Eye lens exposure to IR: current understanding, radiobiology and dose limits

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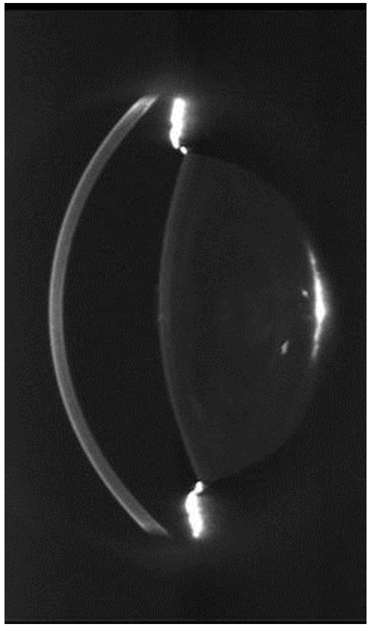
Firenze, January 30th

This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287.

Background

Cataracts are the most frequent cause of blindness worldwide



Courtesy of N. Kleiman

http://vision.ucsf.edu/hortonlab/ResearchProgram%20Pics/kid%20with%20cataract.jpg

https://webeye.ophth.uiowa.edu/eyeforum/atlas/pages/Posterior-subcapsular-cataract-2.html

Multifactorial aetiology: Age related effect; Genetic component (congenital cataracts); Also: Sunlight, alcohol intake, nicotine consumption, diabetes, persistent use of corticosteroids, and *ionising radiation*…

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Radiation protection

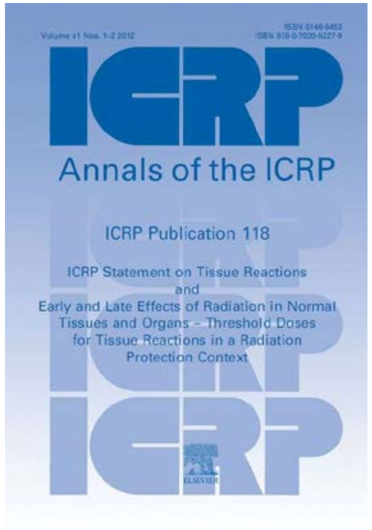
ICRP defines:

- **Deterministic or tissue effects**

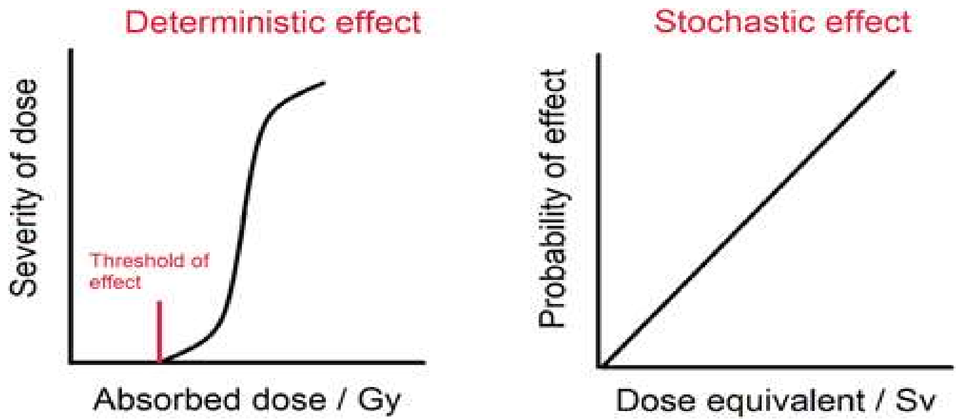
  Those for which there is a defined threshold below which the effect does not occur; severity of effect increases with dose

- **Stochastic effects**

  Those for which there is no threshold, risk (but not severity) increases with dose



http://www.icrp.org/images/P118.JPG



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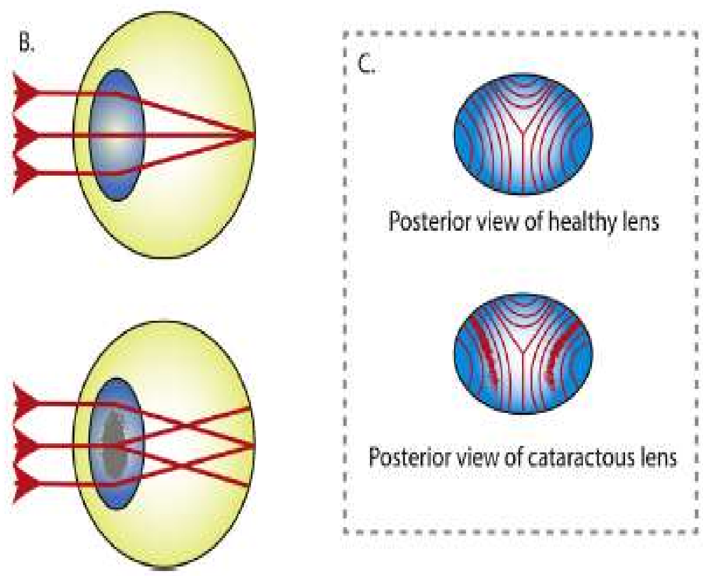
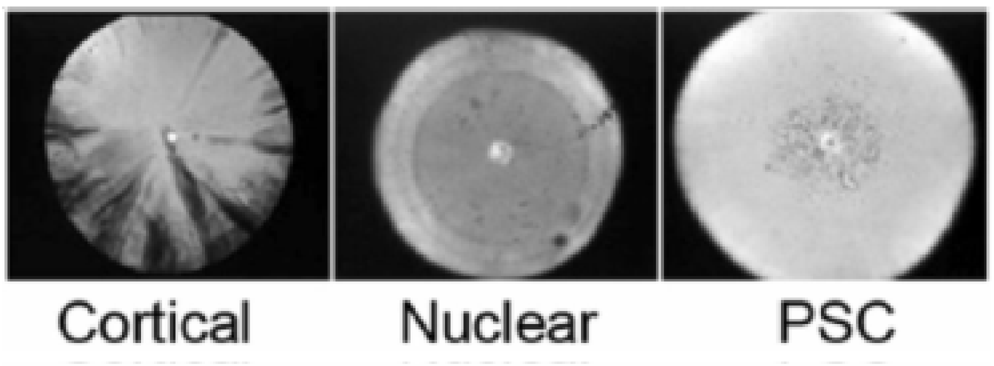
Radiation cataracts

Ionizing radiation is generally (but not exclusively) associated with posterior sub-capsular opacities





Adapted from Beebe , 2008

This and other images without references throughout: Ainsbury *et al.,* 2016

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Lens protection

- **Paradigm:** Radiation cataract is a deterministic, late, effect
- **Recent epidemiological (re)analyses:** The lens is more radiosensitive than previously thought
- ICRP, 2012; EU BSS, 2014:
  - Threshold for radiation cataract ~ 0.5 Gy
  - Occupational lens dose limit 20 mSv y^{-1} (averaged over 5 years, with no single yearly exposure exceeding 50 mSv)
- **But:** This is based on epidemiological data…

http://www.icrp.org/images/P118.JPG

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How does ionising radiation (at low doses) influence cataract development?

