Radiology

High-Voltage Electrical Pulses in Oncology: Irreversible Electroporation, Electrochemotherapy, Gene Electrotransfer, Electrofusion, and Electroimmunotherapy

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This review summarizes the use of high-voltage electrical pulses (HVEPs) in clinical oncology to treat solid tumors with irreversible electroporation (IRE) and electrochemotherapy (ECT). HVEPs increase the membrane permeability of cells, a phenomenon known as electroporation. Unlike alternative ablative therapies, electroporation does not affect the structural integrity of surrounding tissue, thereby enabling tumors in the vicinity of vital structures to be treated. IRE uses HVEPs to cause cell death by inducing membrane disruption, and it is primarily used as a radical ablative therapy in the treatment of soft-tissue tumors in the liver, kidney, prostate, and pancreas. ECT uses HVEPs to transiently increase membrane permeability, enhancing cellular cytotoxic drug uptake in tumors. IRE and ECT show immunogenic effects that could be augmented when combined with immunomodulatory drugs, a combination therapy the authors term *electroimmunotherapy*. Additional electroporation-based technologies that may reach clinical importance, such as gene electrotransfer, electrofusion, and electroimmunotherapy, are concisely reviewed. HVEPs represent a substantial advancement in cancer research, and continued improvement and implementation of these presented technologies will require close collaboration between engineers, interventional radiologists, medical oncologists, and immuno-oncologists.

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Learning Objectives:

After reading the article and taking the test, the reader will be able to:

- Describe the mechanisms by which high-voltage electrical pulses (HVEPs) influence the integrity of the cell membrane when externally applied
- List the two mechanisms that cause the electroporation-induced vascular lock effect
- Discuss the immunogenic potential of electroporation-based ablative modalities

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The first biologic experiment with electrical fields dates to 1754, when Nollet applied electric sparks to human skin and observed the formation of red spots, an effect likely caused by irreversible electroporation (IRE) (1). By the middle of the 20th century, electrical pulses were being investigated for use in water and food sterilization. Doevenspeck, a German engineer, described the nonthermal inactivation of microorganisms by electrical pulses in industrial fish processing, and Zagorul'ko, a Ukrainian food scientist, described electrical breakdown of sugar beet cell membranes for sugar processing (2,3). In the 1950s, research focused on the effect of electrical pulses on cell membranes. In 1967, Sale and Hamilton established the foundational principles of IRE by demonstrating that cell death was not related to temperature rise but instead was primarily related to electrical field parameters (4). Furthermore, they demonstrated increased membrane permeability by detecting leakage of intracellular contents (5). Neumann and Rosenheck coined the term *electroporation* when they observed that the membrane permeability was temporary and that its integrity was eventually restored, a phenomenon now known as reversible electroporation (6). In 1982, Neumann et al demonstrated DNA could be transferred into cells by using high-voltage electrical pulses (HVEPs), a process that is currently referred to as gene electrotransfer (GET) (7). At the same time, Zimmerman et al used reversible electroporation for cell-to-cell fusion, which is now called electrofusion (8). In 1987, the first use of electroporation in oncology occurred when reversible electroporation was used to introduce cytotoxic

Abbreviations

ECT = electrochemotherapy, GET = gene electrotransfer, HVEP = high-voltage electrical pulse, IRE = irreversible electroporation, LAPC = locally advanced pancreatic cancer, OS = overall survival

Summary

Irreversible electroporation, electrochemotherapy, and other electroporation-based therapies represent a treatment paradigm for difficult-to-treat solid tumors; the potent combination of high-voltage electrical pulses with immune cascade–enhancing drugs may offer a bridge between local-regional and systemic treatments in oncology.

Essentials

- Irreversible electroporation is a predominantly nonthermal and adjacent structure–sparring ablation method that has been proven safe and efficient in the treatment of tumors in the liver, pancreas, and prostate.
- Electrochemotherapy uses reversible electroporation to temporarily increase membrane permeability to facilitate the transportation of bleomycin or cisplatin into tumor cells, thereby increasing their cytotoxicity, and has been proven safe and efficient in the treatment of cutaneous and subcutaneous tumors.
- Irreversible electroporation and electrochemotherapy can conceivably induce systemic antitumor T-cell responses that in turn might induce regression in distant untreated metastases, which may be further leveraged in combination with immune-enhancing agents.

agents into malignant cells, a technique currently known as electrochemotherapy (ECT) (9). In ECT, irreversible breakdown of the cell membrane was considered undesirable; thus, IRE was long ignored and avoided in cancer therapy. In 2003, Davalos and Rubinsky (U.S. patent no. 8,048,067) pioneered the idea of using IRE as a nonthermal ablation modality and mathematically demonstrated its capability to ablate substantial tissue volumes while avoiding a thermal effect (10).

Basic Principles of Electroporation

At the cellular level, electrical fields primarily interact with the cell membrane and cause increased membrane permeability. The cell can be considered a conductive body (the cytoplasm) surrounded by a dielectric phospholipid bilayer (the membrane). When HVEPs are applied, the external electrical field alters the resting potential across the cell membrane. If the accumulated transmembrane potential exceeds a critical value, the membrane becomes unstable, and nanoscale membrane defects or "pores" form, hence the term *electroporation*. Formation of pores is initiated by the penetration of water molecules into the lipid bilayer, leading to reorientation of the adjacent lipids, with their polar head groups pointing toward these water molecules (Fig 1, *A*). Even in the absence of HVEPs, unstable pores with nanosecond lifetimes can form; however, when the membrane is exposed to an external electric field, the energy required for penetration of water molecules into the phospholipid bilayer is reduced, and the probability of pore formation increases (11). Pore formation increases membrane permeability and allows entrance of otherwise membrane-impermeant molecules (12). Accumulating evidence suggests that HVEPs also cause membrane permeabilization by inducing chemical changes to membrane lipids and by modulating membrane protein function in voltage-gated channels to allow ion transportation; thus, a more comprehensive term, *electropermeabilization*, is also used (13). Figure 1 shows pore formation, chemical changing of membrane lipids, and protein modulation by HVEPs causing membrane permeabilization.

Electroporation can be either reversible or irreversible (Fig 2). Reversible electroporation occurs when the increase in membrane permeability is transient and the cell regains homeostasis. Electrical pulses usually include eight square wave pulses of 100 μsec, with an amplitude of 100–1000 V. Reversible electroporation is the basis for ECT, GET, and electrofusion. IRE occurs when the magnitude and duration of the electrical pulses overwhelm the adaptive capacity of the cells and result in cell death. For IRE, more pulses (at least 80–100 pulses) and a higher amplitude (up to 3000 V) are required. Electrical field strength and treatment duration determine whether reversible electroporation or IRE occurs (Fig 3) (11).

In addition to these cellular effects, the application of HVEPs to tumor tissue instantaneously but transiently reduces blood flow to near no-flow conditions (Fig 4) (14). This "vascular lock" effect can be explained by two mechanisms: *(a)* direct vasoconstriction through electrical stimulation of precapillary smooth muscle cells followed by indirect sympathetically mediated vasoconstriction of afferent arterioles (15) and *(b)* shape modifications to vascular endothelial cells leading to increased vascular resistance and alteration of endothelial cell-to-cell junctions. Cell-to-cell junction disruption provokes protein leakage, leading to increased interstitial fluid pressure and decreased intravascular pressure (16,17). The vascular lock effect is advantageous, as it decreases washout of applied cytostatics during ECT or of DNA plasmids during GET and reduces bleeding when invasive electrodes (ie, needles) are used (14).

IRE Technique

IRE is a focal ablative technique used for certain solid tumors that are unsuitable for surgery or thermal ablation because of their precarious anatomic location. Although IRE irreversibly injures the membranes of all cells within the target tissue, the preservation of extracellular macromolecules and constitutive connective tissue components spares the structural integrity of the tissue. This characteristic theoretically makes IRE attractive for tumors in the vicinity of vital structures like large blood vessels, intestines, and biliary or urinary tracts.

Mechanism of action for IRE.—When HVEPs exceed a certain threshold, irreversible injury to all cell membranes within the ablation zone will lead to cell death (Fig 5) (18). Cell death by IRE happens through apoptosis or necrosis induced by either permanent membrane disruption or secondary breakdown of the membrane due to abundant transmembrane transfer of electrolytes and adenosine triphosphate, leading to irreparable loss of homeostasis (19,20). The preservation of vital structures after IRE has been investigated in several animal models that were analyzed in a systematic review by Vogel et al (21). Solitary blood vessels remain unchanged 24 hours after ablation. Despite perivascular fibrosis and inflammation observed up to 35 days after treatment, vessel integrity remains intact. Although IRE retains ureter lumen integrity, there is a risk of

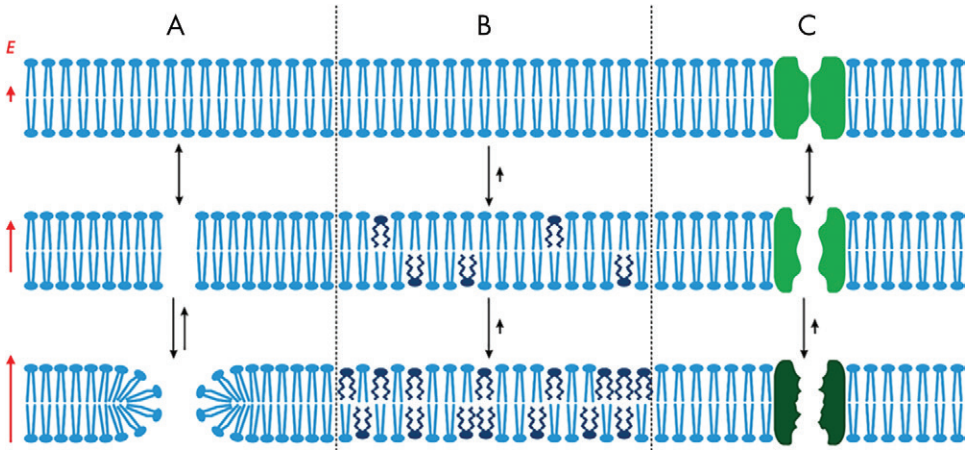

Figure 1: Conceptual scheme of molecular-level mechanisms of electropermeabilization, starting from an intact membrane (top). *A*, Electrical fields induce hydrophilic pores in the lipid bilayer, a process known as electroporation, as shown here in two stages depending on the amplitude of the applied electric field. In the first stage, water molecules penetrate the bilayer and form an unstable hydrophobic pore (middle). In the second stage, adjacent lipids reorient their polar head groups toward the water molecules to form a metastable hydrophilic pore (bottom). *B*, Electrically induced chemical changes can occur to the membrane lipids, such as peroxidation, which deforms the lipid tails and increases permeability of the bilayer to water, ions, and small molecules. *C*, Electrically induced modulation of membrane proteins can occur, especially for a voltage-gated channel that allows ion transportation across the membrane. Arrow lengths for the electric field (E) correspond to field strength (ie, amplitude of electric pulse or pulses). Transitions between states of membrane permeability reflect the transition rate (shorter arrow = slower rate, not drawn to scale between the three mechanisms).

stricture and loss of patency induced by transmural necrosis (22). Clinical outcomes of IRE used to treat tumors in the vicinity of sensitive tissues support these observations and will be discussed in the following sections. Although IRE is predominantly nonthermal, Joule heating of the tissue can occur if too much energy is applied too quickly, leading to thermal damage (23). In the immediate vicinity of the electrodes, thermal cell death usually occurs as a result of an inhomogeneous electrical field distribution and high current density (24). Complications caused by damage to sensitive surrounding structures are often a result of undesirable thermal effects. To minimize thermal damage in the ablation zone, active cooling electrodes were evaluated in porcine livers, reducing tissue temperatures and electric current while maintaining similar lesion sizes (25).

