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Electrochemotherapy – Emerging applications technical advances, new indications, combined approaches, and multi-institutional collaboration

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ABSTRACT

The treatment of tumors with electrochemotherapy (ECT) has surged over the past decade. Thanks to the transient cell membrane permeabilization induced by the short electric pulses used by ECT, cancer cells are exposed to otherwise poorly permeant chemotherapy agents, with consequent increased cytotoxicity. The codification of the procedure in 2006 led to a broad diffusion of the procedure, mainly in Europe, and since then, the progressive clinical experience, together with the emerging technologies, have extended the range of its application. Herein, we review the key advances in the ECT field since the European Standard Operating Procedures on ECT (ESOPE) 2006 guidelines and discuss the emerging clinical data on the new ECT indications. First, technical developments have improved ECT equipment, with custom electrode probes and dedicated tools supporting individual treatment planning in anatomically challenging tumors. Second, the feasibility and short-term efficacy of ECT has been established in deep-seated tumors, including bone metastases, liver malignancies, and pancreatic and prostate cancers (*long-needle variable electrode geometry* ECT), and gastrointestinal tumors (*endoscopic* ECT). Moreover, pioneering studies indicate lung and brain tumors as suitable future targets. A further advance relates to new combination strategies with immunotherapy, gene electro transfer (GET), calcium EP, and radiotherapy. Finally and fourth, cross-institutional collaborative groups have been established to refine procedural guidelines, promote clinical research, and explore new indications.

Introduction

The past decade witnessed an expansion of treatment indications for electrochemotherapy (ECT). This approach was initially aimed to treat superficial, small-size tumors not amenable to surgery or radiation, with substantially greater use in the metastatic setting [1–3]. Reversible tumor electroporation (EP) by short, high-voltage electric pulses results in increased cell permeabilization to bleomycin (BLM) or cisplatin (CDDP), with a locally enhanced cytotoxic effect [1]. ECT started being routinely used in 2006, when the European Standard Operating Procedures of ECT (ESOPE) were released [4] (Fig. 1), and the clinical protocol has

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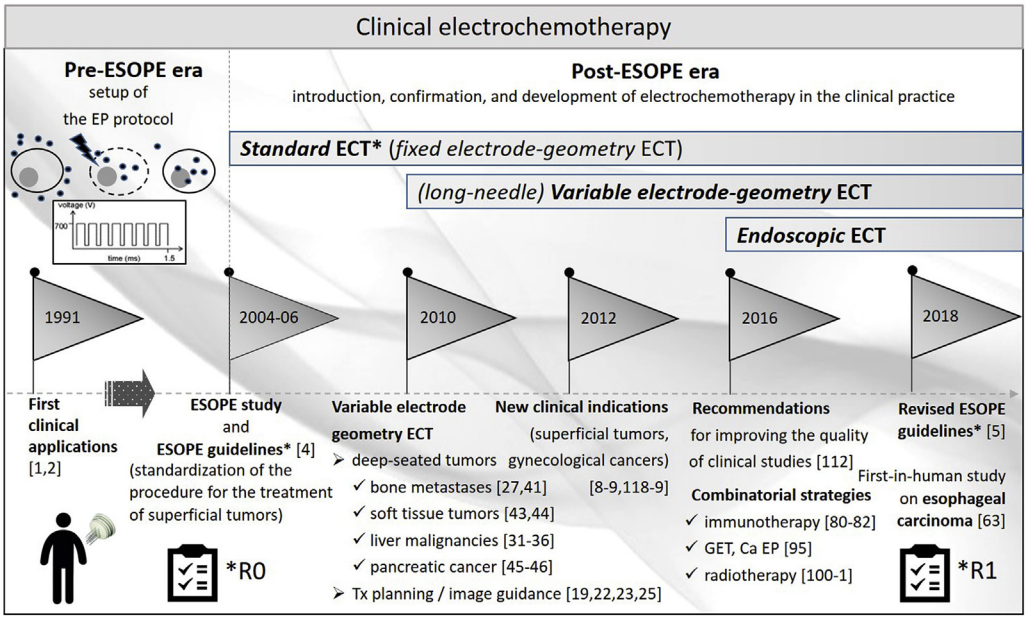

Fig. 1. Development timeline of clinical electrochemotherapy. *Legend*: EP, electroporation; ESOPE, European Standard Operating Procedures of ECT; GET, gene electro transfer; Tx, treatment.

recently been updated [5]. As such, *standard* ECT (i.e., fixed electrode-geometry ECT applied to superficial tumors) became an easy-to-master procedure, and accumulating evidence suggests high local efficacy, good patient tolerability, flexibility of the technique, and repeatability. Although the lack of randomized studies makes quantifying the relative advantages of the procedure over other locoregional palliative treatments difficult, the complete response (CR) rate is 30–65%, and local disease control at 1 year is 30–90% according to different series and treated tumors. Moreover, large multicenter studies consistently reported positive patient reported outcomes [6,7]. Because of the favorable tolerability profile and development of new instrumentation (Fig. 2a), *standard* ECT is being investigated in new settings, such as skin metastases from visceral, hematological, and gynecologic malignancies, non-cancerous skin lesions, and also in combination with systemic immunotherapy [8,9] (Table 1). Interestingly, the development of long, freely placeable needle electrodes (Fig. 2b) has enabled the treatment of large and deep-seated tumors (*long needle variable electrode-geometry* ECT, herein referred to as *variable electrode-geometry* ECT) [10]. Similarly, e*ndoscopic* ECT is gleaming into clinical use for palliation of gastrointestinal cancers by mean of dedicated instrumentation (Fig. 2c). Herein, we provide a description of the emerging ECT applications, including the newest technical developments and provisional data from investigational clinical studies, and explore the potential for combination strategies with immunotherapy, other EP-based approaches, or radiotherapy. Finally, we survey the multi-institutional collaborative efforts in further developing ECT.

