Mechanisms operating after low radiation doses: an explanation for non-linearity and a new therapeutic target

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Outline

- **Introduction to some issues**
  - Saturable LDR effects
  - Adaptive responses, NTE, HRS
  - Uncertainty
  - Individual responses
  - Complexity levels
- **Mechanisms**
  - NTE: photons, exosomes
  - HRS: apoptosis, genomic instability
- **New Targets**
  - Therapy
  - Protection

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background

- Many low dose effects saturate so non-linear dose responses occur
- Large inter-individual variation in response due to lifestyle factors and genetic background
- Adaptive responses (good?) and Genomic instability (bad?) can both occur
- Bystander effects can also be “good” at one level but “bad” at another.
- Low dose hypersensitivity can rid population of damaged cells?
- These “non-targeted effects” (maybe “non-linear” is a better discriptor?) are the focus of this talk
- Need to understand mechanisms to get anywhere!

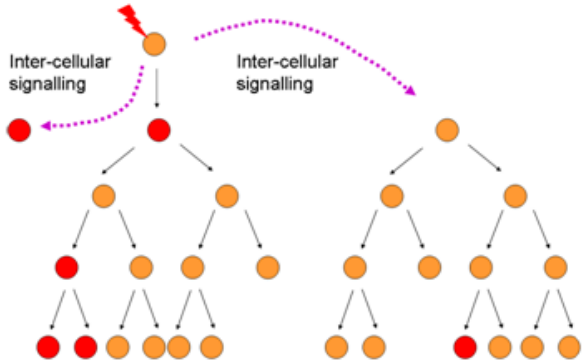
'Non-targeted' radiation effects

Bystander effects

Effects in neighbouring cells

Genomic Instability

Effects in unirradiated descendant cells

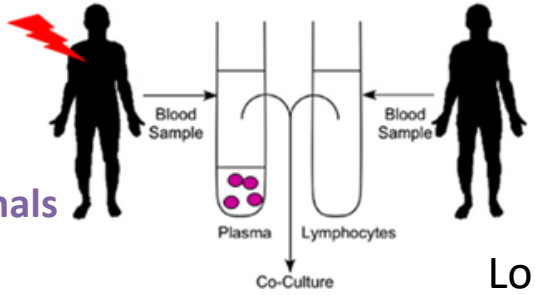


Abscopal effects

Effects in neighbouring tissues

Clastogenic factors

Ex vivo effects in cultured cells



Inter-animal signaling

Effects in neighbouring animals

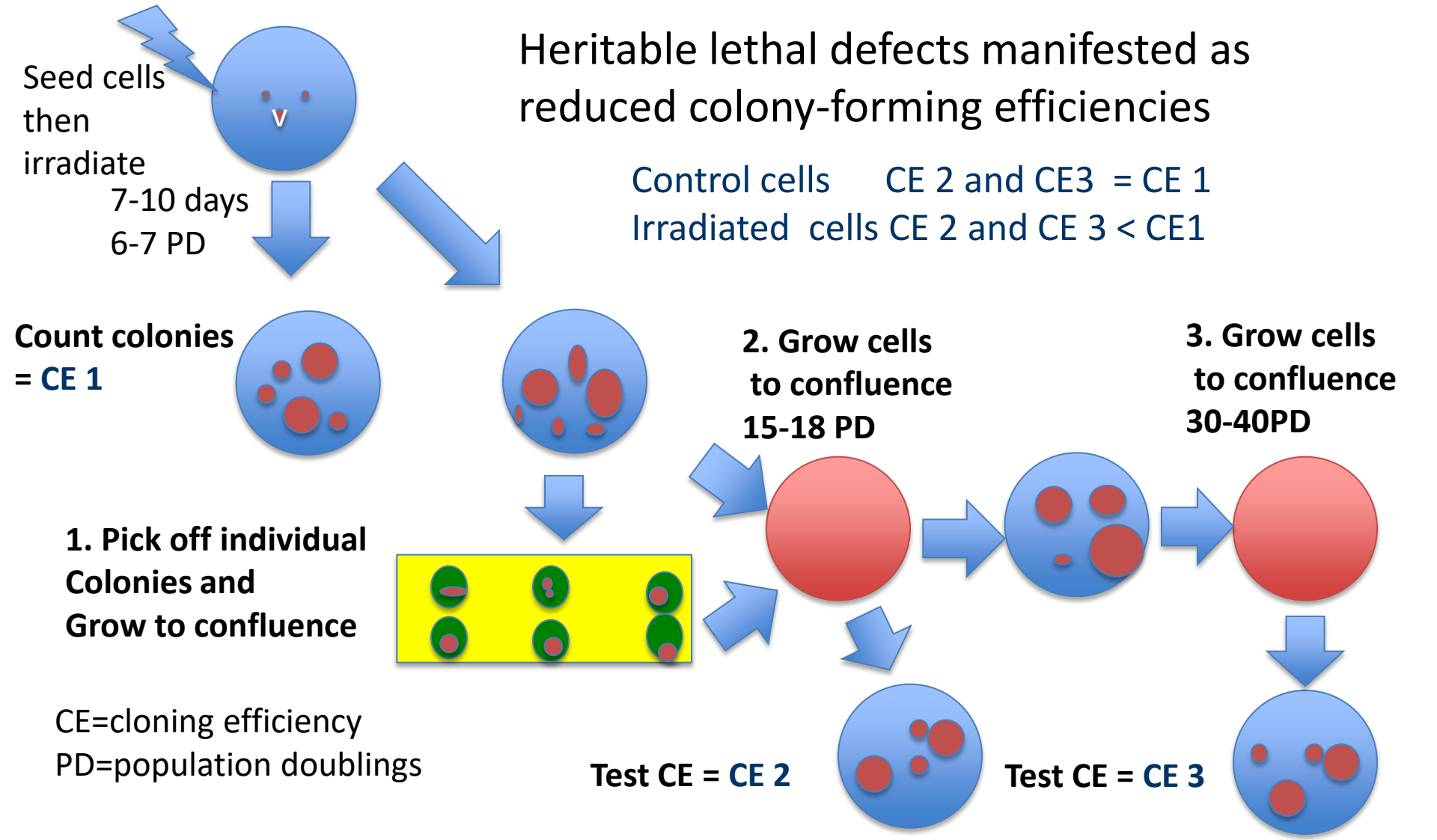
Inflammatory Processes may provide mechanistic link

Long-term effects on innate immune response function may occur

Early days: Lethal mutations and genetic wobble

- The belief: “If an irradiated cell survives and undergoes at least 5 post irradiation divisions, it can be considered to be fully recovered and the progeny will behave as if the progenitors were never irradiated”
- The reality: Irradiated cells acquire and retain the capacity to show late damage, non-clonal mutations and unpredictable effects long after exposure (genomic instability/wobble)
  - Seymour, Mothersill and Alper, 1986
- Explosion during 1990’s leading to the field of Non-targeted effects

Lethal mutations/delayed reproductive death

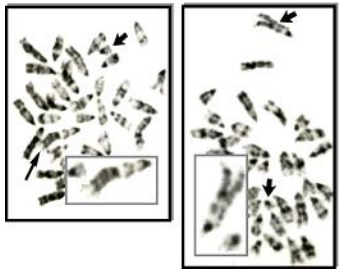


Seymour Mothersill, Alper, 1986

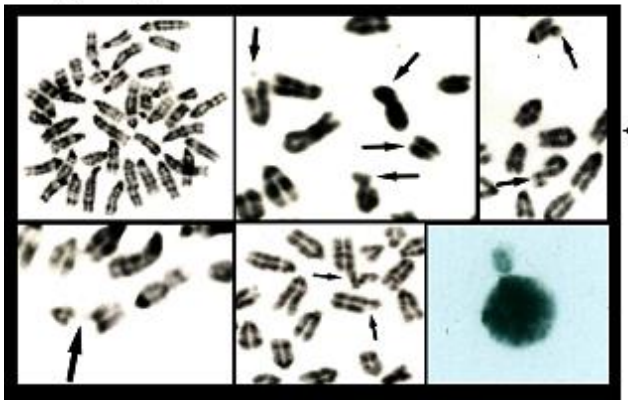
Irradiated stem cell-derived clones

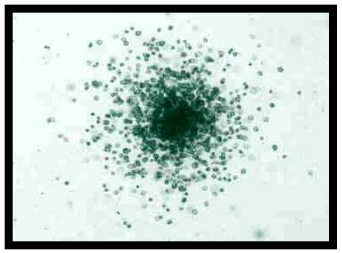
"Expected"

Clonal abnormalities

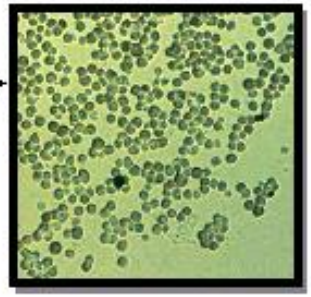


cytogenetic aberrations

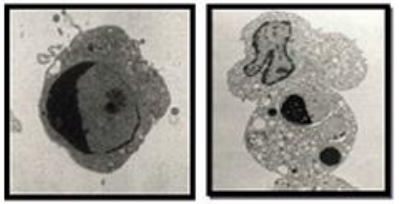




gene mutations



Cell death



Pamfer and Streffer, IJRB, 1989
Kadhim et.al. *Nature* 1992

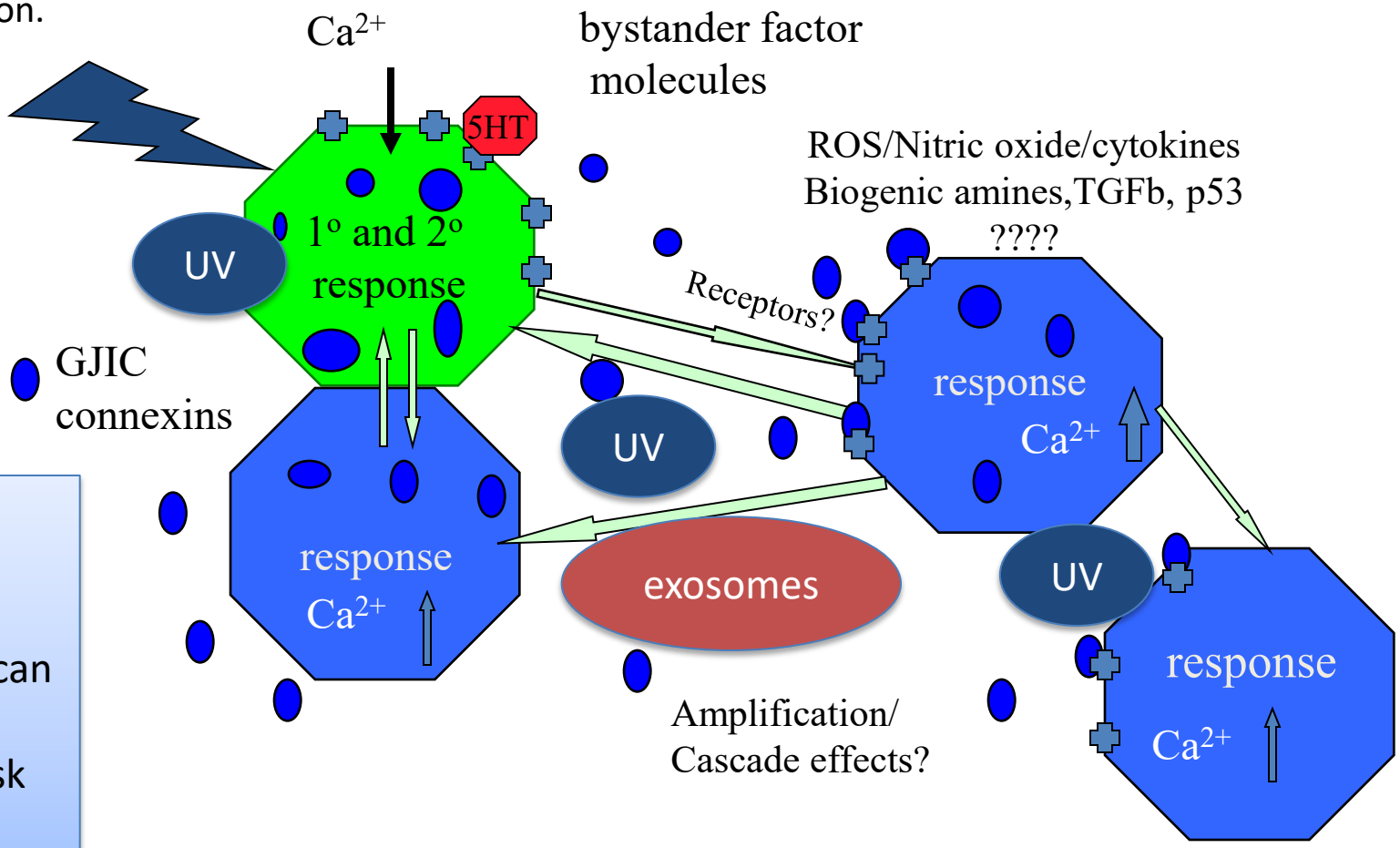
"Unexpected"
'Radiation-induced genomic instability'

Radiation-Induced Bystander Effect

Radiation-induced bystander effect (RIBE): a phenomenon whereby cells that have not been directly traversed or targeted by a primary source of ionizing radiation exhibit characteristics of irradiated cells

The bystander effect

Ionizing radiation, UVA, UVB, ELF-EMF and heavy metals induce affected cell to signal to others. Responses to the signals include apoptosis, micronucleus formation, transformation, mutation, induction of stress and adaptive pathways. Serotonin (5HT), L-type calcium channels (which are 5HT-3 receptors) and Calcium ions known to be involved in signal production.

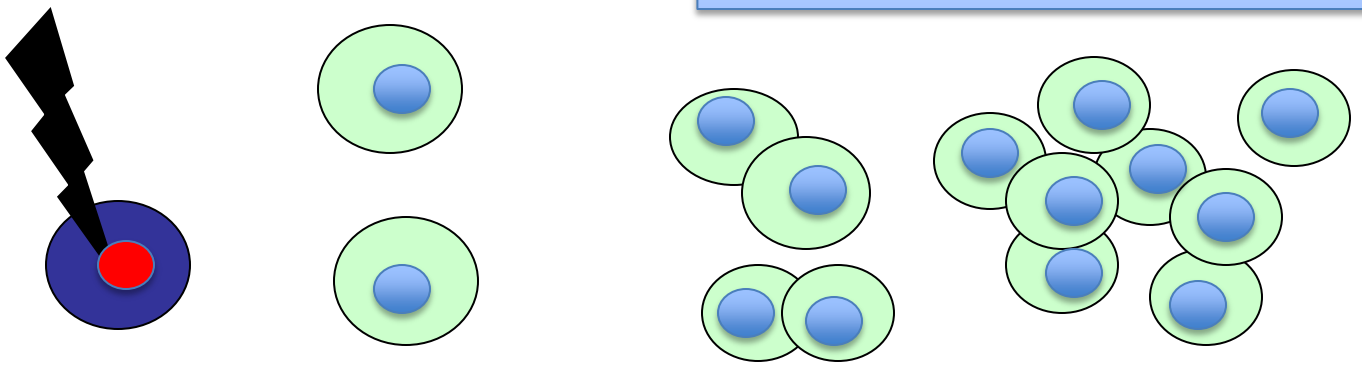


Issue is lots of things cause bystander effects so how can we define what the radiation risk is anymore?