Clinical results of IRE.—The cumulative quality of clinical IRE literature is variable due to largely retrospective reports and prospective phase I or II trials that use different inclusion criteria and outcome measures. While clinical results are largely promising, high-volume prospective registries and randomized controlled trials that directly address the added value of IRE over current standards of care are warranted before widespread adoption into clinical practice can be established. Clinical results per organ are summarized in Tables 1–4.

IRE in the Liver.—In 2011, Thomson et al were among the first to use IRE in a prospective trial setting. Among a total of 69 advanced liver, lung, and kidney tumors, 66% were completely ablated, with the highest percentage achieved in hepatocellular carcinoma (83%), signifying liver tumors as a suitable target for IRE (26) (Table 1). In their ablate-and-resect study (Colorectal Liver Metastatic Disease: Efficacy of Irreversible Electroporation—A Single-arm Phase I Clinical Trial [COLDFIRE 1]), Scheffer et al demonstrated the ability of IRE to cause complete macroscopic tumor nonviability in colorectal liver metastases using vitality staining (27). Hepatic IRE appears to be safe, even when performed near vessels and bile ducts (28,29), with an overall complication rate of 16%, with most complications being needle related (pneumothorax and hemorrhage). IRE treatment requires the insertion of several needles, a disadvantage faced less often with thermal ablation. No deaths have been reported (30). Efficacy results of hepatic IRE vary widely (range, 45.5%–100%), presumably due to the heterogeneity of patient populations and treated tumors, with size being an important factor (31). The results of the prospective COLDFIRE-2 trial, in which 50 patients were treated, showed 76% local tumor progression-free survival after 1 year (32). Because IRE is still relatively new, studies comparing IRE to other ablative therapies have yet to be performed. However, efficacy of thermal ablation is currently higher, with an efficacy around 96% for tumors smaller than 3 cm (33,34). Thus, at this time, IRE should be performed for only truly unresectable and unablatable tumors. Image-guided percutaneous IRE of a liver tumor invading the inferior vena cava is shown in Figure 6.

IRE in the Pancreas.—Because IRE spares vasculature, it is increasingly used to treat locally advanced pancreatic cancer (LAPC). Complication rates for treatment of the pancreas exceed those for treatment of the liver. Furthermore, reported complications tend to be more severe and include portal vein thrombosis, pancreatitis, bile or pancreatic fluid leakage, bile duct strictures, and gastrointestinal bleeding. IRE-related deaths have occurred (35). Complications may be caused by unexpected thermal effects, unwanted healthy pancreatic tissue necrosis, or mechanical effects, like edema leading to biliary and vascular stenosis or occlusion. IRE for pancreatic tumors should be considered a high-risk procedure. The largest retrospective series were published by Narayanan et al (36), Leen et al (37), and Martin et al (38) (Table 2). Most patients were pretreated with chemotherapy, radiation therapy, or both, with the percentage of patients treated ranging from 92% to 100% to 47%, respectively. Median overall survival (OS) from IRE was 14, 27, and 18 months, respectively; median OS from diagnosis varied from 27 months, to not reported, to 23 months, respectively

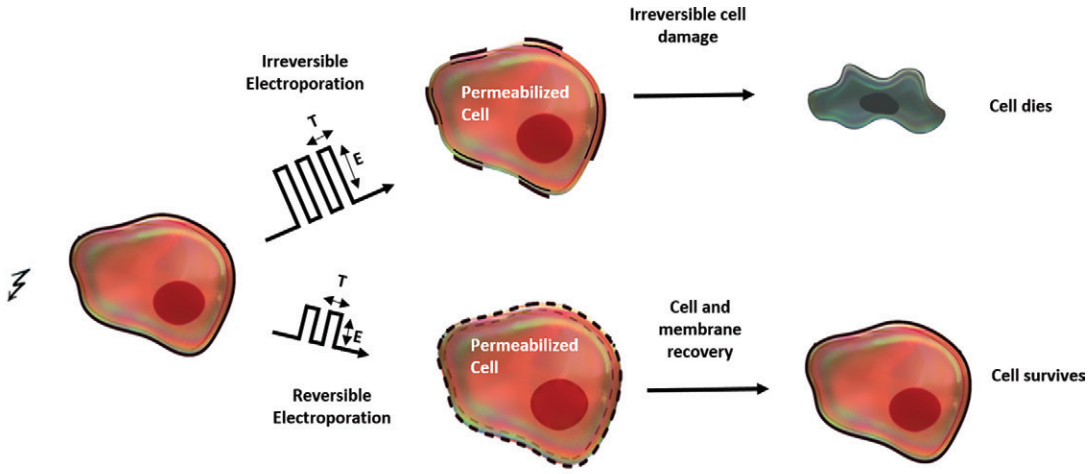

Figure 2: Schematic illustration of reversible and irreversible electroporation (IRE). IRE is the use of short (T) but intense (E) electrical pulses to disrupt the cell membrane and cause cell death. IRE requires that electrical pulses exceed a certain threshold (too high of an electrical field, too long of pulses, or too many pulses) so that cells cannot recover. Reversible electroporation is the use of short (T) but intense (E) electrical pulses in a lesser extent than for IRE. Reversible electroporation requires electrical pulses that are sufficient for permeabilization of the cell membrane but are below a certain threshold to ensure the membrane can recover and the cell will survive.

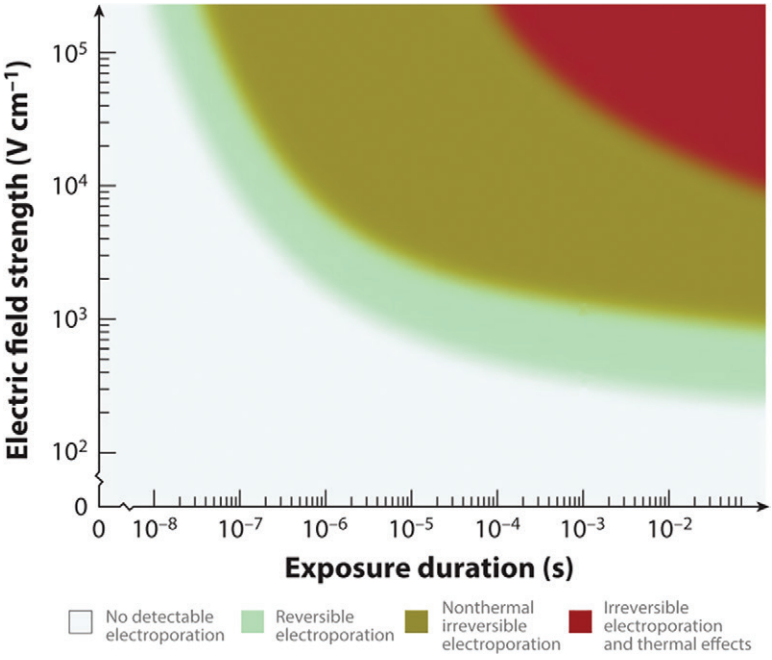

Figure 3: Effect of electrical parameters on membrane permeabilization. Reversible electroporation, irreversible electroporation, and thermal damage as functions of electric field strength and duration.

(36–38). The largest and most recent upfront registered prospective trial was published by Ruarus et al and includes 50 patients: 40 with LAPC and 10 with local recurrence after surgical pancreatic tumor resection (39). All patients were treated percutaneously, and 68% underwent neoadjuvant chemotherapy. Median OS was 17 months after diagnosis and 10 months after IRE (40). Differences in outcome from this prospective trial compared with retrospective cohorts may be explained in part by their retrospective nature leading to immortal time bias and by selection bias.

The OS rates in these studies provide an encouraging nonvariable endpoint and show an additive beneficial effect of IRE compared with standard-of-care chemotherapeutic treatment with FOLFIRINOX (a combination of 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) (median OS, 12–14 months) (41,42). The ability of neoadjuvant chemotherapy to effectively enable selection of patients who are more likely to benefit from IRE was indirectly supported in a prospective series by Månsson et al. The study failed to achieve survival benefit in 24 patients with LAPC who did not undergo neoadjuvant chemotherapy but who were treated with first-line percutaneous IRE (43). Prospective comparative studies with other focal treatment options like stereotactic radiation therapy are currently underway to establish the role of IRE in the treatment spectrum of patients with pancreatic cancer (44).