New technical developments

Electrodes

The efficacy of ECT across tumor histotypes [11–13] has prompted an expansion of its application. New pulse applicators have been designed to access deep-seated and surgically challenging tumors in various ways (Fig. 2) [14–17]. Recently, endovascular balloon catheters containing a non-contact "virtual" electrode (i.e., an electrode having no direct contact with tissue) proved to be safe and effective in an animal model of atrial fibrillation and translation to human medicine can be expected in the near future [18].

Supporting tools

Individual treatment planning is key to *variable electrode-geometry* ECT success. In fact, EP parameters should be set to maximize treatment effect on the target volume, while preserving surrounding healthy tissue trough accurate electrode placement and tailored electric field around tumor. Dedicated supporting tools have been proposed to optimize electrode positioning, simulate treatment outcome on tissues and, ultimately, enable accurate delivery of electric pulses [19–24]. The Pulsar software is a dedicated tool that can be coupled with the most recent version of the electric pulse generator (Cliniporator® VITAE) and calculates optimal electrodes configuration, while reducing their total number. It also estimates the electric field in the target volume and indicates the voltage required for each couple of probes. The Visifield software is a user-friendly web-based tool for automatic 3D visualization of electric field distribution in tissues, based on radiological images (https://www.visifield.com/). The software provides a customized feedback, which includes target tumor delineation, required voltages, and predicted electric currents [25,26]. Throughout the procedure, safe and precise electrode insertion can be supported by an on-site radiologist and, in challenging cases, by navigation (robotic-assisted) tools and electrode tracking systems [19,27,28].