Old and new paradigms

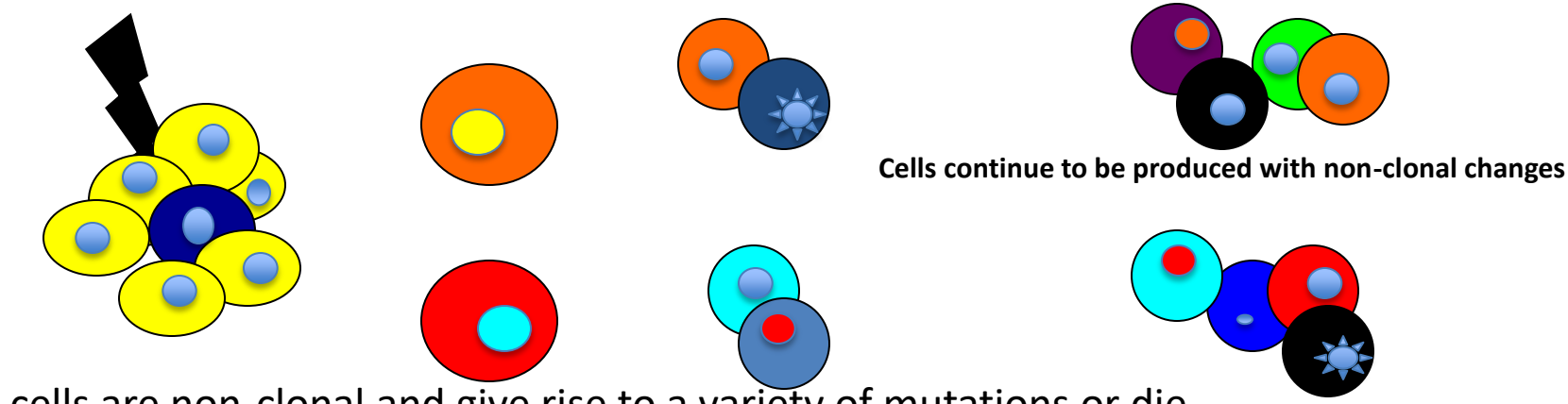
Issue is quantifying risk
when there is spatial and temporal uncertainty

Old **view- clonal outcome**



Progeny are all clonal i.e. identical and mutation is passed to all progeny

New **view-non-clonal, population-determined outcome**



Progeny cells are non-clonal and give rise to a variety of mutations or die

Reported effects

Direct irradiation effects

- Death
- Reproductive failure
- Cellular apoptosis
- Mitochondrial defects
- Proteomic changes
- Signaling defects
- Adaptive responses
- Genetic differences in radiosensitivity

Persistent effects in neighbours or descendant progeny (no further irradiation)

- Death
- Reproductive failure
- Cellular apoptosis
- Mitochondrial defects
- Proteomic changes
- Signaling defects
- Adaptive responses
- Genetic differences in radiosensitivity

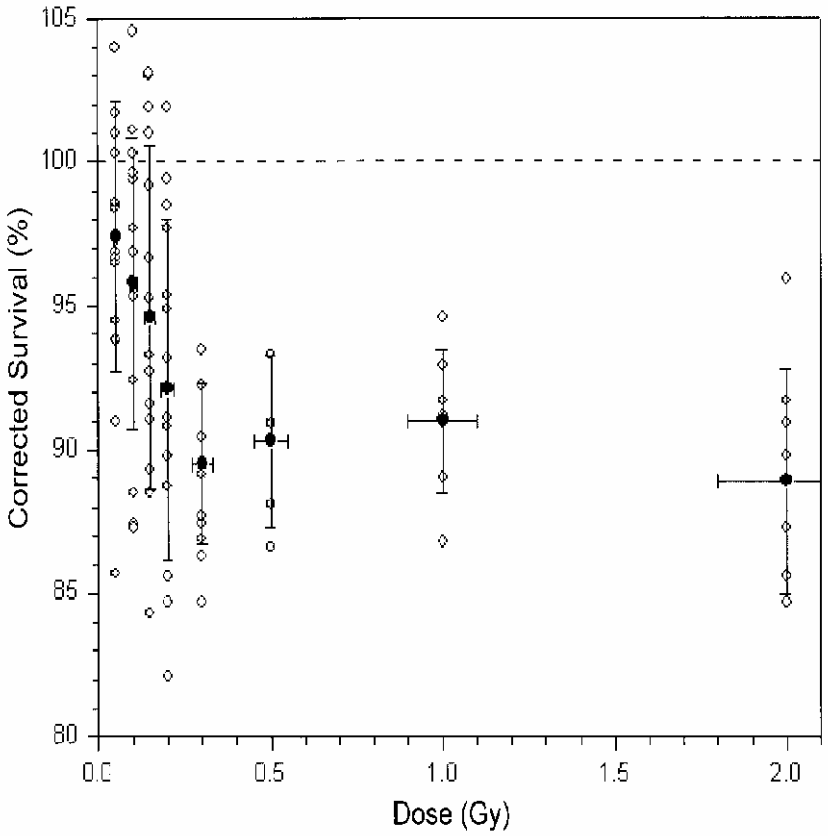


FIG. 1. Survival of a population of V79 cells after the irradiation of a single cell with focused C_K X rays. The data are reported as a function of the nuclear dose delivered to a preselected cell. (V) Measurements from individual experimental dishes (corrected for the control plating efficiency); (v) averages in each dose group. *X* errors are 10% of the delivered dose; *Y* errors are 6 1 standard deviation of the means.

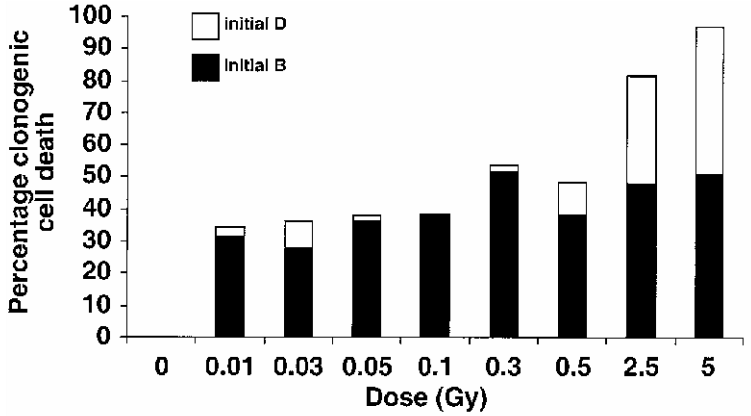
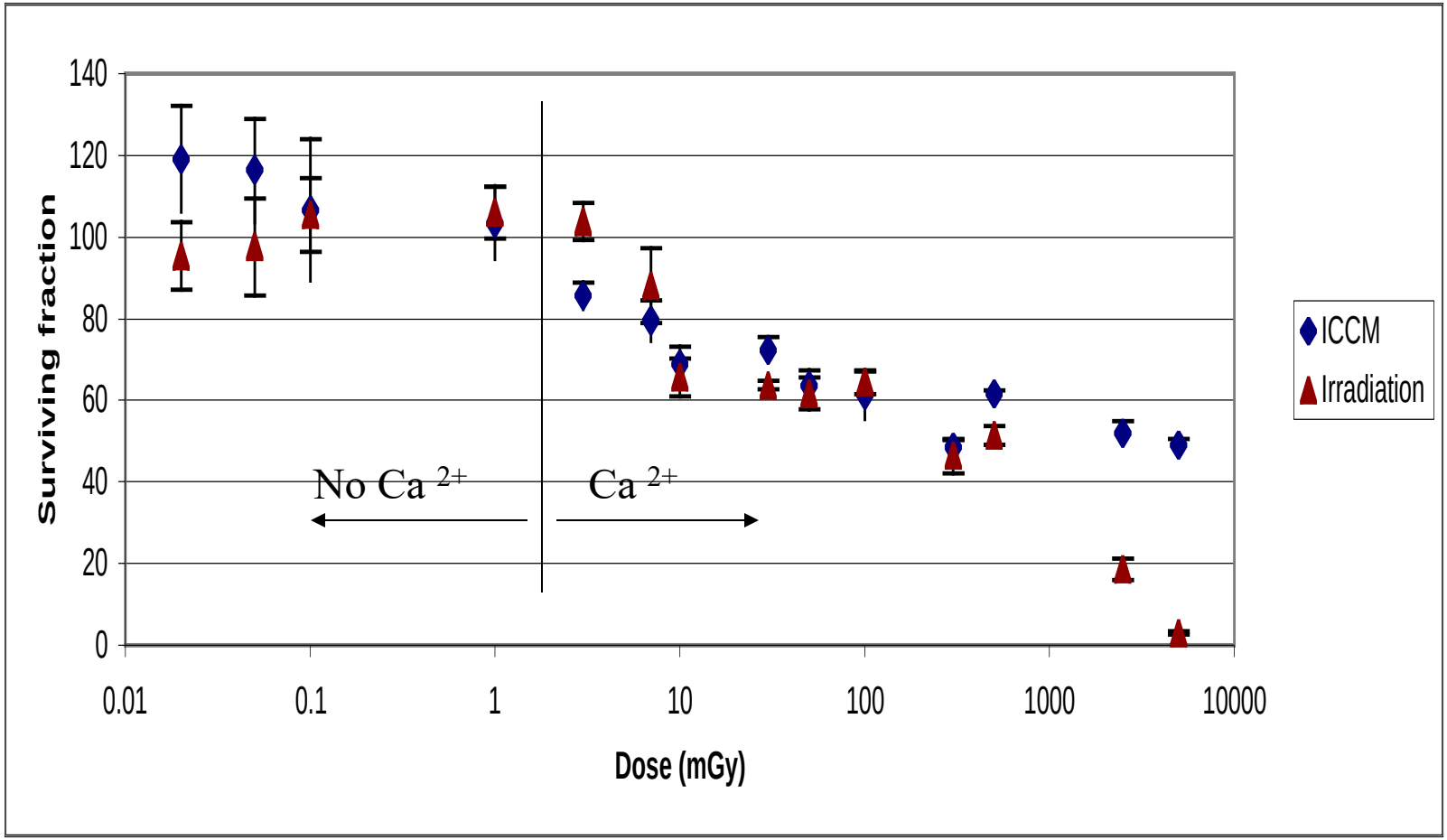


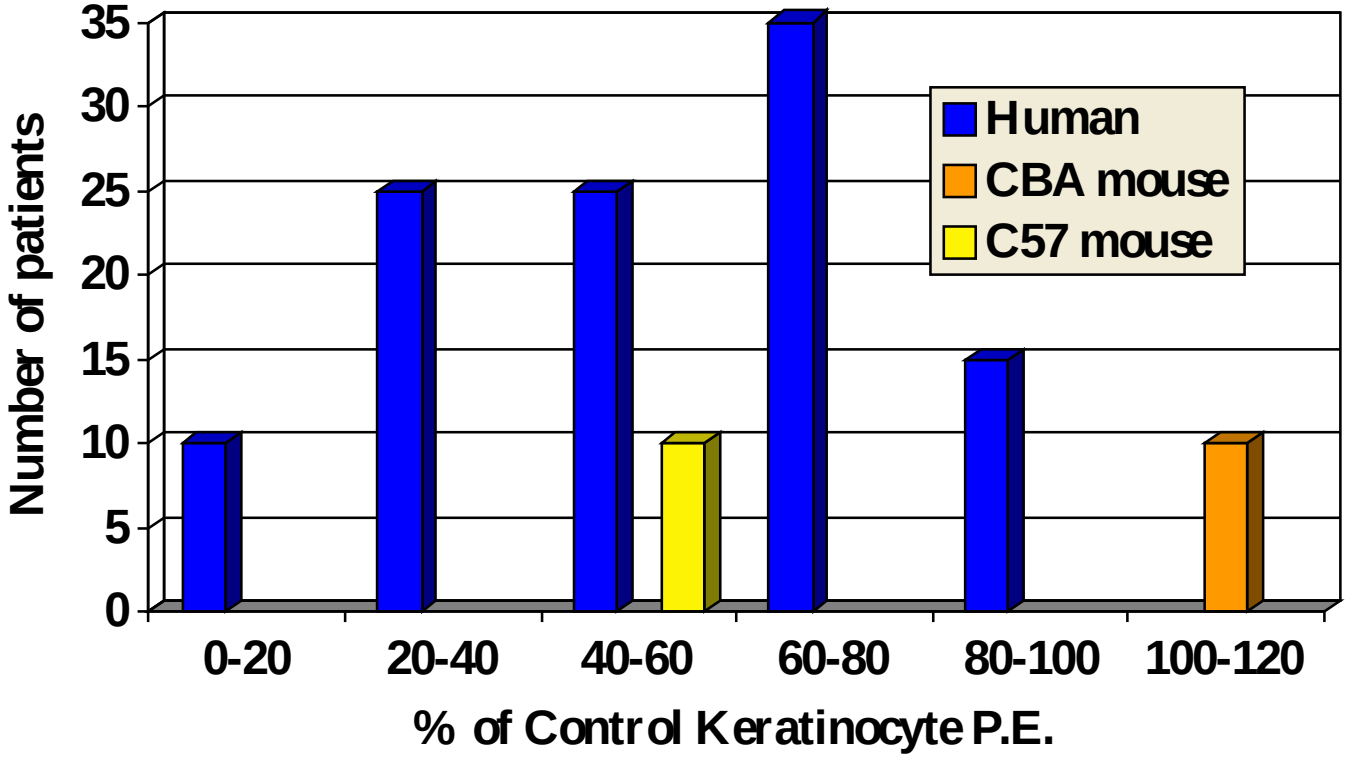
FIG. 1. Clonogenic cell death measured in human keratinocytes. The total bar represents the total death detected after exposure of cells to the radiation dose. The death measured after exposure to ICM (B) is represented by the black portion of the bar, and the remaining death determined by subtraction is represented by the white portion of the bar, giving a value (D) for death not attributable to bystander effects of radiation.

