review

Electrochemotherapy in pancreatic adenocarcinoma treatment: pre-clinical and clinical studies

Sabrina Bimonte[1*], Maddalena Leongito[1*], Vincenza Granata[2], Antonio Barbieri[3], Vitale del Vecchio[3], Michela Falco[3], Aurelio Nasto[1], Vittorio Albino[1], Mauro Piccirillo[1], Raffaele Palaia[1], Alfonso Amore[1], Raimondo di Giacomo[1], Secondo Lastoria[4], Sergio Venanzio Setola[2], Roberta Fusco[2], Antonella Petrillo[2], Francesco Izzo[1]

[1] Division of Abdominal Surgical Oncology, Hepatobiliary Unit, Istituto Nazionale per lo studio e la cura dei Tumori "Fondazione G. Pascale", IRCCS, Naples, Italy

[2] Division of Radiology, Istituto Nazionale per lo studio e la cura dei Tumori "Fondazione G. Pascale", IRCCS, Naples, Italy

[3] S.S.D Sperimentazione Animale, Istituto Nazionale per lo studio e la cura dei Tumori "Fondazione G. Pascale", IRCCS, Naples, Italy

[4] Division of Nuclear Medicine, Department of Diagnostic Imaging and Radiotherapy, Istituto Nazionale Tumori "Fondazione G.Pascale" IRCCS, Naples, Italy

Radiol Oncol 2016; 50(1): 14-20.

Received 17 September 2015
Accepted 13 December 2015

Correspondence to: Dr. Sabrina Bimonte, Division of Abdominal Surgical Oncology, Hepatobiliary Unit, Istituto Nazionale per lo studio e la cura dei Tumori "Fondazione G. Pascale", IRCCS, Via Mariano Semmola, Naples, Italy. E-mail: s.bimonte@istitutotumori.na.it

Disclosure: No potential conflicts of interest were disclosed.

*The first two authors contributed equally to this manuscript

Background. Pancreatic adenocarcinoma is currently one of the deadliest cancers with high mortality rate. This disease leads to an aggressive local invasion and early metastases, and is poorly responsive to treatment with chemotherapy or chemo-radiotherapy. Radical resection is still the only curative treatment for pancreatic cancer, but it is generally accepted that a multimodality strategy is necessary for its management. Therefore, new alternative therapies have been considered for local treatment.

Conclusions. Chemotherapeutic resistance in pancreatic cancer is associated to a low penetration of drugs into tumour cells due to the presence of fibrotic stroma surrounding cells. In order to increase the uptake of chemotherapeutic drugs into tumour cells, electrochemotherapy can be used for treatment of pancreatic adenocarcinoma leading to an increased tumour response rate. This review will summarize the published papers reported in literature on the efficacy and safety of electrochemotherapy in pre-clinical and clinical studies on pancreatic cancer.

Key words: electrochemotherapy; pancreatic carcinoma; bleomycin; pre-clinical study; clinical study; safety; efficacy

Introduction

Pancreatic adenocarcinoma, despite extensive research, remains one of the deadliest cancers with high mortality rate. Surgical resection represents the only curative treatment for this pathology, but the majority of the patients are incurable at initial presentation with metastatic (stage IV) or surgically non-resectable disease (stage III disease).[1-3] At present gemcitabine and paclitaxel (Abraxane) are the best chemotherapeutic agents used for treatment of pancreatic cancer, however, patients develop drug resistance over the time. Thus, new alternative strategies, involving less toxic agents have been considered for local treatment of pancreatic cancer.[4-6] It is of note that chemotherapeutic

TABLE 1. Electrochemotherapy treatment in pancreatic cancer cell lines

Reference	Cell lines	Drugs	IC50 (P value)	Methods	EP parameters
Girelli et al., 2015[17]	PANC1 MiaPaCa2	Bleomycin Cisplatin	< 0.0001 ≤ 0.0001	MTS assay FACS	8 pulses, 100 µs of duration, 5 Hz
Sundararajan, 2014[26]	PANC1 PANC28	Gemcitabine	< 0.0001 ≤ 0.0001	MTS assay	high intensity, low duration (microseconds) pulses; low intensity and long duration pulses (milliseconds).

resistance in pancreatic cancer is associated with low penetration of drugs into tumour cells due to the presence of fibrotic stroma.

