ONKOLOŠKI INŠTITUT
INSTITUTE OF ONCOLOGY
LJUBLJANA

Gene-based immune therapy of solid tumors based on pDNA

Prof.dr. Gregor Serša

gsersa@onko-i.si

Advancements in Cancer Treatment,

21 November 2024 Brussels

Agenda

- Institute of Oncology Ljubljana profile
- Gene therapy
- Electroporation
- Smartgene.si - gene-based cancer treatment modality with a paradigm shift in immunotherapy
- Clinical development –further steps

Institute of Oncology Ljubljana (IOL)

Principal national institution for the comprehensive management of cancer diseases
Public institution

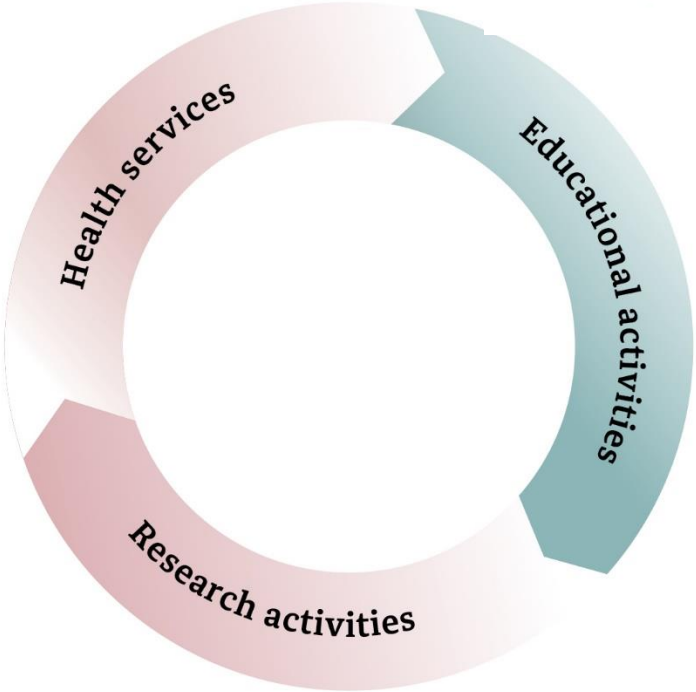
Areas of work

- Prevention, screening
- Diagnostics, **treatment**, rehabilitation and palliative care
- Research and education

National coordination

- **National Cancer Control Programme**
- Cervical and breast cancer **screening program** (upcoming prostate and lung)
- Cancer and Screening **Registries**

Three basic activities of the Institute of Oncology Ljubljana



DPOR | National Cancer Control Programme Slovenia

CANCER REGISTRY REGISTER RAKA SLOVENIJA

Zora
Državni program zgodnjega odkrivanja predrakavih sprememb maternicnega vratu

OECI Organisation of European Cancer Institutes
European Economic Interest Grouping

DORA
SLOVENIAN BREAST CANCER SCREENING PROGRAMME

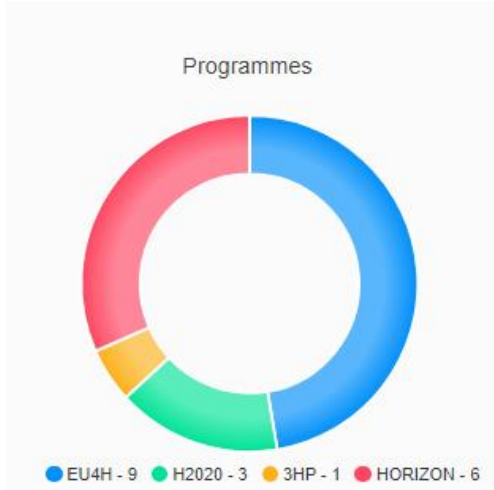
International cooperation and the EU funding landscape

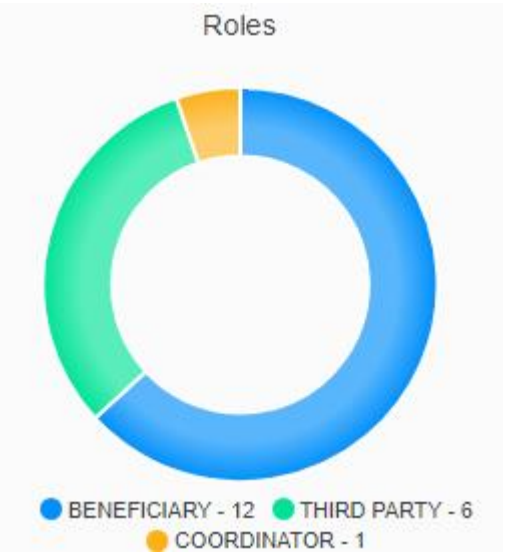
IOL in EU Programmes

- EU4 HEALTH
- Mission Cancer
- Horizon 2020 and Horizon Europe
- Cost Action
- Interreg Slovenia-Italy

IOL role in EU projects

- In Joint Actions IOL act as a **Competent Authority** and coordinate Slovenian partnership and stakeholders
- Coordinator of Twinning ZapCancer project
- Project partner in 22 projects (WP leaders)
- 22 ongoing **clinical studies**





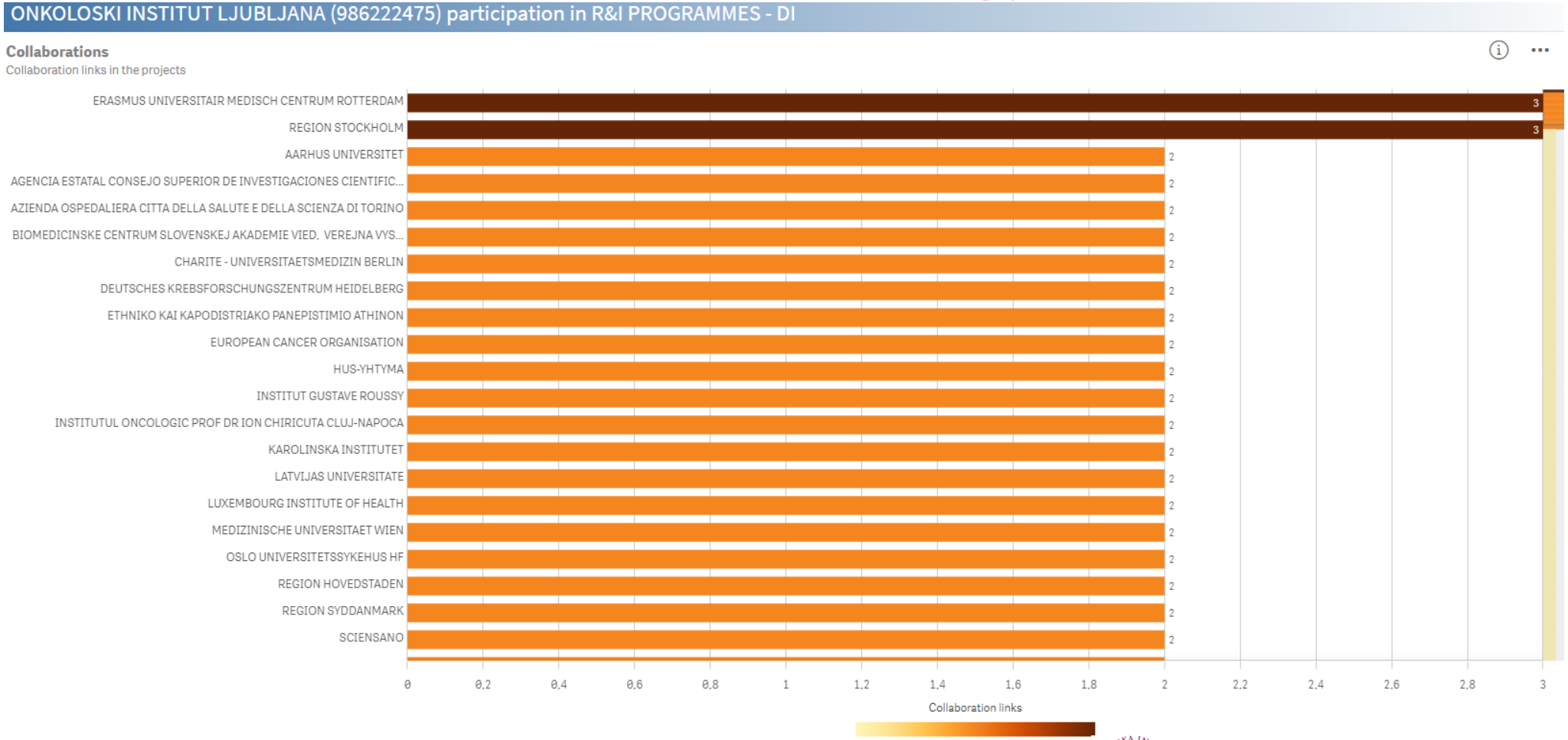
Overview of ongoing R&I projects

CARDIOCARE: An interdisciplinary approach for the management of the elderly multimorbid patient with breast cancer therapy induced cardiac toxicity
4D PICTURE: Design-based Data-Driven Decision-support Tools: Producing Improved Cancer Outcomes Through User-Centred Research
CCI4EU: Comprehensive cancer infrastructures for Europe
JANE(JA): Joint Action on Networks of Expertise on Cancer
PERCH(JA): PartnERship to Contrast HPV
CRANE(JA): Network of Comprehensive Cancer Centres: Preparatory activities on creation of National Comprehensive Cancer Centres and EU Networking
ZAP Cancer Twinning for excellence to advance research in the activation of anti-tumor immune response after electrochemotherapy combined with gene electrotransfer of pDNA encoding ICIs

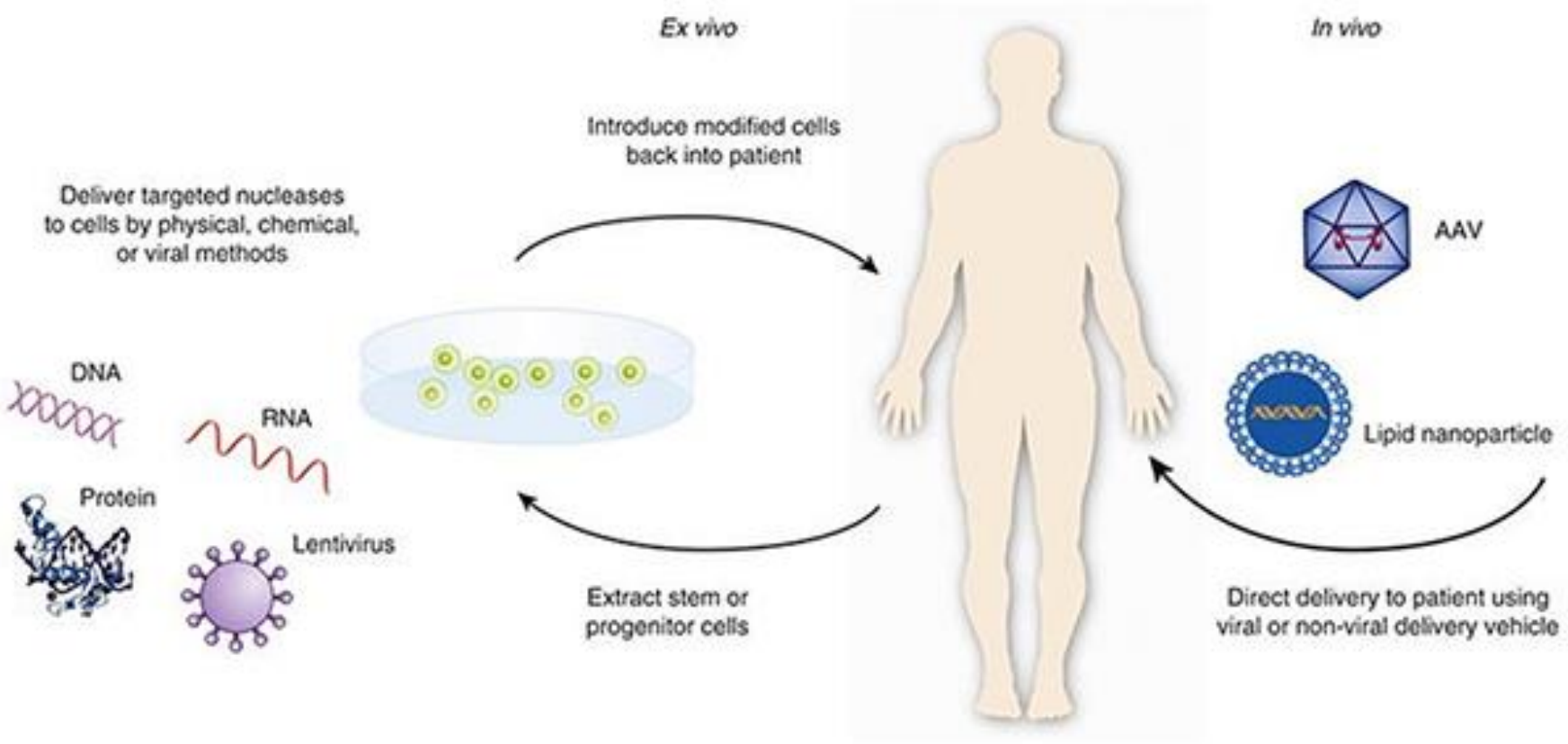
eCAN (JA): Joint Action on strengthening ehealth including telemedicine and remote monitoring for health care systems for cancer prevention and care
smartCARE AG): smart Card Application improving canceR survivors quality of life
CAN.HEAL (AG): Building the EU Cancer and Public Health Genomics platform
HTx: Next Generation Health Technology Assessment to support patient-centred, societally oriented, real-time decision-making on access and reimbursement for health technologies throughout Europe
E-QuoL: e-health tools to promote Equality in Quality of Life for childhood to young adulthood cancer patients, survivors and their families - a PanEuropean project supported by PanCare and Harmonic consortia
EUonQoL: Quality of Life in Oncology: measuring what matters for cancer patients and survivors in Europe
EUCanScreen: Joint Action Project "Implementation of the new European recommendations on cancer screening

EU CanScreen
European Joint Action on Cancer Screening
EU JOINT ACTION JANE NETWORKS OF EXPERTISE ON CANCER
CraNE
4D PICTURE
PERCH PartnERship to Contrast HPV
smartCARE Empowering Cancer Survivors in Europe
can.heal

