Biomarkers of Radiosensitivity.

I am taking biomarkers to encompass:

- Bioassays + genetic markers that can predict radiosensitivity.

+

- bioassays that can monitor radiation exposure.

What do we mean by radiosensitivity

- Main impact of high dose radiation exposure is extensive cell or tissue damage
- For low doses there is unlikely to be extensive cell or tissue damage.
- From chronic exposure or repeat acute exposure can there be accumulative cell or tissue damage.
- What might the sensitive tissues be (eg heart disease)
- Increased risk of carcinogenesis
- Increased ageing – eg senescence or shortened lifespan
- Increased stress response leading to any of the above.

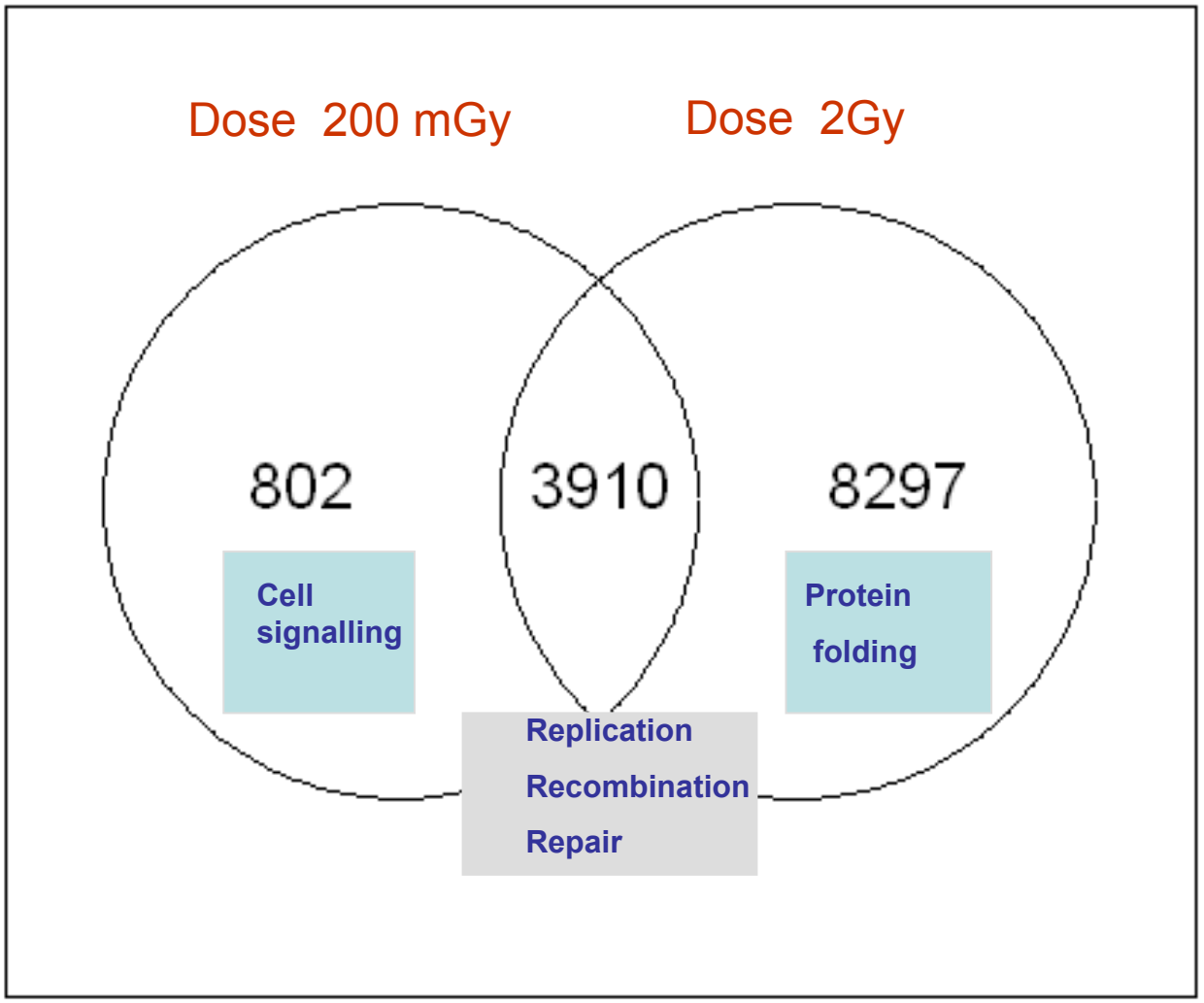
What are the mechanistic processes?

Do they overlap with sensitivity to high dose exposure?

Transcription profiling, proteomics and miRNA analysis suggests that distinct genes/proteins are induced by high versus low doses.

HIGH DOSE AND LOW DOSE TRANSCRIPTOMICS

LYMPHOCYTES OF 106 INDIVIDUALS



Meglicz, Mišovic, Vrieling and Giphart-Gassler, 2010

Total genes 20243

Overlap with high dose exposure?

- Damage response processes likely to be involved in high and low dose exposure.
- ROS production may be significant
- Bystander/signalling – will this be more important
- Will tissue sensitivity remain the same – eg heart – not necessarily
- Cancer/stress/senescence may be more significant.

We should consider processes of significance to high dose exposure plus other processes.

Are individuals sensitive to high doses also sensitive to low dose exposure

- Extreme sensitivity: most individuals fall into the category of defined syndromes (radiation associated syndromes + cancer predisposition syndromes). Diagnosis of these syndromes is the extreme.
- Non-Syndromic individuals with a marked response to radiation exposure – still rare individuals. Known sensitivity to high doses.
- Milder sensitivity – could be relatively common – could be due to multiple subtle changes in genes – or heterozygous individuals

Do any of the above groups have sensitivity to low doses/dose rates

ARE STUDIES WITH HIGH DOSES USEFUL

- Will the response to high dose radiation reflect the response to low dose/dose rate radiation.
- Will the assays developed to predict high dose radiation sensitivity (ie RT) be useful for a consideration of sensitivity to low dose/dose rate radiation?
- Will sensitivity to high doses necessary confer sensitivity to cancer induction or reduce lifespan

