

Radiation induced bystander effects adaptive responses and low dose risk



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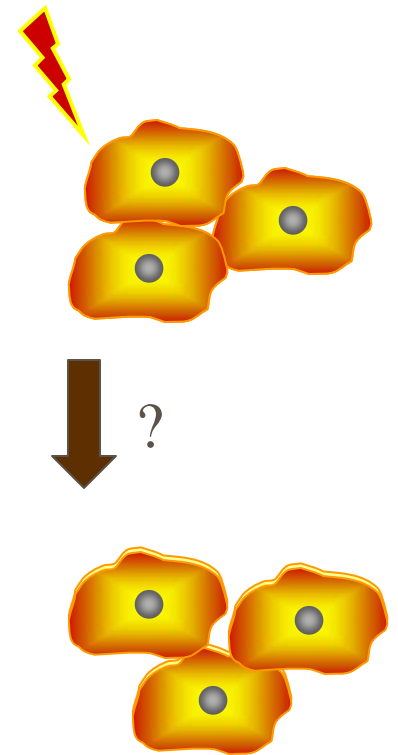



Outline

- Background on the bystander effect
- Phenomenology
- Mechanisms
- Implications for radiation protection
- Implications for therapy
- Data gaps and future approaches

Bystander Effect

- **Communicated damage**
 - Non-linear dose response
 - History - Clastogenic factors
 - Laboratory methods
 - Targeted irradiation
 - Medium transfer
 - Search for the “effector”





Bystander effects - What responses are seen?

- Apoptosis and other forms of cell death
- Induction of early response proteins
- Oxidative stress
- Proliferation
- Genomic instability
- Cytogenetic effects
- Transformation

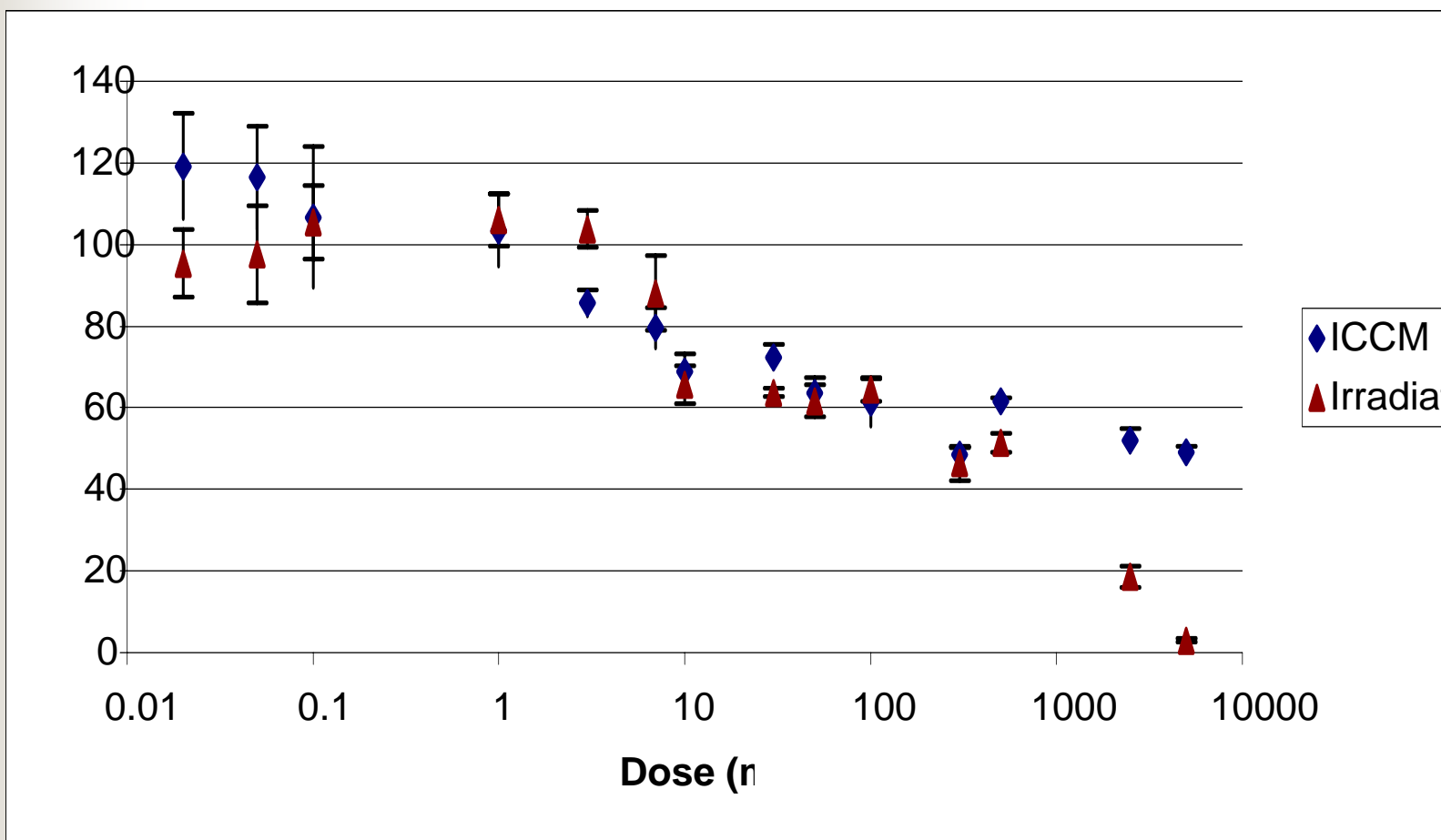


Medium transfer bioassay

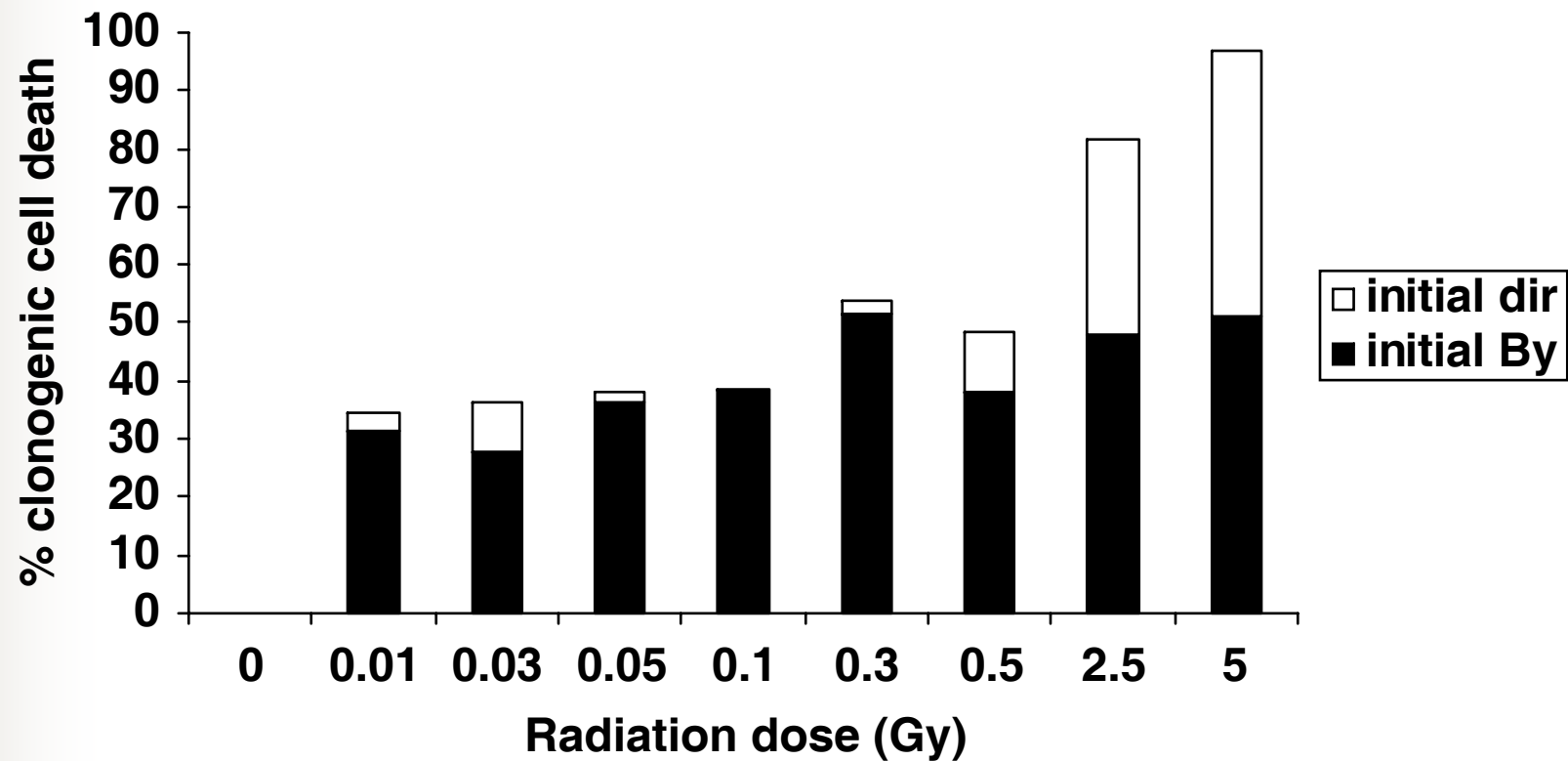
- Cell cultures seeded with a large number of cells are exposed to radiation
- After 1 hr the culture medium is harvested and filtered to remove debris
- The medium is then transferred to unirradiated reporter cells seeded at cloning density
- Samples of medium are reserved for calcium and serotonin assay


Can be applied to tissues or whole animals using explant technique

Bystander and direct dose survival curves over six orders of magnitude ^{60}Co



Direct v bystander effect





Issues in relation to the central role of DNA damage in radiobiology - possible conflicts?