OIL collaboration with leading institutes and clinics in Oncology

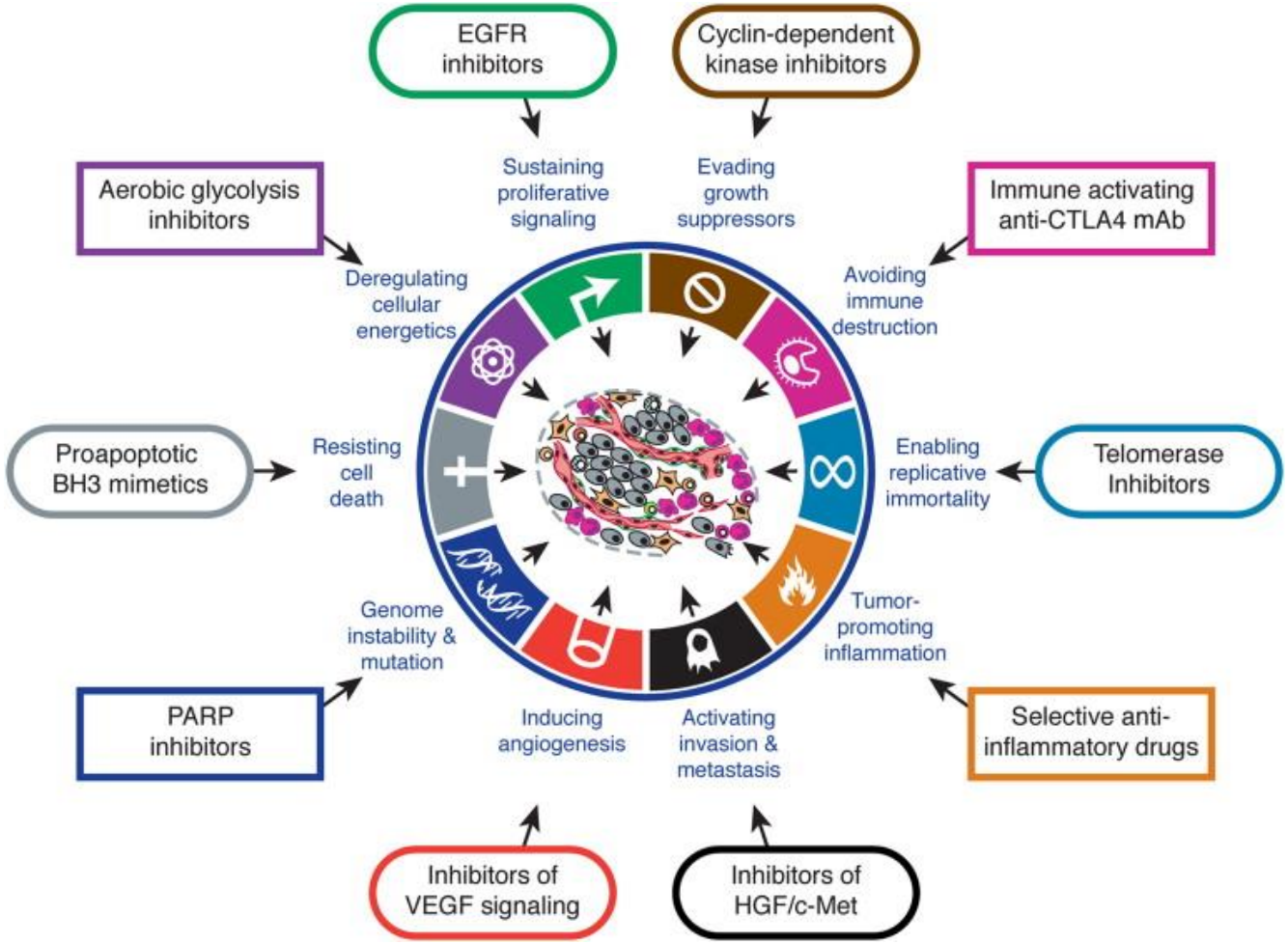


Principles of Gene Therapy



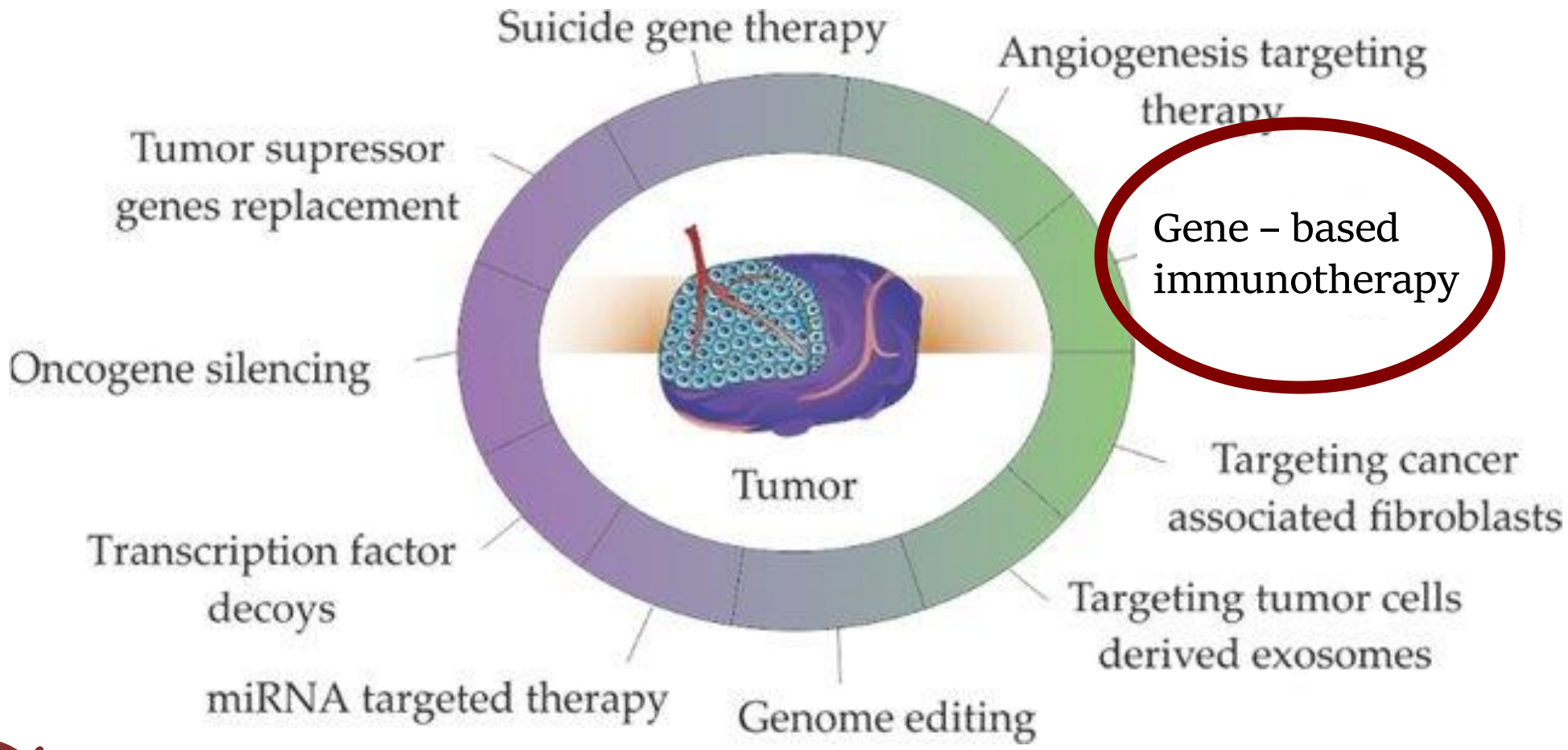
FDA U.S. FOOD & DRUG ADMINISTRATION

Hallmarks and therapeutic targets
Targeted therapies



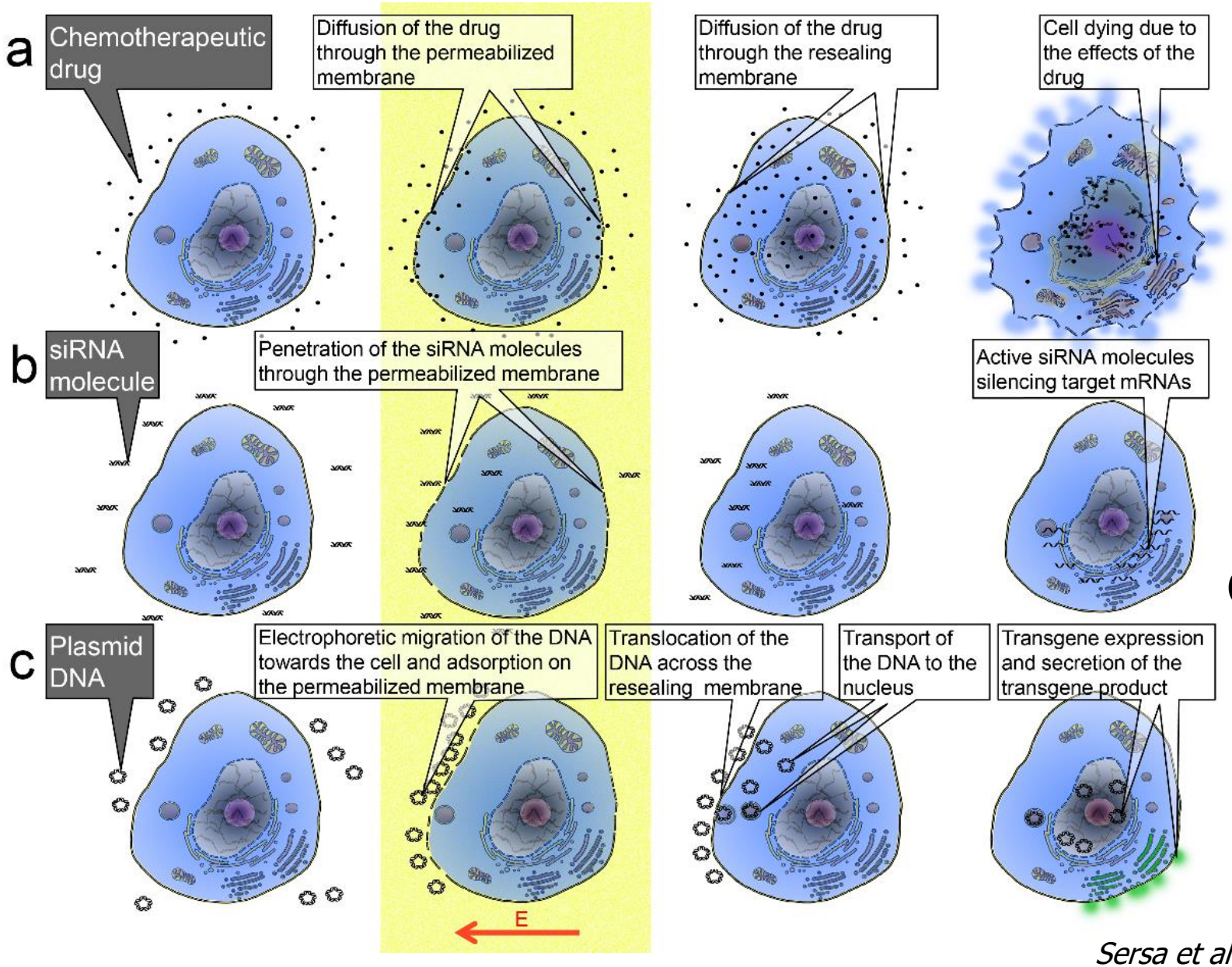
Hanahan in Weinberg, Cell 2011

Gene therapy of cancer - strategies



Roma-Rodrigues et al Pharmaceutics 2020, 12, 233; doi:10.3390/pharmaceutics12030233

Electroporation for drug and gene delivery

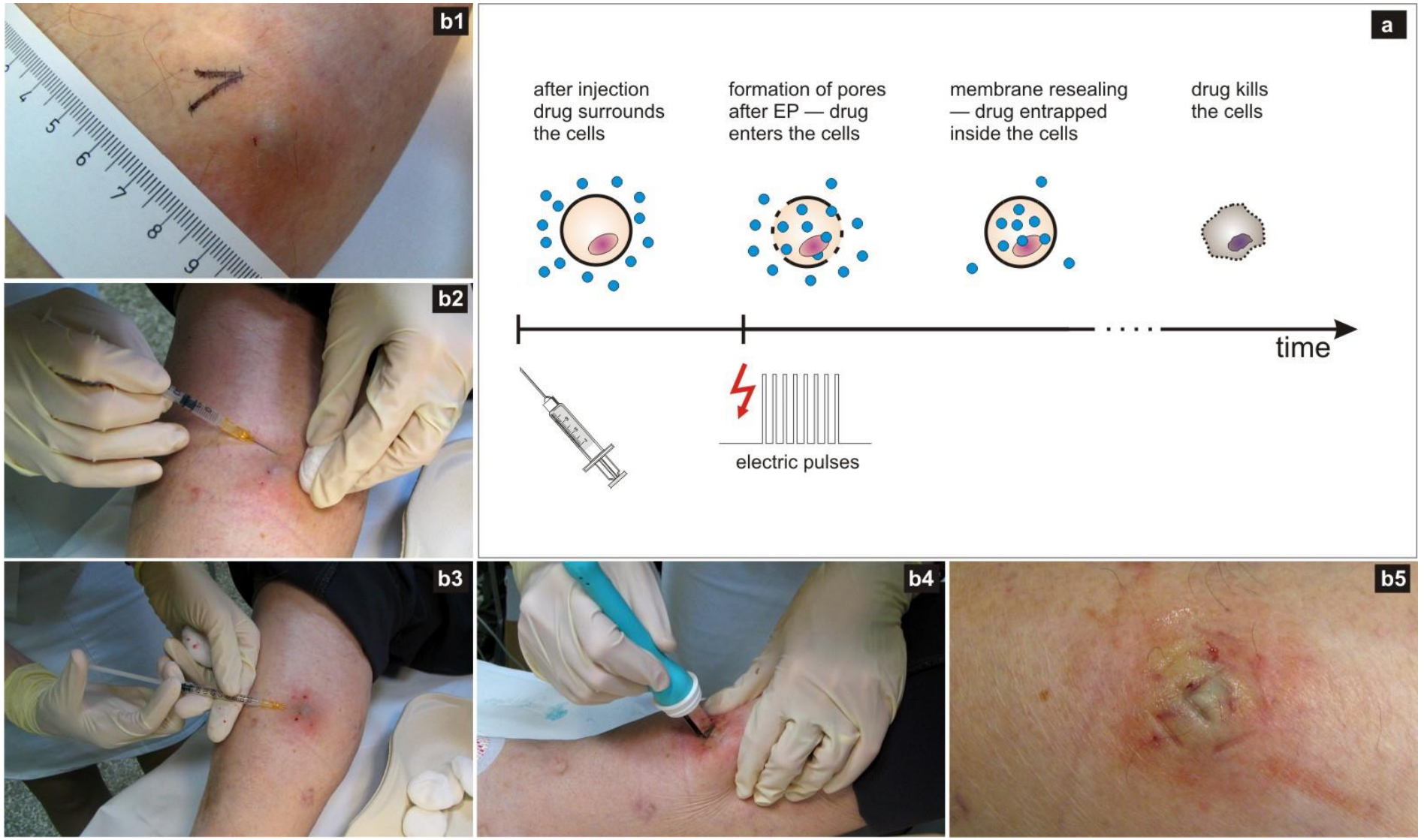


Electrochemotherapy

Gene electrotransfer

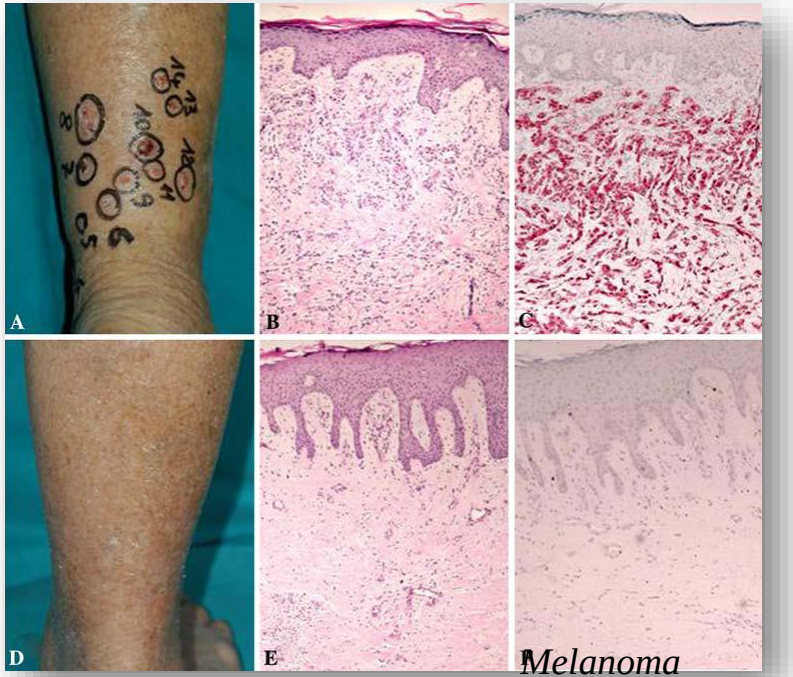
Sersa et al. Cancer Immunol Immunother 2015

Electrochemotherapy - procedure

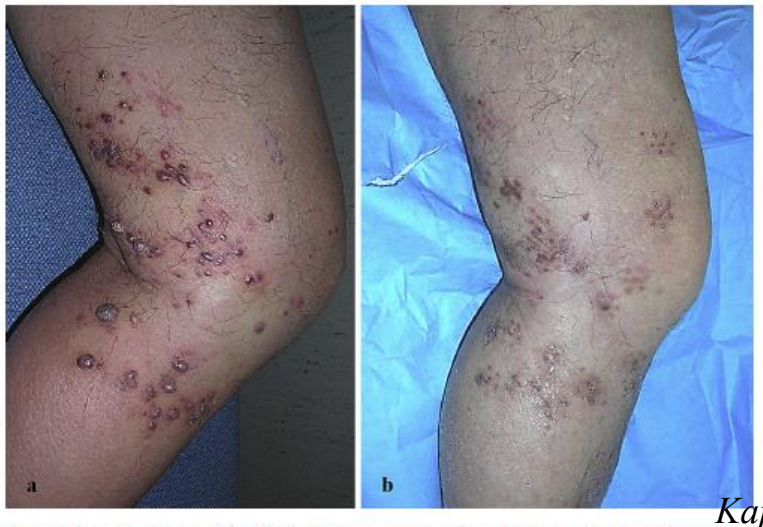


Jarm, Cemazar, Miklavcic & Sersa *Expert Rev. Anticancer Ther.* 10(5), 729–746 (2010)

