Radiation-Induced Neoplastic Transformation *In Vitro*, Hormesis and Risk Assessment

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Neoplastic Transformation *In Vitro*

- Clear cancer-related endpoint (conversion of cells from a non-tumorigenic to a tumorigenic phenotype)
- Has proven to clearly describe high dose radiation carcinogenic effects *in vivo* (dose, dose-rate, LET, chemical promoters, chemical protectors)
- Is proving useful to explore possible mechanisms underlying the shape of the dose-response curve at low doses

Neoplastic Transformation *In Vitro*

- Limited in that the complexity of tissue microenvironment, immunosurveillance etc. is absent
- Limited in terms of difficulty in transforming primary cells, particularly primary human cells
- Limited number of cell systems amenable to quantitative assay of radiation effects

Neoplastic Transformation *In Vitro*

Normal → Preneoplastic → Neoplastic

Most, if not all, *in vitro* systems are examining the transformation from some preneoplastic state to the neoplastic state. Is this relevant?

Neoplastic Transformation *In Vitro*

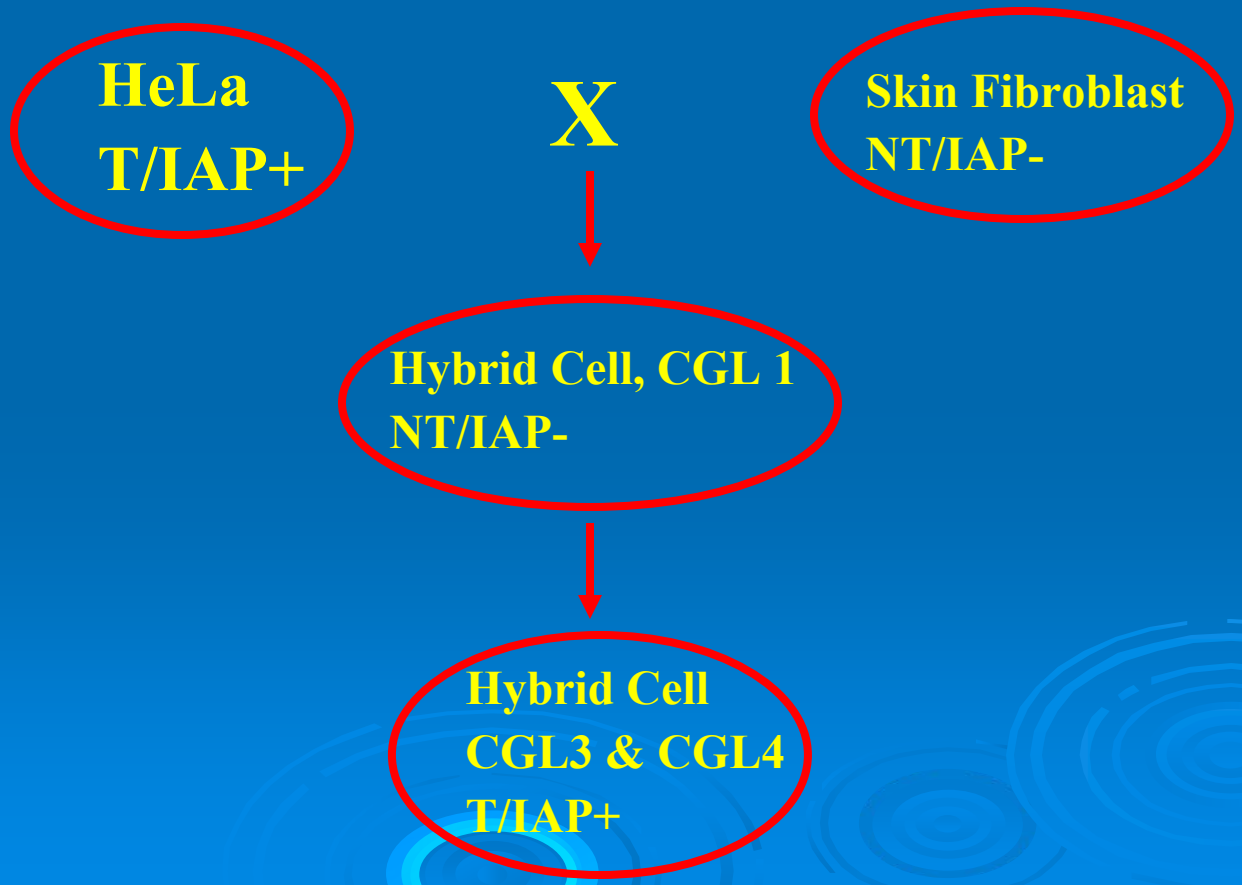
- Most humans are carrying a burden of preneoplastic cells in their bodies
- Given that they are part way down the path to cancer they could be considered a particularly important target
- According to Folkman, most tissues harbor "dormant" tumorigenic cells. It is unclear whether in vitro studies can mimic any possible radiation "activation" of such cells

Neoplastic Transformation *In Vitro*

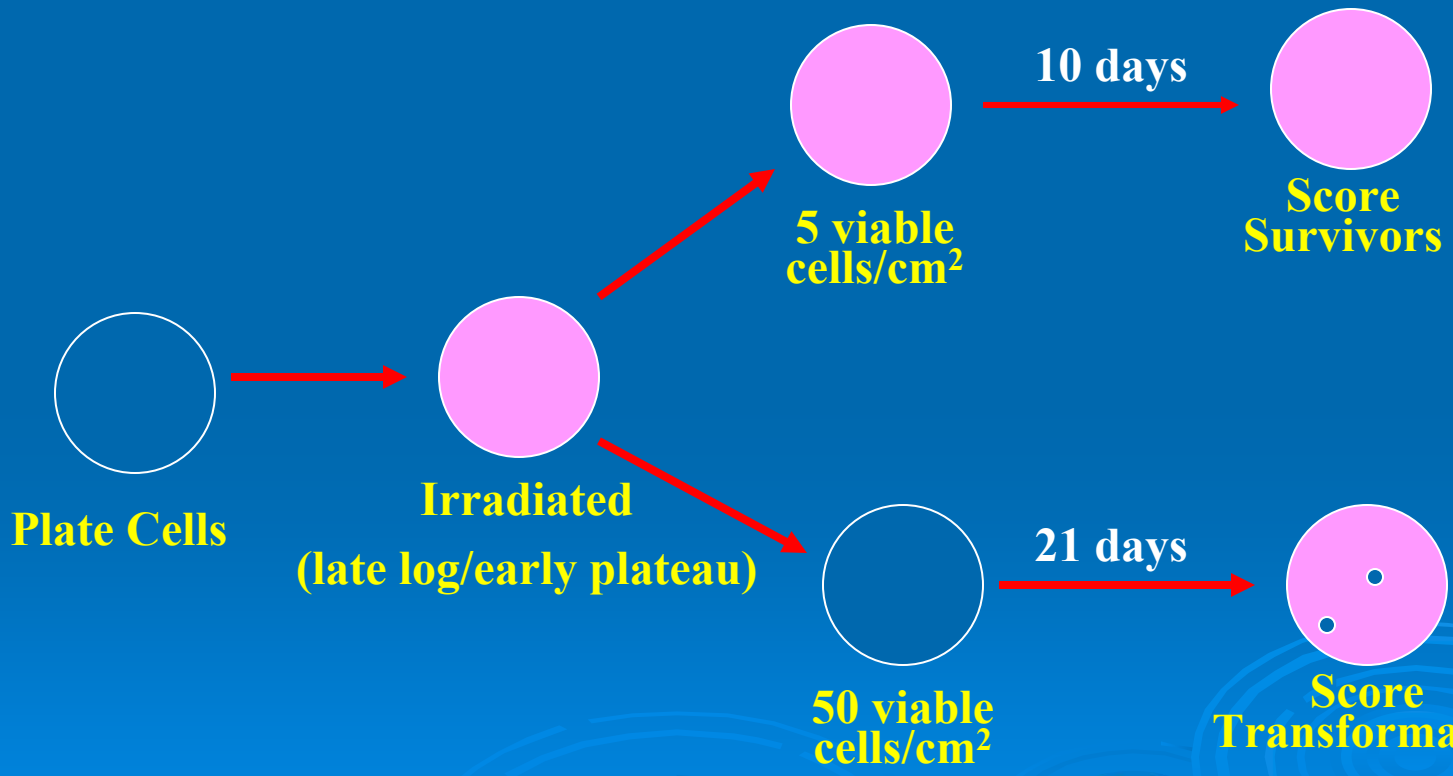
- Amenable to the study of low dose effects, but requires extensive and labor-intensive experimentation to achieve even reasonable statistics
- Two experimental systems (C3H10T1/2 and the HeLa x skin fibroblast human hybrid cell assay) have demonstrated hormetic responses at low doses of low LET radiations and a J-shaped dose-response curve