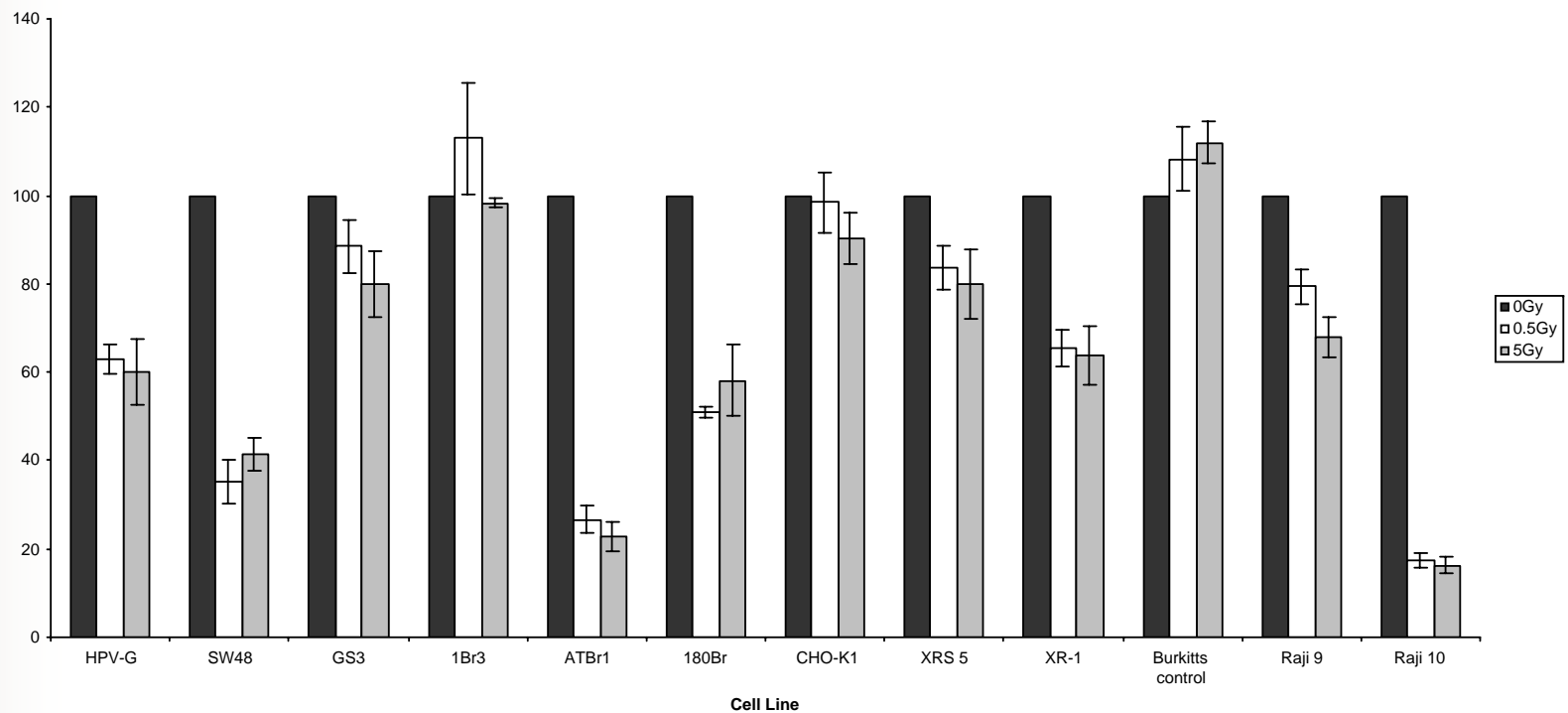
- Effects in cells which were never hit but received signals from hit cells
- No increase in effect with increasing dose, the lowest possible high LET “dose” to a population- 1 track to one cell or a very low acute low LET dose (3mGy) -turns on the population effect
- P53 status of the cells not critical therefore the pathway characteristic of DNA damage response may be circumvented in this situation



Factors suggesting a major involvement of DNA direct damage in producing bystander effects

- Genetic factors are involved in the signaling and response pathways
- DNA repair deficient cells have very toxic responses to bystander signals
- Bystander mechanisms seem to drive genomic instability


Different bystander effects depending on repair ability





Factors not supporting a direct DNA damage involvement

- Lack of a classical dose response
- Induction of large effects in the mGy region
- Negative effect of dose fractionation
- No clear effect of neutron irradiation
- Evidence for effects following EM field exposure



Bystander effects - How are they expressed?

- Initial mechanism similar to a stress response [ROS elevated]
- Long-term perpetuation appears to involve genomic instability type mechanisms
- Final outcome determined mainly by genetic make-up and life-style factors



What is the signal?

- Nature of the signal is unknown
- Destroyed by repeated freeze thaw cycles and destroyed by heating, appears to have a very small size (<400 daltons).

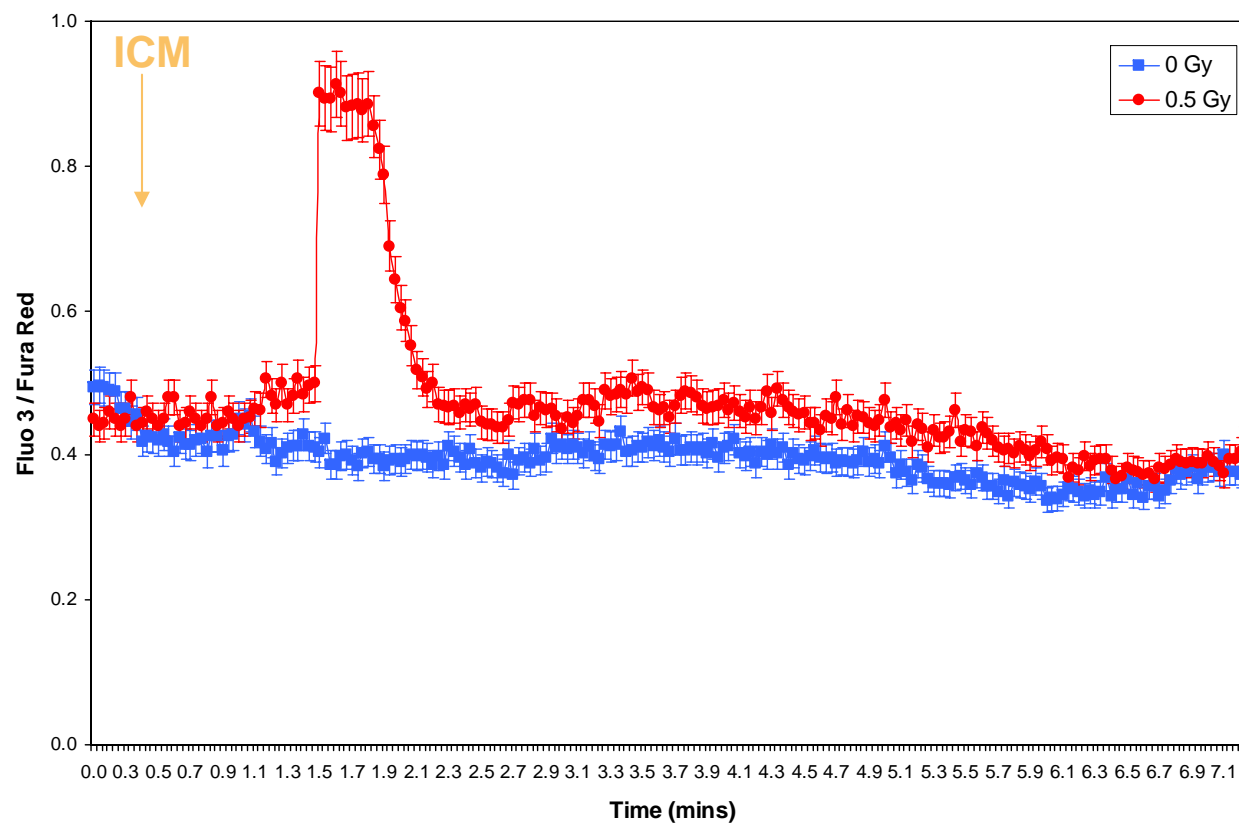


Transduction of the response

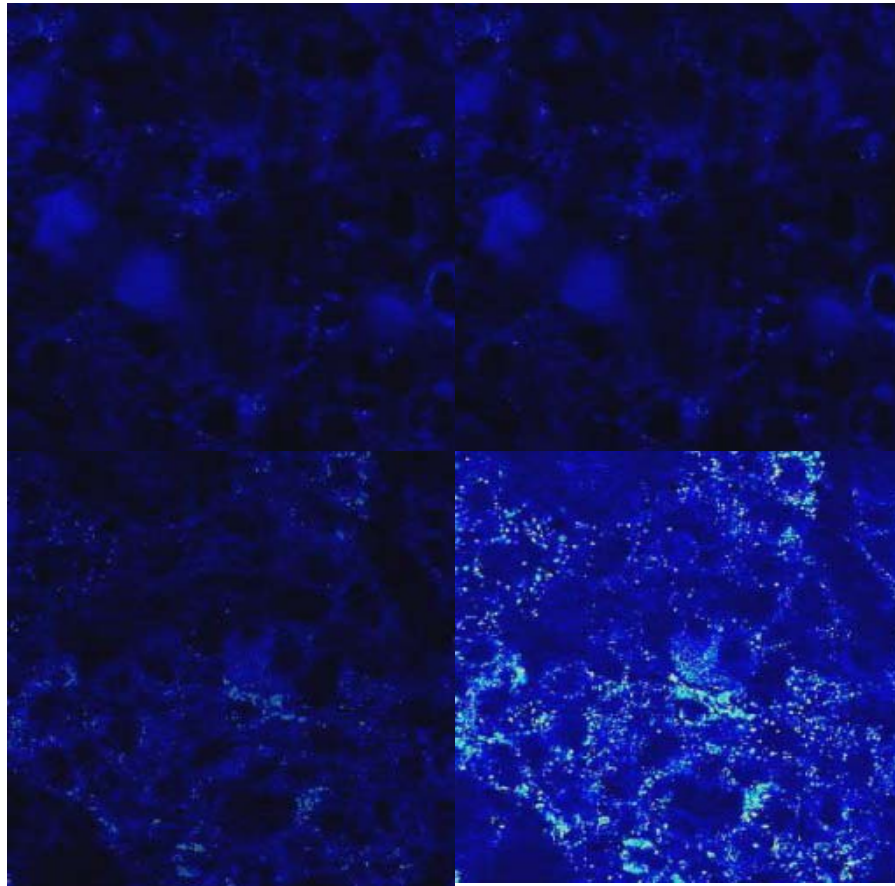
The initial cellular response to the signal in human keratinocytes

- Induction of 2 min calcium flux within in 10sec of receiving ICCM
- Longterm (greater than 6hrs) induction of mitochondrial membrane potential collapse and induction of downstream apoptosis steps
- Longterm induction of oxy-radical production
- p53 independent