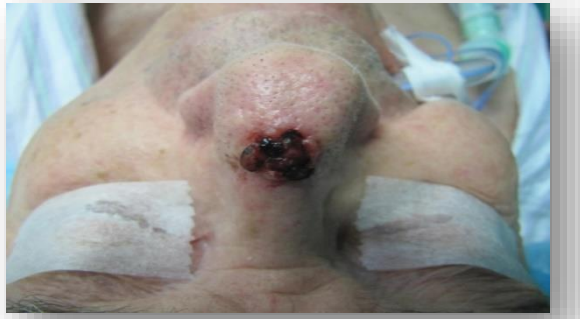
Examples of tumors treated

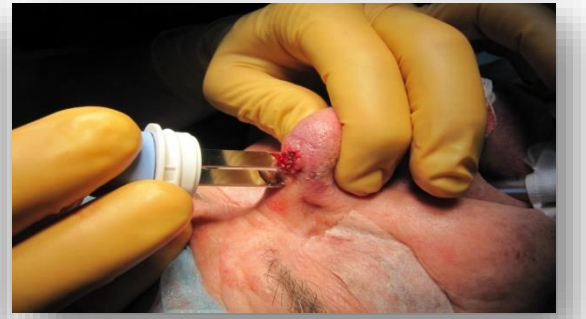


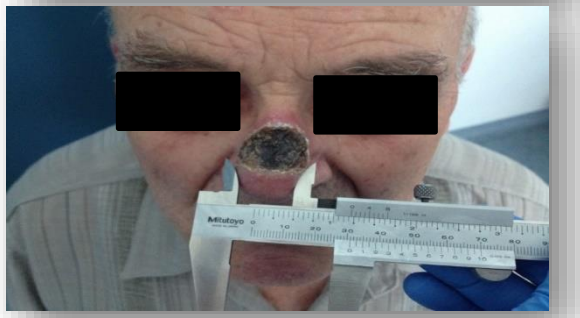
Melanoma

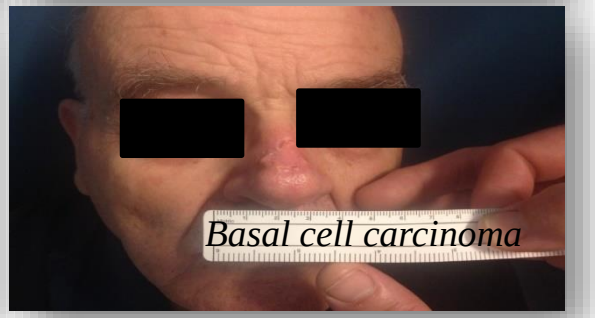


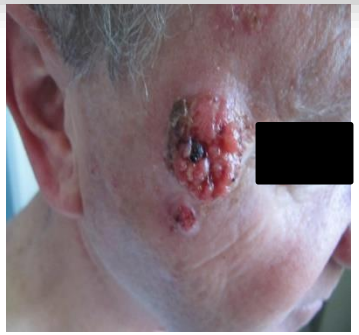
Kaposi's sarcoma

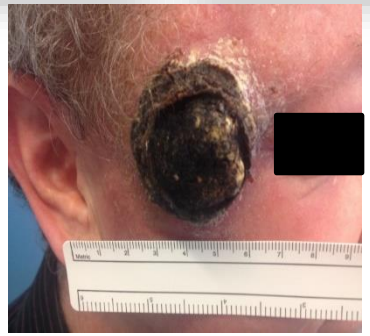


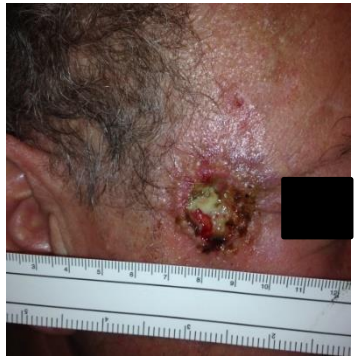


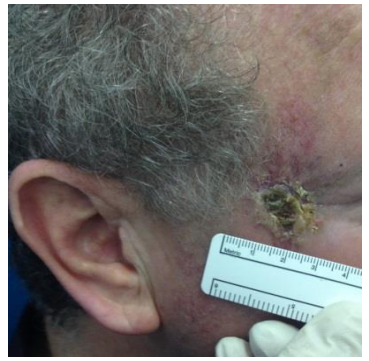








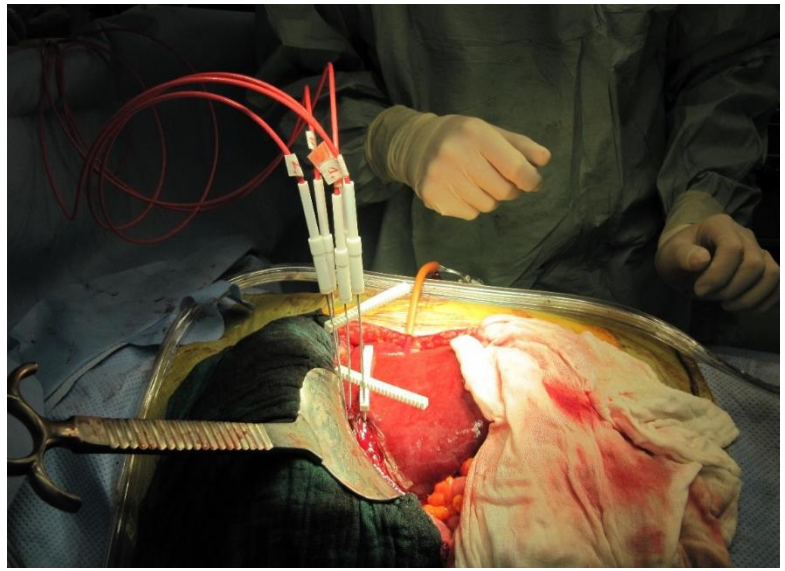


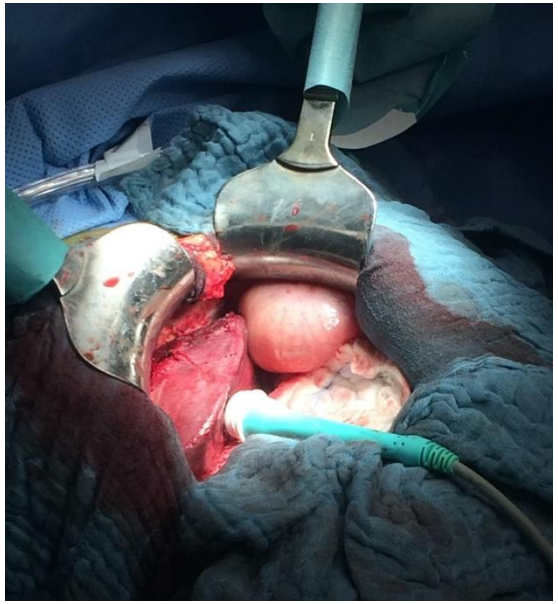


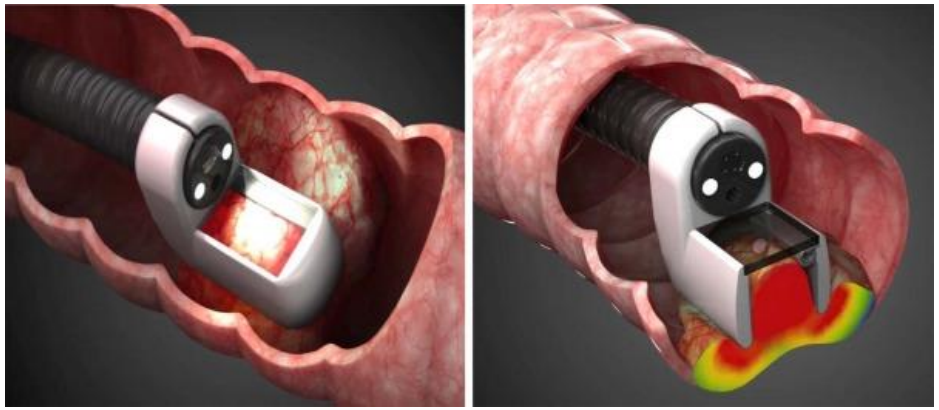
Squamous cell carcinoma

Examples of deep seated tumors: therapeutic approaches

- Intraoperative
- Percutaneous
- Endoscopis
- Laparoscopic

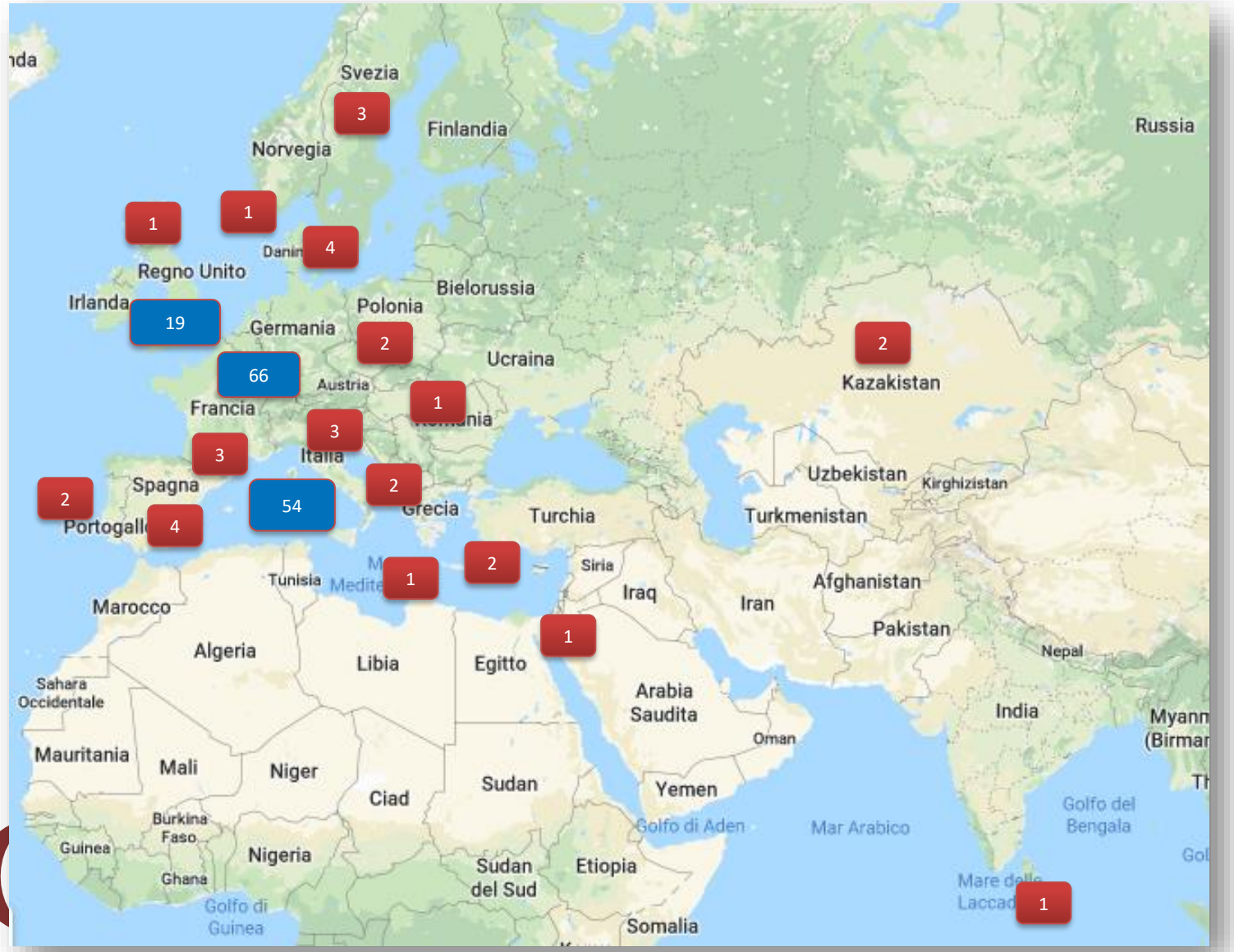






Up to date 03-2022

Centres that utilize electrochemotherapy

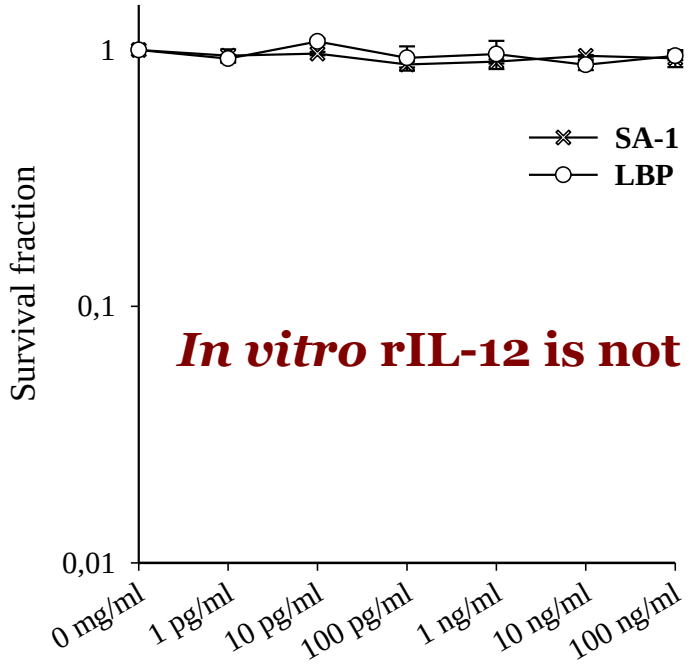


- **182 ECT centres**
- ECT mentioned in national guidelines

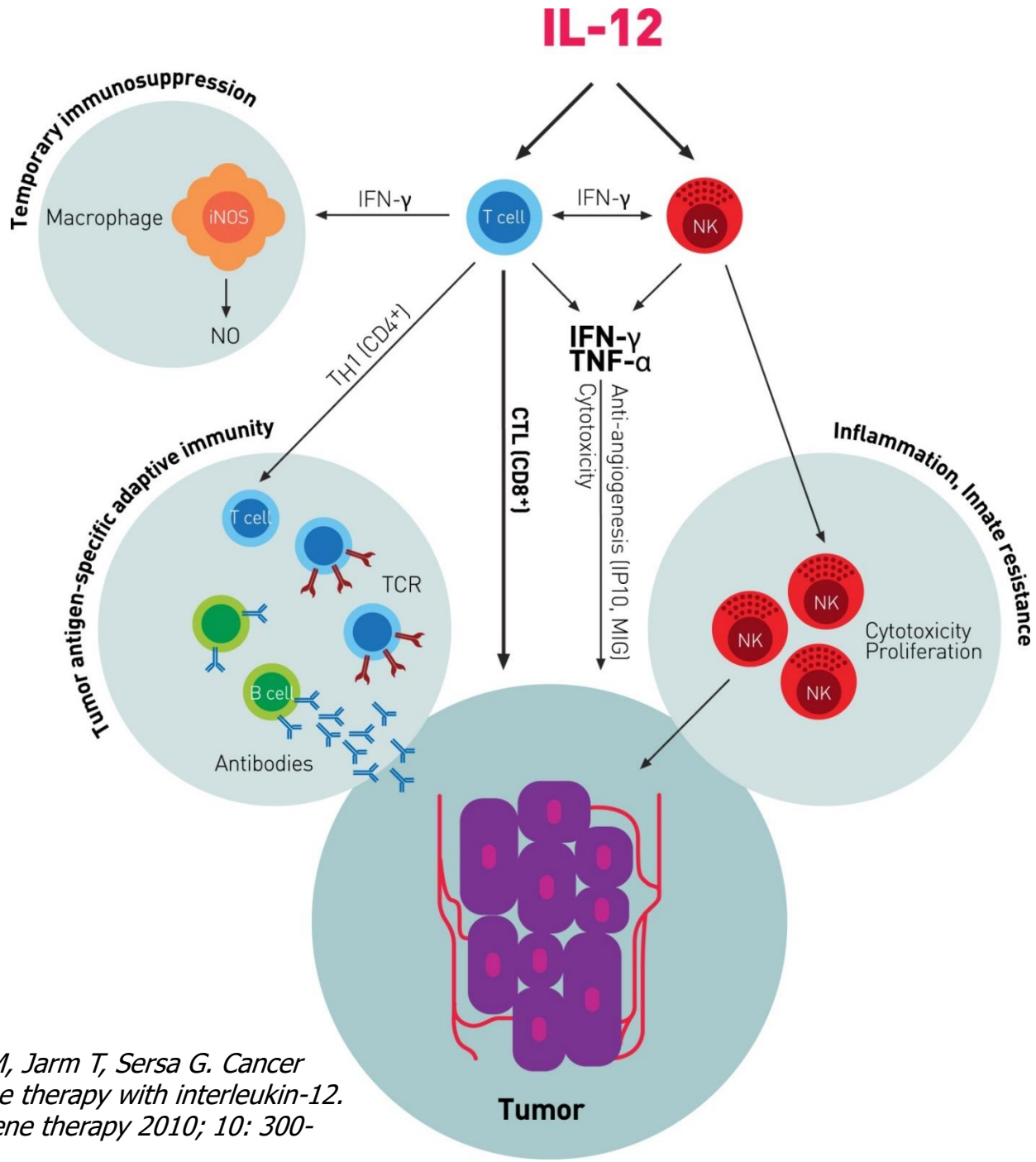
- metastatic melanoma
- breast cancer
- primary basal cell carcinoma (BCC)
- recurrent or metastatic BCC & squamous cell carcinoma
- cutaneous angiosarcoma

Interleukin-12 antitumor effect

IL-12 doesn‘t have direct cytotoxic effect,
IL-12 induces responses of Th1 cells, cytotoxic T lymphocytes and NK cells as well as activates potent anti-angiogenic mechanisms
Clinical studies using recombinant IL-12 resulted in intolerable side effects and were stopped



