Refresher course, topic RC-2 Cellular and molecular effects

Non-targeted biological effects of ionising radiation

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1. Introduction: non targeted effects of ionising radiation

Targeted and non-targeted effects of ionising radiation

Targeted effects

Classical paradigm of radiation biology

- DNA damage occurs during or very shortly after irradiation of the nuclei in targeted cells
- The potential for biological consequences can be expressed within one or two cell generations

Non-targeted effects

New evidence

- Bystander effect
- Radiation-induced genomic instability
- Low dose hypersensitivity
- Adaptive response
- Abscopal (out-of-field) effects
- Clastogenic factors
- Delayed reproductive death
- Induction of genes by radiation

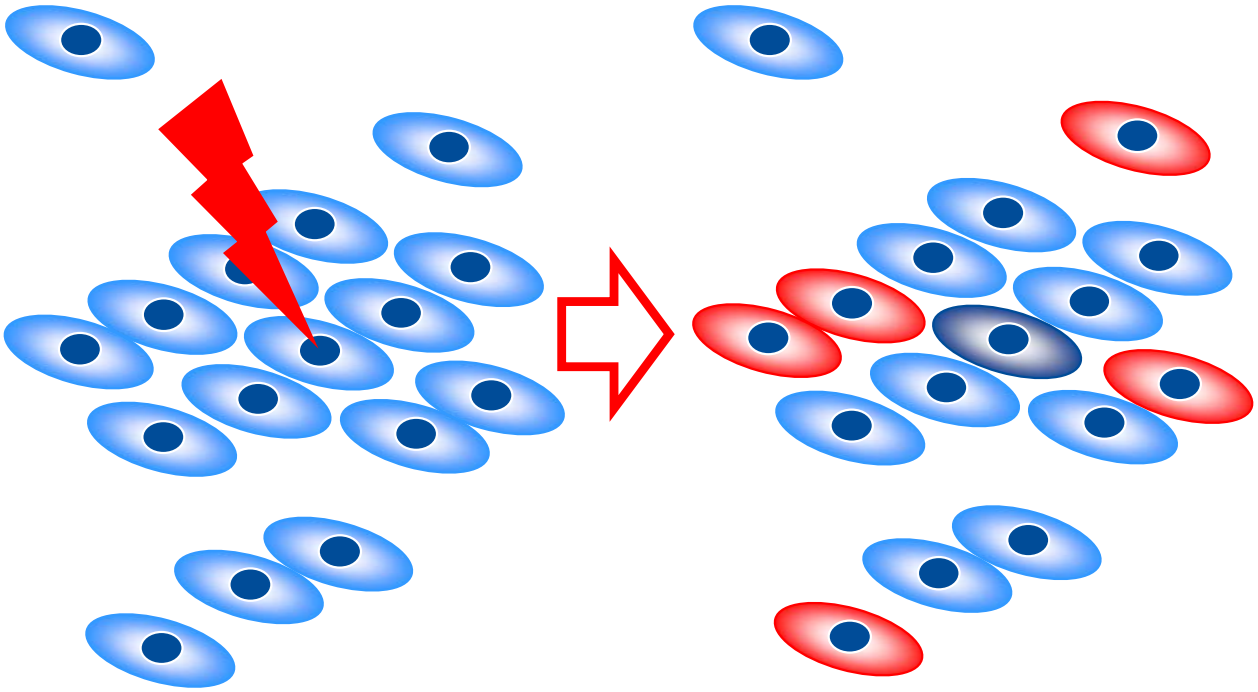
Target theory

- The *target theory* of radiation induced effects (Lea, 1946) postulates that cells contain at least one critical site or *target* that must be hit by radiation in order to kill a cell (or produce an effect).
- Therefore, radiation damage outside of the target should not cause cell death (effect).
- It is widely accepted that nuclear DNA is the critical target for radiation induced cell death (and not death related efefcts).

Non-targeted effects of ionising radiation as a new paradigm of radiation biology

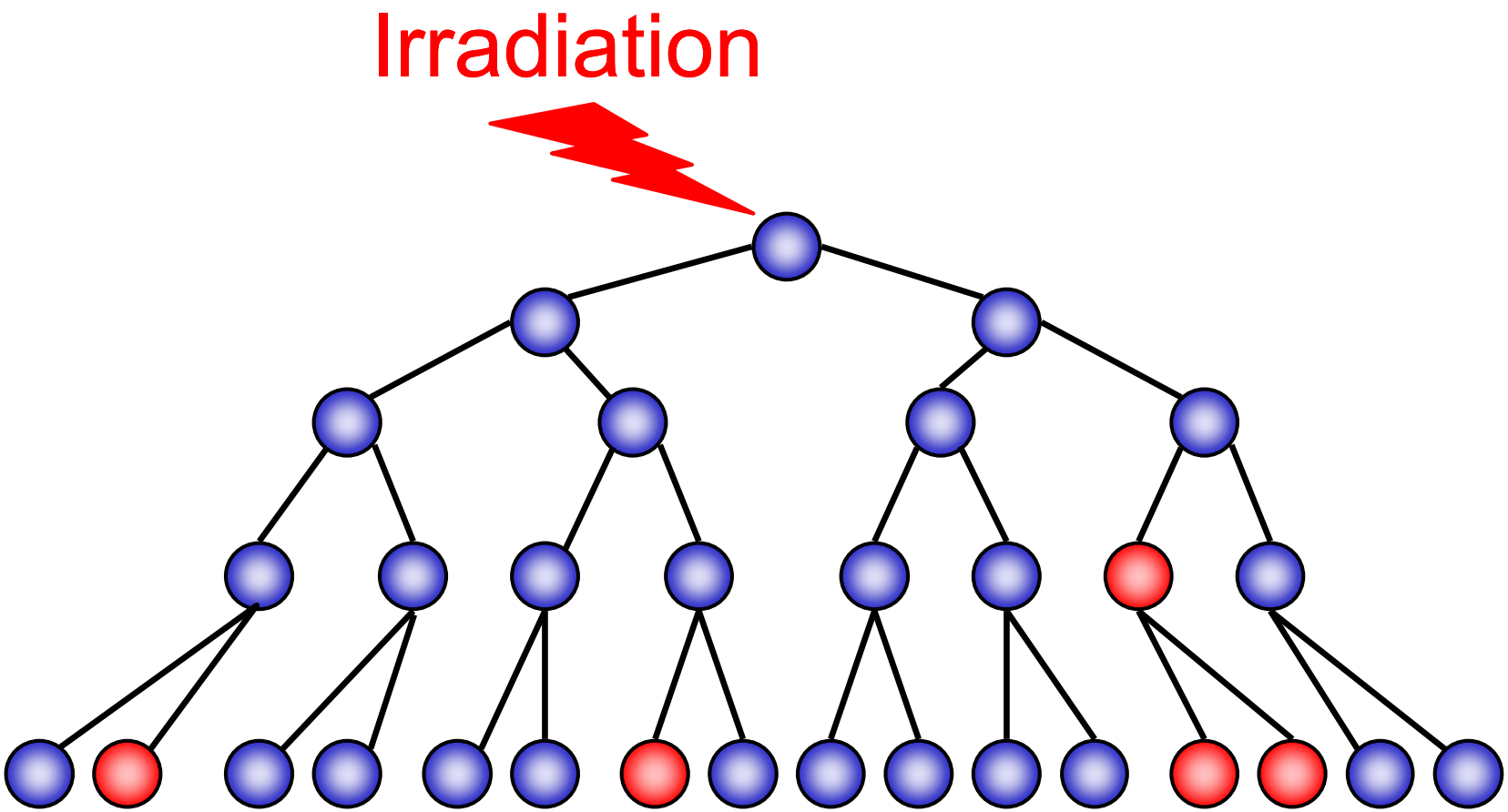
Ward, J. (1999) New paradigms for Low-Dose Radiation Response In *Proceedings of the American Statistical Association Conference on Radiation and Health*. San Diego, California, USA. June 14-17, 1998. *Radiat Res*, **151**:1, 92-117.

Radiation induced bystander effect



The radiation-induced bystander effect is a phenomenon whereby cellular damage is expressed in unirradiated neighboring cells near to an irradiated cell or cells.

Radiation-induced genomic instability



Radiation-induced genomic instability is defined as a persistent elevation in the rate of *de novo* appearance of genetic changes within a clonal population.

Non-targeted *versus* targeted effects

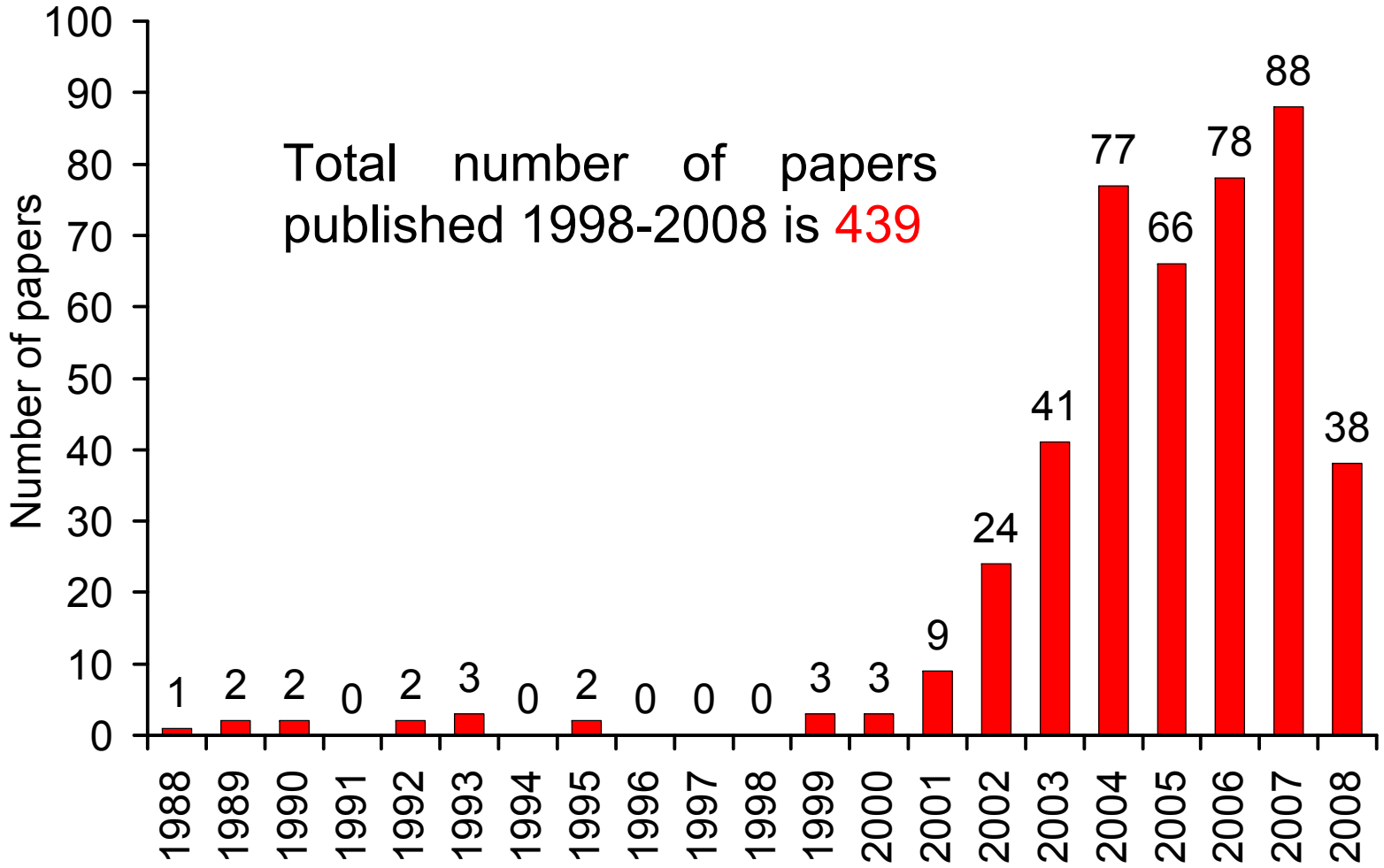
- Non-targeted effects do not contradict to "*target theory*" but increase size of the target in such extent that concept of "target" became meaningless.
- For example, bystander effect increases target *spatially* to the size of cell group, tissue or even organ.
- Genomic instability increases it *temporarily* by prolongation of damage over many cell generations or even transgenerationaly.

Need for a new paradigm of Radiation Biology

- Recent evidence for non-targeted effects suggests a new paradigm for radiation biology that challenges the universality of target theory.
- An essential feature of "non-targeted" effects is that they do not require a direct nuclear exposure by irradiation to be expressed and they are particularly significant at low doses.
- This new radiation biology paradigm should cover both targeted (direct) and non-targeted effects of ionising (and possibly non-ionising) radiation.

Baverstock, K. and Belyakov, O.V. (2005) Classical radiation biology, the bystander effect and paradigms: a reply. *Hum Exp Toxicol*, vol. 24, pp. 537-42.

Number of papers related to radiation induced non-targeted effects, bystander effect and genomic instability referred by Medline



Rationale for the current interest in non-targeted responses

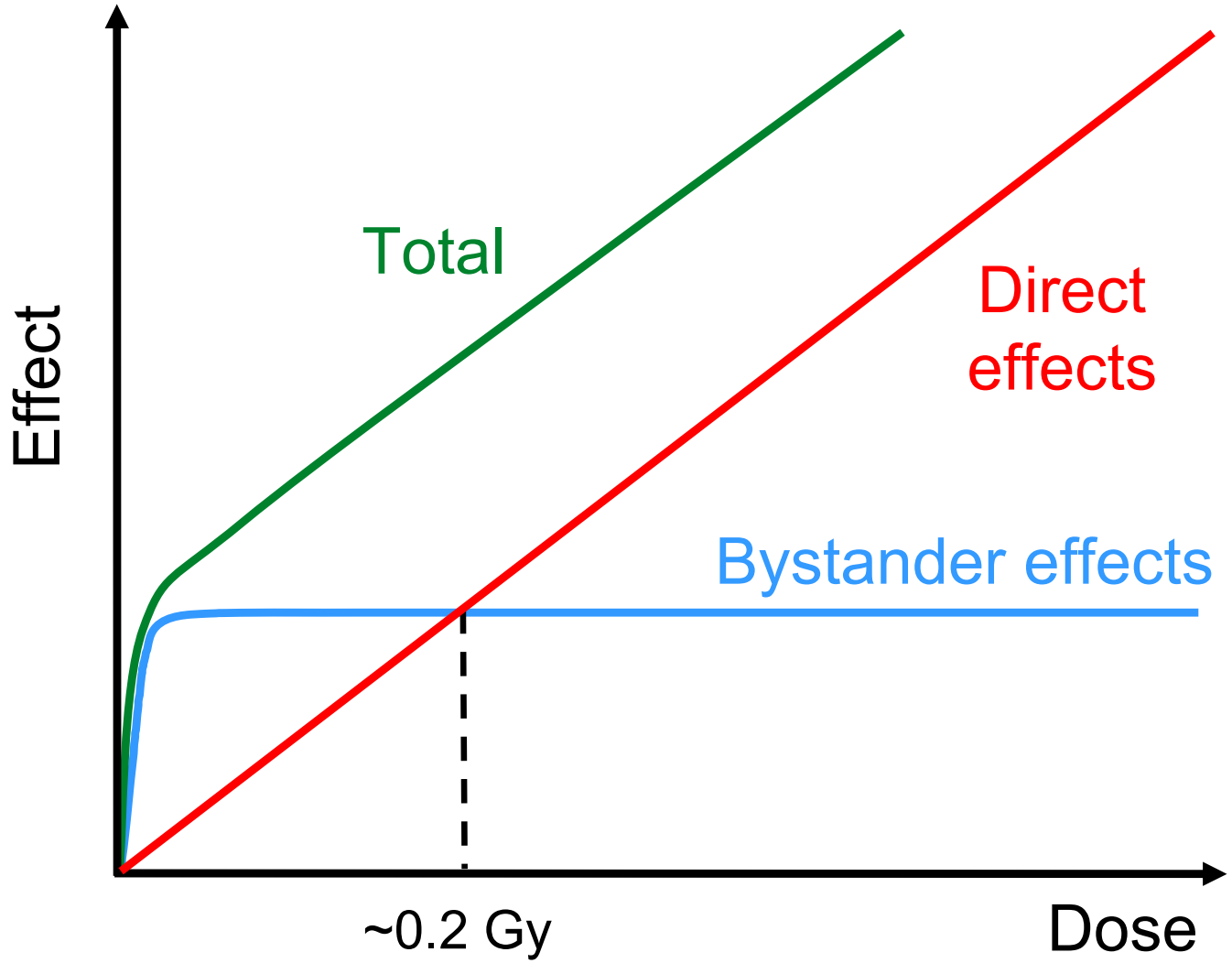
- There is a growing interest in low dose effects.
- Advances in the technical possibilities for precise low dose irradiation such as development of microbeams, imaging and computerized automation.
- Development of more specific and sensitive methods of cellular and molecular biology.
- Change of classic paradigm of radiation biology and challenging the target principle.

2. Bystander effect and genomic instability: evidence and mechanisms

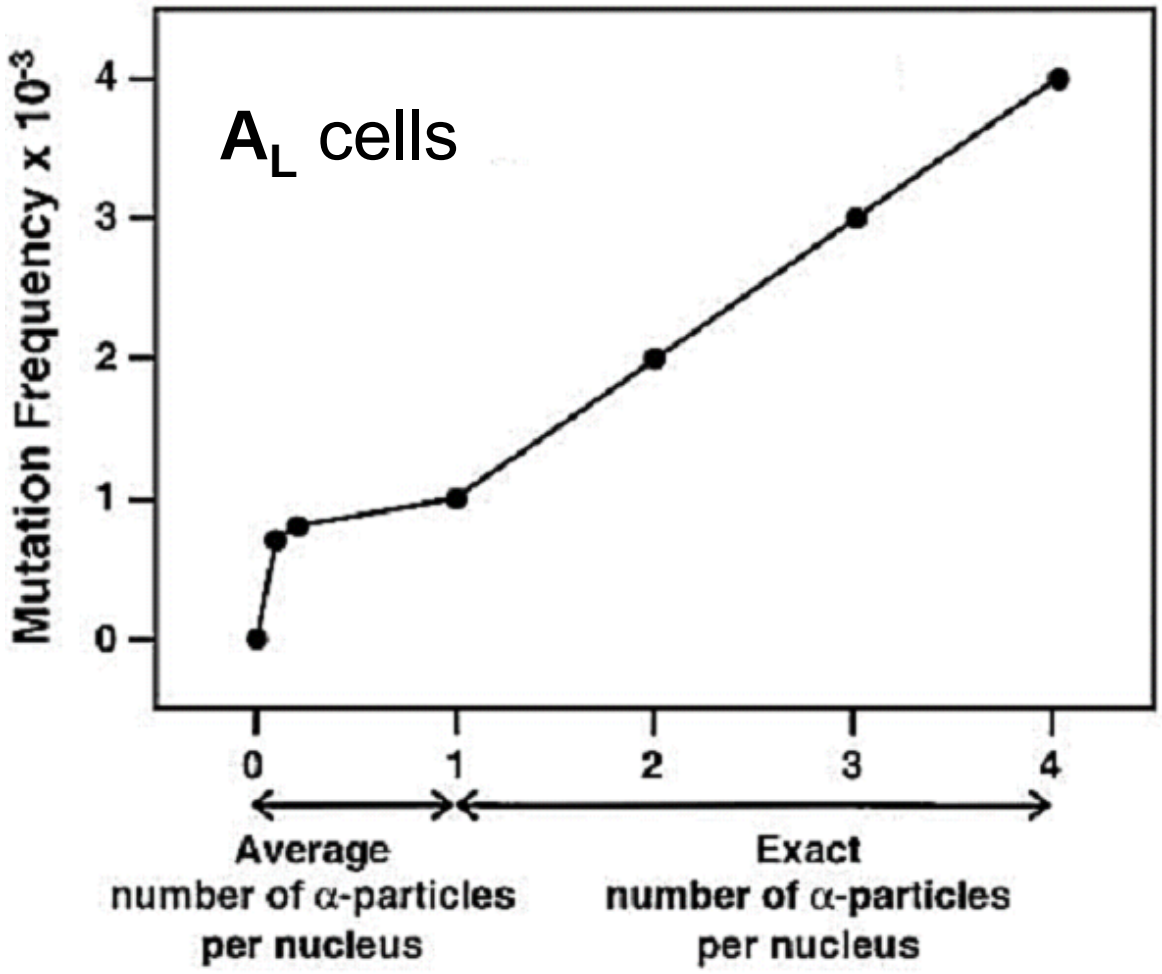
Evidence for radiation induced non targeted effect

- Increased levels of SCE in CHO cells irradiated with low doses of α-particles (Nagasawa and Little, *Cancer Res,* 1992).
- Increased p53 expression in epithelial cells exposed to α-particles (Hickman *et al., Cancer Res,* 1994).
- Extracellular factors involved in SCE following α-particle exposure (Lehnert and Goodwin, *Cancer Res,* 1997).
- Medium from γ-rays irradiated cells reduces the survival of unirradiated cells (Mothersill and Seymour, *Radiat Res,* 2001).
- Bystander effect after microbeam irradiation of a single cell (Belyakov *et al., BJC,* 2001).
- Induction of a bystander mutagenic effect after α-particle microbeam irradiation (Zhou *et al., PNAS,* 2000).
- Increased bystander neoplastic transformation after treatment with medium from irradiated cells (Lewis *et al., Radiat Res,* 2001).
- Bystander effect and genomic instability under *in vitro* (Lorimore *et al., PNAS,* 1998) and *in vivo* conditions (Watson *et al., Cancer Res,* 2000).