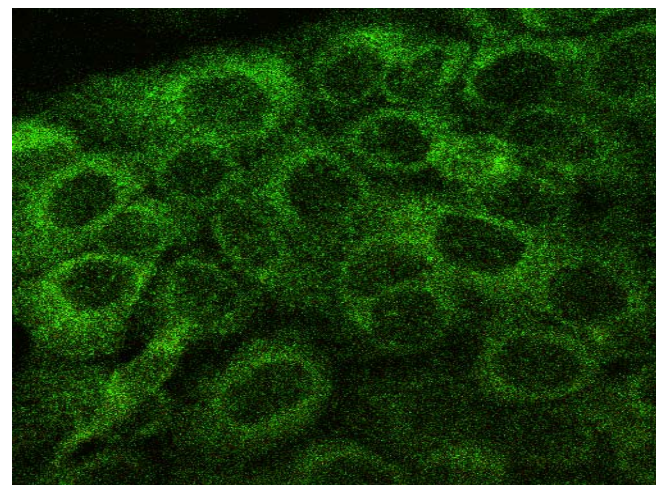
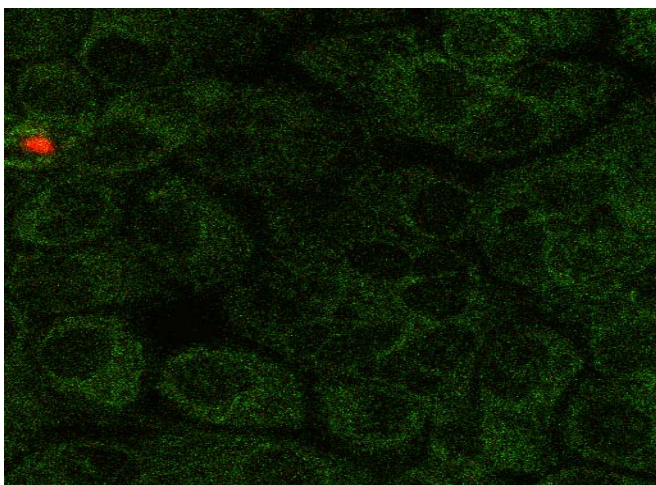
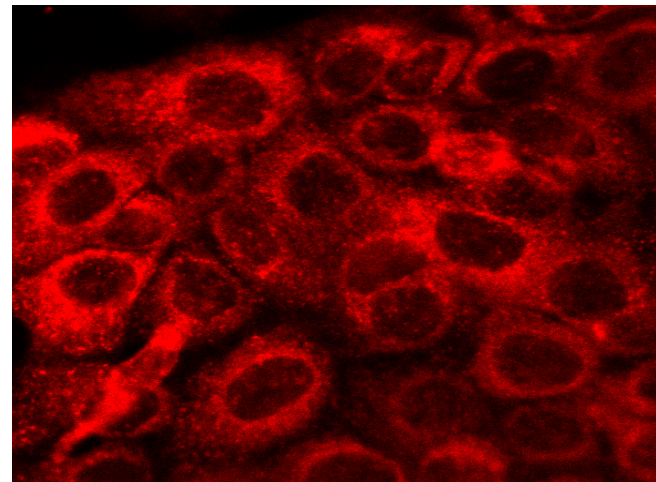
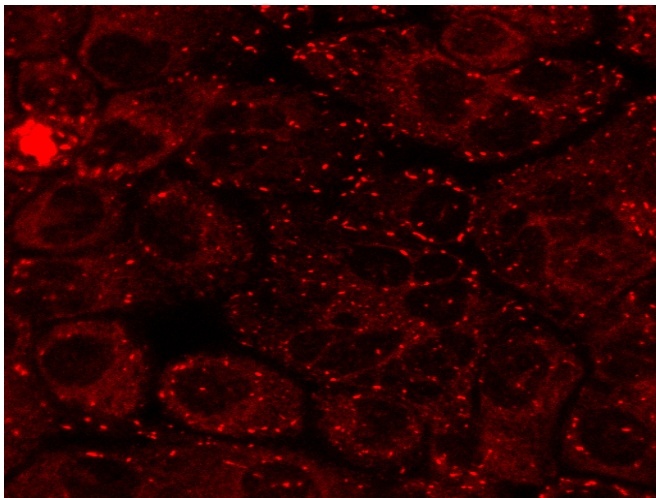
Calcium pulse following addition of 0.5Gy ICCM to cells



Calcium fluorescence following addition of ICCM to cells



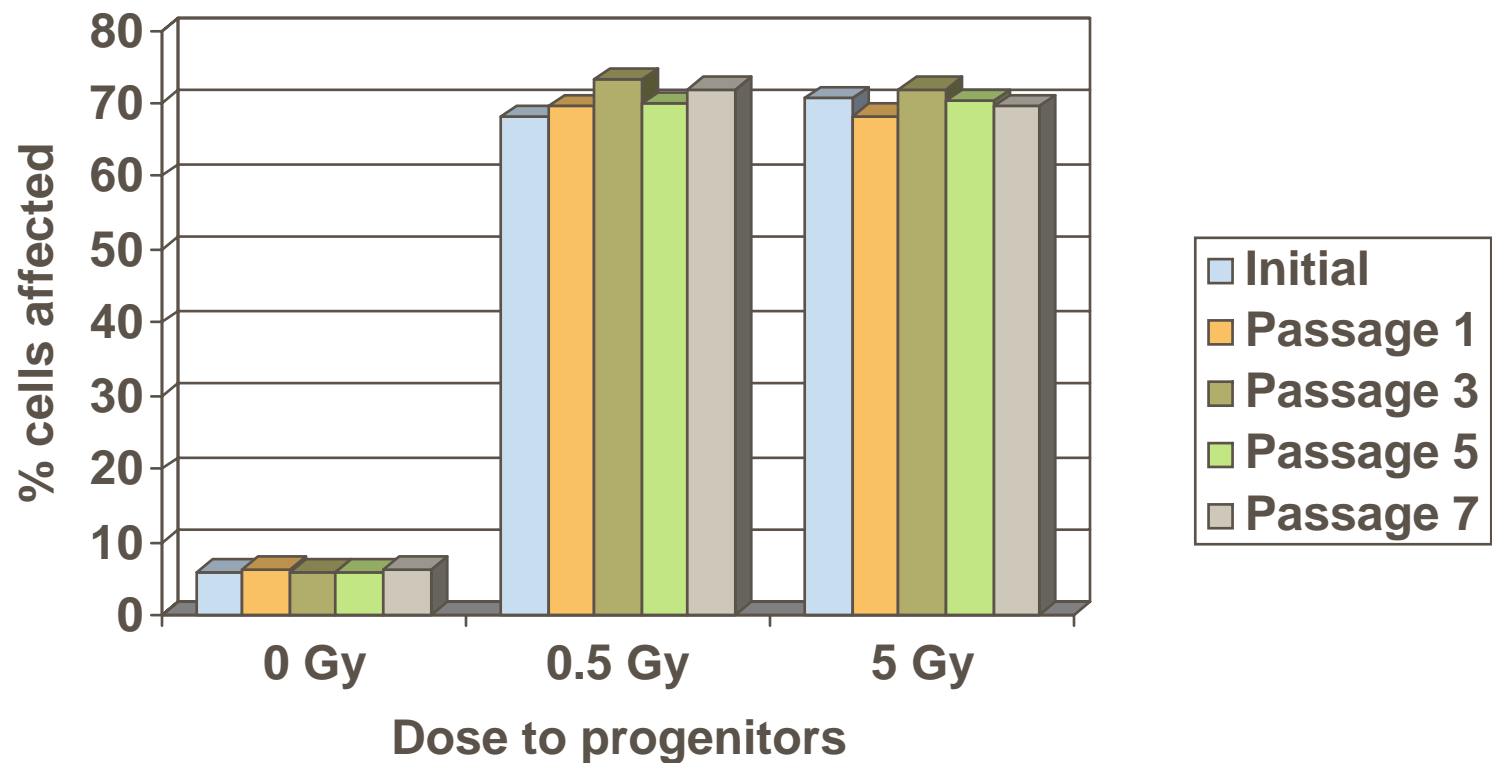
Mitochondrial membrane depolarisation



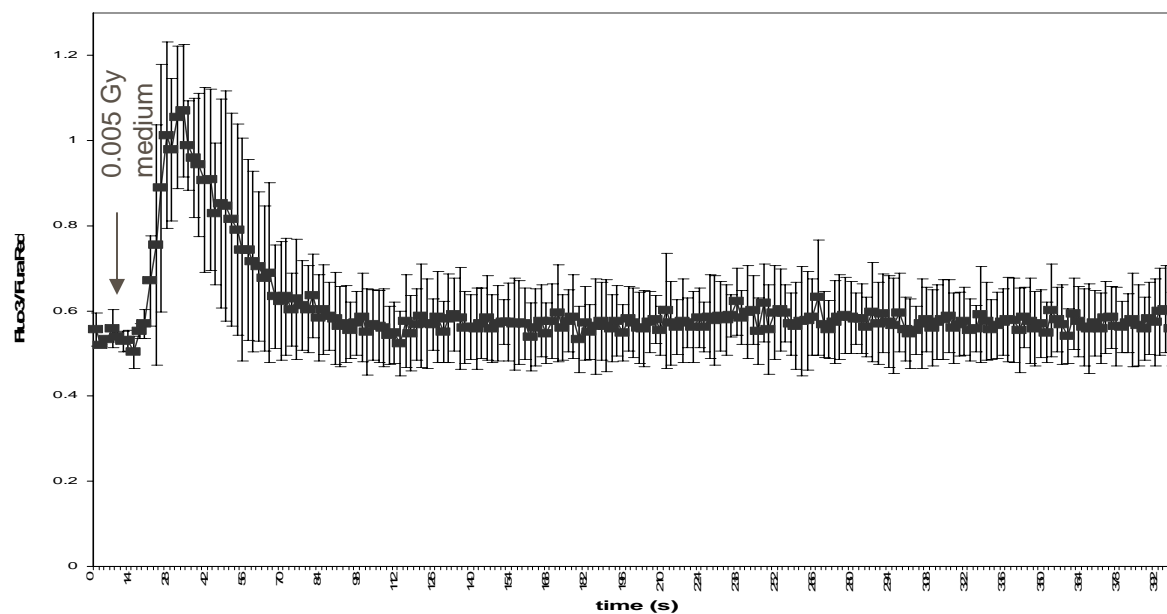
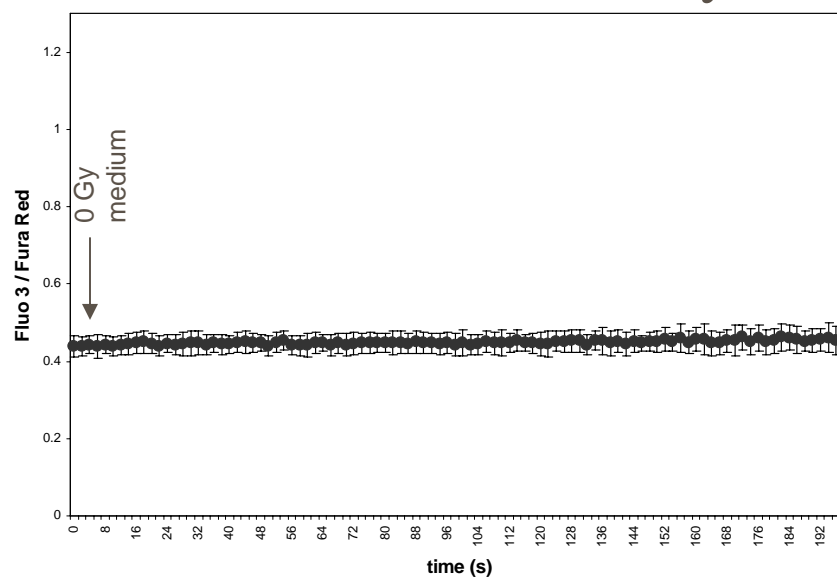
0 Gy

