



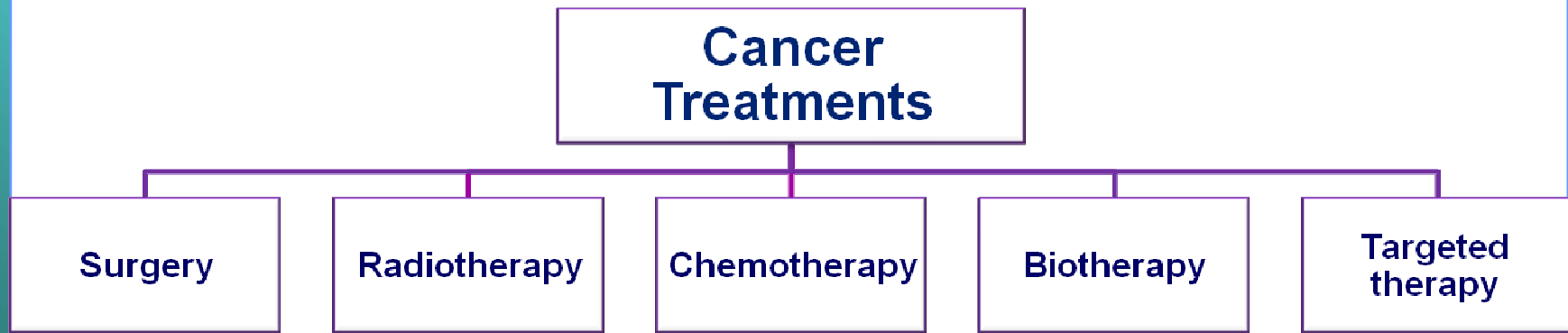
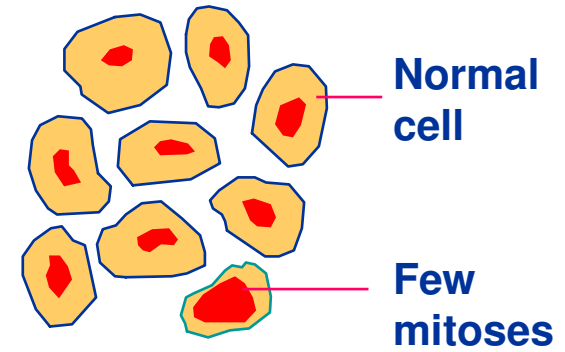
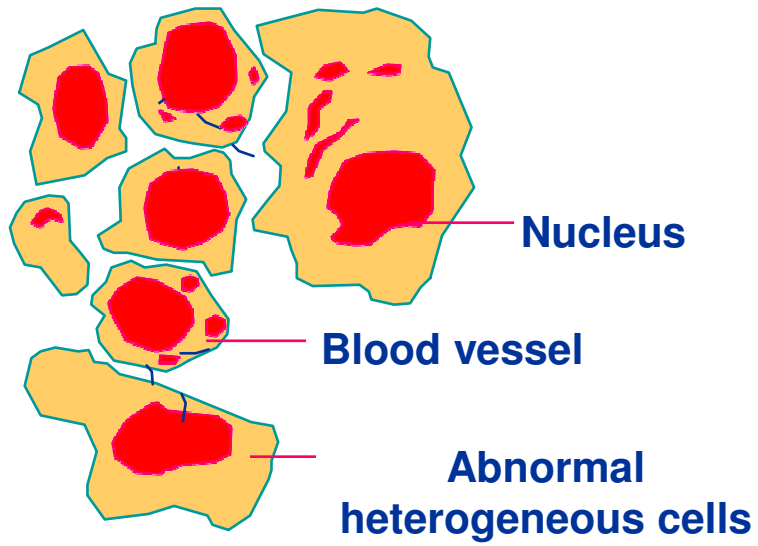
The role of Electroporation in Medicine

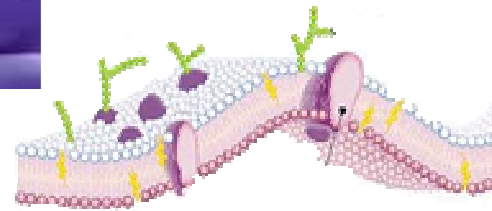
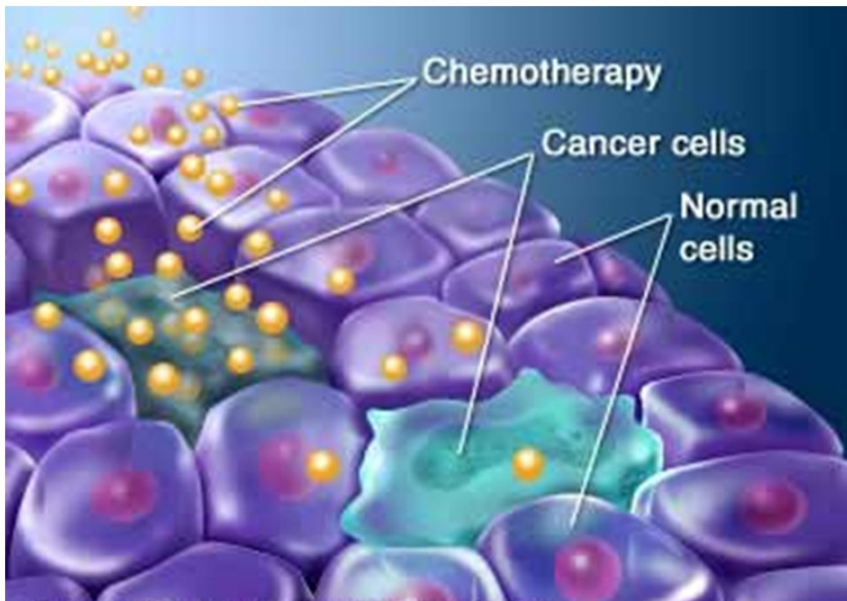
Elham Raeisi, PhD