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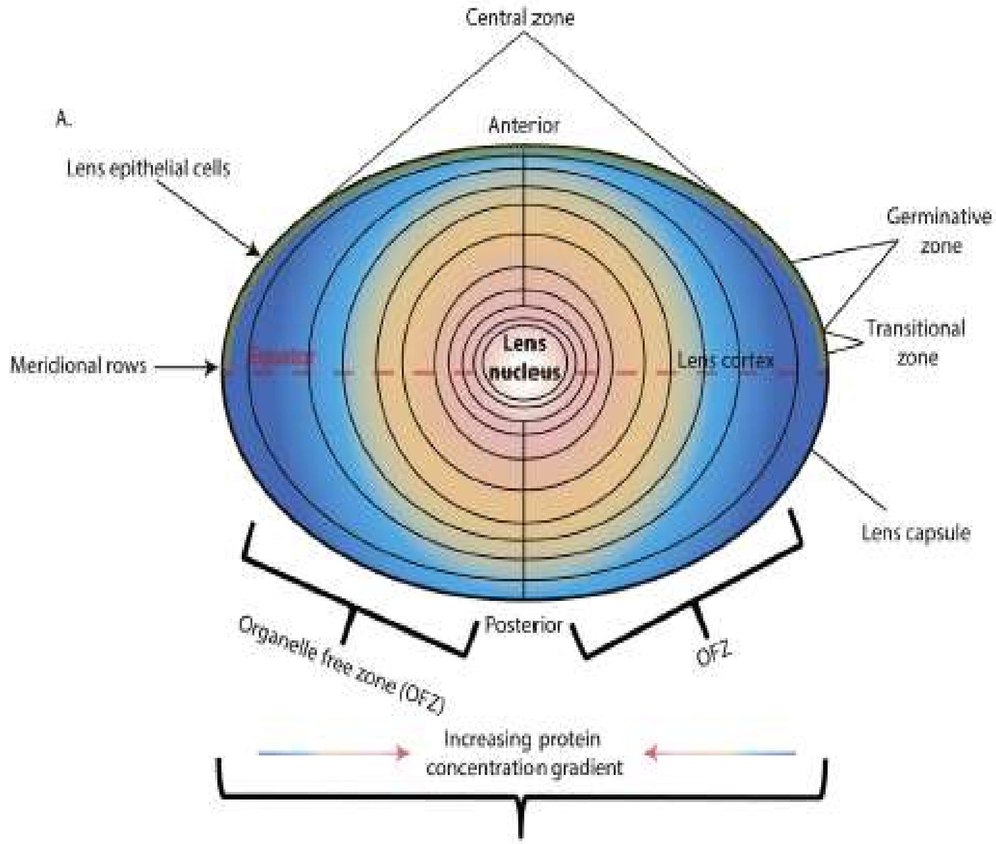
Human Lens

- Diameter ~9-10 mm, thickness ~4.5 mm
- Germinative zone of LEC
- Growth factors

  -> differentiation

  -> lens fibres
- Tight temporal and spatial organisation
- Deregulation -> cataracts



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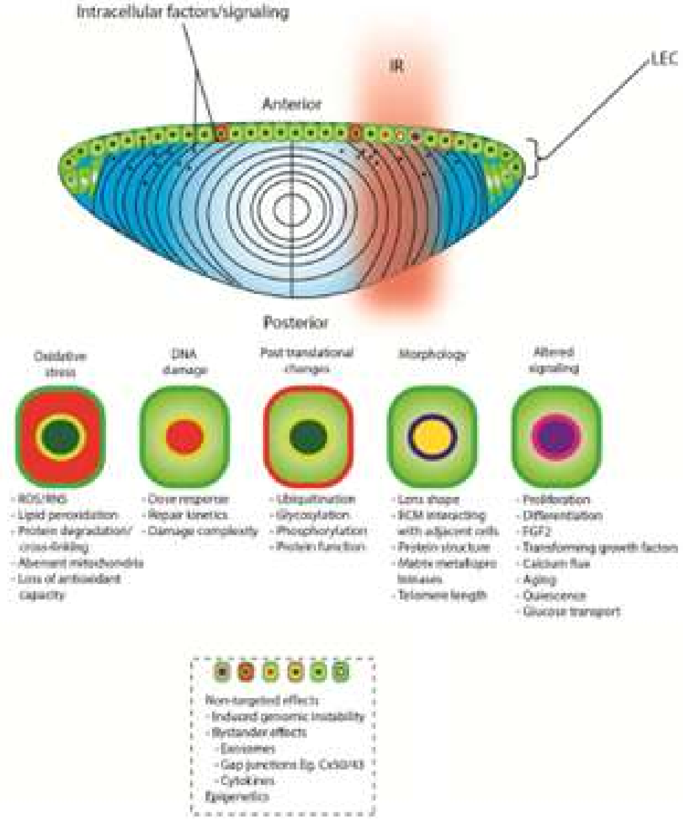
Current mechanistic understanding

Target cells: Germinative Zone on lens epithelium + …

Potential mechanisms might include:

- Oxidative stress
- DNA Damage/Repair/Mis-repair
- Intracellular signalling
- Gene expression
- Cellular proliferation / mobility / migration
- Damage to proteins/ECM/lipids
- Post translational modifications
- Senescence
- Systemic/Non-targeted effects …



Modifying factors: Dose, Dose rate,
Age at exposure, Genetic background …

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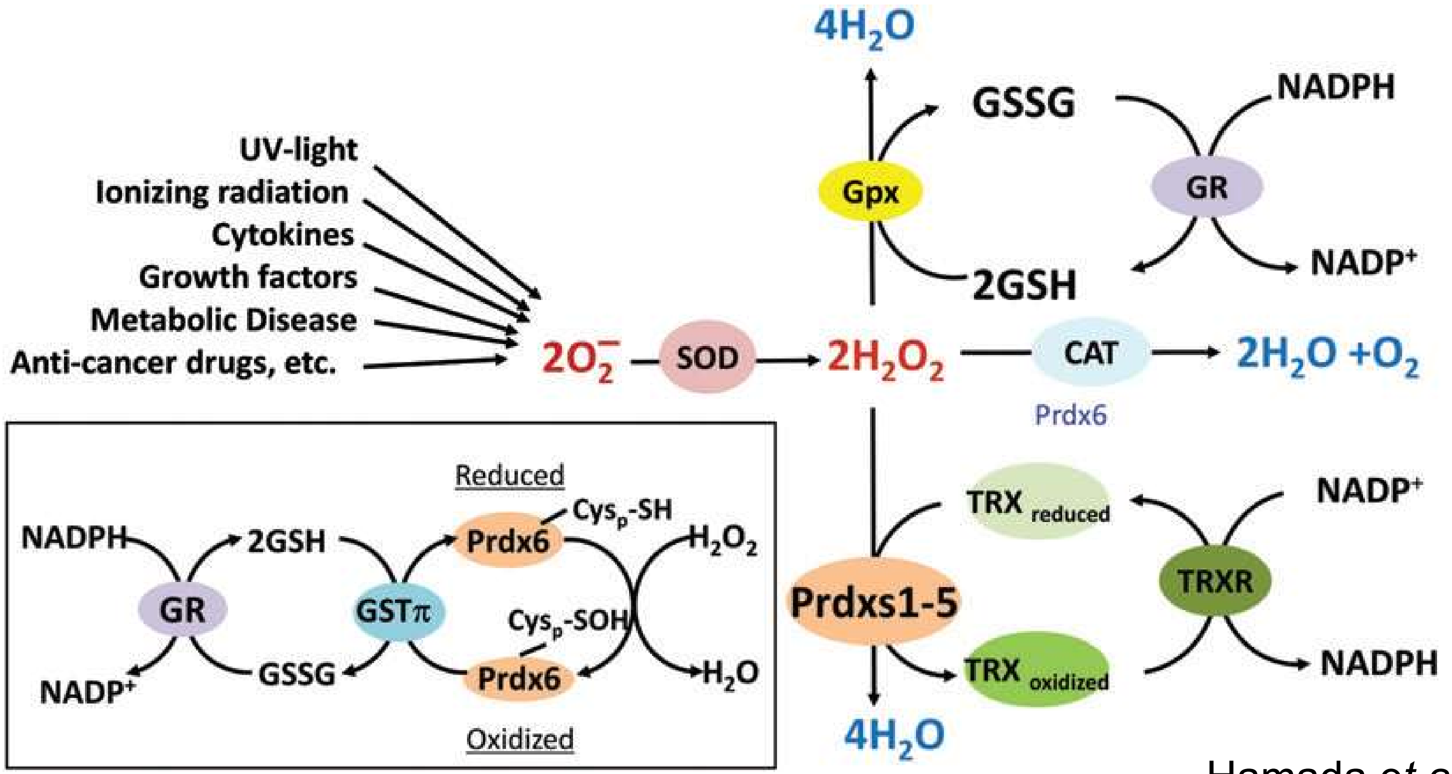
Oxidation