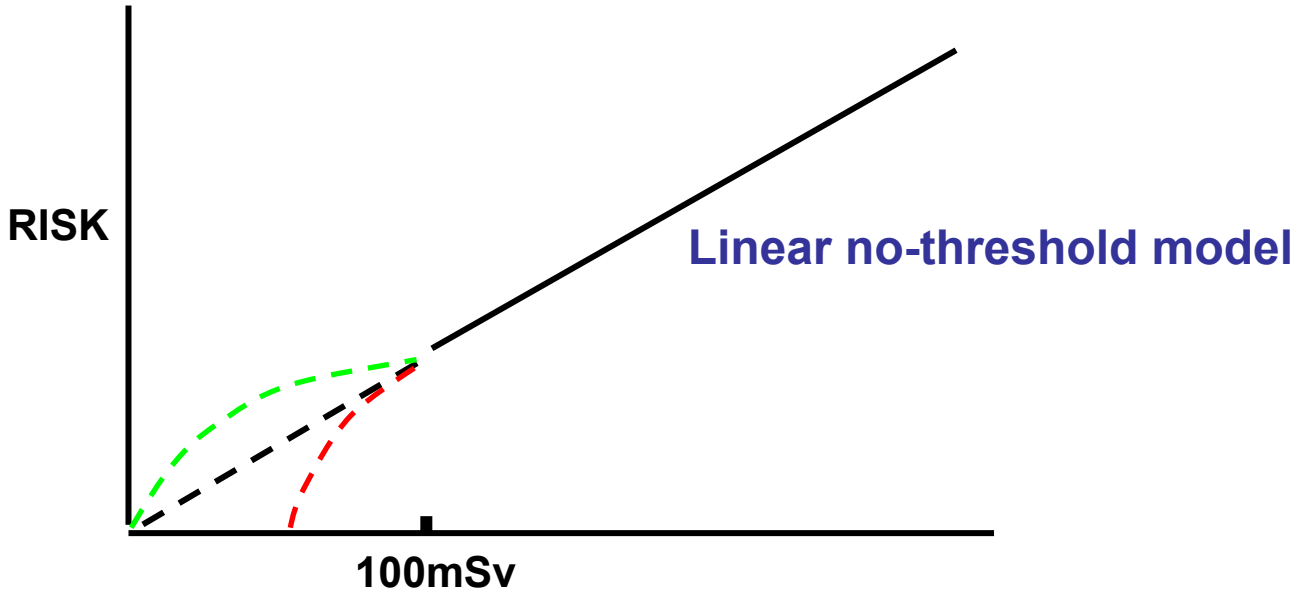
What are the mechanisms/processes : (1)

- DNA damage responses – processes and most genes are known (eg NHEJ proteins, ATM). Do defects in these genes confer sensitivity to low doses/dose rates.
- Impact of low dose effects such as the adaptive response
- Sensitivity to inflammatory responses/cytokine signalling.– processes unclear but gaining understanding . Bystander effect - TGFb
- Carcinogenesis. Have some idea of relevant genes and processes – but uncertainty and few bioassays – what about specificity for IR damage.
- Telomere maintenance and other factors affecting ageing or senescence –
- activation of stress responses/ROS damage

How can we identify biomarkers conferring low dose radiosensitivity.

- To understand the mechanisms that function in response to low doses -Use this information to identify candidate genes and biomarkers
- Transcriptional and protein changes – may suggest mechanism and/or provide biomarkers.
- Develop assays to identify low dose sensitivity (killing or carcinogenesis) and then identify genes.
- Screens for cell lines, mice or other models for low dose sensitivity – identify genes
- Examine whether genes conferring sensitivity to high doses confer sensitivity to low doses (killing or cancer induction).
- Examine candidate genes

Dose response relationships at (very) low doses.



For developing biomarkers/bioassays for assessing radiosensitivity we need not know the shape of the dose response curve but we should consider the response when assessing assays.

Sensitivity of different tissues

- In considering bioassays for sensitivity (and biomarkers) we have to consider distinct tissue responses.
- Eg lymphocytes the easiest cell type to assay but will that reflect responses in other tissues.
- The embryonic brain is highly sensitive – will there be increased sensitivity in eg embryos that are heterozygous for ATM

What assays exist to date: (2)

- CA analysis and dicentrics – CA analysis of lymphocytes.
- Transcriptional changes – eg p21 changes
- DSB markers eg gH2AX foci or 53BP1 foci