Contribution of bystander and direct components to the radiation induced damage



Dose response relationship for direct and bystander mutations

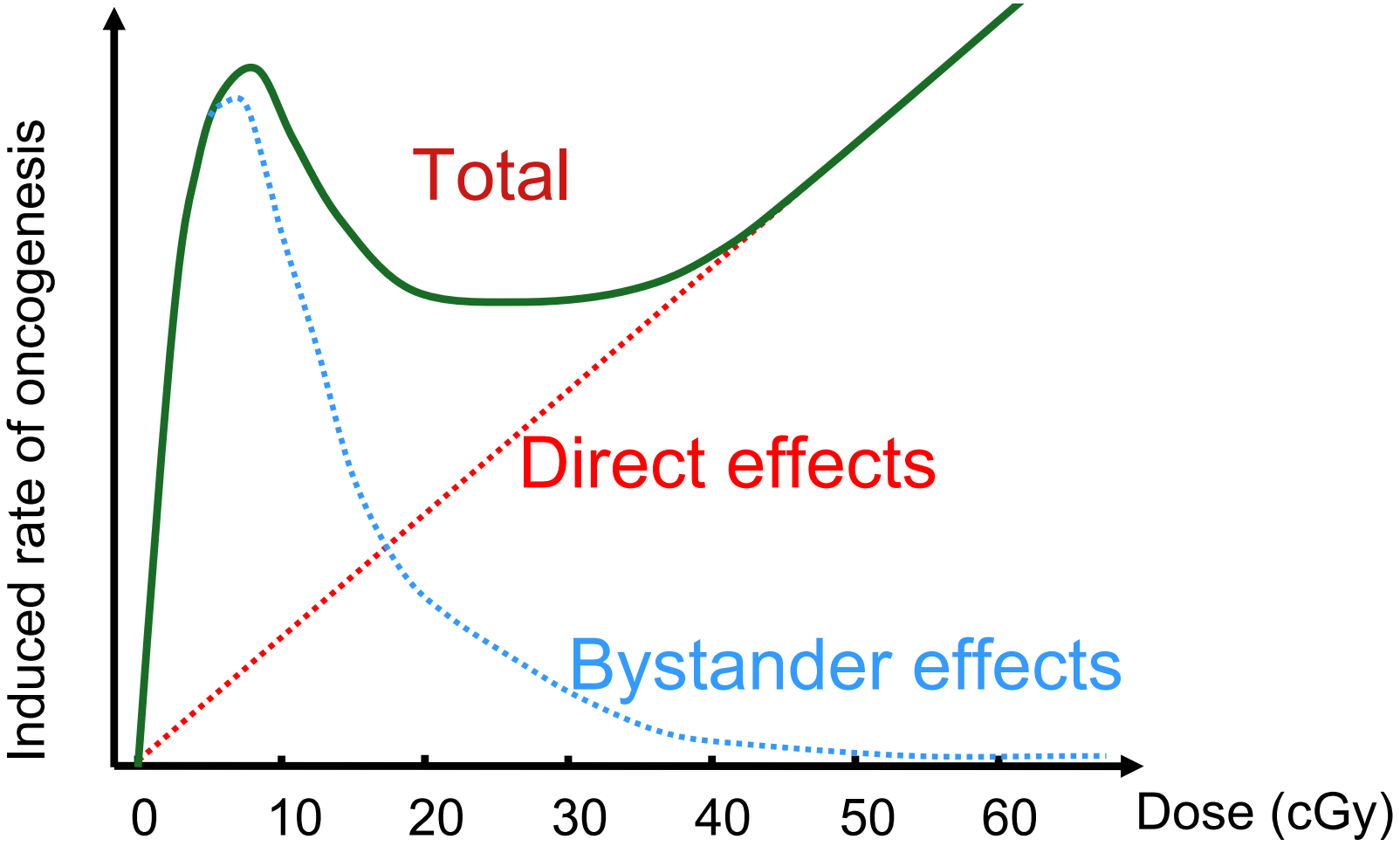


Hall, E.J. and Hei, T.K. (2003) Genomic instability and bystander effects induced by high-LET radiation. *Oncogene*, **22**:45, 7034-7042 (based on the data of Zhu *et al*., *Radiat Res*, 1996; Hei *et al*., *PNAS*, 1997; Zhou *et al*., *PNAS*, 2001)

Mathematical models of bystander effects

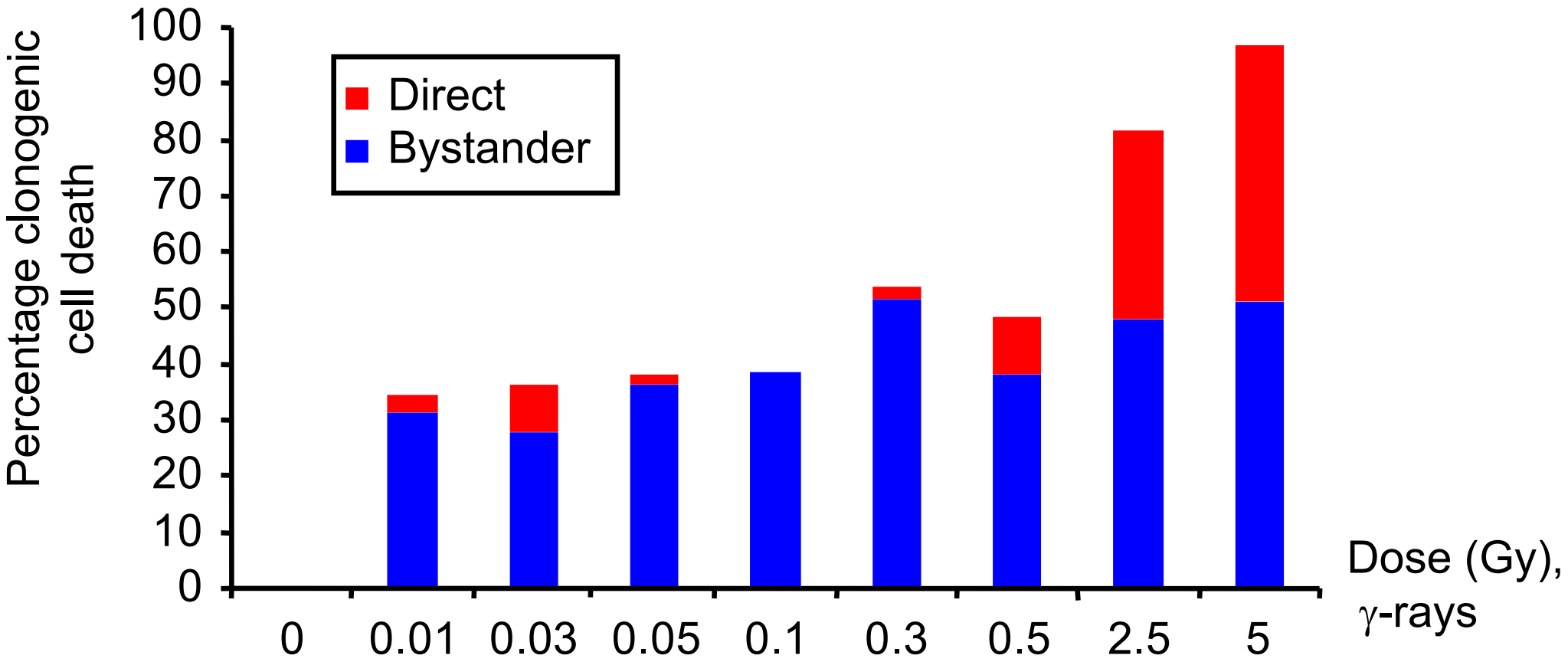
- State-vector model (SVM)
  (Schollnberger, et al., *IJRB*, 2002)
  A biomathematical neoplastic transformation model that includes radioprotective bystander mechanisms. The model successfully simulates experimental data.
- ByStander Diffusion Modell (BSDM)
  (Nikjoo and Khvostunov, *IJRB*, 2003)
  A quantitative model of the radiation-induced bystander effect based on diffusion-type spreading of bystander signal communication between the hit and non-hit cells.
- 3D lattice model
  (Little, et al., *J Theor Biol*, 2005)
  A model for bystander effects, with allowance for spatial position and the effects of cell turnover. It assumes a three-dimensional lattice of points and suitable for tissue modelling.

BaD model, contribution of bystander and direct component to the radiation induced oncogenesis



Brenner, D.J., Little, J.B. and Sachs, R.K. (2001) The bystander effect in radiation oncogenesis: II. A quantitative model. *Radiat Res*, 155:3, 402-8.

What is the relative contribution of “direct" and "bystander" effects to cell death?



Clonogenic cell death measured in human keratinocytes. The whole bar represents the total death after direct exposure. The red portion of the bar represents bystander death measured after exposure to medium from irradiated cells. The remaining death is represented by the blue portion of the bar, giving a value for death not attributable to bystander effect (Seymour and Mothersill, *Radiat Res*, 2000).

Mechanisms of the bystander effects

- Cell type dependent
- Depends on cell proliferative state
- Energy/REDOX metabolism may be involved
- Bystander effect can be induced by low and high LET irradiation
- Different underlying mechanisms
  - Gap junction (GJIC) mediated
  - Medium borne factors mediated

Hypothetical messenger(s)

At least two types of the bystander messenger might exist

Primary

- emitted by targeted cell
- short lived
- unstable
- travels through gap junctions
- water soluble
- non-protein

Long-lived organic radicals

Antioxidants (thiols)

Ca^{2+} or Ip3

cAMP

Secondary

- produced by activated cells
- long lived
- stable
- media borne
- most likely a protein

Lipid hydroperoxidases

Death ligand exfoliation

Cytokines

TNF-α, TGF-β or IL-1

Medium borne primary or secondary messengers

- Reactive oxygen species (H_2O_2/O^{-2}) have been proposed as possible signals involved in bystander responses (Narayanan, *et al.*, *Cancer Res*, 1997; Iyer and Lehnert, *Cancer Res*, 2000)
- Nitric oxide (NO) might play a central role in mediation of bystander effect (Matsumoto, *et al.*, *IJRB*, 2000; Matsumoto, *et al.*, *Radiat Res*, 2001) potentially having a protective value.

Secondary electrons cannot be involved in the bystander effect

- In our research we are using charged particles with energies of 3-4 MeV per nucleon.
- Secondary electrons produced by these particles cannot be involved in the bystander effect because of very short range.
- 7 MeV $^{4}He^{2+}$ maximal calculated energy of secondary electrons would be ≈3.8 keV, which corresponds to a few hundreds of nanometers range. This is much less than size of cell or cell nucleus. Therefore secondary electrons even would not be able to get out of nucleus after it was targeted with microbeam.
- On other hand, hypothetical bystander messenger is proven to be capable of travel for millimeters.

Bystander effect and genomic instability are closely related

- Bystander effect and genomic instability are non-targeted effects of irradiation and might have common mechanisms (Kadhim *et al*., *Mutat Res,* 2004).
- Chromosomal instability could be induced in bystander cells (Lorimore *et al*., *PNAS,* 1998).
- There is a recent evidence that the bystander effect persists for many generations (Lorimore *et al*., *Cancer Res,* 2005).
- This evidence suggests that the initial cross-section for radiation damage is increased by the bystander effect, and cells that are affected by the bystander mechanism may remain at an increased risk of genetic change for many generations.

3. Overview of current bystander effect research

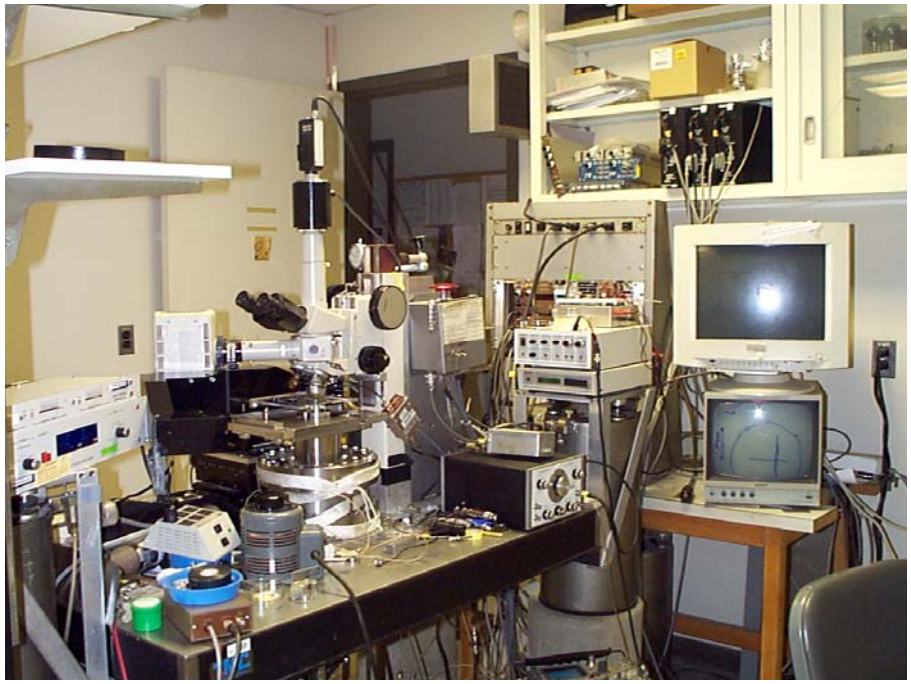
Studies of bystander effects: a *gradual* movement from *in vitro* cell culture towards *in-vivo* system

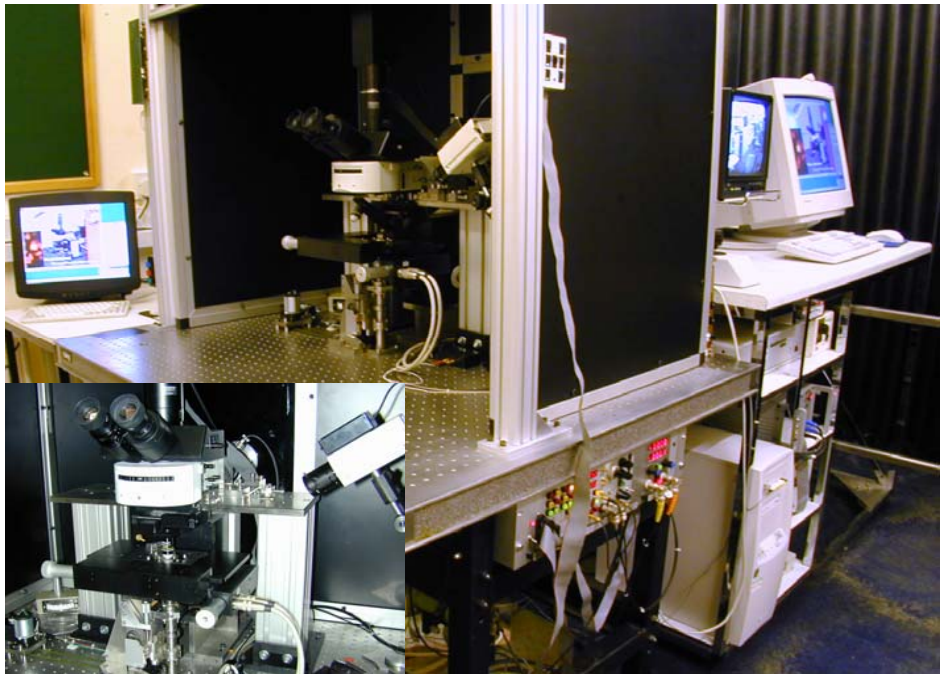
Gray Cancer Institute				CU	STUK	
In vitro Normal human fibroblasts **Broad field irradiation**	***In vitro*** Normal human fibroblasts **Microbeam irradiation**	***In vitro*** Primary porcine and human ureter explant systems **Microbeam irradiation**	**Ex *in vivo*** Primary porcine ureter 3D tissue system ***In situ*** **microbeam irradiation**	**In vivo like** Artificial human 3D tissue systems **Microbeam irradiation**	***In vivo like*** **and** **ex *in vivo*** 3D human tissue skin systems **Microbeam irradiation**	***In vivo*** Mouse with implanted piece of human skin **Microbeam irradiation**
Completed				**Completed**	**In work**	**Project**

Rationale

- Radiation effects at the tissue level under normal conditions prove that individual cells cannot be considered as isolated functional unit within most tissues of a multicellular organism.
- Experimental models, which maintain tissue-like intercellular cell signalling and three-dimensional (3D) structure, are essential for proper understanding of bystander effects.
- The main rationale for our research is that the bystander effect is likely to be natural phenomena which should be studied in an *in vivo* like multicellular system with preserved 3D tissue microarchitecture and microenvironment.
- This necessitates moving from *in vitro* cell culture systems to tissue-based systems.

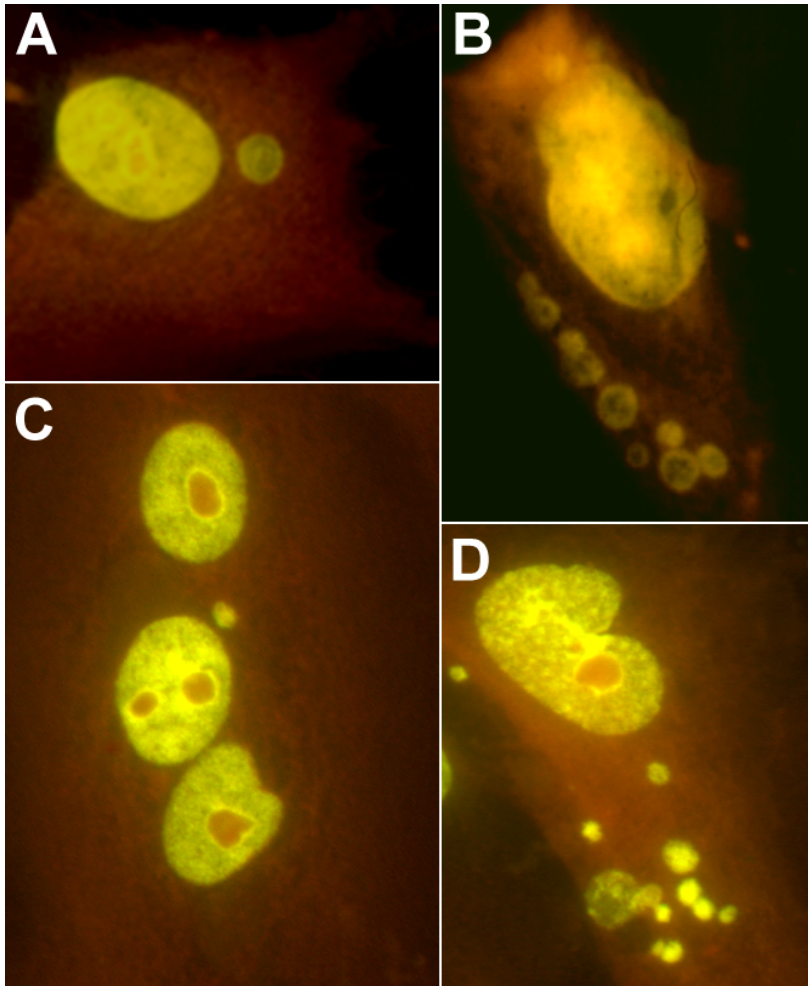
Microbeam technology as a tool for bystander research



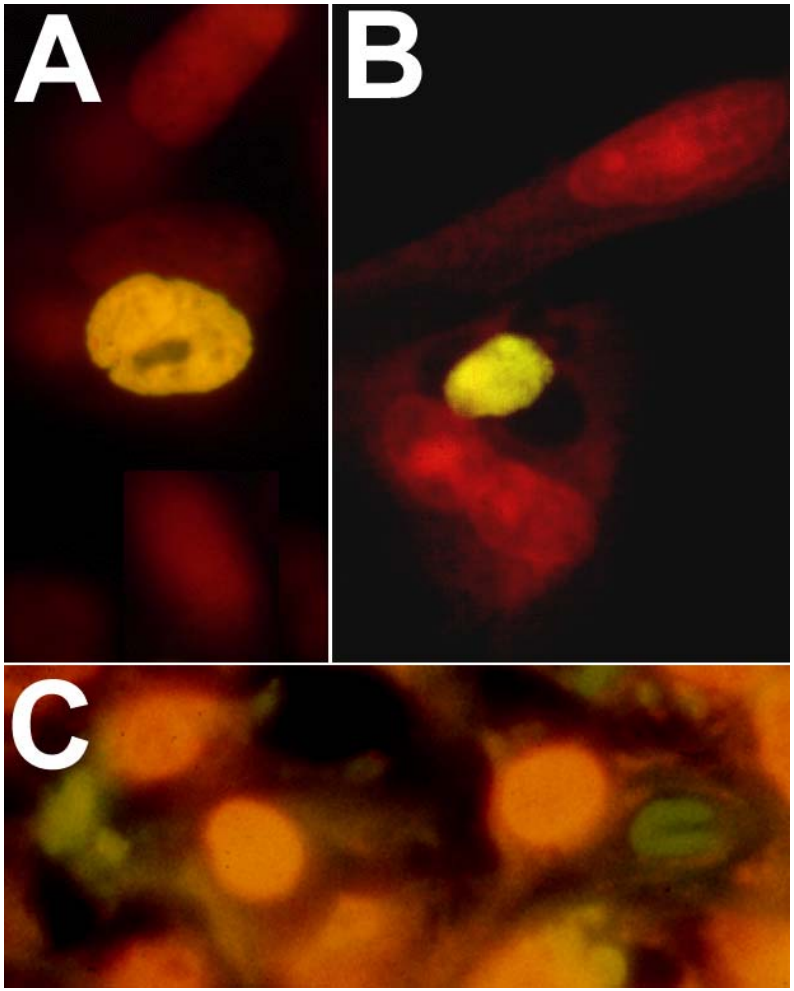


Microbeams are facilities that allow irradiation of individual cells or cell regions with precise numbers of charged particles with *micrometer* precision (see for example: Randers-Pehrson *et al*, *Radiat Res*, 2001; Folkard *et al*, *Int J Radiat Biol*, 1997).

Micronucleated and apoptotic cells



Mironucleated AG01522 fibroblasts (A, B) and urothelial cells (C, D), acridine orange staining.



AG01522 fibroblasts (A and B), porcine urothelium explant outgrowth (C).

Studies of bystander effects in AG01522 normal human fibroblasts

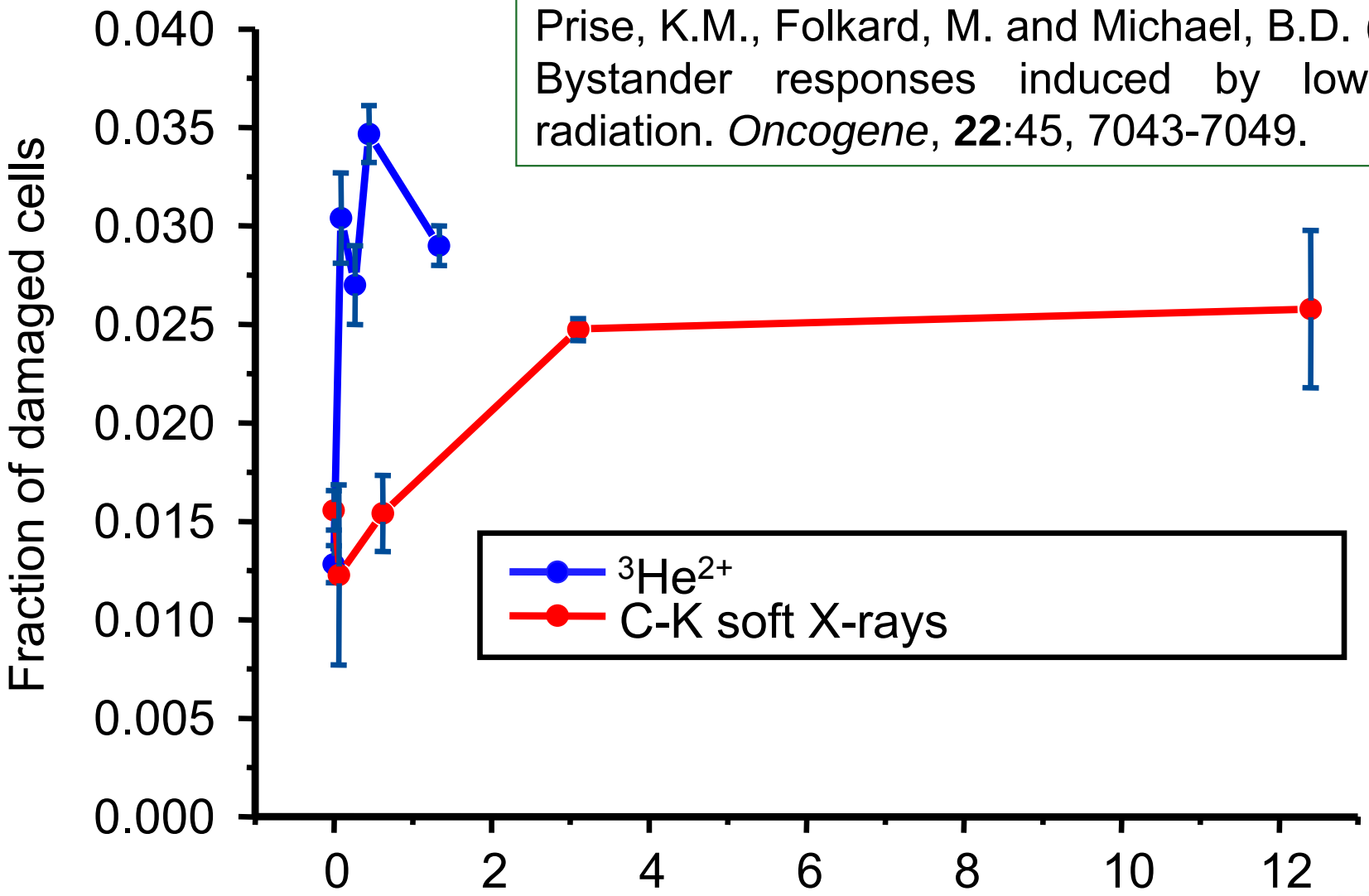
- First direct evidence for a bystander effect.
- Micronucleated and apoptotic cells were scored 3 days after irradiation in AGO1522 primary human fibroblasts.
- Irradiation of 1 fibroblast among a few hundred cells with 1 $^{3}He^{2+}$ particle produced a significant rise in damaged cells from approximately 1% to 3% in the surrounding unirradiated population.
- Further increase of dose does not change the dose response.

Belyakov, O. V., Malcolmson, A. M., Folkard, M., Prise, K. M. and Michael, B. D. (2001). Direct evidence for a bystander effect of ionizing radiation in primary human fibroblasts, *Br J Cancer* **84:5**, 674-679.