ROS: Degradation, cross-linking, aggregation of lens proteins, DNA damage



Hamada *et al.*, 2014

-> Aberrant lens epithelial cell division, cell migration, differentiation…

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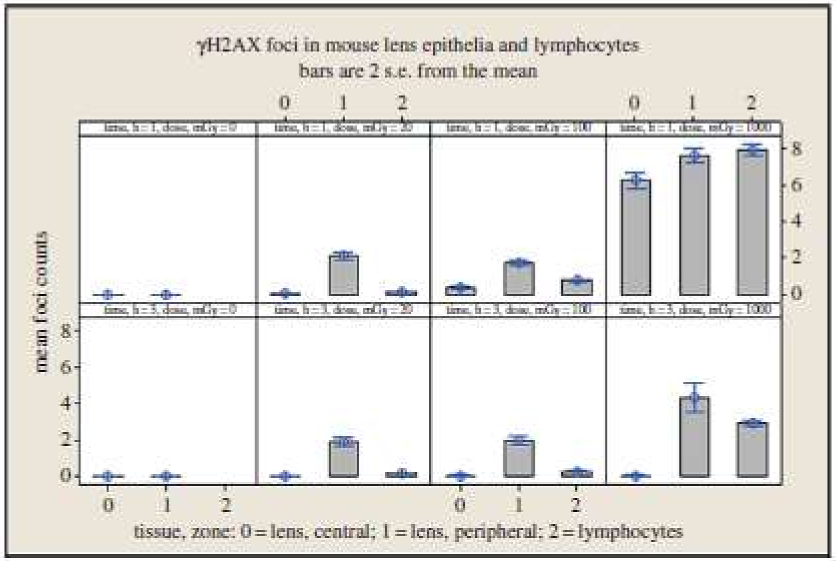
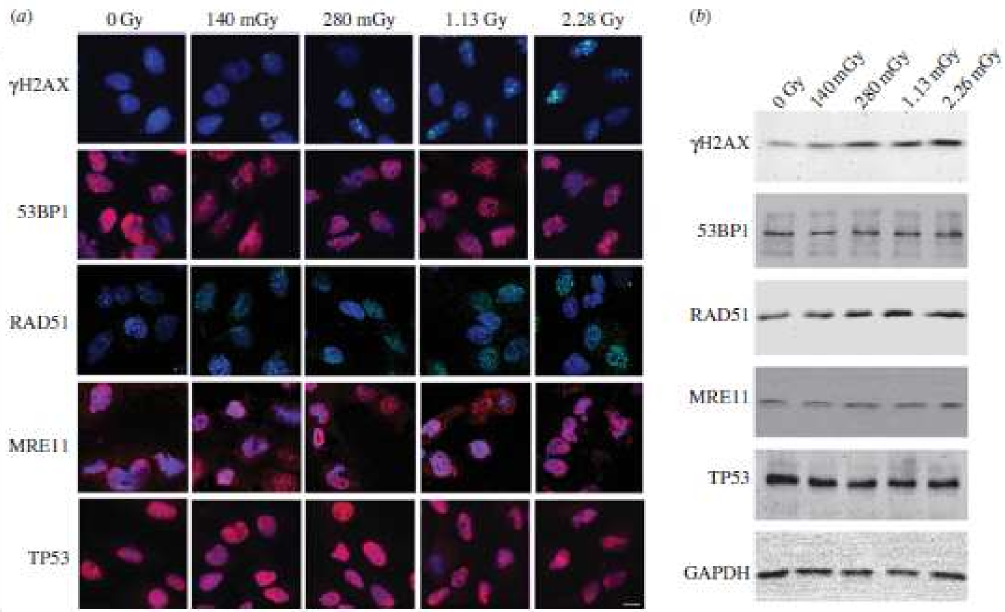
Evidence from a study looking at DNA damage and repair

<u>Markiewicz *et al.*, 2015:</u>

- Low dose, dose-response for DNA damage response in the lens
- Lens is more sensitive than circulating lymphocytes





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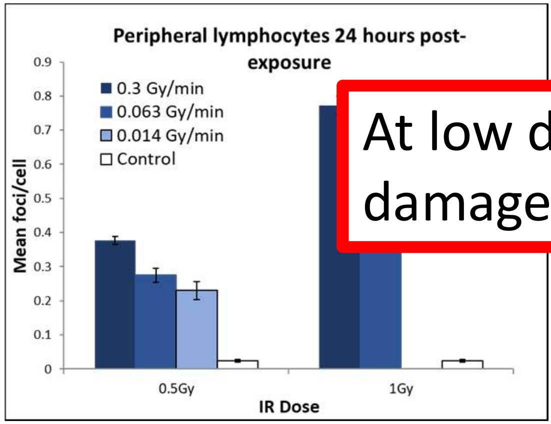
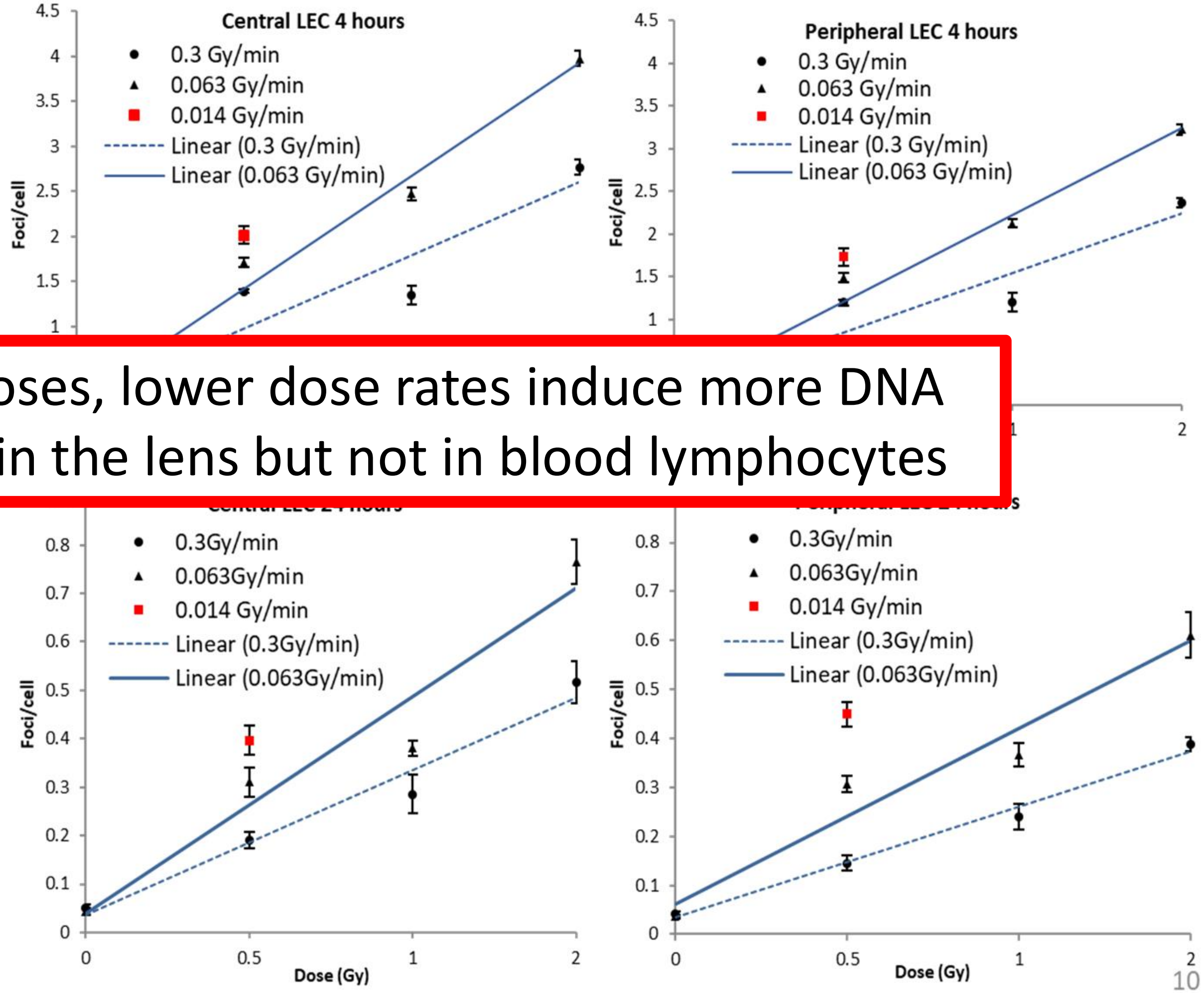
An inverse dose rate effect for DNA damage

