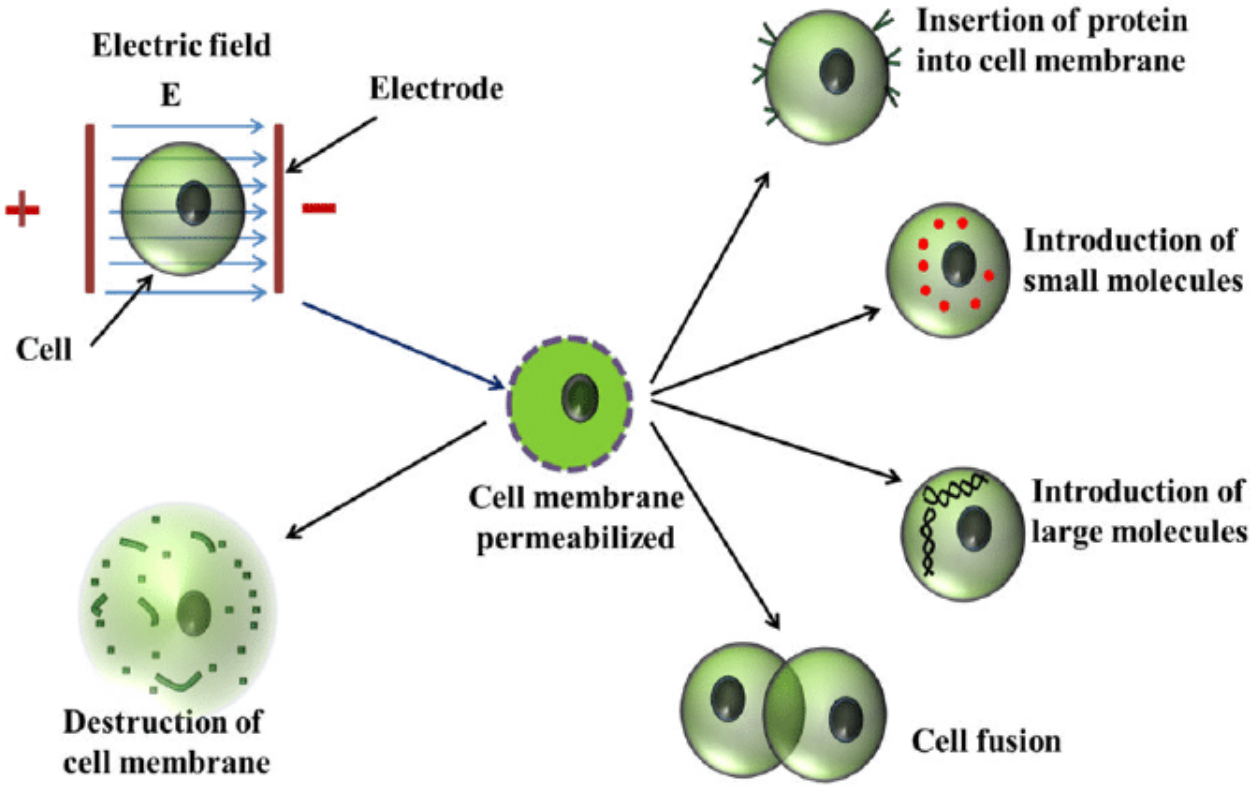
ONKOLOŠKI INŠTITUT
INSTITUTE OF ONCOLOGY
LJUBLJANA

# *Biomedical applications of electroporation*

*Prof. Gregor Serša, Ph.D.*
*Institute of Oncology Ljubljana*
*Department of Experimental Oncology, Slovenia*

# Electric field induces structural changes in plasma membrane

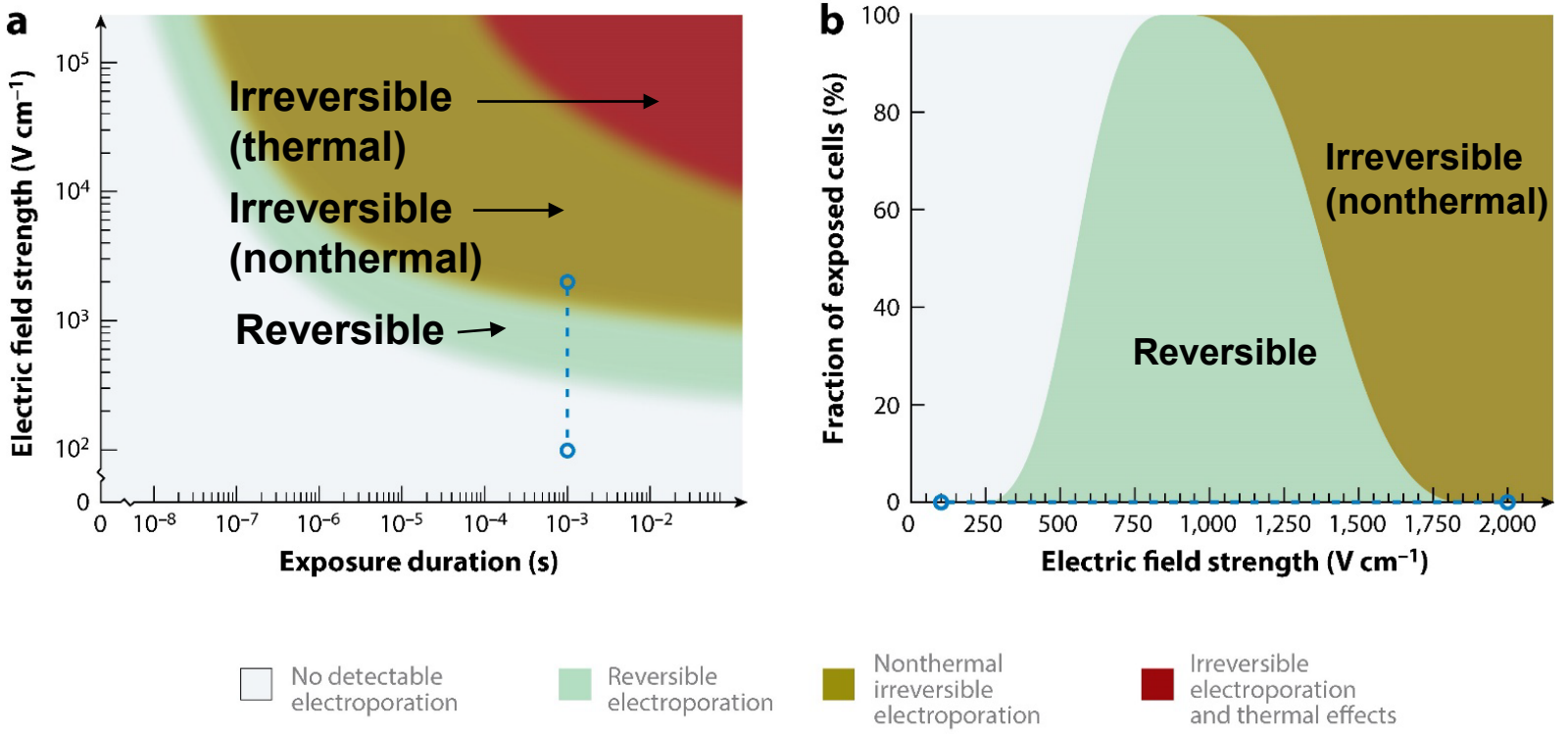
- Cell membrane permeabilization that induces:
  - Destruction of the cell membrane
  - Insertion of proteins into cell membrane
  - Introduction of small molecules
  - Introduction of large molecules
  - Cell fusion



# We distinguish reversible and irreversible electroporation

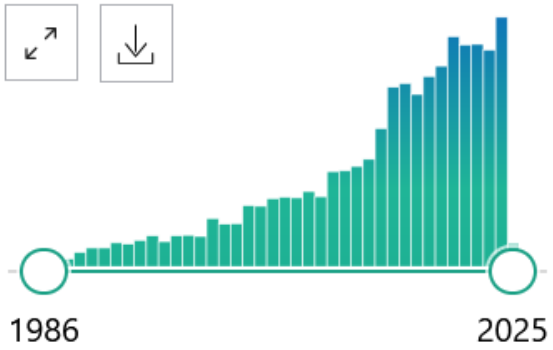
- **Reversible electroporation** preserves cell viability, therefore the electric field, pulse duration and number of pulses needs to be adjusted for specific biomedical application
- **Irreversible electroporation** destructs the cell membrane and this is the means for cell death

  - Depends on:
    - Electric filed strength
    - Exposure duration




Yarmush ML, et al. 2014.
Annu. Rev. Biomed. Eng. 16:295–320

# Biomedical applications of electroporation in different disciplines of medicine




Electroporation, medicine: 380

**Reversible electroporation**

- **Oncology**
  Tumor treatment
- **Infectiology**
  Vaccines
- **Angiology**
  Vascular malformations

**Irreversible electroporation**

- **Oncology**
  Tumor treatment
- **Cardiology**
  Cardiac arrythmias

# The technology is based on application of electric pulses to tissues

Different commercial electroporators are available:

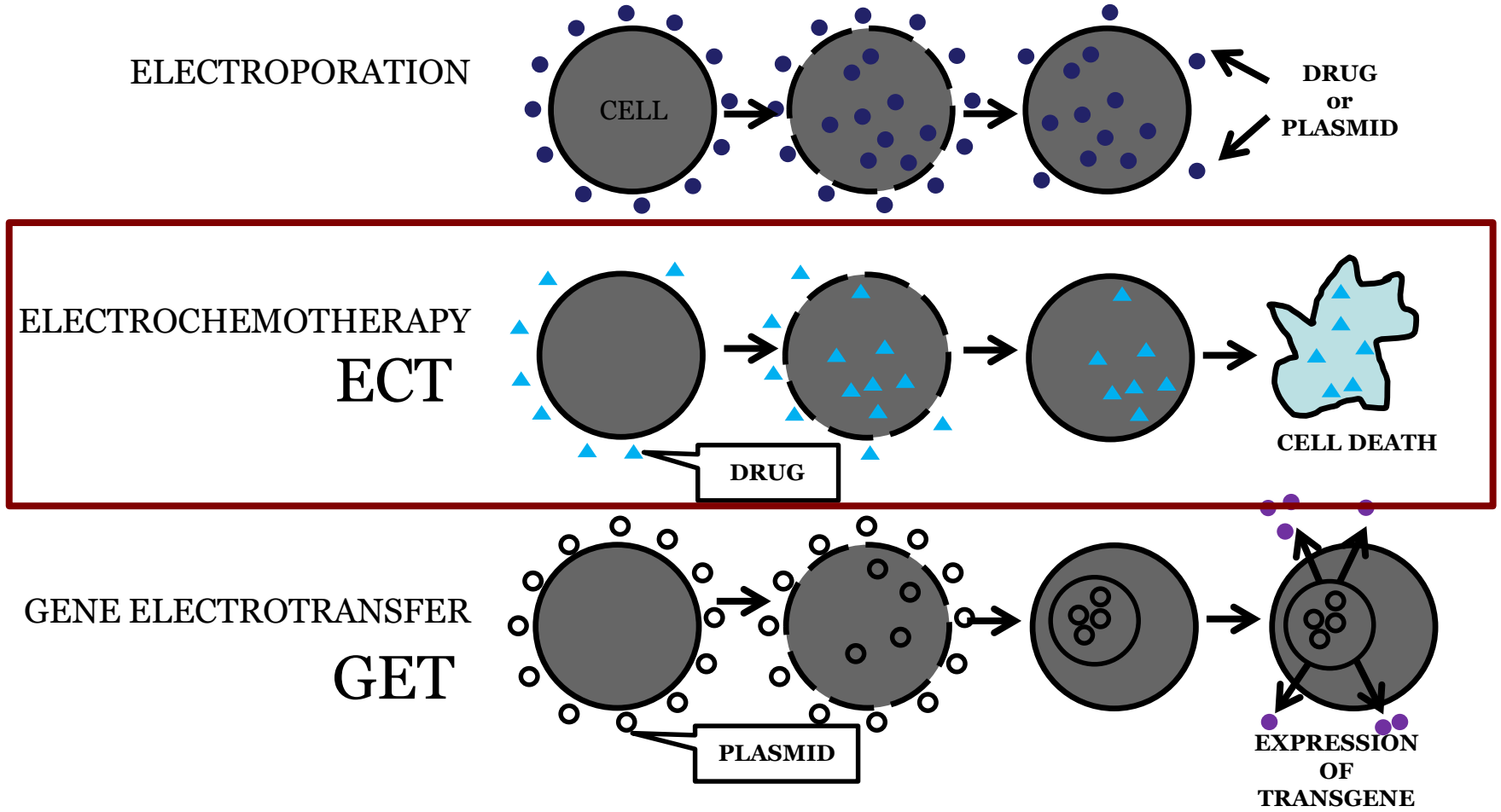
- **BTX:** GEMINI $X^2$
- **Invitrogen:** Neon
- **Intracel:** TSS20
- **IGEA:** Gene Drive
- **Leroy:** Beta-tech B10
- **Societe Jouan**: JOUAN
- **FID GmbH**: FPG 20-1NM4
- **IGEA:** Cliniporator Vitae
- **Pulse Biosciences:** CellFX
- …

http://lbk.fe.uni-lj.si/ic/

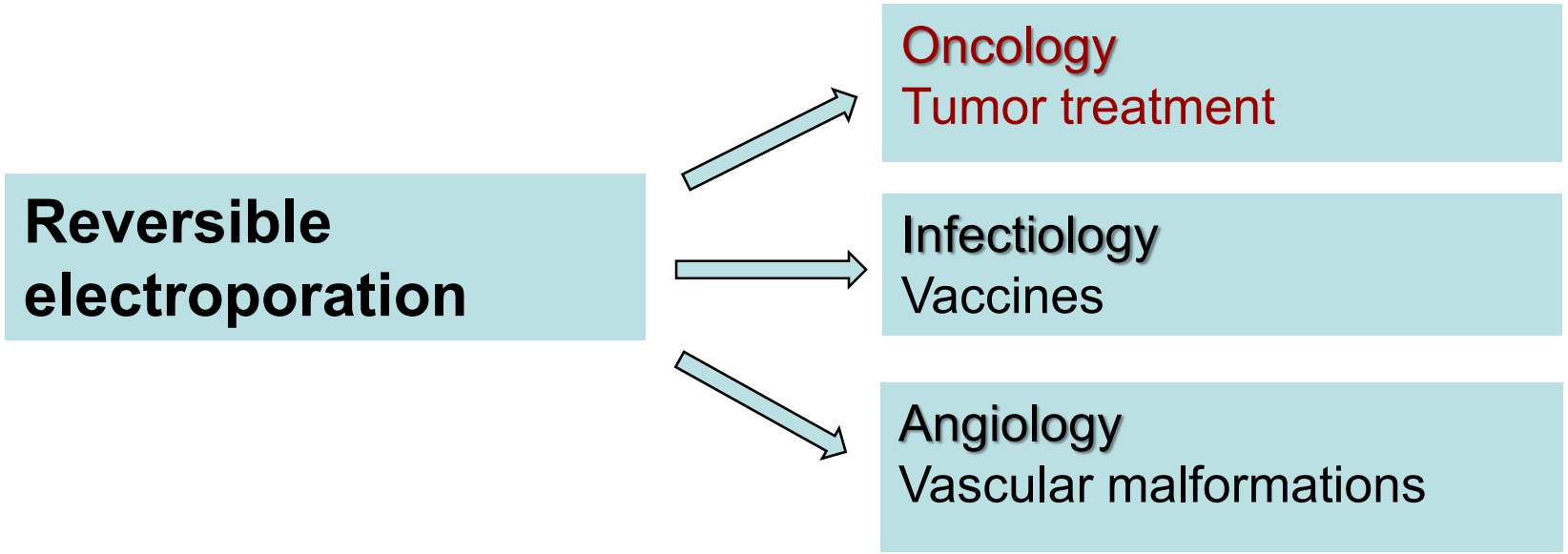

# Presence


254 Hospitals are currently working with the Cliniporator Technology

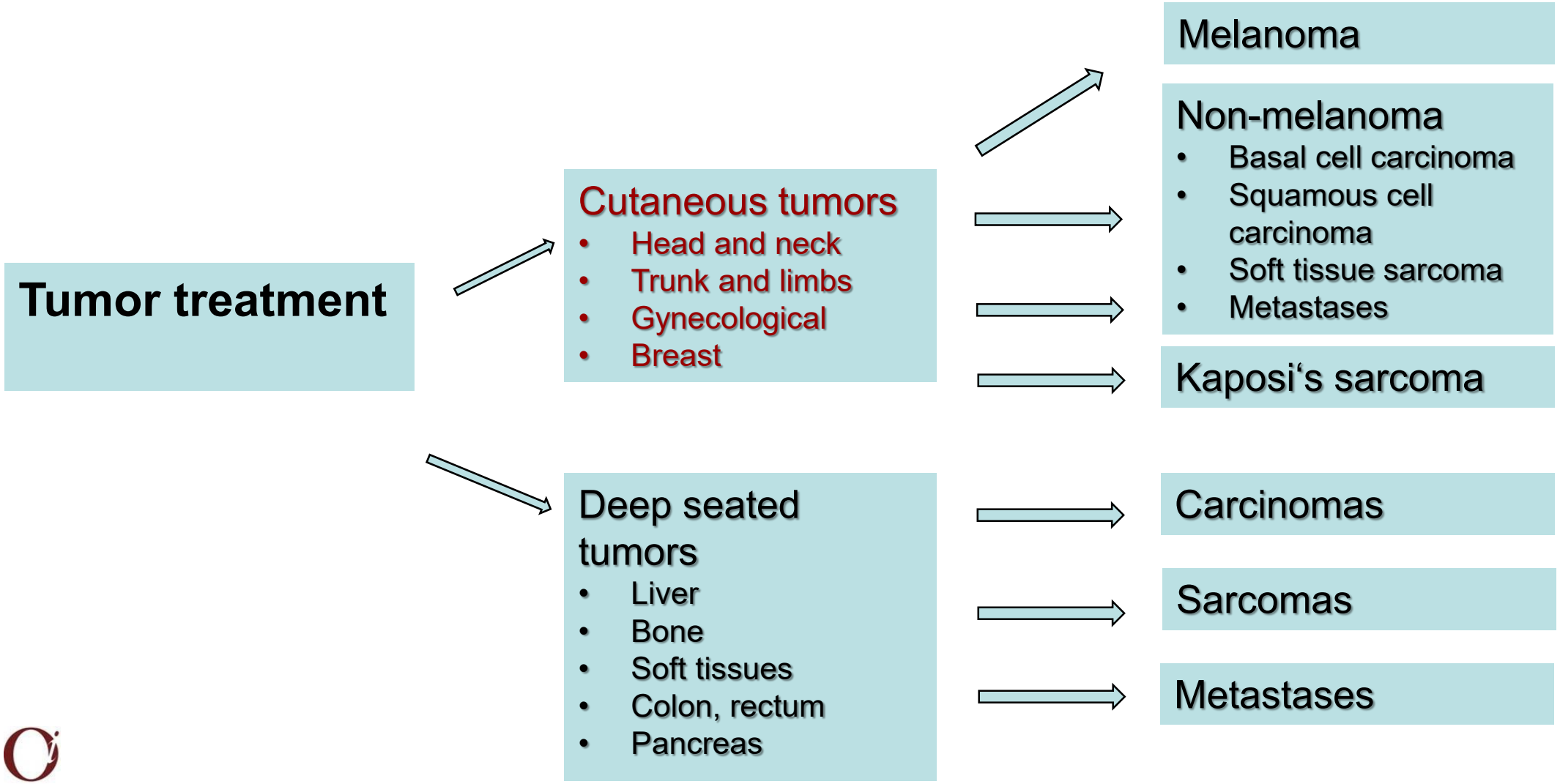
# Reversible electroporation as drug or gene delivery system



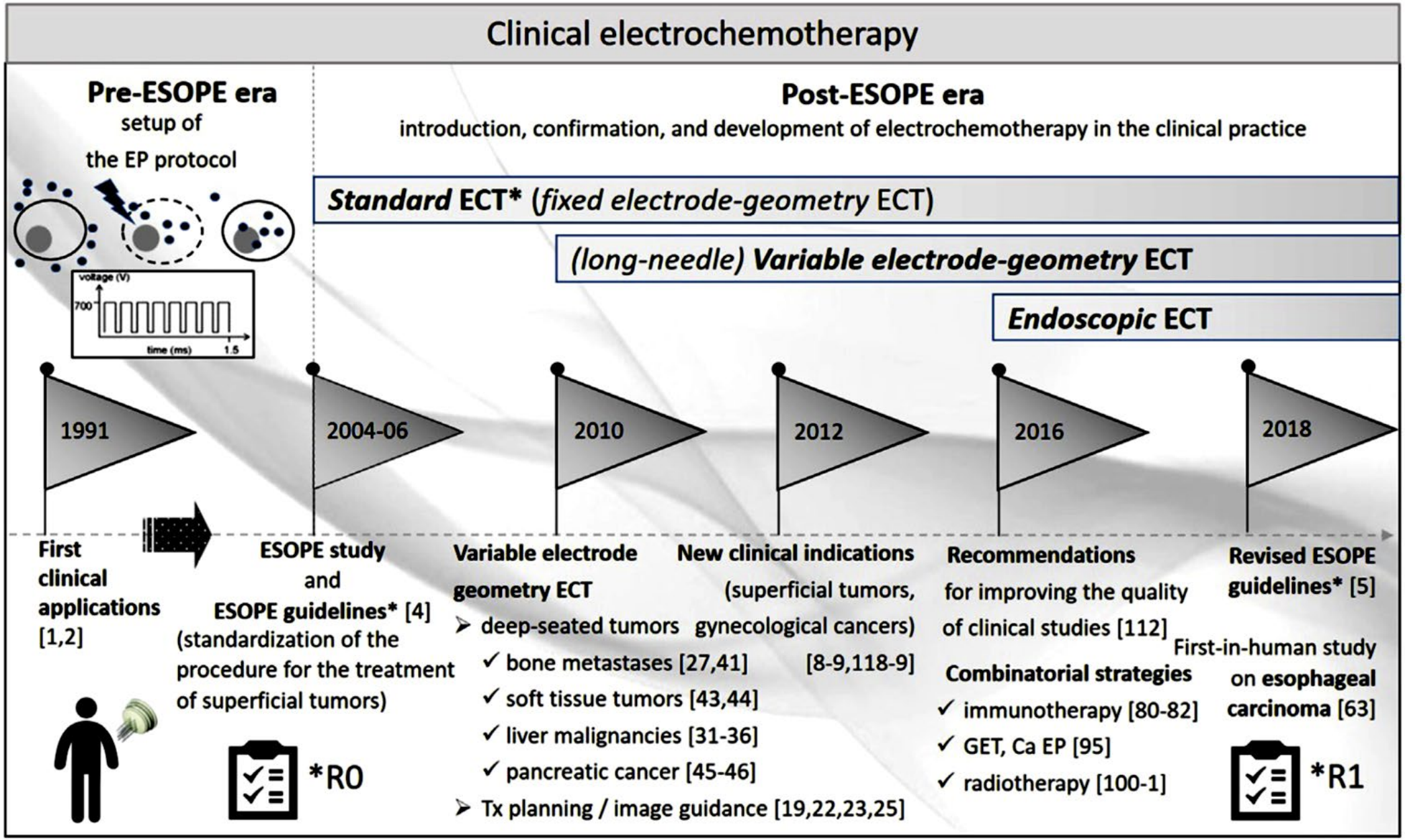
# Clinical applications of reversible electroporation



# Electrochemotherapy and gene electrotransfer in tumor treatment

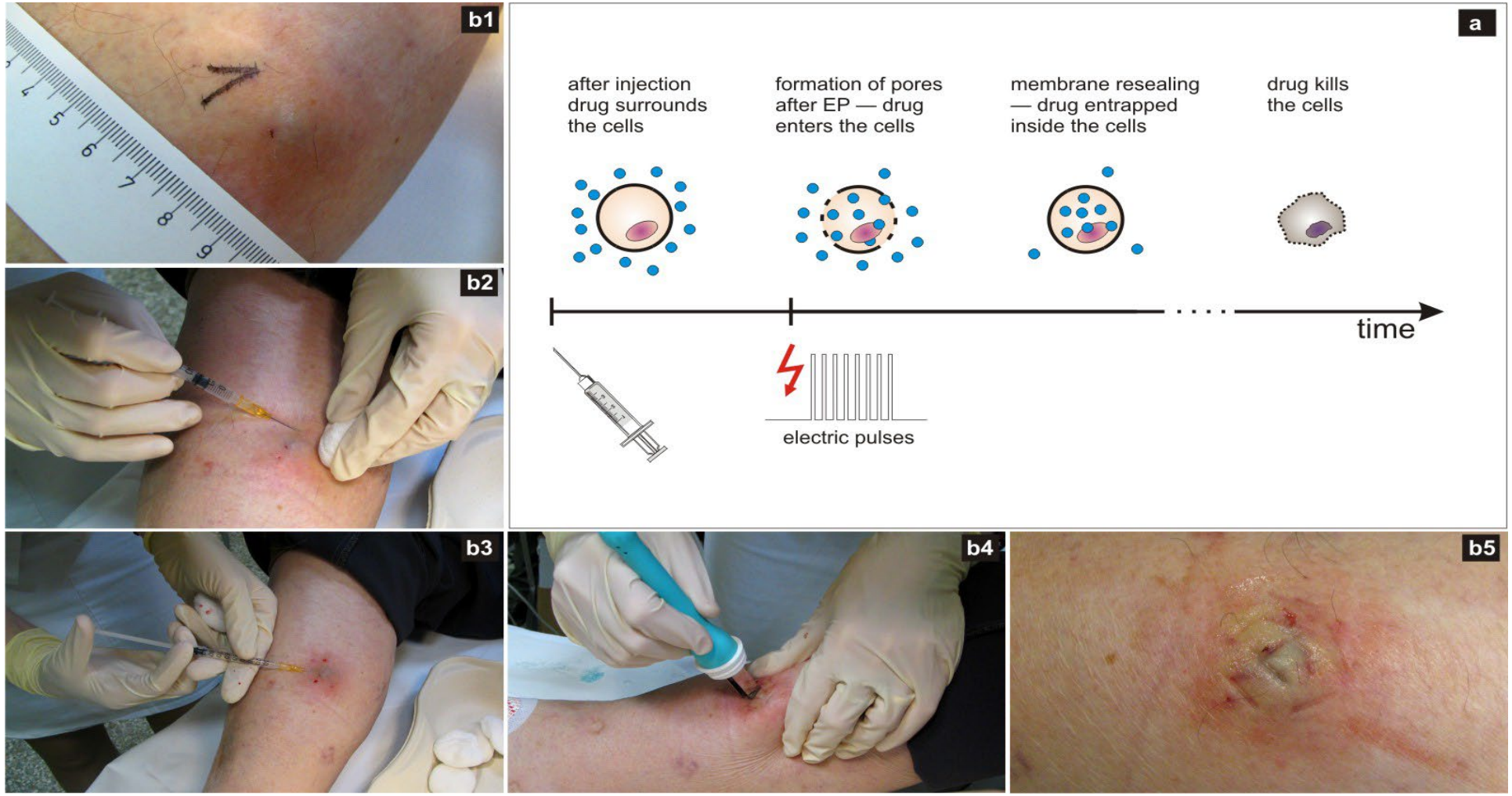


# Timeline of clinical electrochemotherapy




Campana LG et al. EJSO 2019

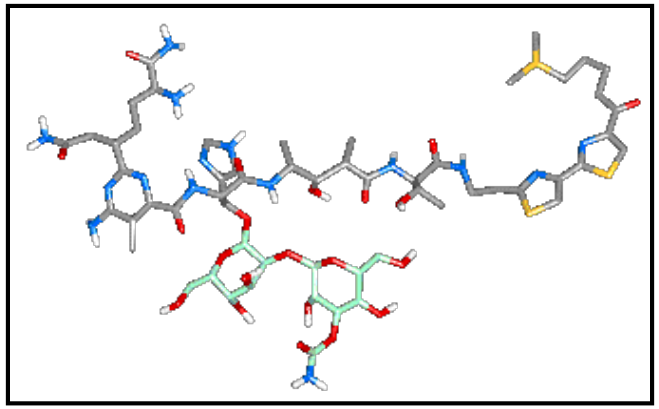
# What is electrochemotherapy ?
# Electrochemotherapy - procedure



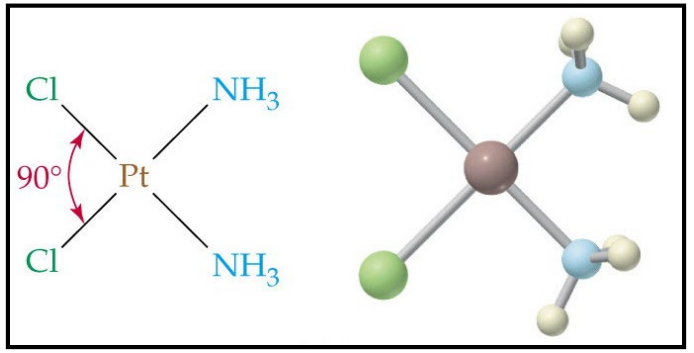

*Marty and Sersa et al. EJC Suppl 2006*

# Which drugs can be used?
# Increased cytotoxicity of chemotherapeutic drugs

- Effective for hydrophilic drugs with hampered transport through the plasma membrane
- Drugs that have clinical applicability:
  - Bleomycin (BLM)
  - Cisplatin (CDDP)
  - Calcium Chloride ($CaCl_2$)



BLM




CDDP

# Electric pulse generator and electrodes for cutaneous tumors

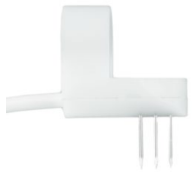

Linear

Hexagonal

Plate

Adjustable Linear

Adjustable Hexagonal



Finger - Orthogonal

Finger - Longitudinal

Single long needle electrodes

# Standard Operating Procedures for ECT

ACTA ONCOLOGICA, 2018
https://doi.org/10.1080/0284186X.2018.1454602

Taylor & Francis
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REVIEW

OPEN ACCESS Check for updates

## Updated standard operating procedures for electrochemotherapy of cutaneous tumours and skin metastases

Julie Gehl[a,b,c], Gregor Sersa[d], Louise Wichmann Matthiessen[c], Tobian Muir[e], Declan Soden[f], Antonio Occhini[g], Pietro Quaglino[h], Pietro Curatolo[i], Luca G. Campana[j,k], Christian Kunte[l,m], A. James P. Clover[f,n], Giulia Bertino[f], Victor Farricha[o], Joy Odili[p], Karin Dahlstrom[q], Marco Benazzo[g] and Lluis M. Mir[r]

[a]Center for Experimental Drug and Gene Electrotransfer (C*EDGE), Department of Clinical Oncology and Palliative Care, Zealand University Hospital, Roskilde, Denmark; [b]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [c]Department of Oncology Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark; [d]Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia; [e]South Tees NHS Foundation Trust, James Cook University Hospital, Middlesbrough, UK; [f]Cork Cancer Research Centre, Western Gateway Building University College Cork, Cork, Ireland; [g]Department of Otolaryngology Head and Neck Surgery, University of Pavia – IRCCS Policlinico San Matteo Foundation, Pavia, Italy; [h]Department of Medical Sciences, Dermatologic Clinic, University of Turin, Turin, Italy; [i]Department of Dermatology and Plastic Surgery, La Sapienza University, Roma, Italy; [j]Department of Surgery Oncology and Gastroenterology (DISCOG), University of Padova, Padova, Italy; [k]Surgical Oncology Unit, Veneto Institute of Oncology IRCCS, Padova, Italy; [l]Department of Dermatologic Surgery and Dermatology, Artemed Fachklinik München, Munich, Germany; [m]Department of Dermatology and Allergology, Ludwig-Maximillian University, Munich, Germany; [n]Department of Plastic Surgery, Cork University Hospital, Cork, Ireland; [o]Melanoma and Sarcoma Unit Department of Surgery, Portuguese Institute of Oncology, Rua Professor Lima Basto, Faculty of Medicine of Lisbon, Lisbon, Portugal; [p]Department of Plastic Surgery, St. George's University Hospitals NHS Foundation Trust, London, UK; [q]Department of Plastic Surgery, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark; [r]Vectorology and Anticancer Therapies, UMR 8203, CNRS, Univ. Paris-Sud, Gustave Roussy, Université Paris-Saclay, Villejuif, France

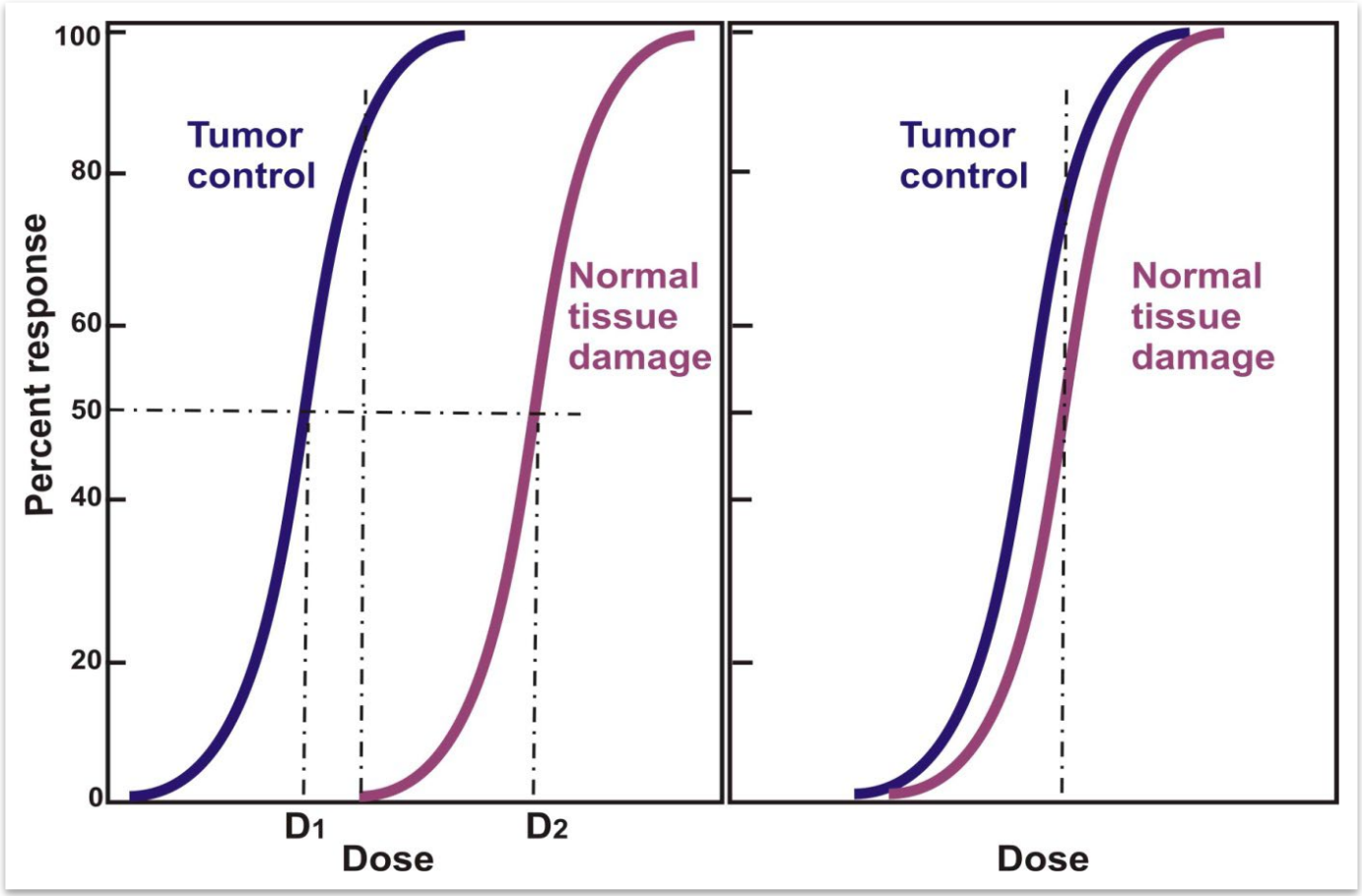
| | Consider local anesthesia / local drug injection | Consider general anesthesia / intravenous drug injection |
|---|---|---|
| Tumour size | ≤ 3 cm | > 3 cm |
| Tumour count | ≤ 7 | > 7 |
| Region suitable for local anesthesia | yes | no |

Deciding treatment strategy based on number and size of tumors to be treated

# Electrochemotherapy as local drug delivery

## Advantages

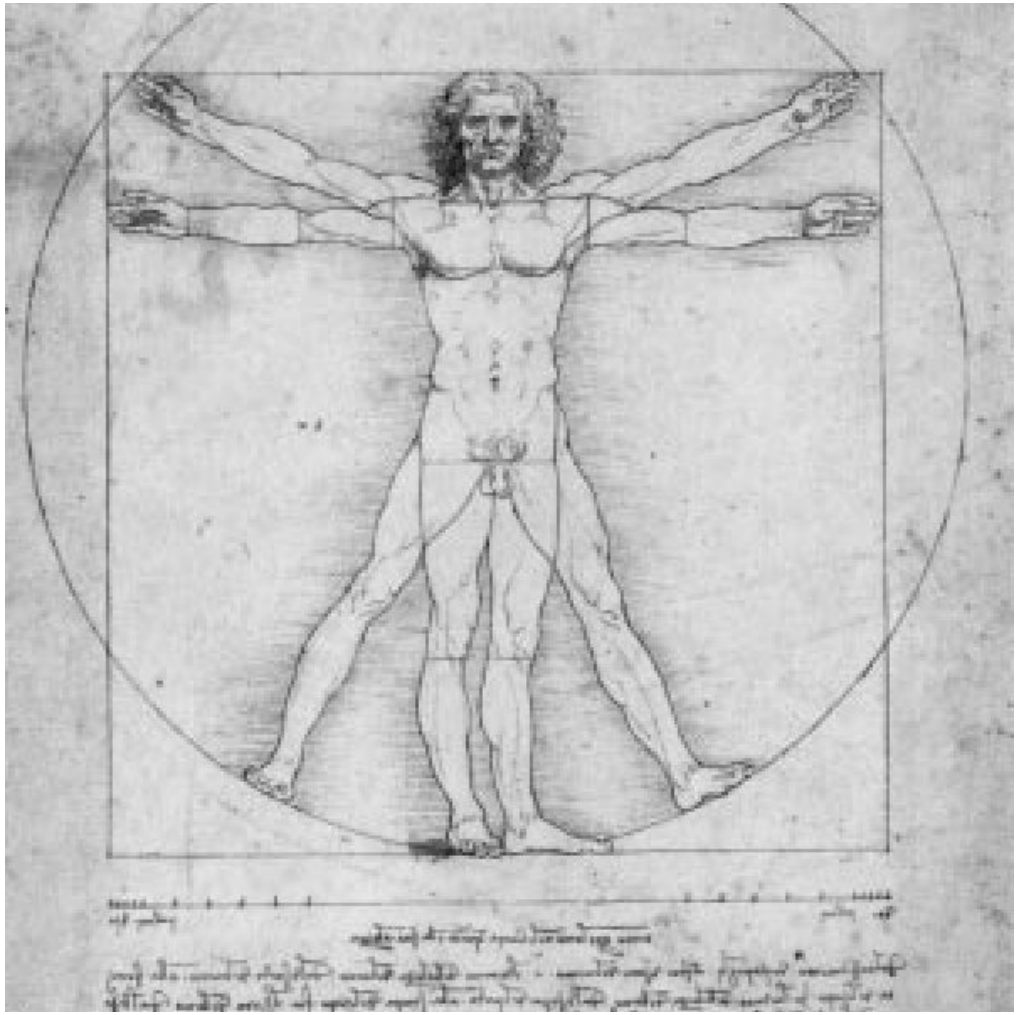
- Increase in therapeutic index:
  - Increase in antitumor efficacy of the administered chemotherapeutic drug.
  - Less side effects, because lower doses are needed for the treatment.