HeLa X Skin Fibroblast Hybrid Cells And The Study of Tumor Suppressor Function

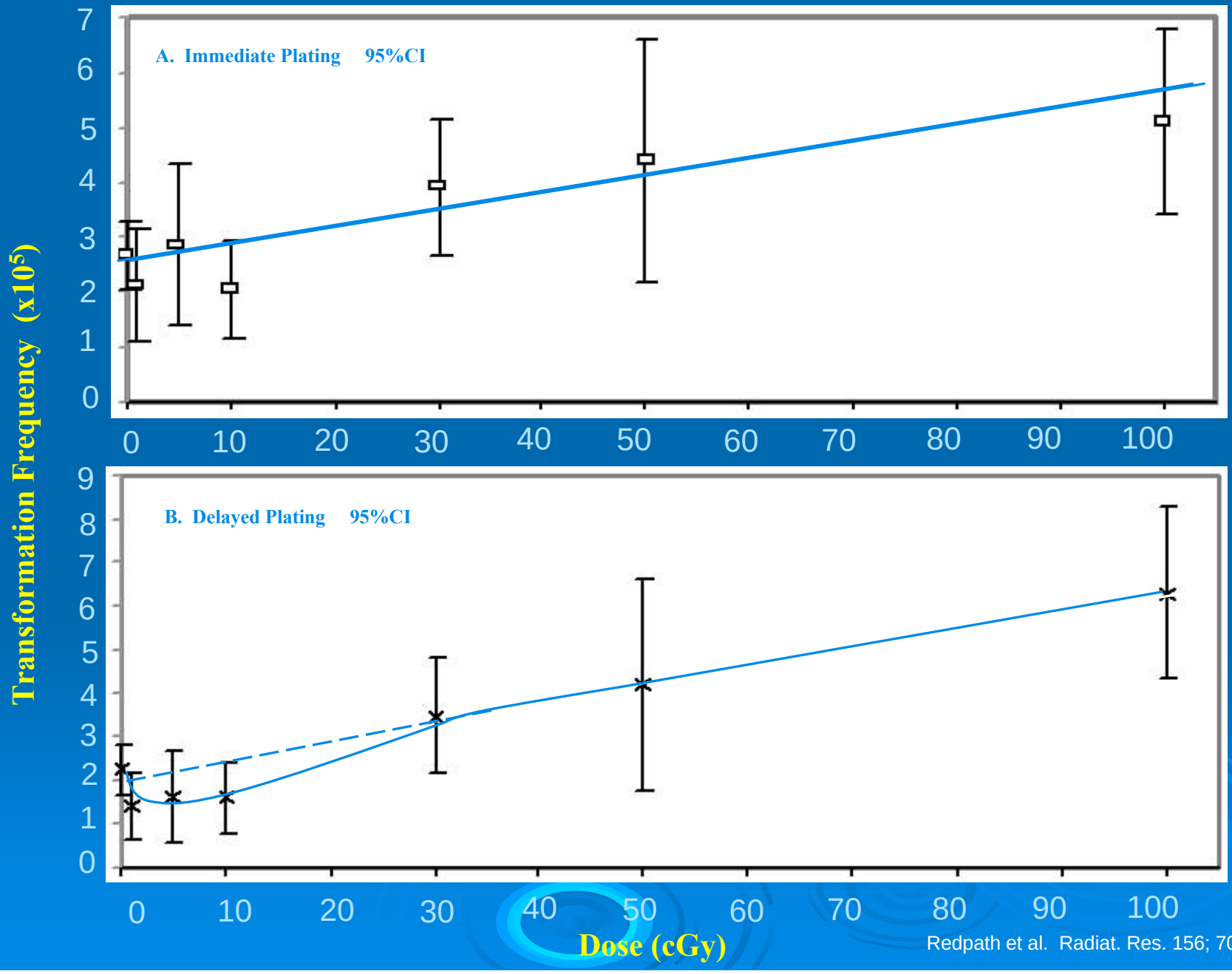
Tumorigenicity and IAP expression are negatively regulated by a suppressor on chromosome 11



Schema Assay of HeLa X Fibroblast Human Hybrid Cell Neoplastic Transformation By Radiation

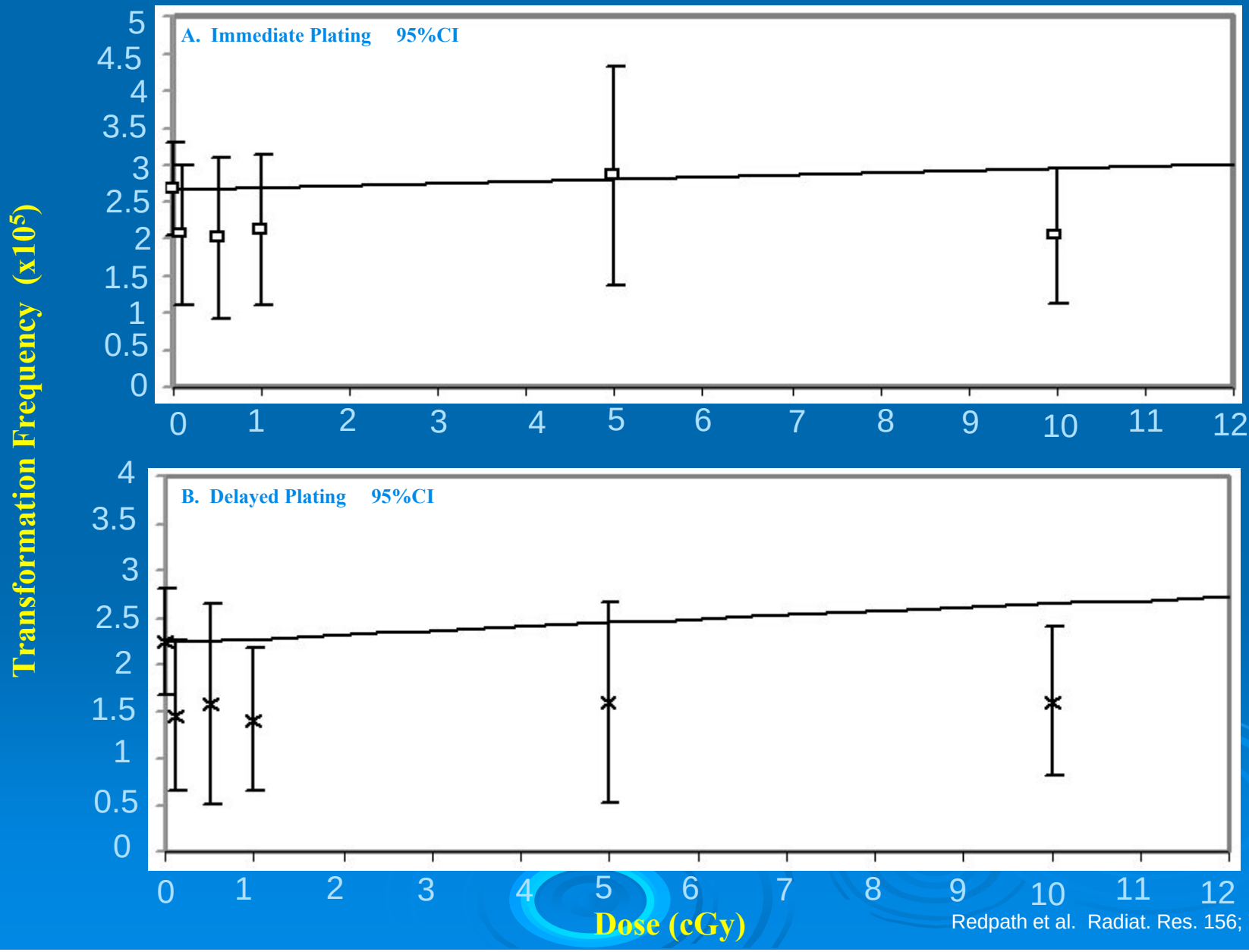


Dose Dependence of Cs-137 γ Radiation Induced Neoplastic Transformation In Vitro.