Barnard *et al.*, 2018:





At low doses, lower dose rates induce more DNA damage in the lens but not in blood lymphocytes

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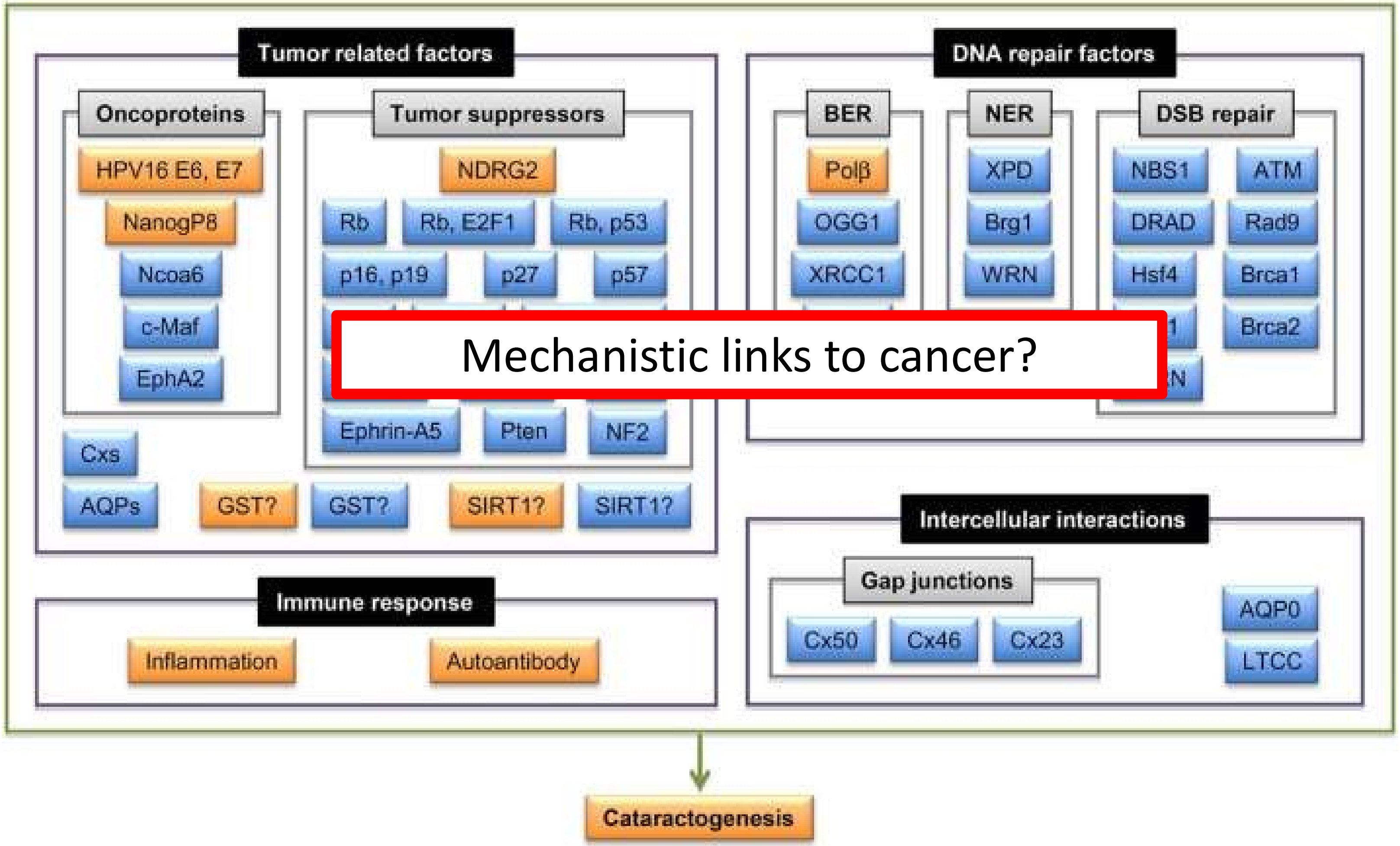
Signalling: Tumour related factors



Hamada and Fujimichi, 2015

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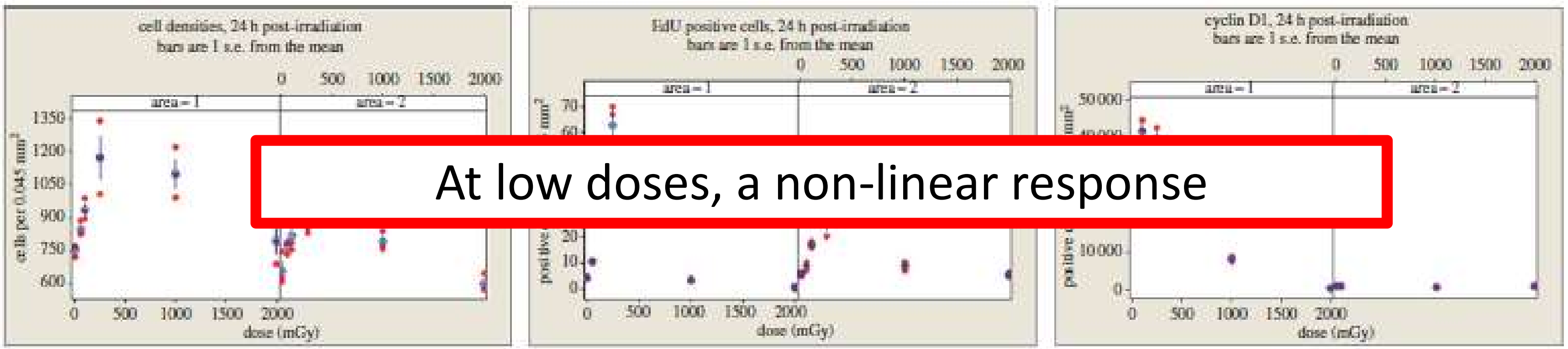
Stimulation of proliferation

<u>Fujimichi and Hamada, 2015:</u> “IR not only inactivates clonogenic potential but also stimulates proliferation of surviving unactivated clonogenic HLE cells”

IR -> abnormal activity

Historical data: Irradiation induces excessive proliferation of rabbit lens epithelial cells; suppression of lens epithelial cell divisions inhibits radiation cataractogenesis in frogs and rats

<u>Markiewicz *et al.* 2014:</u>



At low doses, a non-linear response

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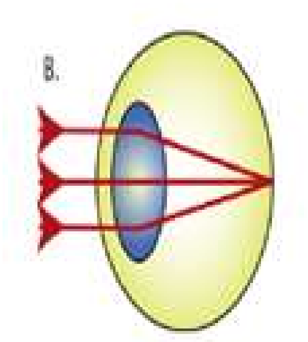
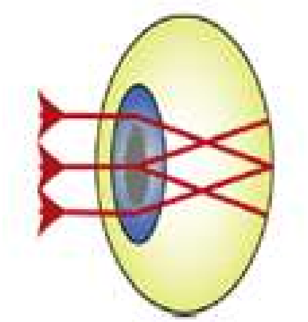
Protein modification





Fujii *et al.*, 2001: Role of post translational modifications? May reduce solubility to alter transparency

Bloemendal *et al*, 2004: Lens crystallins: α-,β- and γ-, form the refractive medium of the lens; proteins e.g. αA- or αB- protect from aggregation

Muranov *et al.*, 2010: Protein changes in irradiated lenses similar to those seen in old age

Wiley *et al.*, 2011: Role of abnormal cellular proliferation, e.g. p53 effect?