Investigational indications

Variable-geometry ECT

Liver tumors

The current management of liver malignancies involves

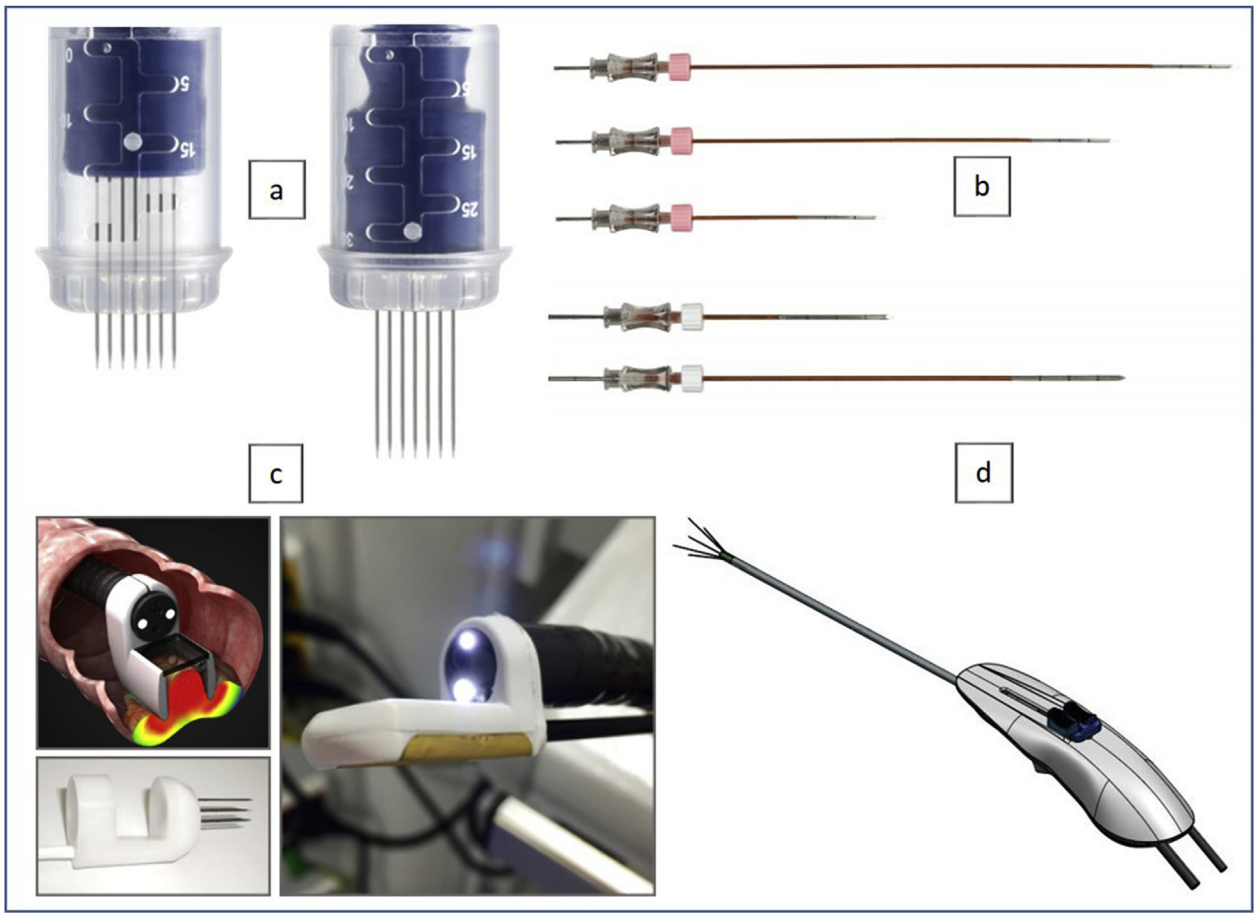

Fig. 2. New electrode probes. (a) Adjustable-length electrode. (b) Long needle electrodes for *variable electrode-geometry* ECT. *Variable electrode geometry* ECT implies the insertion of a variable number (from 2 to 6) of 16- to 30-cm long needle electrodes with an active tip of 3 or 4 cm. (c) Endoscopic electrodes (the "finger" electrode [bottom left image], and the EndoVE [Endoscopic Vacuum Electrode] device). The EndoVE electrode is mounted at the head of an endoscope and uses a vacuum source to draw the tissue in close contact with the electrode, facilitating the capture of tumor tissue and its optimal coverage with electric fields. (d) Prototype of a laparoscopic expandable needle electrode. Expandable electrodes that were first described for treatment of brain metastases rendering them accessible through a single burr hole [92] are now being further developed as minimally invasive laparoscopic expandable devices (courtesy of IGEA S.p.A.).

systemic treatment, surgery, and liver-directed therapies (i.e., embolization, cryo-ablation, radiofrequency ablation, and microwave ablation). The utility of each option depends on tumor burden and anatomical location. The introduction of long freely-placeable needle electrodes (Fig. 2b), and a new pulse generator, which allow customizing the applied voltages according to the distance between electrodes (*variable electrode-geometry* ECT) has allowed to investigate ECT in liver malignancies. Target tumor delineation and treatment planning (based on cross-sectional imaging) are required to determine the number of electrodes and their optimal configuration [15,19,22,26,29]. These features invariably add a level of complexity to the procedure, thus making it more demanding for patients (need for deeper anesthesia) and clinicians (longer treatment duration) compared with *standard* ECT (Suppl. Fig. 1). The first case was performed in 2011, when Edhemovic et al. pioneered *variable electrode-geometry* ECT in a patient with a 34×15 mm metastasis from colorectal cancer located between the inferior vena cava and the main hepatic veins [30]. The procedure was performed intraoperatively, by using 20-cm long, 17-gauge (G)/1.2-mm needle electrodes with a 4-cm non-isolated active tip, under ultrasound (US) guidance. Pulse delivery was synchronized with the electrocardiogram to avoid the induction of cardiac arrhythmias. After 3 months, the pathological examination of the surgical specimen confirmed CR. *Standard* and *variable electrode-geometry* ECT were investigated during laparotomy procedures in 16 patients with 29 metastases from colorectal cancer [31]. There were no intra- or post-operative complications, and radiologically assessed CR rate was 85% (subsequently confirmed in seven patients who underwent resection). In 2017, a pilot study enrolled five patients with colorectal liver metastases who were treated by a 4-cm long, fixed-geometry linear or hexagonal array needle electrode during open surgery [32]. A total of nine metastases (range 6–32 mm) were electroporated, with no treatment-related adverse event. After 1 month, PR at magnetic resonance imaging (MRI) was observed in five tumors (in a single patient) and stable disease in the remaining four metastases (in four patients). ECT has been recently investigated in six Child-A or –B patients with hepatocellular carcinoma for treating portal vein tumor thrombosis at the hepatic hilum. Long needle electrodes were percutaneously placed under US guidance [33]. After 14 months, two patients had complete patency of the portal vein, and three patients had a persistent avascular, non-tumoral thrombus (one patient was lost to follow-up). Djokic et al. conducted the first pilot study with ECT on hepatocellular carcinoma [34] and enrolled 10 patients with 17 metastases

Table 1
Clinical trials on electrochemotherapy registered at ClinicalTrails.gov.

Tumor	Study title	Type	Identifier	Start date	Location	ECT modality	Estimated enrollment
Malignant Melanoma	A phase II, multicentric, open label, non-randomized, interventional study of pembrolizumab in combination with ECT in patients with unresectable melanoma with superficial or superficial and visceral metastases	Phase 2[a]	NCT03448666	Feb 2018	Italy	Standard	53
Pancreatic cancer	A clinical trial using ECT with BLM for the treatment of non-metastatic unresectable pancreatic cancer	Phase 1	NCT03225781	Jul 2017	Italy	Variable Geometry	20
Vulvar carcinoma	Prospective evaluation of clinical efficacy and symptom control using ECT for the inoperable advanced vulva carcinoma	Phase 2	NCT03142061	Jun 2017	Germany	Standard	50
Rectal cancer	Endoscopic assisted ECT in addition to neoadjuvant treatment of locally advanced rectal cancer	Phase 2	NCT03040180	Jan 2017	Denmark	Endoscopic	40
	Treatment of inoperable colorectal cancer with ECT through an endoscopic system	Phase 1	NCT01172860	Mar 2010	Ireland	Endoscopic	10[b]
Capillary malformations	Electrosclerotherapy as a novel treatment option for capillary malformations: a pilot study	Phase 2R[c]	NCT02883023	Nov 2016	Netherlands	Standard	20
Head and neck cancer	ECT on head and neck cancer	Phase 2	NCT02549742	Feb 2014	Denmark	Standard	25
Brain metastases	Electrochemotherapy as a Palliative Treatment for Brain Metastases	Phase 1	NCT01322100	Apr 2011	Denmark	Variable Geometry	16[d]

Legend: BLM, bleomycin; ECT, electrochemotherapy.
[a] Candidate patients must have stage IIIB-IIIC or IV disease with superficial metastases suitable for application of ECT.
[b] Recruitment completed.
[c] Randomized within-patient pilot trial (three regions of interest of the target lesion are randomly allocated to "electrosclerotherapy", BLM alone, or no treatment). Post-treatment evaluation by means of dedicated, disease-specific instruments has been planned.
[d] Recruitment terminated due to slow patient accrual.