IRE in the Kidney.—Thermal ablative treatments are contraindicated for tumors near the renal hilum. Wendler et al published an ablate-and-resect study for pT1a renal cell carcinoma (45). Seven patients with tumors smaller than 4 cm were treated with IRE followed by nephrectomy 4 weeks later. No major complications due to IRE were reported. Resections revealed complete macroscopic coverage of the tumor by the IRE ablation field in 100% of tumors, but pathology showed complete ablation in only four tumors (45). In a prospective phase II trial, 10 renal tumors were treated (mean size, 2.2 cm) (46). Recurrence was detected in only the largest tumor (3.9 cm) 3 months after ablation. Eight patients were discharged the day after treatment, and all but one patient's serum creatinine level returned to the baseline level within 1 week. Other complications observed after IRE of the kidney are pyelonephritis, perinephric

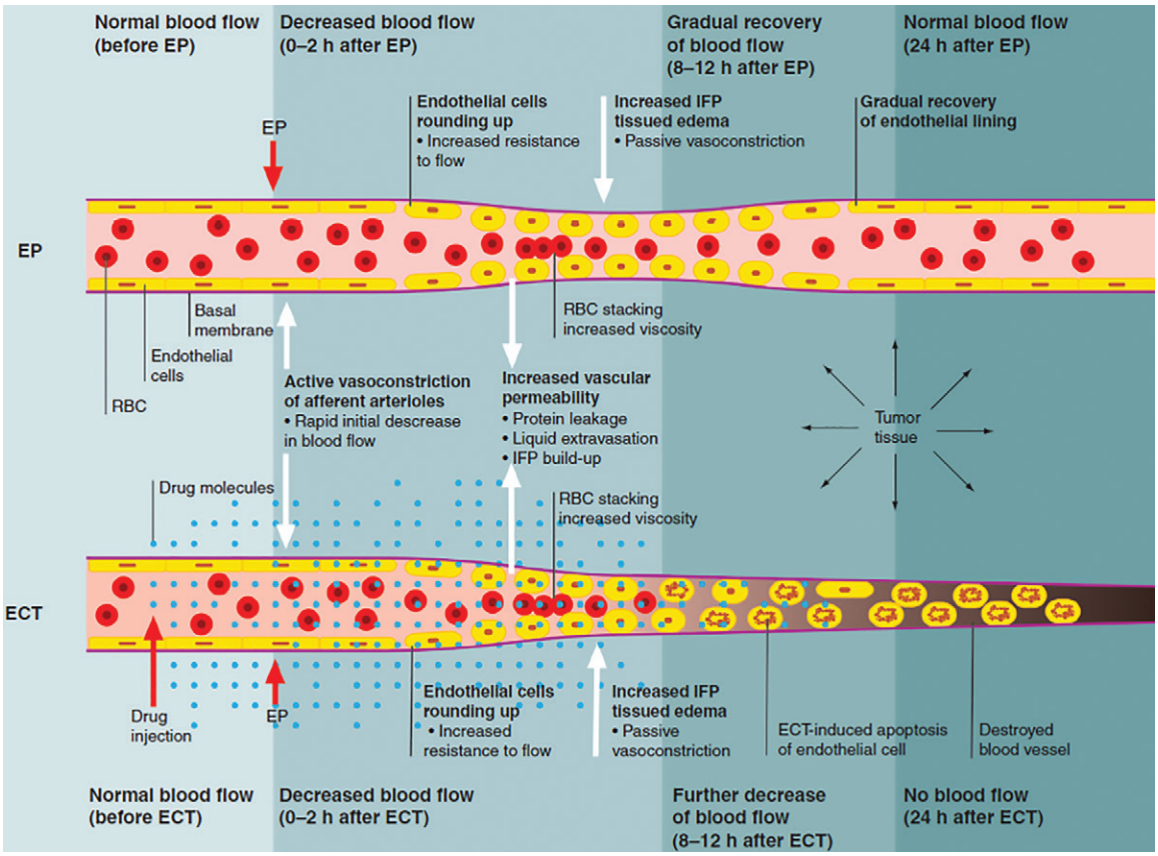

Figure 4: Vascular lock effect in tumors induced by electrical pulses (EPs) and electrochemotherapy (ECT). The effects of EP and ECT are presented at the level of a microcirculatory blood vessel. Application of the drug and electrical pulses is indicated (arrows). The general sequence of physiologic changes and their consequences runs from left to right. IFP = interstitial fluid pressure, RBC = red blood cell.

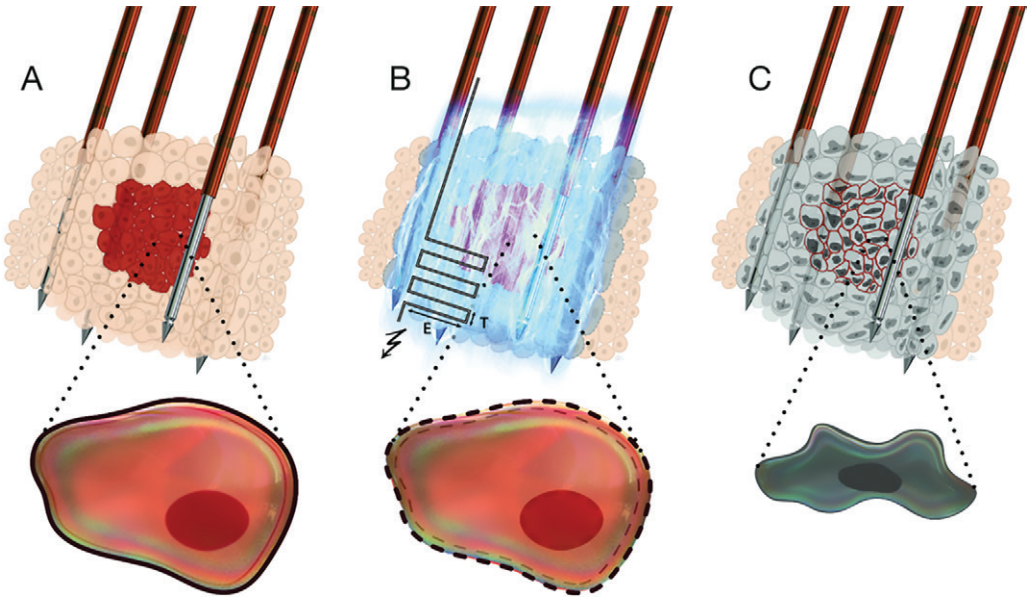

Figure 5: Illustration of irreversible electroporation (IRE). IRE is the use of short but intense electrical pulses to disrupt the cell membrane and cause cell death. The enlargement shows one tumor cell with an intact cell membrane. IRE requires that electrical pulses exceed a certain threshold (too high of an electrical field, too long of pulses, too many pulses) such that cells cannot recover. *A*, Pre-IRE. Needle electrodes are inserted around the tumor (brown) within healthy tissue (beige). *B*, During IRE, multiple short (T) high-voltage (E) electrical pulses cause cell membrane disruption of tissue within the ablation zone (blue), leading to cell death. *C*, Post-IRE. Within the ablation zone (black) there is complete apoptosis or necrosis of the cells. Structural tissue integrity (gray) is preserved. Red circles indicate tumor location before IRE.

Table 1: Irreversible Electroporation Clinical Data in the Liver

Author, Year of Publication, and Reference No.	Study Design	No. of Patients	No. of Lesions	Age (y)*	Tumor Type per Patient and Median Size	Approach	Median Follow-up (mo)	Primary Efficacy (Ahmed et al, 124) (%)	Secondary Efficacy (Ahmed et al, 124) (%)
Bhutiani et al, 2016 (124)	Retrospective	30	30	61	HCC (*n* = 30), 3.0 cm	Open (*n* = 10), laparoscopic (*n* = 20)	6	97	NS
Cannon et al, 2013 (126)	Retrospective	44	48	60	HCC (*n* = 14), CRLM (*n* = 20), Other (*n* = 10); 2.5 cm	Percutaneous (*n* = 28), open (*n* = 14), laparoscopic (*n* = 2)	12	59.5	NS
Frühling et al, 2017 (127)	Prospective	30	38	63	HCC (*n* = 8), CRLM (*n* = 23), other (*n* = 7); 2.4 cm	Percutaneous (*n* = 30)	22.3	65.8 (at 6 months)	NS
Hosein et al, 2014 (128)	Retrospective	28	58	62	CRLM (*n* = 58), 2.7 cm	Percutaneous (*n* = 28)	10.7	97	NS
Kingham et al, 2012 (129)	Prospective (ablate and resect)	28	65	51	HCC (*n* = 2), CRLM (*n* = 21), other (*n* = 5); 1.0 cm	Percutaneous (*n* = 6), open (*n* = 22)	6	93.8	NS
Narayanan et al, 2014 (130)	Retrospective	67	100	24–83†	HCC (*n* = 35), CRLM (*n* = 20), CCC (*n* = 5); 2.7 cm	Percutaneous (*n* = 67)	10.3	NS	NS
Niessen et al, 2015 (131)	Retrospective	25‡	48	59	HCC (*n* = 22), CRLM (*n* = 16), CCC (*n* = 6), other (*n* = 4); 1.7 cm	Percutaneous (*n* = 25)	6	70.8	NS
Niessen et al, 2016 (132)	Retrospective	34‡	65	59	HCC (*n* = 33), CRLM (*n* = 22), CCC (*n* = 5), other (*n* = 5); 2.4 cm	Percutaneous (*n* = 34)	13.9	74.8	NS
Niessen et al, 2017 (133)	Retrospective	71‡	103	64	HCC (*n* = 31), CRLM (*n* = 16), CCC (*n* = 6), other (*n* = 4); 2.3 cm	Percutaneous (*n* = 71)	35.7	68.3	NS
Philips et al, 2013 (134)	Retrospective	60	66	62	HCC (*n* = 13), CRLM (*n* = 23), CCC (*n* = 2), other (*n* = 22); 3.8 cm	Percutaneous (NS) open (NS)	18	NS	NS
Scheffer et al, 2014 (27)	Prospective (ablate and resect)	10§	10	NS	CRLM (*n* = 10), 2.4 cm	Open (*n* = 10)	0	88.9	NS
Thomson et al, 2011 (26)	Prospective	25	63	NS	HCC (*n* = 17), CRLM (*n* = 15), other (*n* = 31); 2.5 cm	Percutaneous (*n* = 25)	3	51.6	56.5

Note.—Efficacy of hepatic irreversible electroporation in prospective and retrospective studies with more than 15 patients. The primary efficacy rate is defined as the percentage of target tumors successfully eradicated after the initial procedure or a defined course of treatment. The term *re-treatment* should be reserved for describing ablation of locally progressive tumors where complete ablation was initially thought to have been achieved based on imaging demonstrating adequate ablation of the tumor (124). CCC = cholangiocarcinoma, CRLM = colorectal liver metastasis, HCC = hepatocellular carcinoma, NS = not specified.

* Unless otherwise indicated, data are medians.

† Data are the range.

‡ Not specified which patients were also included in previous studies.

§ In this ablate-and-resect study, eight of nine treated lesions were visible after staining with 5-triphenyl tetrazolium chloride in complete ablation zone c.