0.005Gy

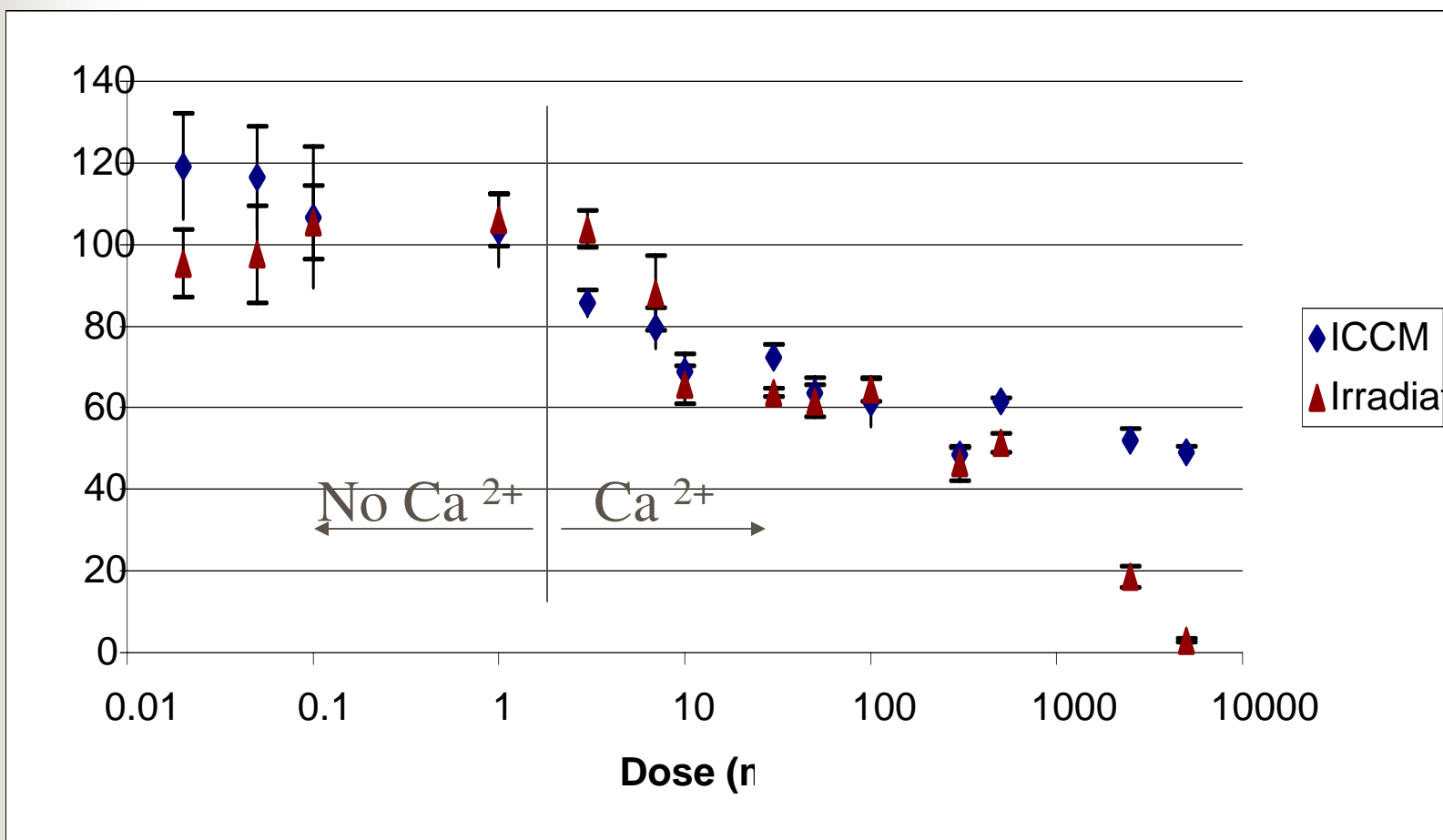
% cells showing increased ROS following ICCM



Signal after exposure to ICCM from 0.005Gy irradiated cells



Bystander and direct dose survival curves
over six orders of magnitude ^{60}Co with calcium data





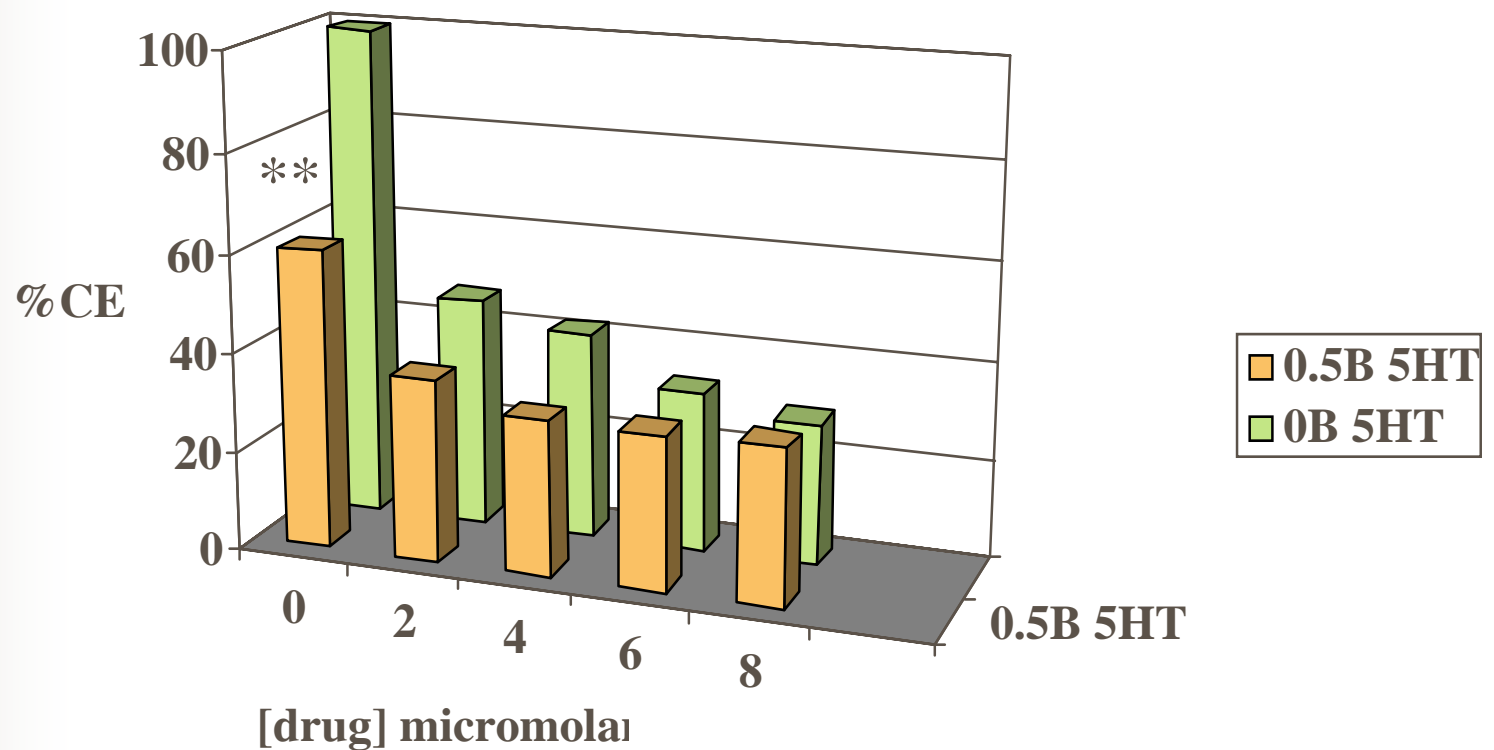
Calcium homeostasis hypothesis

- Calcium influx is the first response in the hit cells and in medium recipients
- Which channels? L channel blockers stop effect
- Serotonin, 1-deprenyl and reserpine all effect calcium homeostasis and all modulate the effect
- Intracellular calcium homeostasis controlled in mitochondria - role of bcl-2?

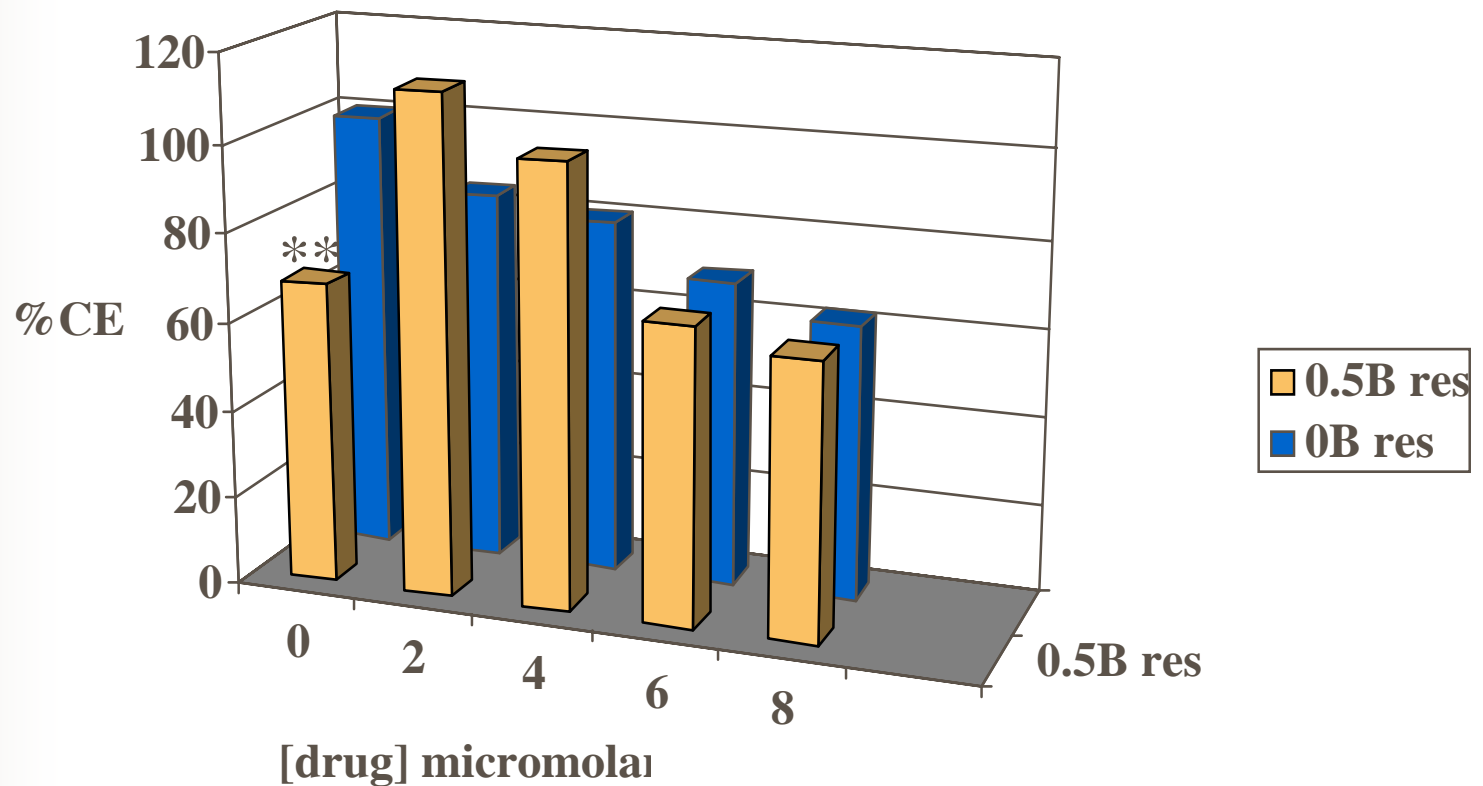
Table1 Peak Fluor 3 /Fura Red ratio value in cultures exposed to 0Gy ICCM or 0.5Gy ICCM in the presence of inhibitors of calcium and ROS. An increase in ratio value indicates an increase in calcium. * p<0.01, ** p<0.05


| | 0Gy ICCM | 0.5Gy ICCM |
|---------------------|-----------------|-------------------|
| No inhibitor | 0.54 ± 0.01 | 1.17 ± 0.02** |
| EGTA | 0.45 ± 0.02 | 0.46 ± 0.01 |
| Verapamil | 0.47 ± 0.02 | 0.46 ± 0.03 |
| Nifedipine | 0.50 ± 0.03 | 0.49 ± 0.04 |
| Thapsigargin | 0.47 ± 0.01 | 0.72 ± 0.02* |
| SOD | 0.55 ± 0.01 | 0.51 ± 0.01 |
| Catalase | 0.54 ± 0.01 | 0.55 ± 0.01 |