Reversible electroporation (EP) is a physical method used to overcome the barrier of the cell membranes by applying short and intense electric field, depending by cell characteristics as shape, size, and cytoskeleton structure and membrane composition. Applications of these pulses create rapid voltage changes that usually reach values between 0.5-1 V. This local polarization, deforms mechanically the membrane by forming a hydrophilic pore with a radius of ≈ 1 nm. It was hypothesized, that primary hydrophobic pores induced the transition to hydrophilic based precisely on a strong and nonlinear local transmembrane voltage. This treatment enables access for extracellular agents into the cells. For this reason EP may be combined with chemotherapeutic agents to improve their uptake, in particular hydrophilic drugs that, differently from lipophilic, are poorly or not permeant; this new cancer treatment modality is named electrochemotherapy (ECT).[7-10] The use of ECT for tumour treatments leads to a local potentiation of chemotherapy by reducing the doses of the drugs, minimizing the side effects, and increasing the efficacy of chemotherapy. In the perspective of pancreatic tumour treatment with ECT, several pre-clinical and clinical studies have been performed. This review will summarize the published papers reported in literature on the efficacy and safety of ECT in preclinical and clinical studies on pancreatic cancer.[11-17]

ECT in treatment of pancreatic cancer: *in vitro* studies

Several studies have been conducted in order to identify the optimal electric field characteristics (amplitude, duration and number of electric pulses and repetition frequency) and the appropriate antineoplastic drugs whose cytotoxicity could be increased by combination with EP.[18,19]

MTT assay allowing to define cellular cytotoxicity, is the method commonly used to screen drugs for feasibility and safety on both electro-permeabilized and not electro-permeabilized cells. It has been shown that, cell death due to the EP procedure was less than 4%, and that more than 90% of cells were permeabilized. Among several substances evaluated in association to EP some of them, such as daunorubicin, doxorubicin, etoposide and paclitaxel, did no show increased cytotoxicity. For carboplatin and cisplatin (CDDP) the efficacy of EP was indexed with a factor 3 and 2.3, respectively, on the IC50 (inhibitory concentration 50%), while bleomycin (BLM) was indexed with a value of 300.[20-22] These data suggest that BLM, by inducing apoptosis of several cancer cell lines, is considered the drug of choice for ECT, while the use of CDDP still remains to be fully explored.[23, 24]

Few studies have been performed on the use of ECT in pancreatic cancer treatment, but the emerging results are very encouraging. In particular, in a recent publication, were reported the feasibility and the safety of ECT for the treatment of pancreatic ductal adenocarcinoma (PDAC), a highly aggressive disease which normally is diagnosed in advanced stage. In this study it was demonstrated that EP represents a safe procedure in the treatment of PDAC and that can potentiate the effect on cytotoxicity of bleomycin and cisplatin in pancreatic tumour cell lines, PANC-1 and Miapaca-2 (Table 1).[17]

New applications of ECT protocol on pancreatic cancer cells have been developed. These techniques combined the use of natural compounds and chemotherapeutics drugs to ECT procedure, in order to reduce cytotoxic drug effects. In particular, two different studies have demonstrated that nanocurcumin, a polymeric nanoparticle-encapsulated curcumin, has better efficacy on pancreatic or breast tumour cell lines respect to normal curcumin and it is able to activate the same molecular pathways.[25,26] Another natural compound that could be used in association with ECT is the epi-

gallocatechin-3-gallate (EGCG), the most abundant catechin found in green tea, in particular combined with BLM. Recently, we showed the efficacy and synergism of EGCG and BLM on the inhibition of pancreatic cancer MiaPaCa-2 cell proliferation by inducing apoptosis.[27]

Several studies are ongoing in our laboratory, to demonstrate that ECT treatment can potentiate the efficacy and synergism of EGCG and BLM in pancreatic cancer cells and in pancreatic cancer mouse model.

