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Journal of Molecular and Genetic Medicine

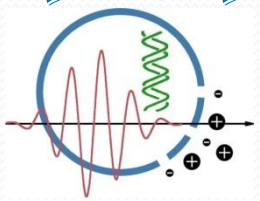
NANOSECOND PULSED ELECTRIC FIELD (NSPEF)-INDUCED MECHANISMS THAT BYPASS CANCER MUTATIONS AND CAUSE CELL DEATH IN CELLS AND TUMORS

STEPHEN J. BEEBE, PHD

FRANK REIDY RESEARCH CENTER FOR BIOELECTRICS

OLD DOMINION UNIVERSITY

NORFOLK VA



OLD DOMINION UNIVERSITY

IDEA FUSION

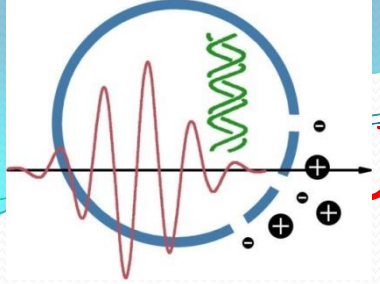
Some Introduction and Concepts

nsPEF Waveforms, Calcium, Mitochondria (ΔΨm) and Effects on Proteins

nsPEFs Conquer Evasion of Apoptosis

Some Perspective about Cancer

nsPEFs Abolish Rat HCC and Disable Evasion of Apoptosis and Immune Surveillance



Using Pulse Power Technology

Cell Manipulations by Pulsed Electric Fields Using Different Pulse Durations

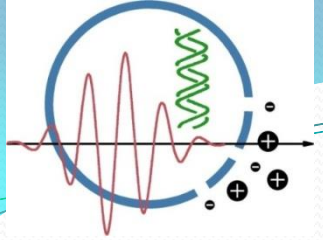
This includes:

Conventional Plasma Membrane Electroporation

Milli- second, Micro- second pulses

Sub-MicroSecond Pulsed Electric Fields

Nanosecond and Pico-second



Pulse Power w/ nsPEFs - Concept 1

Electric Power -stored and released instantaneously into cells and tissues

This produces High Power, low energy, non-thermal conditions

If 1 joule of energy is released all at once in :

1 second = 1 watt

1 microsecond = 1 megawatt

1 nanosecond = 1 gigawatt

100 nanosecond = 10 megawatts

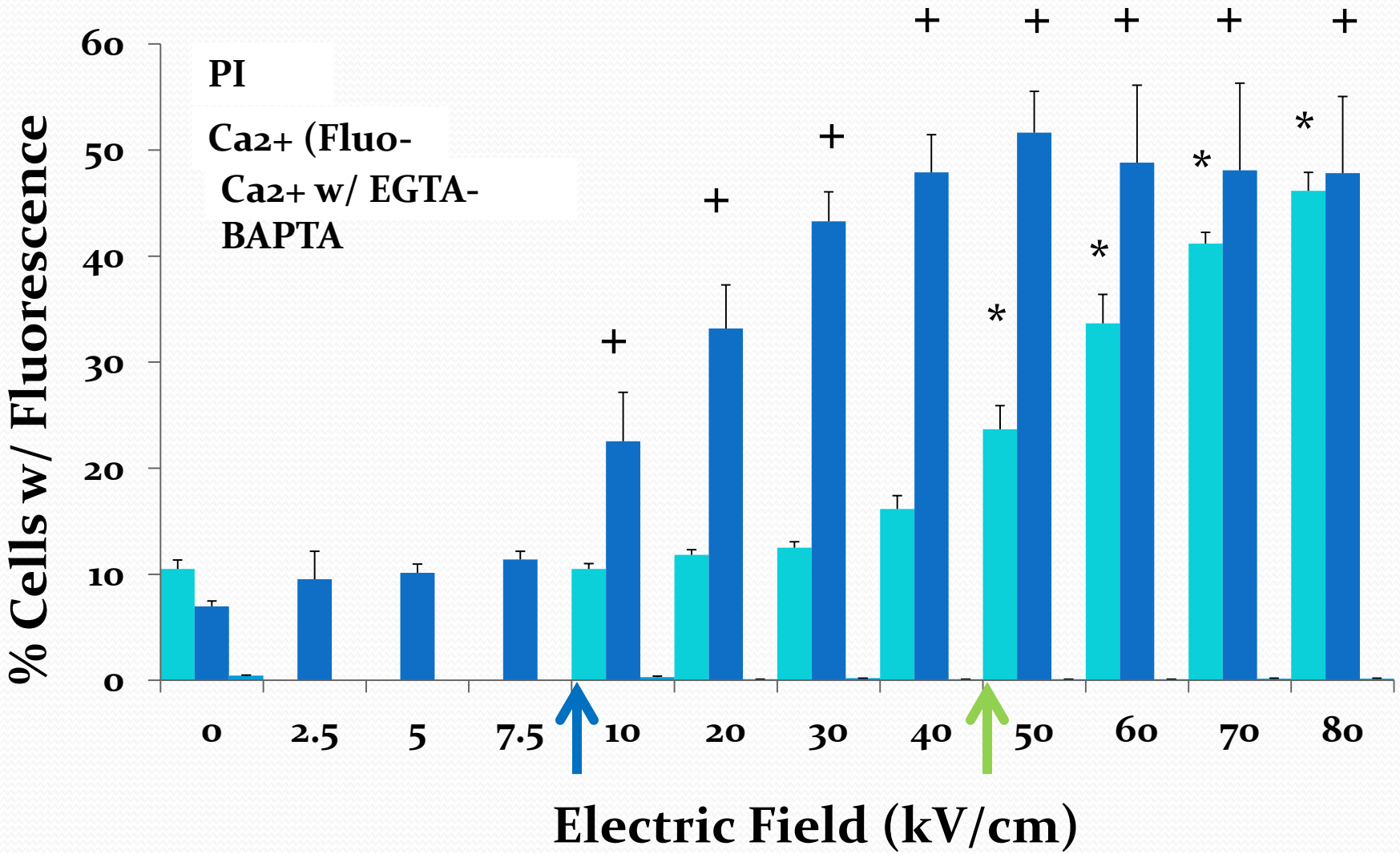
Pulse Power w/ nsPEFs - Concept - 2 Nanopores

High Density Nanoscale Pores in all Cell Membranes

Stewart et al., IEEE Trans Plasma Sci. 2004;32:1696-1708;
Gowrishankar et al., BBRC 2006;341:266-1276;
Vernier et al., BMC Cell Biol. 2006;7:37;
Pakhomov et al., BBRC 2009;385:181-186.

Ca2+ and PI Permeabilization in Jurkat Cells

(10 min post-pulse)

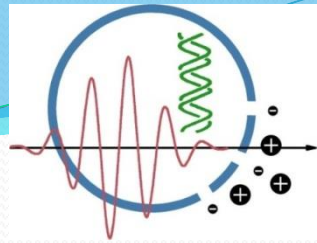


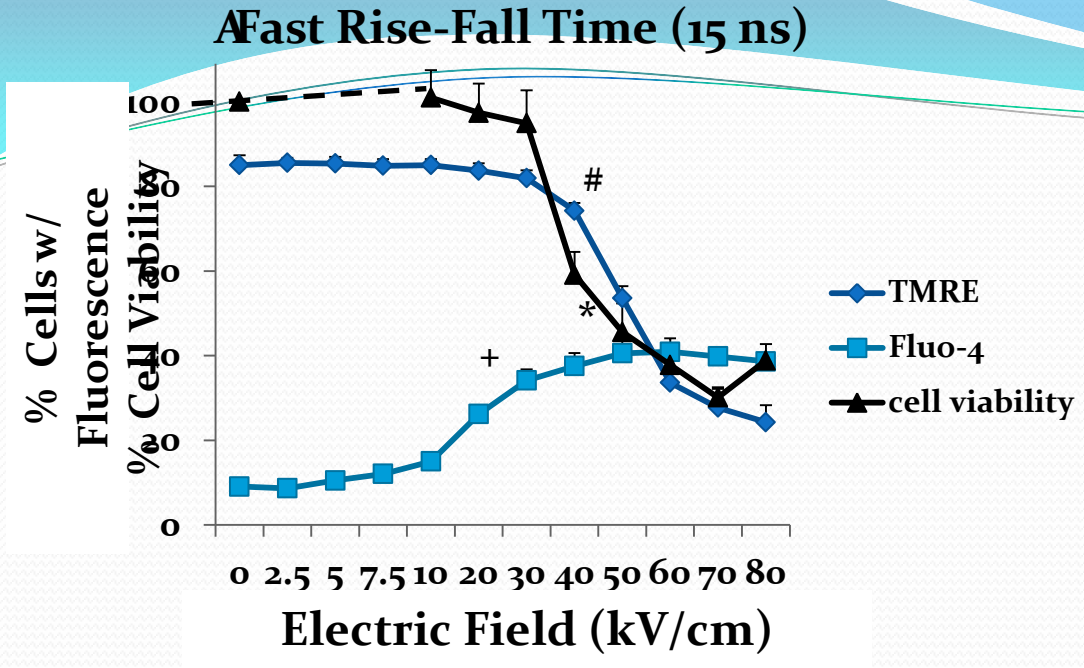
Beebe et al., Cells 2013; 2: 136-

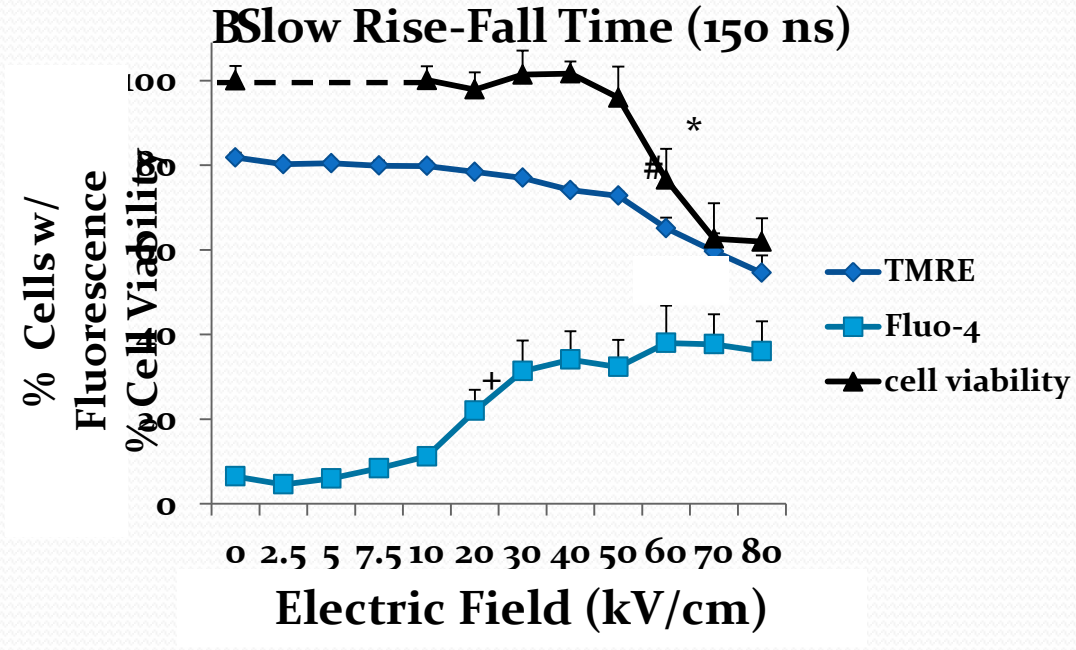
Pulse Power w/ nsPEFs - Concept - 3 Hypothesis

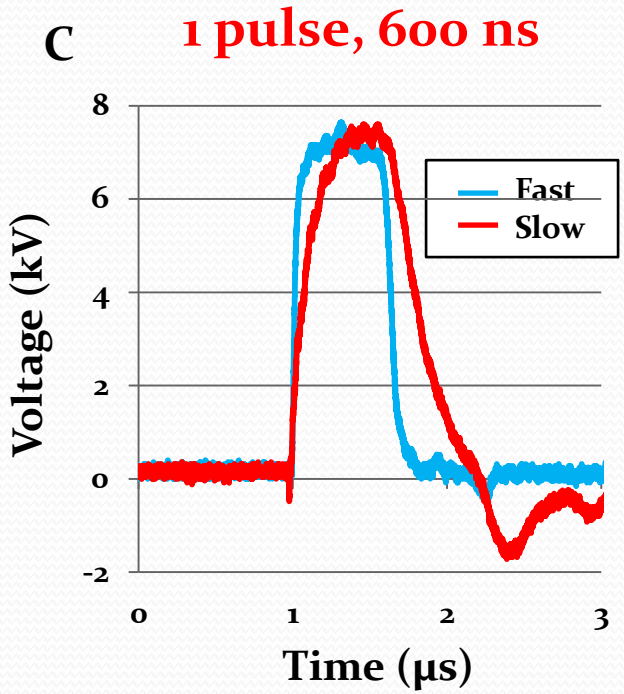
Fast Rise Time (<~70 ns) or High Frequency Component of Sub-Microsecond Pulses Provides Greater Possibilities for Intracellular Effects

Schoenbach et al., Bioelectromagnetics 2001; 22 :440-448.
Beebe et al., PLoS One 2012 ;7 :e51349.
Beebe et al., Cells 2012; 2: 136-162.
Beebe et al., J Nanomedic Nanotechnol. 2013 ;4: 163.