New data: Dose, dose rate and exposed region all impact cellular proliferation and morphology…

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Some genetics data

Mouse models: ATM, RAD9, BRAC1 genes control signalling for DNA damage response signalling; Heterozygosity of these genes known to leads to increased risk of cancers

Worgul *et al.*, 2002:
- Cataracts earliest in homozygotes for Atm, then heterozygotes, then wildtyp
- Severit ls attempting differentiation
- Atm homozygotes/heterozygotes – genetic predisposition to cataracts

Genetic background matters!

Kleiman *et al*., 2007: Cataracts develop earlier and in greater numbers in Atm/Rad9 double heterozygotes

Smilenov *et al.*, 2008: Atm/Rad9/Brca1 double heterozygotes showed increased resistance to apoptosis and increased radiation sensitivity

Humans: e.g. Cataractogenic mutations in human crystallin genes

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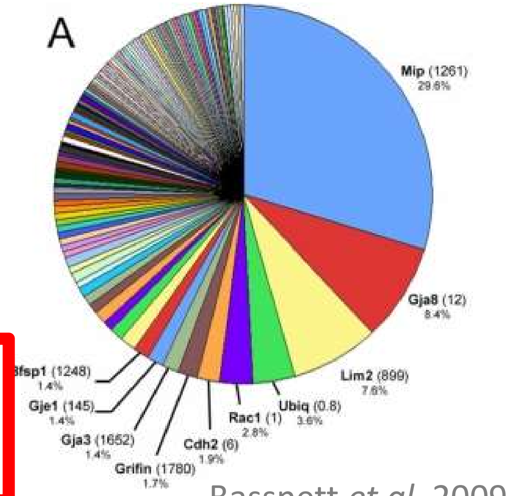
New results: Proteomics and lipidomics – new techniques

E.g. Oxidative stress:

- Proteins: post-translational modifications → aberrant aggregation
- Lipids: lipid peroxidation → elevated hydroperoxides and oxy-derivatives → modifications in lipid-lipid and protein-lipid interactions

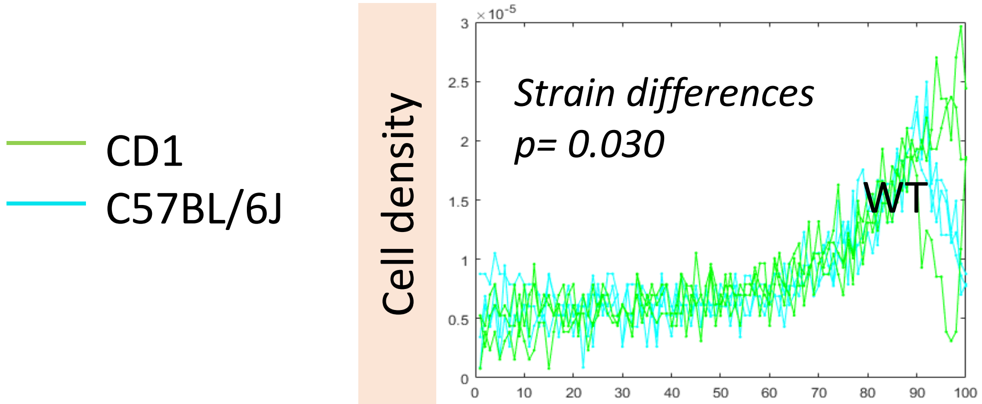
- IR impacts lipid formation
- Genetics influences the impact

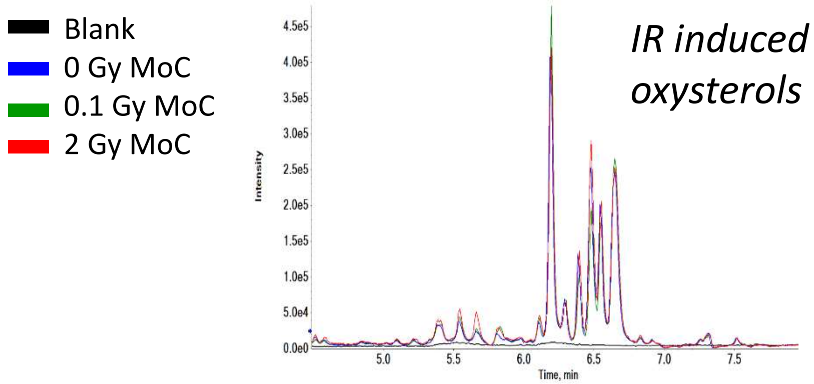


Bassnett *et al.* 2009

Uwineza PhD hypot

Accelerated aging: Ionizing radiation leads to oxidation of major proteins and lipids in the LFCs membrane disrupting eye lens homeostasis





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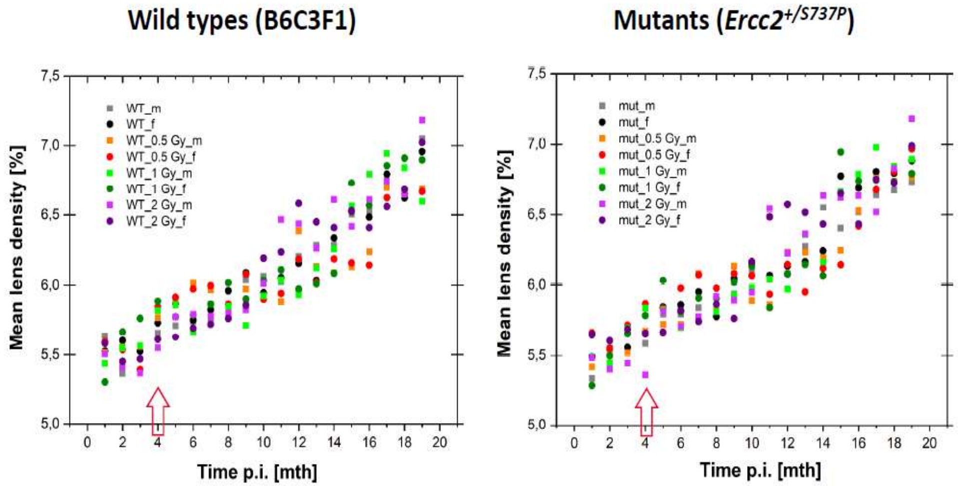
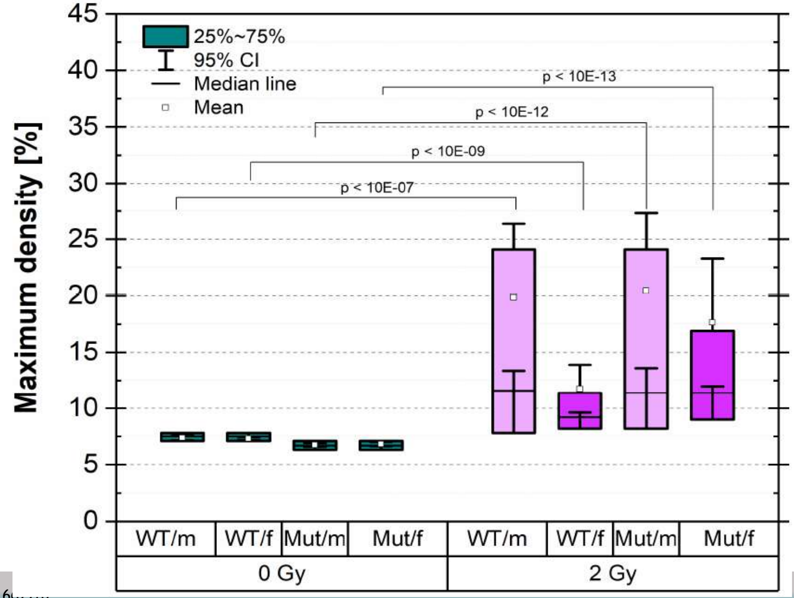
New results: Long term cataractogenesis (1)

Ercc2$^{+/S737P}$ mice:

- Lens density increased with age
- In 10 week old mice, Scheimpflug imaging revealed no radiation-induced or clinically relevant lens opacifications (note: the posterior lens is not fully visible) up to 18 months
- P2 neonates showed a significantly higher incidence of cataracts. About half of the irradiated mice developed a clear cataract with a lens density over 14 %