Taken together, these studies suggest that ECT could be considered as a valid technique for treatment of pancreatic cancer, although more studies will be needed in order to refine the ECT protocols.

Electrochemotherapy in pancreatic cancer: *in vivo* animal models

It is has been reported that (EP), has been used for different types of clinical applications in treatment of cancer: irreversible (IRE) or reversible electroporation combined with chemotherapeutic agents (ECT). IRE is an ablative non-thermal technique which uses a high voltage (maximum 3,000 V) small microsecond pulse lengths (70 to 90 μs) to induce cell membrane permeability which leads to slow/protracted cell death over time. Pre-clinical data supporting both the safety and effectiveness of IRE in treatment of pancreatic cancer have been published. In particular, Bower *et al*. performed an in *vivo* study demonstrating no adverse events of IRE around the portal veins in a large porcine animal model. All pigs have been exposed to a pulse field generated with maximum 3,000 V for 70-90 μs and revealed only mild adhesions, no ascites, and no pancreatic necrosis. This study demonstrates that IRE protocol of the pancreas performed at an optimal voltage is well tolerated, with rapid resolution of pancreatic inflammation and preservation of vascular structures.[28] Similar results were confirmed by Charpentier *et al*. who generated an acute animal model (2 hours survival) and also demonstrated no vascular thrombosis as well as effectiveness with complete ablation in pancreas and liver.[29-31] Another group, showed the feasibility of IRE against pancreatic ductal adenocarcinoma (PDAC). This study demonstrated that IRE treatment had significant antitumour effects and prolonged survival in mice with orthotopic xenografts. Extensive tumour necrosis, reduced tumour cell proliferation and disruption of microvessels, were observed at different days post-IRE.[32] Recently, it has been demonstrated the efficacy of irreversible electroporation in human pancreatic adenocarcinoma by using heterotopic murine model. In this paper, authors optimized IRE parameters and evaluated the effects of IRE on surrounding tissues, recurrence, and biomarker expression changes in recurrent/incompletely electroporated mice tumours.[33,34]

In alternative to use of IRE in treatment of cancer, some pre-clinical studies using different animal models have been performed to investigate the local and systemic effects of ECT in cancer. Sersa *et al.*, in mice models of murine fibrosarcoma SA-1 treated with bleomycin-ECT, described mice tumour destruction due to the immune system activity highly stimulated by ECT, an increased apoptosis of endothelial cells surrounding the tumour, and a reduction of blood flow in the vessels supplying the lesion.[35] Roux *et al.*, by analyzing two tumour mouse models (sarcoma and melanoma) treated with bleomycin-ECT, have demonstrated an increase of local T-dependent response due to a massive recruitment of CD11c and CD11b positive cells in the tumours depending on tumour-associated antigen (TAA) release.[36] However, the parameters and safety of ECT are well calibrated for the treatment of cutaneous and subcutaneous lesions, but not for deep-seated tumours as the pancreatic cancer. So, in order to perform a standardization of ECT protocol for pancreatic tumours, other studies on animal models will be needed. One of the first works on the use of ECT in pre-clinical pancreatic cancer treatment was published in 1998 by Nanda *et al.* In this study, human pancreatic tumours (Pan-4-JCK) implanted subcutaneously in nude mice, were treated with ECT using BLM, mitomycin C or carboplatin. Tumours were monitored for a period of 89 days after the therapy and showed a significant regression (Table 2).[12] Similar results were obtained in another study in which nude mice, xenografted with pancreatic adenocarcinoma cells, were subjected to a different scheme of EP with new electrodes for drugs (doxorubicin, fluorouracil or cisplatin) delivery. Tumour growth analysis performed after 28 days of ECT treatment, revealed a significant regression (Table 2).[37] Another study investigated the use of electrically mediated drug delivery for the treatment of pancreatic adenocarcinoma in a hamster model. Authors showed that treatment of subcutaneous tumours with bleomycin and electric fields resulted in a 100% complete response rate while treatment of tumours induced in the gland, resulted in a 25% complete response rate (Table 2).[13]