In vitro rIL-12 is not cytotoxic

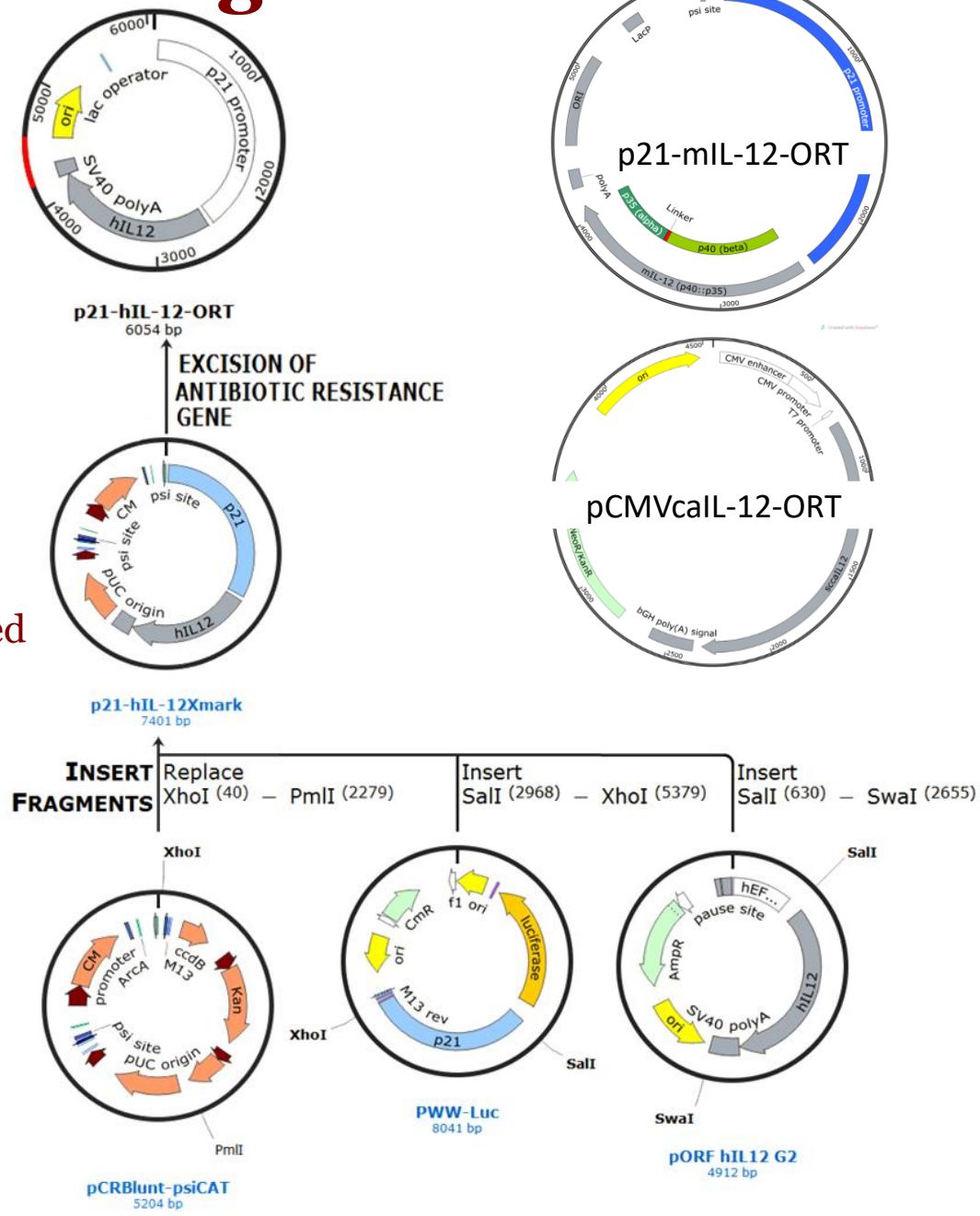


Cemazar M, Jarm T, Sersa G. Cancer electrogene therapy with interleukin-12. Current Gene therapy 2010; 10: 300-311.

Plasmids without antibiotic resistance gene

Needed for selection and production of plasmid DNA in bacteria

- Risk of horizontal gene transfers to environmental and commensal microbes
- Risk of allergic reactions to the residual traces of antibiotic used in the production of plasmids
- Other effects on plasmid performance:
  - inflammatory reactions (CpG islands)
  - lower transfection efficiency (increased size of plasmid)
  - responsible for inefficient and short lived expression
- EMA and FDA recommendations:
  - totally avoiding the use of antibiotic resistance genes in clinically used plasmids
  - use of ones that are not commonly used to treat human infections – kanamycin
    - Operator-represssion titration (ORT) technique was used for preparation of plasmids:
      - p21-mIL-12_ORT
      - pCMV-caIL-12-ORT
      - p21-hIL-12-ORT



Kamensek et al, Heliyon 2022

REPUBLIC OF SLOVENIA
MINISTRY OF EDUCATION, SCIENCE AND SPORT

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

SmartGene.si

Nova generacija genske terapije za zdravljenje raka: od genov do proizvodnje

A new gene-based cancer treatment modality with a paradigm shift in immunotherapy.

2018 – 2021

Javni razpis „Spodbujanje izvajanja raziskovano-razvojnih projektov (TRL 3-6)“
Prednostno področje: Zdravje-medicina
Prednostno podpodročje: Zdravljenje raka

REPUBLIC OF SLOVENIA
MINISTRY OF EDUCATION, SCIENCE AND SPORT

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

SmartGene.si

A new gene based cancer treatment modality with a paradigm shift in immunotherapy.

https://www.smartgene.si/

PARTNERS:

ONKOLOŠKI INŠTITUT
INSTITUTE OF ONCOLOGY LJUBLJANA

COBIK

JAFRAL
passion for biosolutions

University of Ljubljana
Faculty of Electrical Engineering

Clean visions.

univerzitetni klinični center ljubljana
University Medical Centre Ljubljana

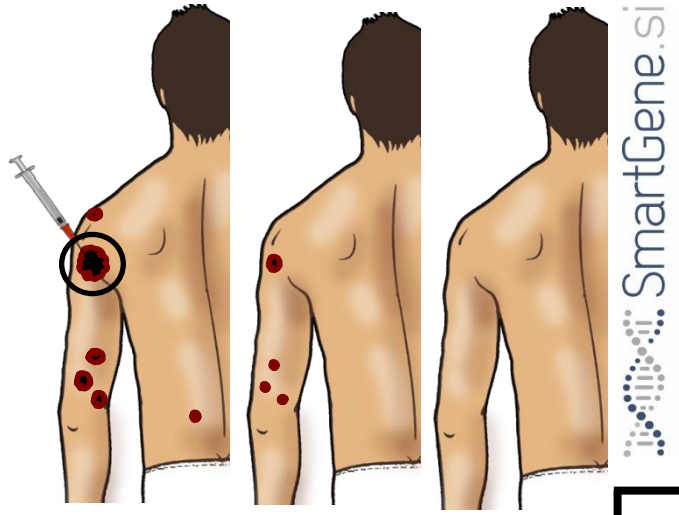
University of Ljubljana
Veterinary Faculty

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

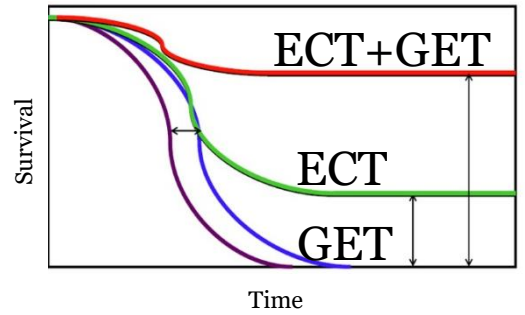
Aim of the platform

TRANSLATION OF GENE THERAPY FOR CANCER INTO THE CLINICS



Standard treatment

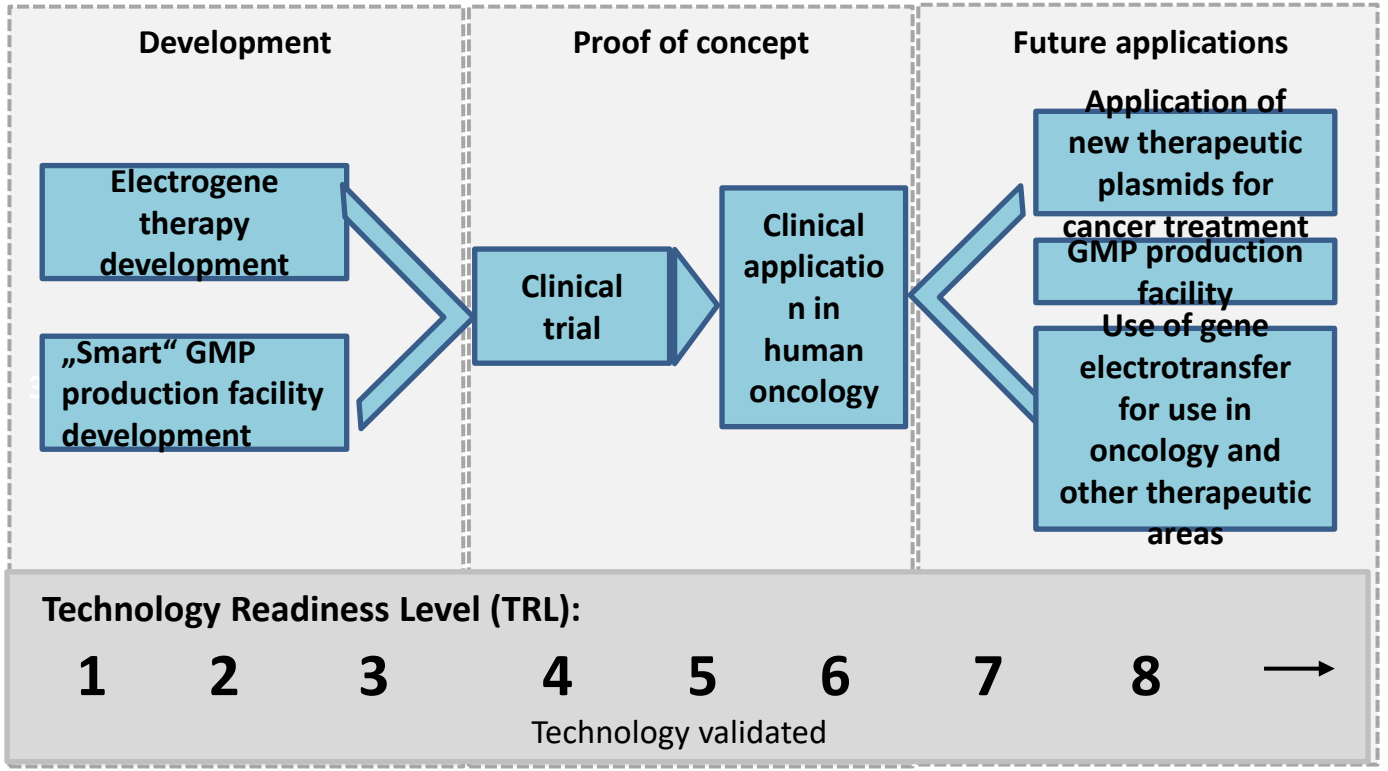
Clinical study phase I/II

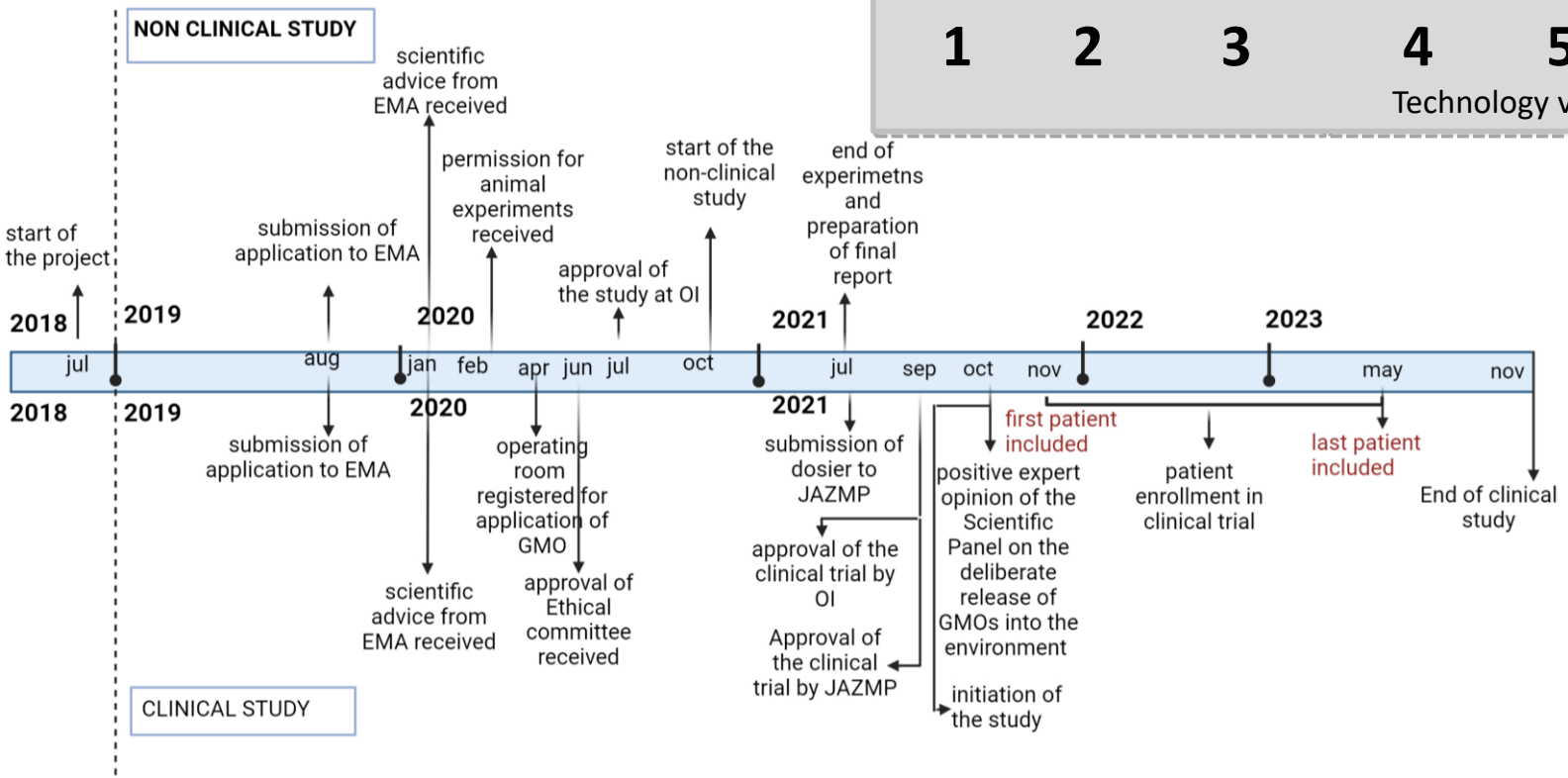


In vivo research

In vitro research

Timeline and TRL of SmartGene.si





Non-clinical study for approval of phase I clinical study

Based on:

- EMA guidelines for advance therapies

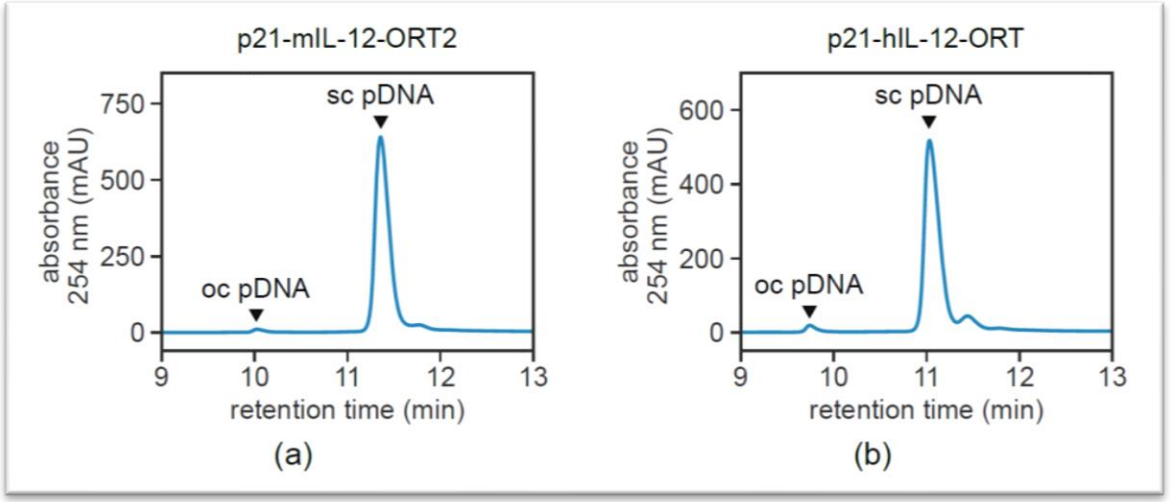
- EMA/CAT/80183/2014 (Quality, preclinical and clinical aspects of gene therapy medicinal products),
- EMA/CAT/852602/2018 (Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials),
- EMEA/CHMP/GTWP/125459/2006 (Guideline 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)