# Possible locations of the tumors

**Cutaneous tumors – locations:**

- Head and neck
- Trunk and limbs
- Gynecological
- Breast

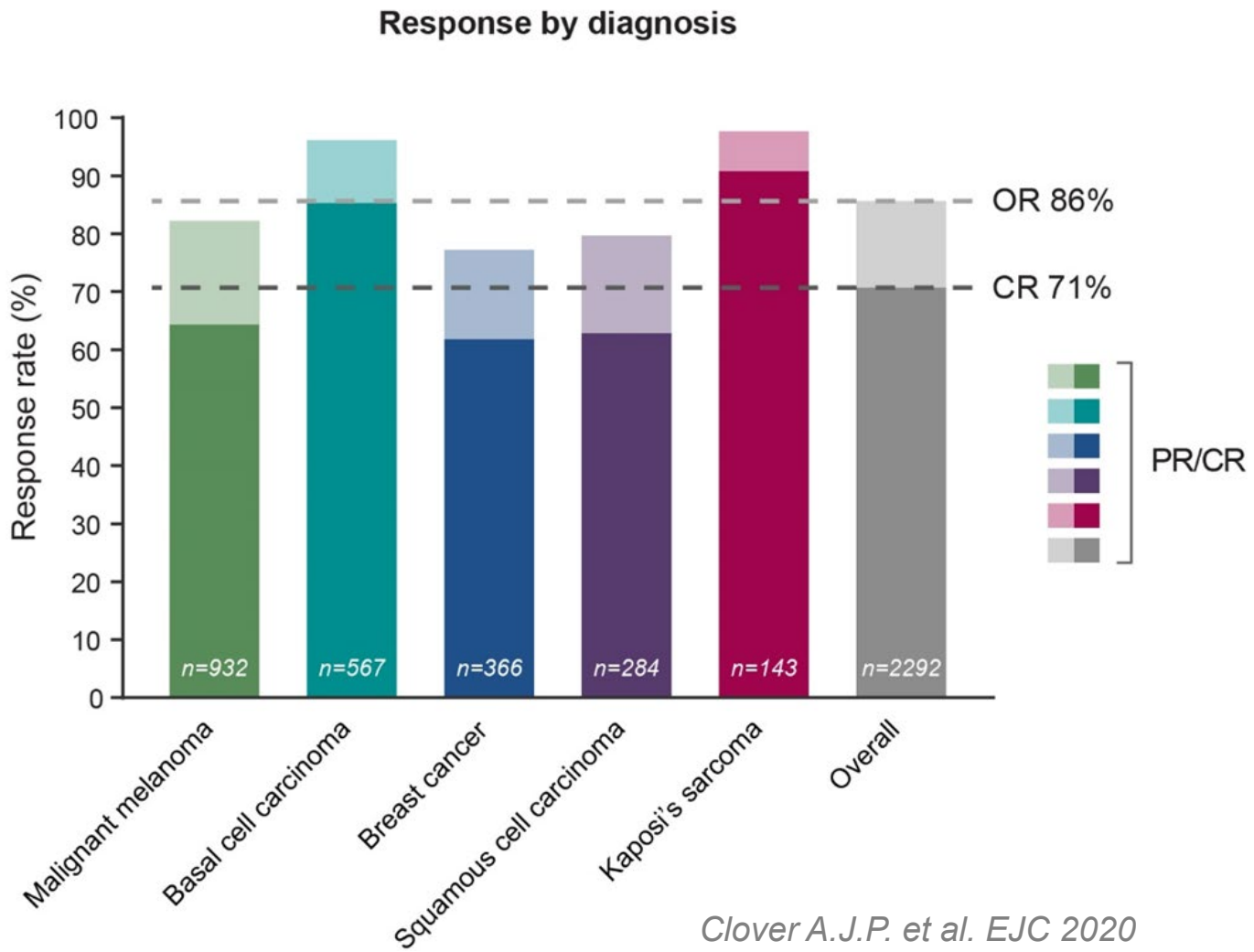


*Leonardo da Vinci*

# Effectiveness of electrochemotherapy on cutaneous tumors

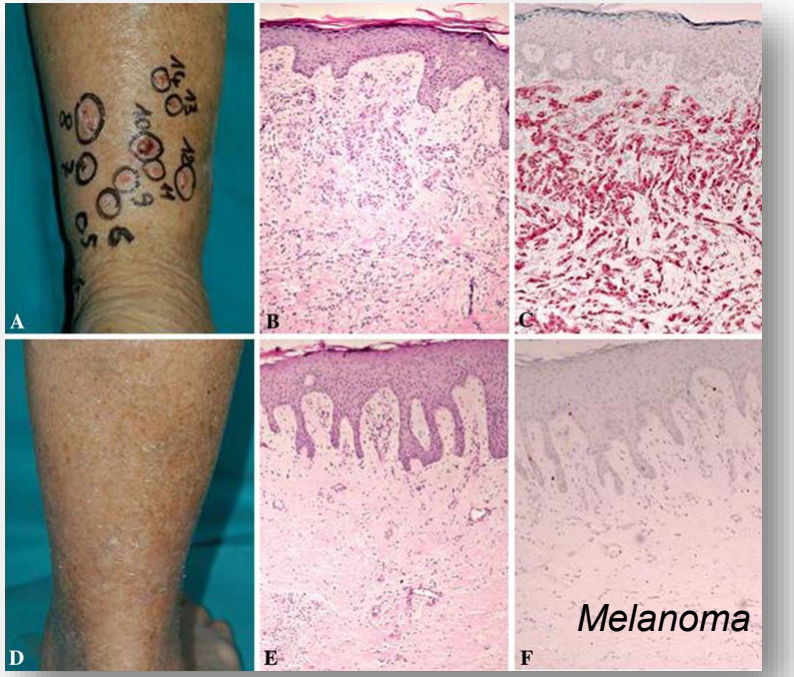
**Which tumors can be treated‘**

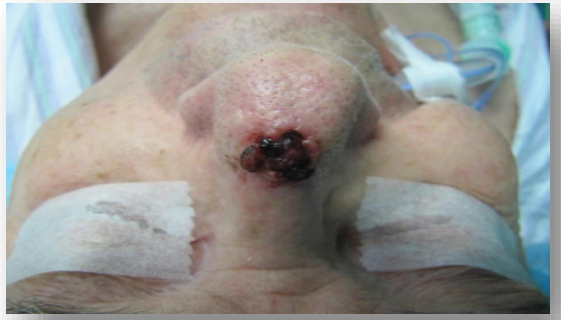
- Malignant melanoma
- Basal cell carcinoma
- Breast cancer
- Squamous cell carcinoma
- Kaposi‘s sarcoma
- And others…..

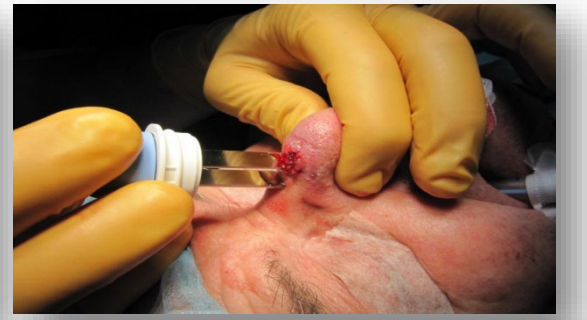


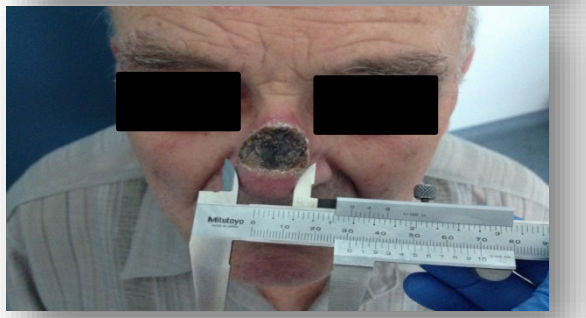

*Clover A.J.P. et al. EJC 2020*

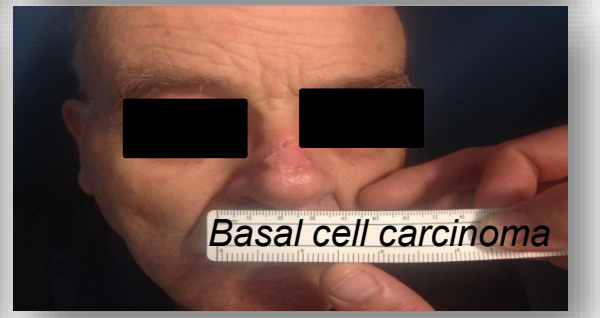
# Examples of tumors treated:

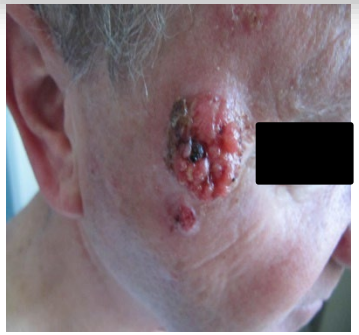


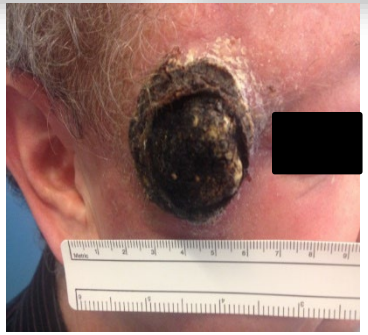



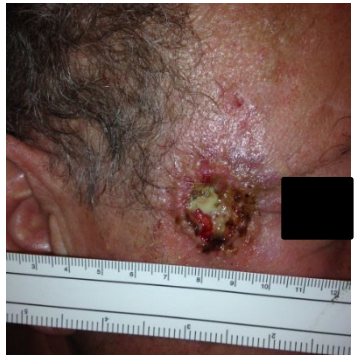


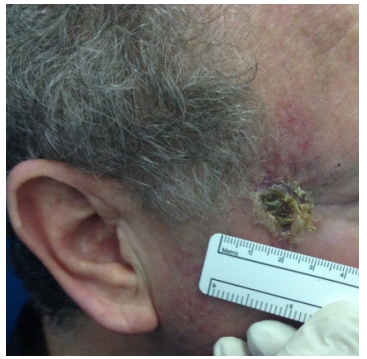




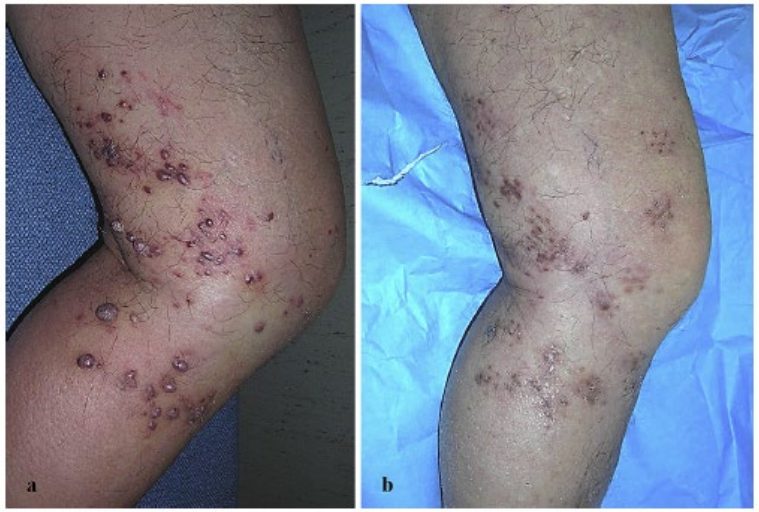









*Squamous cell carcinoma*




*Kaposi‘s sarcoma*

# Recent article on ECT

frontiers | Frontiers in Oncology

TYPE Brief Research Report
PUBLISHED 20 September 2022
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OPEN ACCESS

EDITED BY
Fabrizio Carta,
University of Florence, Italy

REVIEWED BY
Carlos Eduardo Fonseca-Alves,
Paulista University, Brazil
Luca Falzone,
G. Pascale National Cancer Institute Foundation (IRCCS), Italy

*CORRESPONDENCE
Giulia Bertino
giulia.bertino@tin.it

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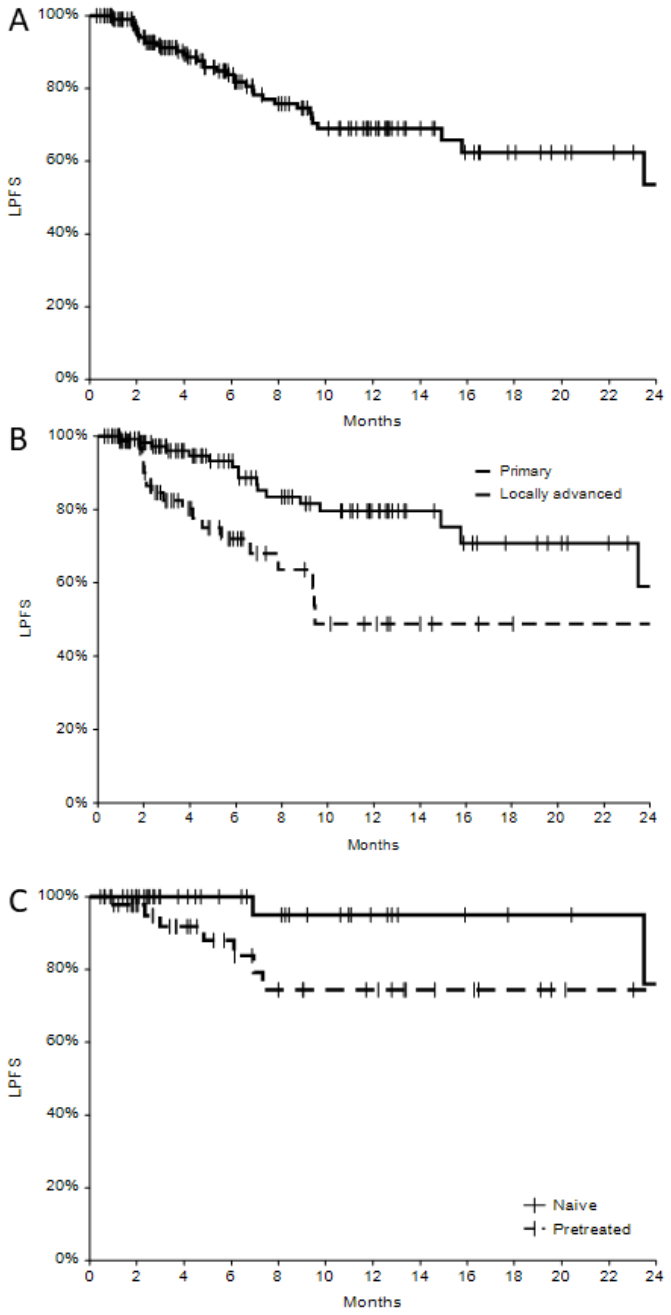
CITATION

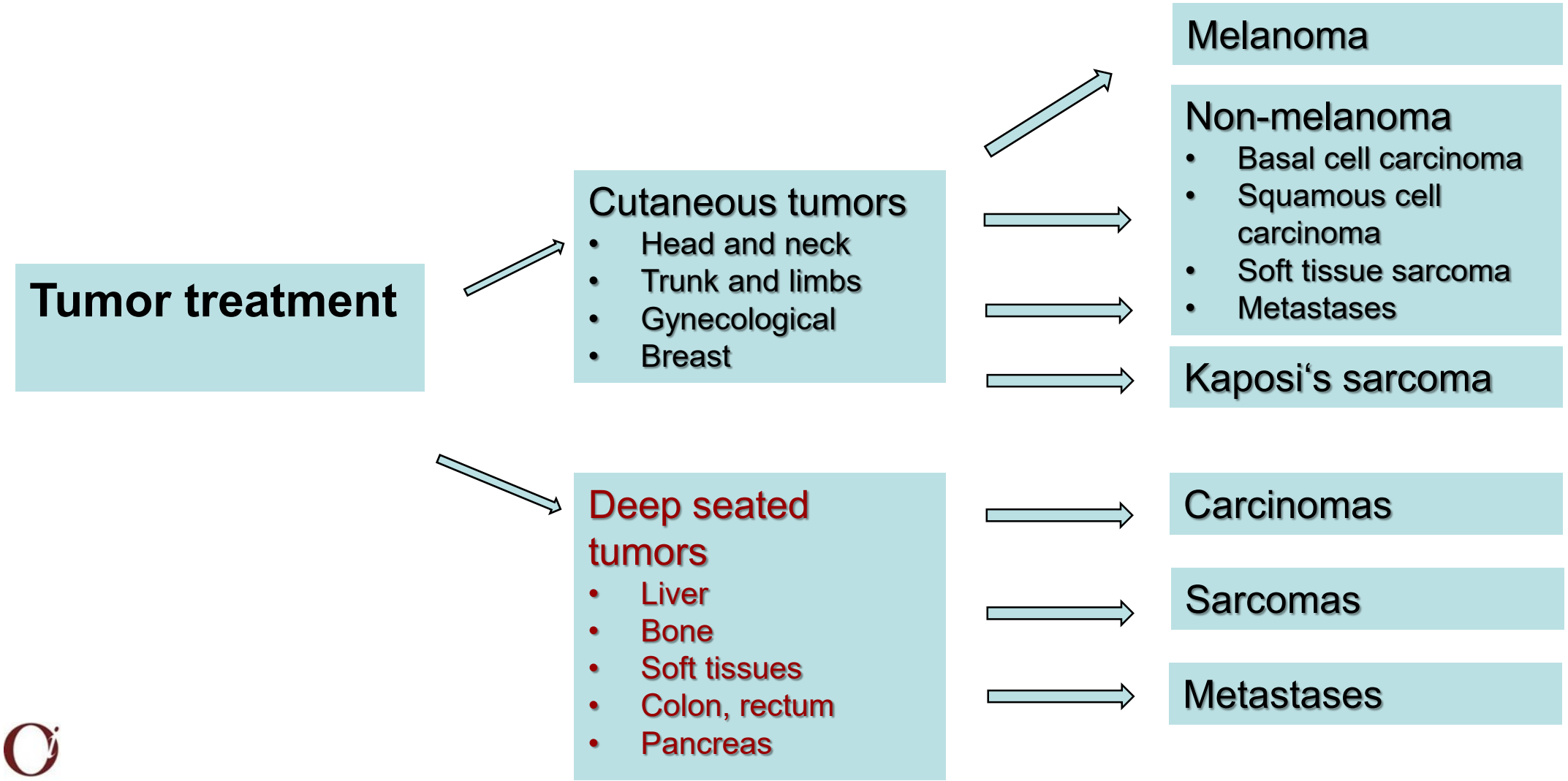
## Electrochemotherapy for the treatment of cutaneous squamous cell carcinoma: The INSPECT experience (2008-2020)

Giulia Bertino[1*], Ales Groselj[2], Luca G. Campana[3], Christian Kunte[4], Hadrian Schepler[5], Julie Gehl[6,7], Tobian Muir[8], James A. P. Clover[9,10], Pietro Quaglino[11], Erika Kis[12], Matteo Mascherini[13], Brian Bisase[14], Giancarlo Pecorari[15], Falk Bechara[16], Paolo Matteucci[17], Joy Odili[18], Francesco Russano[19], Antonio Orlando[20], Rowan Pritchard-Jones[21], Graeme Moir[22], David Mowatt[23], Barbara Silvestri[24], Veronica Seccia[25], Werner Saxinger[26], Francesca de Terlizzi[27] and Gregor Sersa[28,29]



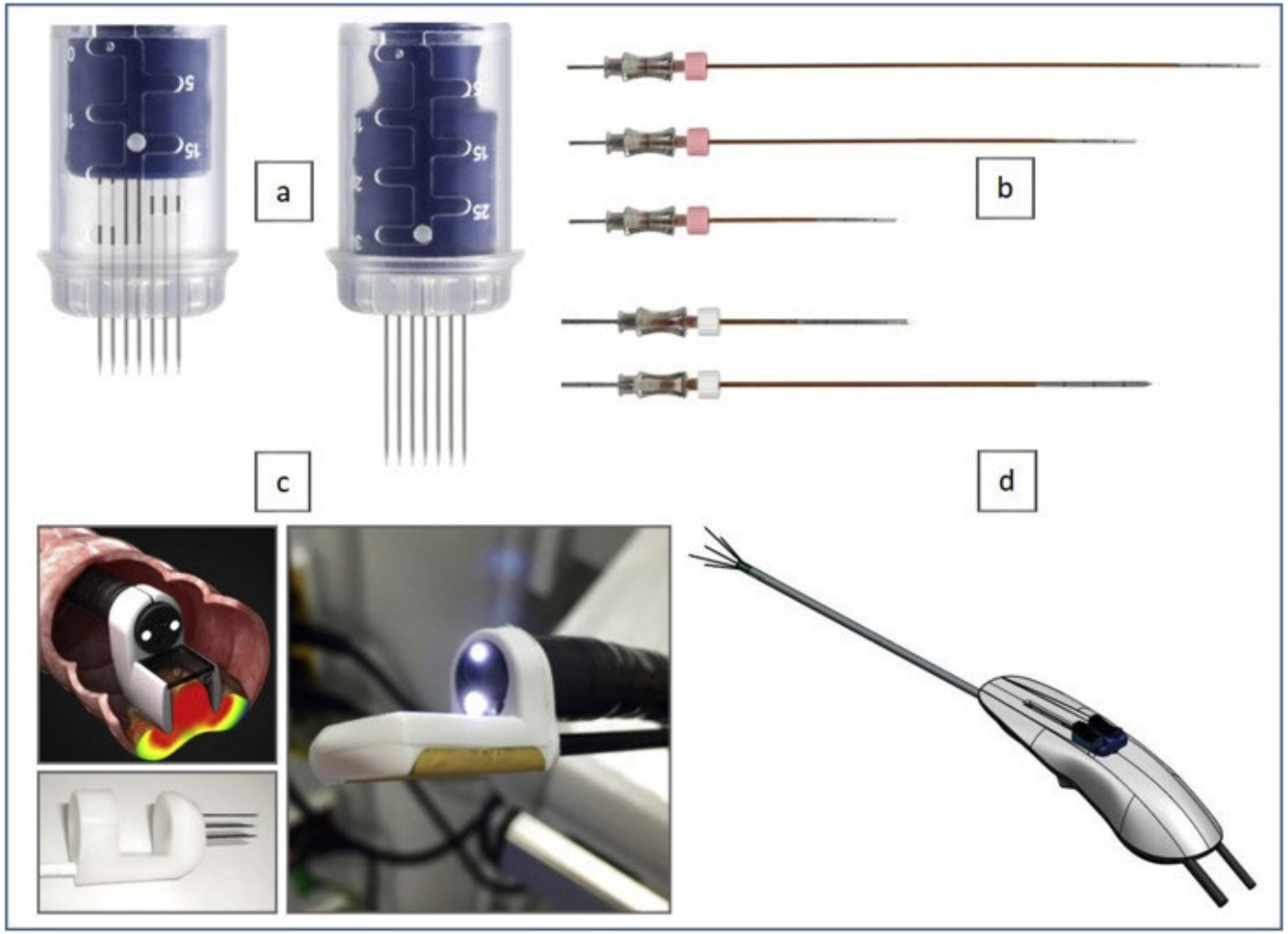

ECT showed antitumor activity and a favorable safety profile in patients with complex cSCC for whom there was no widely accepted standard of care. Better results were obtained in primary and small tumors (<3 cm) using intravenous bleomycin administration.

# Electrochemotherapy and gene electrotransfer in tumor treatment



# The technology is adapted for treatment of deep seated tumors

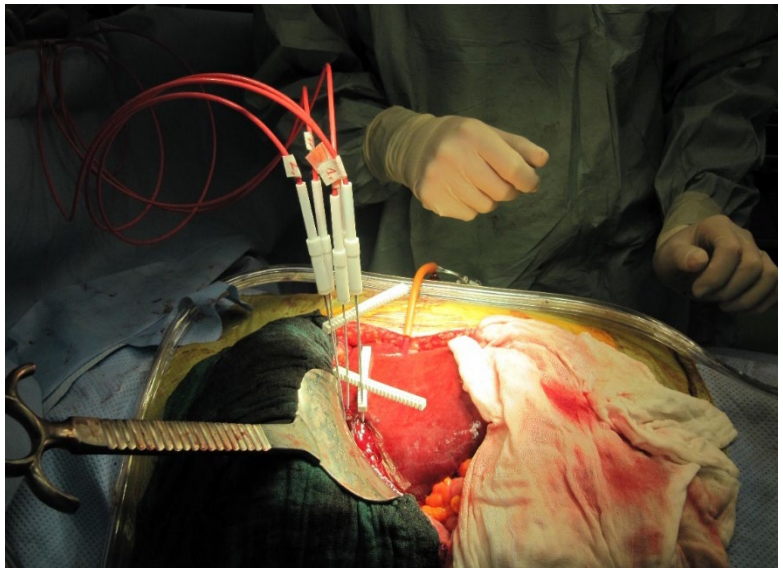
- Electrodes for different uses:
  - A: Intraoperative
  - B: Intraoperative or pecutaneous
  - C: Endoscopic
  - D: Laparoscopic

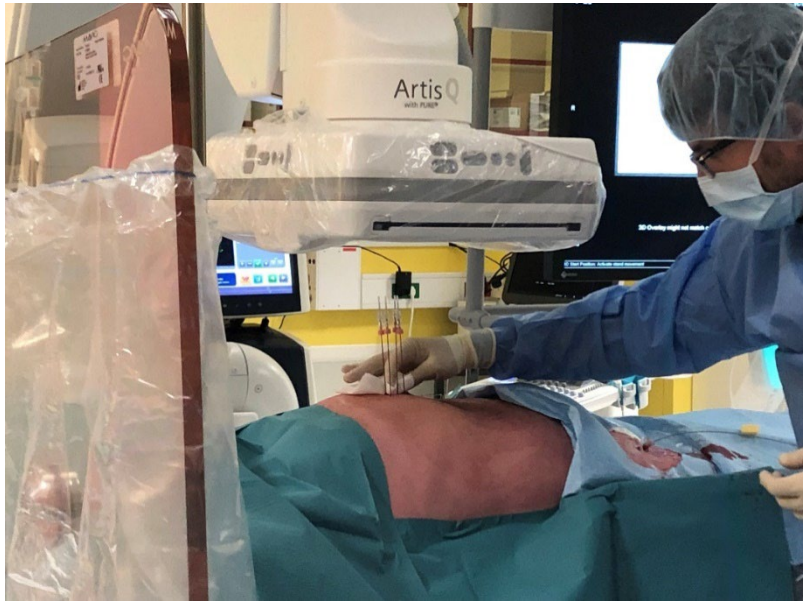


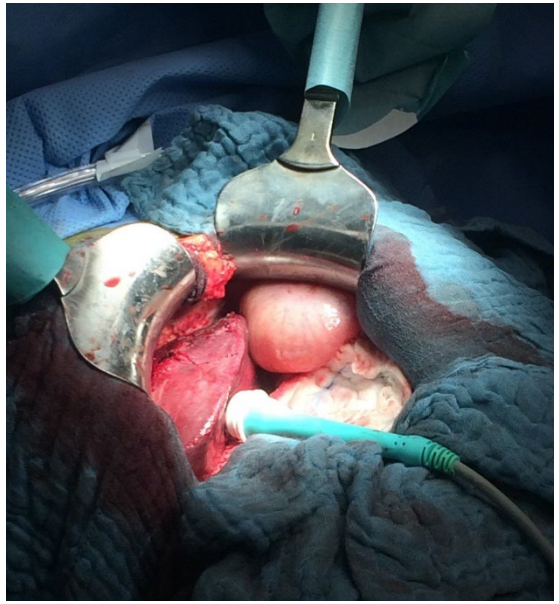

*Campana LG et al. EJSO 2019*

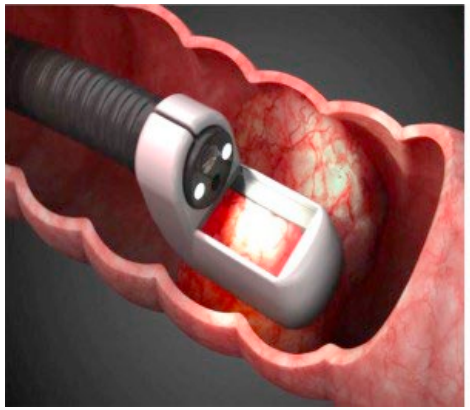
# Examples of therapeutic approaches

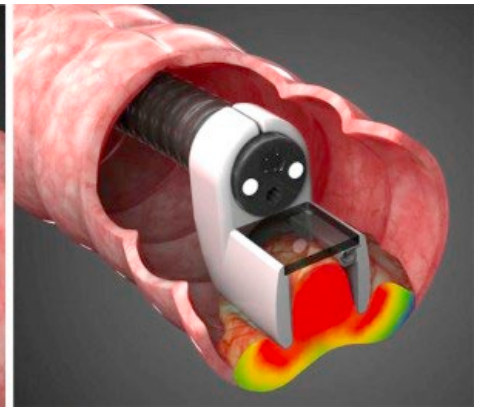
- Intraoperative
- Percutaneous
- Endoscopic
- Laparoscopic



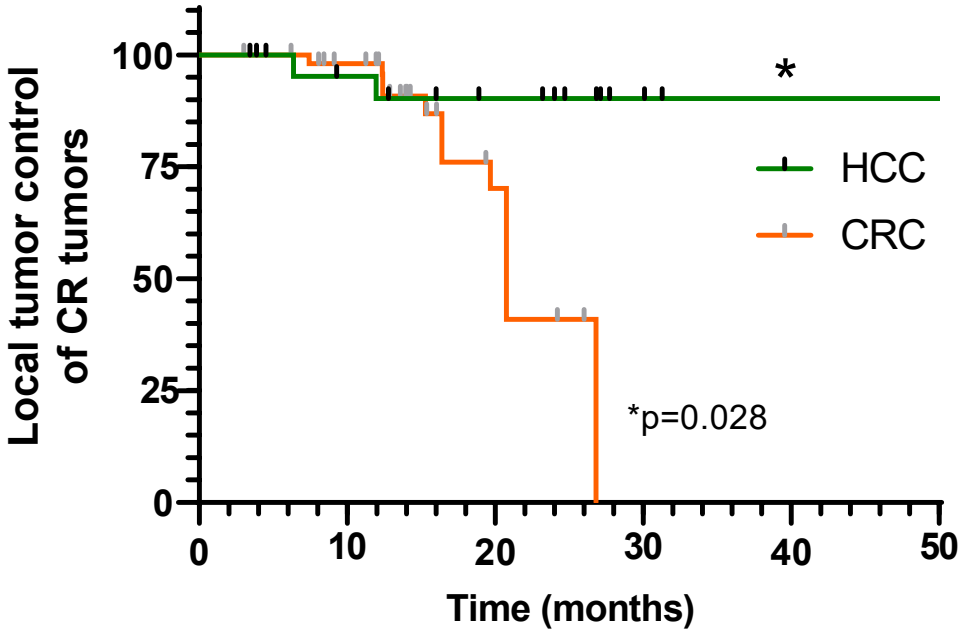




*Hansen F. H. et al. Endosc Int Open. 2020*

# Is it effective?

- Liver
  - Colorectal metastases (CRC)
  - Hepatocellular carcinoma (HCC)
- Bone
- Soft tissue
- Colon, rectum
- Pancreas

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Intraoperative electrochemotherapy of colorectal liver metastases: A prospective phase II study

Ibrahim Edhemovic [a, b, *], Erik Brecelj [a], Maja Cemazar [a, c], Nina Boc [a], Blaz Trotovsek [b, d], Mihajlo Djokic [b, d], Rok Dezman [d], Arpad Ivanecz [e, f], Stojan Potrc [e, f], Masa Bosnjak [a], Bostjan Markelc [a, g], Bor Kos [h], Damijan Miklavcic [h], Gorana Gasljevic [a], Gregor Sersa [a, g, **]

cancers

MDPI

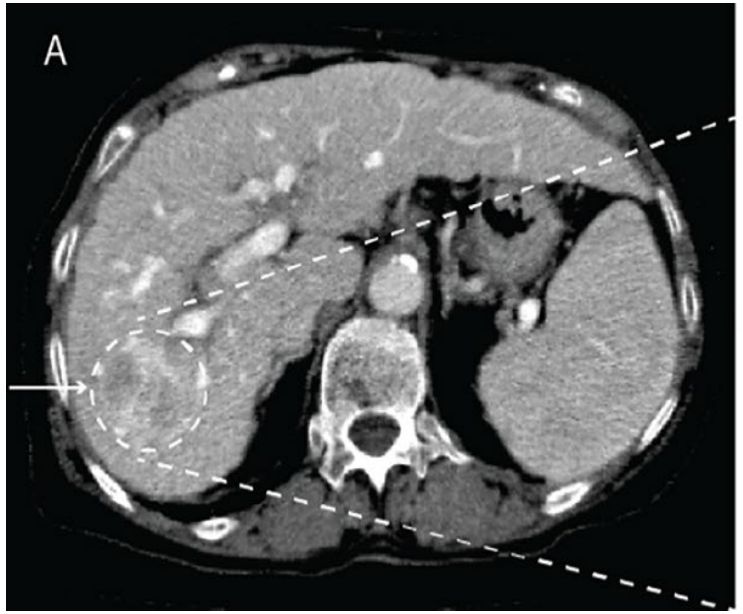
*Article*

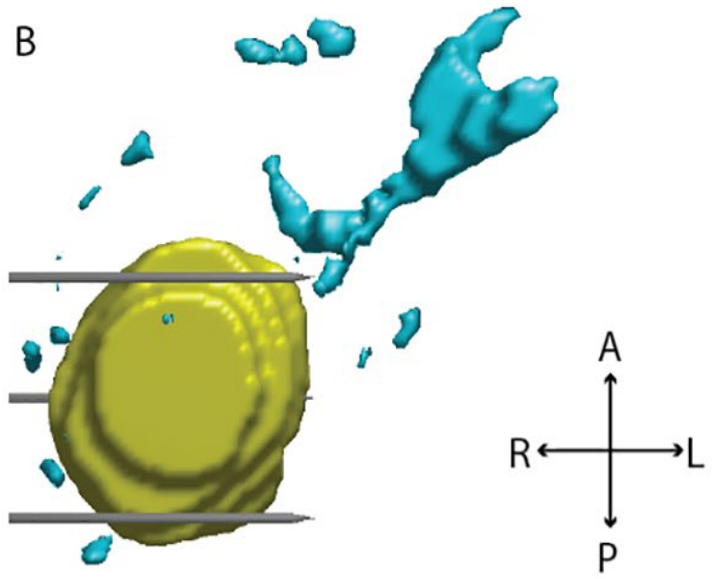
A Prospective Phase II Study Evaluating Intraoperative Electrochemotherapy of Hepatocellular Carcinoma

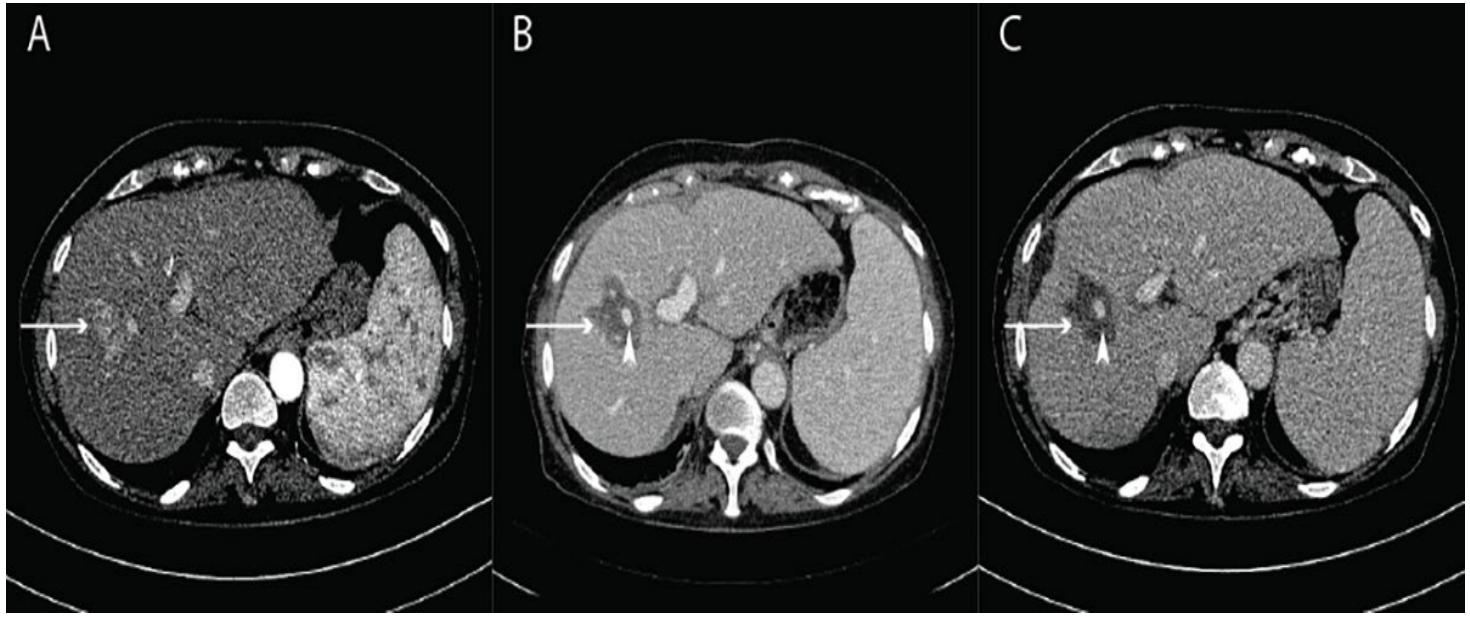
Mihajlo Djokic [1,2], Maja Cemazar [3,4], Masa Bosnjak [3], Rok Dezman [2,5], David Badovinac [1,2], Damijan Miklavcic [6], Bor Kos [6], Miha Stabuc [2,5], Borut Stabuc [2,7], Rado Jansa [2,7], Peter Popovic [2,5], Lojze M. Smid [2,7], Gregor Sersa [3,8,*] and Blaz Trotovsek [1,2,*]

# ECT of liver tumors Conclusions

- Electrochemotherapy of colorectal liver metastases proved to be feasible, safe and efficient treatment modality
- It was shown that ECT has a specific place in difficult to treat metastases, located in the vicinity of major hepatic vessels, not amenable to surgery or radiofrequency ablation.