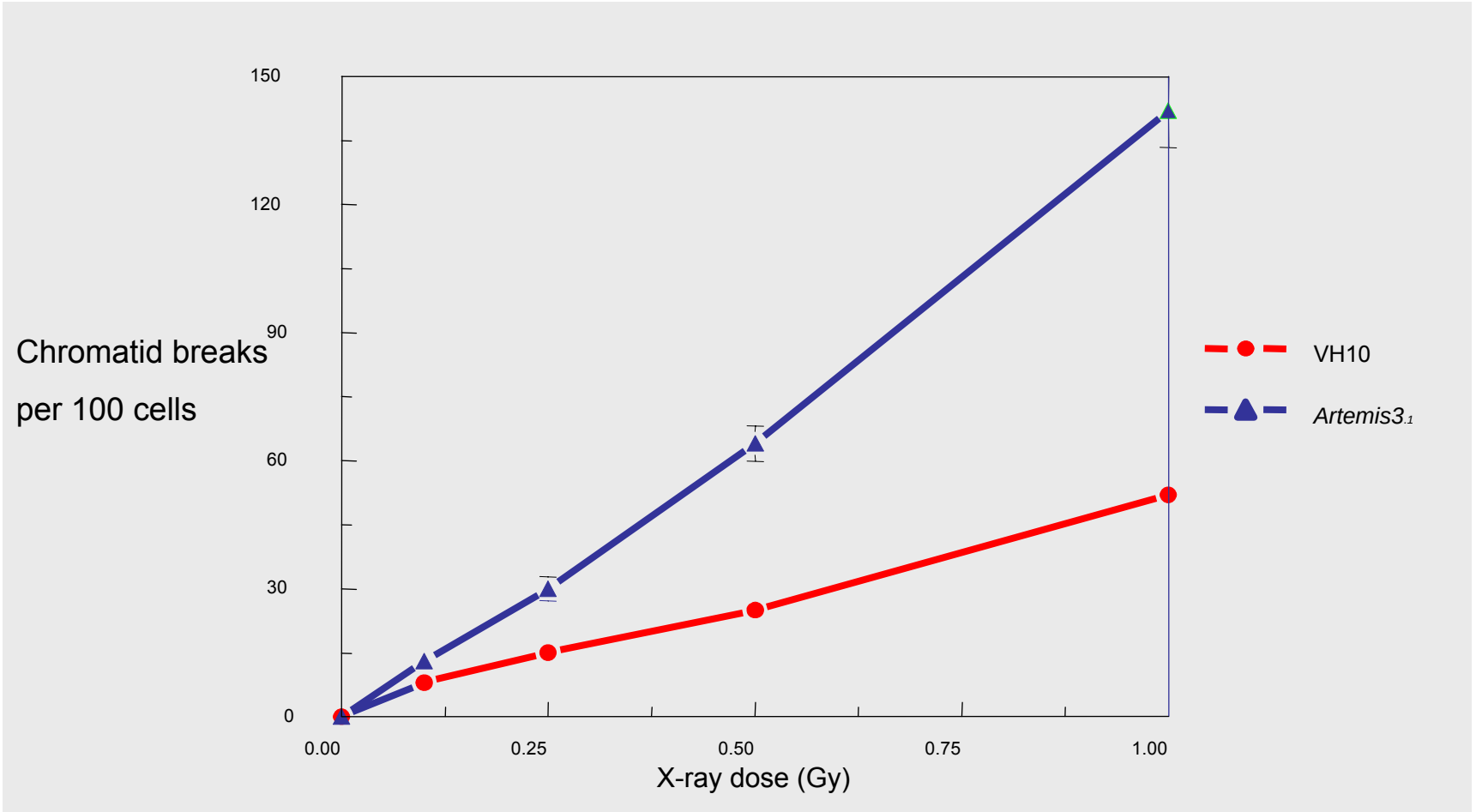
- Apoptosis in lymphocytes.
- Micronucleus assay
- Assays for senescence
- Biomarkers – none evident to date.

CA and dicentric analysis

- Best (gold standard) marker of radiation exposure to date.
- Can be used to identify radiosensitive individuals following in vitro irradiation of lymphocytes but time consuming.
-
- Borgmann and Dikomey have reported correlation between chromosomal radiosensitivity (after 6 Gy but not 3 Gy) and overresponse to RT
- Unlikely to detect minor sensitivity. ?? Any correlation with carcinogenesis.

Dicentrics do not persist in repllicating cells, therefore, unlikely to be accumulative.

CHROMOSOMAL ABERRATIONS DURING G2 IN HUMAN CELLS



F Darroudi et al., 2005

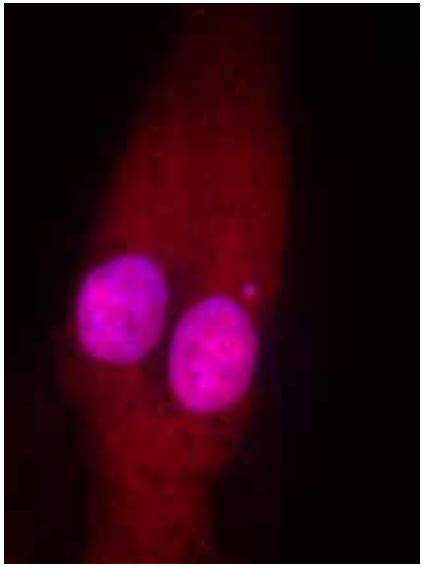
Apoptosis in Lymphocytes

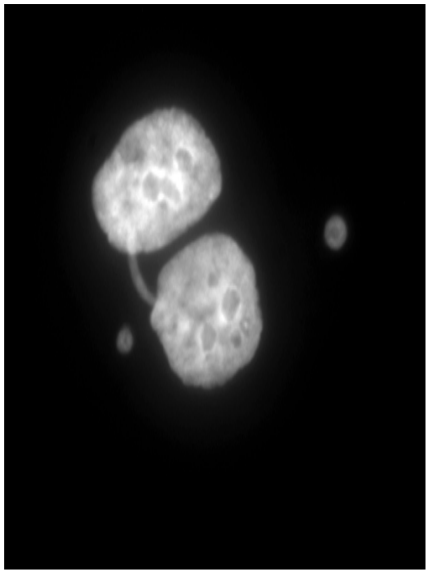
- Azria and Ozsahin have presented evidence that failure to activate apoptosis in CD4 and CD8 lymphocytes irradiated ex vivo correlates with over response to RT.
- Unclear whether this will provide any correlation with low dose exposure