Table 2: Irreversible Electroporation Clinical Data in the Pancreas

Author, Year of Publication, and Reference No.	Study Design	No. of Patients	Median Age (y)	Stage of Disease and Median Largest Tumor Diameter	Approach	Median Follow-up (mo)	Median Overall Survival (mo)	Local Recurrence (%)	Tumor Downstaging Caused by IRE
Belfiore et al, 2017 (114)	Retrospective	29	68.5	LAPC, NS	Percutaneous	29	14.0	3	3 patients
Flak et al, 2019 (115)	Prospective	33	67.1	LAPC, 3.0 cm (88% after chemotherapy or radiation therapy)	Percutaneous (*n* = 32), open (*n* = 1)	9	18.5 (diagnosis), 10.7 (IRE)	NS	3 patients
Kluger et al, 2016 (116)	Retrospective	50	66.5	LAPC T4, 3.0 cm	Open	8.7	12.0 (IRE)	11	NS
Lambert et al, 2016 (117)	Prospective	21	68.2	LAPC, 3.9 cm	Open (*n* = 19), percutaneous (*n* = 2)	NS	10.2	NS	NS
Leen et al, 2018 (37)	Retrospective	75	63.4	LAPC, 3.5 cm (after chemotherapy)	Percutaneous	11.7	27.0 (IRE)	38	3 patients
Månsson et al, 2016 (118)	Prospective	24	65	LAPC, NS (after chemotherapy)	Percutaneous	NS	17.9 (diagnosis), 7.0 (IRE)	58	2 patients
Månsson et al, 2019 (43)	Prospective	24	68	LAPC, 3.0 cm (before chemotherapy)	Percutaneous	NS	13.3 (diagnosis)	33	0
Martin et al, 2015 (38)	Retrospective	150*	62	LAPC, 2.8 cm (after chemo- or radiation therapy)	Open	29	23.2 (diagnosis), 18 (IRE)	2	NS
Narayanan et al, 2016 (36)	Retrospective	50	62.5	LAPC, 3.2 cm ± 1.3† (after chemo- or radiation therapy)	Percutaneous	NS	27 (diagnosis), 14.2 (IRE)	NS	3 patients
Paiella et al, 2015 (119)	Prospective	10	66	LAPC, 3.0 cm	Open	7.6	15.3 (diagnosis), 6.4 (IRE)	NS	NS
Ruarus et al, 2019 (39)‡	Prospective	50	61	LAPC (*n* = 40) and local recurrence (*n* = 10), 4.0 cm (68% after chemotherapy)	Percutaneous	NS	17.0 (diagnosis), 9.6 (IRE)	46	0 patients
Scheffer et al, 2017 (40)	Prospective	25	61	LAPC, 4.0 cm (52% after chemotherapy)	Percutaneous	12 (7–16)§	17.0 (diagnosis), 11.0 (IRE)	NS	NS
Sugimoto et al, 2018 (120)	Prospective	8	64	LAPC, 2.9 cm	Open or percutaneous, NS	17.5	17.5 (diagnosis)	38	0 patients
Vogel et al, 2017 (121)	Prospective	15	NA	LAPC, NS	Open	24	16 (diagnosis)	NS	NS
Yan et al, 2016 (122)	Retrospective	25	58	LAPC, 4.2 cm	Open	3	NS	2	NS
Zhang et al, 2017 (123)	Prospective	21	NA	LAPC, 3.0 cm	Percutaneous	1	NS	NS	NS

Note.—Survival after primary pancreatic irreversible electroporation (IRE) in retrospective studies with more than 15 patients and prospective studies. (Studies using IRE for margin accentuation in combination with surgery and case reports are excluded.) LAPC = locally advanced pancreatic cancer, NS = not specified.

* This study included 200 patients, of which 50 were treated with surgical resection combined with intraoperative IRE for margin accentuation. Results of these 50 patients are not included in this table.

† Data are mean ± standard deviation.

‡ Data are from the Irreversible Electroporation to Treat Locally Advanced Pancreatic Carcinoma trial (or PANFIRE II) study. The first 50% of inclusions were reported earlier in PANFIRE I (40).

§ Data are median, and data in parentheses are the range.

Table 3: Irreversible Electroporation Clinical Data in the Kidney

Author, Year of Publication, and Reference No.	Study Design	No. of Patients	No. of Lesions	Median Age (y)	Tumor Type per Patient and Median Size (cm)	Approach	Adverse Events (CTCAE 4.0)	Oncologic Efficacy	Local Recurrence
Buijs et al (2018) (46)	Prospective	9	10	68	RCC T1a (*n* = 10), 2.2 cm (range, 1.1–3.9 cm)	Percutaneous (*n* = 9)	Grade 1 (*n* = 3), grade 3 (*n* = 2)*	90% without residual tumor on 6-week follow-up scan†	1 patient
Canvasser (2017) (135)	Retrospective	41	42	64	RCC T1a (*n* = 20), benign or indeterminate (*n* = 22); 2.0 cm	Percutaneous	Grade 1 (*n* = 9)‡	93% without residual tumor on 6-week follow-up CT scan	2 patients
Pech et al (2011) (136)	Prospective	6	6	58	RCC (*n* = 6), 2.8 cm	Open (*n* = 6)	None	0% without residual tumor at histopathologic examination 15 minutes after IRE§	NS
Thomson et al (2011) (26)	Retrospective	8	11	NS	RCC (*n* = 7), benign or other (*n* = 4); 3.0 cm	Percutaneous (*n* = 11)	NS‖	25% without residual tumor on 3-month follow-up CT scan	2 patients
Trimmer et al (2015) (137)	Retrospective	20	20	65	RCC T1a (*n* = 13), benign or indeterminate (*n* = 7); 2.2 cm	Percutaneous (*n* = 20)	NS#	90% without residual tumor on 6-week follow-up CT scan	1-year follow-up imaging was available in 6 patients, 1 patient showed recurrent disease
Wendler et al (2018) (45)	Prospective	7	8	NS	RCC T1a (*n* = 7); 2.2 cm	Percutaneous (*n* = 7)	NS**	67% without residual tumor at histopathologic examination 28 days after IRE	NS

Note.— Safety, feasibility, and early oncologic outcome of renal irreversible electroporation (IRE) in prospective and retrospective studies. NS = not specified, RCC = renal cell carcinoma.

* Grade 1 complications: episode of painless hematuria. Perinephric hematoma developed during electrode placement and was visible on images until 1 week after ablation. Painful micturition. Grade 3 complications: increased creatinine level due to partially blocked ureter because of a blood clot. Pyelonephritis with fever.

† This tumor was the largest of the cohort, with a size of 3.9 × 3.9 × 3.7 cm.

‡ There were four patients with asymptomatic perinephric hematoma, two with transient urinary retention, one patient with substantial flank pain, and two patients developed respiratory difficulty.

§ At histopathologic examination, no dead cells were found in the specimens. Time between IRE and resection was only 15 minutes; this is too short to establish any IRE effect.

‖ Common Terminology Criteria for Adverse Events (CTCAE) grades were not specified; however, two patients developed transient hematuria, and one patient had an unplanned insertion of an electrode tip into the adrenal gland that directly caused hypertension and postprocedural hypotension.

CTCAE grades were not specified; however, three patients developed urinary retention, two experienced substantial pain, and two developed perinephric hematomas. All were noted as minor complications.

** CTCAE grades were not specified; however, all seven patients experienced hematuria, and two developed perinephric hematomas. Renal function was retained in all patients.

Table 4: Irreversible Electroporation Clinical Data in the Prostate

Author, Year of Publication, and Reference No.	Study Design	No. of Patients	Median Age (y)	Gleason Score	Pretreatment or Concurrent Treatment	Adverse Events (CTCAE 4.0)	Median Follow-up (mo.)	Functional Outcome (% of patients)	Oncologic Efficacy (no. of patients)
Onik and Rubinsky (2010) (47)	Prospective	16	NS (40–78)*	3+3: *n* = 7 3+4: *n* = 6 4+4: *n* = 3	NS	NS	NS	At 6 months: urinary incontinence, 0%; erectile dysfunction, 0%	Local recurrence, *n* = 0; out-of-field occurrence, *n* = 1†
Van den Bos et al (2016) (52)	Prospective	16	60	3+3: *n* = 8 4+3: *n* = 3 4+4: *n* = 2	Radical prostatectomy 4 weeks after IRE	NS	NS	NS	15 patients showed complete fibrosis or necrosis of ablation zone‡
Van den Bos et al (2018) (50)	Prospective	63	67	3+3: *n* = 9 3+4: *n* = 38 4+3: *n* = 16	Concurrent TURP (*n* = 10)	Grade 1: 24% Grade 2: 11% Grade 3–5: 0%)§	6	At 12 months: urinary incontinence, 0%; erectile dysfunction, 23%	Local recurrence, *n* = 7; out-of-field recurrence, *n* = 4‖
Guenther et al (2019) (51)	Retrospective	429	64	3+3: *n* = 82 3+4/4+3: *n* = 2254+4: *n* = 68 5+3/3+5: *n* = 3	Pretreated with: radical prostatectomy (*n* = 21), radiation therapy (*n* = 28), TURP (*n* = 17), HIFU (*n* = 8), or ADT (*n* = 29)	NS#	12	At 12 months: urinary incontinence, 0%; erectile dysfunction, 3%	Local recurrence, *n* = 20; out-of-field recurrence, *n* = 27**
Valerio et al (2014) (49)	Prospective	34	65	3+3: *n* = 9 3+4: *n* = 19 4+3: *n* = 5 4+4: *n* = 1	NS	Grade 1: 35% Grade 2: 29% Grade 3–5: 0%	6	At 6 months: urinary incontinence, 0%; erectile dysfunction, 5%	Local residual disease, *n* = 6; only one histologic verification. Out-of-field recurrence, NS††
Ting et al (2016) (48)	Prospective	25	67	3+3: *n* = 2 3+4: *n* = 15 4+3: *n* = 8 4+4: *n* = 0	None	Grade 1: 35% Grade 2: 29% Grade 3–5: 0%	6	At 6 months: urinary incontinence, 0%; erectile dysfunction, unknown‡‡	Local recurrent disease, *n* = 0; out-of-field recurrence, *n* = 5 (with histologic verification)

Note.—Safety, feasibility and efficacy of prostate irreversible electroporation (IRE) in prospective and retrospective studies. ADT = androgen deprivation therapy, CTCAE = common terminology criteria for adverse events, HIFU = high-intensity focused US, NS = not specified, TURP = transurethral resection of the prostate.

* Data in parentheses are the range.

† Out-of-field recurrence occurred in untreated prostate tissue outside the IRE ablation zone.

‡ Tumorous tissue outside the ablation zone was found in 15 of the 16 patients.

§ Grade 1: hematuria, dysuria, urgency or frequency complaints, perineal pain. Grade 2: urinary incontinence, urinary tract infections, severe urgency or frequency complaints, epididymitis.

