent tissue properties. The local electric potential depends on the local electrical conductivity and temperature. The electrical conductivity also depends on the local temperature and the electric field, as pore formation due to the local electric field allows for current to flow through cells. Subsequently, temperature generation depends on the electric field magnitude and the local conductivity. Images in panel A were adapted from various sources for instructional purposes.^{100,235}

sistently synchronized with the cardiac refractory period.

To overcome these limitations, Arena *et al.* developed High-Frequency IRE (H-FIRE)¹⁰⁵, which utilizes a series of short (0.5–10 μ s) biphasic pulses. The H-FIRE waveform is constructed of a positive pulse, interphase delay (d1), negative pulse, and interpulse delay (d2), repeated for several cycles to achieve an on-time comparable to IRE (Figure 4B). H-FIRE significantly reduces muscle contraction during treatment and obviates the use of neuromuscular blockers or cardiac synchronization.¹⁰⁶ Further, the shorter pulses are suggested to provide more predictable ablations when the pulse width is below the cell membrane charging time of 1–2 μ s.^{107–108} However, as a consequence of the reduced membrane charging, the EF threshold (EFT) required to induce electroporation increases as pulse width decreases, but thermal heating remains relatively the same.^{109–110} H-FIRE has been used pre-clinically to treat breast¹¹¹, liver¹⁰⁶, brain¹¹², lung¹¹³, and prostate¹¹⁴ cancer with mixed results. To date, H-FIRE has not demonstrated the same tumor ablation capability as IRE, but H-FIRE has been evaluated clinically in prostate cancer, offering a potential reduction in experienced complications.^{115,116} Notwithstanding, H-FIRE has gained prodigious attention for the treatment of cardiac arrhythmias under the name PFA.^{117–129} Between the different groups, H-FIRE (i.e., PFA) is indicated to have been performed in over 100,000 patients as of September 2024, not without appropriate criticism of the lack of transparency for treatment details.

Since PFA primarily induces cell death through permeabilization of the cell membranes, the PEFs minimally affect proteinaceous structures. The nonthermal mechanism is paramount for the control of diseased tissue near critical structures, such as bowels⁹⁷, ducts¹³⁰, mature blood vessels^{131,132}, esophagus¹³³, and nerves^{56,134,135}, where surgical resection and thermal ablation methods are contraindicated. Further, PFA is not influenced by the “heat sink” effect, where blood flow in adjacent vessels dissipates heat, reducing ablation effectiveness and potentially sparing targeted tissue. This allows PFA to completely treat tissue abutting blood vessels. Narayan *et al.* examined the patency of 158 vessels with a mean distance from the treatment lesions of 2.3 mm and noted abnormal changes in 4.4% (7/158) of vessels.¹³² Only 1.4% (2/158) were hemodynamically significant, with many vessels that experienced thrombosis post-treatment already heavily involved before treatment. Tumors

abutted 40 vessels and encased 10 vessels, but 96% (48/50) maintained patency despite being directly within the ablation. Further, Li *et al.* found that neurovascular bundles are not destroyed even when directly treated with ablative PEFs.¹³⁴ Subsequent studies have observed that there may be some degree of thermal damage to the tissue immediately near the treatment electrodes^{136–137}, so careful planning and probe placement are still needed.

Pretreatment planning

Computational modeling is necessary for the successful delivery of PFA¹³⁸, as the entire target tissue must be covered by a critical EF while minimizing collateral damage to nearby critical structures.¹³⁹ Treatment planning includes (Figure 5):

1. Imaging of the treatment area and surrounding structures

Before surgery, the location, size, and geometry of the tissue to be treated are determined with one or more imaging modalities, including contrast-enhanced computed tomography (Ce-CT), positron emission tomography (PET), magnetic resonance imaging (MRI), and 3D-mapping biopsy for prostate cancer (PCa). Except for PCa, Ce-CT is the most used modality due to its availability, high resolution, and ability to rapidly create multi-planar reconstructions of the tumor and surrounding structures.¹⁴⁰ For cancer patients, tumor growth or shifting may cause differences between prior- and intra-procedural images, so Ce-CT also allows for rapid adjustments in the treatment planning and probe position.^{140–142}

2. 3D reconstruction of the anatomy of the treatment area

Multi-planar images are imported into a segmentation software (e.g., 3DSlicer) to separate the tumor, parenchyma, and nearby structures. The geometries are then meshed for importing into finite element analysis software (e.g., COMSOL™).

3. Define the electroporation-dependent material properties for the different tissues

A priori information about the target tissue is needed for accurate treatment modeling. Both the EF

and temperature distributions strongly depend on the tissue-specific electrical properties^{143,144}, which both differ between patients in healthy and malignant tissues and change non-linearly from the electroporation process itself.¹⁴⁵ Results in computational modeling significantly differ when considering electroporation effects^{146,147}, but validated tissue properties are sparse within the literature.

Conventional methods for tissue characterization use *ex vivo* tissue slices with fixed geometries to translate impedance at different applied EF magnitudes to conductivity.^{146,148} Quantifications are often limited to healthy animal tissues due to their availability and can misrepresent the targeted tissue, especially when translating results to tumors. Tissue characterization using patient-derived xenografts is more representative¹⁴⁹, but they can take weeks to grow, are not widely available during treatment planning, and do not replicate *in situ* conditions. Further, even within a specific tumor type, there can be a high degree of tumor tissue heterogeneity between patients and even between tumors at different locations in the body. Translating experimentally found properties to an individual can be unreliable, so improved methods for patient-specific tissue characterizations are greatly needed.¹⁴⁷

In addition to simulating the EF and thermal distributions, it is necessary to know the EFT of the tissues being treated to quantify the lesion coverage. Values for the lethal EFT are variable within the literature due to the lack of validated and standardized protocols. Thresholds gathered *in vitro* using cuvette systems are typically higher than those gathered using 2D or 3D platforms, but *in-situ* data is the most translatable.¹⁵⁰ Pulse widths from nanoseconds to milliseconds will generate ablations, but pulse width negatively correlates with EFTs.^{109,110,151,152}

4. Incorporation of treatment probes within the model and numerical optimization

Intrinsic tissue properties cannot often be changed; thus, treatment parameters (i.e., voltage, probe geometry, and PFA waveform) must be adjusted to find solutions that solve the desired objective. The two main objectives that are usually investigated for PFA are (1) encompassing the target tissue with a lethal EF while (2) minimizing Joule heating and subsequent thermal damage to nearby critical structures.

The number of probes depends on the ability to cover the tumor and margin with a lethal EF. For

deep soft tissue neoplasms, typically 2 to 6 monopolar probes are inserted into or around the neoplasm. For lesions smaller than 2 cm, 3 probes are placed at the periphery of the tumor in a triangle; for lesions between 2–3 cm, 4 probes are placed at the periphery in a square; for lesions larger than 3 cm, 4–6 probes are used, with 1–2 of the probes placed within the lesion and the rest at the periphery.¹⁵³ The distance between electrode pairs should not exceed 2.2 cm, but values have ranged from 0.7 to 2.9 cm in literature. The electrode exposure can vary from 0.5 to 3 cm, but 1.5 cm is the most common. The applied current scales linearly with electrode exposure, and too large of an exposure can trigger the overcurrent on electroporation generators at 50 A. Therefore, if the target is larger than the possible electrode exposure, the deepest portion of the target should be treated first; then, the electrodes can be “pulled back” for subsequent treatments to ensure overlapping and cohesive ablations.

Applied EFs or “voltage-to-distance ratios” (VDRs) typically range from 1200 V/cm to 2000 V/cm for IRE and 2000 V/cm to 3000 V/cm for H-FIRE. Higher VDRs will generate larger ablations at the consequence of increased Joule heating, neuromuscular excitation, and electrochemical effects.

Probe positioning and treatment

IRE has been successfully performed through intraoperatively¹⁵⁴, laparoscopy¹⁵⁵, and percutaneous¹⁵⁶ insertion of treatment probes. For percutaneous insertion, the probes must be carefully inserted under contrast-enhanced ultrasound (ce-US) or ce-CT guidance to prevent puncturing sensitive structures and maintain parallel insertion of the electrodes. Imaging is used to verify correct probe placement and measure the center-to-center probe separation to calculate the VDR. Probes should be placed parallel to each other with no more than 10-degree deviations to prevent irregular ablations and possible incomplete treatment.

Despite the EF coverage ultimately dictating ablation size, clinicians have found that electrical currents between 20 and 40 A during IRE provide sufficient ablations. With the NanoKnife system, 10 pulses are initially delivered to assess the applied current between each electrode pair. Following, if the current is adequate, the rest of the treatment will be delivered. Otherwise, the clinicians will increase or decrease the VDR to achieve the desired current and then deliver the appropriate number

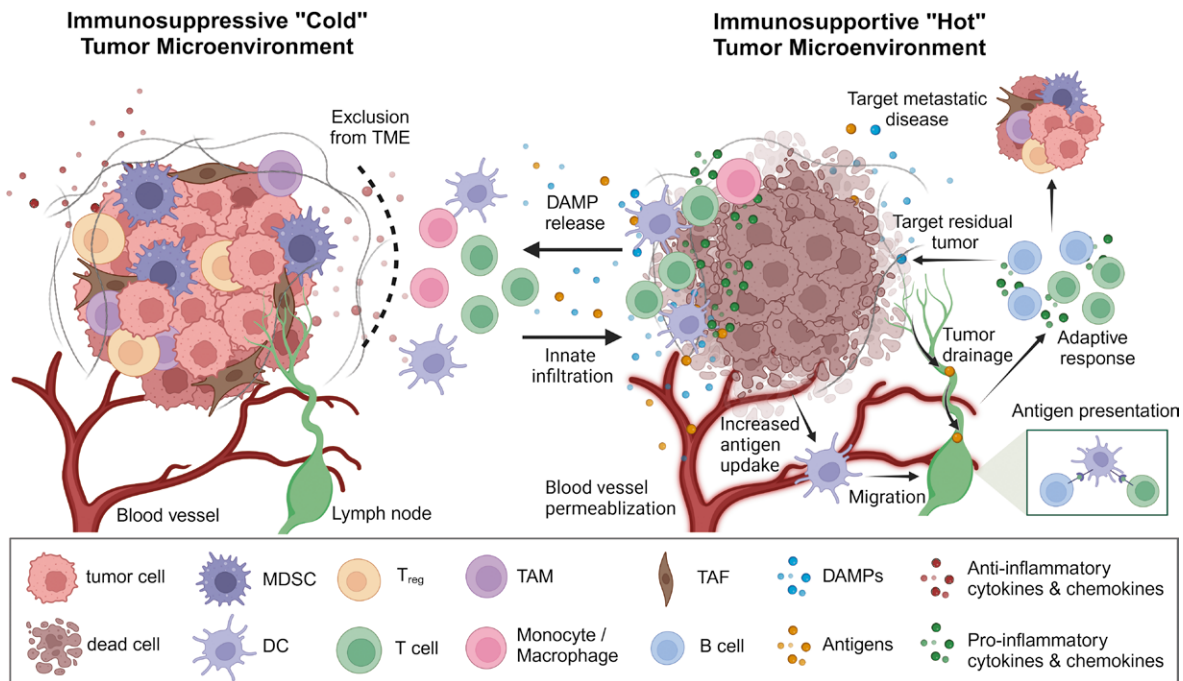


FIGURE 6. Immune response following pulsed field ablation. The tumor microenvironment (TME) evolved through all stages of cancer progression and protects itself through reprogramming immune cells (T regulatory cells [T-reg], myeloid-derived suppressor cells [MDSCs], and tumor-associated macrophages [TAMs]), attracting stromal cells (endothelial cells and fibroblasts) that help deposit a dense extracellular matrix (ECM). This produces an immunosuppressive "cold" tumor that excludes normal immune cells from infiltrating. Pulsed-field ablation indiscriminately kills tumor cells, stromal cells, and immunosuppressive immune cells within the ablation and restructures the ECM. The removal of active immunosuppression, permeabilization of mature blood vessels, and release of Damage Associated Molecular patterns (DAMPs) by IRE entices innate immune cell infiltration. Tumor antigens are released by treated cells, which are either taken up by dendritic cells or drained directly into lymph nodes for antigen presentation. Tumor-specific T- and B-cells mature within the lymph nodes, then antigen-specific T- and B-cells leave the lymph node to potentially remove residual cancer or target distant metastatic disease.

of pulses. An applied potential is only generated between one electrode pair at a time, and the final train of pulses is typically either 70 or 90 pulses between each probe pair.

In addition to cardiac arrhythmia, other absolute contraindications for PFA include the presence of non-removable pacemakers or implantable cardioverter defibrillators, a history of epilepsy or seizures, a history of bleeding disorders, and the presence of anatomical obstacles blocking safe probe insertion.

Post treatment imaging

CT imaging is predominantly used after the procedure to determine treatment success and

to evaluate disease recurrence or remission during follow-ups¹⁵⁷, but ablations are also regularly visualized using PET¹⁴⁰, MRI¹⁵⁷, and US.¹³⁷ Further, both IRE and H-FIRE produce ablations with sharper delineation than other ablation modalities.¹⁵⁸ Histology of ablations demonstrates demarcation between the ablated and live tissue on the order of 1–2 cells.

Cell death and immune activation

Given the complex and nuanced processes involved, the cell death mechanisms following IRE and H-FIRE are still under investigation. Researchers originally attributed necrosis due to

disruption of the osmotic balance as the killing mechanism of electroporation. However, in the late 1990s, it was demonstrated that electroporation not only caused necrosis but also induced delayed cell death following chromosomal DNA fragmentation, which is an explicit indication of late apoptosis.^{159,160}

There is a plethora of competing findings for cell death pathways and mechanisms following PFA, including immunogenic (e.g., necrosis, necroptosis, and pyroptosis) and non-immunogenic (e.g., apoptosis) cell death.^{136,159-163} Each pathway has unique implications for treatment side effects, immune activation, and efficacy.¹⁶⁴ Increasing evidence suggests that H-FIRE induces delayed, regulated cell death while IRE induces immediate, lytic cell death.^{163,165,166} Further, it is suggested that higher EFs are more likely to induce necrosis through membrane hemorrhaging and thermal damage, while lower EFs may permit membrane recovery but induce regulated cell death following ROS generation, DNA damage, mitochondrial damage, ATP loss, osmotic imbalance, or calcium influx.^{29,136,165,166} While apoptosis is frequently highlighted as a key form of cell death in PFA, immediate cell death observed following IRE and H-FIRE often shows characteristics of necrosis. Thus, rather than a single pathway, it is likely a combination of overlapping death mechanisms that lead to the loss of cellular homeostasis.

