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Irreversible electroporation and electrochemotherapy in oncology: State of the art --Manuscript Draft--

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Abstract:	<p>Thermal tumor ablation techniques including radiofrequency, microwave, laser, high-intensity focused ultrasound and cryoablation are routinely used to treated liver, kidney, bone, or lung tumors. However, all these techniques are thermal and can therefore be affected by heat sink effect, which can lead to incomplete ablation, and thermal injuries of non-targeted tissues are possible. Under certain conditions, high voltage pulsed electric field can induce formation of pores in the cell membrane. This phenomenon, called electropermeabilization, is also known as "electroporation". Under certain conditions, electroporation can be irreversible, leading to cell death. Irreversible electroporation has demonstrated efficacy for the treatment of liver and prostate cancer, whereas data are scarce regarding pancreatic and renal cancers. During reversible electroporation, transient cell permeability can be used to introduce cytotoxic drugs into tumor cells (commonly bleomycin or cisplatin). Reversible electroporation used in conjunction with cytotoxic drugs shows promise in terms of oncological response, particularly for solid cutaneous and subcutaneous tumors such as melanoma. Irreversible and reversible electroporation are both not thermal ablation techniques and therefore open a new promising horizon for tumor ablation.</p>

Irreversible electroporation and electrochemotherapy in oncology: State of the art

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Irreversible electroporation and electrochemotherapy in oncology: State of the art

Abstract

Thermal tumor ablation techniques including radiofrequency, microwave, laser, high-intensity focused ultrasound and cryoablation are routinely used to treated liver, kidney, bone, or lung tumors. However, all these techniques are thermal and can therefore be affected by heat sink effect, which can lead to incomplete ablation, and thermal injuries of non-targeted tissues are possible. Under certain conditions, high voltage pulsed electric field can induce formation of pores in the cell membrane. This phenomenon, called electropermeabilization, is also known as “electroporation”. Under certain conditions, electroporation can be irreversible, leading to cell death. Irreversible electroporation has demonstrated efficacy for the treatment of liver and prostate cancer, whereas data are scarce regarding pancreatic and renal cancers. During reversible electroporation, transient cell permeability can be used to introduce cytotoxic drugs into tumor cells (commonly bleomycin or cisplatin). Reversible electroporation used in conjunction with cytotoxic drugs shows promise in terms of oncological response, particularly for solid cutaneous and subcutaneous tumors such as melanoma. Irreversible and reversible electroporation are both not thermal ablation techniques and therefore open a new promising horizon for tumor ablation.

Key-words: Electroporation; Electrochemotherapy; Cancer treatment; Interventional oncology

Abbreviations

DNA: Deoxyribonucleic acid

ECT: Electrochemotherapy

FOLFIRINOX: Combination of folinic acid, fluorouracil, irinotecan hydrochloride, and oxaliplatin

IL: Interleukin

IRE: Irreversible electroporation

IT: Intra tumoral

IV: Intravenous

1. Introduction

1 Thermal tumor ablation techniques include radio-frequency ablation, microwave ablation,
2 Laser ablation, high-intensity focused ultrasound and cryoablation, which can be used for the
3 treatment of a variety of tumors, particularly in the liver, kidney, bone, and lung [1] [2]. During
4 thermal ablation, complete tissue necrosis is achieved by heating or cooling the targeted tissue
5 to a cytotoxic temperature (less than -40°C or more than 60°C). However, thermal ablation of
6 tumors adjacent to large vessels is affected by the cooling effect of the liquid flow in the lumen,
7 called heat sink effect, which can lead to incomplete ablation. In addition, thermal injuries of
8 non-target tissue such as nerves, vessels, bile or pancreatic ducts are possible.

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Electroporation is a non-thermal technique using high-frequency electrical pulses to permeate cell membranes. Irreversible electroporation (IRE) describes the loss of semipermeable barrier properties of membrane cells explaining apoptosis by lethal disruption of the osmotic balance between the cell inside and the environment outside [3, 4]. Electroporation is reversible if disturbances of cell membrane properties are temporary. Both techniques avoid the heat sink effect for tumors near a large vessel and do not cause damage to the extracellular matrix because of lack of cell membrane structure [5].

The purpose of this review was to describe the basic concepts behind electroporation and sum-up current evidence on the applications of reversible electroporation and IRE in oncology.

2 The basics of electroporation

Cell membrane can be considered as a dielectric separating two conductive media (*i.e.*, the cytoplasm and the external medium). When subjected to an external electric field, the cell behaves as a closed capacitor. As a result, the electric field induces a size and position-dependent transmembrane potential, which superimposes to the resting potential, leading to membrane permeabilization or “poration”, when the field-induced transmembrane voltage reaches a critical value [6]. Maximum effects are present at the poles of the cells facing the electrodes when the resulting transmembrane potential reaches a threshold value (close to 200 mV) [7], above which cell membrane permeabilization occurs (Figures 1 and 2). This phenomenon, called electropermeabilization, is also known under the term “electroporation” due to the simplest description of the process based on the formation of pores. At the single cell level, electroporation can be described as different steps including: *i*), induction step, where the field-induced membrane potential difference reaches the critical threshold value; *ii*), expansion

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step, where membrane defects extend as long as the field is present; and *iii*), resealing step, where the membrane repairs, which is mandatory to preserve cell viability [8] [9].

With electrochemotherapy (ECT), tumor cell membrane stays permeable for seconds to minutes after the pulse. This is why ECT can be used in conjunction with the administration of chemotherapy. Chemotherapy can be applied either by intratumoral or intravenous injection during electroporation application. The transfer of drugs both during the pulse, and during the slow resealing, results in a high increase of anticancer drug intracellular concentration, which is multiplied up to 100-fold, explaining why ECT is so effective compared to conventional chemotherapy [10]. Cell death is directly due to the action of cytotoxic drugs such as bleomycin and cisplatin, which are delivered into the cells by electroporation

With IRE, cell death is mainly induced by apoptosis [11], a programmed, regulated, non-inflammatory cell death. Other cell death mechanisms can be present, such as necrosis and immunogenic cell death, including necroptosis and pyroptosis. Impacts of electric pulses depend on electric pulse parameters, cell and tissue types and treatment zones [12]. Cells close to the electrodes are exposed to the highest electric field intensities and generally die by necrosis. Cells present at the margins can undergo reversible electroporation and survive. In that case, it is possible to induce cell death by the addition of a cytotoxic drug that will be transferred into the cells (*i.e.*, by a combination of IRE and electrochemotherapy) [13].

In addition to disruption of the osmotic balance between the inside and the outside of the cell, transiently reduced blood flow to near no-flow conditions can also affect tumoral vascularization [14]. Three mechanisms leading to increased vascular resistance have been described [15]. They include: (*i*), a vasoconstriction induced by electrical stimulation of precapillary smooth muscle cells [16]; (*ii*), a cell-to-cell junction disruption due to modifications of endothelial cell shape; and (*iii*), an increased interstitial fluid pressure due to increased vessel permeability. Chromosomal DNA fragmentation has also been suggested as an explanation for late cell apoptosis [17].