Alexander Xhuvani University

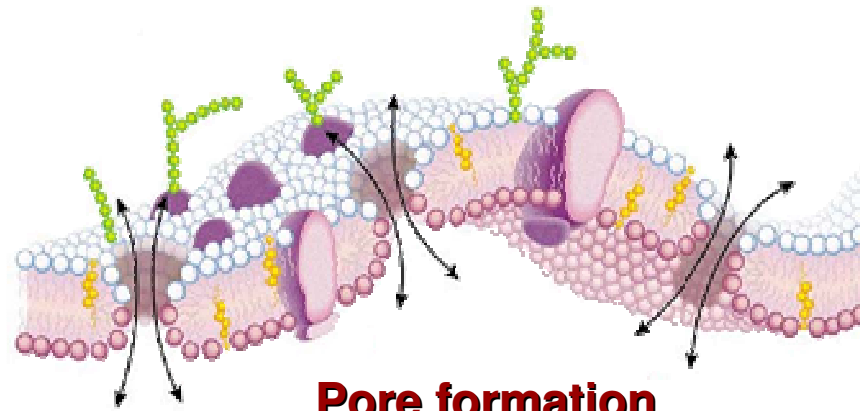
8/07/2016



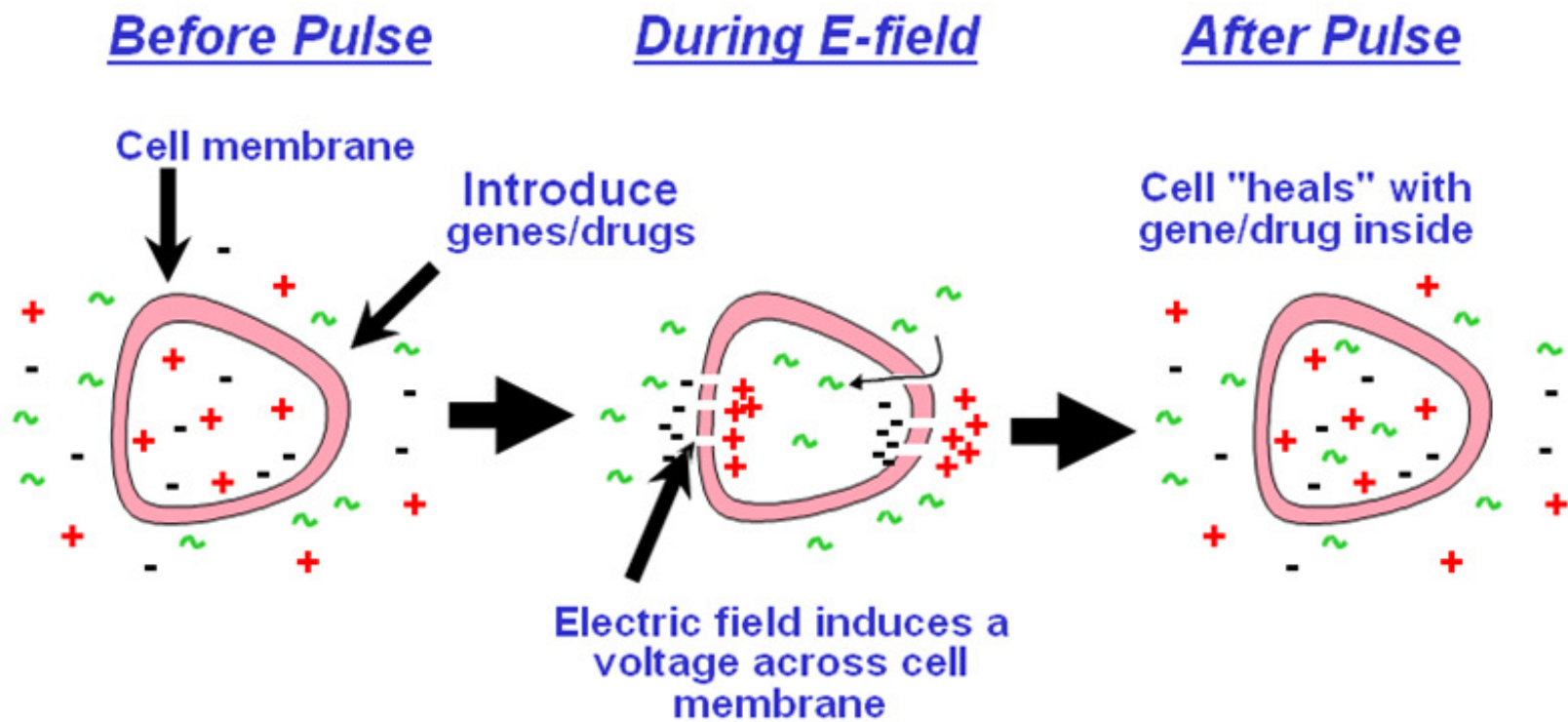




**Cell
membrane**



Electroporation



Mechanism

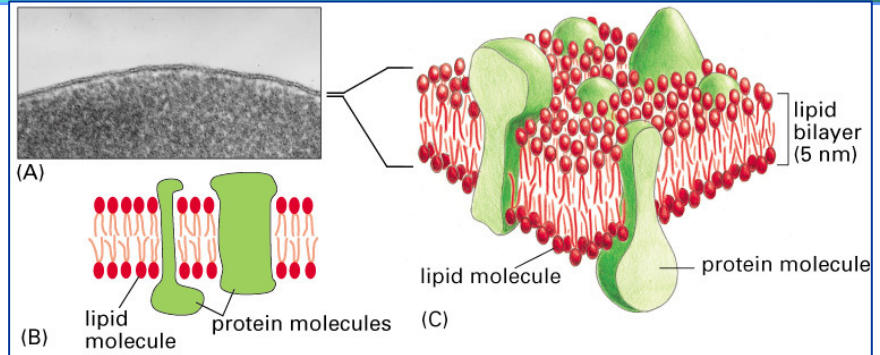
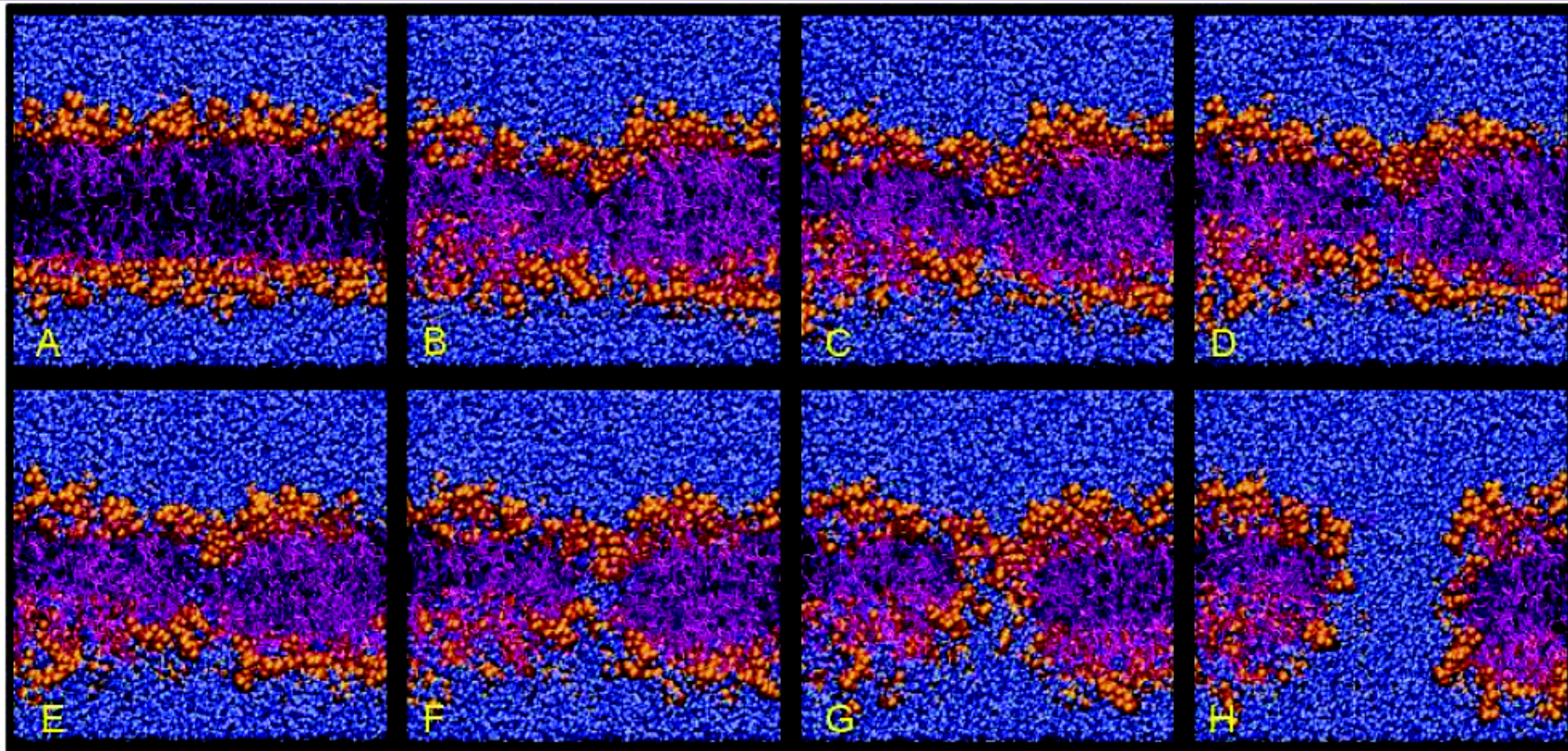
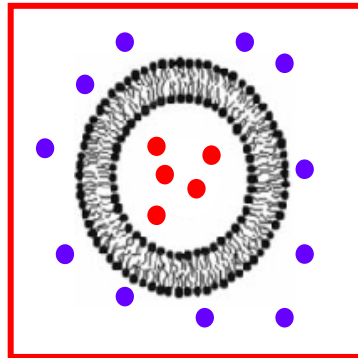


Figure 10-1. Molecular Biology of the Cell, 4th Edition.

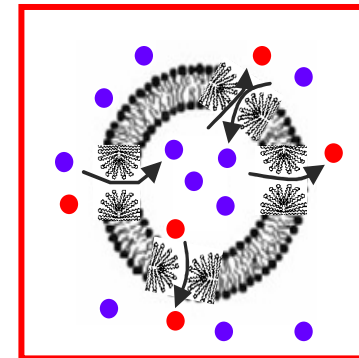


Action of Electroporation



Intact Cell Membrane

Electroporation



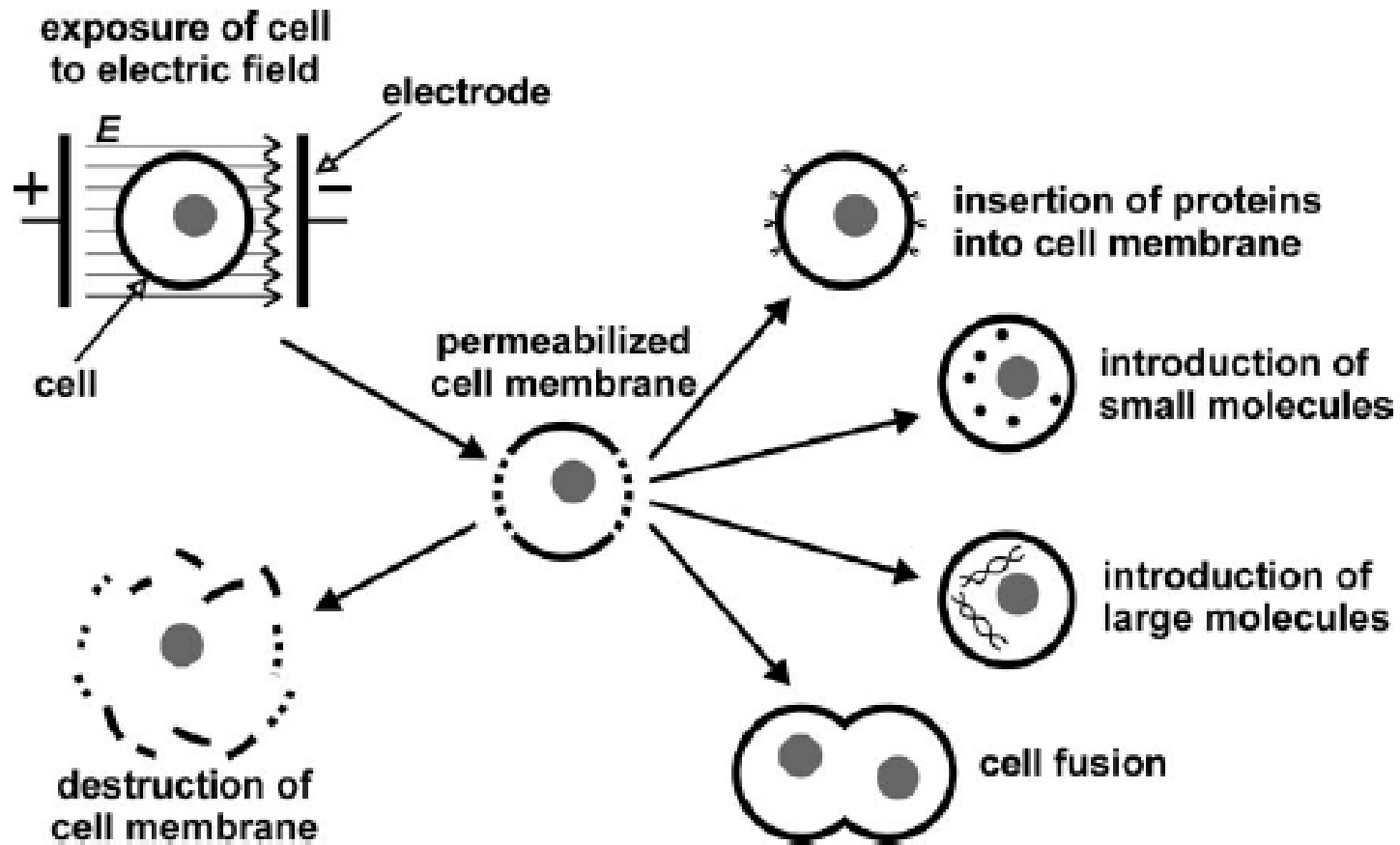
Disruption of Cell Membrane

Results?

Reversible

Irreversible

Application of Electroporation



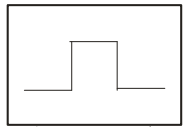
Puc et al. Bioelectrochemistry 2004; 64: 113-24.

Electroporation based Technologies & Treatments

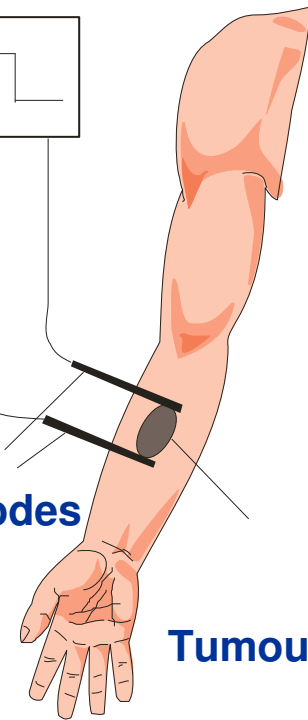
- **Molecular cell biology research**
- **Protein insertion into cell membrane**
- **Cell fusion**
- **Gene expression silencing by siRNA**
- **Electrochemotherapy**
- **Genetherapy based on electro genetransfer**
- **Transdermal drug delivery**
- **Tissue ablation**
- **Biotechnology**
- **Water and liquid food sterilisation**

Electrochemotherapy : chemotherapy + electroporation

Electric pulse generator

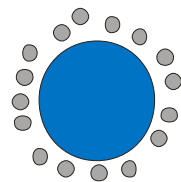


Electrodes



Tumour

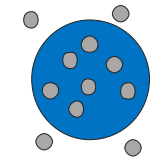
Chemotherapeutic surrounds the cells



Increased membrane permeability allows access to the cytosol

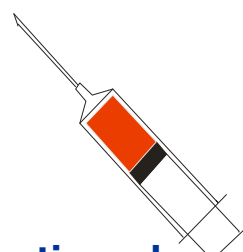


Pores reseal



Electric pulse application

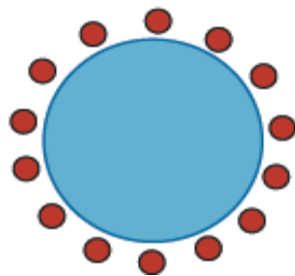
Injecting chemotherapeutic



Sersa et al. Cancer Therapy 2003; 1: 133-42.