TABLE 2. Electrochemotherapy (ECT) in animal models of pancreatic cancer

Reference	Animal models	Cell lines	Methods	Drugs	Effects
Nanda et al., 1998[12]	Nude mice	Pan-4JCK	ECT	Bleomycin Carboplatin Mitomicin C	Tumour regression after 89 days
Dev et al., 2000[37]	Nude mice	BxPc3	ECT	Cisplatin Doxorubicin Fluruoracil	Tumour regression after 28 days
Jaroszeski et al., 1999[13]	Golden Syrian hamster	PC-1	ECT	Bleomycin	100% complete response rate in subcutaneous tumours, 25% response rate in orthotopic tumours

As reported previously, recently Girelli *et al.* have demonstrated the feasibility and the safety of ECT for the treatment of pancreatic ductal adenocarcinoma. In this study, New Zealand non pathological rabbits were subjected to open surgery EP of pancreas and duodenum, according to the ESOPE pulse protocol. Neither systemic nor local toxic effects due to the electroporation procedure were observed, demonstrating the safety of the optimized electric parameters in the treatment of the pancreas *in vivo*.[17]

Taken together these studies suggest that ECT can be used for the local control of non-resectable pancreatic cancer adenocarcinoma (PDAC).

Electrochemotherapy in clinical studies of pancreatic cancer

Recently, different experiences showed the clinical approach of ECT for the treatment of deep-seated tumours as pancreatic cancer diseases[15,38] and liver metastases from colorectal cancer.[39] Specifically for pancreatic cancer, a clinical phase I/II study on patients with locally advanced disease, is ongoing at the National Cancer Institute, "*G. Pascale Foundation*" of Naples.[38] Patients are enrolled in this study by using the following inclusion criteria: age between 18 and 80 years; good mental health; life expectancy ≥ 3 months; diagnosis of pancreatic adenocarcinoma or pancreatic neuroendocrine tumours, confirmed by histological analysis; locally advanced disease [stage III]. In this study, were not included patients with one or more of the following conditions: pregnancy positive test for women, significant heart disease, coagulation disturbances, and allergy to bleomycin, lung and kidney dysfunction, concomitant presence of distant metastases. It is important to underline that all patients received systemic chemotherapy (GEMOX or FOLFIRINOX). Subsequently, to choose the patients suitable to receive ECT treatment, were performed clinical and radiological examinations (CT, MRI and PET). By using functional MRI parameters, it was observed a significant reduction of viable tumour tissue in ECT treated target area. Results from PET analysis, indicated that the uptake of ^{18}FDG during post-operative PET examination was lower in respect to pre-operative evaluations. No serious side effects for the patients were observed. In addition, pain reduction of patients (evaluated by VAS-score) was reported immediately after the ECT treatment compared to pre-operative status. Preliminary data on feasibility and safety of the ECT treatment on patients with locally advanced cancer were reported by Granata et *al.*[15] For a significant number of patients, a reduced diameter and tumourigenicity of the lesions associated with good clinical parameters were reported.

These data suggest that ECT can be safely performed in locally advanced pancreatic tumours.

ECT *vs* IRE in treatment of patients with unresectable pancreatic cancer

It is of note that multi-modality therapy, including chemotherapy, surgery and/or radiation therapy represent the optimal treatment option for patients with pancreatic adenocarcinoma especially stage II disease. Since the incidence of more advanced staged disease (stage III and stage IV) is becoming higher over the time, only a small percentage of patients who are diagnosed with pancreatic adenocarcinoma are eligible for definitive surgical resection. Due to this high incidence, alternative techniques have been developed in order to improve quality-of-life especially in patients with stage III pancreatic adenocarcinoma. Radiofrequency ablation (RFA) has been studied as possible therapy centered on thermal techniques, but the reported morbidity rates were high in the majority of these

TABLE 3. Clinical studies on irreversible electroporation (IRE) in pancreatic cancer

