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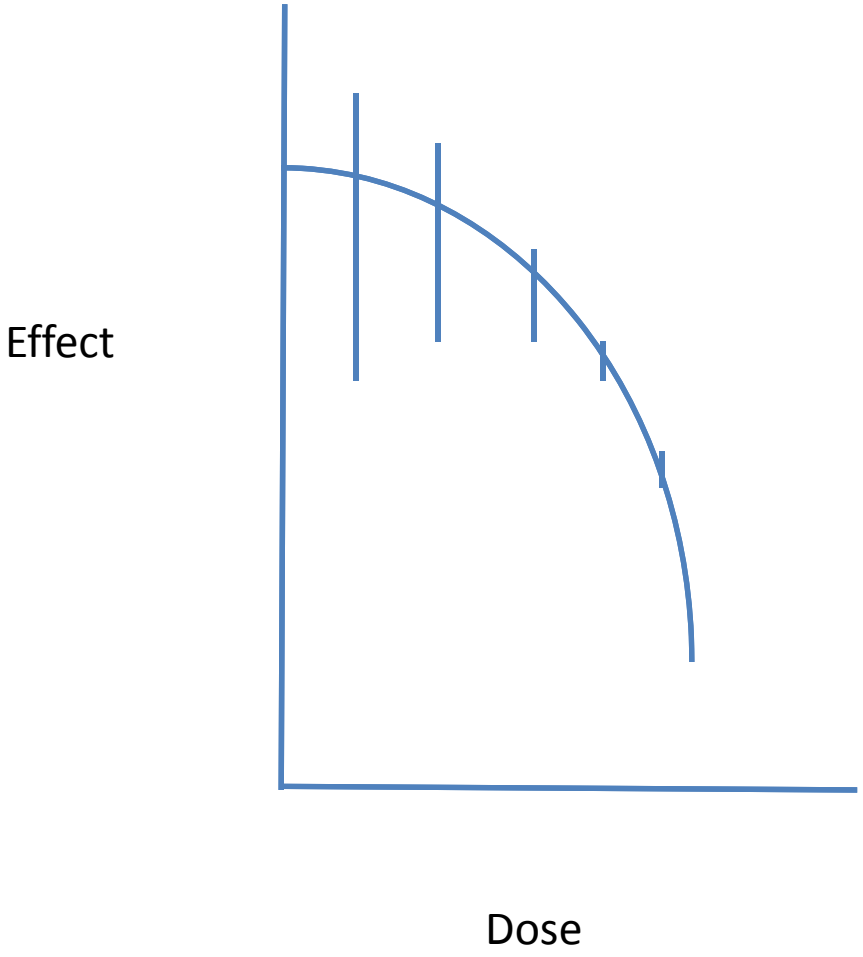
Canada

Low dose effects, stress, adaptive responses and hormesis

Colin's Nutshell!
The Issue: Acceptability of Uncertainty



Deviations about the mean
much greater in low dose
part of survival or dose response curve leading to uncertainty

Causes are physical associated with dose distribution and deposition and
biological associated with transduction of response at Individual and population/ ecosystem level

Challenges in Radiobiology: The data gaps and the data availability

- Low versus high dose ---------- LNT
- Chronic versus acute ----------DDREF
- Internal versus external---------TF and CR
- Alpha versus gamma----------RBE
- Ecosystem versus individual----Biomarkers
- Epigenetics versus genetics ----NTE
- F_0 versus F_n----------TGE

Goals of our laboratory

- To reduce uncertainty by understanding what determines the ultimate outcome when radiation interacts with a population be it cells or individuals
- To understand the mechanisms involved in low dose and chronic effects response transduction
  - Role of NTE
  - Role of radiation quality

Stories!

- Our old work – pre and post conditioning
- Mechanistic work – what seems important
- Radium data – in vivo adaptive response
- Our students' work (on posters)
  - Michelle Le – UV photons start BE
  - Cris Fernandez-Palomo - IRR associated with loss of BE
  - Jason Cohen – "priming dose" can be given after irradiation

1993 Experiment

5Gy+3hrs+10Gy
not the same as
10Gy+3hrs+ 5Gy

Immediate and
delayed death
levels are different

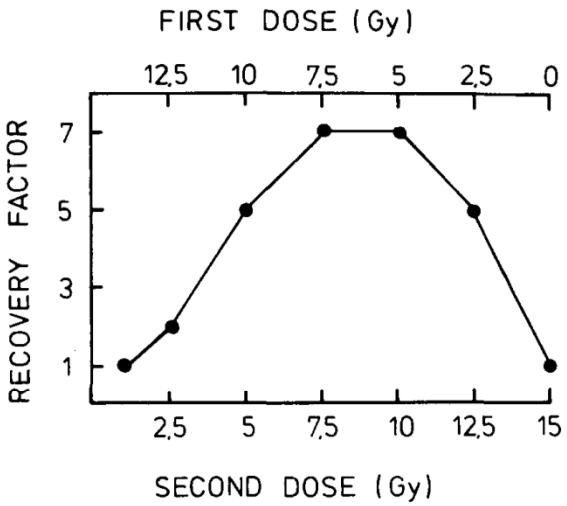


Fig. 3. Plot of the recovery factors obtained when CHO-KI cells were exposed to a total of 15 Gy given as two doses separated by 3 h where the size of 1st and 2nd doses was varied as indicated.

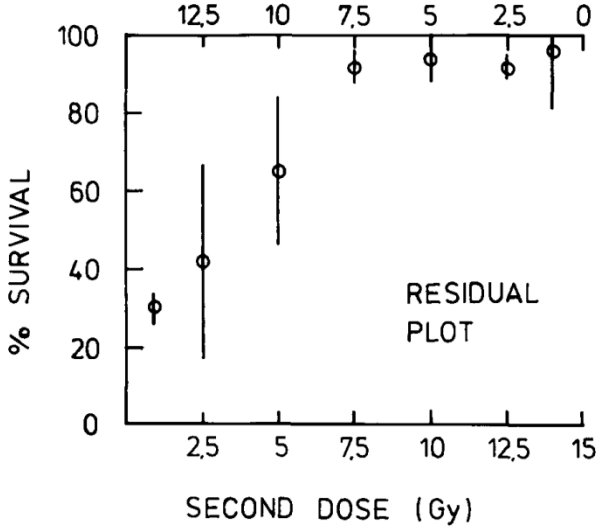


Fig. 4. Plot of the residual % survival obtained from the experiments presented in fig 3. Error bars represent the SEM over 9 points.

ing efficiencies is also much greater, as shown by the size of the standard errors.

Effect of azacytidine on the production of shoul-

Recovery of the radiation survival-curve shoulder in CHO-KI, XRS-5 and revertant XRS-5 populations.Mutat Res. 1993 Feb;285(2):259-66. Mothersill C, Seymour CB.

Old “post-conditioning” data

Bottom line

“a real dose of radiation is needed to induce this protection and that treatment with ICCM is not as effective”

Bystander and delayed effects after fractionated radiation exposure. Mothersill C, Seymour CB.
Radiat Res. 2002 Nov;158(5):626-33.

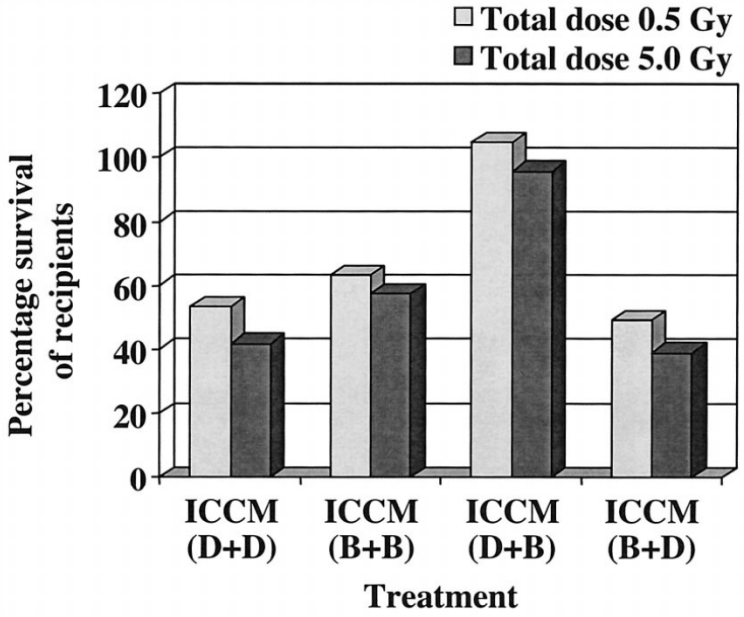


FIG. 2. Selected data plotted from Tables 3a and 3b comparing the results for experiments in which ICCM was harvested and transferred to recipients that had never been exposed. The harvested ICCM was generated after two real doses of ^{60}Co γ radiation (D+D), two ICCM “doses” (B+B), a first real dose followed by ICCM generated from a second dose (D+B), or ICCM generated from a first dose followed by a real second dose (B+D). In all cases, the time between treatments was 3 h. In the figure, D indicates a real radiation dose and B indicates that the cells received the appropriately generated ICCM (bystander medium).