3. Irreversible electroporation in human oncology

3.1 IRE procedure

To date, IRE has been used for the treatment of liver, pancreatic, renal and prostatic cancers. Patients should be referred after multidisciplinary team discussion and must be advised about treatment alternatives. IRE can be performed percutaneously under imaging guidance or during

1 open surgery. No specific prophylactic treatment is given before the procedure. Chemotherapy
2 is commonly stopped at least three weeks before the procedure. IRE must be performed under
3 general anesthesia with complete muscle paralysis, using high frequency jet ventilation that
4 limits diaphragm muscle movement to 2 mm. Three-dimensional tumor measures, including at
5 least a 5-mm tumor-free margin, have been used to determine the number and position of the
6 electrodes. Electrodes are inserted with a 5-mm tumor-free margin and an interelectrode
7 distance of 1.5–2.4 cm. An active tip length of 15–40 mm can be applied. Ten test pulses of 90
8 μ sec and 1500 V/cm are then delivered between all electrode pairs to evaluate tissue
9 conductivity. Voltage settings are then adjusted to achieve a target current of 20–40 A. A total
10 of 90 pulses are finally administered between each pair of electrodes. If necessary, a pullback
11 is performed to treat the whole tumor.
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22 **3.2 Liver cancer**

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25 After the first study by Thomson et al. that described IRE ablation of liver tumors [18], several
26 reports have suggested IRE as a useful ablation modality for hepatic malignancies adjacent to
27 major blood vessels and/or the biliary tree, where thermal ablation techniques are associated
28 with risk of complications and heating effect [19] [20, 21], [22]. In a meta-analysis including
29 nine studies and 300 patients undergoing IRE for liver tumors, major complications were
30 reported in seven patients including four hepatic abscesses, one bile duct dilatation, one
31 arrhythmia, and one portal vein thrombosis [23]. Yet, as in every other ablative techniques, the
32 main limiting factor is the tumor size, with treatment efficacy declining with increasing lesion
33 size [18], and 3 cm remains a consensual threshold [24]. In 2021, a new meta-analysis including
34 25 studies (15 prospective and 10 retrospective), summed-up the results of IRE for liver ablation
35 in 776 patients with 354 liver metastases, 285 hepatocellular carcinomas, and 100
36 cholangiocarcinomas [19]. Pooled overall survival and progression-free survival at 6, 12, and
37 24 months were 93.28% and 79.72%, 81.29% and 64.19%, and 61.47% and 49.05%,
38 respectively. The overall adverse event rate was 23.7%, with 6.9% of serious adverse events
39 (*i.e.*, grades C-F according to the Society of Interventional Radiology classification).
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51 Table 1 provides a summary of the main studies that reported the use of IRE for the treatment
52 of liver cancers. Figures 3 and 4 illustrate the use of IRE for the treatment of liver tumors.
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58 **3.3 Pancreatic cancer**

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1 Taking into account its mechanism of action, IRE can be used to destroy pancreatic tumors
2 while not affecting surrounding structures such as mesenteric and portal vessels, or biliary tract
3 even if the tumor encases these structures. In these latter situations, the use of other ablative
4 methods could be unsafe and are not recommended [25]. Currently, IRE is mainly used for the
5 treatment of stage III locally advanced pancreatic adenocarcinoma [26], although researchers
6 have reported the use of IRE for the treatment of stage IV pancreatic adenocarcinoma with liver
7 metastases [27].
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12 The reported rate of serious adverse events during or after IRE varies among studies, ranging
13 from 7.2% [28] to 48% [29], but mortality due to IRE ranges between 0% and 2% [26]. A
14 systematic review reported complication rate and mortality rate directly related to the IRE
15 procedure of respectively 15% and 9% and 3% and 0% for open and percutaneous
16 approaches [30]. Abdominal pain is the most commonly reported adverse event with an
17 incidence of 70%. Frequent serious adverse events are pancreatitis, biliary tract leakage,
18 pancreatic leakage, duodenal leakage, duodenal ulcer, pneumothorax, hematoma, and deep
19 vein thrombosis [31]. Therefore, IRE of pancreatic tumors seems to be more frequently
20 associated with complications by comparison with IRE of hepatic ones. However, several
21 studies have demonstrated encouraging survival outcomes [32]. In this regard, Martin et al.
22 reported significant improvement in overall survival from 13 to 20 months, local progression-
23 free survival from 6 to 14 months, and distant progression-free survival from 9 to 15 months
24 in favor of IRE in a propensity-matched study comparing patients receiving IRE after
25 induction chemotherapy with a retrospective cohort of patients receiving chemotherapy alone
26 in locally advanced pancreatic adenocarcinomas [32]. However, only a small proportion of
27 patients included in the control arm received FOLFIRINOX regimen, making it difficult to
28 translate these results into up-to-date practice [32]. Table 2 provides a summary of the main
29 studies that reported the use of IRE for the treatment of pancreatic cancers. Figure 5 illustrates
30 the use of IRE for the treatment of locally advanced pancreas adenocarcinoma.
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47 To date, IRE can be used to shrink a pancreatic tumor, in association with radiotherapy
48 in palliative treatment or to reduce the rate of R1-R2 (microscopic-macroscopic residual tumor)
49 resections. However, the indications of IRE remain debated [33, 34]. To date, there is a
50 consensus for using IRE after at least three months of chemotherapy to identify patients with
51 aggressive tumor and early metastatic progression, allowing exclusion of patients who would
52 not benefit from IRE. In addition, it is currently admitted that a combination of systemic
53 chemotherapy and IRE should be considered because of potential synergistic effects [35]. In
54 this area, chemotherapy could be more effective after reversible electroporation, as stated by
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1 the concept of ECT. However, it should be noted that ECT alone has been infrequently
2 evaluated in locally advanced pancreatic cancer [36, 37]. This lack of data also concerns
3 neuroendocrine pancreatic tumor, of which only a few cases have been reported [38].
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5 To date, IRE of pancreatic tumors seems effective and relatively safe. Further efforts
6 are however needed to better address patient selection, as well as the place of IRE in the current
7 therapeutic armamentarium, and patients should be treated after inclusion in prospective studies
8 and randomized trials [26].
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12 **3.4 Renal cancer**

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14 Since the recommendations of the American Urology Association and the European
15 Society for Medical Oncology issued in 2019 and 2017 respectively [39, 40], image-guided
16 thermal ablation has been considered as a treatment option for small localized renal cancer (<
17 3 cm). However, thermal ablation with radiofrequency, microwaves or cryoablation can cause
18 damage to nearby vital structures in such as vascular pedicles or ureter [41]. As a consequence,
19 the use of IRE has been suggested for the treatment of renal cancer. However, it should be noted
20 that eventhough IRE hypothetically spares the ureter, there is a possible risk of stricture,
21 especially for treatment settings of 2000 V/cm or higher, which may cause transmural necrosis
22 [42].
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34 Data regarding the efficacy of renal IRE remain scarce. Pech et al. reported the first use
35 of IRE for the treatment of renal cell carcinoma in six patients [43]. Over the last ten years,
36 there were fewer than 10 published case series on the safety and feasibility of IRE in renal cell
37 carcinoma. The largest study was performed by Canvasser et al. [44]. According to this study,
38 71% of patients were discharged one the same day of the procedure and no major (Clavien
39 grade II or higher) complications occurred. However, because of the lack of systematic
40 histopathological analysis of tumors (only 25 out of 42 tumors [60%] had a pretreatment
41 biopsy), the significance of the oncological results is questionable [45]. Longer follow-up and
42 randomized prospective studies are therefore required to validate the oncological efficacy of
43 IRE in the treatment of renal cell carcinoma. Table 3 provides a summary of the main studies
44 that reported the use of IRE for the treatment of renal cancers.
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56 **3.5 Prostate cancer**

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1 The first use of IRE in prostate cancer was reported in an animal model [46]. Histopathological
2 analysis revealed that the neurovascular bundle and blood vessels remained preserved, and that
3 there was no damage to the urethra or rectum after IRE [46, 47]. Onik et al. showed that at two
4 weeks after IRE, the ablated area was primarily replaced by collagenous tissue in dogs [47].
5 The first human prostate IRE was performed in localized prostate cancer to assess safety and
6 feasibility [48]. Neal et al. treated two patients by IRE before radical prostatectomy.
7 Pathological analysis showed that there was no viable prostate cancer within the ablation zone,
8 which had been replaced by tissue necrosis and inflammatory response [48]. The two most
9 important studies in terms of population were published by Blazeovski et al. and Wang et al
10 including respectively 50 and 117 patients [49, 50]. Low and intermediate-risk prostate cancers
11 according to the d'Amico criteria are the main indication. The technique seems safe with no
12 Clavien–Dindo Grade III–IV adverse event observed. A risk of recurrence or residual tumor
13 of 3% at 6 months and 2.7% at 12-months was demonstrated [49, 50]. Disease-free survival,
14 defined as avoidance of whole gland therapy or metastasis/death related to prostate cancer was
15 96.75% at 3 years [49].