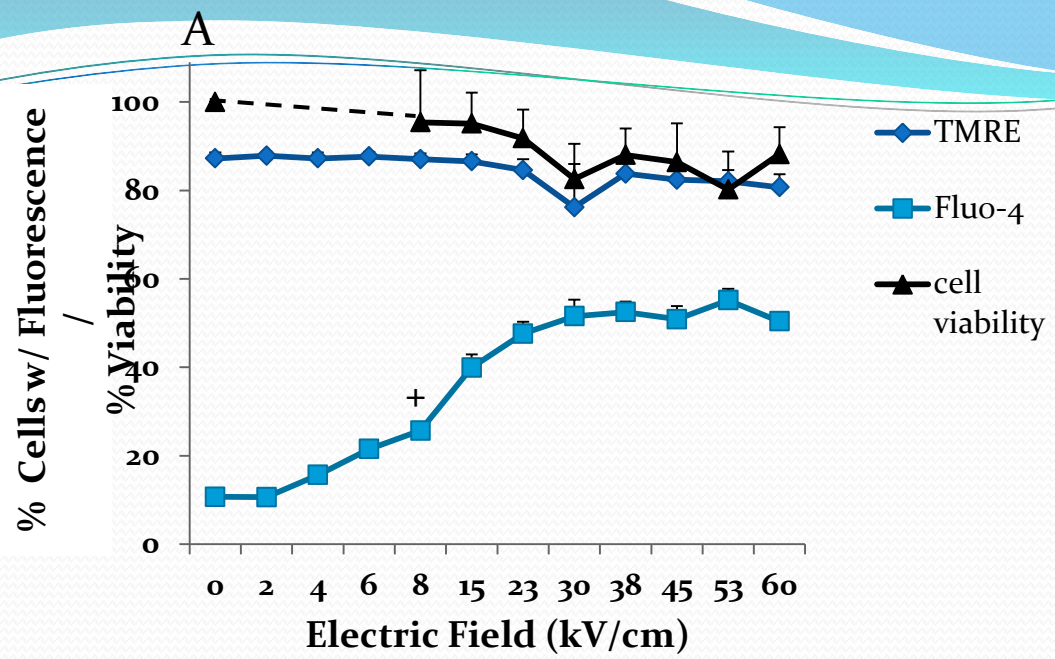


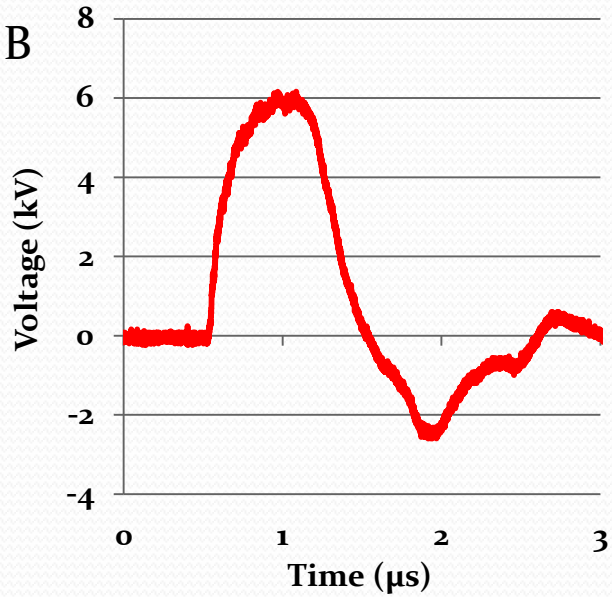






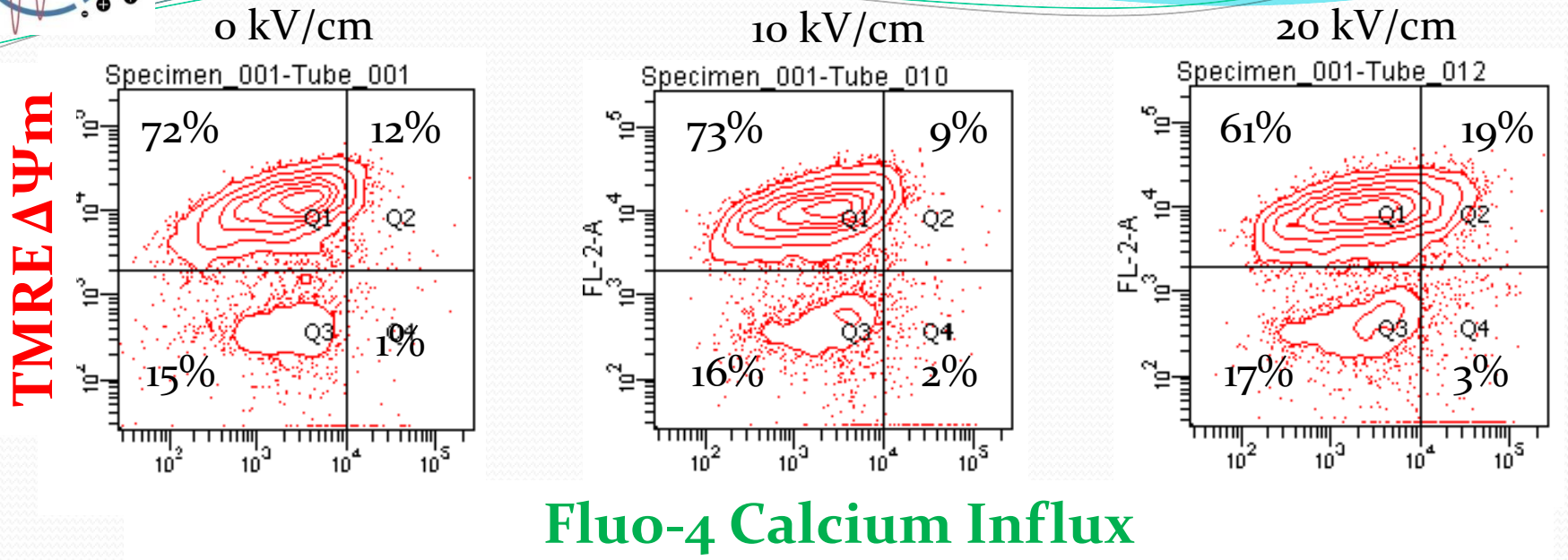
Beebe et al., PLoS One 2012; 7: 51349

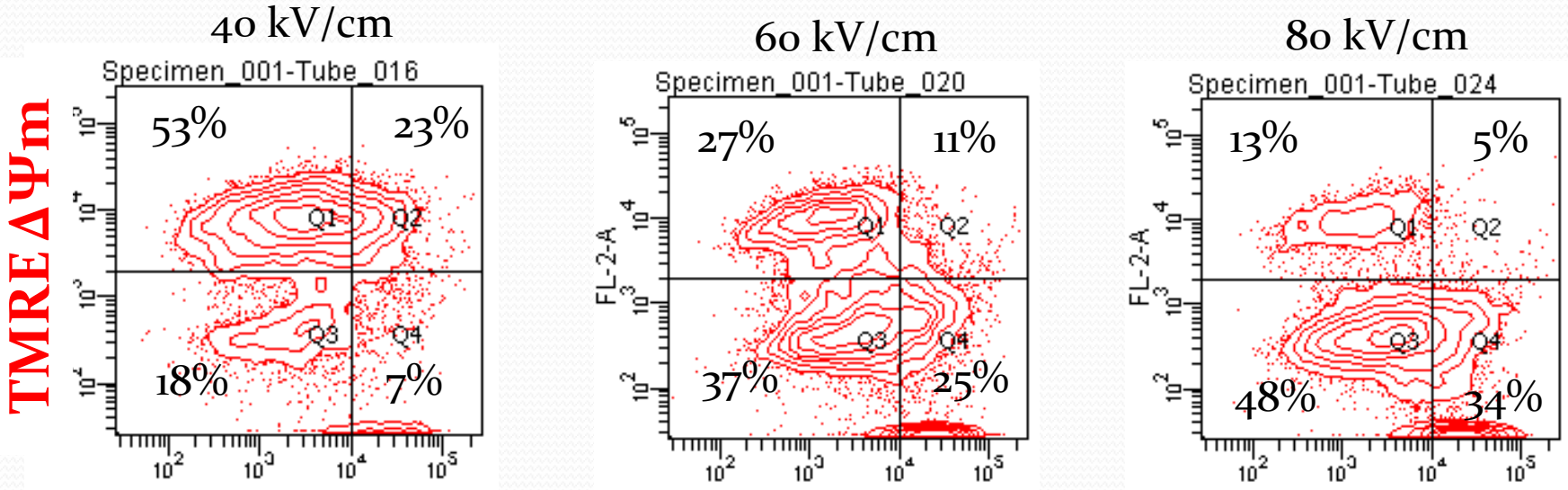




Beebe et al., Cells 2012; 2: 136-162.

Fast Rise-Fall Time, Matched Load

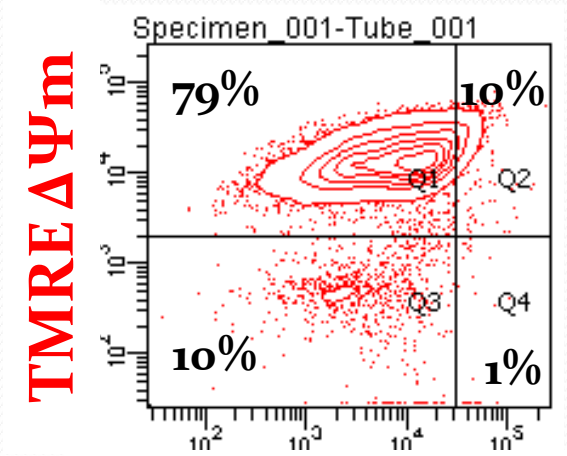




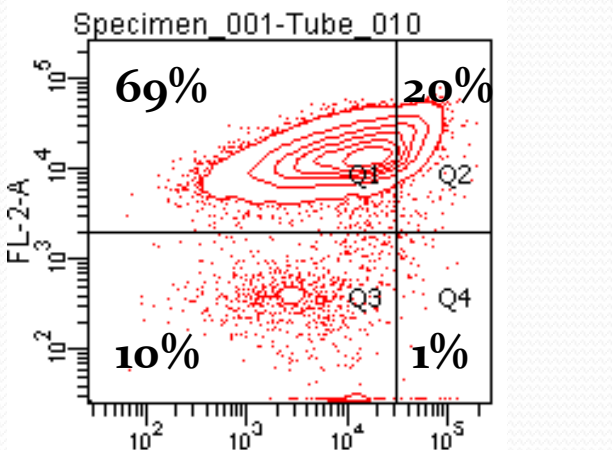
Sain and Beebe,

Slow Rise-Fall Time, Unmatched Load

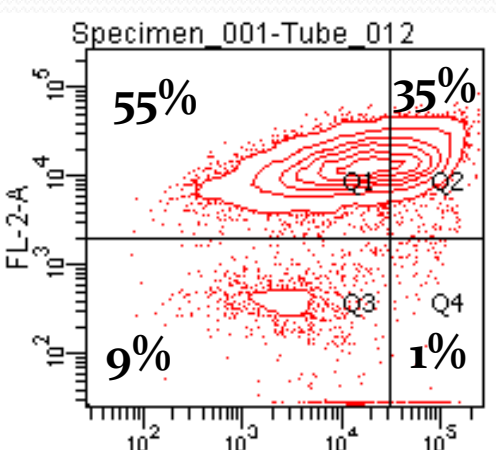
0 kV/cm



7.5 kV/cm

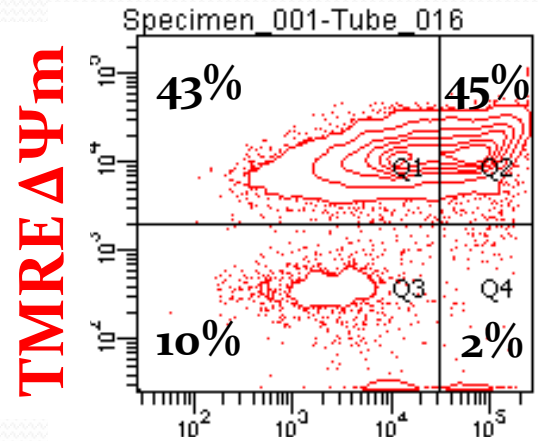


15 kV/cm

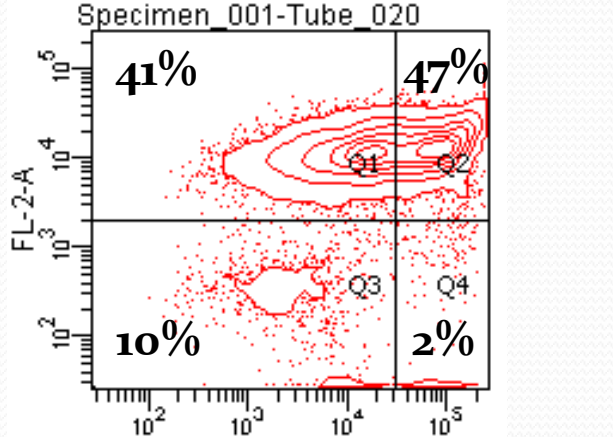


Fluo-4 Calcium Influx

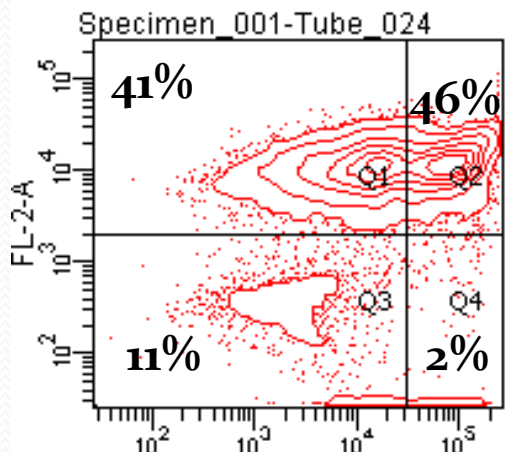
30 kV/cm



45 kV/cm



60 kV/cm



Fluo-4 Calcium Influx

Sain and Beebe,

nsPEF-induced Decrease in ΔΨm is Ca2+ Dependent

[(1) Effects on Proteins (2) Not Poration of Inner Mitochondria Membrane]

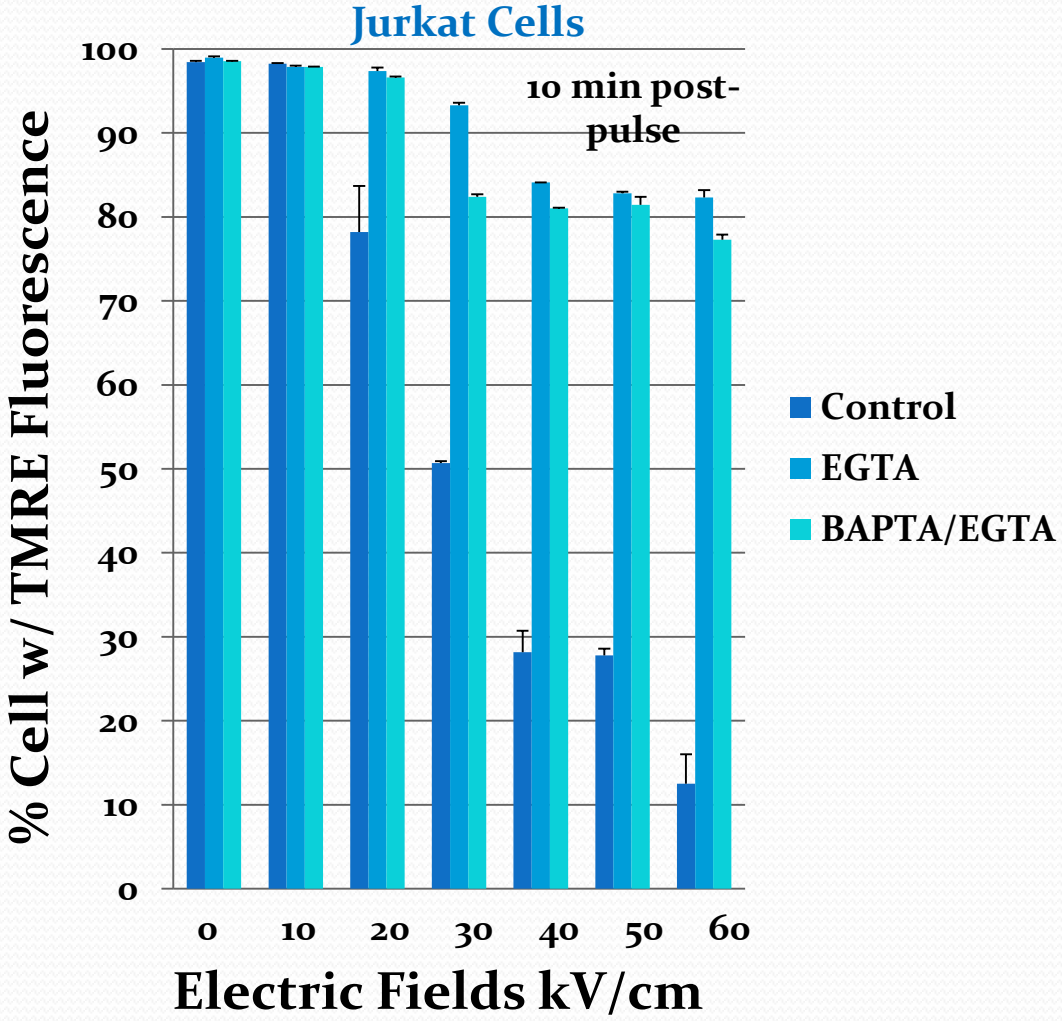
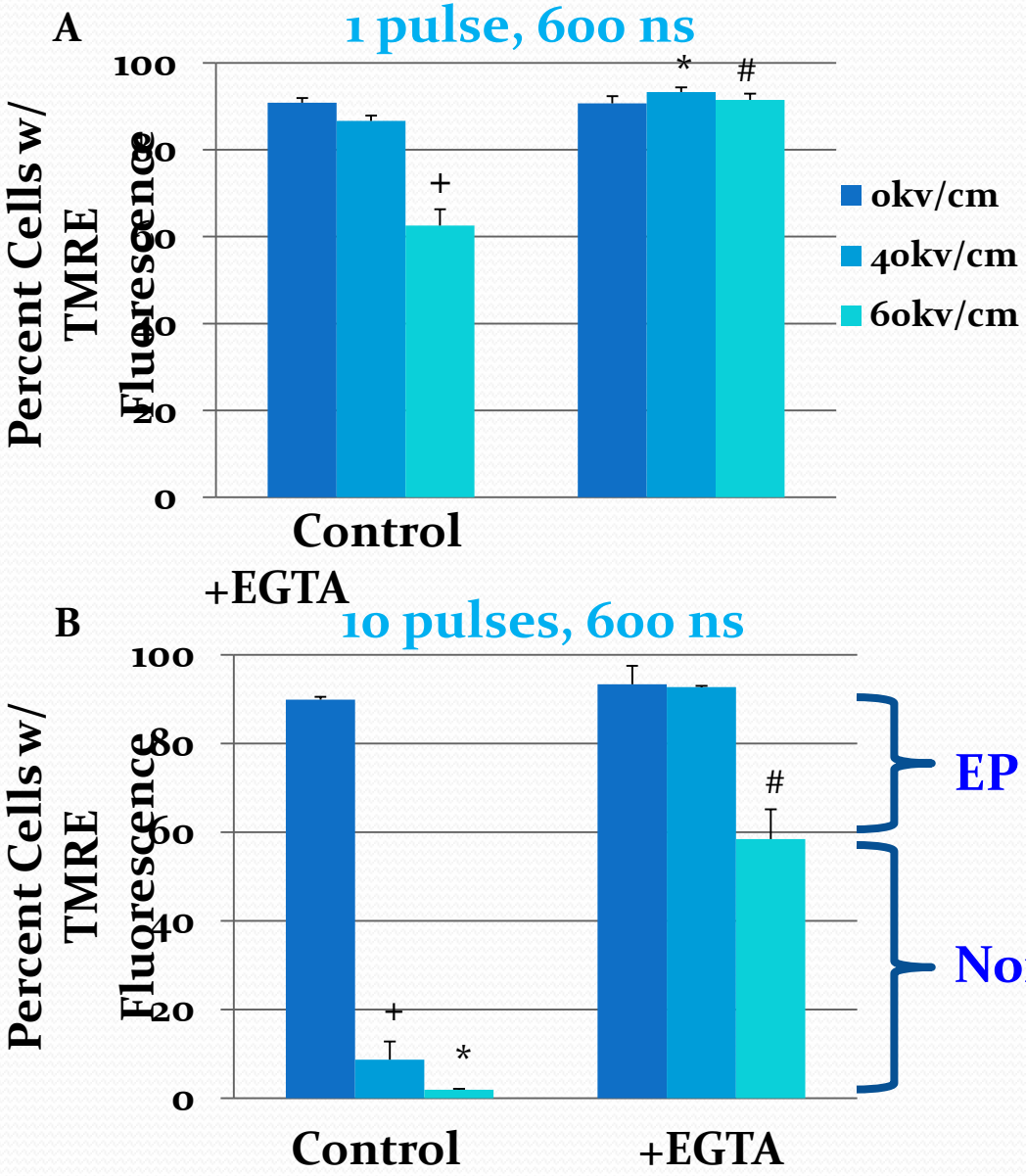
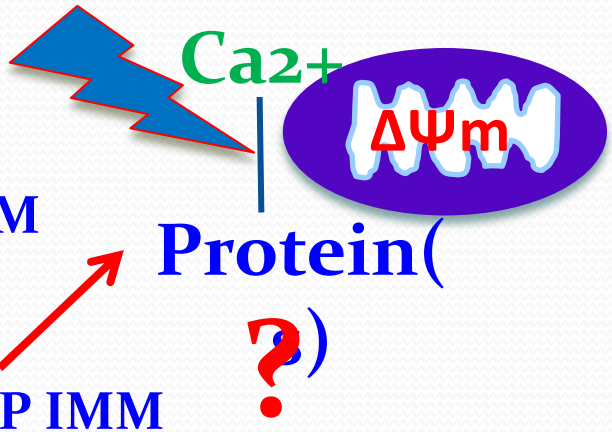


