



Italian National Agency for New Technologies,
Energy and Sustainable Economic Development



Sumcastec

Ultra-short Pulsed Electric fields: A first study on Cancer Stem Cells

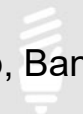
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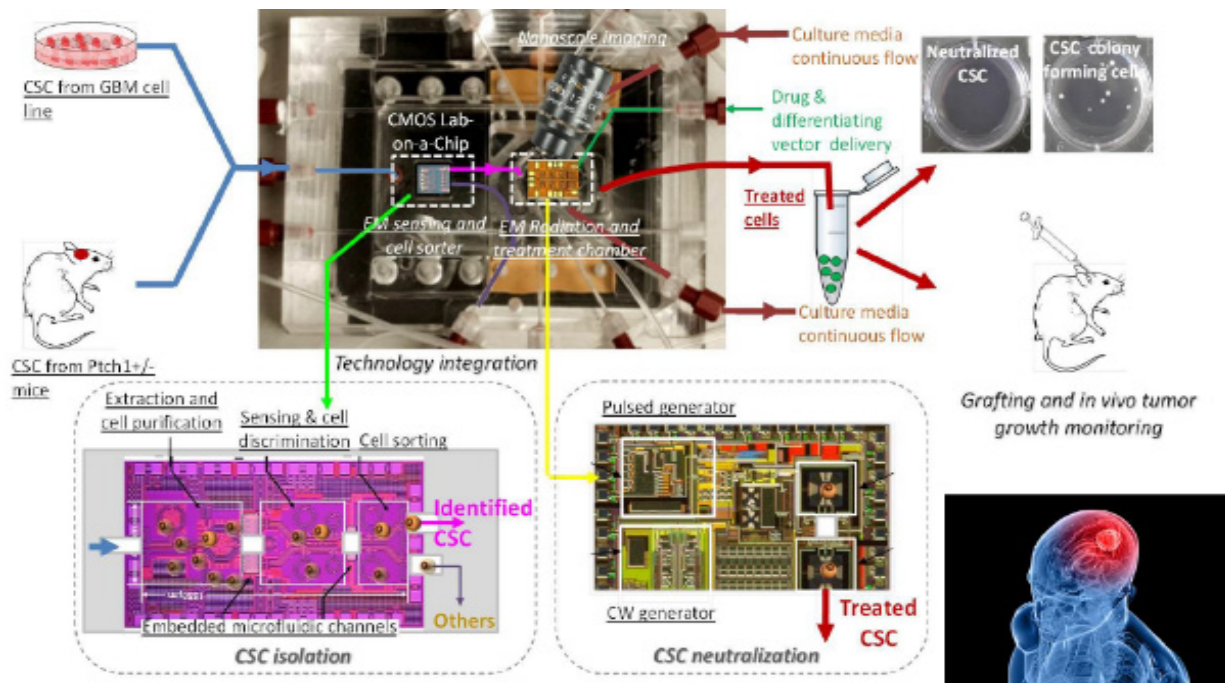


Workshop, Bangor University, UK, July 13, 2018

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SUMCASTEC

Semiconductor based Micromanipulation of Cancer Stem Cells



EM based on-chip:

- discrimination
- neutralization
- imaging of **CSCs**

Different EMF exposures under test: PEF, CW-MW



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European Commission

Horizon 2020
European Union funding
for Research & Innovation



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DI PADOVA



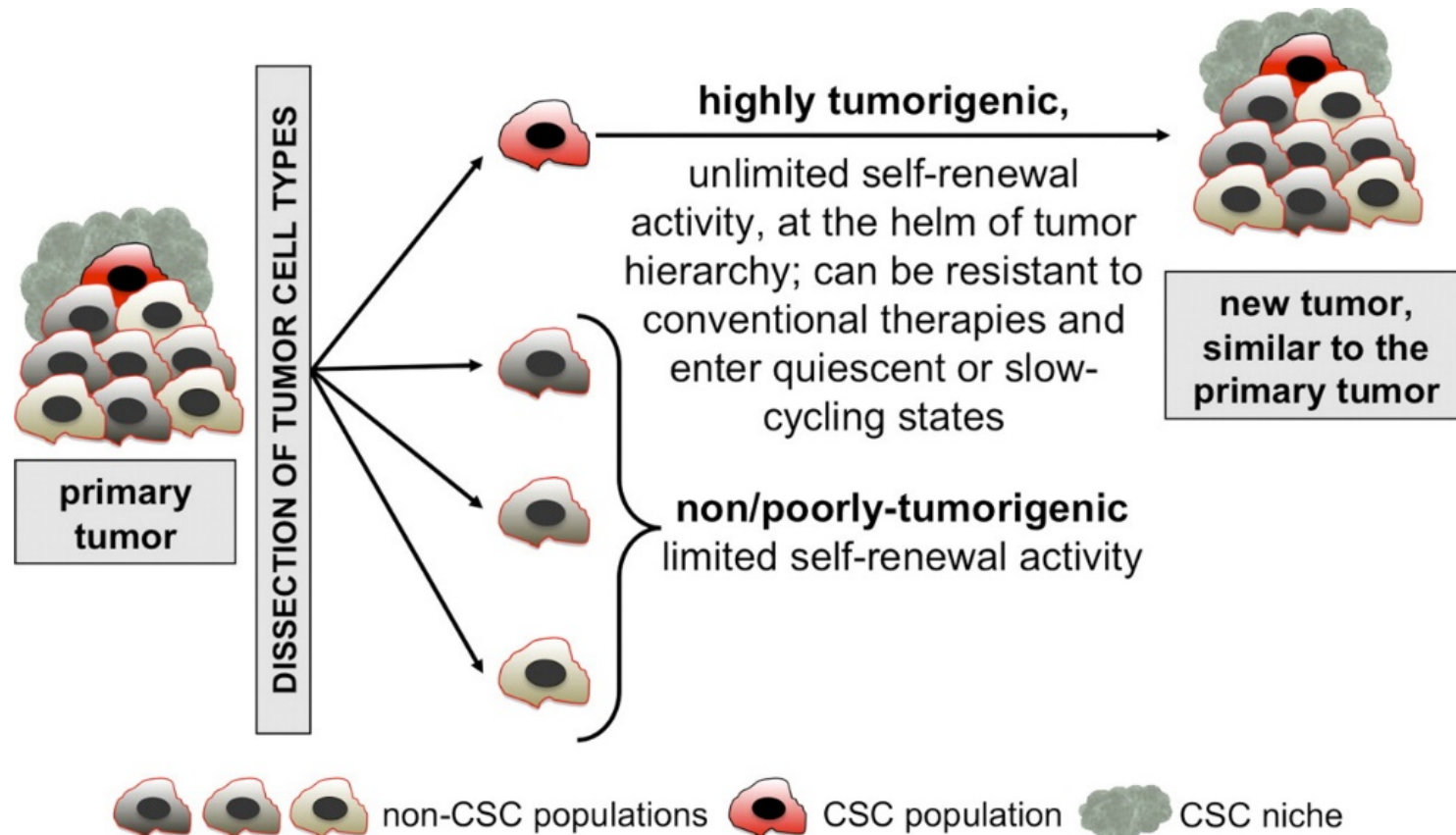
PRIFYSGOL
BANGOR
UNIVERSITY



Ingredients

- ❑ Cancer Stem Cells (CSCs)
- ❑ Ultra-Short Electric Pulses
 - nsPEF
 - μ sPEF
- ❑ Biological targets
 - CSC vs normal cell (astrocytes)
- ❑ Biological outcomes
 - Viability/proliferation (trypan blue exclusion test)
 - Cell cycle (flow cytometry)
 - Level of CD133 (flow cytometry)

Cancer Stem Cells (CSCs)

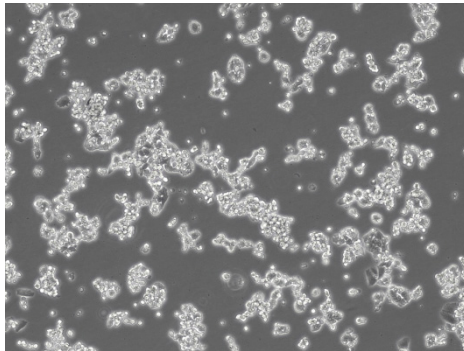


reference

Cancer Stem Cells (CSCs)