Two published datasets showing that NTE saturate at very low acute doses and that once “on” do not increase or diminish with dose. They are Best described as a “reset” of the system’s tolerance

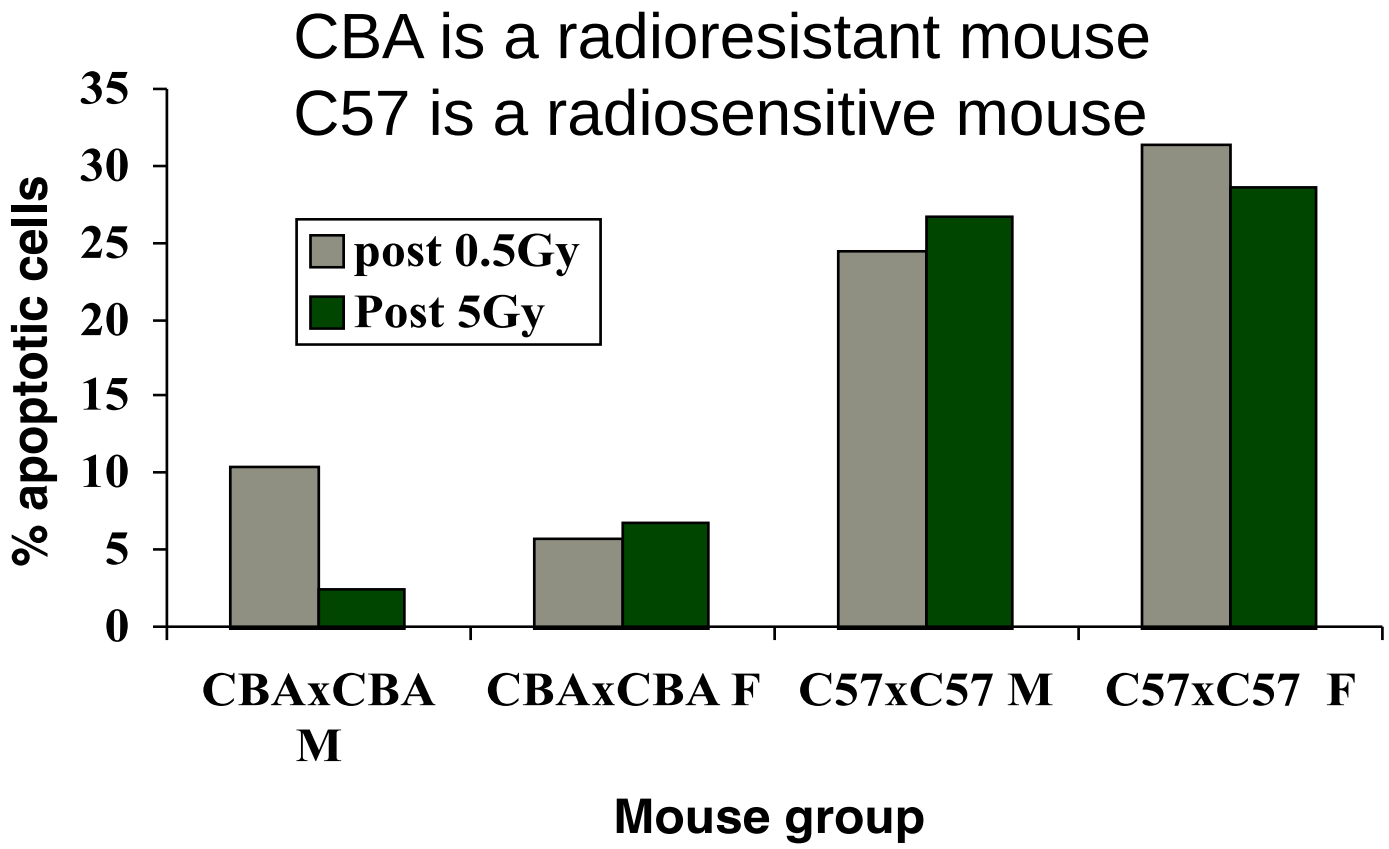
Bystander and direct dose survival curves
over six orders of magnitude ^{60} Co with calcium data



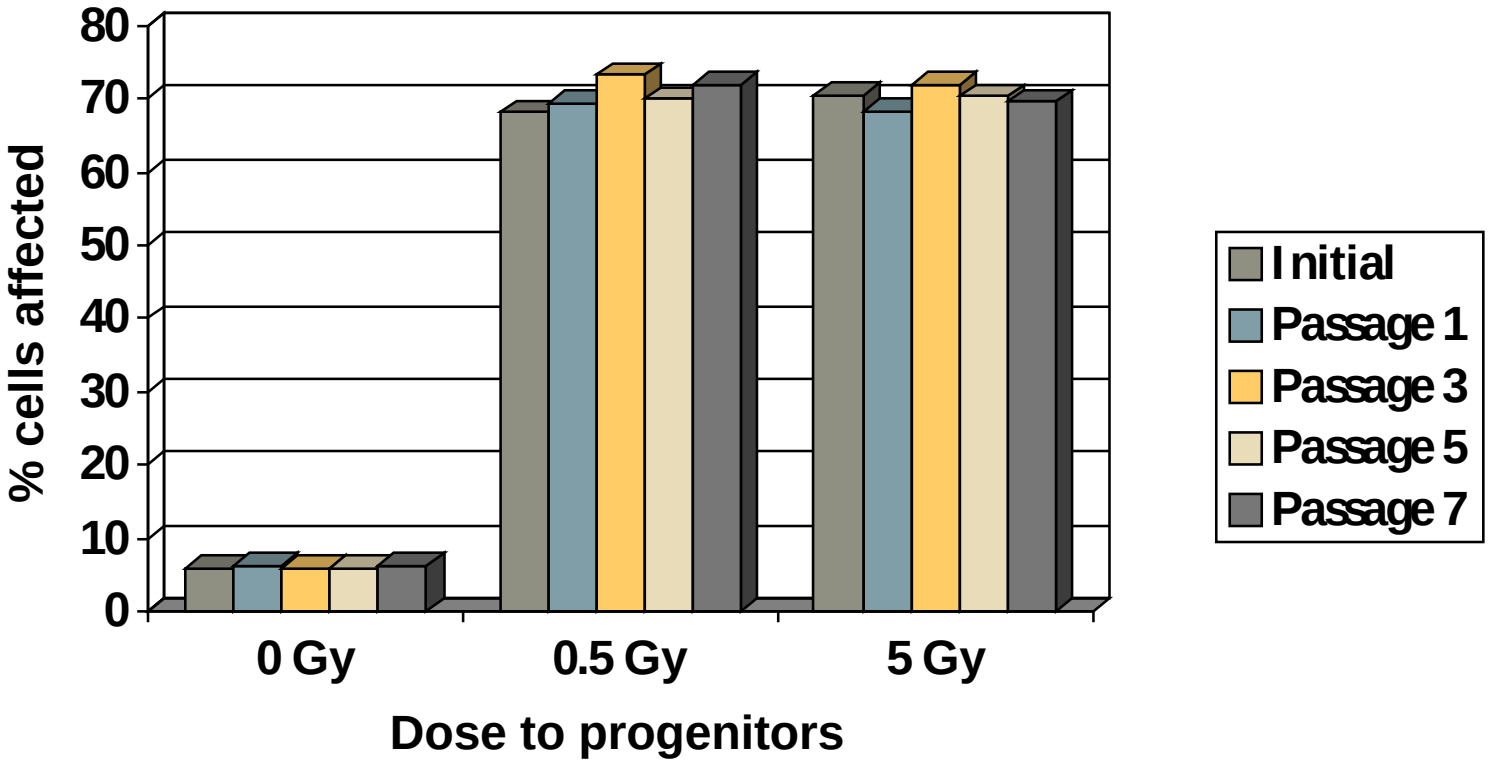
Individual variation in the cytotoxic properties of bystander medium