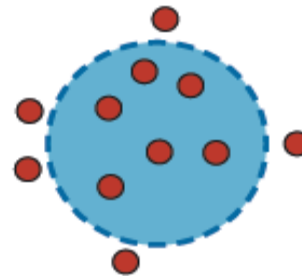
Protocol

Chemotherapeutic surrounds the cells



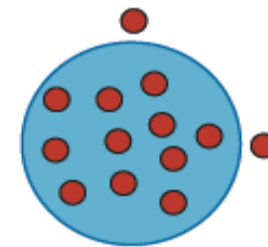
fase 1

Increased membrane permeability allows access to the cytosol



fase 2

Pores resealing



fase 3



Electric pulses application

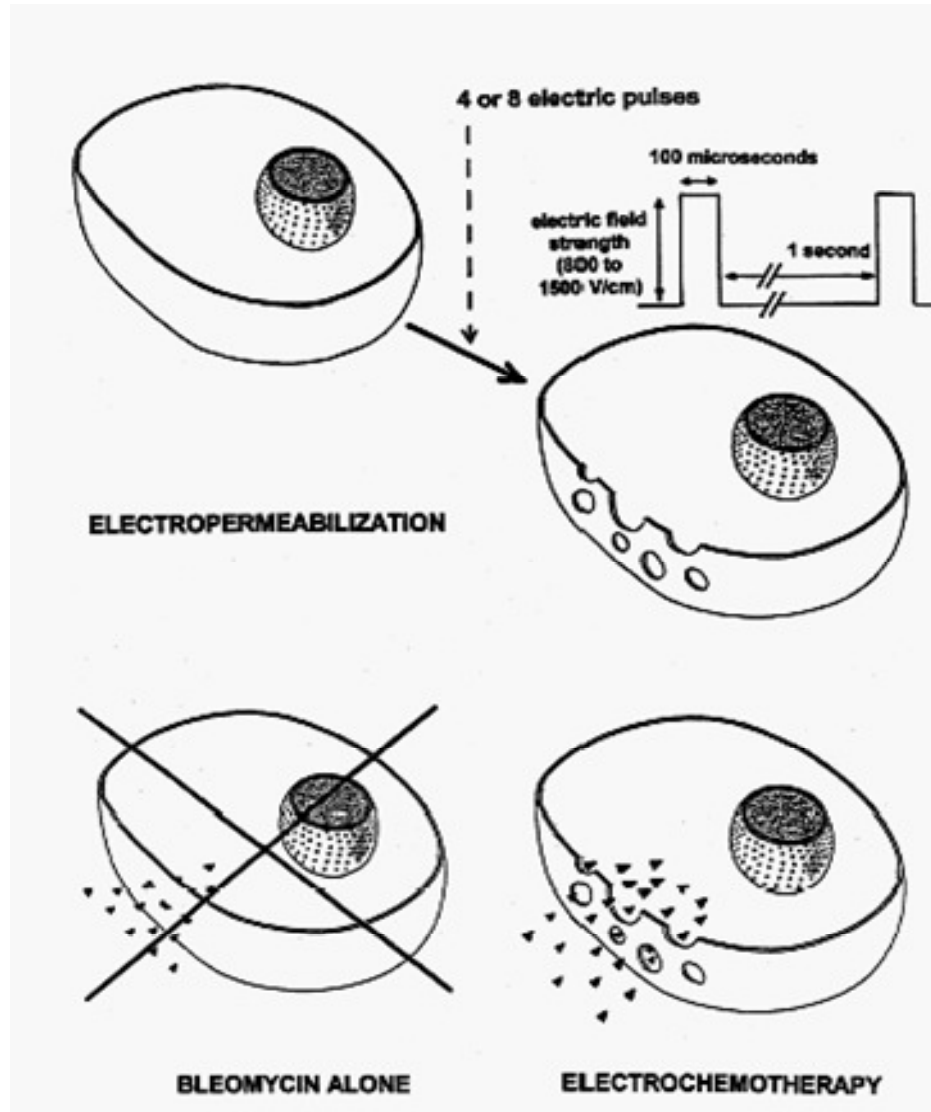
Time



Injecting chemotherapeutic drug

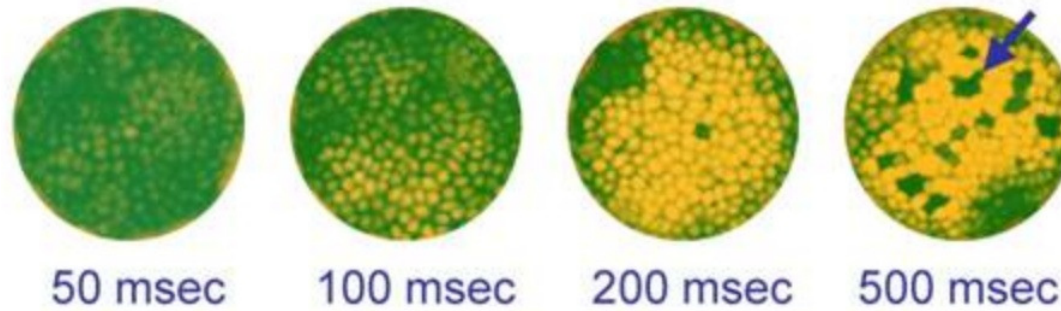
Therapeutic mechanism 1

Permeability

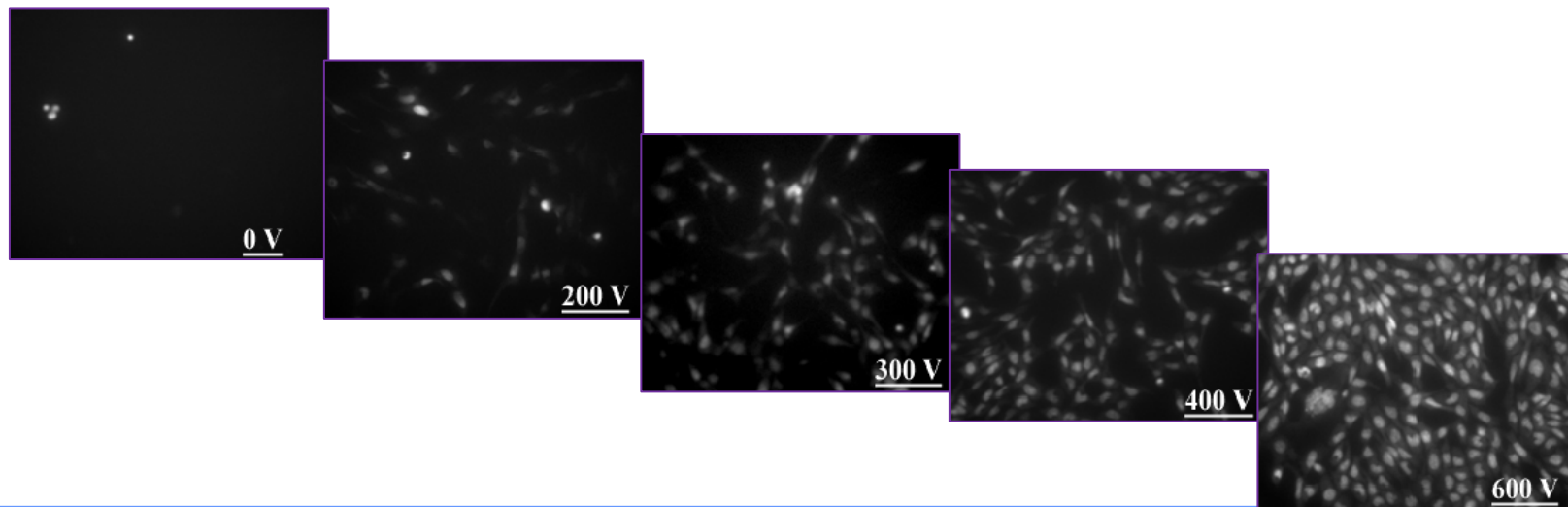


In vitro

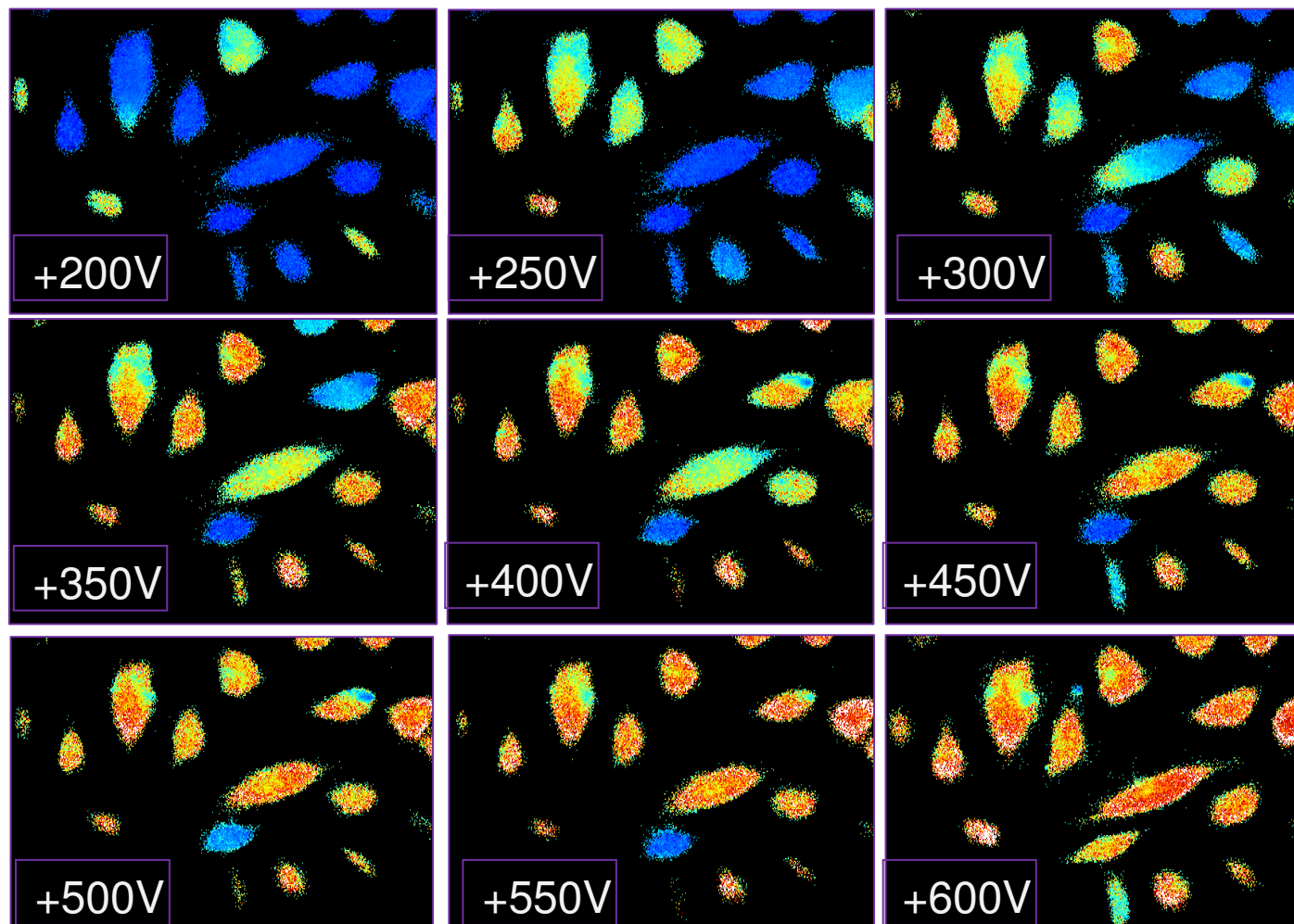
Lucifer yellow



Propidium Iodide

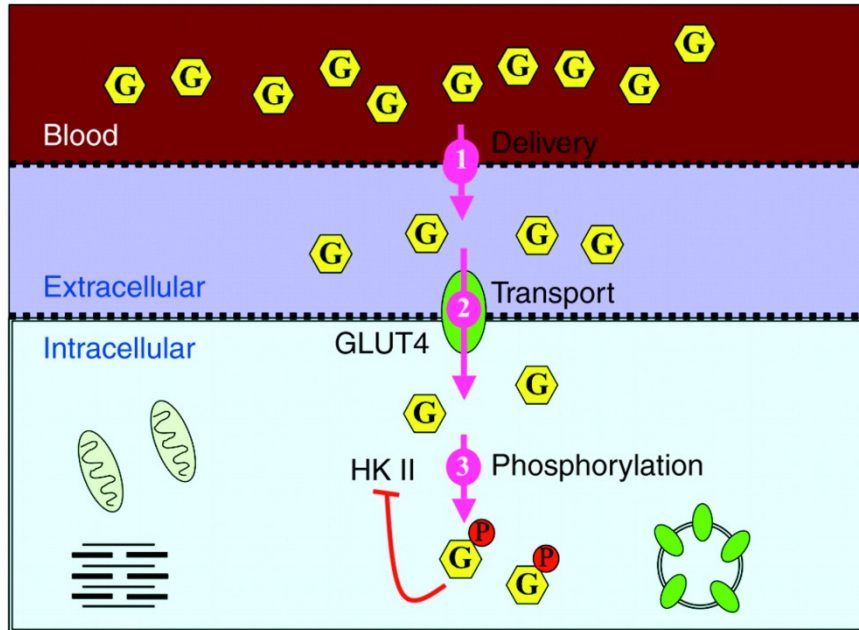


Fura-2AM



0,00nM 500,00nM

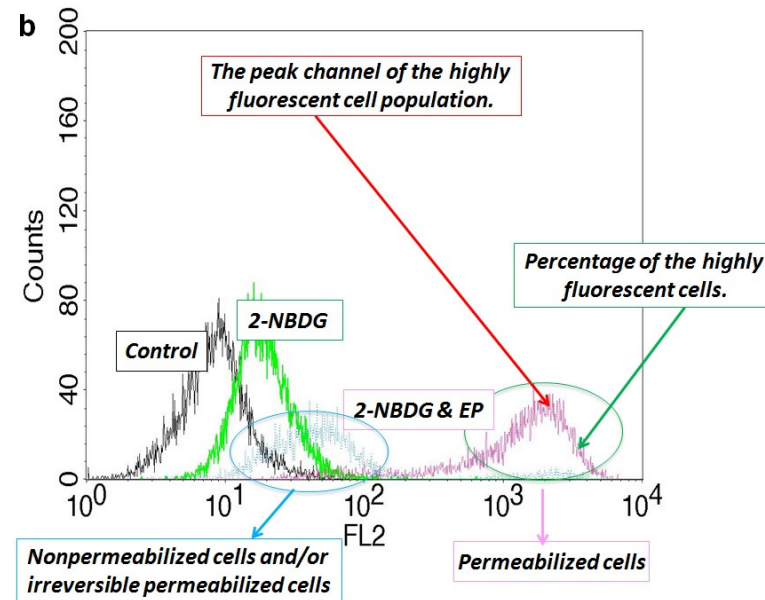
Glucose derivatives



Wasserman et al. J Exp Biol 2011; 214: 254-62.

