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

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Conflicts of interest are listed at the end of this article.

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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
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 ablational modality and mathematically demonstrated its capability to ablative 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 trans-

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
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 ablative-and-resect study (Colorectal Liver Metastatic Disease: Efficacy of

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.
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

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(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.

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

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

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

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

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

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.

† 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

<table>
<thead>
<tr>
<th>Author, Year of Publication</th>
<th>Study Design</th>
<th>No. of Patients</th>
<th>Median Age (y)</th>
<th>Gleason Score</th>
<th>Pretreatment or Concurrent Treatment</th>
<th>Functional Outcome</th>
<th>Oncologic Efficacy</th>
<th>Adverse Events (CTCAE 4.0)</th>
<th>follow-up (mo.)</th>
<th>Functional Outcome (%) of Patients</th>
<th>Oncologic Efficacy (% of Patients)</th>
<th>Adverse Events (CTCAE 4.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onik and Rubinsky (2010)</td>
<td>Prospective</td>
<td>16 NS (60–70)</td>
<td>3+3: n = 7</td>
<td>3+4: n = 6</td>
<td>4+4: n = 3</td>
<td>Nonspecified</td>
<td>NS</td>
<td>0%</td>
<td>6</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Van den Bos et al (2016)</td>
<td>Prospective</td>
<td>16 60</td>
<td>3+3: n = 8</td>
<td>3+4: n = 9</td>
<td>4+3: n = 2</td>
<td>Nonspecified</td>
<td>NS</td>
<td>0%</td>
<td>12</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Van den Bos et al (2018)</td>
<td>Prospective</td>
<td>63 67</td>
<td>3+3: n = 9</td>
<td>3+4/4+3: n = 8</td>
<td>4+4: n = 16</td>
<td>Nonspecified</td>
<td>NS</td>
<td>0%</td>
<td>6</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Guenther et al (2019)</td>
<td>Retrospective</td>
<td>429 64</td>
<td>3+3: n = 9</td>
<td>3+4: n = 19</td>
<td>4+4: n = 5</td>
<td>Nonspecified</td>
<td>NS</td>
<td>0%</td>
<td>12</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Valerio et al (2014)</td>
<td>Prospective</td>
<td>34 65</td>
<td>3+3: n = 2</td>
<td>3+4: n = 15</td>
<td>4+3: n = 8</td>
<td>Nonspecified</td>
<td>NS</td>
<td>0%</td>
<td>6</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Ting et al (2016)</td>
<td>Prospective</td>
<td>25 67</td>
<td>3+3: n = 2</td>
<td>3+4: n = 15</td>
<td>4+4: n = 8</td>
<td>Nonspecified</td>
<td>NS</td>
<td>0%</td>
<td>6</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

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.
* Grade 1: short-term transient urologic symptoms outside the IRE ablation zone.
* Grade 2: transient urologic symptoms outside the IRE ablation zone, mild procedure-related discomfort.
* Grade 3: procedure-related symptoms and pain persisting for >1 month. Procedure-related symptoms of this severity may not be associated with recurrence.
* Grade 4: severe procedure-related symptoms and pain persisting for >1 month. Procedure-related symptoms of this severity may be associated with recurrence.
* Grade 5: more severe or prolonged symptoms and pain that are not related to the ablation procedure, such as life-threatening complications.
* Maximum follow-up was 72 months. Recurrence was determined by a rise in prostate-specific antigen (PSA) level with corresponding findings on multiparametric MRI scans.
* 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 proprocedural and postprocedural existing erectile dysfunction. This rate is probably affected by low baseline erectile function in this cohort.
tive, 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 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 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 fluoroethylglucose 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.
eralized muscular contractions. Therefore, general anesthesia is required to attain complete muscle relaxation (58). If uncontrollable 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 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.
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.

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 different 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 (EOPNE) 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

**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

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**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.
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/m² 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 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).

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.
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 electroprotection 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 1 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.
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–

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

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**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 (T1) high-voltage (E) and long-duration (T2) 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.
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 spectrum 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 counteracting 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 electroportion-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 electroportion (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 electotransfer, 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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