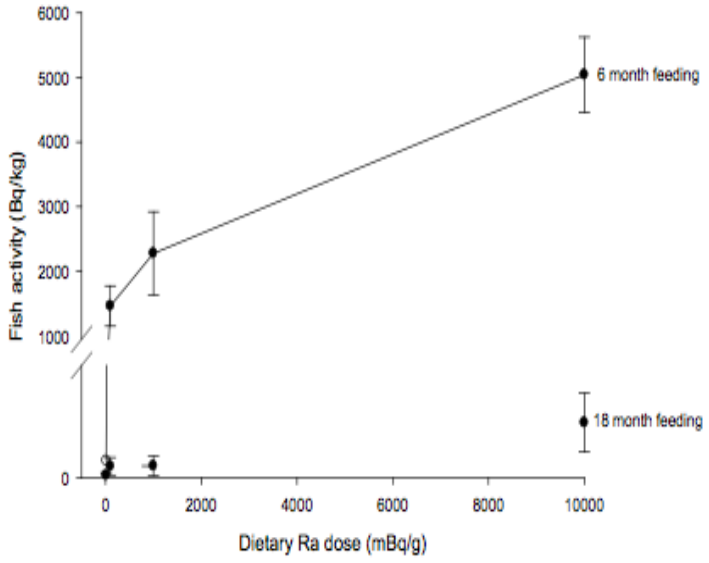
Apoptosis data for mouse strains

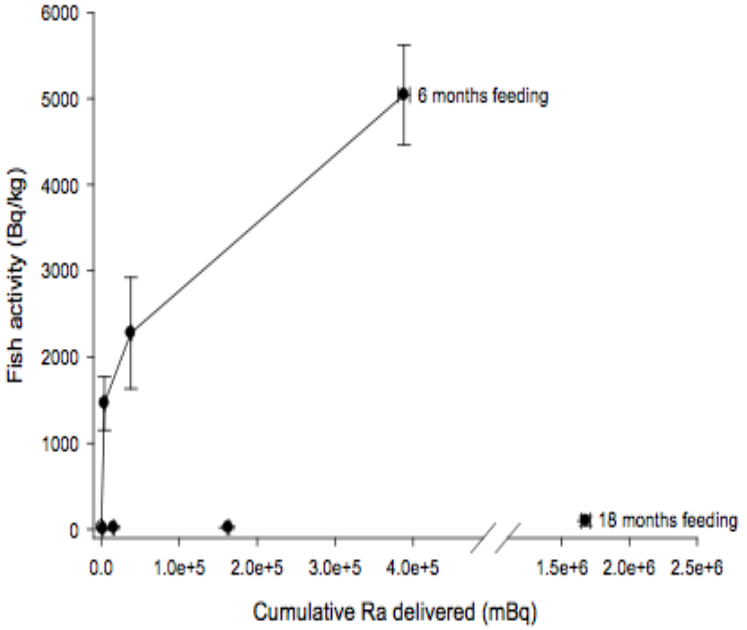


% cells showing increased ROS following ICCM

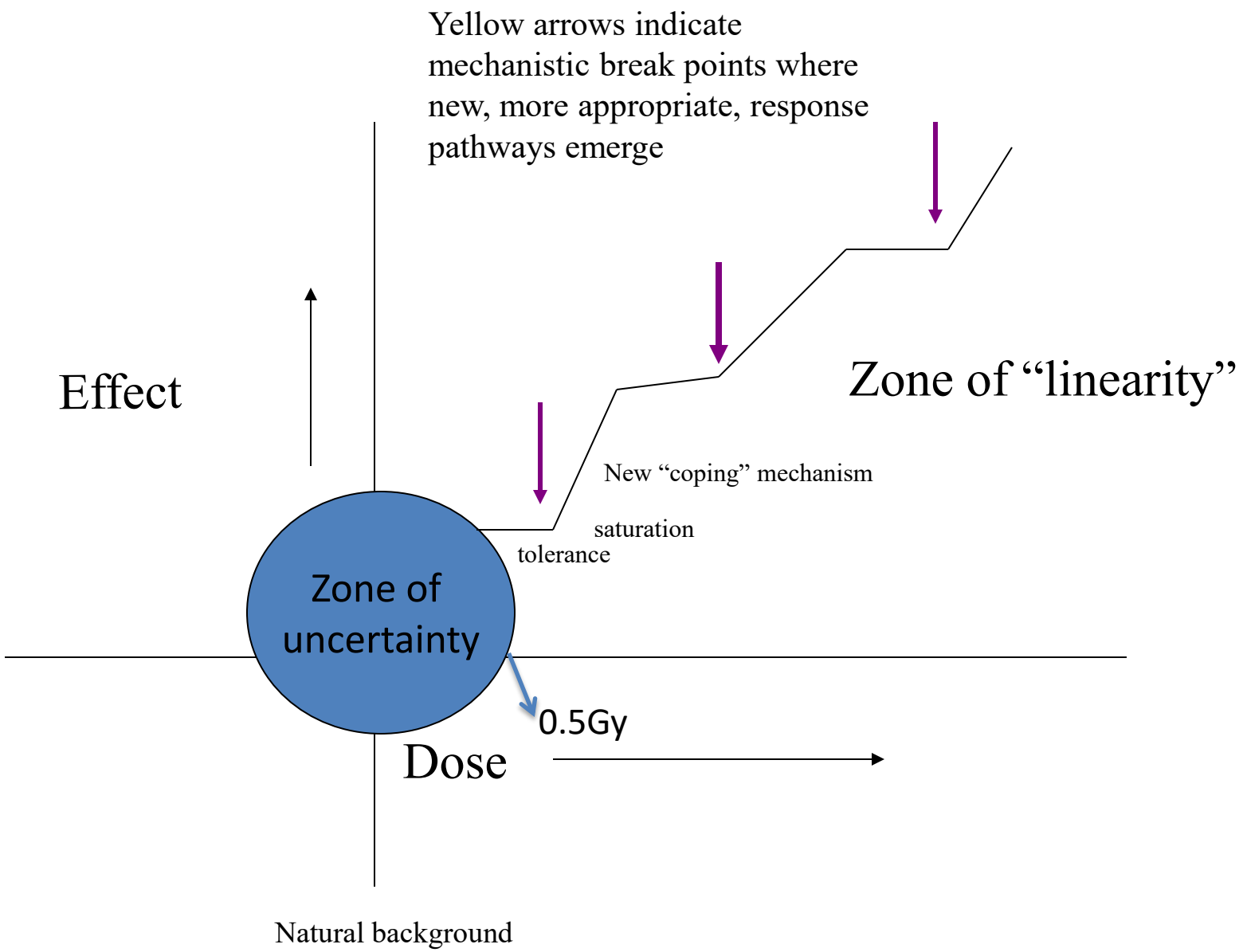


Comparison of 6 and 18 months showing loss of accumulated Ra-226 at 18 months

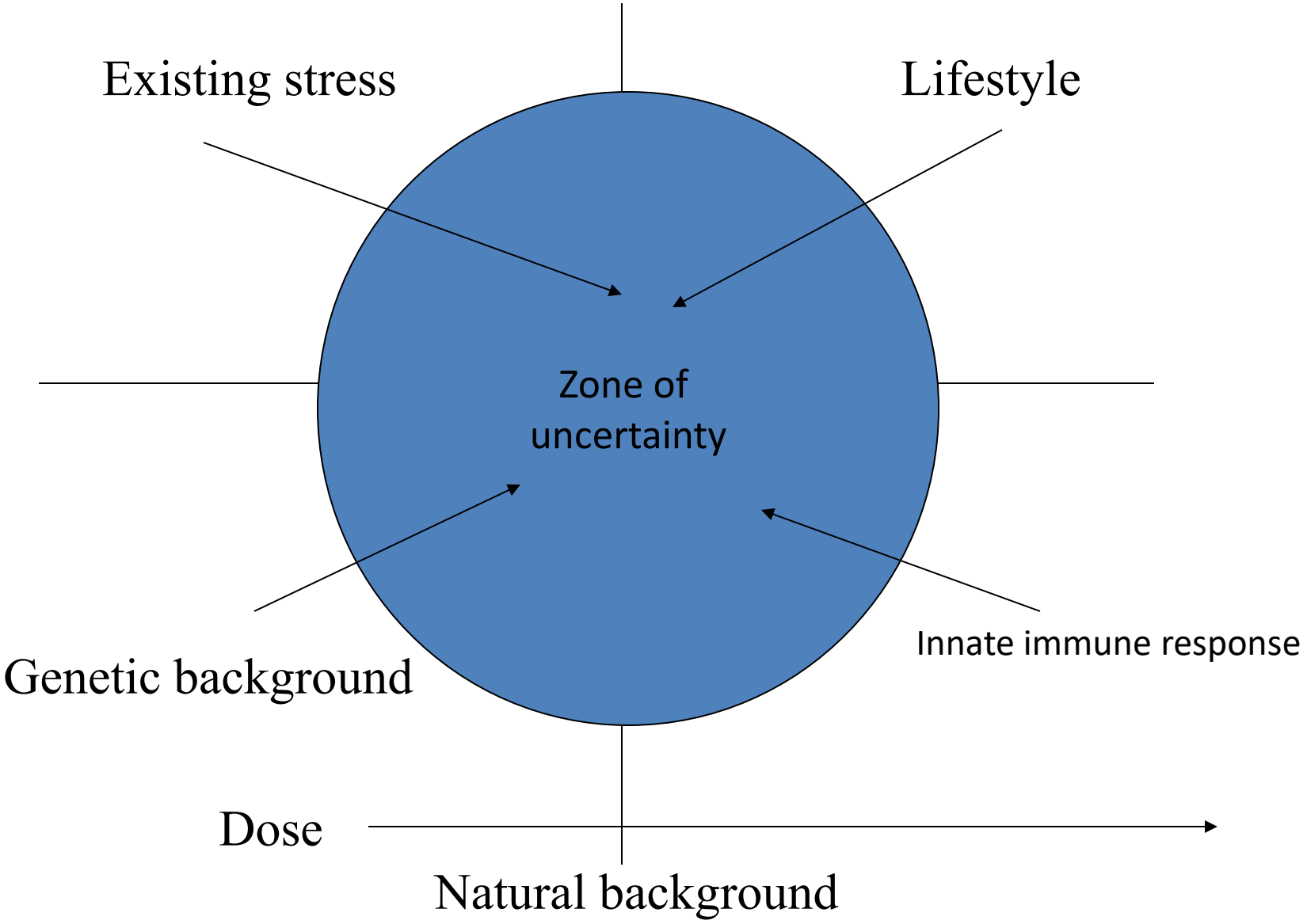




Proposed dose response relationship for radiation-induced effects



Factors influencing outcome in the zone of uncertainty



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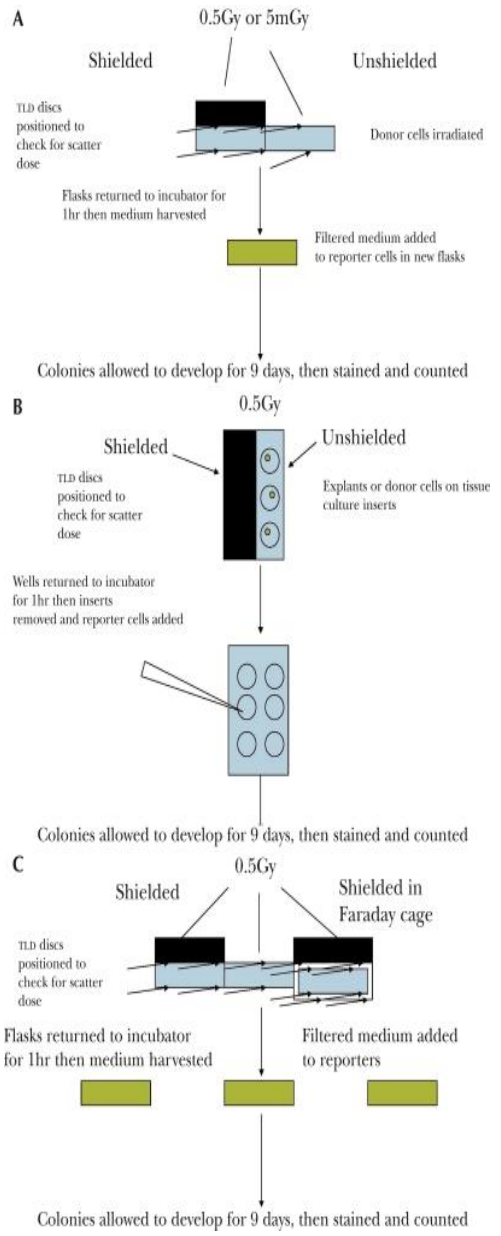
Lots of data and lots of loose ends

- Little thought given to how ionising radiation leads to non-targeted effects
- Ionising radiation involves energy deposition. Leads to ionisation and excitation.
- Excitation important after low doses and low radiation energy exposures – seldom considered.
- Possibility of a physical component to the actual signal?
- First suggested by Irma Mosse in 2006 because melanin was found to prevent the bystander effect (Marozik et al)
- Early evidence from our lab in 2007 (Faraday cage reduced BE) and in 2011 (Fish experiment in separate aquariums)
- How does it all fit?

Early indications

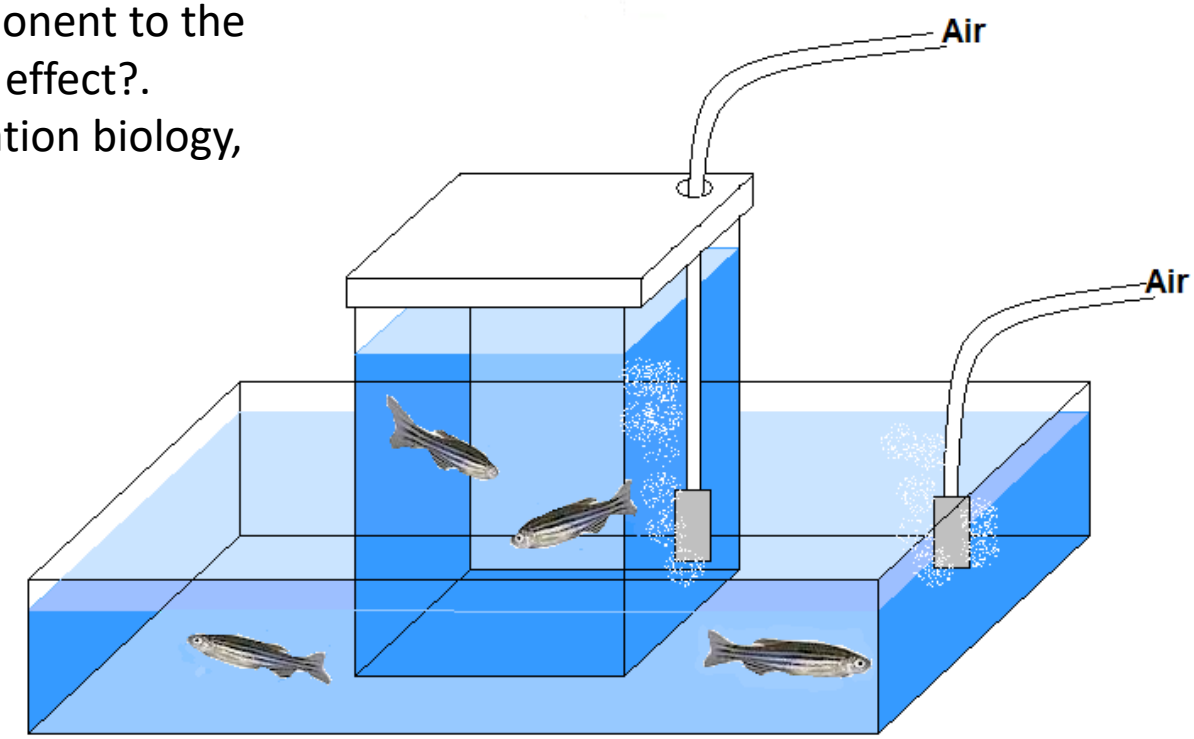
Mothersill C, Moran G, McNeill F, et al. A Role for Bioelectric Effects in the Induction of Bystander Signals by Ionizing Radiation? *Dose-Response*. 2007;5(3):214-229. doi:10.2203/dose-response.06-011.Mothersill.



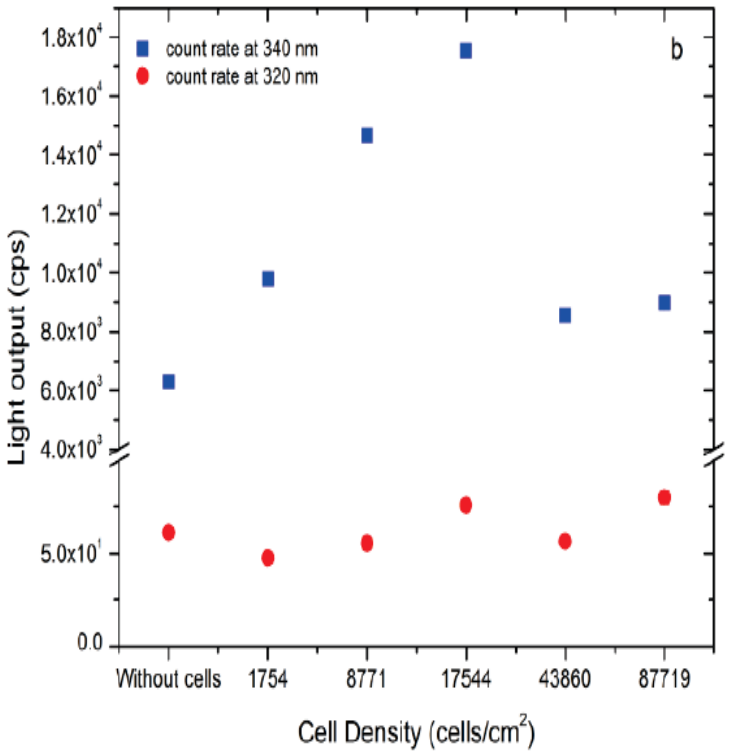
Medium Transfer not required

The fish experiment

Mothersill, C., Smith, R. W., Fazzari, J., McNeill, F., Prestwich, W., & Seymour, C. B. (2012). Evidence for a physical component to the radiation-induced bystander effect?. International journal of radiation biology, 88(8), 583-591.



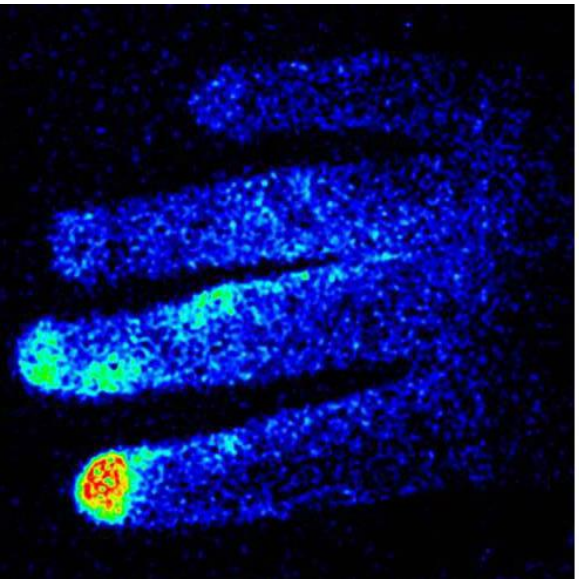
New Mechanism detected



Ahmad, PhD thesis, Chapter 4 p 79

- Photon emission detected from HPV-G cells irradiated with Yttrium-90 (beta emitter)
  - Dr. Bilal Ahmad, former PhD student in Medical Physics department 2014
- Investigate the potential effects of emitted UV photons upon bystander cells