Figure Unpublished
Beebe et al., Cells 2013; 2: 136-

Rat N1-S1 Hepatocellular Carcinoma Cells

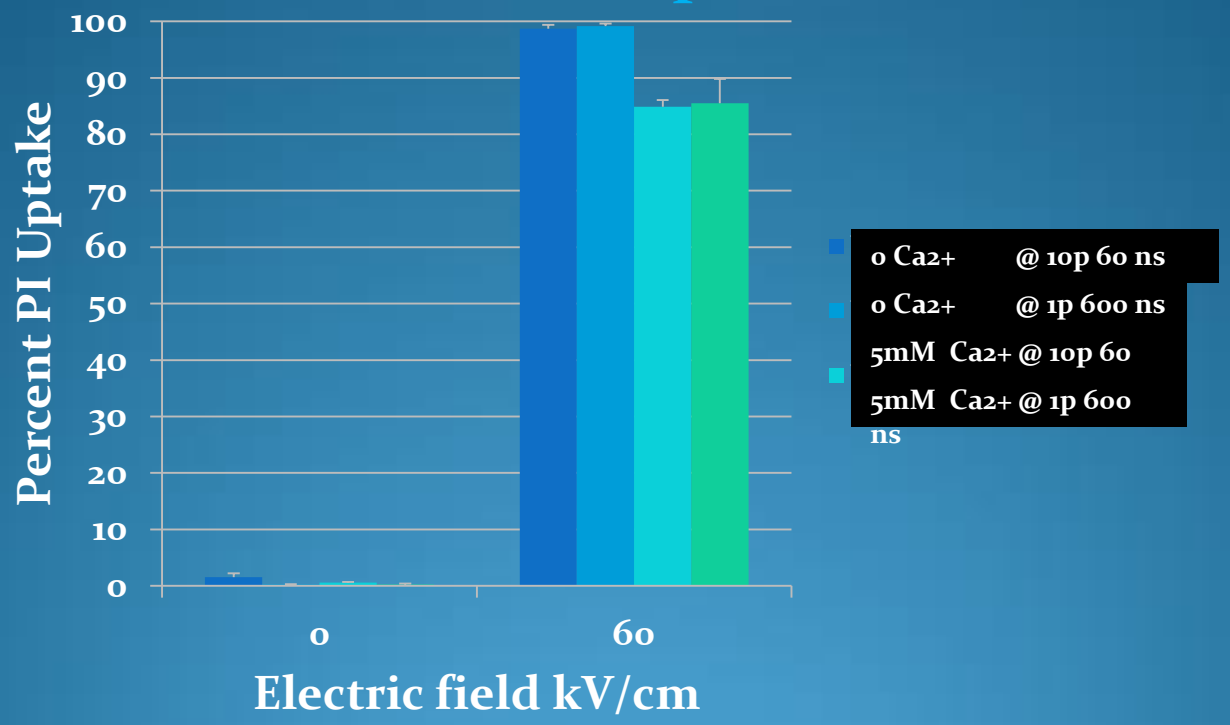
(Ca2+ dependent decrease in ΔΨm – not poration effect)





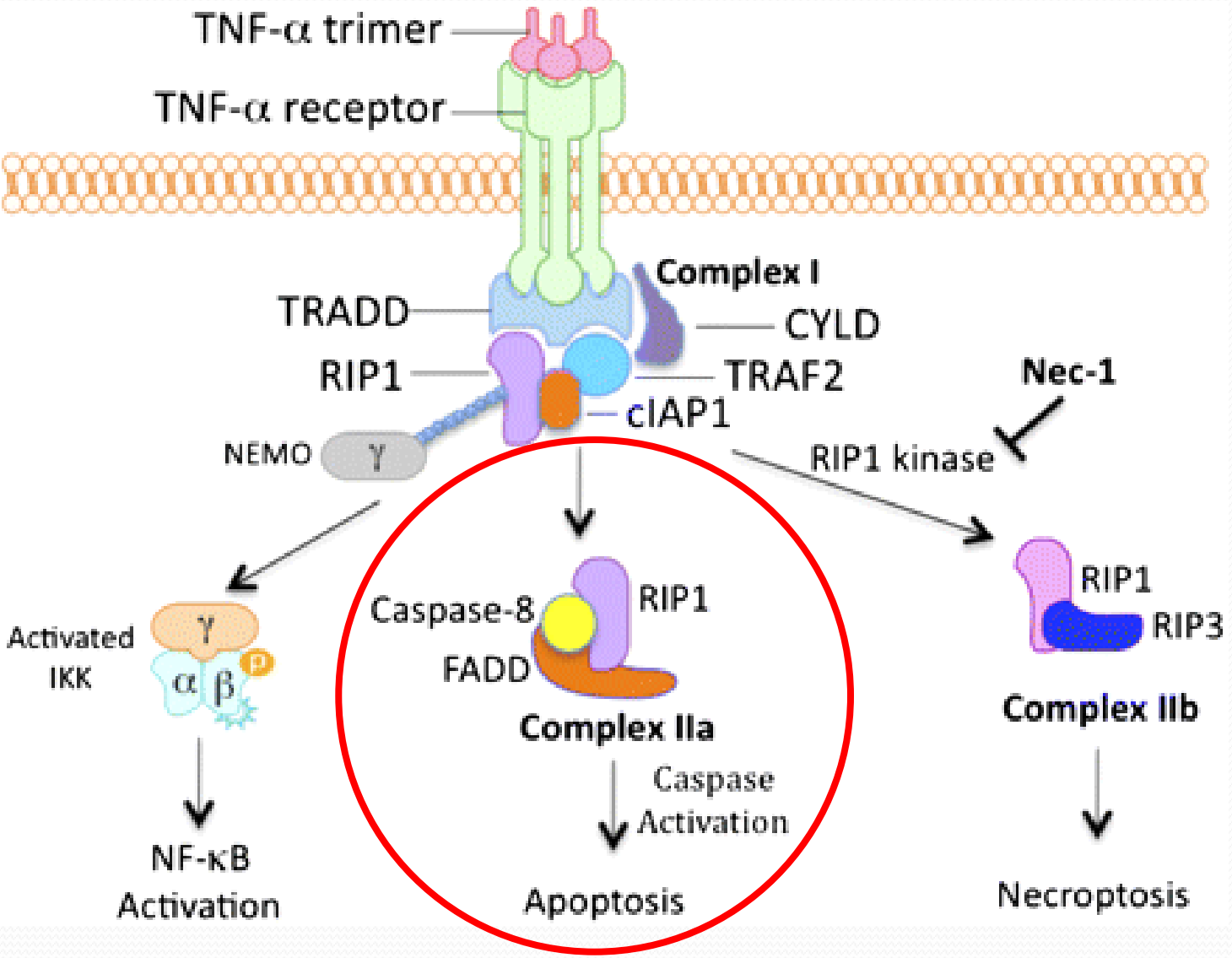
Sain, Harlow and Beebe, Unpublished

Plasma Membrane Permeabilization is not Ca2+ Dependent



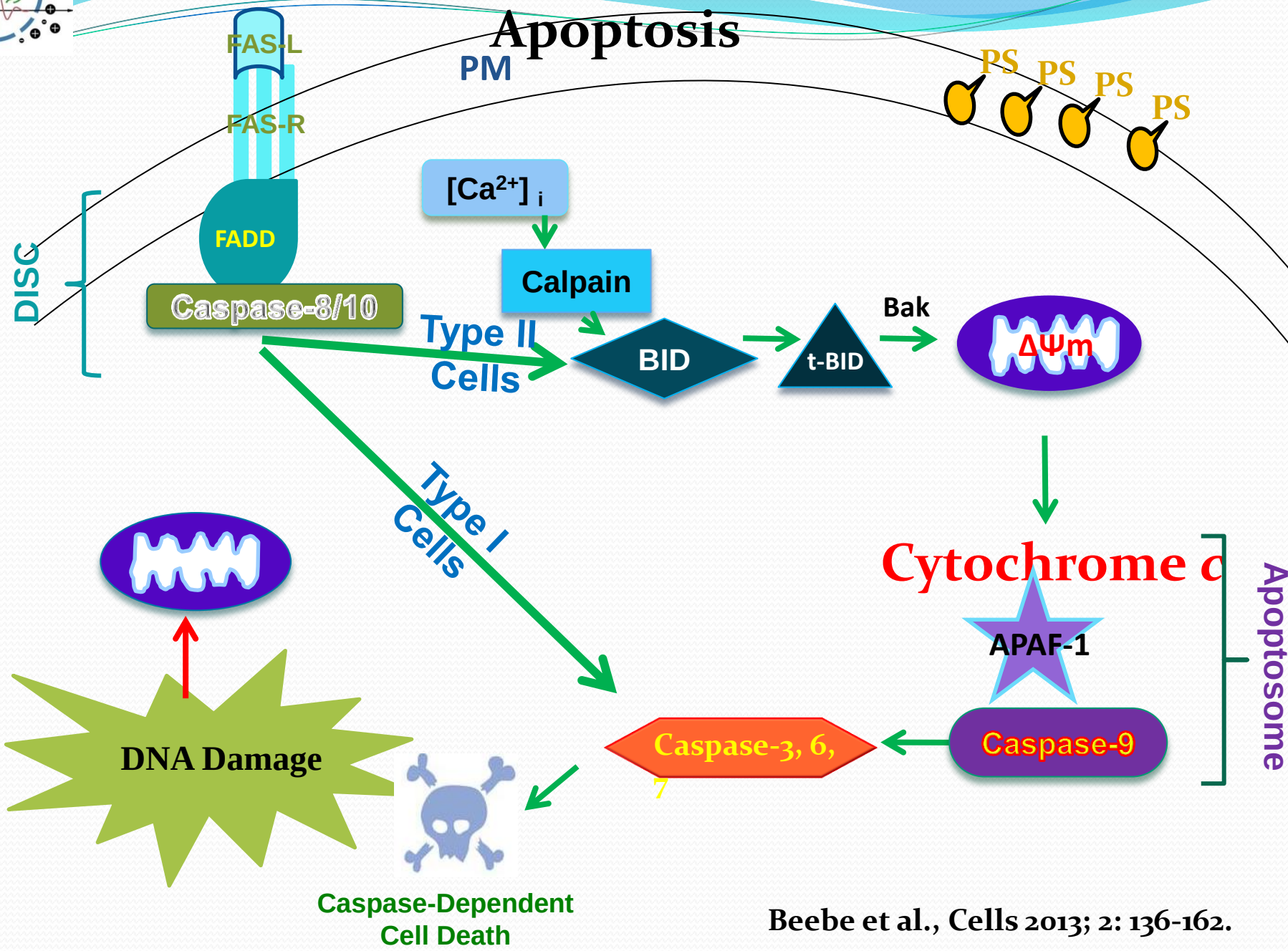
Beebe, Bioelectrochemistry, in

Signaling Complexes induced by TNFα mediate NfκB activation, apoptosis and necroptosis



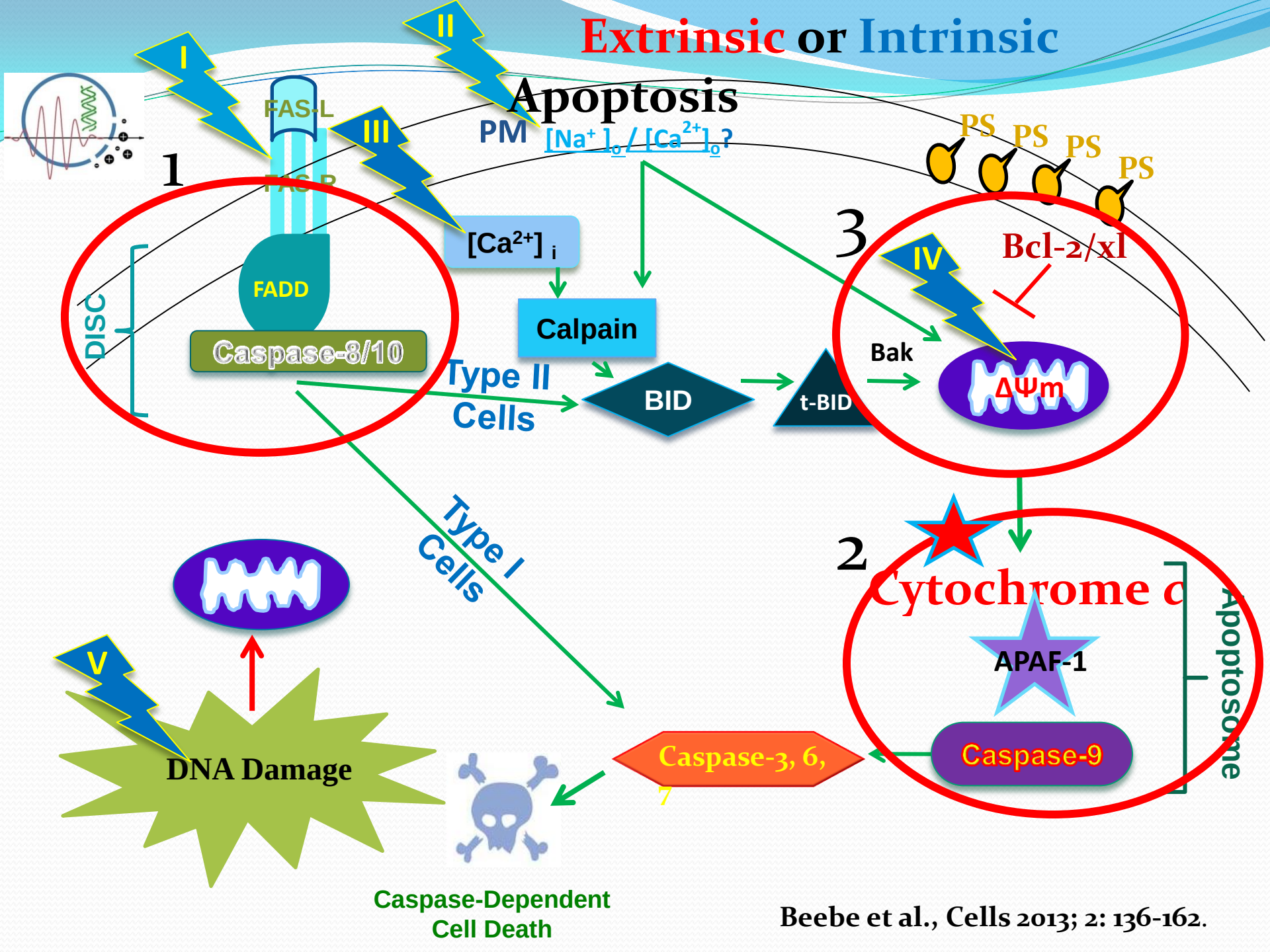
Yuan and Kroemer Genes & Dev 2010; 24:2592-2602