Bystander effect of 5HT with 0.5Gy



Bystander effect of reserpine and 0.5Gy





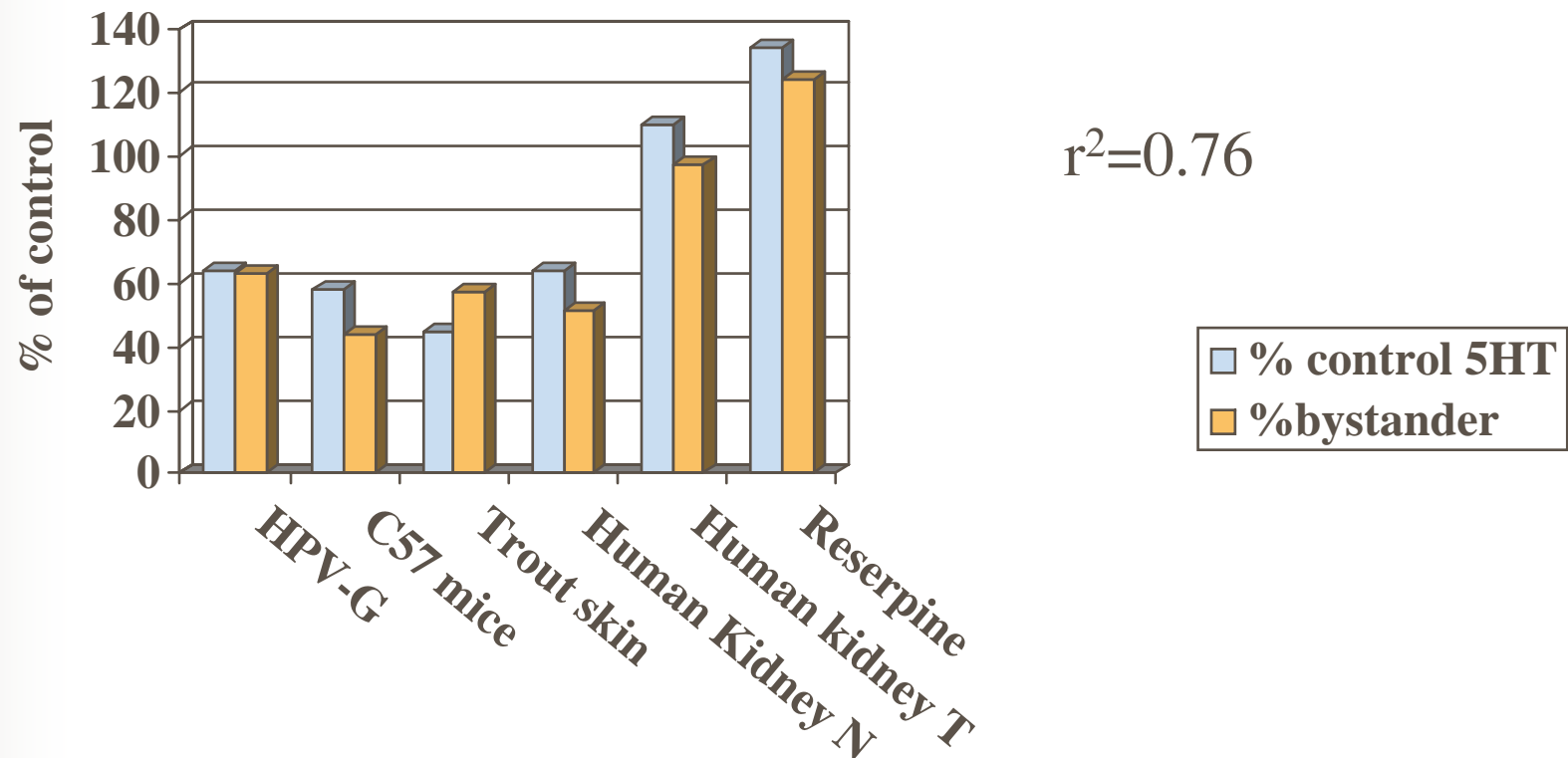
Cell colonies pretreated with reserpine

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

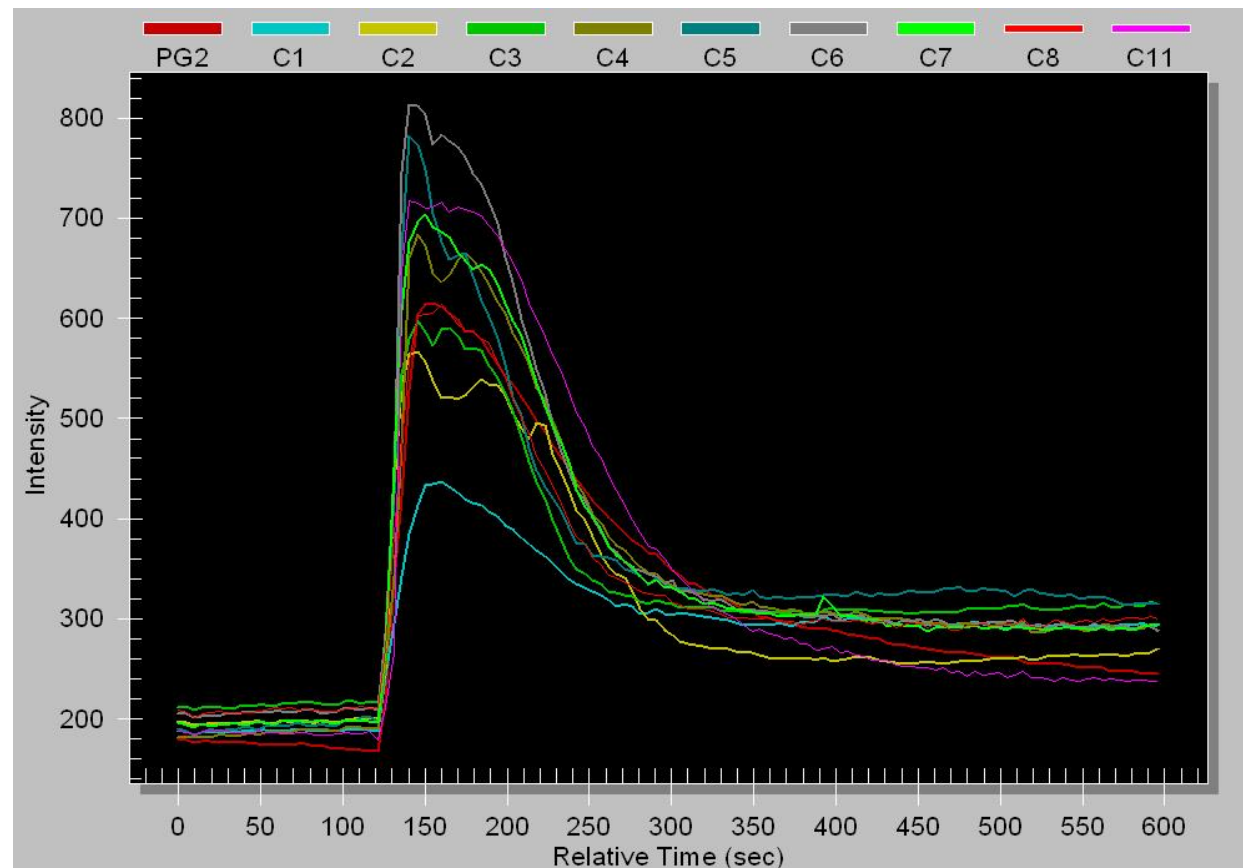
Control
Bystander medium

+10nM
Reserpine

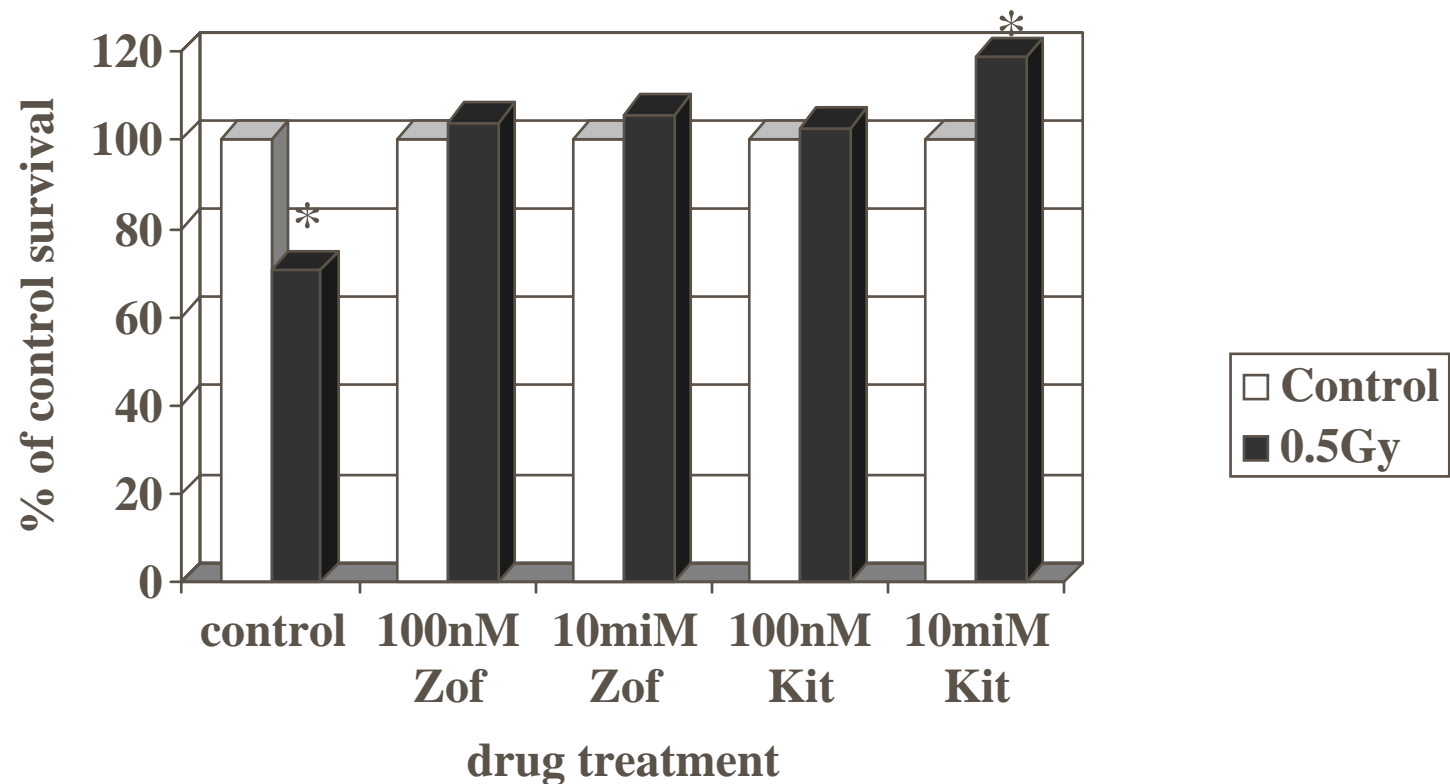
Serotonin depletion following irradiation, and the bystander effect