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27 Because conceptually, IRE of prostate tumor should be considered as partial prostate
28 ablation, it should be used in patients who require only focal therapy. About 10–15% of patients
29 treated with IRE have new or other lesions outside the ablation zone on follow-up [49, 51].
30 Fusion of ⁶⁸Ga-PSMA-positron emission tomography and multiparametric magnetic resonance
31 imaging could be useful to detect potential multifocal tumor [52]. However, more than imaging,
32 advances in genetic and epigenetic markers seem very promising [53]. Table 4 provides a
33 summary of the main studies that reported the use of IRE for the treatment of prostate cancer.
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42 **3.6 Other applications**

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45 Disadvantages of thermal ablation techniques for bone are the high risk of permanent nerve
46 injury. Consequently, IRE might be an accurate alternate option for bone lesions close to neural
47 structures. An animal study showed regeneration of initially damaged axons with complete
48 recovery of function after IRE [54]. However, a more recent study demonstrated that even at
49 low voltage settings, nerve root injury can occur [55]. Clinical data regarding IRE for the
50 treatment of bone tumor are very scarce and it is therefore premature to recommend the use
51 IRE in clinical practice [56].

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58 Regarding malignant lung tumor, a prospective study reported poor efficacy of IRE [57].
59 The authors hypothesized that the IRE energy deposit is highly sensitive to air exposure [57].
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1 For lymph node or pelvic recurrence after rectal cancer, single case reports have suggested
2 potential utility for selected patients [58] [59] [60].
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5 **4. Electrochemotherapy**

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9 The proof of concept of ECT was conducted with bleomycin, where the cytotoxicity of this
10 drug was enhanced 700-fold after electroporation, compared to the drug alone [61]. In addition
11 to being effective, the method appeared to be safe. The side effects of the drug were highly
12 limited due to direct and local delivery by electric pulses, which were applied directly to the
13 cancerous tissue, placed between the electrodes. This method has been used for more than 10
14 years and in more than 150 centers and clinics throughout Europe, particularly for the treatment
15 of melanoma. Therefore, ECT is now an alternate approach to classical chemotherapy by
16 potentiating the effectiveness of cytotoxic drugs [62, 63].
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20 Subsequent to the efforts of several groups with the support of the European Union (FP5
21 Cliniporator, FP6 Esope), the treatment was standardized in the framework of the European
22 Standard Operating Procedure on Electrochemotherapy (ESOPE) multicenter trial, first
23 released in 2006 [64] and recently updated [65]. The objective of ESOPE was to validate the
24 clinical applications of electroporation of cells in cutaneous and subcutaneous tumors
25 (electrochemotherapy and electrogenetherapy) and to establish standard operating procedures
26 for their rapid dissemination in Europe. The protocol consists of associating cytotoxic drug
27 injection with the application of calibrated electric field pulses delivered locally at the tumor
28 site. The cytotoxic drugs (bleomycin or cisplatin) are injected either intravenously or directly
29 into the tumor. A series of eight pulses of 100 μ s (frequency up to 5 kHz) is applied at a 1300
30 V to electrode width (cm) with plate or needle electrodes [66].
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34 ECT has been shown to be an effective treatment in the palliative management of
35 unresectable recurrent disease in solid cutaneous and subcutaneous tumors with overall
36 response rates of 80-90%. ECT is also now recognized as a loco-regional therapy for
37 disseminated cutaneous and subcutaneous tumor lesions, improving the patient's quality of life.
38 The National Institute for Health and Care Excellence prepared an interventional procedure
39 overview about the safety and efficacy of ECT for primary basal cell carcinoma and primary
40 squamous cell carcinoma, which are the most common types of skin cancer, generally slow-
41 growing but capable of causing extensive tissue destruction or spreading to other parts of the
42 body. With the relevant technology (design of pulses generators and electrodes) it has become
43 possible to specifically target certain tissues within the body, regardless of the molecules [67].
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1 In 2021, Petrelli et al. published a contemporary systematic review and meta-analysis of the
2 impact of ECT in metastatic cutaneous melanoma [68]. Based on 27 studies, more than 1000
3 individuals and 5000 tumors, the authors showed that ECT is associated with a 77% overall
4 response rate, and that intravenous and intratumoral administration of bleomycin are equally
5 effective. The International Network for Sharing Practices of ECT registry was queried for
6 adults over 90 years old with skin cancers/cutaneous metastases of any histotype who had
7 undergone bleomycin-ECT. Other applications, particularly for liver tumor, were tested. In a
8 preliminary study on colorectal liver metastases, ECT was feasible and safe [69]. A subsequent
9 phase II study for colorectal liver metastases treated by ECT demonstrated a 75% complete
10 response rate [70]. ECT was also tested in patients with hepatocarcinoma when other curative
11 treatment options had failed [71] [72] [73]. A feasibility study on locally advanced pancreatic
12 cancer was recently published on five patients [74]. From a multicenter, prospective study on
13 102 patients, ECT should also be considered as an alternative and safe treatment for metastatic
14 bone disease as it has appeared to be a valid tool for their treatment with effectiveness on both
15 pain relief and local tumor control [75].
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29 **5 Ongoing developments**

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33 There is a lot of ongoing research on electroporation with 292 studies registered on the
34 website clinicaltrials.gov

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36 (<https://clinicaltrials.gov/ct2/results?cond=&term=electroporation&cntry=&state=&city=&dis>
37 [t=](#)) at the 29th of September 2022. On the basis of the numerical study of Arena et al.
38 demonstrating that short pulses (500 ns–2 μ s) of alternating polarity was more favorable for
39 predictable tissue ablation in heterogeneous tissues than long monopolar pulses [76], research
40 has been focused on what is now called high-frequency irreversible electroporation. Ability of
41 these waveforms to generate nonthermal ablations in animals without muscle contractions has
42 been demonstrated [77, 78, 79]. The first application of high-frequency irreversible
43 electroporation in prostate cancer was demonstrated in 40 men without ECG synchronization
44 [80].
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53 Calcium electroporation is a new application of ECT. The method uses of high voltage
54 electric pulses to introduce supraphysiological calcium concentrations into cells. This induces
55 acute adenosine triphosphate depletion and a fall in mitochondrial membrane potential, which
56 efficiently induces cell death [81]. European Standard Operating Procedure on
57 Electrochemotherapy-equivalent protocols has been issued for calcium electroporation [82].
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1 Calcium electroporation can help to overcome the drawbacks of standard ECT, particularly
2 those due to drugs (side effects, cost, storage) and is promising in clinics [83] [84].

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4 In an attempt to increase systemic antitumor effectiveness of ECT, gene electrotransfer
5 with immunomodulatory effect could be used as adjuvant treatment [85]. The first
6 electroporation-mediated gene transfer experiment was published 40 years ago by Neumann et
7 al. [86], and since then, the translation to clinical application has benefited from increased
8 knowledge of the mechanisms involved in the electrotransfer of nucleic acids [87, 88]. In
9 tumors (*i.e.*, for clinical developments), the first evidence of gene electrotransfer was reported
10 on mice by Rols et al. in 1998 [89]. In 2008, Daud et al. reported the first clinical trial utilizing
11 *in vivo* DNA electroporation of plasmid interleukin (IL)-12 electroporation in patients with
12 metastatic melanoma [90]. Two (10%) of 19 patients with nonelectroporated distant lesions and
13 no other systemic therapy showed complete regression of all metastases, whereas eight
14 additional patients (42%) showed disease stabilization or partial response [90].