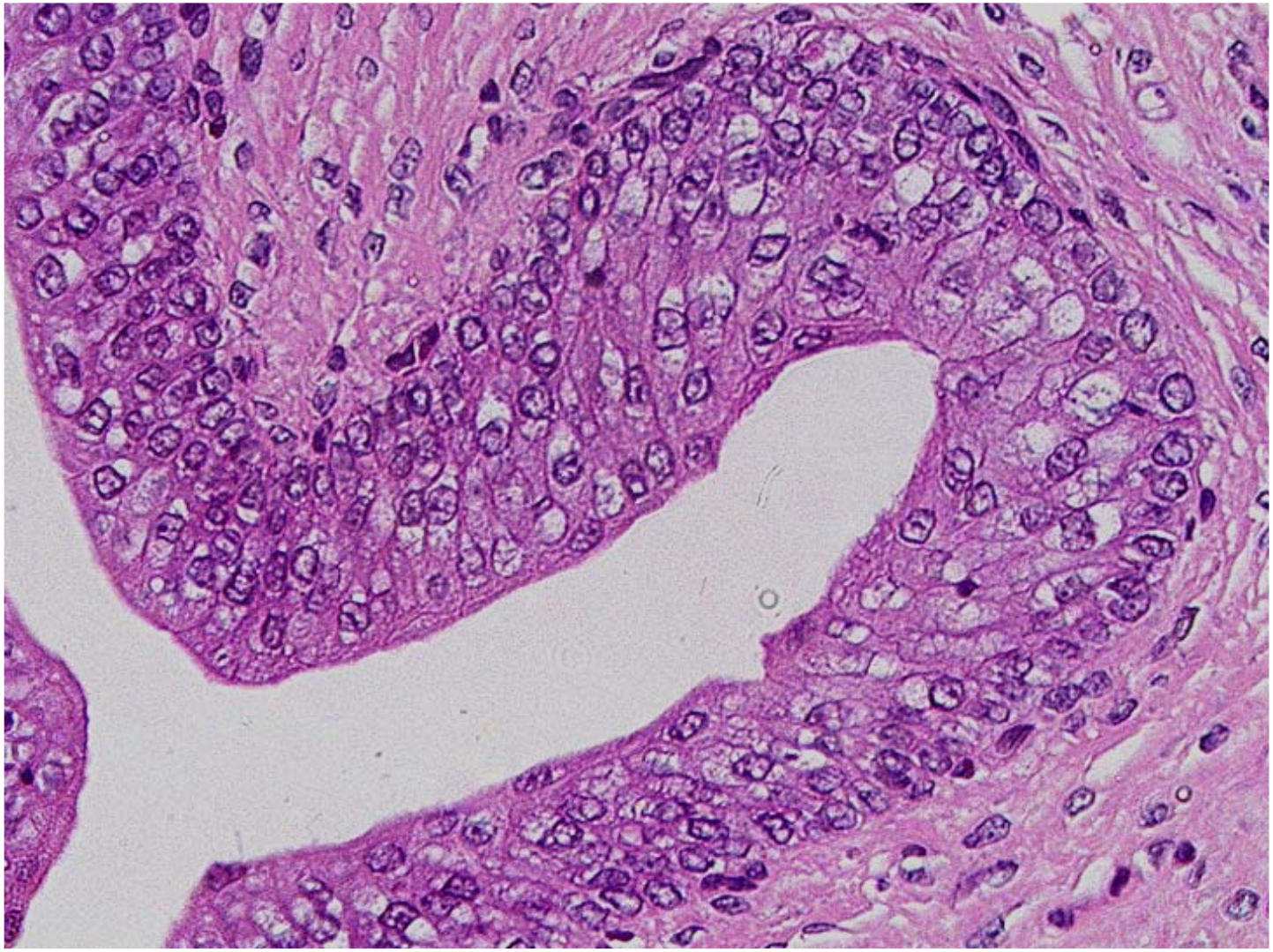
Prise, K.M., Belyakov, O.V., Folkard, M. and Michael, B.D. (1998) Studies of bystander effects in human fibroblasts using a charged particle microbeam. *Int J Radiat Biol*, **74:6,** 793-8.

Bystander effect in human fibroblasts after $^{3}He^{2+}$ microbeam and ultra soft X-ray microprobe irradiation of a single cell

Prise, K.M., Folkard, M. and Michael, B.D. (2003) Bystander responses induced by low LET radiation. *Oncogene*, **22**:45, 7043-7049.



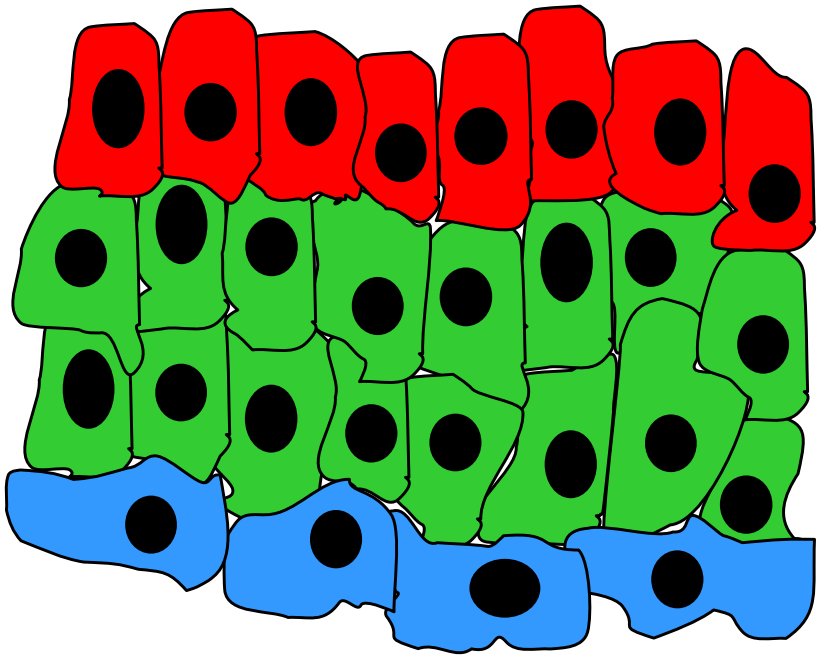
Porcine ureter section



4 μm paraffin section, Haemotoxylin-Eosin staining

Ureter tissue microarchitecture

Lamina propria



Basal cell layer, dividing

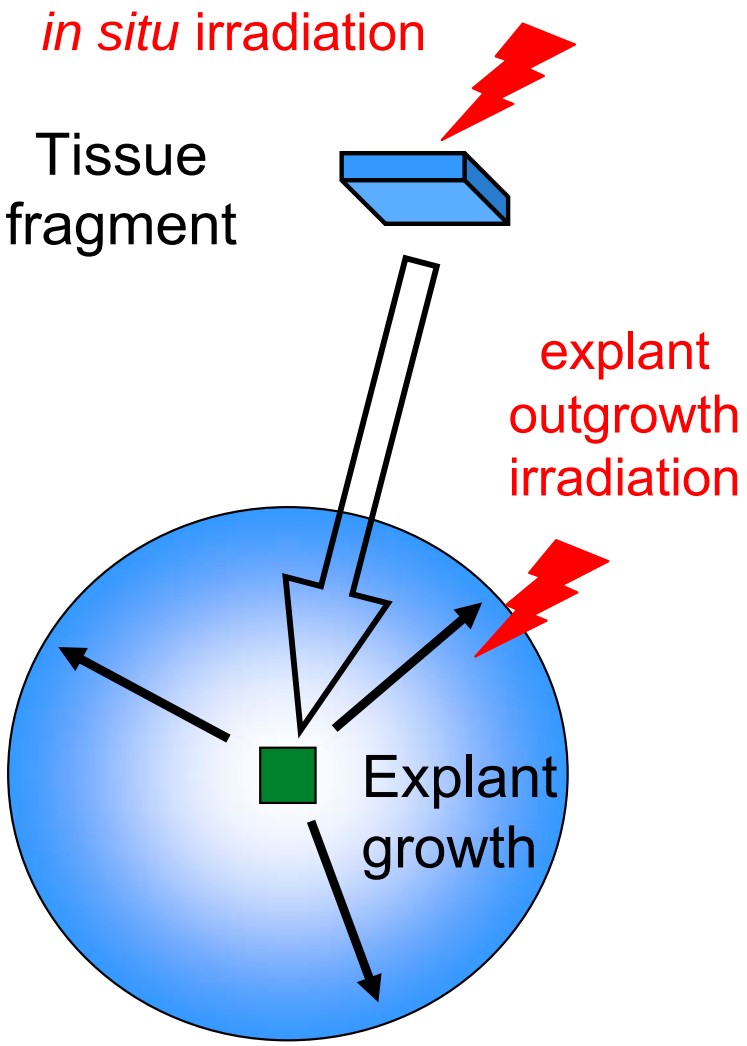
2-3 intermediate cell layers - semi-differentiated, non-dividing

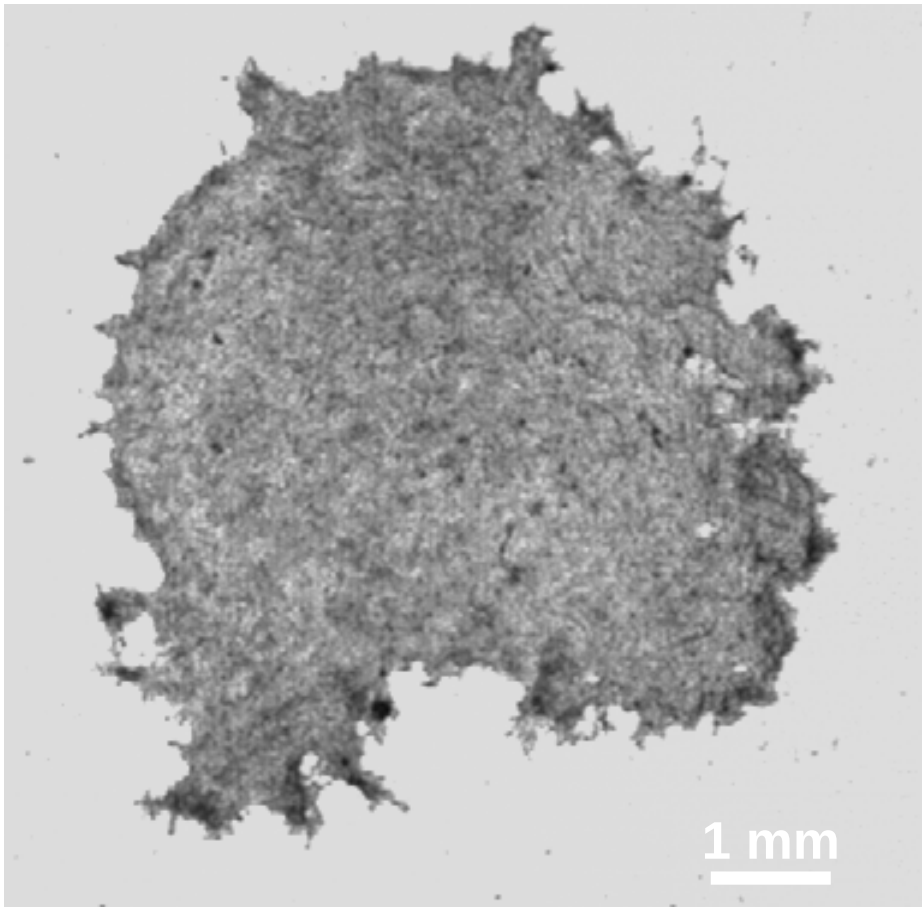
Superficial cell layer - differentiated

Cell movement

Lumen

Primary explant technique





Human urothelial explant outgrowth

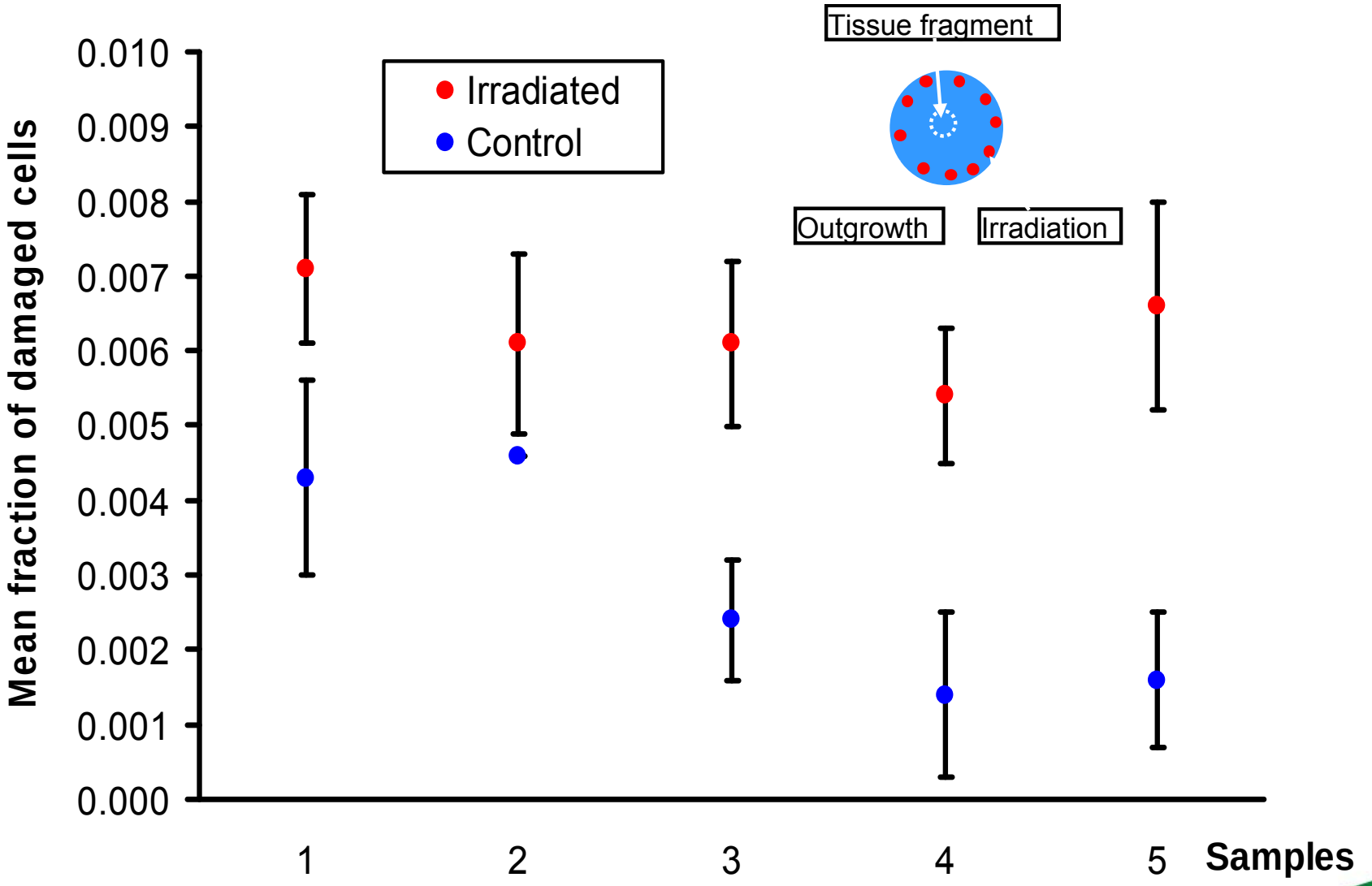
Outgrowth is a 2D representation of 3D tissue microarchitecture including *in vivo* like differentiation pattern.

A proliferation-dependent bystander effect in urothelial explants

- A significant bystander-induced effect was observed only when the periphery of the explant outgrowth (consisting of proliferating cells) was targeted.
- Approximately 2000-6000 additionally damaged cells were produced after irradiation of a few cells initially.
- This finding suggests a cascade mechanism of cell damage induction.
- The fraction of damaged cells did not exceed 1-2% of the total number of the cells within the explant outgrowth.
- The bystander-induced damage depends on the proliferation status of the cells and can be observed with this *in vivo* like explant model.

Belyakov, O.V., Folkard, M., Mothersill, C., Prise, K.M. and Michael, B.D. (2003) A proliferation-dependent bystander effect in primary porcine and human urothelial explants in response to targeted irradiation. *Br J Cancer*, **88**:5, 767-74.

Fraction of damaged cells after microbeam irradiation at the *periphery* of urothelial explant outgrowth, 10 cells have been irradiated at the edge of each explant (10 $^{3}He^{2+}$ particles/cell)

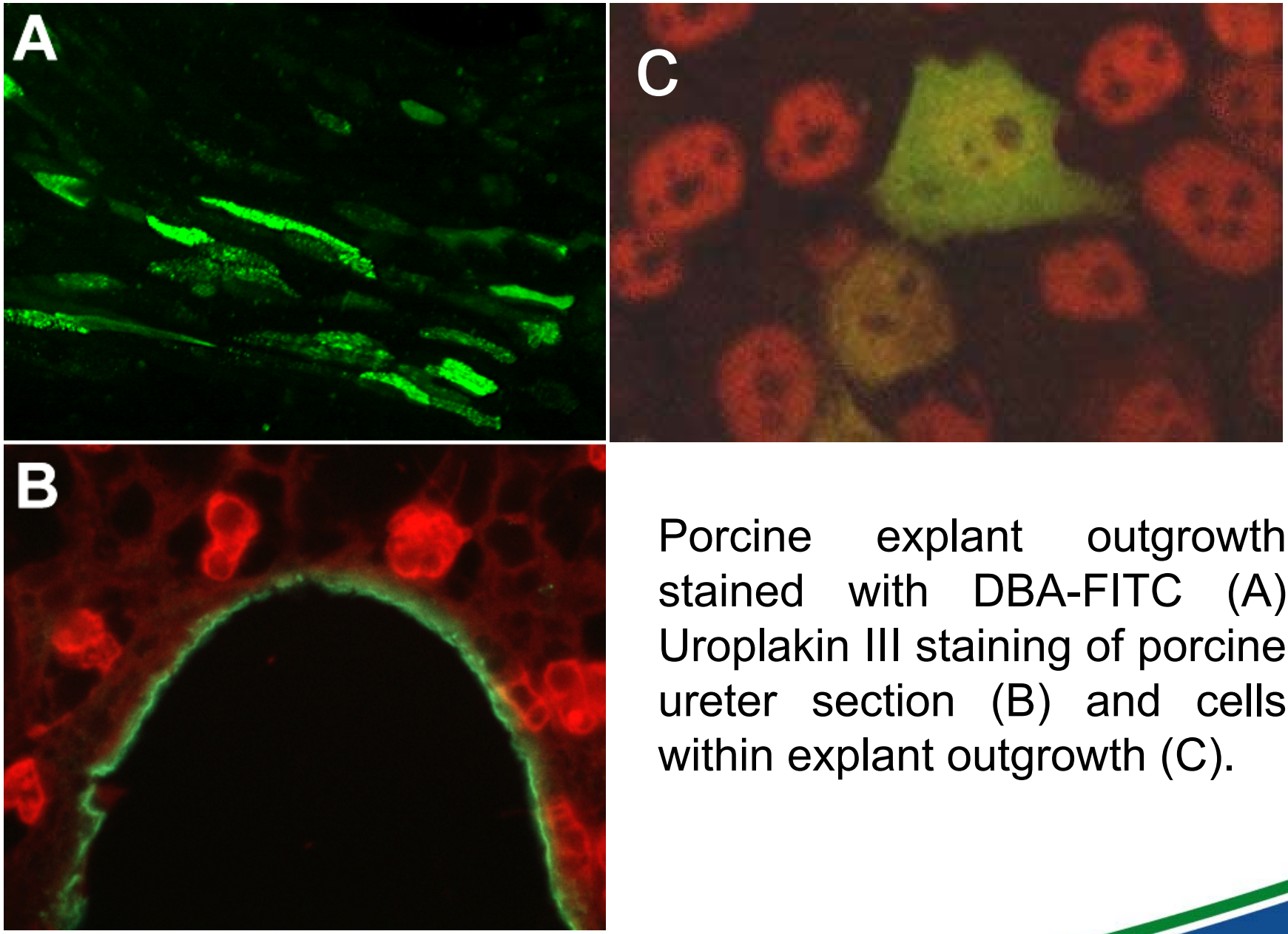


Bystander-induced differentiation in porcine ureter tissue models following *in situ* microbeam irradiation

- A single 2 μm location on ureter tissue section was pre-irradiated with 10 3He2+ particles (5 MeV; LET 75 keV/μm).
- Differentiation was estimated using antibodies to Uroplakin III, a specific marker of terminal urothelial differentiation.
- Micronucleation and apoptosis involve only a small fraction of cells (typically 1-2% of total cell number).
- Irradiated samples demonstrate about 10-15% additional differentiation in comparison to control. By far the biggest bystander response has a protective role rather than a damaging one by switching on differentiation.

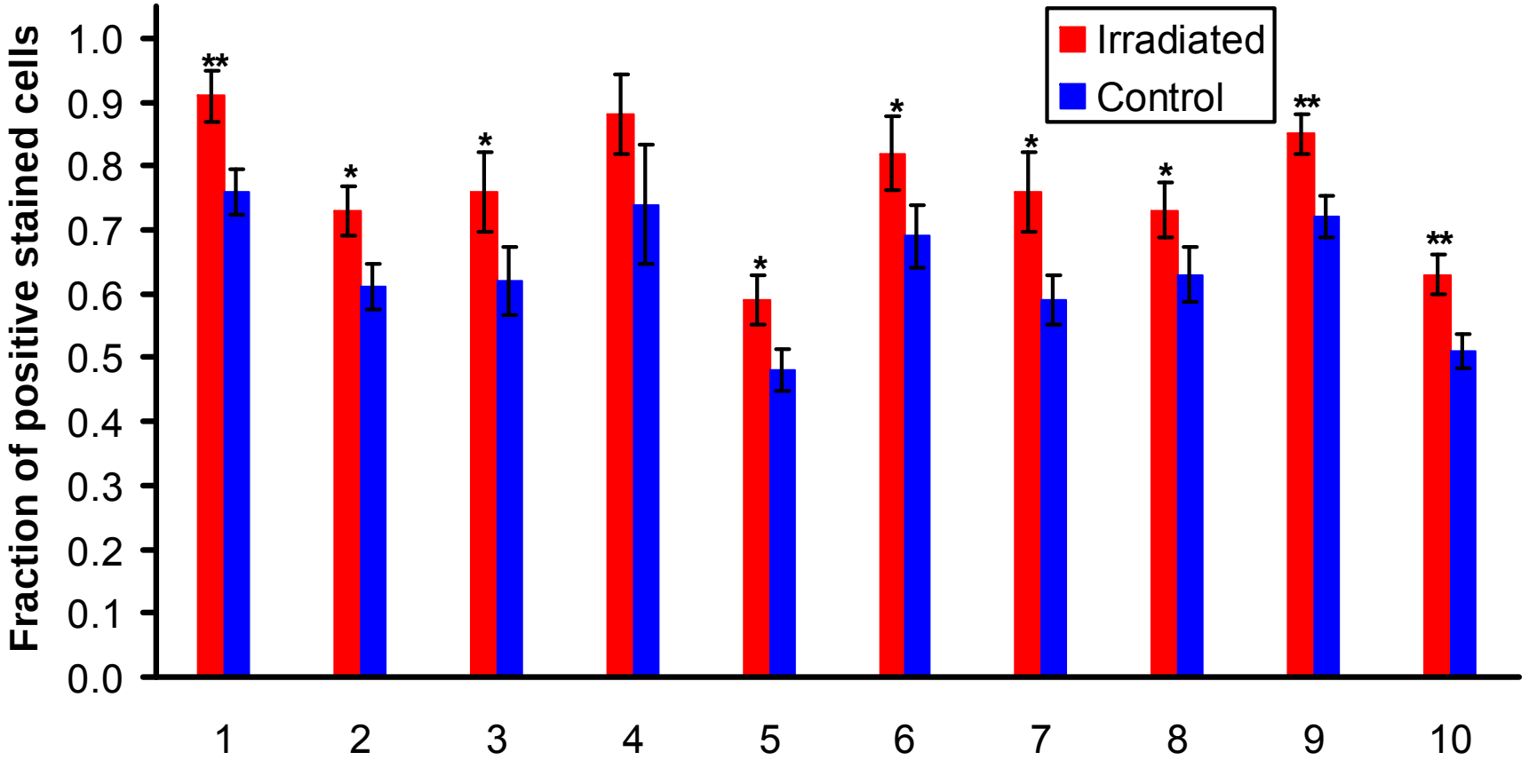
Belyakov, O.V., Folkard, M., Mothersill, C., Prise, K.M. and Michael, B.D. (2006) Bystander-induced differentiation: A major response to targeted irradiation of a urothelial explant model. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **597**:1-2, 43-49.