I. PFA reduces the anti-inflammatory cell populations within the tumor microenvironment

In many solid tumors, multiple cell populations contribute to the immunosuppressive “cold” TME (Figure 6), including differentiated cancer cells, cancer stem cells, tumor-associated fibroblasts (TAFs), and immunosuppressive immune cells (ISICs) (e.g., tumor-associated macrophages [TAMs], myeloid-derived suppressor cells [MDSCs], and regulatory T-cells [T_{reg}]).¹⁶⁷ Further, the epigenetic and cellular composition of tumors can vary between patients, between different tumors within a patient, and even at different locations within the same tumors¹⁶⁸, making it challenging to provide single-target therapeutics. PFA acts indiscriminately on proliferating and non-proliferating cells¹⁶⁹ within the critical EFT. Therefore, recalcitrant (e.g., cancer stem cells^{170,171}) and immunosuppressive cells (TAMs, MDSCs, TAFs, and T_{regs} ^{111,172,173}) are removed in addition to bulk tumor cyoreduction.

II. PFA effectively reverses the stroma-induced immunosuppression

PFA ablation alters the physical properties of the TME through reduction of the extracellular matrix density and rigidity^{174,175} and increases tumor-associated blood vessel permeability.^{47,48,137,175,176} These both reduce tumor-associated hypoxia that impedes leukocyte function.¹⁷⁵ Increases in microvascular density are indicated after treatment^{174,175}, but this may be attributed to transient decreases in vascular junction integrity and subsequent increases in the expression of junction proteins to regain microvasculature function. The preservation of mature vasculature patency while increasing permeability allows for infiltration of leukocytes and transport of TAAs to tumor-draining lymph nodes²³⁸. These results are not replicated in other focal ablation therapies, indicating that IRE may uniquely modulate the TME. Regeneration of the ablation site by parenchymal cells is also indicated at 1–2 weeks post ablations¹⁷⁷, but underlying tissue disease or chemoembolization may prevent the healing process.⁹⁵

III. PFA induces a pro-inflammatory TME and activates the adaptive immune system

In addition to reducing anti-inflammatory cell populations, PFA actively promotes an immune-supportive TME. Damage associated molecular patterns (DAMPs) are released by electroporated cells and recognized by the innate immune system for generating early inflammation.^{111,175} Tumor-associated antigens (TAAs) are also released and evaginated by dendritic cells and macrophages for antigen presentation.¹⁷⁸ Unlike with thermal ablation modalities, DAMPs and TAAs released by electroporated cells are presumably not destroyed due to the lack of sufficient thermal heat to denature proteins, potentially allowing for the priming of mature T-cells with receptors directed at the *in situ* protein motif.¹⁷⁹

Although PFA treatment success is not predicted by the induction of an anti-tumor immune response, both *in vivo* and clinical data suggest a correlation between immune activation and progression-free survival (PFS) and overall survival (OS). He *et al.* demonstrated the disparity in patient OS when gating by immune activation; when separating patients into high and low T-lymphocyte responses, there was 70–80% and 0% survival at 30 months post-IRE, respectively.⁷⁴ Goboers *et al.*

found that T-cell activation correlated with pretreatment tumor sizes and suggested that antigen release may correlate with the extent of ablation.¹⁷³ Larger ablation volumes would presumably induce more TAA and DAMP release while generating a larger variety of cell death mechanisms to create a robust immune response. They also found a decrease in circulating dendritic cell populations indicative of activation-induced migration to lymph nodes and treated tissue, which was supported by the activated T-cells expressing specific receptors against prostate cancer-associated antigens.

IV. PFA can be combined with immunotherapies

To consistently generate persistent peripheral anti-tumor immune activation, current research aims to adjust pulsing waveforms to generate more inflammatory cell death modalities or combine treatment with adjuvant immunotherapies. The combination of IRE and immune checkpoint inhibitors (ICIs), such as anti-CTLA4, anti-PDL1, and anti-PD1, have positive results in both mice and humans.^{172,175,178,180} He *et al.* presented promising results when combining IRE with anti-PD1 in Stage III locally advanced pancreatic cancer, achieving an overall survival of 44.3 months versus 23.4 months for IRE alone.¹⁸⁰ Further, they did not observe differences in adverse side effects between the two treatment groups, demonstrating that ICIs may offer a significant increase in IRE efficacy without additional side effects. Primary tumor ablation with IRE in a PCa mouse model, followed by anti-CTLA4 and anti-PD1 immune checkpoint inhibitors, induced a significant increase in both tissue-resident and circulating memory cytotoxic T-cells with T-cell receptors targeting PCa-specific antigen, SPAS-1.¹⁷⁸ Subsequently, this work indicated that a tumor vaccine effect was achieved by the tissue-resident and circulating memory cytotoxic CD8⁺ T-cells, limiting the reintroduction of new PCa. A recent direct comparison of IRE with cryoablation (CA) and thermal ablation further demonstrated that anti-PD1 synergizes best with IRE, leading to longer tumor-free survival, increased infiltration of CD8⁺ T-cells, and protection against tumor reintroduction.¹⁸¹ Due to the modulation of the immunosuppressive TME, the efficacy of dendritic cell vaccination is improved after IRE.¹⁸²

Despite promising results, local and distant tumor recurrence still occurs. A potential reason for the eventual tumor recurrence is that major histocompatibility complex I (MHC I) downregulation

occurs 30–100% in many cancer types, with pancreatic cancer having a suppression rate of 40–100%.^{183,184} IRE clearly benefits from an induced immune response, but without antigen presentation for T-cell recognition, the local and metastatic micro-tumors are hidden from the heightened immune response and eventually repopulate local and distant sites. Lin *et al.* demonstrated the potential for combining IRE with autologous $\gamma\delta$ T-cells, which can recognize and lyse cancers in an MHC-unrestricted manner. Patient $\gamma\delta$ T-cells were isolated from the blood, expanded, and then reintroduced after IRE through at least 2 cycles.¹⁸⁵ Patients with multiple infusions survived longer after treatment (17 months) than patients with a single infusion (13.5 months) or IRE alone (11 months). Further, IRE has been combined with natural killer (NK) cells^{186–189}, which recognize cells that have downregulated MHC I receptors.¹⁹⁰ Despite only evaluating the efficacy at 1-mo post-treatment, a randomized study of 92 LAPC patients found that the IRE-NK group achieved an overall response of 71.7% compared to IRE alone with 56.5%.¹⁸⁸

Prostate (PCa)

PCa is a leading cause of cancer-related deaths among men¹⁹¹, and the contemporary treatment for localized PCa is active surveillance, radical prostatectomy, and radiation therapy. Routine prostate examinations are becoming increasingly popular, resulting in earlier detection of manageable small-volume neoplasms. While whole-gland approaches have historically offered the best possible oncological outcome for local disease, low- to intermediate-risk patients may not benefit from radical treatments, as damage to the neurovascular bundle, external sphincter, bladder neck, urethra, and rectum are often associated with genitourinary dysfunction which could include impotence, incontinence, pain, loss of rectal control, and loss of sensation. IRE offers a valuable treatment option for these patients, as the negative side effects can be circumvented while still achieving sufficient oncological outcomes. Further, IRE can be successfully delivered to any region of the gland (apex, middle, or base) with similar disease control¹⁹², while other focal ablation therapies are known to be preferential for certain areas.^{193,194}

The first evaluation of IRE in the prostate was performed by Onik *et al.* in 2007 in six healthy canine prostates.⁶¹ Histology revealed a fine demarcation between the unaffected and necrotic

prostate tissue, spanning only a few cells. When directly including the urethra within the ablation, necrotic glandular tissue abutted urethral structures without necrosis within the sub-mucosa. Vessel patency was also preserved when deliberately treating the neurovascular bundle, though variable endothelial and fibrinoid necrosis was observed. The authors expressed that nerves within the neurovascular bundles did not appear to be affected, with no evidence of ganglion cell death. Following, Onik *et al.* performed the first human clinical trial for IRE, involving 16 patients with low- to moderate-risk prostate cancer in a series of outpatient procedures.⁶² All patients were continent immediately after IRE, and all patients who were potent before the procedure were still potent after the procedure. Two patients who had bilateral areas treated required 6 months for a full return of potency. Color Doppler US showed intact flow within the neurovascular bundle immediately after the procedure, and postoperative biopsies taken from the area of previously known cancer in 15 patients showed no evidence of cancer.

A disadvantage of focal ablation therapies is the possible presence of multi-focal disease that is not initially diagnosed through imaging or biopsy. As PCa is frequently multi-focal, IRE application to multiple segments or the entire prostate gland can extend its coverage. A multi-center randomized clinical trial evaluated the control of focal and extended IRE in 106 low- to intermediate- risk patients.⁵⁶ A similar total rate of recurrence was observed, but the extended ablation cohort experienced lower recurrence away from the lesion site. Guenter *et al.* also presented encouraging results from a large retrospective assessment of 429 patients with low ($n = 25$), intermediate ($n = 88$), and high-risk ($n = 312$) prostate cancer.¹⁹⁵ Patients were treated focally ($n = 123$), sub-whole-gland ($n = 154$), whole gland ($n = 134$), or for recurrent disease after previous treatment with other modalities ($n = 63$). During a maximum follow-up time of 72 months, 3 (12%), 18 (20.4%), and 26 (8.3%) recurrent cancers were observed in the low-, intermediate-, and high-risk groups, respectively. Urinary continence was preserved in all patients. Ten patients developed a temporary decrease in erectile function, with 4 patients experiencing a decrease longer than a year. Scheltema *et al.* recently released their longer-term (60 months) oncologic and functional evaluation following IRE as a primary treatment in 229 patients (International Society of Urologic Pathologists [ISUP] grade 1–4).¹⁹⁶ The long-term follow-up confirmed earlier findings that IRE

provides acceptable local and distant oncological control with lower loss of continence and potency than radical treatments.

Radiotherapy is a well-established therapy for PCa; however, one in five patients recur with significant disease, forming a difficult-to-treat patient sub-population. Recently, IRE has been evaluated in patients with recurrent PCa, specifically following prostatectomy and radiotherapy.¹⁹⁷⁻¹⁹⁹ Mid-term oncological and safety results demonstrate that IRE can be delivered safely to ISUP 1–5 recurrent patients, with similar in-field oncologic responses to *in situ* treatment.¹⁹⁷

Dong *et al.* were the first to demonstrate the feasibility of tumor ablation using H-FIRE in humans.¹¹⁵ They treated 40 PCa patients using a 5 μ s pulse width without ECG synchronization and with moderately lower muscle relaxants than conventional treatments. No muscle contractions or abnormalities were observed during H-FIRE delivery, with all patients able to move ~10 hours after treatment. Lesions were clearly visible on MRI at 4 weeks post-treatment. At a median follow-up of 6 months, no major complications were experienced, with sexual function and urinary continence preserved in all patients. A recent multi-center non-randomized prospective clinical study treated 109 patients with low ($n = 27$) and intermediate ($n = 82$) risk PCa using an unspecified H-FIRE waveform.¹¹⁶ One hundred patients underwent a 6-month biopsy, with clinically significant prostate cancer in the treatment zone and out of the treatment zones for 1 and 5 patients, respectively. Urinary continence was maintained in 99.1% of patients, and emergent sexual dysfunction was experienced in 9% of patients.

Pancreas (PC)

Pancreatic cancer is currently the 3rd deadliest malignancy and possesses an insidious prognosis due to its surreptitious progression, with over 80% of patients unfortunately presenting stage III locally advanced pancreatic cancer (LAPC) or metastatic disease at diagnosis. Poor outcomes for LAPC are attributed to diffuse cancer infiltration, the sclerotic and immunosuppressive tumor microenvironment, and significant involvement of sensitive structures. This precludes surgical resection in > 80% of patients. The intervention of unresectable PC consists of chemoradiation, which has not meaningfully increased survival, with a median overall survival of 9.3–11.8 months after

diagnosis.^{200,201} IRE provides perhaps one of the largest benefits to patients with LAPC, and numerous clinical evaluations are published yearly, demonstrating its safety and efficacy. Further, multiple studies have evaluated IRE to treat margins after pancreatectomy in borderline resectable pancreatic cancers (BRPCs), termed margin accentuation (MA), when negative margins are not expected.

Martin *et al.* and Narayanan *et al.* published the first clinical series on the treatment of PC using IRE.^{156,202} Martin *et al.* treated 27 patients with IRE either *in situ* (n = 19) or for MA following surgical resection (n = 8). They achieved 100% ablation of the primary tumor evaluated at the 90-day follow-up. Nine patients experienced 18 complications, with most being potentially associated with the open surgery approach and 4 being possible device-related complications. In parallel, Narayanan *et al.* treated 11 patients with LAPC and 3 with metastatic disease using a percutaneous approach. Ten of the 11 LAPC patients were still alive at 14 months post-treatment, but the 3 metastatic patients did not benefit from IRE with a median overall survival of 4 months. Contrast-enhanced CT immediately and 24 hours after treatment showed that vascular patency was preserved in all patients. Martin *et al.* subsequently treated 200 Stage III LAPC patients treated with either *in situ* (n = 150) or for MA following surgical resection (n = 50).²⁰³ All patients had initially undergone induction chemotherapy, and 52% were additionally given chemoradiation therapy for a median of 6 months before IRE. At a median follow-up of 29 months, 58 patients developed recurrences (6 local recurrences) with a median progression-free survival of 12.4 months. MA had a higher median overall survival than IRE alone (28.3 vs. 23.2 months). Twenty patients (40%) experienced 49 complications in the MA group, and 54 patients (36%) experienced 100 complications in the *in situ* group, with the most common complications being gastrointestinal complaints. Ten severe complications were experienced after treatment. The same group published their results on another prospective multi-institutional assessment with 152 additional patients treated.⁶⁷ *In situ* IRE was successfully delivered to all patients with tumors ranging from 1 to 5.4 cm in diameter with a median follow-up of 19 months. There were 9 local recurrences and 27 distant recurrences, resulting in a median progression-free survival of 22.8 months and a median overall survival of 30.7 months. Nineteen patients experienced severe adverse events, with the most common complications being gastrointestinal or hepatic related. In

both studies, the liver was the most common site of distant recurrence.