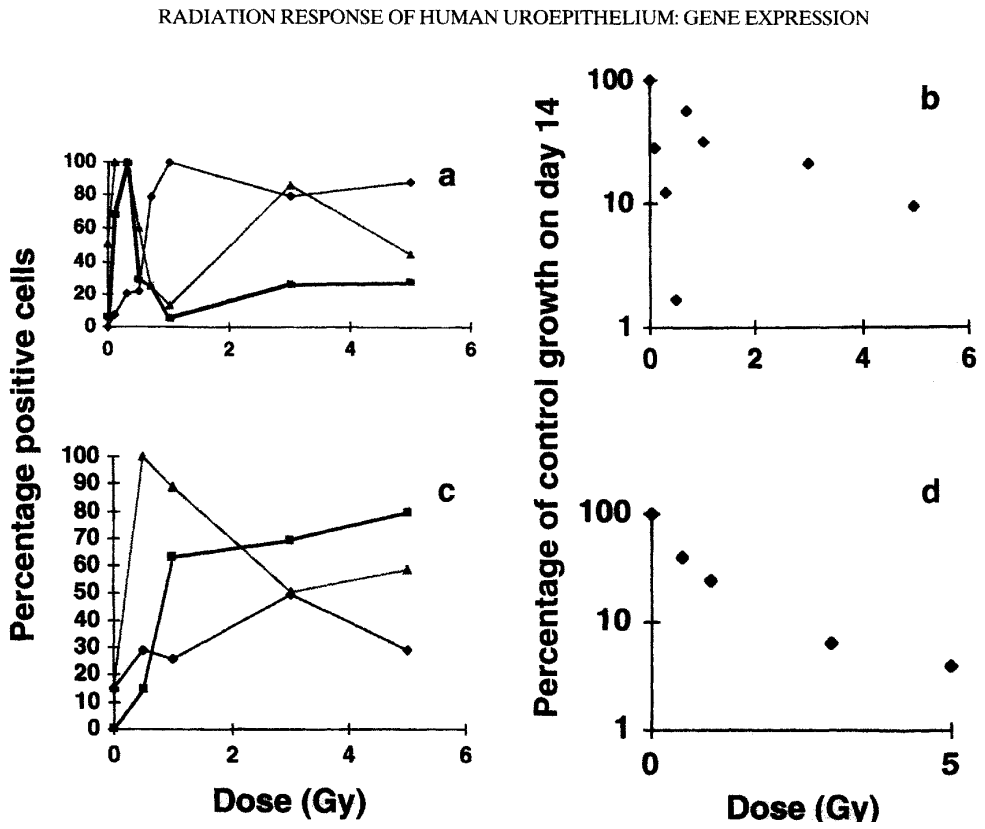
Adaptive response with BM

TABLE 1
Clonogenic Survival of HPV-G Cells

Dose	Direct radiation	ICCM
0 Gy	100 (33 ± 1.1)	100 (31 ± 2)
0.5 Gy	74 ± 3%*	68 ± 2%*
5 Gy	15 ± 2%*	66 ± 3%*
0 Gy + 0 Gy	100 (32 ± 1)	100 (33 ± 1)
5 mGy + 0.5 Gy	101 ± 2%*	79 ± 6%*
0.5 Gy + 5 Gy	53% ± 6*	80 ± 26%*

This study has shown that a low priming dose of ICCM 24hrs before has the ability to induce an adaptive response in HPV-G cells subsequently exposed to a challenge dose of ICCM. NAC inhibits effect of ICCM Maguire et al, 2007

Genetic basis of dose response



Normal urothelium from 15 human Patients. show clear division into those Inducing radioresistance around 0.5Gy and those who do not, p53, bcl2 and cmyc ratios critical

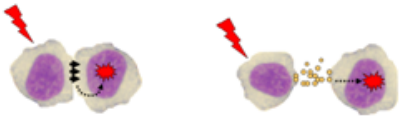
Mechanistic studies

Aimed at discovering the mechanisms of critical importance in the low dose part of the survival curve