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16 Several other reports have shown that intratumoral administration of different vectors carrying
17 the IL-12 gene generates a strong systemic therapeutic effect in a number of models including
18 metastatic digestive tumors, venereal tumors and in cutaneous tumors [91, 92].

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20 In IRE, evidence that the combination of partial IRE and gene electrotransfer IL-12 can also
21 induce antitumor immunity has been reported [93]. Studies evaluating IRE's effects on the
22 immune system are nonetheless contradictory. Some publications have reported evidence for
23 an immune response that is stronger after IRE in comparison with the one induced by surgery
24 in an osteosarcoma rat model [94], in immunocompetent mice, in comparison with
25 immunodeficient mice bearing renal carcinoma tumors [95]. In contrast, another study did not
26 give evidence for infiltration of immune cells in IRE-treated tissues [96]. A possible
27 explanation is that the extent of the effect of IRE on immune response could be conditioned by
28 pulse parameters [97]. Immune response following IRE or ECT could potentialize the immune
29 response induced by immunotherapy. There is evidence that immunogenic effects of IRE
30 outperform other ablative techniques [98] and can be enhanced by immunotherapy [99]. To
31 underline the interest of this concept, Geboers et al. in 2020 suggested the word
32 "electroimmunotherapy" [100] to describe the combination of IRE and immunomodulatory
33 drugs. Substantial benefits of combining IRE with anti-PD1 were recently shown *in vivo* on
34 pancreatic ductal adenocarcinoma [101]. Other clinical studies investigating a combination of
35 IRE and allogeneic natural killer cell therapy demonstrated promising results on pancreatic
36 cancer and hepatocellular carcinoma [99, 102, 103]. Further prospective clinical data are needed
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2 to provide robust evidence on the efficacy and safety of electroimmunotherapy as compared to
3 today's standards of care in these different cancers.
4

5 **6 Conclusion**

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9 IRE and ECT are emerging techniques associated with promising oncological results. IRE is
10 associated with minimal morbidity for localized or locally advanced liver, kidney, pancreas and
11 prostate cancers. ECT can be used for some tumors such as melanoma. However, it should still
12 be considered an ongoing studied treatment performed only as part of prospective clinical
13 studies and/or randomized trials comparing these methods versus standard of care with long-
14 term follow-up before use in routine clinical practice.
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37 **Human rights**

38 The authors declare that the work described has been performed in accordance with the
39 Declaration of Helsinki of the World Medical Association revised in 2013 for experiments
40 involving humans.
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47 **Informed consent and patient details**

48 The authors declare that this report does not contain any personal information that could lead
49 to the identification of the patients.
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58 **Disclosure of interest**

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25 26 27 **Figure legends**

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31 Figure 1. Drawing illustrates the different applications of electroporation. Depending on the
32 electric field intensity value E , cell membrane can be permeabilized either reversibly
33 ($E_{rev} < E < E_{irrev}$) or irreversibly ($E > E_{irrev}$). This phenomenon led to the development of
34 different applications such as the delivery of cytotoxic drugs (ECT) or of nucleic acids (EGT),
35 cell fusion, and, for irreversible membrane permeabilization to direct cell death (IRE).
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42 Figure 2. Drawing illustrates the basics of electroporation. A: Schematic representation of
43 square wave electric pulses. B: Schematic representation of the effect of the external electric
44 field applied on a cell. The external electric field induces a change in the resting transmembrane
45 potential, which is uniform along the membrane (blue arrows represent its gradient). The value
46 of the induced change depends on shape f of the cell and the conductivity of the media $g(l)$. r is
47 the radius of the cell, E is electric field strength, and $q(M)$ is the angle between the direction of
48 the field and the cell surface at the point M (red arrows have different directions, with lengths
49 mimicking their value). C: Representative fluorescence image of propidium iodide (PI) in a
50 cell, a dye fluorescing only inside the cell and thereby reflecting its electroporation-mediated
51 influx into the cell. The cell was electroporated by a train of 10 pulses, with a duration T of 100
52 μ s and electric field strength E of 1000 V/cm.
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2 Figure 3. A 64-year-old man with hepatocellular carcinoma (HCC) treated using irreversible
3 electroporation. The close situation of the tumor with the left portal branch led to use of
4 electroporation more than other ablative techniques.
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7 A, CT image in the axial plane before treatment shows HCC (arrows) adjacent to left portal
8 branch.
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11 B, CT image in the coronal plane shows needle positioning. Five needles are placed, including
12 one in the center and four to surround the target.
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15 C, Maximum intensity projection CT image in the axial plane shows needles around the HCC.
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18 D, CT image in the axial plane obtained immediately after electroporation shows destruction of
19 the tumor (arrows). Note the permeability of the portal division (arrowheads). The lesion is
20 considered as in complete response two years after this procedure. Neither portal thrombosis
21 nor biliary tract lesion was observed during follow-up.
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26 Figure 4 A 59-year-old man with liver metastasis from colon carcinoma treated using
27 irreversible electroporation.
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30 A, CT image in the coronal plane obtained during the portal venous phase of enhancement
31 shows hypovascular metastasis (black arrows). The metastasis is close to the right colon (white
32 arrows) and a previous attempt to displace the adjacent colon failed, likely due to local
33 postoperative adhesions.
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37 B, Maximum intensity projection CT image in the axial plane shows needle placement (only
38 three are visible).
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41 C, CT image in the coronal plane shows needle positioning, four needles surround the
42 metastasis and one is in the center of the metastasis.
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45 D, CT image in the axial plane obtained immediately after irreversible electroporation shows a
46 ring of inflammation (white arrows) around the metastasis. Of note, no colon injury is visible.
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49 E, CT image in the axial plane obtained six months after irreversible electroporation shows
50 shrinking of the metastasis (arrows) and normal colon.
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53 Figure 5. A 57-year-old woman with locally advanced pancreatic ductal adenocarcinoma
54 treated using irreversible electroporation.
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57 A, CT image in the axial plane shows tumor (arrows) of the pancreatic head. The biliary drain
58 was removed before irreversible electroporation..
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1 B, Maximum intensity projection CT image in the axial plane shows needle positioning. Four
2 needles are placed around the pancreatic tumor. White arrow indicates magnetic positioning
3 guidance system (Imactis).
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5 C, CT image in the coronal plane shows needle positioning, four needles surround the tumor.
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7 D, CT image in the axial plane obtained immediately after irreversible electroporation shows
8 haziness of abdominal fat (arrowheads) and gas around the treatment zone (arrows). The gas is
9 due to hydrolytic process of the water induced by the high current used.
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Hightlights

1. Irreversible electroporation leads to cell apoptosis and can be used to treat liver, kidney, pancreatic or prostate tumors with effective tumor necrosis.
2. Reversible electroporation allows delivering cytotoxic drugs such as bleomycin and cisplatin, and inducing cell death by apoptosis, a process called electrochemotherapy.
3. Electrochemotherapy can be used to treat solid cutaneous and subcutaneous tumors, such as melanoma, basal cell carcinoma and squamous cell carcinoma.
4. Electric fields used in irreversible electroporation and electrochemotherapy respect collagenic structure of the surrounding tissue offering promising options for tumor ablation particularly those in complex anatomic localizations.