□ Medulloblastoma cell line: D283 (ATCC HTB-185)

D283

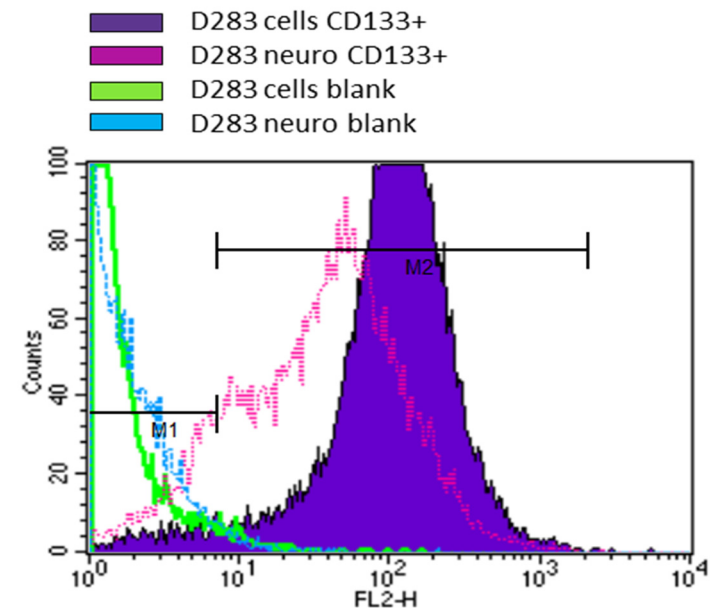


Medulloblastoma	
Age	6 years
Gender	male
Ethnicity	Caucasian
Tumorigenic	Yes
Derived from metastatic site: peritoneum	

□ Flow cytometric analysis to assess level of CD133 a primary marker for CSCs

□ Results confirmed by Stem Flow kit/CD133 staining

- **High levels** of proliferation and self renewal markers
- **Very low levels** of neuronal and astrocytes differentiation markers



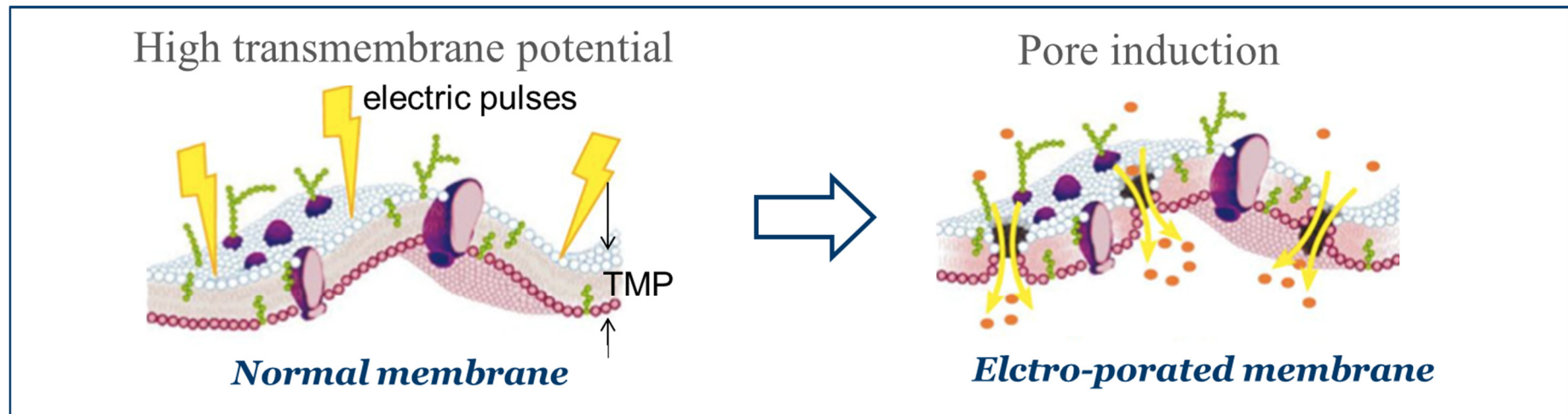
Ultra-short electric pulses: biological evidences

Last 30 years of pulsed E fields: Orłowski, Belehradek, Paoletti, Mir et al., 1988

⇒ ms and μ s time scale (trapezoidal, kV/m, used in ECT, EGT, IRE biotechnological applications)

⇒ ns time scale down to hundred of ps (trapezoidal/Gaussian MV/m, cancer ablation, neuronal and muscles stimulations, calcium control etc.,)

Davalos et al., 2005; Marty et al., 2006, Daud et al., 2008; Sack et al., 2010; Nuccitelli et al., 2009; Pakhomov et al., 2014; De Menorval et al., 2016; Merla et al., 2017; Sozer et al., 2017; Garcia-Sanchez et al., 2018.



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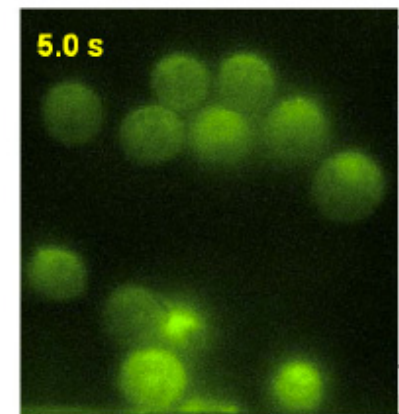
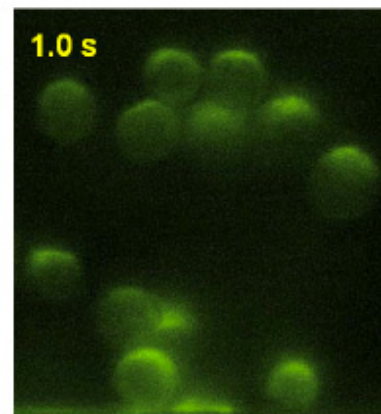
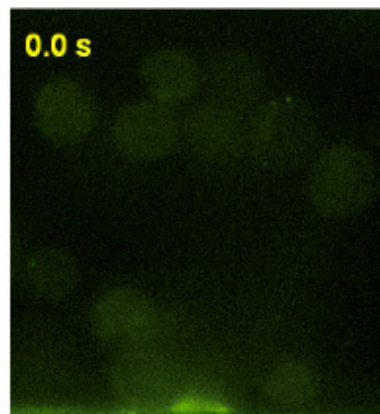
Davalos et. al., 2005; Marty et al., 2006, Daud et al., 2008; Sack et al., 2010; Nuccitelli et al., 2009; Pakhomov et al., 2014; De Menorval et al., 2016; Merla et al., 2017; Sozer et al., 2017; Garcia-Sanchez et al., 2018.

Experimental evidence

- Lipid oxidation
- Membrane channels (Ca²⁺ modulation)
- Membrane receptors (ROS)
- Gap-junctions
- Mechanical stress
- Cytoskeleton



E.B. Sozer, et al., Sci Rep. 2017.



(2006) 37.

Ultra-short electric pulses: applications

CLINICAL PRACTICE

μ s pulsed electric field (μ sPEF)

- *E field amplitude: tens of kV/m*
- *duration: few μ s up to few ms*

MEDICAL TREATMENTS

- *electrochemotherapy (cancer)*
– *thousand of treated patients in EU*
Gothelf et al., Cancer Treat. Rev., 2003
- *gene-therapy*
Heller et al., Cancer, 1996

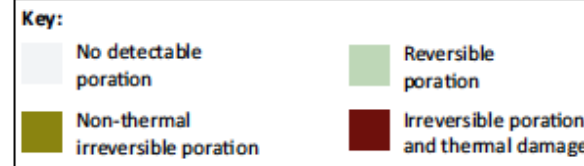
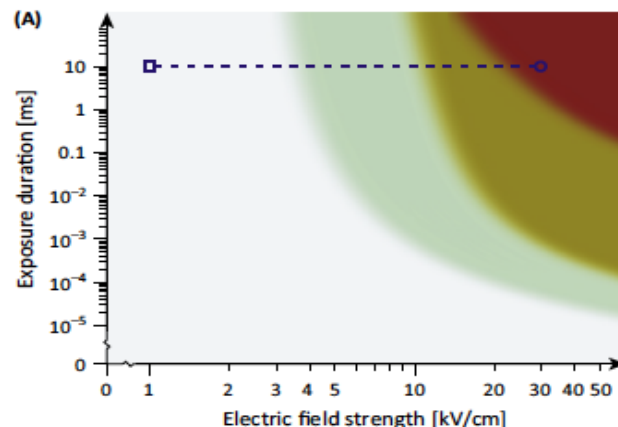
THE NEW FRONTIER

ns pulsed electric field (nsPEF)