'Non-targeted' radiation effects

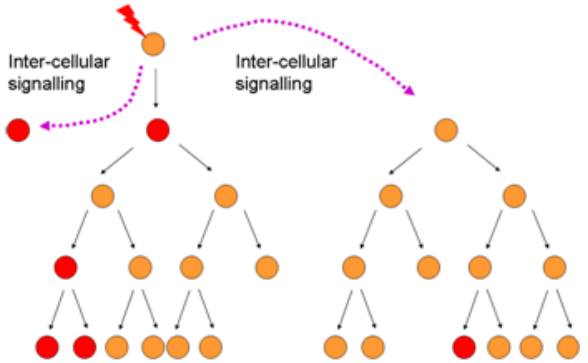
Bystander effects

Effects in neighbouring cells



Genomic Instability

Effects in unirradiated descendant cells

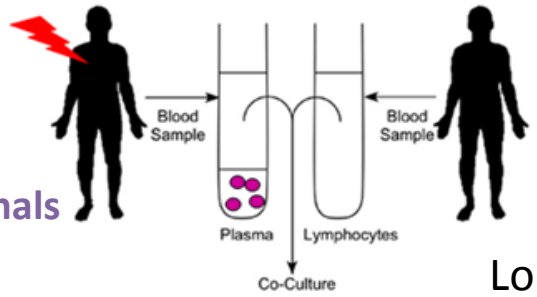


Abscopal effects

Effects in neighbouring tissues

Clastogenic factors

Ex vivo effects in cultured cells



Inter-animal signaling

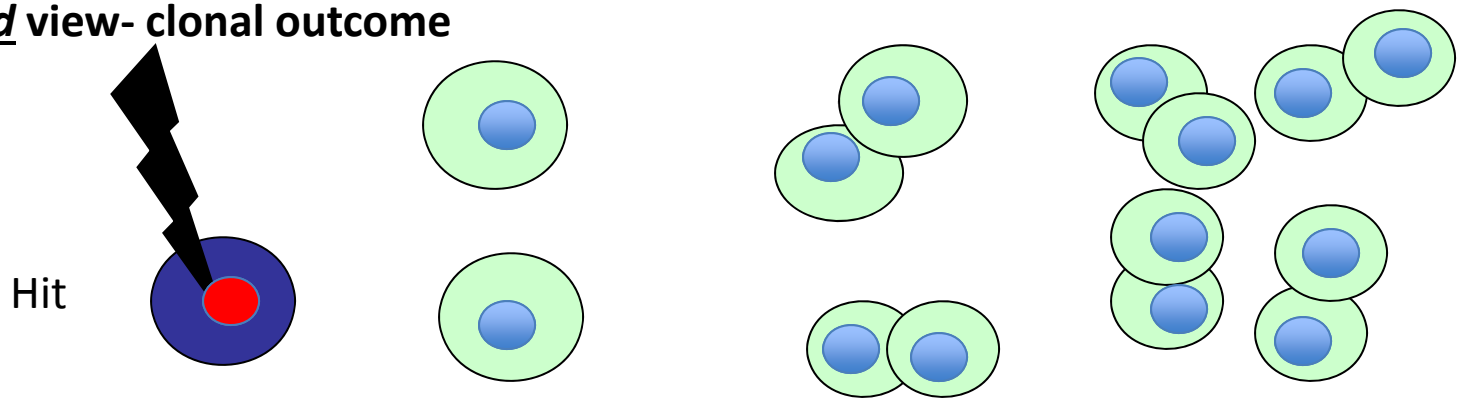
Effects in neighbouring animals

Inflammatory Processes may provide mechanistic link

Long-term effects on innate immune response function may occur

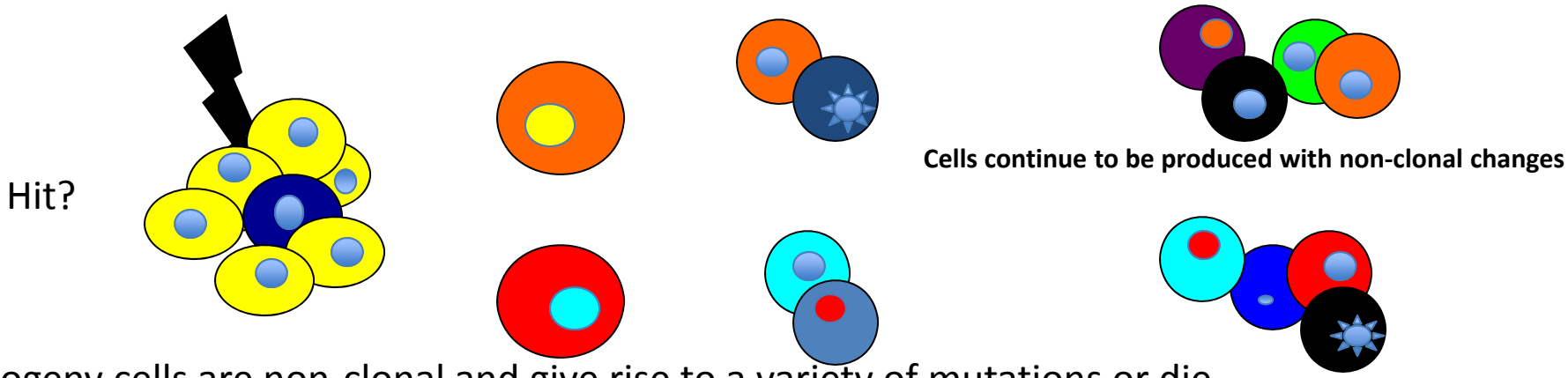
The link between bystander effects and genomic instability – twin pillars of the new paradigm

Old **view- clonal outcome**



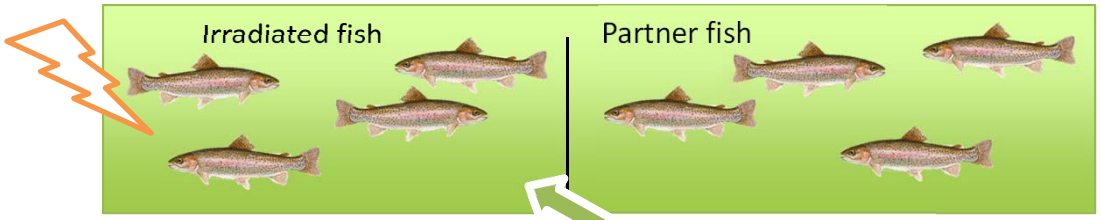
Progeny are all clonal i.e. identical and mutation is passed to all progeny

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



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

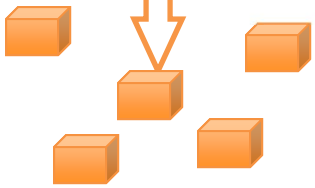
Measuring bystander response to radiation *in vivo* (adapted from Mothersill et al 2006)



Irradiate or sham irradiate fish, allow to swim with unexposed partner for 2hrs

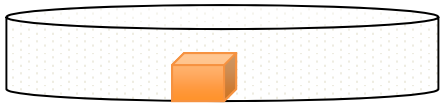
Unexposed fish introduced into water from irradiated or sham fish After 2hrs. Dissect tissues

Do proteomics



Explant pieces taken from skin, fin, gill, spleen and kidney

Do tissue culture

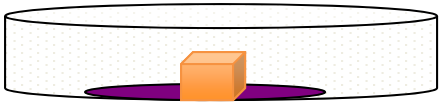


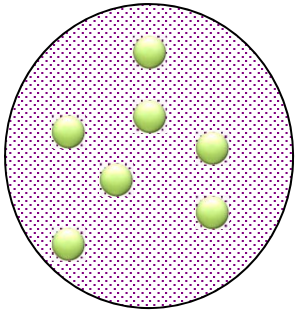
Culture of explants for 2 days

Harvest culture medium for calcium flux, ELISA and clonogenic assays

Grow up culture

examine explant outgrowth do immunocytochemistry





Add medium to unirradiated clonogenic cell line

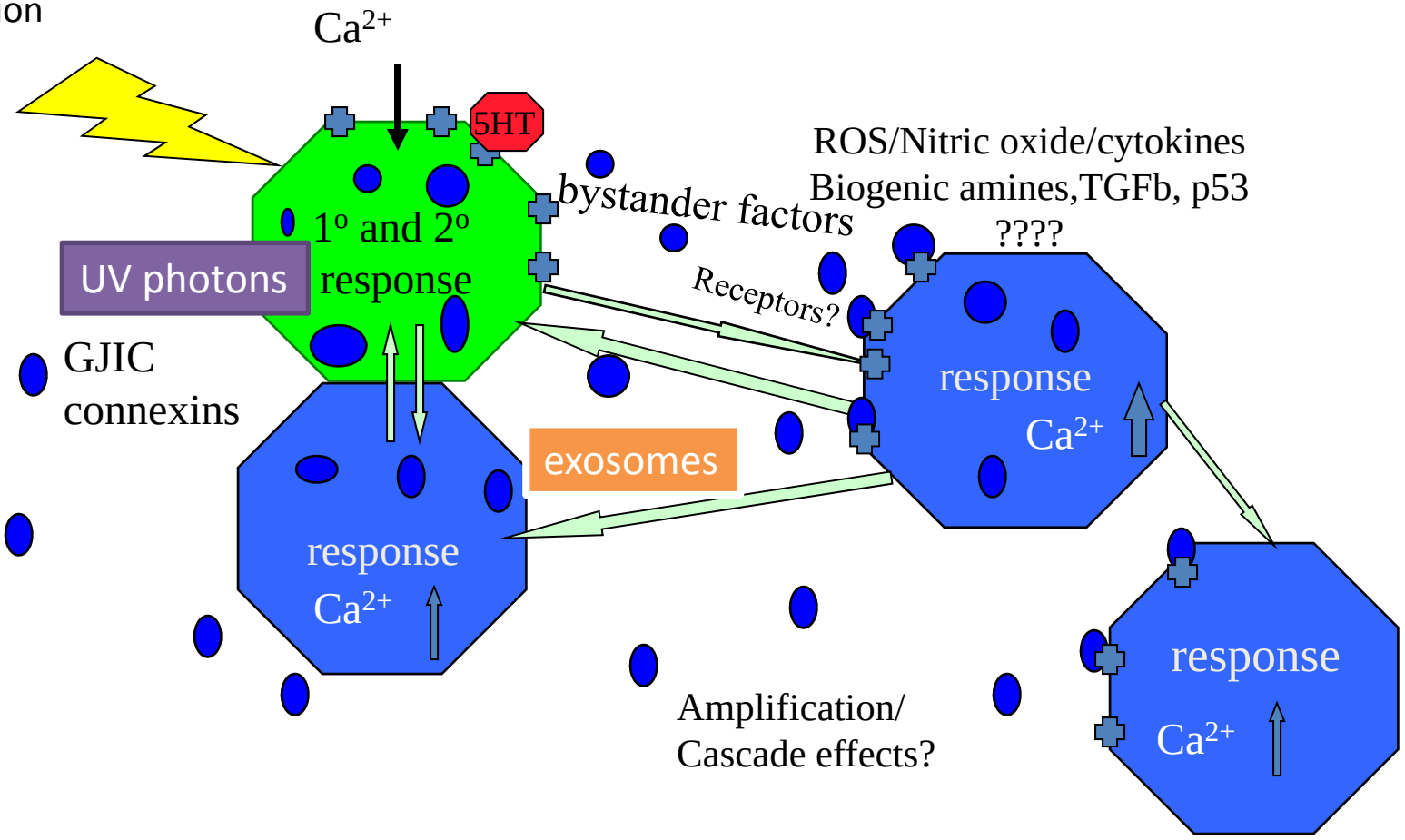
determine surviving fraction by counting colonies after 10 days