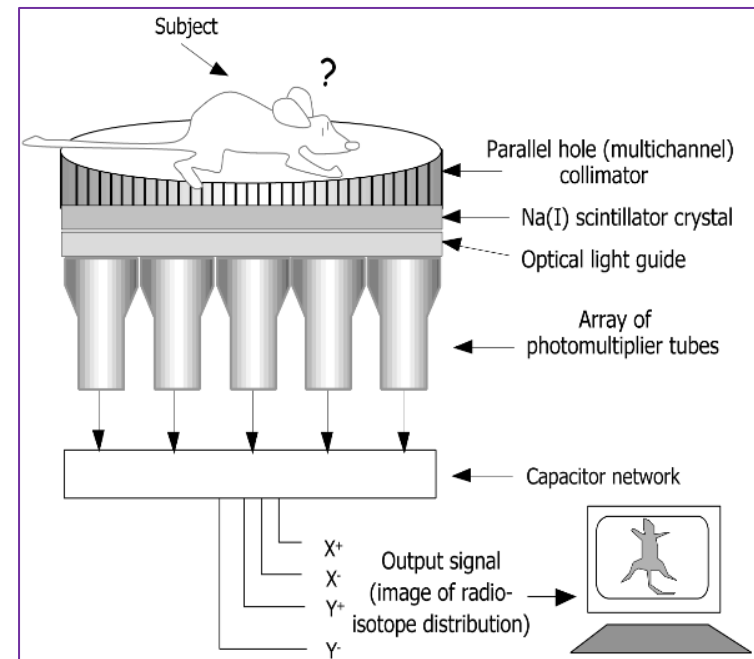
Raeisi E & Mir LM. J Membrane Biol 2012; 245: 226-32.

Raeisi E, Lemoigne Y, Mir LM. IFMBE Proceeding 2016; 46:226-32.