‖ In 71% of treated patients confirmed with follow-up biopsy.

Mild in 19% of patients (hematuria, urinary retention, dysuria). Moderate in 3.7% of patients (prostatitis, proctitis, epididymitis, urinary tract infection). Severe in 1.4% of patients (permanent urinary retention, rectoprostatic fistula, bladder perforation, severe prostatitis).

** Maximum follow-up was 72 months. Recurrent prostate cancer was determined by a rise in prostate-specific antigen (PSA) level with corresponding findings on multiparametric MRI scans, prostate-specific antigen membrane (PSMA) PET/CT scans, or both.

†† Recurrent prostate cancer was determined by a rise in PSA value or suspicious findings on multiparametric MRI scans.

‡‡ There were 53% of patients who experienced erectile dysfunction, but no distinction was made between postprocedural and preprocedural existing erectile dysfunction. This rate is probably affected by low baseline erectile function in this cohort.

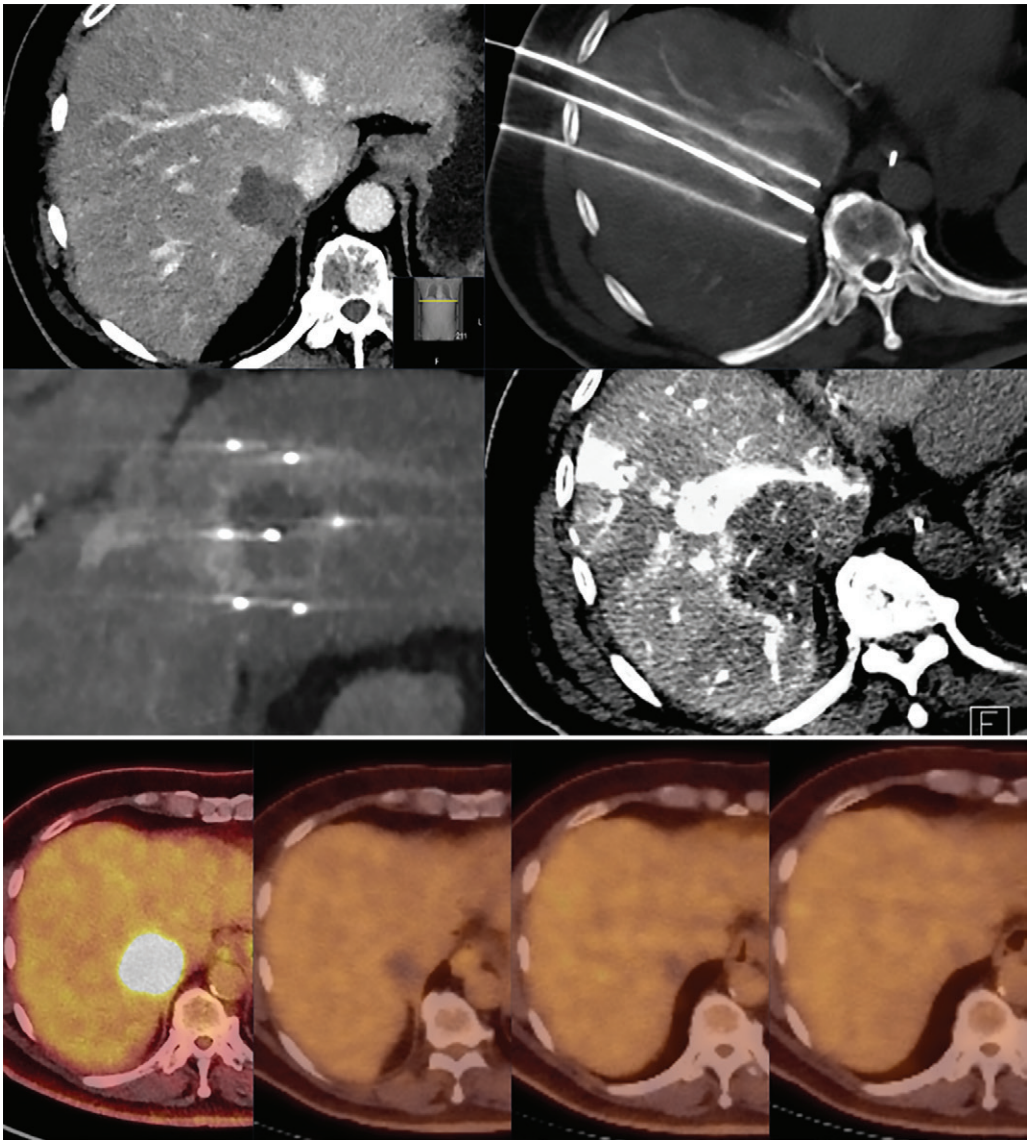

Figure 6: Image-guided percutaneous irreversible electroporation (IRE) in a 56-year-old man with a chemotherapy-naive solitary colorectal liver metastasis invading the inferior vena cava. Upper left: Transverse contrast-enhanced CT scan shows tumor invading the inferior vena cava. Upper right: Transverse contrast-enhanced CT scan shows three IRE needles around the tumor. Middle left: Coronal contrast-enhanced CT scan shows seven IRE needles around the tumor. Middle right: Transverse contrast-enhanced CT scan obtained after IRE shows the ablation zone exceeding the original tumor volume. Bottom row: Four transverse fluorine 18 fluorodeoxyglucose PET/CT images show the same tumor before treatment (left) and 3 (middle left), 6 (middle right), and 12 (right) months after treatment. The patient was treated in the setting of the prospective Colorectal Liver Metastatic Disease: Efficacy of Irreversible Electroporation–A Single-arm Phase II Clinical Trial (or COLDFIRE-2) (NCT02082782) and did not receive any systemic neoadjuvant or induction therapy.

hematoma, transient hematuria, and urinary retention (Table 3). On the basis of these studies, IRE appears safe for small renal masses up to 4 cm. However, the consensus is that current evidence is still inadequate in quality and quantity; therefore, IRE for this indication should only be used in the context of research.

IRE in the Prostate.—IRE has the potential to reduce treatment side effects encountered after conventional therapy for prostate cancer, such as damage to the urethra and neurovascular bundles. The first-in-humans clinical trial on IRE was conducted for this indication and was published in 2010 by Onik and Rubinsky (47). In 16 patients, all postprocedural biopsy results were negative, while continence and potency were preserved. Thereafter, several phase I and II trials were performed with IRE used for localized prostate cancer (Table 4) (48–52). These studies demonstrated IRE was a safe and effective treatment modality with promising functional outcomes regarding potency and continence preservation. Effectiveness of IRE for prostate cancer was demonstrated in an ablate-and-resect study by van den Bos et al with 16 patients where histopathologic analysis after radical prostatectomy showed necrotic or fibrotic tissue and no residual tumor within the ablation zone (52). The largest prospective cohort study of IRE for prostate cancer included 63 patients (50). Overall quality-of-life scores transiently deteriorated in the first weeks after treatment due to postprocedural hematuria, dysuria, urinary urgency or frequency, or perineal pain in 24% of patients and due to urinary incontinence, urinary tract infections, epididymitis, or urinary retention in 11% of patients. The sole quality-of-life domain deterioration that persisted was erectile function, which showed a mild decrease after 6 months. No serious adverse events were reported. In-field and whole-prostate oncologic control were 84% and 76%, respectively. Prospective long-term data are needed before IRE can be established as an effective treatment modality for tumor ablation in the setting of prostate cancer.

Technical Treatment Specifications and Considerations for IRE

Treatment planning and positioning.—The success of IRE is dependent on coverage of the entire tumor volume with a sufficiently high electrical field while minimizing damage to healthy and critical tissue. The exact threshold depends on the tissue

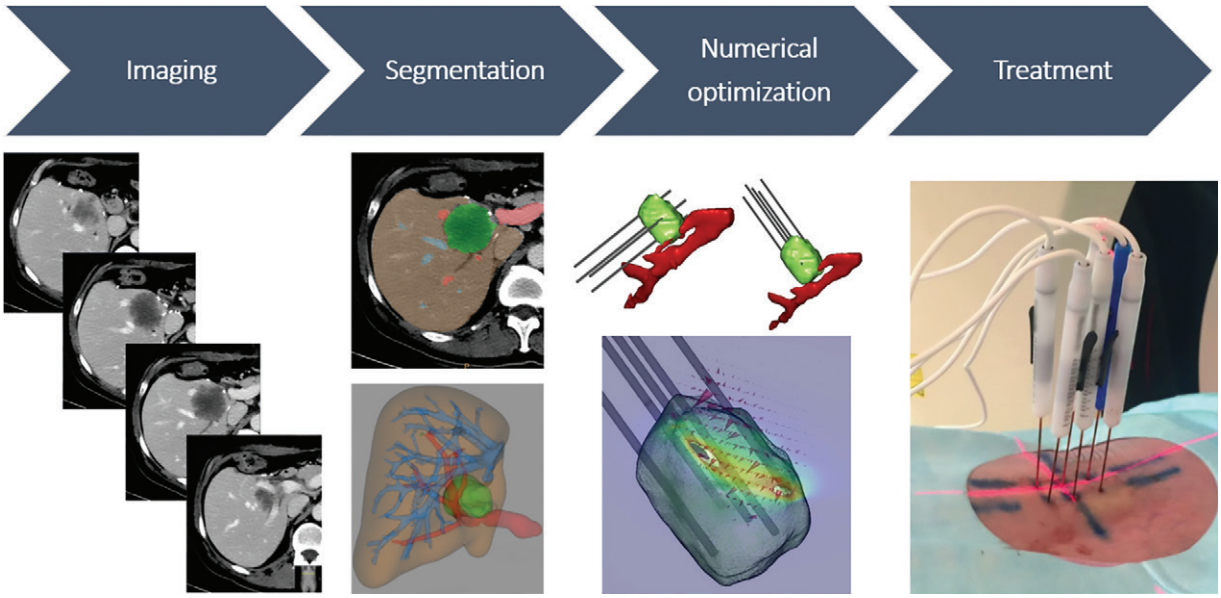

Figure 7: Schematic illustration of treatment planning workflow in a 58-year-old man. First, contrast material–enhanced transverse CT images are used to diagnose and locate a liver tumor on top of the portal vein bifurcation. Next, images are segmented with anatomic three-dimensional reconstruction. Then, numeric optimization of the electrical field and treatment planning are performed with the web-based tool Visifield *(https://www.visifield.com/)*, numeric modeling is performed with COMSOL Multiphysics software (COMSOL, Stockholm, Sweden), and a code was developed in MATLAB (Mathworks, Natick, Mass) to automatically segment CT images and build a patient-specific three-dimensional model of the tumor and surrounding tissue. Electrodes are inserted into the model by the user based on his or her experience and with respect to target tissue position on the medical images. Interpolation functions are used to specify the conductivity at each point of the model and to correct for changing conductivity values during electroporation. The MATLAB code automatically processes the increases of the conductivity of the tissue at each point in the model as a function of the local electric field. The electric field is computed iteratively until conductivity reaches a steady state. After voltages on all electrode pairs are computed, the total coverage of the target tissue and volumes of surrounding tissues covered with electrical fields above the irreversible threshold are determined. Visifield software generates a report on optimal electrode positioning and electrical pulse parameters settings (54). Finally, six irreversible electroporation needle electrodes are placed with percutaneous CT guidance.

type, but in general, electrical fields higher than 600 V/cm are recommended (53). An effective way to optimize treatment outcome is through patient-specific treatment planning consisting of medical image segmentation and numeric modeling for optimization of the electrical field. A freely available web-based tool can be used to automatically generate a three-dimensional model of the target tissue from uploaded CT images and to optimize electrode positioning and electric pulse parameters via visualization of the electrical field distribution through numeric modeling (Fig 7) (54). The optimized treatment plan can be executed manually or with a navigation system, such as robotic needle positioning (55). Further refinement of such three-dimensional modeling tools will likely enhance the efficacy of IRE by improving the prediction of treatment outcome. Figure 8, *F,* shows typical IRE needle electrodes.