Reference	No. of patients	Stage of pancreatic cancer	Results
Bagla *et al.*, 2012[54]	78	Stage III	No residual disease and a decreasing cancer antigen 19-9 level.
Mansson *et al.*, 2014[55]	5		No serious treatment-related adverse events were observed.
Paiella *et al.*,2015[56]	10	Stage III	Overall survival of 7.5 months
Martin *et al*, 2013[57]	54	Stage III	Improvement in local progression-free survival (14 vs. 6 months, p = 0.01), distant progression-free survival (15 vs. 9 months, p = 0.02), and overall survival (20 vs. 13 months, p = 0.03).
Martin *et al*, 2014[58]	48	Stage III	No significant vascular complications were seen, and of the high-grade complications, bleeding (2), biliary complications (3) and DVT/PE (3) were the most common.

DVT/PE = deep vein thrombosis and pulmonary embolism

published studies.[40-44] In addition, anatomy of pancreas represents a significant obstacle to other thermal ablation techniques including cryoablation, high intensity focal ultrasonography, and MWA which to date have not been as well studied as RFA. To bypass the problems relative to thermal techniques, irreversible electroporation (IRE) has been introduced to treat pancreatic cancer, since it does not use thermal energy and does not damage blood vessels and bile ducts.[45-49] Recent studies have demonstrated the safety and palliation with encouraging improvement in overall survival. It has also been demonstrated that for patients with LAPC (stage III), the addition of IRE to conventional chemotherapy and radiation therapy results in substantially prolonged survival compared with historical controls.[50] Table 3 summarizes clinical studies on IRE in pancreatic cancer.

It is important to underline, that recent studies have shown that a small area of thermal effect of IRE is likely present immediately adjacent to the probe.[51] In addition, treating deep seated tumours either during open surgery or percutaneously in liver or other organs due to high voltage (up to 3000 V) and consequently high currents (up to 50 A) delivered pulses could potentially interfere with cardiac activity.[52] Moreover, one limitation of IRE remains tissue heterogeneity and the unique settings based on tumour histology and prior induction therapy. For this reason, based on our knowledge, IRE could not be considered a standard-of-care practice for treatment of locally advanced pancreatic cancer. As previously described, preliminary studies indicate that ECT represents a feasible and safe treatment modality in patients with locally advanced pancreatic adenocarcinoma. Differently from IRE, ECT protocols for pancreatic cancer uses a lower voltage and lower currents of delivered pulses. In this way the risk of interference with cardiac activity of patients is lower than those induced by IRE protocols. A recent observational study on the effects of ECT in colorectal liver metastases treatment, demonstrated that in patients after ECT treatment, were found in ECG signals recorded during early post-operative care, no major changes in functioning of the heart or pathological morphological changes.[53]

Conclusions

IRE and ECT represent new non-thermal techniques with high interest in treatment of locally advanced pancreatic cancer. IRE applies a higher voltage leading to cell death by apoptosis rather than necrosis. Despite the exact mechanism by which IRE induces apoptosis is still unclear, it seems to induce permanent nanopore formation and consequent ion disruption. As previously reported, although IRE is known as non-thermal technique, studies provided evidence that induces a small area of thermal effect near the probe. One complication for patients treated with IRE is a significant musculature contraction as consequence of high voltage induced. On the other hand, ECT protocol for pancreatic cancer uses a lower voltage and lower currents of delivered pulses. No side effects or major complications have been recorded for ECT treatment of patients with pancreatic cancer, although clinical studies need to be improved.

Taken together, these data suggest that IRE and ECT are promising techniques for treatment of pancreatic cancer, although both require more investigation in the future.

Authors' contributions

BS and LM performed preparation of the manuscript; BA, DV, FM performed experimental support; AV, PM, AA, DR, NA, GV, PA, SL, SS, FR performed bibliographic research. AN and IF were responsible for coordination of this study. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to specially thank Massimiliano Spinelli Data Manager of S.S.D. Animal Sperimentation, from Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione Giovanni Pascale", IRCCS, Italia, for kind help in providing informatics assistance.