- *E field amplitude: MV/m*
- *duration: 1 ns to about 100 ns*

MEDICAL TREATMENTS

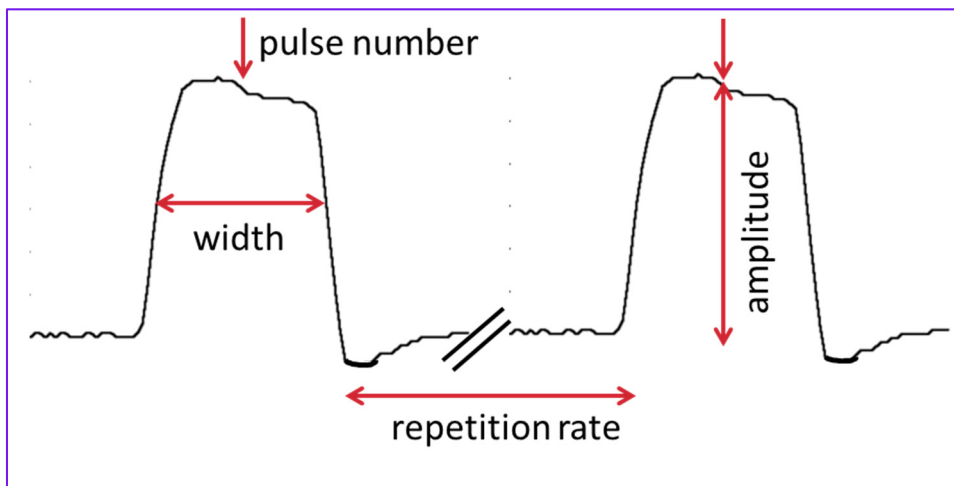
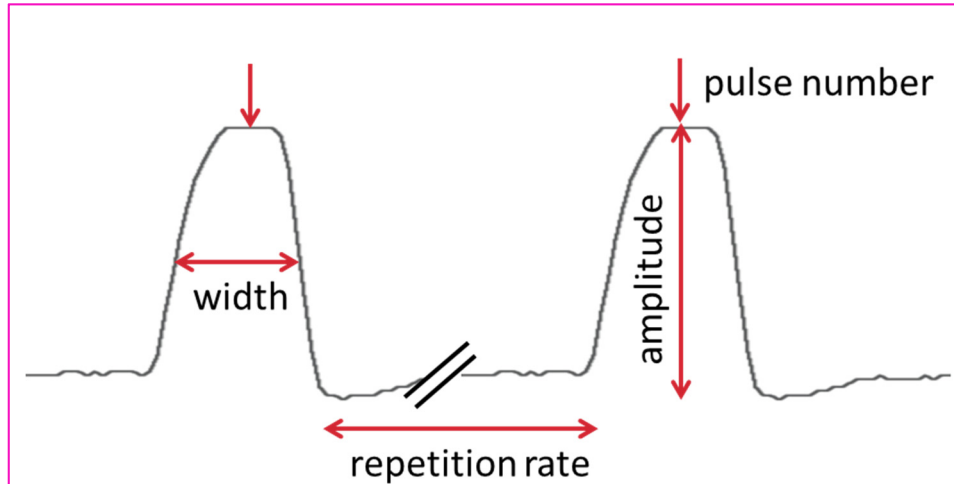
- *cancer therapy*
Nuccitelli et al., Int. J. Cancer, 2009
- *gene expression enhancement*
Schoenbach et al., Proc. IEEE, 2004
- *wound healing*
Zhang et al., ABB, 2008
- *Ca dependent cell functions control*
- *biosensing*
Kolb et al., Biomicorfl., 2012
- *neuromuscular stimulation*
Pakhomov et al., Arc. Bioch. Biophys., 2007
- *neurophysiology application*
Pakhomov et al., Arc. Bioch. Biophys., 2007
- *cardiac stimulation*
Wang et al., Biophys. J., 2009
Joshi et al., Phys.Rev. E, 2007



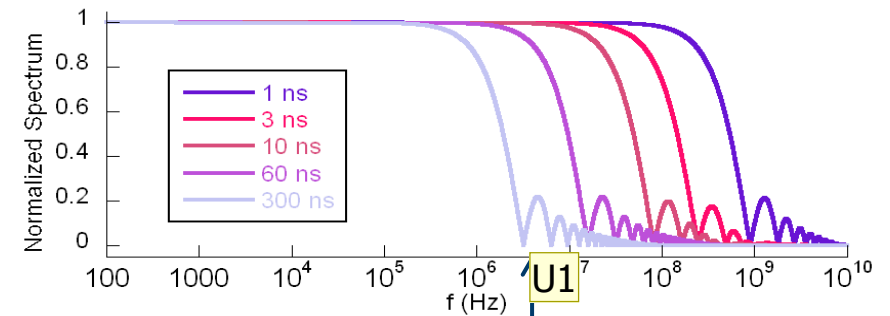
Kotnik et al., Trends in Biotechnology, 2015

Ultra-short electric pulses

Time domain



Frequency domain *n-seconds*



SPG1

width= 200 ns **PEF1**
amplitude= 1.1 MV/m
repetition rate= 1 Hz
Pulse number= 8

width= 300 ns **PEF2**
amplitude= 1.1 MV/m
repetition rate= 1 Hz
Pulse number= 8

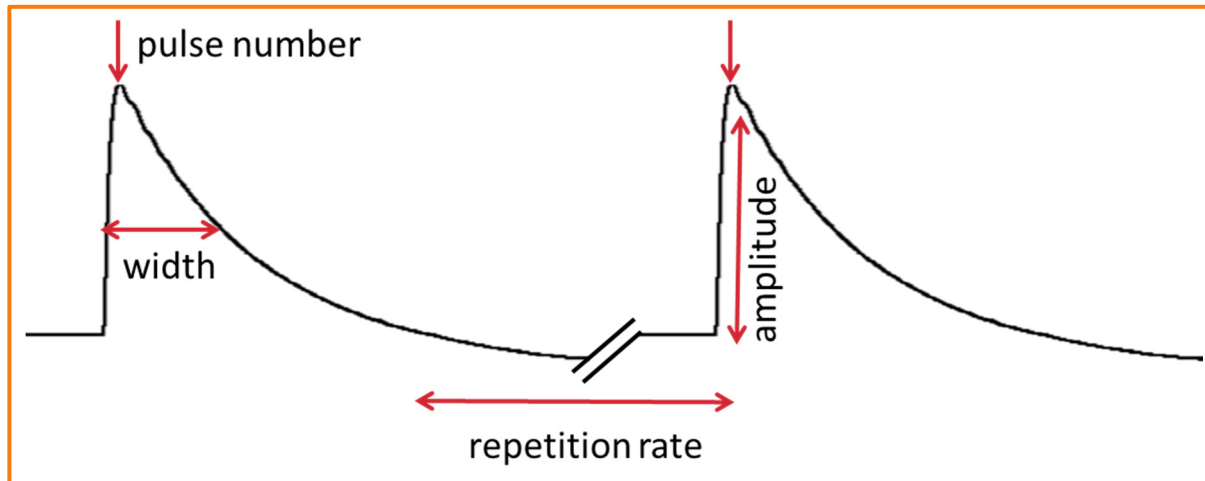
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U1