Many clinical studies have evaluated IRE following inductive chemotherapy. A randomized trial demonstrated the additive effect of IRE with or without chemotherapy.²⁰⁴ Specifically, combinatorial treatment patients had higher OS (20.3 vs. 16.2 months). Similarly, the PANFIRE-2 trial found IRE following induction chemotherapy provided a benefit to OS (17 vs. 12.4 months).¹⁴⁰ A recent prospective randomized clinical trial compared the safety and efficacy of IRE (n = 34) to MRI-guided stereotactic ablative body radiotherapy (SABR, n = 34) following induction FOLFIRINOX.²⁰⁵ There were no differences in OS (12.5 vs. 16.1 months), PFS (9.5 vs. 8.5 months), or number of complications. Distant tumor-free survival was higher following IRE (13.2 vs. 8.5 months), but this could be due to a higher percentage of patients receiving adjuvant therapy following IRE. He *et al.* analyzed the SEER and SYUCC databases to compare the efficacy and long-term safety of IRE (n = 206) following induction chemotherapy against chemotherapy alone (n = 3444)²⁰⁶ and found that IRE following induction chemotherapy had a higher OS (18 vs. 8 months) and PFS (7.7 vs. 4.1 months). Recently, Suraju *et al.* compared resection (n = 40), MA (n = 13), *in situ* IRE (n = 14), and unresected (n = 35) in BRPC and LPAC patients who received neoadjuvant chemotherapy.²³⁶ Despite having a higher number of patients with LAPC in the MA group, they experienced a non-significantly higher OS and PFS compared to resectable patients; the median OS from diagnoses were 30 months for MA, 28 months for *in situ* IRE, 27 months for resection, and 14 months for the unresected group. Neoadjuvant chemoradiation, IRE, and resection were independently associated with decreased risk of mortality, and IRE with an open approach had fewer severe complications than pancreatectomy.

Liver

Liver cancer is the fifth most fatal malignancy globally, with hepatocellular carcinoma (HCC) comprising over 80% of primary liver tumors.²⁰⁷ Additionally, the liver is a frequent site of metastasis, especially from colorectal cancer; at least 25% of colorectal cancer patients develop liver metastases (CRLM), accounting for a substantial proportion of secondary liver tumors.²⁰⁸ Standard treatment approaches for HCC and CRLM, including chemoradiation and surgical resection, are often

limited, and up to 80% of patients are deemed ineligible for resection due to tumor burden, anatomical location, or proximity to critical structures. Following hepatectomy, critical structures like the single remaining portal vein, central bile duct, and one or two major hepatic veins limit further resection, as removal or damage to these could compromise liver function. If further resection of these structures is not feasible, then focal ablation offers an effective treatment, but thermal ablation strategies are limited due to the associated “heat sink” effects and potential damage to critical structures.

Thus, IRE has been an increasingly effective method for treating tumors near these structures.^{155,209} Ma *et al.* demonstrated that percutaneous IRE is a safe and effective treatment for HCC abutting the diaphragm.²¹⁰ They successfully ablated 36/39 tumors with no major complications and achieved a median 20.4 months to local tumor progression. The COLDFIRE-I ablate and resect clinical trial demonstrated the feasibility and safety of IRE to treat CRLM in 10 patients.²¹¹ The subsequent COLDFIRE-II trial further demonstrated the efficacy and safety of IRE in 51 patients with a total of 76 CRLMs.²³⁷ The 1-year local-progression-free (LPF) rate was 68%, and following repeated procedures in 8 patients, local control was achieved in 37/50 (74%) patients. The median overall survival from treatment was 32 months. Fruhling *et al.* further demonstrate that IRE was a safe ablation modality in 149 patients with HCC (n = 53) and CRLM (n = 71) when other treatment options are unsuitable.²¹² At 12 months, they achieved local ablation success of 40.3% in HCC patients and 25.4% in CRLM patients. This translated to a median OS of 35 months and 27 months for HCC and CRLM patients, respectively. Three patients experienced severe complications, with one death due to thromboembolism. In a subsequent analysis of the patient population, they found that smaller decreases in resistance and larger tumor sizes were associated with earlier recurrence in CRLM but not HCC patients.²¹³

In an evaluation of IRE as a salvage treatment, Hitpass *et al.* demonstrated that IRE is a safe option when resection and thermal ablation are unsuitable.⁸⁴ All tumors were located adjacent to the sole remaining intrahepatic blood vessels and bile ducts, but IRE was successfully delivered with a 5 mm margin in 31/32 lesions across 23 patients, with one incomplete ablation. The local progression-free rate was 64% and 57.4%, and the intrahepatic progression-free rate was 36.4% and 19.5% at 12 and 36 months, respectively. Altogether, five

patients were tumor-free at the last follow-up. No vessel injury or thrombosis was observed, and only minor complications occurred, including moderate segmental cholestasis, which spontaneously resolved. Recently, Narayanan *et al.* confirmed that IRE is a safe and viable option for the treatment of unresectable CLRLMs close to the portal and hepatic veins, inferior vena cava, bile duct, and gallbladder.²¹⁴ They achieved a median OS of 40.4 months with only minor complications. In a recent randomized non-inferiority clinical trial, Zhang *et al.* compared IRE (n = 78) to radiofrequency ablation (RFA) (n = 78) for the treatment of malignant liver tumors.²¹⁵ They demonstrated that IRE was not inferior to RFA, with comparable tumor ablation rates (94.9% vs. 96%), similar complication rates, and similar 6-mo recurrence rates (13.3% vs. 19.7%) between IRE and RFA. In a direct comparison of IRE to RFA and MWA in a propensity score-matched population of early HCC, Wada *et al.* found 2-year local tumor progressions of 0%, 45%, and 25% for IRE, RFA, and MWA, respectively.²¹⁶

A majority of HCC develops in patients with underlying pathologies, and the possibility of damaging diseased hepatic parenchyma (e.g. Child-Pugh B/C) has the associated risk of severe liver failure and mortality.²¹⁷ Bhutiani *et al.* compared the tolerability and efficacy of IRE and microwave ablation for treating HCC patients with moderate Child-Pugh B liver dysfunction.²¹⁸ They found that both modalities had comparable success rates, but IRE was better tolerated with a significantly lower length of stay and 90-day readmission rate.

Kidney

Small renal cell carcinoma (RCC) has traditionally been treated with surgical resection, with radical nephrectomy being the most common treatment. IRE has yet to be fully established for the treatment of renal tumors, but it may be considered when surgical resection or thermal ablation is not an option. Thomson *et al.* treated 7 patients with RCC using IRE.⁹⁵ Transient hematuria was observed in two patients with treatments near the center of the kidney, which resolved in under 24 hours. Follow-up CT at 3 months confirmed successful ablations in 71.4% (5/7) of patients, with the other 2 receiving a second IRE procedure. The first large cohort of patients with renal tumors treated with IRE was reported by Trimmer *et al.*, in which 20 patients with T1a renal carcinoma (n = 13), indeterminate

masses (n = 5), or benign masses (n = 2) underwent CT-guided IRE.⁸⁶ All ablations were initially technically successful, as verified with ce-CT, but two patients required salvage therapy at 2 weeks due to incomplete ablation. All 15 patients imaged at 6 months had no evidence of recurrence, and only one patient was observed to experience recurrence at 1 year after IRE.

Despite initial data supporting the feasibility and safety of IRE, a few clinical studies have found suboptimal short- and mid-term disease control. Canvasser *et al.* found that the initial treatment was successful in 93% (39/42) of tumors, but the 2-year local-recurrence-free rate was 83%⁸⁹, which is unfavorably compared to contemporary local-recurrence-free rates of >97% for partial nephrectomy of tumors < 3.0 cm. Further, the first prospective Phase II clinical trial (IRENE) found “complexities in the overall procedure”.⁹² All tumors were resected after treatment to assess the lesion. Four patients had no residual tumor, while 3 had microscopic residual tumor due to incomplete ablation. Dai *et al.* found similar results in a retrospective study of 47 patients with 48 tumors, with 45.8% (22/44) being biopsy-proven RCC.²¹⁹ At a median follow-up of 50.4 months, their 5-year local recurrence-free rate was 81.4% in biopsy-confirmed RCC patients and 81.0% in all patients.

None of the studies observed major complications, supporting the safe initial use of IRE for RCC. While the safety profile after IRE is compelling, if it is concluded that IRE does not present a significant advantage over conventional therapies, patient selection for IRE could include those with central renal tumors near blood vessels and collecting systems in which the nonthermal mode of ablation can be exploited. Min Wah *et al.* evaluated the safety and efficacy of CT-guided IRE in 26 patients with 30 biopsy-proven RCCs near vital structures of the kidney.⁹⁶ Nearby structures included the colon (n = 11), ureter (n = 11), and renovascular pedicles (n = 7). They specified that the initial technical success of 73.3% was due to an early operator’s learning curve, and 7/8 of the residual tumors were treated with CA to achieve a technical success rate of 97%. They state that one patient was not retreated due to an unexpected stroke at 4 months post-IRE. The 2- and 3-year recurrence-free survival was 91% for both time points. Six patients experienced minor complications, and 1 patient experienced a major complication (Clavien-Dindo III), as the patient developed post-proximal ureteral stricture that required long-term retrograde ureteric stenting.

Lung

Lung cancer is the deadliest and most prevalent cancer globally, with few curative treatment options. Central tumors near the central bronchial structures and large blood vessels are especially challenging to treat with surgical resection and thermal ablation modalities. IRE can potentially spare critical structures, but current oncological outcomes are lacking.

Thomson *et al.* treated 1 patient with 1 non-small-cell carcinoma and 3 patients with 5 colorectal lung metastases.⁹⁵ None of these patients treated with IRE had a satisfactory tumor response, and they all presented with progressive disease when assessed by the 3-mo time point. A biopsy from one of the patients showed coagulative necrosis in a portion of the tumor with viable cancerous tissue at the margin of the treated lesion. All four patients experienced transient ventricular arrhythmia, one patient presented transient supraventricular tachycardia, and one patient required cardioversion as a response to atrial fibrillation. Pneumothorax was observed in two out of the four patients which resolved spontaneously. Usman *et al.* reported on the use of IRE to treat two patients with lung neoplasms that had been previously deemed unresectable.⁹⁸ One of the patients presented with an increase of the right suprahilar mass with ce-CT, suggesting tumor growth reported 2 months after the procedure. Moderate parenchymal hemorrhage was observed during the procedure, and at the 9-month follow-up, it was suggested that the tumor had invaded the trachea. The cancer continued to progress, and the patient succumbed to the disease within a year post-IRE. The other patient was reported to still be alive 2.5 years after the procedure, with no major complications described. The authors explain that challenges still remain with using IRE to treat lung tumor masses due to the heterogeneity, geometry, and low density of lung tissue. It is clear that further research is needed to optimize IRE treatment of lung cancer through collaboration between engineers and clinicians. It can be argued that these studies were limited because the probes themselves were not designed for lung treatments, and thus, surgical probes need to be tailored for this particular application.

Kodama *et al.* determined that electroporation applied through an endobronchial catheter is a feasible technique for the treatment of parabronchial tumors in a pig lung tumor model.²²⁰ The ablations measured on gross pathology were signifi-

cantly smaller than the treatment-related changes measured on CT, contrasting observations in other organ systems. Using FEM, they predicted EFs sufficient to induce irreversible electroporation (500–2000 V/cm) within a 1 cm circumference around the probe, which was reflected by extensive ablations seen in gross histology. However, large blood vessels and airways significantly affected the EF distribution, reducing the local EF in portions of the tumor below the lethal EFT. Lastly, they found that electroporation does not affect the patency of the treated bronchi.

Cardiac

Catheter-based PFA is emerging as a promising alternative to thermal techniques (RFA & CA) in treating cardiac arrhythmias due to the better safety profile and similar efficacy.^{221,222} The rapid success of PFA in the clinic has led many research groups and companies to develop their own probes and electroporation systems (Figure 8), often keeping technical details and treatment parameters secret. Direct electric currents were first used to treat cardiac arrhythmias in the 1980s; however, the continuous application of the EF caused electrical arcing, barotrauma, and proarrhythmic effects. Lavee *et al.* were the first to utilize IRE for atrial ablation in 5 pigs, which mitigated the previous complications experienced with direct current applications¹²⁴ and achieved sharp transmural with no evidence of thermal damage. Subsequently, preclinical and clinical studies have demonstrated that PFA selectively ablates cardiac tissue while minimally affecting peri-atrial tissue, such as the esophagus and phrenic nerve²²³, and lowers the risk of pulmonary vein stenosis compared to thermal ablation. Recently, the results from multiple large clinical trials have been released.

The first and most studied PFA catheter is the multi-electrode pentaspline catheter.¹²² The Impulse, PEFCAT, PEFCAT2, and PersAFONE trials demonstrated the initial feasibility and safety of this catheter for treating paroxysmal and persistent AF in relatively small cohorts.²²⁴ Recently, the MANIFEST-PF¹¹⁷ and MANIFEST-17k²²⁵ clinical trials provide compelling safety and efficacy results in larger patient cohorts and across more centers. The MANIFEST-PF trial included 24 centers and 1,758 patients to determine the acute effectiveness and safety of PFA and found that PFA achieved complete acute pulmonary vein isolation in 99.9% of patients on immediate electroanatomical

mapping. The 1-year recurrence rates were 31% for the total cohort, 27% for paroxysmal AF, and 42% for persistent AF. The MANIFEST-17k trial evaluated the safety of PFA across at 106 centers across 20 countries in 17,642 patients with paroxysmal (57.8%) and persistent (35.2%) AF. At a median of 15 months follow-up, no esophageal damage, pulmonary vein stenosis, or persistent phrenic nerve palsy were reported. Major complications were reported in 0.98% of patients, with the most common being pericardial tamponade (0.36%), vascular events (0.30%), stroke (0.12%), hemolysis-related acute renal failure (0.03%), and death (0.03%). Two of the deaths (0.01%) were procedure-related from irreversible neurological damage; post-procedural brain MRI was performed in 96 asymptomatic patients to determine the rate of silent cerebral lesions (SCLs), of which 9.4% of patients showed abnormalities. Further, the recent ADVENT trial demonstrated the non-inferiority of PFA using the pentaspline catheter in a randomized, single-blind prospective comparison to conventional thermal ablation (RFA or CA) in 707 paroxysmal AF patients^{221,222} evaluating the safety and 1-year recurrence rates of pulsed-field ablation against thermal ablation (RFA or CA). Urbanek *et al.* found similar results in 400 patients and achieved similar 1-year success rates between CA and pentaspline PFA in both paroxysmal AF (83.1% CA *vs.* 80.3% PFA) and persistent AF (71% CA *vs.* 66.8% PFA).¹²⁶

The PULSED AF pivotal trial evaluated the circular-lasso-type 9-electrode catheter in 150 paroxysmal and 150 symptomatic persistent AF patients.¹²⁵ They achieved 100% acute pulmonary vein isolation rates for both groups, but at the 90-day follow-up, the recurrence rate was already 30.5% and 37.7% for the paroxysmal and persistent AF groups, respectively. The 1-year recurrence rates did not increase much from the 90-day rates, with 33.8% for the paroxysmal AF and 44.9% for the persistent AF patients. Two severe adverse effects occurred due to treatment (0.7%): one cerebrovascular accident occurred the same day as treatment and one pericardial effusion that required draining.