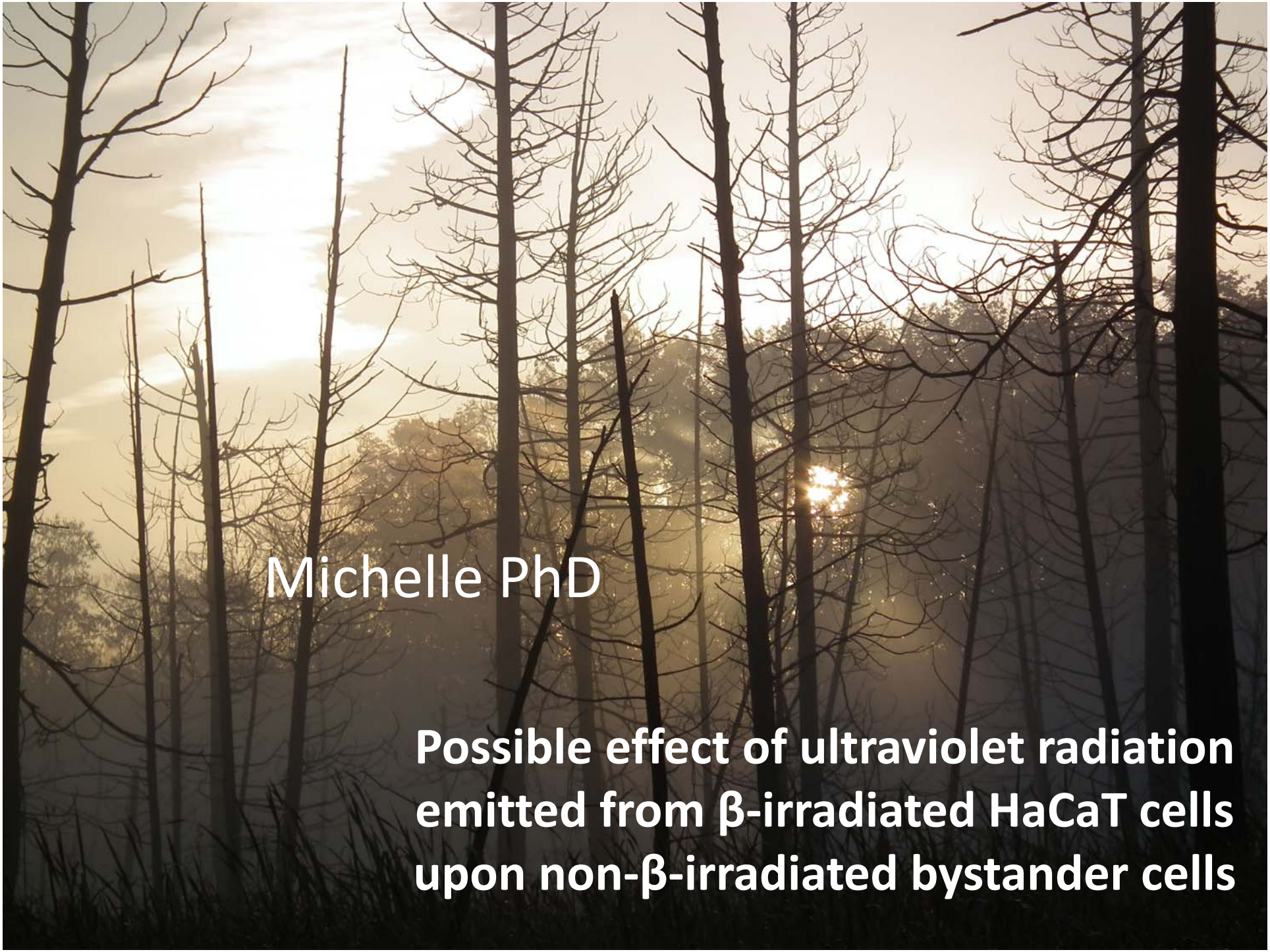
Published stuff we know!

- Bystander mechanism perpetuates genomic instabiity
- Calcium pulse occurs within 30 secs after 5mGy acute dose and up
- Serotonin binding to cell membrane receptor which is a voltage gated calcium, triggers calcium flux
- Excitation decay UV photon emission appears to initiate signal production pathway
- P53 and cytokines involved in transducing response to signals
- Response depends on genotype and p53 status, signal production independent of these
- Proteome reveals changes in energy, oxidative stress and structural proteins

The bystander effect

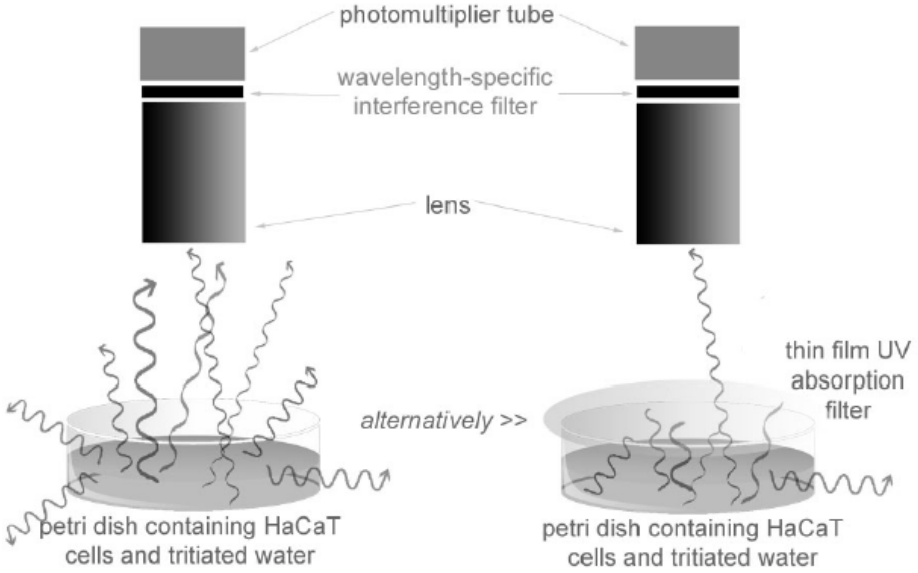
Ionizing radiation, UVA, UVB, ELF-EMF and heavy metals induce affected cell to signal to others. Responses to the signals include apoptosis, micronucleus formation, transformation, mutation, induction of stress and adaptive pathways. Serotonin (5HT), L-type calcium channels (which are 5HT-3 receptors)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



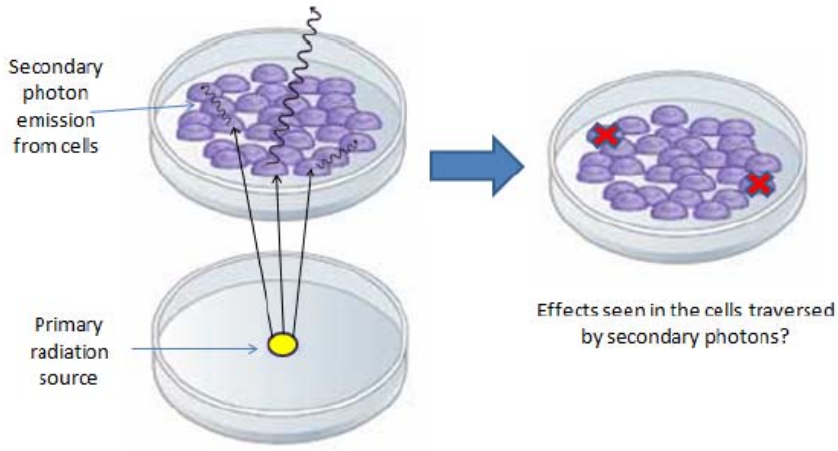

Michelle PhD
Possible effect of ultraviolet radiation emitted from β-irradiated HaCaT cells upon non-β-irradiated bystander cells