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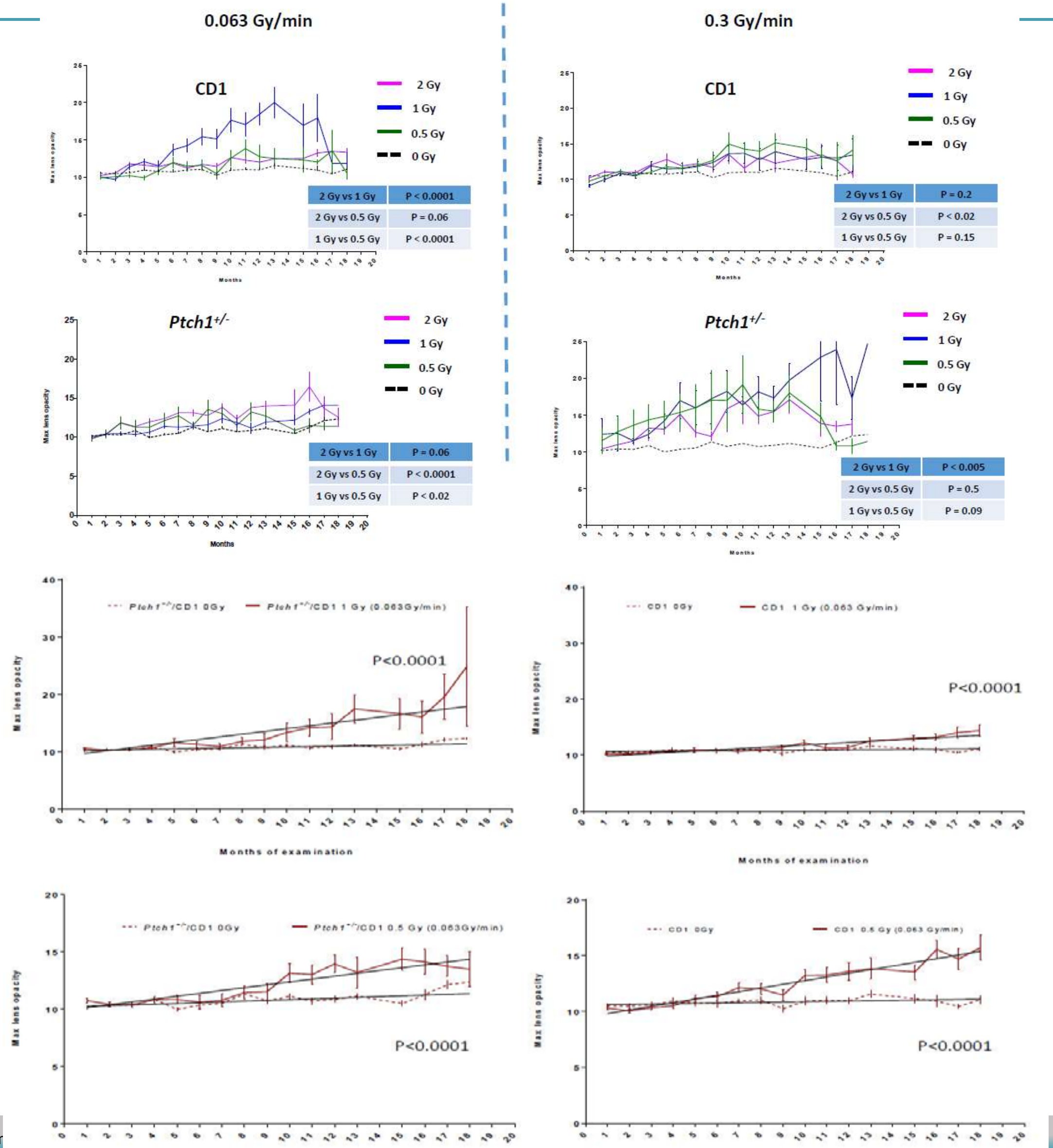
New results: Long term cataractogenesis (2)

Ptch1$^{+/-}$ mice:

- Adult mice: Dose effect was dependent on strain; dose rate effect for Ptch mice only
- P2 irradiated mice showed a clear effect of age at irradiation in accelerating cataractogenesis in *Ptch1*$^{+/-}$ and significantly in WT mice on CD1 background, but not on C57BL/6 background



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LD Lens Rad

Summary of 'imaging' results

- **Significant effects** of **dose** and **dose rate** have been detected in some models
- But, most lens densities at 19 months post exposure were below the LOCS III criterio … man
- **Age** and **geneti** … to cataract risk
- For most mouse … outweighs the effect of radiatio …
- Ptch1$^{+/-}$ P2 irrad… diation is strongly influenced by genetic background, **clear interaction effects for all strains**

- Dose
- Dose rate
- Genetics
- Age
- The interaction of all these factors

… are all important

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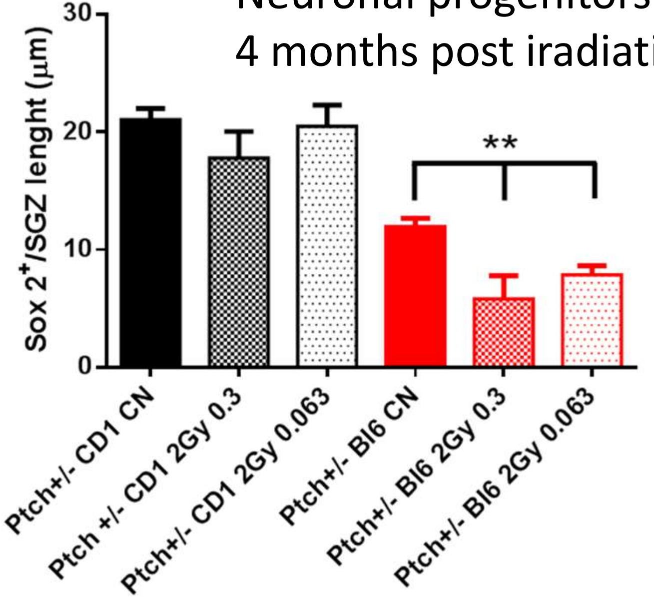
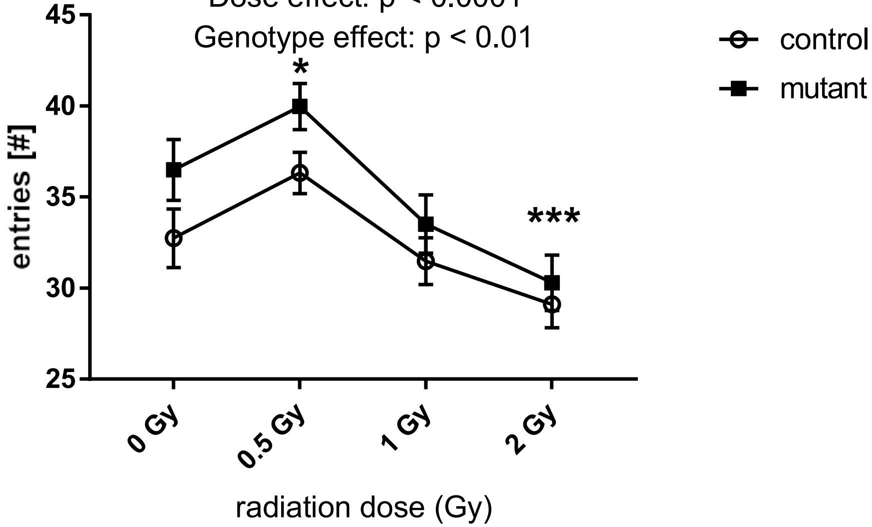
Results: Lens as a global biomarker

Hippocampal neurogenesis and behavioural testing

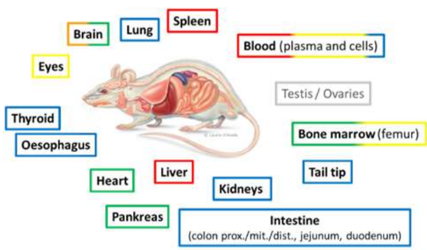
Neuronal progenitors
4 months post iradiation