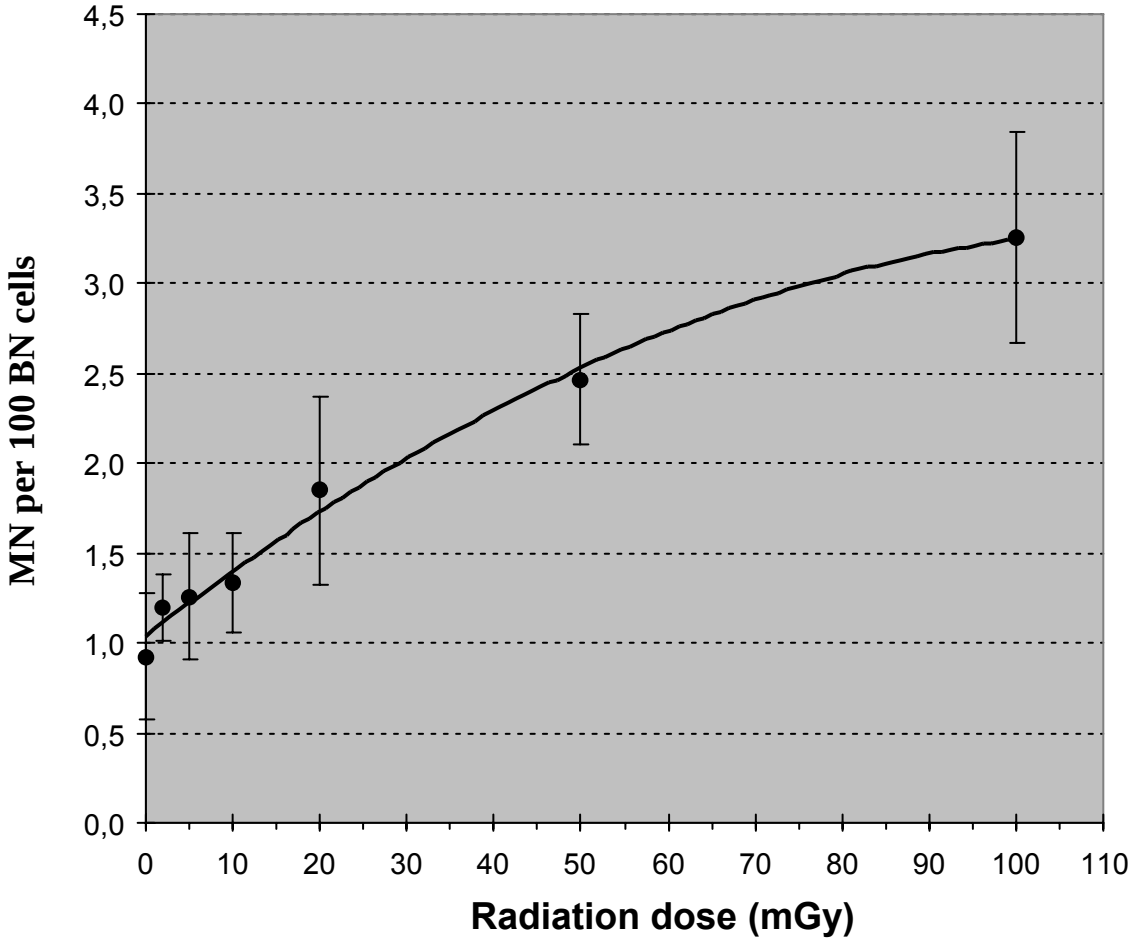
Transcriptional analysis

- Differences in transcripts have been identified after high dose and low dose irradiation. Distinct transcript changes have been observed after low (200 mGy) dose IR
- P21 (CDKN1) is one of the most sensitive changes after high doses and qRT-PCR approaches have been developed to detect changes in over responding cells/patients/mice:
- Can be high increase in p21 or failure to increase p21.
- Identifying the transcripts activated by low doses can a) help the identification of potential biomarkers and b) allow the development of bioassays (eg qRT-PCR assays)
- NB: not all transcript changes are necessarily functional – but can still correlate with endpoints such as sensitivity