Extrinsic or Intrinsic Apoptosis



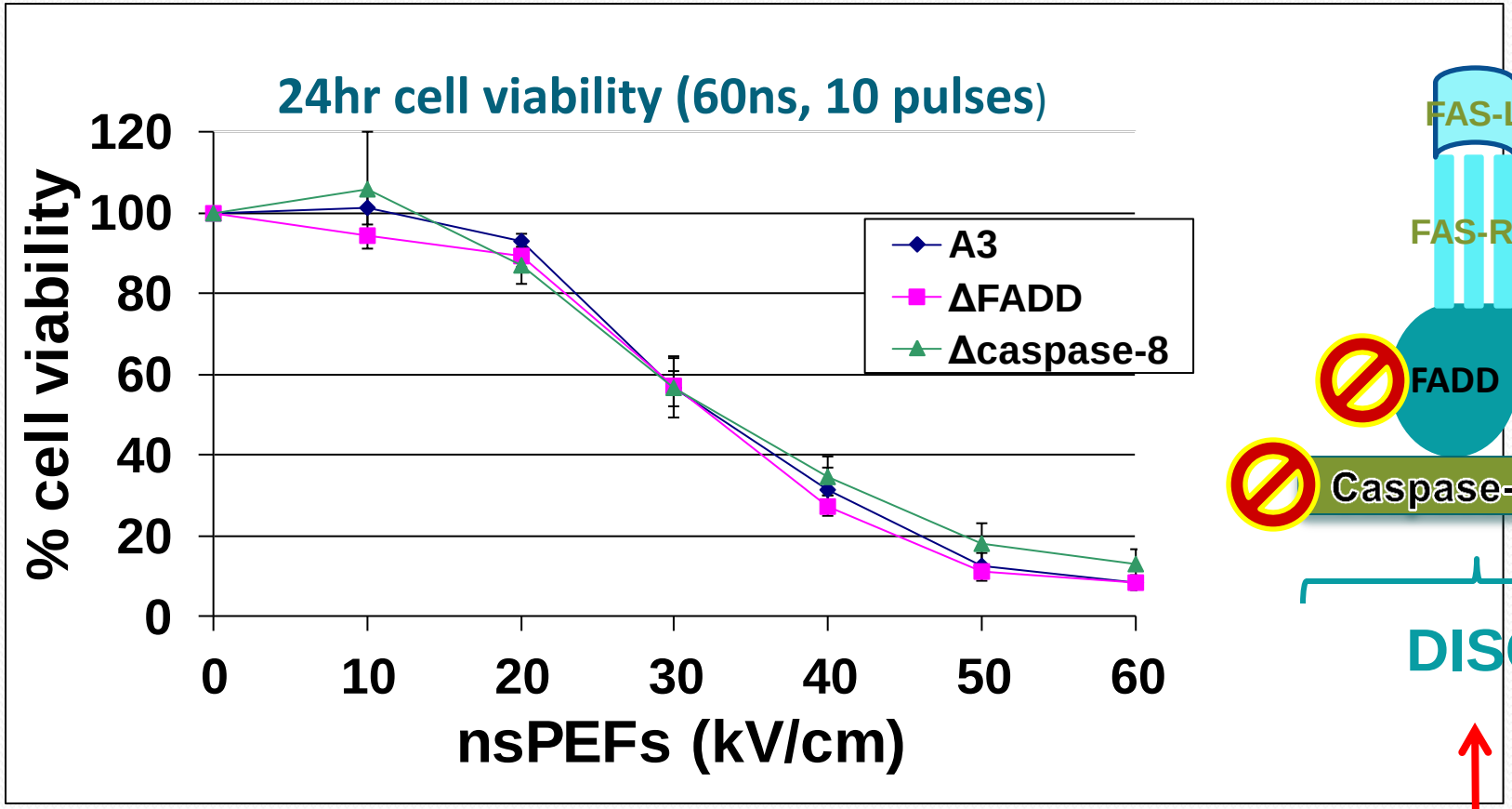
Beebe et al., Cells 2013; 2: 136-162.

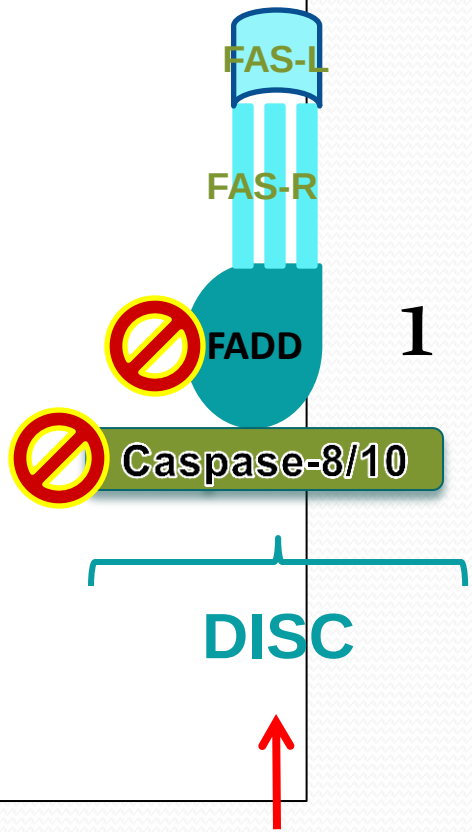
Extrinsic or Intrinsic



Beebe et al., Cells 2013; 2: 136-162.

nsPEF-Induced Cell Death Does Not Require The DISC (ΔCaspase-8 / Δ FADD)

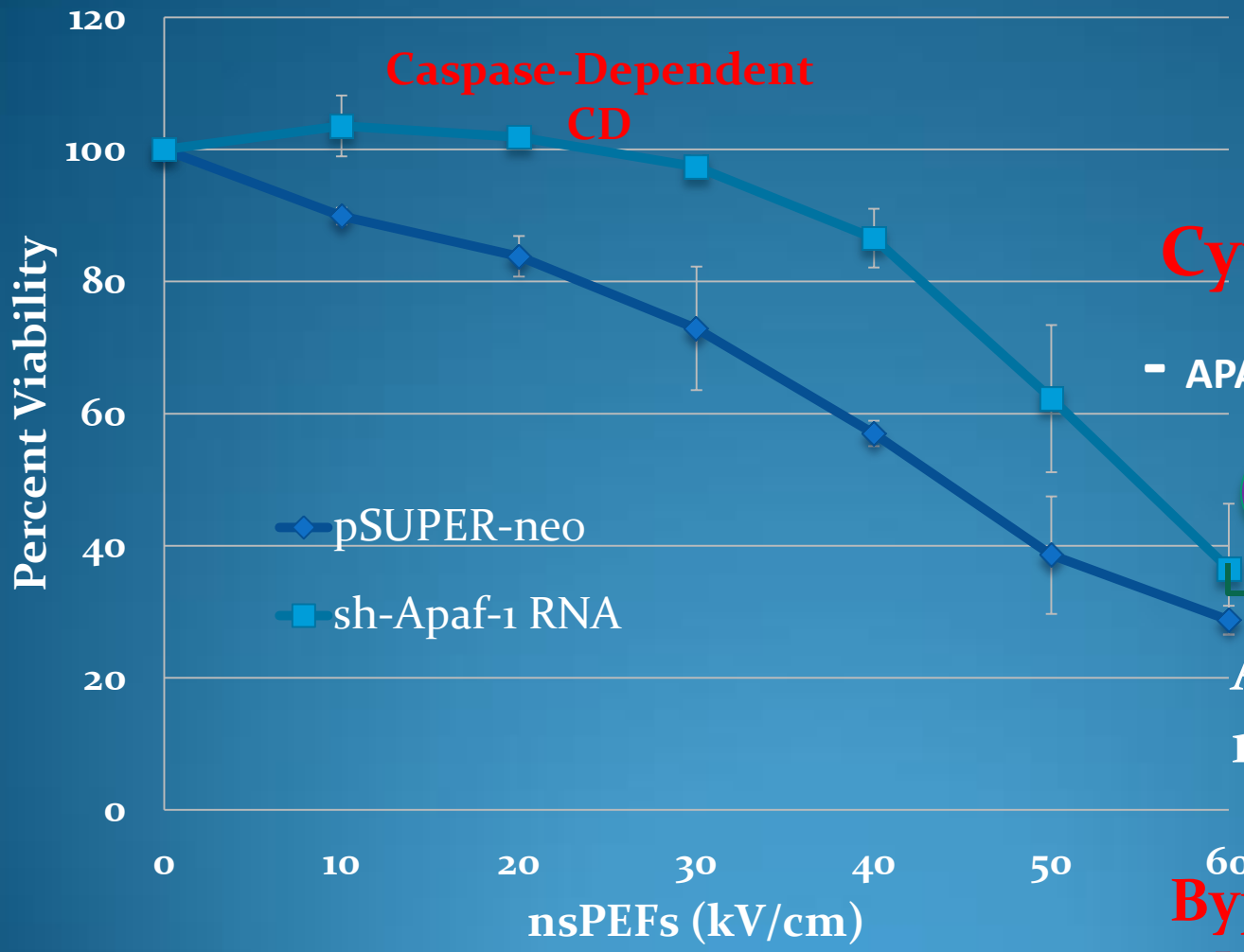




Bypasses Cancer Mutations @

Ren et al., BBRC 2012; 421: 808-112.

APAF-1 Deficient Jurkat Cells Require Higher Electric Fields to Induce Cell Death



Cytochrome *c*

- APAF-1

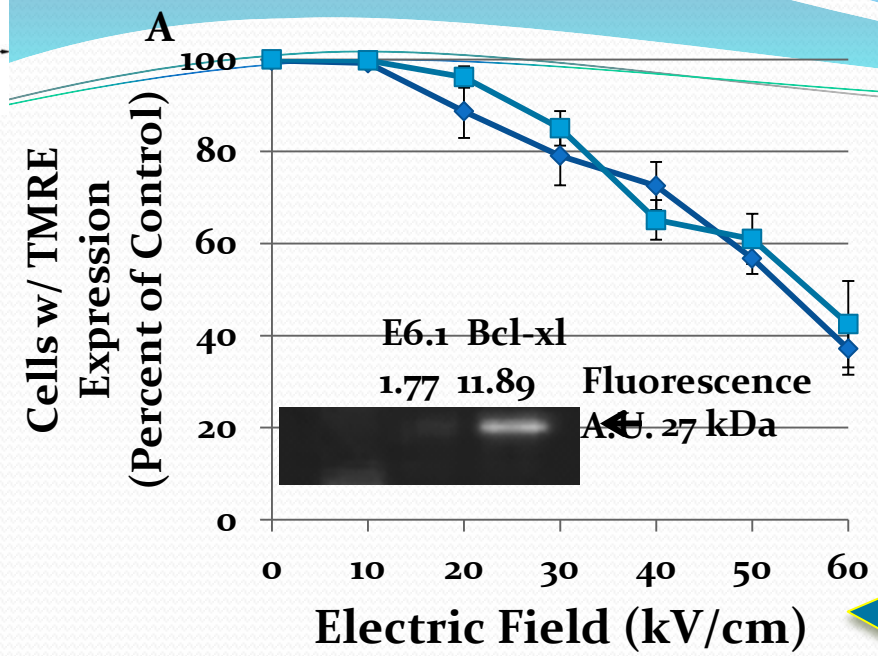
2

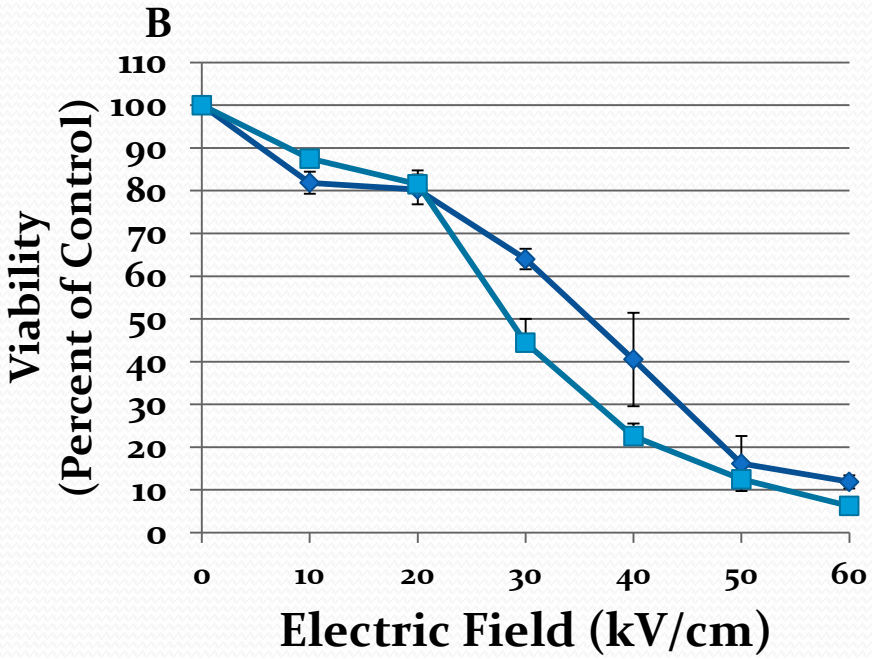
Caspase-9

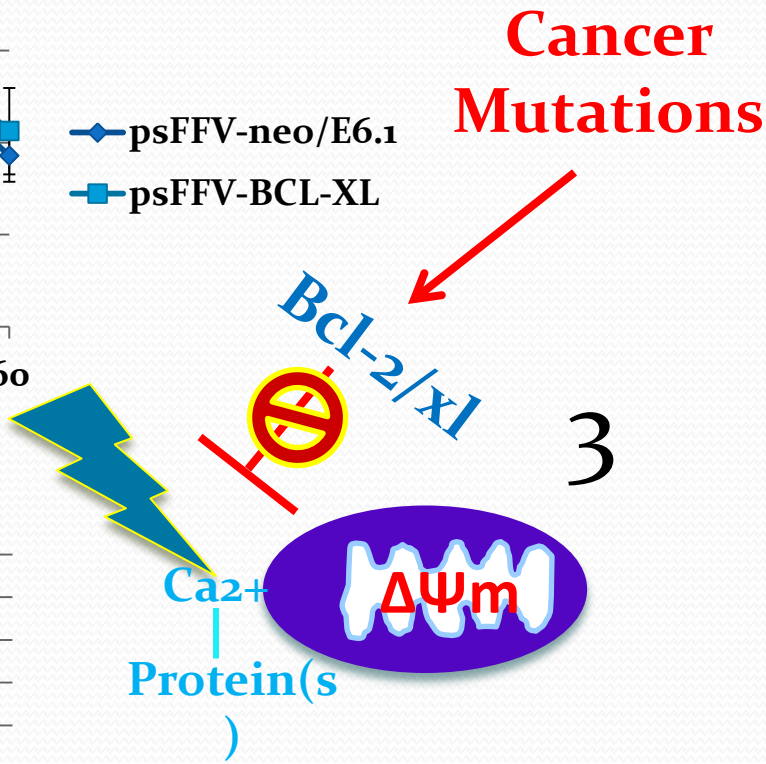
Apoptosome

Bypasses Cancer Mutations @ IAPs

Ren et al., BBRC 2012; 421: 808-112.