Experimental Design

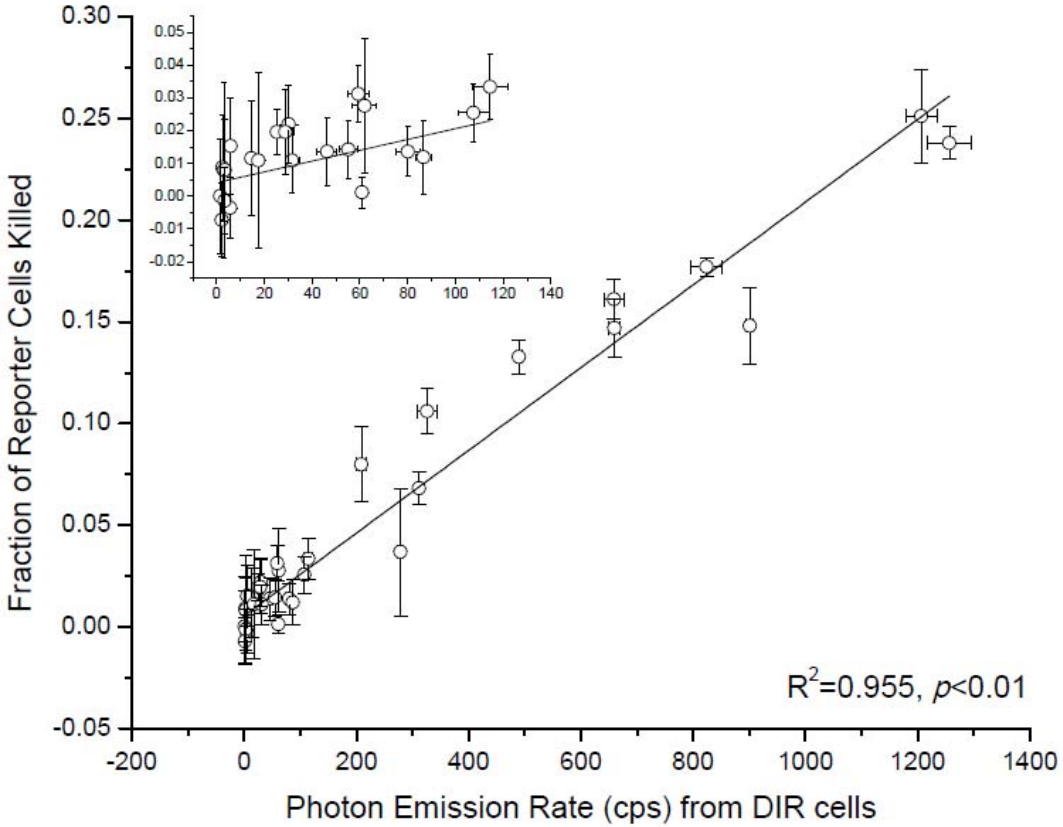
- **Photon Quantification**
  - Quantified ultraviolet photon flux emitted from tritium-irradiated cells
    - Observed significant photon emission upon beta-irradiation of human keratinocyte (HaCaT) cells



- **Clonogenic Survival of Bystander Cells**
  - Placed bystander cells directly superior to directly-irradiated cells to receive emitted UV photons
  - Then assessed clonogenic survival in bystander cells



Strong association between photon emission from directly-irradiated cells and bystander cell killing



- Linear regression: $R^2 = 0.955$

HRS/IRR and NTE

Cris PhD

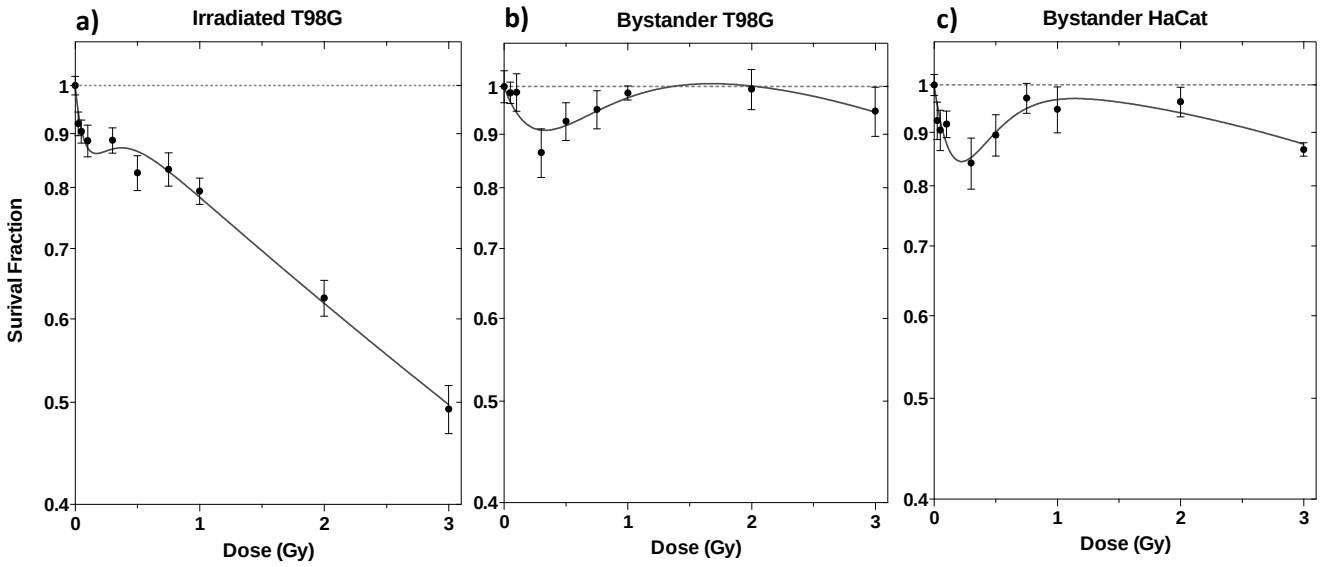


Figure 2. Survival fraction of irradiated and unirradiated (bystander) cells. The solid line represents the best fit for the IR Model for each plot. (a) Clonogenic survival of donor T98G cells irradiated with a cesium-137 source. (b) Clonogenic survival of T98G cells grown in ICCM from irradiated T98G. (c) Clonogenic survival of HaCat cells grown in ICCM from irradiated T98G. (Irradiated T98G n=18; Bystander T98G & HaCat, n=9 each; Error bars=SEM)