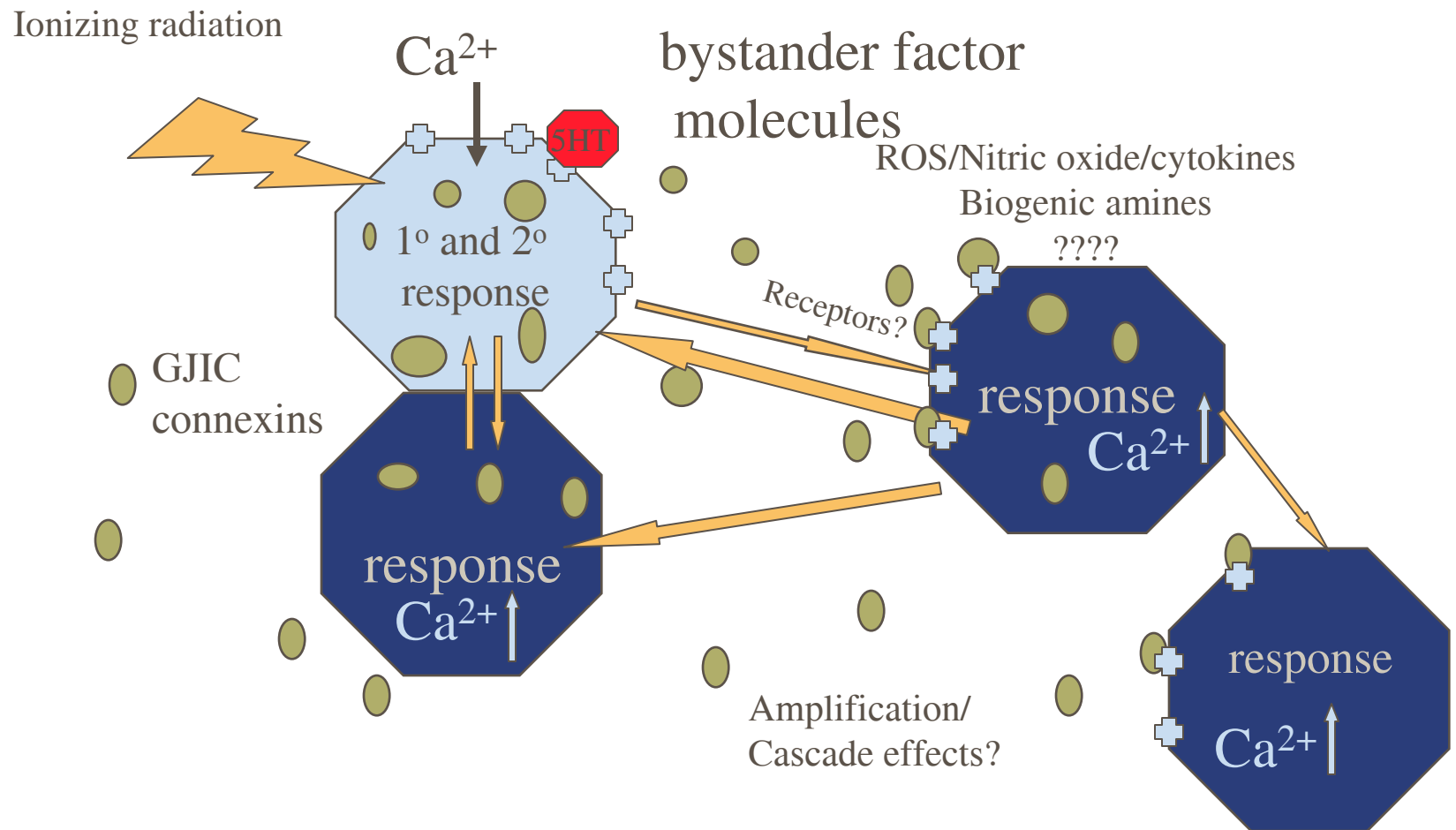
Calcium flux induced in HPV-G cells by 8micromolar 5HT



Effect of 5HT 3 receptor inhibitors Zofran and Kitryl,



The bystander effect





Is the effect relevant in vivo??

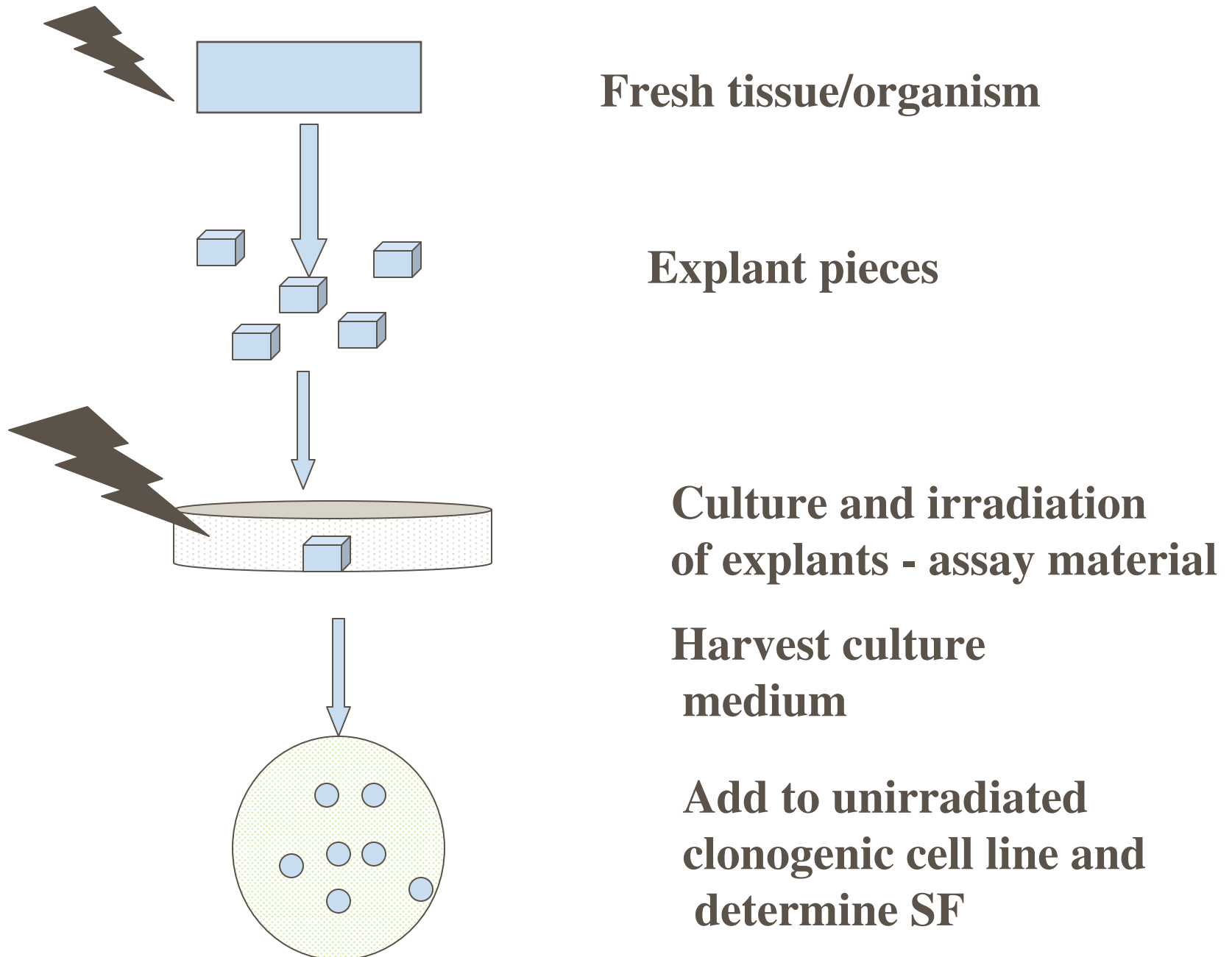
- Evidence from fresh human tissue irradiated ex vivo
- Evidence from Mice irradiated in vivo to low total body doses
- Evidence from bloods taken from radiotherapy patients showing variation during therapy
- Fish model



Methods for detecting signals in tissues

- Media harvest from exposed explants or whole tissues
- Detection of signals using reporter cells which are exposed only to media from exposed samples
- Endpoints include growth, apoptosis, protein expression, calcium fluxes and mitochondrial responses

Measuring bystander response *in vitro* or *in vivo*

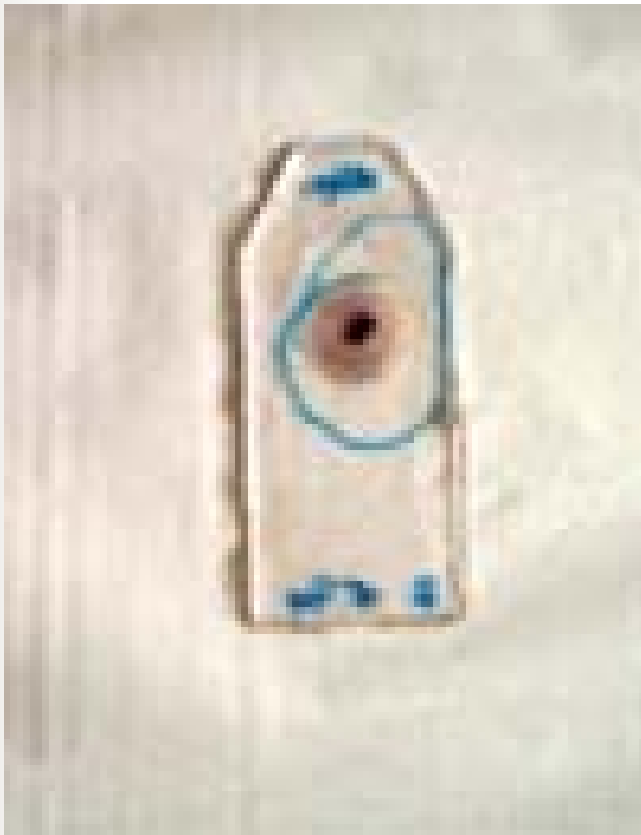




Human data

- 300 normal human urothelial samples show wide variation between subjects
- 50 samples from benign prostate where blood samples from the same patient were available show correlation between response of both tissues
- Data for radiotherapy patients' blood showing changes in bystander effect during therapy
- New data from nephrectomy patients show normal tissue signals following ex vivo irradiation but none from tumour cells