Ablation monitoring during IRE.—To achieve complete ablation, delivered current is generally between 20 and 40 A. During treatment, the ablation zone appears as a hypoechoic (US) or hypoattenuated (CT) area, which correlates reliably with the pathologically defined zone of cell death (56,57). US and CT are therefore used to ensure that the ablation zone encompasses the tumor with a good margin.

Anesthetic management during IRE.—The HVEPs pose specific intraprocedural challenges. Electroporation allows ion transportation over the cellular membrane, which elicits generalized muscular contractions. Therefore, general anesthesia is required to attain complete muscle relaxation (58). If uncontrolled ion transportation occurs in cardiac tissue, arrhythmias or even fibrillation may occur (59). IRE is therefore contraindicated in patients with ventricular arrhythmias. Arrhythmias can largely be prevented by synchronizing pulse delivery with the absolute refractory period of the heart (50 msec after each R wave). Arrhythmias can still occur when using electrocardiographically synchronized pulsing, but they are often mild and self-limiting (58). Nevertheless, it is strongly recommended to preventively attach the patient to an external defibrillator. High-frequency IRE (or H-FIRE) is a technique that uses high-frequency bipolar electrical pulses and has been proposed to reduce muscle contractions. Both preclinical (19) and clinical (60) results seem promising.

Future directions for IRE.—Besides inducing local tumor destruction, IRE may result in a systemic effect by inducing a systemic immune response. Unlike in surgery, the treated malignancy is not removed from the body. The cell remnants release damage-associated molecular pattern molecules and remain available for uptake by phagocytes. Because the larger vessels remain intact, activated antigen-presenting cells can infiltrate the lesion and transport tumor fragments to draining lymph nodes, where adaptive immune activation can take place (61). Hypothetically, IRE can induce a durable and systemic antitumor T-cell response that in turn might

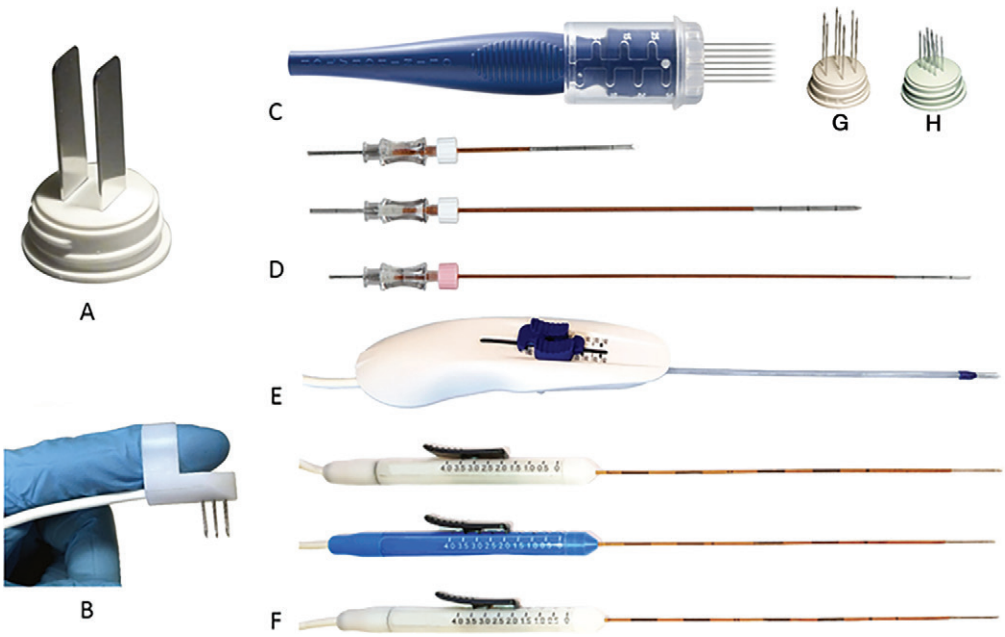

Figure 8: Types of electrochemotherapy (ECT) and irreversible electroporation (IRE) electrode probes. *A*, A noninvasive plate electrode used for superficial exophytic tumors. *B*, A finger electrode used for tumors in difficult to reach locations, like the oropharynx. *C*, An adjustable probe with needle electrodes has a hexagonal configuration *(G)*, which is used for larger infiltrating tumors, and a linear configuration *(H)*, which is used for small subcutaneous tumors. *D*, ECT needle electrodes used for deep-seated tumors. *E*, A minimally invasive ECT probe with expandable needle electrodes in the tip meant to be used laparoscopically (50 cm long) for liver tumors and endoscopically (20 cm long) for brain tumors. *F*, Typical IRE needle electrodes (blue = activator needle) used for deep-seated tumors.

induce regression in distant untreated metastases, a phenomenon known as the abscopal effect (62). In effect, IRE serves as in vivo tumor vaccination. Systemic tumor-specific T-cell responses are also observed after thermal ablation (63). However, the tumor-infiltrative immune effects of IRE seem to be more robust (64,65). Furthermore, a recent in vitro study showed that IRE induces more protein and antigen release than does cryo- or heat ablation and vastly outperforms both in terms of T-cell activation (66).

Many cancer types induce immune dysfunction by downregulation of the tumor-specific T-cell response and upregulation of immune-suppressive regulatory T cells, T-helper cells, and cytokines that could conceivably be overcome by IRE treatment (Fig 9) (67). To test this hypothesis, Scheffer et al have monitored T cells in the peripheral blood of patients with LAPC treated with IRE (68). Their findings confirm a transient decrease in systemic regulatory T-cell rates and a simultaneous transient upregulation of PD-1+ checkpoint rates on CD4+ and CD8+ T cells. Accordingly, a boost in tumor antigen–specific T-cell response was found after IRE in five of 10 patients, and although this increase was not significant ($P = .055$), there was a tendency for these patients to have better OS. Pandit et al contributed to the accumulation of evidence by demonstrating a decrease in systemic regulatory T-cell rates after intraoperative IRE in 11 patients with LAPC (69). These studies suggest the manifestation of an immunogenic window after IRE that can be further leveraged in combination with immune-stimulating agents. This approach is further discussed in the Electroimmunotherapy section of this article.

Electrochemotherapy

ECT uses reversible electroporation to temporarily increase membrane permeability to facilitate the transportation of typically poorly penetrating chemotherapeutic drugs into tumor cells to increase their cytotoxicity (Fig 10).

Mechanism of action for ECT.—Three principal mechanisms of action for ECT have been identified: *(a)* increased membrane permeability, *(b)* vascular effects, and *(c)* involvement of the immune response. The first and predominant mechanism enables the anticancer drugs to directly access their target cytosol and cellular DNA. Bleomycin and cisplatin have been identified as the cytostatics of choice, since ECT potentiates the cytotoxicity of bleomycin up to 5000-fold and that of cisplatin up to 12-fold (70). The second mechanism is two-fold and is especially advantageous in well-vascularized tumors. As discussed earlier, the vascular lock effect prolongs drug entrapment for several hours. Additionally, ECT causes endothelial cell death in afferent tumor vessels and subsequent blockage of tumor blood flow (71). This vascular disruption leads to tumor ischemia. The third mechanism relates to ECT-induced immunogenic cell death, which facilitates the release of damage-associated molecular pattern molecules and antigen shedding (72), which in turn can induce a strong priming of anticancer immunity (73). Like IRE, ECT may convert the tumor into an in situ vaccine. The combination of ECT with immune-stimulating agents awaits investigation.

Clinical results of ECT.—Effectiveness of ECT has been demonstrated in melanoma, Kaposi sarcoma, and breast, renal cell, and basal cell carcinoma (74). The multi-institutional European Standard Operating Procedures of Electrochemotherapy (ESOPE) study reported an objective response rate of 85% (complete remission + partial remission defined as tumor decrease >50%) in skin cancers. Only minor side effects were reported (muscle contractions and pain sensation) (75). Some patients experience increased severe pain after treatment, which is predicted by tumor size, previous irradiation, and a high pain score before ECT (76). A meta-analysis on the effectiveness of ECT for primary and metastatic tumors found a mean objective response rate of 84% and a complete response rate of 59%, both

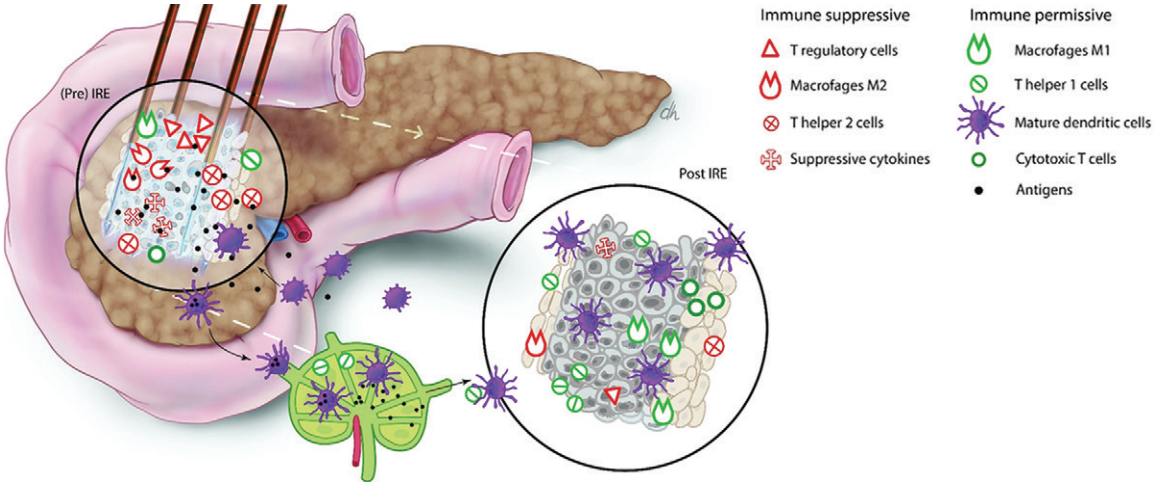

Figure 9: Illustration shows immune reaction enhancement and suppression of the immunosuppressive tumor microenvironment with irreversible electroporation (IRE) in a pancreatic tumor. The pre-IRE tumor is surrounded by immune-suppressive infiltrates. After IRE is applied, apoptotic cell remnants release antigens that are recognized and taken up by dendritic cells. The mature dendritic cells migrate to the lymph nodes where T-cell cross priming takes place and effector T cells migrate back to local and distant tumors to induce a tumor suppressive immune response and ultimately cause tumor regression.