UMR8203; 08/07/2018

Ultra-short electric pulses

Time domain

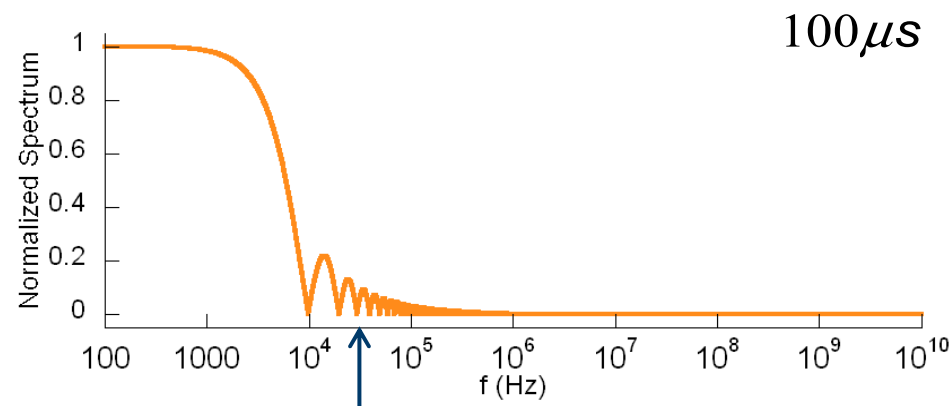


width= 40 μ s *PEF3, 4*
amplitude= 0.35 MV/m
repetition rate= 1 Hz
Pulse number= 2; 5