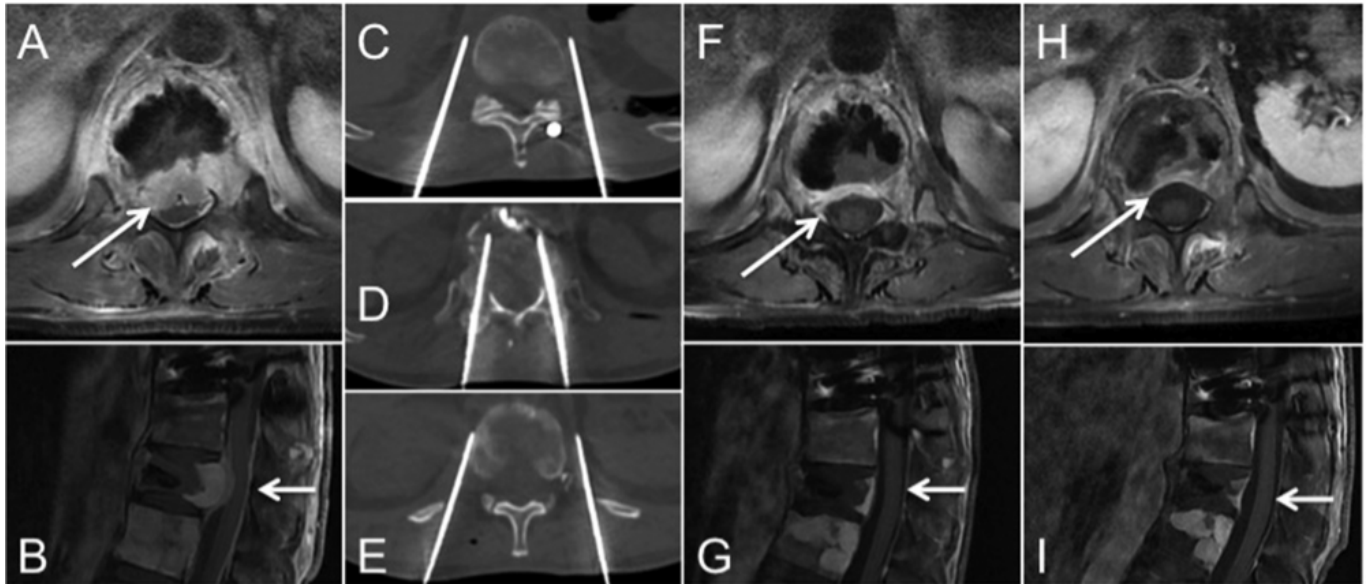
# Is it effective?

- Liver
  - Colorectal metastases
  - Hepatocellular carcinoma
- Bone
- Soft tissue
- Colon, rectum
- Pancreas

**Table 3.** Response to treatment according to RECIST and PERCIST criteria, and pain pre- and post-ECT (at early and at late follow-up).

| | RECIST | | PERCIST | | Pain | Before ECT | | Early FU | | Late FU | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | | N | % | N | % | N | % |
| CR | 3 | 9% | 5 | 14% | no | 4 | 11% | 8 | 27% | 7 | 47% |
| PR | 6 | 16% | 8 | 22% | mild | 3 | 8% | 13 | 43% | 5 | 33% |
| SD | 22 | 59% | 5 | 14% | moderate | 18 | 47% | 6 | 20% | 1 | 7% |
| PD | 6 | 16% | 19 | 50% | severe | 13 | 34% | 3 | 10% | 2 | 13% |

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; ECT = electrochemotherapy; FU = follow-up.




European Journal of Cancer 186 (2023) 62–68

Available online at www.sciencedirect.com

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Original Research

## Electrochemotherapy in radiotherapy-resistant epidural spinal cord compression in metastatic cancer patients

Frederic Deschamps [a,b,*], Lambros Tselikas [a], Steven Yevich [a], Baptiste Bonnet [a], Charles Roux [a], Adrian Kobe [a], Benjamin Besse [c], Kevin Berthelot [d], Amelie Gaudin [e], Lluis M. Mir [b], Thierry de Baere [a]

Journal of MEDICAL IMAGING and RADIATION ONCOLOGY

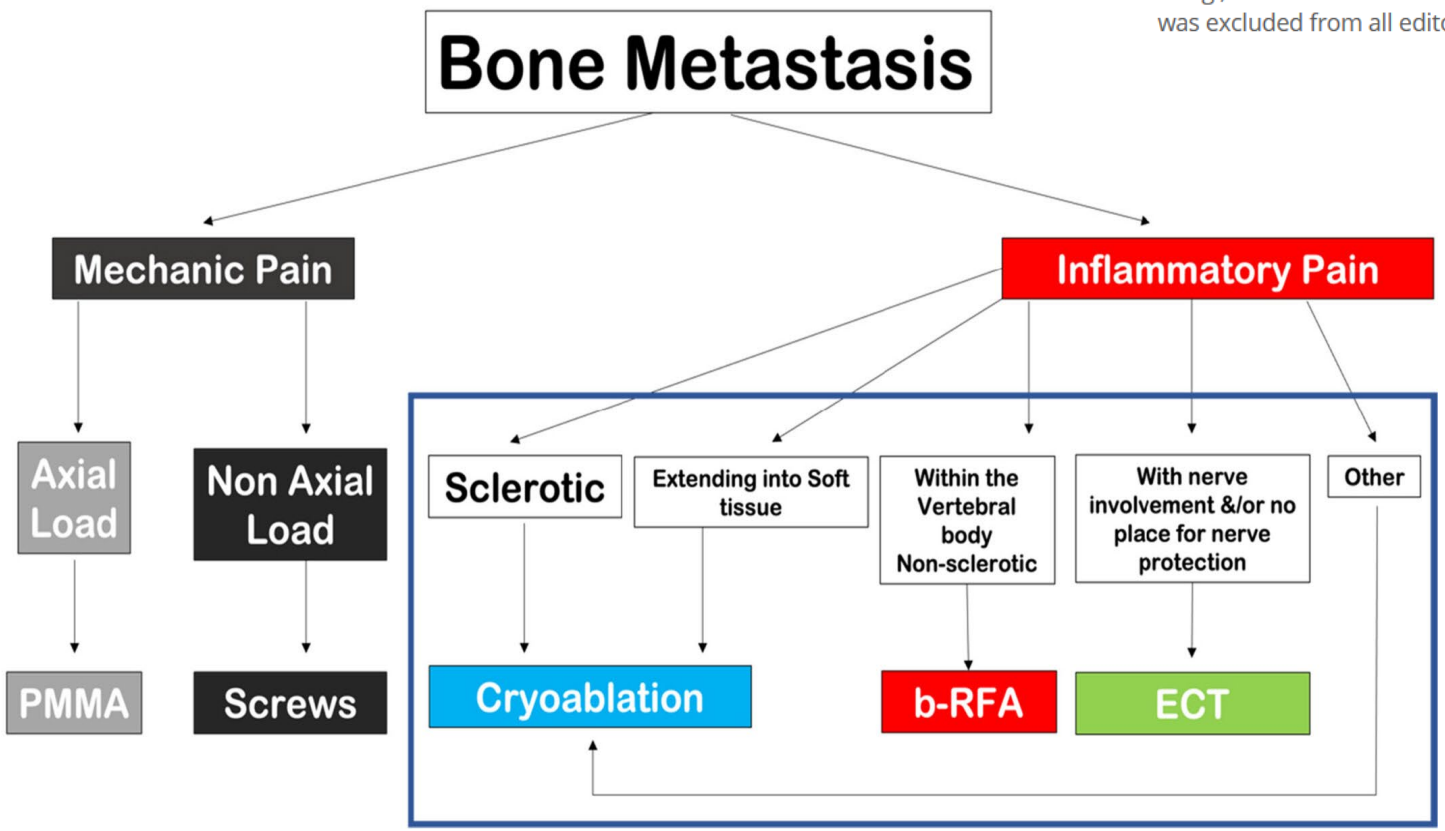
Radiation Oncology—Review Article | Full Access

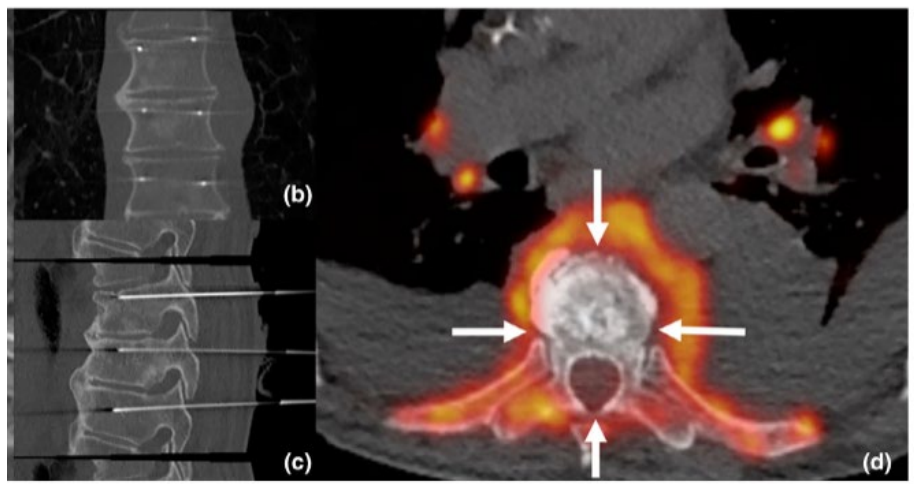
# Interventional management of malignant bone tumours

Roberto Luigi Cazzato, Julien Garnon, Jack William Jennings, Afshin Gangi

First published: 24 September 2023 | https://doi.org/10.1111/1754-9485.13587

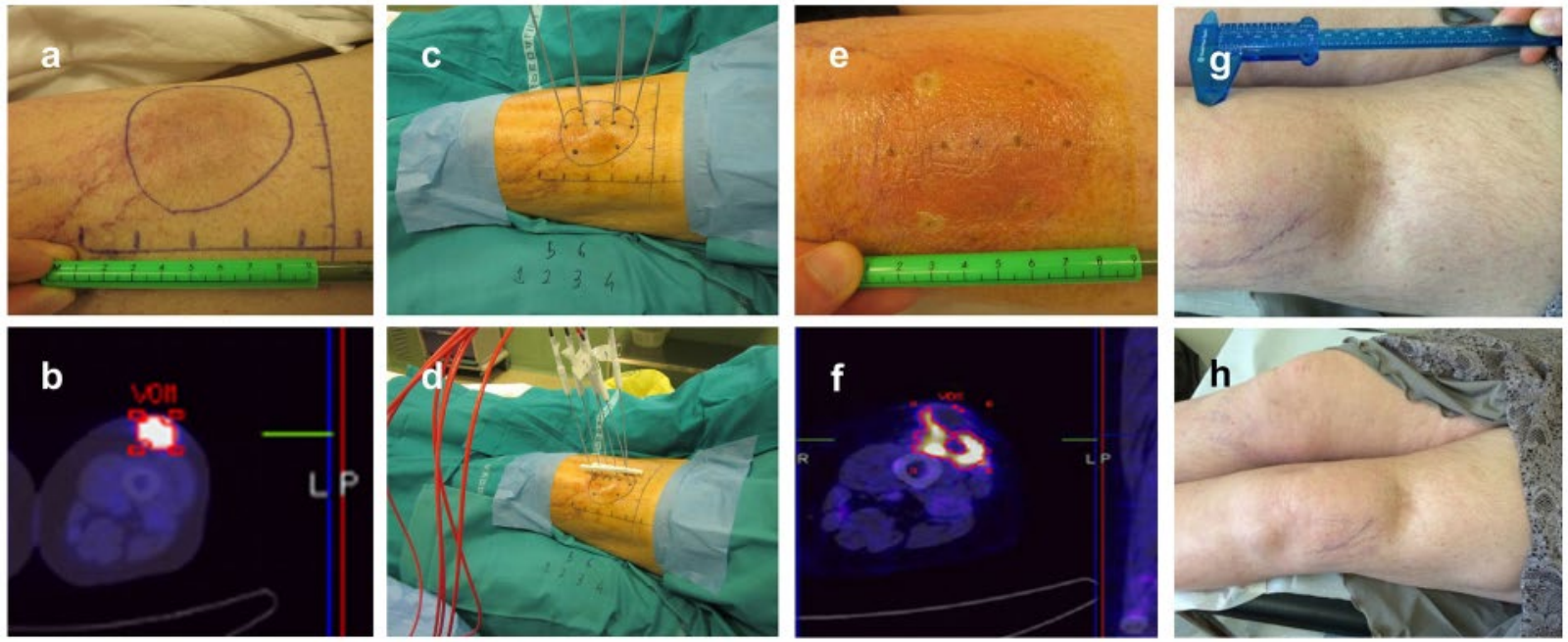
**RL Cazzato:** MD, PhD; **J Garnon** MD, PhD; **JW Jennings** MD, PhD; **A Gangi** MD, PhD.
Gangi, Afshin is an Editorial Board member of JMIRO and a co-author of this article. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication.



# Is it effective?

- Liver
  - Colorectal metastases
  - Hepatocellular carcinoma
- Bone
- Soft tissue
- Colon, rectum
- Pancreas




SCIENTIFIC REPORTS
nature research

OPEN

## Ablation of soft tissue tumours by long needle variable electrode-geometry electrochemotherapy: final report from a single-arm, single-centre phase-2 study

Andrea Simioni[1,11], Sara Valpione[2,3,11], Elisa Granziera[4], Carlo Riccardo Rossi[5], Francesco Cavallin[6], Romina Spina[4], Elisabetta Sieni[7,8], Camillo Aliberti[9], Roberto Stramare[10] & Luca Giovanni Campana[5*]

patient experienced grade-3 ulceration and infection. One-month $^{18}$F-FDG-SUV decreased by 86%; CRR was 63% (95% CI 44–79%). Local control was durable in 24 of 30 patients (two-year LPFS, 62%). Patients reported an improvement in "usual activities", "anxiety/depression", and "overall health" scores. VEG-ECT demonstrated encouraging antitumour activity in soft-tissue malignancies; a single course of treatment produced high and durable responses, with low complications.

# Is it effective?

- Liver
  - Colorectal metastases
  - Hepatocellular carcinoma
- Bone
- Soft tissue
- Colon, rectum
- Pancreas

Table 2 Treatments.

| Patient | Country | Treatment number | Bleomycin (IE) | | Pulses (n) | Highest current (A) | Treated tumor surface | Treatment duration (min) | Comment to treatment | Treatment outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ireland | 1 | 31,500 | IV | 9 | 7 | >50% | 80 | | Partial response |
| | | 2 | 31,500 | IV | 12 | 11 | ~ 100% | 18 | | Complete response |
| 2 | Ireland | 1 | 20,000 | IV | 17 | 16 | >25% | 25 | | Partial response |
| | | 2 | 20,000 | IV | 31 | | ~ 100% | 39 | | Complete response |
| 3 | Ireland | 1 | 15,000 | IV | 8 | 4 | >75% | 22 | Voltage decreased to 650 V due to discomfort from muscular contractions. | Partial response |
| 4 | Ireland | 1 | 25,000<br>5,000 | IV<br>IT | 11 | 16 | >25% | 32 | | Partial response |
| 5 | Denmark | 1 | 27,450 | IV | 34 | 13 | >25% | 62 | | Partial response |
| 6 | Denmark | 1 | 27,000 | IV | 7 | 21 | >50% | 48 | Voltage decreased to 900 V due to lack of capacity of the cliniporator. | Partial response |
| | | 2 | 27,000 | IV | 18 | 21 | >75% | 24 | Voltage decreased to 900 V due to lack of capacity of the cliniporator. | Partial response |
| 7 | Denmark | | | | | | | | | Complete response |

IV, intravenous; IT, i

Original article

Thieme

**Electrochemotherapy for colorectal cancer using endoscopic electroporation: a phase 1 clinical study**

OPEN ACCESS

Authors
Hanne Falk Hansen[1], Michael Bourke[2], Trine Stigaard[3], James Clover[2], Martin Buckley[4], Micheal O'Riordain[5], Des C. [6], Helle Hjorth Johannesen[7], Rasmus Hvass Hansen[7], Hanne Heebøll[7], Patrick Forde[2], Henrik Loft Jakobsen[3], sen[8], Jacob Rosenberg[3,10], Declan Soden[2], Julie Gehl[1,9,10]

**Results** Seven patients aged 62 to 88 years with multiple comorbidities were included and had one or two treatments each. Post-treatment scans showed tumor responses in the treated areas and no damage to surrounding tissues. Only a few grade one adverse events were reported. Three patients had preoperative rectal bleeding, of which two reported cessation of bleeding and one reported decreased bleeding.

# Is it effective?

- Liver
  - Colorectal metastases
  - Hepatocellular carcinoma
- Bone
- Soft tissue
- Colon, rectum
- Pancreas

Journal of *Clinical Medicine* MDPI

*Article*

## Clinical Phase I/II Study: Local Disease Control and Survival in Locally Advanced Pancreatic Cancer Treated with Electrochemotherapy

Francesco Izzo [1], Vincenza Granata [2], Roberta Fusco [3,*], Valeria D'Alessio [3], Antonella Petrillo [2], Secondo Lastoria [4], Mauro Piccirillo [1], Vittorio Albino [1], Andrea Belli [1], Salvatore Tafuto [5], Antonio Avallone [6], Renato Patrone [7] and Raffaele Palaia [1]

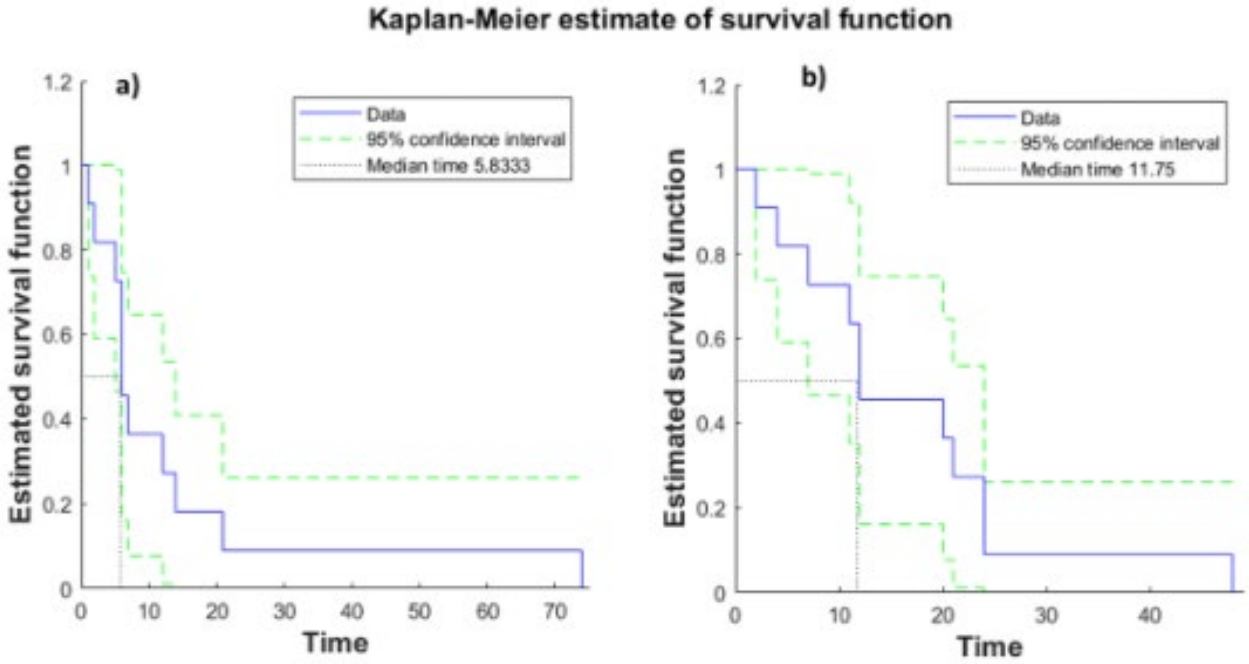
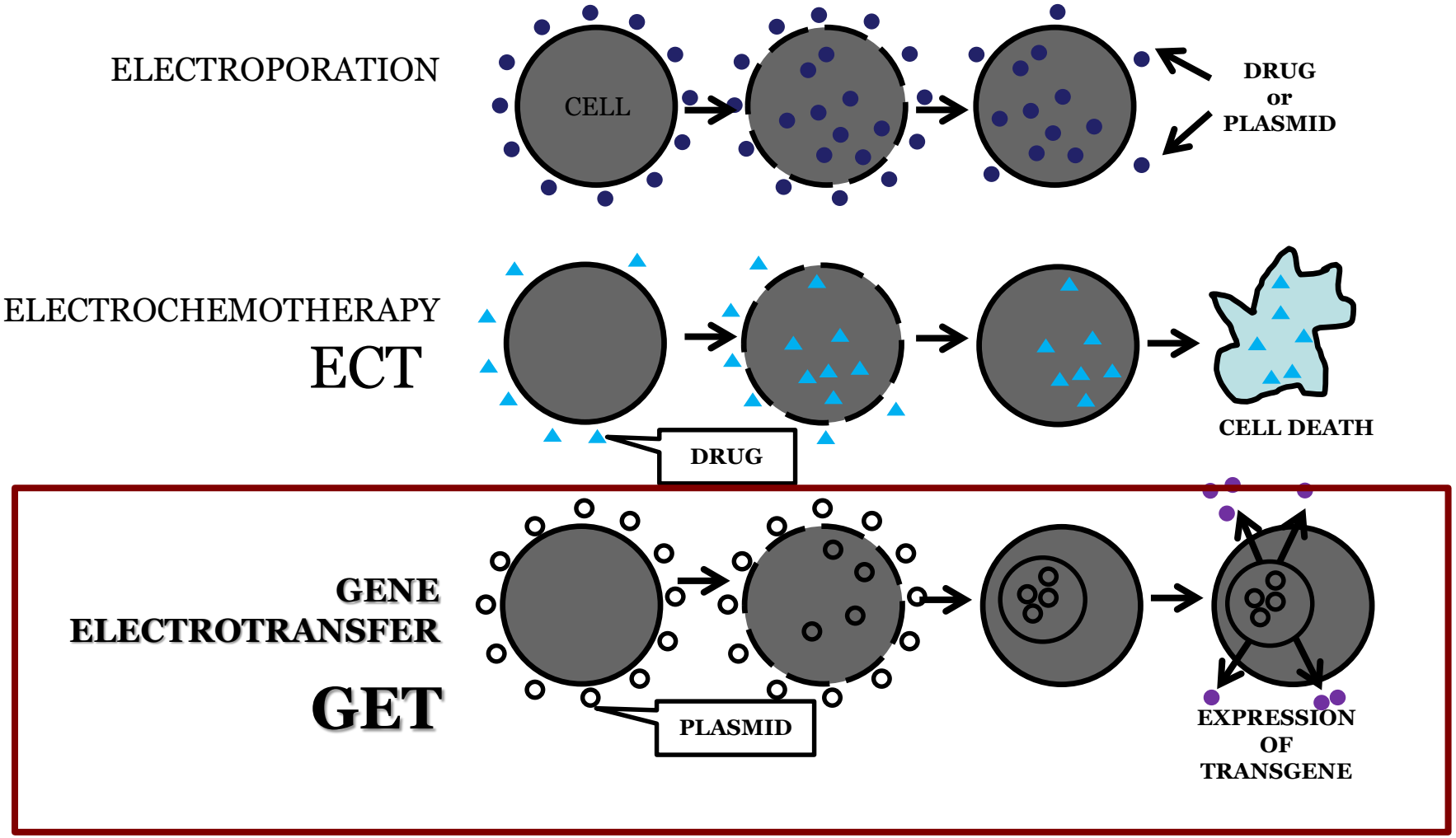


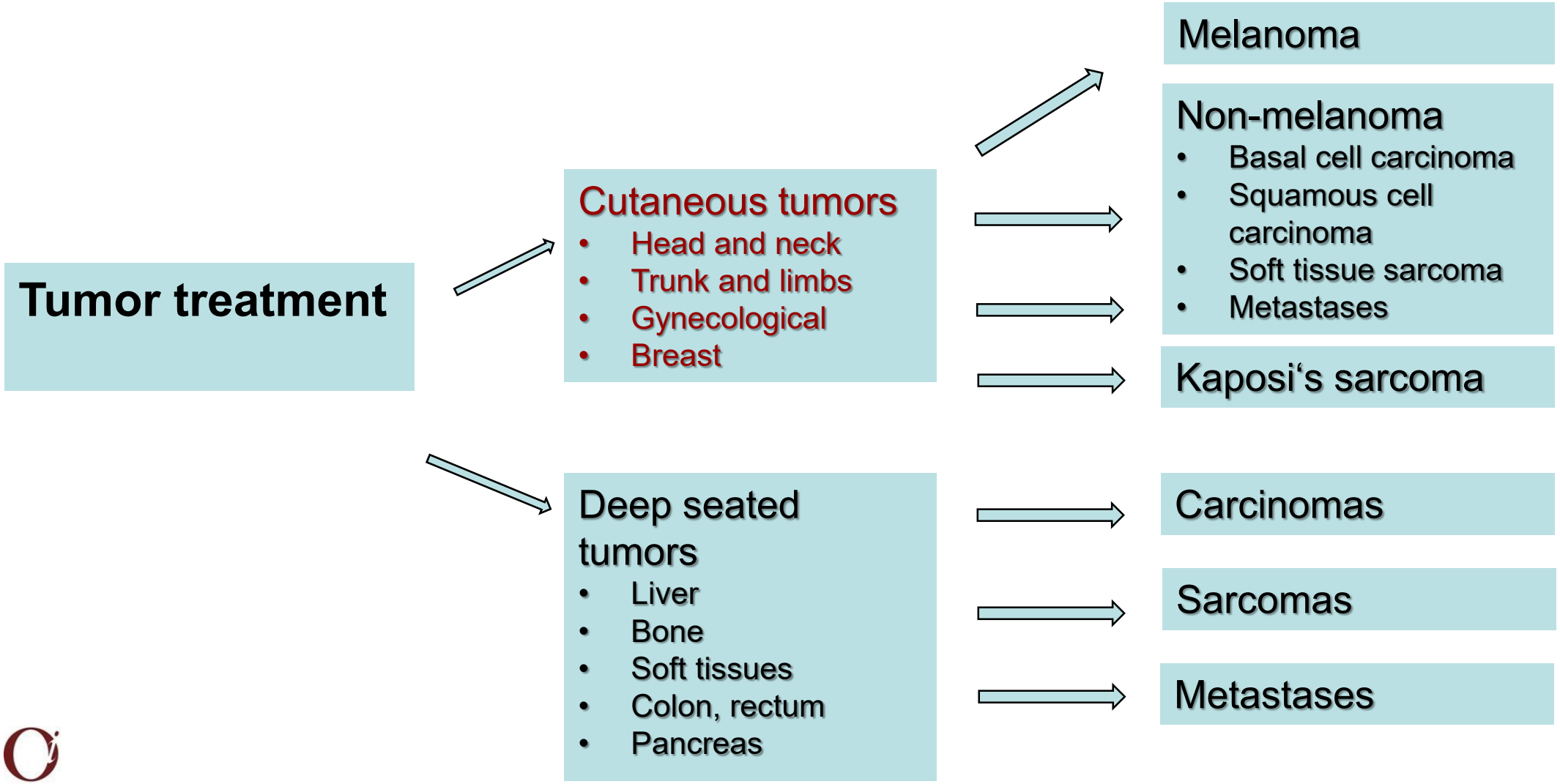

**Figure 3.** Overall survival curve (months) by Kaplan–Meier analysis in the two groups treated, respectively, with fixed (**a**) and variable geometry (**b**).

survival estimates were calculated by means of a Kaplan-Meier analysis. Results. At 1 month after ECT, 76% of patients were in partial response (PR) and 20% in stable disease (SD). Six months after ECT, 44.0% patients were still in PR and 12.0% in SD. A LDCR of 56.0% was reached six months after

# Reversible electroporation as drug or gene delivery system



# Electrochemotherapy and gene electrotransfer in tumor treatment



# Gene electrotransfer in treatment of cutaneous tumors

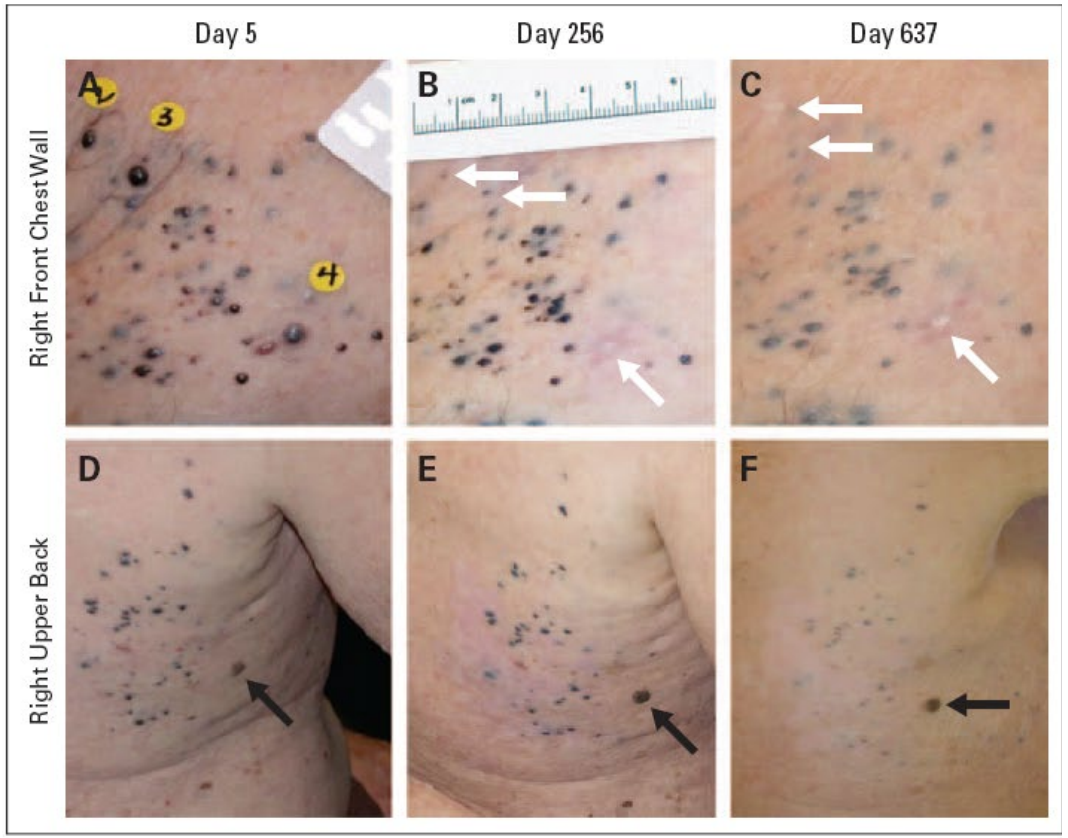
## Melanoma in transit metastases

- Plasmid coding for interleukin 12 (IL-12)
- Intratumoral plasmid injection
- Application of electric pulses

- Effectiveness
  - Regression of treated tumors
  - Regression of regional untreated metastases

Phase I Trial of Interleukin-12 Plasmid Electroporation in Patients With Metastatic Melanoma

*Adil I. Daud, Ronald C. DeConti, Stephanie Andrews, Patricia Urbas, Adam I. Riker, Vernon K. Sondak, Pamela N. Munster, Daniel M. Sullivan, Kenneth E. Ugen, Jane L. Messina, and Richard Heller*




*J Clin Oncol 26.* 

# **Tavokinogene** telseplasmid

- Plasmid coding for interleukin 12 (IL-12)
- Used in multiple preclinical and clinical studies

## Completed clinical trials

| Intervention/treatment | Condition or disease | Phase |
|---|---|---|
| Biological: Tavokinogene telseplasmid<br>Device: OncoSec Medical System | Head and Neck Squamous Cell Carcinoma | Phase 2 |
| Biological: Tavokinogene telseplasmid<br>Device: OncoSec Medical System | Merkel Cell Carcinoma | Phase 2 |

## Currently ongoing clinical trials

| Intervention/treatment | Condition or disease | Phase |
|---|---|---|
| Biological: **Tavokinogene** telseplasmid<br>Biological: Pembrolizumab<br>Device: ImmunoPulse | Stage III/IV Melanoma | Phase 2 |
| Biological: **Tavokinogene** telseplasmid<br>Biological: Pembrolizumab<br>Device: Immunopulse<br>Drug: nab paclitaxel | Triple Negative Breast Cancer | Phase 2 |
| Drug: **Tavokinogene** telseplasmid<br>Drug: Nivolumab<br>Device: OncoSec Medical System | Melanoma | Phase 2 |

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

### Intratumoral delivery of tavokinogene telseplasmid yields systemic immune responses in metastatic melanoma patients

A. Algazi[1], S. Bhatia[2], S. Agarwala[3], M. Molina[4], K. Lewis[5], M. Faries[6], L. Fong[1], L. P. Levine[1], M. Franco[1], A. Oglesby[1], C. Ballesteros-Merino[7], C. B. Bifulco[7], B. A. Fox[7], D. Bannavong[8], R. Talia[8], E. Browning[8], M. H. Le[8], R. H. Pierce[8], S. Gargosky[8], K. K. Tsai[1], C. Twitty[8] & A. I. Daud[1*]

## Highlights

- Intratumoral pIL-12 electroporation (Tavo) results in an ORR of 35.7% with CR in 17.9%.
- 46% of patients have regression in at least 1 uninjected lesion.
- 25% of patients have regression in all uninjected lesions.
- Upregulation of immune activation and co-stimulation but also adaptive resistance.