Biophotons

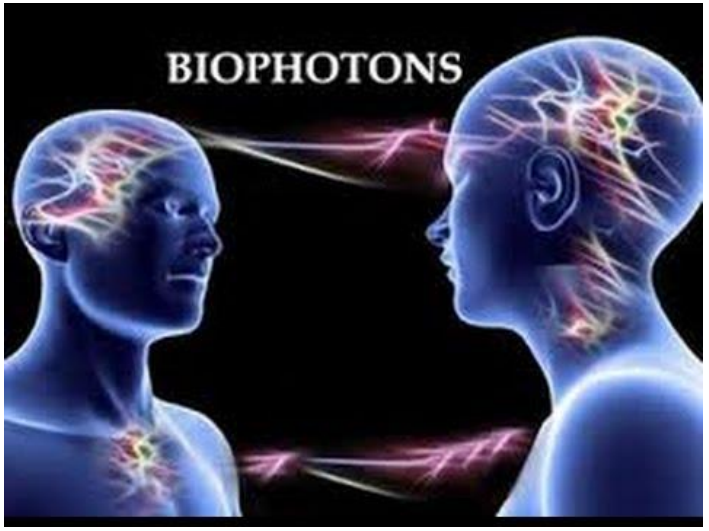


Science

imaging

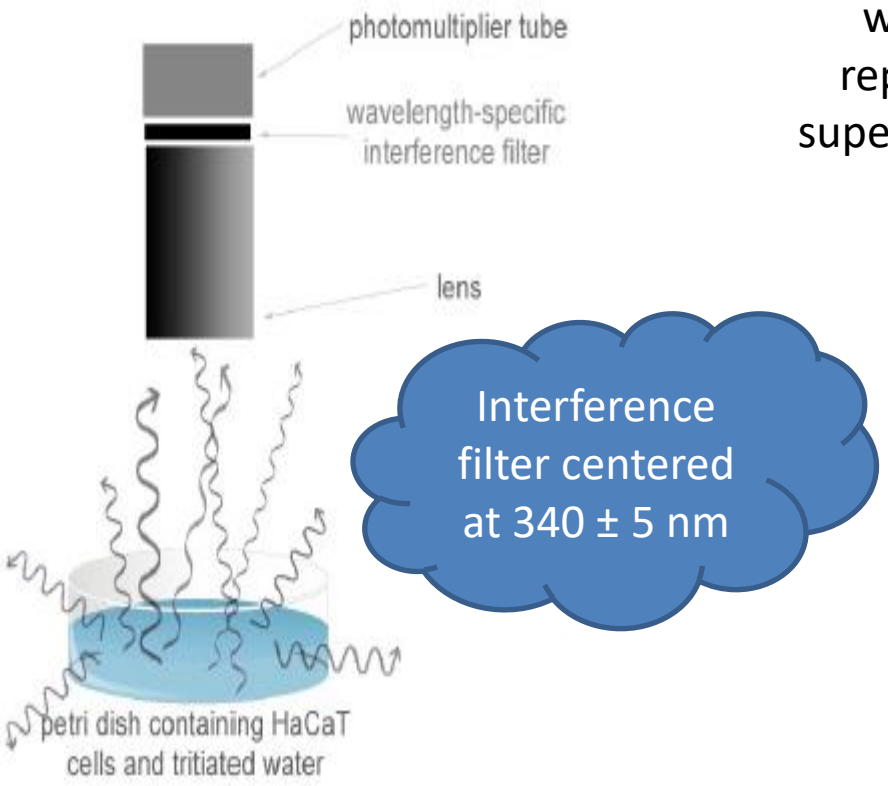
Pseudoscience

Basis of conscious connections



Assessing UV emission and bystander cell survival

Photon Quantification



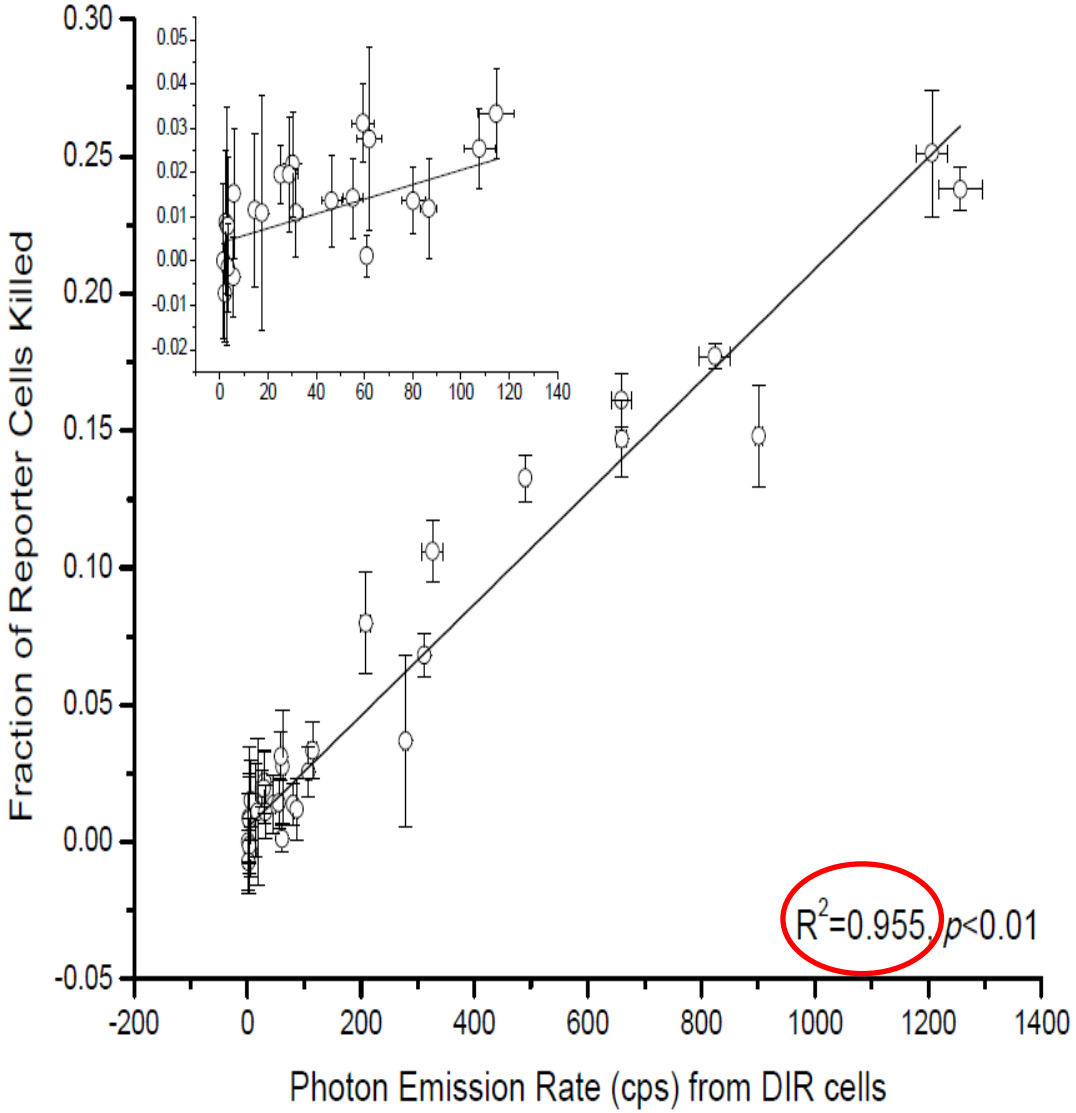
Bystander cell survival

1. Irradiation of HaCaT cells with tritium (^3H) while reporter cells sit <1.5 cm superior to irradiated culture for 24 hours

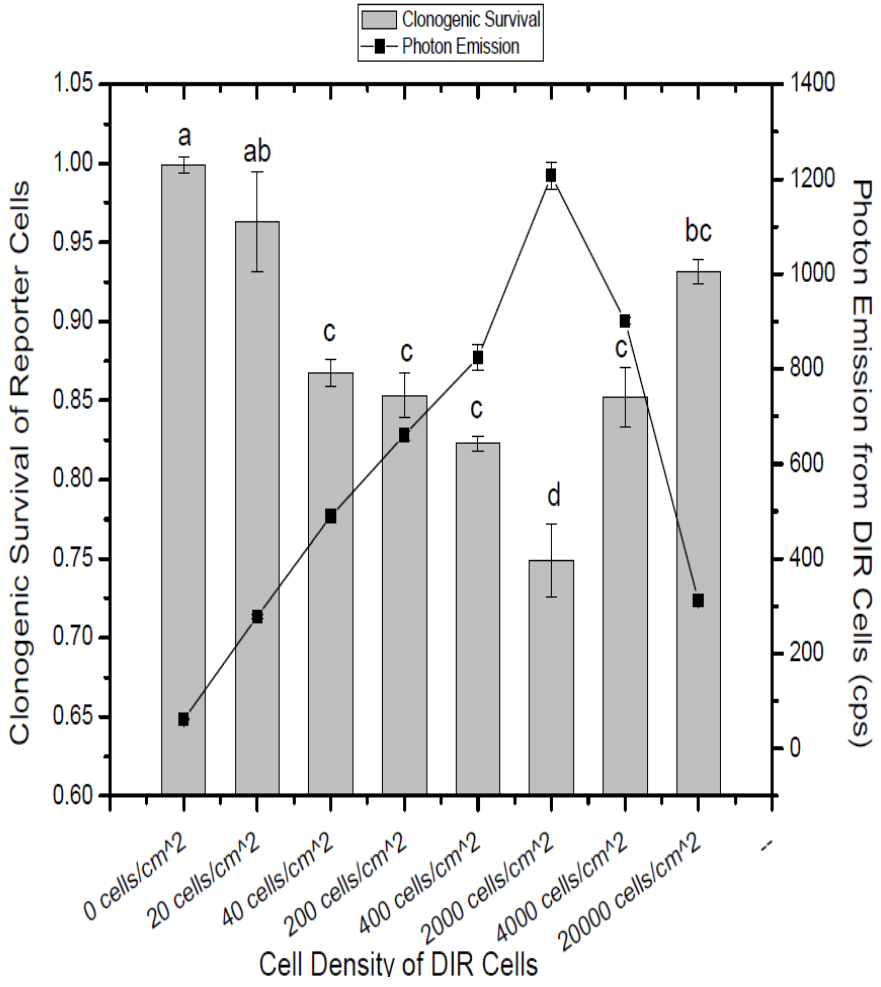
2. Incubate reporter cells at 37˚C, 5% CO_2 for an additional 7-8 days

3. Assess clonogenic survival using assay developed by Puck and Marcus (1956)

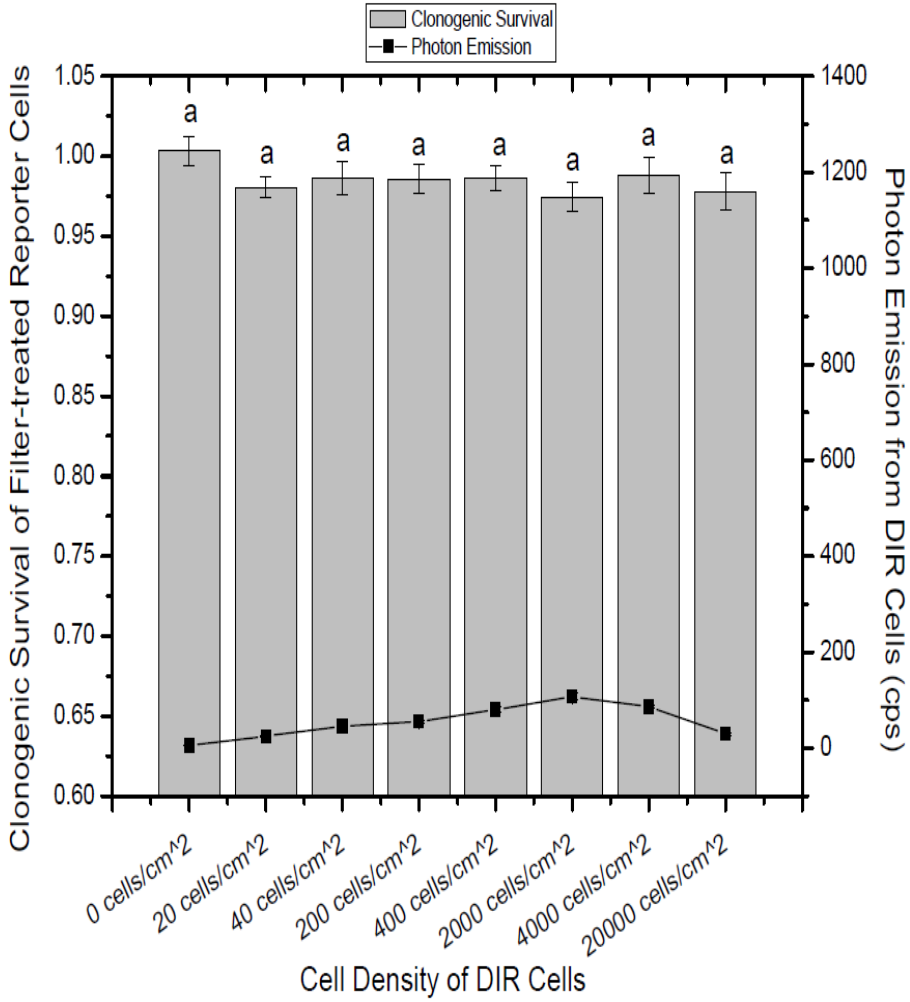
Strong relationship between cell death and photon flux