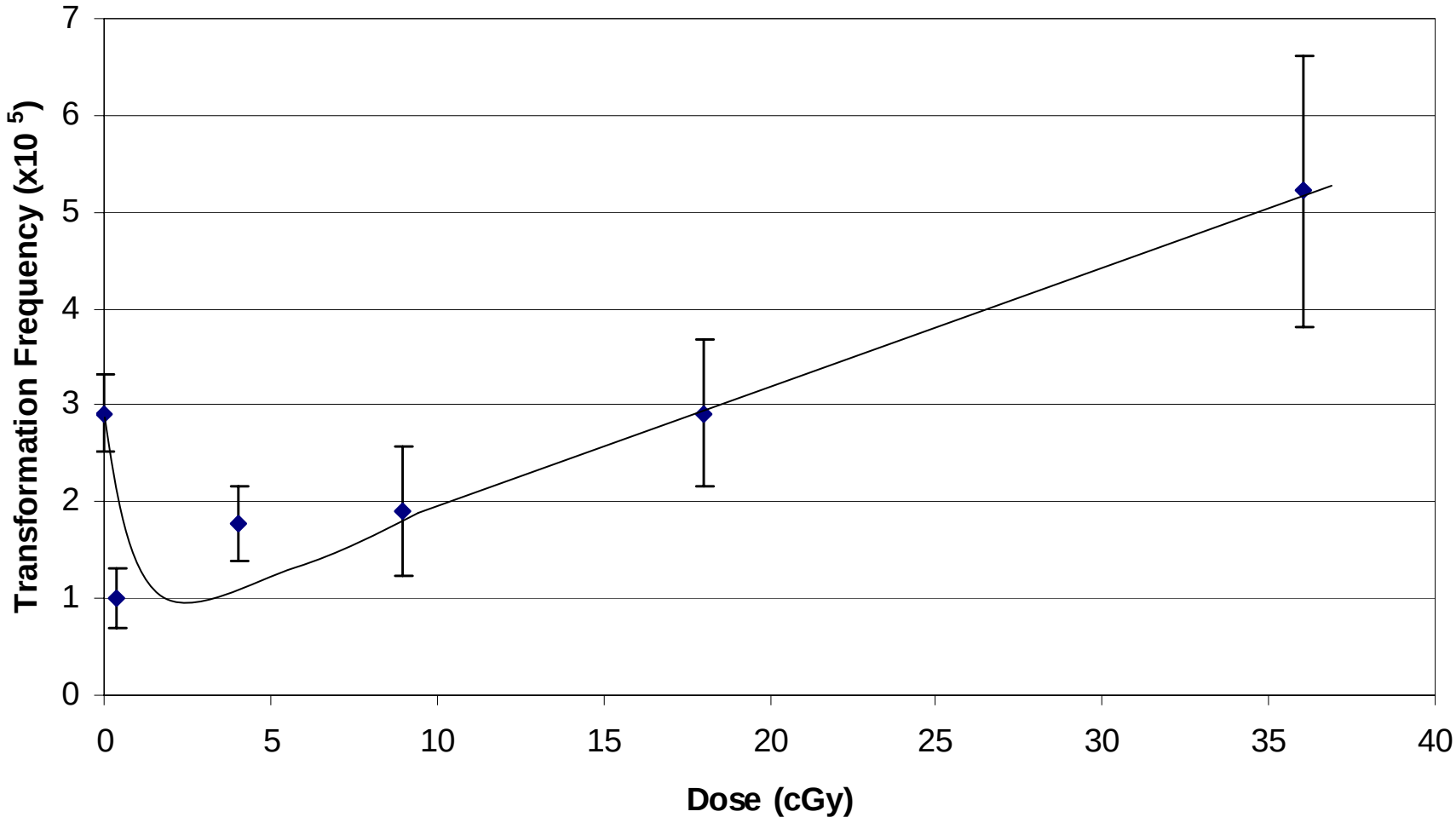
Redpath et al. Radiat. Res. 156; 700-707, 2001

Dose Dependence of Cs-137 γ Radiation Induced Neoplastic Transformation In Vitro: Low Dose Deviation from Linear Extrapolation from High Doses.