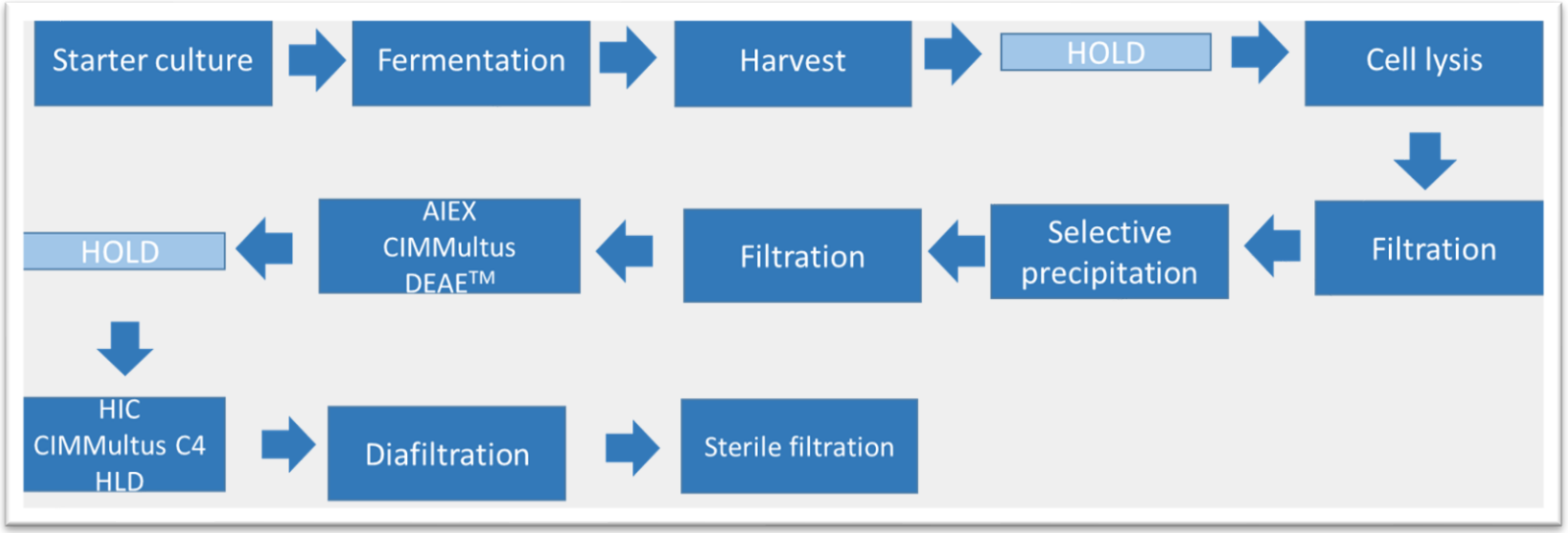
Plasmid manufacturing

- Development and validation of analytical and potency methods



Kos, S et al, 2021

- Development of manufacturing process



Plasmid manufacturing

- Design and set up of „Smart“ GMP facility.
- GMP manufacturing

Test / Analysis	Method	Preliminary specification for release
Visual inspection	Ph Eur. 2.9.20.	Clear colorless liquid without visible particles
pH	Ph. Eur. 2.2.3.	5.0-7.0
Sub visible particles	Ph. Eur 2.9.19., USP <787>	≤ 3000 for particles ≥ 10 µm ≤ 300 for particles ≥ 25 µm
Osmolality	Ph. Eur. 2.2.35., USP <785>	250-350 mOsm/L
Activity	HEK-Blue™ IL-12 reporter system for IL-12 and ELISA	HEK-Blue IL-12 reporter: Report result ELISA: 1000 – 5000 ng IL-12/mg plasmid
Concentration	Fluorimetric detection of dsDNA	1.8-2.2 mg/mL
Plasmid homogeneity	AIEX HPLC	≥ 94% sc pDNA
Identity	PCR	Size of specific PCR product
Purity	260/280 nm	1.7-1.9
Endotoxin	Ph. Eur. 2.6.14., USP<85>	< 10 EU/mg pDNA
Residual RNA	qPCR; revers transcription	< 100 ng/mg pDNA
Residual HC gDNA	qPCR	< 20 µg/mg pDNA
HCP	ELISA[2]	< 100 ng/mg pDNA
Sterility	Ph. Eur. 2.6.1., USP <71>	Negative
Identity	Sequencing	Identical

pharmaceutics

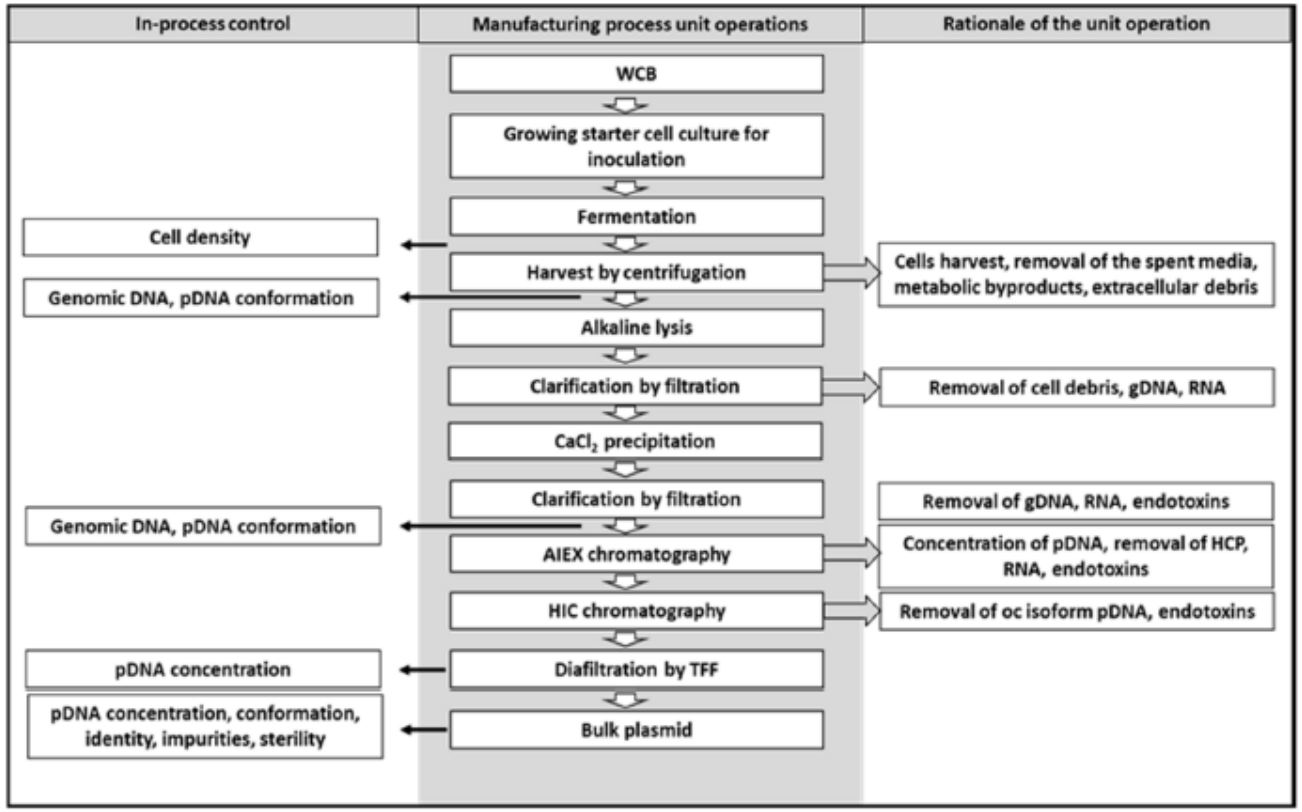
MDPI

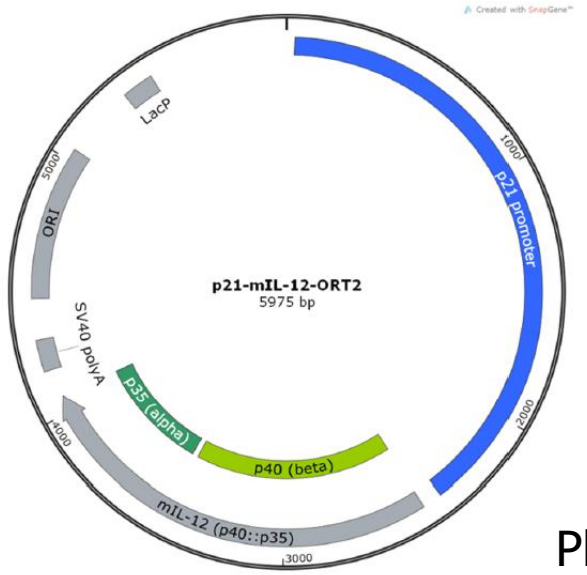
Article

Non-Clinical In Vitro Evaluation of Antibiotic Resistance Gene-Free Plasmids Encoding Human or Murine IL-12 Intended for First-in-Human Clinical Study

Spela Kos [1,†], Masa Bosnjak [1,2,†], Tanja Jesenko [1,3], Bostjan Markelc [1,4], Urska Kamensek [1,5], Katarina Znidar [1], Urska Matkovic [1], Andrej Rencelj [1,3], Gregor Sersa [1,4], Rosana Hudej [6], Aneja Tuljak [6], Matjaz Peterka [6] and Maja Cemazar [1,7,*]

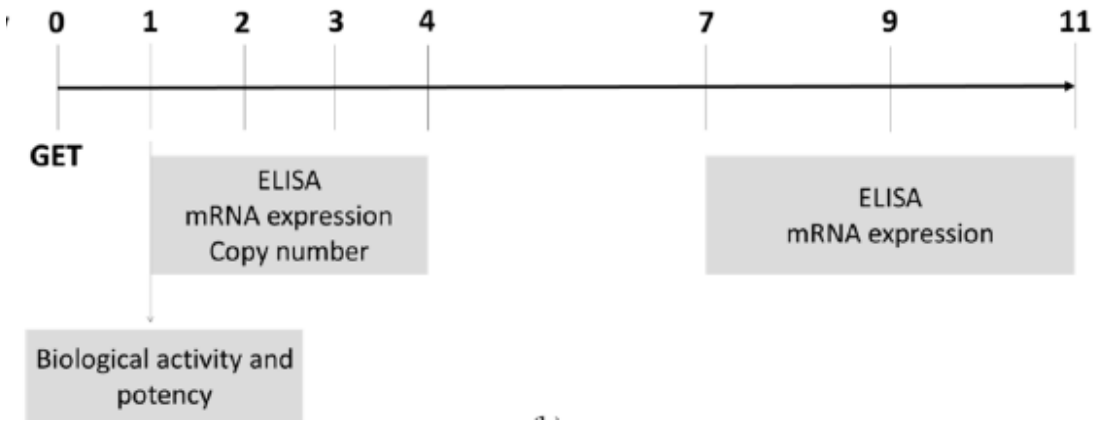
Production process





Plasmid

In vitro experiments



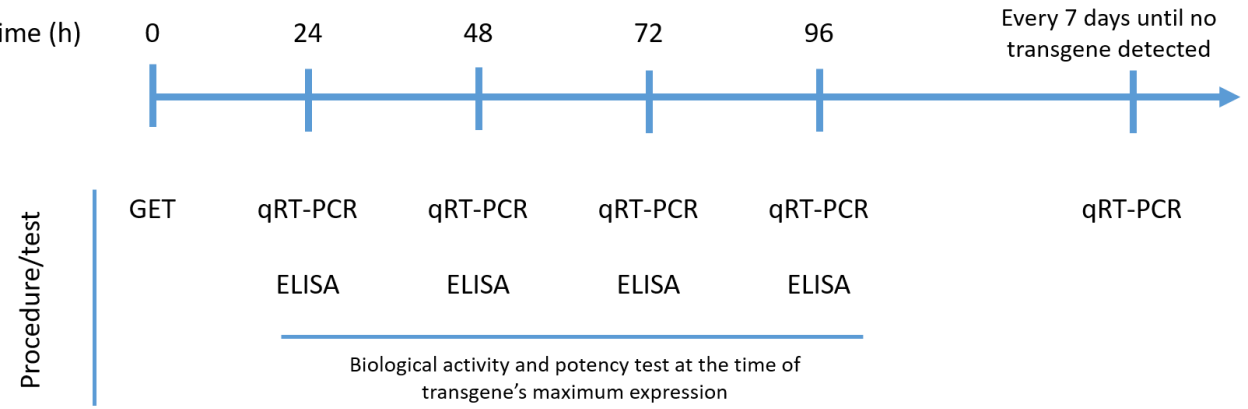
Pharmaceutics 2021, *13*, 1739. https://doi.org/10.3390/pharmaceutics13101739

Non-clinical study

In vitro studies were used to determine the biological activity of the proteins produced from plasmids phIL12 and pmIL12, the expression level of the transgenes and the copy number of plasmid DNA in the cells.

All *in* vitro studies with phIL12 were performed on the human pharyngeal squamous cell carcinoma cell line FaDu

All *in vitro* studies with pmIL12 were performed on the murine colon cancer cell line CT26



Time line of in vitro experiments

Non-clinical study **SMG-01**

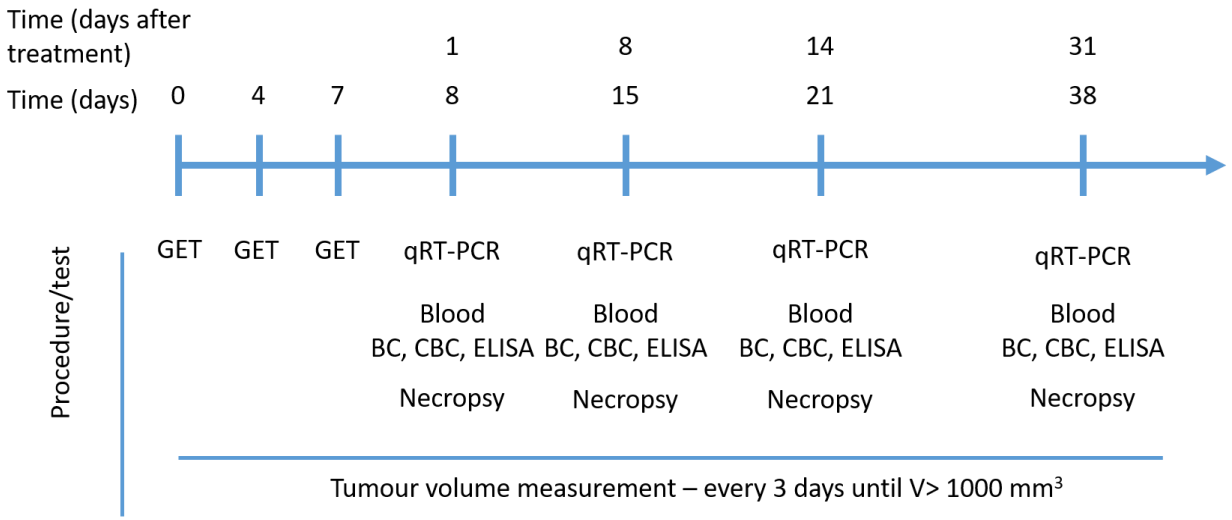
SmartGene.si

REPUBLIC OF SLOVENIA
MINISTRY OF EDUCATION, SCIENCE AND SPORT

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

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

OPEN

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

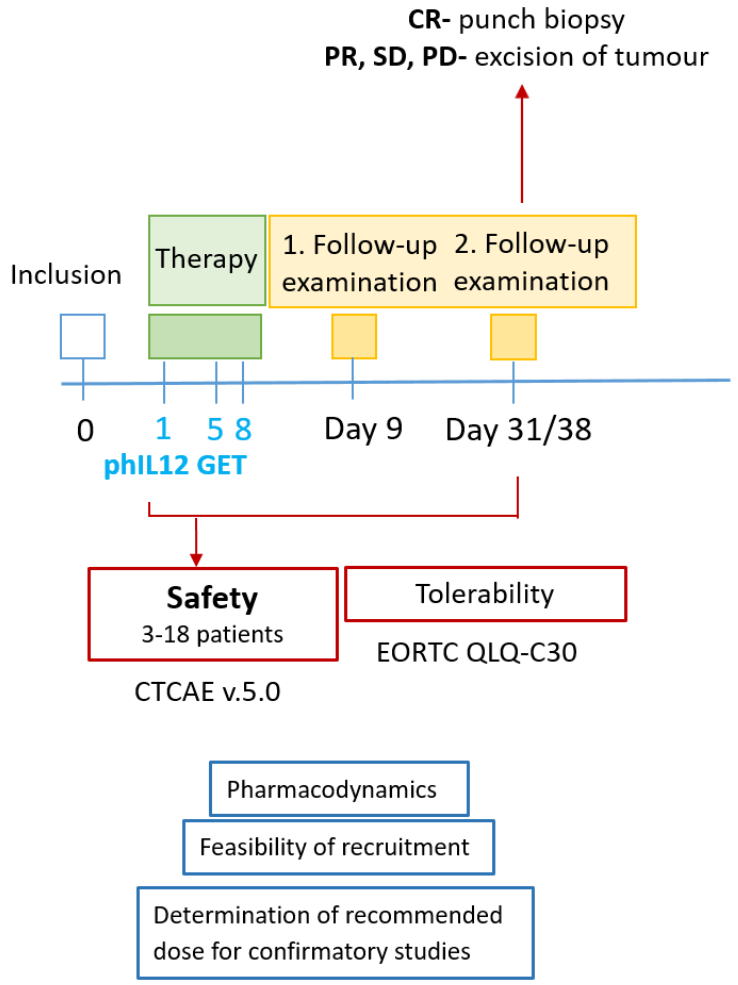
REPUBLIC OF SLOVENIA
MINISTRY OF EDUCATION, SCIENCE AND SPORT

SmartGene.si

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

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 was needed.
- The design (3 + 3 design) and the corresponding sample size were usual for phase I trials in oncology.
- Descriptive statistics was be used.