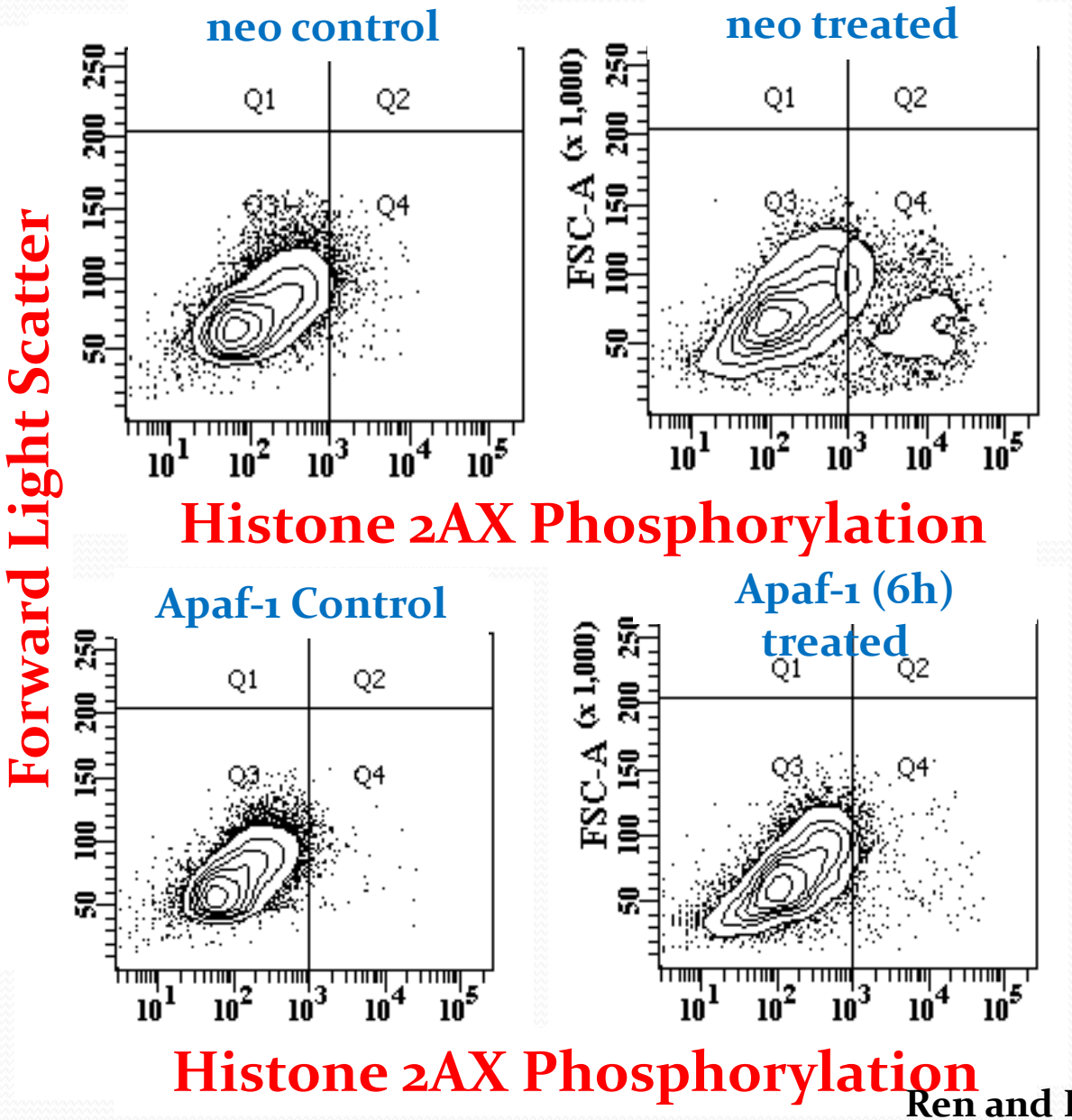




3

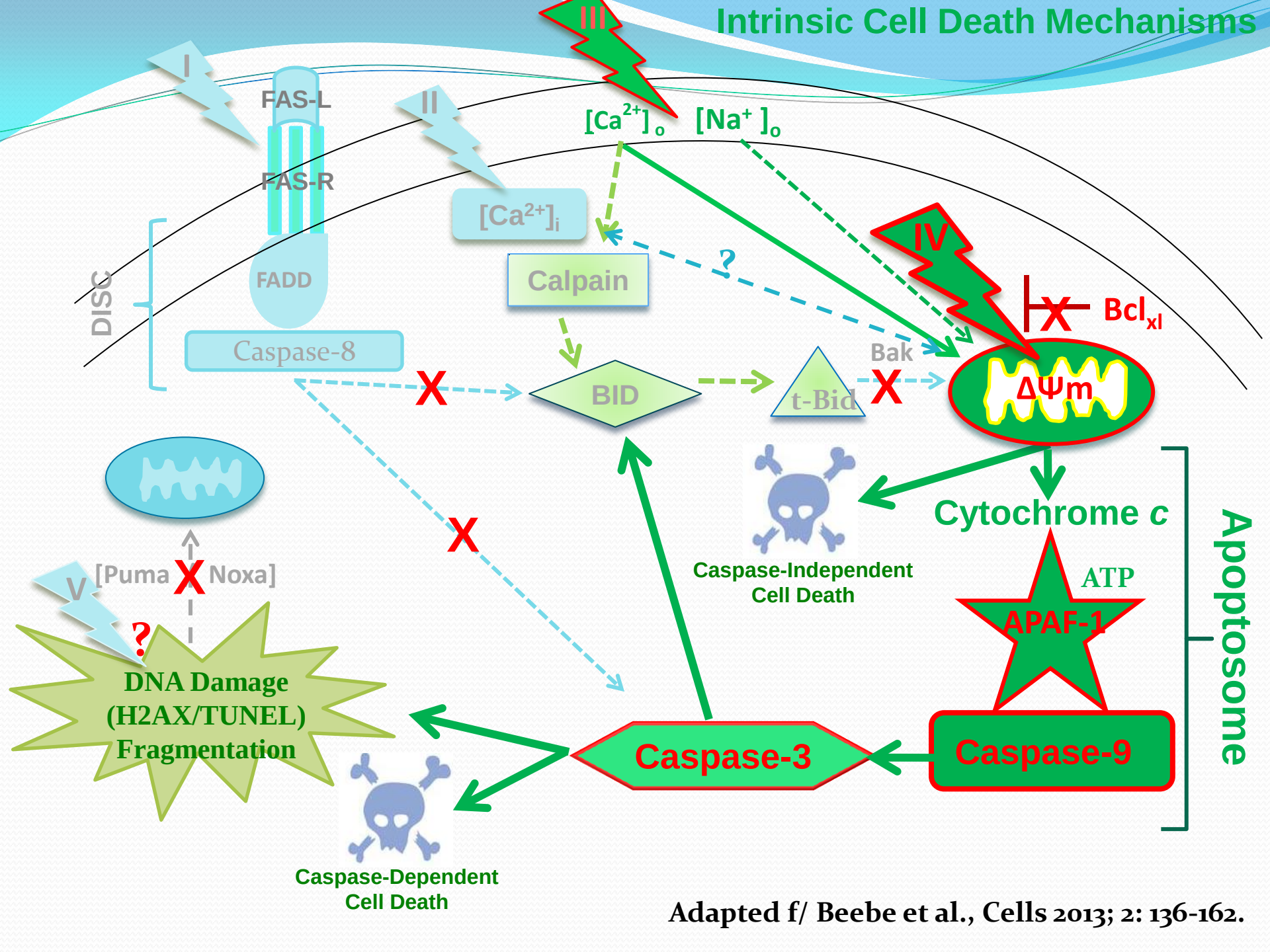
Ren, Harlow and Beebe, Unpublished

nsPEF-induced DNA Damage is Caspase-dependent



Ren and Beebe, unpublished

Intrinsic Cell Death Mechanisms



Adapted f/ Beebe et al., Cells 2013; 2: 136-162.

In Vitro Summary and Conclusions

Pulse Shape is a Determinant of Effects on ΔΨm and Viability

NsPEF-induced Decrease in ΔΨm is Ca2+ Dependent

Influx of Ca2+ Necessary, but not Sufficient, for Drop in ΔΨm and CD

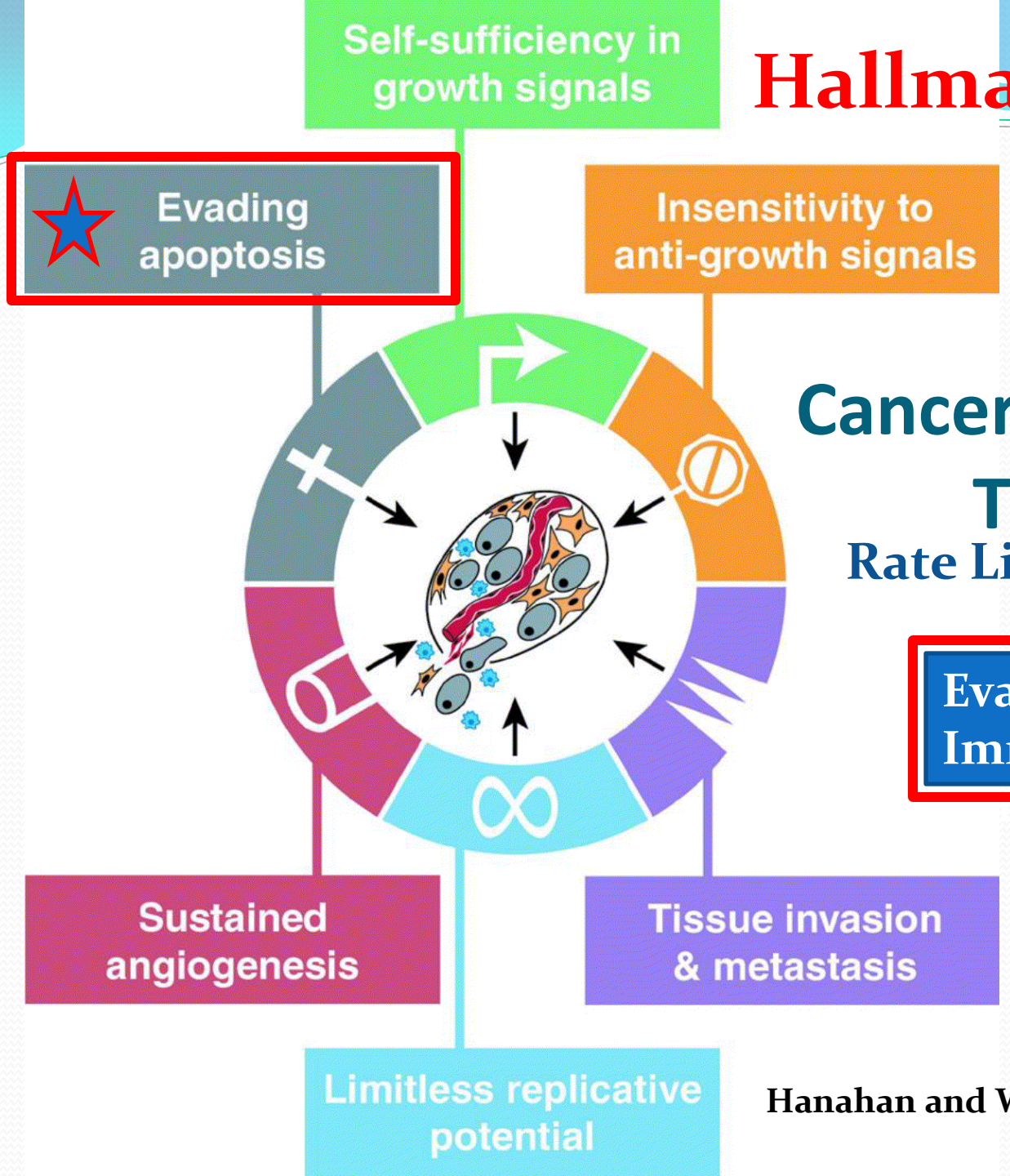
Primary Decrease in ΔΨm is Not Due to Poration of IMM

Decrease in ΔΨm May be Due to Effects on Protein(s)

nsPEF-induced DNA Damage is Caspase-Dependent

nsPEF Bypass Cancer Mutations @ DR, Caspases and Mitochondria

Hallmarks of Cancer



Cancer Diagnosis and Treatment

Rate Limiting-Stochastic Events

Evasion of Immune

Hanahan and Weinberg Cell 2000; 100: 57-70
Cell. 2011; 144: 646-674.

Cancer Genome Landscapes

Vogelstein et al., Science. 2013; 339: 1546-1558.

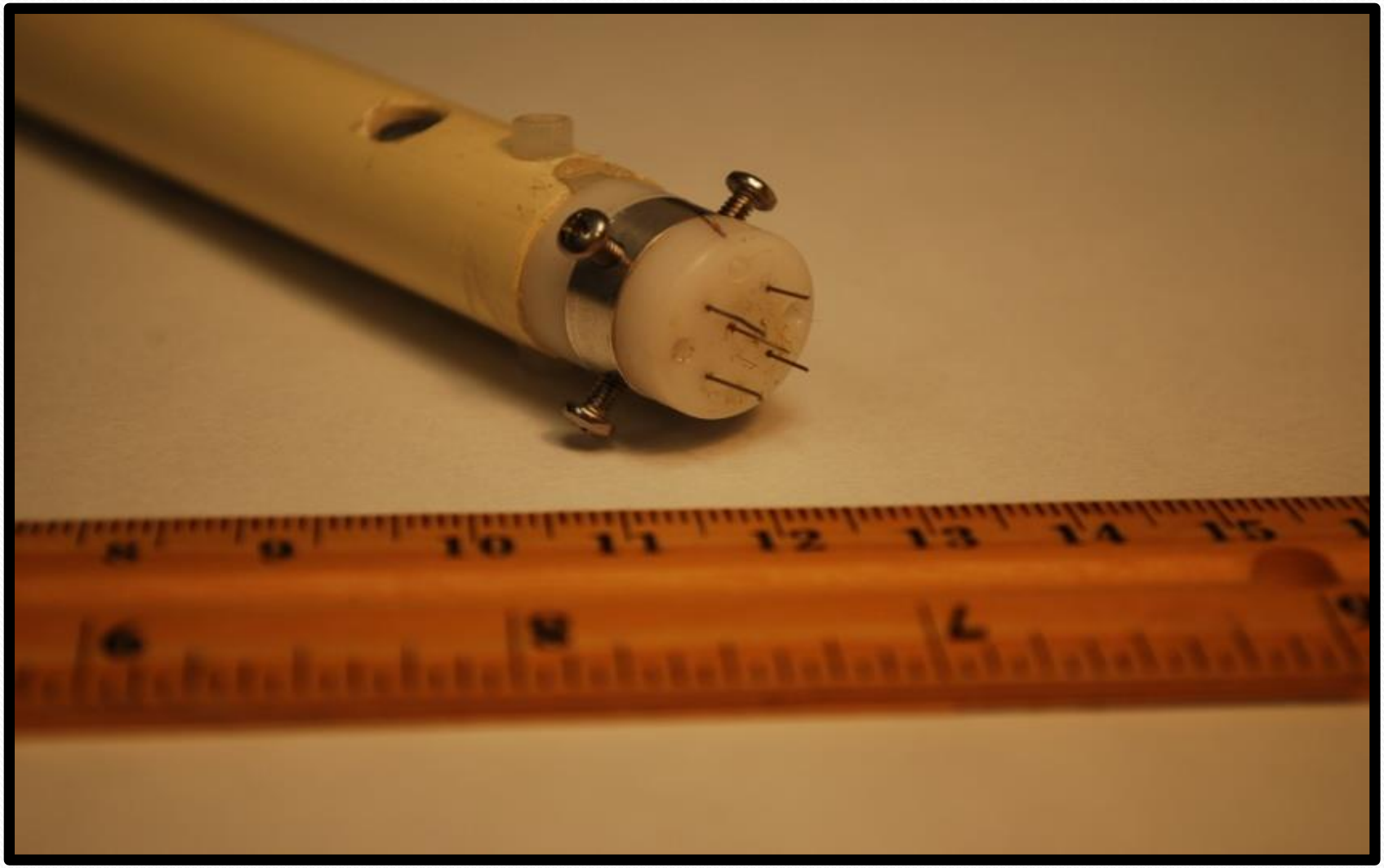
A Concept for Cancer

Not an Invading Army – Cancer Comes from Within Us

A Criminal Gang	Cancer
Within the local community	Microenvironment
Coerces the local population	Supporting host cells
Uses their resources	Growth, vascularization
Thwart the authorities	Evades immune surveillance

Expanded f/Drake (Neuberg S.) Nat Med 2011;17:757; Beebe SJ, J Nanomed & Biotherapeutic Discovery 4:e134

Electrode Design: 5 Needle Array



Kolb JF, unpublished

NsPEF Ablation of Rat N1S1 Orthotopic HCC

Conditions:

Pulse Duration: 100 ns

Electric Field: 50 kV/cm

Pulse Number: 100, 300, 500 or 1000

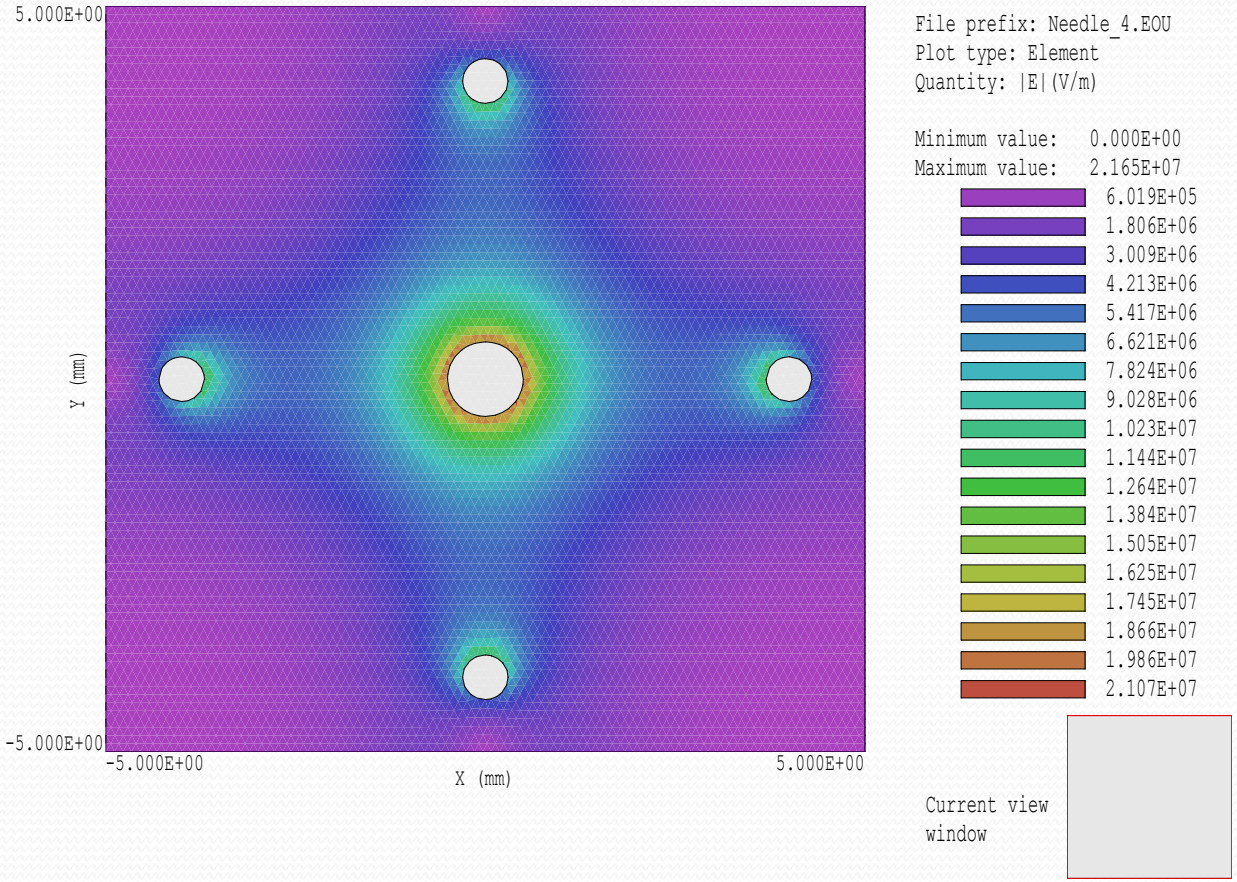
Treatments: 1

Electrodes: 5 Needle Array

Chen R et al., Eur J Cancer 2014, in press

Electric Field Simulation

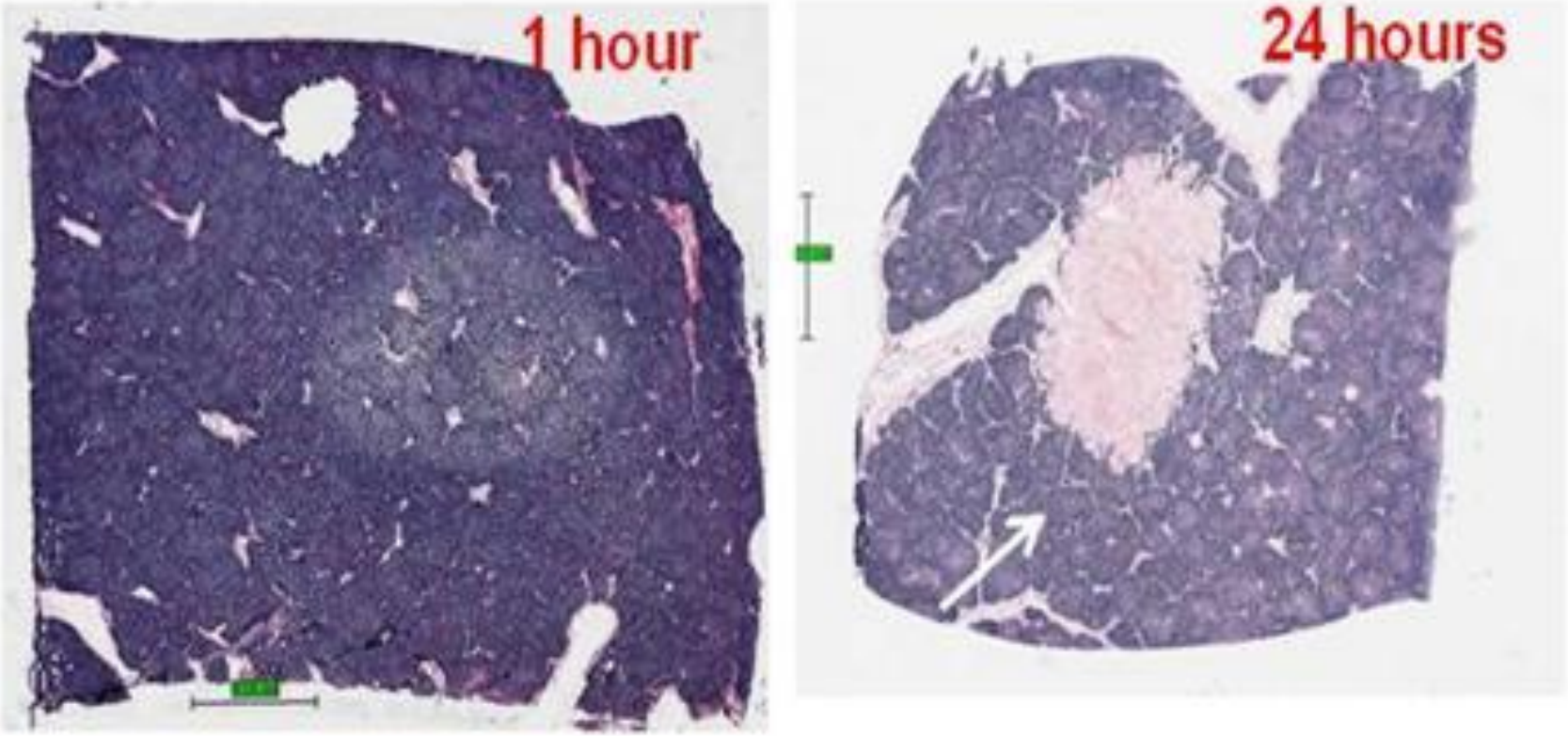
5 Needle Array



Zhuang J, Kolb JF, Chen X, Beebe SJ, Unpublished

Histopathological Analysis of Porcine Liver

30 pulses, 100ns and 10-12kV/cm



NADH activity using Nitro Blue Tetrazolium

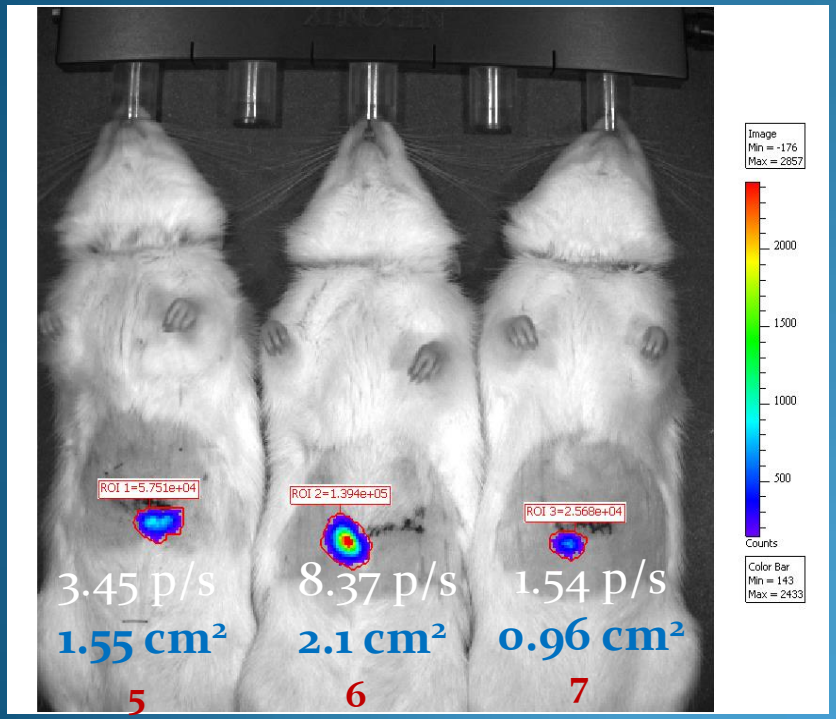
Viable: Purple Non-viable: Pink

Long G et al., IEEE EMB, 2011; 58: 2161-

Luminescence of N1S1/Luciferase Cells in Rat Liver

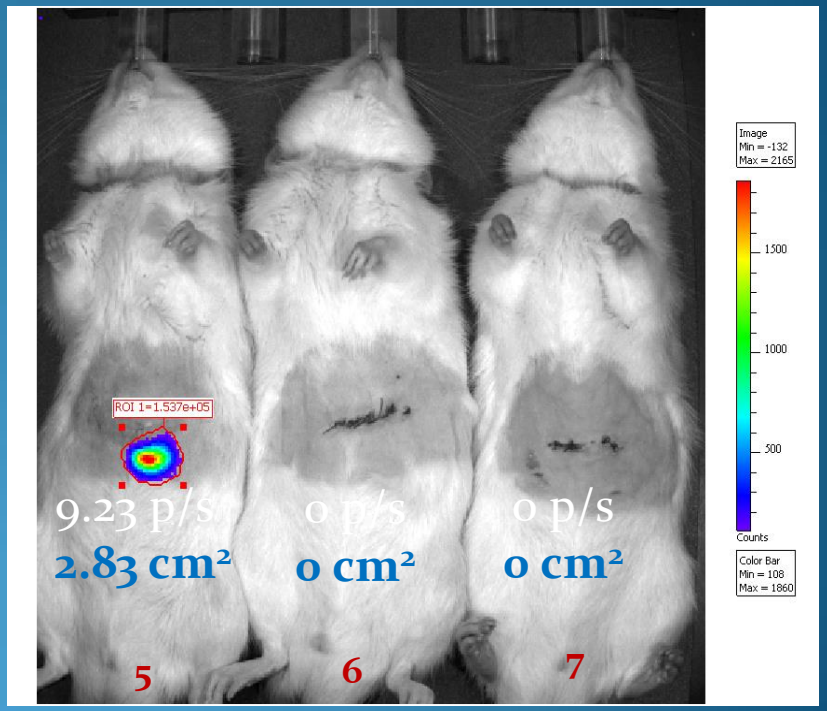
Before and After Treatment with 100 ns, 50 kV/cm

Day -1



100p 300p 1000p

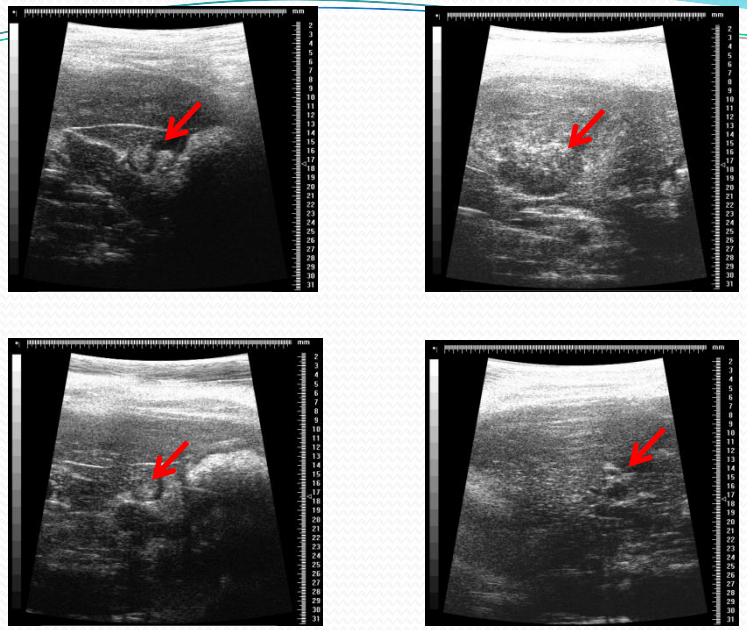
Day +6



100p 300p 1000p

Sain NM and Beebe SJ,

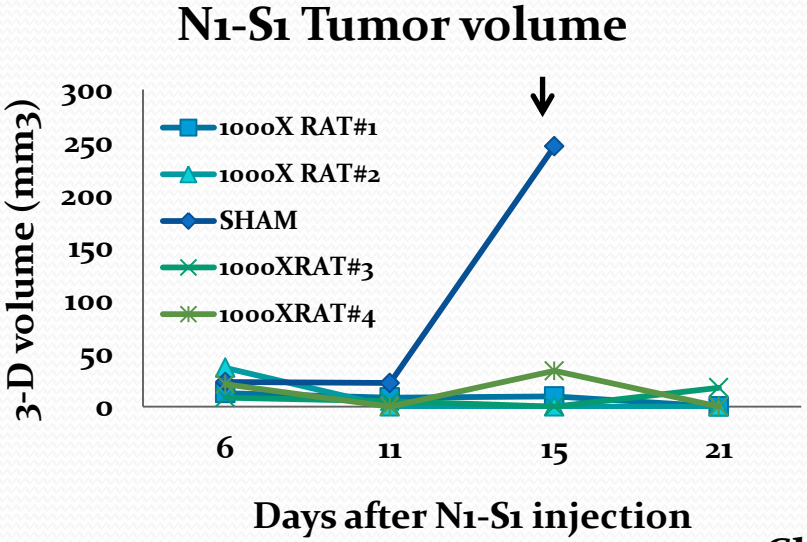
Orthotopic Rat N1S1 HCC



Sham

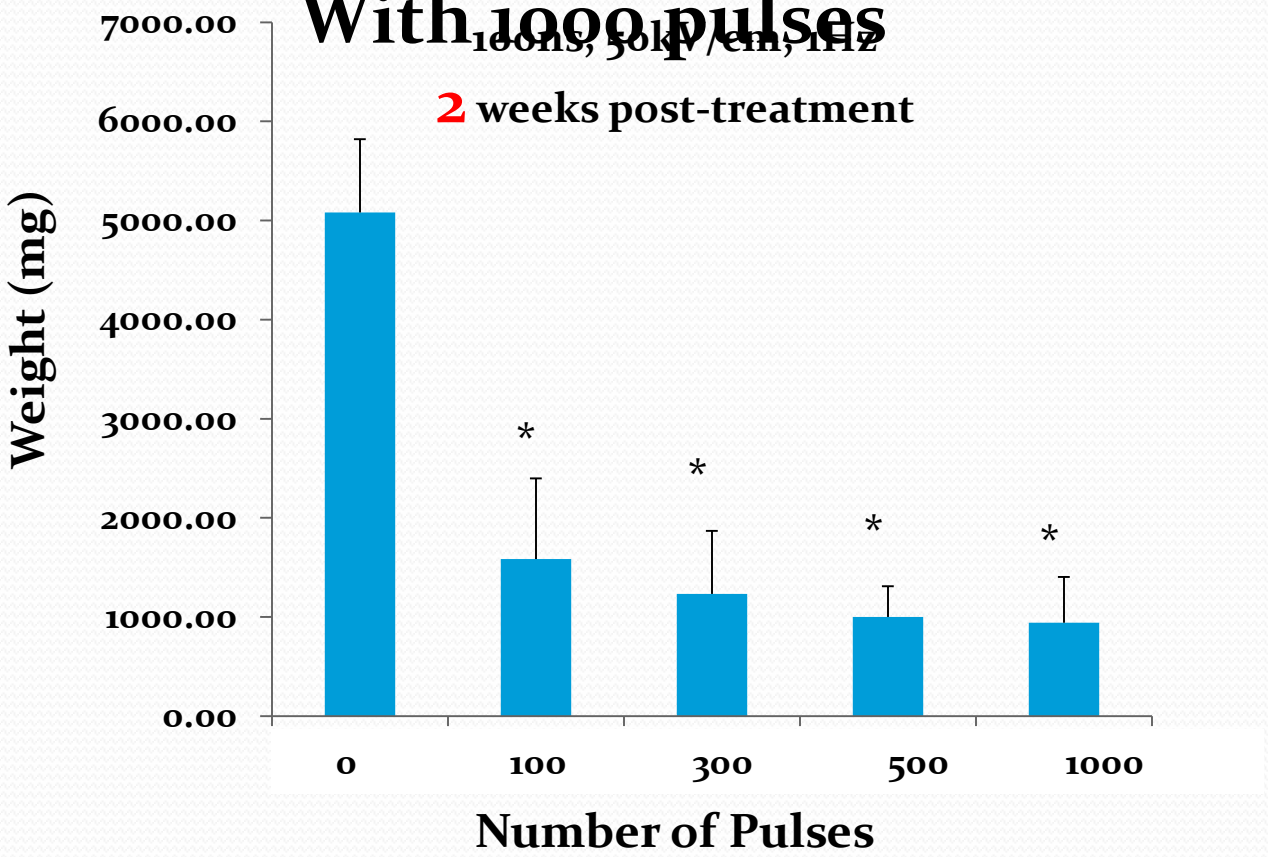
1000X

Day 6 Day 15



Chen R et al., Eur J Cancer 2014, in press

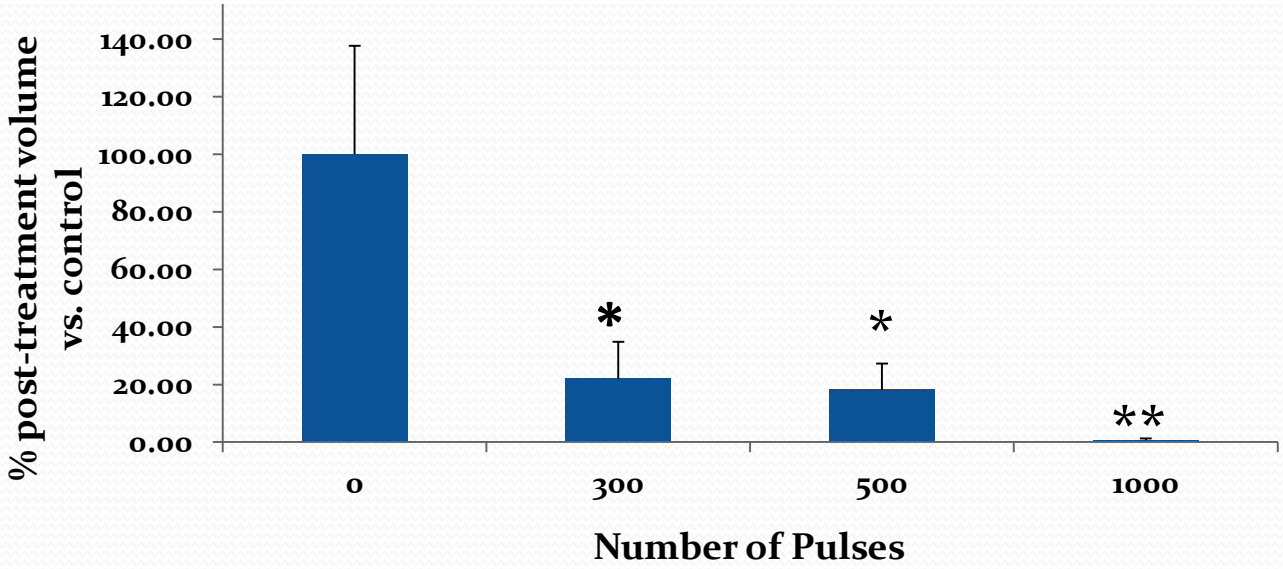
NsPEFs Eliminate Orthotopic Rat N1-S1 HCC Tumors With 1000 pulses