MICRONUCLEI (MN) INDUCTION USING CYCLING HUMAN FIBROBLASTS

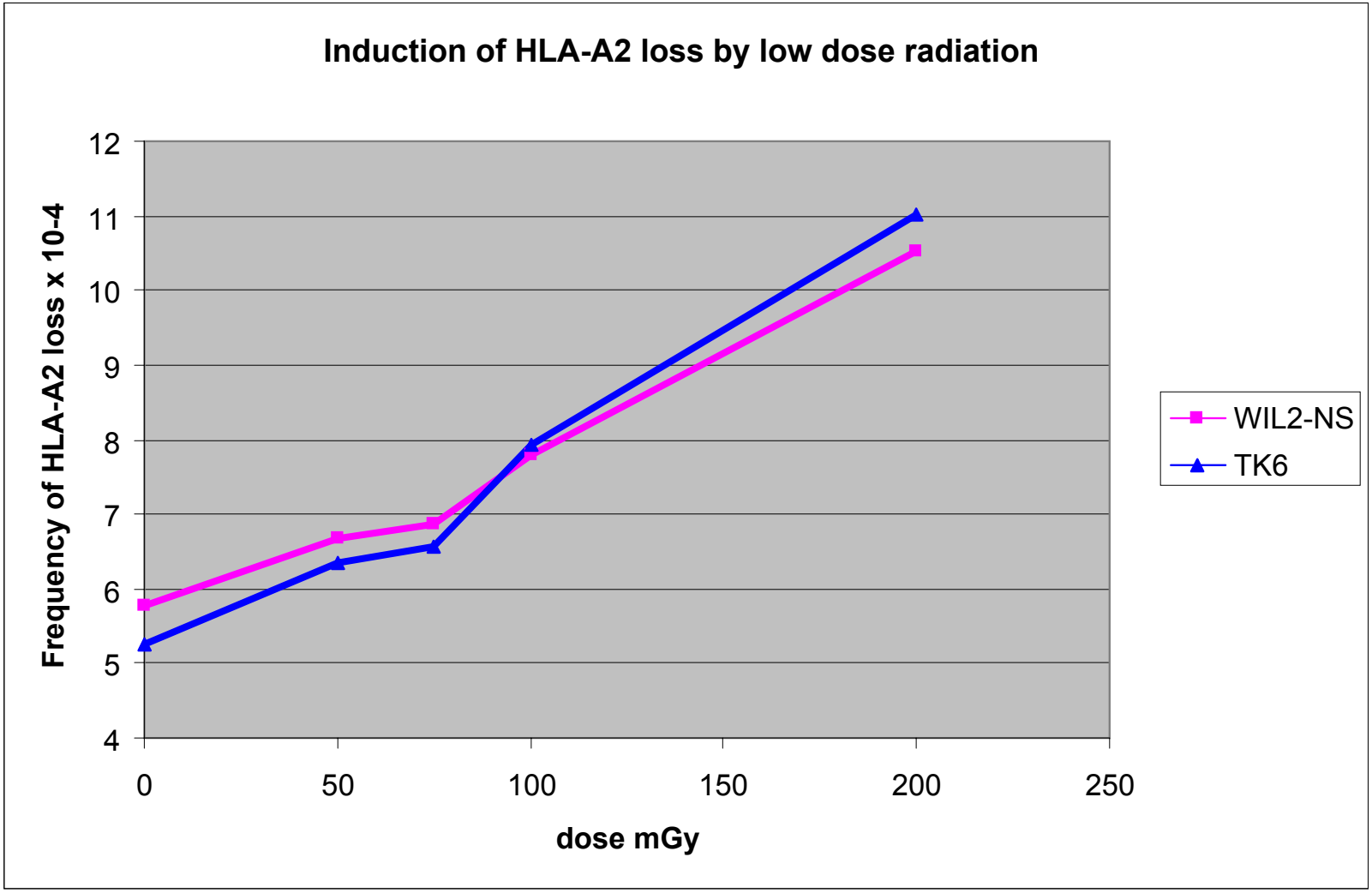






J. Boei et al., 2010

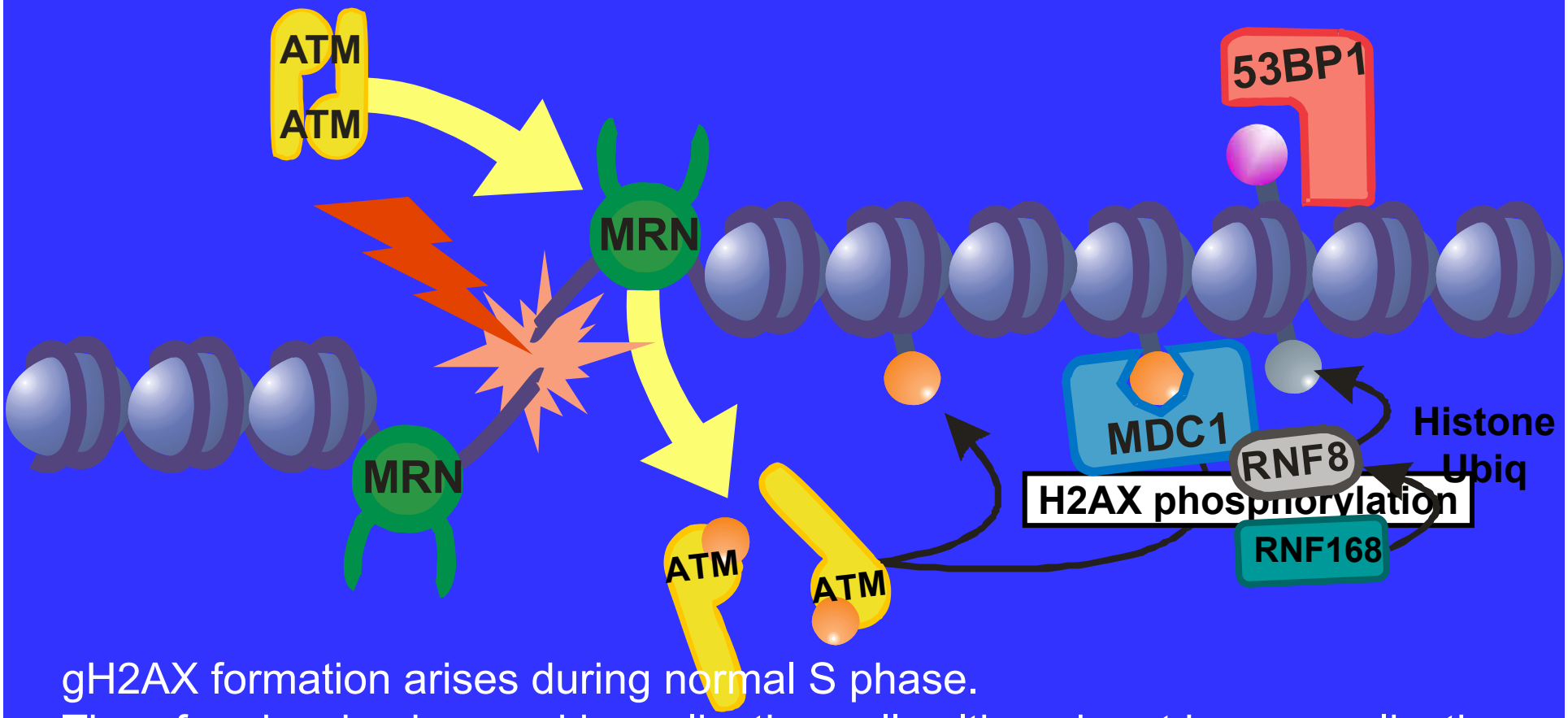
LOH INDUCTION IN HUMAN LYMPHOBLASTOID CELLS (HLA-A2 loss)



J. Boei et al., 2010

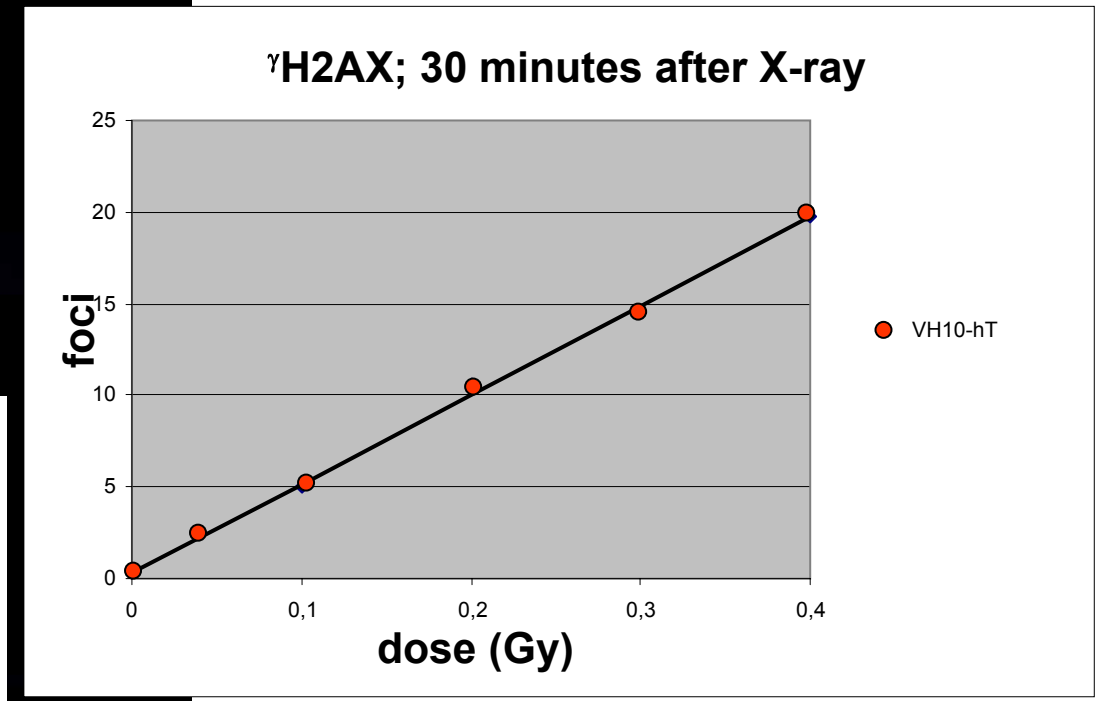
Exploitation of DNA damage response signalling – gH2AX analysis