Effect is abolished following use of an UV absorption filter

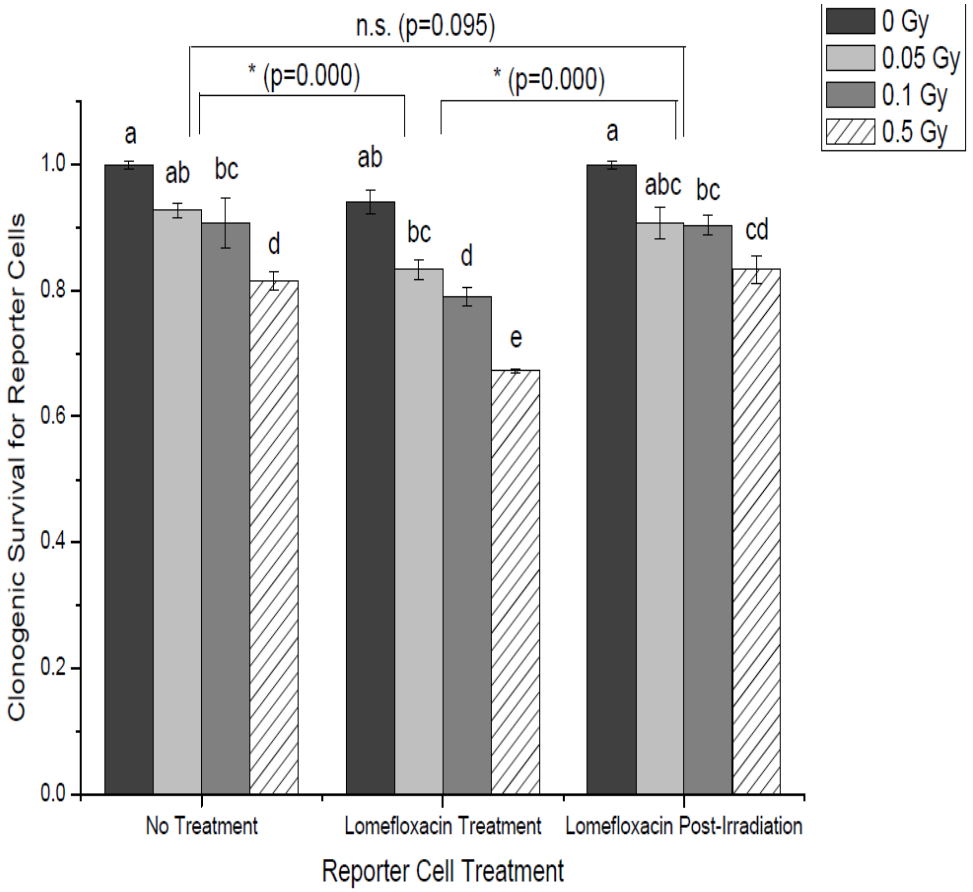


Without filter

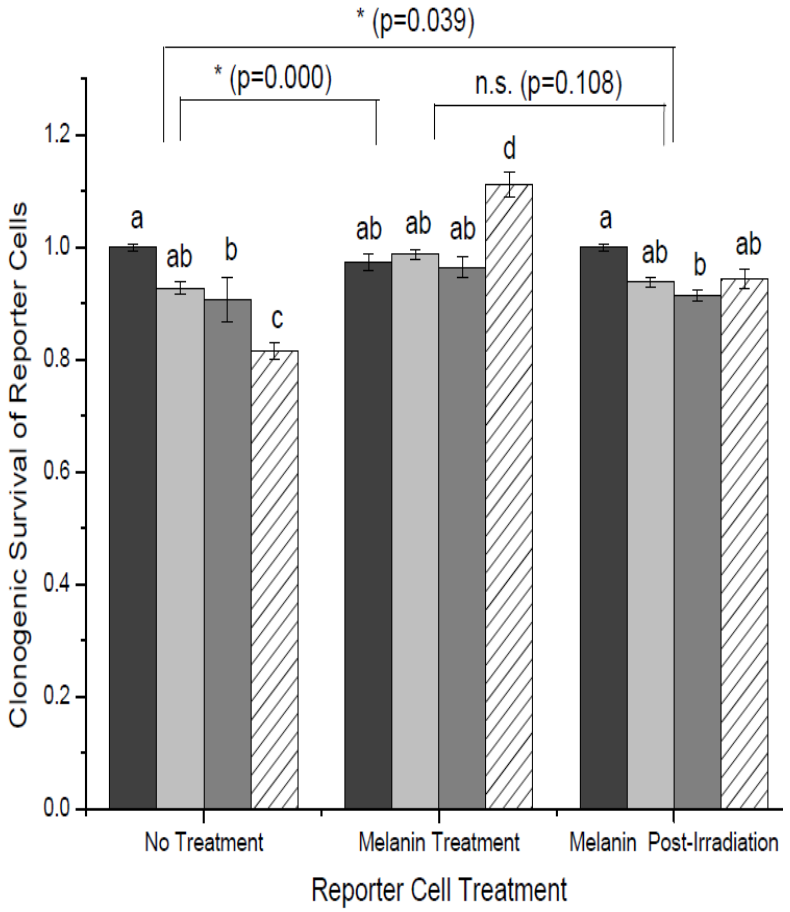


With filter

Effect magnitude can be modulated by photosensitizers and photoprotectors

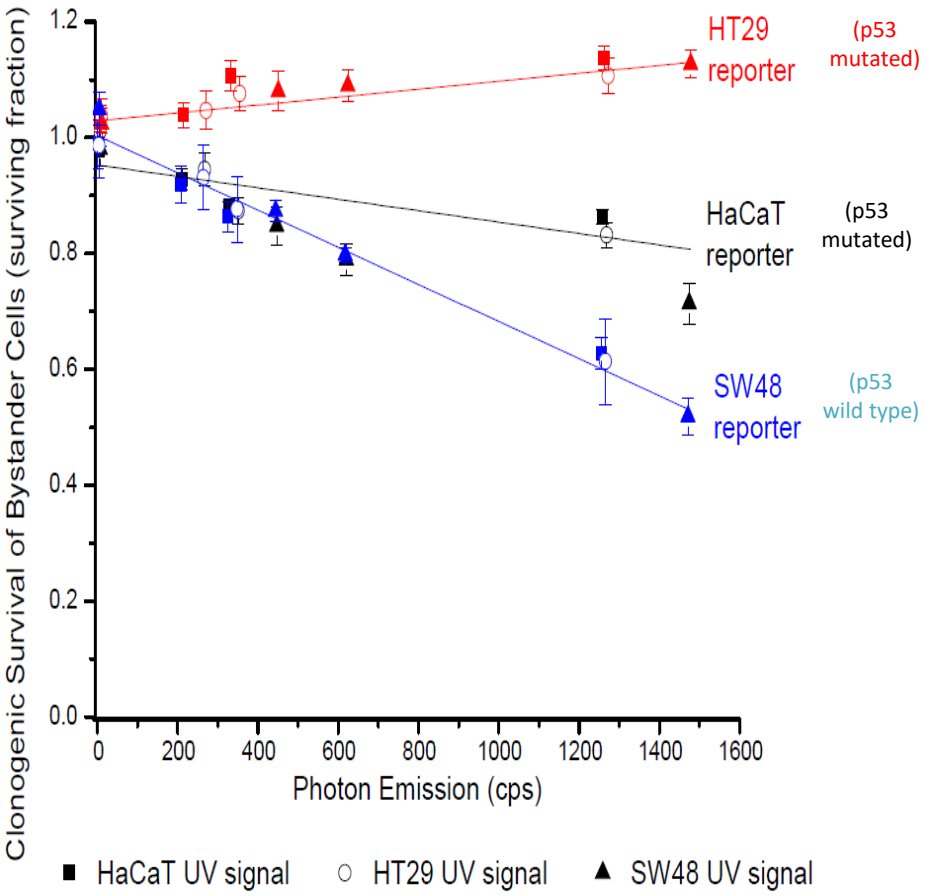


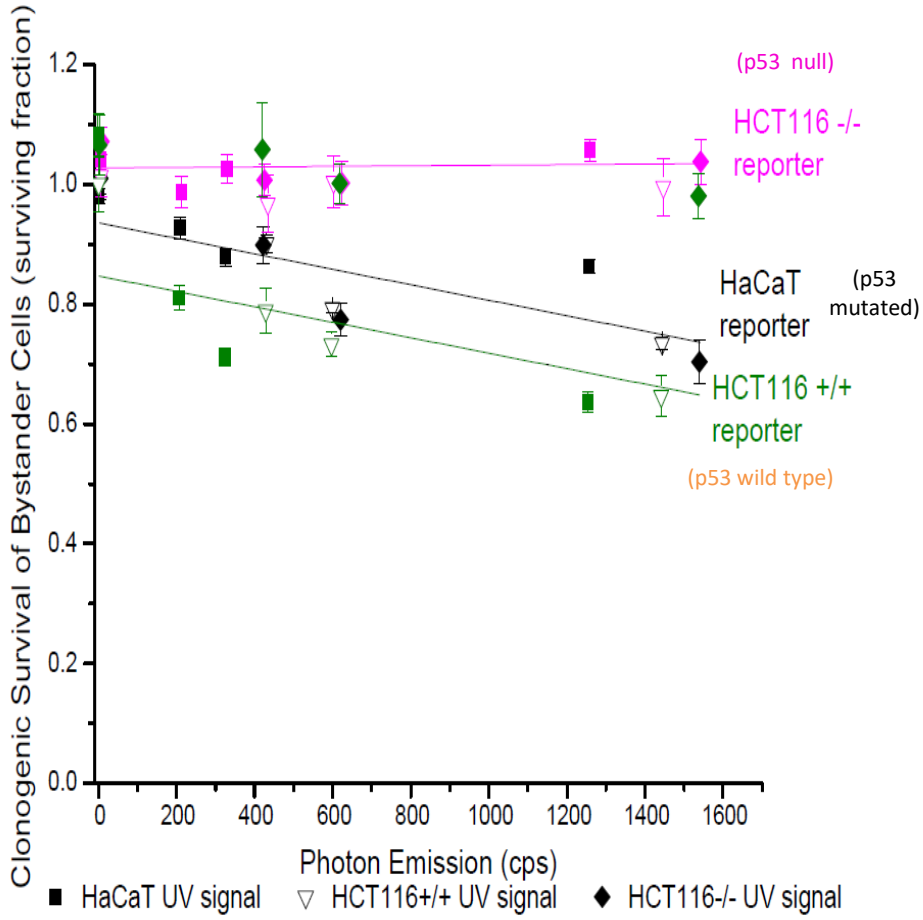
Photosensitizer



Photoprotector

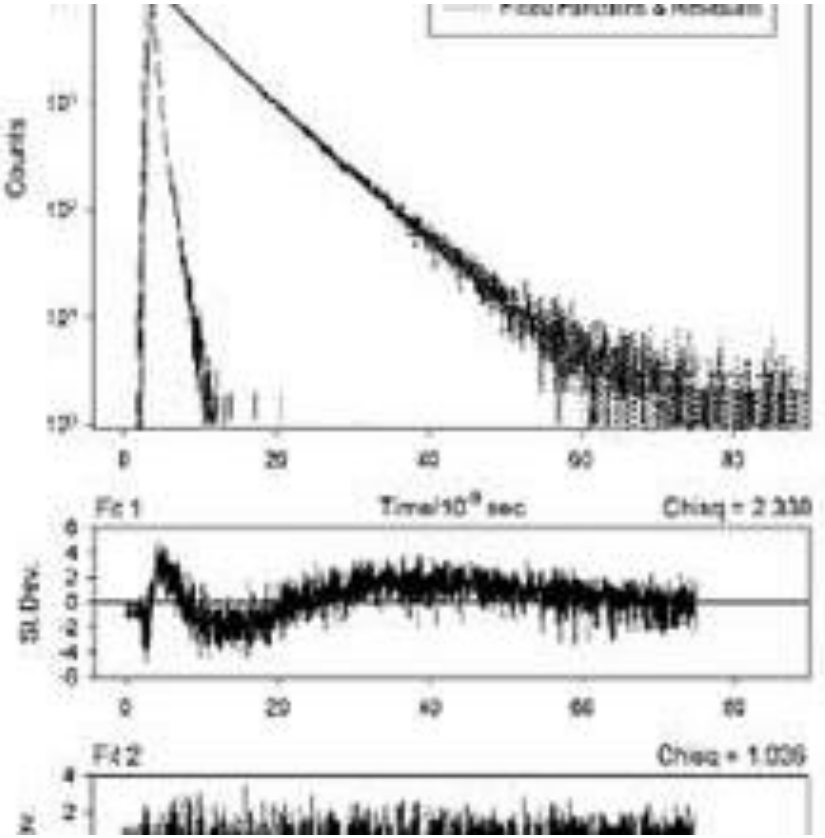
Response to UV signaling is dependent upon p53 status





Excitation decay leading to radioluminescence is the likely mechanism

- So it seems that UV (or light) could be the initial signal emitted due to the interaction of ionising radiation with biological material. This raises fascinating questions about absorbers and emitters and downstream biochemistry and bioenergetics

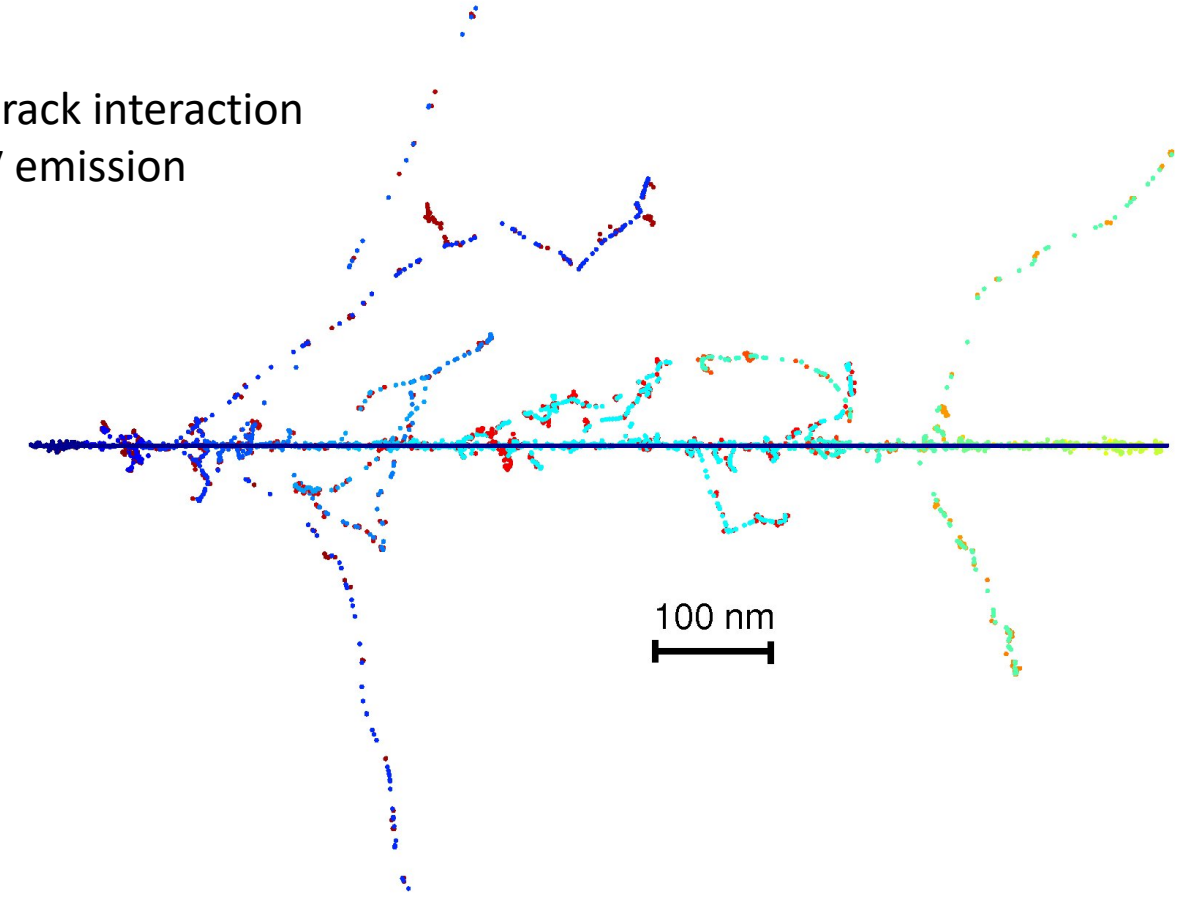


What we now know!

- Photons in the UVA range are produced by cells directly exposed to ionising radiation (Amhad et al, 2013)
- Using a single photon counting system gated to collect at 340nm photons numbers correlated with dose to the cells and with bystander effect (BE) in unirradiated cells exposed to the photons (Le et al, 2014)
- Blocking photons from reaching bystanders using absorbing film or photo absorber melanin prevented BE (Le et al, 2015)
- p53 status not important for photon production but is important for expression of BE in reporter cells but cell lines vary in the quantitative production of photons (Le et al, 2016)
- Non-targeted effects (bystander and genomic instability) are produced in cells receiving harvested medium from UVA exposed cells [Koch's hypothesis style proof!] (O'Reilly and Mothersill, 1997) Whiteside and McMillan 2009)

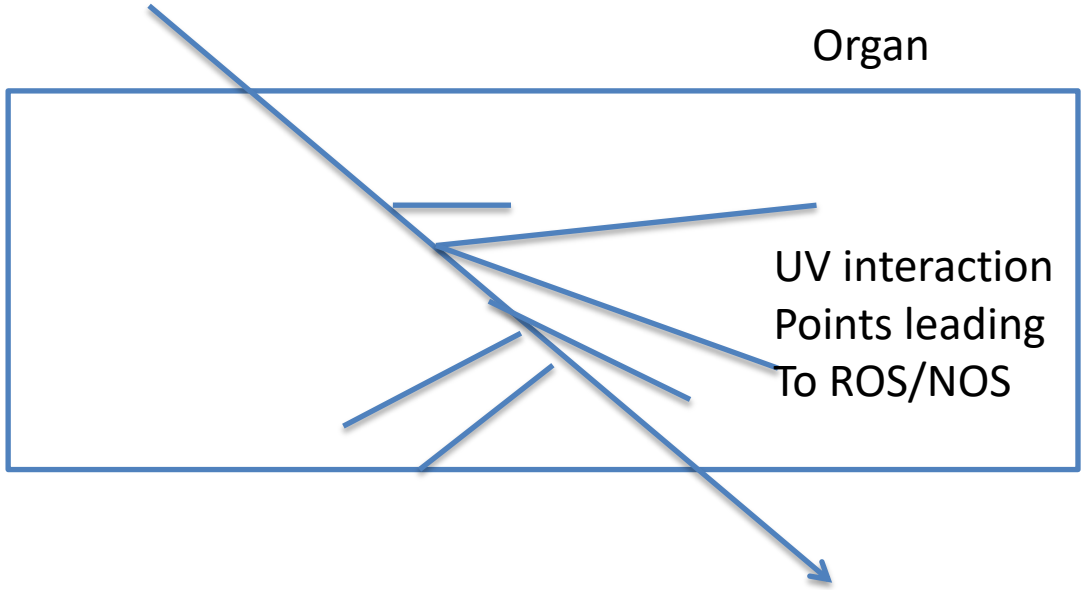
Ionising track in matter

Each ionising track interaction will lead to UV emission



What this does inside an organ

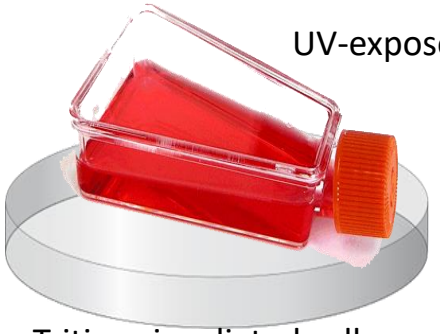
Track of ionising radiation



However…….

- Reports in the literature claimed the bystander “factor” in medium was exosomes or microvesicles!
- Albanese and Dainiak first suggested this in 2002
- Kadhim group 2012-2016 and Lyng group in 2013 found evidence for exosome mediated transmission
- So what about our electromagnetic signal?