In vivo

Radiolabeled bleomycin



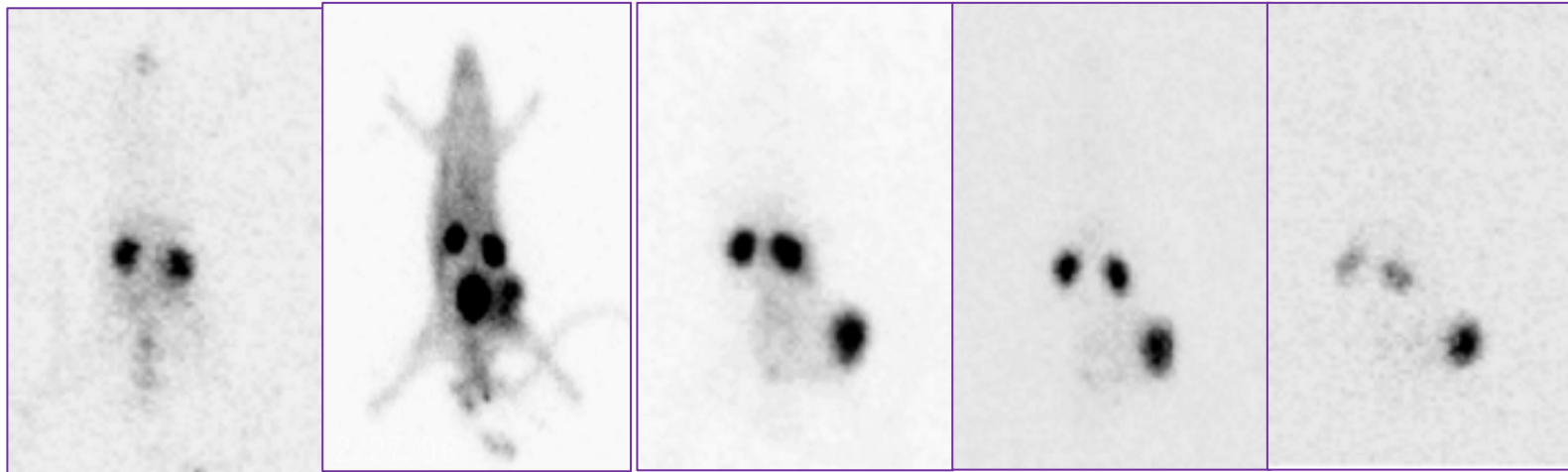
No pulse

1 h

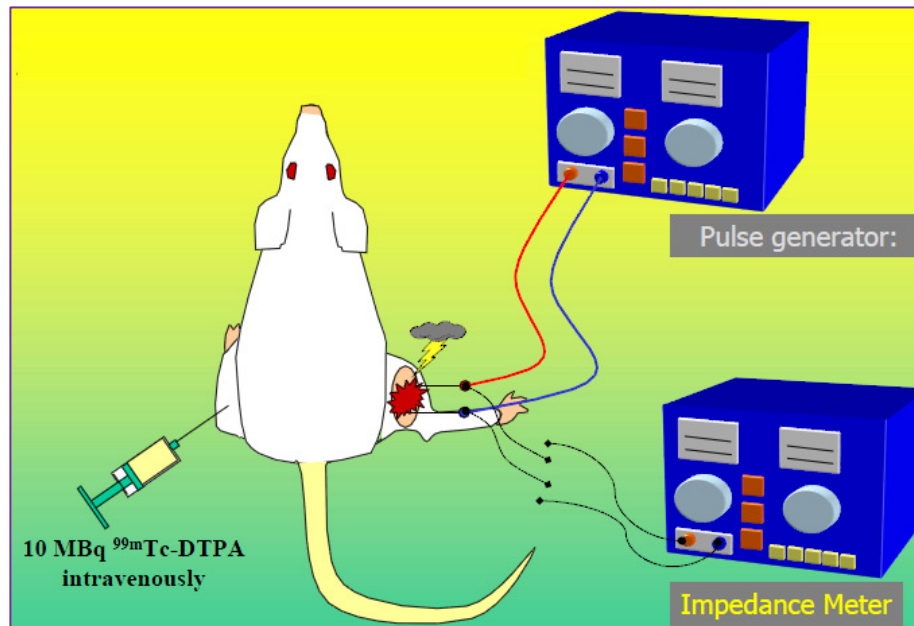
1 day

3 day

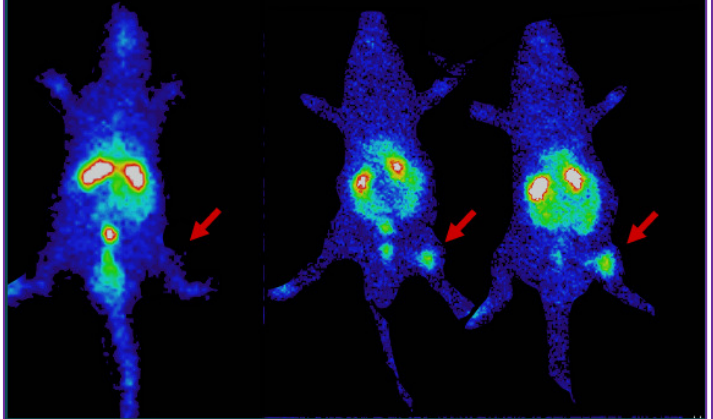
7 day



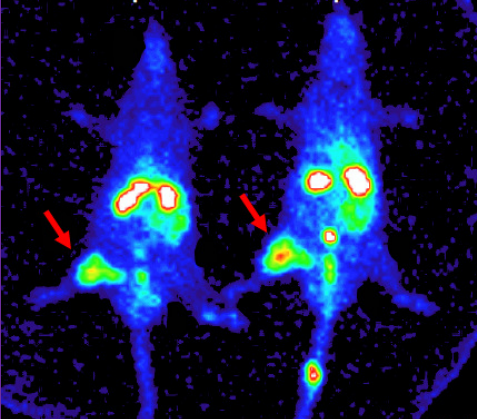
⁹⁹Tc-DTPA



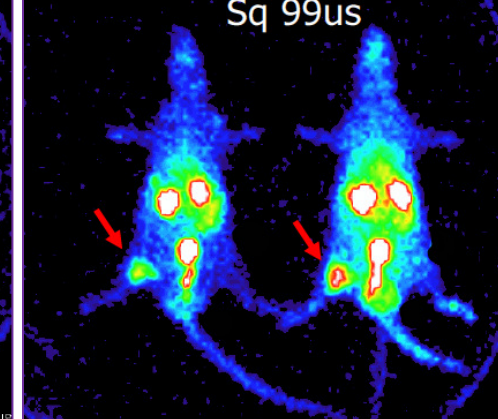
200 V/cm 99us 200, 400 V/cm 1.0 ms



8 pulses 12 pulses

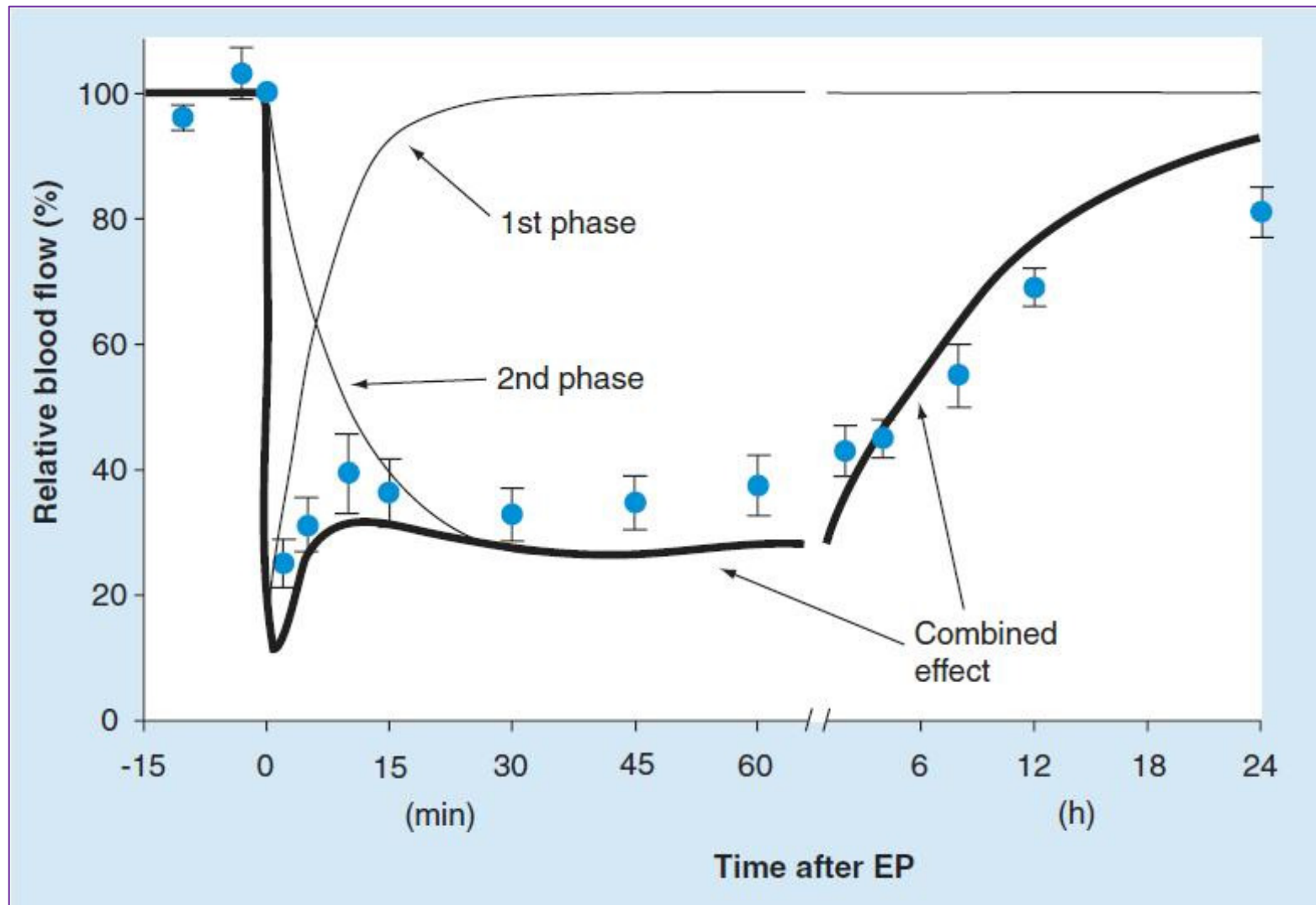


1200V/cm 1600 V/cm
Sq 99us

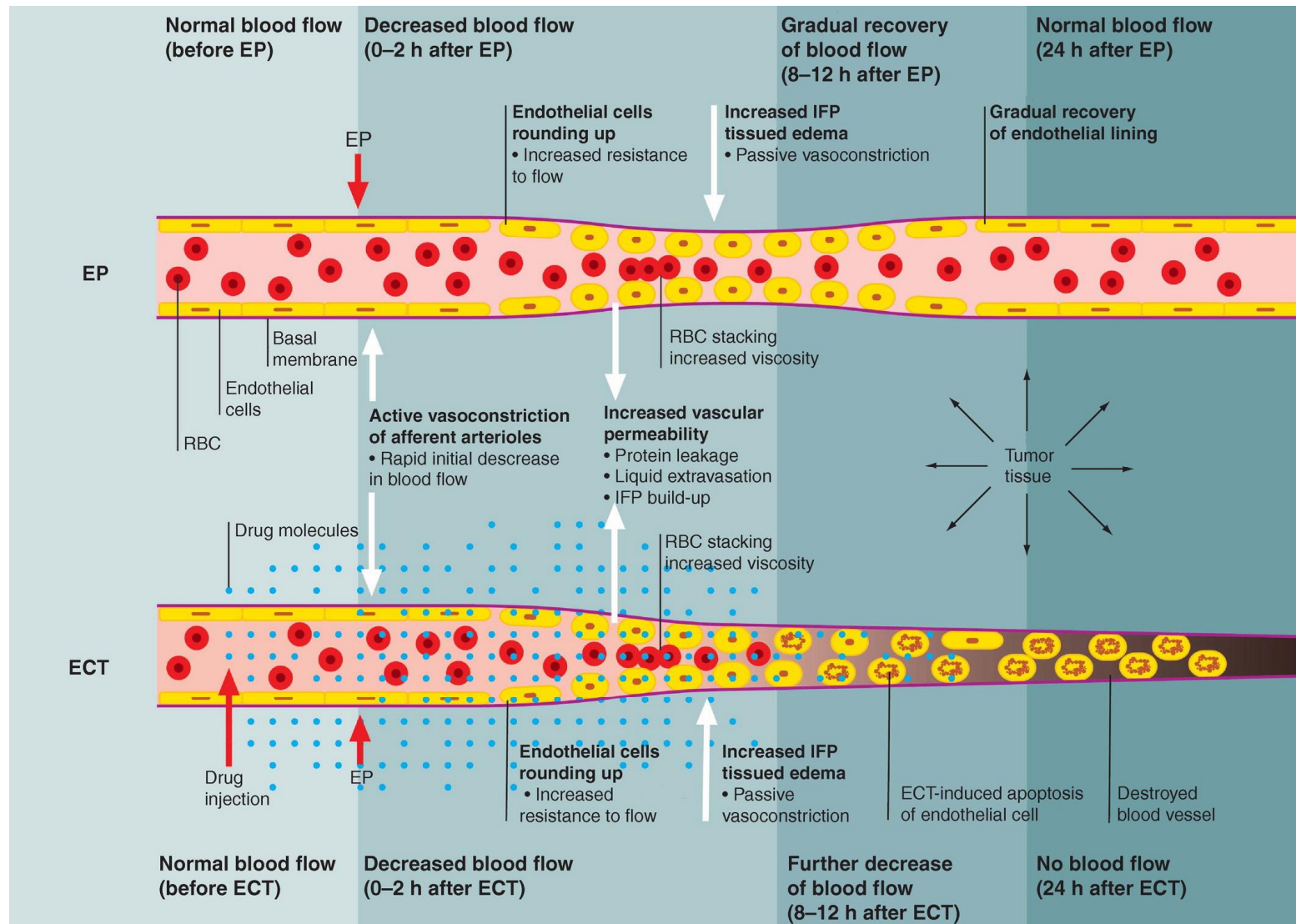


Grafstrom et al. Cancer Biother Radiopharm 2006; 21: 623-35.

Therapeutic mechanism 2: *Vascular effect 1*



Vascular effect 2



Electrical factors influencing the efficiency of ECT

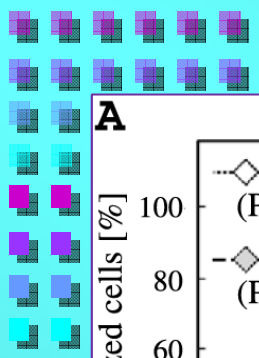
Pulse shape

Pulse amplitude

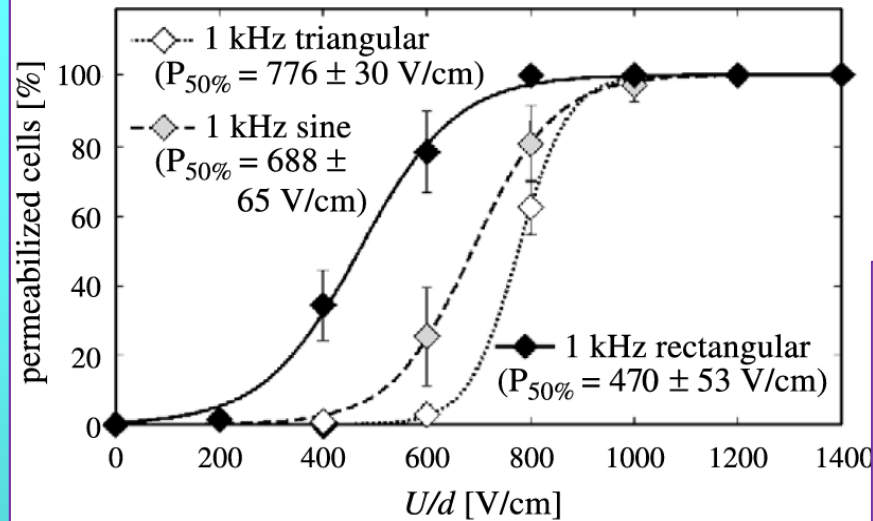
Pulse duration

Number of pulse

Pulse frequency

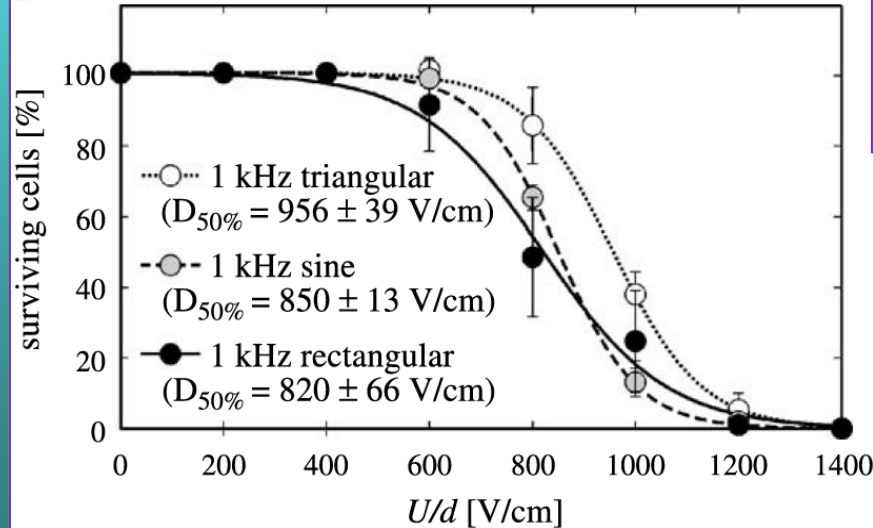


A

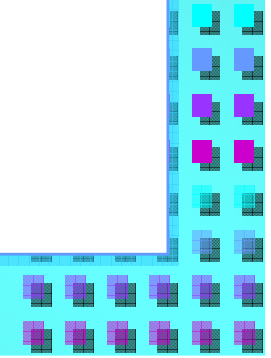
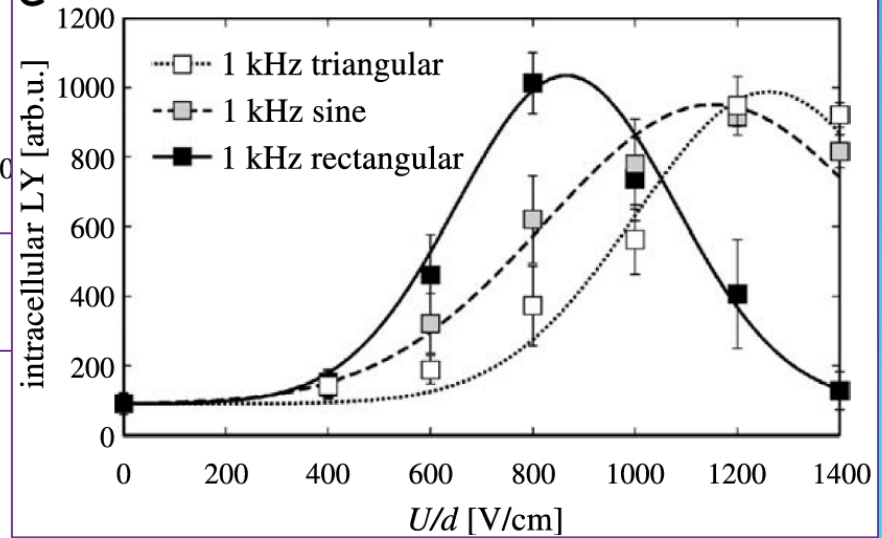


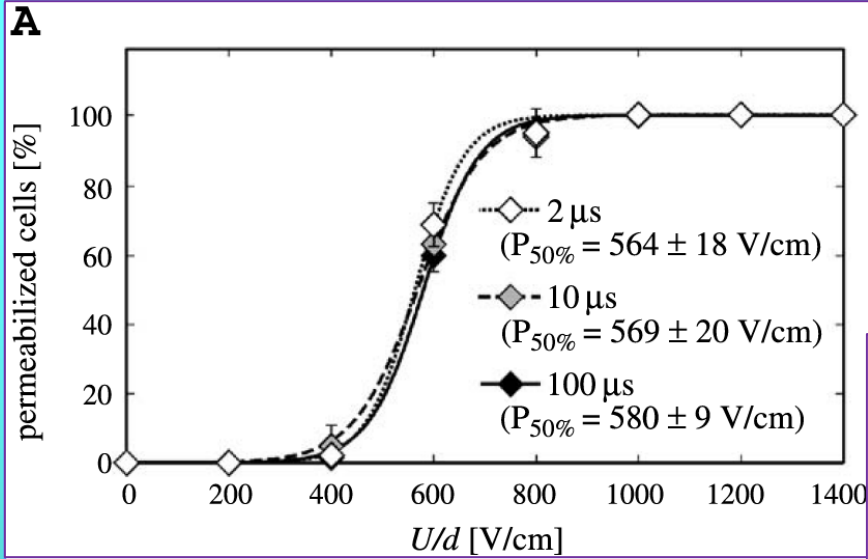
Pulse shape

B

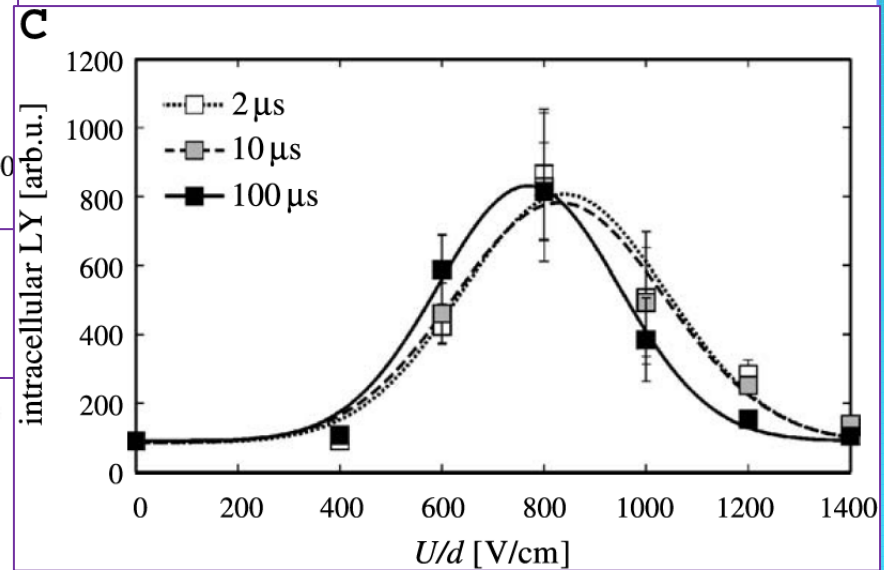
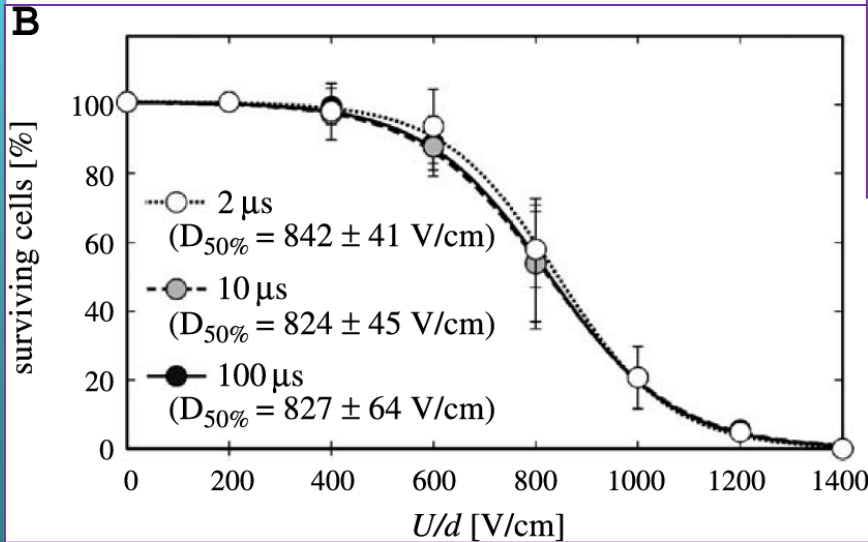