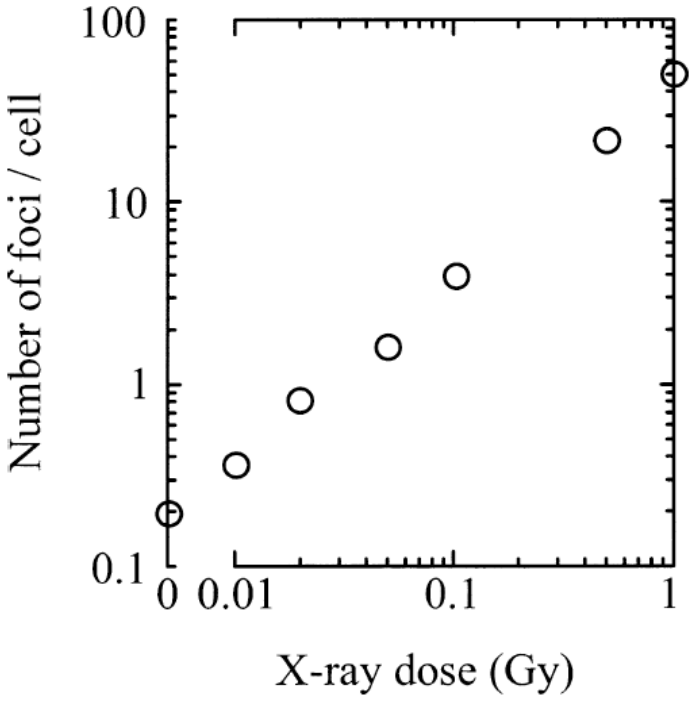
The ATM signalling mediator hierarchy



gH2AX formation arises during normal S phase.
Therefore is a background in replicating cells although not in non-replicating cells.
53BP1 has a lower background in S phase cells and can be clearer

DNA DAMAGE SIGNALLING
PHOSPHORYLATION OF H2AX (FOCI)





Suzuki et al. Radiation Res (2006)

J. Boei et al., 2010

gH2AX foci analysis

- Excellent for diagnosis of syndromic patients – also for identification of radiosensitive individuals with marked sensitivity but non-syndromic.
- Also reasonable for examining exposure up to 24 h post IR
- Can be used with fibroblasts or lymphocytes.
- Is amenable to high through put analysis (programmes have been developed).
- Will discuss results with high dose exposure then low doses

Future considerations.

- These findings correlate with those of Borgmann and Dikomey – who found that chromosomal radiosensitivity of lymphoctyes (after 6 gy but not 3 Gy) correlated with over response to RT.
- Can we expect the over response to RT to correlate with over response to chronic low dose irradiation (to some degree yes).

Sensitivity for assessing the response to acute low dose.

- Markus Lobrich has examined gH2AX foci in patients undergoing CT scanning of thorax and abdomen.
- Saw elevated foci in lymphoctyes taken from patients that depended on dose-length – ie body size + body length
- Kinetics of loss of foci was similar to that observed in fibroblasts given higher doses.
- One patient identified who over responded to RT

This patient showed markedly slow repair in fibroblasts also.

This suggests that an over response to a high dose correlates with DSBs persisting longer after exposure to low dose

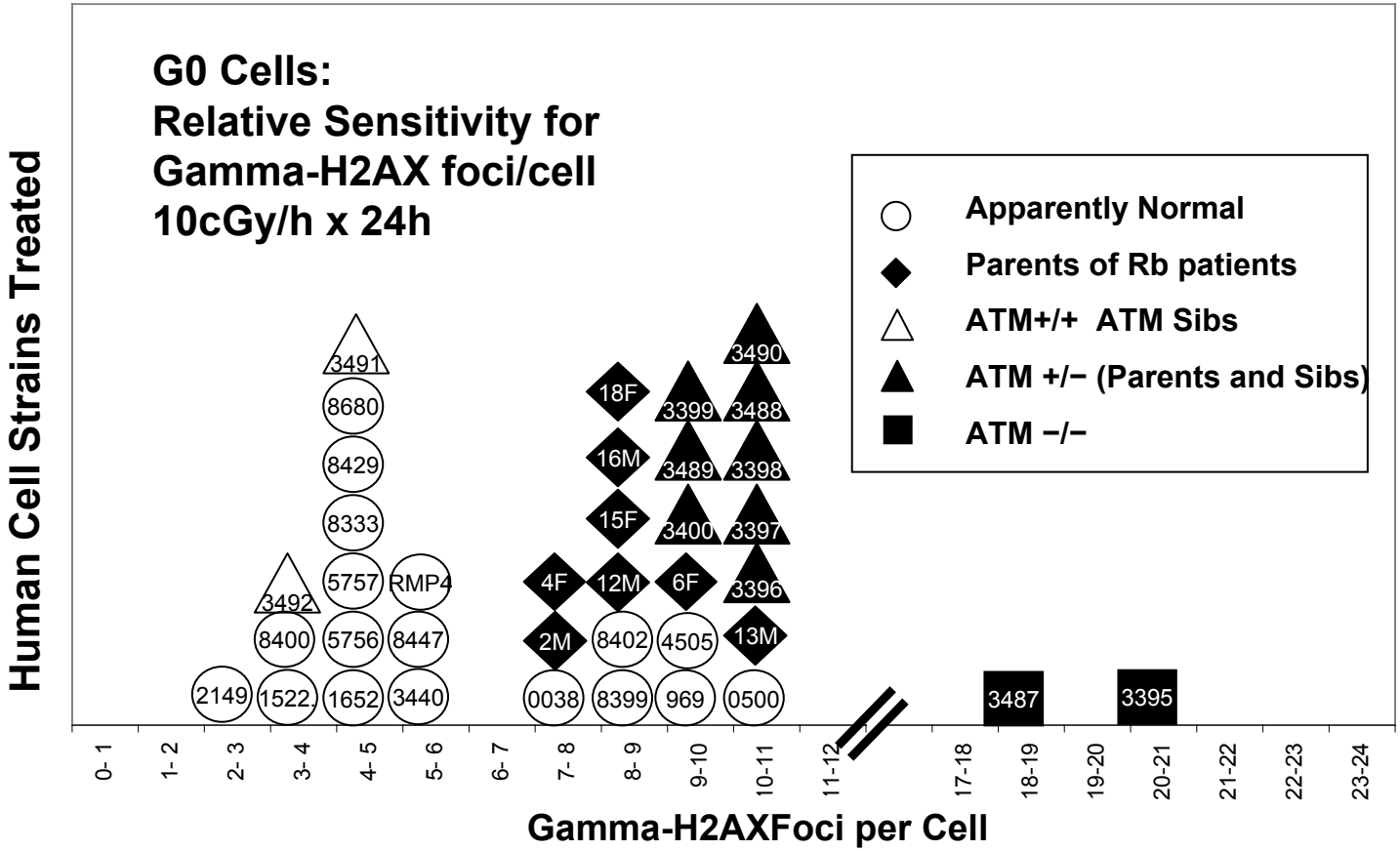
What does this tell us:

- Impaired repair can be detected in lymphocytes after low doses.
- Can identify highly radiosensitive individuals – but these may only be a fraction of over responding patients.
- Was the sensitivity (ie slow DSB repair) to CT doses harmful for this patient? Eg will it activate a stress response – lead to inaccurate repair?

Can gH2AX foci predict over responders to chronic low dose IR

- Joel Bedford used gH2AX foci analysis following chronic low dose exposure of non-replicating fibroblasts

Used 10 cGy/h for 24 h. Then analysis of accumulated DSBs in Control, ATM-/- and ATM+/- cells (patient cells) and Rb+/- individuals



Kato, et al, DNA Repair 6, 818-829 (2007)

- AT cells have very high number of unrepaired DSBs.
- Hets can be distinguished from most controls. Shown also with mouse cells and the controls all look normal
- ~25-30% controls looked like AT hets.
- Distribution bimodal and not linear.

What does this tell us:

- Since AT hets can be clearly distinguished, it says that modest sensitivity can be identified.
- If 30% “normals” fall into this category, then there are more sensitive individuals.
- This does not correlate with RT over response – does it indicate sensitivity to low dose rate or carcinogenicity.

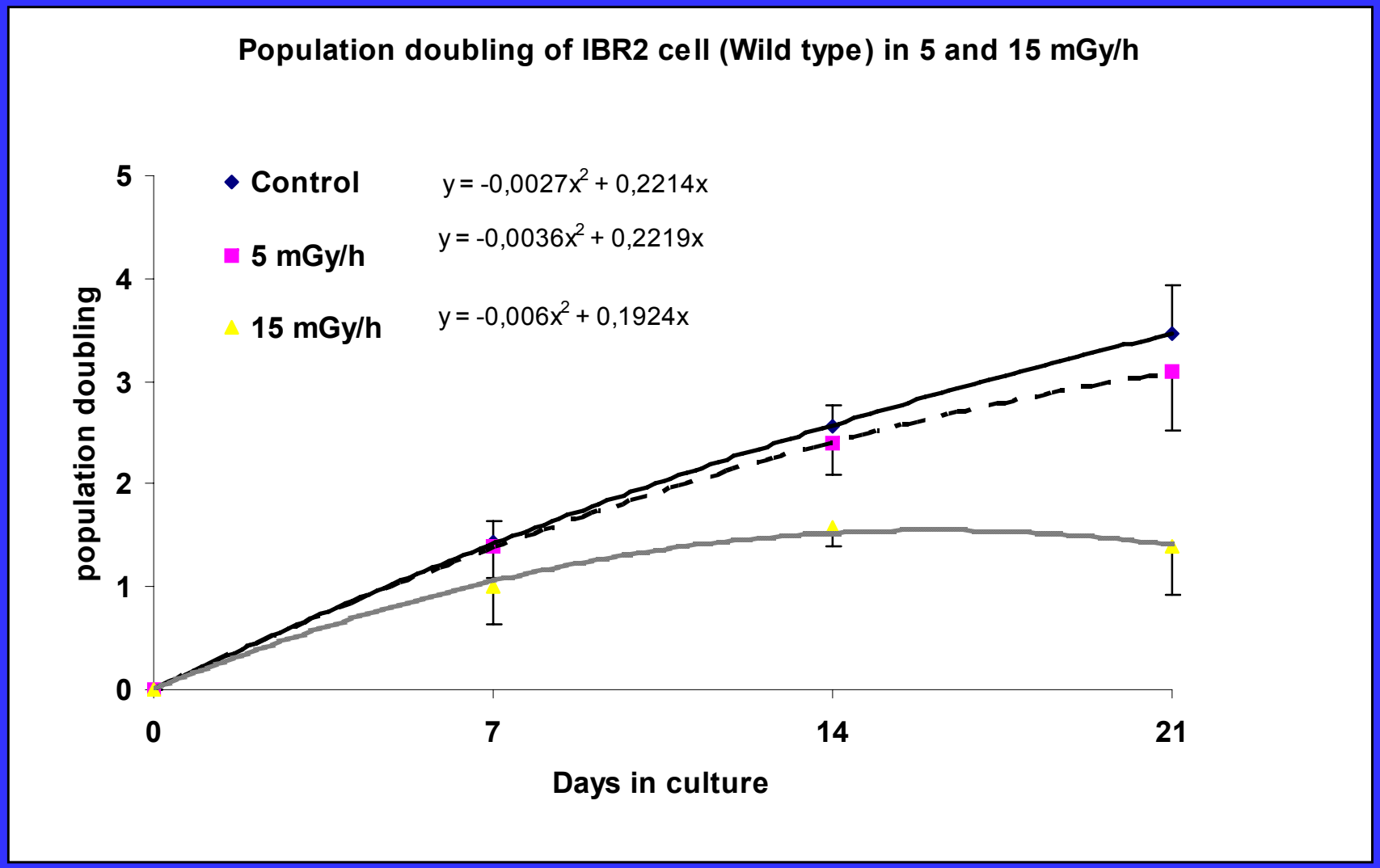
- In a collaborative study with Mats Harms-Ringdahl and Siamak Haghoost, we have observed gH2AX foci accumulating in ATM, Artemis-/- and LIGIV syndrome patients after chronic low dose irradiation (5 and 15 mG/hr) but hets have not yet been examined.