Markers of urothelial differentiation



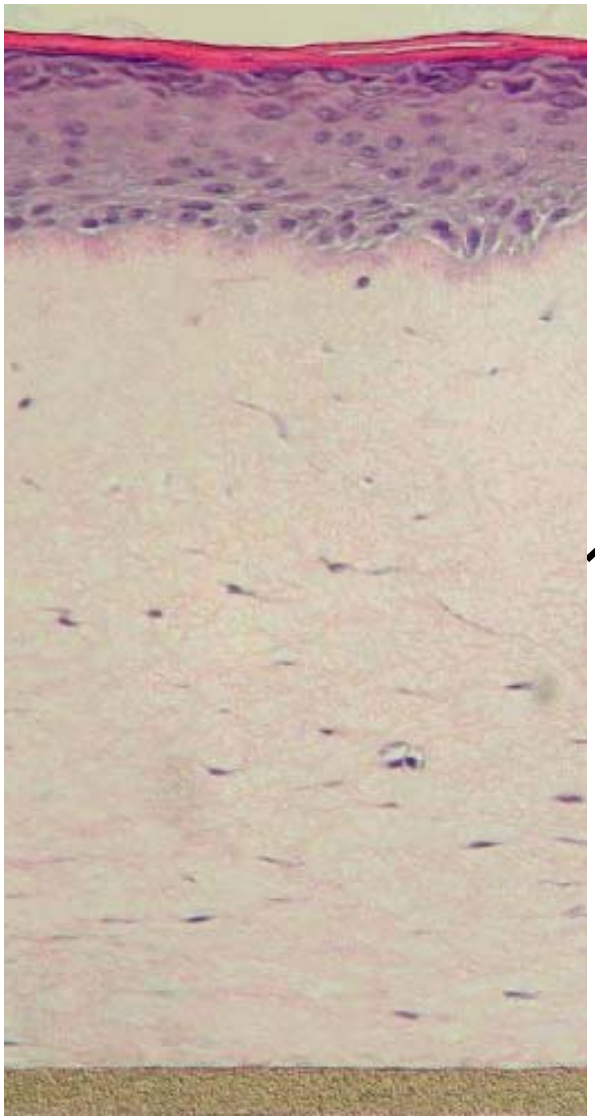
Porcine explant outgrowth stained with DBA-FITC (A) Uroplakin III staining of porcine ureter section (B) and cells within explant outgrowth (C).

Fraction of differentiated cells measured with Uroplakin III immunostaining in porcine urothelial explant outgrowths

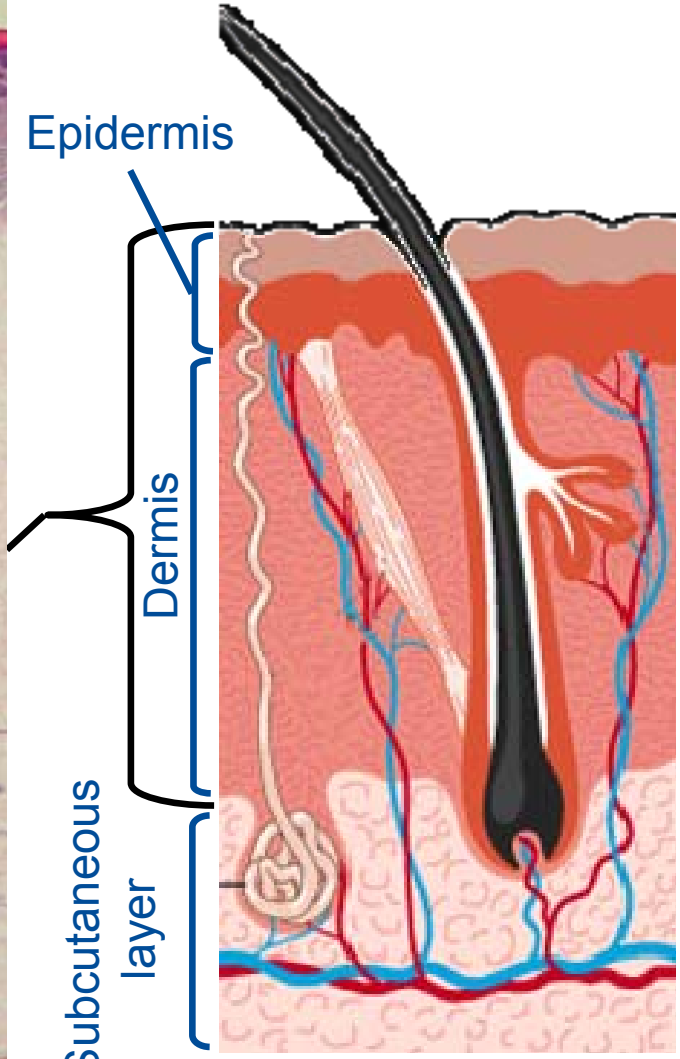


Error bars represent standard error of the means.
Significance tests were made using Student's *t*-test
($^{*}P<0.05$; $^{**}P<0.01$).

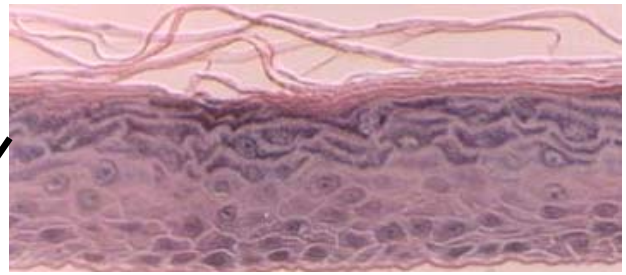
Artificial human skin tissue system



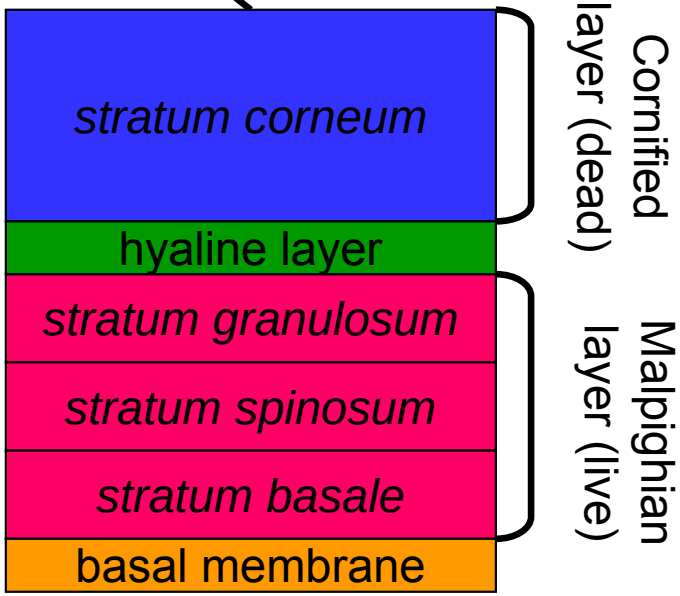
EpiDermFT



Scheme of human skin

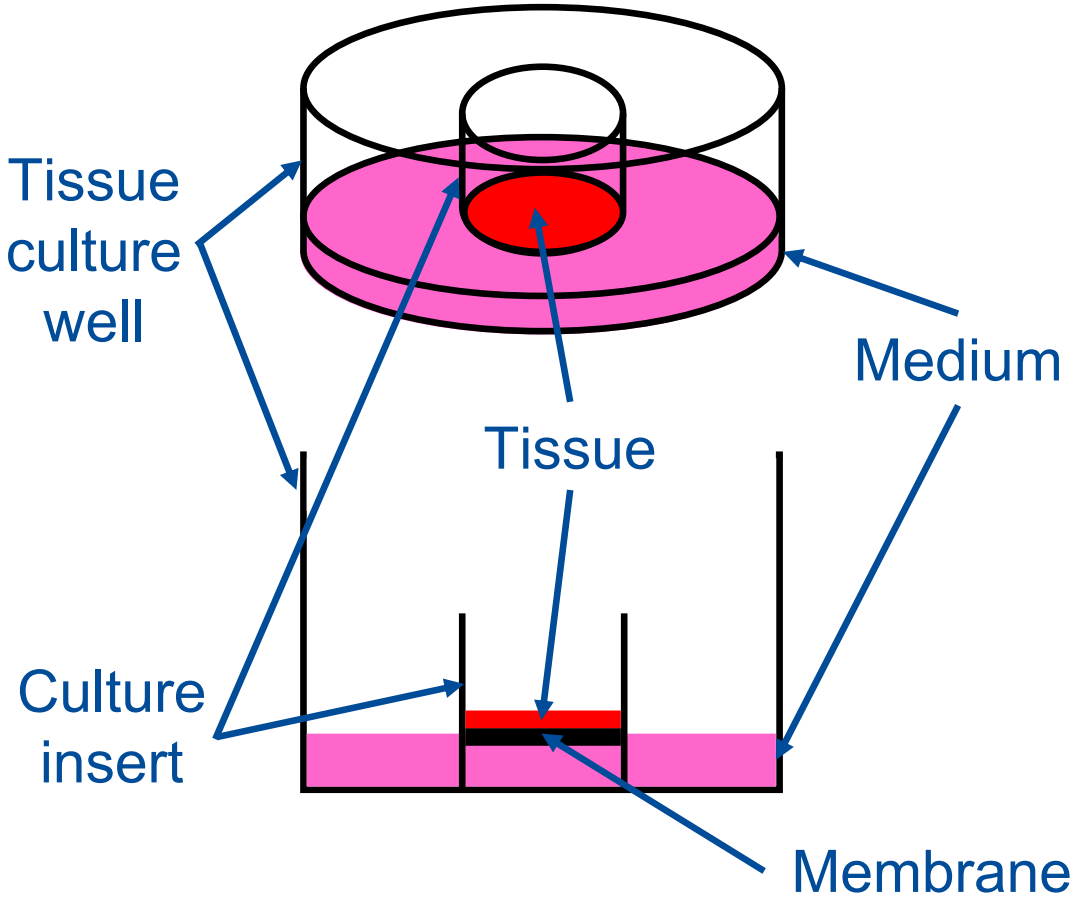


EpiDerm, EPI-200

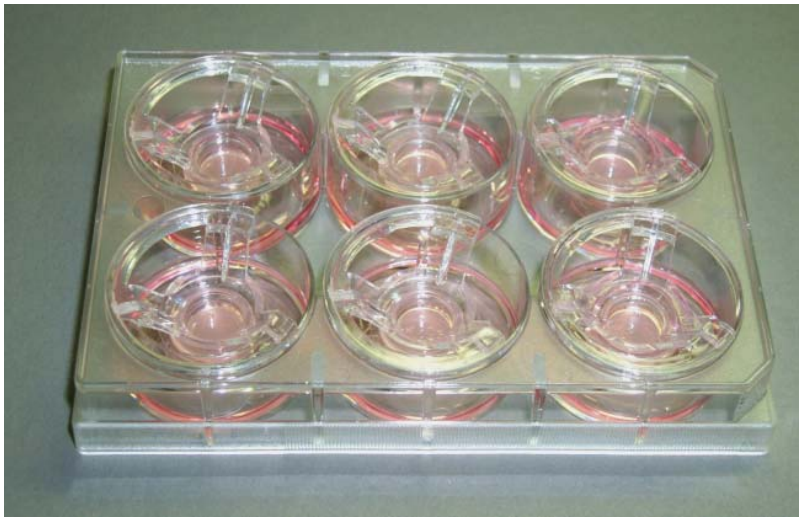


Scheme of epidermis

Cultivation



Schematic representation of the Air-Liquid Interface tissue culture technique



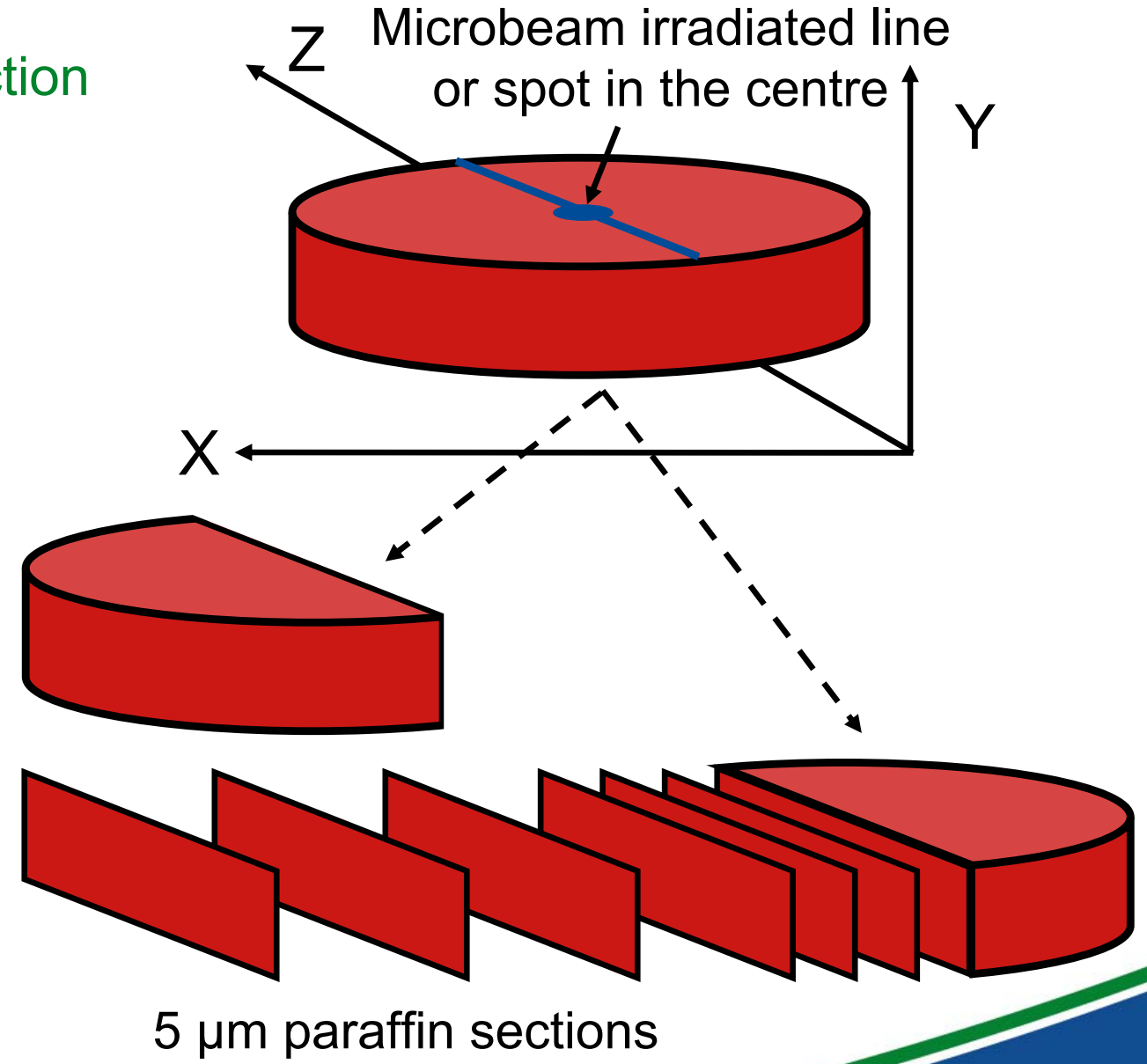
EpiAirway (AIR-100-SNP)

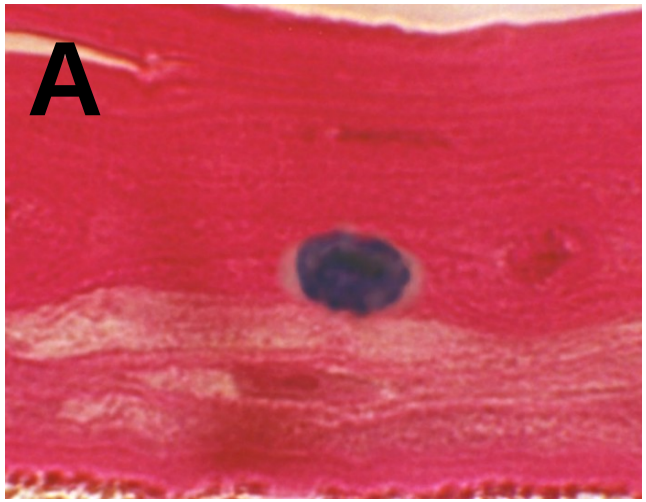
EpiDerm (EPI-212)

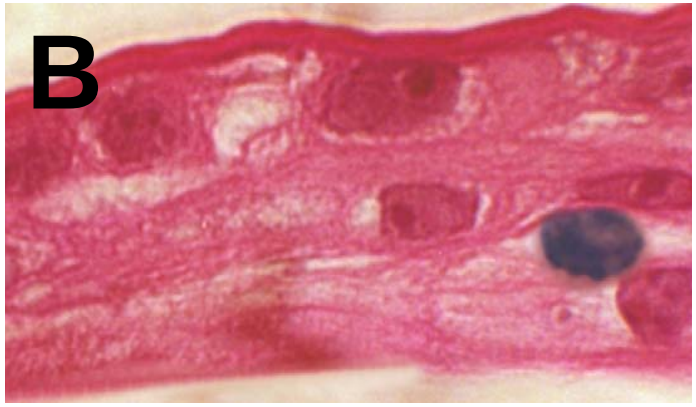
Distance-dependent assay after microbeam irradiation

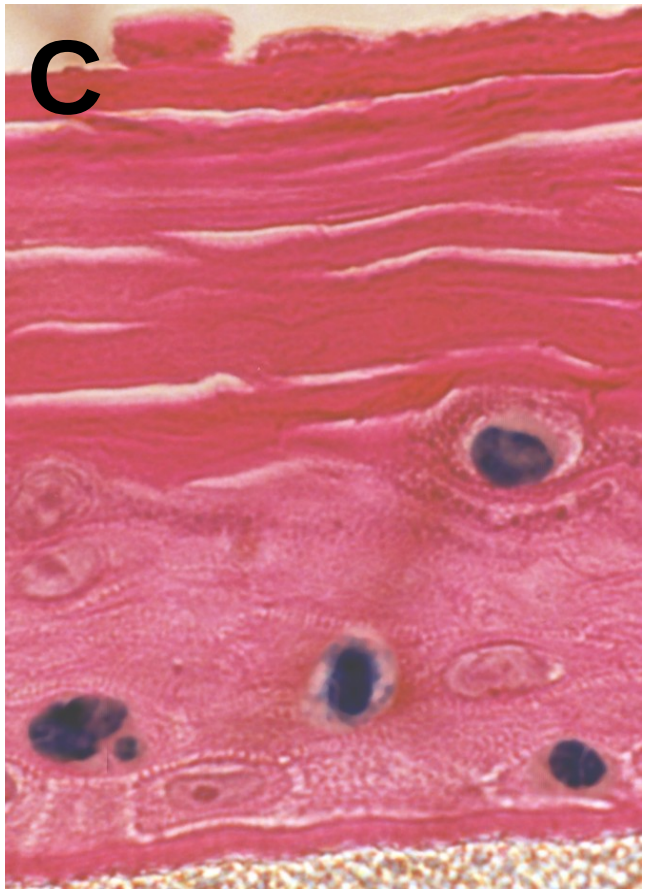
Paraffin histological section preparation

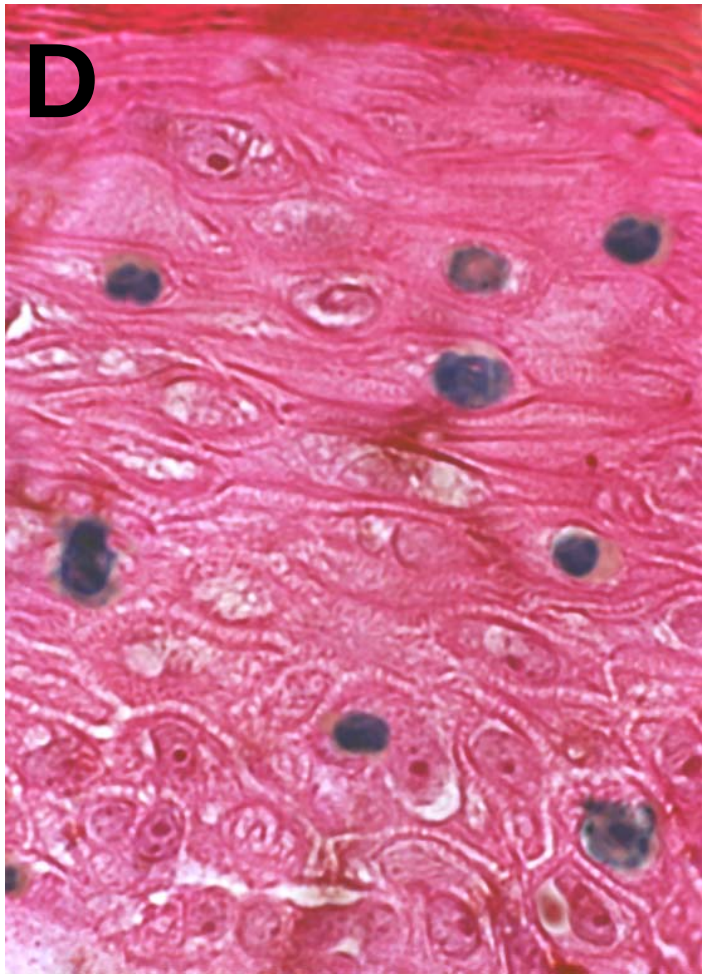
- Incubation for 1-3 days.
- Fixation in 10% neutral buffered formalin.
- Tissue is cut in half along line of irradiation.
- Paraffin embedding.
- Sample is to be cut in series or levels along X axis.











Bystander apoptosis

Bystander induced apoptosis in artificial human skin systems stained with Derma TACS apoptosis kit. Positive apoptotic cells appear blue.

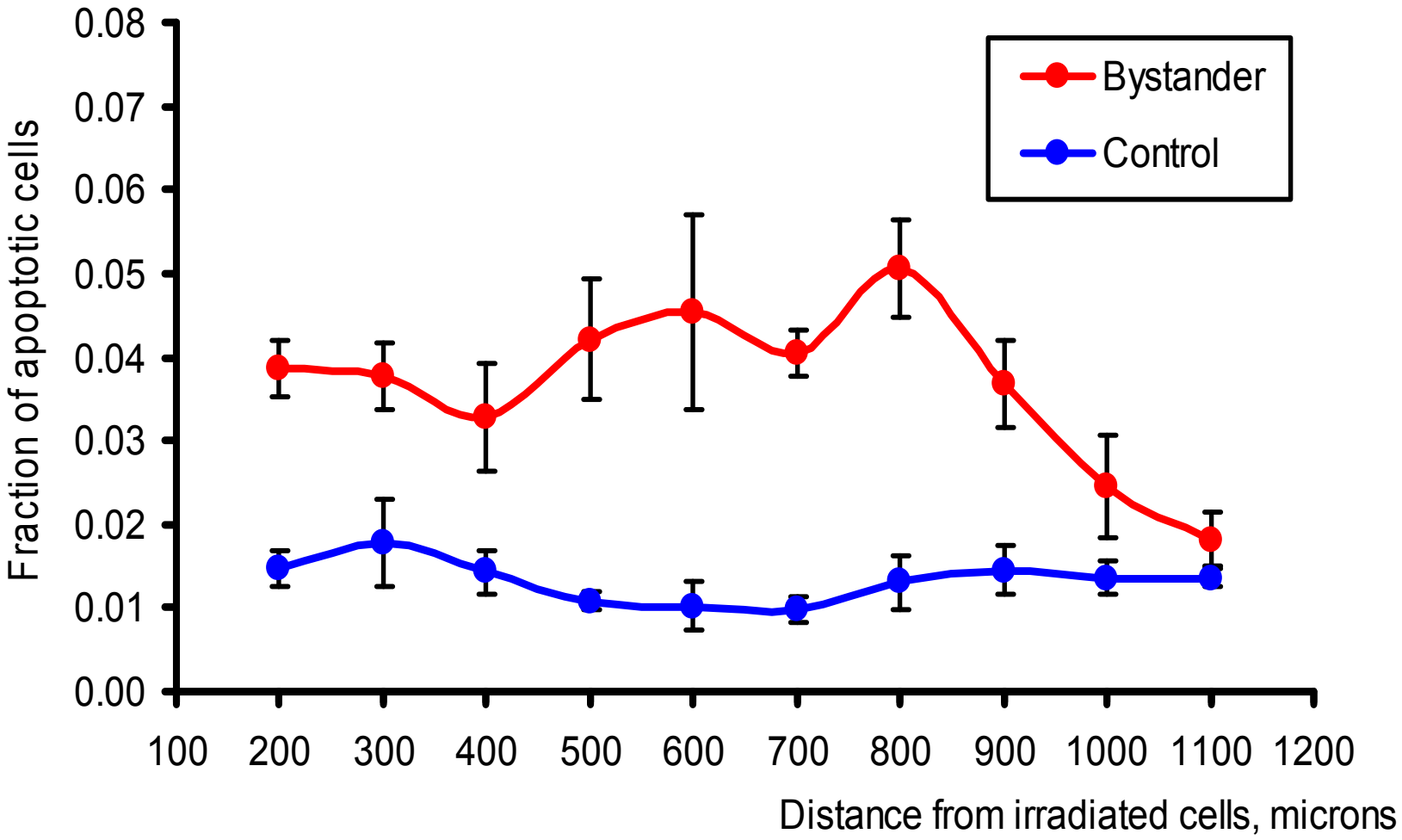
- EPI-201 (A)
- EPI-200-3s (B)
- EPI-200 (C)
- EFT-100 (D)

Bystander effect propagates up to 1 mm away from the irradiated site

- Artificial skin models were irradiated along a straight line across tissue sample (8 mm) every 100 (or 20) μм with α-particles (~7.2 MeV).
- Fractions of micronucleated and apoptotic cells were estimated.
- Mean fraction of bystander apoptotic cells was 3.7±0.6% in irradiated cells and 1.3±0.3% in control.
- Using distance-dependent assay we demonstrated for the first time that bystander effect can be propagated up to 1 mm in tissue after irradiation with α-particle microbeam.