References

1. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 2010; **46:** 765-81.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61:** 69-90.
3. Krejs GJ. Pancreatic cancer: epidemiology and risk factors. *Dig Dis* 2010; **28:** 355-8.
4. Cunningham D, Chau I, Stocken DD, Valle JW, Smith D, Steward W, et al. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2009; **27:** 5513-8.
5. Burris HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15:** 2403-13.
6. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364:** 1817-25.
7. Neumann E, Kakorin S, Toensing K. Fundamentals of electroporative delivery of drugs and genes. *Bioelectrochem Bioenerg* 1999; **48:** 3-16.
8. Orlowski S, Mir LM. Cell electropermeabilization: a new tool for biochemical and pharmacological studies. *Biochim Biophys Acta* 1993; **1154:** 51-63.
9. Gehl J. Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. *Acta Physiol Scand* 2003; **177:** 437-47.
10. Mir LM, Orlowski S, Belehradek J Jr, Paoletti C. Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses. *Eur J Cancer* 1991; **27:** 68-72.
11. Sersa G, Novakovic S, Miklavcic D. Potentiation of bleomycin antitumor effectiveness by electrotherapy. *Cancer Lett* 1993; **69:** 81-4.
12. Nanda GS, Sun FX, Hofmann GA, Hoffman RM, Dev SB. Electroporation enhances therapeutic efficacy of anticancer drugs: treatment of human pancreatic tumor in animal model. *Anticancer Res* 1998; **18(3A):** 1361-6.
13. Jaroszeski MJ, Illingworth P, Pottinger C, Hyacinthe M, Heller R. Electrically mediated drug delivery for treating subcutaneous and orthotopic pancreatic adenocarcinoma in a hamster model. *Anticancer Res* 1999; **19(2A):** 989-94.
14. Liu X, Tian X, Wang F, Ma Y, Kornmann M, Yang Y. BRG1 promotes chemoresistance of pancreatic cancer cells through crosstalking with Akt signalling. *Eur J Cancer* 2014; **50:** 2251-62.
15. Granata V, Fusco R, Piccirillo M, Palaia R, Petrillo A, Lastoria S, et al. Electrochemotherapy in locally advanced pancreatic cancer: Preliminary results. *Int J Surg* 2015; **18:** 230-6.
16. Miklavcic D, Sersa G, Brecelj E, Gehl J, Soden D, Bianchi G, et al. Electrochemotherapy: technological advancements for efficient electroporation-based treatment of internal tumors. *Med Biol Eng Comput* 2012; **50:** 1213-25.
17. Girelli R, Prejano S, Cataldo I, Corbo V, Martini L, Scarpa A, et al. Feasibility and safety of electrochemotherapy (ECT) in the pancreas: a pre-clinical investigation. *Radiol Oncol* 2015; **49:** 147-54.
18. Jaroszeski MJ, Dang V, Pottinger C, Hickey J, Gilbert R, Heller R. Toxicity of anticancer agents mediated by electroporation in vitro. *Anticancer Drugs* 2000; **11:** 201-8.
19. Cadossi R, Ronchetti M, Cadossi M. Locally enhanced chemotherapy by electroporation: clinical experiences and perspective of use of electrochemotherapy. *Future Oncol* 2014; **10:** 877-90.
20. Miklavcic D, Mali B, Kos B, Heller R, Sersa G. Electrochemotherapy: from the drawing board into medical practice. *Biomed Eng Online* 2014; **13:** 29.