HRS/IRR Model

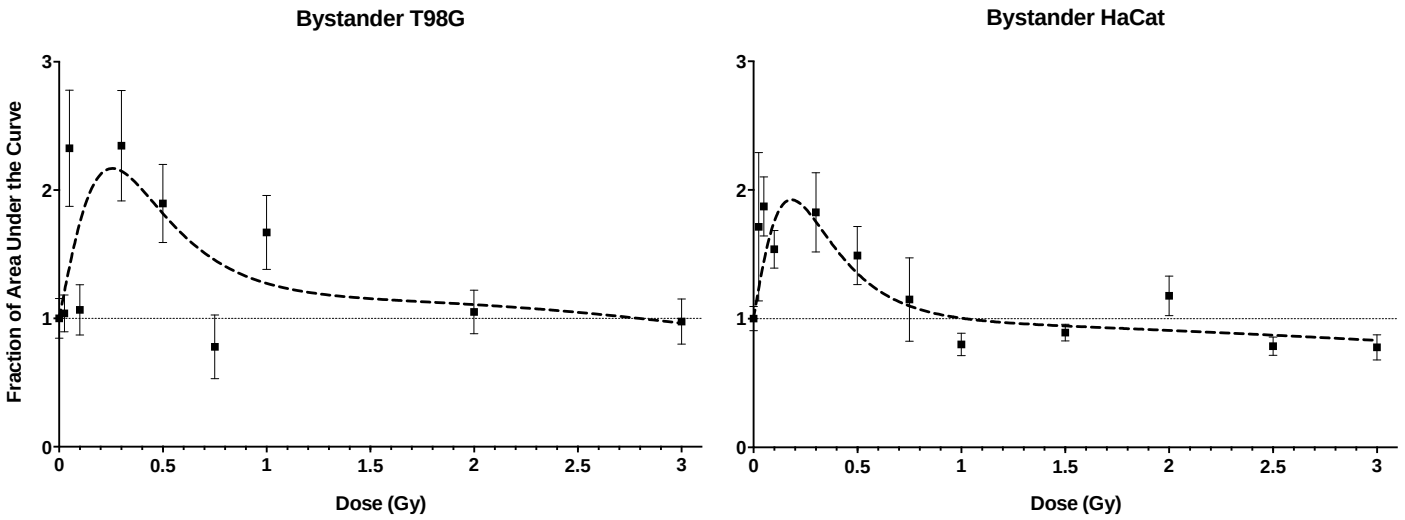
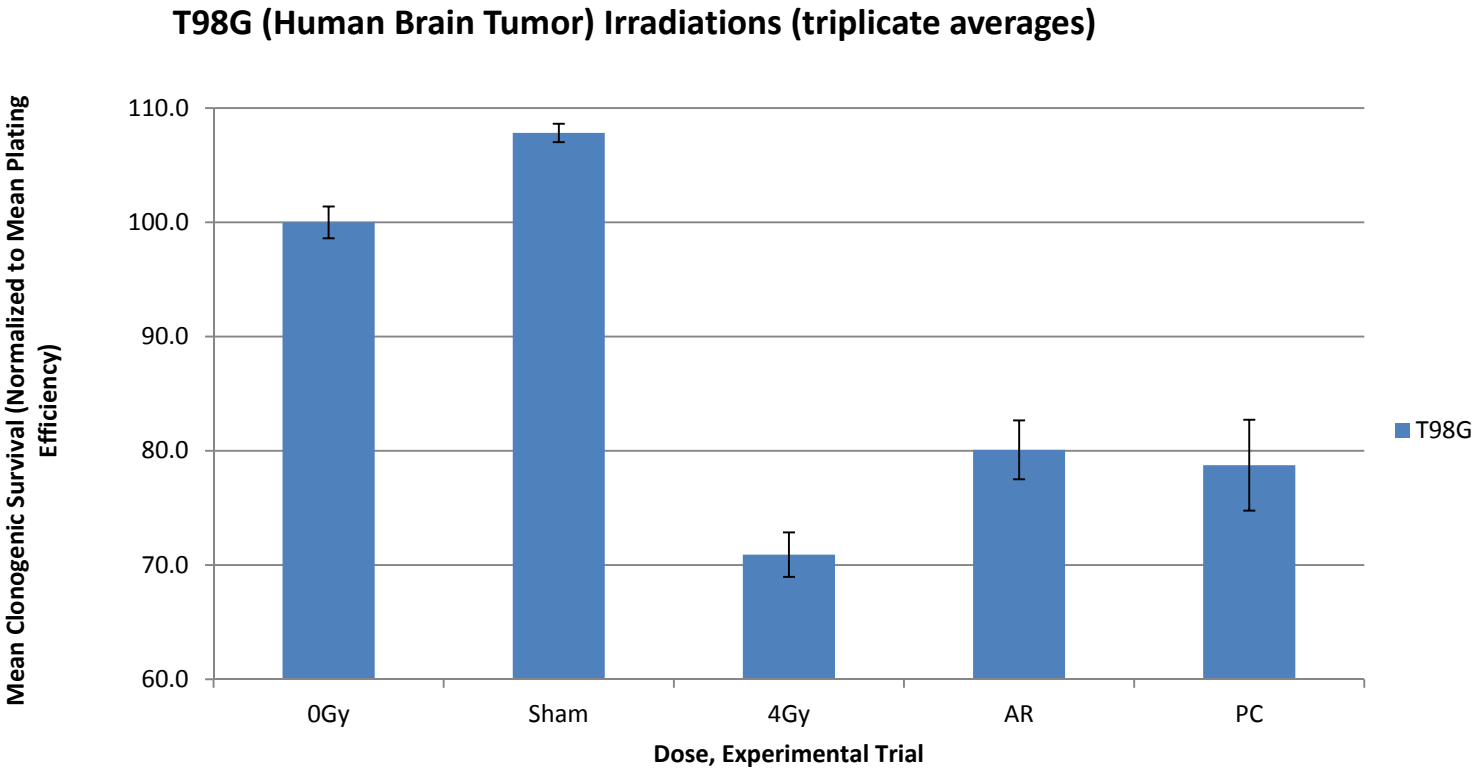


Figure 3. Calcium influx on bystander cells induced by ICCM from irradiated T98G.
Fura 2/AM was used to perform ratiometric calcium measurements on T98G and HaCat cells. The data show the influx of calcium ions through the cellular membrane triggered by the addition of ICCM from irradiated T98G. The data was plotted as Total Peak Area Under the Curve for each dose. The data have been normalized to the control (0 Gy) in terms of 'Fraction of Area' to allow the best fit for the IR Model , which is shown as a dashed line (n=10; Error bars= SEM)