Explant technique



Original tissue explant
with cells stained in situ

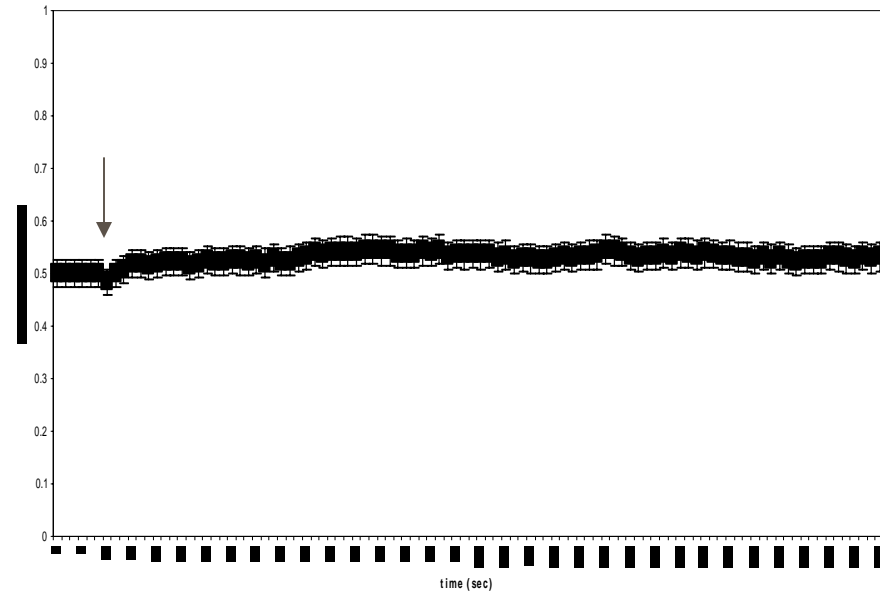


Mouse data

- Bladders taken from mice given 0.5 Gy TBI or irradiation to bladder explants ex vivo.
- CBA/Ca strain is radiation resistant, C57Bl/6 is radiosensitive
- Apoptotic cascade induced in cells exposed to signals from the sensitive mice only

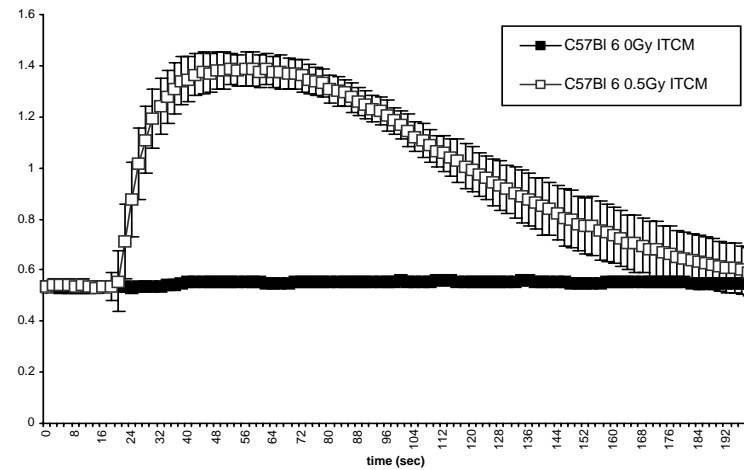
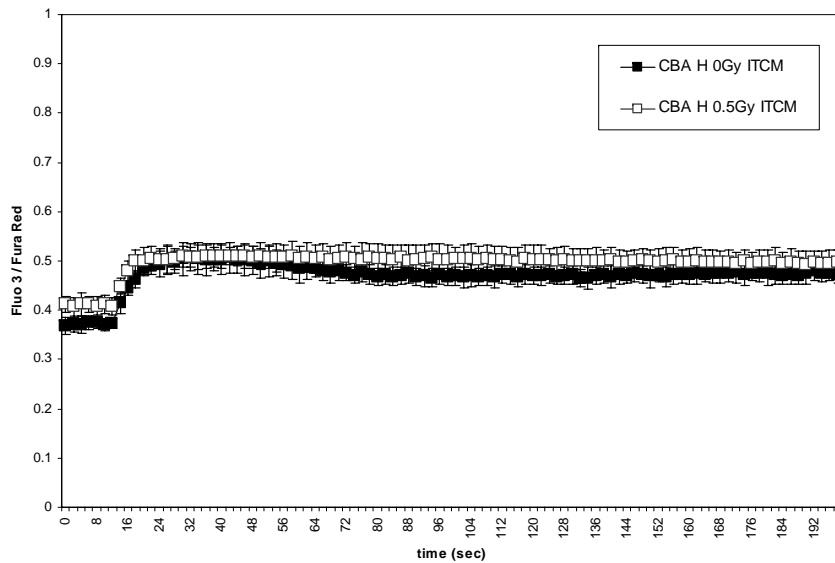
Calcium ratios in control and 0.5Gy TBI CBA/Ca and C57BL/6 mice

Medium from unirradiated tissues from both strains

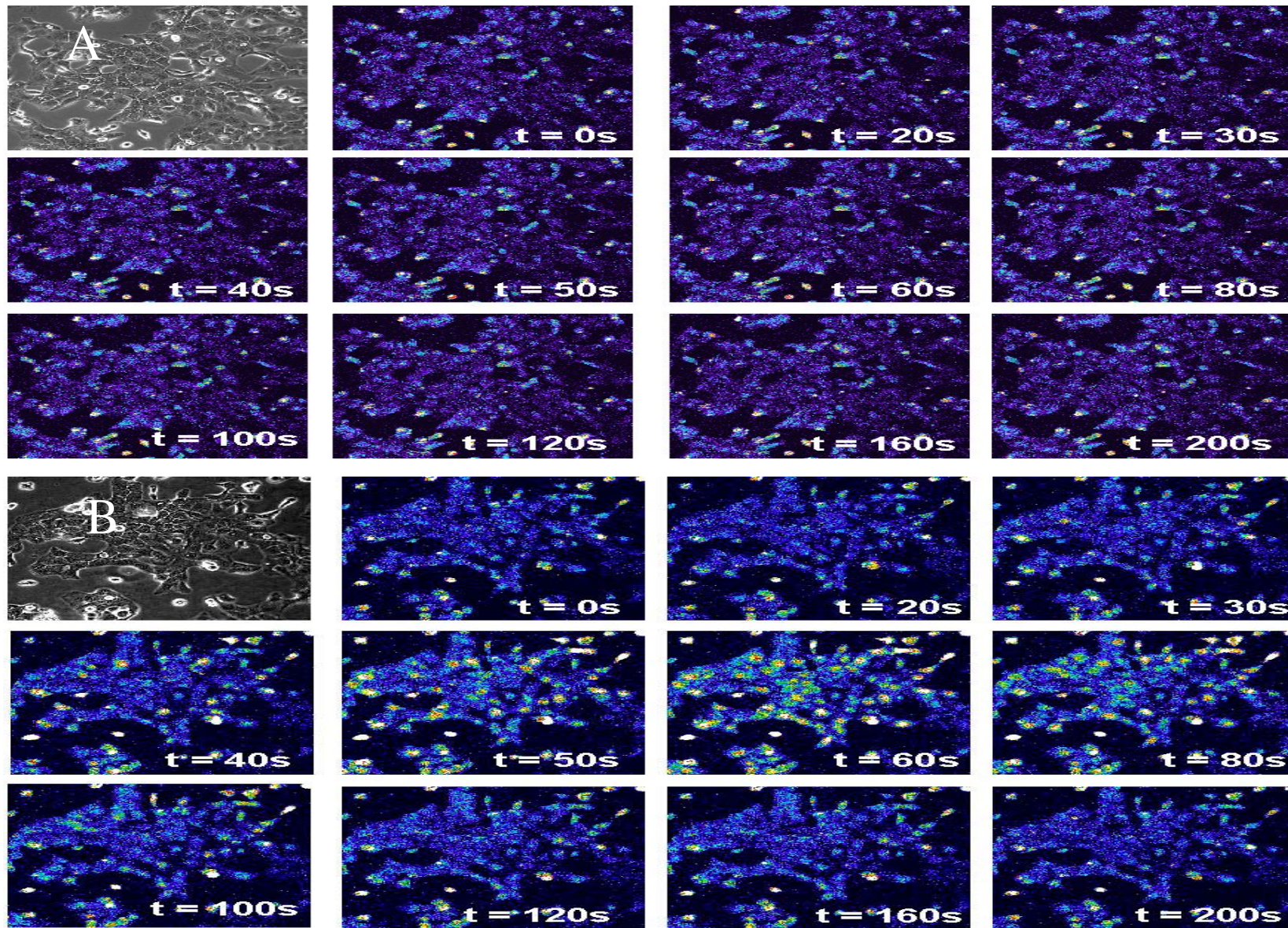


C57BL/6

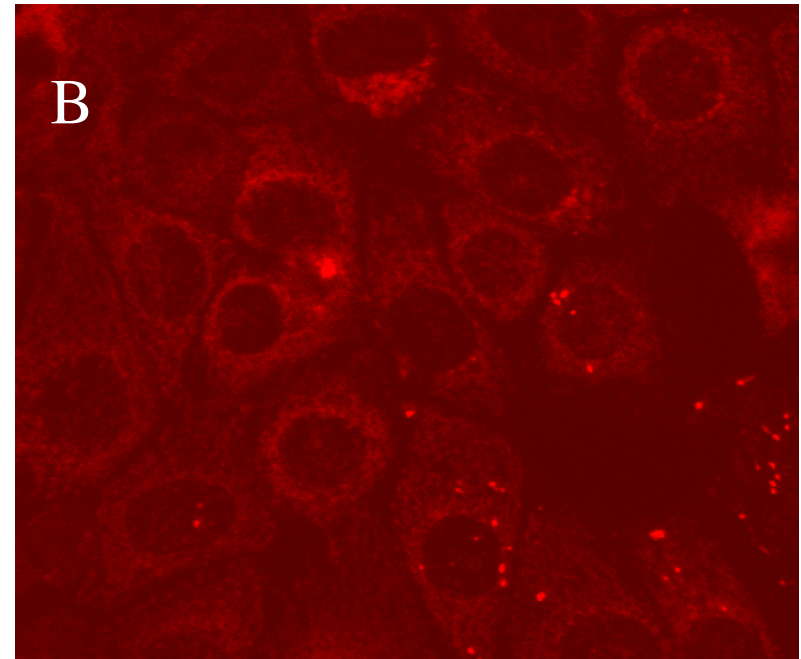
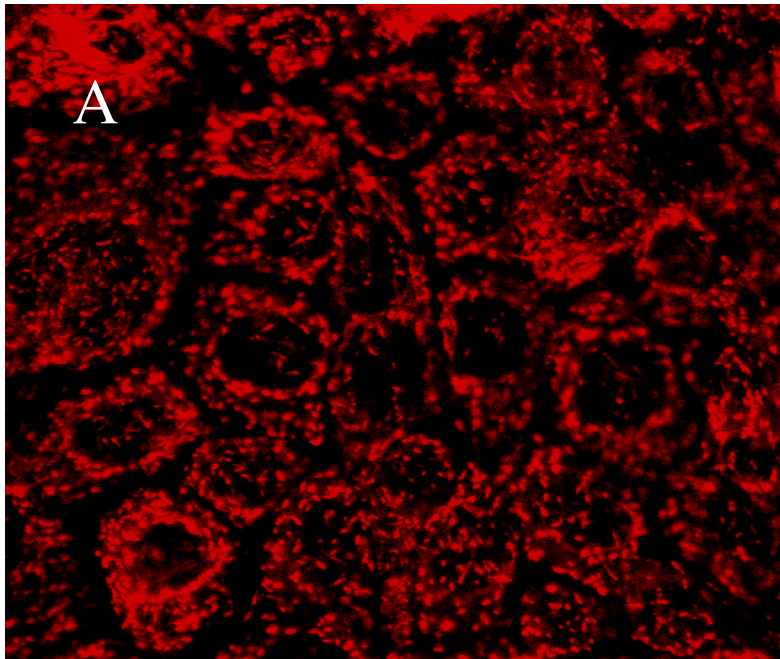
CBA/Ca