(median tumor size 24 mm). Radiological evaluation after 3–6 months showed CR in eight of 10 patients and 15 of 17 tumors, with no relevant toxicity. Finally, Tarantino et al. explored ECT in perihilar cholangiocarcinoma (Klatskin tumor). The treatment was safely applied either percutaneously or intraoperatively. Three of five patients achieved CR, and two of them remained locally disease-free after 30 and 18 months, respectively [35]. Overall, these experiences support the feasibility of ECT in patients with liver malignancies. With regard to treatment safety near major blood vessel, an *ex-vivo* histopathological study on surgical specimens demonstrated tumor necrosis in the ECT area with preservation of liver vessels larger than 5 mm [36]. Also, pre-/post-operative Holter electrocardiographic monitoring in 10 patients who were treated for liver metastases did not find clinically relevant cardiac arrhythmias [37]. On these bases, ECT can be envisioned a promising tool in interventional oncology for treating hepatic tumors that are unresectable or in proximity to vessels, where the efficacy of thermal ablation is impaired by the so-called "heat sink" effect [10,38].

Bone metastases

Metastatic bone disease is a major health care issue, affecting 4.9 million individuals in the United States [39]. Prostate, breast, lung, kidney, and thyroid cancers account for approximately 80% of cases. In addition to standard anticancer treatment, available options include local (i.e., surgery, radiotherapy, percutaneous thermal ablation with cementoplasty, embolization, and focused ultrasound) and systemic (i.e., radiopharmaceuticals, bone-targeted agents) therapies as well as analgesics. Surgery provides structural stability, but may be technically challenging and associated with prolonged recovery. Radiotherapy ameliorates bone pain in 80–90% of patients, but tolerance of surrounding tissues, resistance of some histotypes, and weakening of the healthy bone may represent an issue. Notably, *in vivo* preclinical tests support the safety and selectivity of EP [40]. In these experiments, the permeabilization of bone tumors was achieved with no impairment of bone stability and osteogenic activity. Conversely, the heterogeneous structure of bone tissue did not affect pulse delivery and tumor EP. Notably, the application of electric pulses to the meninges covering the spinal cord in an animal model did not cause relevant structural changes at electron microscopy and these findings support the safety of ECT at tumor margins.

Treatment of bone tumors in humans is performed by 15-G/1.8-mm needle electrodes [15] (Fig. 2b). The first experiences have been conducted at the Rizzoli Institute of Bologna (Suppl. Fig. 1) [27,41]. A patient with metastatic spinal melanoma was treated using four needle probes inserted at L5 level, through a mini-open surgery and left laminectomy. Their placement was monitored through fluoroscopy and neuronavigation control under general anesthesia. Positron-emission tomography-computed tomography (PET-CT) assessment indicated a near CR, which lasted 6 months and was associated with improvement of pain. Between July 2009 and June 2017, 55 patients underwent ECT, with the first 29 in the frame of a phase-II study. The patients had metastases of the pelvis or appendicular skeleton smaller than 6 cm and no associated fracture. The procedure was tolerable, and no intra- or early post-operative complications were reported. After a mean 7-month follow-up, 20 (84%) of the 24 evaluable patients indicated a 50% or greater decrease in bone pain. Treatment-related adverse events were observed in three patients with advanced, highly pretreated tumors. These included a fracture of the proximal femur following the second ECT, a neurogenic bladder in a patient with a metastasis of the sacrum, and an extensive soft-tissue necrosis of the leg in a patient with a metastasis of the proximal tibia. Overall, ECT is

tolerable in well-selected patients with bone metastases, provided it is applied at referral centers. The Italian Society of Orthopedics and Traumatology (SIOT) has included this therapy in the guidelines for the management of unresectable tumors of the sacrum [42] and a prospective registry of the treated cases has been set up in Italy by the ReinBone (Registry of ECT in bone) group.

Soft tissue metastases

In 2010, Miklavcic et al. reported the first patient with a deep-seated soft-tissue metastasis from melanoma on a lower limb who was treated by *variable electrode-geometry* ECT [43]. A numerical treatment planning provided individualized electrode configuration and electric field strength. The tumor was approached percutaneously by four long needle electrodes. Despite only partial response, this work set the ground for numerical treatment planning-based ECT. A phase-II study in patients with deep-seated and large (>3 cm) soft-tissue tumors has closed the accrual at the Veneto Institute of Oncology of Padua, and results are expected in the next year [15]. Following pre-operative radiological imaging and pre-treatment planning, the electric pulses were applied with 17-G/1.2-mm needle electrodes under US guidance [44].