21. Gehl J, Skovsgaard T, Mir LM. Enhancement of cytotoxicity by electropermeabilization: an improved method for screening drugs. *Anticancer Drugs* 1998; **9:** 319-25.
22. Poddevin B, Orlowski S, Belehradek J Jr, Mir LM. Very high cytotoxicity of bleomycin introduced into the cytosol of cells in culture. *Biochem Pharmacol* 1991; **42(Suppl):** S67-75.
23. Sersa G, Cemazar M, Miklavcic D. Antitumor effectiveness of electrochemotherapy with cis-diamminedichloroplatinum(II) in mice. *Cancer Res* 1995; **55:** 3450-5.
24. Larkin JO, Collins CG, Aarons S, Tangney M, Whelan M, O'Reily S, et al. Electrochemotherapy: aspects of preclinical development and early clinical experience. *Ann Surg* 2007; **245:** 469-79.
25. Bisht S, Feldmann G, Soni S, Ravi R, Karikar C, Maitra A. Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): a novel strategy for human cancer therapy. *J Nanobiotechnology* 2007; **5:** 3.
26. Kishore B, Khare P, Gupta RJ, Bisht S, Majumdar K. Hemoglobin E disease in North Indian population: a report of 11 cases. *Hematology* 2007; **12:** 343-7.
27. Bimonte S, Leongito M, Barbieri A, Del Vecchio V, Barbieri M, Albino V, et al. Inhibitory effect of (-)-epigallocatechin-3-gallate and bleomycin on human pancreatic cancer MiaPaca-2 cell growth. *Infect Agent Cancer* 2015; **10:** 22.
28. Bower M, Sherwood L, Li Y, Martin R. Irreversible electroporation of the pancreas: definitive local therapy without systemic effects. *J Surg Oncol* 2011; **104:** 22-8.
29. Charpentier KP, Wolf F, Noble L, Winn B, Resnick M, Dupuy DE. Irreversible electroporation of the pancreas in swine: a pilot study. *HPB (Oxford)* 2010; **12:** 348-51.
30. Martin RC. Use of irreversible electroporation in unresectable pancreatic cancer. *Hepatobiliary Surg Nutr* 2015; **4:** 211-5.
31. Charpentier KP, Wolf F, Noble L, Winn B, Resnick M, Dupuy DE. Irreversible electroporation of the liver and liver hilum in swine. *HPB (Oxford)* 2011; **13:** 168-73.
32. Jose A, Sobrevals L, Ivorra A, Fillat C. Irreversible electroporation shows efficacy against pancreatic carcinoma without systemic toxicity in mouse models. *Cancer Lett* 2012; **317:** 16-23.
33. Philips P, Li Y, Li S, St Hill CR, Martin RC. Efficacy of irreversible electroporation in human pancreatic adenocarcinoma: advanced murine model. *Mol Ther Methods Clin Dev* 2015; **2:** 15001.
34. Philips P, Li Y, Martin RC 2nd. Low-energy DC current ablation in a mouse tumor model. *Meth Mol Biol* 2014; **1121:** 257-65.
35. Sersa G, Cemazar M, Snoj M. Electrochemotherapy of tumours. *Curr Oncol* 2009; **16:** 34-5.
36. Roux S, Bernat C, Al-Sakere B, Ghiringhelli F, Opolon P, Carpentier AF, et al. Tumor destruction using electrochemotherapy followed by CpG oligodeoxynucleotide injection induces distant tumor responses. *Cancer Immunol Immunother* 2008; **57:** 1291-300.
37. Dev SB, Hofmann GA, Nanda GS. Treatment of human pancreatic tumors xenografted in nude mice by chemotherapy combined with pulsed electric fields. *Methods Mol Med* 2000; **37:** 277-83.