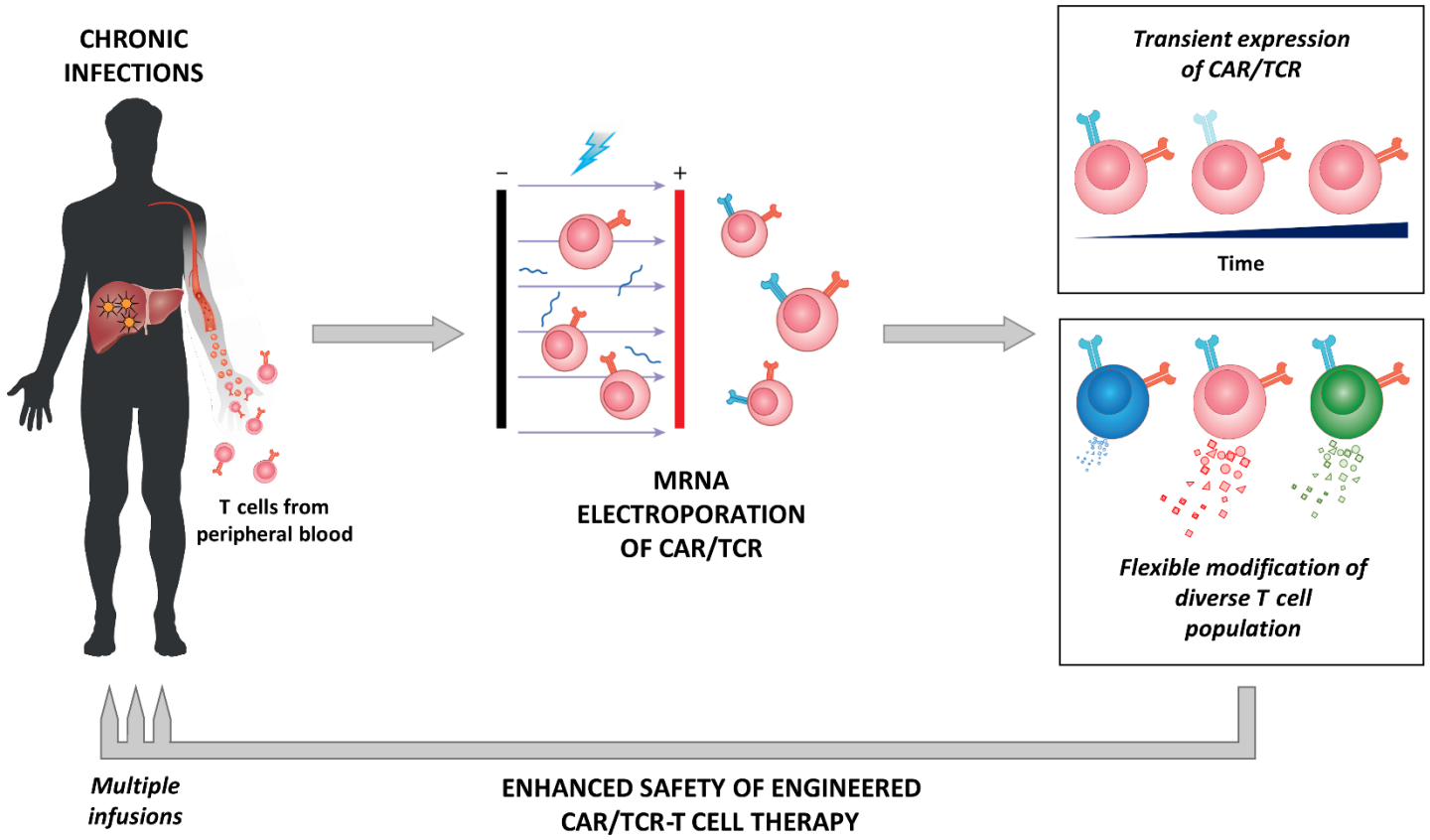
CLINICAL CANCER RESEARCH | CLINICAL TRIALS: IMMUNOTHERAPY

### Intratumoral Plasmid IL12 Expands CD8$^+$ T Cells and Induces a CXCR3 Gene Signature in Triple-negative Breast Tumors that Sensitizes Patients to Anti–PD-1 Therapy AC

Melinda L. Telli[1], Hiroshi Nagata[2], Irene Wapnir[3], Chaitanya R. Acharya[2], Kaitlin Zablotsky[1], Bernard A. Fox[4], Carlo B. Bifulco[4], Shawn M. Jensen[4], Carmen Ballesteros-Merino[4], Mai Hope Le[5], Robert H. Pierce[5], Erica Browning[5], Reneta Hermiz[5], Lauren Svenson[5], Donna Bannavong[5], Kim Jaffe[5], Jendy Sell[5], Kellie Malloy Foerter[5], David A. Canton[5], Christopher G. Twitty[5], Takuya Osada[2], H. Kim Lyerly[2,6,7], and Erika J. Crosby[2]

# CAR- and TCR-T cell–based therapy

- Advantages of electroporation:
- Relatively high viability and transfection efficiency
- High expression of transgene
- Simple, safe and inexpensive




Bertoletti A. and Tanoto T. A., Biotechnol Progress. 2021

Zhang et al. BMC Biotechnology (2018) 18:4
DOI 10.1186/s12896-018-0419-0

BMC Biotechnology

METHODOLOGY ARTICLE Open Access

## Optimized DNA electroporation for primary human T cell engineering

Zhang Zhang[1†], Shunfang Qiu[1,2†], Xiaopeng Zhang[1*] and Wei Chen[1*]

Received: 26 June 2020 | Revised: 11 August 2020 | Accepted: 13 August 2020
DOI: 10.1002/btpr.3066

REVIEW

BIOTECHNOLOGY PROGRESS

## Optimization of electroporation and other non-viral gene delivery strategies for T cells

Emily Harris | Jacob J. Elmer

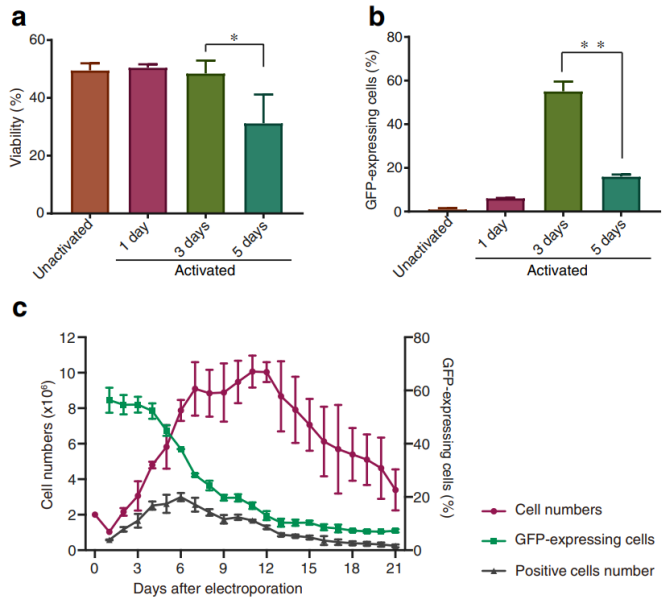
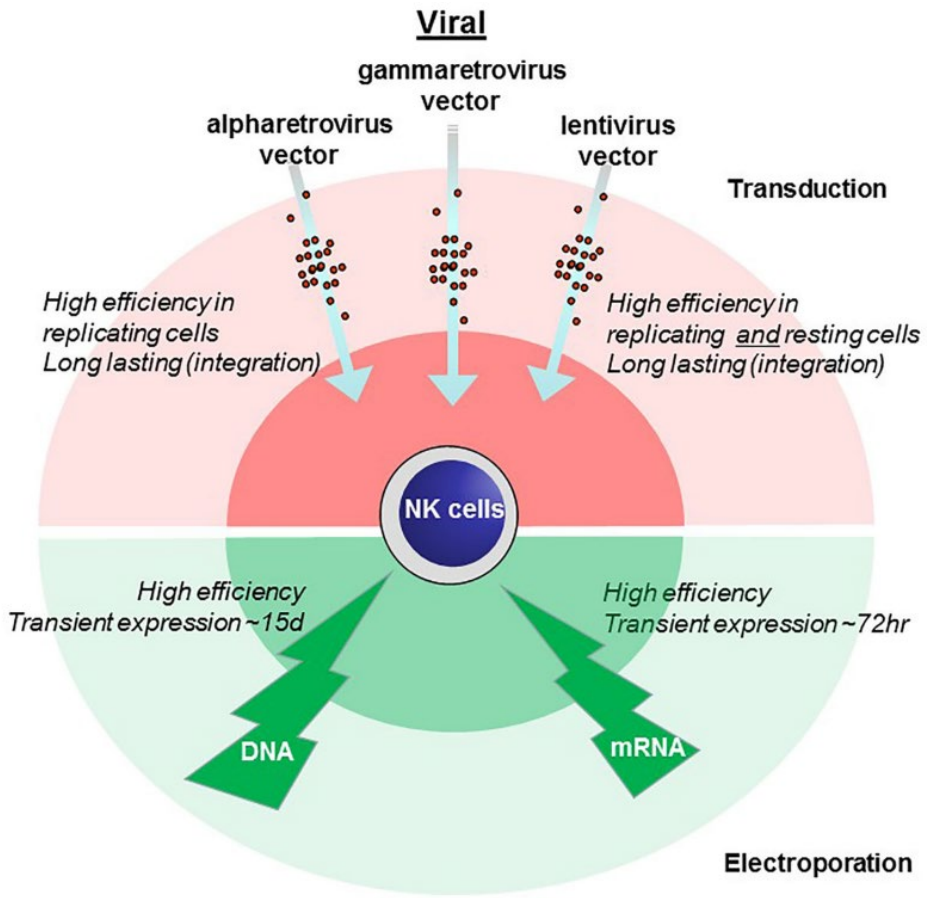



**Fig. 1** Activation and culturing time affect the efficiency of T cell electroporation. **a**, **b** Cell viability and percentage of positively transfected cells at 24 h after electroporation. **c** Change in the percentage of positively transfected cells (green line) and cell proliferation (red line) after electroporation. Positive cell number (gray line) = percentage of positive cells × viable cell number. Error bars in all figures represent standard deviation

- Enables generation of CAR-NK cells with transient expression of antitumor transgene




Schmidt P. et al., Frontiers in Immunology 2021

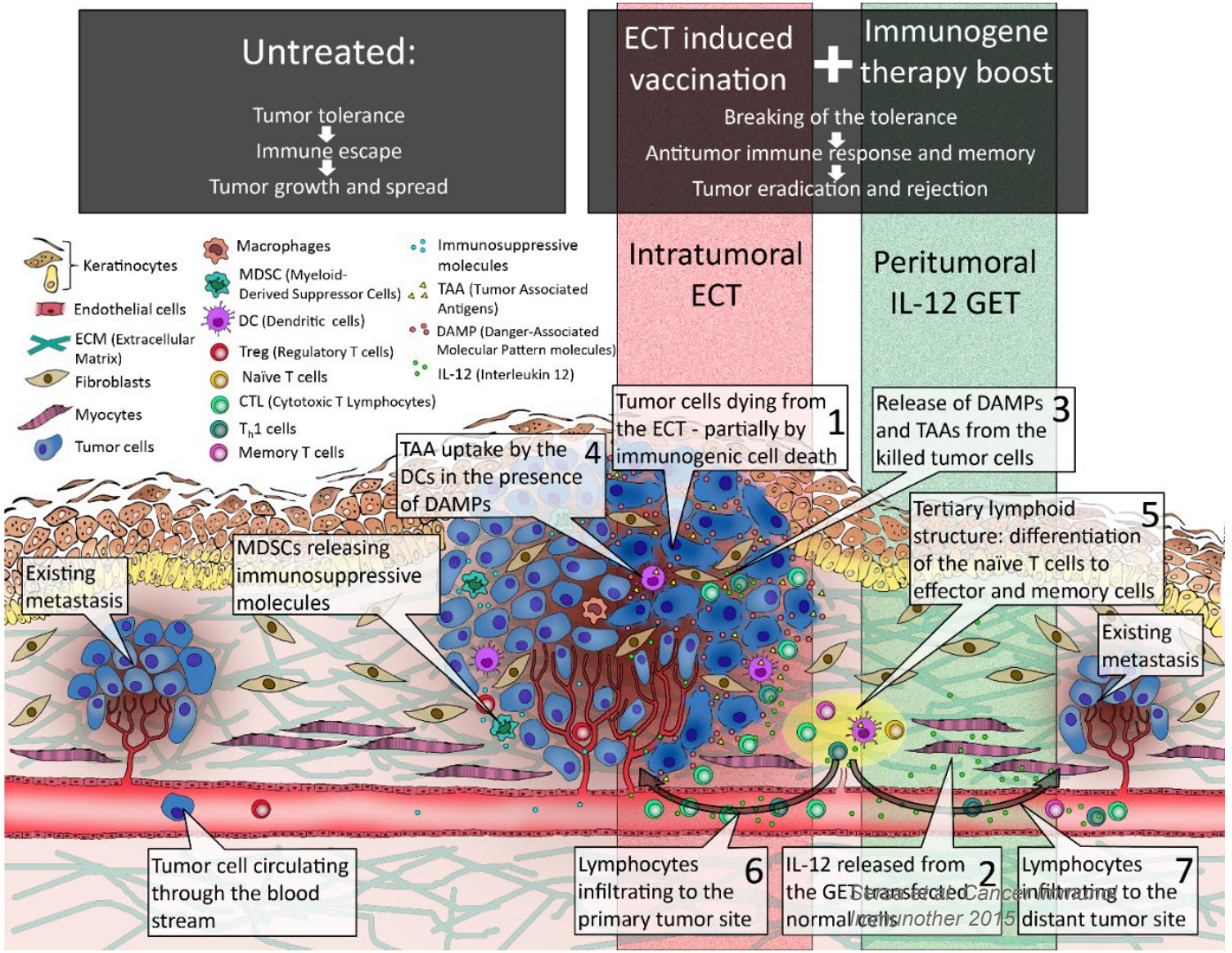
**TABLE 1** Optimized parameters for the electroporation of T cells. (n.r. = not reported, Expression (Exp) = episomal (Epi), genomic (G), or integrated via Sleeping Beauty Transposon (SB), *Lonza Nucleofector Manual)

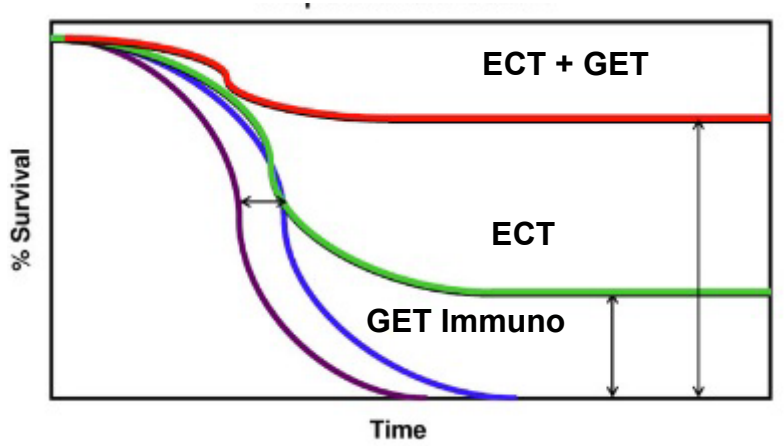
| Species | Cell type | DNA/ RNA | Plasmid/transgene | Exp | Mass (μg) | Cell # ($\times 10^6$) | Voltage | Pulse time | % Transf. cells | Cell viability | Ref# |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | Primary T cells | pDNA | pMaxGFP | Epi | n.r. | 5–10 | n.r. | n.r. | 51% | 58% | * |
| | | | pCMV-EGFPN1 | Epi | 10 | 2 | Nucleofector T-27 | | 32% | 76% | 82 |
| | | | pEFGFP-N3 | Epi | 20 | 60 | 310 mV | Exp | 20% | n.r. | 83 |
| | | | pEFGFP-C1 | Epi | 20 | 5 | 290 V | Exp | 16% | 19% | 84 |
| Human | Jurkat | pDNA | pMaxGFP | Epi | 2 | 1 | Nucleofector CL-120 | | 89% | 93% | |
| | | | Pegfp-Actin | Epi | 20 | 1 | 250 V | Exp | 88% | n.r. | 85 |
| | | | pT2-GFP + SB100X | SB | 4 | 1 | Nucleofector X-005 | | 35% | 80% | 86 |
| | | | Pzap-CV2 | Epi | 5 | 5 | 1,400 V | 30 ms | 33% | n.r. | 87 |
| | | | Multiple BCL2/MYC | Epi | 50 | 20 | 500 V | Exp | n.r. | 60% | 88 |
| | Primary T cells | pDNA | GFP | Epi | 1,000 | 20 | 2,200 V | 20 ms | 81.3% | 90% | 63 |
| | | | GFP | Epi | 7.5 | 1 | 1,600 V | 3x10ms | 50% | 65% | 89 |
| | | | Luc siRNA | Epi | 5 | 5 | Nucleofector U-15 | | 47.5% | 80.5% | 60 |
| | | | pMaxGFP | Epi | 20,000 | 10 | Nucleofector T7 | | 40% | 55% | 90 |
| | | | pMaxGFP | Epi | 1 | 2 | 500 V | 20 ms | 40% | 50% | 91 |
| | | | pT2-GFP + SB100X | SB | 4 | 10 | Nucleofector U-14 | | 30% | 50% | 88 |
| | | | Multiple CRISPR | G | 6.5 | 2 | 1,550 V | 3x10ms | 20% | n.r. | 92 |
| | | | EGFP | Epi | 20 | 30 | 300 V | Exp | 16.3% | 30% | 93 |
| | | mRNA | CD19-CAR | Epi | 50 | 10 | 500 V | 2 ms | 94% | 80% | 65 |
| | | | TCR | Epi | 150 | 80 | 550 V | 5 ms | 93% | n.r. | 94 |
| | | | ErbB2/CEA in pGEM4Z | Epi | 150 | 140 | 500 V | 5 ms | 83.0% | n.r. | 95 |
| | | | GFP | Epi | 6 | 1 | 1,600 V | 3x10ms | 80% | 60% | 91 |
| | | | pGEM4Z/GFP/A64 | Epi | 10 | 5 | 300 V | Exp | 70% | n.r. | 96 |
| | | | pGEM4Z/GFP/A64 | Epi | 20 | 50 | 300 V | Exp | 57% | 87% | 97 |
| | | | Multiple ZFNs | Epi | 10 | 5 | Nucleofector T-20 | | 37% | n.r. | 98 |

tially be safer, less expensive, and more expedient. Electroporation is currently the most promising non-viral method in terms of safety and efficiency, but additional optimization experiments and clinical trials must be completed before it can be directly compared with retroviral CAR gene delivery. Nonetheless, these studies show that non-viral

Harris E and Elmer JJ, Biotechnol Progress. 2021

# Proposed model of *in situ* vaccination with ECT, boosted by immunogene therapy with IL-12




Gregor Sersa, Justin Teissie, Maja Cemazar, Emanuela Signori, Urska Kamensek, Guillermo Marshall & Damijan Miklavcic


Cancer Immunology, Immunotherapy

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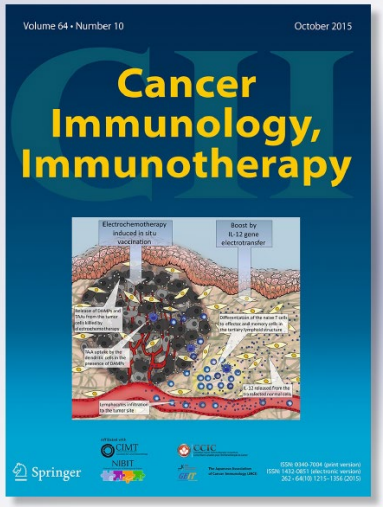
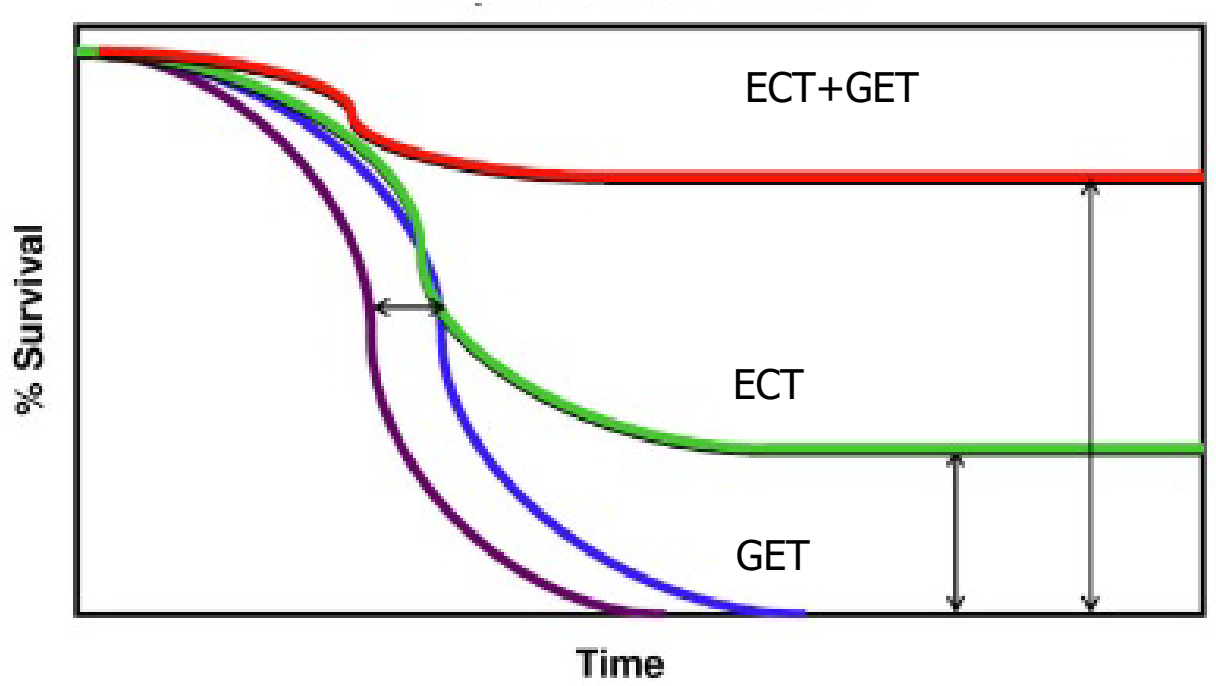
Cancer Immunol Immunother (2015)
64:1315-1327
DOI 10.1007/s00262-015-1724-2

# ECT as *in situ* vaccination boosted with IL-12 GET

- For the abscopal effect we need
  - Immunostimulation
  - Immuno Checkpoint Blockade
- The immunostimulation can provide higher response rate and possibly also the effect on distant metastases, with influence on survival.



# Future directions

- **Optimization** of gene therapy schedule protocol

- **Translation** in clinical practice: project „Next Generation of Cancer Gene Therapy: From Genes to Production and Clinics“


Onkološki inštitut Institute of Oncology Ljubljana 80 let years

University of Ljubljana Faculty of Electrical Engineering

COBIK Centre of Excellence for Biosensors, Instrumentation and Process Control

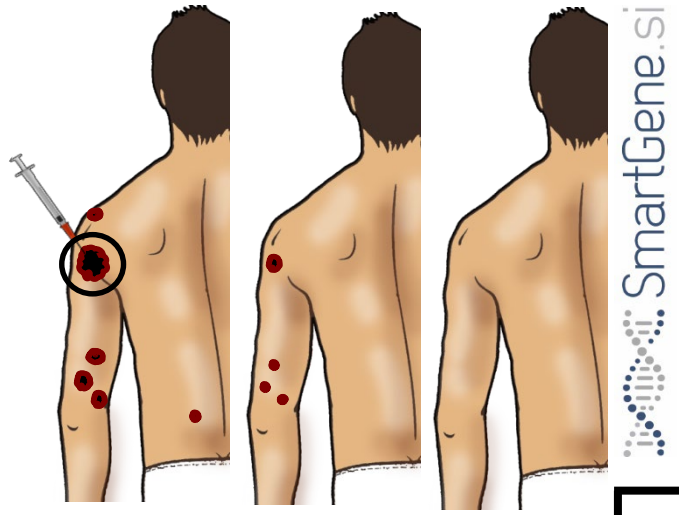
JAFRAL passion for biosolutions

Iskra pio d.o.o.

REPUBLIC OF SLOVENIA MINISTRY OF EDUCATION, SCIENCE AND SPORT

EUROPEAN UNION EUROPEAN REGIONAL DEVELOPMENT FUND INVESTING IN YOUR FUTURE

# Development of a platform for translation of gene therapy for cancer into the clinics

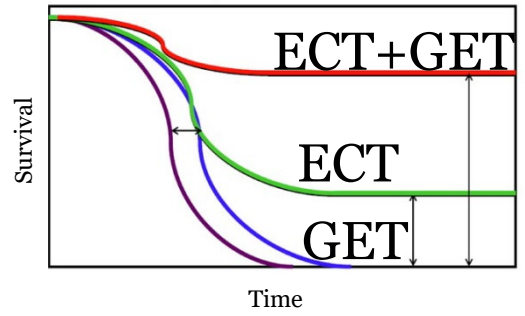



SmartGene.si

Standard treatment

Clinical study phase I/II

*In vivo* research

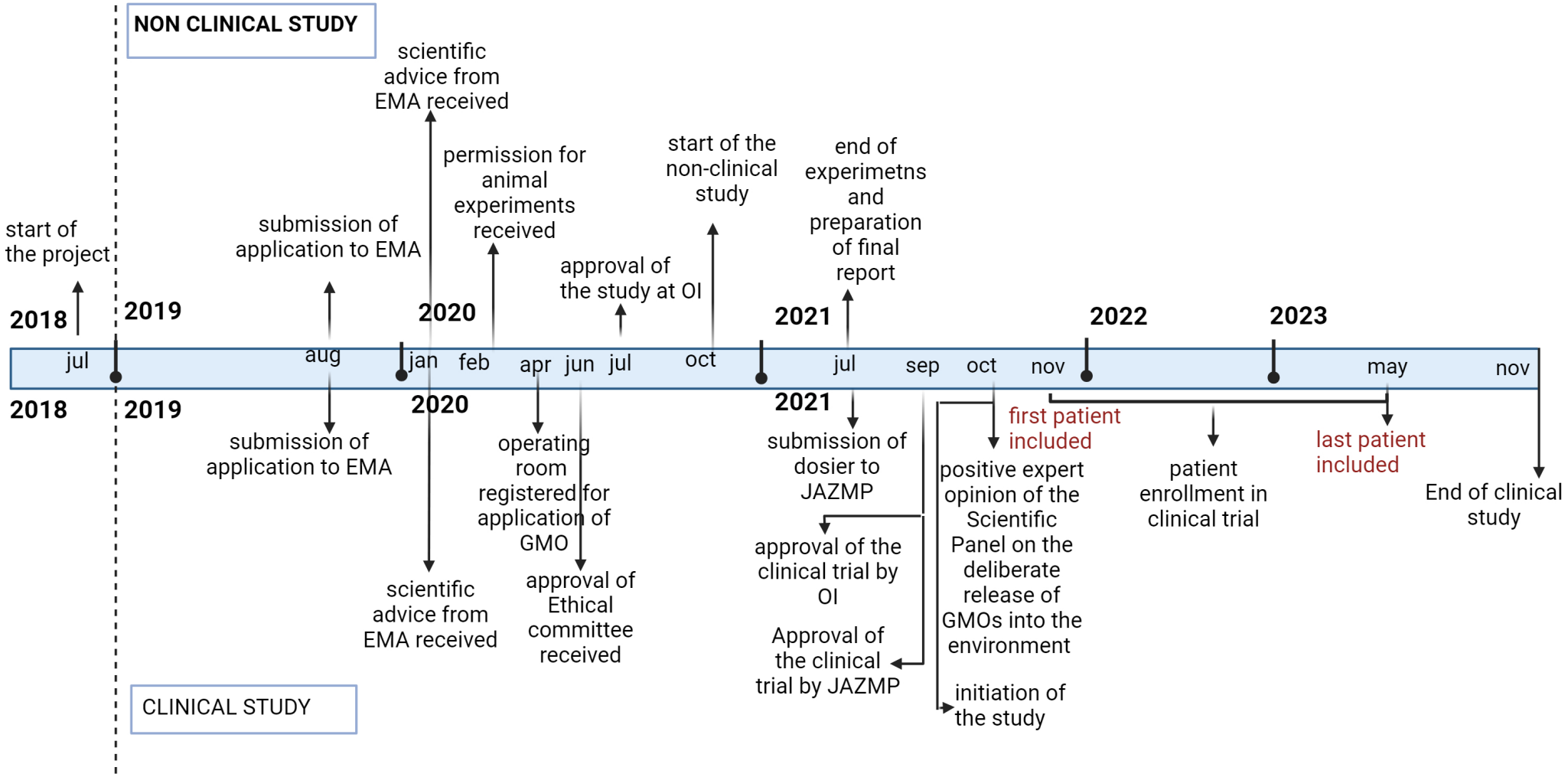



*In vitro* research

# SmartGene.si: A platform for development, manufacturing and clinical testing of DNA based therapeutics

- Construction of DNA vector containing therapeutic genes
- Preclinical testing on cell culture and mice models
- Electroporation device and method for plasmid DNA delivery to cells.
- Development and validation of analytical and potency methods
- Development of manufacturing process
- Design and set up of „Smart“ GMP facility.
- GMP manufacturing
- Clinical study

# Time-line of the project



# Non-clinical study for approval of phase I clinical study

Based on:

- EMA guidelines for advance therapies

- EMA/CAT/80183/2014 (Quality, preclinical and clinical aspects of gene therapy medicinal products),
- EMA/CAT/852602/2018 (Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials),
- EMEA/CHMP/GTWP/125459/2006 (Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products),
- EMA/CPMP/ICH/286/1995 (ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals),
- EMA/CHMP/ICH/646107/2008 (ICH guideline S9 on nonclinical evaluation for anticancer pharmaceuticals),
- EMEA/273974/2005 (Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors),
- CPMP/BWP/3088/99 (Note for Guidance on the Quality, Preclinical and Clinical aspects of gene transfer medicinal products),
- CPMP/SWP/1042/99 Rev 1 Corr (Guideline on repeated dose toxicity),
- Reflection paper: Expectations for Biodistribution (BD) Assessments for Gene Therapy (GT) Products.

## Scientific advice

- EMA/CHMP/SAWP/19705/2020 based on our question and presentation of our study and meeting with the EMA experts.
- Slovenian GLP guidelines (Uradni list RS, št. 38/00 in 2/04)

# SmartGene.si clinical trial completed

European Journal of Surgical Oncology 51 (2025) 109574

Contents lists available at ScienceDirect

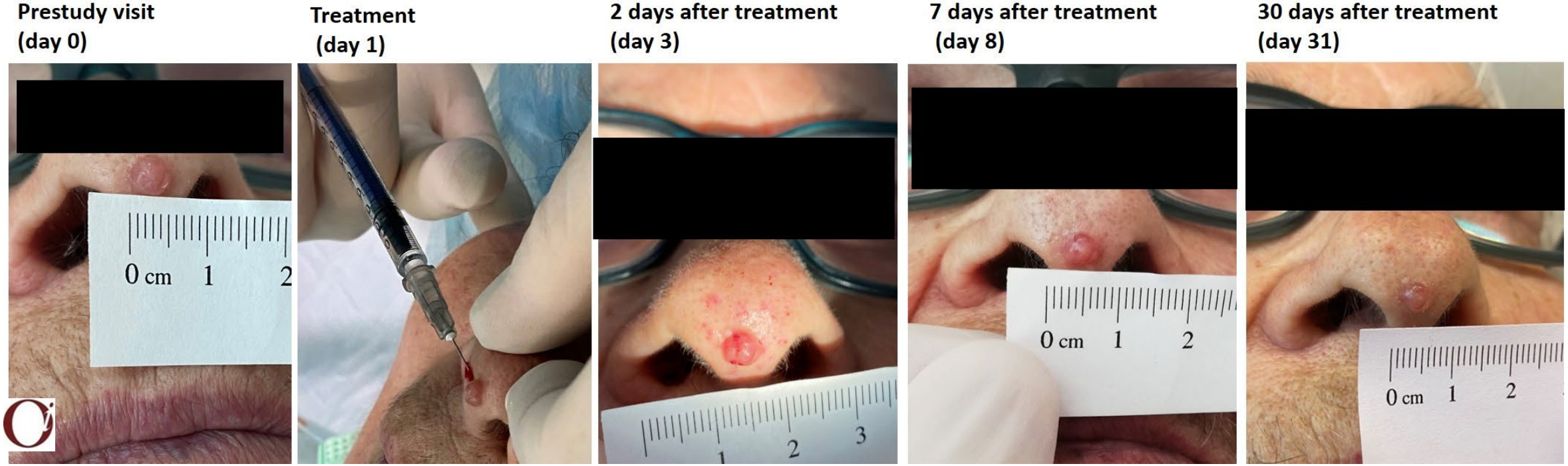
European Journal of Surgical Oncology

journal homepage: www.ejso.com

## Phase I trial of phIL12 plasmid intratumoral gene electrotransfer in patients with basal cell carcinoma in head and neck region

Primoz Strojan [a,b], Tanja Jesenko [a,b], Masa Omerzel [a,c], Crt Jamsek [d], Ales Groselj [d], Ursa Lampreht Tratar [a,e], Bostjan Markelc [a,f], Gorana Gasljevic [a,g], Alojz Ihan [b], Frenk Smrekar [h], Matjaz Peterka [i], Maja Cemazar [a,j,**], Gregor Sersa [a,c,*]

## Treatment with intratumoral IL-12 of basal cell carcinoma




*ClinicalTrials.gov identifier (NCT number):* ***NCT05077033***

# Non-clinical study SMG-01

SmartGene.si

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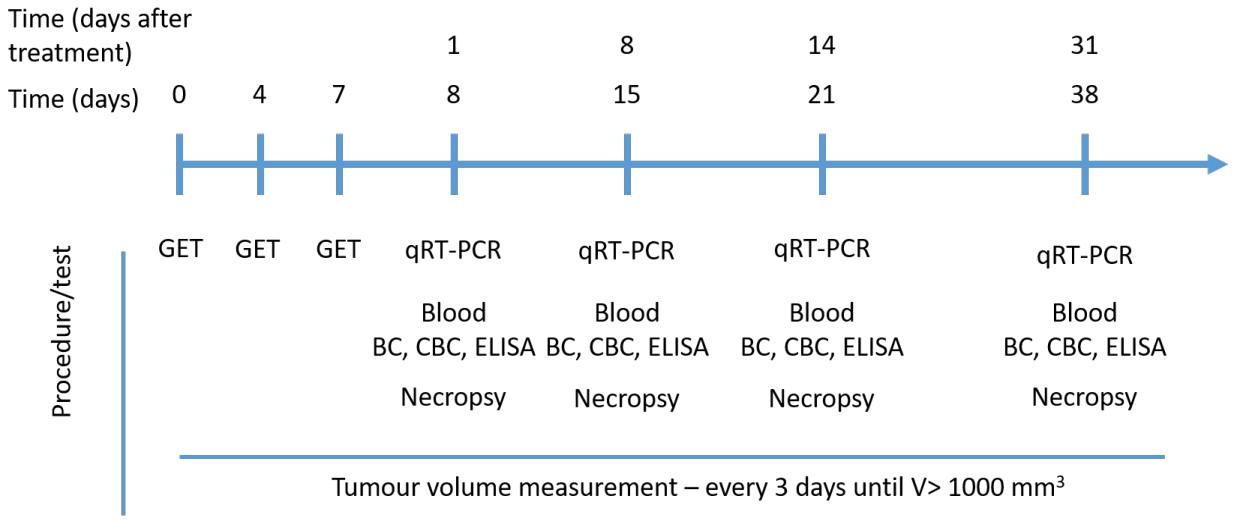
In vivo studies were performed to determine the efficacy (pharmacodynamics), pharmacokinetics, toxicity, tolerability and immunogenicity of the **pmIL12 plasmid.** The studies were performed in CT26 murine tumours.