Chen R et al., Eur J Cancer 2014, in press

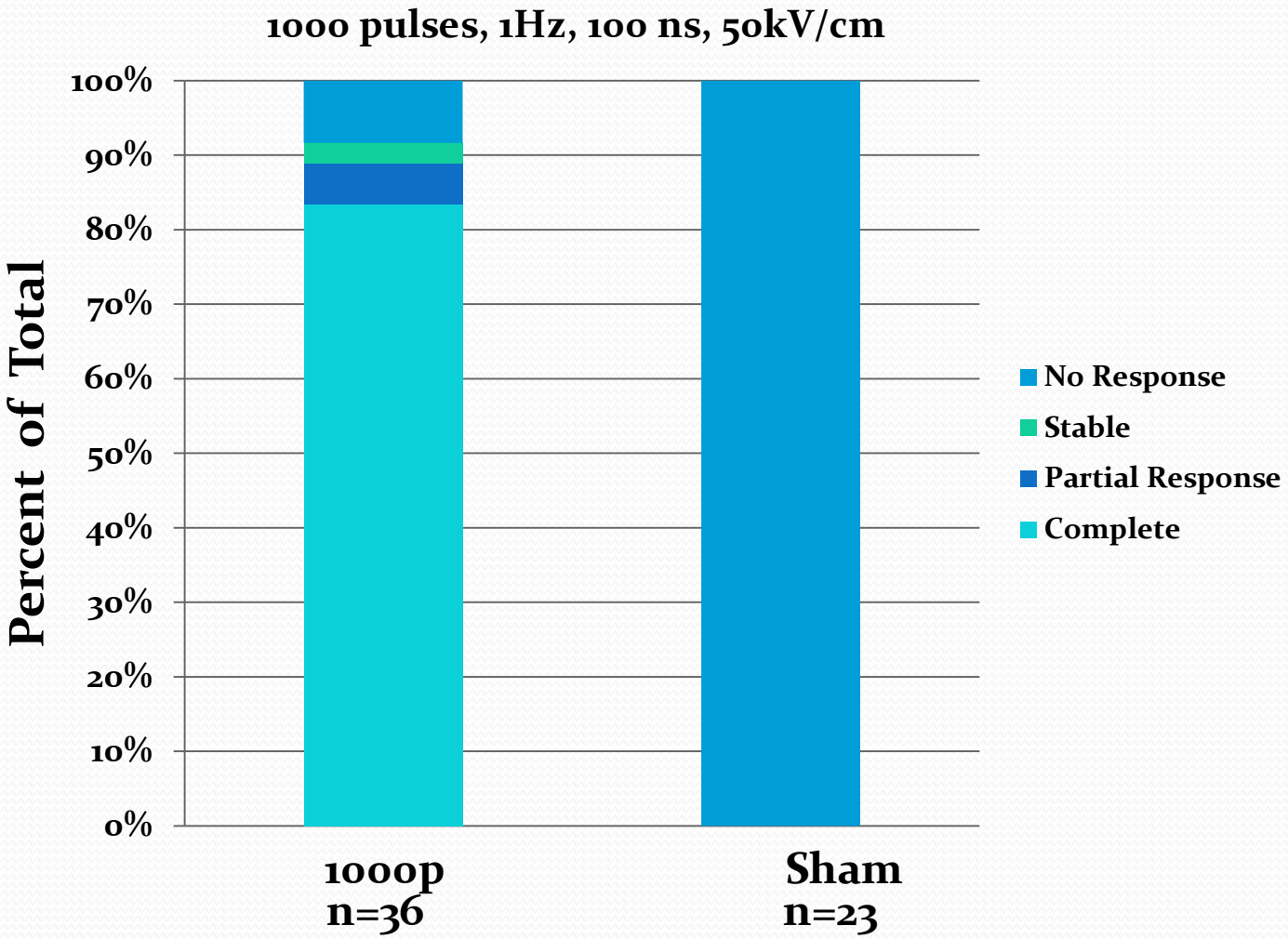
Orthotopic Rat N1S1 Hepatocellular Carcinoma Treated with nsPEFs [100 ns, 50 kV/cm, 1 Hz]

Tumor Volumes 6 Weeks Post-Treatment Percent Change vs. Sham Treatment



Chen R et al., Eur J Cancer 2014, in press

NsPEFs Eliminate 80-90% of Rat N1-S1 HCC Tumors



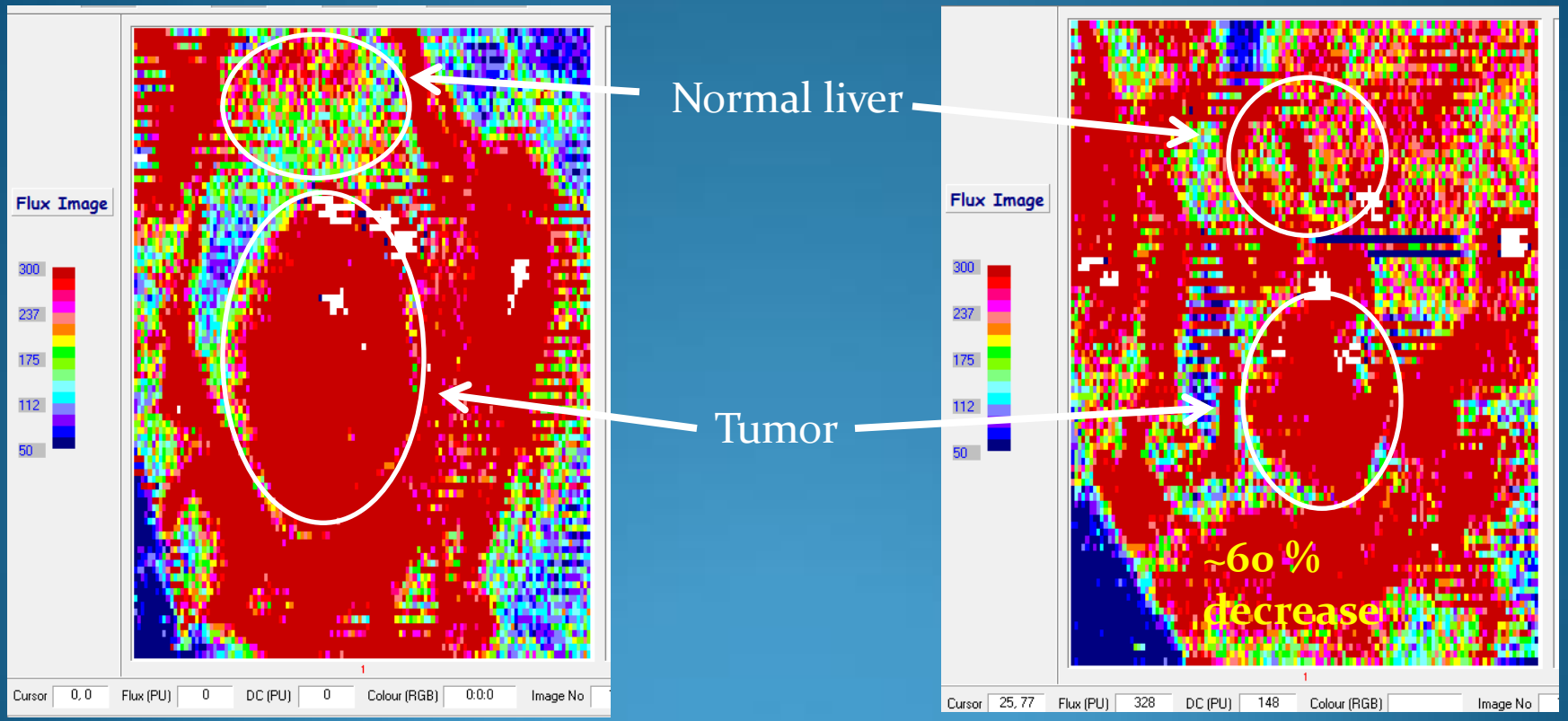
Chen R et al., Eur J Cancer 2014, in press

Journal of Molecular and Genetic Medicine

nsPEFs Decrease Blood Flow – Laser Doppler

Before

After

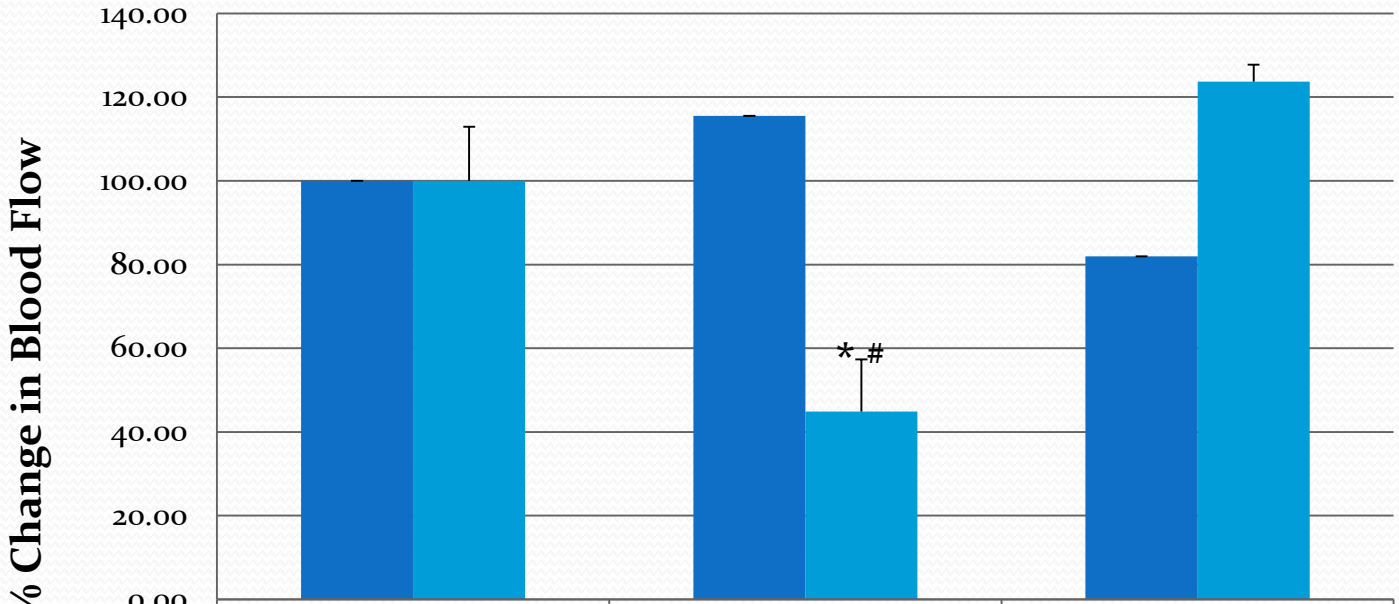


Before Treatment

After Treatment
1000p x 100ns x 50kV/cm

Chen R, Sain NM and Beebe SJ,

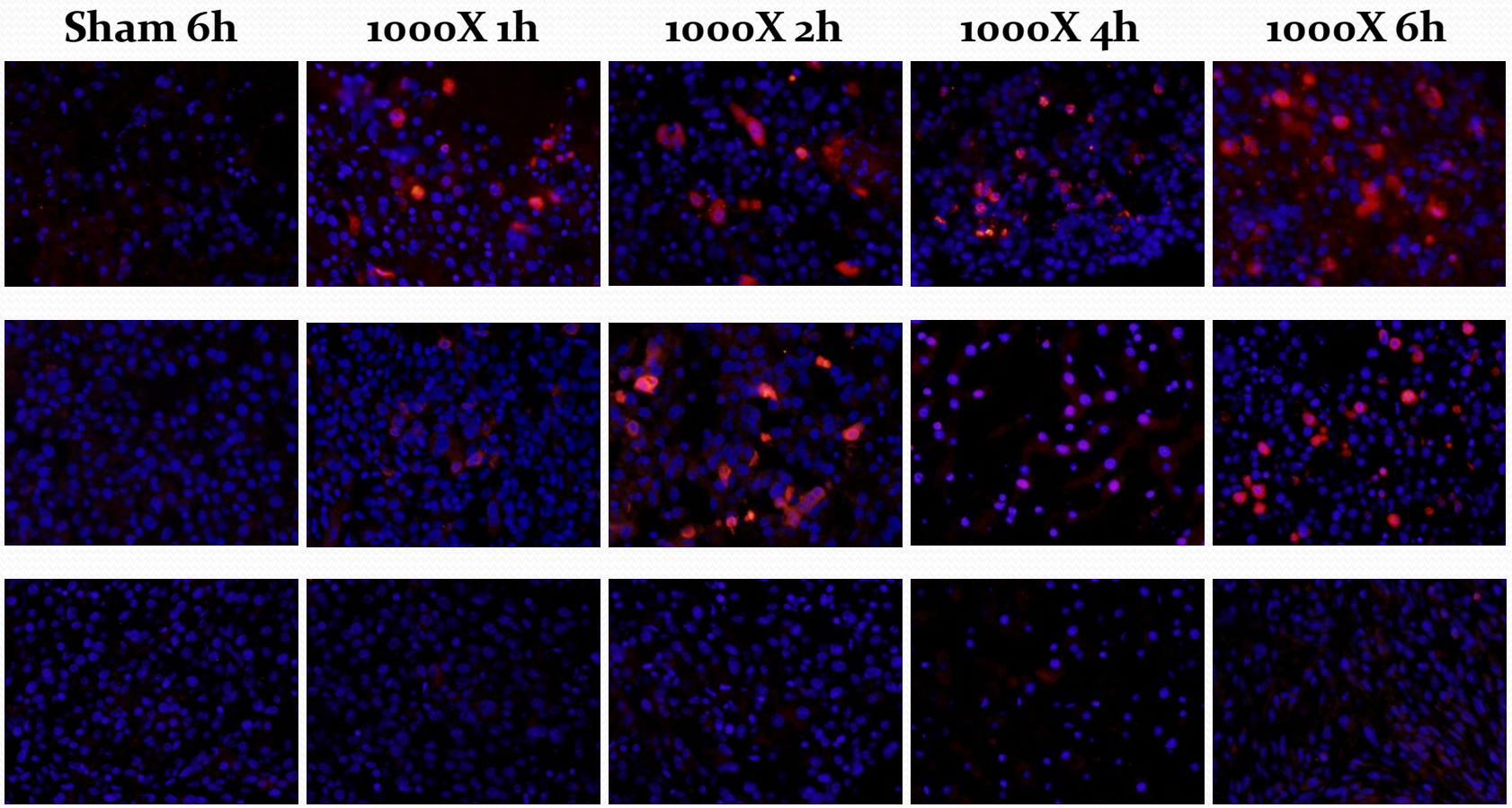
Transient, Tumor-Specific Blood Flow Change



	before treatment	after treatment	7 days post-treatment
Sham (n=3)	100.00	115.48	81.97
1000X (n=7)	100.00	44.85	123.73

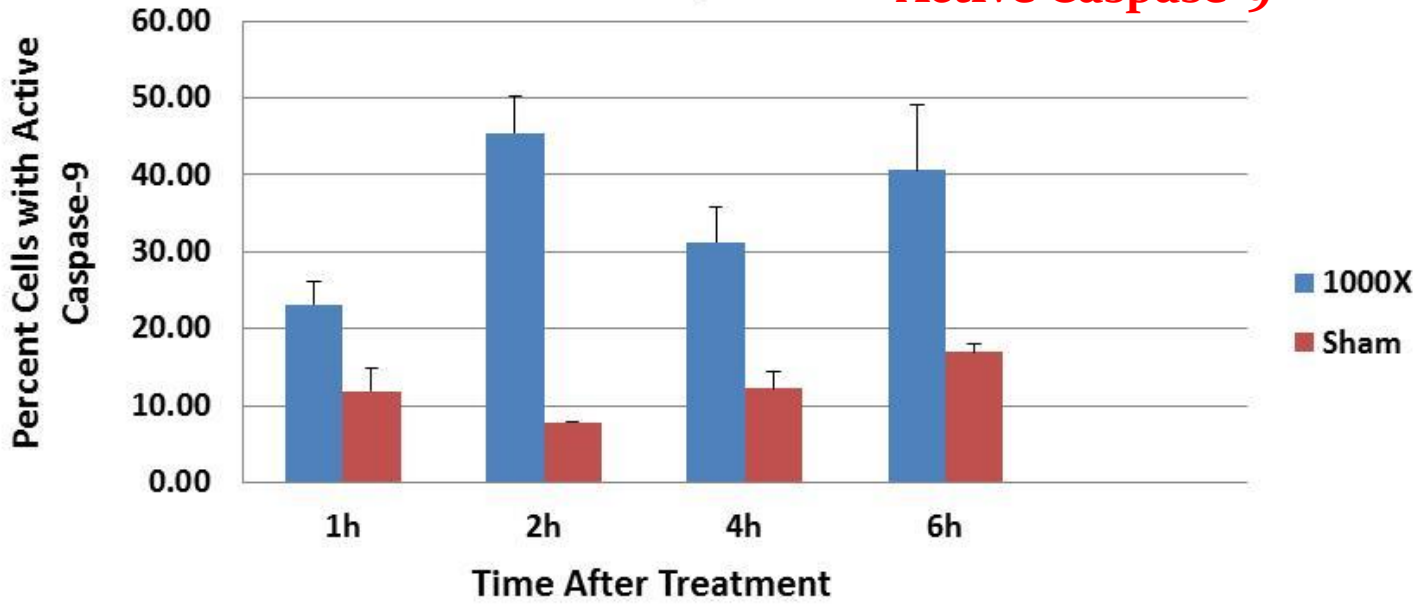
R Chen et al., Surgery: Current Research 2013, S12:

Intrinsic Caspase Activation in N1S1 Tumors

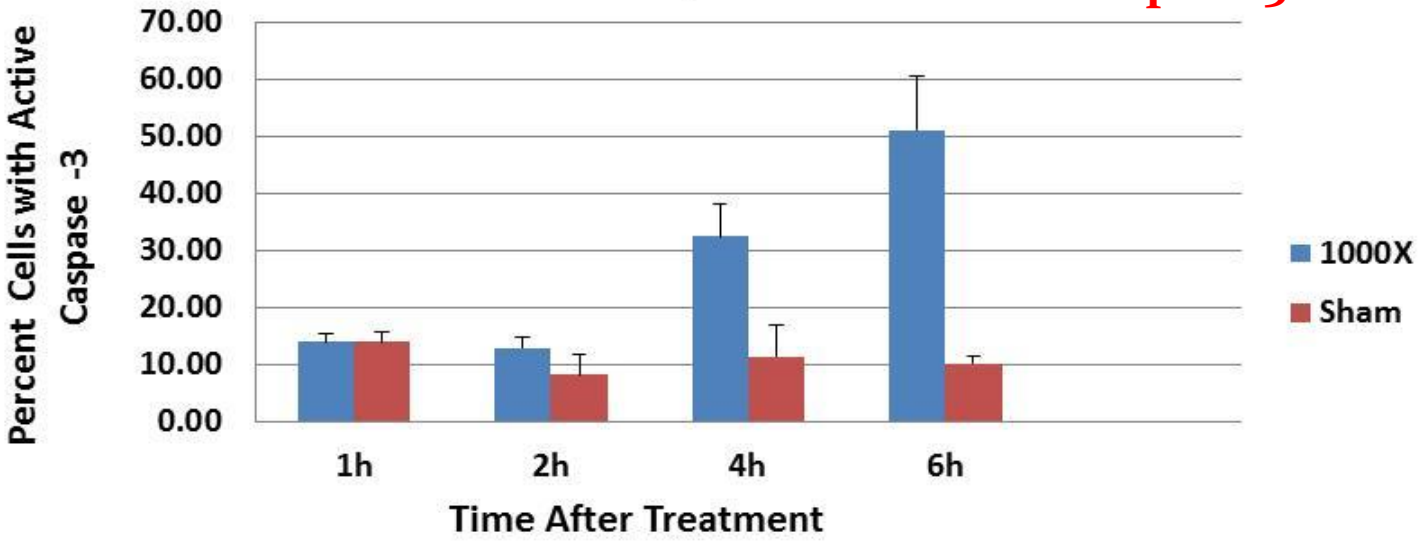


Chen R et al., Eur J Cancer 2014, in press

Active Caspase-9

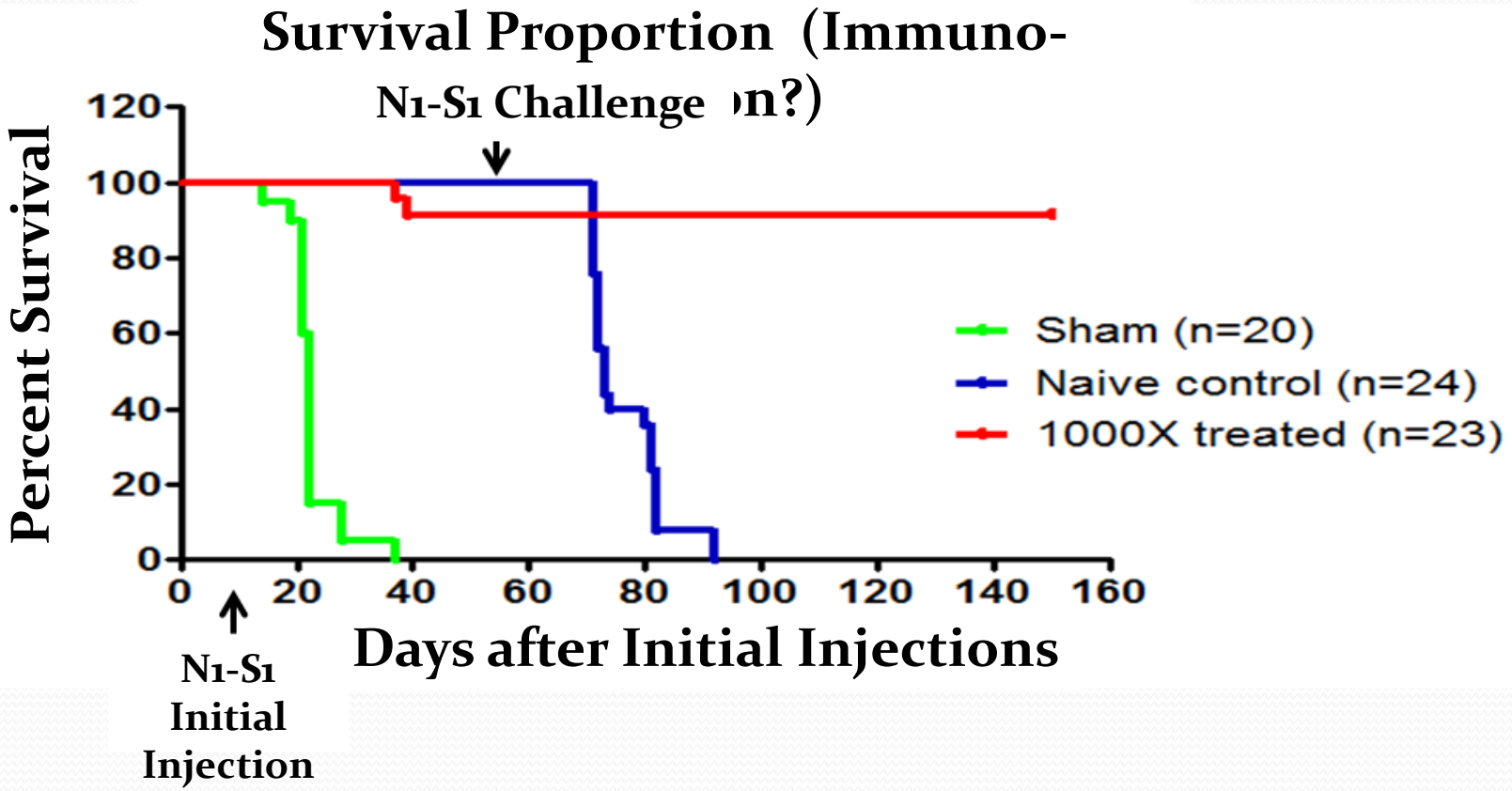


Active Caspase-3



Chen R et al., Eur J Cancer 2014, in press

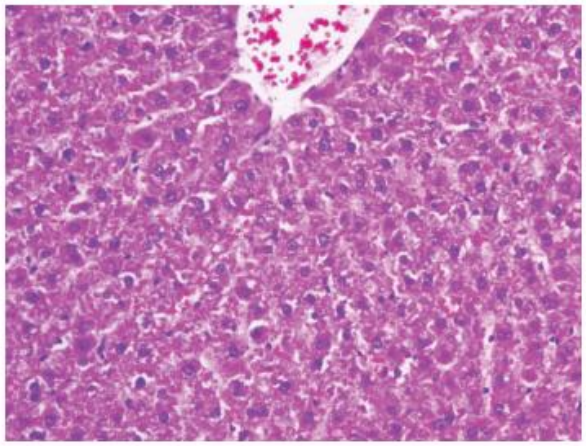
NsPEF Ablation Induces a Vaccine-Like Protective Effects Against N1-S1 HCC



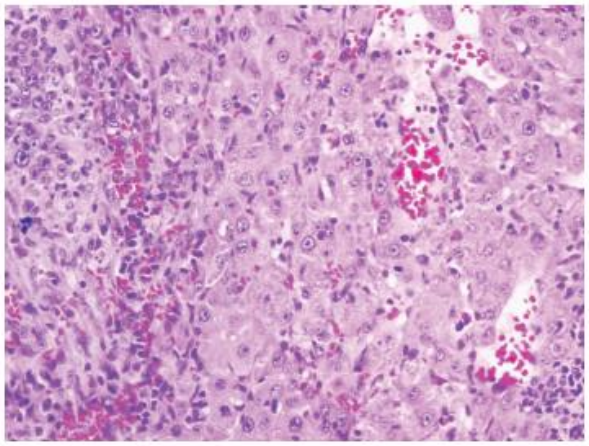
Chen R et al., Eur J Cancer 2014, in press

NsPEFs Induce a Primary Immune Response in N1S1 HCC Tumors
14 Days after Treatment

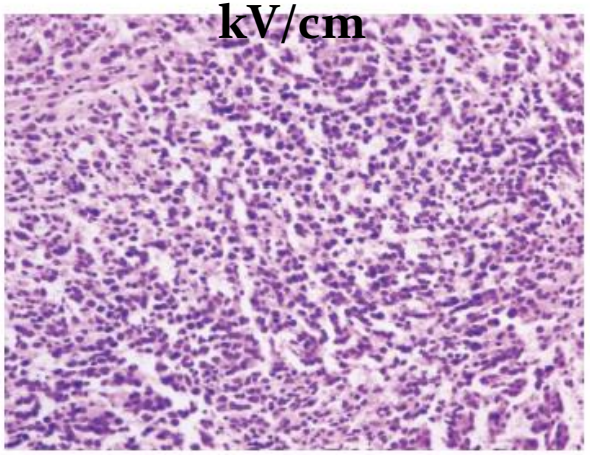
Normal Liver



Sham

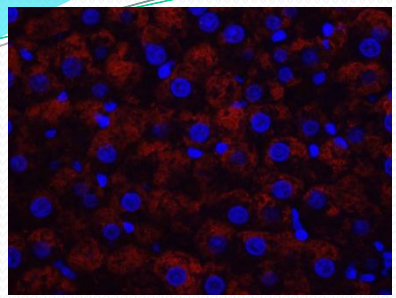


1000 X 100 ns X 50 kV/cm

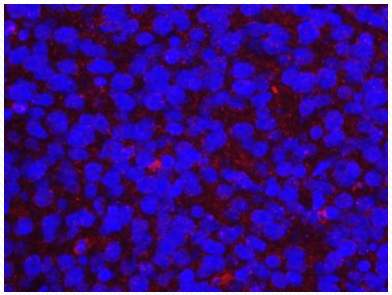


R Chen et al., Surgery: Current Research 2013, S12:

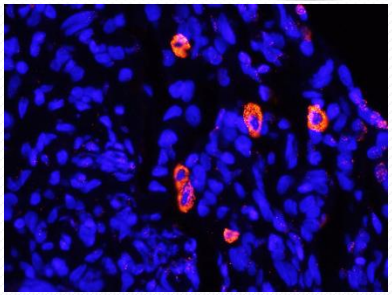
Granzyme B



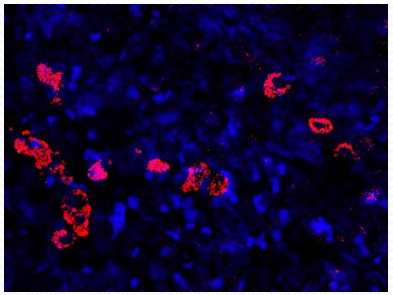
Normal Liver



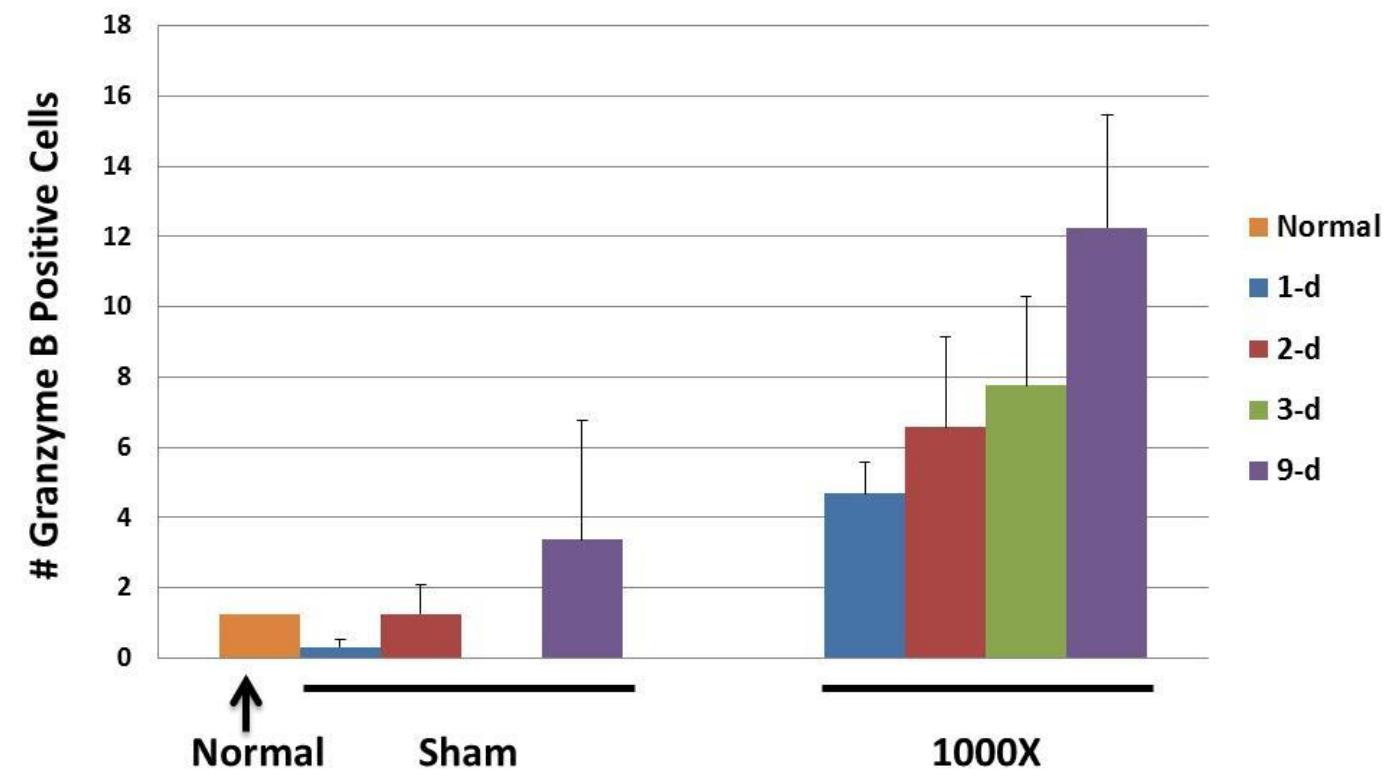
Sham, 1d



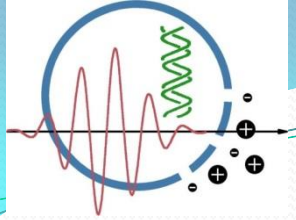
1000ps, 3d



1000ps, 9d



Chen R et al., Eur J Cancer 2014, in press



OLD DOMINION UNIVERSITY
IDEA FUSION

Pulse Power with nsPEFs Ablates 80-90% of N1-S1 HCC Tumors

Ablation with 1 Treatment without Recurrence

Induces Caspase-Dependent and –Independent Cell Death

Induces Transient Decrease in Tumor Blood Flow

Provides a Post-Ablation Protective Vaccine-Like Effect

Activates Innate and/or Adaptive Immune Responses

Advantages with nsPEFs

1. Targets multiple cell death mechanisms
2. Well defined treatment zones
3. Targets mitochondria and PMs – bypasses cancer mutations
4. Broad cell death specificity (tumor & host cells, cancer stem cells)
5. Local infarction of small vessels
6. Minimal local and systemic side effects
7. Possibly enhances immune surveillance
8. No need to block muscle contractions

Acknowledgments

Who Did the Work

Dr. Ru Chen — *In Vivo*

Dr. Wei Ren — *In Vitro*

Ms Nova M Sain — *In Vitro* and *In Vivo*

Ms K. Tyler Harlow — *In Vitro* and *In Vivo*

Peter Shires and Richard Heller

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Mr. Frank Reidy (Research Center for Bioelectrics)

Journal of Molecular and Genetic Medicine

- Drug Designing: Open Access
- Journal of Molecular Biomarkers & Diagnosis

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