Table 1. Main studies reporting liver tumors treated using irreversible electroporation

Study [Ref #]	Study design	Number of patients	HCC/ Metastasis/ Others*	Mean tumor size (cm)	Progression-free survival (follow-up duration in months)	Overall survival (follow-up duration in months)	Major complication rate	Minor complication rate
Kingham et al. 2012 [104]	R	28	2/25/1	1	94.3% (6)	N.R.	0%	7%
Cannon et al. 2013 [105]	P	44	14/30/0	1.1	59.5% (12)	N.R.	0%	20%
Hosein et al. 2014 [106]	R	29	0/29/0	2.7	18% (24)	62% (24)	0%	7%
Niessen et al. 2015 [107]	P	25	10/9/6	1.7	70.8% (6)	N.R.	N.R.	N.R.
Bhutiani et al. 2016 [108]	P	30	30/0/0	3	97% (6)	11%	0%	56%
Niessen et al. 2016 [109]	P	34	34/0/0	2.4	74.8% (6)	97% (12)	18%	23%
Granata et al. 2016 [110]	P	20	20/0/0	2	91.7% (6)	100% (6)	0%	10%
Distelmaier et al. 2017 [111]	P	29	5/21/3	N.R.	N.R.	N.R.	0%	28%
Langan et al. 2017 [112]	R	40	7/32/1	1.3	86.3% (24)	N.R.	0%	35%
Niessen et al. 2017 [113]	P	71	31/36/0	1.8	68.3% (36)	50.7% (36)	7%	10%
Frühling et al. 2017 [114]	P	30	8/22/0	2.4	65.8% (6)	56.6% (12)	1%	20%
Sutter et al. 2017 [115]	R	58	58/0/0	2.4	70% (12)	96.5% (12)	5%	14%
Mafeld et al. 2018 [116]	R	52	20/29/3	2.7	44% (12)	90% (12) 65% (24) 52% (36)	6%	11%
Martin et al. 2018 [117]	R	26	26/0/0	2.7	75% (12)	65% (12)	4%	12%
Schicho et al. 2018 [118]	R	24	0/24/0	2.2	N.R.	79.1% (12) 52.2% (24) 25% (36)	N.R.	N.R.
Kalra et al. 2019 [119]	R	21	0/21/0	2.6	71% (6)	61.9% (12)	0%	43%
Yang et al. 2019 [120]	P	22	13/0/9	4.73	50% (12)	66.7% (12)	0%	100%
Stillsröm et al. 2019 [121]	R	42	20/20/2	2	63% (12)	N.R.	5%	20%
Cornelis et al. 2020 [122]	R	25	0/25/0	2.1	48.3% (12) 40.5% (24)	82.8% (12) 61.3% (24) 26.8% (36)	16%	8%
Fang et al. 2021 [38]	R	69	33/24/12	N.R.	N.R.	N.R.	4%	N.R.
Meijerink et al. 2021 [123]	P	51	0/76/0	2.2	76% (12)	78% (31)	22% (including one death)	25%

R indicates retrospective; P indicates prospective; HCC indicates hepatocellular carcinoma; Others indicates cholangiocarcinoma and epitheloid hemangioendothelioma; NR indicates not reported.

Only studies including at least 20 patients are reported.

Table 2. Main studies reporting liver tumors treated using electrochemotherapy

Study [Ref#]	Study design	Number of patients	HCC/ Metastasis	Mean tumor size (cm)	Drug	Complete response (follow-up duration in months)	Partial response (follow-up duration in months)
Edhemovic et al. 2014 [70]	P	16	0/29	2.3	Bleomycin IV	85% (6)	15% (N.R.)
Coletti et al. 2017 [124]	P	5	0/9	2.0	Bleomycin IV	33% (6)	0% (6)
Tarantino et al. 2017 [125]	P	6	6/0	3.4 (all with portal thrombosis)	Bleomycin IV	100% (6 or 9)	0% (6)
Djokic et al. [71]	P	10	17/0	2.4	Bleomycin IV	80% (6)	20% (6)
Djokic et al. 2020 [72]	P	24	32/0	2.5	Bleomycin IV	79.2% (6)	16.6% (6)

IV indicates intravenous administration; R indicates retrospective; P indicates prospective; HCC indicates hepatocellular carcinoma; NR indicates not reported.
Only studies including at least five patients are reported.

Table 3. Main studies reporting irreversible electroporation (IRE) and electrochemotherapy (ECT) for the treatment of pancreatic adenocarcinoma

Study [Ref#]	Study design	Number of patients	Mean tumor size (cm)	Overall survival (months)	Major complication rate	Minor complication rate	Mortality rate
Irreversible electroporation							
Martin et al. 2013 [32]	P	54	3.2	20	24%	55.5%	2%
Martin et al. 2015 [33]	R	200	2.8	24.	18.5%	50.5%	2%
Lambert et al. 2016 [126]	P	21	3.8	10.2	23.8%	N.R.	0%
Yan et al. 2016 [127]	R	25	4.2	NR	36%	16%	0%
Narayanan et et al. 2017 [128]	R	50	3.2	27	20%	N.R.	0%
Scheffer et al. 2017. [129]	P	25	3.6	11	40%	40%	0%
Liu et al. 2019 [130]	P	54	4.9	16.2 20.3 if IRE+ chemotherapy	7.4%	81%	0%
Ruarus et al. 2020 [29]	P	50	3.7	11.6 14.9 if IRE + FOLFIRINOX	42%	28%	2%
Van Veldhuisen et al. 2020 [35]	P	30	5.3	17.0	20%	23%	0%
Electrochemotherapy							
Granata et al. 2015 [36]	P	13	5.1	N.R.	23%	0%	0%
Granata et al. 2017 [131]	P	19	5.1	N.R.	N.R.	N.R.	N.R.

IRE indicates irreversible electroporation; R indicates retrospective; P indicates prospective; N.R. indicates not reported. For irreversible electroporation, only studies including at least 20 patients are reported. For electrochemotherapy, only studies including at least 15 patients are reported

Table 4. Main studies reporting irreversible electroporation for the treatment of renal cancer

Study [Ref#]	Study design	Number of patients	Mean tumor size (cm)	Overall survival at 2 years	Major complication rate (>grade 3)	Technical success
Trimmer et al. 2015 [132]	P	20	2.2	N.R.	0%	90%
Canvasser et al. 2017 [44]	P	41	2.0	100%	0%	93%
Dai et al. 2021 [133]	R	47	2.2	90.6%	0%	N.R.

R indicates retrospective; P indicates prospective; N.R. indicates not reported. Only studies including at least 20 patients are reported.

Table 5. Main studies reporting irreversible electroporation for the treatment of prostate cancer

Study [Ref#]	Study design	Number of patients	In-field recurrence (%)	Functional outcomes	Complications (n; %)
Valerio et al. 2014 [134]	R	34	16%	Continence, 100%, Preserved potency, 95%	Grade I: 12 (35%) Grade II:10 (29%) Grade III: 0 (0%)
Ting et al. 2016 [135]	P	32	0%	Continence, 100% Preserved potency, 100%	Grade I: 5 (20%) Grade II: 0 (0%) Grade III: 1(4%)
Murray et al. 2016 [136]	R	25	16%	Continence, 91% Preserved potency, 92%	Grade I: 6 (22%) Grade II:7 (29%) Grade III: 1(7%)
Van den Bos et al. 2016 [137]	P	63	16%	Continence, 100% Preserved potency, 77%	Grade I: 15 (24%) Grade II:7 (11%) Grade III:0 (0%)
Colletini et al. 2019 [138]	P	30	18%	Continence, 97%, Preserved potency, 79%	Grade I: 6 (20%) Grade II: 3 (10%) Grade III: 1 (3%)
Blazeski et al. 2020 [49]	P	123	3% (excluding initial 32 patients)	Continence, 99%, Preserved potency, 93%	Grade I: 272 (22%) Grade II: 11 (9%) Grade III: 0 (0%)
Wang et al. 2022 [50]	P	191	6% (out of 100 patients)	Median international prostate symptom score, 4.5 (IQR: 2.0, 9.5) Median international index of erectile function score, 2.0 (IQR: 0.5, 12.5)	Grade I; 33 (30%) Grade II; 7 (6%) Grade III; 1 (1%)