C

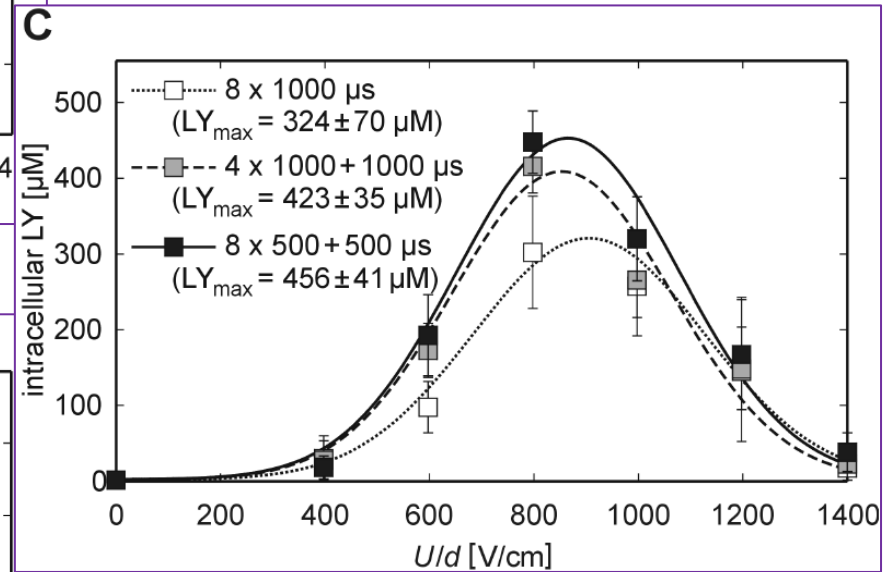
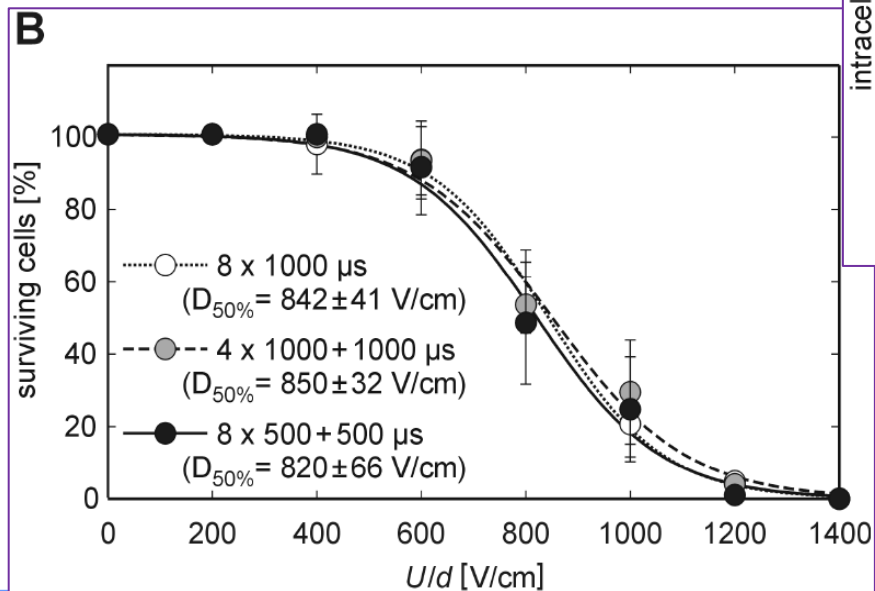
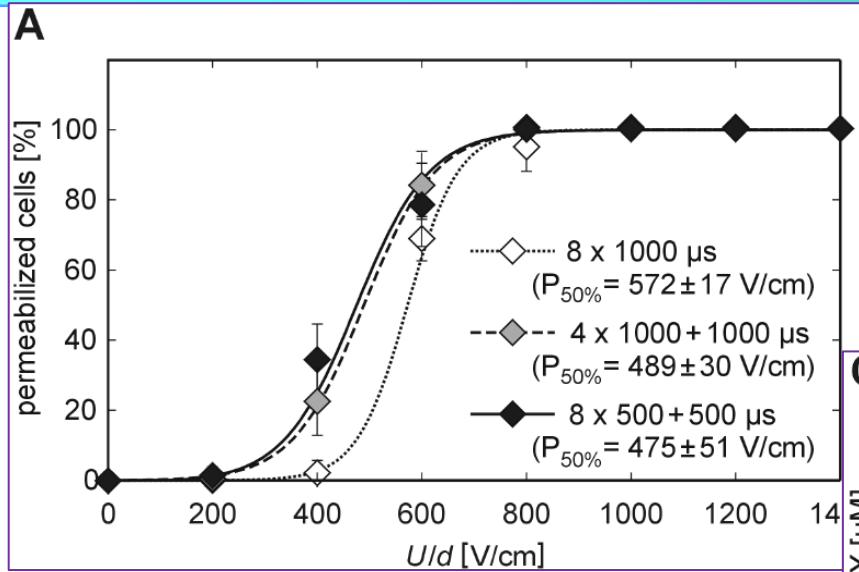




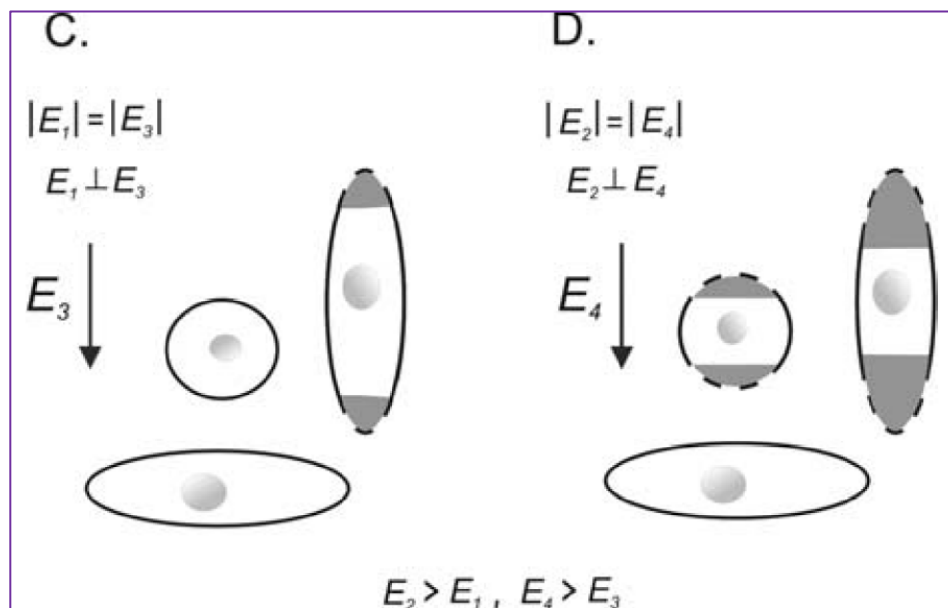
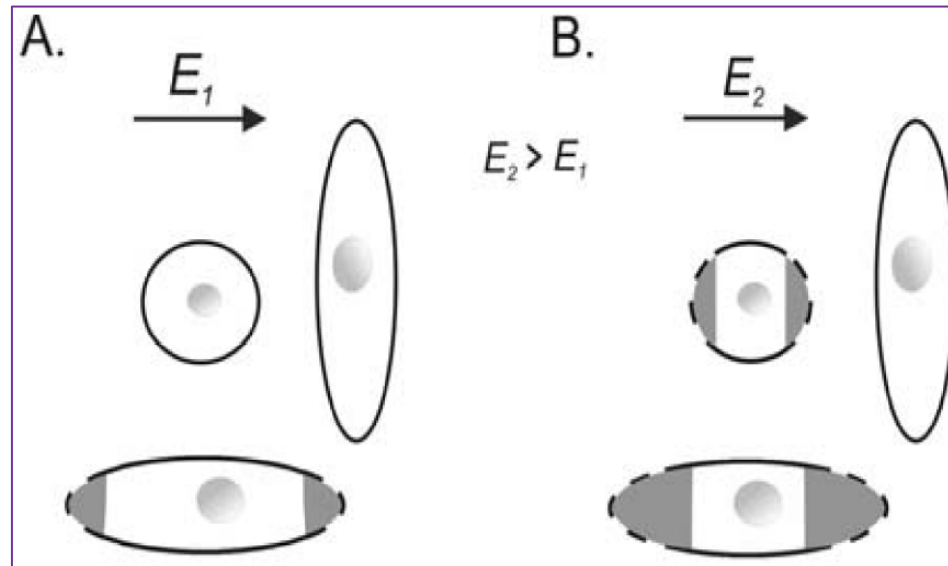
Pulse duration



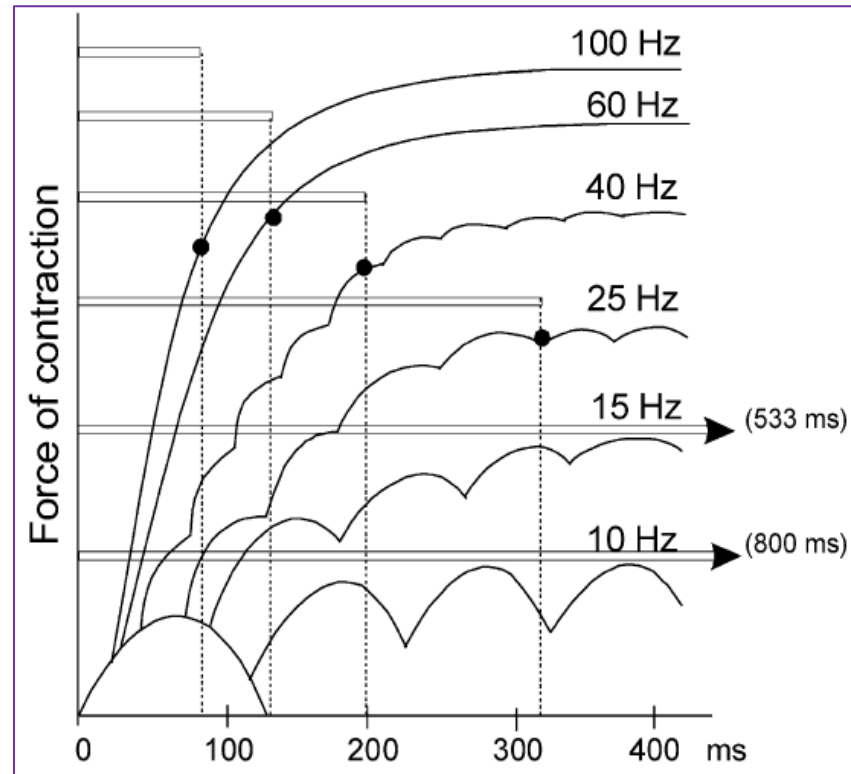
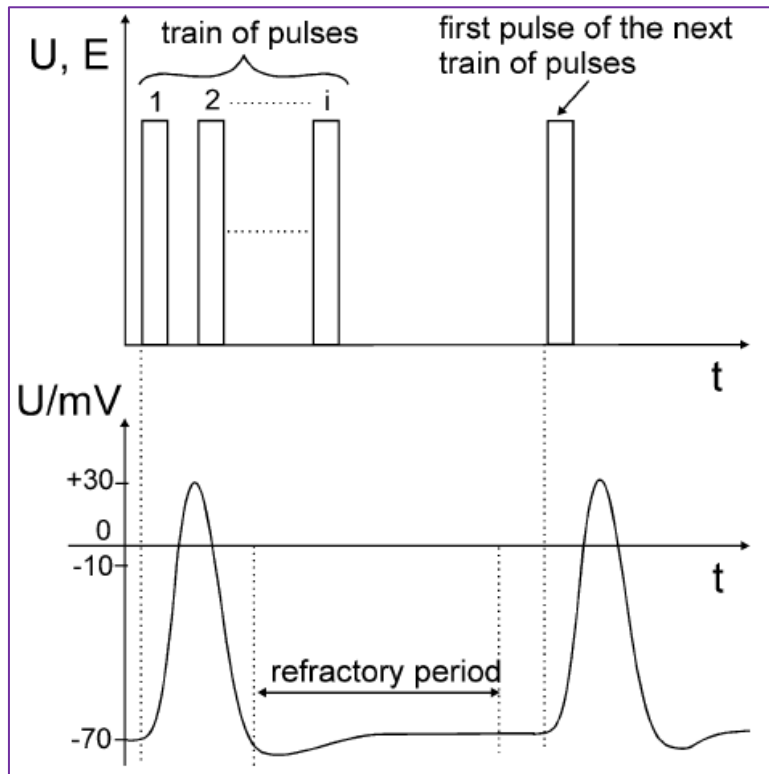
Pulse amplitude