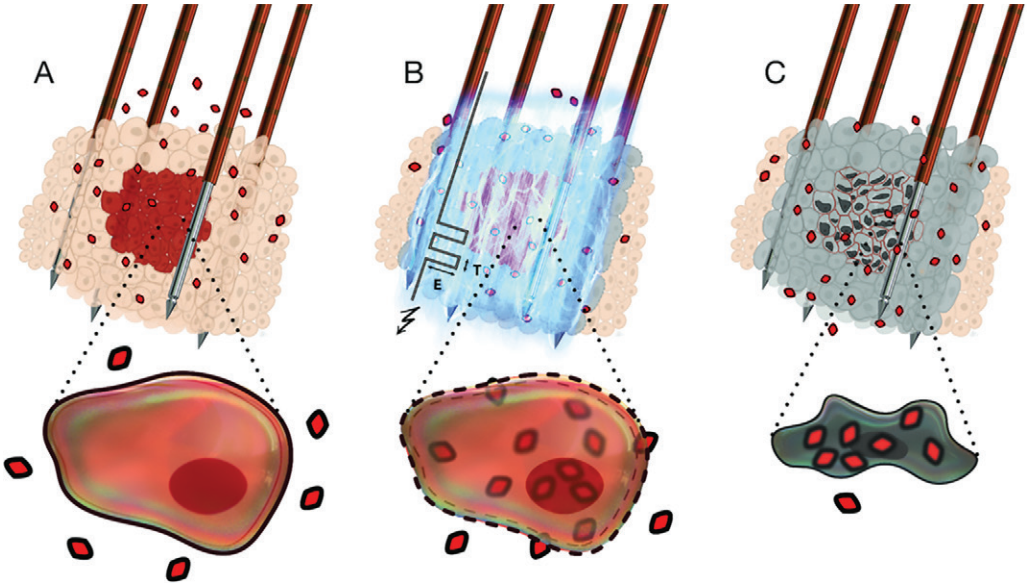

Figure 10: Illustration shows electrochemotherapy (ECT). ECT is the use of short and intense electrical pulses to increase the intracellular concentration of chemotherapeutic drugs in tumor cells. Cell membrane permeabilization permits the drugs to enter the cell and induce cell death. *A*, Before ECT. Top: Needle electrodes are inserted in and around a tumor (brown) within healthy tissue (beige). Bottom: Enlargement shows one tumor cell with an intact cell membrane that hinders chemotherapy particles (red) from entering the cell. *B*, During ECT. Reversible electroporation of tissue within the ablation zone (blue) by short-duration (T) high-voltage (E) electrical pulses causes reversible cell membrane disruption (pore formation) and migration of chemotherapy particles through the membrane and into the tumor cell. *C*, After ECT. Tumor cells recover membrane integrity but die due to uptake of chemotherapy particles (black). Structural tissue integrity (gray) is preserved. Tumor location before ECT is indicated by brown lines.

independent of treated tumor type (77). The high tumor response rate and the limited effect on surrounding healthy tissues allows for the potential of repetitive treatment, making ECT an appealing oncologic treatment (78). The procedure is increasingly introduced into European clinical guidelines, including advanced melanoma (79) and primary squamous carcinoma (80). Standard operating procedures were updated in 2018, as ECT is now clinically used to treat cutaneous larger-sized metastases of all histologic types (76).

Technical treatment specifications and considerations for ECT.—ECT is delivered under local or general anesthesia, and the chemotherapeutic drug is administered intratumorally (1 mg/mL cisplatin or 1000 IU/mL bleomycin) or intravenously (15,000 IU/m^2 bleomycin). Intratumoral injection is guided by tumor volume; the recommended dose should fill the entire tumor volume with the drug. The correct dose for intravenous administration of bleomycin is based on body surface area (in square meters). The route of administration depends on the

number and size of tumors, as well as on patient features like pulmonary and renal function (75,76). The most frequently applied modality is intravenous bleomycin for 8 minutes under general anesthesia followed by application of electric pulses over a 40-minute period. Patients with locally advanced tumors can undergo up to seven treatment sessions with an interval of at least 4 weeks. However, given the heterogeneity of treated tumors, the treatment strategy should be individualized and guided by treatment response, patient tolerance, and optimal combination with other therapies (72).

Electrode types for ECT.—All ECT electrodes are characterized by a fixed geometry. There are two types of fixed geometry electrodes: *(a)* plate (contact) electrodes and *(b)* needle electrodes, with lengths that can range from 5 to 30 mm (Fig 8, *A–E*). Plate electrodes are placed over the tumor and are used for superficial exophytic tumors. Conversely, needle electrodes are inserted percutaneously (or intraoperatively during laparotomy) to treat deep-seated tumors (81).

Anesthetic management during ECT.—Given the lower amplitude and number of pulses compared with IRE, complete muscle relaxation and electrocardiographic synchronization are not necessary during pulse delivery in superficial tumors (76). On the contrary, for deep-seated tumors, these precautions must be taken by any means. Intraoperative anesthetic management depends on disease extent and anatomic location along with electrode type. General anesthesia is best suited for deep-seated and superficial tumors of the face, scalp, and oropharynx to ensure patient comfort and to maintain airway control (82). In most other superficial locations, ECT can be safely performed with the patient under propofol sedation while spontaneous ventilation is maintained and analgesia is provided through neuraxial or regional anesthesia (72).

Future directions for ECT.—Efforts are being made to translate the application of ECT from easily accessible cutaneous tumors to deep-seated tumors. Preliminary results show that ECT for deep-seated tumors is feasible, safe, and effective for tumor load reduction (83). Clinical case reports on ECT in the setting of locally advanced pancreatic carcinoma and perihilar cholangiocarcinoma show improved survival and minimal complications (84,85). To date, five prospective studies on ECT for liver tumors have been performed. Radiologic complete response rates varied from 55% to 88% in 39 patients, and partial response rates varied from 12% to 15% (81,86–89). A prospective feasibility study on palliative ECT for bone metastases achieved better than 50% pain relief in 84% of 29 patients (90). Painful spinal metastases from malignant melanoma can be treated with ECT and decreased the visual analog scale pain score from 10 to 2 in 1 month (91). Overall, efficacy data for deep-seated tumors seem promising but remain of limited value, as current studies have mainly included patients in whom all standard treatment options have failed. Also, no studies have compared ECT with competing therapies, such as radiation therapy or thermal ablation. As with IRE, systemic immune activation has been observed in animal studies (92). Immunogenic cancer cell death is responsible for the generation of tumor-specific T cells that can kill remaining cancer cells. This ECT-driven immune response may not be strong enough on its own to affect distant tumors, but preclinical evidence suggests that immune-stimulating agents combined with ECT could potentiate the local effect and be used to simultaneously treat distant nodules (73).

Experimental Techniques: GET, Electrofusion, and Electroimmunotherapy

Gene electrotransfer.—GET uses HVEPs to deliver protein-encoding DNA into cells to alter their properties. In oncology, GET can be used to transport DNA into tumor cells or healthy surrounding cells to induce immune stimulation and anticancer properties. First, a DNA plasmid saline solution is injected into the tumor under high hydrostatic pressure. This mode of administration increases GET efficiency up to 500-fold when compared with low-pressure administration. Optimal dose has yet to be determined but is dependent on tumor volume. Electrodes subsequently deliver short (microseconds) and intense electrical pulses for cell membrane permeabilization and are followed by longer (milliseconds) less intense pulses to electrophoretically drive DNA into the cells (Fig 11). The produced protein can exert distant therapeutic effects (93). The main drawback of GET for clinical use remains its low efficiency. Upscaling from small-animal models to human tumor volumes is challenging. Because of its low immunogenicity in early clinical studies, GET has not yet achieved widespread acceptance for use in humans (94). However, recent clinical outcomes are promising, and ongoing trials might re-establish the value of GET in oncology.

The two most-developed GET applications in oncology are cytokine therapy and DNA vaccination, both at the junction of electroporation and immunology. Electroporation-based cytokine therapy uses HVEPs to transport cytokine-encoding plasmids into tumor cells. Animal studies report local and systemic antitumor effects after GET in conjunction with interleukin-12 plasmid in a variety of tumors (95). Clinical evaluation of GET with an interleukin-12 plasmid in 24 patients with metastatic melanoma yielded clinically important tumor necrosis and T-cell infiltration. In addition, 10% of patients with nonelectroporated distant lesions showed complete regression of all metastases and 42% displayed stable disease or partial response, indicating a systemic effect (96).

For DNA vaccination, DNA plasmids encoding an antigen of interest are administered intramuscularly or intradermally to protect the body against cancer cells expressing this antigen by generating a population of tumor-specific B and T cells (97). HVEPs enhance DNA delivery into the cell. Furthermore, GET generates greater-than-expected immune responses from increased DNA uptake alone and improves the capacity to mount systemic adaptive antitumor immune responses (73). Clinical outcomes achieved by DNA vaccination facilitated through GET are positive. Phase I trials in which DNA plasmids were injected intramuscularly in patients with melanoma (98) or cervical cancer (99) were generally tolerated well and demonstrated clinically observable tumor clearance and durable CD8+ T-cell responses with high levels of interferon-γ production in up to 72% of patients.