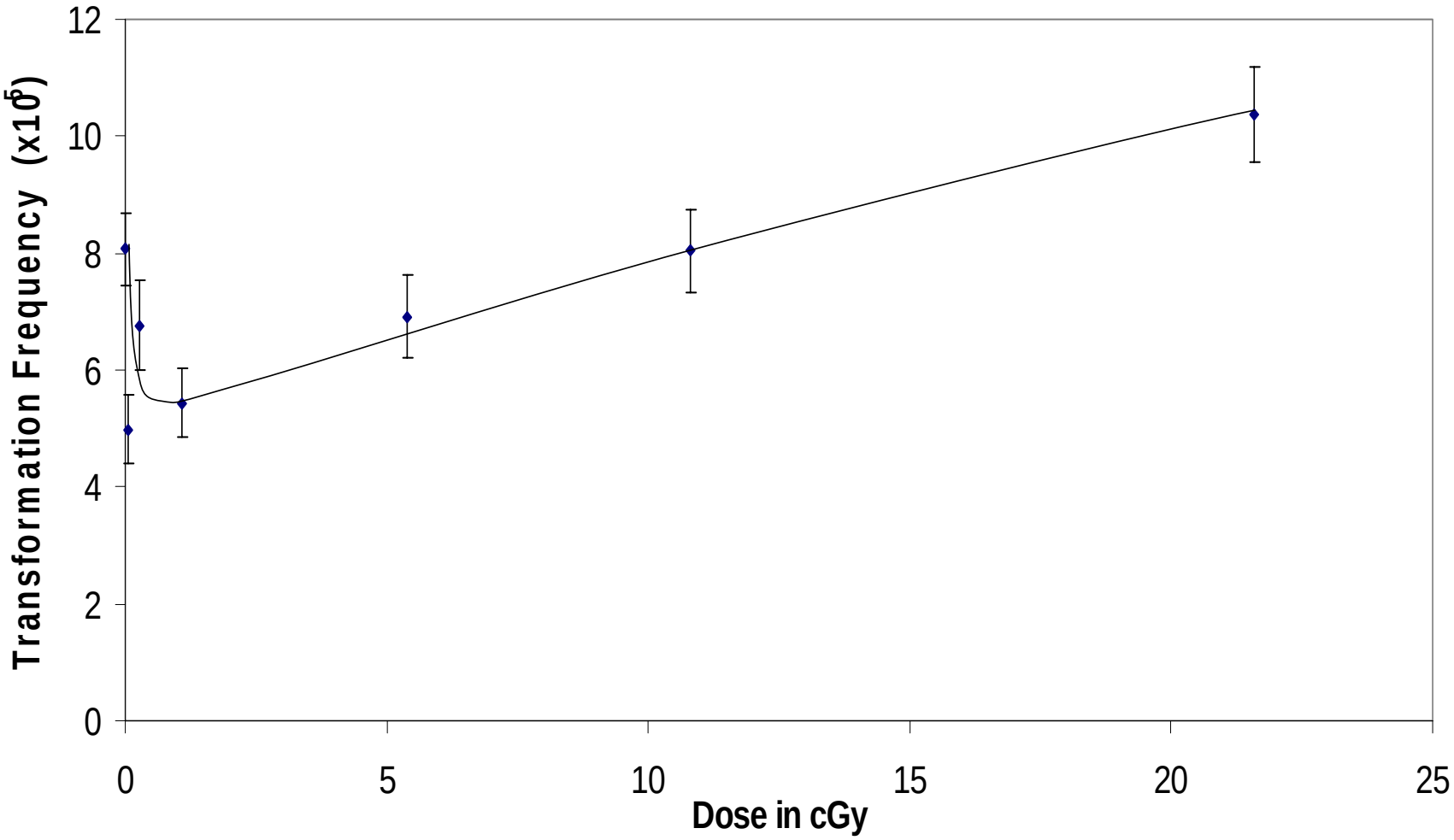


Redpath et al. Radiat. Res. 156; 700-707, 2001

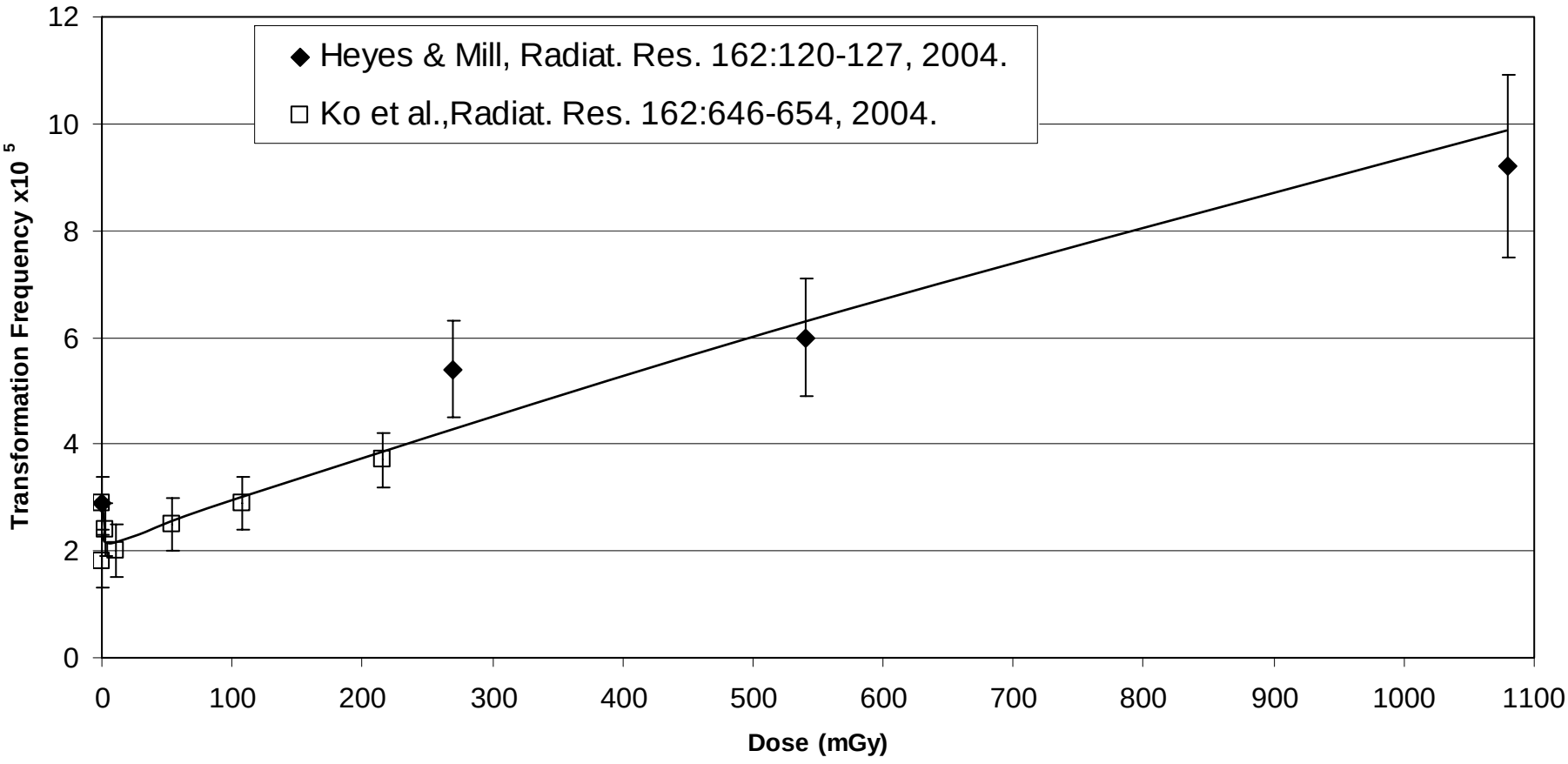
Dose-response curve for neoplastic transformation of human hybrid cells by 60 kVp X-rays



Dose-response curve for neoplastic transformation of human hybrid cells by 28 kVp X-rays



Dose-response curve for neoplastic transformation of human hybrid cells by 28 kVp X-rays: Two data sets normalized to the same spontaneous frequency



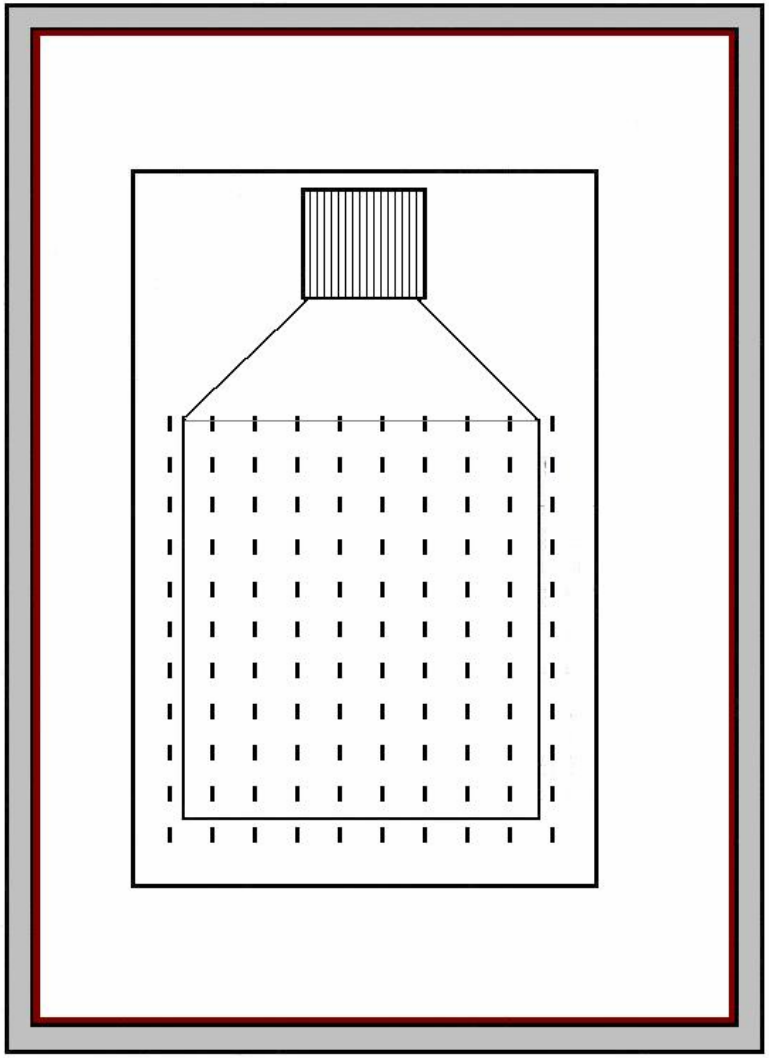
Neoplastic Transformation In Vitro

- In summary, we have routinely observed J-shaped dose-response curves following HDR treatment and are interested in mechanisms underlying this shape
- Studies with the human hybrid cell system indicate that there may be at least two phenomena involved
- Low dose hyper-radiosensitivity of a transformation-sensitive subpopulation (G2?)
- Induction of DNA repair. Future studies will examine the roles of NHEJ and HR using RNAi.

Neoplastic Transformation In Vitro

- We have recently performed dose-rate studies using an I-125 irradiator that is allowing us to look at dose-rates <2.0 mGy/min of ca. 30 keV photons with the aim of comparing the data with that seen at high doses for high dose-rates of similar energy (28 kVp) mammography x-rays (Heyes and Mill, Rad. Res. 162:120, 2004; Ko et al., Rad. Res. 162:646, 2004).