New Post –conditioning experiments

Jason –
Co-op student

AR and PC are the same – T98G cells

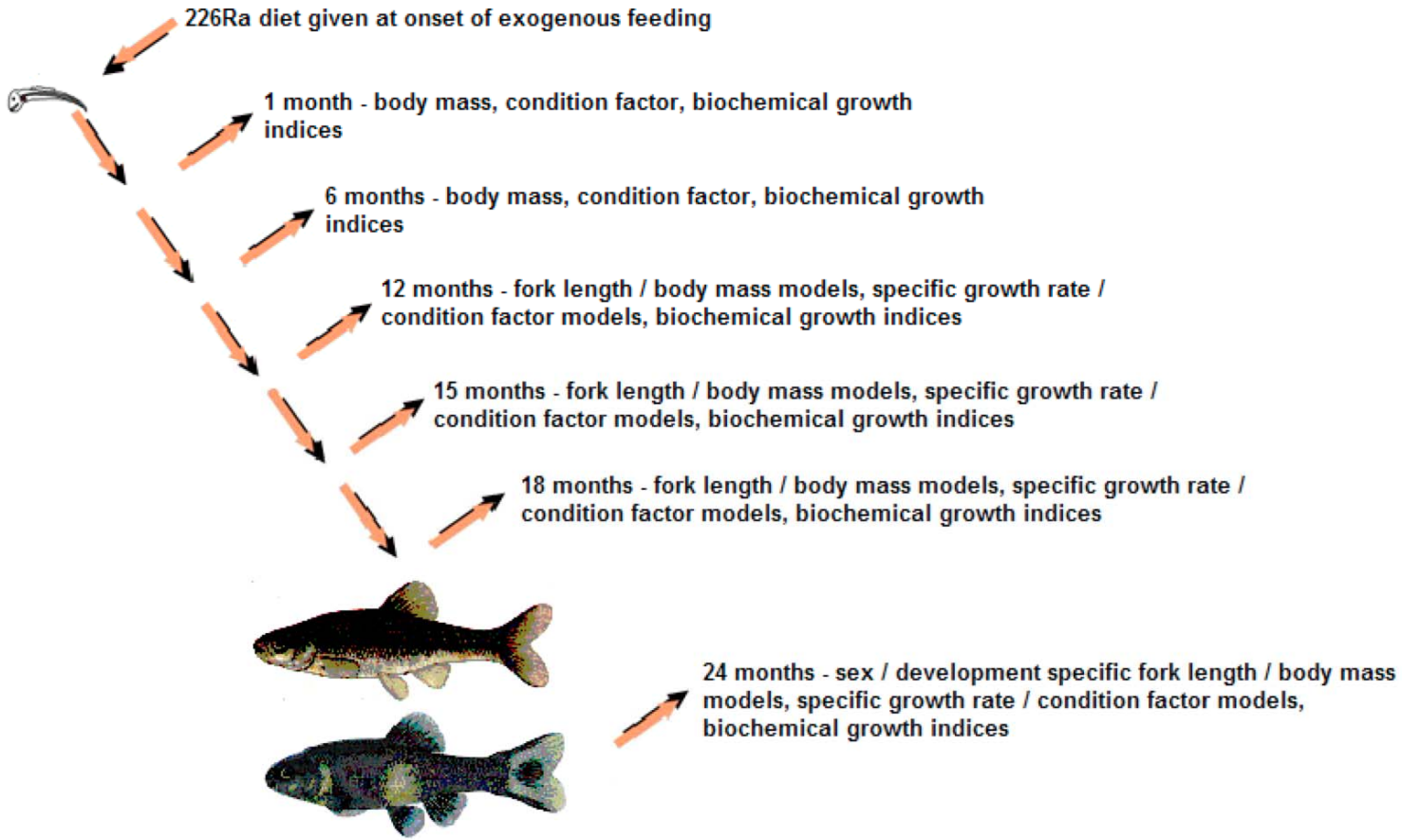


Radium depuration with time

CNSC/NSERC funded work

Schematic of Experiment

Figure 1



Calculated CF from Dr Lariviere

Averaged concentration factor (CF) calculated for various fish age

	Fish age (months)				
Food activity (Bq kg^{-1})	1 (n=2)	6 (n=16)	18 (n=8)	24 (n=16)	Average
10	3.1	0.375[a]	0.692	0.92	1.27
100	0.1	10.06	0.2321	0.099	2.62
1 000	0.0295	1.70825	0.02788	0.0174	1.78
10 000	0.0112	0.378143	9.865×10^{-3}	4.55×10^{-3}	0.10

a. Only two fishes test had activities above DL.

Concentration factor falls as fish age and as dose in food increases. Radium actually lost from older fish

Radium purging (depuration)

- Confirms data seen based on a small pilot study
- Suggests a modification of calcium transport mechanisms
- Supports the pattern of adaptive effects during chronic exposures (Hinton, Stuart, Mitchel and others)
- Means ^{226}Ra uptake in this fish species at these low concentrations is probably not an issue


Reproductive and transgenerational effects
Acute and chronic data

Reproduction in ^{226}Ra treated fish and mice

- Chronic feeding with radium spiked food/water
- Several attempts with fathead minnows failed as the fish would not breed. Zebrafish in progress.
- Very successful mouse breeding expt to F_4 [collaboration with Marilyn Stuart AECL (now CNL)]
- No indication of any impacts on reproduction of the environmentally relevant doses tested (10mBq – 10Bq per g/ml)

Acute x-ray study with salmonids

- Fish exposed at early life stages to a single 0.5Gy x-ray and maintained at Alma hatchery
- F0 and F1 stages showed effects on proteome, stress signaling and biochemical indices but no impacts on growth or reproduction
- F2 assays just completed – NO IMPACTS AT ALL
- F0 data published, F1 and F2 to come!

Irradiation of rainbow trout at early life stages results in legacy effects in adults.

Mothersill C, **Smith** RW, Saroya R, Denbeigh J, Rowe B, Banevicius L, Timmins R, Moccia R, Seymour CB.

Int J Radiat Biol. 2010 Oct;86(10):817-28.

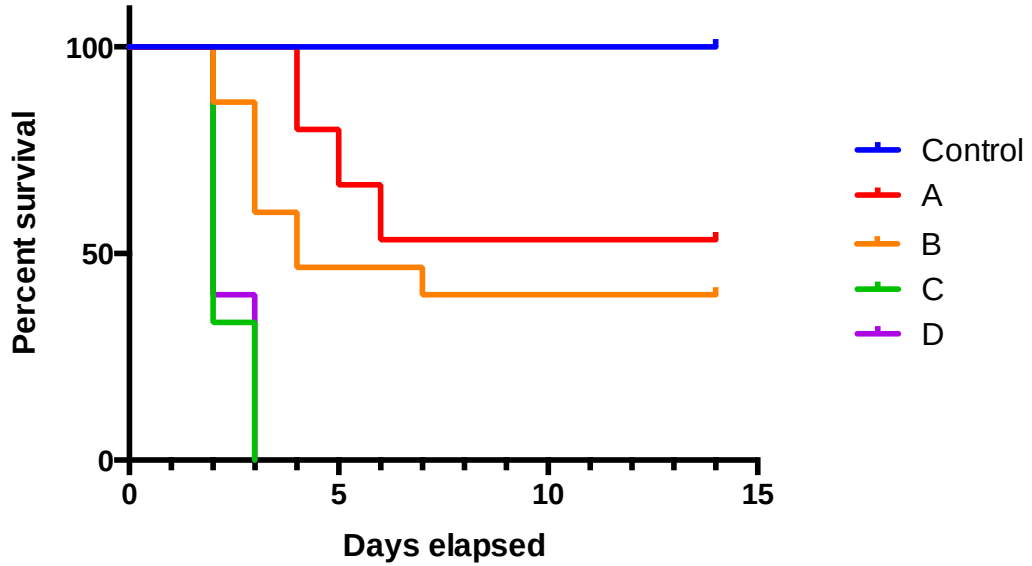
Transmission of effects in vivo

In vivo mortality endpoint in fish collaboration with Stirling and HWU

- Problem with in vivo work is there is no mortality endpoint for radiation at environmentally relevant doses
- To try to get one fish were exposed to a pathogen instead of radiation
- Sublethally exposed (SLE), swim buddies of SLE and controls were exposed to a lethal dose
- Results show protection of swim buddies as well as SLE group
- Paper in press in FEMLS

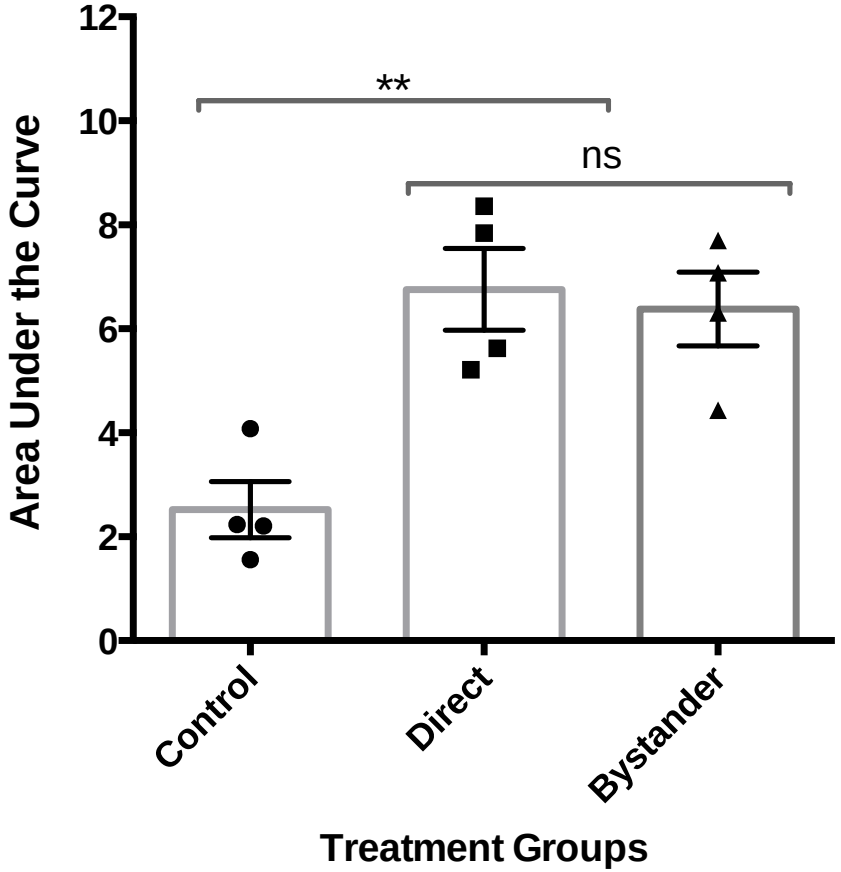
Mortality data

Exposure to signals from recovered fish protected naïve fish against a lethal dose of pathogen