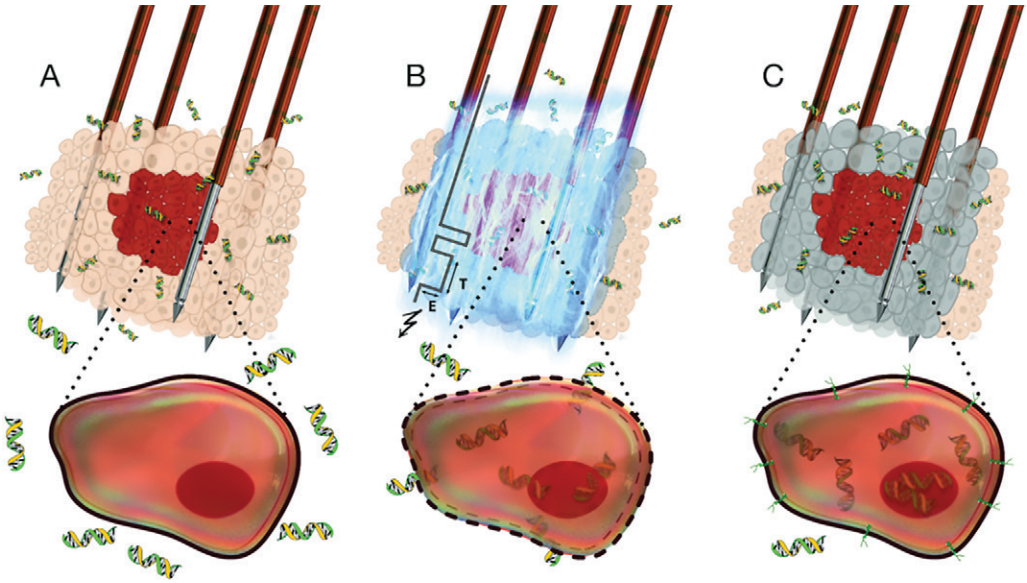

Figure 11: Illustration of gene electrotransfer (GET). GET is the use of short but intense electrical pulses to permeabilize the cell membrane combined with a long-duration and low-voltage electrical pulse to drive DNA plasmids to and across the cell membrane and into the cell. DNA enters the cell and can modify cell properties by encoding for a protein of interest. *A*, Before GET. Needle electrodes are inserted around a tumor (brown) within healthy tissue (beige). The enlargement shows one tumor cell with an intact cell membrane that hinders DNA (green) from entering the cell. *B*, During GET. Reversible electroporation of all tissue within the ablation zone (blue) with short-duration (T) high-voltage (E) and long-duration (T) low-voltage (E) electrical pulses causes reversible cell membrane disruption (pore formation) and transportation of DNA plasmids toward the membrane and into the tumor cell. *C*, After GET. Cell viability and structural tissue integrity are preserved (gray). Cells recover membrane integrity, and transported DNA plasmids in the cell nucleus lead to changed cell properties (green receptors).

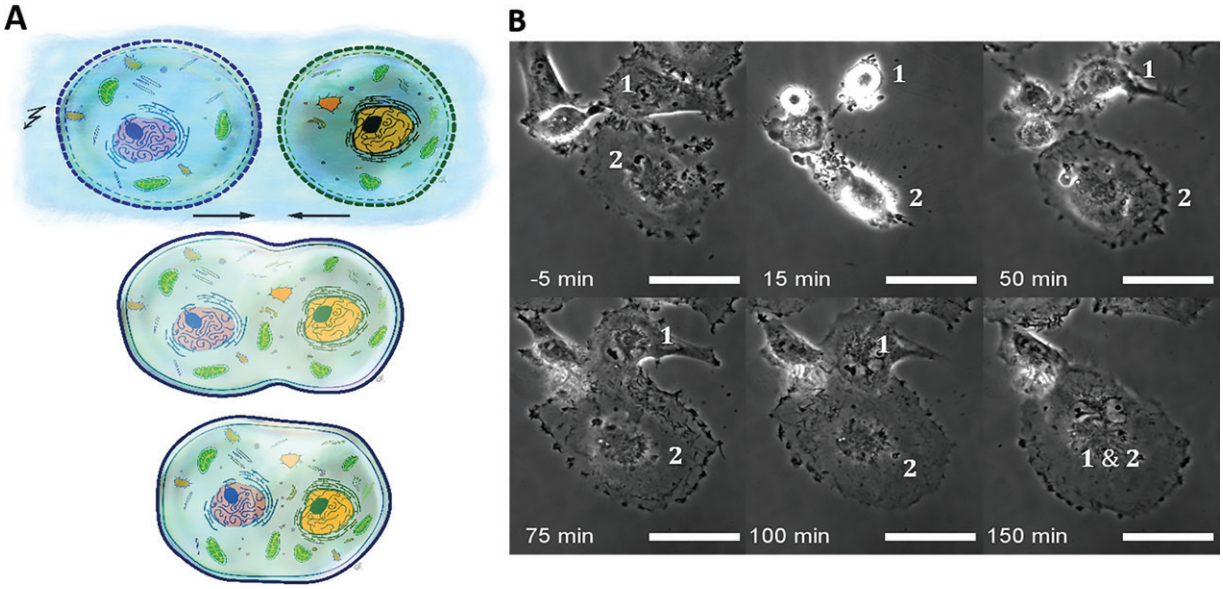

Figure 12: Examples of electrofusion. *A*, Illustration shows electrofusion is most efficiently achieved by very short (nanosecond) electrical pulses that make cell membranes permeable and cause cells to enter a fusogenic state. When cells are in close physical contact, fusion can occur. The created cell will be a fused product of both original cells. *B*, Microscopic images show electrofusion of two glioblastoma cells (1 and 2) after ten 100-μsec 1000 V/cm electrical pulses. Pulses were delivered at 0 minutes. Scale bar is 50 μm.

A randomized, double-blind placebo-controlled phase II trial of VGX-3100, a vaccine for human papillomavirus subtypes 16 and 18 facilitated by HVEPs in women with high-grade cervical dysplasia, reported potent antigen-specific CD8+ T-cell responses and showed great efficacy in almost 50% of patients (100). The first clinical trial combining GET with intradermal injection of DNA plasmids yielded less efficient anti–prostate-specific antigen antibody production compared with intramuscular administration in patients with prostate cancer (101). Two studies used HER2 carcinoembryonic antigen DNA vaccines in patients with several tumor types and detected both humoral and cellular immune responses but found no evidence of tumor antigen–

specific immune responses (102). On the basis of this early clinical evidence, GET has the potential to become a valuable tumor treatment, especially if the yield of antibody production can be further increased. Several clinical trials evaluating GET in the treatment of melanoma, cutaneous lymphoma, and Merkel cell, cervical, colorectal, and prostate cancer are currently underway (103).

***Electrofusion.*—**Electrofusion is the use of electrical pulses to make the cell membrane permeable and bring it into a "fusogenic" state, allowing fusion between cells near each other. The created cell is a fusion product of both original cells. The highest electrofusion yield for cells is achieved with nanosecond HVEPs (104). A schematic electrofusion event and a microscopy picture of two fusing glioblastoma cells are shown in Figure 12. In oncology, electrofusion is used to create immune-enhancing therapies, such as cancer cell vaccines. This involves the fusion of dendritic cells with live cancer cells. These fusion products can express a wide spectru5m of tumor-associated antigens, stimulating both cytotoxic and helper T cells. Their therapeutic antitumor effect has been demonstrated in vitro and in vivo (105). In the majority of clinical trials, electrofusion of dendritic and cancer cells as a monotherapy has had limited efficacy but may prove to be more efficacious when combined with other immunotherapies (106) or with GET for the production of costimulatory cytokines to obtain a synergistic immune effect (107).

***Electroimmunotherapy.*—**Increasing evidence shows that HVEPs alone induce immunologic effects in both normal and cancer tissues by the induced release of damage-associated molecular pattern molecules and through the exposure of calreticulin on the cell surface, attracting dendritic cells (108). The immune response following HVEP application is synergistic with the response elicited by tumor cell death through IRE or ECT. Clinical evidence for ablation to induce counteractive pro-oncogenic effects similarly exists and has been linked to aggressive tumor development and worse patient outcomes (109). However, an increasing number of studies suggest that the immunogenic effects of IRE outperform those of other ablative techniques (65,66) and can be further enhanced by immunomodulatory drugs (110–113). We suggest using the new term, *electroimmunotherapy*, to describe the use of IRE in combination with the administration of immunomodulatory drugs. In vivo research demonstrated substantial benefits of combining IRE ablation with anti-PD1 checkpoint blockade therapy (113). Immunocompetent mice with orthotopic pancreatic ductal adenocarcinoma showed significant ($P < .0001$) prolonged survival after combination therapy with IRE and anti-PD1 compared with IRE and anti-PD1 monotherapies. About 40% of the mice showed a durable response and rejected tumor cell rechallenge 60 days after treatment because of substantial infiltration of CD8+ T cells. Clinical studies investigating the combination of IRE and allogeneic natural killer cell therapy demonstrated higher median OS in stage III and IV pancreatic cancer (112), as well as higher median OS and a decline in circulating tumor cells in stage III and IV hepatocellular carcinoma (110,111). More clinical data are needed to determine the efficacy and safety of electroimmunotherapy, but early results are promising.

Conclusion

Clinical and preclinical data show substantial potential for electroporation-based therapies to advance cancer treatment. Although limited in number, early clinical results are encouraging; electrochemotherapy (ECT) has been established as a reliable option in the palliative treatment of cutaneous cancers, and irreversible electroporation (IRE) has been proven safe and effective for pancreatic, liver, and prostate cancer. Soon, we expect IRE and ECT to become important players in interventional oncology, and the conduction of high-volume prospective registries and randomized controlled trials will accelerate the implementation process. Additionally, it will be essential for the optimization of IRE and ECT outcomes to elucidate the exact effects of all individually adjustable parameters (ie, duration, length, and number of pulses; interelectrode distance; voltage; configuration; and number of needle electrodes) on the ablation zone and immune reaction in different tissue types. Gene electrotransfer, electrofusion, and especially electroimmunotherapy have the potential to become clinically relevant therapies and require close collaboration between interventional radiologists, medical oncologists, engineers and immuno-oncologists. If smart combinations of immune-enhancing or cytotoxic drugs with IRE or ECT prove to trigger an antitumor immune effect and provoke deep, durable responses, high voltage electrical pulses may one day provide the bridge between local and systemic treatment.

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