I-125 IRRADIATOR



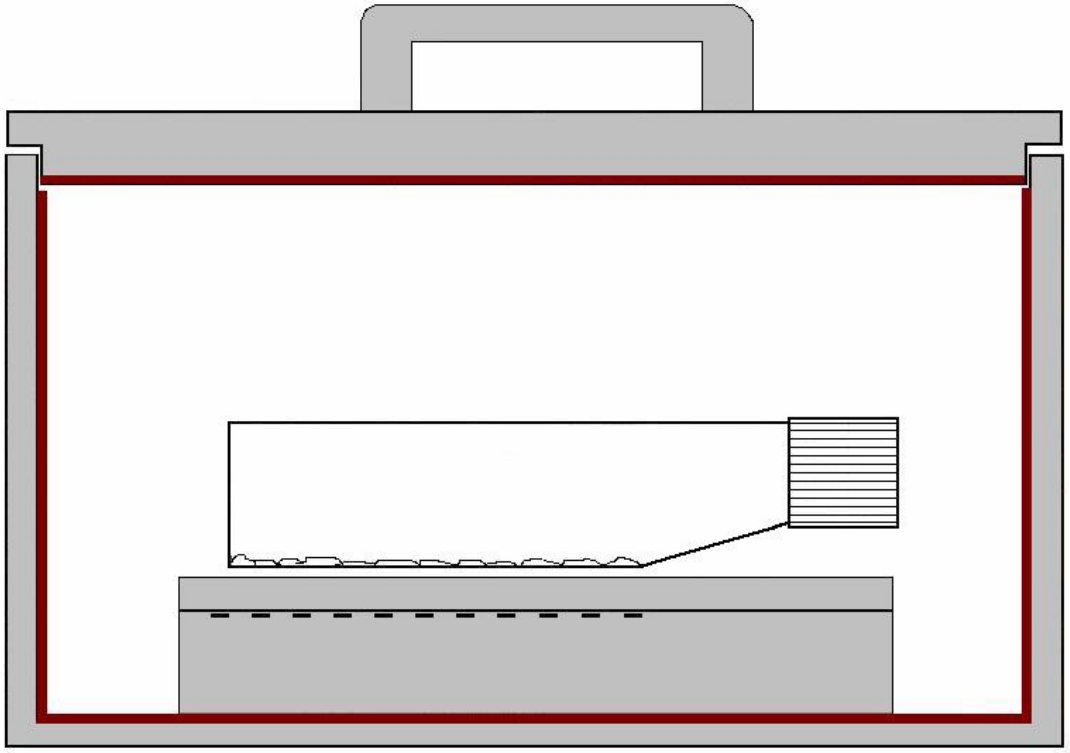
Plan

6mm Pb-lined lucite box

10 x 11 seed array

Initial activity =
1.0 mCi/seed

Initial dose rate =
2.2 mGy/min

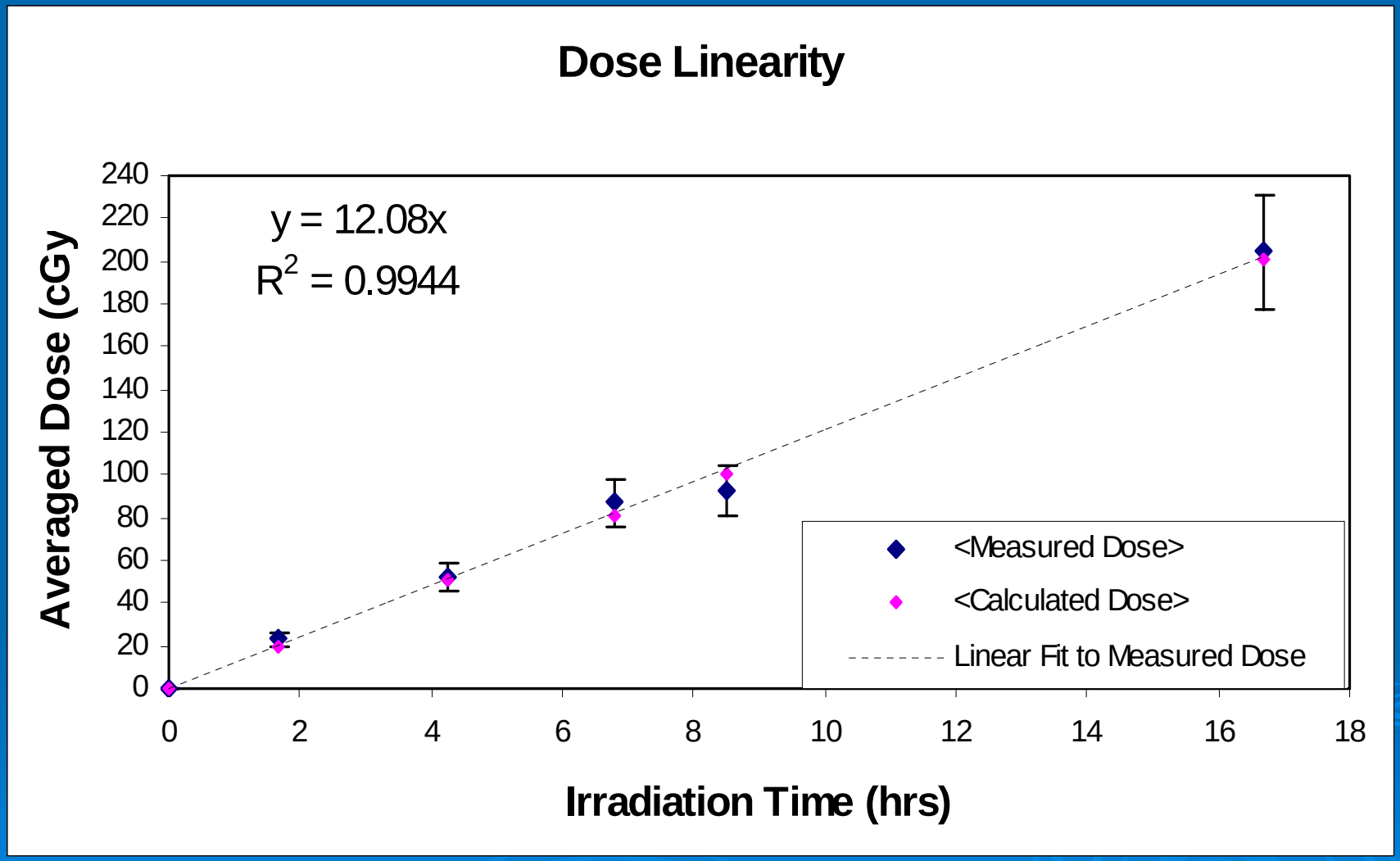


Elevation

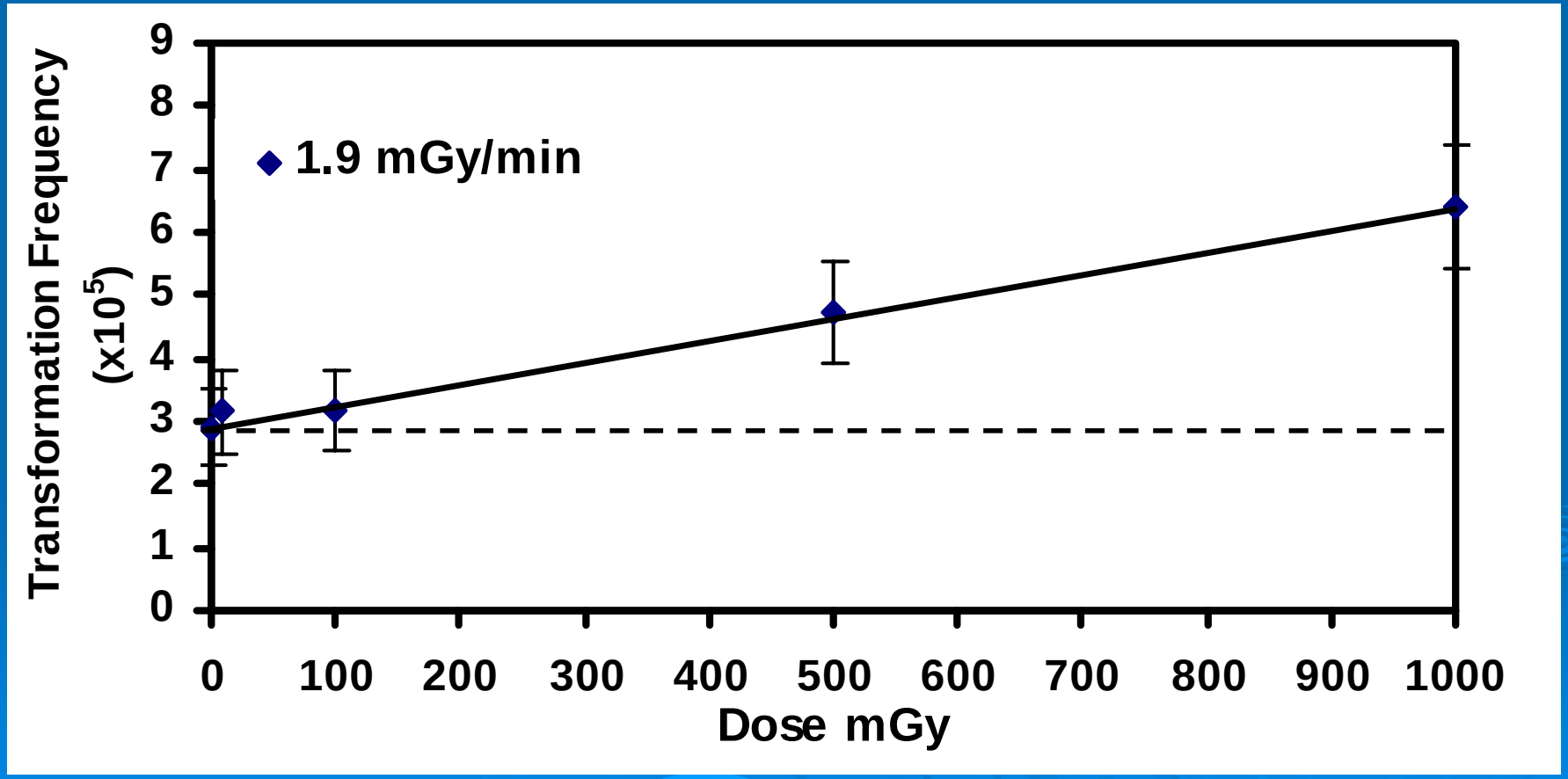
T-75 Flask

0.5 cm forward scatter

2 cm backward scatter


Dose Linearity
y = 12.08x
R2 = 0.9944
Averaged Dose (cGy)
240
220
200
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
Irradiation Time (hrs)
<Measured Dose>
<Calculated Dose>
Linear Fit to Measured Dose

Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates



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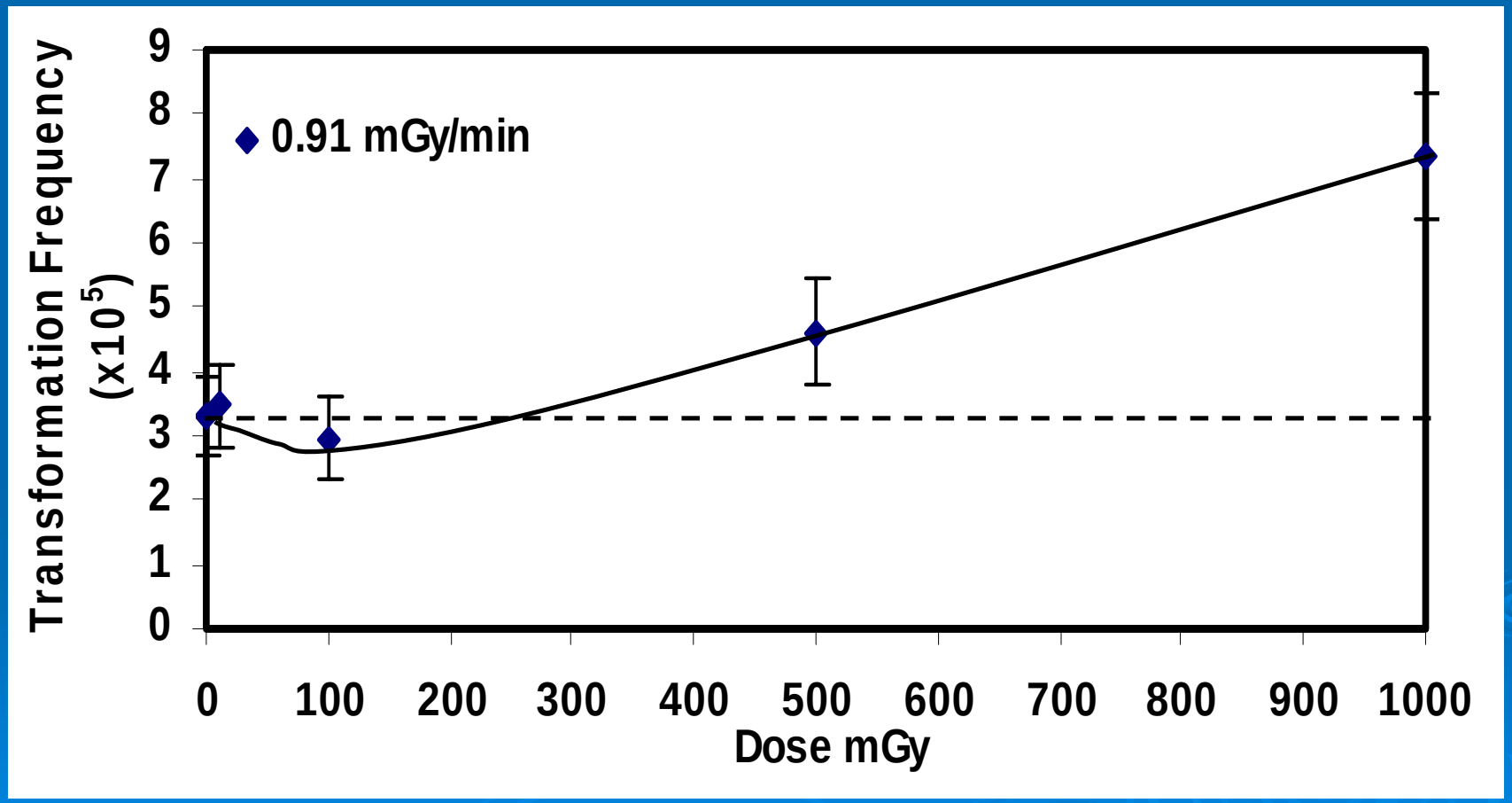

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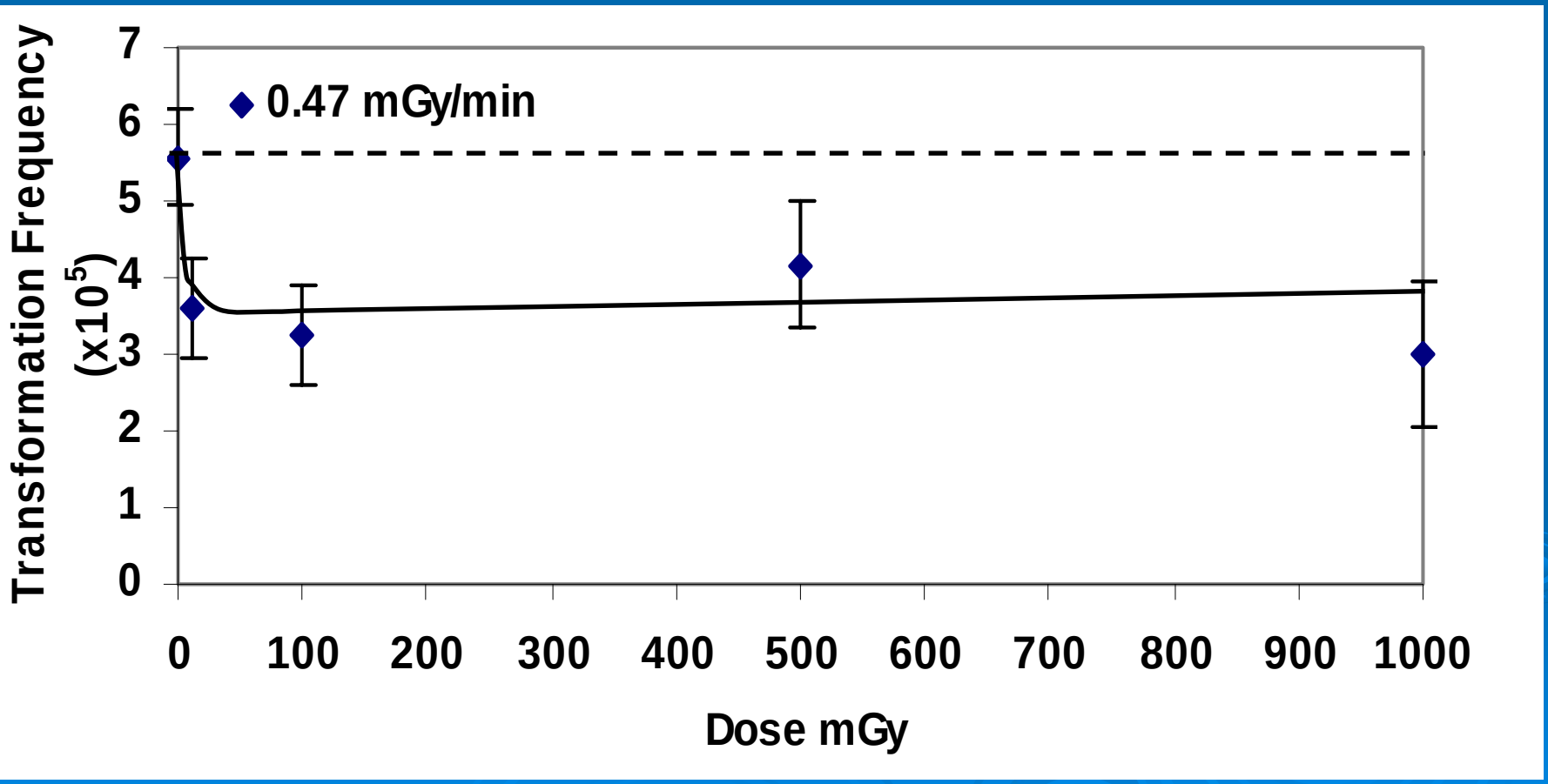