Time line of in vivo experiments




scientific reports

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## Non-clinical evaluation of pmIL12 gene therapy for approval of the phase I clinical study

Bostjan Markelc[1,2,12], Tanja Jesenko[1,3,12], Simona Kranjc Brezar[1,3], Masa Omerzel[1,4], Ursa Lampreht Tratar[1,5], Andrej Rencelj[1], Urska Matkovic[1], Katarina Znidar[1], Spela Kos[1], Kristina Levpuscek[1,3], Ziva Pisljar[1,3], Ursa Kesar[1,3], Tilen Komel[1,6], Tim Bozic[1], Aneja Tuljak[7], Rosana Hudej[7], Matjaz Peterka[7], Urska Kamensek[1,8], Andrej Cör[9,10], Gorana Gasljevic[1,11], Alenka Nemec Svete[5], Natasa Tozon[5], Gregor Sersa[1,2] & Maja Cemazar[1,10]

Immunotherapeutic drugs are promising medicines for cancer treatment. A potential candidate for immunotherapy is interleukin-12 (IL-12), a cytokine well known for its ability to mediate antitumor activity. We developed a plasmid encoding human IL-12 devoid of an antibiotic resistance gene (phIL12). For the approval of phase I clinical trials in basal cell carcinoma (BCC), the regulatory agency requires non-clinical in vivo testing of the pharmacodynamic, pharmacokinetic and toxicological properties of the plasmid. As human IL-12 is not biologically active in mice, a mouse ortholog of the plasmid phIL12 (pmIL12) was evaluated. The evaluation demonstrated the antitumor effectiveness of the protein accompanied by immune cell infiltration. The plasmid was distributed throughout the body, and the amount of plasmid diminished over time in all organs except the skin around the tumor. The therapy did not cause any detectable systemic toxicity. The results of the non-clinical evaluation demonstrated the safety and efficacy of the pmIL12/phIL12 GET, and on the basis of these results, approval was obtained for the initiation of a phase I clinical study in BCC.

**Keywords** Plasmid DNA, Interleukin 12, Electroporation, Gene electrotransfer, CT26 colorectal carcinoma

# OUR PRODUCT: **plasmid encoding IL-12 for first in human use**

Plasmid DNA coding for IL-12

Therapy and tumor inducible promoter

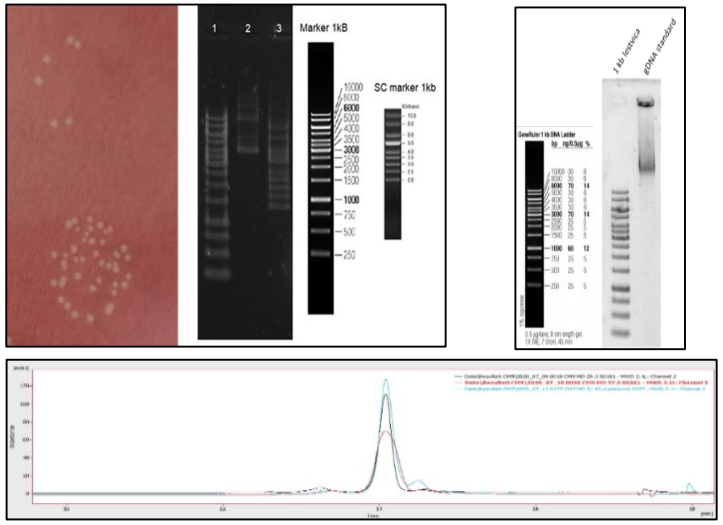
Antibiotic gene resistance free

Compliant with the EMA guidelines

# Production of the drug product

## Experience in:

- Development and validation of analytical and potency methods
- Development of manufacturing process

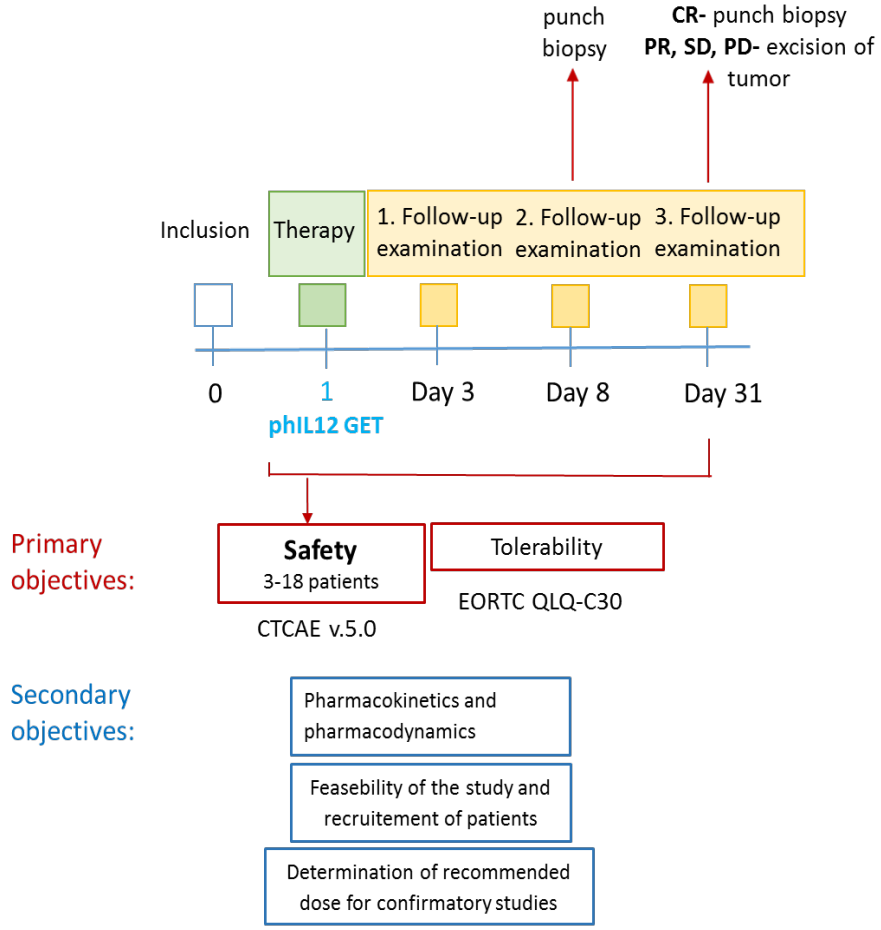



## GMP facility and manufacturing

# ALL THE PROCESS WAS WITH THE AIM TO TEST THE PRODUCT (PLASMID FOR IL-12) IN FIRST IN HUMAN CLINICAL TRIAL in Europe

The design of the trial was approved by EMA „scientific advice“ and JAZMP, and National Ethical Committee

Study completed in June 2023




*Groselj et al. Radiol Oncol 2022*

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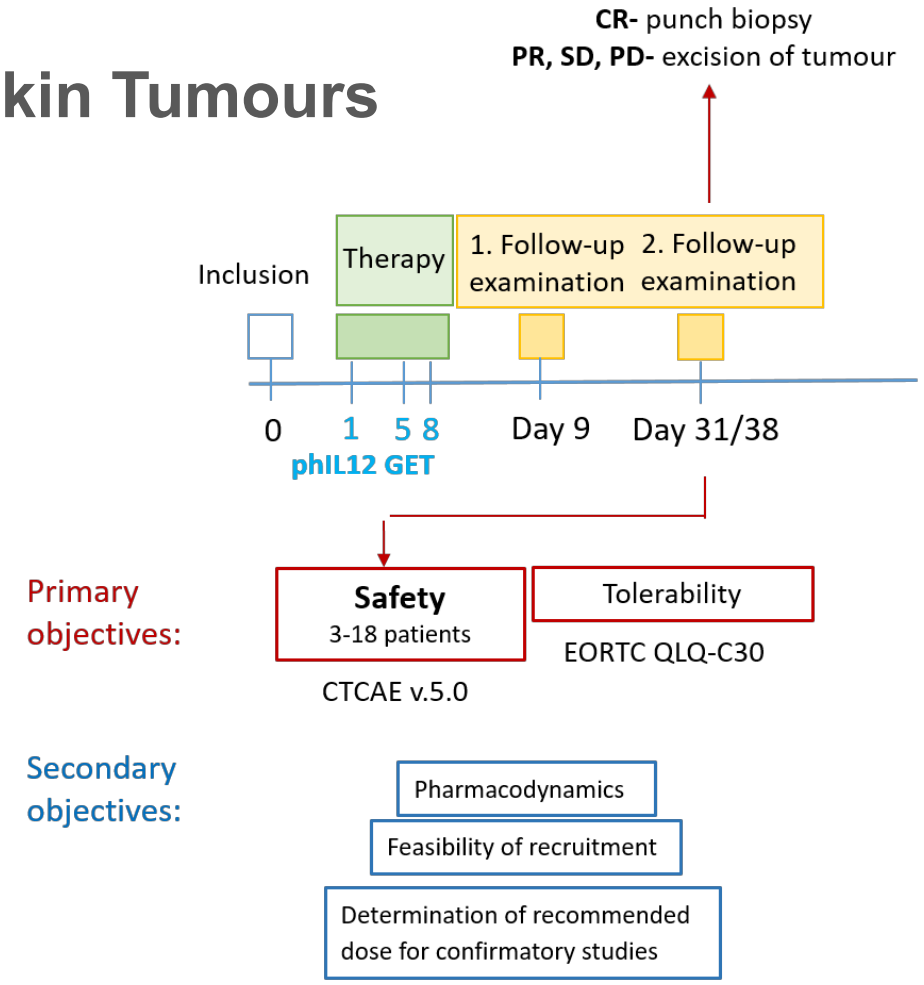
# Phase I: **Treatment of the Head and Neck Skin Tumours with Interleukin 12 Gene Electrotransfer**

- Phase I exploratory study.
- Basal cell carcinoma in head and neck region.

- Dose escalation study: adapted 3+3 design.
  3-6 patients/dose level; 3 dose levels (3-18 patients); 0,5, 1 and 2 mg; volume of injection: 1/4 of tumor volume.
- Exploratory study; therefore, no formal sample size calculation is needed.
- The design (3 + 3 design) and the corresponding sample size are usual for phase I trials in oncology.
- Descriptive statistics will be used.

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# Objectives of the study protocol

TABLE 1. Primary objectives

| Primary objective | Definition of objectives | Timepoint of objectives evaluation |
|---|---|---|
| Assessment of the safety of intratumoral phIL12 GET | Assessment of adverse events in accordance with the CTCAE v5 criteria | From the beginning of therapy until the follow-up examination on day 30 after the treatment (day 1, 3, 8 and 31) |
| Assessment of the tolerability of intratumoral phIL12 GET | Assessment of patient reported outcome by the quality of life questionnaire EORTC QLQ-C30 | A follow-up examination on day 0, 8 and 31 |

CTCAE = Common Terminology Criteria for Adverse Events; GET = gene electrotransfer

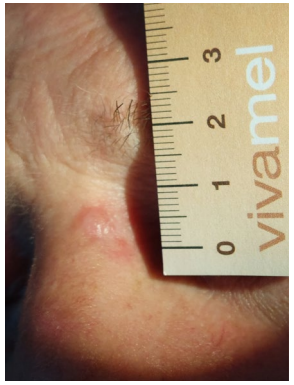
TABLE 2. Secondary objectives

| Secondary objective | Definition of objectives | Timepoint of objectives evaluation |
|---|---|---|
| Pharmacokinetics and biodistribution. | Determination of serum levels of IL-12 cytokine. | A follow-up examination according to clinical trial protocol (day 0, 3, 8 and 31). |
| Pharmacodynamics | Determination of tumor IL-12 and IFN-γ levels in tumor biopsies.<br>Determination of plasmid DNA in tumor biopsies. | A follow-up examination according to clinical trial protocol (day 8 and 31). |
| Feasibility of recruitment | Evaluation of the appropriateness and execution of the treatment and follow up procedures. | During recruitment, execution of the treatment and follow up. |
| Determination of recommended dose for confirmatory studies | Measurement of pharmacodynamics data and selection of the phIL12 dose that produces IL-12 expression in the tumors with best biological activity, infiltration of the immune cells and no toxicity. | Based on all measurements during follow up. |

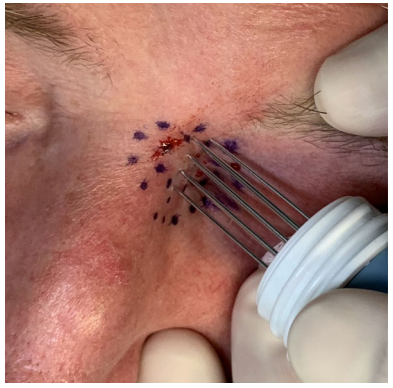
# Safety and tolerability

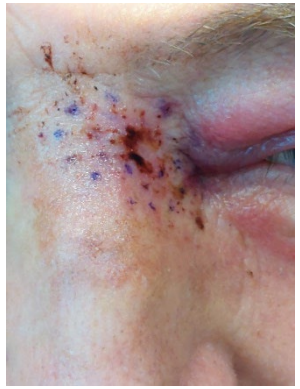
<u>Safety</u>

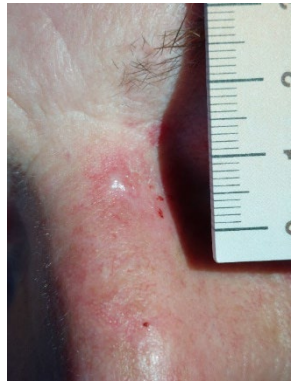
- AE:
  - 1 patient: mild pain 2 days after GET
  - 1 patient: edema in the treatment area 2 days after GET

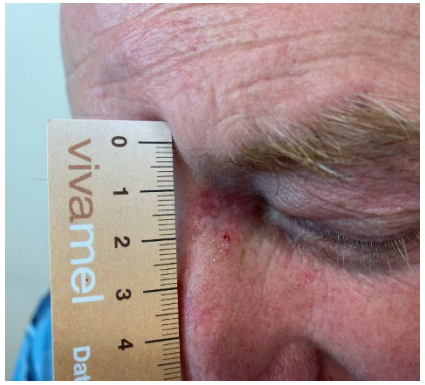


Before GET


Application of electric pulses


2 days after GET


7 days after GET



1 month after GET

- SAE: NO

# Tolerability

- Well tolerable

Table 2: Patients' self evaluation of health and quality of life by EORTC QLQ-C30 (max value: excellent 7).

| | | Health | | | Quality of life | | |
|---|---|---|---|---|---|---|---|
| **Cohort** | Patient | Before treatment | Day 7 | Day 31 | Before treatment | Day 7 | Day 31 |
| **1 phIL12: 0.5 mg/ml** | SMG 01 | 6 | 6 | 6 | 6 | 6 | 6 |
| | SMG 02 | 4-5 | 4-5 | 5 | 5 | 5 | 6 |
| | SMG 03 | 6 | 6 | 6 | 6 | 6 | 6 |
| **2 phIL12: 1 mg/ml** | SMG 04 | 5 | 5 | 7 | 5 | 5 | 7 |
| | SMG 05 | 5 | 7 | 7 | 6 | 7 | 7 |
| | SMG 06 | 4 | 4 | 4 | 4 | 4 | 4 |
| **3 phIL12: 2 mg/ml** | SMG 07 | 5 | 6 | 6 | 7 | 7 | 7 |
| | SMG 08 | 5 | 5 | 4 | 5 | 5 | 4 |
| | SMG 09 | 7 | 7 | 7 | 7 | 7 | 7 |
| | Median | 5 | 6 | 6 | 6 | 6 | 6 |

- Health slightly better after treatment, no changes in quality of life

# Secondary objectives

**I. Pharmacokinetics and biodistribution**

Determination of serum levels of IL-12 cytokine.

**II. Pharmacodynamics**

Determination of tumour IL-12 and IFN-γ levels in tumour biopsies.

Determination of plasmid DNA in tumour biopsies.

**III. Feasibility of recruitment**

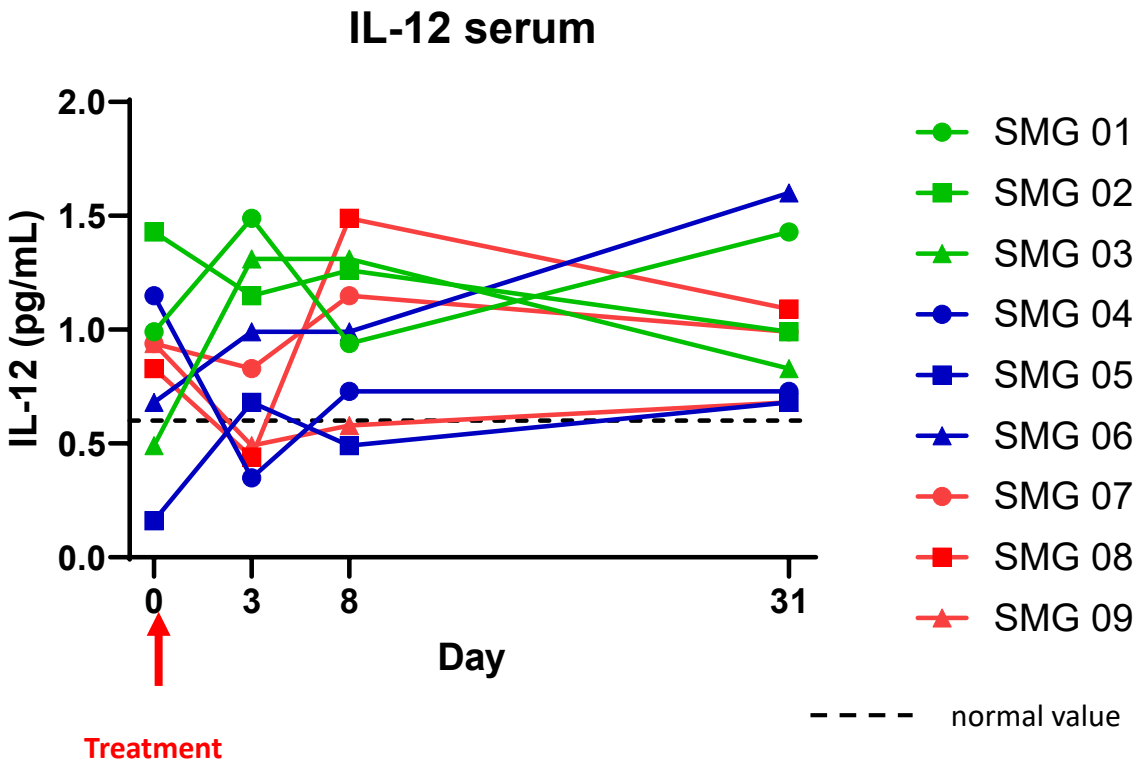
Evaluation of the appropriateness and execution of the treatment and follow up procedures.

**IV. Determination of recommended dose for confirmatory studies**

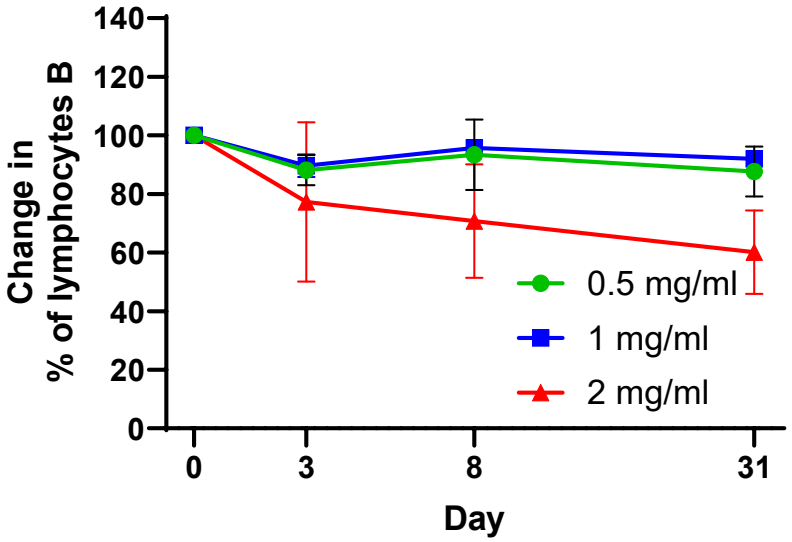
Measurement of pharmacodynamics data and selection of the phIL12 dose that produces IL-12 expression in the tumours with best biological activity, infiltration of the immune cells and no toxicity.

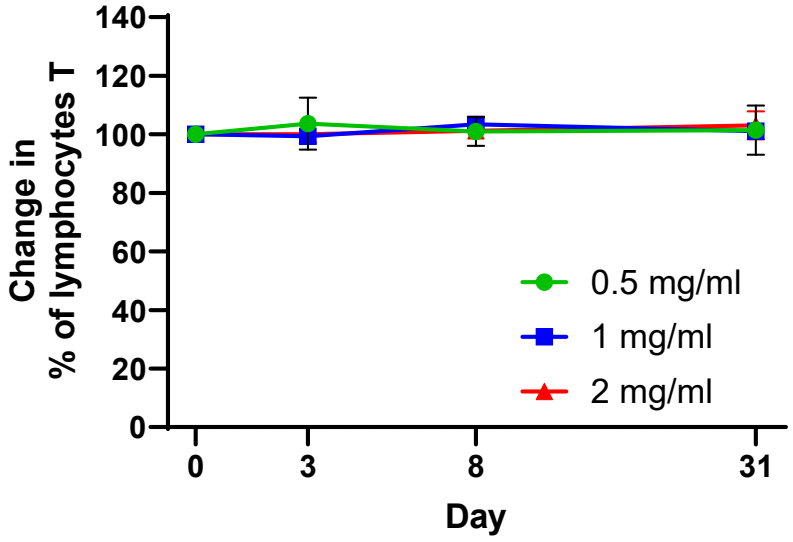
# I. Pharmacokinetics and biodistribution

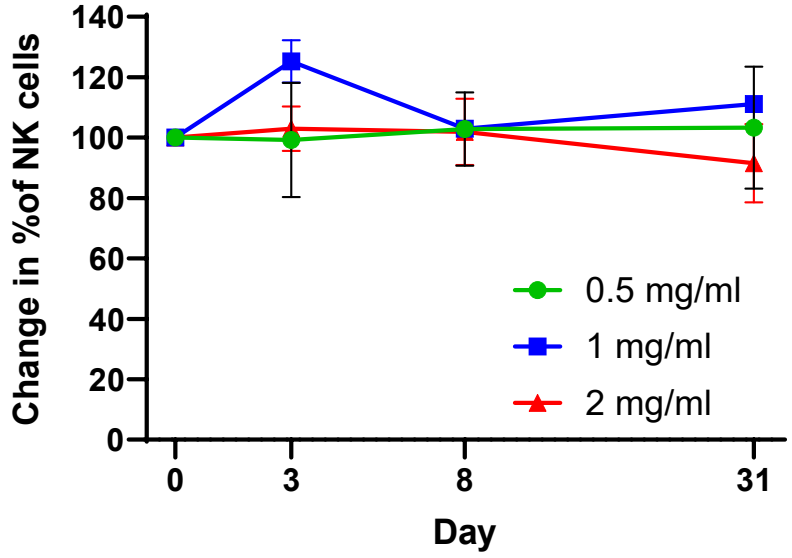
## Determination of serum levels of IL-12 cytokine

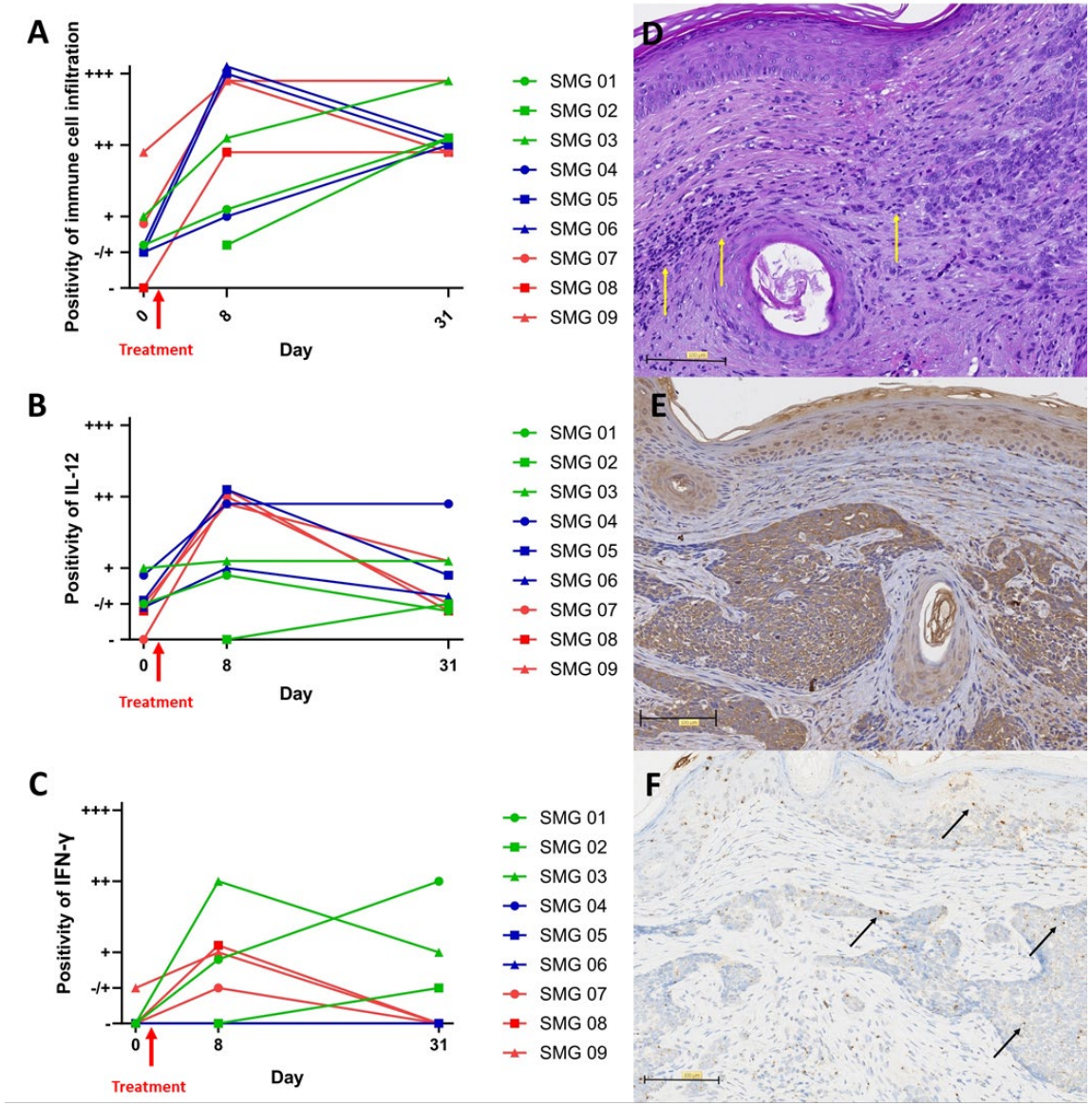


# Effects on serum levels of B lymphocytes, T lymphocytes and NK cells






Graphs showing the evaluation of immune cell infiltration (A), IL-12 (B) and IFN-γ (C) staining together with the representative figures of patient SMG 08 on day 8 after the treatment (D,E,F; Yellow arrows presenting infiltration of immune cells; black arrows showing the positive cells for IFN-γ)

# Immunoscore

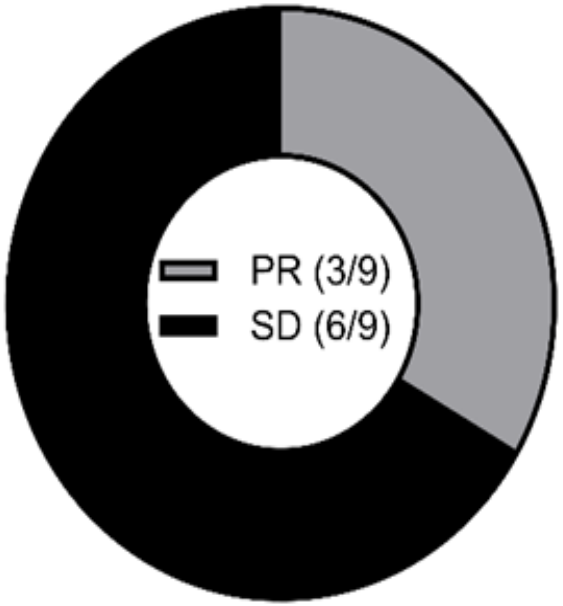
| Time point | | HE | IL-12 | IFN-γ | Sum | Immunoscore | Concentration of phIL12 (mg/mL) |
|---|---|---|---|---|---|---|---|
| Day 8 | SMG01 | 1 | 1 | 1 | 3 | | |
| | SMG02 | 0.5 | 0 | 0 | 0.5 | | |
| | SMG03 | 2 | 1 | 2 | 5 | 8.5 | 0.5 mg/ml |
| | SMG04 | 1 | 2 | 0 | 3 | | |
| | SMG05 | 3 | 2 | 0 | 5 | | |
| | SMG06 | 3 | 1 | 0 | 4 | 12 | 1 mg/ml |
| | SMG07 | 3 | 2 | 0.5 | 5.5 | | |
| | SMG08 | 2 | 2 | 1 | 5 | | |
| | SMG09 | 3 | 2 | 1 | 6 | 16.5 | 2 mg/ml |

Table presenting the immunoscore of three different parameters: Immune cell infiltration, IL-12 and IFN-γ positivity for all patients at day 8 and 31 after IL-12 GET.

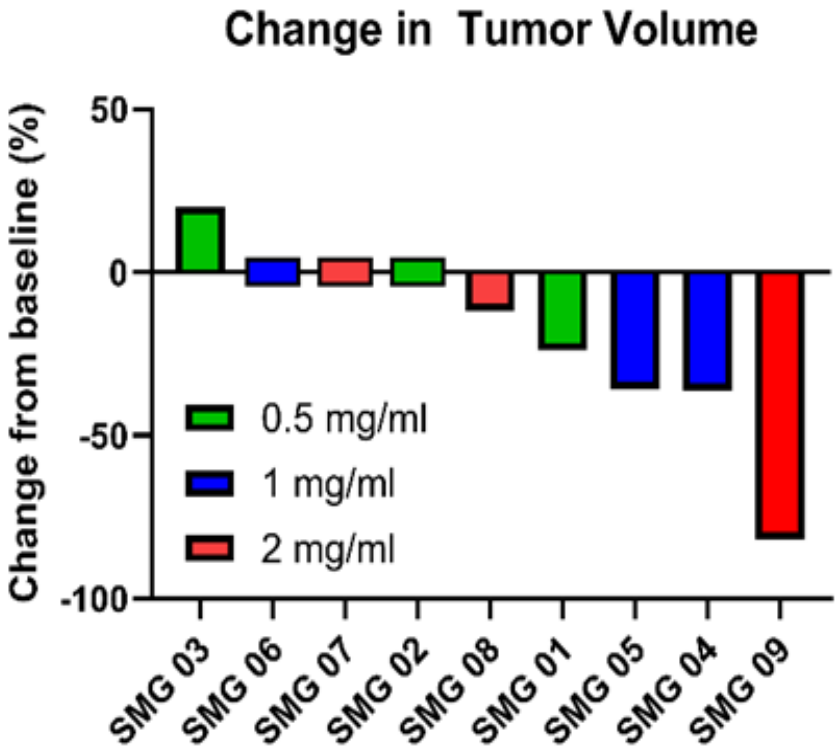
An immunoscore was calculated based on the positivity of all three parameters—HE, IL-12, and IFN-γ staining. The analysis revealed that the 2 mg/ml groups exhibited the highest immunoscore on day 8 compared to the other two groups.

# Response rate

A




B



# Results of the clinical trial were just published

European Journal of Surgical Oncology 51 (2025) 109574

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journal homepage: www.ejso.com

## Phase I trial of phIL12 plasmid intratumoral gene electrotransfer in patients with basal cell carcinoma in head and neck region

Primoz Strojan [a,b], Tanja Jesenko [a,b], Masa Omerzel [a,c], Crt Jamsek [d], Ales Groselj [d], Ursa Lampreht Tratar [a,e], Bostjan Markelc [a,f], Gorana Gasljevic [a,g], Alojz Ihan [b], Frenk Smrekar [h], Matjaz Peterka [i], Maja Cemazar [a,j,**], Gregor Sersa [a,c,*]

[a] Institute of Oncology Ljubljana, Slovenia
[b] Faculty of Medicine, University of Ljubljana, Slovenia
[c] Faculty of Health Sciences, University of Ljubljana, Slovenia
[d] Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana, Slovenia
[e] Veterinary Faculty, University of Ljubljana, Slovenia
[f] Biotechnical Faculty, University of Ljubljana, Slovenia
[g] Medical Faculty, University of Maribor, Slovenia
[h] JAFRAL d.o.o., Slovenia
[i] COBIK-Centre of Excellence for Biosensors, Instrumentation and Process Control, Slovenia
[j] Faculty of Health Sciences, University of Primorska, Slovenia

ARTICLE INFO

*Keywords:*
Phase I clinical trial
phIL12 plasmid
Interleukin-12
Gene electrotransfer
Basal cell carcinoma

ABSTRACT

*Introduction:* In the treatment of cancer, immunomodulatory approaches are developed to support the organism in fighting cancer or to enhance the immunomodulatory effects of local ablative techniques. To this end, we conducted an interventional, open-label, single-arm Phase I trial to evaluate the safety and tolerability of intratumoral phIL12 plasmid DNA gene electrotransfer as primary objectives.

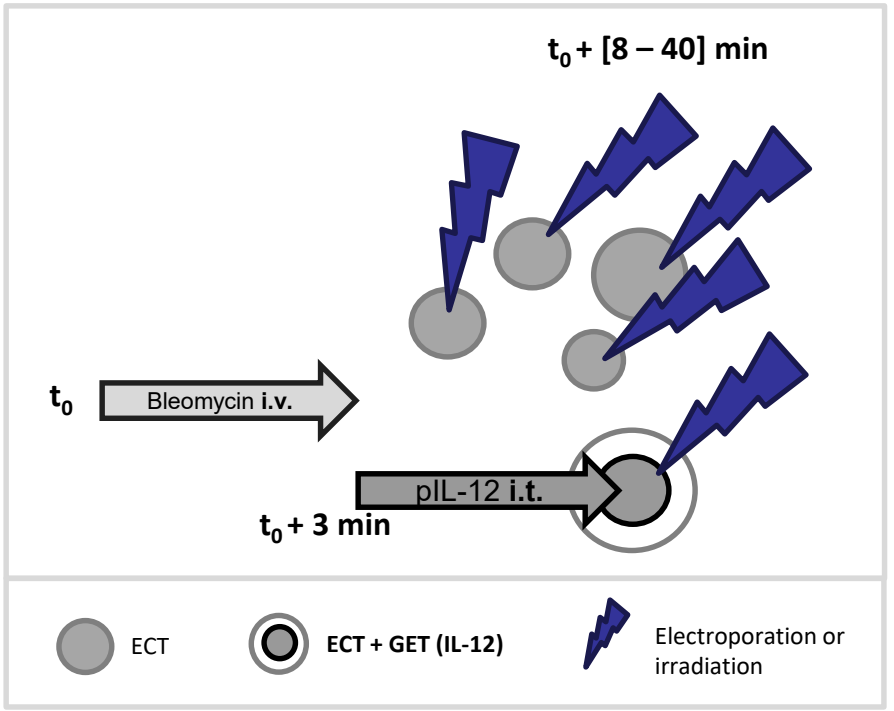
*Methods:* The study was dose-escalating with 3 consecutive cohorts of 3 patients per phIL12 dose level (0.5 mg/ml, 1 mg/ml or 2 mg/ml) according to a matched 3 + 3 design. Recruitment of patients was staggered. The waiting period was 30 days after treatment of the previous patient, based on the expected duration of acute and subacute toxicity.