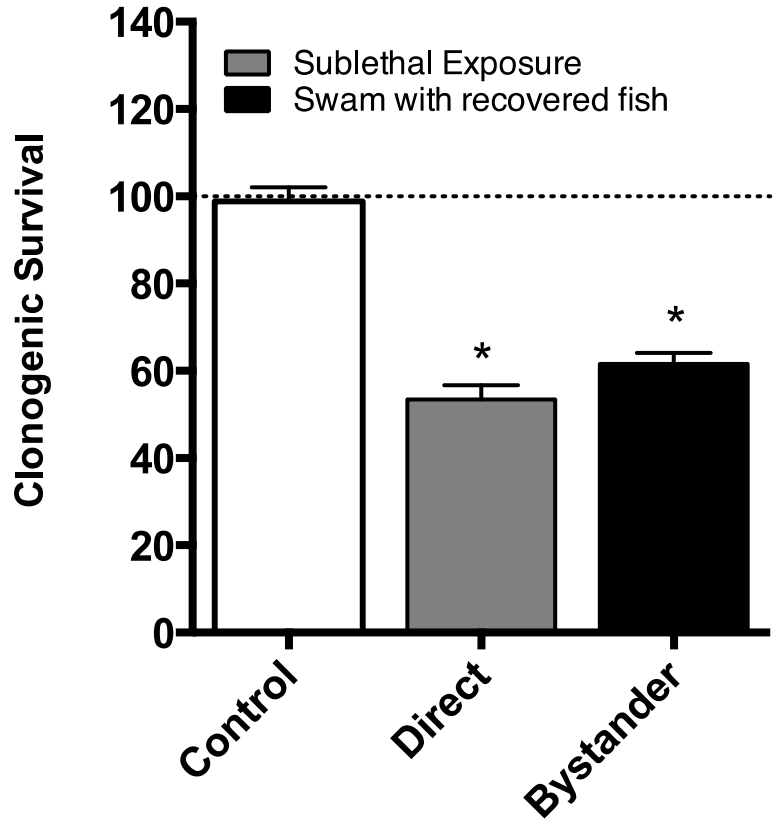


A = Sub-lethally exposed and recovered then lethally exposed group
B= swim buddies to group A
C = lethal challenge only
D= swim buddies to lethal challenge only (added at same time as B)

Results 2.1: calcium signal strength



Results 3: reporter assay data




Summary

Population based response after low dose exposure?

- Are non-targeted effects a reflection of population level regulation to optimise population fitness (tissue or individual level)?
- Is the function of radiation-induced bystander signaling to co-ordinate behaviour at higher hierarchical levels of organisation?
- Quorum sensing in bacteria is an example of this at the population level as are hormones at the organism level
- Can we exploit signal production as a population level biosensor?

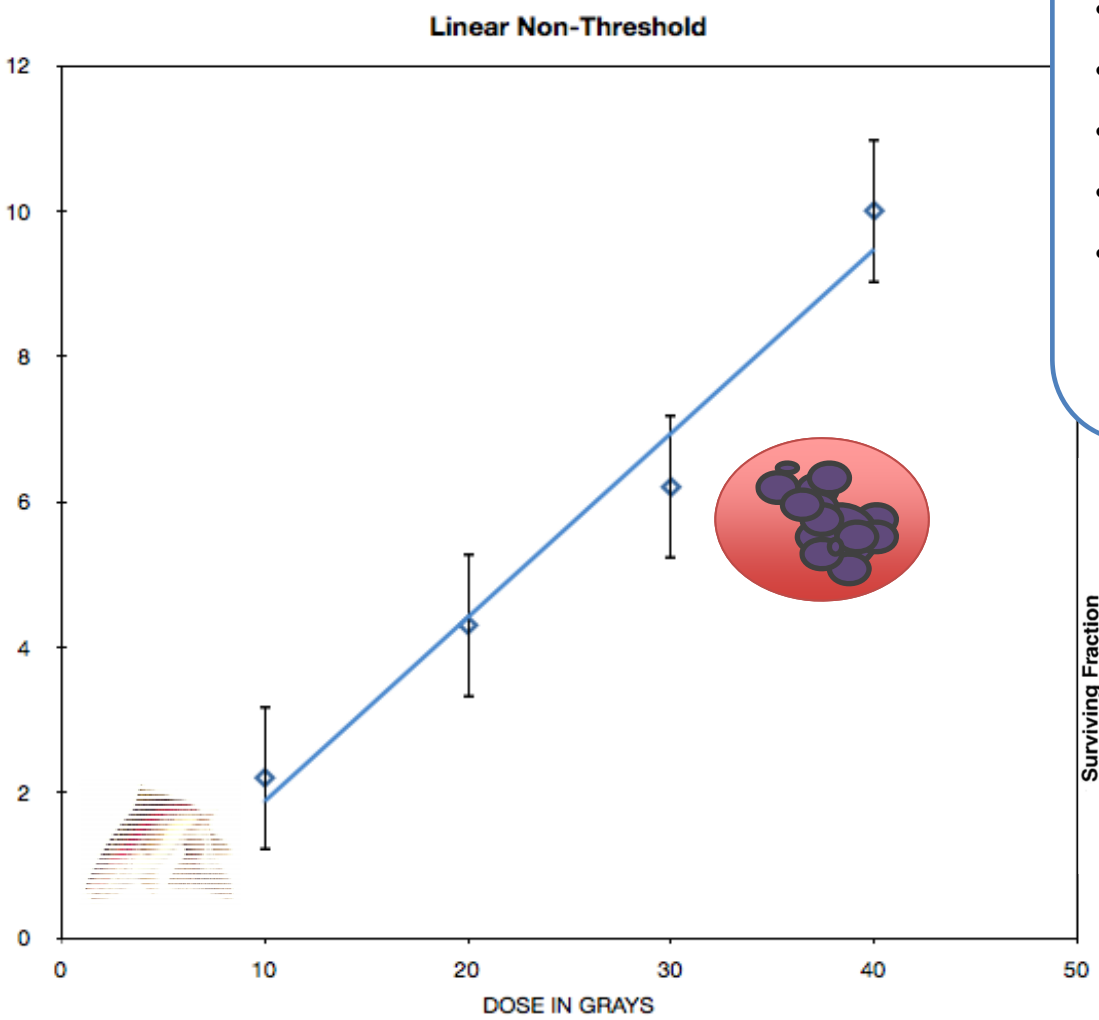
Low dose effects are different because the cells/tissues/individuals can cope

- Adaptive effects – not only strict radiobiological adaptive response but long-term evolutionary acclimation
- Hormetic effects – low dose of radiation is beneficial leading to non-linear dose responses for a variety of endpoints
- Homeostatic effects- systems accommodate and adjust to low dose induced perturbations
- Genetic and environmental factors more important than dose

Danger of taking a framework from one field in radiobiology and applying it elsewhere

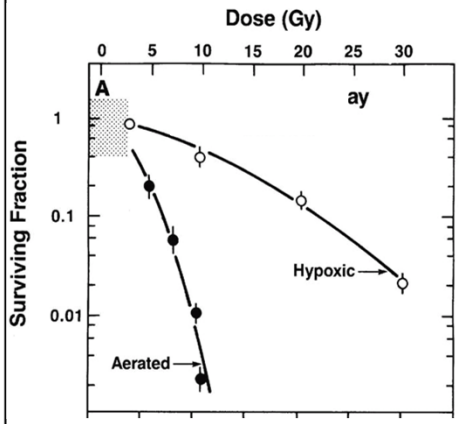
Low dose issues

- Whole Organism
- Population
- Ecosystem
- Trans-generational
- cancer/adverse effect in population is the concern



High dose issues

- Cellular Response
- Micro-environment
- Therapeutic Advantage
- Consistent effect
- Individual is of concern




Opposite bystander effect induced by the low-dose hyper-radiosensitive region in C6 and F98 rat glioma cell lines
Fernandez-Palomo, C.[a], Seymour, C.[a], Mothersill, C.[a]
[a]McMaster University, Hamilton, Ontario, Canada
McMaster University
General Information
Introduction
Aim
Materials and Methods
Results & Discussion
Figure 1 – F98 Cells
Figure 2 – C6 Cells
Conclusions
Cristian