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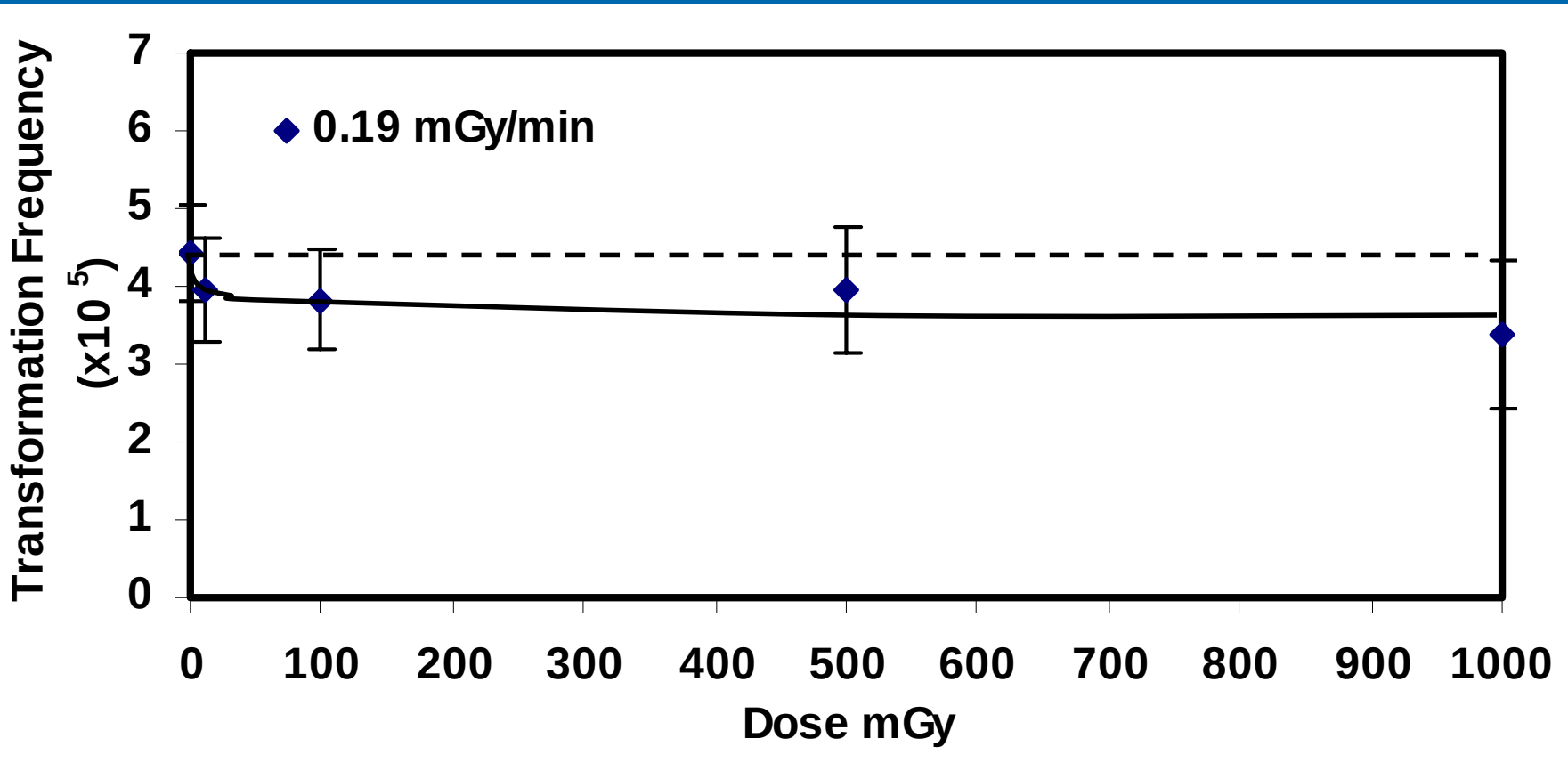

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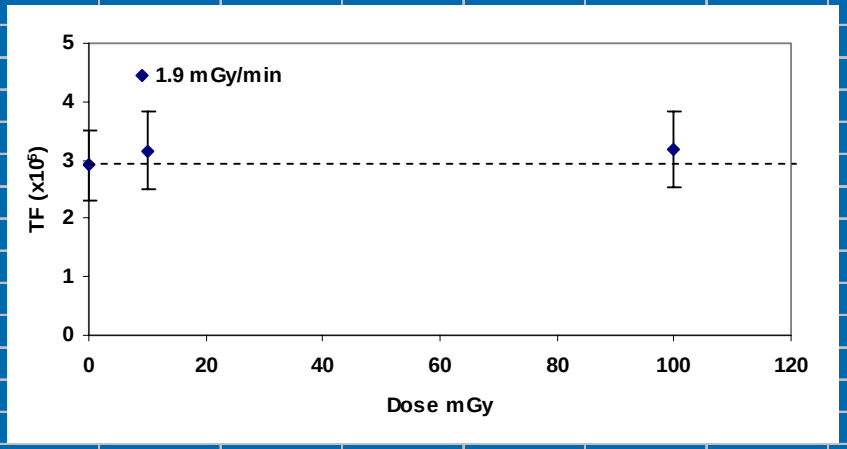
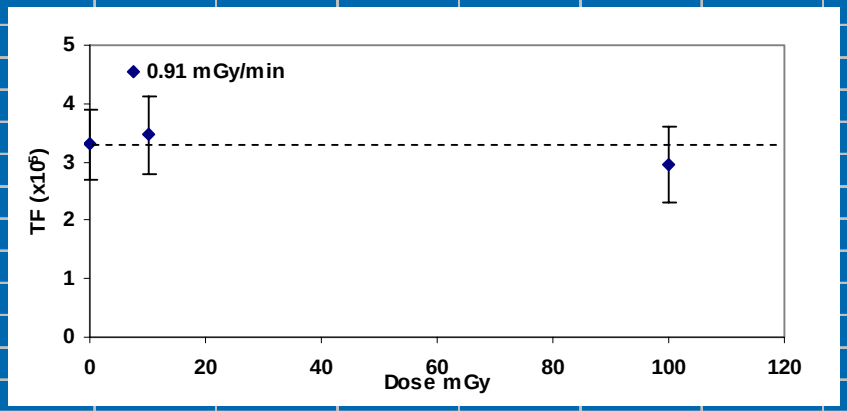
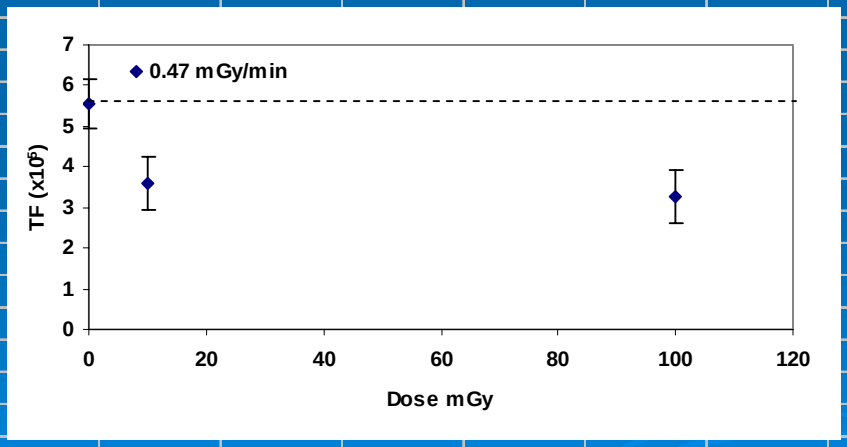
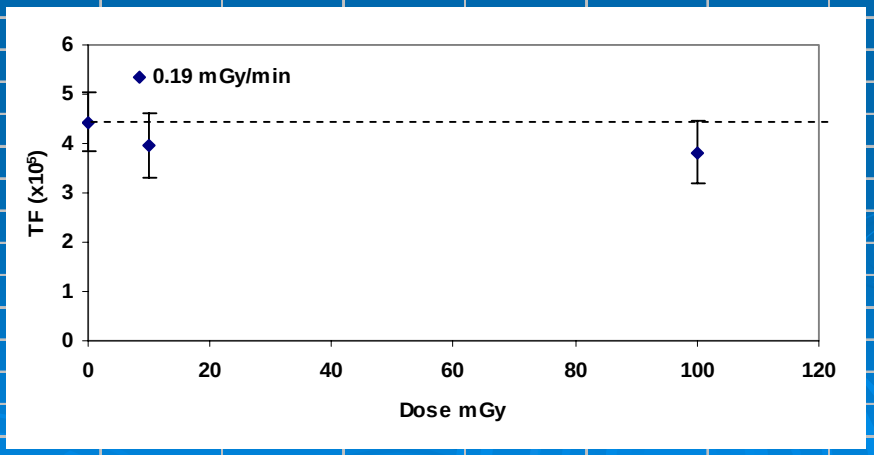
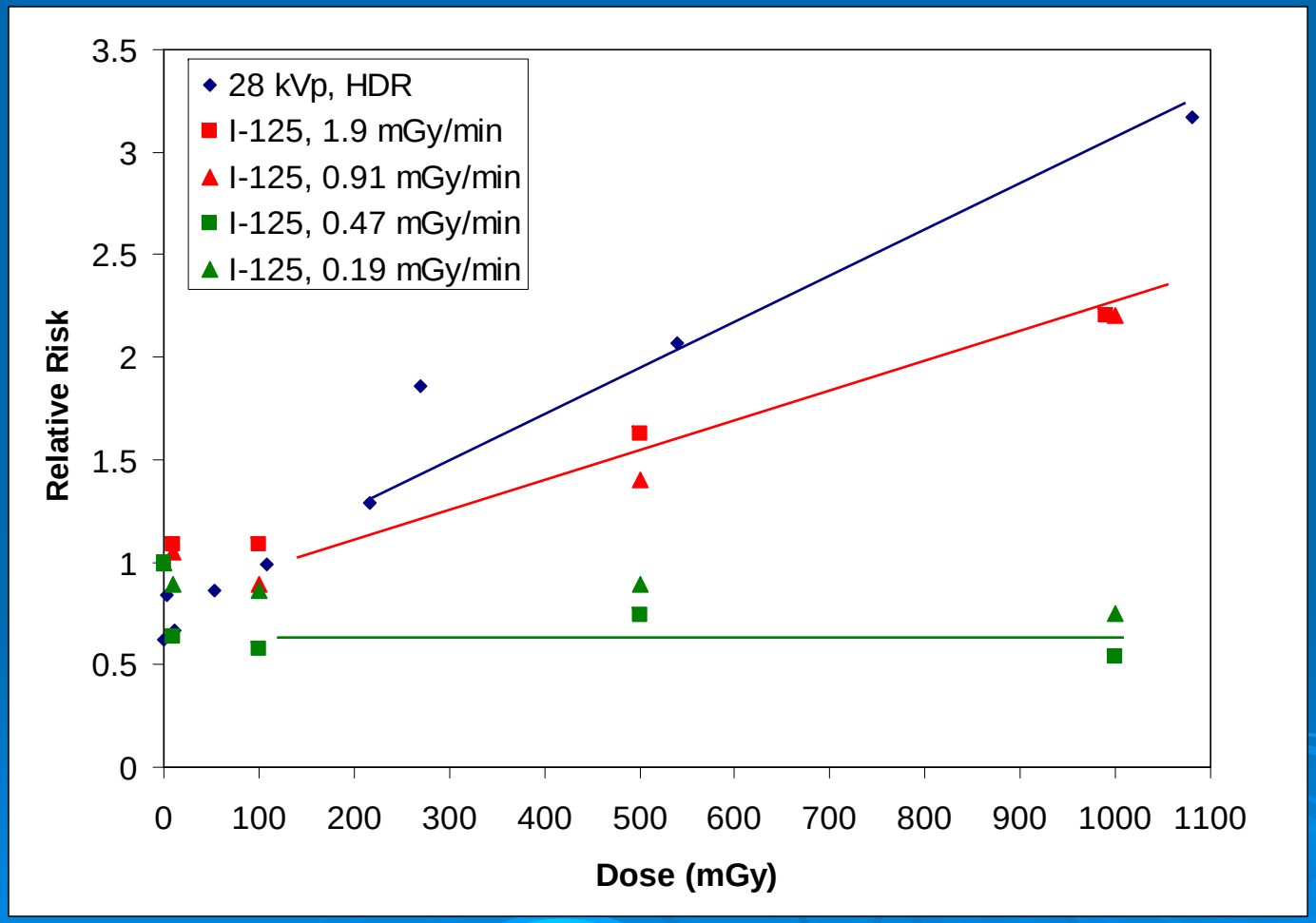