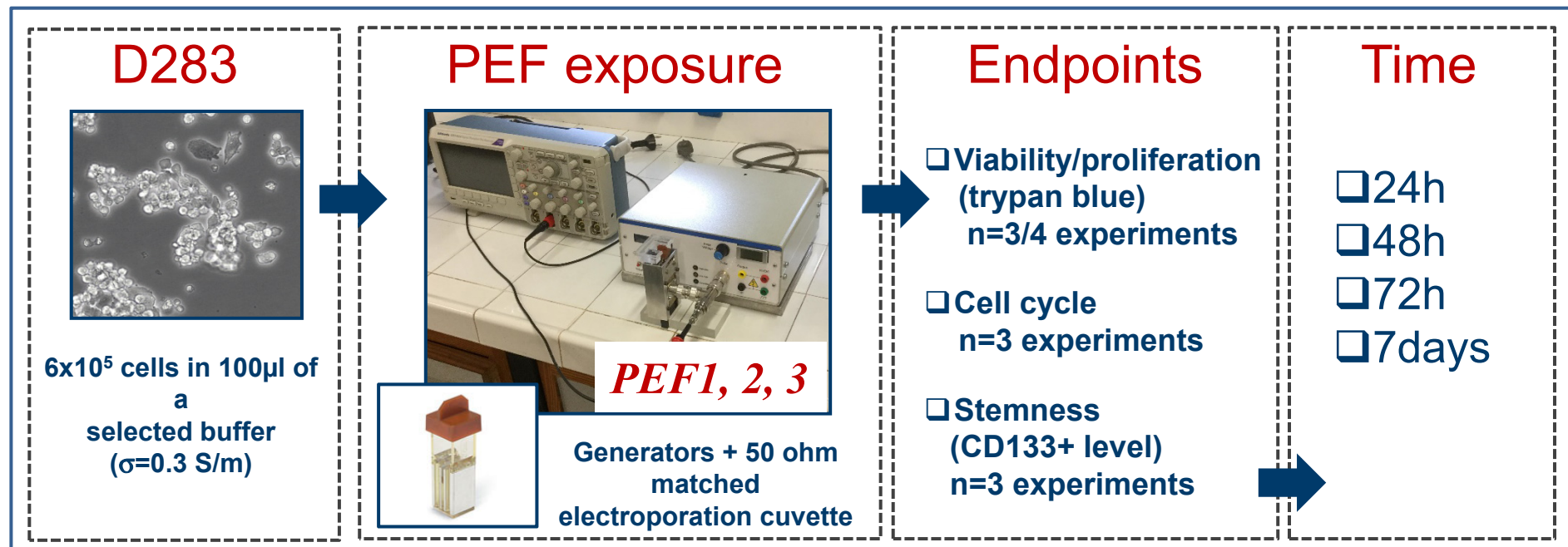
Shafner



Frequency domain

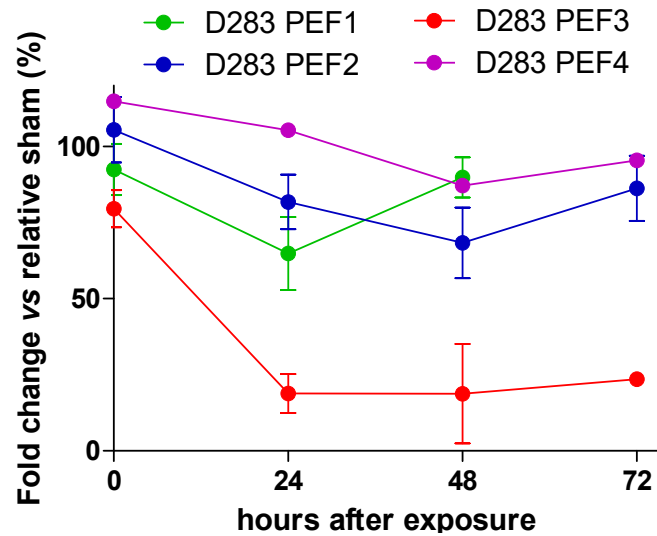


Experimental Protocols

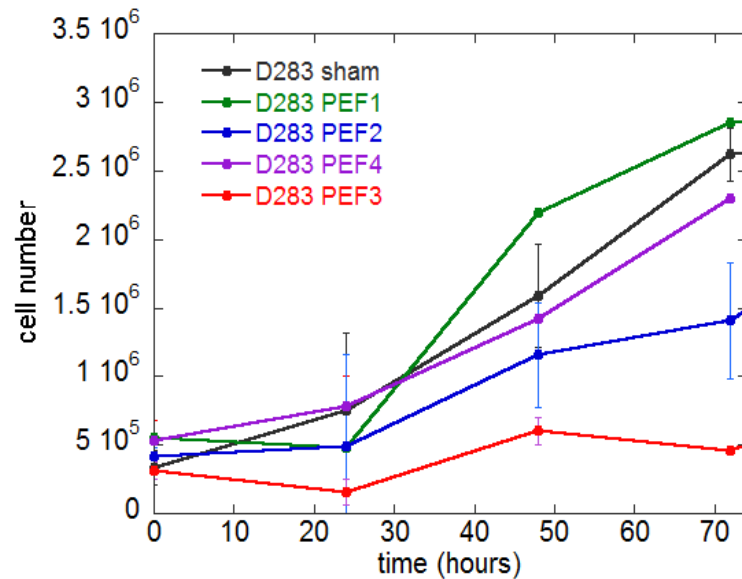


D283 viability and proliferation

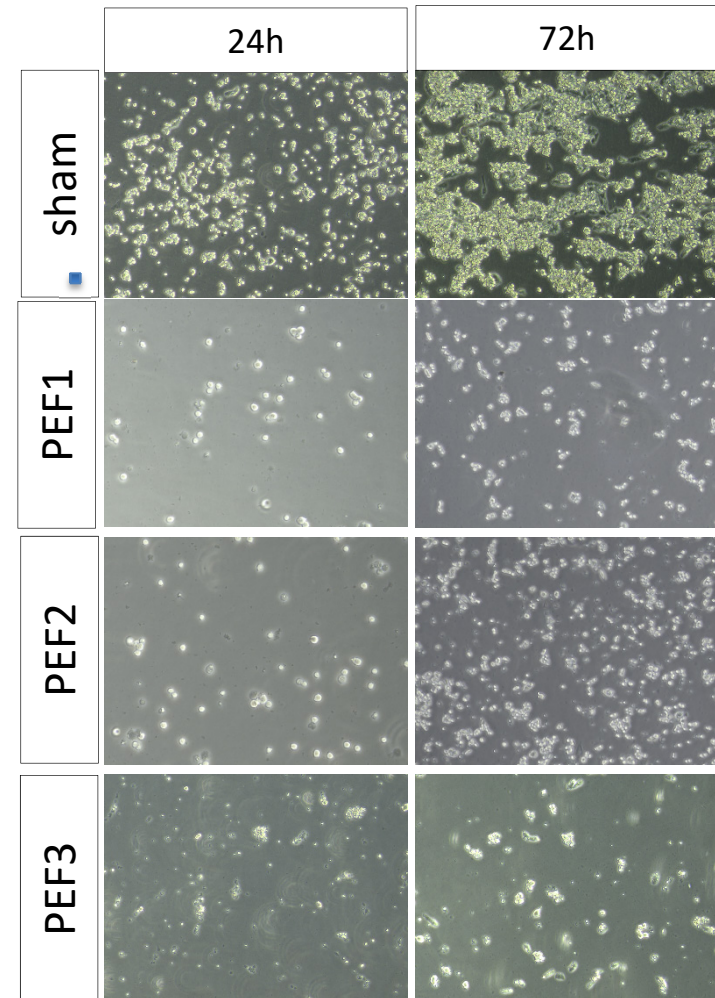
Trypan blue assay



viability

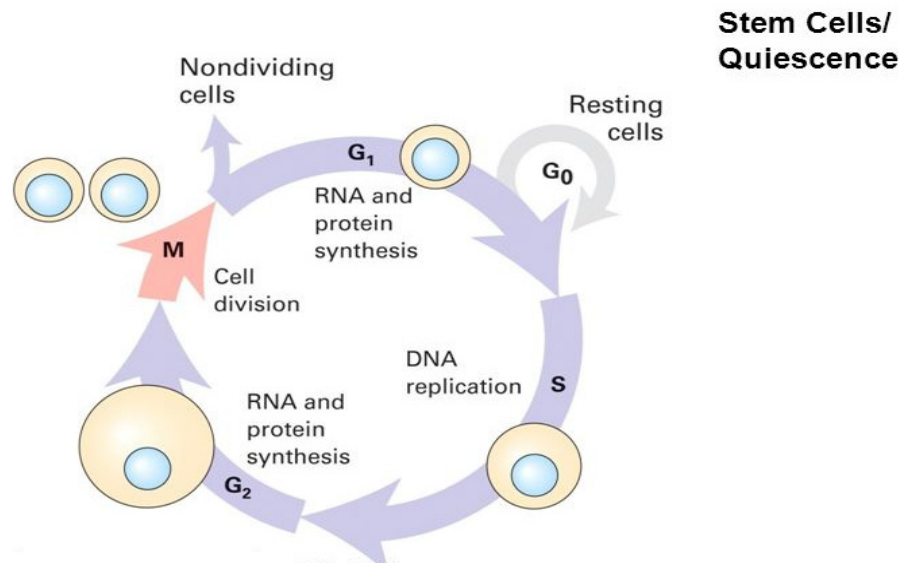


proliferation



Cell cycle

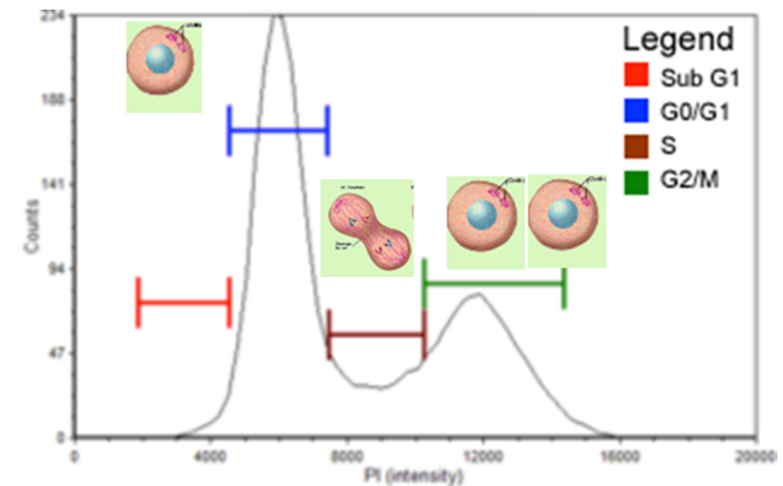
The cell cycle



- G₀ ⇒ resting cells (quiescent)
- G₁ ⇒ preparation to division
- S ⇒ DNA replication
- G₂/M ⇒ preparation/cell division

The quiescence state is the survival strategy of CSCs responsible for tumor recurrences and relapses

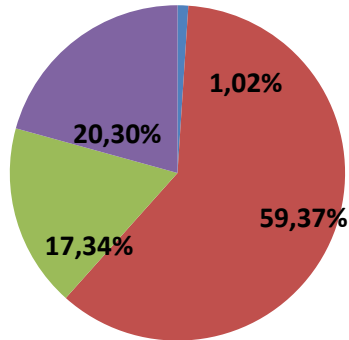
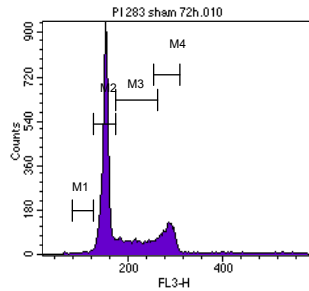
Cell cycle evaluation by flow cytometry



Propidium iodide (PI) binds to double stranded DNA by intercalating between base pairs.

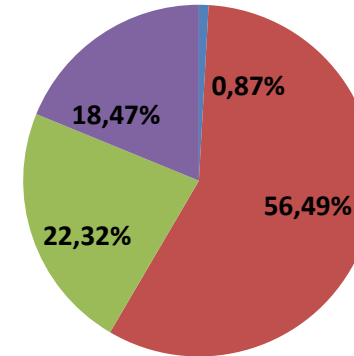
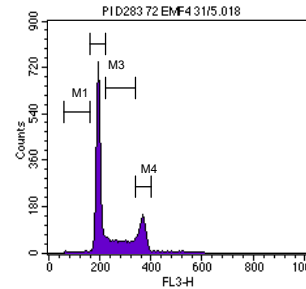
Cell cycle:72h

SHAM



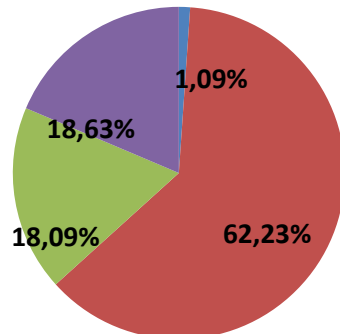
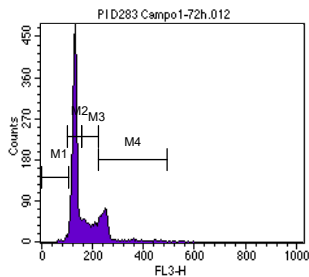
- SG_1 (M1)
- G0/G1 (M2)
- S (M3)
- G2/M (M4)

PEF4



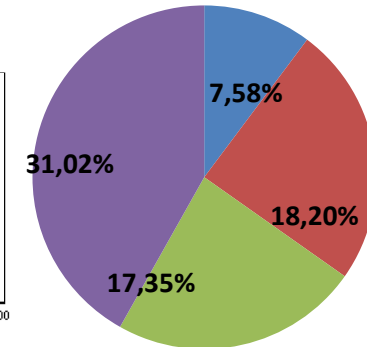
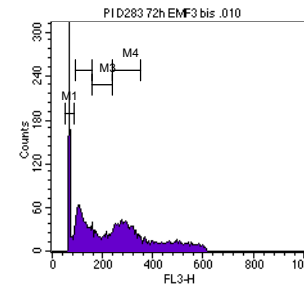
- SG_1 (M1)
- G0/G1 (M2)
- S (M3)
- G2/M (M4)

PEF1



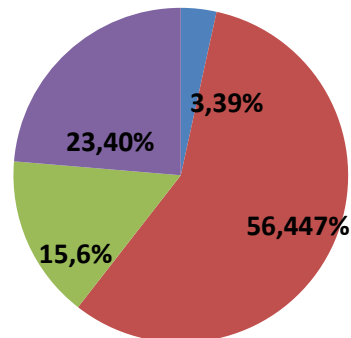
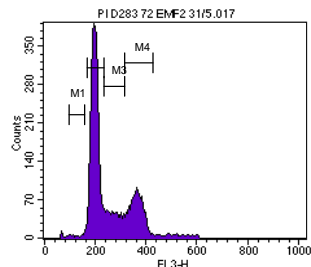
- SG_1 (M1)
- G0/G1 (M2)
- S (M3)
- G2/M (M4)

PEF3



- SG_1 (M1)
- G0/G1 (M2)
- S (M3)
- G2/M (M4)