The SPHERE PER-AF trial is a randomized, 2-arm prospective study evaluating a large-tip catheter dual PFA and RFA ablation system against a control RFA system.²²⁶ They found that PFA had significantly lower energy application times, transpired ablation times, and skin-to-skin procedural times. At a 1-year follow-up, 73.8% and 65.8% of patients were arrhythmia-free for the large-tip

catheter and control system, respectively, with no major complications observed in either group.

The insPIRE and admIRE trials investigated the safety and efficacy of using a variable-loop circular catheter (VLCC).^{227,228} The insPIRE trial investigated the safety and efficacy of the VLCC in 226 patients with paroxysmal AF. The 12-month freedom from symptomatic arrhythmia was 79%. Pre- and post-treatment MRI imaging detected SCLs in 4 of the first 6 patients. After adjusting treatment to include a 10-second pause between PFA applications and strictly adhering to the anticoagulation regimen, SCLs were found in 4 of the remaining 33 patients. All the SCLs were asymptomatic and resolved spontaneously. The VLCC can be used for guidance, stimulation/recording of cardiac signals, and applying PFA, so the admIRE trial investigated the use of the VLCC for real-time non-fluoroscopic procedural guidance and lesion indexing in 277 patients with paroxysmal AF. They achieved 97.5% success on first-pass per vein isolation, with 100% of veins ultimately isolated. At 12 months, they found similar efficacy to patients treated without fluoroscopy (75% vs. 72.7%), demonstrating that treatments can be delivered without fluoroscopy, which can potentially speed up procedures, minimizing procedure-related complications and exposure to X-rays.

Collectively, these results indicate that H-FIRE is a safe and effective method for pulmonary isolation, but high acute pulmonary isolation rates have not necessarily translated to long-term freedom from disease. Nevertheless, PFA has similar, if not slightly better, efficacy than thermal ablation, but currently, methods are still needed to generate deeper and wider transmural lesions to prevent recurrence.

Multiple preclinical and early clinical evaluations have also demonstrated the feasibility of PFA for the treatment of ventricular arrhythmias (VAs).²²⁹⁻²³¹ VAs pose a unique challenge due to the thickness of the tissue and frequent scar tissue, making it challenging to develop deep lesions. PFA is indicated to better penetrate through scar tissue²³¹⁻²³³, allowing for treatment of tissue that other focal ablation therapies cannot reach and for redo ablations. Peich *et al.* evaluated focal PFA in 21 patients with ventricular premature complexes and 23 patients with scar-related ventricular tachycardia.²³⁴ Using the highest energy setting (25A), they achieve 81% and 52% success for the premature complex and tachycardia patients, respectively, at a mean follow-up of 116 days.

Concluding remarks

It has almost been 300 years since the earliest recording of electrically mediated tissue damage by Jean-Antoine Nollet in 1754. He observed the formation of red spots, presumably due to IRE, following the application of high voltages to human and animal skin. Only 20 years ago was IRE again described as a viable option for controlled tissue destruction. In such a short period, it has significantly impacted the treatment of soft tumors and cardiac tissue. However, there are still multiple areas of improvement:

(1) Factors influencing electroporation at the cellular and tissue level are still not fully understood, and there is still a large gap in knowledge on the precise mechanisms of cell death following different PFA procedures. PFA is unique compared to every other focal therapy, and understanding genetic and proteomic changes following treatment is paramount for developing synergistic therapies.

(2) Accordingly, the dynamics of tumor micro-environmental changes following PFA have only recently started being investigated.

(3) Electroporation-dependent tissue properties for many tissues and tumors are not available, and there are currently no guidelines on appropriate methods for gathering and validating data. This limits confidence in computational models for predicting ablation outcomes before treatment.

(4) Inserting and maintaining multiple probes is the most technically challenging and time-consuming aspect of IRE treatments. Improved methods for delivering PEFs will presumably help increase the adoption of PFA and decrease operating room times.

(5) While ablations can be measured soon after treatment, there are no clinically ready methods for real-time ablation progression or temperature monitoring. The lack of real-time feedback can lead to unnecessary thermal damage and avoidable complications.

(6) Due to the multifaceted nature of PFA, optimized waveforms for oncology and cardiology have yet to be developed.

Therefore, it is important for industry, clinicians, and researchers to work together to allow for independent analysis and validation of data. If clinicians are aware of the capabilities and limitations of PFA procedures, tissues that were once considered untreatable and unresectable may now find a legitimate contender with IRE.

References

- Gabriel B, Teissie J. Generation of reactive-oxygen species induced by electroporation of Chinese hamster ovary cells and their consequence on cell viability. *Eur J Biochem* 1994; **223**: 25-33. doi: 10.1111/j.1432-1033.1994.tb18962.x
- Maccarrone M, Rosato N, Agro AF. Electroporation enhances cell membrane peroxidation and luminescence. *Biochem Biophys Res Commun* 1995; **206**: 238-45. doi: 10.1006/bbrc.1995.1033
- Maccarrone M, Bladergroen MR, Rosato N, Agro AF. Role of lipid peroxidation in electroporation-induced cell permeability. *Biochem Biophys Res Commun* 1995; **209**: 417-25. doi: 10.1006/bbrc.1995.1519
- Bonnafous P, Vernhes MC, Teissie J, Gabriel B. The generation of reactive-oxygen species associated with long-lasting pulse-induced electroporation of mammalian cells is based on a non-destructive alteration of the plasma membrane. *Biochim Biophys Acta Biomembranes* 1999; **1461**: 123-34. doi: 10.1016/S0005-2736(99)00154-6
- Rems L, Viano M, Kasimova MA, Miklavčič D, Tarek M. The contribution of lipid peroxidation to membrane permeability in electroporation: a molecular dynamics study. *Bioelectrochemistry* 2019; **125**: 46-57. doi: 10.1016/j.bioelechem.2018.07.018
- Balantič K, Weiss VU, Pittenauer E, Miklavčič D, Kramar P. The role of lipid oxidation on electrical properties of planar lipid bilayers and its importance for understanding electroporation. *Bioelectrochemistry* 2023; **153**: 108498. doi: 10.1016/j.bioelechem.2023.108498
- Rems L, Kasimova MA, Testa I, Delemotte L. Pulsed electric fields can create pores in the voltage sensors of voltage-gated ion channels. *Biophys J* 2020; **119**: 190-205. doi: 10.1016/j.bpj.2020.05.030
- Geboers B, Scheffer HJ, Graybill PM, Ruarus AH, Nieuwenhuizen S, Puijk RS, et al. High-voltage electrical pulses in oncology: Irreversible electroporation, electrochemotherapy, gene electrotransfer, electrofusion, and electroimmunotherapy. *Radiology* 2020; **295**: 254-72. doi: 10.1148/RADJOL.2020192190
- Neumann E, Rosenheck K. Permeability changes induced by electric impulses in vesicular membranes. *J Membr Biol* 1972; **10**: 279-90. doi: 10.1007/BF01867861
- DeBruin KA, Krassowska W. Modeling electroporation in a single cell. I. Effects of field strength and rest potential. *Biophys J* 1999; **77**: 1213-24. doi: 10.1016/S0006-3495(99)76973-0
- Lee RC, Gaylor DC, Bhatt D, Israel DA. Role of cell membrane rupture in the pathogenesis of electrical trauma. *J Surg Res* 1988; **44**: 709-19. doi: 10.1016/0022-4804(88)90105-9
- Jacobs EJ, Graybill PM, Jana A, Agashe A, Nain AS, Davalos R V. Engineering high post-electroporation viabilities and transfection efficiencies for elongated cells on suspended nanofiber networks. *Bioelectrochemistry* 2023; **152**: 108415. doi: 10.1016/j.bioelechem.2023.108415
- Böckmann RA, De Groot BL, Kakorin S, Neumann E, Grubmü H. Kinetics, statistics, and energetics of lipid membrane electroporation studied by molecular dynamics simulations. *Biophys J* 2008; **95**: 1837-50. doi: 10.1529/biophysj.108.129437
- Neumann E, Kakorin S. Membrane electroporation: chemical thermodynamics and flux kinetics revisited and refined. *Eur Biophys J* 2018; **47**: 373-87. doi: 10.1007/S00249-018-1305-3
- Saulis G. Pore disappearance in a cell after electroporation: theoretical simulation and comparison with experiments. *Biophys J* 1997; **73**: 1299-309. doi: 10.1016/S0006-3495(97)78163-3
- Ottlakan A, Lazar G, Olah J, Nagy A, Vass G, Vaset M, et al. Current updates in bleomycin-based electrochemotherapy for deep-seated soft-tissue tumors. *Electrochem* 2023; **4**: 282-90. doi: 10.3390/electrochem4020019
- Mir LM, Orłowski S, Belehradek J, Paoletti C. Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses. *Eur J Cancer Clin Oncol* 1991; **27**: 68-72. doi: 10.1016/0277-5379(91)90064-K
- Spiliotis AE, Holländer S, Rudzitis-Auth J, Wagenpfeil G, Eisele R, Nika S, et al. Evaluation of electrochemotherapy with bleomycin in the treatment of colorectal hepatic metastases in a rat model. *Cancers* 2023; **15**: 1598. doi: 10.3390/cancers15051598
- Pakhomova ON, Gregory B, Semenov I, Pakhomov AG. Calcium-mediated pore expansion and cell death following nanoelectroporation. *Biochim Biophys Acta Biomembr* 2014; **1838**: 2547-54. doi: 10.1016/j.bbmem.2014.06.015
- Szewczyk A, Gehl J, Daczewska M, Sacko J, Krog Frandsen S, Kulbacka J. Calcium electroporation for treatment of sarcoma in preclinical studies. *Oncotarget* 2018; **9**: 11604-18. doi: 10.18632/oncotarget.24352
- Falk H, Matthiessen LW, Wooler G, Gehl J. Calcium electroporation for treatment of cutaneous metastases; a randomized double-blinded phase II study, comparing the effect of calcium electroporation with electrochemotherapy. *Acta Oncol* 2018; **57**: 311-9. doi: 10.1080/0284186X.2017.1355109
- Frandsen SK, Gissel H, Hojman P, Tramm T, Eriksen J, Gehl J. Direct therapeutic applications of calcium electroporation to effectively induce tumor necrosis. *Cancer Res* 2012; **72**: 1336-41. doi: 10.1158/0008-5472.CAN-11-3782
- Plaschke CC, Gehl J, Johannesen HH, Fisher BM, Kjaer A, Lomholt AF, et al. Calcium electroporation for recurrent head and neck cancer: a clinical phase I study. *Laryngoscope Investig Otolaryngol* 2019; **4**: 49-56. doi:10.1002/liv.2.233
- Kraemer MM, Tsimpaki T, Berchner-Pfannschmidt U, Bechrakis NE, Seitz B, Fiorentz M. Calcium electroporation reduces viability and proliferation capacity of four uveal melanoma cell lines in 2D and 3D cultures. *Cancers* 2022; **14**: 2889. doi:10.3390/cancers14122889
- Frandsen SK, Gibot L, Madi M, Gehl J, Rols MP. Calcium electroporation: Evidence for differential effects in normal and malignant cell lines, evaluated in a 3D spheroid model. *PLoS One* 2015; **10**: e0144028. doi: 10.1371/journal.pone.0144028
- Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH. Gene transfer into mouse lymphoma cells by electroporation in high electric fields. *EMBO J* 1982; **1**: 841-5. doi: 10.1002/j.1460-2075.1982.tb01257.x
- Rosazza C, Haberl Meglic S, Zumbusch A, Rols MP, Miklavcic D. Gene electrotransfer: a mechanistic perspective. *Curr Gene Ther* 2016; **16**: 98-129. doi: 10.2174/1566523216666160331130040
- Alex A, Piano V, Polley S, Stuver M, Voss S, Ciossani G, et al. Electroporated recombinant proteins as tools for in vivo functional complementation, imaging and chemical biology. *Elife* 2019; **8**: e48287. doi: 10.7554/eLife.48287.001
- Batista Napotnik T, Polajžer T, Miklavčič D. Cell death due to electroporation – a review. *Bioelectrochemistry* 2021; **141**: 107871. doi: 10.1016/j.BIOELECHEM.2021.107871
- Saulis G, Saule R. Size of the pores created by an electric pulse: microsecond vs millisecond pulses. *Biochim Biophys Acta* 2012; **1818**: 3032-9. doi: 10.1016/j.bbamem.2012.06.018
- Runas KA, Malmstadt N. Low levels of lipid oxidation radically increase the passive permeability of lipid bilayers. *Soft Matter* 2015; **11**: 499-505. doi: 10.1039/c4sm01478b
- Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014; **2014**: 360438. doi: 10.1155/2014/360438
- Vernier PT, Levine ZA, Wu YH, Joubert V, Ziegler MJ, Mir LM, et al. Electroporating fields target oxidatively damaged areas in the cell membrane. *PLoS One* 2009; **4**: e7966. doi: 10.1371/journal.pone.0007966
- Leguèbe M, Silve A, Mir LM, Poignard C. Conducting and permeable states of cell membrane submitted to high voltage pulses: mathematical and numerical studies validated by the experiments. *J Theor Biol* 2014; **360**: 83-94. doi: 10.1016/j.jtbi.2014.06.027
- Graybill PM, Davalos R V. Cytoskeletal disruption after electroporation and its significance to pulsed electric field therapies. *Cancers* 2020; **12**: 1132. doi: 10.3390/cancers12051132
- Steuer A, Schmidt A, Labohá P, Babica P, Kolb JF. Transient suppression of gap junctional intercellular communication after exposure to 100-nanosecond pulsed electric fields. *Bioelectrochemistry* 2016; **112**: 33-46. doi: 10.1016/j.bioelechem.2016.07.003
- Steuer A, Wende K, Babica P, Kolb JF. Elasticity and tumorigenic characteristics of cells in a monolayer after nanosecond pulsed electric field exposure. *Eur Biophys J* 2017; **46**: 567-80. doi: 10.1007/s00249-017-1205-y