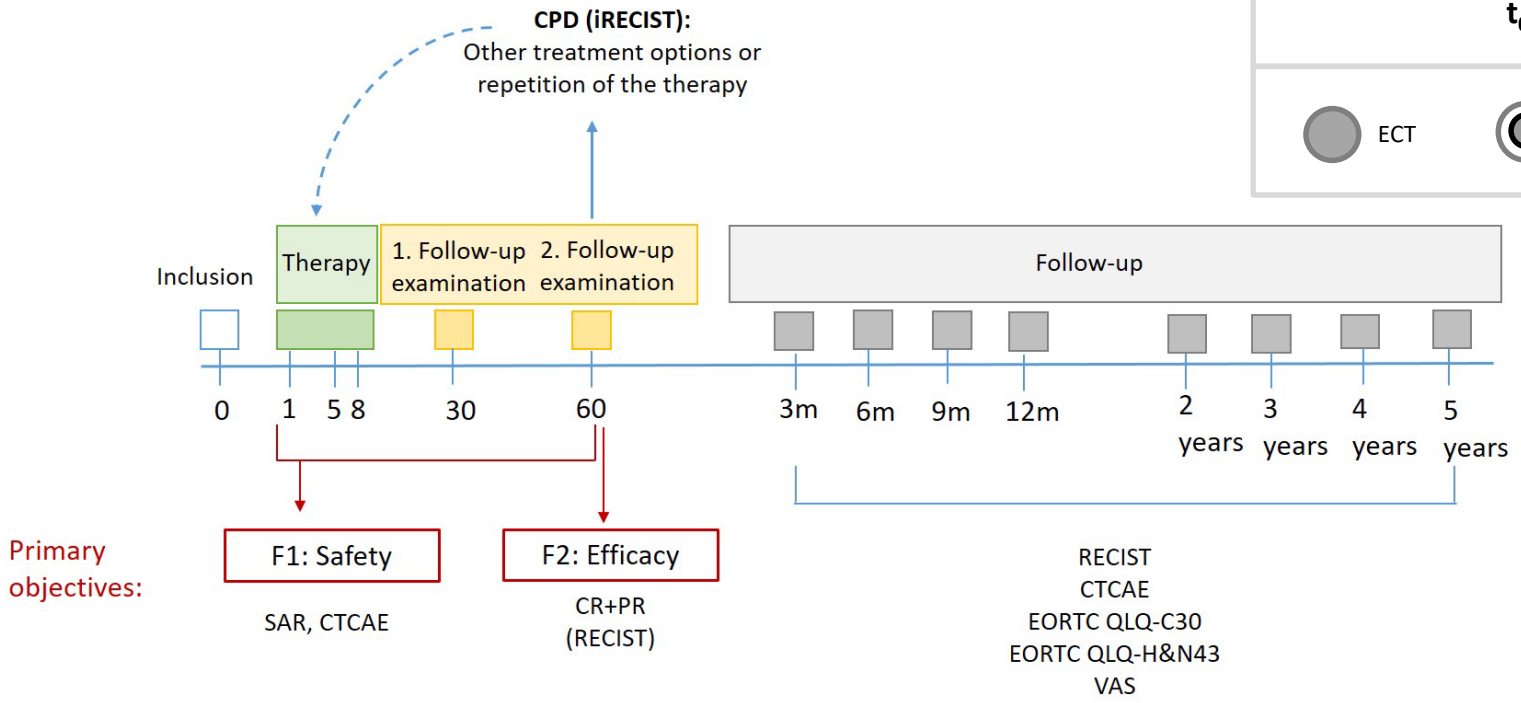
*Results:* The results of this phase I clinical trial in basal cell carcinoma demonstrated the feasibility and safety of the phIL12 plasmid by gene electrotransfer. We were able to demonstrate that phIL12 gene electrotransfer induced local IL-12 production, which was accompanied with IFN-γ expression. Triggering of the immune response was demonstrated by increased infiltration of immune cells and some antitumor effect. Based on these data, we would recommend the use of a concentration of 2 mg/ml of the plasmid in future trials.

*Conclusion:* The trial lays the foundation for future Phase II clinical trials in which phIL12 gene electrotransfer is used in combination with local tumor-ablative approaches, such as electrochemotherapy or radiotherapy.

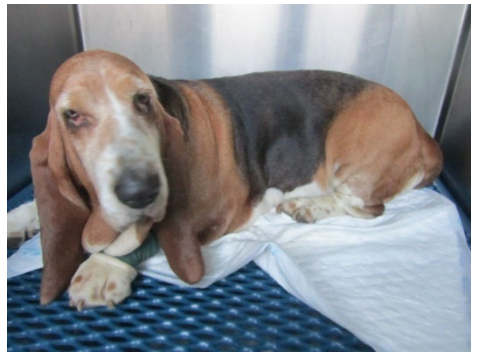
# Clinical development –further steps

**Clinical trial (Phase I/II):** Treatment of the Head and Neck Skin Tumors with the combination of electrochemotherapy or radiotherapy and gene electrotransfer of Interleukin 12

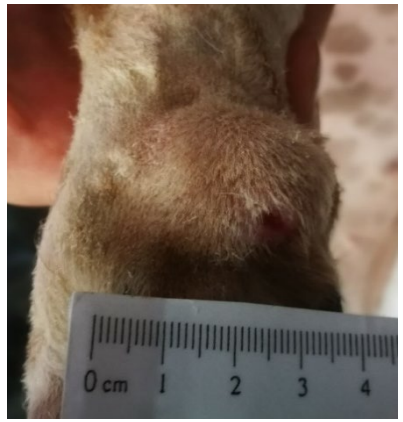


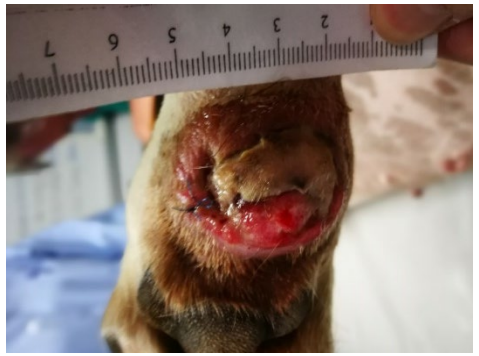
# Intratumoral application of pcaIL-12 & ECT bleomycin



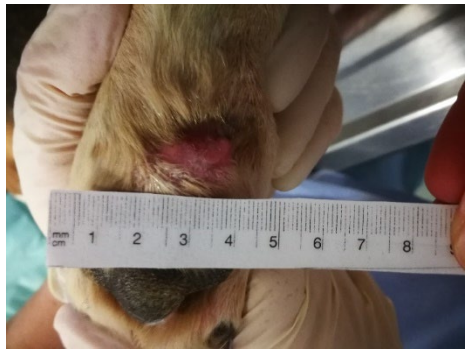
- Basset hound
- female
- 6 years



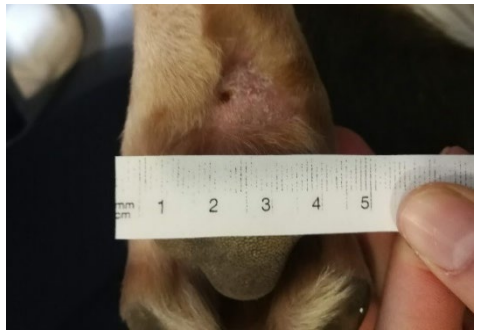
- Mastocytoma
- Right sole of hind leg 2.6 $cm^3$
- Two treatments 4 weeks apart
- (PR 0.4 $cm^3$ after first th.)
- Patohistological grade (II)



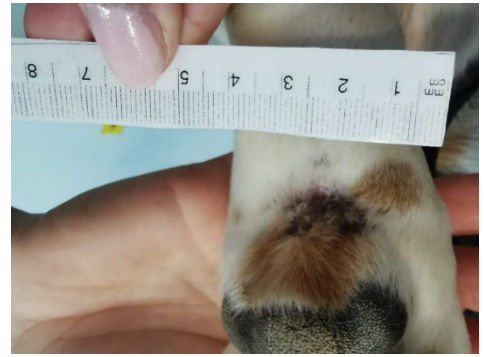
1 week after the first therapy



1 month after the second therapy



2 months after the second therapy

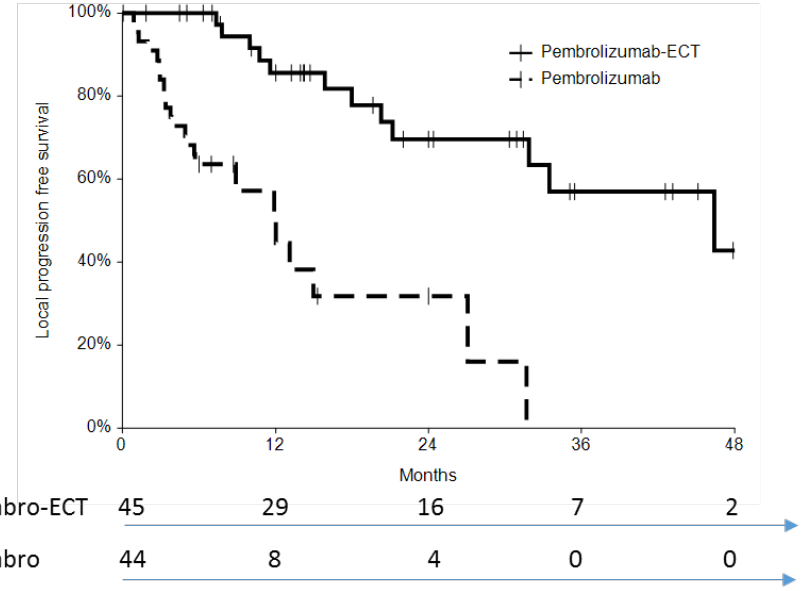


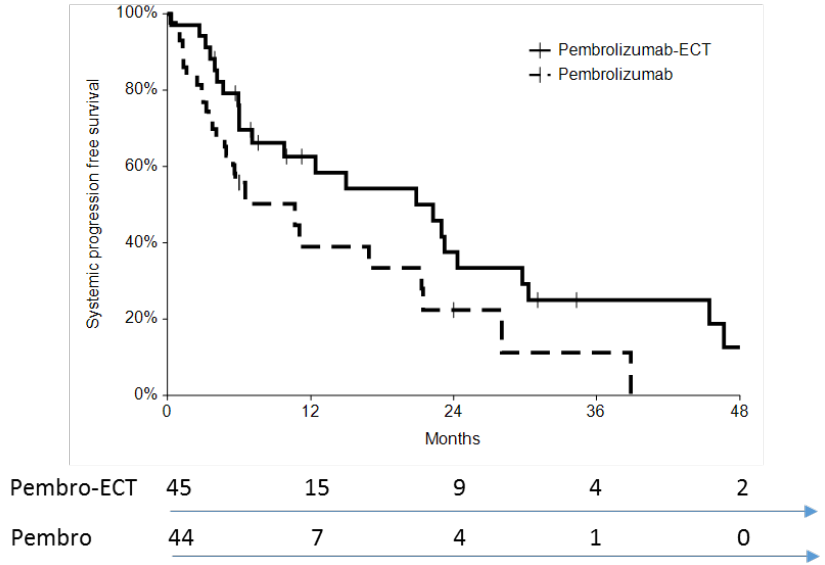
6 months after the second therapy

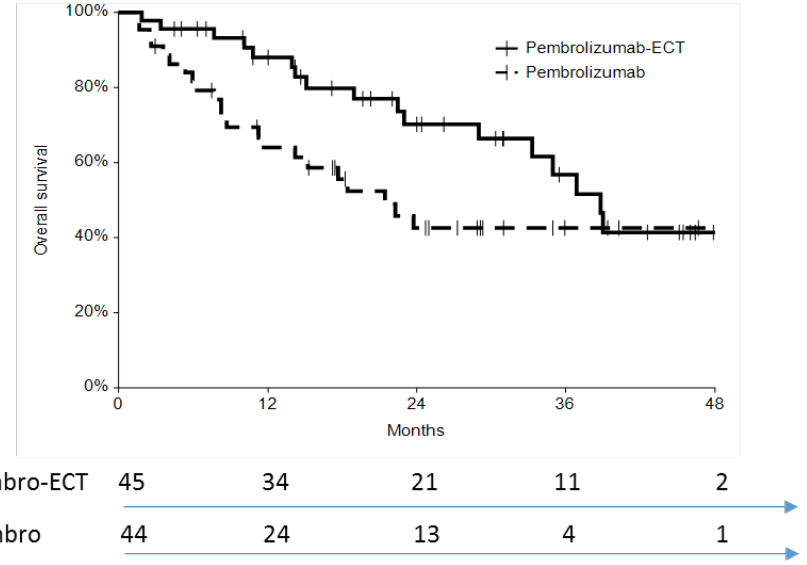
Lampreht Tratar U, Milevoj N, Cemazar M, et al .Int Immunopharmacol. 2023 May 20;120:110274. doi: 10.1016/j.intimp.2023.110274.

# Combined treatment of ECT and Pembrolizumab

- Advanced skin melanoma
- ECT contributed to response with immune checkpoint inhibitors

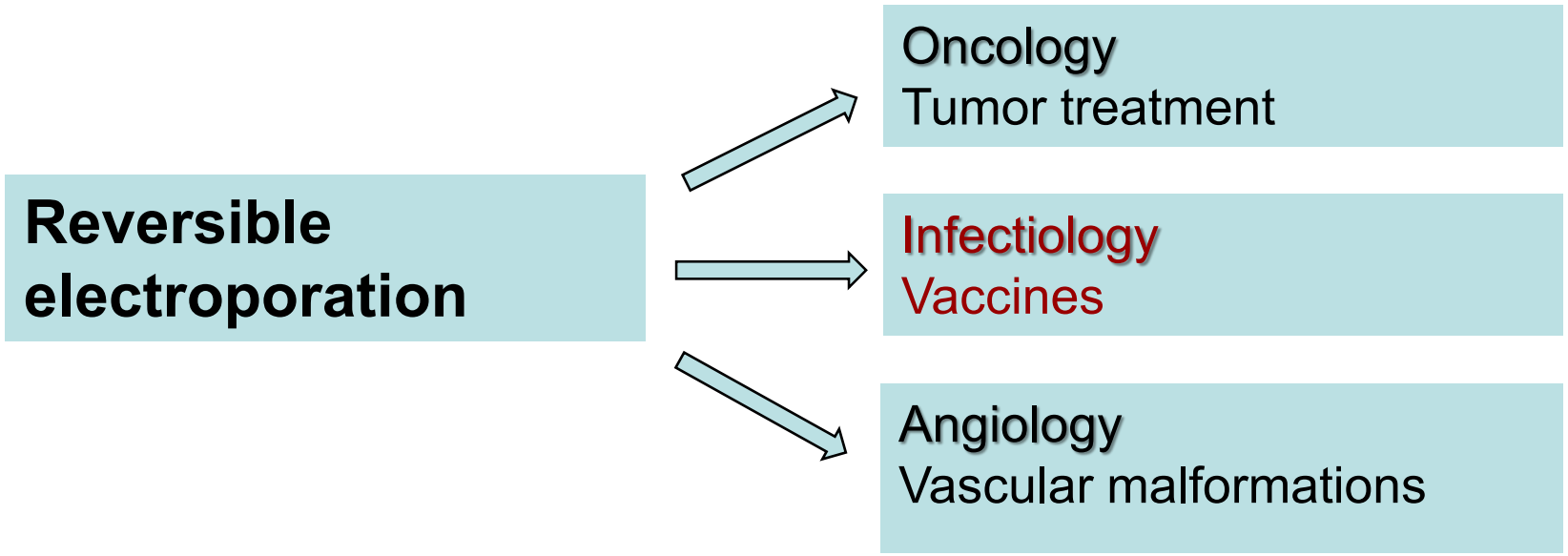


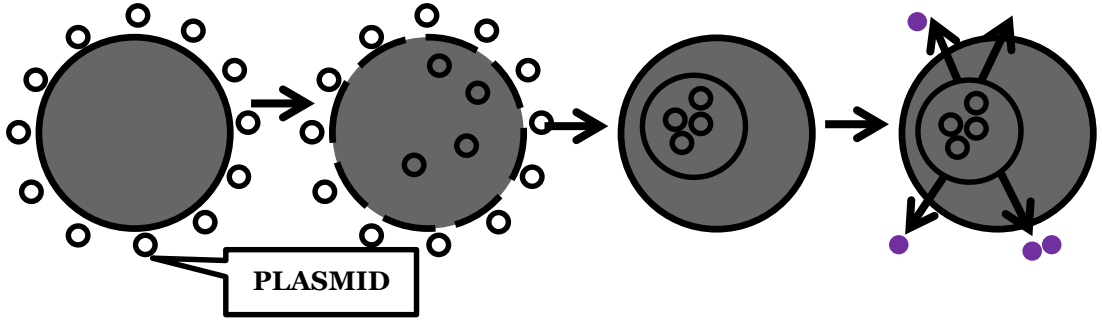

*Campana LG et al., Cancers 2021*

# Clinical applications of reversible electroporation

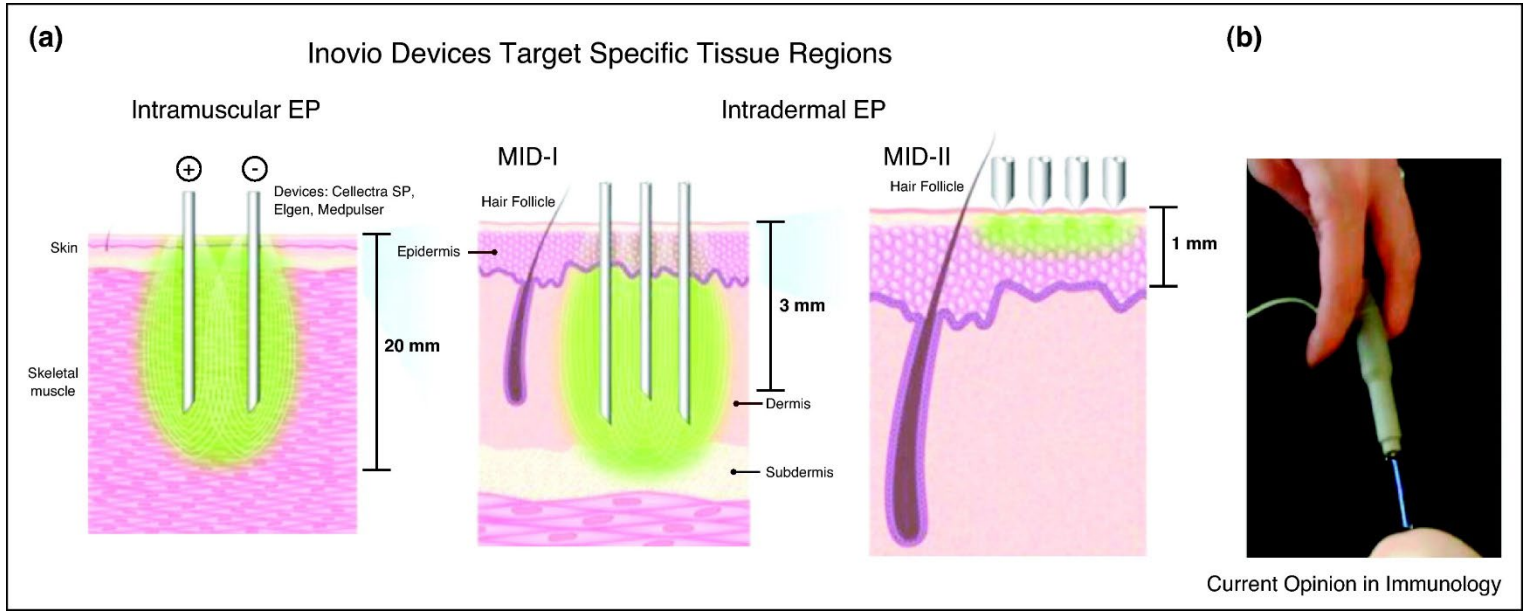


# Electroporation for delivery od DNA vaccines

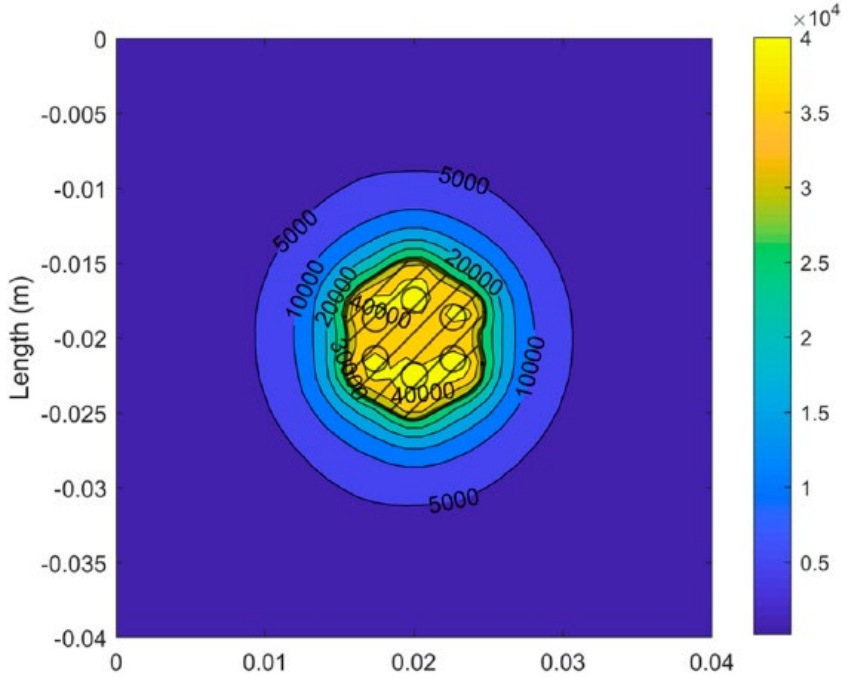
- GET – Gene Electrotransfer




- Target tissue becomes the producer of the transfected gene
  - Skin
  - Muscle

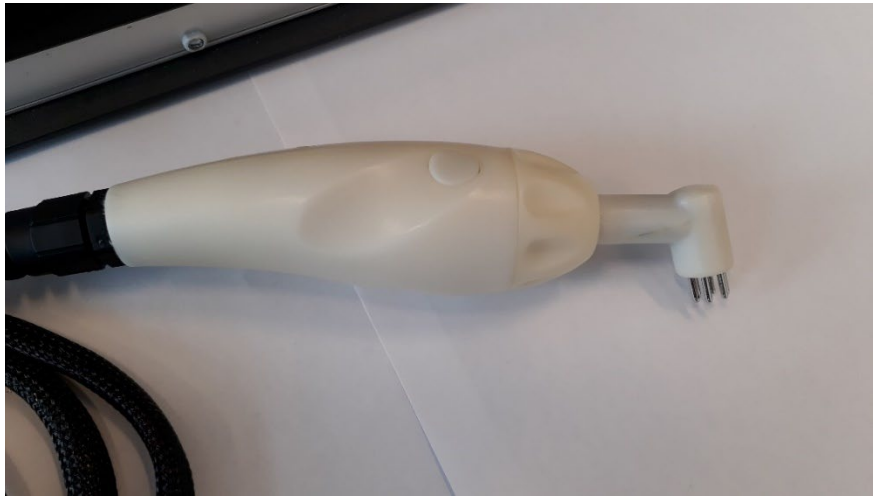


# Electric pulse generator for skin




*pharmaceutics*

MDPI



*Article*

## Design, Development, and Testing of a Device for Gene Electrotransfer to Skin Cells In Vivo

Aleksandra Cvetkoska [1], Janja Dermol-Černe [1], Damijan Miklavčič [1], Simona Kranjc Brezar [2], Boštjan Markelc [2], Gregor Serša [2] and Matej Reberšek [1,*]

# Reversible electroporation

- DNA Vaccines
  - Cancer vaccines
  - Infectious diseases

Molecular Therapy
Original Article

## COVID-eVax, an electroporated DNA vaccine candidate encoding the SARS-CoV-2 RBD, elicits protective responses in animal models

Antonella Conforti,[1,2,16] Emanuele Marra,[1,16] Fabio Palombo,[1,3,16] Giuseppe Roscilli,[1,16] Micol Ravà,[4,16] Valeria Fumagalli,[4,6,16] Alessia Muzi,[1] Mariano Maffei,[2] Laura Luberto,[1] Lucia Lione,[1] Erika Salvatori,[1] Mirco Compagnone,[3] Eleonora Pinto,[1] Emiliano Pavoni,[1] Federica Bucci,[1] Grazia Vitagliano,[1] Daniela Stoppoloni,[1] Maria Lucrezia Pacello,[1] Manuela Cappelletti,[1] Fabiana Fosca Ferrara,[1] Emanuela D'Acunto,[1] Valerio Chiarini,[1] Roberto Arriga,[1] Abraham Nyska,[5] Pietro Di Lucia,[4] Davide Marotta,[4,6] Elisa Bono,[4] Leonardo Giustini,[4] Eleonora Sala,[4,6] Chiara Perucchini,[4] Jemma Paterson,[7] Kathryn Ann Ryan,[7] Amy-Rose Challis,[7] Giulia Matusali,[8] Francesca Colavita,[8] Gianfranco Caselli,[9] Elena Criscuolo,[6] Nicola Clementi,[6,10] Nicasio Mancini,[6,10] Rüdiger Groß,[11] Alina Seidel,[11] Lukas Wettstein,[11] Jan Münch,[11] Lorena Donnici,[12] Matteo Conti,[12] Raffaele De Francesco,[12,13] Mirela Kuka,[4,6] Gennaro Ciliberto,[13] Concetta Castilletti,[8] Maria Rosaria Capobianchi,[8] Giuseppe Ippolito,[8] Luca G. Guidotti,[4,6,17] Lucio Rovati,[9,14,17] Matteo Iannacone,[4,6,15,17] and Luigi Aurisicchio[1,2,3,17]

[1]Takis Biotech, Via Castel Romano 100, 00128 Rome, Italy; [2]Evvivax Biotech, Via Castel Romano 100, 00128 Rome, Italy; [3]Neomatrix Biotech, Via Castel Romano 100, 00128 Rome, Italy; [4]Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, 20132 Milan, Italy; [5]Sackler School of Medicine, Tel Aviv University, Haharuv 18, PO Box 184, Timrat 36576, Israel; [6]Vita-Salute San Raffaele University, 20132 Milan, Italy; [7]National Infection Service, Public Health England (PHE), Porton Down, Salisbury, Wiltshire SP4 0JG, UK; [8]National Institute for Infectious Diseases Lazzaro Spallanzani, Via Portuense 292, 00149 Rome, Italy; [9]Rottapharm Biotech s.r.l., Via Valosa di Sopra 9, 20900 Monza, Italy; [10]Laboratory of Microbiology and Virology, IRCCS San Raffaele Scientific Institute, 20132 Milan, Italy; [11]Institute of Molecular Virology, Ulm University Medical Center, Meyerhofstr. 1, 89081 Ulm, Germany; [12]INGM-Istituto Nazionale di Genetica Molecolare "Romeo ed Erica Invernizzi," Milan, Italy; [13]National Cancer Institute Regina Elena, Via Elio Chianesi 53, 00144 Rome, Italy; [14]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy; [15]Experimental Imaging Centre, IRCCS San Raffaele Scientific Institute, 20132 Milan, Italy

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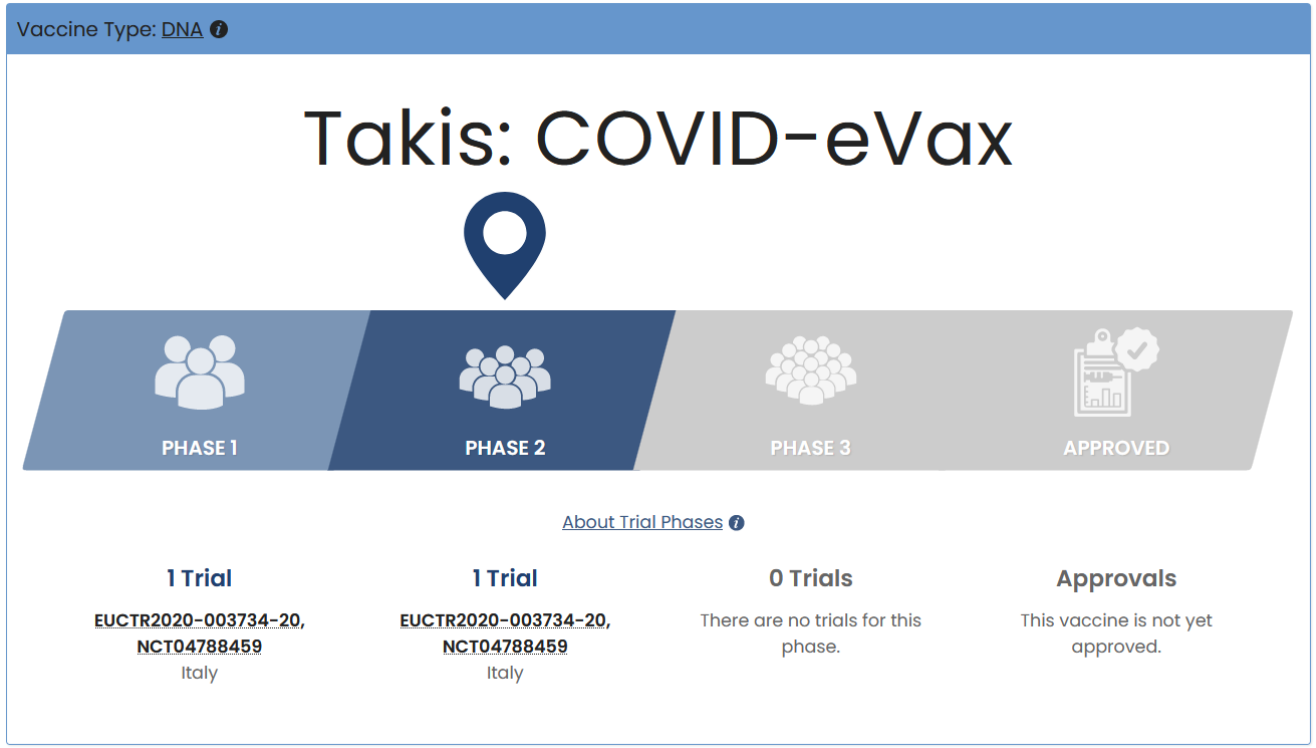
Journal of Experimental & Clinical Cancer Research

REVIEW Open Access

## Cancer DNA vaccines: current preclinical and clinical developments and future perspectives

Alessandra Lopes, Gaëlle Vandermeulen† and Véronique Préat*†



# Development of T cell therapy strategies for hepatitis B virus induced hepatocellular carcinoma

## Conclusion

Immunotherapy of HBV-HCC using T cells engineered to transiently recognize HBV epitopes is a treatment strategy with a good safety profile and promising efficacy in patients with either primary HBV-HCC or HBV-HCC recurrence after liver transplantation. While it is still necessary to evaluate

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Advance access publication 24 December 2021
**Review**

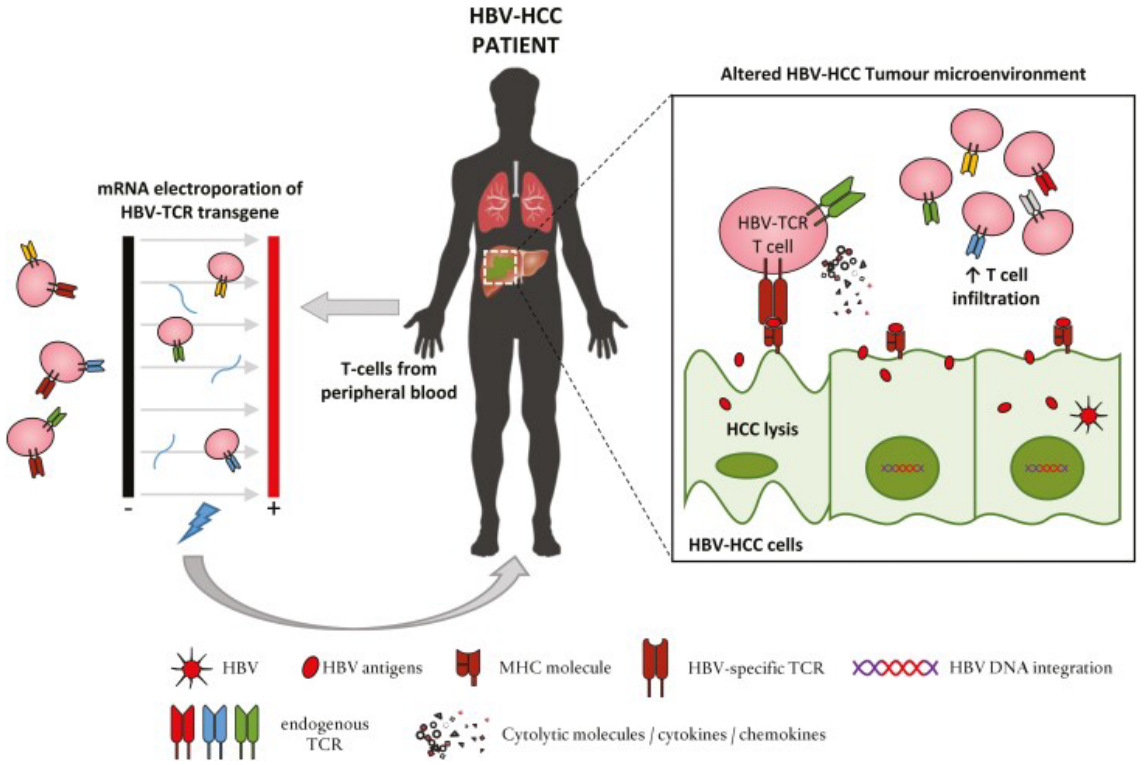
OXFORD

## HBV-HCC treatment with mRNA electroporated HBV-TCR T cells

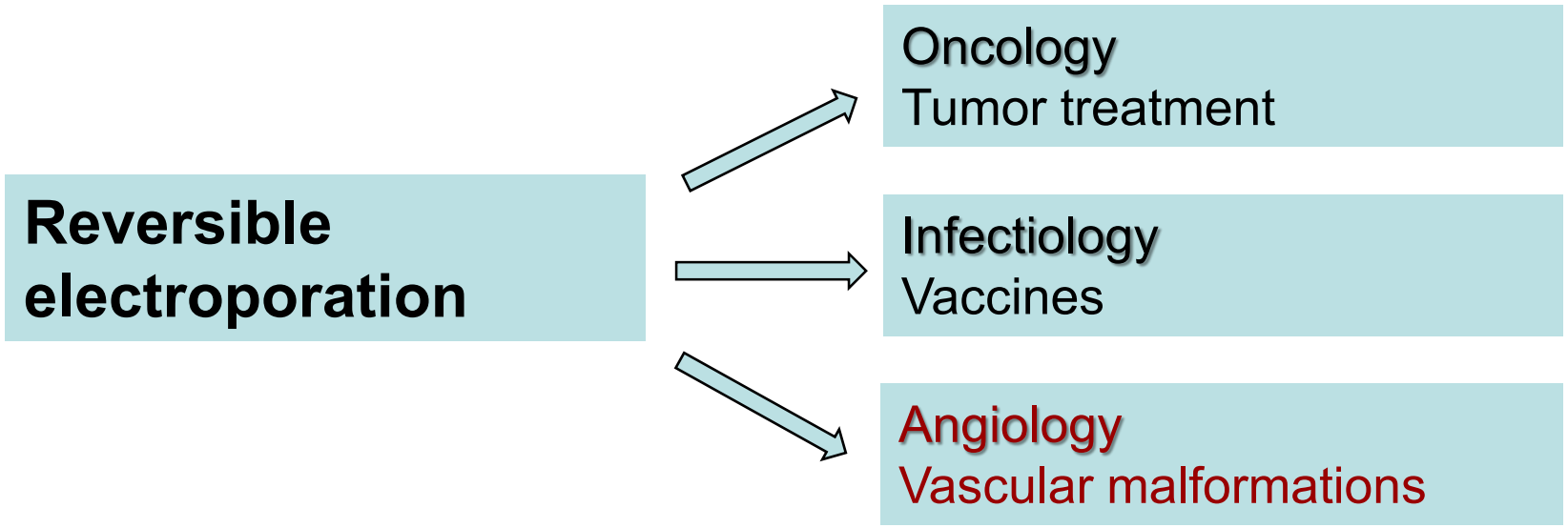
**Anthony T. Tan**[1] **and Antonio Bertoletti**[1,2,*]