PEF2



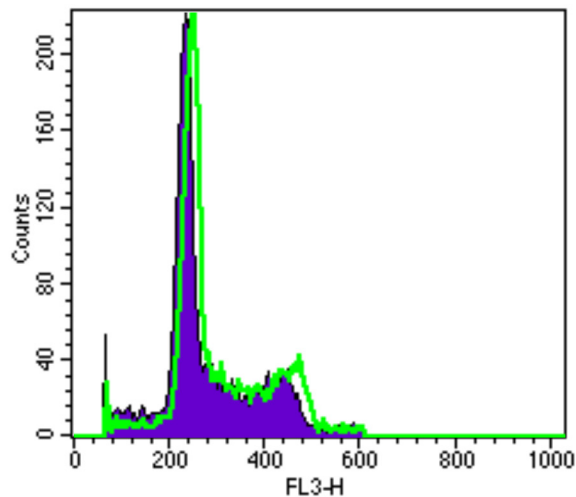
- SG_1 (M1)
- G0/G1 (M2)
- S (M3)
- G2/M (M4)

Similar trends for D283 cell cycles under PEF exposures at 24 and 48 h

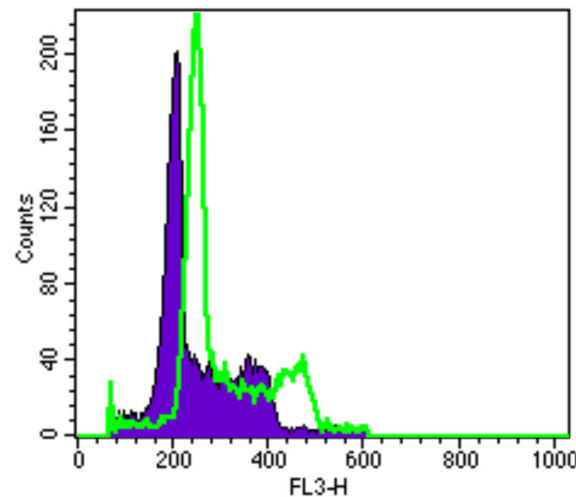
Cell cycle

PI *after 7 days* from exposure to different E pulses regimes (μs , ns)