Pancreatic cancer

Surgical resectability is a key issue in pancreatic cancer, because the majority of patients present with locally advanced disease. This factor prompted researchers to investigate ECT as a potential treatment. Thirteen patients were enrolled in a phase-I/II study and underwent ECT with BLM during open surgery [45]. No intra- and post-operative relevant complications were reported after intensive radiological follow-up with contrast-enhanced US (CEUS), CT, and MRI. Three patients had a splenic infarction, without evidence of thrombosis of the splenic vessels. The authors reported the value of functional imaging tools in MRI (i.e., perfusion and diffusion derived parameters) as well as Choi and PET Response Criteria in Solid Tumors (PERCIST) for early assessment of tumor response, in keeping with preclinical results with MRI in electroporated brain tissue [46,47]. A phase-I study is enrolling patients with locally advanced disease who achieve disease stability with chemotherapy (Table 1).

Prostate cancer

Focal therapies [i.e., irreversible electroporation (IRE), cryosurgery, and high-intensity focused US (HIFU)] represent a valuable alternative to surgery for prostate cancer. ECT has been pioneered in a patient with a 2.4-cm recurrent carcinoma infiltrating the external urethral sphincter [48]. The treatment was applied as an adjunct to androgen deprivation therapy and was preferred to radical prostatectomy and radiotherapy to avoid functional side effects (i.e., incontinence and impotence). The tumor was approached through the perineum with four needle probes arranged in a square fashion and inserted under rectal US guidance. An i.v. bolus of BLM was followed by the application of a train of eight 100 μs-long electric pulses. The total procedural time was 80 min, and the urinary catheter was maintained for 12 days. Post-interventional MRI at 24 h, 8 weeks, and 6 months showed only mild tissue edema and no evidence of disease. There was no impairment in functional outcomes.

Brain tumors

The treatment of intracranial tumors with ECT is still in its preclinical phase [49–52]. By using an 8-needle expandable device, which may enter through a single burr hole and can be deployed to cover a larger area, researchers have tested ECT in a rat model inoculated with glia-derived tumor cells [53]. BLM was injected intracranially, and EP consisted of a train of 32 pulses, each of 100 V, 0.1-ms duration, and 1 Hz repetition frequency. During the procedure, the electrode was placed only once, and planned target volume measured approximately 100 mm^3 (gross tumor volume 1–34 mm^3). Nine of 13 rats (69%) displayed CR at MRI over 2–3 weeks, with no long-term adverse effects in healthy brain tissue [47,54]. Notably, EP has proved to reversibly disrupt the blood-brain barrier [53,55]. These encouraging results prompted researchers to design a device suitable for human application [56] and to develop statistical models to predict and reliable methods to verify permeabilization of the blood-brain barrier [24].

Endoscopic ECT

Gastrointestinal cancers

The newly available customized electrodes (Fig. 2c) provide excellent maneuverability in confined anatomical spaces and make gastrointestinal cancer a suitable target [14–17,29]. Patients with unresectable rectal cancer are initially managed with long-course chemoradiotherapy, including capecitabine, infusional/bolus fluorouracil (5-FU) with leucovorin, or oxaliplatin-based regimens [57]. If deemed unsuitable for surgical resection, patients can still undergo salvage chemotherapy with irinotecan, targeted drugs, and immunotherapy, but only one of five patients can be spared from extensive surgery [58]. Conversely, the treatment of the patients who fail salvage therapy is definitely palliative and, somehow, still undefined. Unfortunately, the uncontrolled growth of tumor within the rectum can be highly debilitating, and several treatments have been proposed, including endorectal brachytherapy [59], transarterial chemo-embolization [60], hypofractionated radiotherapy combined with local hyperthermia, capecitabine, oxaliplatin and metronidazole, escalated radiotherapy, and radiofrequency ablation. Similarly, the management of inoperable esophageal cancer represents an unmet need. In fact, over 50% of patients present with stage III/IV disease, and the majority of them are candidates for symptomatic treatment only [61]. Although many palliative methods exist (i.e., endoscopic dilatation, laser therapy, stent placement, external radiotherapy, and brachytherapy), none can be considered totally satisfactory. *Endoscopic* ECT lends itself as a potentially effective local treatment for these cases.

Besides an isolated experience with the endoscopic application of low-level electric currents alone in patients with malignant stenosis of the esophagus [62], only the recent development of a dedicated pulse applicator [the endoscopic vacuum electrode (EndoVE), Fig. 2c], stimulated the interest for *endoscopic* ECT [14]. The first in-human phase-I study has been conducted in Denmark in six patients with advanced esophageal carcinoma [63]. Treatment was performed under general anesthesia, and an electrocardiogram triggering monitor was used to prevent cardiac arrhythmias. The duration of the procedure varied from 24 to 59 min, and the number of applied pulses ranged from 9 to 48. There were no major safety issues, and an endoscopic visual response was reported in all cases (confirmed by PET-CT in four patients). Two phase-I studies in Ireland are currently investigating the EndoVE device in patients with colorectal and esophageal cancers (Clinicaltrials.gov NCT01172860; EudraCT: 2015-005246-59). The procedure was performed under general sedation or general anesthesia. An i.v. bolus of BLM (15,000 IU/m^2) was followed by a train of eight 100-μs long, 1000 V/cm electric pulses at 1-Hz repetition frequency. No serious adverse events were reported so far. Post-treatment MRI did not show toxicity of surrounding tissues, and an endoscopic and radiological response was evident in all cases (Suppl. Fig. 2). The tumor burden was decreased by more than 50% after a single treatment and a further application was performed on residual disease (unpublished data). Notably, a

reduction of tumor bleeding was observed in patients with rectal cancer, due to the well-known antivascular effect of ECT [64].