Publication of the study protocol

sciendo Radiology and Oncology | Ljubljana | Slovenia | www.radioloncol.com RADIOLOGY AND ONCOLOGY

study protocol

Treatment of skin tumors with intratumoral interleukin 12 gene electrotransfer in the head and neck region: a first-in-human clinical trial protocol

Ales Groselj[1,2], Masa Bosnjak[3,4], Tanja Jesenko[2,3], Maja Cemazar[3,5], Bostjan Markelc[3,6], Primoz Strojan[2,7], Gregor Sersa[3,6]

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

Radiol Oncol 2022; 56(3): 398-408.

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. 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.

Inclusion and exclusion criteria

TABLE 3. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Histologically or cytologically confirmed, previously untreated cutaneous basal cell carcinoma located in the head and neck region	Other malignancy at the time of inclusion
Solitary tumors, with largest diameter up to 3 cm, in the region where curative (R0) surgery is feasible	Lesions not suitable for treatment with GET (invasion into the bone, infiltration of large vessels)
Age 18-years or older	A life-threatening infection and/or severe heart failure and/or liver failure and/or other life-threatening systemic diseases
Life expectancy > 3 months	Significantly reduced lung function, which requires the determination of DLCO. Patients should not be treated if DLCO is abnormal
Physical performance in accordance with the Karnofsky scale ≥ 70 or < 2 in accordance with World Health Organization (WHO) scale	Treatment with immunosuppressive drugs, steroids and other drugs that would affect poor wound healing
The patient must be capable of understanding the treatment procedure and possible adverse events, which may arise during treatment	Age under 18-years
The patient must be capable of signing the informed consent to participate in the clinical study (voluntary and conscientious consent after education)	Major disruptions in the coagulation system (who does not respond to the standard therapy – replacement of vitamin K or freshly frozen plasma)
Prior to inclusion in the trial, the patient must be presented at a multidisciplinary advisory team meeting	A chronic decline in the kidney function (creatinine > 150 µmol/L)
	Epilepsy
	Pregnancy and breast-feeding
	The patient's incapability of comprehending the purpose or course of the trial, or not agreeing to be included in the trial
	Patients unwilling or unable to comply with the protocol requirements and scheduled visits

DLCO = Diffusing Capacity of the Lungs for carbon monoxide; GET = gene electrotransfer

Treatment procedure: Preparation of plasmid for injection

- The plasmid was prepared according to a GMP procedure approved by the Slovenian Medicine Agency, based on EMA recommendations.

- Dilution and preparation of the plasmid solution in the syringe for injection is carried out in a surgical room registered for work with GMOs.

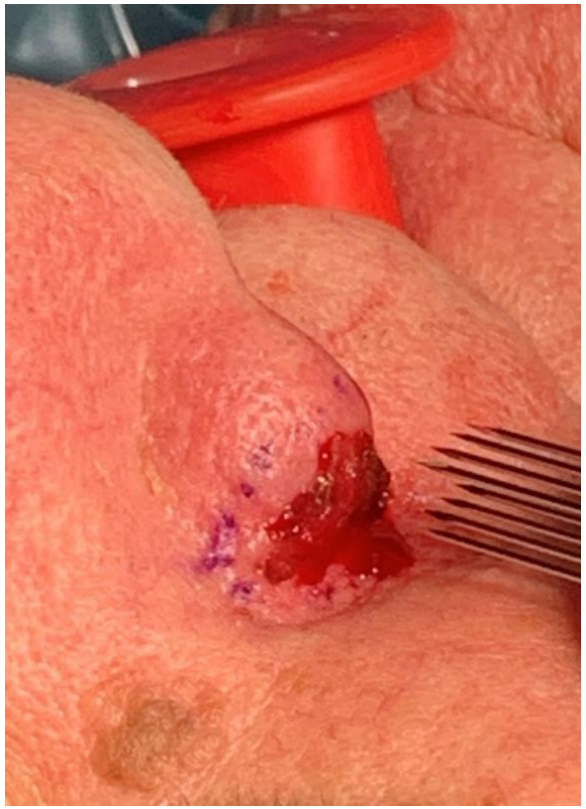
REPUBLIC OF SLOVENIA
MINISTRY OF EDUCATION, SCIENCE AND SPORT

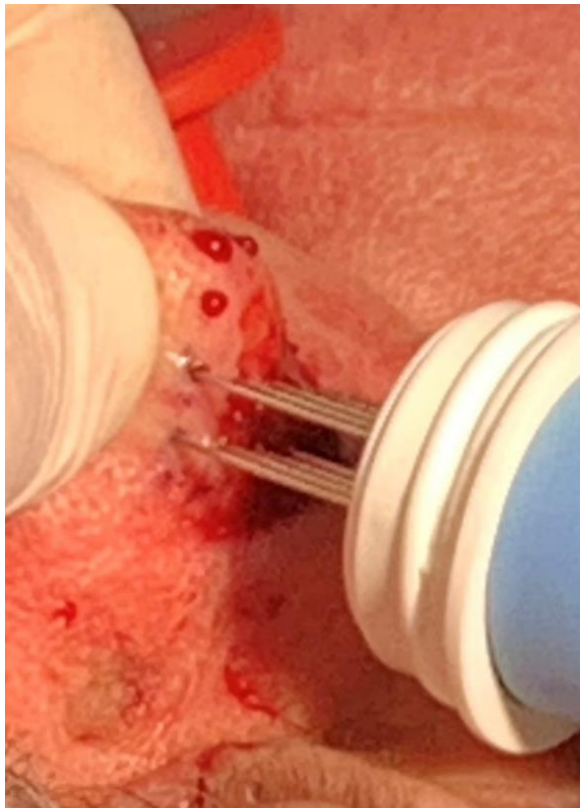
SmartGene.si

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

Treatment procedure: application of electric pulses – gene electrotransfer

- The electric pulses were applied by parallel needle electrodes and the pulses are generated by an IGEA generator
- The electric pulses were delivered in such a number of pulses that the entire tumour volume was covered





REPUBLIC OF SLOVENIA
MINISTRY OF EDUCATION, SCIENCE AND SPORT

SmartGene.si

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

Primary objectives

SAFETY

Assessment of the safety of intratumoral phIL12 GET.

Assessment of adverse events in accordance with the CTCAE v5.0 criteria.

TOLERABILITY

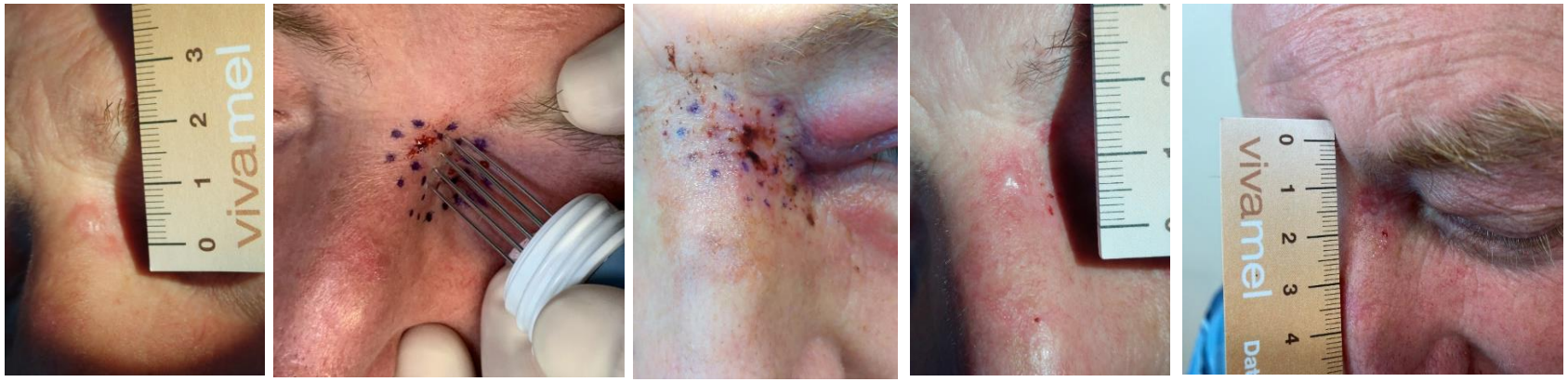
Assessment of the tolerability of intratumoral phIL12 GET.

Assessment of patient reported outcome by the quality of life questionnaire EORTC QLQ-C30.

Safety and tolerability

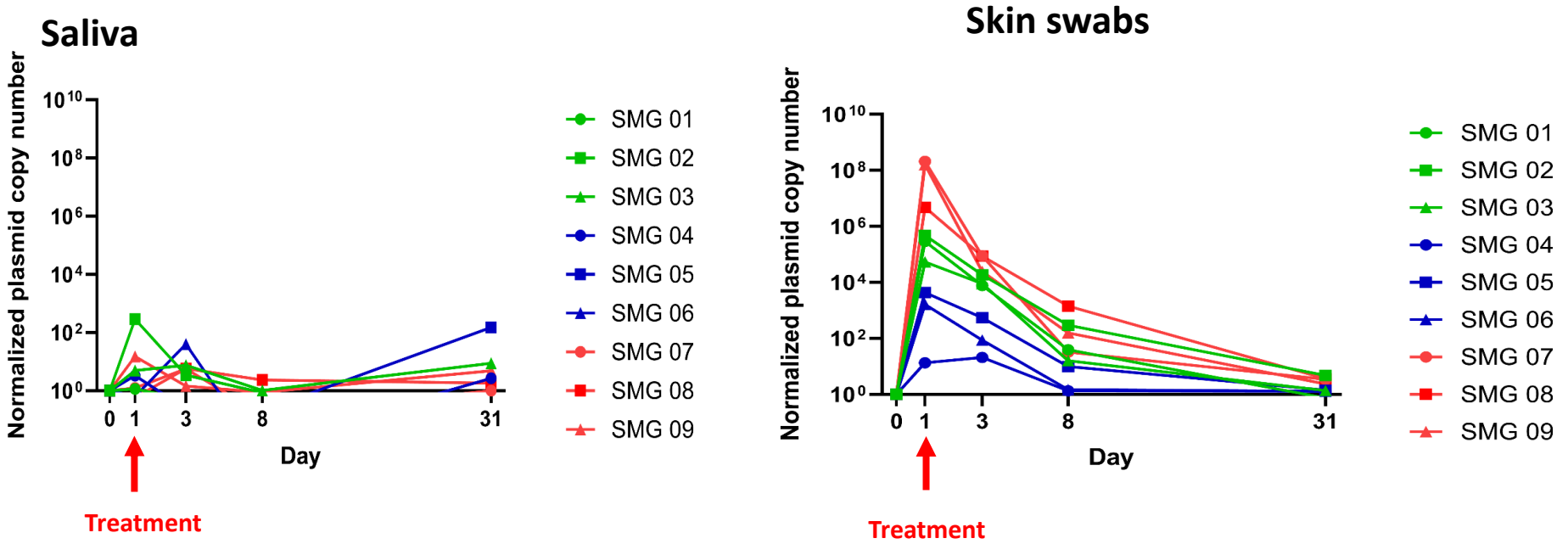
Safety
AE:
- 1 patient: mild pain 2 days after GET
- 1 patient: edema in the treatment area 2 days after GET

SAE: NO



Before GET | Application of electric pulses | 2 days after GET | 7 days after GET | 1 month after GET

- No safety concerns based on complete blood count and biochemistry



Safety and tolerability

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

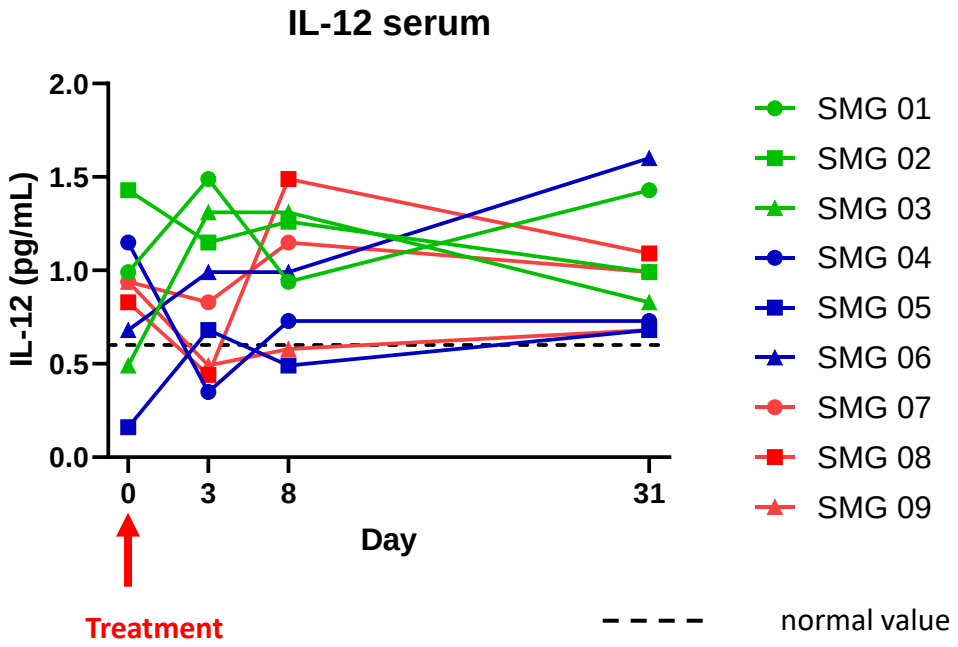
Evaluation of the appropriateness and execution of the treatment and follow up procedures.

IV. Determination of recommended dose for confirmatory studies

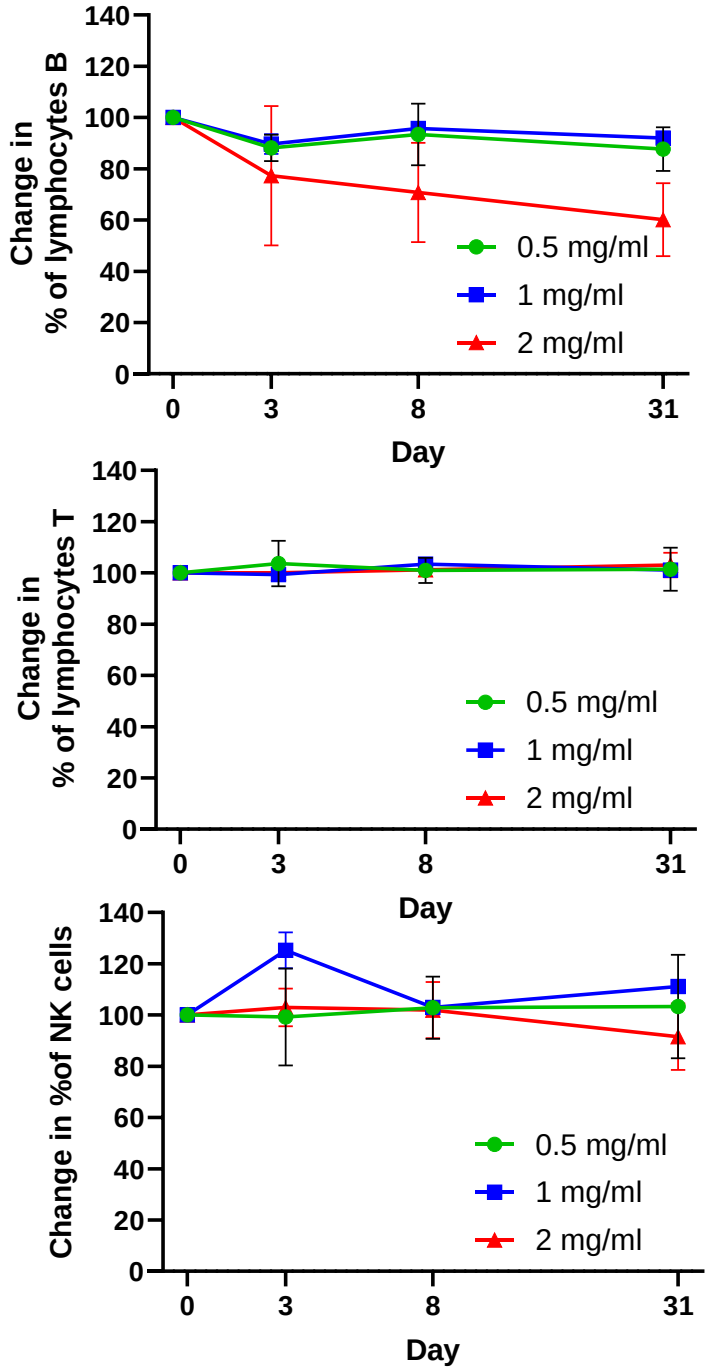
Measurement of pharmacodynamics data and selection of the phIL12 dose that produces IL-12 expression in the tumours with best biological activity, infiltration of the immune cells and no toxicity.