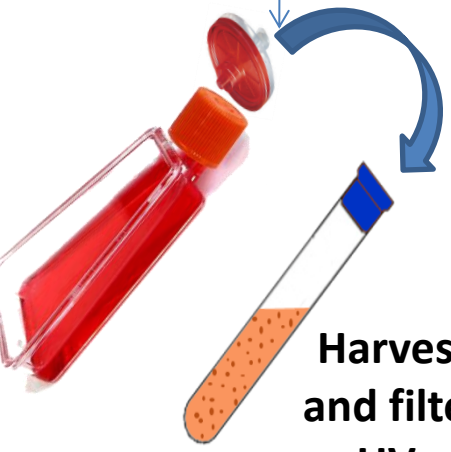
Exosome Work: experimental methods



UV-exposed bystander cells

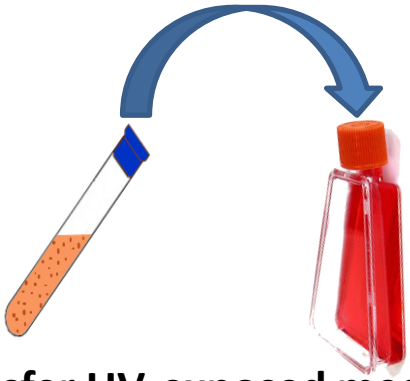
Tritium-irradiated cells

Incubate for 24 hours



Harvest and filter UV-exposed medium

Ultracentrifuge UV-exposed medium for 90 minutes at 4°C



Transfer UV-exposed medium directly onto clonogenic reporter cells

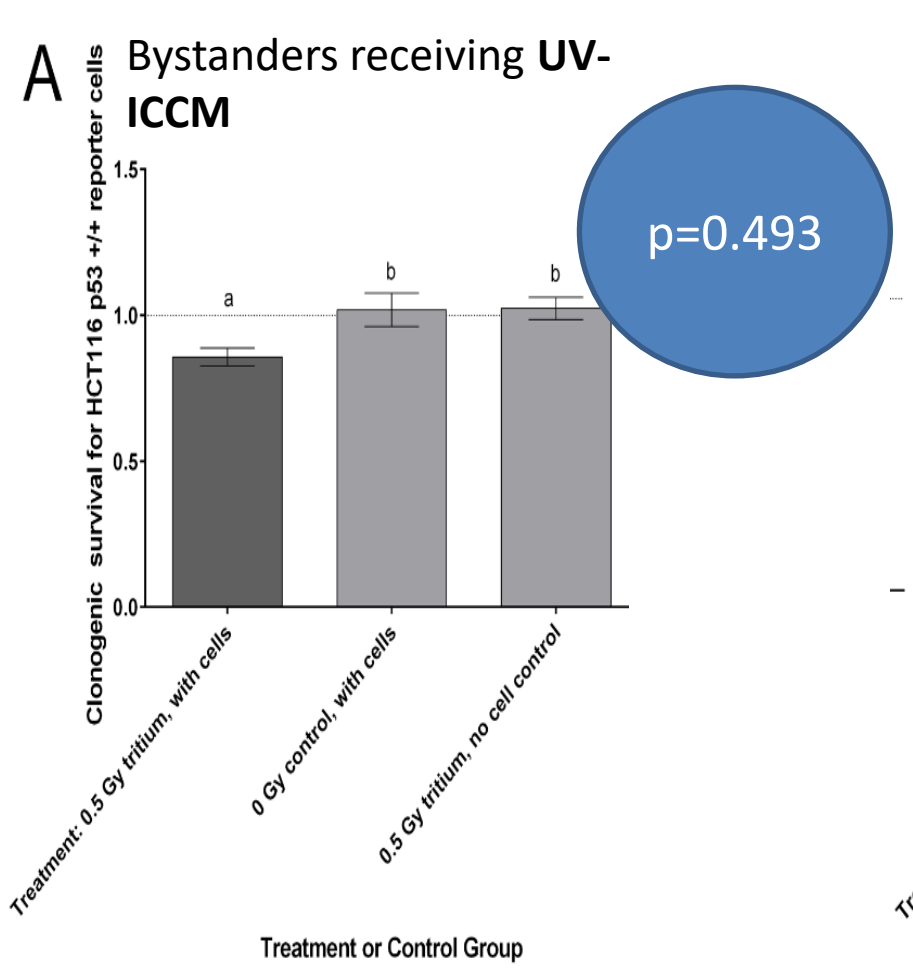
Resuspend pellet containing exosomes in

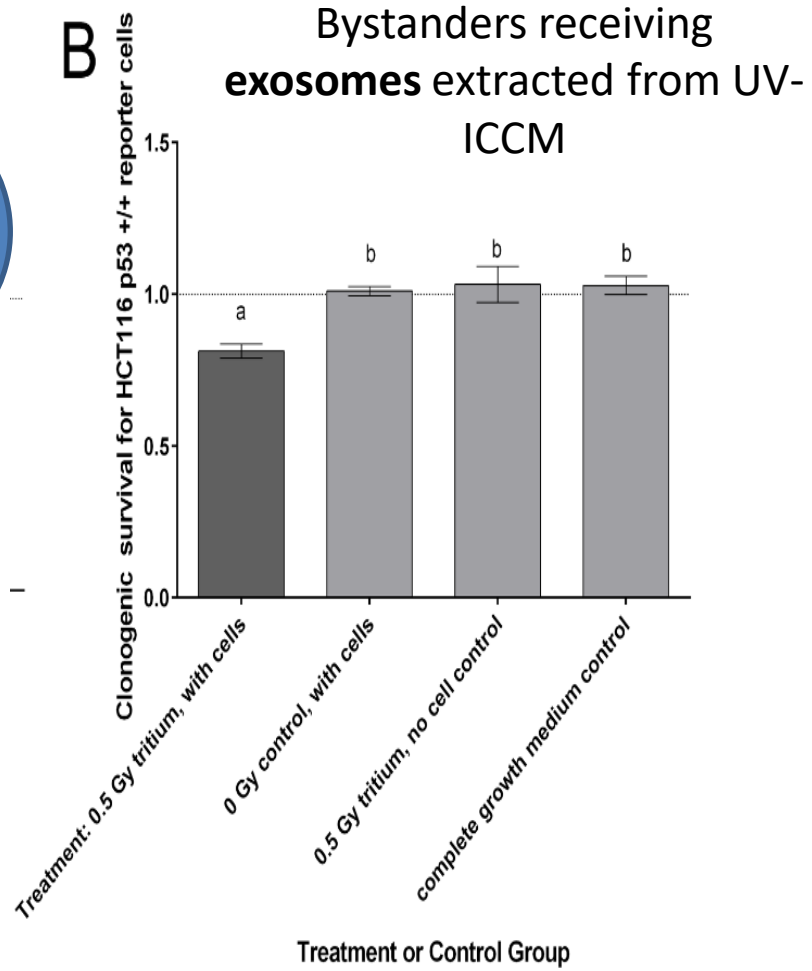
Transfer exosome fraction onto JC-1 incubated cells for detection of mitochondrial membrane depolarization

Transfer exosome fraction onto clonogenic reporter

Cell death is induced following exosome transfer to bystanders

No significant difference between bystander medium or exosomes extracted from the medium

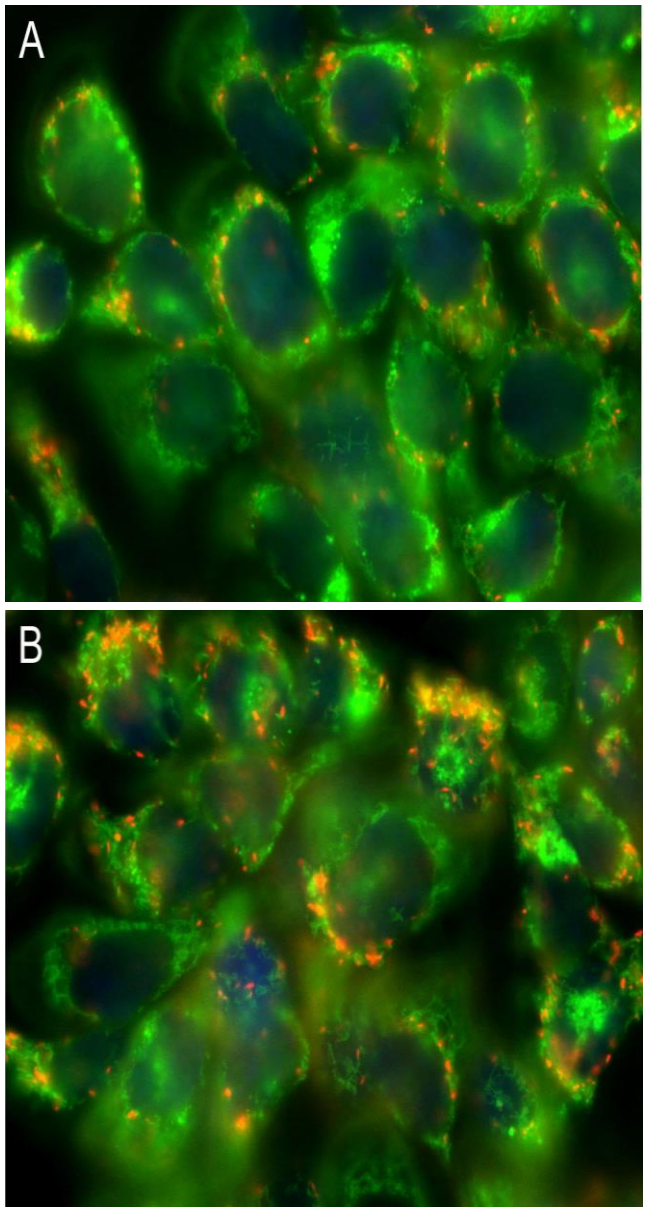


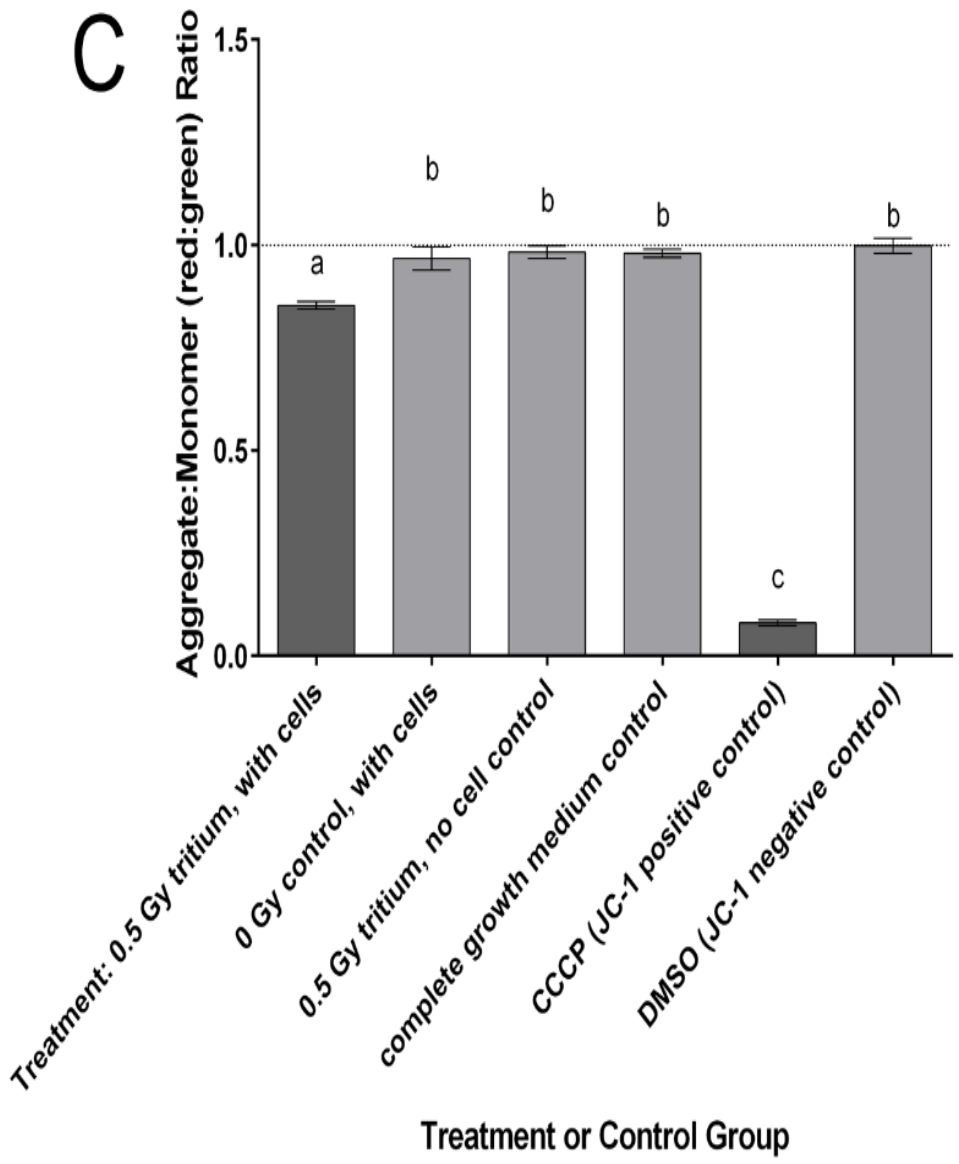


Mitochondrial membrane depolarization is induced following exosome transfer to bystander cells

Exosomes from UV-ICCM

Exosomes from control CCM



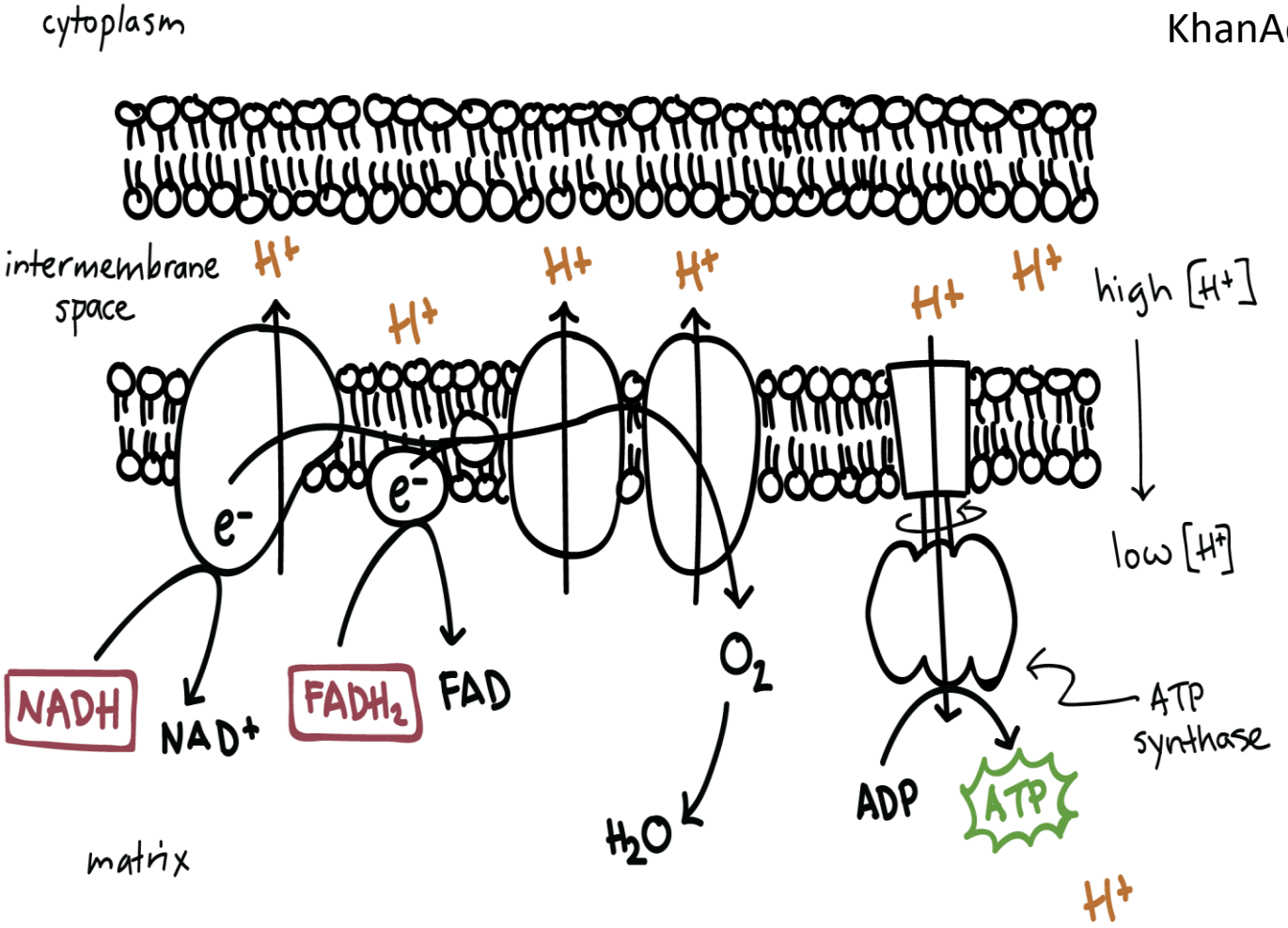


Investigation of mitochondrial activity

- Key question is what are the photons doing ?
- How is the excitation energy affecting the cellular response in the bystanders?
- Since we knew mitochondrial activity was affected, we decided to look at events in the electron transport chain