Important because it says extreme sensitivity patients (including hypomorphic ligase Iv patients) accumulate unrepaired DSBs under chronic low dose rate irradiation

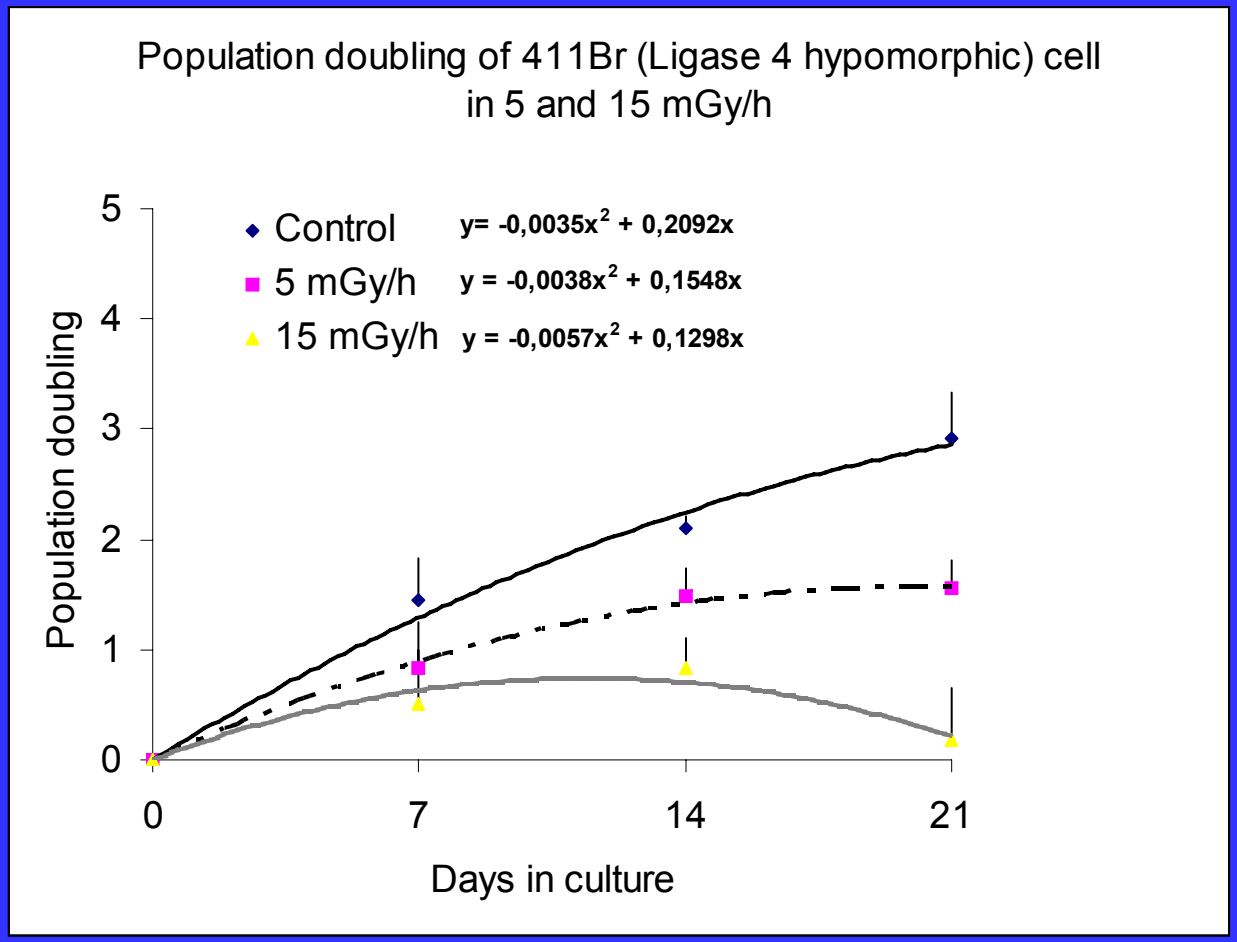
Activation of stress response/senescence by low doses IR

Mats Harms-Rindahl and Siamak Haghoost have exposed primary fibroblasts to chronic low dose IR to examine senescence

Control cells exposure ot 5 and 15 mG/H show enhance premature senescence or loss of proliferation



See elevated senescence in a repair deficient line from a LIG4 Syndrome patients



Mouse studies.

- Low doses (below 2 Gy) have not indicated any cancer induction.
- Genes conferring sensitivity have been identified eg Rb and the Ptch mouse
- In embryonic ventricular zone, elevated apoptosis after 50 mGy or 100 mGy and this is greater in LigIVm/m mice – but no obvious increase in LigIV+/m mice.

Bystander response

- Is it activated in vivo
- Distinction to cytokine signalling.
- Does it place an importance on ROS damage?
- How does it suggest biomarkers – now can we exploit our knowledge of the bystander response

Future directions.

- Have any biomarkers or assays been developed/identified?
- What are the end points we should consider: tissue damage, inflammatory response, stress response, senescence, cancer induction.
- Is the bystander effect operative – if so how does that inform for biomarkers
- Do we make the assumption that the damage will be accumulative.
- What are the best approaches to pursue.

(NB our remit is to identify biomarkers not examine the mechanisms)

Future directions:

- Identifying assays that will monitor sensitivity in cells/tissues/mice is worthwhile.
- Transcriptional profiling/proteome changes – are they likely to provide biomarkers?
- specific screens to search for sensitivity to low dose exposure
- Examination of whether damage response genes (the extreme) confer sensitivity? Rare but important for patients and informative for mechanisms?
- Is gH2AX analysis after low doses a possibility. If possible, then other aspects of the damage response could be exploited.
- Use of Hets to identify sensitivity.

Suitable future assays.

- Use of lymphocytes ex vivo??.
- Micronucleus assay – it is sensitive to low doses and could detect sensitivity in cells– but will it predict sensitivity at the patient level – it requires replication for detection
- Proliferation following chronic irradiation – not predictive but could be informative.
- Activation of stress responses.
- Epidemiology??
- Mouse studies – how can they provide most iinformation