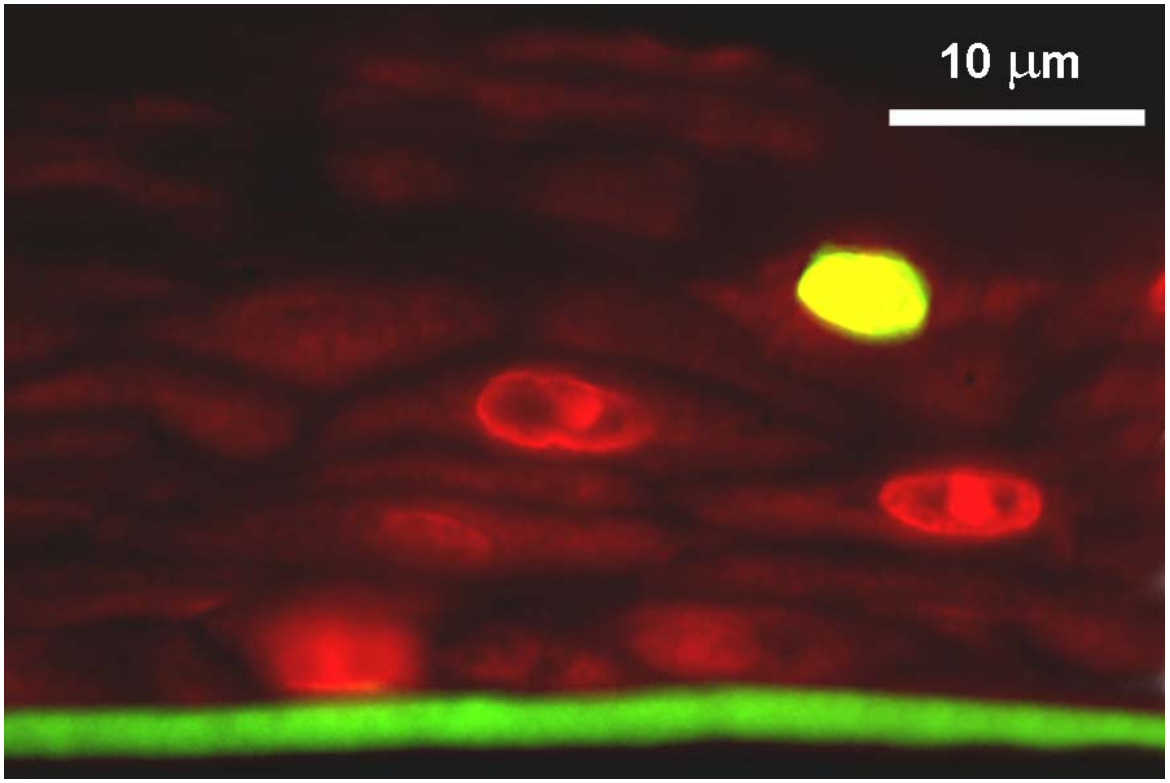
Belyakov, O.V., Mitchell, S.A., Parikh, D., Randers-Pehrson, G., Marino, S.A., Amundson, S.A., Geard, C.R. and Brenner, D.J. (2005) Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away. *Proc Natl Acad Sci U S A*, **102**:40, 14203-8.

Bystander apoptosis in EPI-200 artificial human tissue after microbeam irradiation



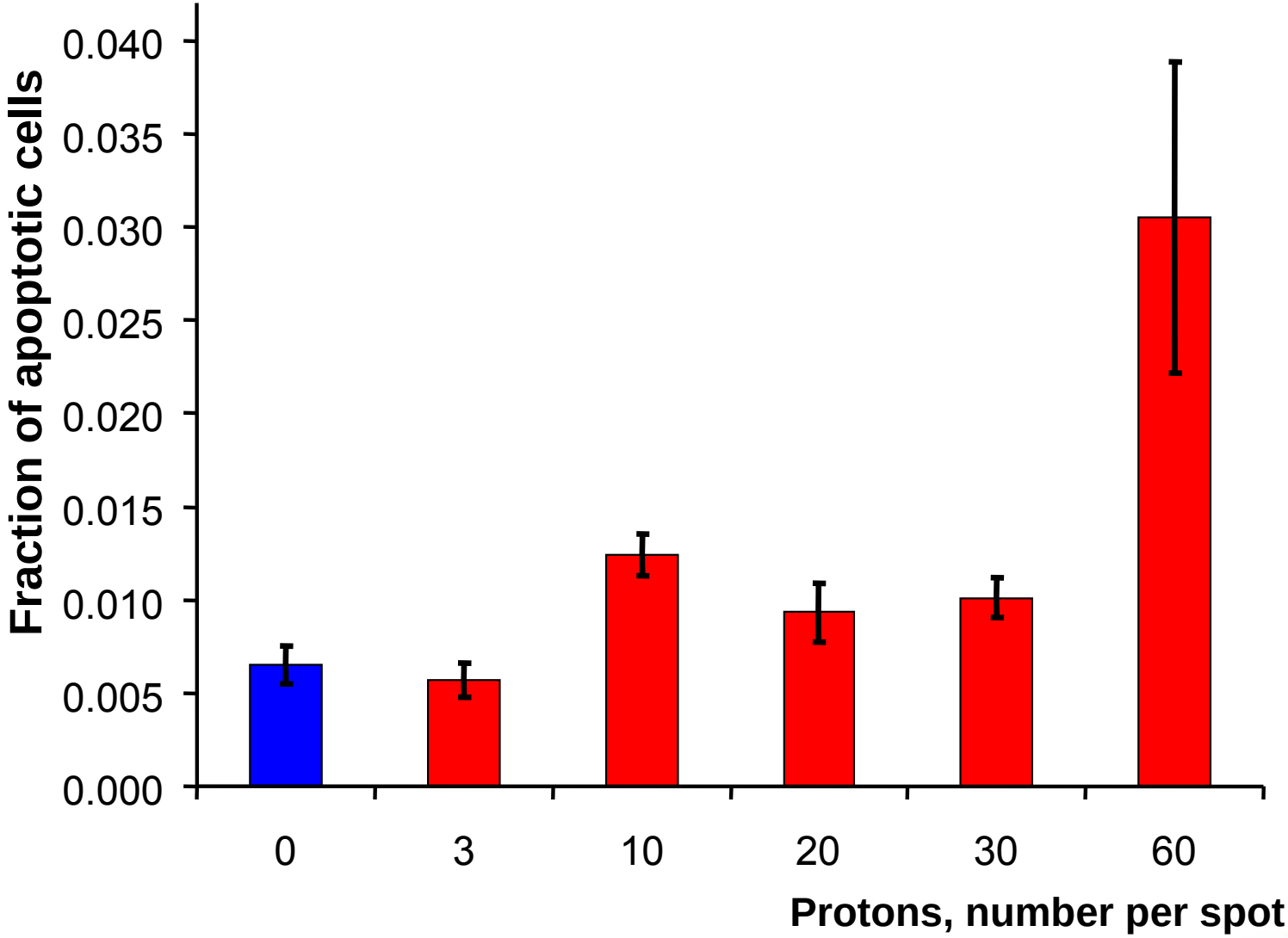
Experimental setup

- Microbeam irradiation of a single 2 µm spot with protons and $^{3}He^{2+}$ ions.
- *In situ* apoptosis assay with 3'-OH DNA end-labelling based technique.
- Studies of bystander-induced differentiation under *in situ* conditions using morphological measurements in underdeveloped EPI-201 model.

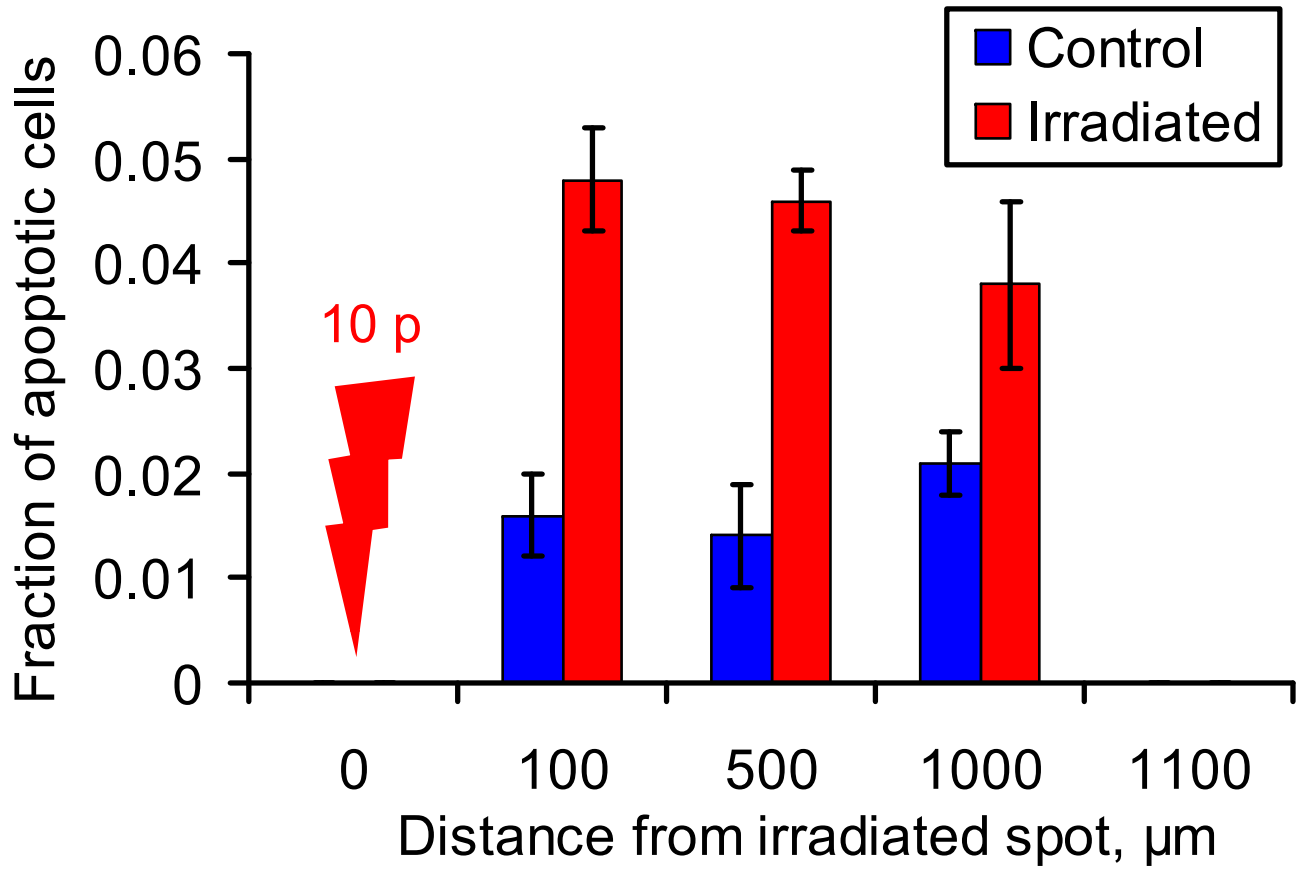


EPI-200, 4 µm paraffin section, 3' OH DNA end-labelling, positive apoptotic cell are green, fluorescent microscope.

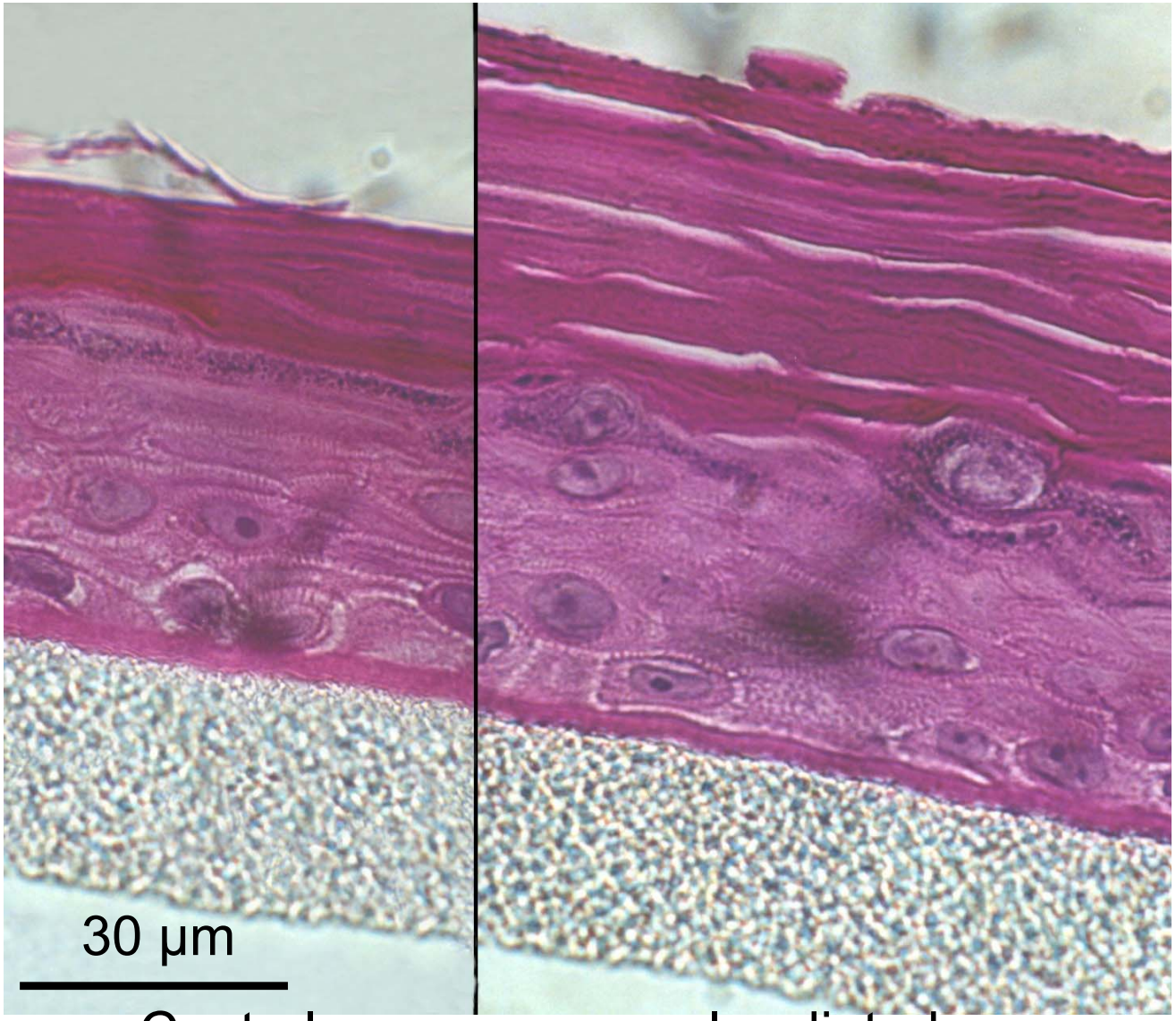
Dose-effect dependency for bystander induced apoptosis in EPI-200 artificial human skin models after microbeam irradiation with protons



Bystander apoptosis in EPI-200 artificial human skin after spot microbeam irradiation with 10 protons



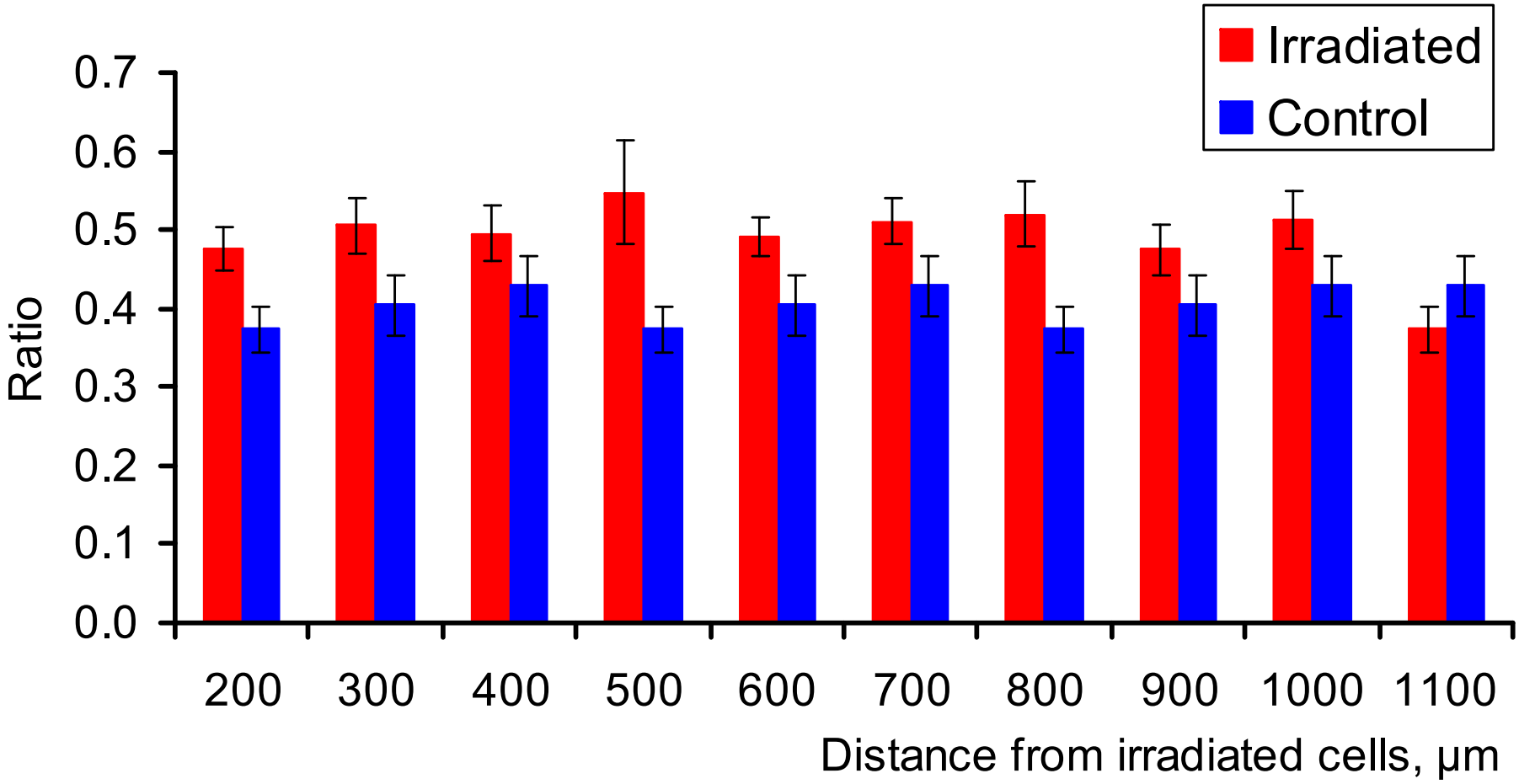
Changes in bystander differentiation pattern after microbeam irradiation EPI-201, 3 days after irradiation



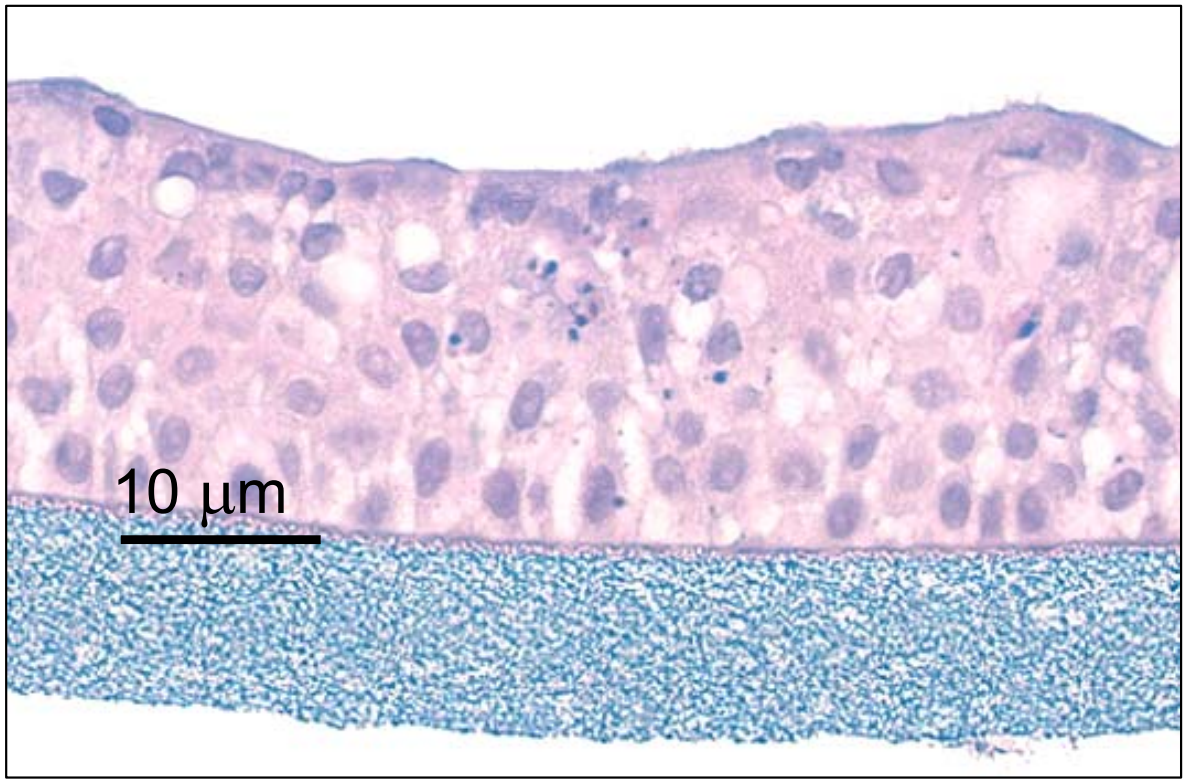
Cornified layer (terminally differentiated cells)

Malpighian layer (non-differentiated, live cells)

Microbeam irradiation increases ratio “cornified layer / total thickness”