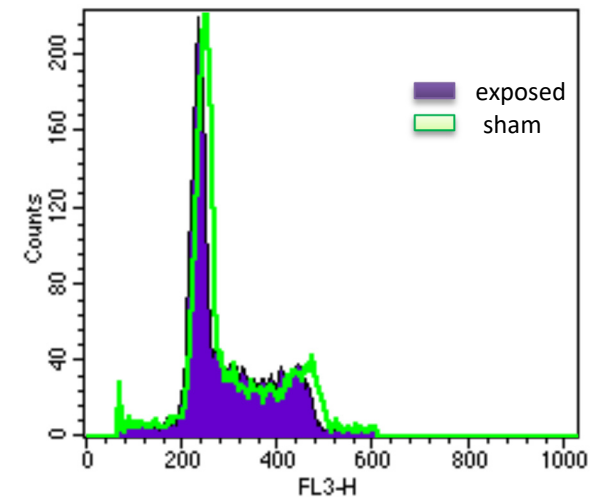
SHAM vs PEF1



SHAM vs PEF2



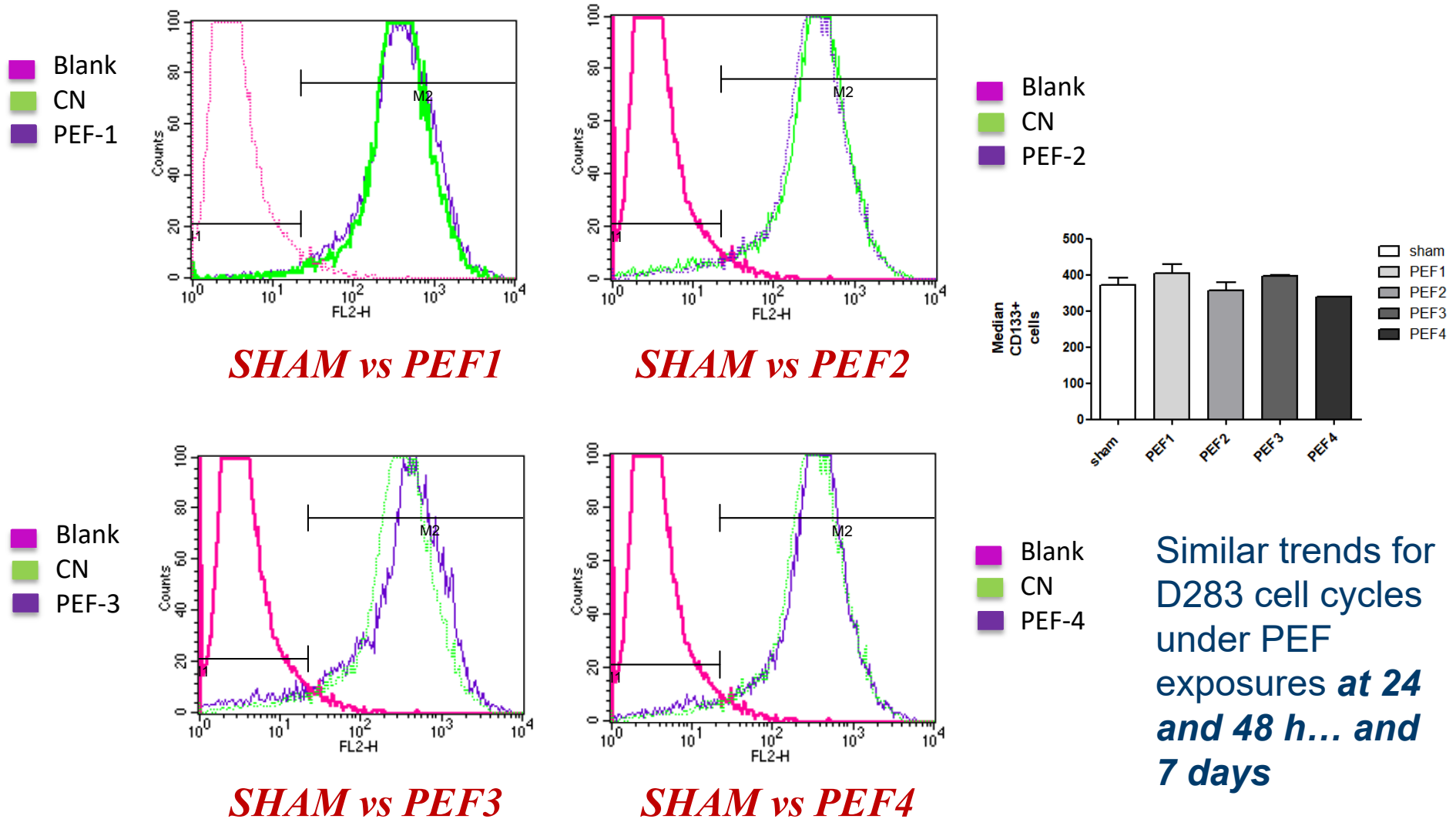
SHAM vs PEF3



Cell cycle is *only reversibly effected* by PEFs exposure

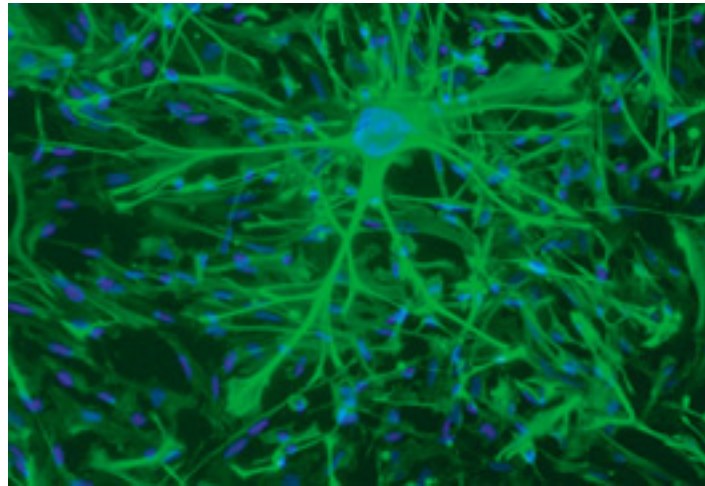
Stemness marker: CD133

Flow cytometry assay: CD133 72h after PEFs

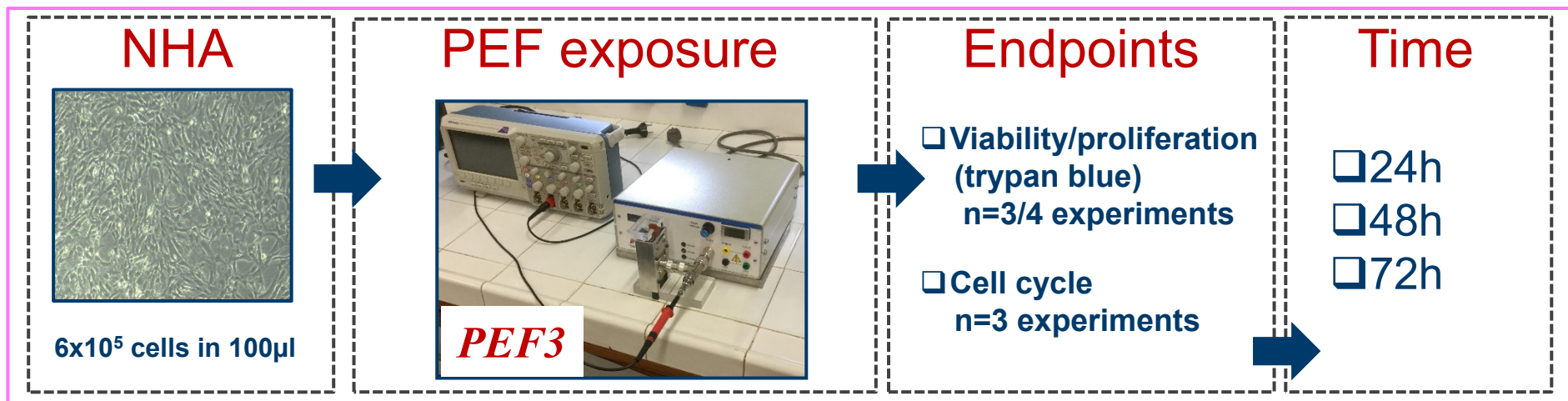


CSCs vs astrocytes

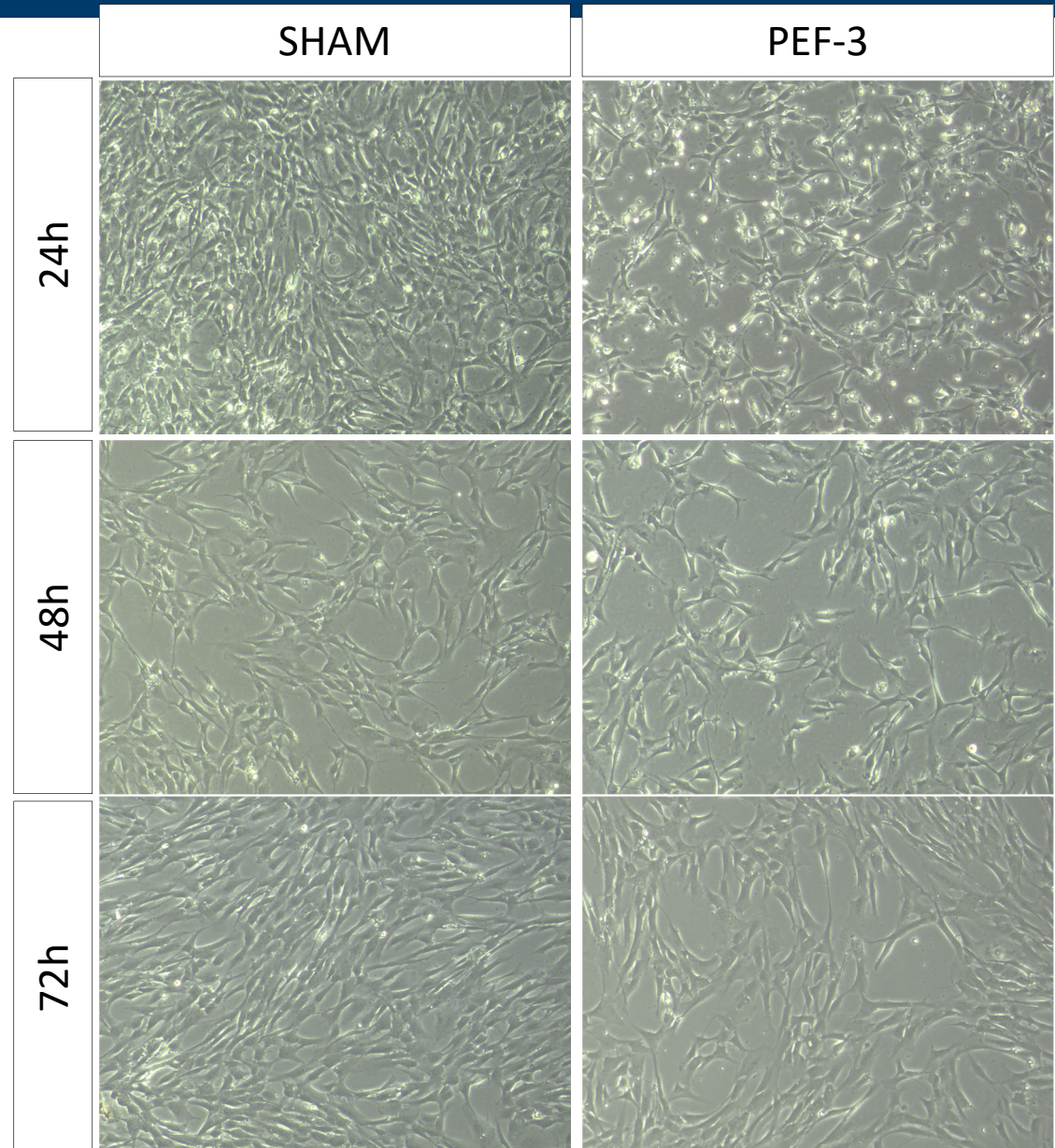
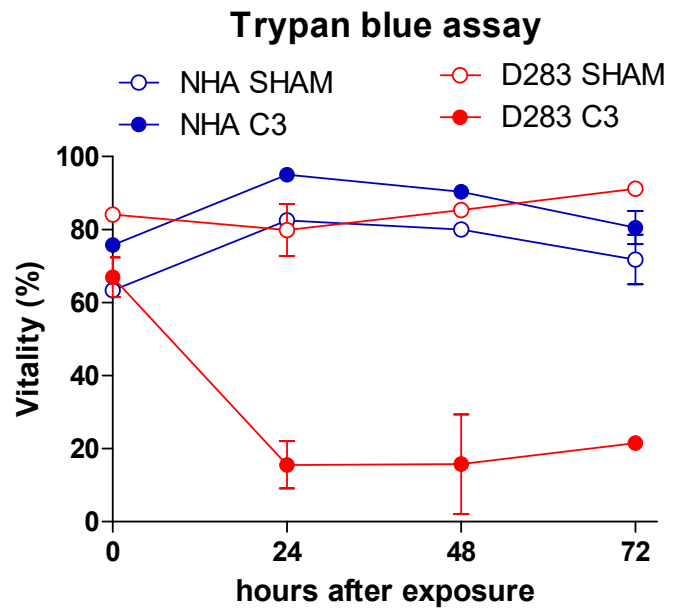
Normal Human Astrocytes (LONZA CC-3186)



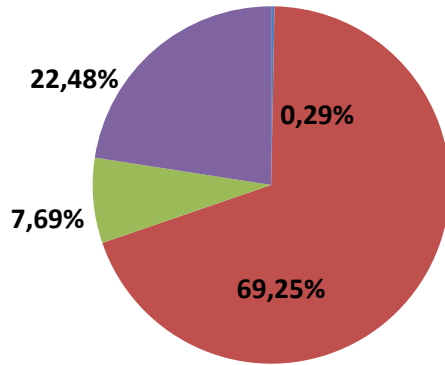
Human astrocytes stained for GFAP (green) and DAPI (blue)