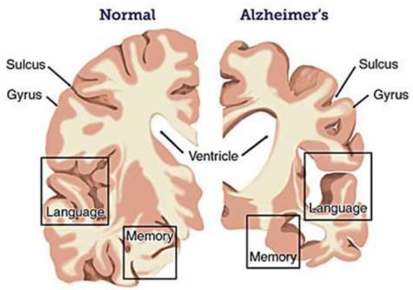


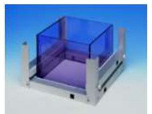
Y-maze entries (8 min)
Dose effect: $p < 0.0001$
Genotype effect: $p < 0.01$



INSTRA*
Dalke *et al.*, 2018







Open field

Prepulse inhibition

Social Discrimination

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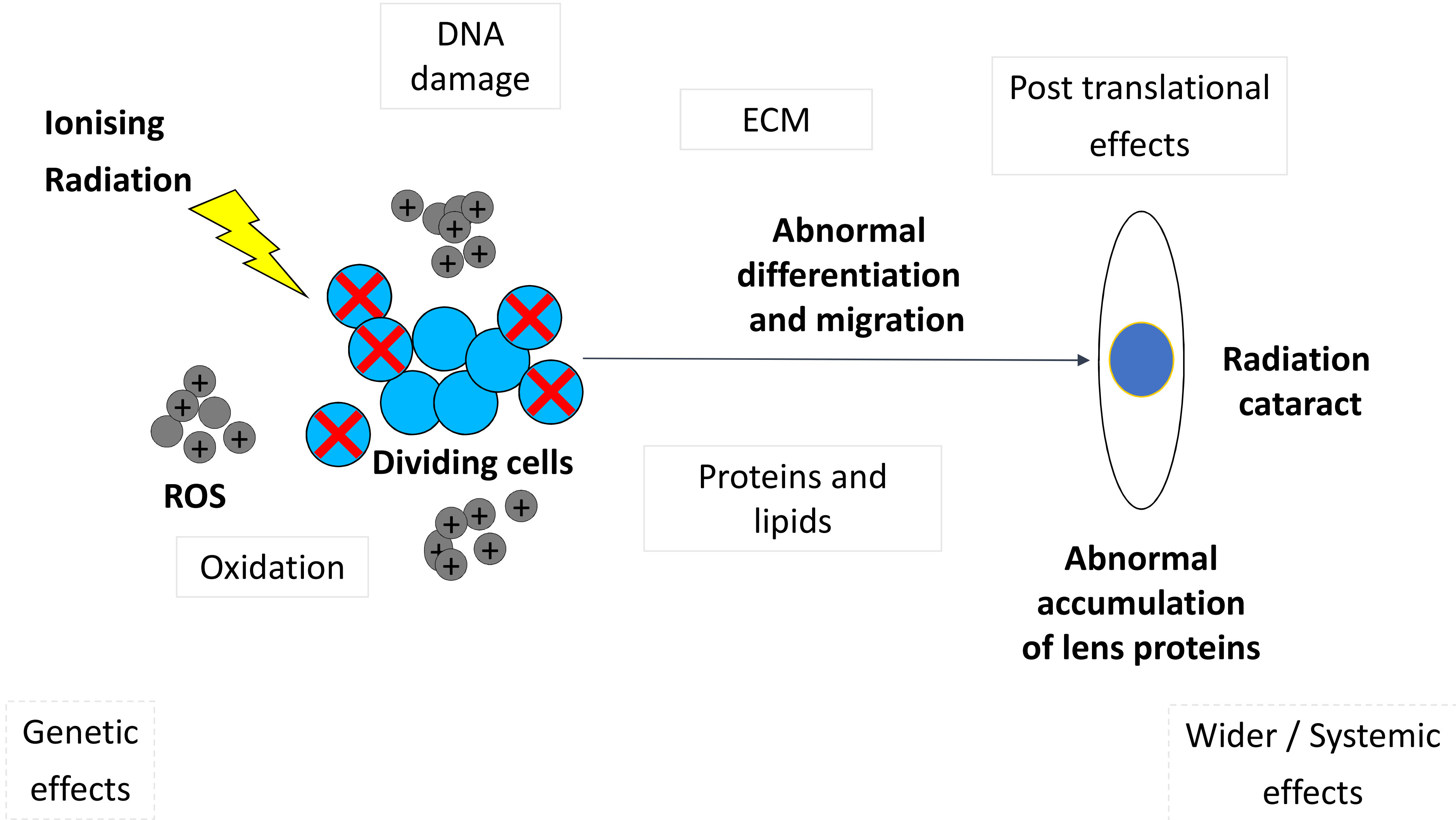
(Very basic) summary of current (incomplete) mechanistic hypothesis



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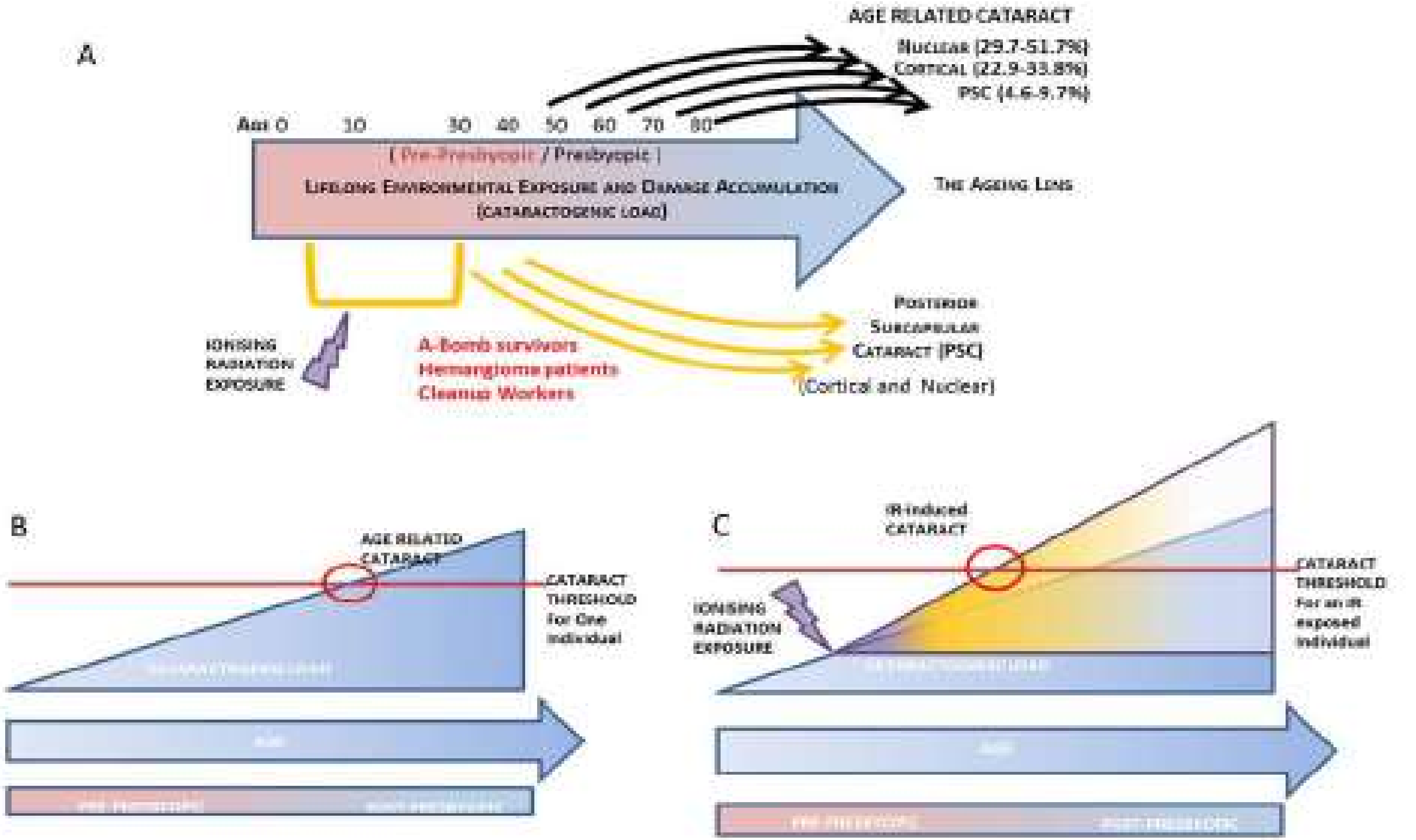
Uwineza et al., 2019: Cataractogenic load