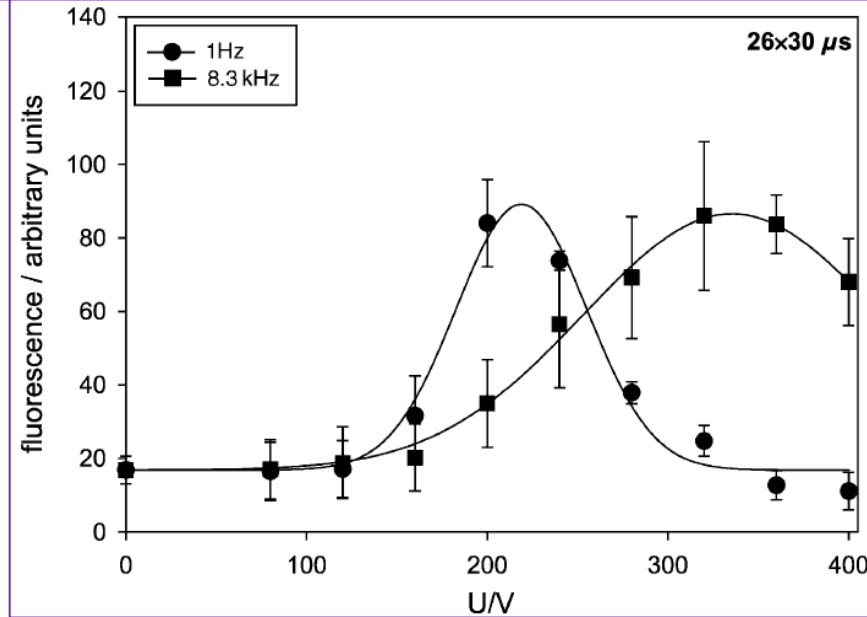
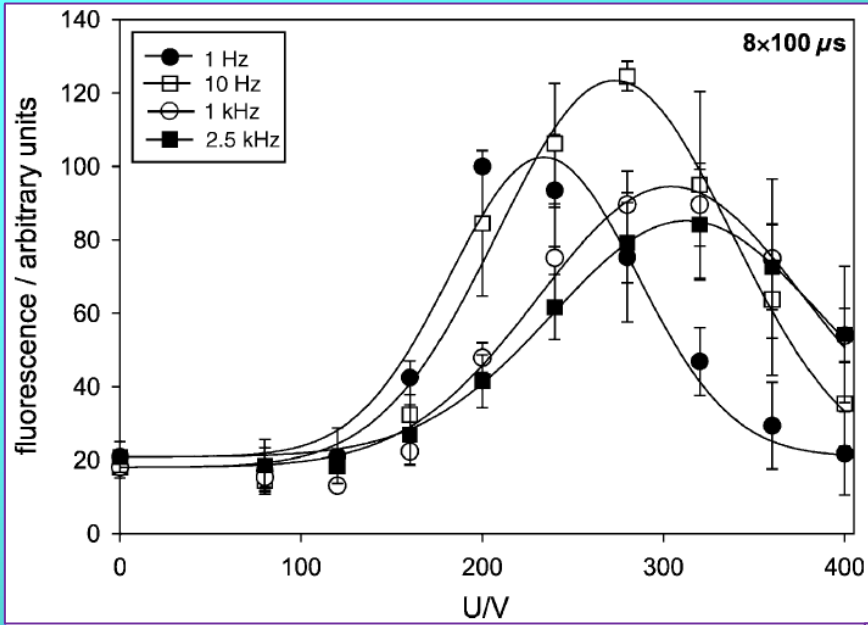
Cells shape & size



Pulse frequency 1



Pulse frequency 2



In vitro studies

| Tumor type | Tumor location | Animal | Bleomycin adm. |
|---|------------------------------------|---------------|-----------------------|
| Adenocarcinoma of the breast | Spontaneous | Mice | im |
| Ehrlich ascites tumor | Implanted sc | Mice | iv |
| Fibrosarcoma SA-1 | Implanted sc | Mice | iv |
| Hamster pancreatic adenocarcinoma | Implanted in the pancreas or sc | Hamsters | it |
| Hepatocellular carcinoma | Implanted in the tongue | Rats | sc |
| Hepatocellular carcinoma | Implanted sc | Rats | im |
| Human rhabdomyosarcoma | Implanted im | Nude rats | it |
| Human uterine leiomyosarcoma | Implanted sc | Mice | it |
| KB human oral epidermoid carcinoma cell line | Implanted sc | Mice | im |
| LPB sarcoma | Implanted sc | Mice | im or iv |
| Murine B16 melanoma | Implanted sc | Mice | im, iv or it |
| Murine bladder carcinoma | Implanted sc | Mice | ip or im |
| Murine colon adenocarcinoma | Implanted sc | Mice | it, iv or ip |
| Murine Lewis lung tumor | Implanted sc | Mice | iv |
| N32 glioma tumors | Implanted sc | Rats | iv |
| Rat adenocarcinoma of colon | Implanted in the liver | Rats | it |
| Rat glioma cell line RG2 | Implanted in the brain | Rats | iv |
| Rat hepatoma cells | Implanted in the liver | Rats | it |
| Rat squamous cell carcinoma | Implanted in the tongue | Rats | sc |
| Soft-tissue sarcomas | Spontaneous | Cats | iv |
| VX2 carcinoma cells | Implanted in the liver | Rabbits | iv |

Clinical studies: melanoma tumors

| Reference | # of patients | # of nodules | Response | | | | |
|------------------------------|---------------|--------------|----------|---------|---------|---------|----------|
| | | | PD (%) | NC (%) | PR (%) | CR (%) | OR (%) |
| <i>Bleomycin i.v.</i> | | | | | | | |
| Rudolf 1995 | 2 | 24 | 1 (4) | 1 (4) | 0 | 22 (92) | 22 (92) |
| Heller 1996 | 3 | 10 | 0 | 5 (50) | 2 (20) | 3 (30) | 5 (50) |
| Mir 1998 | 7 | 30 | 1 (3) | 2 (7) | 3 (10) | 24 (80) | 27 (90) |
| Rols 2000 | 4 | 55 | 0 | 4 (7) | 46 (84) | 5 (9) | 51 (93) |
| Sub-total | 16 | 119 | 2 (2) | 12 (10) | 51 (43) | 54 (45) | 105 (88) |

Bleomycin i.t.

| | | | | | | | |
|---------------|----|-----|--------|---------|---------|----------|----------|
| Glass 1996 | 5 | 23 | 0 | 1 (4) | 4 (17) | 18 (78) | 22 (96) |
| Heller 1998 | 12 | 84 | 0 | 1 (1) | 8 (10) | 75 (89) | 83 (99) |
| Gehl 2000 | 1 | 9 | 0 | 0 | 0 | 9 (100) | 9 (100) |
| Rodriguez2001 | 2 | 13 | 0 | 2 (15) | 8 (62) | 3 (23) | 11 (85) |
| Byrne 2005 | 21 | 52 | 5 (10) | 10 (19) | 4 (8) | 33 (63) | 37 (71) |
| Kubota 1998 | 1 | 8 | 0 | 0 | 0 | 8 (100) | 8 (100) |
| Sub-total | 42 | 189 | 5 (3) | 14 (7) | 24 (13) | 146 (77) | 170 (90) |

Cisplatin i.v.

| | | | | | | | |
|-------|---|----|--------|---------|---------|--------|---------|
| Sersa | 9 | 27 | 3 (11) | 11 (41) | 10 (37) | 3 (11) | 13 (48) |
|-------|---|----|--------|---------|---------|--------|---------|

Cisplatin i.t.

| | | | | | | | |
|--------------|-----------|------------|---------------|----------------|-----------------|-----------------|-----------------|
| Sersa 1998 | 2 | 13 | 0 | 0 | 0 | 13 (100) | 13 (100) |
| Sersa 2000 | 10 | 82 | 5 (6) | 6 (7) | 5 (6) | 66 (80) | 71 (87) |
| Sersa 2003 | 14 | 211 | 16 (8) | 24 (11) | 23 (11) | 148 (70) | 171 (81) |
| Snoj 2005 | 1 | 1 | 0 | 0 | 1 (100) | 0 | 1 (100) |
| Sub-total | 27 | 307 | 21 (7) | 30 (10) | 29 (9) | 227 (74) | 256 (83) |
| Total | 94 | 642 | 31 (5) | 67 (10) | 114 (18) | 430 (67) | 544 (85) |

Clinical studies: non-melanoma tumors

| Reference | Histology | # of patients | # of nodules | Response | | | | |
|------------------------------|--------------------------|---------------|--------------|----------|---------|---------|---------|----------|
| | | | | PD (%) | NC (%) | PR (%) | CR (%) | OR (%) |
| <i>Bleomycin i.v.</i> | | | | | | | | |
| Belehradek1993 | HN SCC | 8 | 37 | 0 | 8 (22) | 6 (16) | 23(62) | 29 (78) |
| Mir 1998 | HN SCC | 13 | 77 | 21 (27) | 8 (10) | 15 (19) | 33 (43) | 48 (62) |
| Glass 1996 | Basal cell carcinoma | 2 | 6 | 0 | 0 | 4 (67) | 2 (33) | 6 (100) |
| Heller 1996 | Breast adeno ca. | 1 | 2 | 0 | 0 | 0 | 2 (100) | 2 (100) |
| Domenge 1996 | Breast adeno ca. | 1 | 7 | 7 (100) | 0 | 0 | 0 | 0 |
| Domenge 1996 | Salivary gland adeno ca. | 1 | 20 | 0 | 0 | 0 | 2 (100) | 2 (100) |
| Sersa 2000 | Hypernephroma | 1 | 1 | 0 | 1 (100) | 0 | 0 | 0 |
| Sub-total | | 27 | 150 | 28 (19) | 17 (11) | 25 (17) | 80 (53) | 105 (70) |

Bleomycin i.t.

| | | | | | | | | |
|----------------|--------|----|----|---|--------|---------|---------|----------|
| Heller 1998 | SCC | 1 | 1 | 0 | 0 | 1 (100) | 0 | 1 (100) |
| Panje 1998 | HN SCC | 8 | 8 | 0 | 2 (25) | 2 (25) | 4 (50) | 6 (75) |
| Alegretti 2001 | HN SCC | 4 | 4 | 0 | 0 | 2 (50) | 2 (50) | 4 (75) |
| Rodriguez 2001 | HN SCC | 2 | 2 | 0 | 0 | 2 (100) | 0 | 2 (100) |
| Burian 2003 | HN SCC | 12 | 12 | 0 | 0 | 2 (17) | 10 (83) | 12 (100) |

Clinical studies: non-melanoma tumors

| Reference | Histology | # of patients | # of nodules | Response | | | | |
|------------------------------|--------------------------|---------------|--------------|----------|---------|---------|---------|----------|
| | | | | PD (%) | NC (%) | PR (%) | CR (%) | OR (%) |
| <i>Bleomycin i.v.</i> | | | | | | | | |
| Belehradek1993 | HN SCC | 8 | 37 | 0 | 8 (22) | 6 (16) | 23(62) | 29 (78) |
| Mir 1998 | HN SCC | 13 | 77 | 21 (27) | 8 (10) | 15 (19) | 33 (43) | 48 (62) |
| Glass 1996 | Basal cell carcinoma | 2 | 6 | 0 | 0 | 4 (67) | 2 (33) | 6 (100) |
| Heller 1996 | Breast adeno ca. | 1 | 2 | 0 | 0 | 0 | 2 (100) | 2 (100) |
| Domenge 1996 | Breast adeno ca. | 1 | 7 | 7 (100) | 0 | 0 | 0 | 0 |
| Domenge 1996 | Salivary gland adeno ca. | 1 | 20 | 0 | 0 | 0 | 2 (100) | 2 (100) |
| Sersa 2000 | Hypernephroma | 1 | 1 | 0 | 1 (100) | 0 | 0 | 0 |
| Sub-total | | 27 | 150 | 28 (19) | 17 (11) | 25 (17) | 80 (53) | 105 (70) |

Bleomycin i.t.

| | | | | | | | | |
|----------------|--------|----|----|---|--------|---------|---------|----------|
| Heller 1998 | SCC | 1 | 1 | 0 | 0 | 1 (100) | 0 | 1 (100) |
| Panje 1998 | HN SCC | 8 | 8 | 0 | 2 (25) | 2 (25) | 4 (50) | 6 (75) |
| Alegretti 2001 | HN SCC | 4 | 4 | 0 | 0 | 2 (50) | 2 (50) | 4 (75) |
| Rodriguez 2001 | HN SCC | 2 | 2 | 0 | 0 | 2 (100) | 0 | 2 (100) |
| Burian 2003 | HN SCC | 12 | 12 | 0 | 0 | 2 (17) | 10 (83) | 12 (100) |