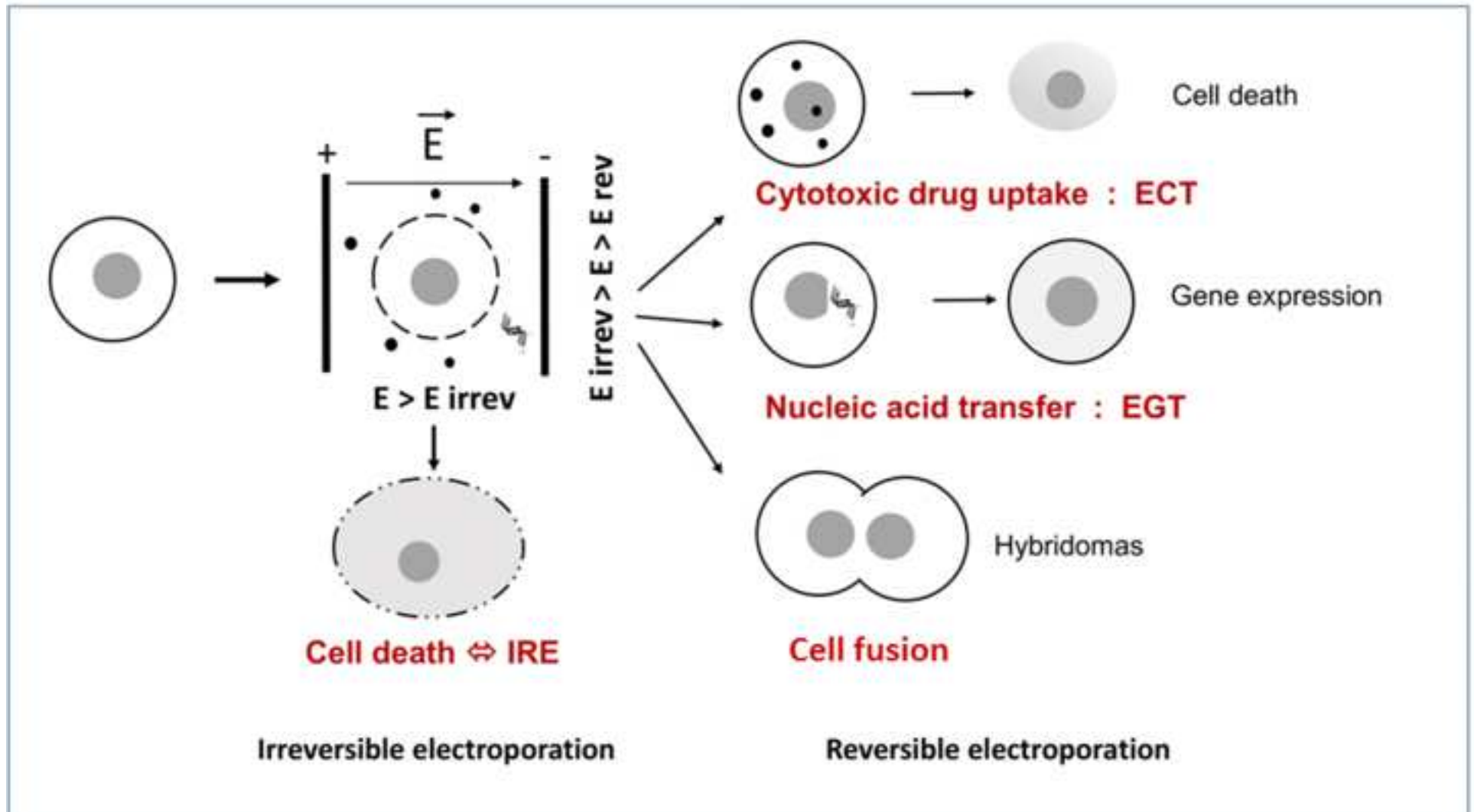
R indicates retrospective; P indicates prospective; N.R. indicates not reported. Only studies including at least 20 patients are reported.

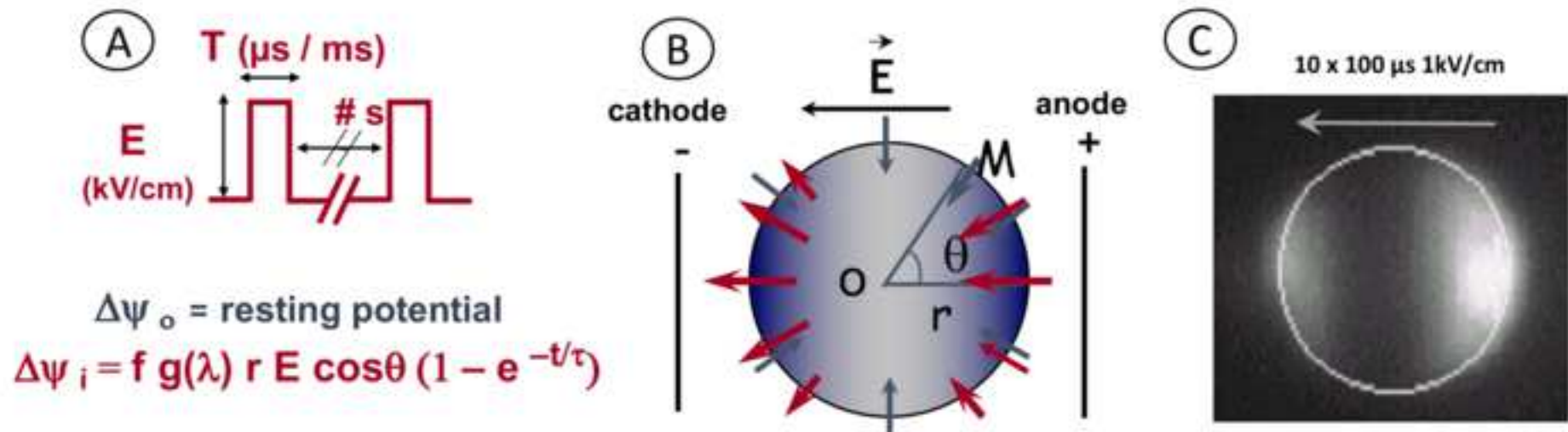
Table 6. Main studies reporting electrochemotherapy for the treatment of skin tumors.