Combined approaches

Immunotherapy, gene electro transfer (GET), calcium EP, and radiotherapy provide exciting opportunities for innovative therapeutic strategies in combination with ECT. A further approach combining IRE on the target tumor and simultaneous administration of chemotherapy, which can take advantage of the reversible EP effect around the tumor, has also been postulated [55,65].

Immunotherapy

Following the advent of new immunotherapies, research efforts are now focused on converting the local effect of ECT into a systemic one [66,67]. Previous studies reported that ECT-mediated tumor regression is dramatically impaired in immunodeficient mice [1,68]. Moreover, preclinical and clinical experiences, particularly in melanoma patients, have characterized the local immune infiltrate at the ECT site [69–74]. In addition, EP can induce an *immunogenic cell death* through the liberation of ATP and HMGB1 molecules and the translocation of calreticulin [69], which act as damage-associated molecular patterns (DAMP) signals towards the immune system [75–77] (Fig. 1). Moreover, the massive liberation of tumor antigens together with DAMPs can activate the antigen-presenting dendritic cells [72,73,78]. Whether combining ECT and immunotherapy may represent an effective strategy for harnessing tumor infiltrating lymphocytes, overcoming tumor-induced tolerogenic mechanisms [78,79], and elicit an effective (possibly systemic) immune response remains to be established [67,70]. In addition, the complexity of the immune network, the number of immunotherapies, and the ECT parameters (i.e., chemotherapy, route of administration, and number and strength of pulses) make the task of defining an optimal schedule highly challenging. ECT and CTLA4 or PD1 inhibitors have been evaluated by two retrospective studies in melanoma patients, which indicate their safety and increased combined efficacy, and suggest T regulatory (T-reg) cell levels as a potentially predictive biomarker (Table 2) [80,81]. In 2016, Theuric et al. reported on 45 patients with melanoma treated with ipilimumab and concurrent local therapy (either radiation or ECT) [82]. Notably, despite the heterogeneity of local therapies included in the analysis, the authors observed a longer overall survival in patients who received the combined treatment compared with 82 matched patients who received ipilimumab alone. Anti-PD-1 therapy can be considered a safe and effective choice in elderly patients with massive skin metastases; nonetheless, ECT represents a tolerable complementary approach [83–85]. The sequential administration of pembrolizumab and ECT is currently being tested in a phase-II multicenter trial (Table 1). In patients with limited tumor burden, the combination of ECT with intralesional immunotherapies [talimogene laherparepvec (T-VEC), velimogene aliplasmid (Allovectin-7), interleukin-2 (IL-2), and rose Bengal (PV-10)] can also be conceived [86].

Gene electro transfer (GET)

GET delivers nucleic acids (i.e., oligonucleotides, siRNAs, and plasmid DNA) into the tissue of interest. Its combination with ECT has been tested in animal models, in which researchers used different types of GET, mainly based on interleukin-12 (IL-12), due to its antiangiogenic and immune effects [87,88]. In veterinary oncology, a combination of ECT using either CDDP or BLM and GET with IL-12 has been performed in client-owned dogs with spontaneous tumors, observing enhanced antitumor effectiveness [89,90]. A 2008 phase-I in-human clinical trial investigated the i.t. delivery of a plasmid encoding IL-12 alone in 19 patients with superficially metastatic melanoma [91]. The treatment, applied only to some of the cutaneous metastases, evoked a systemic immune response and in some cases determined PR (8 of 19 patients) or CR (2 of 19 patients) of distant, non-treated lesions. On these bases, the association of ECT and GET can be envisioned as an intriguing

Table 2
Combination of electrochemotherapy with systemic immunotherapy in metastatic melanoma.

Author (year)	No of pts	Immuno-therapy	Local treatment	Local response ORR (CRR),%	Systemic response ORR (CRR)	Local toxicity (CTAE)	Notes
Mozzillo (2015)	15	Ipilimumab[a]	ECT (i.v.[b] BLM)	67 (27)	60% (0)	G2 in 20%of pts	T-reg cells Significantly reduced in responders
Heppt (2016)	33	Ipilimumab[a] (n = 28) – Pembrolizumab[c] (n = 3) Nivolumab[d] (n = 2)	ECT (i.v.[b]/i.t.[e] BLM)	66.7 (15.2)	19.2% (–) (ipilimumab) – 40.0% (–) (anti-PD1)	≥G3 in 15.2% of pts	TTDP:2 months – TTDP:5 months
Theuric (2016)	45	Ipilimumab[a]	ECT[e] RT[f]	n.r.	37.8% (6.7%)	16.7%–21.4%[g] of pts	The addition of local treatment prolonged OS compared to 82 pts who received ipilimumab alone
Karaca (2017)	1	Nivolumab[d]	ECT[h]	CR	n.e.	No AEs	LDFS, 4 years

Legend: AEs, adverse events; EC, electrochemotherapy; LDFS, local disease-free survival; ORR, overall response rate; CRR, complete response rate; CTCAE, common terminology criteria for adverse events; T-reg, T-regulatory cells; OS, overall survival; n.e., not evaluable; n.r., not reported.

[a] 3 mg/kg body weight every 3 weeks for four cycles.

[b] (15 mg/m^2).

[c] 2 mg/kg every 3 weeks.

[d] 3 mg/kg every 2 weeks until disease progression or intolerable toxicity.

[e] ECT treatment parameters (n = 4 patients) were not reported.

[f] RT included conventional local irradiation of lymph nodes (n = 20 patients), bone (n = 17 patients), skin (n = 12 patients), mediastinum (n = 3 patients), liver (n = 1 patients), lung (n = 1 patient), or selective internal radiotherapy (SIRT) of liver metastases (n = 1 patient).