[1]Emerging Infectious Diseases, Duke-NUS Medical School, Singapore
[2]Singapore Immunology Network, Agency for Science and Technology (A*STAR), Singapore

***Correspondence:** Antonio Bertoletti, Programme in Emerging Infectious Diseases Programme, Duke-NUS Medical School, 8 College Road, Singapore 169857, Singapore. Email: antonio@duke-nus.edu.sg



# Clinical applications of reversible electroporation



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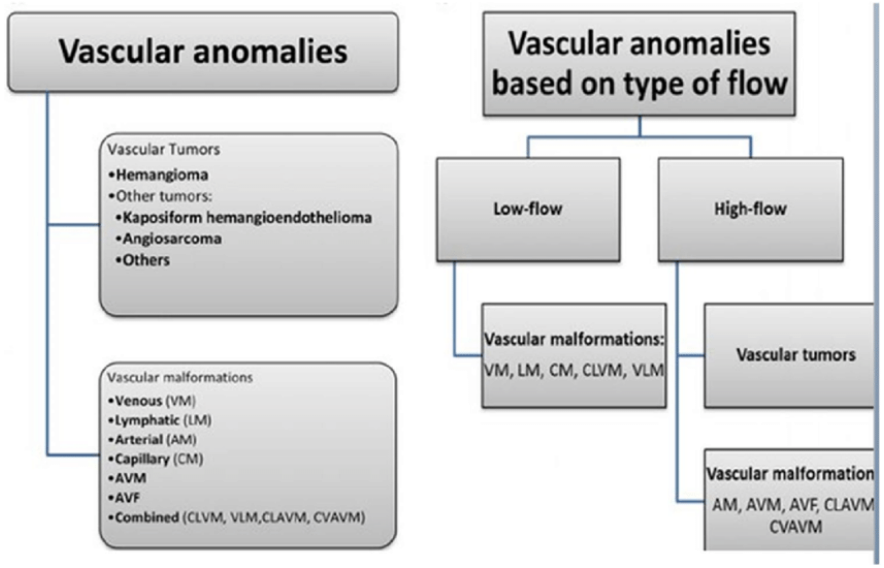
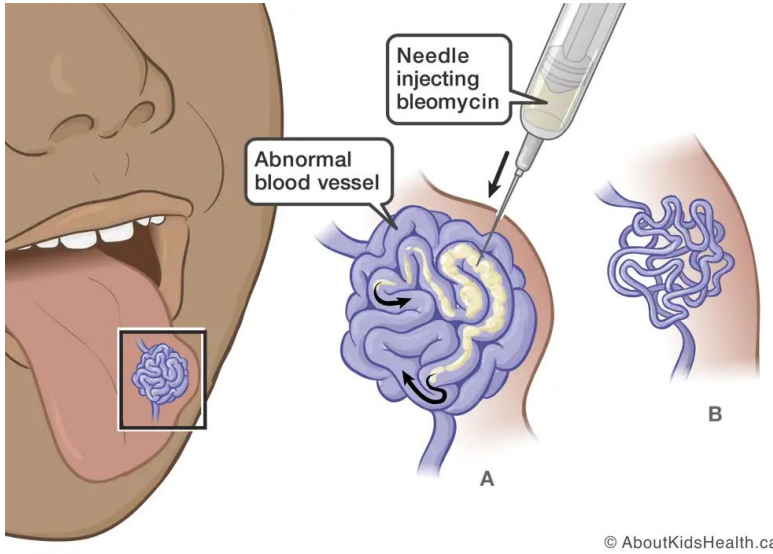
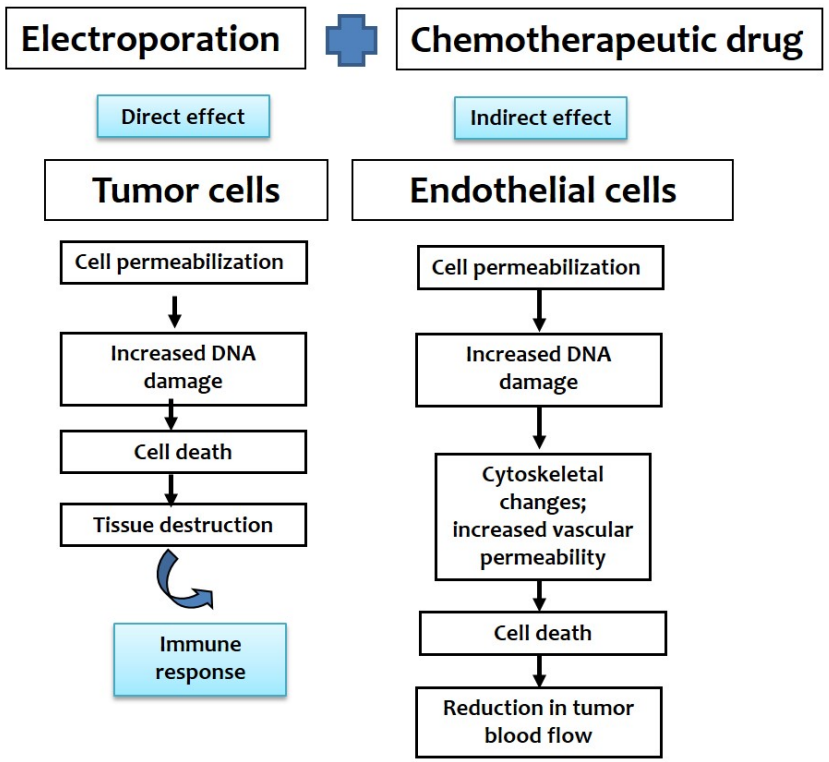
*review*

# Bleomycin electrosclerotherapy (BEST) for the treatment of vascular malformations. An International Network for Sharing Practices on Electrochemotherapy (InspECT) study group report

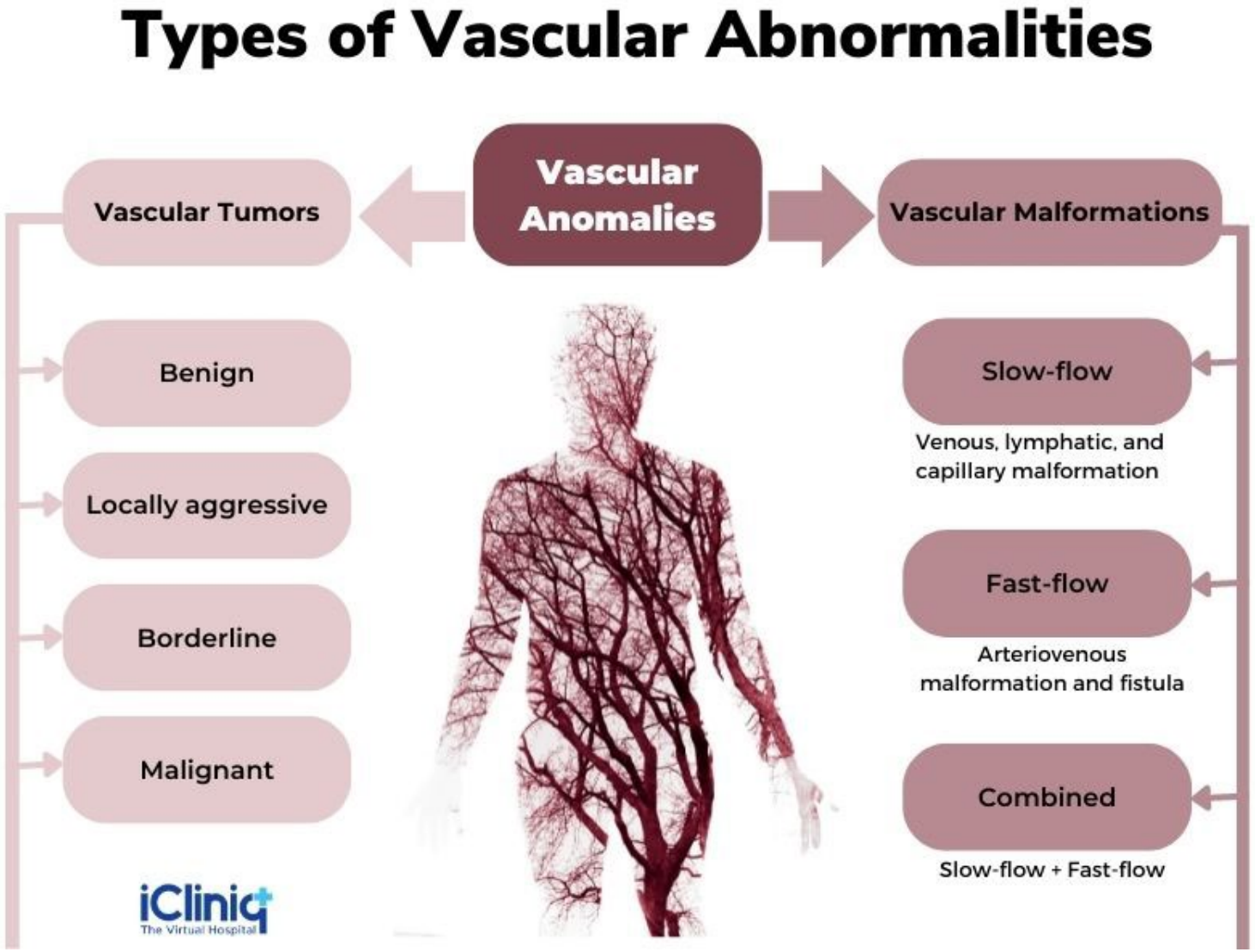
Tobian Muir[1], Giulia Bertino[2], Ales Groselj[3,4], Lakshmi Ratnam[5], Erika Kis[6], Joy Odili[7], Ian McCafferty[8], Walter A Wohlgemuth[9], Maja Cemazar[10,11], Aljosa Krt[11], Masa Bosnjak[10], Alessandro Zanasi[13], Michela Battista[13], Francesca de Terlizzi[13], Luca G Campana[14], Gregor Sersa[10,15]



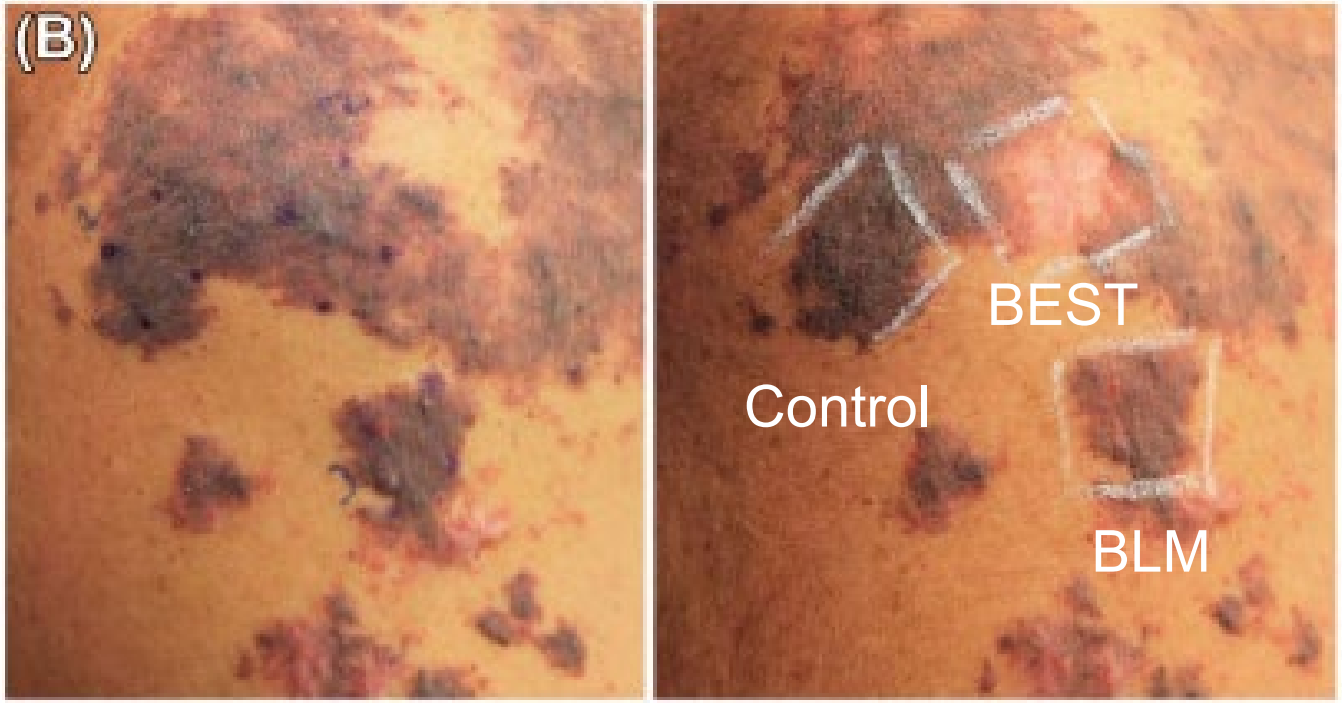



# Similarities between tumor vasculature and vascular malformations



# The first record of electrosclerotherapy




Patient 2; ROI 1: control, ROI 2: EST, ROI 3: bleomycin.

## Electrosclerotherapy as a Novel Treatment Option for Hypertrophic Capillary Malformations: A Randomized Controlled Pilot Trial

Sophie E.R. Horbach, MD, PhD,* Albert Wolkerstorfer, MD, PhD,† Folkert Jolink, MD,* Paul R. Bloemen, BEng,‡ and Chantal M.A.M. van der Horst, MD, PhD*

BACKGROUND Bleomycin sclerotherapy is ineffective for treating capillary malformations (CMs) because bleomycin cannot adequately be injected into the small-diameter capillary lumina. Electrosclerotherapy (EST) might be a new treatment modality for CMs, as it combines bleomycin sclerotherapy and "electroporation"—an electric field applied to the tissue. Electroporation disrupts the transmembrane potential, facilitating bleomycin transportation across the vessel wall, hypothetically leading to targeted drug delivery and increased effectiveness of bleomycin in CMs.

OBJECTIVE To explore the efficacy, safety, and feasibility of EST for CMs in a randomized within-patient controlled pilot study.

MATERIALS AND METHODS Fifteen regions of interest (ROI) within the hypertrophic CMs of 5 patients were randomly allocated to EST, bleomycin injection, or no treatment. Outcome was assessed after 7 weeks by the patient and a blinded outcome assessor using the patient-observer scar assessment score (POSAS), global assessment of change (GAC), colorimetry, and laser speckle contrast imaging.

RESULTS Color and hypertrophy of all ROIs treated with EST significantly improved, based on the POSAS (medians patient −11; observer −13), GAC, and colorimetry (ΔE 3.4–16.5) scores.

CONCLUSION This pilot study demonstrates the first proof of concept for electrosclerotherapy as a new treatment modality for CMs. Further research is warranted.

*The authors have indicated no significant interest with commercial supporters. The equipment for this study was provided by IGEA medical. IGEA medical was not involved in the study design, execution, analyses, and writing of the manuscript. This study was approved by the local institutional review board of the Academic Medical Center of the University of Amsterdam, the Netherlands, and was conducted conforming to the Declaration of Helsinki. The study protocol was published and registered at clinicaltrials.gov (NCT02883023) on August 29, 2016 and the Netherlands National Trial Register (NTR6169) on November 15, 2016.*

*Dermatologic Surgery 2020*

# Bleomycin electrosclerotherapy - BEST

## Bleomycin electrosclerotherapy in therapy-resistant venous malformations of the body

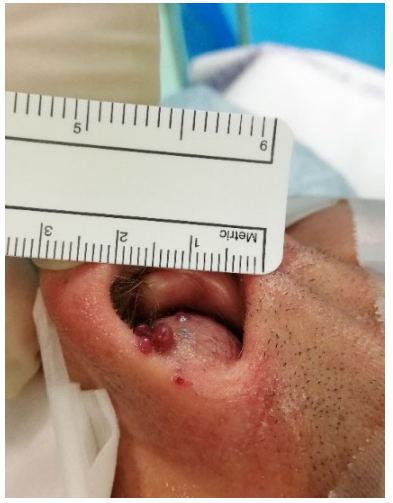
Walter A. Wohlgemuth, PhD,[a] Rene Müller-Wille,[b] Lutz Meyer,[c] Moritz Wildgruber,[d] Moritz Guntau,[a] Susanne von der Heydt,[e] Maciej Pech,[f] Alessandro Zanasi,[g] Lilit Flöther,[h] *and* Richard Brill,[a] *Halle (Saale), Göttingen, Eberswalde, Munich, Berlin, and Magdeburg, Germany*

ORIGINAL ARTICLE

## Bleomycin Electrosclerotherapy Treatment in the Management of Vascular Malformations

Kostusiak, Milosz MBBS, MRCS, BSc; Murugan, Srinivasen MBBS, FRCS (Glasgow); Muir, Tobian MBChB, MMed (Plast Surg)*

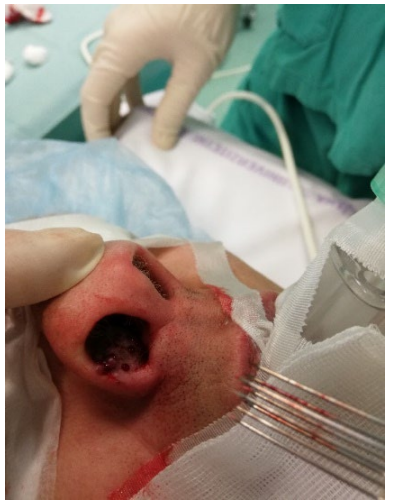
*Patient treated at the Institute of Oncology Ljubljana*



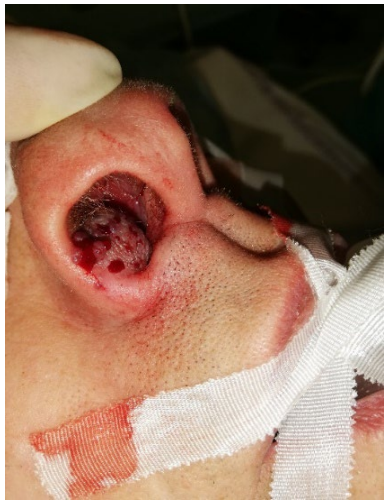
Tumor measurement

Intratumoral bleomycin

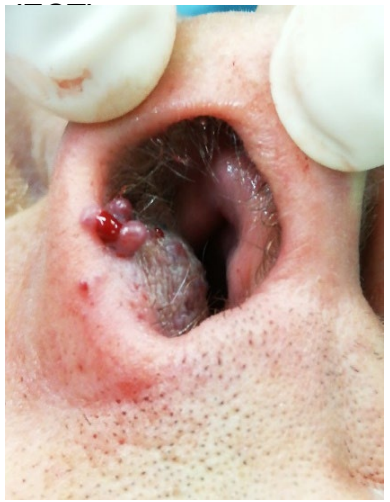
Electrochemotherapy



Immediately after ECT



Immediately after ECT



5 min after ECT

*Patient treated at the Institute of Oncology Ljubljana*

# Bleomycin Electrosclerotherapy

- High-flow head and neck vascular malformation
- Selective artery catheterization and angiography of the right facial artery to adequately visualize tumor vascularisation
- Low dose intratumoral BLM injection
- 15 applications of electric pulses
- Complete remission after 18 months

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TYPE Brief Research Report
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EDITED BY
Zhao-Jun Liu,
University of Miami, United States

REVIEWED BY
Wayne Yakes,
The Yakes Vascular Malformation Center, United States
Maria Paola Belfiore,
University of Campania Luigi Vanvitelli, Italy

*CORRESPONDENCE
Ales Groselj
ales.groselj@kclj.si

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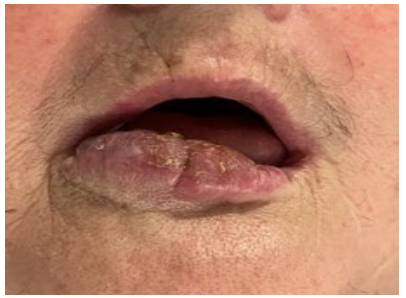
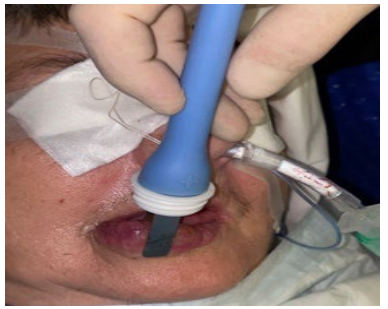
## Combining superselective catheterization and electrochemotherapy: A new technological approach to the treatment of high-flow head and neck vascular malformations

Aljosa Krt[1], Maja Cemazar[2,3], Dimitrij Lovric[4], Gregor Sersa[2,5], Crt Jamsek[6] and Ales Groselj[6,7]*

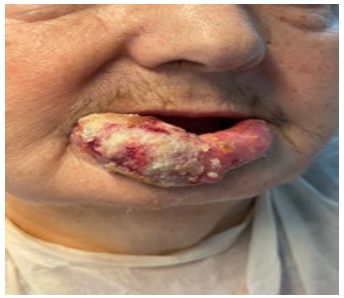
[1]Department of Otorhinolaryngology, Izola General Hospital, Izola, Slovenia, [2]Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia, [3]Faculty of Health Sciences, University of Primorska, Izola, Slovenia, [4]Department of Radiology, University Medical Centre Ljubljana, Ljubljana, Slovenia, [5]Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia, [6]Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia, [7]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia



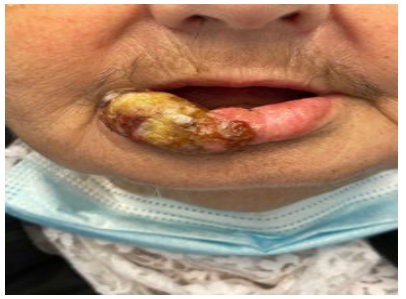
Before treatment



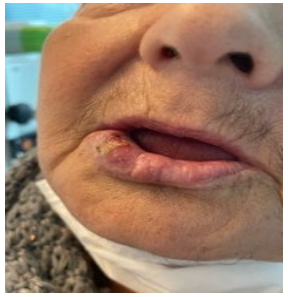
Treatment



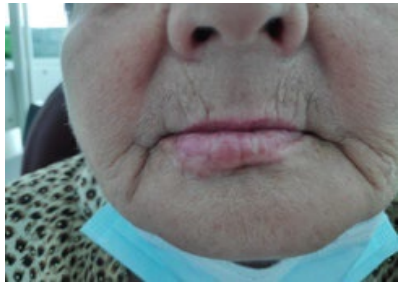
3 weeks



5 weeks



10 weeks



18 months

# Attempt to standardize the treatment

The group of clinicians within the InspECT consortium, in collaboration with other experts in the field of vascular malformations treatment, have prepared this document, a Current Operating Procedure (COP). It is a proposal for the clinical standardisation of BEST using the Cliniporator® as the electrical pulse generator with its associated electrodes. The electrical parameters considered in this protocol are those validated by the European Standard Operating Procedures for Electrochemotherapy (ESOPE) with the Cliniporator®.[23] After validation of these COP in the clinical application, a standard operating procedure would need to be prepared.

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review

## Current Operating Procedure (COP) for Bleomycin ElectroScleroTherapy (BEST) of low-flow vascular malformations

Tobian Muir[1], Walter A Wohlgemuth[2], Maja Cemazar[3,4], Giulia Bertino[5], Ales Groselj[6,7], Lakshmi A Ratnam[8,9], Ian McCafferty[10], Moritz Wildgruber[11,12], Bernhard Gebauer[13], Francesca de Terlizzi[14], Alessandro Zanasi[14], Gregor Sersa[3,15]

[1] South Tees NHS Foundation Trust, Middlesbrough TS4 3BW, United Kingdom
[2] Clinic and Policlinic of Radiology, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany
[3] Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[4] Faculty of Health Sciences, University of Primorska, Isola, Slovenia
[5] Department of Otolaryngology Head Neck Surgery, University of Pavia, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo Foundation, Pavia, Italy
[6] Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia
[7] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[8] St George's University Hospitals NHS Foundation Trust, London, United Kingdom
[9] City St George's University of London, School of Health & Medical Sciences, London, United Kingdom
[10] Birmingham Women's and Children's Hospital NHS Foundation Trust, Birmingham, United Kingdom
[11] Department of Radiology, University Hospital, LMU Munich, München, Germany
[12] Interdisziplinäres Zentrum für Gefäßanomalien (IZGA), University Hospital, LMU Munich, München, Germany
[13] Diagnostic and Interventional Radiology, Charité Universitätsmedizin Berlin, Germany
[14] IGEA S.p.A., Clinical Biophysics Lab. Carpi, Modena, Italy
[15] Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia

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Correspondence to: Prof. Gregor Serša, Ph.D., Institute of Oncology Ljubljana, Department of Experimental Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia. E-mail: gsersa@onko-i.si

Dr. Tobian Muir and Prof. Walter A Wohlgemuth contributed equally to preparation of the manuscript and share first authorship.

Disclosure: AZ and FdT are IGEA employees. The other authors do not declare potential conflicts of interest.

# Current Operating Procedures for BEST

## Indications for BEST of vascular malformations

- Patients with a low-flow vascular malformation (venous, lymphatic, capillary, mixed type) suitable for BEST, i.e.: injection of bleomycin and safe placement of electrodes into the vascular malformation are technically feasible.
- Patients with a low-flow malformation poorly responding or recurring after previous treatment(s).

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## Contraindications for BEST of vascular malformations

- Pregnancy and lactation.
- In adults, previous bleomycin exposure with a cumulative dose greater than 100 000 IU.
- In children, previous bleomycin exposure greater than 1300 IU/kg (taking into account the increasing weight of the child).
- In case of abnormal respiratory results/chest pathology (including previous severe or long COVID) in consultation with a pulmonologist, special care is required, and bleomycin exposure may be contraindicated.
- In patients with impaired renal function, the dose of bleomycin should be reduced at least by 1/3.
- Known allergy or hypersensitivity to bleomycin.
- Presence of significant central venous drainage precluding sclerotherapy.

# Current Operating Procedures for BEST

## Recommended preparation and dosing of intralesional injection

| | Concentration | Preparation for injection | Maximal dose per session | Cumulative dose in all sessions |
|---|---|---|---|---|
| **Bleomycin and contrast** | 1 000 IU/ml in NaCl solution | 1 part of solution in 3 parts of Contrast Medium (250 IU/ml) | 10 000 IU in adults<br>200 IU/kg in children<br>Divide the total dose into anticipated number of sessions<br>The interval between sessions should be at least 8 weeks | 100 000 IU in adults<br>1 300 IU/kg in children -divided by number of sessions |
| **Foamed bleomycin** | 1 000 IU/ml in NaCl solution | 1 ml albumin; 1 ml plain 1% lidocaine; 8 000 IU bleomycin; contrast agent may be added; orthogonal 3 way tap connection; 5 ml air- or according to local practice | As above | As above |

Bleomycin has confusing unit nomenclature and care should be taken to ensure predictable dosing. Historically, bleomycin dosage is described in terms of mg potency, where 1 mg-potency corresponds to 1 Unit or 1 000 International Units. Because 1 mg potency is not always equivalent to 1 mg weight, the International Unit measure is preferred.

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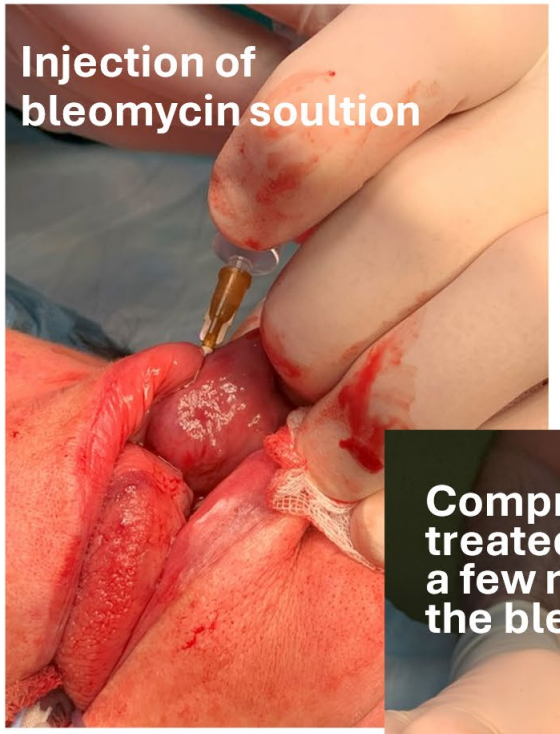
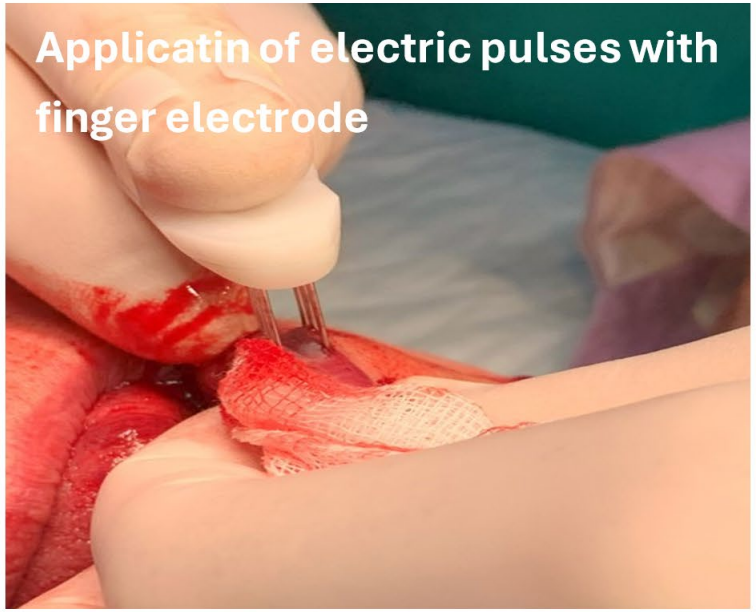
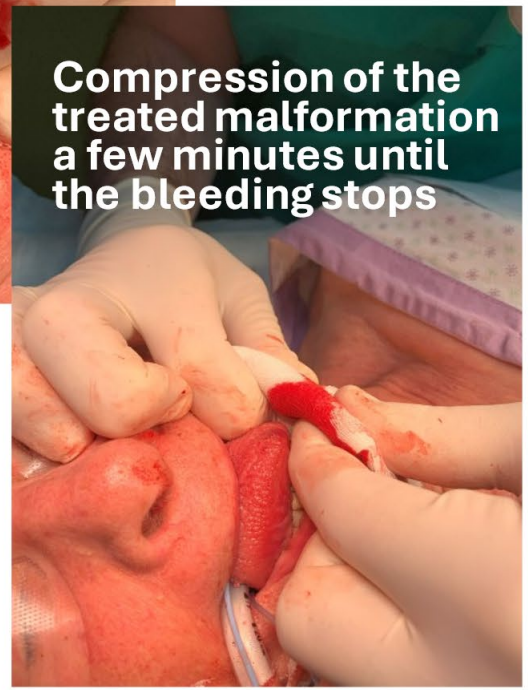
# Current Operating Procedures for BEST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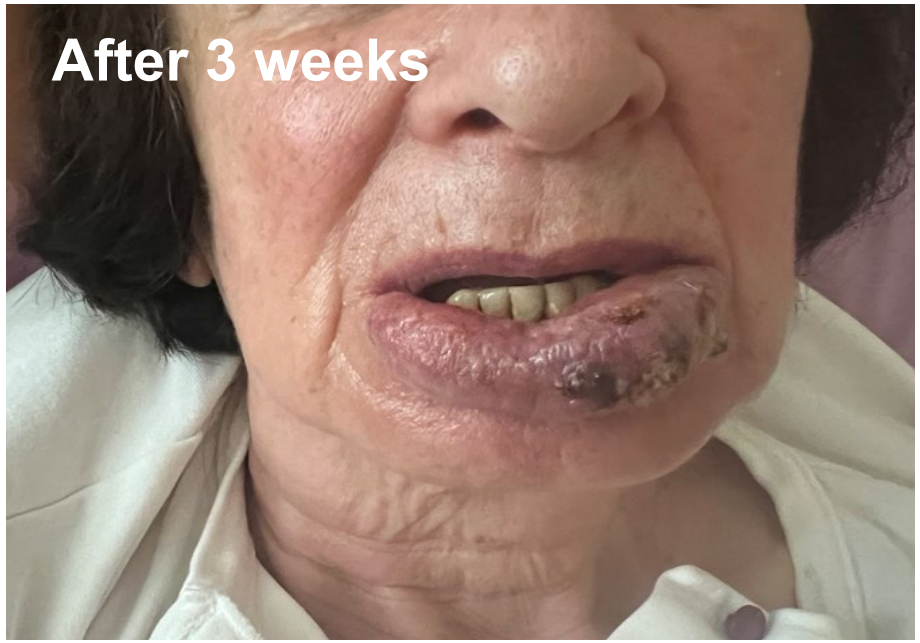
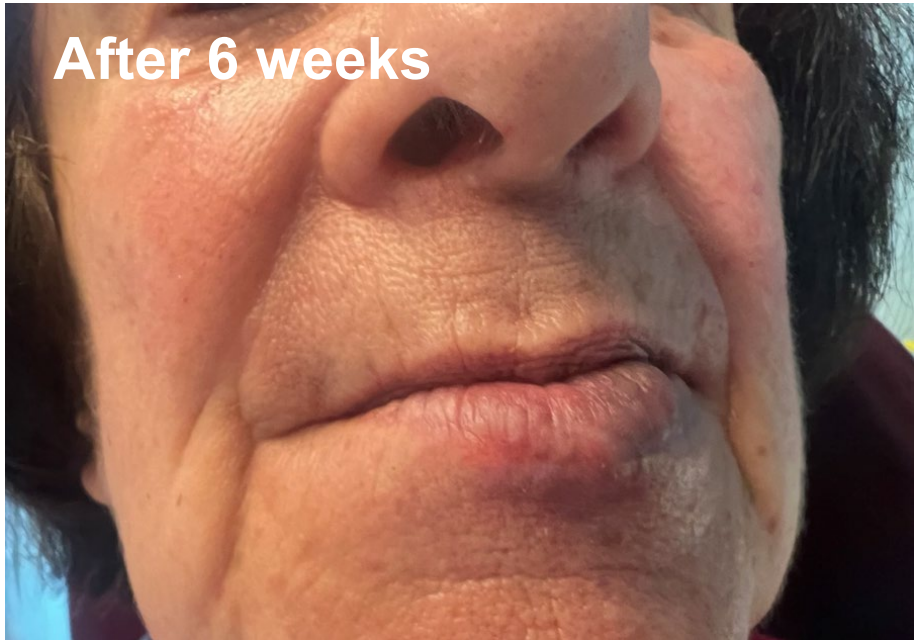
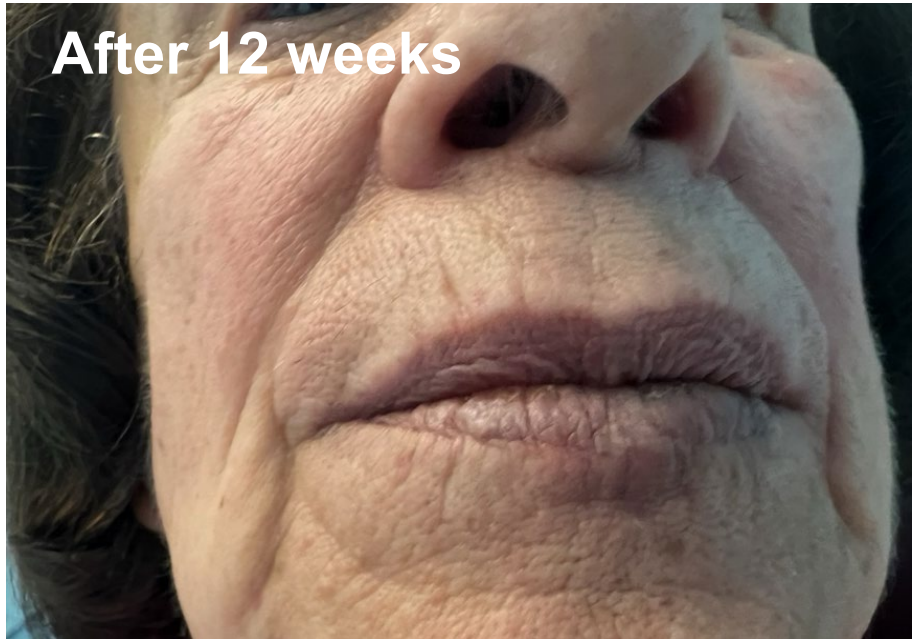
Before treatment with BEST