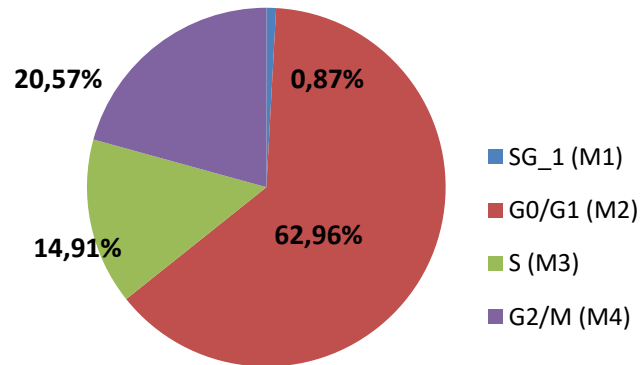
Viability



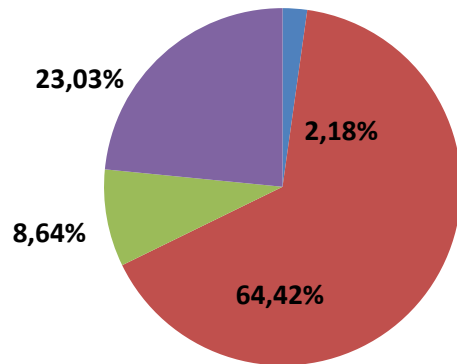
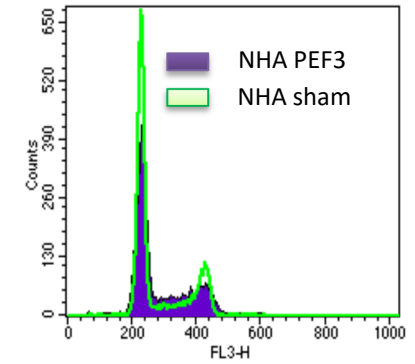
Cell Cycle



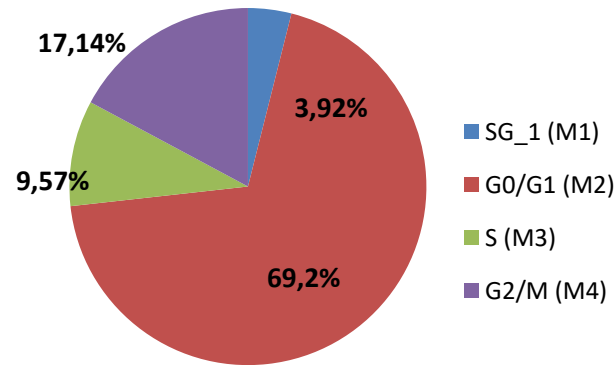
SHAM 24H



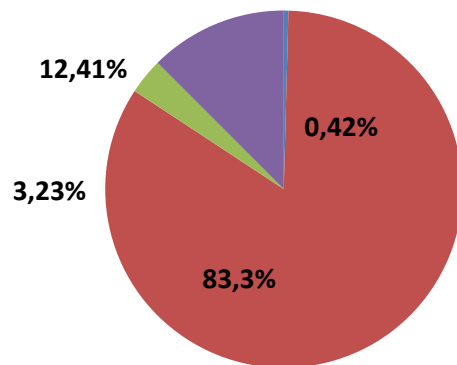
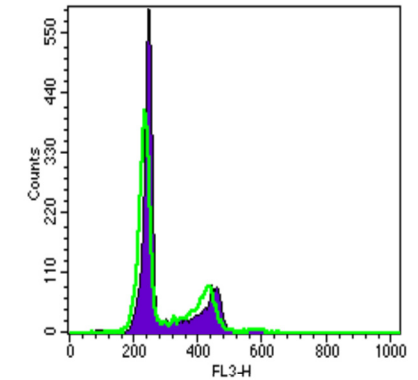
PEF3 24H



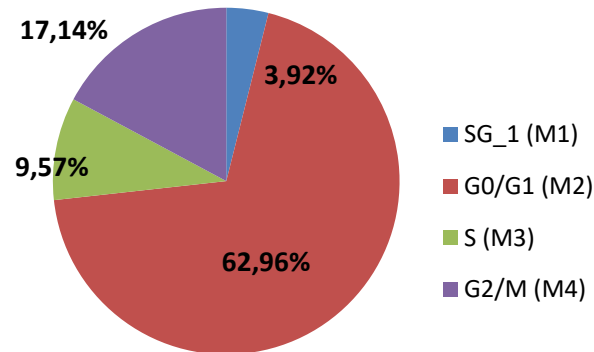
SHAM 48H



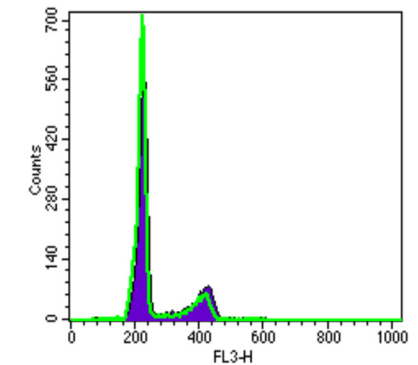
PEF3 48H



SHAM 72H

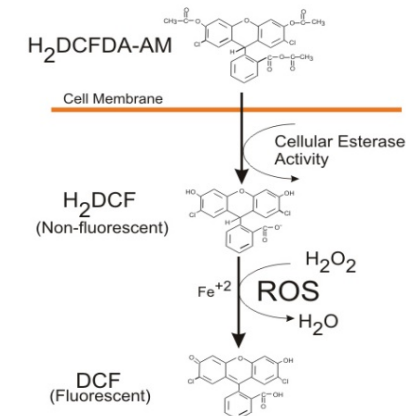
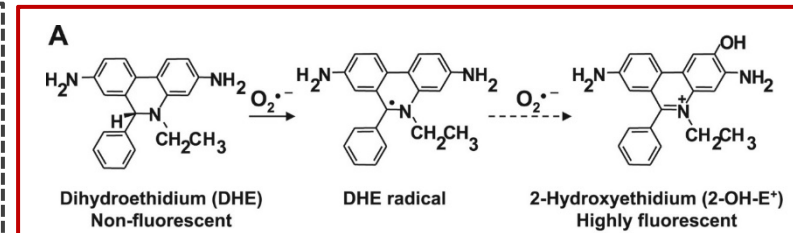
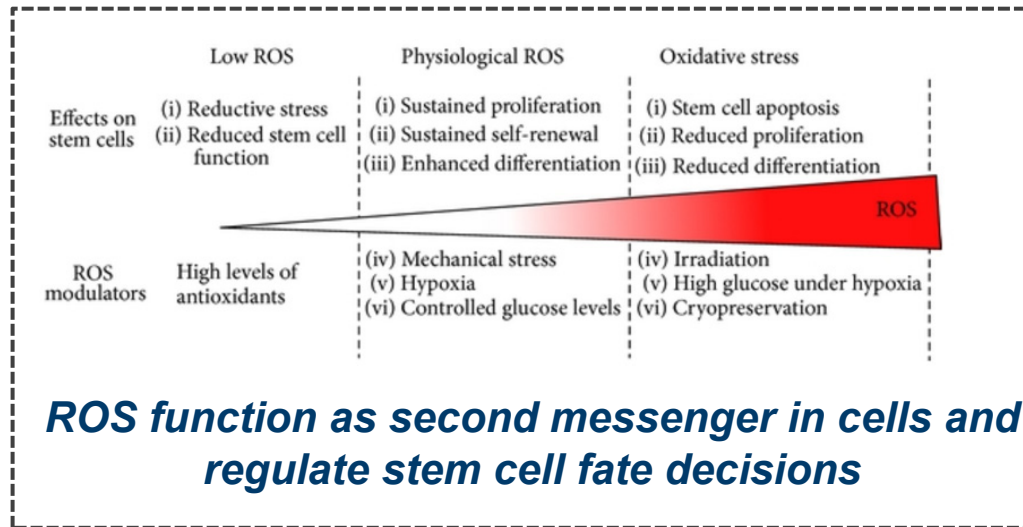


PEF3 72H



Work in progress

ROS evaluation (flow cytometry) under ultrashort pulses exposure



Assessment of cell electroporation (YOPRO-1 uptake by flow cytometry)

Assessment of an « equivalent » electric energy able to induce comparable results between μ sPEF and nsPEF

Combine RX-PEF3 exposure on MB and NHA cells (24 after PEF use X-rays 8 Gy or less) in order to avoid cell recovery

Conclusions

- ❑ PEF3 exposure affects D283 viability and proliferation but not the NHA one
- ❑ PEF3 exposure affects D283 cell cycle but not the NHA one
- ❑ The effects on cycle are reversible
- ❑ Exposure to different PEFs exposure protocols does not change stemness status in D283 cells
- ❑ The best strategy to target CSCs seems to induce their exit from quiescence by the PEFs exposure
- ❑ ***Mechanisms for the observed selective action have to be deeply investigated***