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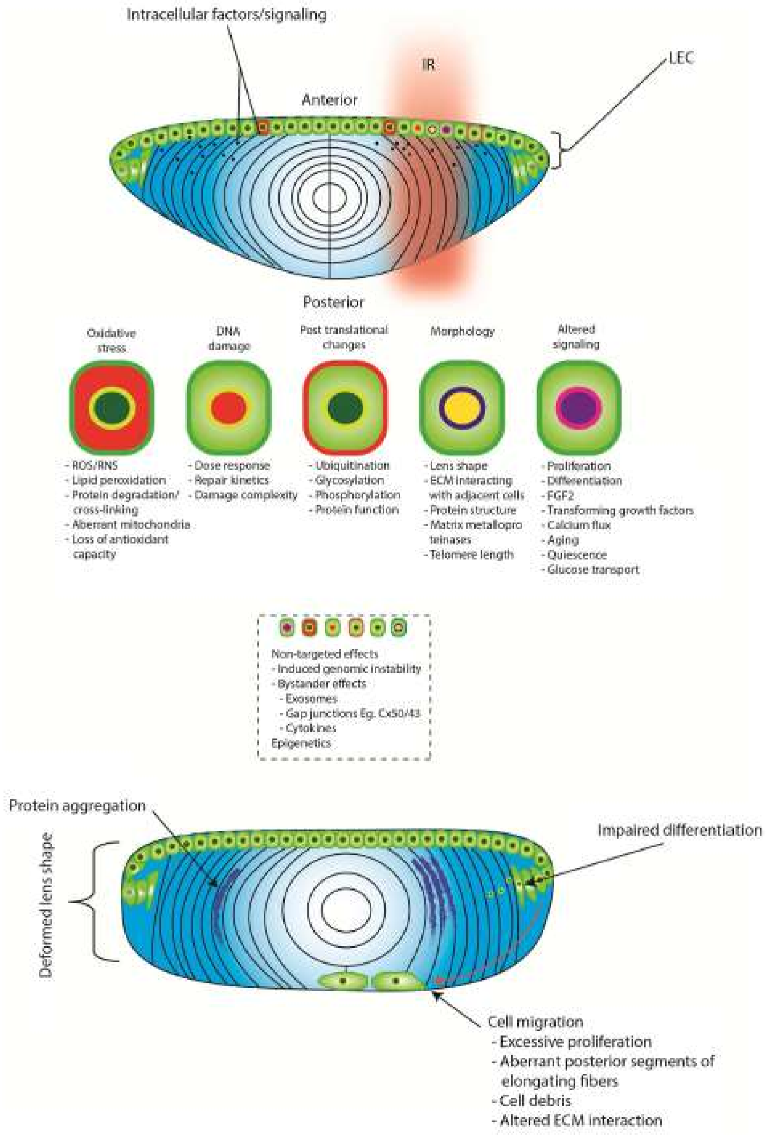
What do we know?

- Understanding of lens biology (structure, physiology, process of fibre cell formation)
- Epidemiology: IR is associated with posterior sub-capsular cataracts…
- High dose response
- Involvement of genomic damage of lens epithelial cells
- Morphological changes: Number of potential competing/parallel mechanisms
- Genetic background
- Age dependence (radiation acceleration)
- Cataract detection/assessment



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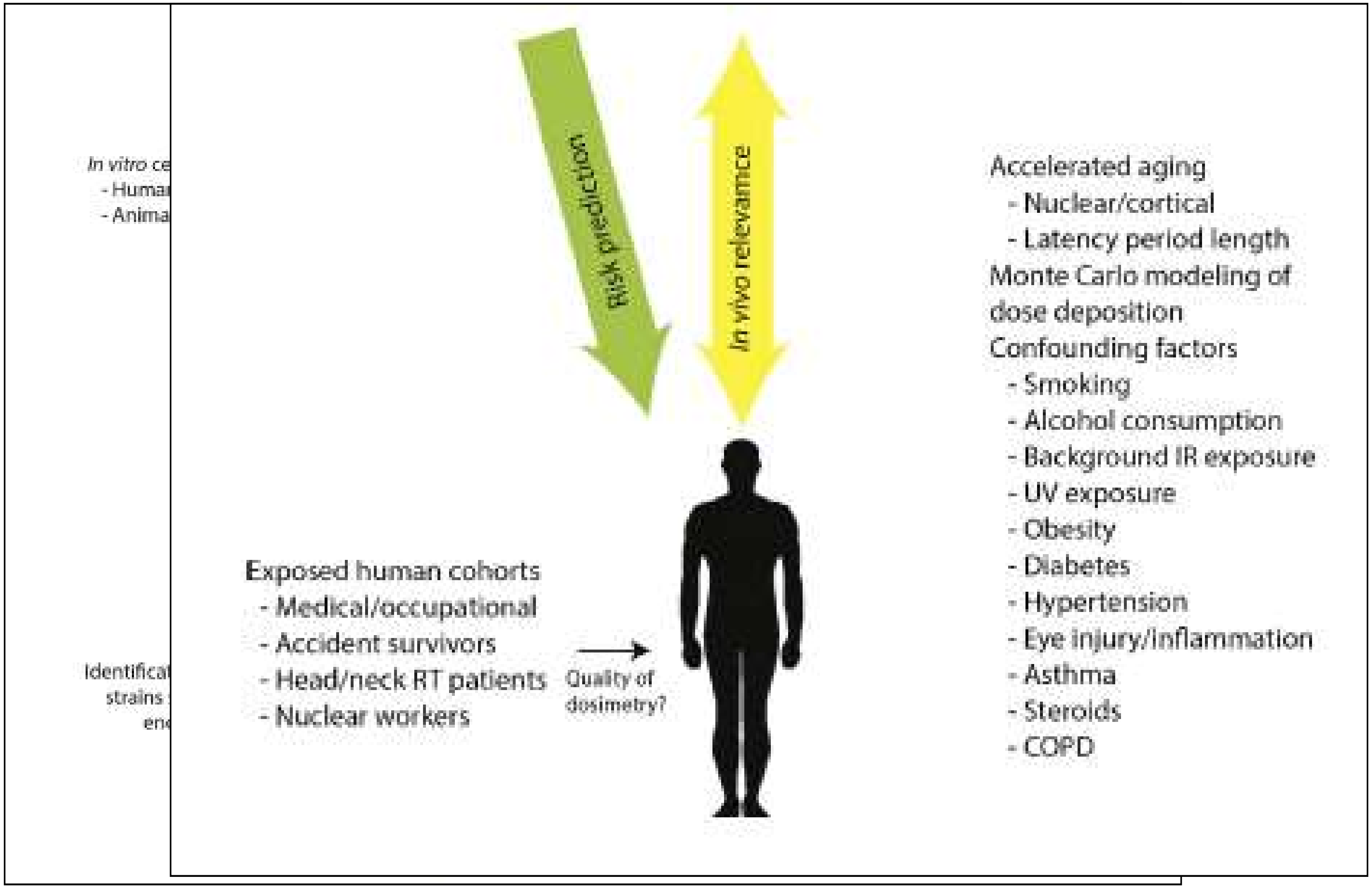
What don’t we know?



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‘Take home’ messages

- Current regulations are based on (mostly) high dose population studies
- The mechanistic link between low dose radiation and cataract is still under investigation (but some excellent studies in progress!)
- ICRP must make pragmatic recommendations to protection radiation workers and the public *in spite of* a lack of complete information
- EU occupational lens dose limit: 20 mSv y^{-1} (averaged over 5 years, with no single yearly exposure exceeding 50 mSv)

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Project partners and AB members

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Thank you for listening!

Questions / comments?

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https://www.researchgate.net/project/LDLensRad-the-European-CONCERT-project-starting-in-2017-Towards-a-full-mechanistic-understanding-of-low-dose-radiation-induced-cataracts

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