After 3 weeks

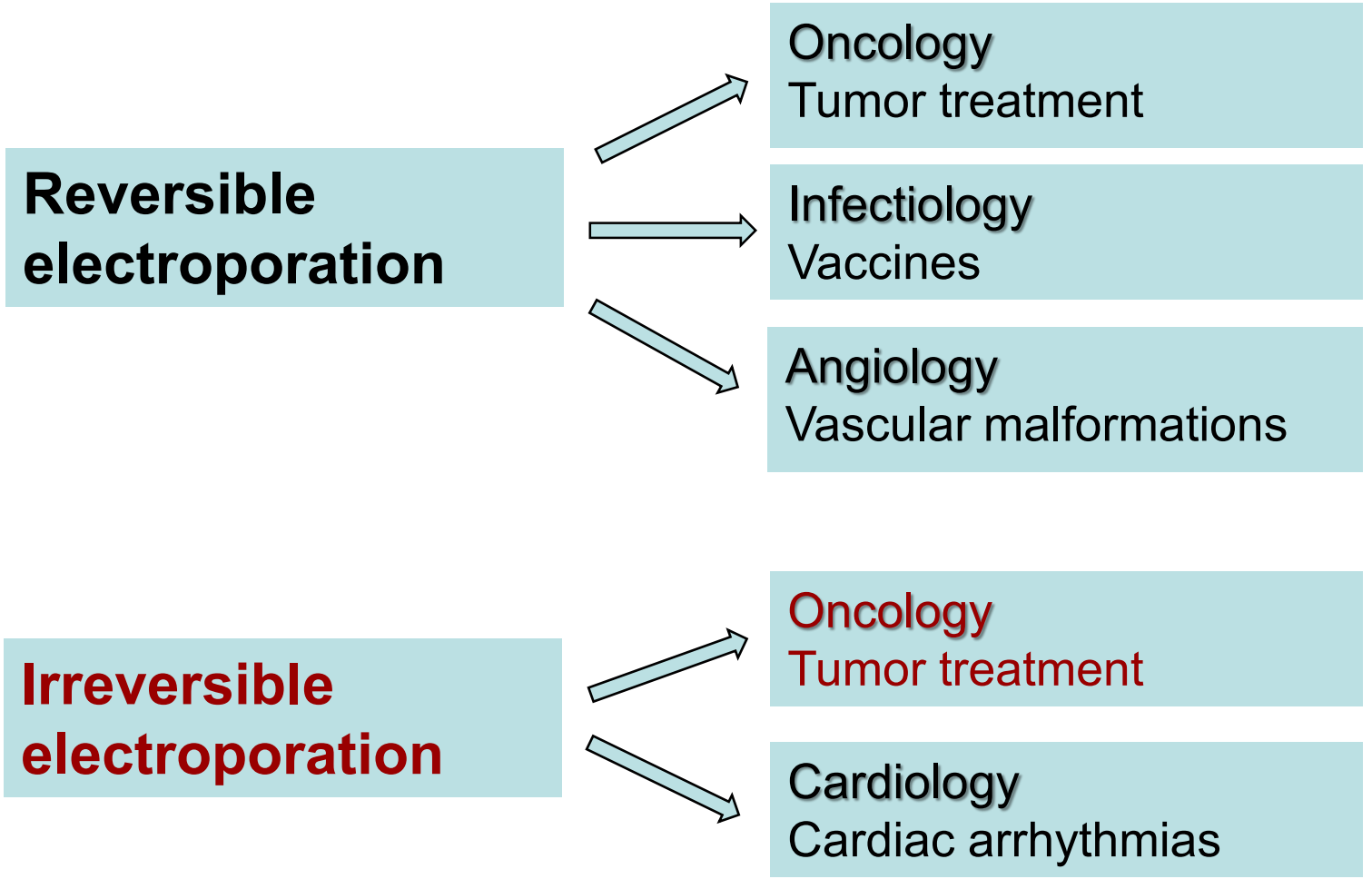

After 6 weeks


After 12 weeks

# Open questions for BEST standardization:

- Patient referral and suitability for BEST treatment
- Local or general anesthesia
- Safety of BLM administration
- Route of BLM administration
- Bleomycin dose
- Volume of drug solution
- Interval between BLM injection and application of el. pulses
- Choice of electrodes
- Coverage of the vascular malformation with el. pulses

# Biomedical applications of electroporation in different disciplines of medicine

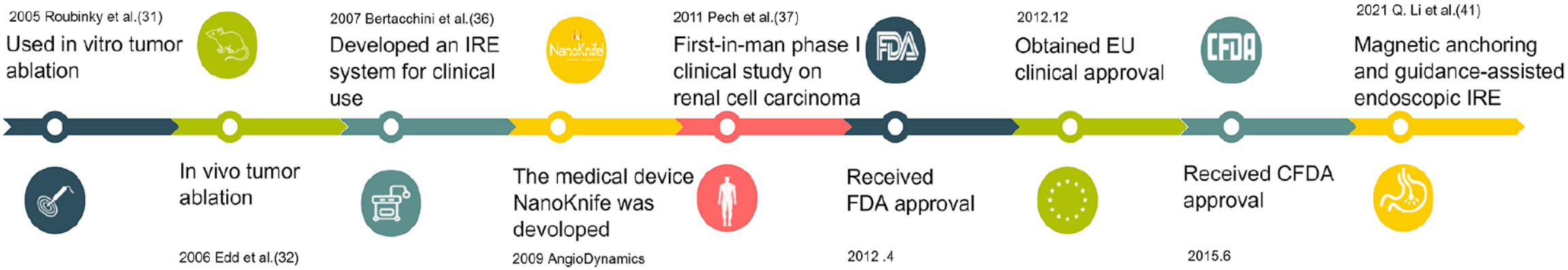


# Development of irreversible electroporation - IRE

Review > Front Immunol. 2022 Jan 7;12:811726. doi: 10.3389/fimmu.2021.811726.
eCollection 2021.

## Irreversible Electroporation: An Emerging Immunomodulatory Therapy on Solid Tumors

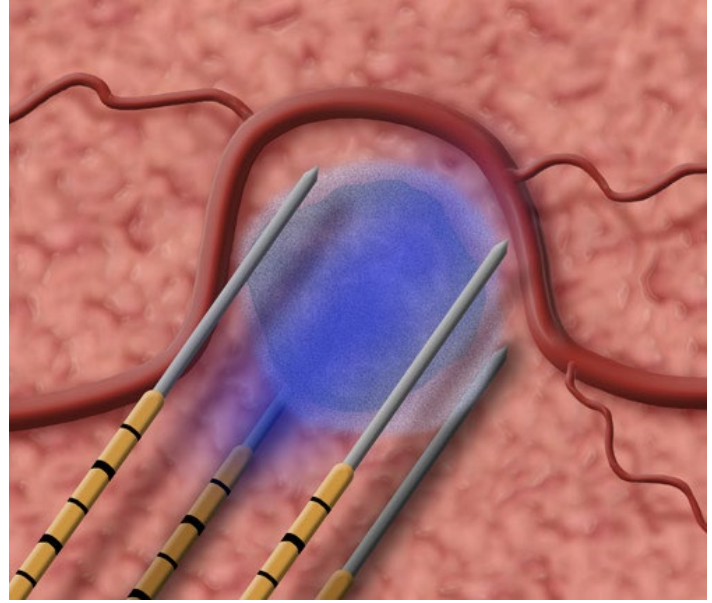
Nana Zhang [1 2 3], Zhuoqun Li [2 3 4], Xuan Han [2 3 4], Ziyu Zhu [1], Zhujun Li [1], Yan Zhao [1], Zhijun Liu [1 2 3], Yi Lv [1 2 3 4]



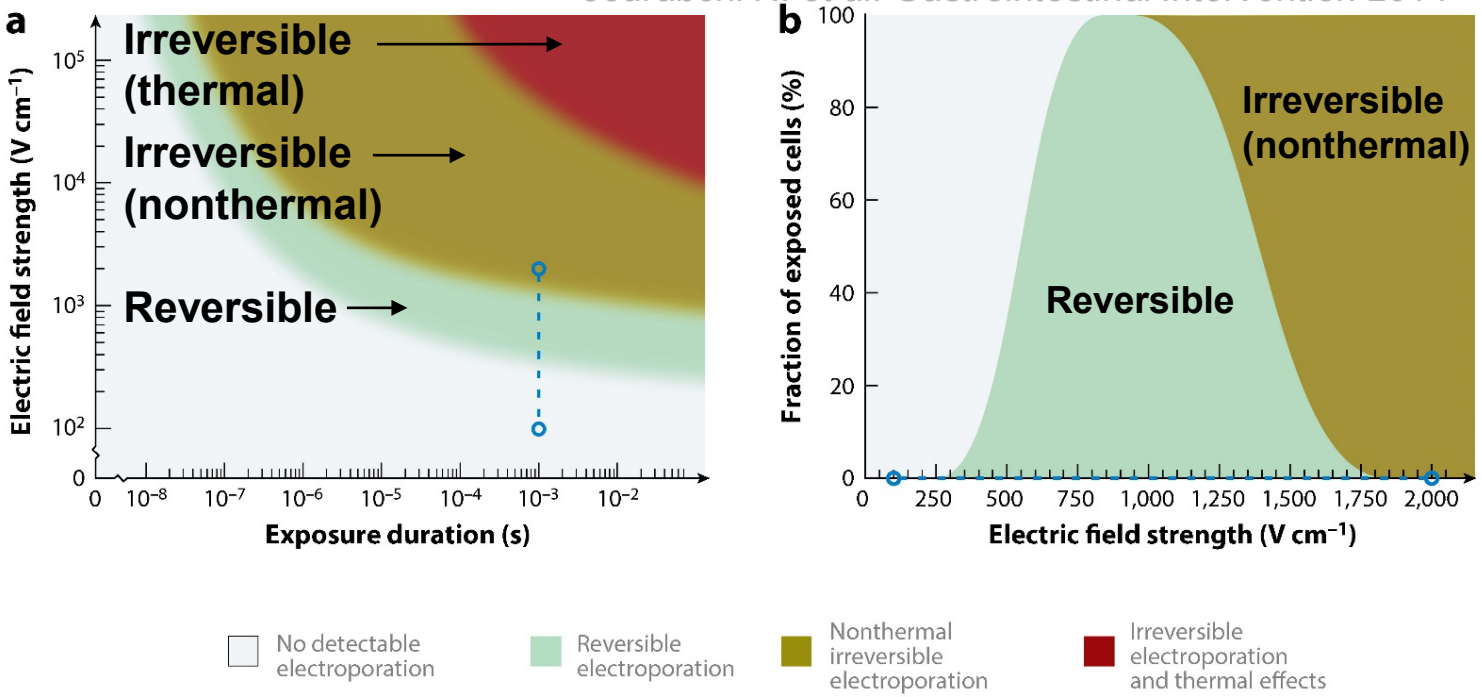
# Applications of IRE treatment

- Liver tumors
  - Hepatocellular carcinoma
  - Colorectal liver metastases
  - Cholangiocarcinoma
- Pancreatic tumors
- Adenocarcinoma of prostate
- Kidney
- Lung

Schematic representation of IRE treatment of a tumor:



Jourabchi N, et al. Gastrointestinal Intervention 2014




Yarmush ML, et al. 2014.
Annu. Rev. Biomed. Eng. 16:295–320

# Colorectal liver metastases

**Table 1.** Indications and contraindications for IRE of CRLM.

| Indications | Relative contraindication | Absolute contraindication |
|---|---|---|
| Patient<br>• Oligometastatic disease in poor surgical candidates<br>• ECOG 0–2, ASA 1–2, CCI $\leq$ 8 | Patient<br>• Atrial fibrillation<br>• Correctable coagulopathy<br>• Limited extrahepatic disease<br>• ASA of 3 | Patient<br>• Ventricular arrhythmia<br>• Pacemaker or implantable cardioverter defibrillator (ICD)<br>• Uncorrectable coagulopathy<br>• ASA >3, ECOG >2<br>• Prior history of epilepsy or seizures |
| Anatomic<br>• Near major bile ducts<br>• Near bowel<br>• Near major vessels or other structures which may lead to a significant heat-sink effect<br>• Tumors $\leq$ 3 cm<br>• Other locations considered unfavorable for partial hepatectomy or thermal ablation | Anatomic<br>• Bilioenteric sphincter * (needs IV antibiotics)<br>• Superficial lesions<br>• Metal stents<br>• Tumors 3–5 cm | Anatomic<br>• Intrahepatic bile duct dilation<br>• Exophytic tumor due to risk of seeding<br>• Tumors >5 cm |

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## Irreversible electroporation for colorectal cancer liver metastasis: a review

Yilun Koethe[a], Nicole Wilson[b] and Govindarajan Narayanan[b,c,d]

[a]Department of Interventional Radiology, Oregon Health and Science University, Portland, OR, USA; [b]Herbert Wertheim College of Medicine, Florida International University, Miami, FL, USA; [c]Miami Cancer Institute, Baptist Health South Florida, Miami, FL, USA; [d]Miami Cardiac and Vascular, Baptist Health South Florida, Miami, FL, USA

**ABSTRACT**

Irreversible electroporation (IRE) ablation is gaining popularity over the last decade as a nonthermal alternative to thermal ablation technologies such as radiofrequency ablation (RFA) and Microwave ablation (MWA). This review serves as a practical guide for applying IRE to colorectal cancer liver metastases (CRLM) for interventional radiologists, oncologists, surgeons, and anesthesiologists. It covers patient selection, procedural technique, anesthesia, imaging, and outcomes.

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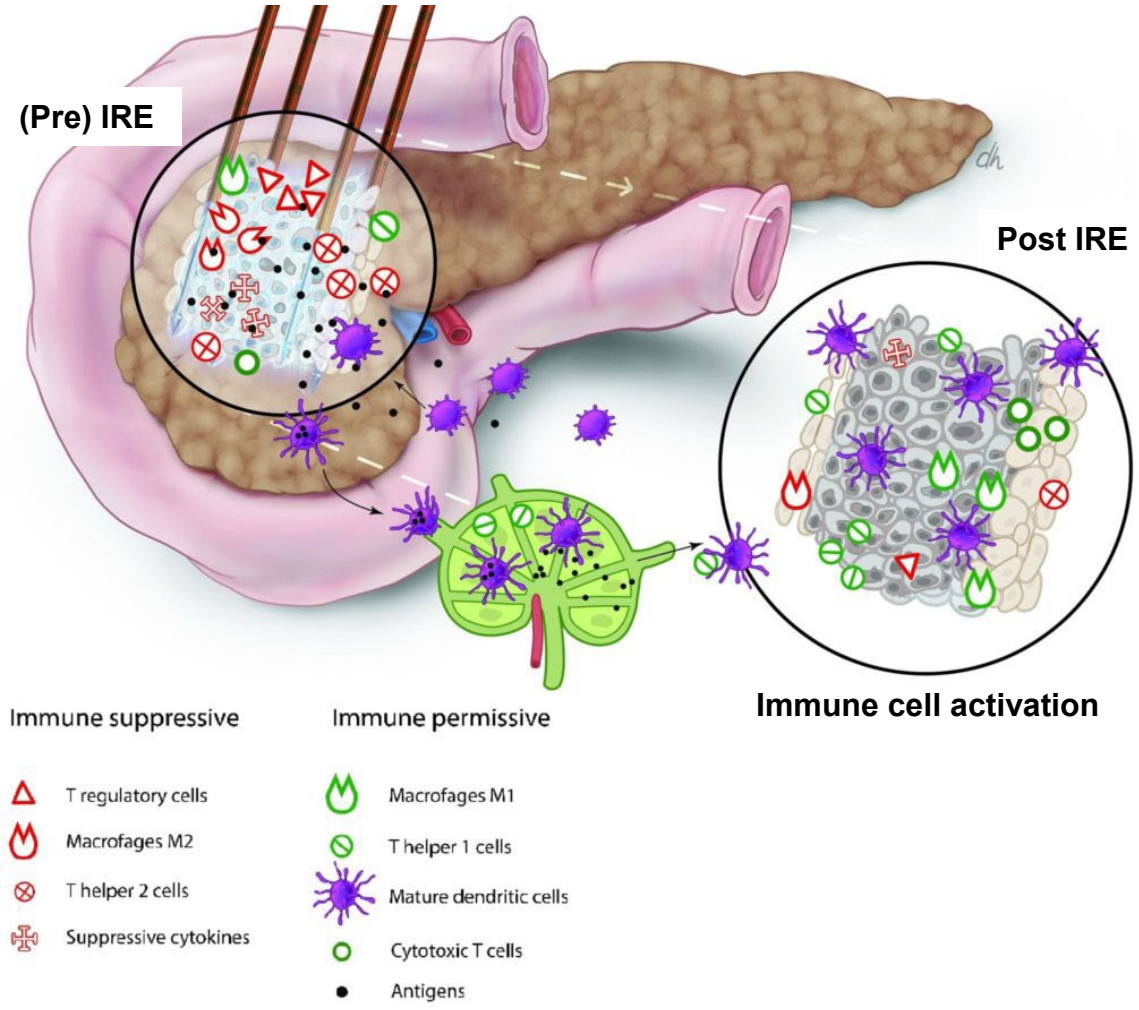
**KEYWORDS**
IRE; MCRC; ablation; colon cancer; metastasis

IRE extends ablation as a therapeutic option for many patients who are not RFA or MWA candidates. The improved survival outcomes from the CLOCC trial have been extrapolated to IRE in clinical practice. Unfortunately, because IRE is typically reserved for patients who aren't RFA or MWA candidates, a direct, matched and unbiased comparison between IRE and thermal ablation is not feasible. In addition, most IRE studies are small, observational, and include a heterogeneous population of liver tumors including HCC, CRLM, and other metastasis, making analysis limited to CRLM patients difficult.

# Pancreatic cancer




*Timmer E.F.F. et al. Techniques in Vascular and Interventional Radiology 2020*

Journal of *Clinical Medicine*

MDPI

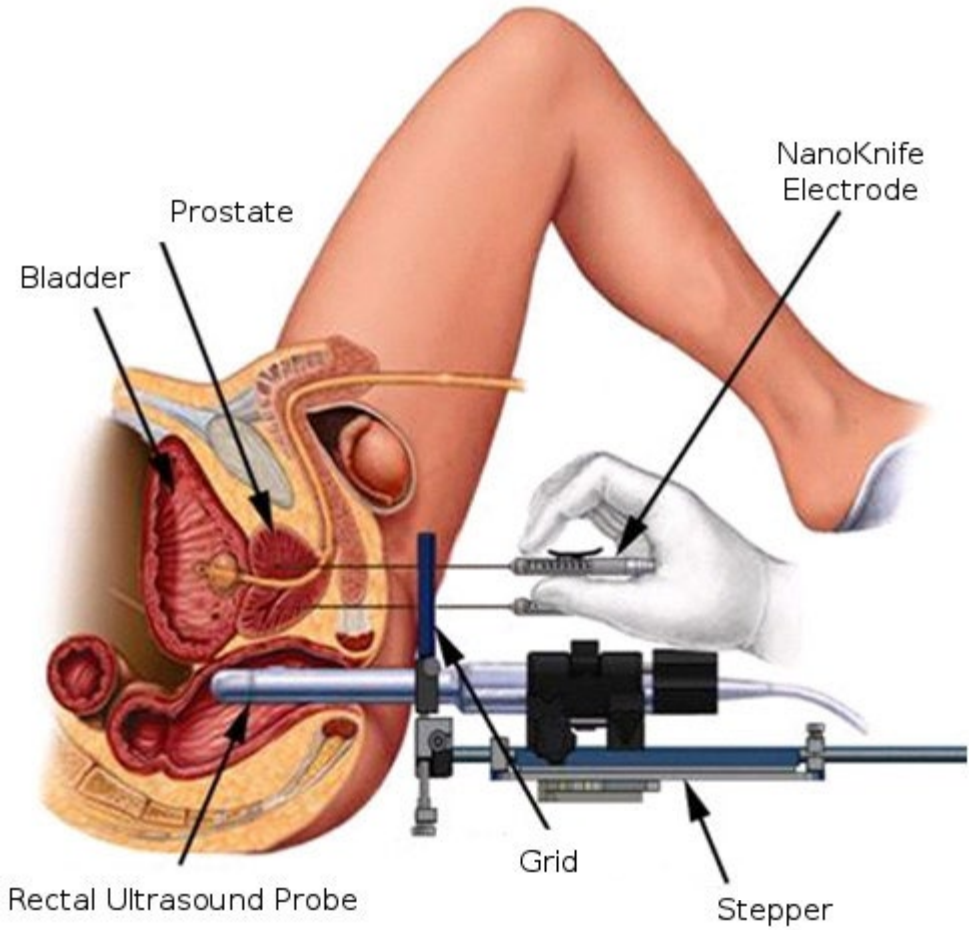
*Review*

## Irreversible Electroporation (IRE) in Locally Advanced Pancreatic Cancer: A Review of Current Clinical Outcomes, Mechanism of Action and Opportunities for Synergistic Therapy

Zainab L. Rai [1,2,3,*], Roger Feakins [3], Laura J. Pallett [4], Derek Manas [5] and Brian R. Davidson [1,3]

**Abstract:** Locally advanced pancreatic cancer (LAPC) accounts for 30% of patients with pancreatic cancer. Irreversible electroporation (IRE) is a novel cancer treatment that may improve survival and quality of life in LAPC. This narrative review will provide a perspective on the clinical experience of pancreas IRE therapy, explore the evidence for the mode of action, assess treatment complications, and propose strategies for augmenting IRE response. A systematic search was performed using PubMed regarding the clinical use and safety profile of IRE on pancreatic cancer, post-IRE sequential histological changes, associated immune response, and synergistic therapies. Animal data demonstrate that IRE induces both apoptosis and necrosis followed by fibrosis. Major complications may result from IRE; procedure related mortality is up to 2%, with an average morbidity as high as 36%. Nevertheless, prospective and retrospective studies suggest that IRE treatment may increase median overall survival of LAPC to as much as 30 months and provide preliminary data justifying the well-designed trials currently underway, comparing IRE to the standard of care treatment. The mechanism of action of IRE remains unknown, and there is a lack of data on treatment variables and efficiency in humans. There is emerging data suggesting that IRE can be augmented with synergistic therapies such as immunotherapy.

# Prostate cancer




*Ting F. et al. Prostate Cancer and Prostatic Diseases 2016*

*life*

MDPI

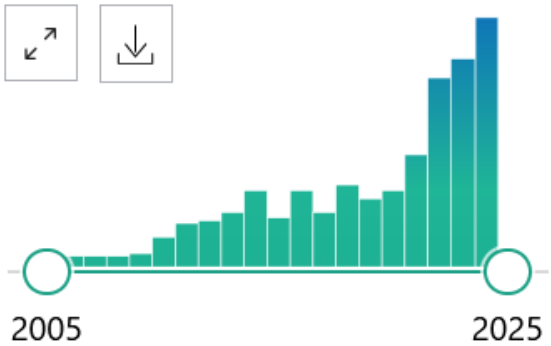
*Review*

## Irreversible Electroporation for Prostate Cancer

Sean Ong [1,2], Matthew Leonardo [2,3], Thilakavathi Chengodu [1], Dominic Bagguley [1,2] and Nathan Lawrentschuk [1,2,4,*]

**Abstract:** Although it can be lethal in its advanced stage, prostate cancer can be effectively treated when it is localised. Traditionally, radical prostatectomy (RP) or radiotherapy (RT) were used to treat all men with localised prostate cancer; however, this has significant risks of post-treatment side effects. Focal therapy has emerged as a potential form of treatment that can achieve similar oncological outcomes to radical treatment while preserving functional outcomes and decreasing rates of adverse effects. Irreversible electroporation (IRE) is one such form of focal therapy which utilises pulsatile electrical currents to ablate tissue. This modality of treatment is still in an early research phase, with studies showing that IRE is a safe procedure that can offer good short-term oncological outcomes whilst carrying a lower risk of poor functional outcomes. We believe that based on these results, future well-designed clinical trials are warranted to truly assess its efficacy in treating men with localised prostate cancer.

# Biomedical applications of electroporation in different disciplines of medicine




Pulsed electric field cardiac ablation: 89

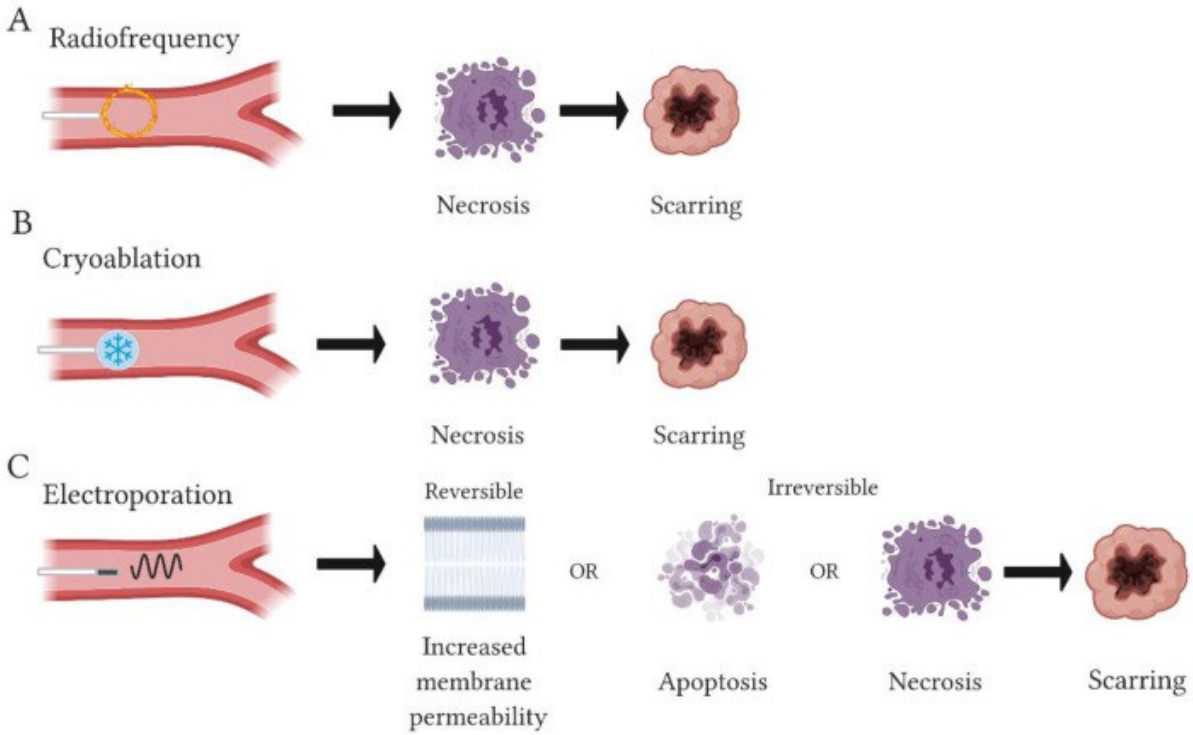
**Reversible electroporation**

- Oncology
  Tumor treatment
- Infectiology
  Vaccines
- Angiology
  Vascular malformations

**Irreversible electroporation**

- Oncology
  Tumor treatment
- Cardiology
  Cardiac arrhythmias

# IRE in arrhythmia-related cardiovascular disease




McBride S. et al., Clinical medicine 2021

Journal of *Clinical Medicine*

MDPI

*Review*

## Ablation Modalities for Therapeutic Intervention in Arrhythmia-Related Cardiovascular Disease: Focus on Electroporation

**Shauna McBride [1], Sahar Avazzadeh [1], Antony M. Wheatley [1], Barry O'Brien [2], Ken Coffey [2], Adnan Elahi [3,4], Martin O'Halloran [3] and Leo R. Quinlan [1,5,*]**

**Abstract:** Targeted cellular ablation is being increasingly used in the treatment of arrhythmias and structural heart disease. Catheter-based ablation for atrial fibrillation (AF) is considered a safe and effective approach for patients who are medication refractory. Electroporation (EPo) employs electrical energy to disrupt cell membranes which has a minimally thermal effect. The nanopores that arise from EPo can be temporary or permanent. Reversible electroporation is transitory in nature and cell viability is maintained, whereas irreversible electroporation causes permanent pore formation, leading to loss of cellular homeostasis and cell death. Several studies report that EPo displays a degree of specificity in terms of the lethal threshold required to induce cell death in different tissues. However, significantly more research is required to scope the profile of EPo thresholds for specific cell types within complex tissues. Irreversible electroporation (IRE) as an ablative approach appears to overcome the significant negative effects associated with thermal based techniques, particularly collateral damage to surrounding structures. With further fine-tuning of parameters and longer and larger clinical trials, EPo may lead the way of adapting a safer and efficient ablation modality for the treatment of persistent AF.

# Technological advancements

Catheter ablation with pulmonary vein isolation is currently the most effective method of AF treatment. With technological advancements, we can expect improvements and new approaches to increase the availability and safety of this invasive AF treatment.

CARDIOVASCULAR SYSTEM

Slovenian Medical Journal

## Catheter ablation for the treatment of atrial fibrillation – development of various technical modalities

Zdravljenje atrijske fibrilacije s katetrsko ablacijo – razvoj različnih tehničnih možnosti

Jernej Štublar,[1,2] David Žižek,[2] Matevž Jan,[3] Tomaž Jarm,[1] Damijan Miklavčič[1]

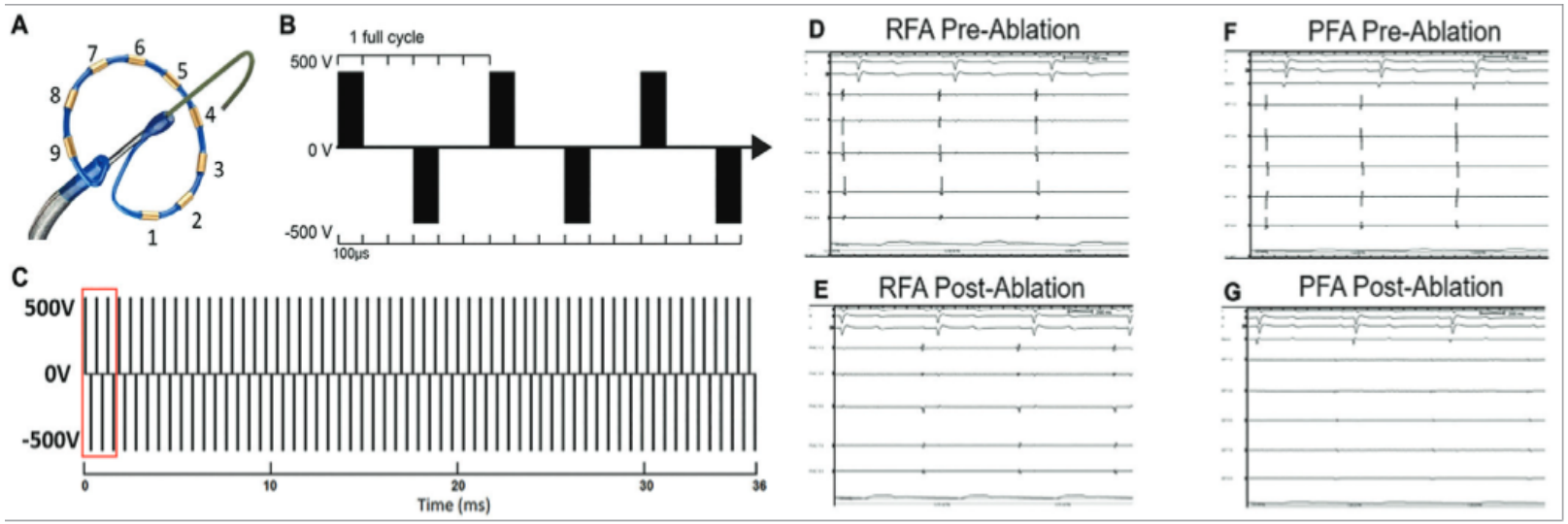
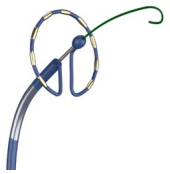
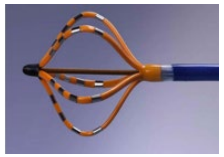



**Figure 5:** Multi-electrode PVAC GOLD catheter, used to supply IRE pulses (A). A schematic representation of the amplitude and duration of supplied biphasic pulses (B). Display of the duration of one ablation, composed of 60 biphasic pulse cycles (C). Display of intracardiac electric signals in a pulmonary vein before (D, F) and after (E, G) ablation with irreversible electroporation (46). Permission to use the images has been obtained.

# PFA technology landscape for cardiac ablation

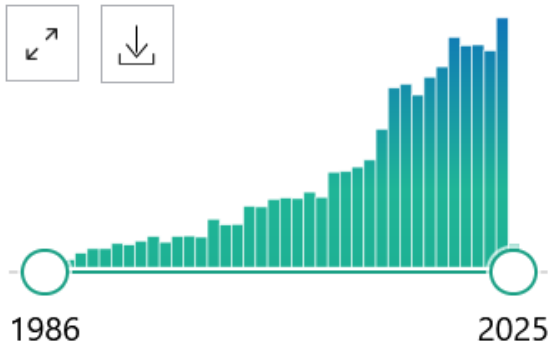
| Anatomical Solutions | Medtronic | Boston Scientific | Biosense Webster | Affera | Adagio | Kardium | Abbott Utrecht Group |
|---|---|---|---|---|---|---|---|
| **Device** | PulseSelect<br>*Loop catheter* | Farawave<br>*Pentaspline catheter* | Varipulse | Sphere-PVI | iCLAS<br>*PFCA* | Globe<br>*Spherical Array* | Circular catheter |
| |  |  |  |  |  |  |  |
| **Ongoing Trials** | PULSED AF IDE | ADVENT IDE<br>FARA FREEDOM | admIRE IDE | SPHERE PVI<br>(FIH) | PsAF<br>(FIH) | PULSE-EU | FIH |

| Focal Solutions | Boston Scientific | Affera | Galaxy Medical | Acutus |
|---|---|---|---|---|
| **Device** | Farapoint | Sphere-9<br>*Lattice-Tip catheter* | CENTAURI generator | AcQFORCE |
| |  |  |  |  |
| **Ongoing Trials** | PERSAFONE II<br>PERSAFONE III | Sphere-9 IDE<br>Sphere-Per-AF IDE | PFA-AF | SPACE-AF<br>ECLIPSE-AF |

*Information gathered from public sources*

With courtesy of Medtronic

# Biomedical applications of electroporation are gaining more attention and is fast developing technology




Electroporation; 380

**Reversible electroporation**

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  Vaccines
- Angiology
  Vascular malformations

**Irreversible electroporation**

- Oncology
  Tumor treatment
- Cardiology
  Cardiac arrhythmias

EU JOINT ACTION JANE2 NETWORKS OF EXPERTISE ON CANCER

NETWORK OF EXPERTISE ON CANCER
HI-TECH MEDICAL RESOURCES

EU JOINT ACTION JANE2 NETWORKS OF EXPERTISE ON CANCER
Co-funded by the European Union

https://jane-project.eu/

## NoE on Hi-tech medical resources:

1. Nuclear Medicine
2. Radiomics
3. Innovative Radiotherapies
4. Innovative Surgery
5. **Physical Methods of Ablation**
6. Cell Therapies
7. Ex-vivo Testing of Agents

RFA (radiofrequency ablation)
MWA (microwave ablation)
HIFU (high focussed ultrasound)
Cryotherapy
Electroporation based therapies
- Irreversible electroporation
- Electrochemotherapy

https://jane-project.eu/

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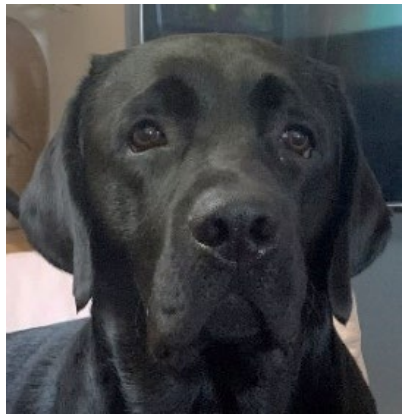
Saša Kupčič
Jaka Vrevc Žlajpah

## Thank you for your attention!



# Electroporation-Based Technologies and Treatments

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