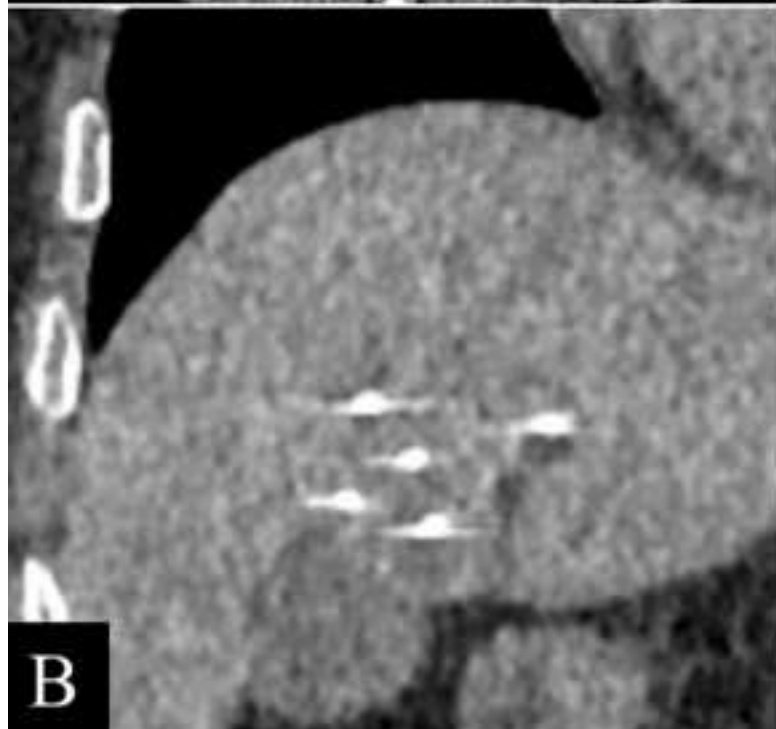
Study [Ref#]	Drug injection modality	Number of patients	Number of lesions	Complete response (%)	Partial response (%)	Rate of recurrence (%) and follow-up (months)
Jamsek et al. 2020 [139]	IV	28	42	98	2	21% (20-55)
Rotuno et al. 2018 [140]	IV	10	26	83	17	N.R.
Campana et al. 2017 [141]	IV/IT	84	185	63	36	20% (60)
Clover et al. 2020 [142]	IV/IT	298	567	85	11	N.R.
Heller et al. 1998 [143]	IT	20	54	94	6	0% (28)
Peycheva et al. 2001	IT	99	194	96		93% (36)
Clover et al. 2020 [144]	IT	45	65	92	8	7.5% (60)
Montuori et al. 2018 [145]	IT	11	15	100	0	0% for 9 patients (3–23)
Peycheva et al. 2004 [146]	IT	85	85	100	0	20% for short pulse, 0% for long pulse (12)

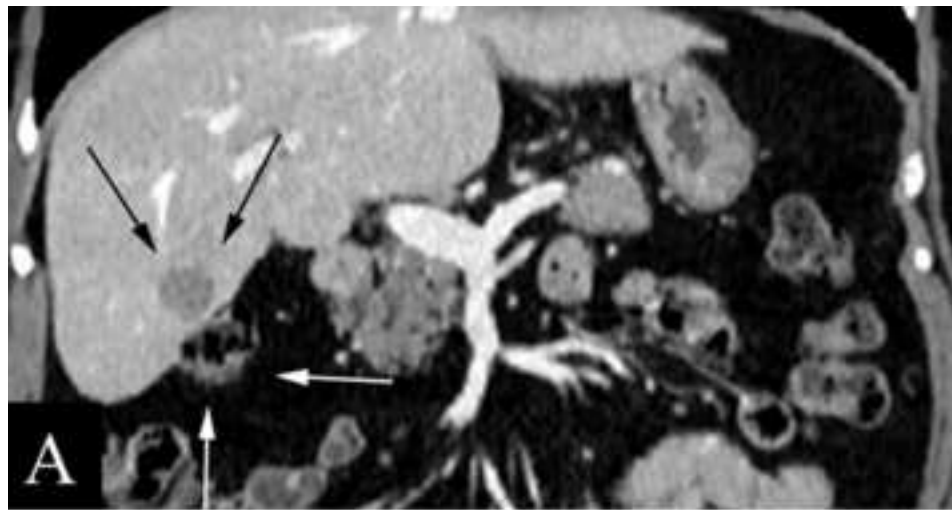
Only studies including at least 10 patients are reported.

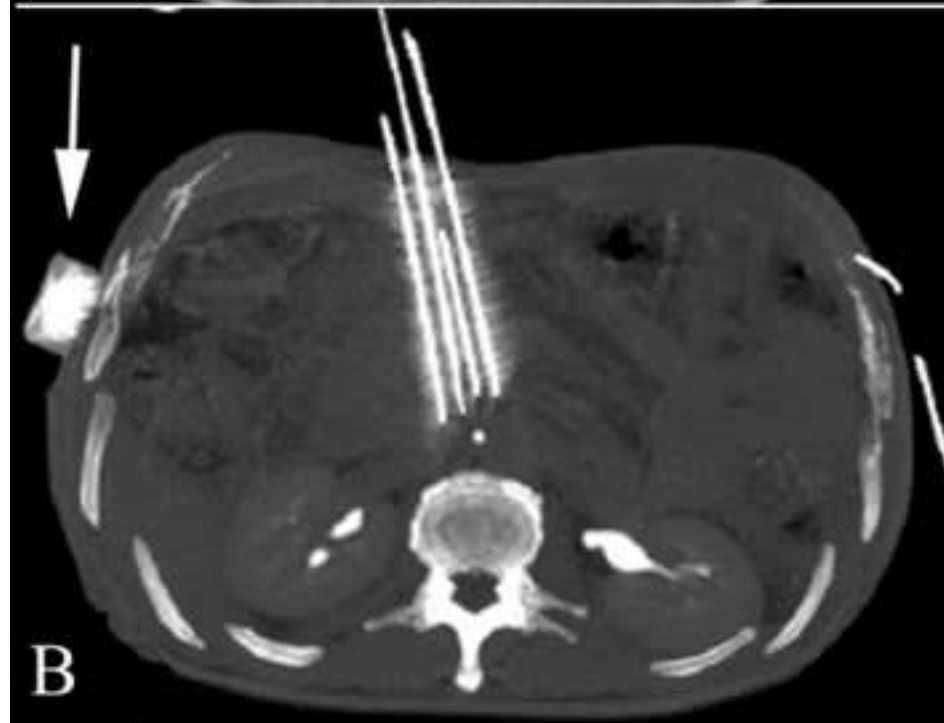
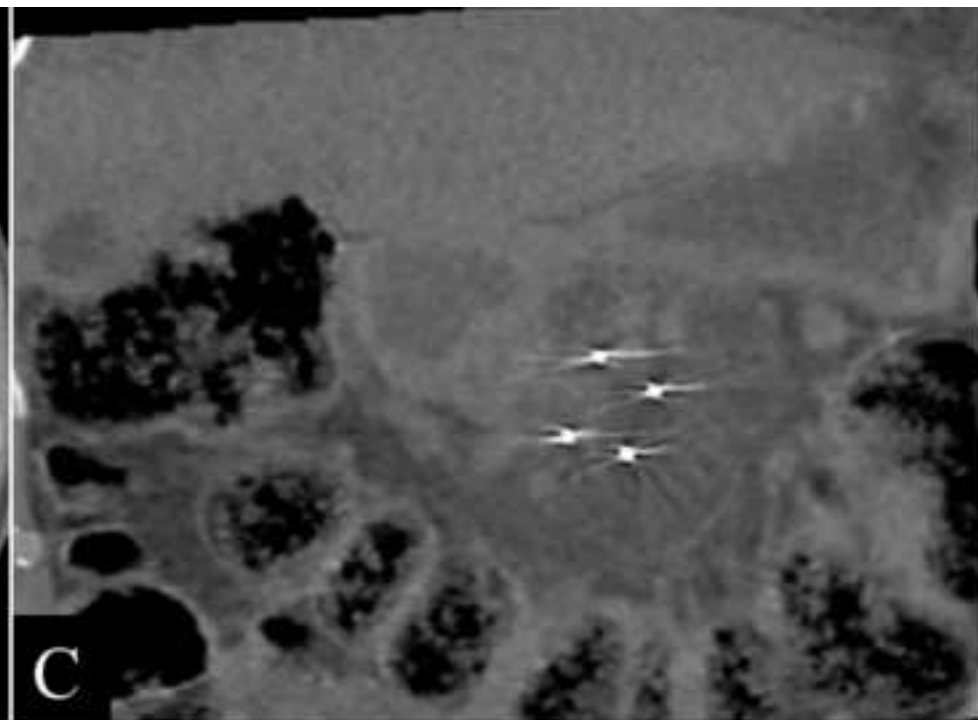
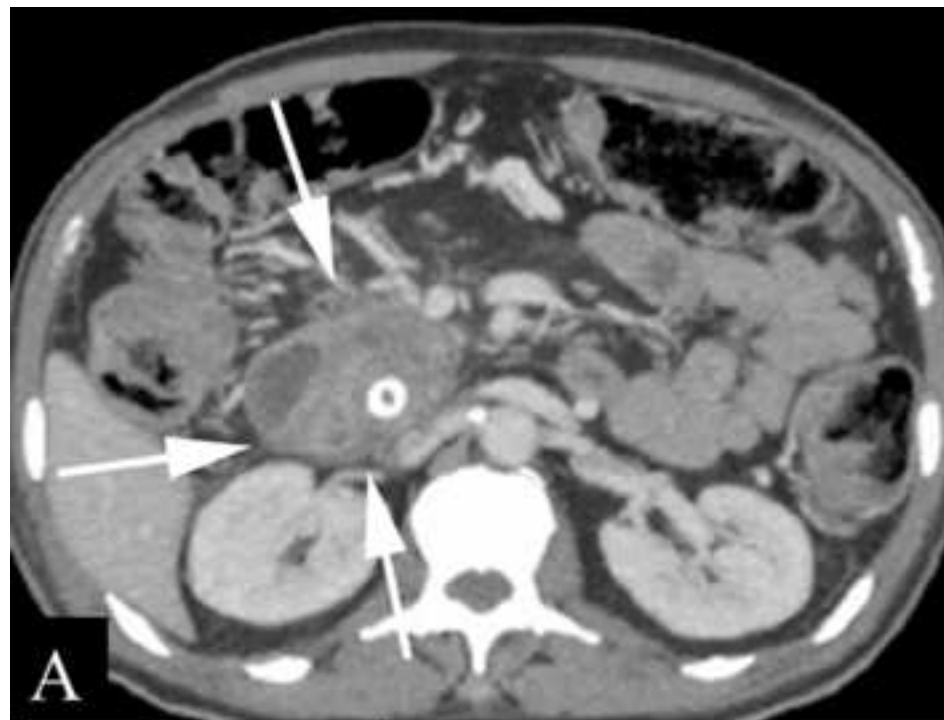
IV indicates intravenous; IT indicates intra tumoral; N.R. indicates not reported.