[g] G3 local toxicities were reported after RT on skin (16.7% of patients) or lymph nodes (21.4% of patients).

[h] ECT (a single cycle with i.v. BLM) was performed between the 9th and 10th nivolumab administration on a 4 × 3 cm axillary mass that progressed on anti-PD-1 therapy while other metastases disappeared (biphenotypic response). The patient was previously treated with adjuvant IFN, temozolomide, vemurafenib, and ipilimumab.

combined modality to capitalize upon local tumor response to achieve systemic antitumor immunization [92].

Calcium electroporation

Calcium EP is a novel, safe, and inexpensive investigational antitumor treatment, in which BLM or CDDP is replaced by calcium, which provokes necrotic cell death by internalization of large quantities of calcium. In eukaryotic cells, the concentration of intracellular calcium is low and can be drastically increased by manipulation of cell permeability [93]. The result of calcium internalization is tumor necrosis due to acute ATP depletion, but also antivascular effects may play a role [94]. The antitumor effectiveness of calcium EP has been demonstrated *in vitro*, *in vivo*, and in early clinical experiences [93,95,96]. The first randomized phase-II study enrolled seven patients with superficially metastatic melanoma or breast cancer who were randomly assigned to i.t. calcium (0.5–1 ml/cm^3 of tumor volume of a 9 mg/ml solution) or i.t. BLM (0.5–1 ml of a 1000 IU/ml solution), followed by the application of pulses. The calcium EP objective response rate was comparable to ECT with i.t. BLM (72% and 84%, respectively, $p = 0.5$), with less ulceration (38% vs. 68%). After 6 months, local recurrence was observed in 2/18 and 3/19 tumors, respectively [96]. A case report on a patient with melanoma supports the feasibility of the sequenced combination of ECT and calcium EP [95].

Radiotherapy

There is evidence in murine tumor models that EP-mediated delivery of CDDP and BLM increases their radiosensitising effect, whereas ECT-induced hypoxia (due to its antivascular effects [64]) does not hamper radiation efficacy [97–99]. Notwithstanding, there are no human studies available, and this strategy was sporadically applied in isolated patients with locally advanced tumors, with favorable results [100,101]. In a phase-II study in patients with pre-irradiated chest wall recurrence from breast cancer, ECT was highly effective, although there were some concerns regarding skin toxicity and local pain in case of further ECT applications [102]. Carefully designed protocols and further clinical investigation are warranted to establish the safety of this combined approach.

Multi-institutional collaboration

The constitution of multi-institutional groups has promoted interdisciplinary collaboration and has stimulated clinical research on ECT through greater coordination of initiatives and resources. The International Network for Sharing Practices on ECT (InspECT) is an open, independent group of researchers founded in 2008, including 25 European centers from nine countries and promotes registry-based studies (https://insp-ect.eu) [103]. Members adopted the ESOPE protocol [4,5] and share the “Copenhagen Agreement”, which regulates membership policies, clinical practice, and research activity. Past focus areas included treatment of skin metastases [104], management of post-procedural pain [105], treatment of melanoma and breast cancer [106,107], and evaluation of ECT with de-escalated doses of BLM [108]. The current research portfolio includes projects on basal cell carcinoma and angiosarcoma, combination with immunotherapy, individuation of predictive biomarkers, and development of an expert-based consensus on ECT application in melanoma patients. Other disease-oriented collaborative networks include the European Research on ECT in Head and Neck Cancer group (EURECA) [7,109], the Italian Senologic Group for ECT (Gisel) [110,111], and Reinbone, which is focused on osteoncology.

Discussion

The last decade witnessed a rapid incremental development of ECT [13,15,21,43,112]. Based on treatment set up and supporting equipment, ECT can now be categorized into three different modalities. Among these, *standard* ECT represents a consolidated locoregional therapy for the containment of superficial tumors and their symptomatic control, which is supported by abundant literature and international guidelines [11–13,113–117]. Concomitantly, the range of its application has progressively expanded towards new types of cancer such as, for instance, vulvar carcinoma. In addition to *standard* ECT, *variable electrode-geometry* and *endoscopic* ECT, although at an early stage of clinical development, are appearing in the clinic (Fig. 1). In this phase of transition and research opportunities, at least four major advancements can be identified: the development of custom electrodes (Fig. 2) and supporting tools, the investigation of new ECT modalities on deep-seated cancers, the development of combination strategies, and the constitution of cross-institutional research groups.

The new investigational indications for *standard* ECT include skin metastases from visceral, hematological, and gynecologic malignancies. Although the clinical experience is still sparse, accumulating evidence suggest that, under certain circumstances, ECT allows to control tumor growth locally and may provide a clinical benefit in well-selected patients, particularly in women with recurrent vulvar cancer [9,118], or in patients with peristomal tumor recurrences [8,10,119].