38. Tafuto S, von Arx C, De Divitiis C, Tracey Maura C, Palaia R, Albino V, et al. Electrochemotherapy as a new approach on pancreatic cancer and on liver metastases. *Int J Surg* 2015; **21(Suppl 1):** S78-82.

39. Edhemovic I, Gadzijev EM, Brecelj E, Miklavcic D, Kos B, Zupanic A, et al. Electrochemotherapy: a new technological approach in treatment of metastases in the liver. *Technol Cancer Res Treat* 2011; **10:** 475-85.

40. Girelli R, Frigerio I, Salvia R, Barbi E, Tinazzi Martini P, Bassi C. Feasibility and safety of radiofrequency ablation for locally advanced pancreatic cancer. *Br J Surg*. 2010; **97:** 220-5.

41. Girelli R, Frigerio I, Giardino A, Regi P, Gobbo S, Malleo G, et al. Results of 100 pancreatic radiofrequency ablations in the context of a multimodal strategy for stage III ductal adenocarcinoma. *Langenbecks Arch Surg* 2013; **398:** 63-9.

42. Giardino A, Girelli R, Frigerio I, Regi P, Cantore M, Alessandra A, et al. Triple approach strategy for patients with locally advanced pancreatic carcinoma. *HPB (Oxford)* 2013; **15:** 623-7.

43. Cantore M, Girelli R, Mambrini A, Frigerio I, Boz G, Salvia R, et al. Combined modality treatment for patients with locally advanced pancreatic adenocarcinoma. *Br J Surg* 2012; **99:** 1083-8.

44. Matsui Y, Nakagawa A, Kamiyama Y, Yamamoto K, Kubo N, Nakase Y. Selective thermocoagulation of unresectable pancreatic cancers by using radiofrequency capacitive heating. *Pancreas* 2000; **20:** 14-20.

45. Young SJ. Irreversible electroporation and the pancreas: What we know and where we are going? *World J Gastrointest Surg* 2015; **7:** 138-44.

46. Scheffer HJ, Nielsen K, de Jong MC, van Tilborg AA, Vieveen JM, Bouwman AR, et al. Irreversible electroporation for nonthermal tumor ablation in the clinical setting: a systematic review of safety and efficacy. *J Vasc Interv Radiol* 2014; **25:** 997-1011.

47. Trueba-Arguinarena FJ, de Prado-Otero DS, Poves-Alvarez R. Pancreatic adenocarcinoma treated with irreversible electroporation Case Report: first experience and outcome. *Medicine (Baltimore)* 2015; **94:** e946.

48. Gall TM, Thompson Z, Dinneen EP, Sodergren M, Pai M, Frampton AE, et al. Surgical techniques for improving outcomes in pancreatic ductal adenocarcinoma. *Expert Rew Gastroenter Hepatol* 2014; **8:** 241-6.

49. Weiss MJ, Wolfgang CL. Irreversible electroporation: a novel pancreatic cancer therapy. *Curr Probl Cancer* 2013; **37:** 262-5.

50. Martin RG, II, McFarland K, Ellis S, Velanovich V. Irreversible electroporation in locally advanced pancreatic cancer: potential improved overall survival. *Ann Surg Oncol* 2013; **20:** 443-9.

51. Long G, Bakos G, Shires PK, Gritter L, Crissman JW, Harris JL, et al. Histological and finite element analysis of cell death due to irreversible electroporation. *Technol Cancer Res Treat* 2014; **13:** 561-9.

52. Ball C, Thomson KR, Kavnoudias H. Irreversible electroporation: a new challenge in "out of operating theater" anesthesia. *Anesth Analg* 2010; **110:** 1305-9.

53. Mali B, Gorjup V, Edhemovic I, Brecelj E, Cemazar M, Sersa G, et al. Electrochemotherapy of colorectal liver metastases - an observational study of its effects on the electrocardiogram. *Biomed Eng Online* 2015; **14(Suppl 3):** S5.

54. Bagla S, Papadouris D. Percutaneous irreversible electroporation of surgically unresectable pancreatic cancer: a case report. Journal of vascular and interventional radiology: *J Vasc Interv Radiol* 2012; **23:** 142-5.

55. Mansson C, Bergenfeldt M, Brahmstaedt R, Karlson BM, Nygren P, Nilsson A. Safety and preliminary efficacy of ultrasound-guided percutaneous irreversible electroporation for treatment of localized pancreatic cancer. *Anticancer Res* 2014; **34:** 289-93.

56. Paiella S, Butturini G, Frigerio I, Salvia R, Armatura G, Bacchion M, et al. Safety and feasibility of irreversible electroporation (IRE) in patients with locally advanced pancreatic cancer: results of a prospective study. *Digest Surg* 2015; **32:** 90-7.

57. Martin RC, McFarland K, Ellis S, Velanovich V. Irreversible electroporation in locally advanced pancreatic cancer: potential improved overall survival. *Ann Surg Oncol* 2013; **20(Suppl 3):** S443-9.

58. Martin RC, Philips P, Ellis S, Hayes D, Bagla S. Irreversible electroporation of unresectable soft tissue tumors with vascular invasion: effective palliation. *BMC Cancer* 2014; **14:** 540.