38. Thompson GL, Roth C, Tolstykh G, Kuipers M, Ibey BL. Disruption of the actin cortex contributes to susceptibility of mammalian cells to nanosecond pulsed electric fields. *Bioelectromagnetics* 2014; **35**: 262-72. doi: 10.1002/bem.21845
39. Graybill PM, Jana A, Kapania RK, Nain AS, Davalos R V. Single cell forces after electroporation. *ACS Nano* 2021; **15**: 2554-68. doi: 10.1021/acsnano.0c07020
40. Thompson GL, Roth CC, Dalzell DR, Kuipers MA, Ibey BL. Calcium influx affects intracellular transport and membrane repair following nanosecond pulsed electric field exposure. *J Biomed Opt* 2014; **19**: 055005. doi: 10.1117/1.jbo.19.5.055005
41. Kanthou C, Kranjc S, Sersa G, Tozer G, Zupanic A, Cemazar M. The endothelial cytoskeleton as a target of electroporation-based therapies. *Mol Cancer Ther* 2006; **5**: 3145-52. doi: 10.1158/1535-7163.MCT-06-0410
42. Harkin DG, Hay ED. Effects of electroporation on the tubulin cytoskeleton and directed migration of corneal fibroblasts cultured within collagen matrices. *Cell Motil Cytoskeleton* 1996; **35**: 345-57. doi: 10.1002/(SICI)1097-0169(1996)35:4<345::AID-CM6>3.0.CO;2-5
43. Thompson GL, Roth CC, Kuipers MA, Tolstykh GP, Beier HT, Ibey BL. Permeabilization of the nuclear envelope following nanosecond pulsed electric field exposure. *Biochem Biophys Res Commun* 2016; **470**: 35-40. doi: 10.1016/j.bbrc.2015.12.092
44. Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. *Nature* 2010; **463**: 485-92. doi: 10.1038/nature08908
45. Rols MP, Teissié J. Experimental evidence for the involvement of the cytoskeleton in mammalian cell electroporation. *Biochim Biophys Acta* 1992; **1111**: 45-50. doi: 10.1016/0005-2736(92)90272-N
46. Partridge BR, Kani Y, Lorenzo MF, Campelo SN, Allen IC, Hinckley J, et al. High-frequency irreversible electroporation (H-FIRE) induced blood-brain barrier disruption is mediated by cytoskeletal remodeling and changes in tight junction protein regulation. *Biomedicines* 2022; **10**: 1384. doi: 10.3390/biomedicines10061384
47. Arena CB, Garcia PA, Sano MB, et al. Focal blood-brain-barrier disruption with high-frequency pulsed electric fields. *Technology* 2014; **2**: 206-13. doi: 10.1142/s2339547814500186
48. Garcia PA, Rossmeis JH, Robertson JL, Olson JD, Johnson AJ, Ellis TL, et al. 7.0-T magnetic resonance imaging characterization of acute blood-brain barrier disruption achieved with intracranial irreversible electroporation. *PLoS One* 2012; **7**: e50482. doi: 10.1371/journal.pone.0050482
49. Stacey M, Fox P, Buescher S, Kolb J. Nanosecond pulsed electric field induced cytoskeleton, nuclear membrane and telomere damage adversely impact cell survival. *Bioelectrochemistry* 2011; **82**: 131-4. doi: 10.1016/j.bioelechem.2011.06.002
50. Xiao D, Tang L, Zeng C, Wang J, Luo X, Yao C, et al. Effect of actin cytoskeleton disruption on electric pulse-induced apoptosis and electroporation in tumour cells. *Cell Biol Int* 2011; **35**: 99-104. doi: 10.1042/cbi20100464
51. Davalos R V, Mir LM, Rubinsky B. Tissue ablation with irreversible electroporation. *Ann Biomed Eng* 2005; **33**: 223-31. doi: 10.1007/s10439-005-8981-8
52. Edd JF, Horowitz L, Davalos R V, Mir LM, Rubinsky B. In vivo results of a new focal tissue ablation technique: Irreversible electroporation. *IEEE Trans Biomed Eng* 2006; **53**: 1409-15. doi: 10.1109/TBME.2006.873745
53. Al-Sakere B, André F, Bernat C, Connault E, Opolon P, Davalos RV, et al. Tumor ablation with irreversible electroporation. *PLoS One* 2007; **2**: e1135. doi: 10.1371/JOURNAL.PONE.0001135
54. Bertacchini C, Margotti PM, Bergamini E, Lodi A, Ronchetti M, Cadossi R. Design of an irreversible electroporation system for clinical use. *Technol Cancer Res Treat* 2007; **6**: 313-20. doi: 10.1177/15330346070060040
55. Gielchinsky I, Lev-Cohain N. Focal irreversible electroporation for localized prostate cancer – oncological and safety outcomes using mpMRI and transperineal biopsy follow-up. *Res Rep Urol* 2023; **15**: 27-35. doi: 10.2147/RRU.5393243
56. Zhang K, Teoh J, Laguna P, Dominguez-Escrig J, Barret E, Ramon-Borja JC, et al. Effect of focal vs extended irreversible electroporation for the ablation of localized low- or intermediate-risk prostate cancer on early oncological control: a randomized clinical trial. *JAMA Surg* 2023; **158**: 343-9. doi: 10.1001/jamasurg.2022.7516
57. Prabhakar P, Avudaiappan AP, Sandman M, Eldefrawy A, Caso J, Narayanan G, et al. Irreversible electroporation as a focal therapy for localized prostate cancer: a systematic review. *Indian J Urol* 2024; **40**: 6-16. doi: 10.4103/iju.iju_370_23
58. van den Bos W, Jurhill RR, de Bruin DM, Savci-Heijink CD, Postema AW, Wagstaff PG, et al. Histopathological outcomes after irreversible electroporation for prostate cancer: results of an ablate and resect study. *J Urol* 2016; **196**: 552-9. doi: 10.1016/j.juro.2016.02.2977
59. Valerio M, Dickinson L, Ali A, Ramachandran N, Donaldson I, McCartan N, et al. Nanoknife electroporation ablation trial: a prospective development study investigating focal irreversible electroporation for localized prostate cancer. *J Urol* 2017; **197**: 647-54. doi: 10.1016/j.juro.2016.09.091
60. van den Bos W, Scheltema MJ, Siriwardana AR, Kalsbeek AMF, Thompson JE, Ting F, et al. Focal irreversible electroporation as primary treatment for localized prostate cancer. *BJU Int* 2018; **121**: 716-24. doi: 10.1111/bju.13983
61. Onik G, Mikus P, Rubinsky B. Irreversible electroporation: Implications for prostate ablation. *Technol Cancer Res Treat* 2007; **6**: 295-300. doi: 10.1177/153303460700600405
62. Onik G, Rubinsky B. Irreversible electroporation: first patient experience focal therapy of prostate cancer. In: *Irreversible electroporation*. [internet]. 1970: 235-47. [cited 2024 Oct 15]. doi: 10.1007/978-3-642-05420-4_10. Available at: <https://www.researchgate.net/publication/225882920>
63. Valerio M, Stricker PD, Ahmed HU, Dickinson L, Ponsky L, Shnier R, et al. Initial assessment of safety and clinical feasibility of irreversible electroporation in the focal treatment of prostate cancer. *Prostate Cancer Prostatic Dis* 2014; **17**: 343-7. doi: 10.1038/pcan.2014.33
64. Van den Bos W, De Bruin D, Veelo D, Postema AW1, Muller BG1, Varkarakis IM, et al. Quality of life and safety outcomes following irreversible electroporation treatment for prostate cancer: results from a phase II study. *J Cancer Sci Ther* 2015; **7**: 312-21. doi: 10.4172/1948
65. Tian G, Guan J, Chu Y, Zhao Q, Jiang T. Immunomodulatory effect of irreversible electroporation alone and its cooperating with immunotherapy in pancreatic cancer. *Front Oncol* 2021; **11**: 712042. doi: 10.3389/fonc.2021.712042
66. Kwon D, McFarland K, Velanovich V, Martin RCG. Borderline and locally advanced pancreatic adenocarcinoma margin accentuation with intraoperative irreversible electroporation. *Surgery* 2014; **156**: 910-20. doi: 10.1016/j.surg.2014.06.058
67. Holland MM, Bhutiani N, Kruse EJ, Weiss MJ, Christein JD, White RR, et al. A prospective, multi-institution assessment of irreversible electroporation for treatment of locally advanced pancreatic adenocarcinoma: initial outcomes from the AHPBA pancreatic registry. *HPB (Oxford)* 2019; **21**: 1024-31. doi: 10.1016/j.hpb.2018.12.004
68. Belfiore MP, Ronza FM, Romano F, Ianniello GP, De Lucia G, Gallo C, et al. Percutaneous CT-guided irreversible electroporation followed by chemotherapy as a novel neoadjuvant protocol in locally advanced pancreatic cancer: our preliminary experience. *Int J Surg* 2015; **21(Suppl 1)**: S34-9. doi: 10.1016/j.ijso.2015.06.049
69. Spiliopoulos S, Reppas L, Filippiadis D, Delvecchio A, Conticchio M, Memeo R, et al. Irreversible electroporation for the management of pancreatic cancer: current data and future directions. *World J Gastroenterol* 2023; **29**: 223-31. doi: 10.3748/wjg.v29.i2.223
70. Stephens K, Philips PP, Egger ME, Scoggins CR, McMasters KM, Martin RCG. Multi-institutional review of adverse events associated with irreversible electroporation in the treatment of locally advanced pancreatic cancer. *Surgery* 2024; **175**: 704-11. doi: 10.1016/j.surg.2023.08.042
71. Shuiqing H, Sheng L. Is irreversible electroporation (IRE) an effective and safe ablation method for local advanced pancreatic cancer: a meta-analysis. *Health Sciences Review* 2022; **3**: 100029. doi: 10.1016/j.hsr.2022.100029
72. Woeste MR, Wilson KD, Kruse EJ, Weiss MJ, Christein JD, White RR, et al. Optimizing patient selection for irreversible electroporation of locally advanced pancreatic cancer: analyses of survival. *Front Oncol* 2022; **11**: 817220. doi: 10.3389/fonc.2021.817220
73. Pishvaian MJ, Blais EM, Brody JR, Lyons E, DeArbeloa P, Hendifar A, et al. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. *Lancet Oncol* 2020; **21**: 508-18. doi: 10.1016/S1470-2045(20)30074-7