Credit authors statement

Conceptualization JP Tasu

Methodology JP Tasu, MP Rolls

Validation JP Tasu, D Tougeron, MP Rolls

Formal Analysis Not applicable

Investigation Not applicable

Data Curation Not applicable

Writing - Original Draft JP Tasu, MP Rolls

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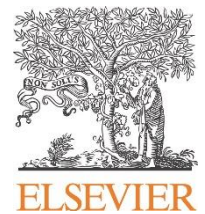
Visualization JP Tasu

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Author responsibilities, integrity, ethics

Article title : Applications of electricity in oncology; A state of the art of irreversible



Human and animal rights

- The authors declare that the work described has been carried out in accordance with the [Declaration of Helsinki](#) of the World Medical Association revised in 2013 for experiments involving humans as well as in accordance with the EU Directive [2010/63/EU](#) for animal experiments.
- Not applicable.

Informed consent and patient details

- The authors declare that this report does not contain any [personal information](#) that could lead to the identification of the patient(s).
- Not applicable
- The authors declare that they obtained a written [informed consent](#) from the patients and/or volunteers included in the article. The authors also confirm that the personal details of the patients and/or volunteers have been removed.
- Not applicable

Disclosure of interest

- The authors declare that they have no known [competing financial](#) or [personal relationships](#) that could be viewed as influencing the work reported in this paper.
- The authors declare the [following financial](#) or [personal relationships](#) that could be viewed as influencing the work reported in this paper:

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- All authors attest that they meet the current International Committee of Medical Journal Editors ([ICMJE](#)) criteria for Authorship.

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The authors would like to thank Mr Jeffrey Arsham for his useful English review of this document.

Reviewers' Comments:

The authors have significantly improved their manuscript. However, the paper is much too long.

In addition, there is a problem with the numbering of the sections 2. History of high voltage pulses in medicine and 2- The basics of Electroporation
Corrections done all along the text

The authors should consider making an introduction that discusses the use of electroporation in the more general spectrum of percutaneous ablative treatments, addressing the current use of RFA, microwave ablation and cryotherapy and why electroporation shows promises. They should references RFA, microwave ablation and cryotherapy with, but not exhaustively, the following references

Introduction was rewritten and the first paragraph completely removed and changed for;
Thermal tumor ablation techniques include radio-frequency (RF), microwave (MW), laser, high-intensity focused ultrasound (HIFU) and cryoablation which can be used in the treatment of tumors of the liver, kidney, bone, and lung [1] [2]. Complete tissue necrosis is achieved by heating or cooling the targeted tissue to a cytotoxic temperature, less than -40°C or more than 60°C . However, thermal ablation of tumors adjacent to large vessels is affected by the cooling effect of the liquid flow in the lumen, called heat sink effect, which can lead to incomplete ablation. In addition, thermal injuries of non-target tissue such as nerves, vessels, bile or pancreatic ducts are possible.
Electroporation is a non-thermal technique using high-frequency electrical pulses to permeate cell membranes. Irreversible electroporation (IRE) describes the loss of semipermeable barrier properties of wall cells explaining apoptosis by lethal disruption of the osmotic balance between the cell inside and the environment outside [3, 4]. Electroporation is reversible if disturbances of cell membrane properties are temporary. Both techniques avoid the heat sink effect for tumors near the vessels and do not cause damage to the extracellular matrix because of lack of cell membrane structure [5].

References were added as requested

Young S, Rivard M, Kimyon R, Sanghvi T. Accuracy of liver ablation zone prediction in a single 2450MHz 100 Watt generator model microwave ablation system: An in human study. *Diagn Interv Imaging*. 2020;101:225-233.

Tomita K, Iguchi T, Matsui Y, Uka M, Umakoshi N, Hiraki T. Pneumodissection during percutaneous renal cryoablation resulting in massive subcutaneous and mediastinal emphysema. *Diagn Interv Imaging* 2022;103:231-232.

They authors should consider deleting ALL historical references, which will help reduce the number of references and be more straight to the point with a section that briefly explains the concepts of reversible and irreversible electroporation
Done

Section 2.1 and 2.2 should be deleted or restricted to two sentences that can be placed in the Introduction
Done

In the section entitled "Liver cancers", the authors should add the following reference

Garnon J, Auloge P, Dalili D, Cazzato RL, Gangi A. Percutaneous irreversible electroporation of porta hepatis lymph node metastasis. *Diagn Interv Imaging* 2021;102:53-54.
This reference was already present in the previous version in the paragraph "other applications". Considering that the topic of this report is not liver, we have maintained the reference in the chapter on node treatment.

Suggest adding the following references in the corresponding sections of the paper

Vroomen LGPH, Petre EN, Cornelis FH, Solomon SB, Srimathveeravalli G. Irreversible electroporation and thermal ablation of tumors in the liver, lung, kidney and bone: What are the differences? *Diagn Interv Imaging* 2017 ;98(9):609-617.
Added

Cornelis FH, Korenbaum C, Ben Ammar M, Tavoraro S, Nouri-Neuville M, Lotz JP. Multimodal image-guided electrochemotherapy of unresectable liver metastasis from renal cell cancer. *Diagn Interv Imaging* 2019;100:309-311.

The 2 references were added in the text (introduction for the first, liver for the second)

Tables are fine

Figures should be checked because there is figure 4f but no figure 4e
Numeration was corrected.

The authors would like to greatly thank the reviewer for his useful comments.