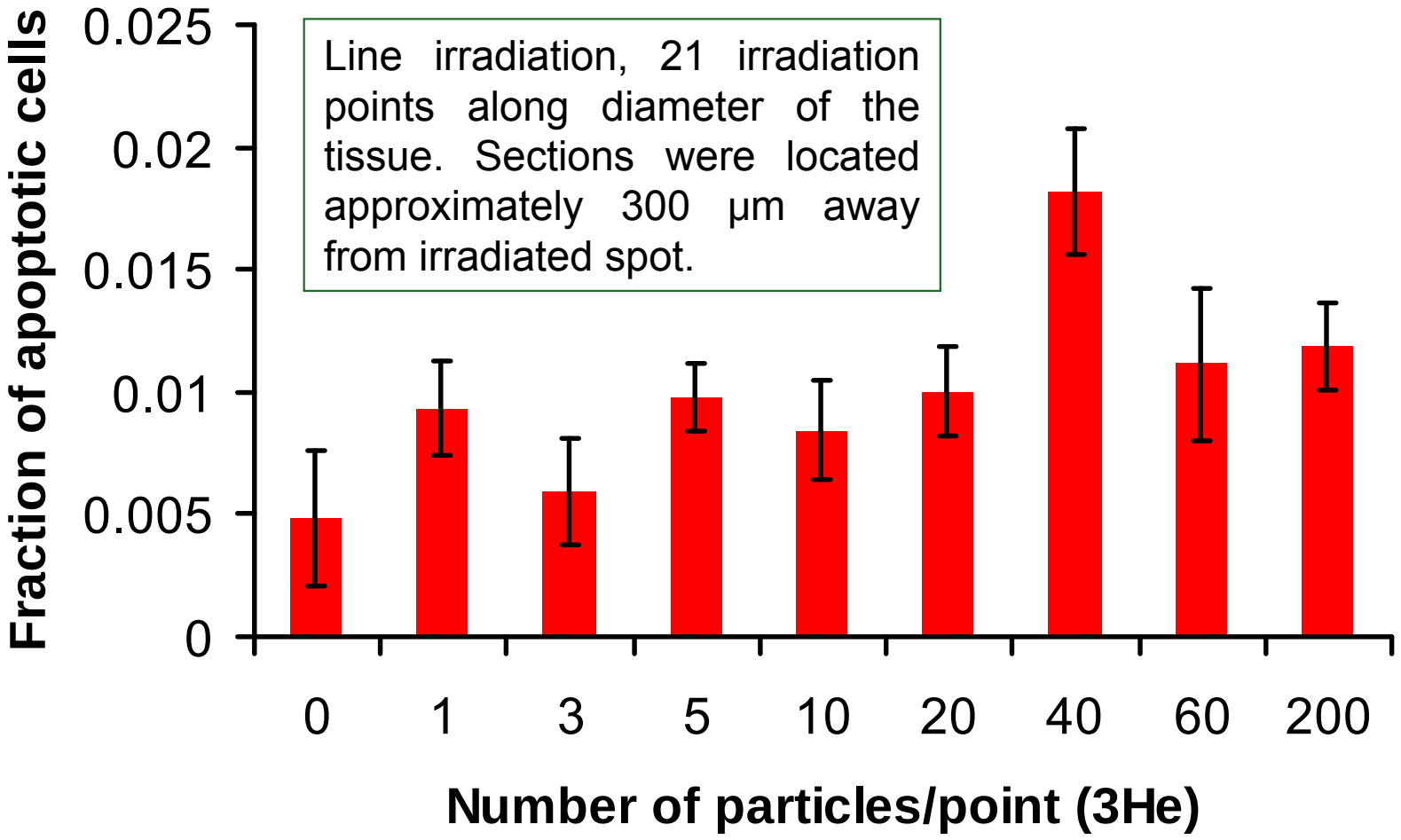
MatTek artificial tracheal/bronchial epithelial tissue system



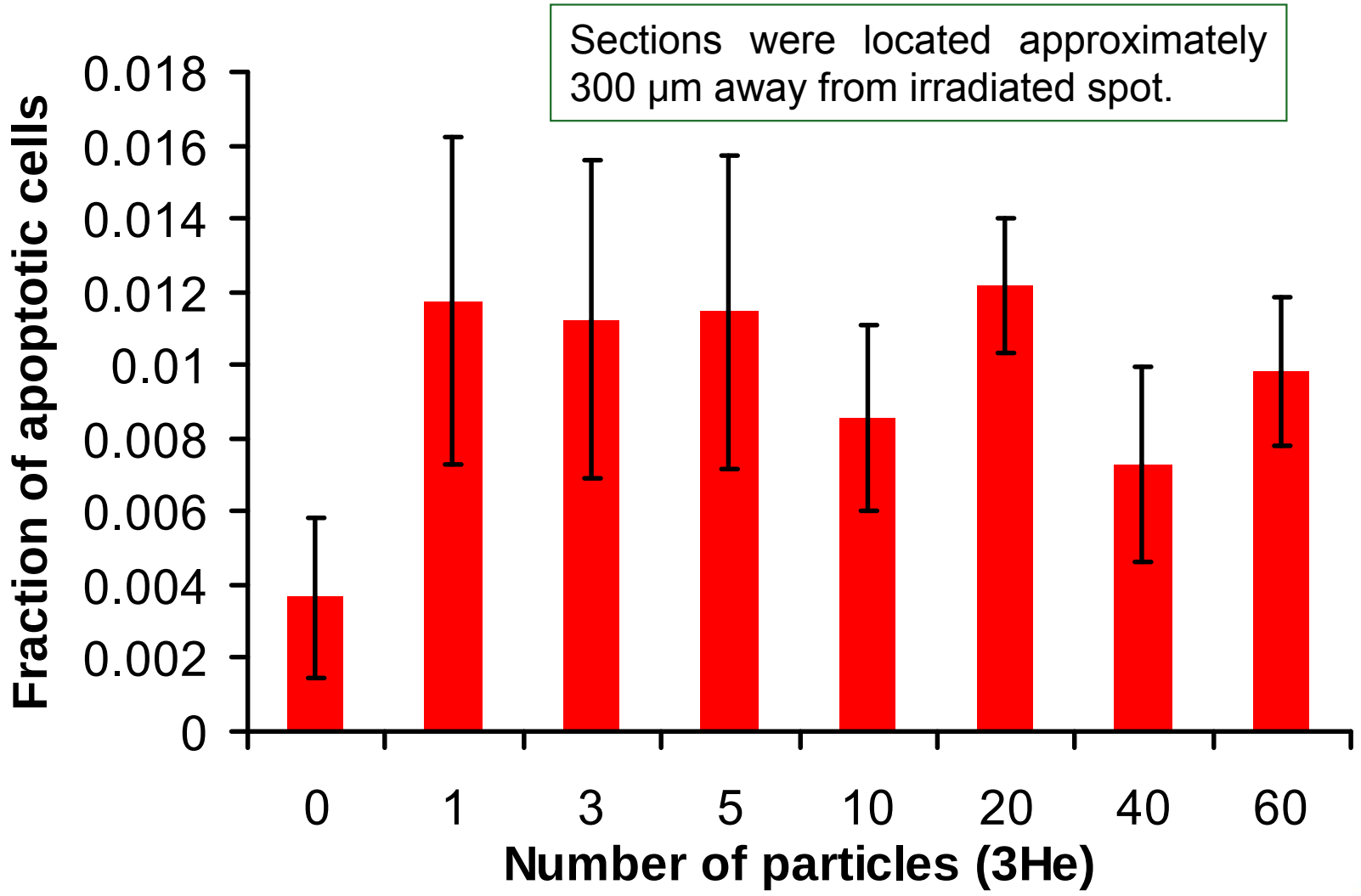
MATTEK CORPORATION

4 μm paraffin section, Haematoxylin - Eosin staining

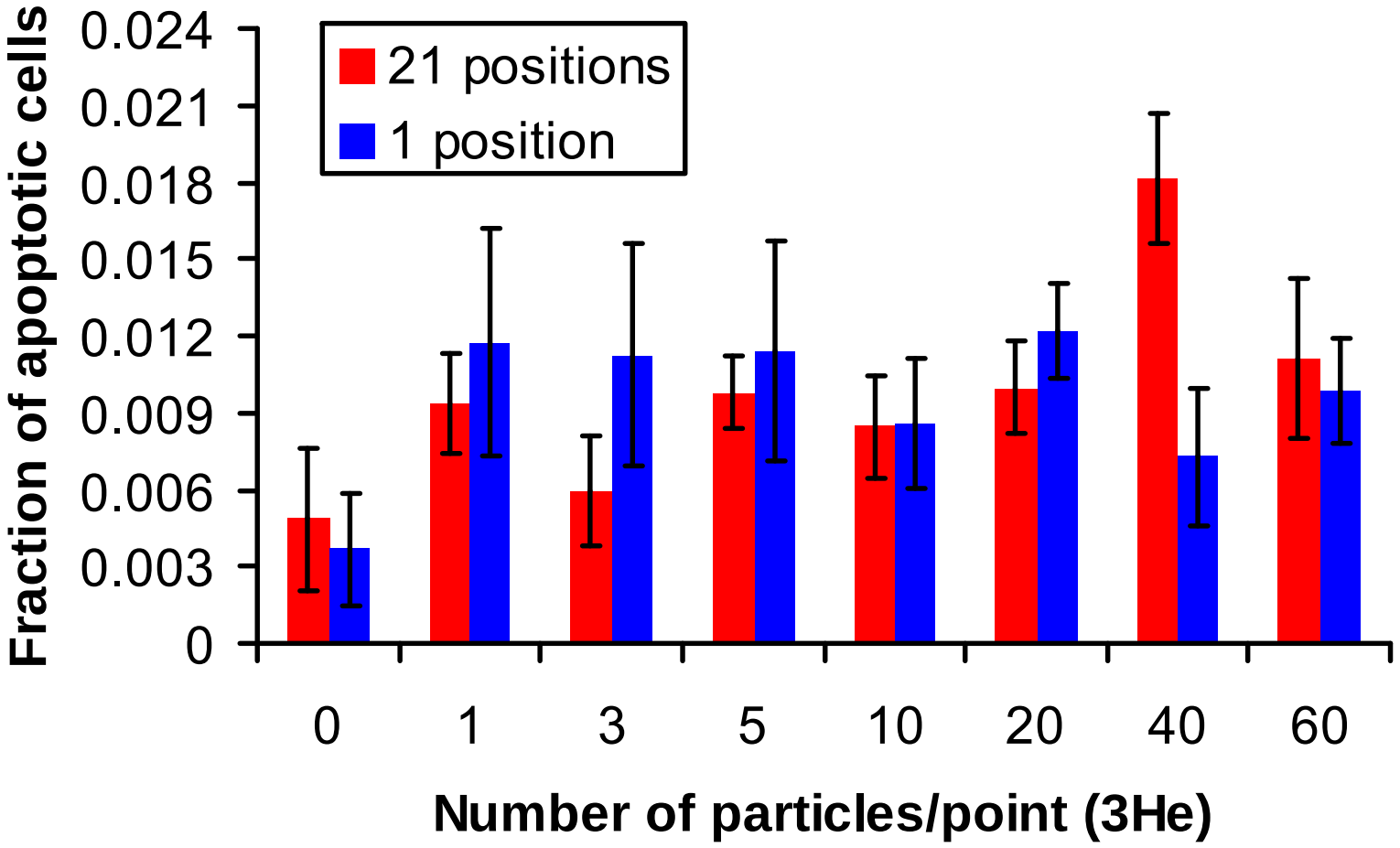
Bystander induced apoptosis following line $^{3}He^{2+}$ microbeam irradiation



Bystander induced apoptosis following single spot $^{3}He^{2+}$ microbeam irradiation



Bystander induced apoptosis following line and spot $^{3}He^{2+}$ microbeam irradiation



4. Hypothesis, summary and possible implications

Hypothesis - bystander effect is a protective mechanism

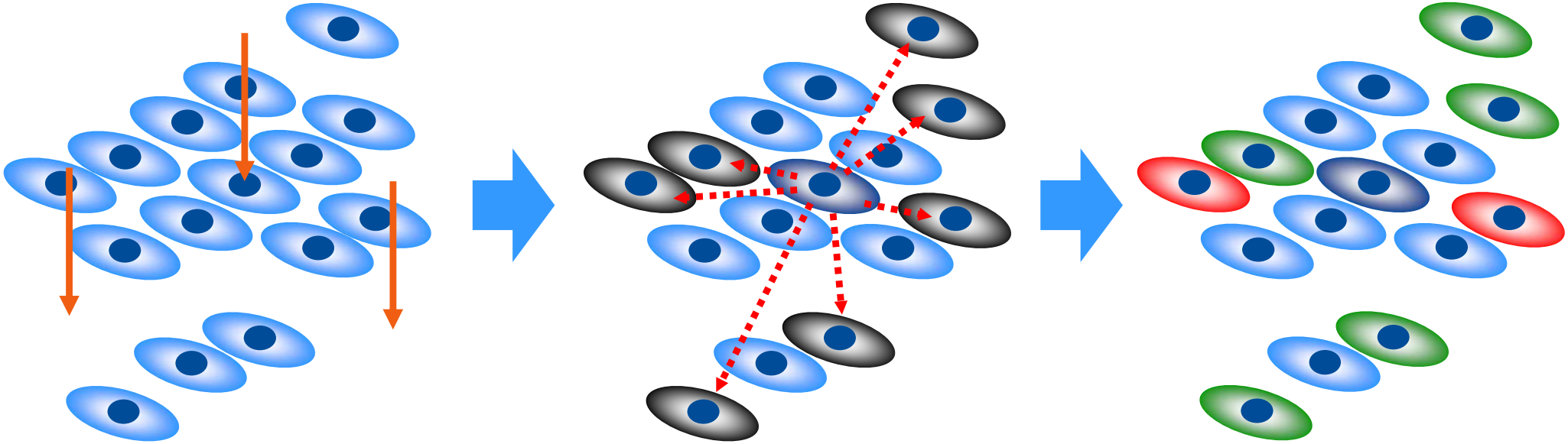
- Remove potentially damaged *functional group* of cells to decrease risk of transformation.
- Maximal at low doses when a small fraction of cells is exposed.
- Normal tissue microarchitecture amplifies the response.
- Apoptosis is an important contributor.
- Irreversible differentiation is a major pathway of removing potentially damaged cells from proliferating population.

A general scheme of radiation induced bystander effect in tissue systems

Sparse irradiation

Bystander signal

Tissue response



Track

Intercellular communication

Targeted cell

Potentially damaged cell

Premature differentiated cell

Apoptotic cell

Summary

- Bystander response measured as increase in apoptosis, and differentiation was observed in cell cultures, explants and 3D tissue models.
- Bystander induced apoptosis is propagated over large distances in 3D tissue.
- Tissue sample acts as a single unit in response to microbeam irradiation. A cascade mechanism of bystander effect induction might be involved.
- It is tempting to suggest that the bystander response has the function of eliminating potentially damaged cells in the vicinity of radiation induced DNA damage by apoptosis and increased differentiation.

Implications for Radiation Protection

- Non-targeted effects could be important in several radiation related areas.
- It might contribute to better estimation of cancer risk from domestic radon exposure and uranium in drinking water.
- Effects of HZE (high-charge-and-energy) particles during space missions.
- High energy radiotherapy outcome.
- Health effects of air crew and nuclear power station personnel.
- In particular, bystander effect is potentiality significant for radiation protection issues and may have implications for the applicability of the Linear-No-Threshold (LNT) model in extrapolating radiation risk data into the low-dose region.

Significance of the bystander effects for radiotherapy

- The spectrum of secondary malignancies in radiotherapy patients may suggest some contribution of the bystander effect (Hall, *Cancer J*, 2000).
- Microbeam radiation therapy (Thomlinson, *et al.*, *Cell Mol Biol (Noisy-le-grand),* 2000) is a new technology of cancer treatment, which might utilise non-targeted effects.
- Finding of a significant bystander induced differentiation after microbeam irradiation would suggest a potential value of the bystander effect for differentiation therapy of cancer treatment; see review of (Beere and Hickman, *Anticancer Drug Des,* 1993).

5. Future trends in non-targeted research

Experimental systems: opportunities

Currently available

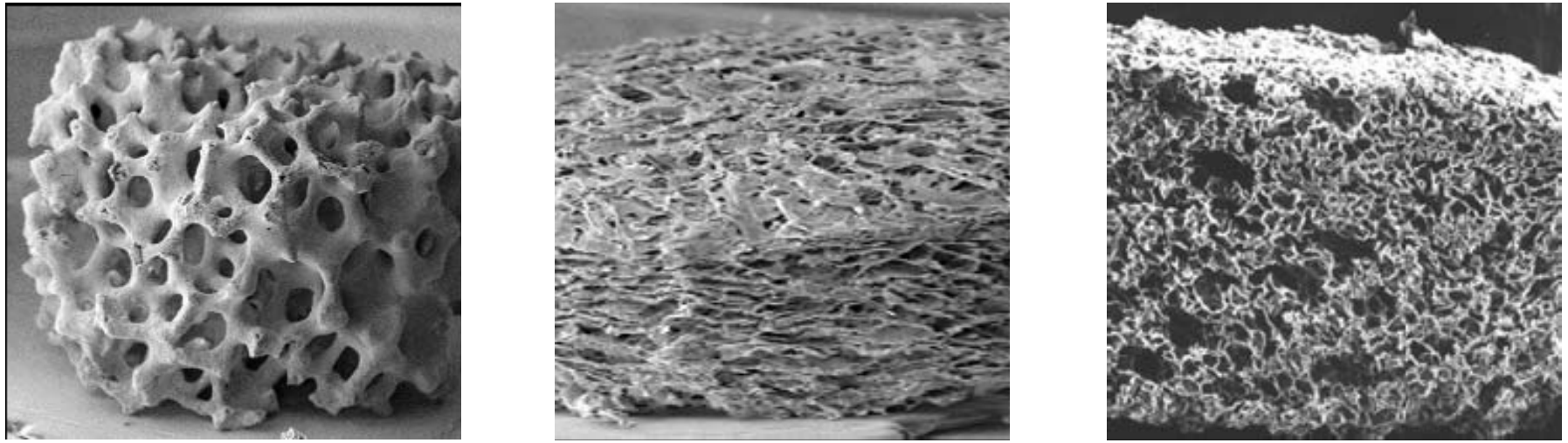
- Primary explant techniques
- Artificial human skin tissue systems
- Tissue scaffolding
- …

Future directions

- Adaptation of the “window chamber technique” for radiobiological experiments
- Tissue transplants, for example, piece of human tissue grafted on a nude mice
- …

Tissue scaffolding

- Allows to use conventional cells cultures to form tissue-like 3D microarchitecture.
- Easy to handle, cells could be easily inoculated and extracted with conventional cell culture techniques.
- Preparation of histological sections and non invasive 3D deep tissue imaging is possible.
- Stable, highly reproducible model.

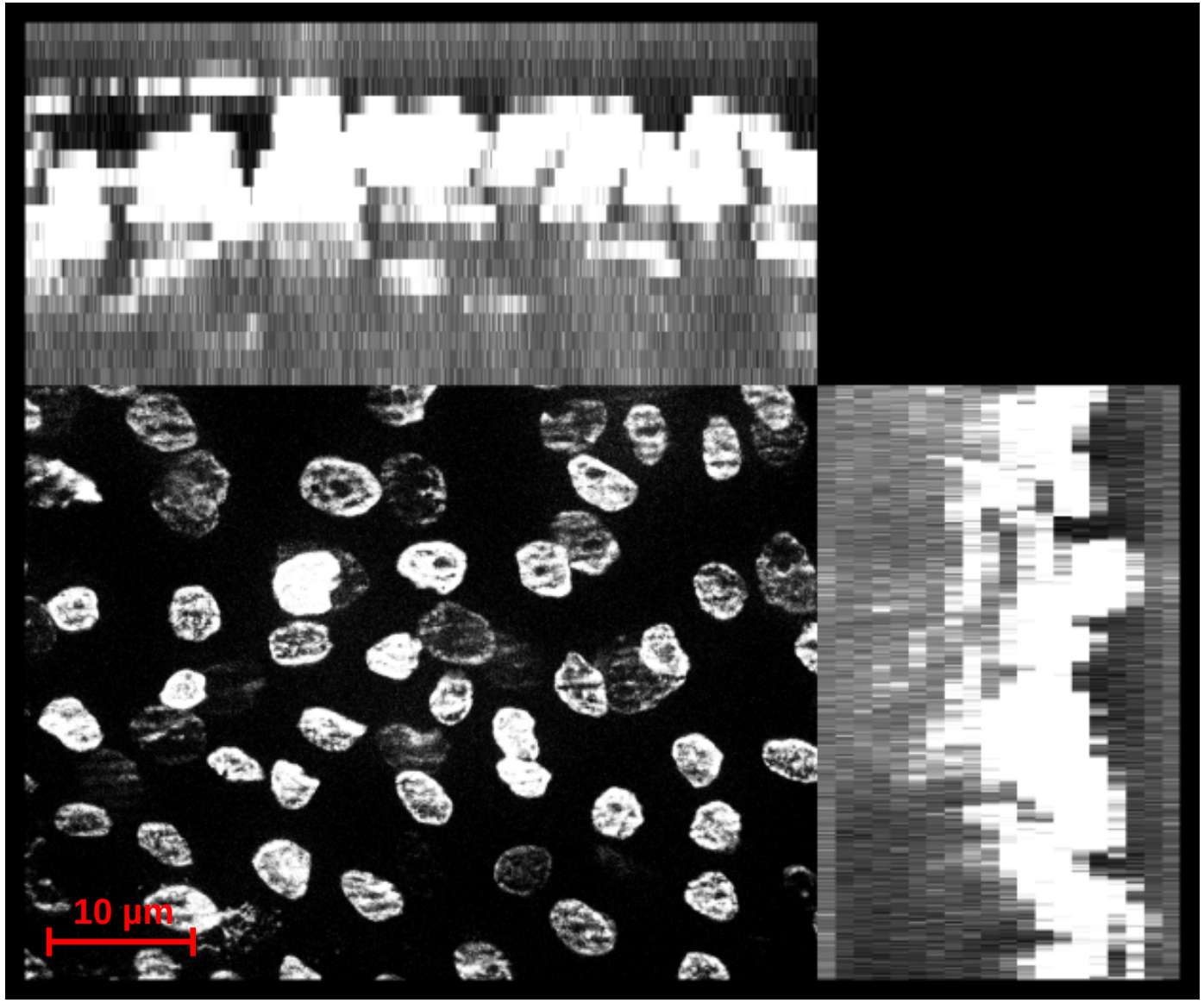


The BD Three Dimensional (3D) Scaffolds: 3D Calcium Phosphate Scaffold (left), 3D Collagen Composite (centre) and OPLA® (Open-Cell Poly-Lactic Acid [right]) scaffolds.

Endpoints

- All models are suitable for histological examination and consequent histoimmunochemistry.
- Deep tissue non-invasive imaging techniques are under development (confocal, 3-photon imaging, Zeiss ApoTome systems).
- Non-destructive life tissue examinations are possible.
- Mutations (?) and epigenetic changes.
- Genomic instability and bystander effect.
- Markers of proliferation and differentiation.
- Malignant conversion (?).
- Progression to invasive cancer (using transformed cell lines and tissue scaffolding or co-culture techniques).

Non-invasive deep tissue imaging



Non-invasive deep fixed and unfixed tissue imaging using Zeiss ApoTome system.

Priorities

- The main priority is a shift from *in vitro* cell systems towards *in vivo* (or at least 3D) tissue models.
- Possible use of human cell lines (with tissue scaffolds), tissue transplants, window chambers technique and other *in vivo* human model systems.
- Low dose irradiation can be performed with broad and microbeam charged particle and X/γ-ray facilities.

Constraints

- Significant inter-individual variability (in case of explants).
- Tissue models typically contain several types of cells, role of tissue microenvironment is significant.
- Cells in tissues are in different proliferation and differentiation states.
- 3D tissue difficult to irradiate quantitatively with existing charge-particle microbeams because of low range (typically tenths of micrometers).
- 3D tissue studies would require new methods of non-invasive deep tissue imaging to preserve 3D microarchitecture and study spatial distribution.