74. He C, Wang J, Sun S, Zhang Y, Li S. Immunomodulatory effect after irreversible electroporation in patients with locally advanced pancreatic cancer. *J Oncol* 2019; **2019**: 9346017. doi: 10.1155/2019/9346017
75. Dai Z, Wang Z, Lei K, Liao J, Peng Z, Lin M, et al. Irreversible electroporation induces CD8+ T cell immune response against post-ablation hepatocellular carcinoma growth. *Cancer Lett*. 2021; **503**: 1-10. doi: 10.1016/j.canlet.2021.01.001
76. Sugimoto K, Kakimi K, Takeuchi H, Fujieda N, Saito K, Sato E, et al. Irreversible electroporation versus radiofrequency ablation: comparison of systemic immune responses in patients with hepatocellular carcinoma. *J Vasc Interv Radiol* 2019; **30**: 845-53.e6. doi: 10.1016/j.jvir.2019.03.002
77. Lu LC, Shao YY, Chan SY, Hsu CH, Cheng AL. Clinical characteristics of advanced hepatocellular carcinoma patients with prolonged survival in the era of anti-angiogenic targeted-therapy. *Anticancer Res* 2014; **34**: 1047-52. doi: 10.1016/j.jvir.2019.03.002
78. Dai Z, Wang Z, Lei K, Liao J, Peng Z, Lin M, et al. Irreversible electroporation induces CD8+ T cell immune response against post-ablation hepatocellular carcinoma growth. *Cancer Lett* 2021; **503**: 1-10. doi: 10.1016/j.canlet.2021.01.001
79. Narayanan G, Koethe Y, Gentile N. Irreversible electroporation of the hepatobiliary system: current utilization and future avenues. *Medicina (Kaunas)* 2024; **60**: 251. doi: 10.3390/medicina60020251
80. Yang Y, Qin Z, Du D, Wu Y, Qiu S, Mu F, et al. Safety and short-term efficacy of irreversible electroporation and allogenic natural killer cell immunotherapy combination in the treatment of patients with unresectable primary liver cancer. *Cardiovasc Intervent Radiol* 2019; **42**: 48-59. doi: 10.1007/s00270-018-2069-y
81. Guo X, Du F, Liu Q, Guo Y, Wang Q, Huang W, et al. Immunological effect of irreversible electroporation on hepatocellular carcinoma. *BMC Cancer* 2021; **21**: 443. doi: 10.1186/s12885-021-08176-x
82. Eller A, Schmid A, Schmidt J, May M, Brand M, Saake M, et al. Local control of perivascular malignant liver lesions using percutaneous irreversible electroporation: initial experiences. *Cardiovasc Intervent Radiol* 2015; **38**: 152-9. doi: 10.1007/s00270-014-0898-x
83. Beyer LP, Pregler B, Michalik K, Niessen C, Dollinger M, Müller M, et al. Evaluation of a robotic system for irreversible electroporation (IRE) of malignant liver tumors: initial results. *Int J Comput Assist Radiol Surg* 2017; **12**: 803-9. doi: 10.1007/s11548-016-1485-1
84. Hitpass L, Distelmaier M, Neumann UP, Schöning W, Isfort P, Keil S, Kuhl CK, et al. Recurrent colorectal liver metastases in the liver remnant after major liver surgery - IRE as a salvage local treatment when resection and thermal ablation are unsuitable. *Cardiovasc Intervent Radiol* 2022; **45**: 182-9. doi: 10.1007/s00270-021-02981-4
85. Buijs M, Zondervan PJ, de Bruin DM, van Lienden KP, Bex A, van Delden OM. Feasibility and safety of irreversible electroporation (IRE) in patients with small renal masses: results of a prospective study. *Urolo Oncol* 2019; **37**: 183.e1-183.e8. doi: 10.1016/j.urolonc.2018.11.008
86. Trimmer CK, Khosla A, Morgan M, Stephenson SL, Ozayar A, Cadeddu JA. Minimally invasive percutaneous treatment of small renal tumors with irreversible electroporation: a single-center experience. *J Vasc Interv Radiol* 2015; **26**: 1465-71. doi: 10.1016/j.jvir.2015.06.028
87. Narayanan G, Doshi MH. Irreversible electroporation (IRE) in renal tumors. *Curr Urol Rep* 2016; **17**: 1-7. doi: 10.1007/s11934-015-0571-1
88. Hilton A, Kourounis G, Georgiades F. Irreversible electroporation in renal tumours: a systematic review of safety and early oncological outcomes. *Urologia* 2022; **89**: 329-37. doi: 10.1177/03915603221077590
89. Canvasser NE, Sorokin I, Lay AH, Morgan MSC, Ozayar A, Trimme C, et al. Irreversible electroporation of small renal masses: suboptimal oncologic efficacy in an early series. *World J Urol* 2017; **35**: 1549-55. doi: 10.1007/s00345-017-2025-5
90. Pech M, Janitzky A, Wendler JJ, Morgan MSC, Ozayar A, Trimmer C, et al. Irreversible electroporation of renal cell carcinoma: a first-in-man phase I clinical study. *Cardiovasc Intervent Radiol* 2011; **34**: 132-8. doi: 10.1007/s00270-010-9964-1
91. Deodhar A, Monette S, Single GW, Hamilton WC Jr, Thornton R, Maybody M, et al. Renal tissue ablation with irreversible electroporation: preliminary results in a porcine model. *Urology* 2011; **77**: 754-60. doi: 10.1016/j.urol.2010.08.036
92. Wendler JJ, Pech M, Fischbach F, Jürgens J, Friebe B, Baumunk D, et al. Initial assessment of the efficacy of irreversible electroporation in the focal treatment of localized renal cell carcinoma with delayed-interval kidney tumor resection [Irreversible Electroporation of Kidney Tumors Before Partial Nephrectomy [IRENE] trial - an ablate-and-resect pilot study]. *Urology* 2018; **114**: 224-32. doi: 10.1016/j.urology.2017.12.016
93. Neal RE, Garcia PA, Kavnoudias H, Rosenfeldt F, Mclean CA, Earl V, Bergman J, et al. In vivo irreversible electroporation kidney ablation: experimentally correlated numerical models. *IEEE Trans Biomed Eng* 2015; **62**: 561-9. doi: 10.1109/TBME.2014.2360374
94. Deodhar A, Monette S, Single GW, Hamilton WC Jr, Thornton R, Maybody M, et al. Renal tissue ablation with irreversible electroporation: preliminary results in a porcine model. *Urology* 2011; **77**: 754-60. doi: 10.1016/j.urol.2010.08.036
95. Thomson KR, Cheung W, Ellis SJ, Federman D, Kavnoudias H, Loader-Oliver D, et al. Investigation of the safety of irreversible electroporation in humans. *J Vasc Interv Radiol* 2011; **22**: 611-21. doi: 10.1016/j.jvir.2010.12.014
96. Min Wah T, Lenton J, Smith J, Bassett P, Jagdev S, Ralph C, et al. Irreversible electroporation (IRE) in renal cell carcinoma (RCC): a mid-term clinical experience. *Eur Radiol* 2021; **31**: 7491-9. doi: 10.1007/s00330-021-07846-5
97. Sorokin I, Canvasser N, Johnson B, Lucas E, Cadeddu JA. Irreversible electroporation for renal ablation does not cause significant injury to adjacent ureter or bowel in a porcine model. *J Endourol* 2021; **35**: 873-7. doi: 10.1089/END.2020.0856
98. Usman M, Moore W, Talati R, Watkins K, Bilfinger T V. Irreversible electroporation of lung neoplasm: a case series. *Med Sci Monit* 2012; **18**: C543-7. doi: 10.12659/MSM.882888
99. Rieke J, Jürgens JHW, Deschamps F, Tselikas L, Uhde K, Kosiek O, et al. Irreversible electroporation (IRE) fails to demonstrate efficacy in a prospective multicenter phase II trial on lung malignancies: The ALICE Trial. *Cardiovasc Intervent Radiol* 2015; **38**: 401-8. doi: 10.1007/s00270-014-1049-0
100. Garcia PA, Kos B, Rossmeis JH, Pavliha D, Miklavčič D, Davalos R V. Predictive therapeutic planning for irreversible electroporation treatment of spontaneous malignant glioma. *Med Phys* 2017; **44**: 4968-80. doi: 10.1002/mp.12401
101. Narayanan G. Irreversible electroporation. *Semin Intervent Radiol* 2015; **32**: 349-55. doi: 10.1055/s-0035-1564706
102. Cannon R, Ellis S, Hayes D, Narayanan G, Martin RCG. Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. *J Surg Oncol* 2013; **107**: 544-9. doi: 10.1002/jso.23280
103. Aycock KN, Davalos RV. Irreversible electroporation: background, theory, and review of recent developments in clinical oncology. *Bioelectricity* 2019; **1**: 214-34. doi: 10.1089/BIOE.2019.0029
104. Mali B, Jarm T, Jager F, Miklavčič D. An algorithm for synchronization of in vivo electroporation with ECG. *J Med Eng Technol* 2005; **29**: 288-96. doi: 10.1080/03091900512331332591
105. Arena CB, Sano MB, Rossmeis JH, Caldwell JL, Garcia PA, Rylander MN, et al. High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction. *Biomed Eng Online* 2011; **10**: 102. doi: 10.1186/1475-925X-10-102
106. Partridge BR, O'Brien TJ, Lorenzo MF, Coutermarsh-Ott SL, Barry SL, Stadler K, et al. High-frequency irreversible electroporation for treatment of primary liver cancer: a proof-of-principle study in canine hepatocellular carcinoma. *J Vasc Interv Radiol* 2020; **31**: 482-91.e4. doi: 10.1016/j.jvir.2019.10.015
107. Bhonsle S, Arena C, Sweeney D, Davalos R. Mitigation of impedance changes due to electroporation therapy using bursts of high-frequency bipolar pulses. *Biomed Eng Online* 2015; **14(Suppl 3)**: S3. doi: 10.1186/1475-925X-14-S3-S3
108. Siddiqui IA, Latouche EL, DeWitt MR, Swet JH, Kirks RC, Baker EH, et al. Induction of rapid, reproducible hepatic ablations using next-generation, high frequency irreversible electroporation (H-FIRE) in vivo. *HPB (Oxford)* 2016; **18**: 726-34. doi: 10.1016/j.hpb.2016.06.015
109. Jacobs IV EJ, Campelo SN, Charlton A, Altreuter S, Davalos R V. Characterizing reversible, irreversible, and calcium electroporation to generate a burst-dependent dynamic conductivity curve. *Bioelectrochemistry* 2024; **155**: 108580. doi: 10.1016/j.bioelechem.2023.108580

110. Aycock KN, Vadlamani RA, Jacobs EJ, Imran KM, Verbridge SS, Allen IC, et al. Experimental and numerical investigation of parameters affecting high-frequency irreversible electroporation for prostate cancer ablation. *J Biomech Eng* 2022; **144**: 061003. doi: 10.1115/1.4053595
111. Ringel-Scaia VM, Beitel-White N, Lorenzo MF, Brock RM, Huie KE, Coutermarsh-Ott S, Eden K, et al. High-frequency irreversible electroporation is an effective tumor ablation strategy that induces immunologic cell death and promotes systemic anti-tumor immunity. *EBioMedicine* 2019; **44**: 112-5. doi: 10.1016/j.ebiom.2019.05.036
112. Campelo SN, Lorenzo MF, Partridge B, Alinezhadbalalami N, Kani Y, Garcia J, et al. High-frequency irreversible electroporation improves survival and immune cell infiltration in rodents with malignant gliomas. *Front Oncol* 2023; **13**: 1171278. doi: 10.3389/fonc.2023.1171278
113. Hay AN, Aycock KN, Lorenzo M, David K, Coutermarsh-Ott S, Salameh Z, et al. Investigation of high frequency irreversible electroporation for canine spontaneous primary lung tumor ablation. *Biomedicines* 2024; **12**: 2038. doi: 10.3390/biomedicines12092038
114. Xing R, Ji S, Li X, Gong T, Jiang Q. High-frequency irreversible electroporation ablation for the prostate in Beagle dogs. *Transl Androl Urol* 2024; **13**: 2016-26. doi: 10.21037/tau-24-108
115. Dong S, Wang H, Zhao Y, Sun Y, Yao C. First human trial of high-frequency irreversible electroporation therapy for prostate cancer. *Technol Cancer Res Treat* 2018; **17**: 1-9. doi: 10.1177/1533033818789692
116. Wang H, Xue W, Yan W, Yin L, Dong B, He B, et al. Extended focal ablation of localized prostate cancer with high-frequency irreversible electroporation: a nonrandomized controlled trial. *JAMA Surg* 2022; **157**: 693-700. doi: 10.1001/jamasurg.2022.2230
117. Ekanem E, Reddy VY, Schmidt B, Reichlin T, Neven K, Metzner A, et al. Multi-national survey on the methods, efficacy, and safety on the post-approval clinical use of pulsed field ablation (MANIFEST-PF). *Europace* 2022; **24**: 1256-66. doi: 10.1093/europace/euac050
118. Reddy VY, Neuzil P, Koruth JS, Petru J, Funosako M, Cochet H, et al. Pulsed field ablation for pulmonary vein isolation in atrial fibrillation. *J Am Coll Cardiol* 2019; **74**: 315-26. doi: 10.1016/j.jacc.2019.04.021
119. Reddy VY, Anic A, Koruth J, Petru J, Funasako M, Minami K, et al. Pulsed field ablation in patients with persistent atrial fibrillation. *J Am Coll Cardiol* 2020; **76**: 1068-80. doi: 10.1016/j.jacc.2020.07.007
120. Loh P, Van Es R, Groen MHA, Neven K, Kassenberg W, Wittkamp FHM, et al. Pulmonary vein isolation with single pulse irreversible electroporation: a first in human study in 10 patients with atrial fibrillation. *Circ Arrhythm Electrophysiol* 2020; **13**: E008192. doi: 10.1161/CIRCEP.119.008192
121. Reddy VY, Anter E, Rackauskas G, Peichl P, Koruth JS, Petru J, et al. Lattice-tip focal ablation catheter that toggles between radiofrequency and pulsed field energy to treat atrial fibrillation: a first-in-human trial. *Circ Arrhythm Electrophysiol* 2020; **13**: E008718. doi: 10.1161/CIRCEP.120.008718
122. Reddy VY, Koruth J, Jais P, Petru J, Timko F, Skalsky I, et al. Ablation of atrial fibrillation with pulsed electric fields: an ultra-rapid, tissue-selective modality for cardiac ablation. *JACC Clin Electrophysiol* 2018; **4**: 987-95. doi: 10.1016/j.jacep.2018.04.005
123. Kueffer T, Madaffari A, Muehl A, Maurhofer J, Stefanova A, Seiler J, et al. Pulsed-field- vs. cryo- vs. radiofrequency ablation: one-year recurrence rates after pulmonary vein isolation in patients with paroxysmal atrial fibrillation. *EP Europace* 2023; **25(Suppl 1)**: euaad122.157. doi: 10.1093/europace/euad122.157
124. Lavee J, Onik G, Mikus P, Rubinsky B. A novel nonthermal energy source for surgical epicardial atrial ablation: Irreversible electroporation. *Heart Surgery Forum* 2007; **10**: 96-101. doi: 10.1532/HSF98.20061202
125. Verma A, Haines DE, Boersma LV, Sood N, Natale A, Marchlinski FE, et al. Pulsed field ablation for the treatment of atrial fibrillation: PULSED AF pivotal trial. *Circulation* 2023; **147**: 1422-32. doi: 10.1161/CIRCULATIONAHA.123.063988
126. Urbanek L, Bordignon S, Schaack D, Chen S, Tohoku S, Efe TH, et al. Pulsed field versus cryoballoon pulmonary vein isolation for atrial fibrillation: efficacy, safety, and long-term follow-up in a 400-patient cohort. *Circ Arrhythm Electrophysiol* 2023; **16**: 389-98. doi: 10.1161/CIRCEP.123.011920
127. Tabaja C, Younis A, Hussein AA, Taigen TL, Nakagawa H, Saliba WJ, et al. Catheter-based electroporation: a novel technique for catheter ablation of cardiac arrhythmias. *JACC Clin Electrophysiol* 2023; **9**: 2008-23. doi: 10.1016/j.jacep.2023.03.014
128. Bradley CJ, Haines DE. Pulsed field ablation for pulmonary vein isolation in the treatment of atrial fibrillation. *J Cardiovasc Electrophysiol* 2020; **31**: 2136-47. doi: 10.1111/jce.14414
129. Sugrue A, Maor E, Ivorra A, Vaidya V, Witt C, Kapa S, et al. Irreversible electroporation for the treatment of cardiac arrhythmias. *Expert Rev Cardiovasc Ther* 2018; **16**: 349-60. doi: 10.1080/14779072.2018.1459185
130. Ueshima E, Schattner M, Mendelsohn R, Gerdes H, Monette S, Takaki H, et al. Transmural ablation of the normal porcine common bile duct with catheter-directed irreversible electroporation is feasible and does not impact duct patency. *Gastrointest Endosc* 2018; **87**: 300.e1-6. doi: 10.1016/j.gie.2017.05.004
131. Maor E, Ivorra A, Leor J, Rubinsky B. The effect of irreversible electroporation on blood vessels. *Technol Cancer Res Treat* 2007; **6**: 307-12. doi: 10.1177/153303460700600407
132. Narayanan G, Bhatia S, Echenique A, Suthar R, Barbery K, Yrizarry J. Vessel patency post irreversible electroporation. *Cardiovasc Intervent Radiol* 2014; **37**: 1523-9. doi: 10.1007/s00270-014-0988-9
133. Koruth JS, Kuroki K, Kawamura I, Brose R, Viswanathan R, Buck ED, et al. Pulsed field ablation versus radiofrequency ablation: esophageal injury in a novel porcine model. *Circ Arrhythm Electrophysiol* 2020; **13**: E008303. doi: 10.1161/CIRCEP.119.008303
134. Li W, Fan Q, Ji Z, Qiu X, Li Z. The effects of irreversible electroporation (IRE) on nerves. *PLoS One* 2011; **6**: e18831. doi: 10.1371/journal.pone.0018831
135. Moshkovits Y, Grynberg D, Heller E, Maizels L, Maor E. Differential effect of high-frequency electroporation on myocardium vs. non-myocardial tissues. *Europace* 2023; **25**: 748-55. doi: 10.1093/europace/eaac191
136. Faroja M, Ahmed M, Appelbaum L, Ben-David E, Moussa M, Sosna J, et al. Irreversible electroporation ablation: is all the damage nonthermal? *Radiology* 2013; **266**: 462-70. doi: 10.1148/radiol.12120609/-/DC1
137. Appelbaum L, Ben-David E, Sosna J, Nissenbaum Y, Goldberg SN. US findings after irreversible electroporation ablation: radiologic-pathologic correlation. *Radiology* 2012; **262**: 117-25. doi: 10.1148/radiol.11110475
138. Kos B, Voigt P, Miklavcic D, Moche M. Careful treatment planning enables safe ablation of liver tumors adjacent to major blood vessels by percutaneous irreversible electroporation (IRE). *Radiol Oncol* 2015; **49**: 234-41. doi: 10.1515/raon-2015-0031
139. Kranjc M, Kranjc S, Bajd F, Serša G, Serša I, Miklavčič D. Predicting irreversible electroporation-induced tissue damage by means of magnetic resonance electrical impedance tomography. *Sci Rep* 2017; **7**: 10323. doi: 10.1038/s41598-017-10846-5
140. Ruarus AH, Vroomen LGPH, Geboers B, van Veldhuisen E, Puijk RS, Nieuwenhuizen S, et al. Percutaneous irreversible electroporation in locally advanced and recurrent pancreatic cancer (PANFIRE-2): a multicenter, prospective, single-arm, phase II study. *Radiology* 2020; **294**: 212-20. doi: 10.1148/radiol.2019191109
141. Geboers B, Timmer FEF, Ruarus AH, Pouw JEE, Schouten EAC, Bakker J, et al. Irreversible electroporation and nivolumab combined with intratumoral administration of a toll-like receptor ligand, as a means of in vivo vaccination for metastatic pancreatic ductal adenocarcinoma (Panfire-iii). A phase I study protocol. *Cancers* 2021; **13**: 3902. doi: 10.3390/cancers13153902
142. Geboers B, van der Lei S, Kloppenborg LTE, Boon RM, Timmer FE, Puijk RS, et al. Transcatheter CT arteriography-guided irreversible electroporation of locally advanced pancreatic adenocarcinoma: a pictorial essay. *J Med Imaging Radiat Oncol* 2023; **67**: 428-34. doi: 10.1111/1754-9485.13535
143. Miklavcic D, Semrov DS, Mekid H, Mir LM. A validated model of in vivo electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy. *Biochim Biophys Acta* 2000; **1523**: 73-83. doi: 10.1016/S0304-4165(00)00101-X
144. Ivorra A, Rubinsky B. In vivo electrical impedance measurements during and after electroporation of rat liver. *Bioelectrochemistry* 2007; **70**: 287-95. doi: 10.1016/j.bioelechem.2006.10.005
145. Pavšelj N, Bregar Z, Cukjati D, Batiuskaite D, Mir LM, Miklavčič D. The course of tissue permeabilization studied on a mathematical model of a subcutaneous tumor in small animals. *IEEE Trans Biomed Eng* 2005; **52**: 1373-81. doi: 10.1109/TBME.2005.851524
146. Beitel-White N, Lorenzo MF, Zhao Y, Brock RM, Coutermarsh-Ott S, Allen IC, et al. Multi-tissue analysis on the impact of electroporation on electrical and thermal properties. *IEEE Trans Biomed Eng* 2021; **68**: 771-82. doi: 10.1109/TBME.2020.3013572