Mitochondrial Electron Transport

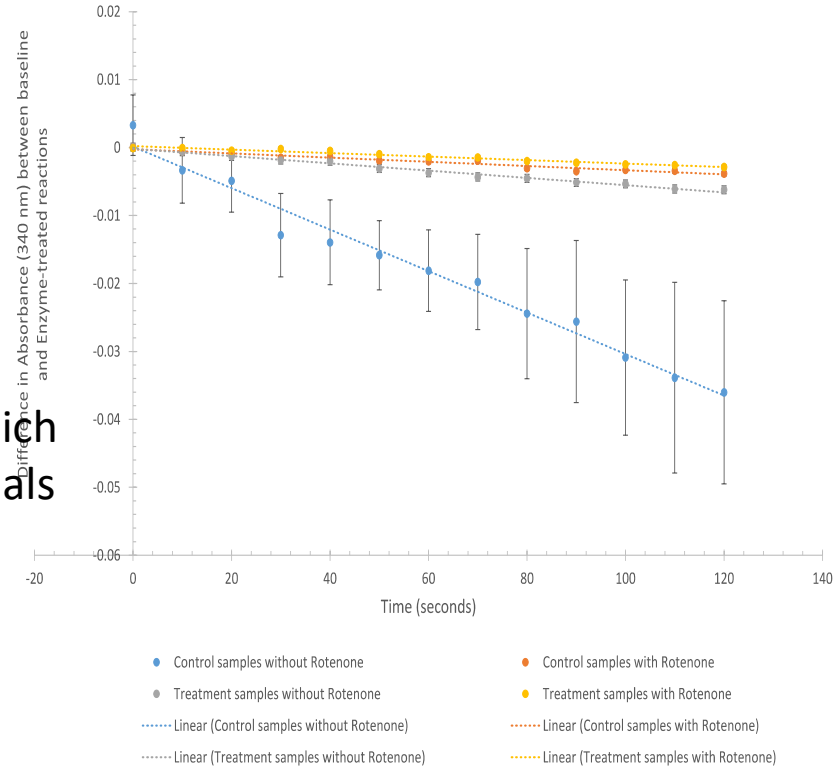
From KhanAcademy.org



Complex I activity

$$NADH + H^+ + UQ1 \xrightarrow{I} NAD^+ + UQ1\,H_2$$

Activity of complex I was assayed in mitochondria isolated from unirradiated control cells and from bystander cells which Received electromagnetic bystander signals



Activity of complex I was Completely suppressed by the Electromagnetic signals

Figure 1: Complex I activity. Oxidation of NADH demonstrated by a decrease in absorbance at 340 nm over a 2 minute duration. Each data point represents data acquired from three different mitochondrial protein samples (biological replicates) tested in triplicate (3 technical replicates). Errors bars represent standard error for n=9.

Conclusion from ETC study

- Mitochondrial effects mediated through inhibition by the EM bystander signal of complex I and V activity result in altered ATP production
- This will have knock-on effects for repair processes and metabolic activity in the cells receiving the EM signal

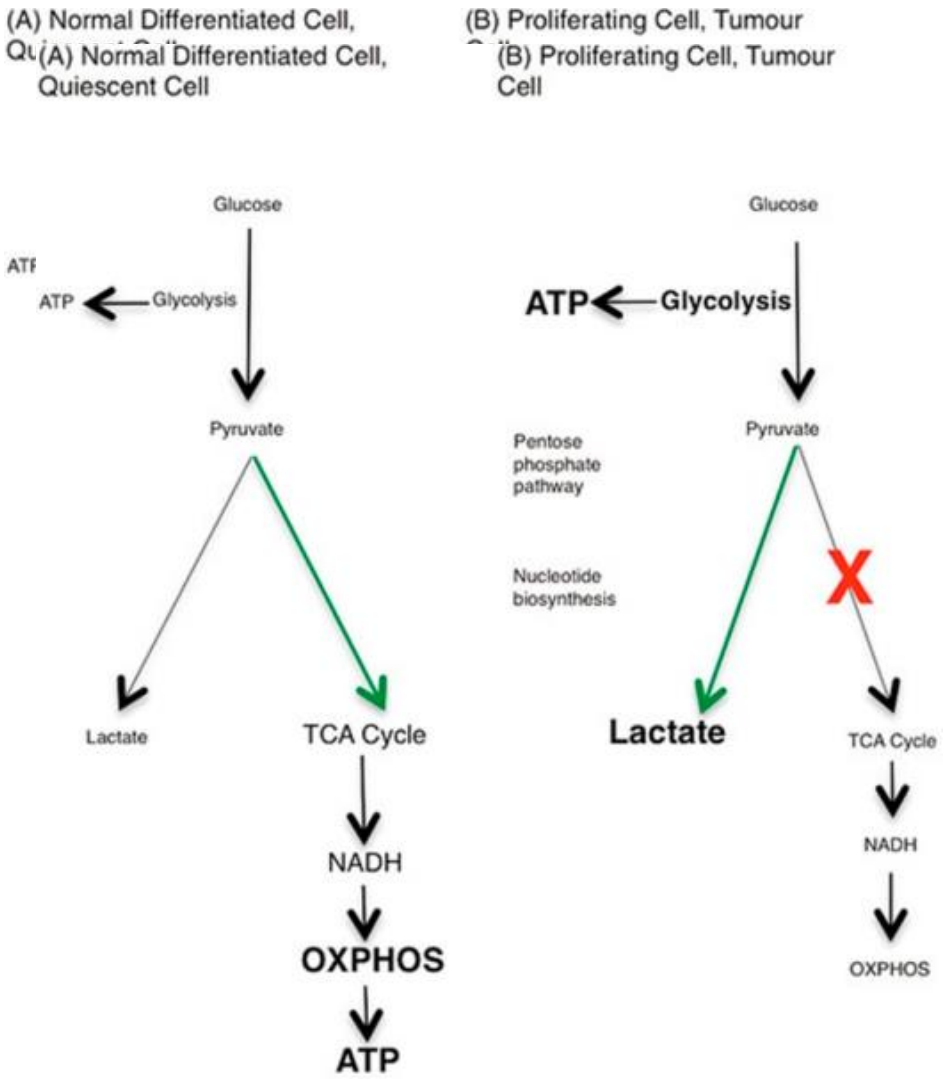
Biophotons appear to be key players

- They are produced by irradiated cells
- They have peak energies of 340nm (UVA) and 400nm (blue light)+smaller peak in the red (around 600nm)
- Quantity is directly related to number of cells and dose
- They can *by themselves*, induce NTE and do this by modifying the contents of exosomes secreted by cells as a means of communication
- Harvested exosomes from biophoton-exposed cells can turn on NTE

Our current hypothesis

- Biophotons are emitted from excitation decay in directly irradiated cells or *organic material even if "dead"*
- Biophotons trigger exosome release in cells that receive photon energy possibly as a result of the mitochondrial ETC biochemistry
- Exosomes delivered to other cells in the system
- Exosomes contain information leading to system level response
- These exosomes contain information leading to system level response which may be sub-optimal –i.e. the target is widened or protective i.e. a defense mechanism is triggered.

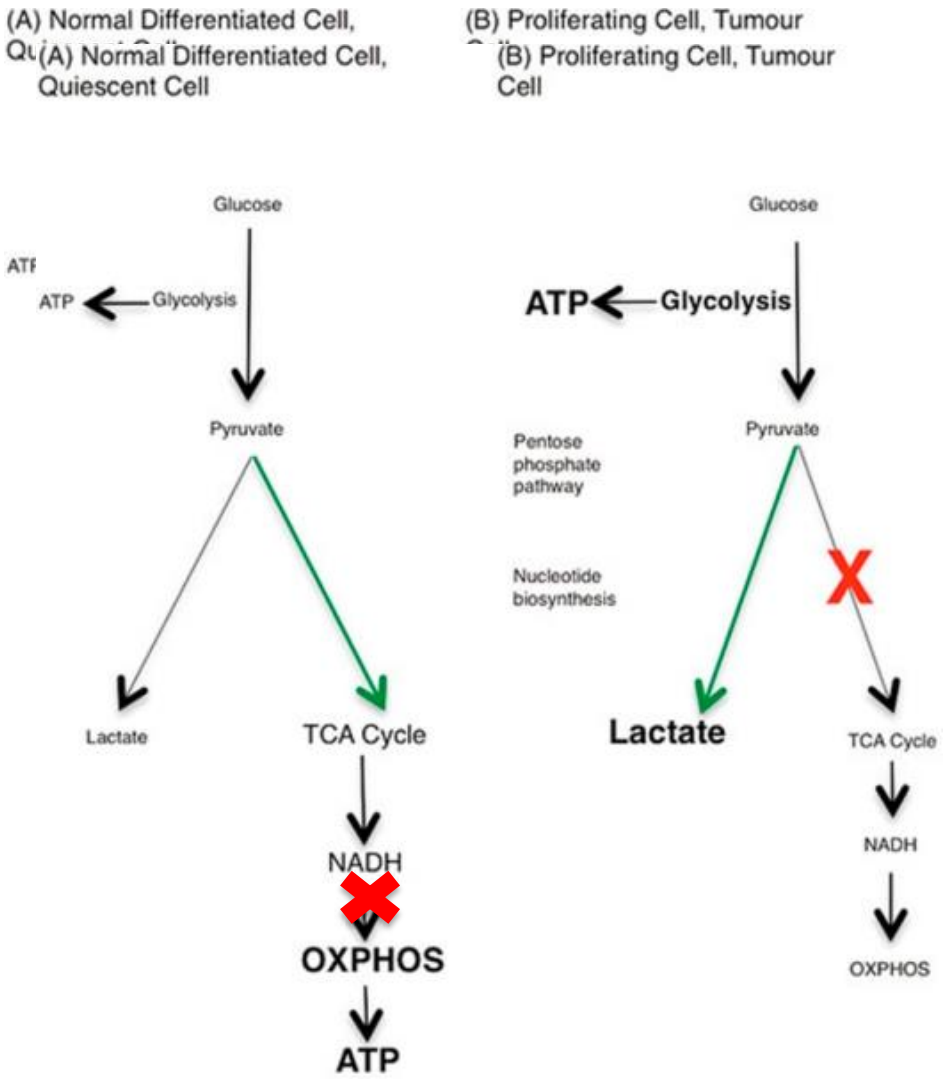
Warburg Biochemistry



Warburg: Key points

- Warburg thought cancer arose from mitochondrial malfunction
- Idea dismissed when cancer cells were shown to have mitochondrial OXPHOS activity. Idea was they just preferred anaerobic pathway
- Now however cancer is thought to result from failure of cells to pass a block at pyruvate i.e the idea that loss of aerobic metabolism is a cause not a consequence is back in favour
- We say there can be more than one block and a complex 1 block could, if present allow carcinogenesis

Warburg Biochemistry



Warburg story

Otto Warburg's contributions to current concepts of cancer metabolism

Willem H. Koppenol, Patricia L. Bounds & Chi V. Dang

Nature Reviews Cancer volume 11, pages 325–337 (2011)

J Cancer. 2016; 7(7): 817–822.
Published online 2016 Apr 26. doi: 10.7150/jca.14274
PMCID: PMC4860798
PMID: 27162540
Warburg Effect - a Consequence or the Cause of Carcinogenesis?
Slobodan Devic ✉

The future

- UV/Exosome theory published in Plos 1 in 2016
- Mitochondrial ETC published in Environmental Research in 2017
- Proof that gamma radiation can also lead to biophoton production submitted to IJRB
- Now we need to find out what is in the exosomes (pilot data suggests inflammatory response and FOXO pathways involved
- What are the impacts of dose rate and radiation quality on the biophoton emission and exosome content. Also what are the impacts of cell type, underlying genetics and (micro) environmental factors?
- Also working to determine what is absorbing the photons and why the activity of ETC complexes I and V in mitochondria are suppressed and what are the implications for normal and tumour cell metabolism.

Outline

- **Introduction to some issues**
  - Saturable LDR effects
  - Adaptive responses, NTE, HRS
  - Uncertainty
  - Individual responses
  - Complexity levels
- **Mechanisms**
  - NTE: photons, exosomes
  - HRS: apoptosis, genomic instability
- **New Targets**
  - Therapy
  - Protection

So What……

- So non-targeted effects occur and UV is at least a triggering event if not the bystander signal itself.
- BE appear to drive genomic instability and generate a higher than normal frequency of, or tolerance for mutations
- What does this mean for therapy, carcinogenesis and mutation rates in human and non-human populations?

What NTE do to radiation protection and why they matter

- Dissociate radiation energy deposition in a target (e.g. DNA) from the effects
- Opens the way for big effects after small doses
- Opens the way for diffuse and unpredictable effects at the level of the organ, organism, population and ecosystem which are not linked simply to the dose delivered to a particular “target”
- Link radiation induced oxidative stress to biological effect
- Could explain the conditions seen in Atomic Veterans, Gulf War veterans and CFIDS/ME sufferers
- Also allow for hormetic and adaptive effects

Relevance for carcinogenesis

- NTE raise the tolerance for mutations in a system so rogue cells may not be eliminated and de novo mutations may occur at a higher frequency – good for evolution
- Genomic instability persists and is transgenerational so de novo mutations created with every cell division
- BE leads to communication of damage to un-hit cells but lots of evidence that BE turns on defensive mechanisms
- Bottom line – relevance unknown!

The Future - Practical

- Our research represents a radical paradigm shift which opens up new possibilities for treatment of sufferers
  - Can we harness “good” exosomes and supply them to sufferers?
  - Can we bypass the complex 1 block and restore normal ATP production?
  - Can we develop an assay based on mitochondrial function to diagnose patients with likely problems?

QUESTIONS?