6. Non-targeted effects and radiation protection

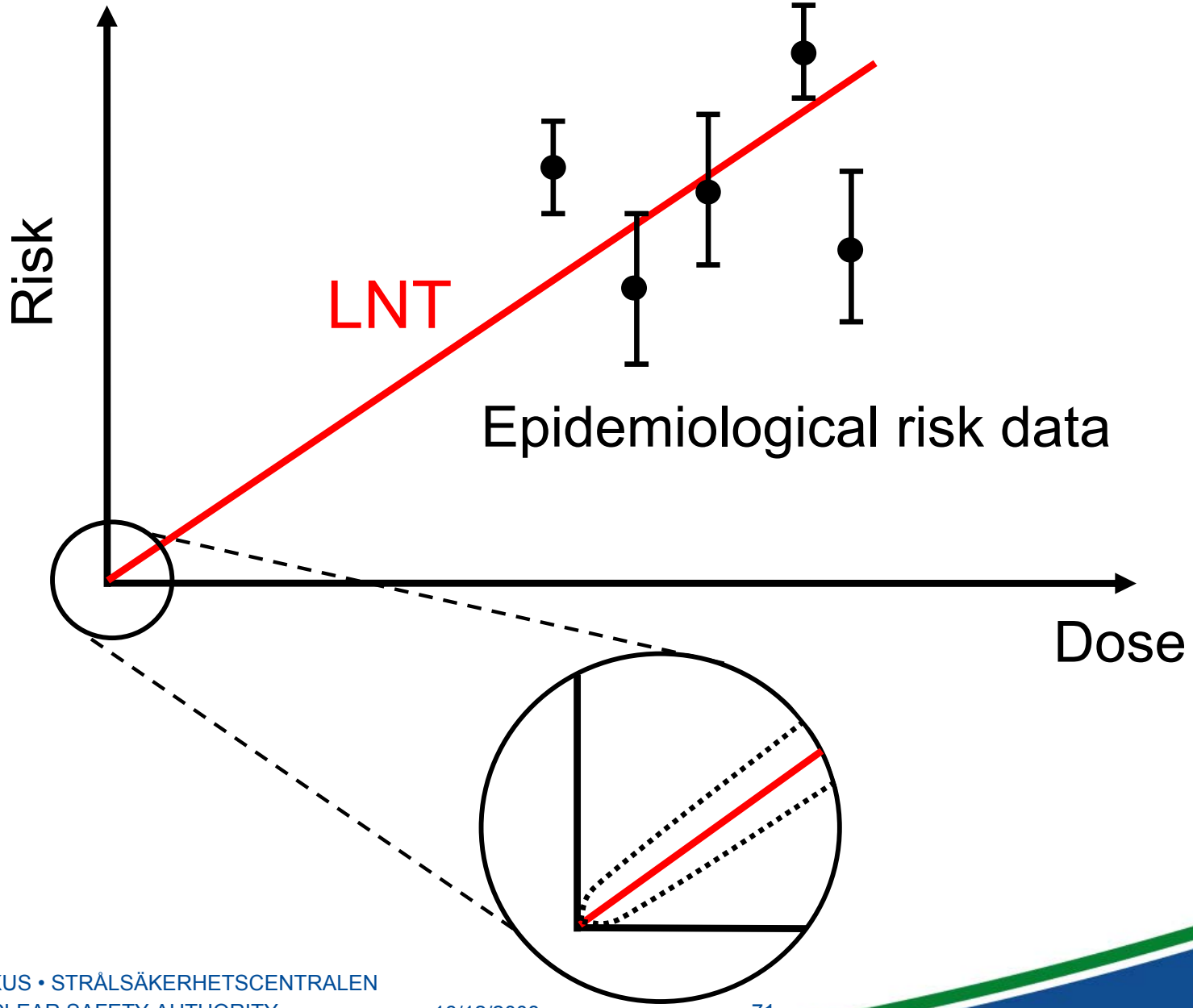
System of radiation protection

- Present estimations of radiation risk is based on direct epidemiological evidence, as well as on radiation biology research.
- The system is designed to protect against both deterministic and stochastic effects.
- Linear-Non-Threshold (LNT) model is used for estimation of long-term health effects including carcinogenesis and genetic effects.
- A dose and dose-rate correction factor is used to relate the effects of acute exposures to chronic exposures (DDREF).
- Radiation dose is used as a surrogate for risk.
- The effects produced by different types of radiation are assumed to be qualitatively the same.
- Doses can be summed to predict overall risk.

Challenges of the present radiation protection system

- The main objective of the system is to protect the individual. The protection system is generally applicable, in the same fashion, to all age groups, males and females.
- The protection system include the principles of justification, optimisation and exposure restrictions.
- There is a broad international agreement among governmental bodies that the current system of radiation protection is effective, robust and adequately protects people and the environment.
- There are, however, scientific challenges that may bring into question various aspects of the current approach, and which may have significant policy, regulatory and operational implications.
- These challenges include non-targeted effects.

LNT and uncertainties in extrapolation of radiation risk



Key question

Do non-targeted effects increase or decrease low dose risk in relation to LNT?

The bystander effect might be harmful

- The bystander-induced mutagenesis

  Nagasawa and Little, *Rad Res,* 1999

  Zhou *et al., Radiat Res*, 2000; Zhou *et al., PNAS*, 2001

- Bystander-induced transformation

  Lewis *et al., Radiat Res,* 2001

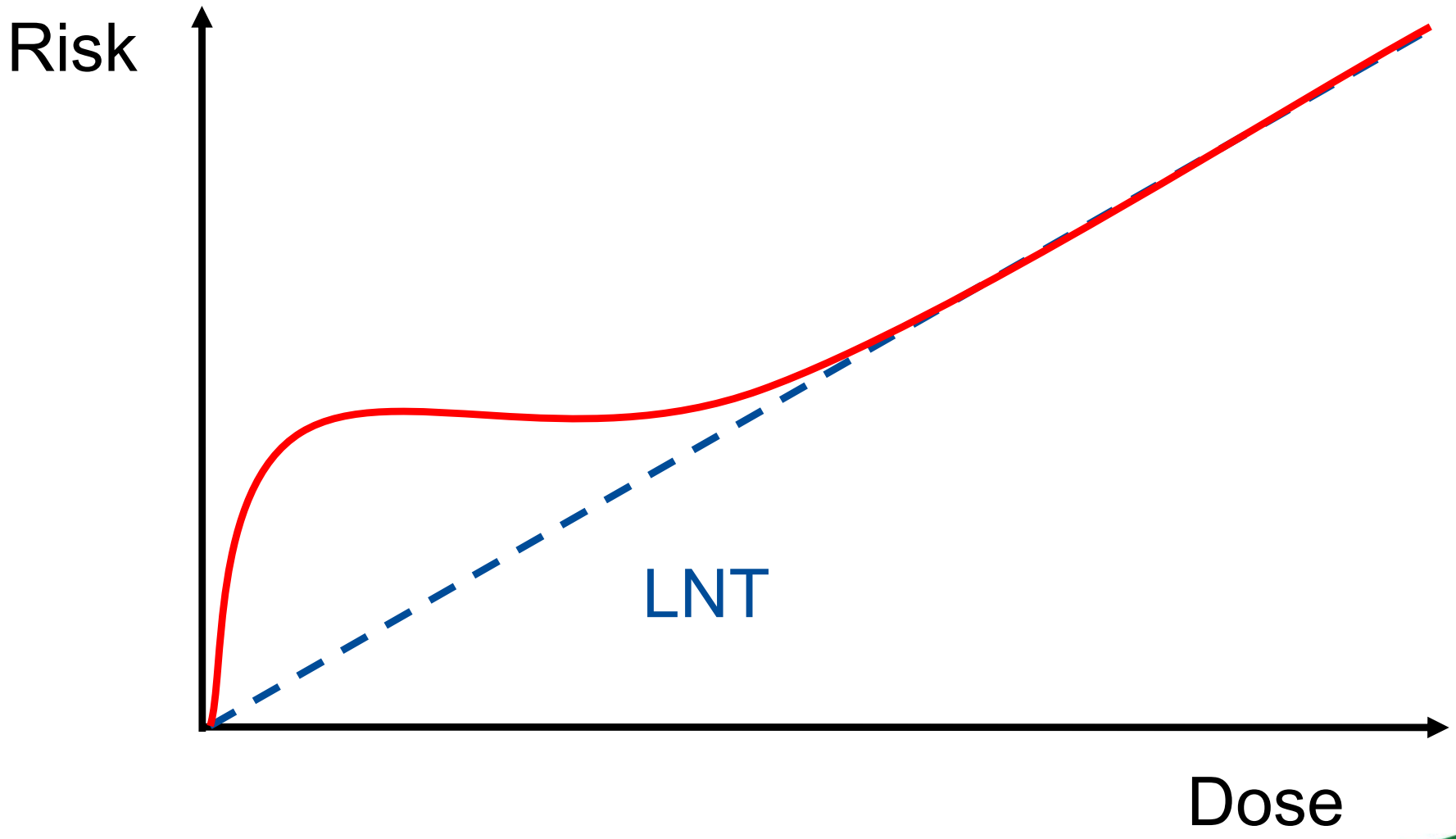
  Sawant *et al., Radiat Res*, 2001

- Chromosomal instability could be induced in bystander cells

  Lorimore *et al., PNAS,* 1998

  Watson *et al*., *Cancer Res*, 2000

The risk at low doses might be *greater* than predicted by LNT



The bystander effect might be protective

- A gross bystander induced differentiation in the urothelial explant outgrowth after microbeam irradiation

  Belyakov *et al., Mut Res,* 2006

- Cell survival is increased after treatment with medium from irradiated cells

  Matsumoto *et al*., *Radiat Res*, 2001

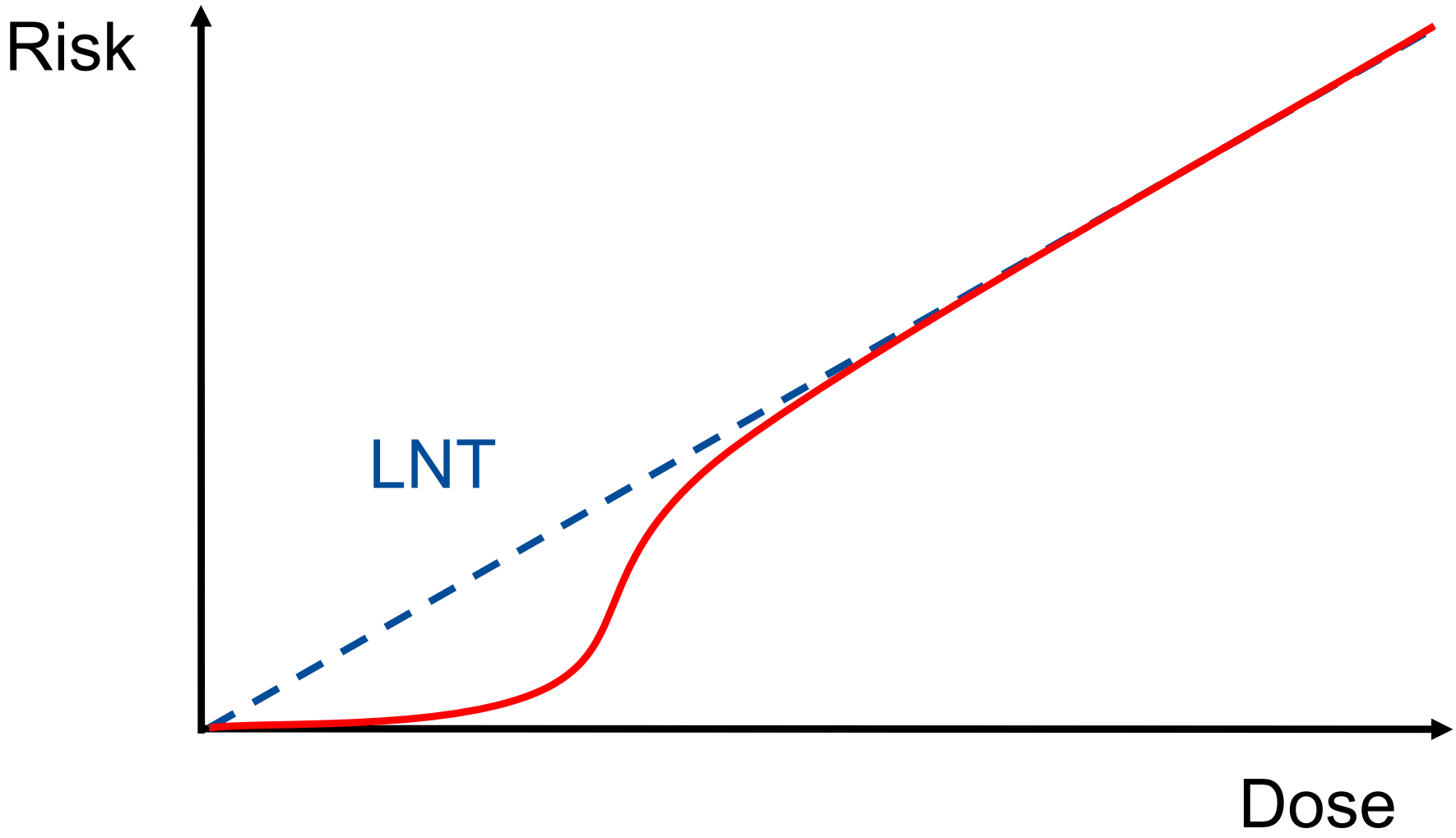
- Increase in cell proliferation after low doses of α-particle exposure

  Iyer and Lehnert, *Cancer Res*, 2000

- Bystander effect is a mechanism of tissue integrity maintenance

  Barcellos-Hoff and Brooks, *Rad Res*, 2001

The risk at low doses might be *less* than predicted by LNT



Summary

	RISK	
Bystander effects:		
cell death	-	+
mutation		+
chromosomal damage	-	+
malignant transformation		+
premature differentiation	-	
Other non-targeted effects:		+
genomic instability	-	
adaptive responses		

Implications for radiation protection

- The observation of the non-targeted effects are preliminary in nature, and the applicability of any conclusion derived from *in vitro* studies to *in vivo* situation is still uncertain.
- The risk at low doses might be *greater* or *less* than predicted by a linear extrapolation of the high dose.
- However, non-targeted effects will clearly result in an overall risk, which is a *non-linear* function of dose.
- It would be *premature* to consider revising current risk calculations on the basis of current studies of bystander phenomena.
- On other hand, the LNT model is important for radiation protection as a simple method to optimise procedures and regulations. However, it should not be mistaken as a scientific model directly derived from the present state of knowledge of the processes involved in radiation risk estimations.

7. The way forward: the NOTE project

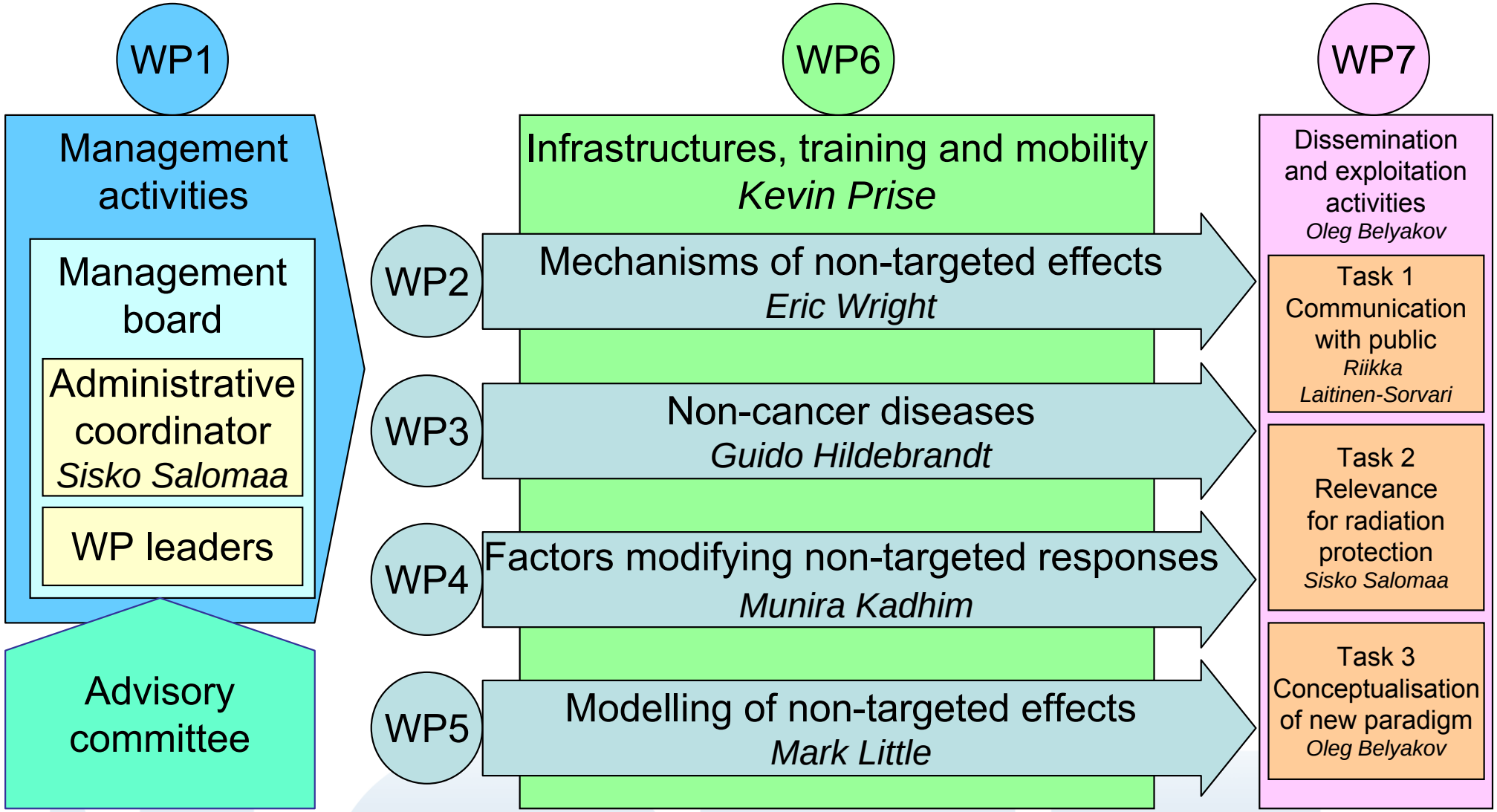
Non-targeted effects of ionising radiation (NOTE)

European Integrated project, 2006-2010

NOTE
– TOWARDS A NEW PARADIGM

NOTE team: 20 partner organisations from the EU, Norway and Canada, 133 scientists and 6 advisers

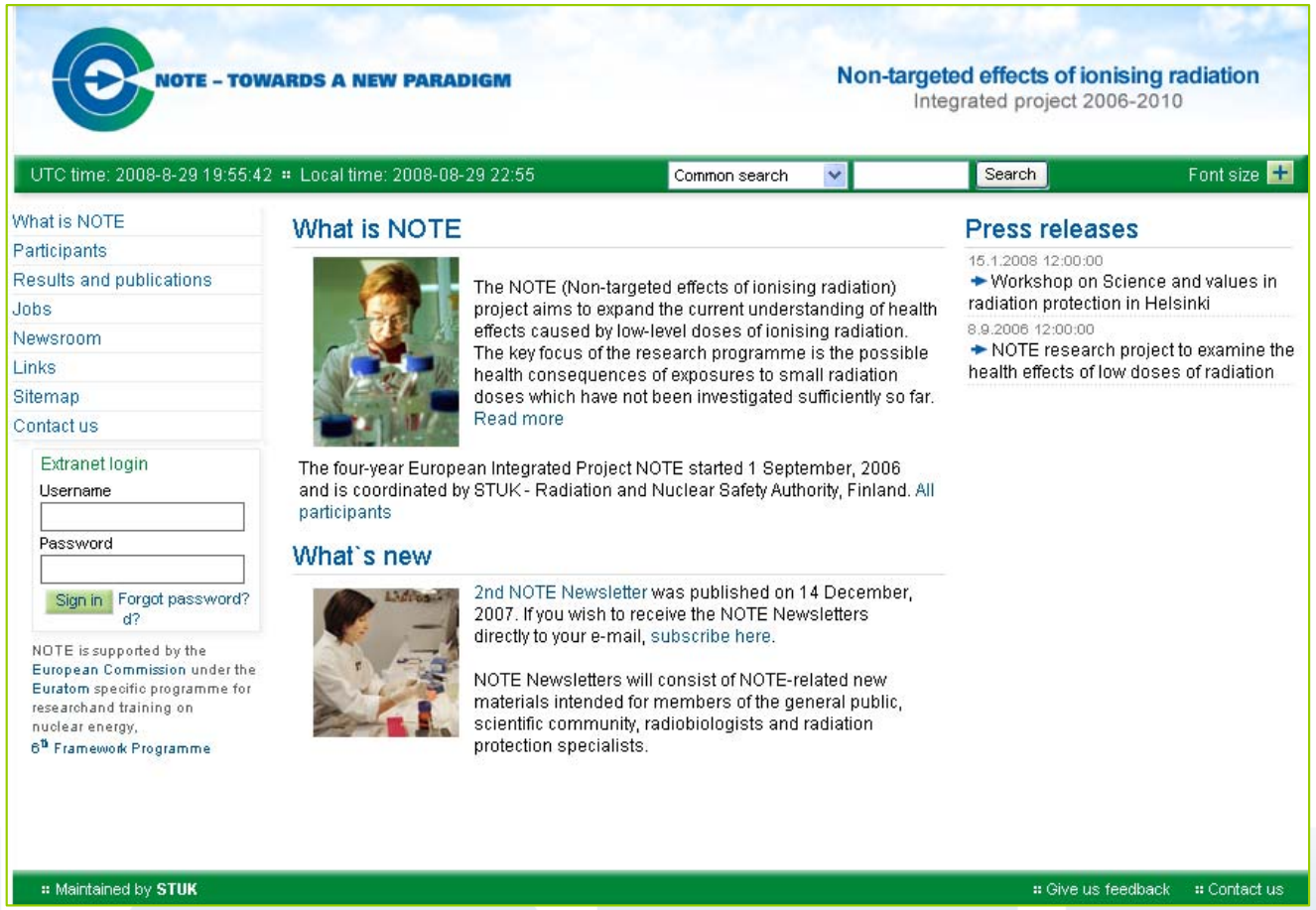
NOTE 1st annual meeting, 17-20 September 2007, Aldemar Knossos Royal Village Hotel, Crete, Greece.


WP1
Management activities
Management board
Administrative coordinator
Sisko Salomaa
WP leaders
Advisory committee
WP6
Infrastructures, training and mobility
Kevin Prise
WP2
Mechanisms of non-targeted effects
Eric Wright
WP3
Non-cancer diseases
Guido Hildebrandt
WP4
Factors modifying non-targeted responses
Munira Kadhim
WP5
Modelling of non-targeted effects
Mark Little
WP7
Dissemination and exploitation activities
Oleg Belyakov
Task 1
Communication with public
Riikka Laitinen-Sorvari
Task 2
Relevance for radiation protection
Sisko Salomaa
Task 3
Conceptualisation of new paradigm
Oleg Belyakov

General objectives of the NOTE IP

- To investigate the mechanisms of non-targeted effects, in particular, bystander effects, genomic instability and adaptive response.
- To investigate if and how non-targeted effects modulate the cancer risk in the low dose region, and whether they relate to protective or harmful functions.
- To investigate if ionising radiation can cause non-cancer diseases or beneficial effects at low and intermediate doses.
- To investigate individual susceptibility and other factors modifying non-targeted responses.
- To assess the relevance of non-targeted effects for radiation protection and to set the scientific basis for a modern, more realistic, radiation safety system.
- To contribute to the conceptualisation of a new paradigm in radiation biology that would cover both the classical direct (DNA-targeted) and non-targeted (indirect) effects.