147. Jacobs EJ, Aycock KN, Santos PP, Tuohy JL, Davalos R V. Rapid estimation of electroporation-dependent tissue properties in canine lung tumors using a deep neural network. *Biosens Bioelectron* 2024; **244**: 115777. doi: 10.1016/j.bios.2023.115777
148. Beitel-White N, Bhonsle S, Martin RCG, Davalos RV. Electrical characterization of human biological tissue for irreversible electroporation treatments. *Annu Int Conf IEEE Eng Med Biol Soc* 2018; **2018**: 4170-3. doi: 10.1109/EMBC.2018.8513341
149. Brock RM, Beitel-White N, Coutermarsh-Ott S, Grider DJ, Lorenzo MF, Ringel-Scaia VM, et al. Patient derived xenografts expand human primary pancreatic tumor tissue availability for ex vivo irreversible electroporation testing. *Front Oncol* 2020; **10**: 843. doi: 10.3389/fonc.2020.00843
150. Kos B, Mattison L, Ramirez D, Cindrič H, Sigg DC, Iazzo PA, et al. Determination of lethal electric field threshold for pulsed field ablation in ex vivo perfused porcine and human hearts. *Front Cardiovasc Med* 2023; **10**: 1160231. doi:10.3389/fcvm.2023.1160231
151. Rao X, Chen S, Alfadh Y, Chen X, Sun L, Yu L, Zhou J, et al. Pulse width and intensity effects of pulsed electric fields on cancerous and normal skin cells. *Sci Rep* 2022; **12**: 18039. doi: 10.1038/s41598-022-22874-x
152. Fesmire CC, Williamson RH, Petrella RA, Kaufman JD, Topasna N, Sano MB. Integrated time nanosecond pulse irreversible electroporation (INSPIRE): assessment of dose, temperature, and voltage on experimental and clinical treatment outcomes. *IEEE Trans Biomed Eng* 2024; **71**: 1511-20. doi:10.1109/TBME.2023.3340718
153. Yun JH, Fang A, Khorshidi F, Habibollahi P, Kutsenko O, Etezadi V, et al. New developments in image-guided percutaneous irreversible electroporation of solid tumors. *Curr Oncol Rep* 2023; **25**: 1213-26. doi: 10.1007/s11912-023-01452-y
154. Martin II R, McFarland K, Ellis S, Velanovich V. Irreversible electroporation in locally advanced pancreatic cancer: potential improved overall survival. *Ann Surg Oncol* 2013; **20(Suppl 3)**: S443-9. doi: 10.1245/s10434-012-2736-1
155. Cannon R, Ellis S, Hayes D, Narayanan G, Martin RCG. Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. *J Surg Oncol* 2013; **107**: 544-9. doi: 10.1002/jso.23280
156. Narayanan G, Hosein PJ, Arora G, Barbery KJ, Froud T, Livingstone AS, et al. Percutaneous irreversible electroporation for downstaging and control of unresectable pancreatic adenocarcinoma. *J Vasc Interv Radiol* 2012; **23**: 1613-21. doi: 10.1016/j.jvir.2012.09.012
157. Akinwande O, Ahmad SS, Van Meter T, Schulz B, Martin RCG. CT Findings of patients treated with irreversible electroporation for locally advanced pancreatic cancer. *J Oncol* 2015; **2015**: 680319. doi: 10.1155/2015/680319
158. Lee EW, Thai S, Kee ST. Irreversible electroporation: a novel image-guided cancer therapy. *Gut Liver* 2010; **4(Suppl 1)**: S99-104. doi: 10.5009/gnl.2010.4.S1.S99
159. Hofmann F, Ohnimus H, Scheller C, Strupp W, Zimmermann U, Jassoy C. Electric field pulses can induce apoptosis. *J Membr Biol* 1999; **169**: 103-9. doi: 10.1007/s002329900522
160. Piñero J, López-Baena M, Ortiz T, Cortés F. Apoptotic and necrotic cell death are both induced by electroporation in HL60 human promyeloid leukaemia cells. *Apoptosis* 1997; **2**: 330-6. doi: 10.1023/A:1026497306006
161. Lee EW, Chen C, Prieto VE, Dry SM, Loh CT, Kee ST. Advanced hepatic ablation technique for creating complete cell death: Irreversible electroporation. *Radiology* 2010; **255**: 426-33. doi: 10.1148/radiol.10090337
162. Lee EW, Loh CT, Kee ST. Imaging guided percutaneous irreversible electroporation: ultrasound and immunohistological correlation. *Technol Cancer Res Treat* 2007; **6**: 287-93. doi: 10.1177/153303460700600404
163. Mercadal B, Beitel-White N, Aycock KN, Castellvi Q, Davalos RV, Ivorra A. Dynamics of cell death after conventional IRE and H-FIRE treatments. *Ann Biomed Eng* 2020; **48**: 1451-62. doi: 10.1007/s10439-020-02462-8
164. Brock RM, Beitel-White N, Davalos RV, Allen IC. Starting a fire without flame: the induction of cell death and inflammation in electroporation-based tumor ablation strategies. *Front Oncol* 2020; **10**: 1235. doi: 10.3389/fonc.2020.01235
165. Polajžer T, Miklavčič D. Immunogenic cell death in electroporation-based therapies depends on pulse waveform characteristics. *Vaccines* 2023; **11**: 1036. doi: 10.3390/vaccines11061036
166. Peng W, Polajžer T, Yao C, Miklavčič D. Dynamics of cell death due to electroporation using different pulse parameters as revealed by different viability assays. *Ann Biomed Eng* 2024; **52**: 22-35. doi: 10.1007/s10439-023-03309-8
167. de Visser KE, Joyce JA. The evolving tumor microenvironment: from cancer initiation to metastatic outgrowth. *Cancer Cell* 2023; **41**: 374-403. doi: 10.1016/j.ccell.2023.02.016
168. Liu M, Bertolazzi G, Sridhar S, Mulder K, Syn N, Hoppe MM, et al. Spatially-resolved transcriptomics reveal macrophage heterogeneity and prognostic significance in diffuse large B-cell lymphoma. *Nat Commun* 2024; **15**: 2113. doi: 10.1038/s41467-024-46220-z
169. Ivey JW, Latouche EL, Sano MB, Rossmeisl JH, Davalos R V, Verbridge SS. Targeted cellular ablation based on the morphology of malignant cells. *Sci Rep* 2015; **5**: 17157. doi: 10.1038/srep17157
170. Ivey JW, Wasson EM, Alinezhadbalalami N, Kanitkar A, Debinski W, Sheng Z, et al. Characterization of ablation thresholds for 3D-cultured patient-derived glioma stem cells in response to high-frequency irreversible electroporation. *Research* 2019; **2019**: 8081351. doi: 10.34133/2019/8081351
171. Rolong A, Schmelz EM, Davalos RV. High-frequency irreversible electroporation targets resilient tumor-initiating cells in ovarian cancer. *Integr Biol* 2017; **9**: 979-87. doi: 10.1039/c7ib00116a
172. Ma Y, Xing Y, Li H, Yuan T, Liang B, Li R, et al. Irreversible electroporation combined with chemotherapy and PD-1/PD-L1 blockade enhanced antitumor immunity for locally advanced pancreatic cancer. *Front Immunol* 2023; **14**: 1193040. doi: 10.3389/fimmu.2023.1193040
173. Geboers B, Scheltema MJ, Jung J, Bakker J, Timmer FEF, Cerutti X, et al. Irreversible electroporation of localised prostate cancer downregulates immune suppression and induces systemic anti-tumour T-cell activation – IRE-IMMUNO study. *BJU Int* 2024. doi:10.1111/bju.16496
174. He C, Huang X, Zhang Y, Lin X, Li S. T-cell activation and immune memory enhancement induced by irreversible electroporation in pancreatic cancer. *Clin Transl Med* 2020; **10**: E39. doi: 10.1002/ctm2.39
175. Zhao J, Wen X, Tian L, Xu C, Wen X, Melancon MP, et al. Irreversible electroporation reverses resistance to immune checkpoint blockade in pancreatic cancer. *Nat Commun* 2019; **10**: 899. doi: 10.1038/s41467-019-08782-1
176. Markelc B, Čemažar M, Serša G. Effects of reversible and irreversible electroporation on endothelial cells and tissue blood flow. In: *Handbook of electroporation*. Springer International Publishing; 2017: 607-20. doi: 10.1007/978-3-319-32886-7_70
177. Monleón E, Lucía Ó, Güemes A, López-Alonso B, Arribas D, Sarnago H, et al. Liver tissue remodeling following ablation with irreversible electroporation in a porcine model. *Front Vet Sci* 2022; **9**: 1014648. doi: 10.3389/fvets.2022.1014648
178. Burbach BJ, O'Flanagan SD, Shao Q, Young KM, Slaughter JR, Rollins MR, et al. Irreversible electroporation augments checkpoint immunotherapy in prostate cancer and promotes tumor antigen-specific tissue-resident memory CD8+ T cells. *Nat Commun* 2021; **12**: 3862. doi: 10.1038/s41467-021-24132-6
179. Shao Q, O'Flanagan S, Lam T, Roy P, Pelaez F, Burbach BJ, et al. Engineering T cell response to cancer antigens by choice of focal therapeutic conditions. *Int J Hyperthermia* 2019; **36**: 130-8. doi: 10.1080/02656736.2018.1539253
180. He C, Sun S, Zhang Y, Li S. Irreversible electroporation plus anti-pd-1 antibody versus irreversible electroporation alone for patients with locally advanced pancreatic cancer. *J Inflamm Res* 2021; **14**: 4795-807. doi: 10.2147/JIR.S331023
181. Jiang M, Shao Q, Slaughter J, Bischof J. Irreversible electroporation has more synergistic effect with anti-PD-1 immunotherapy than thermal ablation or cryoablation, in a colorectal cancer model. *Adv Ther* 2024; **7**: 2400068. doi:10.1002/adtp.202400068
182. Yang J, Eresen A, Shangguan J, Ma Q, Yaghmai V, Zhang Z. Irreversible electroporation ablation overcomes tumor-associated immunosuppression to improve the efficacy of DC vaccination in a mice model of pancreatic cancer. *Oncol Immunology* 2021; **10**: 1875638. doi: 10.1080/2162402X.2021.1875638
183. Dhatchinamoorthy K, Colbert JD, Rock KL. Cancer immune evasion through loss of MHC class I antigen presentation. *Front Immunol* 2021; **12**: 636568. doi: 10.3389/fimmu.2021.636568