I. Pharmacokinetics and biodistribution

Determination of serum levels of IL-12 cytokine

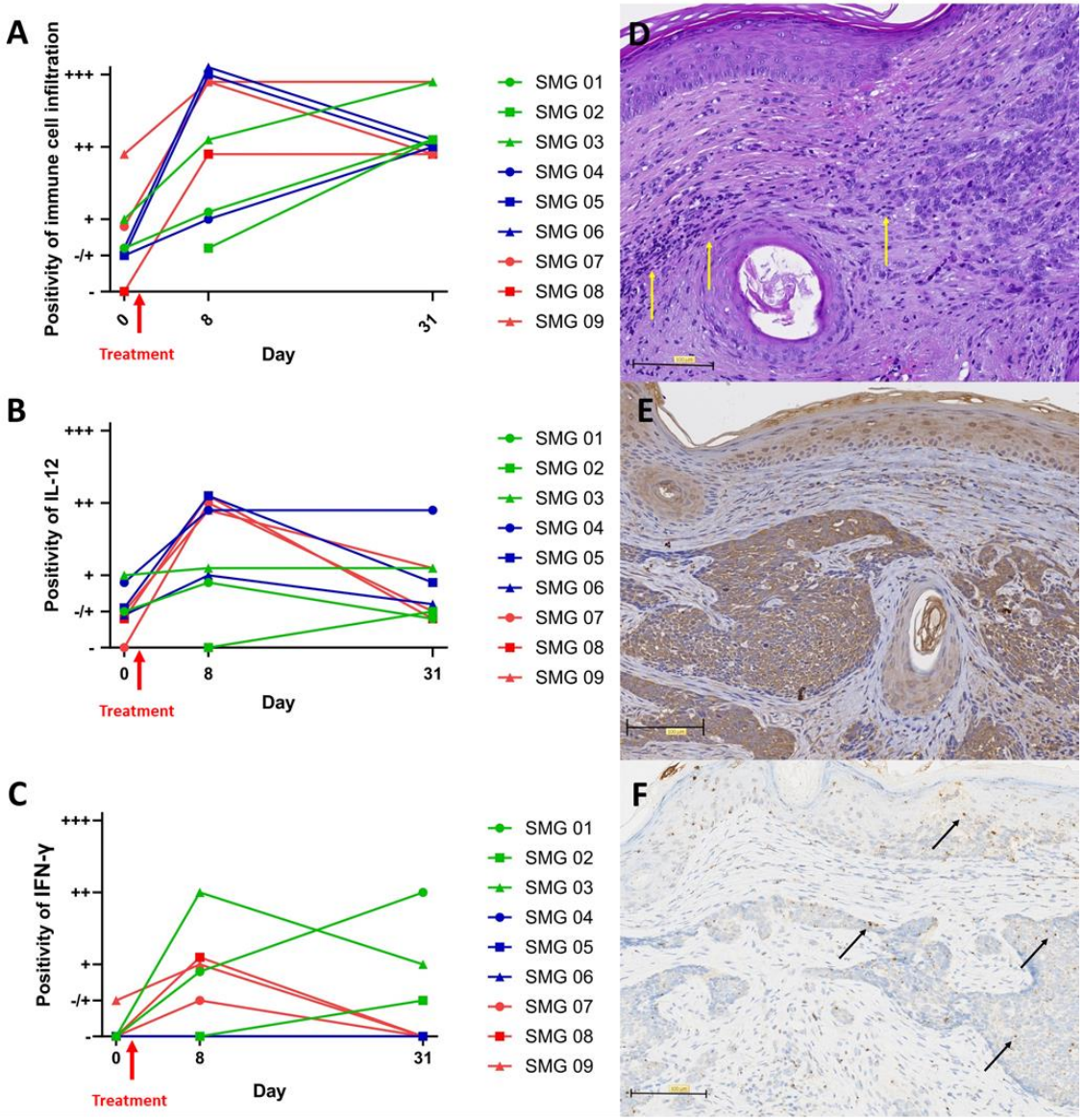


Effects on serum levels of B lymphocytes, T lymphocytes and NK cells



II. Pharmacodynamics

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



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

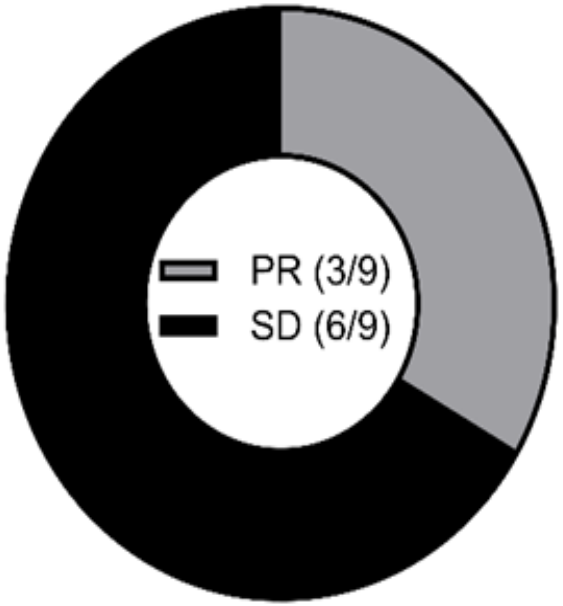
Time point		HE	IL-12	IFN-γ	Sum	Immunoscore	Concentration of phIL12 (mg/mL)
Day 8	SMG01	1	1	1	3		
	SMG02	0.5	0	0	0.5		
	SMG03	2	1	2	5	8.5	0.5 mg/ml
	SMG04	1	2	0	3		
	SMG05	3	2	0	5		
	SMG06	3	1	0	4	12	1 mg/ml
	SMG07	3	2	0.5	5.5		
	SMG08	2	2	1	5		
	SMG09	3	2	1	6	16.5	2 mg/ml

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.

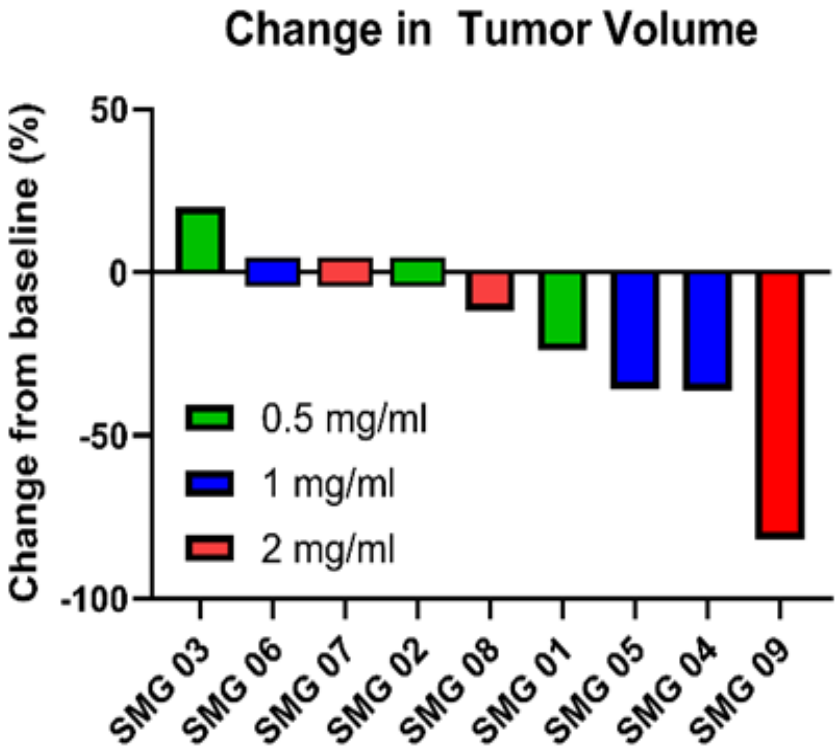
An immunoscore was calculated based on the positivity of all three parameters—HE, IL-12, and IFN-γ staining. The analysis revealed that the 2 mg/ml groups exhibited the highest immunoscore on day 8 compared to the other two groups.

Response rate

A



B



III. Feasibility of recruitment

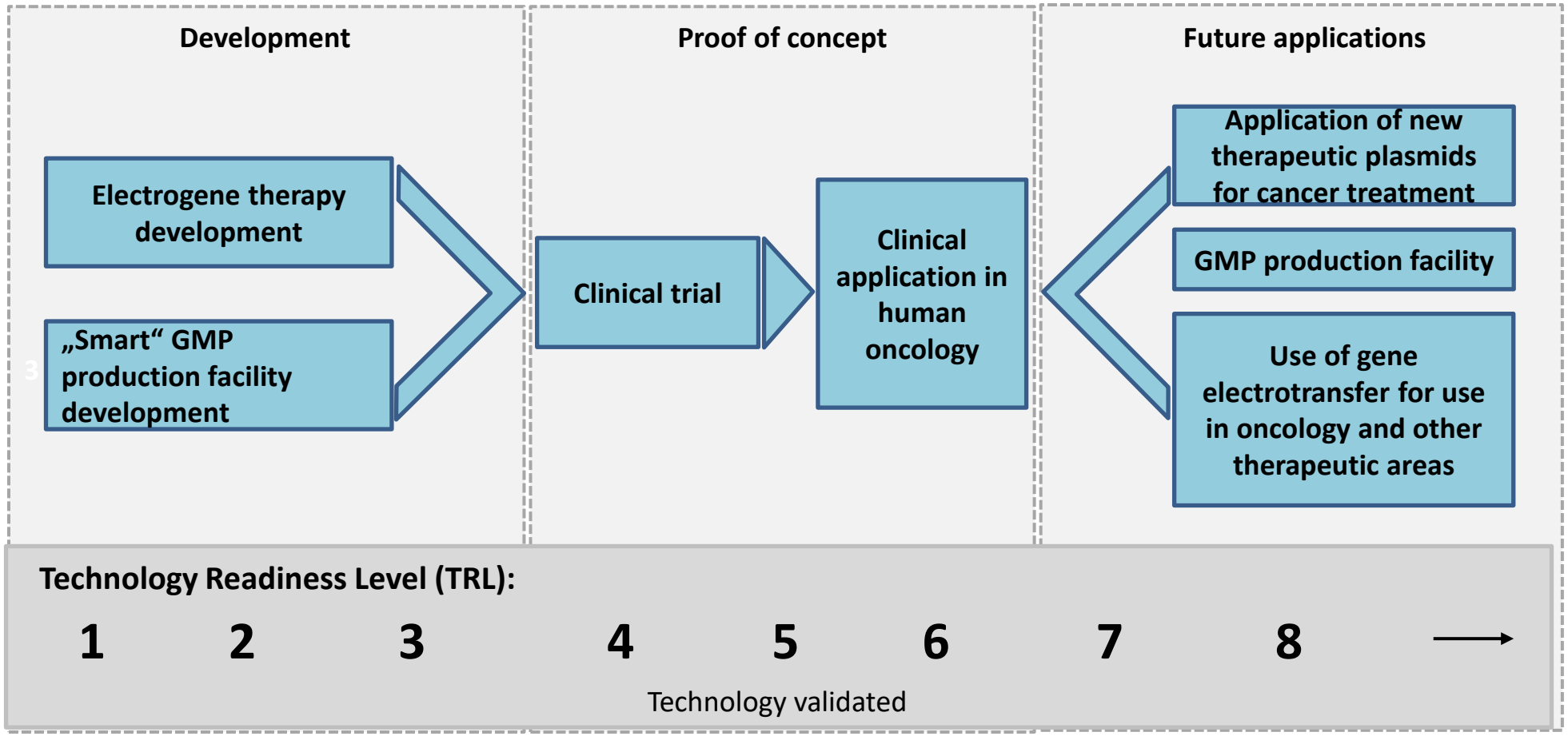
Evaluation of the appropriateness and execution of the treatment and follow up procedures.

- minor problems with recrruitment of patients due to tight follow up schedule
- no problem with treatment procedure and follow up once the patients were included
- no patient lost to follow up

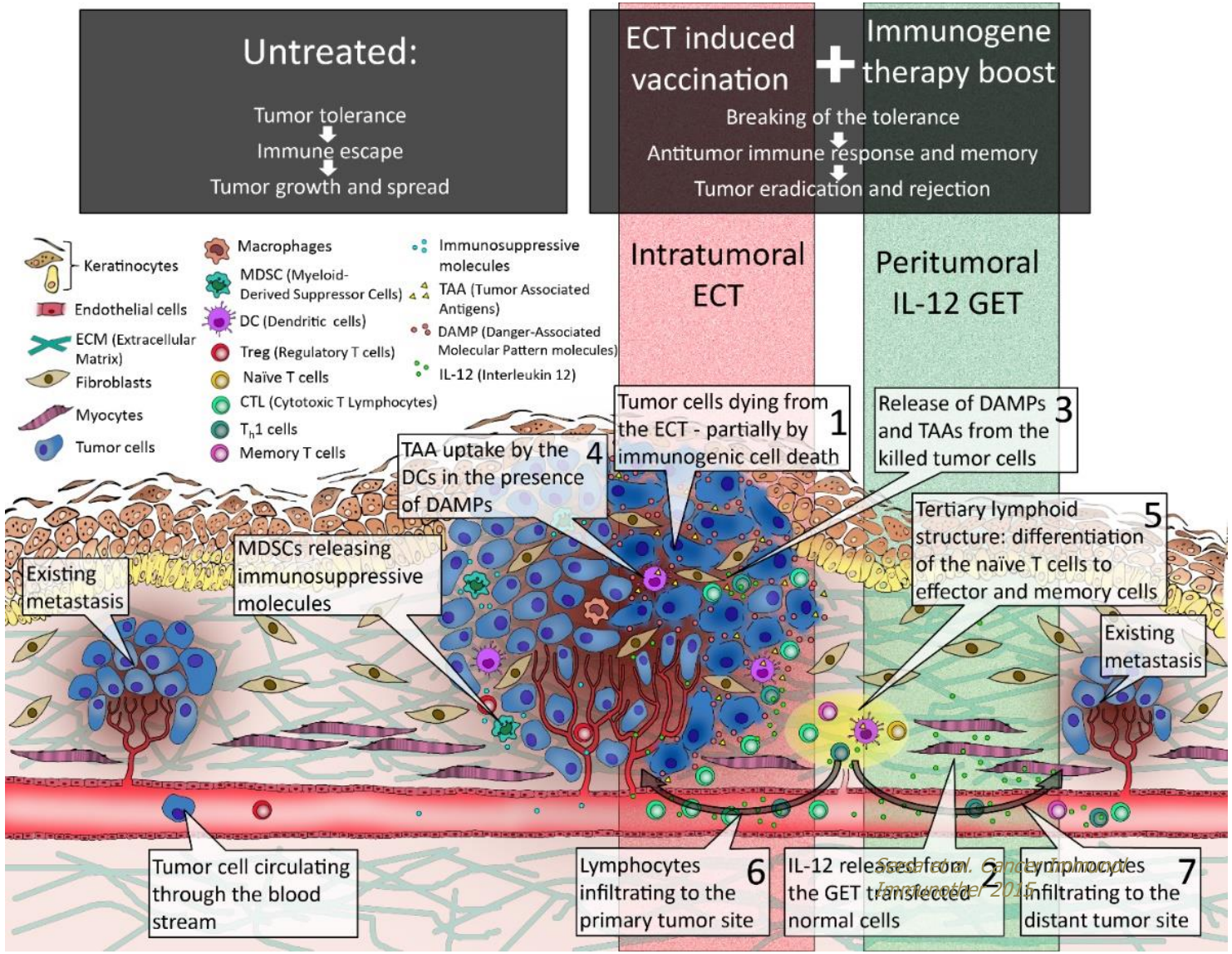
IV. Determination of recommended dose for confirmatory studies

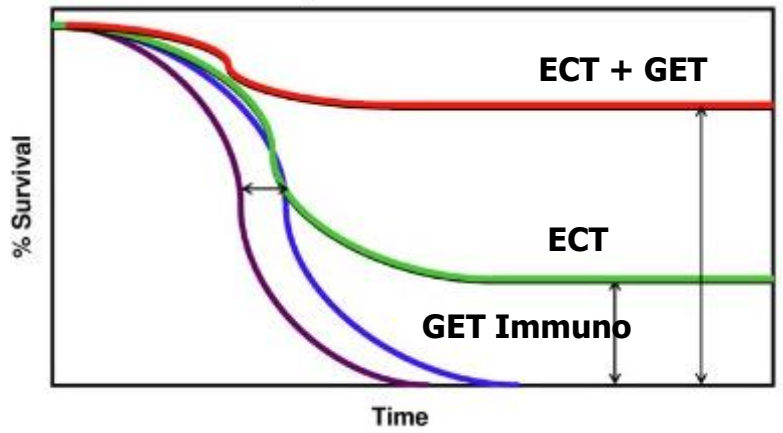
Measurement of pharmacodynamics data and selection of the phIL12 dose that produces IL-12 expression in the tumours with best biological activity, infiltration of the immune cells and no toxicity: **2 mg/ml**

The future – scaling up and going beyond IL-12!