NOTE website: http://www.note-ip.org/



NOTE newsletters

NOTE Newsletter

NOTE – TOWARDS A NEW PARADIGM

Non-targeted effects of ionising radiation
Integrated project 2006-2010

Issue 2; 14 December, 2007

In this issue

- NOTE DIP2 highlights
- 1st NOTE Annual meeting
- Future meetings
- OECD-NEA workshop
- 2nd Systems Biology workshop
- New paradigm workshop
- 3rd European IRPA Congress in June 2010
- Collaboration
- Periodic Reporting to EC

NOTE DIP2 highlights

Final revised version of the DIP2 - Detailed implementation plan for the months 13-30 (1 September, 2007-28 February, 2009) was prepared and submitted to the EC on 7 December, 2007. Addressing low doses and promoting experimentalist - modeller interaction continue to be important themes. The NOTE Management Board will develop a strategy for moving towards the new paradigm and this will be also reflected in the next internal RTD call. The paradigm workshop in Ireland in autumn 2008 will be a major milestone for NOTE. Read more on the highlights of DIP2:

Editor's NOTE

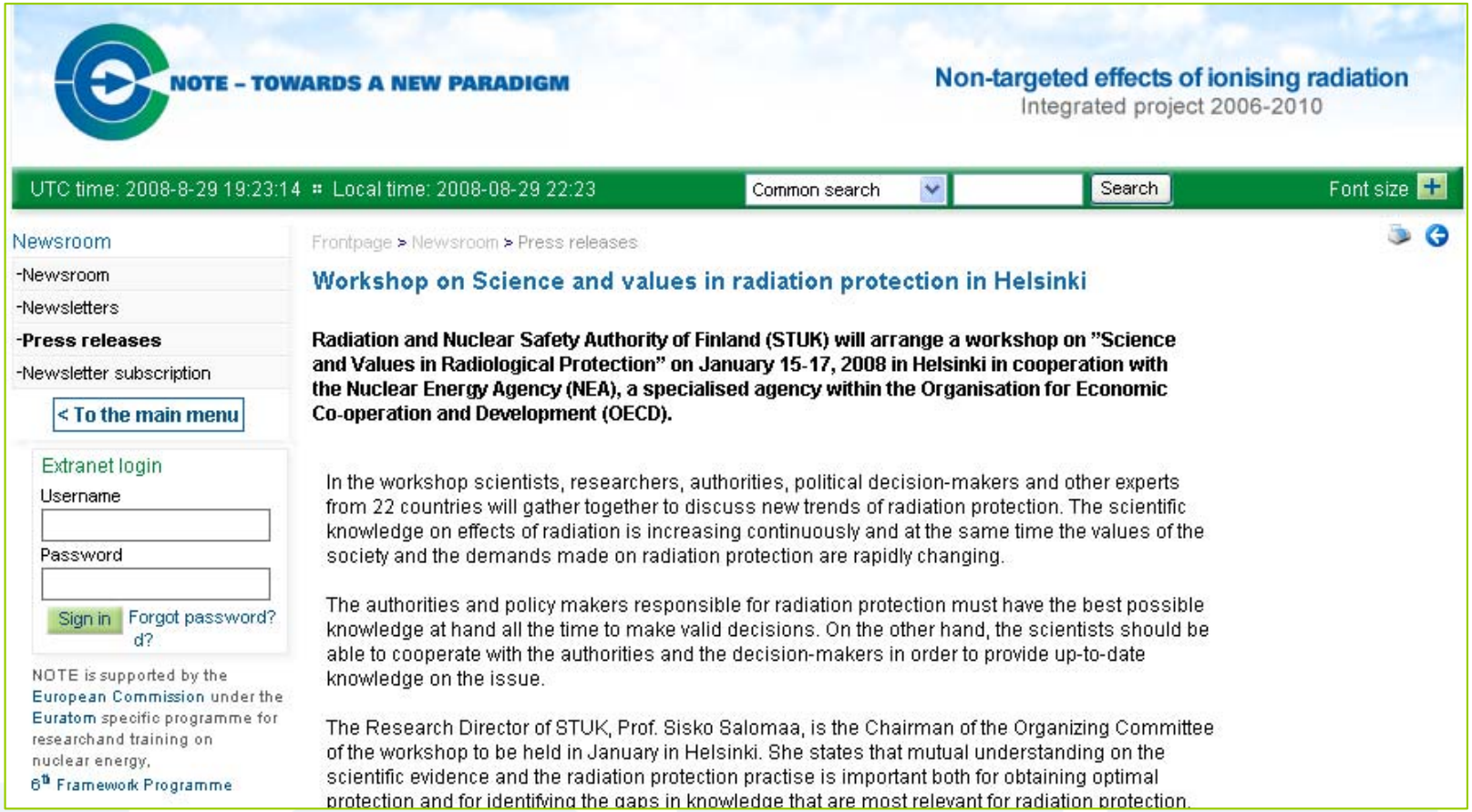
1st Annual review of the NOTE Integrated Project took place 20 November 2007 in Brussels, Belgium. According to Dr. George-Neale Kelly, EC project officer: "The review process went very well and there was a broad consensus that the project is proceeding extremely well".

In the review process, the Commission was assisted by following independent experts: Prof. William H. Morgan, University of Maryland, USA; Prof. Dudley Goodhead, MRC Medical Research Council, UK and Dr. Wolfgang Weiss; Federal Office for Radiation Protection, Germany.

From the NOTE side Management Board

Next newsletters: months 26, 30, 36 and 42 during DIP3.

NOTE press releases



44 papers published/accepted in 2006-2008

NOTE – TOWARDS A NEW PARADIGM

Non-targeted effects of ionising radiation
Integrated project 2006-2010

UTC time: 2008-8-29 18:22:45 :: Local time: 2008-08-29 21:22 | Common search | Search | Font size

Papers published

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NOTE is supported by the European Commission under the Euratom specific programme for researchand training on nuclear energy, 6th Framework Programme

Frontpage > Papers published

Papers published

Research peer reviewed publications

1 Ryan, L.A., Smith, R.W., Seymour, C.B. and Mothersill, C.E. (2008) Dilution of irradiated cell conditioned medium and the bystander effect. *Radiat Res*, **169**:2, 188-96. **Ref. No 45**. *Without NOTE acknowledgment.* Link to abstract

2 Ryan, L.A., Seymour, C.B., O'Neill-Mehlenbacher, A. and Mothersill, C.E. (2008) Radiation-induced adaptive response in fish cell lines. *J Environ Radioact*, **99**:4, 739-47. **Ref. No 43**. *Without NOTE acknowledgment.* Link to abstract (Medline).

3 Gow, M.D., Seymour, C.B., Byun, S.H. and Mothersill, C.E. (2008) Effect of dose rate on the radiation-induced bystander response. *Phys Med Biol*, **53**:1, 119-32. **Ref. No 76**. *Without NOTE acknowledgement.* Link to abstract (Medline).

4 Friedland, W., Paretzke, H.G., Ballarini, F., Ottolenghi, A., Kreth, G. and Cremer, C. (2008) First steps towards systems radiation biology studies concerned with DNA and chromosome structure within living cells. *Radiat Environ Biophys*, 47:1, 49-61. **Ref. No 4**. Link to abstract (Medline).

5 Shao, C., Folkard, M. and Prise, K.M. (2008) Role of TGF-beta1 and nitric oxide in the bystander response of irradiated glioma cells. *Oncogene*, **27**:4, 434-40. **Ref. No 25**. Link to abstract (Medline)

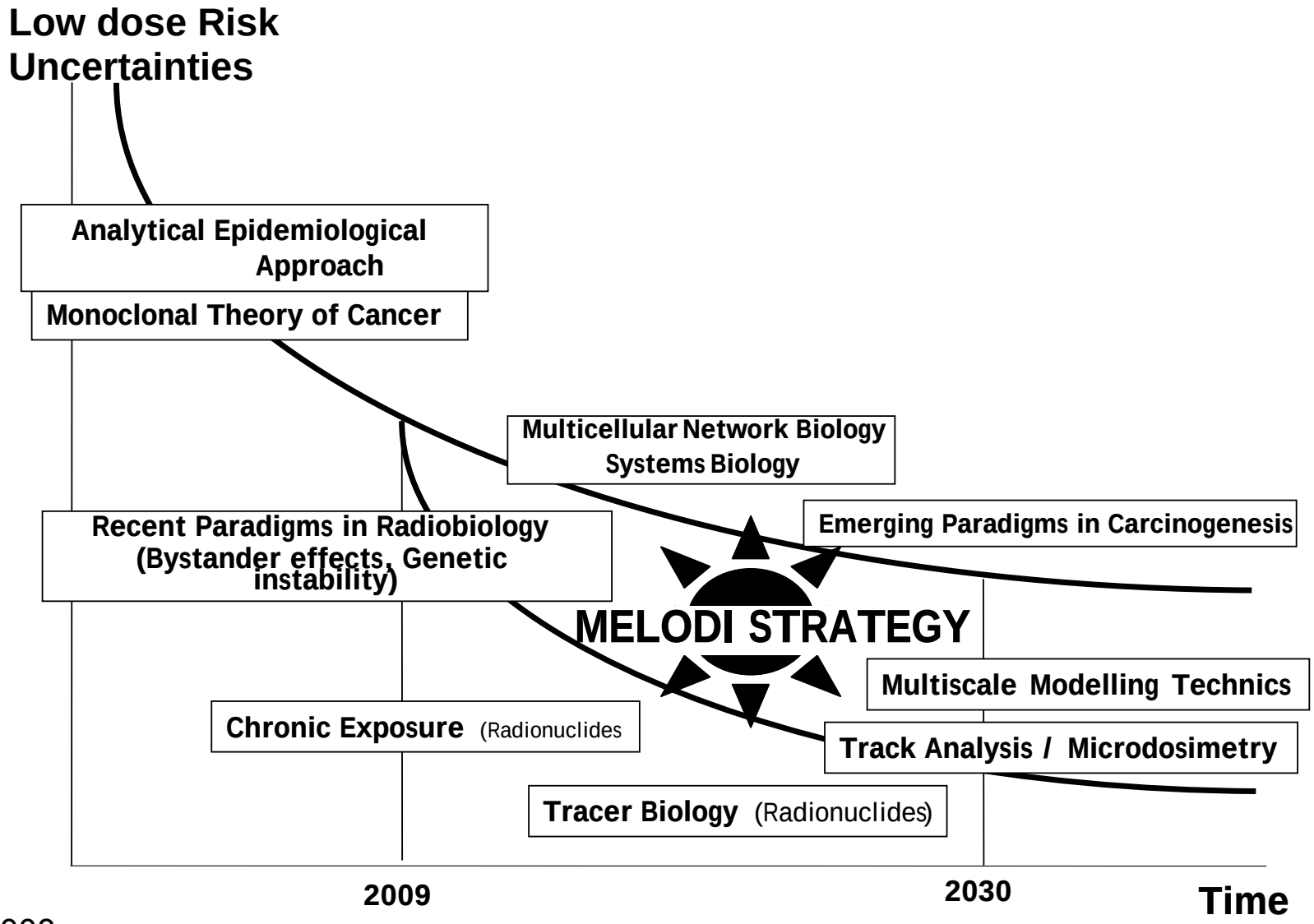
6 Little, M.P., Heidenreich, W.F., Moolgavkar, S.H., Schollnberger, H. and Thomas, D.C. (2008) Systems biological and mechanistic modelling of radiation-induced cancer. *Radiat Environ Biophys*, **47**:1, 39-47. **Ref. No 5**. Link to abstract (Medline).

8. Beyond the NOTE: the MELODI initiative

"High Level and Expert Group" (HLEG) on European Low Dose Risk Research

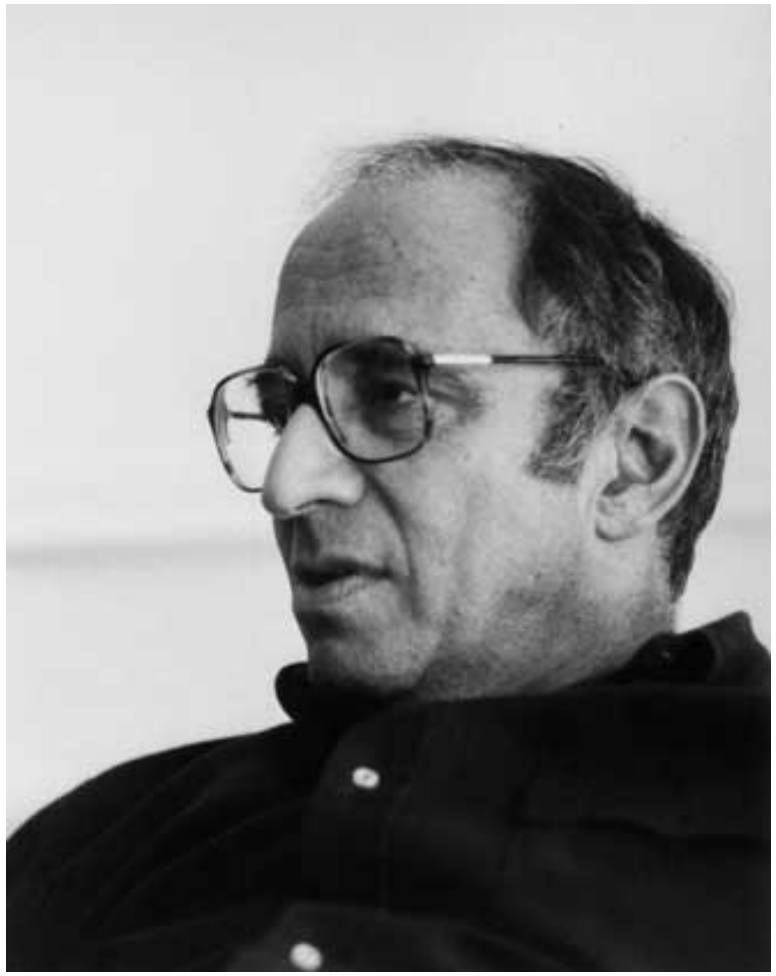
- Formulate and agree the policy goals to be addressed.
- Develop a strategic research agenda and road map.
- Specify elements of and next steps for establishing a sustainable operational framework for low dose risk research in Europe
- Draft HLEG report is open for consultation till 30 November 2008 (http://www.hleg.de).
- Final report will be published in January 2009 taking account of comments.
- The next step would be establishment of governance structure and detailed Strategic Research Agenda (SRA) and the road map.

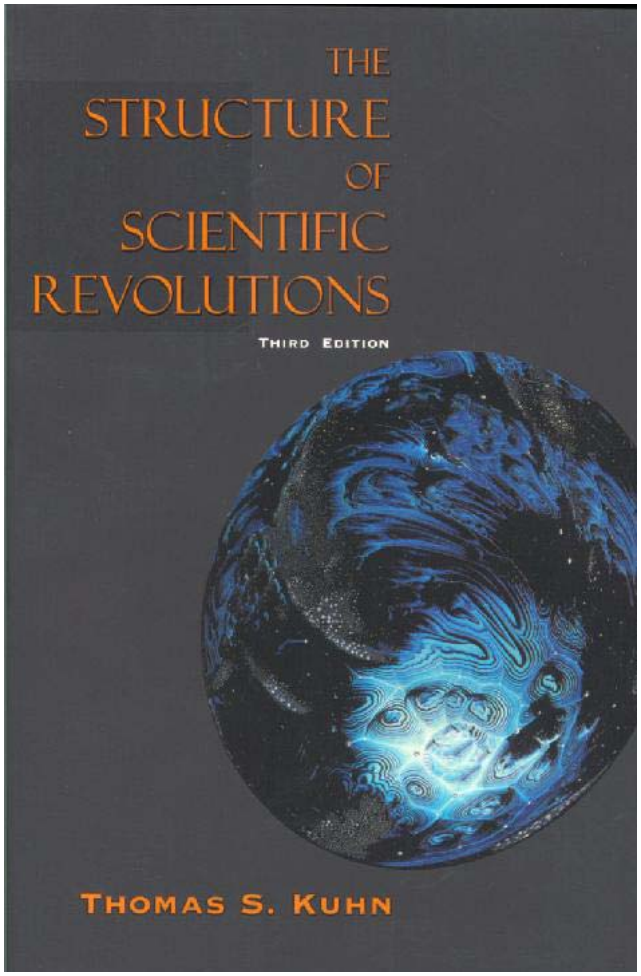
Multidisciplinary European LOw Dose Initiative (MELODI)



9. Change of radiobiological, risk and radiation protection paradigms

“Scientific paradigm” and “paradigm shift”



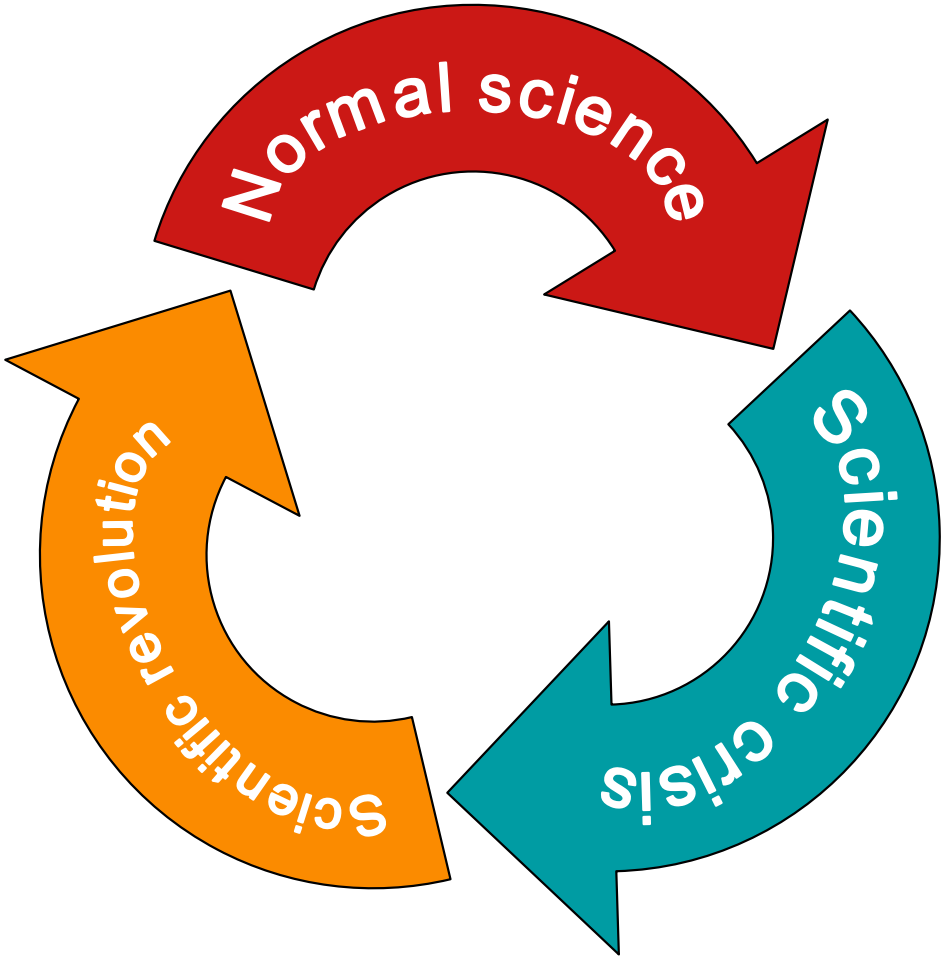


Thomas Samuel Kuhn, 1922-1996 (left); Kuhn, T.S. (1970) The Structure of Scientific Revolutions. Chicago: University of Chicago Press, 1970 (right).

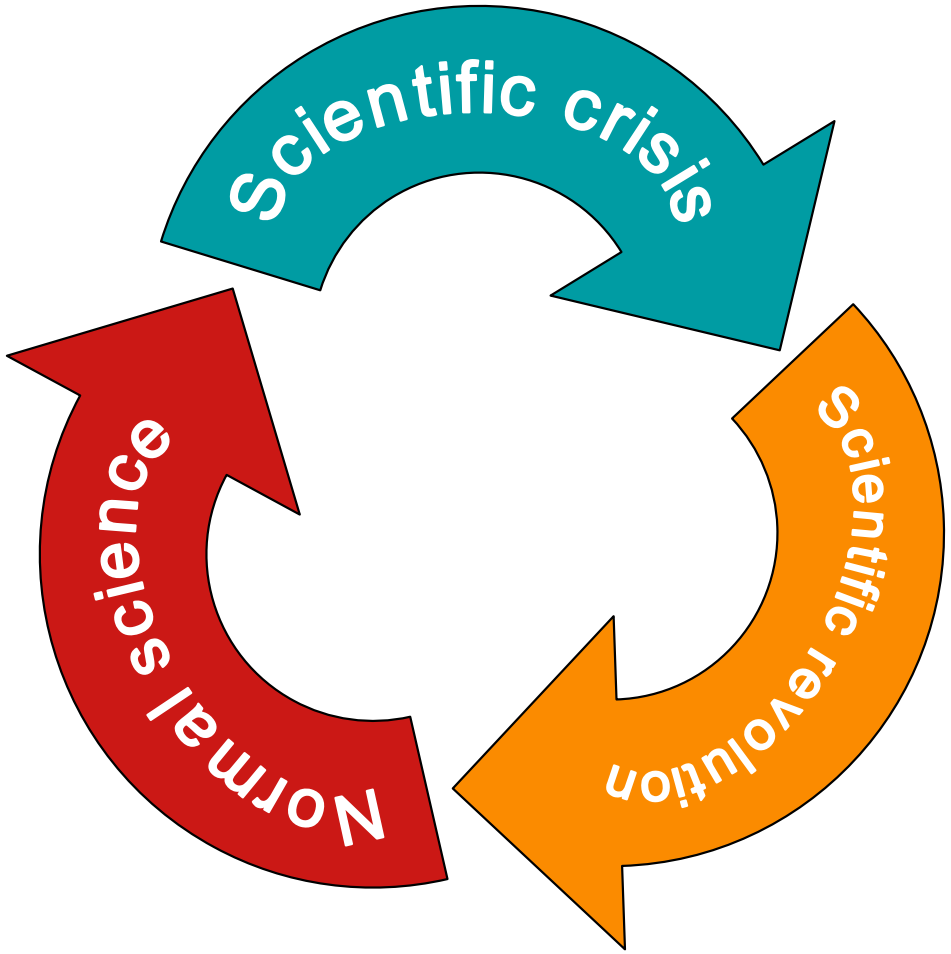
Scientific paradigm

- Kuhn introduced the term paradigm, which he described as essentially a set of basic statements shared by scientists or a set of agreements about how problems are to be understood.
- Paradigms are essential to scientific inquiry.
- A paradigm guides the research efforts of scientific communities, and it is this criterion that most clearly identifies a field as a science.
- The typical developmental pattern of a mature science is the successive transition from one paradigm to another through a process of revolution.

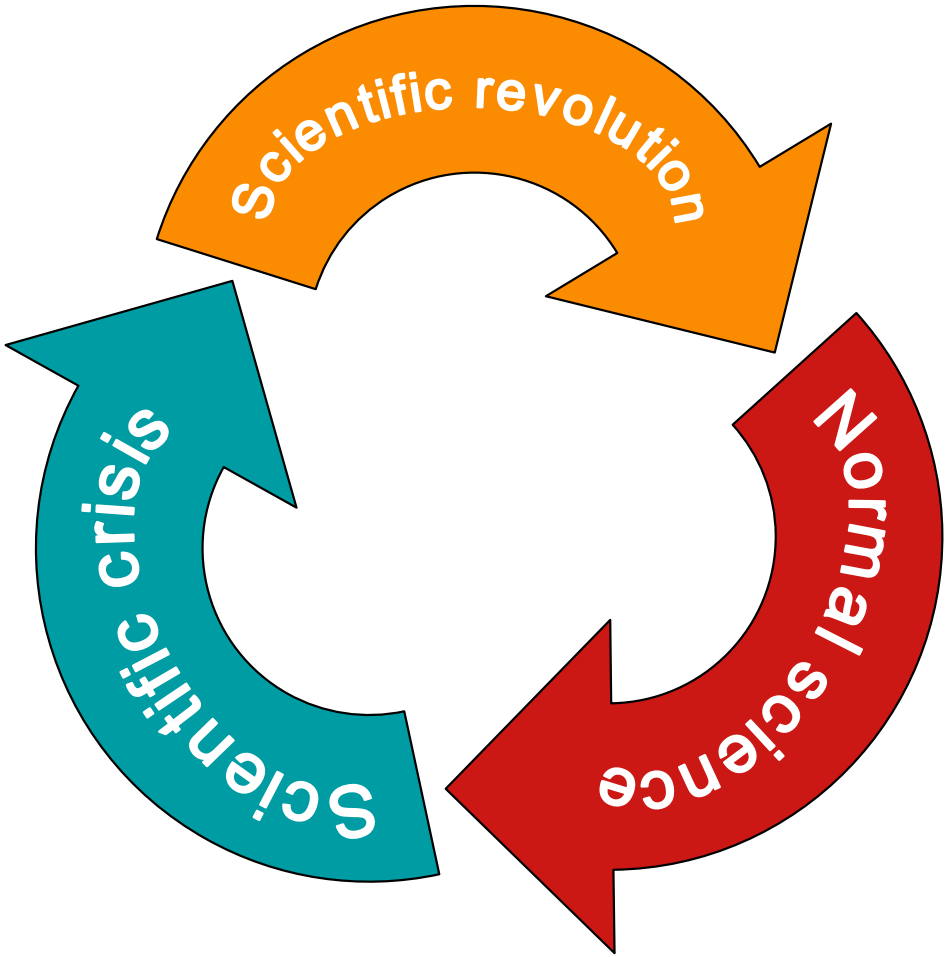
Development of science is cyclic



Development of science is cyclic



Development of science is cyclic



Paradigmatic changes in radiation biology, radiation risk and radiation protection

- This distinction was introduced recently by Prof. Sisko Salomaa in a document, describing NOTE project research strategy.
- There are different paradigms of radiation biology, radiation risk and radiation protection.
- Radiobiological paradigm describes how radiation acts on cells and tissues, it centers on phenomenology and mechanisms.
- Risk paradigm is connected with of qualitative and quantitative estimation of radiation induced health effects, its based mainly on epidemiological evidence.
- Radiation protection paradigm is a pragmatic system for protection of public and environment from harmful effects exposure to ionising radiation, its based not only on science but on values as well.

10. Conclusions and acknowledgements

Conclusions

- The current system of radiation protection is robust and protect people well from deterministic and stochastic effects of ionising radiation.
- However, recent discovery of non-targeted effects of ionising radiation indicates that the current radiation protection might be too conservative.
- Linear-Non-Threshold (LNT) model is challenged by non-targeted effects of ionising radiation.
- Health risks associated with non-targeted effects seems to be non-linear.
- Non-targeted effects is constituted paradigm shift in radiation biology, however, respective changes in risk and radiation protection paradigms might take future 20-30 years.
- For that more specific targeted research will be required.

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