184. Cornel AM, Mimpfen IL, Nierkens S. MHC class I downregulation in cancer: underlying mechanisms and potential targets for cancer immunotherapy. *Cancers* 2020; **12**: 1-33. doi: 10.3390/cancers12071760
185. Lin M, Zhang X, Liang S, Luo H, Alnaggar M, Liu A, et al. Irreversible electroporation plus allogenic Vy9V62 T cells enhances antitumor effect for locally advanced pancreatic cancer patients. *Signal Transduct Target Ther* 2020; **5**: 215. doi: 10.1038/s41392-020-00260-1
186. Alnaggar M, Lin M, Mesmar A, Liang S, Qaid A, Xu K, et al. Allogenic natural killer cell immunotherapy combined with irreversible electroporation for stage IV hepatocellular carcinoma: Survival outcome. *Cell Physiol Biochem* 2018; **48**: 1882-93. doi: 10.1159/000492509
187. Eresen A, Yang J, Scotti A, Cai K, Yaghamai V, Zhang Z. Combination of natural killer cell-based immunotherapy and irreversible electroporation for the treatment of hepatocellular carcinoma. *Ann Transl Med* 2021; **9**: 1089. doi: 10.21037/atm-21-539
188. Pan Q, Hu C, Fan Y, Wang Y, Li R, Hu X. Efficacy of irreversible electroporation ablation combined with natural killer cells in treating locally advanced pancreatic cancer. *J BUON*. 2020; **25**: 1643-49. PMID: 32862617.
189. Lin M, Liang S, Wang X, Liang Y, Zhang M, Chen J, et al. Short-term clinical efficacy of percutaneous irreversible electroporation combined with allogeneic natural killer cell for treating metastatic pancreatic cancer. *Immunol Lett* 2017; **186**: 20-27. doi: 10.1016/j.imlet.2017.03.018
190. Paul S, Lal G. The molecular mechanism of natural killer cells function and its importance in cancer immunotherapy. *Front Immunol* 2017; **8**: 1124. doi: 10.3389/fimmu.2017.01124
191. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin* 2024; **74**: 203. doi: 10.3322/caac.21820
192. Blazeovski A, Scheltema MJ, Yuen B, Masand N, Nguyen TV, Delprado W, Shnier R, et al. Oncological and quality-of-life outcomes following focal irreversible electroporation as primary treatment for localised prostate cancer: a biopsy-monitored prospective cohort. *Eur Urol Oncol* 2020; **3**: 283-90. doi: 10.1016/j.euo.2019.04.008
193. Sivaraman A, Barret E. Focal Therapy for prostate cancer: an "à la carte" approach. *Eur Urol* 2016; **69**: 973-5. doi: 10.1016/j.eururo.2015.12.015
194. Ganzer R, Arthanareeswaran VKA, Ahmed HU, Cestari A, Rischmann P, Salomon G, et al. Which technology to select for primary focal treatment of prostate cancer? European Section of Urotechnology (ESUT) position statement. *Prostate Cancer Prostatic Dis* 2018; **21**: 175-86. doi: 10.1038/s41391-018-0042-0
195. Guenther E, Klein N, Zapf S, Weil S, Schlosser C, Rubinsky B, et al. Prostate cancer treatment with irreversible electroporation (IRE): safety, efficacy and clinical experience in 471 treatments. *PLoS One* 2019; **14**: e0215093. doi: 10.1371/journal.pone.0215093
196. Scheltema MJ, Geboers B, Blazeovski A, Doan P, Katelaris A, Agrawal S, et al. Median 5-year outcomes of primary focal irreversible electroporation for localised prostate cancer. *BJU Int* 2023; **131**(Suppl 4): 6-13. doi: 10.1111/bju.15946
197. Geboers B, Scheltema MJ, Blazeovski A, Katelaris A, Doan P, Ali I, et al. Median 4-year outcomes of salvage irreversible electroporation for localized radio-recurrent prostate cancer. *BJU Int* 2023; **131**(Suppl 4): 14-22. doi: 10.1111/bju.15948
198. Scheltema MJ, van den Bos W, Siriwardana AR, Doan P, Katelaris A, Agrawal S, et al. Feasibility and safety of focal irreversible electroporation as salvage treatment for localized radio-recurrent prostate cancer. *BJU Int* 2017; **120**: 51-8. doi: 10.1111/bju.13991
199. Yaxley WJ, Gianduzzo T, Kua B, Oxford R, Yaxley JW. Focal therapy for prostate cancer with irreversible electroporation: oncological and functional results of a single institution study. *Investig Clin Urol* 2022; **63**: 285-93. doi: 10.4111/icu.20210472
200. Park W, Chawla A, O'Reilly EM. Pancreatic cancer: a review. *JAMA* 2021; **326**: 851-62. doi: 10.1001/jama.2021.13027
201. Wang ZQ, Zhang F, Deng T, Zhang L, Feng F, Wang FH, et al. The efficacy and safety of modified FOLFIRINOX as first-line chemotherapy for Chinese patients with metastatic pancreatic cancer. *Cancer Commun* 2019; **39**: 26. doi: 10.1186/s40880-019-0367-7
202. Martin RCG, McFarland K, Ellis S, Velanovich V. Irreversible electroporation therapy in the management of locally advanced pancreatic adenocarcinoma. *J Am Coll Surg* 2012; **215**: 361-9. doi: 10.1016/j.jamcollsurg.2012.05.021
203. Martin RCG, Kwon D, Chalikhonda S, Sellers M, Kotz E, Scoggins C, et al. Treatment of 200 locally advanced (Stage III) pancreatic adenocarcinoma patients with irreversible electroporation safety and efficacy. *Anna Surg* 2015; **262**: 486-94. doi: 10.1097/SLA.0000000000001441
204. Liu S, Qin Z, Xu J, Zeng J, Chen J, Niu L, et al. Irreversible electroporation combined with chemotherapy for unresectable pancreatic carcinoma: a prospective cohort study. *Onco Targets Ther* 2019; **12**: 1341-50. doi: 10.2147/OTT.S186721
205. Timmer FEF, Geboers B, Ruarus AH, Vroomen LGPH, Schouten EAC, van der Lei S, et al. MRI-guided stereotactic ablative body radiotherapy versus CT-guided percutaneous irreversible electroporation for locally advanced pancreatic cancer (CROSSFIRE): a single-centre, open-label, randomised phase 2 trial. *Lancet Gastroenterol Hepatol* 2024; **9**: 448-59. doi: 10.1016/S2468-1253(24)00017-7
206. He C, Huang X, Zhang Y, Cai Z, Lin X, Li S. Comparison of survival between irreversible electroporation followed by chemotherapy and chemotherapy alone for locally advanced pancreatic cancer. *Front Oncol* 2020; **10**: 6. doi: 10.3389/fonc.2020.00006
207. Oh JH, Jun DW. The latest global burden of liver cancer: a past and present threat. *Clin Mol Hepatol* 2023; **29**: 355-7. doi: 10.3350/cmh.2023.0070
208. Martin J, Petrillo A, Smyth EC, Shaïda N, Khwaja S, Cheow HK, et al. Colorectal liver metastases: current management and future perspectives. *World J Clin Oncol* 2020; **11**: 761-808. doi: 10.5306/wjco.v11.i10.761
209. Niessen C, Thumann S, Beyer L, Pregler B, Kramer J, Lang S, et al. Percutaneous irreversible electroporation: long-term survival analysis of 71 patients with inoperable malignant hepatic tumors. *Sci Rep* 2017; **7**: 43687. doi: 10.1038/srep43687
210. Ma Y, Chen Z, Liang B, Li R, Li J, Li Z, et al. Irreversible electroporation for hepatocellular carcinoma abutting the diaphragm: a prospective single-center study. *J Clin Transl Hepatol* 2022; **10**: 190-6. doi: 10.14218/JCTH.2021.00019
211. Scheffer HJ, Nielsen K, van Tilborg AAJM, Vieveen JM, Bouwman RA, Kazemier G, et al. Ablation of colorectal liver metastases by irreversible electroporation: results of the COLDFIRE-I ablate-and-resect study. *Eur Radiol* 2014; **24**: 2467-75. doi: 10.1007/s00330-014-3259-x
212. Frühling P, Stillström D, Holmquist F, Nilsson A, Freedman J. Irreversible electroporation of hepatocellular carcinoma and colorectal cancer liver metastases: a nationwide multicenter study with short- and long-term follow-up. *EJSO* 2023; **49**: 107046. doi: 10.1016/j.ejso.2023.107046
213. Frühling P, Stillström D, Holmquist F, Nilsson A, Freedman J. Change in tissue resistance after irreversible electroporation in liver tumors as an indicator of treatment success - a multi-center analysis with long term follow-up. *EJSO* 2024; **50**: 108508. doi: 10.1016/j.ejso.2024.108508
214. Narayanan G, Gentile NT, Eyshi J, Schiro BJ, Gandhi RT, Peña CS, et al. Irreversible electroporation in treating colorectal liver metastases in proximity to critical structures. *J Vasc Interv Radiol* 2024; **35**: 1806-13. doi: 10.1016/j.jvir.2024.08.021
215. Zhang X, Zhang X, Ding X, Wang Z, Fan Y, Chen G, et al. Novel irreversible electroporation ablation (Nano-knife) versus radiofrequency ablation for the treatment of solid liver tumors: a comparative, randomized, multicenter clinical study. *Front Oncol* 2022; **12**: 945123. doi: 10.3389/fonc.2022.945123
216. Wada T, Sugimoto K, Sakamaki K, Takahashi H, Kakegawa T, Tomita Y, et al. Comparisons of radiofrequency ablation, microwave ablation, and irreversible electroporation by using propensity score analysis for early stage hepatocellular carcinoma. *Cancers* 2023; **15**: 732. doi: 10.3390/cancers15030732
217. Schlageter M, Terracciano LM, D'Angelo S, Sorrentino P. Histopathology of hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 15955-64. doi: 10.3748/wjg.v20.i43.15955
218. Bhutiani N, Philips P, Scoggins CR, McMasters KM, Potts MH, Martin RCG. Evaluation of tolerability and efficacy of irreversible electroporation (IRE) in treatment of Child-Pugh B (7/8) hepatocellular carcinoma (HCC). *HPB* 2016; **18**: 593-9. doi: 10.1016/j.hpb.2016.03.609
219. Dai JC, Morgan TN, Steinberg RL, Johnson BA, Garbens A, Cadeddu JA. Irreversible electroporation for the treatment of small renal masses: 5-year outcomes. *J Endourol* 2021; **35**: 1586-92. doi: 10.1089/end.2021.0115

220. Kodama H, Vroomen LG, Ueshima E, Reilly J, Brandt W, Paluch LR, et al. Catheter-based endobronchial electroporation is feasible for the focal treatment of peribronchial tumors. *J Thorac Cardiovasc Surg* 2018; **155**: 2150-9.e3. doi: 10.1016/j.jtcvs.2017.11.097
221. Reddy VY, Gerstenfeld EP, Natale A, Whang W, Cuoco FA, Patel C, et al. Pulsed field or conventional thermal ablation for paroxysmal atrial fibrillation. *N Engl J Med* 2023; **389**: 1660-71. doi: 10.1056/nejmoa2307291
222. Reddy VY, Mansour M, Calkins H, d'Avila A, Chinitz L, Woods C, et al. Pulsed field vs conventional thermal ablation for paroxysmal atrial fibrillation: recurrent atrial arrhythmia burden. *J Am Coll Cardiol* 2024; **84**: 61-74. doi: 10.1016/j.jacc.2024.05.001
223. Neven K, Van Es R, Van Driel V, van Wessel H, Fidder H, Vink A, et al. Acute and long-term effects of full-power electroporation ablation directly on the porcine esophagus. *Circ Arrhythm Electrophysiol* 2017; **10**: e004672. doi: 10.1161/CIRCEP.116.004672
224. Reddy VY, Dukkkipati SR, Neuzil P, Anic A, Petru J, Funasako M, et al. Pulsed field ablation of paroxysmal atrial fibrillation: 1-year outcomes of IMPULSE, PEFCAT, and PEFCAT II. *JACC Clin Electrophysiol* 2021; **7**: 614-27. doi: 10.1016/j.jacep.2021.02.014
225. Ekanem E, Neuzil P, Reichlin T, Kautzner J, van der Voort P, Jais P, et al. Safety of pulsed field ablation in more than 17,000 patients with atrial fibrillation in the MANIFEST-17K study. *Nat Med* 2024; **30**: 2020-9. doi: 10.1038/s41591-024-03114-3
226. Anter E, Mansour M, Nair DG, Sharma D, Taigen TL, Neuzil P, et al. Dual-energy lattice-tip ablation system for persistent atrial fibrillation: a randomized trial. *Nat Med* 2024; **30**: 2303-10. doi: 10.1038/s41591-024-03022-6
227. Duytschaever M, De Potter T, Grimaldi M, Anic A, Vijgen J, Neuzil P, et al. Paroxysmal atrial fibrillation ablation using a novel variable-loop biphasic pulsed field ablation catheter integrated with a 3-dimensional mapping system: 1-year outcomes of the multicenter insPIRE study. *Circ Arrhythm Electrophysiol* 2023; **16**: E011780. doi: 10.1161/CIRCEP.122.011780
228. Reddy VY, Calkins H, Mansour M, Wazni O, Di Biase L, Bahu M, et al. Pulsed field ablation to treat paroxysmal atrial fibrillation: safety and effectiveness in the ADMIRE pivotal trial. *Circulation* 2024; **150**: 1174-86. doi: 10.1161/CIRCULATIONAHA.124.070333
229. van Zyl M, Ladas TP, Tri JA, Yasin OZ, Ladejobi AO, Tan NY, et al. Bipolar electroporation across the interventricular septum: electrophysiological, imaging, and histopathological characteristics. *JACC Clin Electrophysiol* 2022; **8**: 1106-18. doi: 10.1016/j.jacep.2022.06.002
230. Koruth JS, Kuroki K, Iwasawa J, Viswanathan R, Brose R, Buck ED, et al. Endocardial ventricular pulsed field ablation: a proof-of-concept preclinical evaluation. *Europace* 2020; **22**: 434-9. doi: 10.1093/europace/euaz341
231. Younis A, Buck E, Santangeli P, Tabaja C, Garrott K, Lehn L, et al. Efficacy of pulsed field vs radiofrequency for the reablation of chronic radiofrequency ablation substrate. *JACC Clin Electrophysiol* 2024; **10**: 222-34. doi: 10.1016/j.jacep.2023.09.015
232. Im S II, Higuchi S, Lee A, Morrow B, Schenider K, Speltz M, et al. Pulsed field ablation of left ventricular myocardium in a swine infarct model. *JACC Clin Electrophysiol* 2022; **8**: 722-31. doi: 10.1016/j.jacep.2022.03.007
233. Sandhu U, Alkukhun L, Kheiri B, Hodovan J, Chiang K, Splanger T, et al. In vivo pulsed-field ablation in healthy vs. chronically infarcted ventricular myocardium: biophysical and histologic characterization. *Europace* 2023; **25**: 1503-9. doi: 10.1093/europace/eaac252
234. Peichl P, Bulava A, Wichterle D, Schlosser F, Stojadinović P, Borišincová E, et al. Efficacy and safety of focal pulsed-field ablation for ventricular arrhythmias: two-centre experience. *Europace* 2024; **26**: euae192. doi: 10.1093/europace/eaee192
235. Garcia PA, Pancotto T, Rossmeis JH, Henao-Guerrero N, Gustafson NR, Daniel GB, et al. Non-thermal irreversible electroporation (N-TIRE) and adjuvant fractionated radiotherapeutic multimodal therapy for intracranial malignant glioma in a canine patient. *Technol Cancer Res Treat* 2011; **10**: 73-83. doi: 10.7785/tcrt.2012.500181
236. Suraju MO, Su Y, Chang J, Katwala A, Nayyar A, et al. Impact of irreversible electroporation on survival among patients with borderline resectable/locally advanced pancreatic cancer: A single center experience. *Surgical Oncology Insight* 2024; **1**: 100075. doi: 10.1016/j.soi.2024.100075.
237. Meijerink MR, Ruarus AH, Vroomen LG, Puijk RS, Geboers B, et al. Irreversible electroporation to treat unresectable colorectal liver metastases (COLDFIRE-2): A phase II, two-center, single-arm clinical trial. *Radiology* 2021; **299**: 470 - 480. doi: 10.1148/RADOL.2021203089.
238. Esparza S, Jacobs E, Hammel J, Michelhaugh SK, Alinezhadbalamani, Nagai-Singer M, et al. Transient Lymphatic Remodeling Follows Sub-Ablative High-Frequency Irreversible Electroporation Therapy in a 4T1 Murine Model. *Annals of Biomedical Engineering* 2025; **3674**. doi.org/10.1007/s10439-024-03674-y.