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



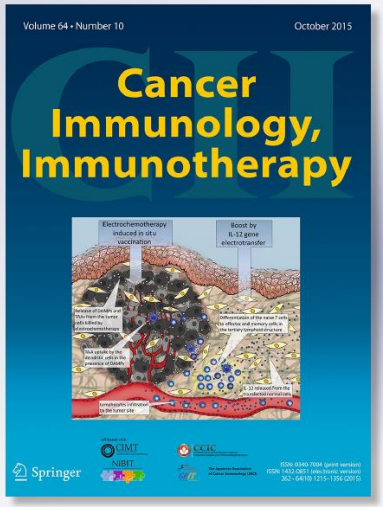


Gregor Sersa, Justin Teissie, Maja Cemazar, Emanuela Signori, Urska Kamensek, Guillermo Marshall & Damijan Miklavcic

Cancer Immunology, Immunotherapy

ISSN 0340-7004
Volume 64
Number 10

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



WHO initiative – One Health

World Health Organization

The One Health Triad

Antibiotic resistance
Zoonotic diseases
Cancer

By Alexander G. Watts - https://followtheoutbreak.wordpress.com/2013/10/16/opinion-do-we-need-to-induce-stress-in-the-one-health-paradigm/, Fair use, https://en.wikipedia.org/w/index.php?curid=53699284

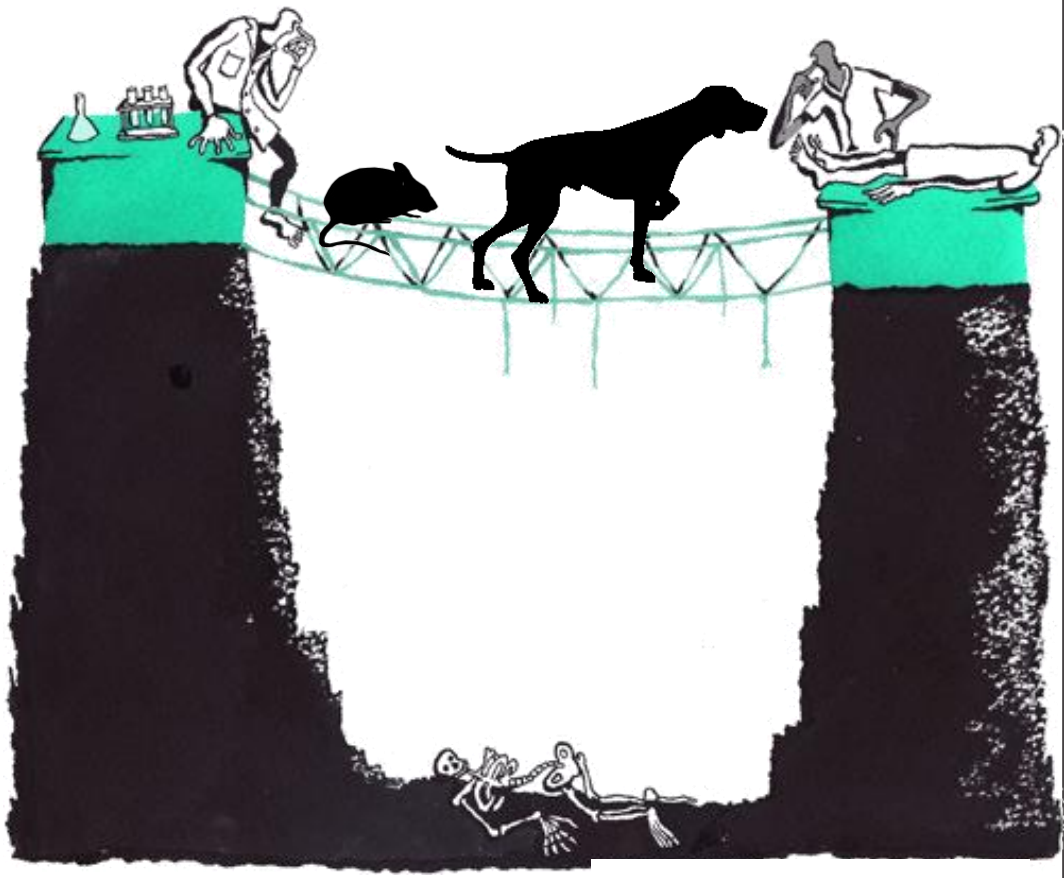
Dog – good translational model

Living environment
Environmental risk factors

Tumor homology
Epidemiology
Tumor characteristics
Clinical signs

Large research group
Europe: 55 milion dogs
World: ~600 milion dogs

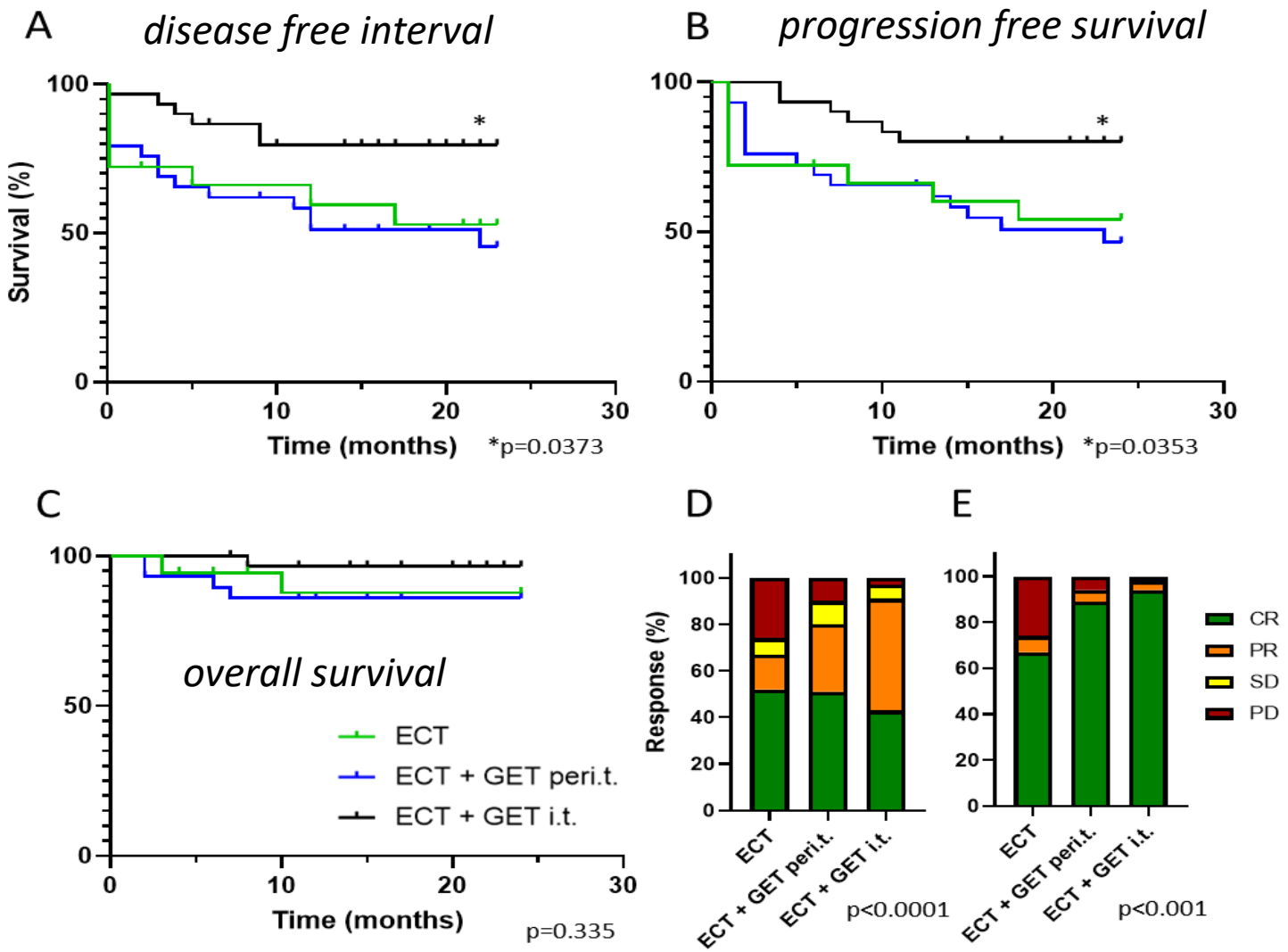
Life expectancy
Long-term side effects



Lack of gold standards for therapy
Faster and more humane evaluation of new therapies

Pathogenesis studies
Breed specific tumor type
Similar genetic mutations (colorectal cancer)

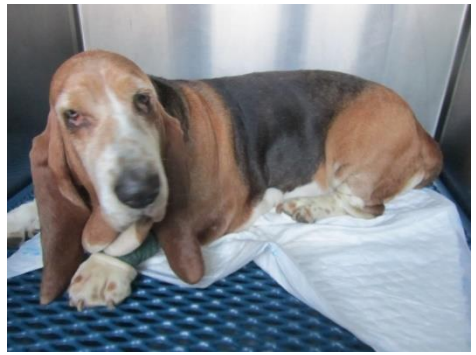
Response according to the patient



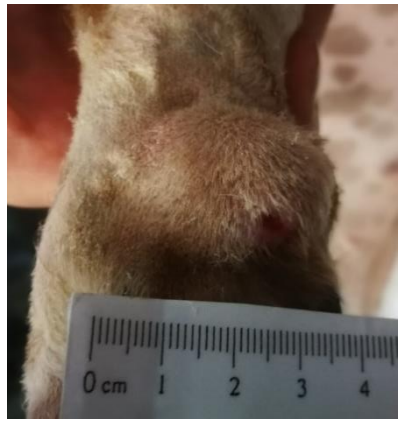
disease-free interval (DFI) - the time from CR onset to the time of relapse of the disease (MCT) or the end of observation period
progression free survival (PFS) - the time from treatment initiation until disease (MCT) progression
overall survival (OS) - the period from the enrolment of the patient until the death due to the disease (MCT).

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

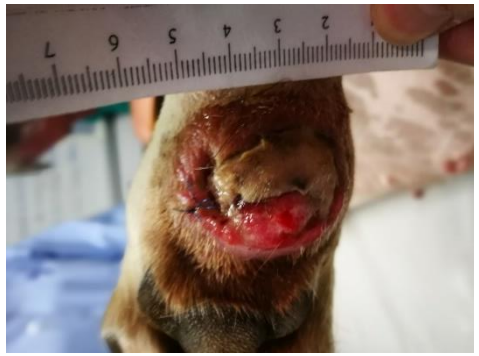
Intratumoral application of pcaIL-12 & ECT bleomycin



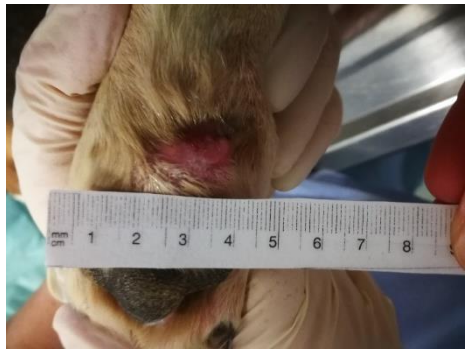
- Basset hound
- female
- 6 years



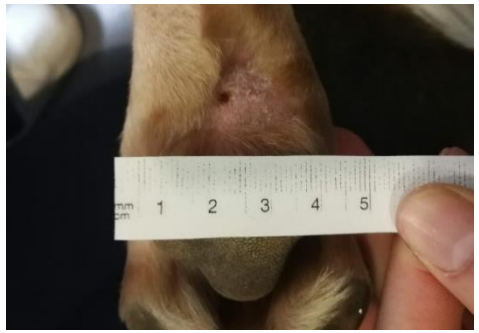
- Mastocytoma
- Right sole of hind leg 2.6 cm^3
- Two treatments 4 weeks apart
- (PR 0.4 cm^3 after first th.)
- Patohistological grade (II)



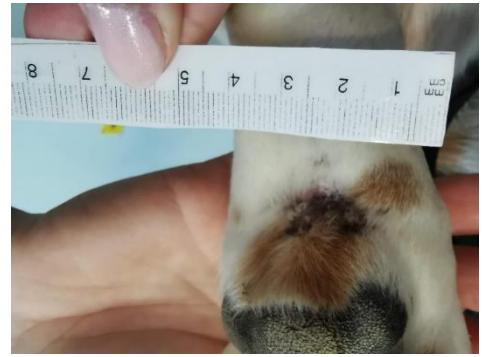
1 week after the first therapy



1 month after the second therapy



2 months after the second therapy

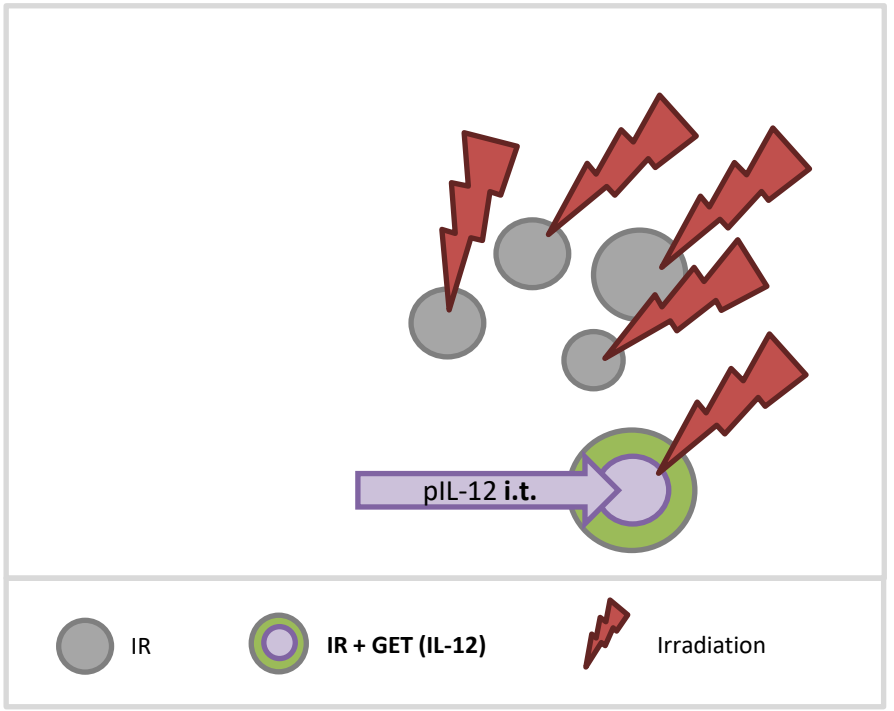


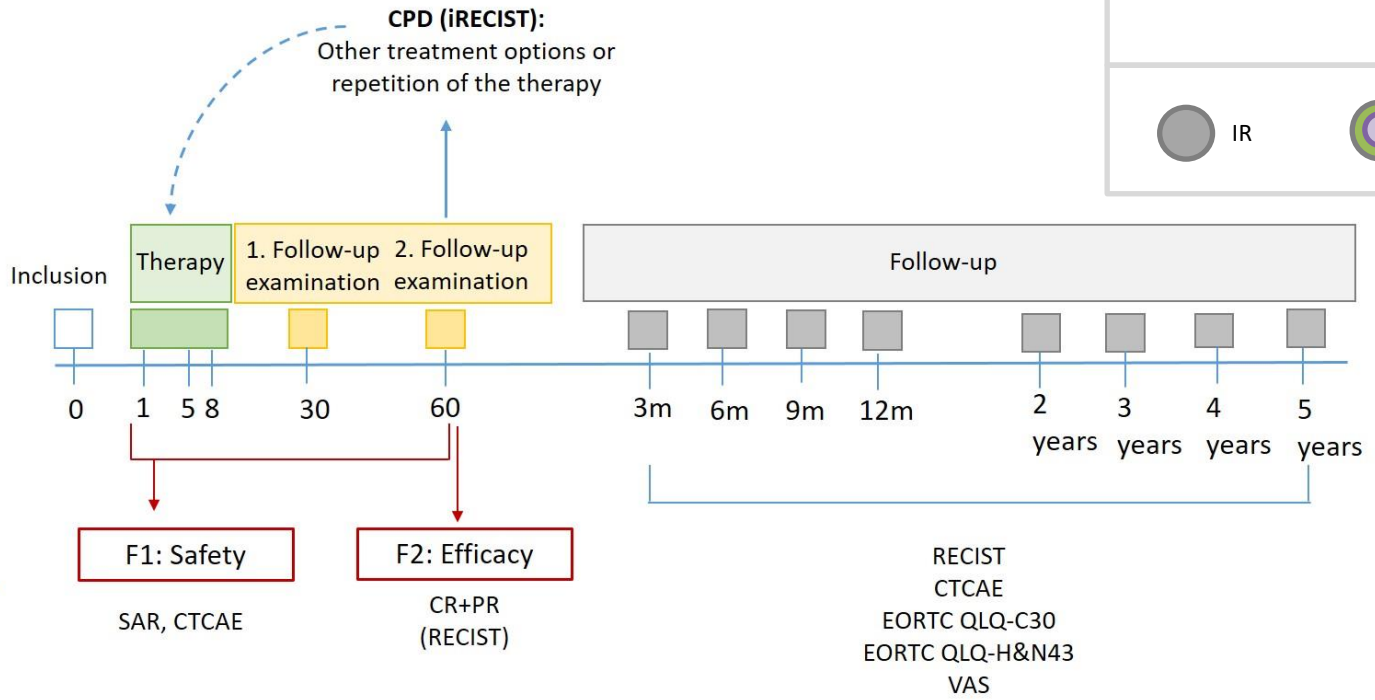
6 months after the second therapy

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

Clinical development –further steps

Clinical trial (Phase I/II): Treatment of the Head and Neck Skin Tumours with the Combination of radiotherapy and gene electrotransfer of Interleukin 12





Acknowledgements

Thanks to all of my co-workers:

Thank you for your attention!

prof. dr. Maja Čemažar
dr. Maša Bošnjak
dr. Urša Lampreht Tratar
dr. Simona Kranjc Brezar
doc. dr. Urška Kamenšek
dr. Boštjan Markelc
dr. Katja Uršič Valentinuzzi
dr. Špela Kos
dr. Tanja Jesenko
dr. Katarina Žnidar
dr. Tim Božič
dr. Tilen Komel
Živa Modic
Urša Kešar
Teja Valant
Mira Lavrič
Ajda Medved
Iva Šantek
Saša Kupčič