Gehl & Geertsen Melanoma Research 2000



1st clinical trial, at IGR Berehradek et al. *Cancer* 1993

Before



During



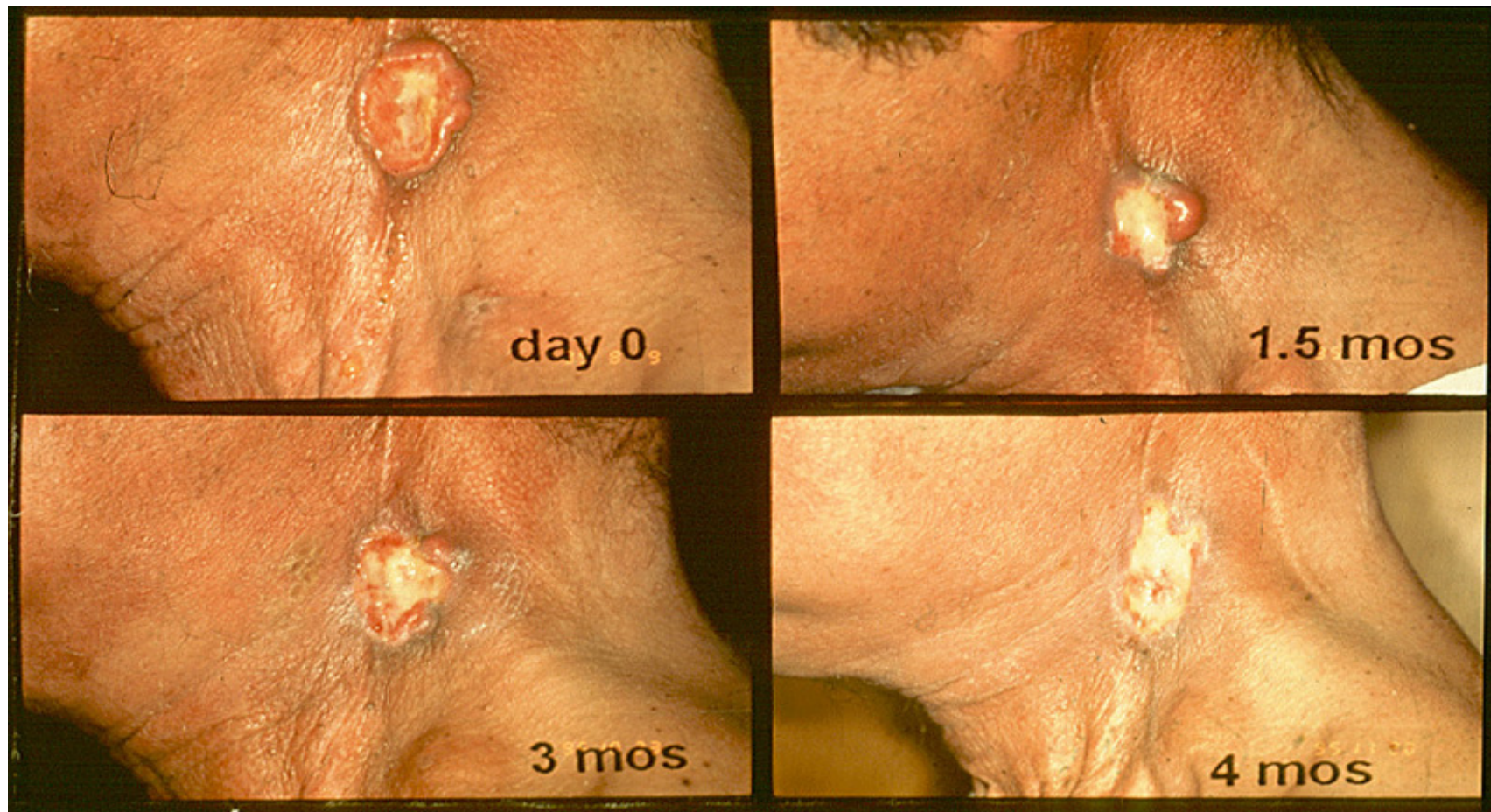
30 days later



3 days later



Skin metastasis of squamous cell carcinoma of the supraglottis



Skin metastasis of malignant melanoma



Before treatment



One year after ECT

SKIN METASTASIS OF BREAST CANCER

ECT with BLM i.v., hexagonal electrode,, single session– Cork Cancer Centre (IR)



Melanoma: metastasis

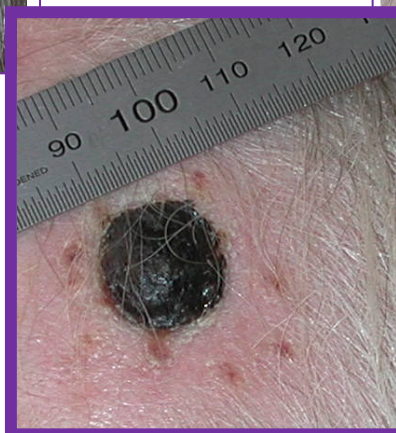
ECT with BLM i.v., hexagonal electrode, single session –
Herlev Hospital, Copenhagen (DK)



Before ECT



10 weeks



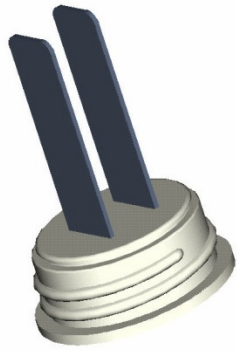
Kaposi's sarcoma

ECT with BLM iv, hexagonal electrode, multi-sessions – Institute Gustave-Roussy, Paris

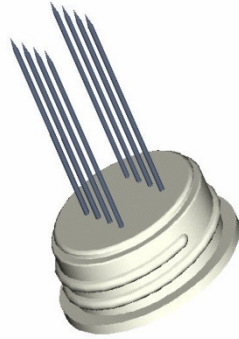
Before ECT



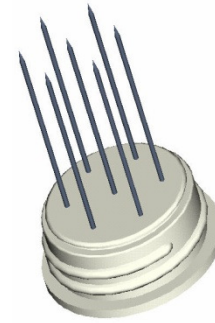
After l'ECT



PLATE



LINEAR NEEDLE

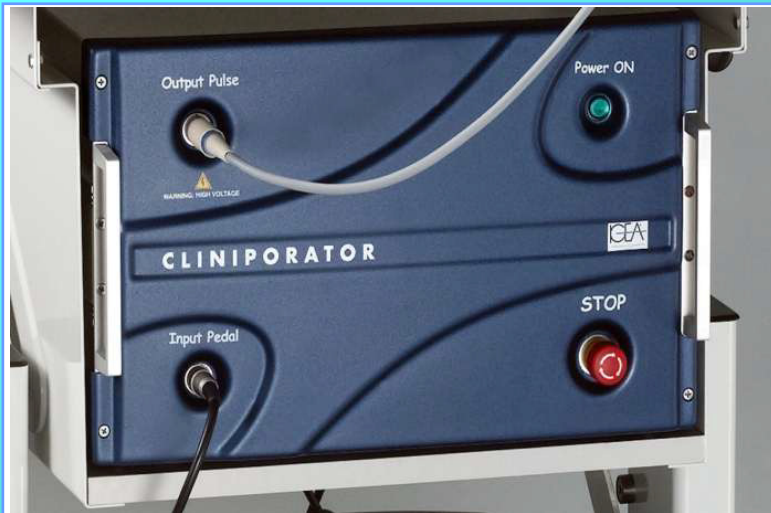


HEXAGONAL NEEDLE

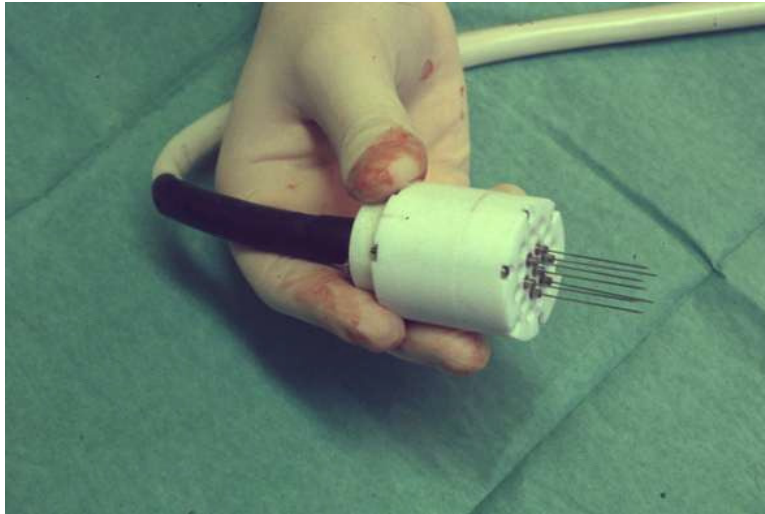


HANDLES



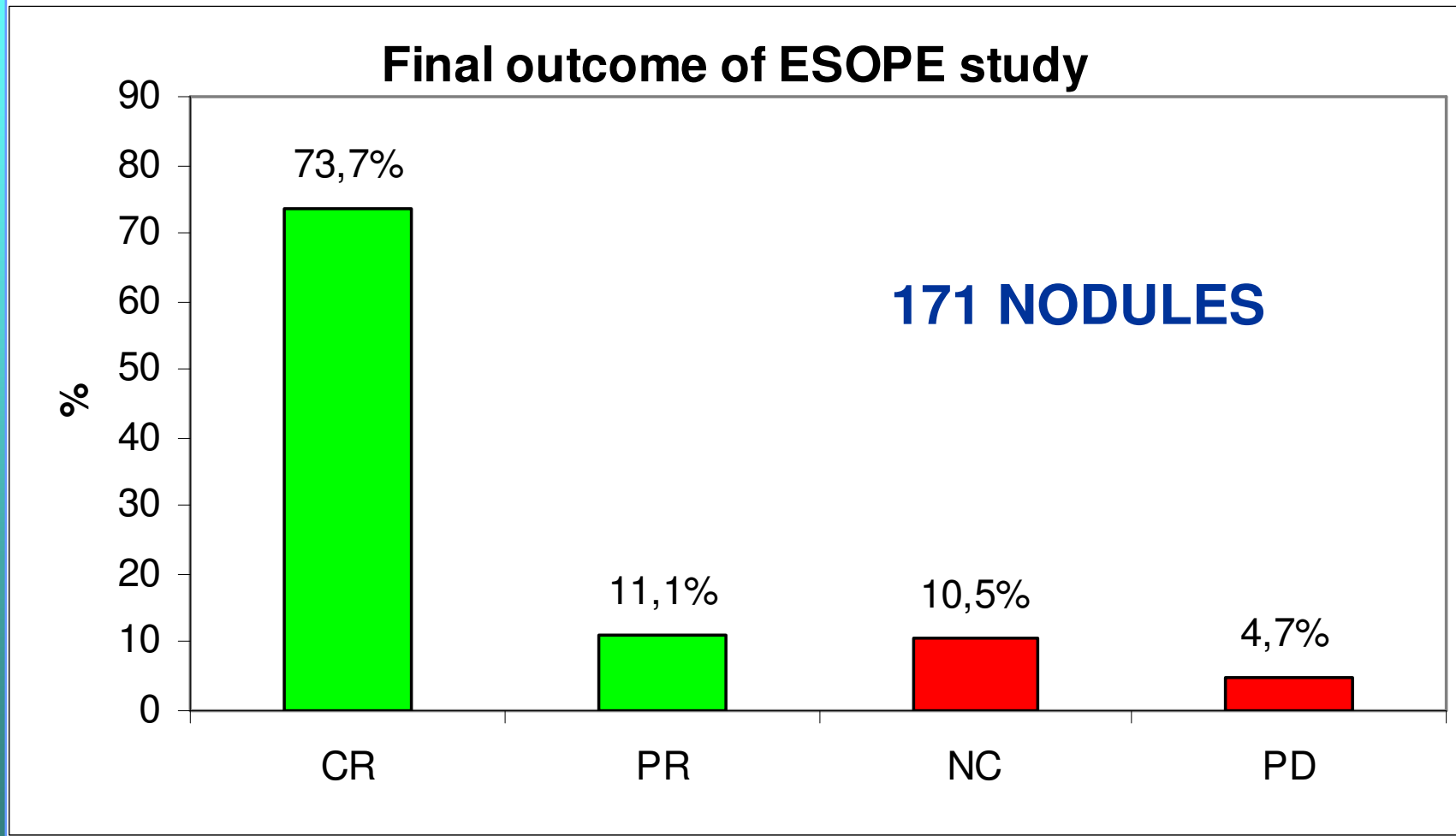


Using hexagonal centered array of needles to treat tumors transplanted in rabbit liver



Objective Response Rate: 85%

No Response: 15%



Disadvantage

Pain in muscles

Not for tumor in depth

For solid (85%) & small tumors

Advantages

Easy & effective for nodules in cutaneous & subcutaneous

Treatment of hemorrhagic or painful nodules

Lower hospitalization costs

Less side effects



**Thank you for
your attention**