A further advance relates to the introduction of adjustable, freely placeable, customized, and endoscopic electrodes (Fig. 2). These devices provide adequate means for targeting a range of new lesions, and, in theory, also brain, lung, and bladder cancers [10,120,121]. Among these, bone metastases represent a devastating event, being associated with pain, disability, and complications. Animal studies have demonstrated bone structural integrity after EP, and the first in-human experiences confirm ECT feasibility along with a beneficial impact on bone pain. Notably, the treatment of spinal metastases could benefit from a “transpendicular” approach (i.e., electrode insertion through vertebral pedicles) to avoid surgical laminectomy, thus further reducing the invasiveness of the procedure, and preserving bone stability [122]. Overall, the published information supports the investigation of ECT in patients without bone instability or neurological symptoms. Additionally, the patients with a pathological fracture could be managed by surgical stabilization and intraoperative ECT, while reserving radiation as a rescue option. Similarly, *variable electrode-geometry* ECT has enabled targeting liver and pancreatic tumors percutaneously, during open laparotomy procedures, and also laparoscopically (Fig. 2d). For these approaches to be applied at the level of care, their feasibility needs broader confirmation, and clinical research is underway (Table 1). Until recently, the treatment of gastrointestinal tumors with EP has been limited by anatomical constraints, inherent technical limitations, and lack of clinical data supporting its feasibility. The initial clinical experiences are promising, but further studies are needed to confirm ECT safety and to clarify its palliative benefit (e.g., reduction of stenting procedures and impact on quality of life). A phase-II study in patients with locally advanced rectal cancer is underway in Denmark to investigate the effect of preoperative *endoscopic* ECT in addition to neoadjuvant chemotherapy on surgical outcomes and local immune infiltration. From a technological standpoint, the development of endoluminal balloon catheters, which enable non-contact application of electric pulses, may open new avenues in this field [18].

The third major advance relates to the advent of modern cancer immunotherapy and to the new insights on tumor microenvironment following application of ECT. Taken together, these findings

Fig. 3. First European Society of Surgical Oncology (ESSO) Course on Electrochemotherapy of Cutaneous and Deep Seated Tumors (Ljubljana, 22–23 October 2018). This educational event provided information about the basic principles and applications of electrochemotherapy in oncology through presentation of clinical cases, discussion of the clinical benefit of treatment application, and demonstration of treatment procedure on patients. (a) Live surgery session on electrochemotherapy in liver metastases; (b) intraoperative electrode placement into liver parenchyma; (c) intraoperative ultrasound examination for electrode tracking and treatment verification.

provide exciting opportunities to conceive new combination approaches with checkpoint inhibitors [123]. A study from Cork University Hospital is currently evaluating the sequential administration of ipilimumab and ECT in metastatic melanoma (Enhanced Malignant Melanoma Immunological Engagement, EMMIE-IPI/BMS Protocol Number CA184-426) and an Italian phase-II study is investigating the combination of *standard* ECT with pembrolizumab (Table 1). Similarly, other EP-based approaches (i.e., GET, calcium EP, and IRE), and radiotherapy may represent suitable partner therapies. Consequently, ECT is expected to move from a mere last-resort option toward the role of component of new multimodal treatment strategies [80–82,124,125].

Cross-institutional collaboration will be fundamental to conduct high quality research on these new treatments and to improve clinical practice. An effort to level up the quality of ECT clinical studies has been made through specific recommendations and a dedicated checklist [112]. The recently established collaborative groups (i.e., InspECT, EURECA, Gisel, and ReinBone) are actively contributing through different projects and programs. The recent update of procedural guidelines of *standard* ECT [5], as well as research on de-escalated chemotherapy [108,126] and management of postoperative pain [105], represent some prominent examples of rapid translatability of research findings into clinical practice. In this regard, it is worth noting that, based on recent pharmacokinetics insights in elderly patients, ECT procedure can now be extended up to 40 min following BLM infusion (instead of 20 min according to the original ESOPE [4]), thus allowing to treat more widespread tumors and also some challenging cancers by the new, more sophisticated ECT modalities.

Because these novel ECT approaches are still in the early phase of development, they should however remain in the research realm until conclusive proof of benefit is available. The evaluation of therapeutic medical devices is complex, and potential challenges include device modification, technical maturation, contextual factors, and the complexity of the disease [10,127]. Of great importance for clinical application, as in *standard* ECT, are careful patient selection, multidisciplinary discussion, and meticulous anesthesiological evaluation. Moreover, it is essential to adopt consistent and reproducible methods for assessing response to treatment, particularly in deep-seated tumors [46].

In conclusion, the number of cancers potentially amenable to ECT has expanded. The widespread use of *standard* ECT and the introduction of *variable electrode geometry* and *endoscopic* ECT together with the new combination strategies has enlarged the number of patients who might benefit from this therapy. For these emerging treatments to be rigorously investigated and possibly consolidated at the level of care, specific training of clinical staff through cross-institutional networking and international schools (Fig. 3) as well as the conduction of high-quality histotype-oriented clinical trials are necessary. Alignment of practice across ECT centers, along with adoption of shared clinical outcome and their standardized reporting will enable the full realization of the potential of emerging ECT applications to benefit patients.

Disclosures of interests

All authors take full responsibility for the content of the present publication; they confirm that the article reflect their view point and medical experience. The content of the manuscript is not influenced by any pharma company. Authors did not receive any compensation for authoring the manuscript. DM holds patents which are licensed to electrochemotherapy device manufacturer IGEA S.p.A.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2018.11.023.

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Glossary

BLM: bleomycin
CDDP: cisplatin
CR: complete response
ECT: electrochemotherapy
GET: gene electro transfer
EP: electroporation
ESOPE: European Standard Operating Procedures of Electrochemotherapy
IRE: irreversible electroporation
i.t.: intratumoral
i.v.: intravenous
LPFS: local progression-free survival
PR: partial response
Standard ECT: fixed electrode-geometry electrochemotherapy
Variable-geometry ECT: long-needle variable electrode-geometry electrochemotherapy