Real time calcium flux for Control and CBA/Ca mice (A) and C57BL 6 0.5Gy TBI (B)



Mitochondrial membrane potential decrease in C57BL/6 0.5Gy TBI



Fish data



Truly truly in vivo!!!!

What do bystander effects do to radiation protection?

- Dissociate
 - Dose from effect
 - Effect from harm
 - Harm from risk
- Enables the concept of a “zone of uncertainty” where outcome can be assessed relative to the context in which the dose is delivered



So Bystander effects are BAD?

- Nothing is black and white!
 - Our reporter assay responds by inducing cell death
 - Genetic background predetermines response options
 - Lifestyle factors such as smoking decrease apoptosis following exposure to bystander signals
 - Magnitude of dose is not as important as response to dose
 - Bystander factors following chronic or repeated exposures appear to be very complex

Bottom line: *effect* does not equate with *harm*



Link to adaptive response

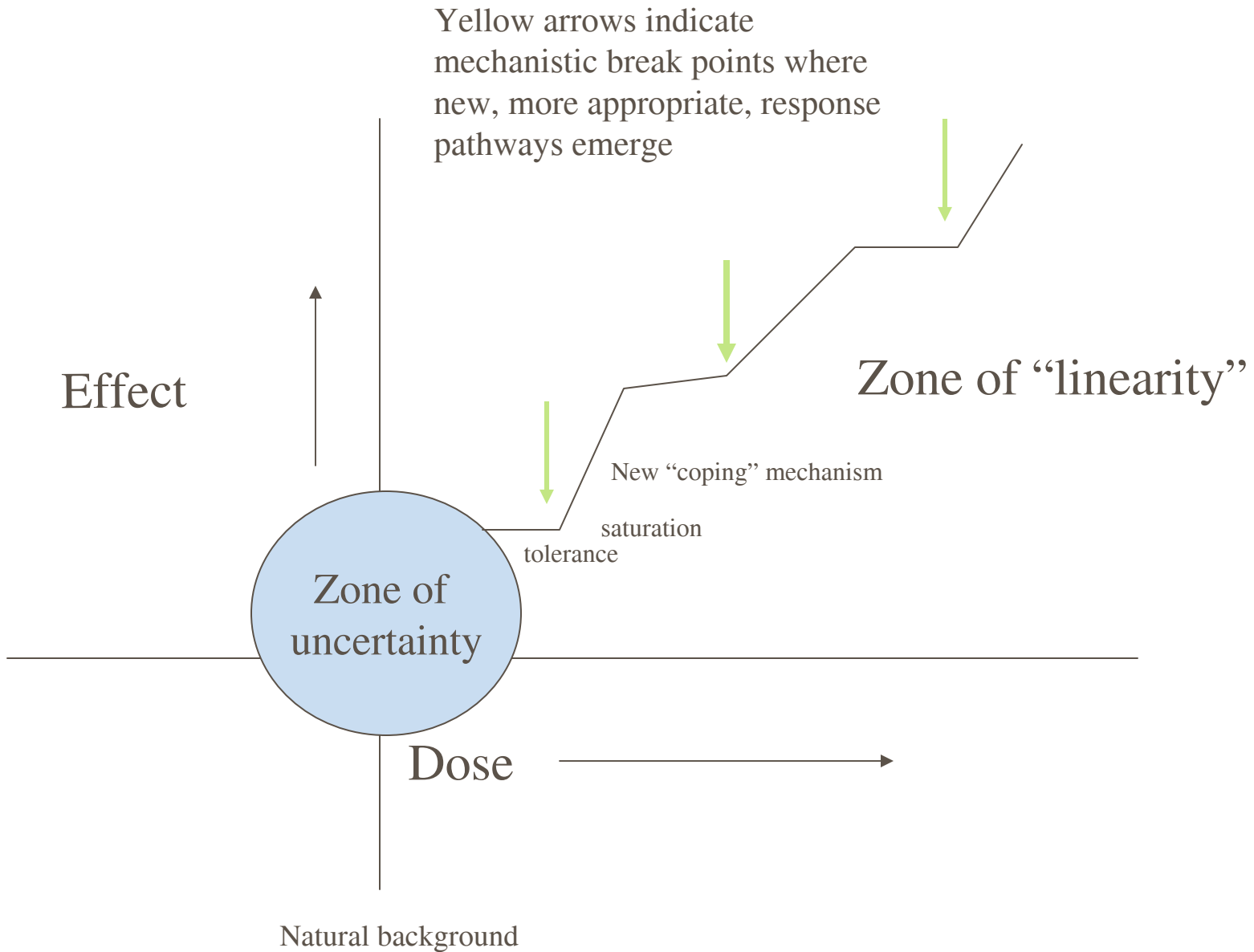
- Low dose hypersensitivity and bystander effects are mutually exclusive [published data for 13 cell lines)
- Adaptive response appears to sector with bystander effect -ie get a bystander effect get an adaptive response (4 cell lines tested so far)
- Pre-treatment of cells with bystander medium induces resistance to an actual dose.
- Bcl-2 induction appears to be the key.



Therapeutic possibilities

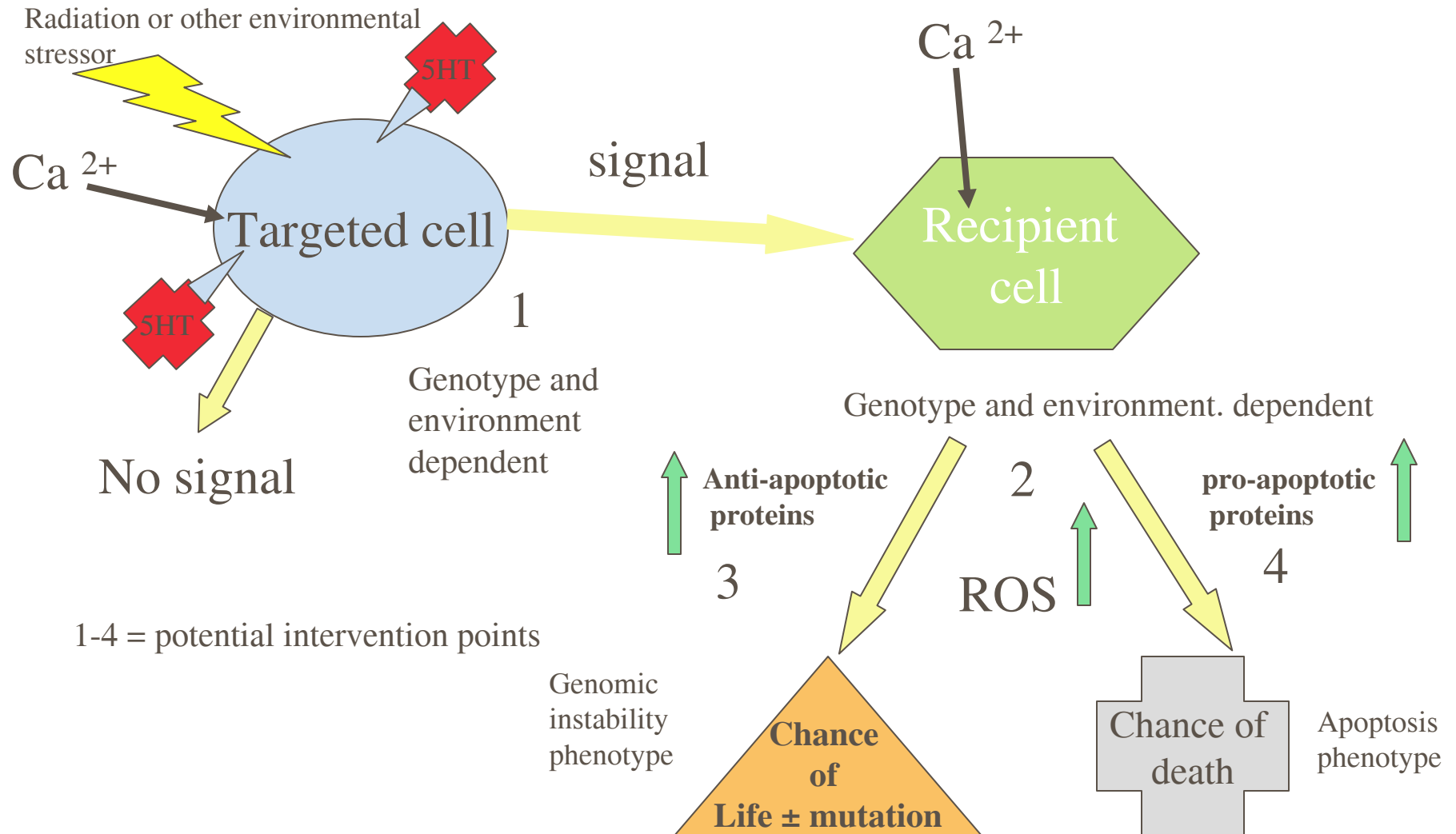
- Harness the effect to sensitise tumours or to collapse supporting tissue?
- Use the assay for predictive testing?
- Integrate the bystander biology into treatment planning?
- Therapy for non-malignant conditions?

Proposed dose response relationship for radiation-induced effects



Possible model for expression of bystander effects in biota

With intervention points for protective/therapeutic strategies





Data Gaps

- Information about the mechanisms involved in **SIGNAL GENERATION**
- What determines **RESPONSE CHOICE**
- Relevance to low dose **RISK**
- **MULTIPLE STRESSOR** relevance



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