Neoplastic Transformation In Vitro: Relative Risk vs. Dose and Dose-Rate



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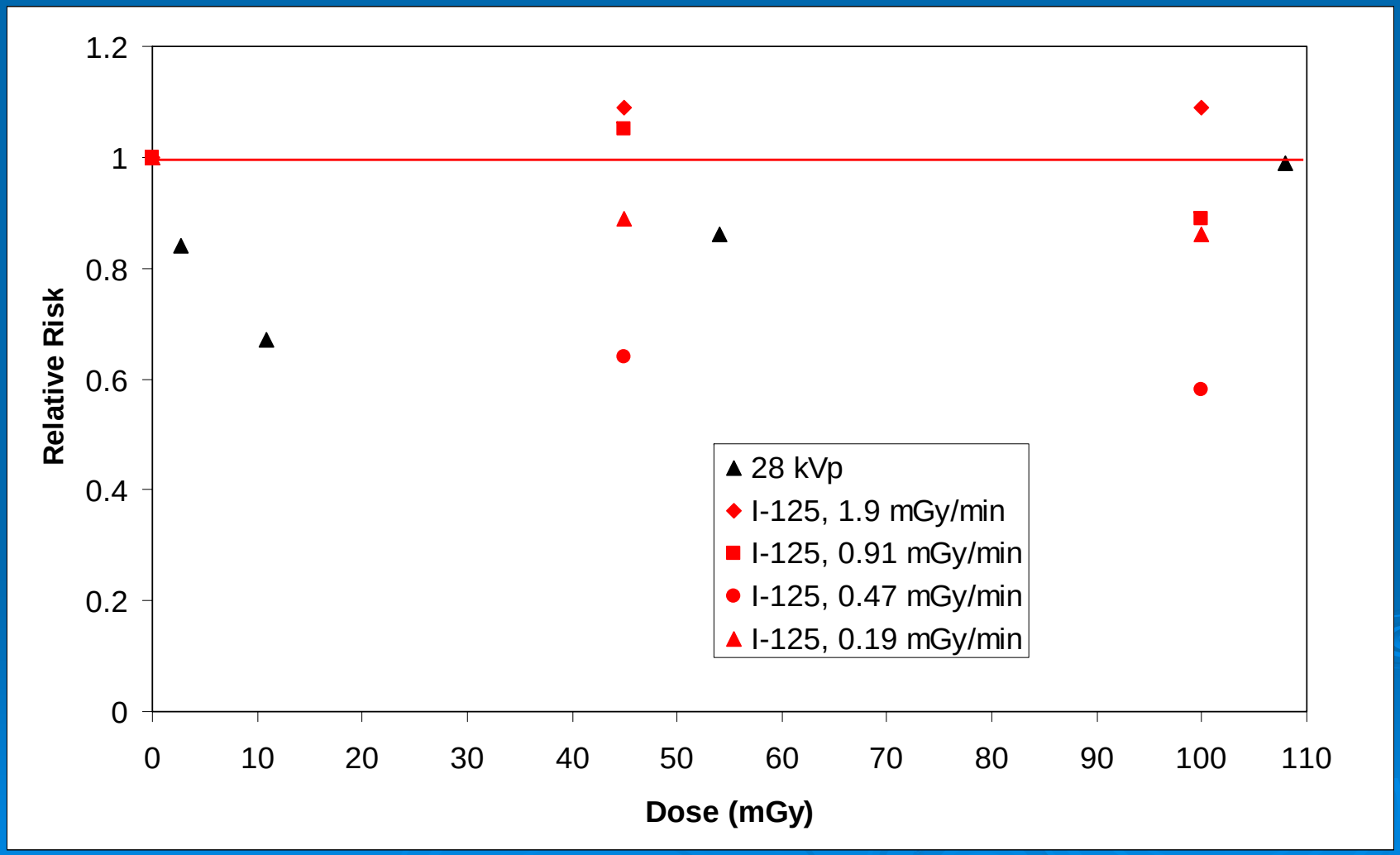


Neoplastic Transformation In Vitro

- Not unexpectedly, the data show that as the dose-rate is decreased the effectiveness of the radiation in inducing neoplastic transformation decreases.
- At dose-rates of 0.19 and 0.47 mGy/min no induction of transformation is seen up to a dose of 1000 mGy, and the data suggest that there may be a suppression of transformation at these dose-rates, i.e. certainly do not conform to the LNT model.

Neoplastic Transformation *In Vitro*

- Now to the $64,000 question! Is there any evidence for J-shaped dose-response curves in the animal and human epidemiological data?
- The answer is a qualified yes, i.e. there are trends in this direction but these are not significant.

Low Dose Radiation Carcinogenesis In Vivo

- Several *in vivo* studies of the effects of low doses of low LET radiation cannot rule out the notion of a threshold dose.
- Ullrich and Storer (1979) indicated thresholds of 0.22 Gy for myeloid leukemia, and 0.10 Gy for ovarian, pituitary and Harderian gland tumors, fit the data as well as the LNT model.

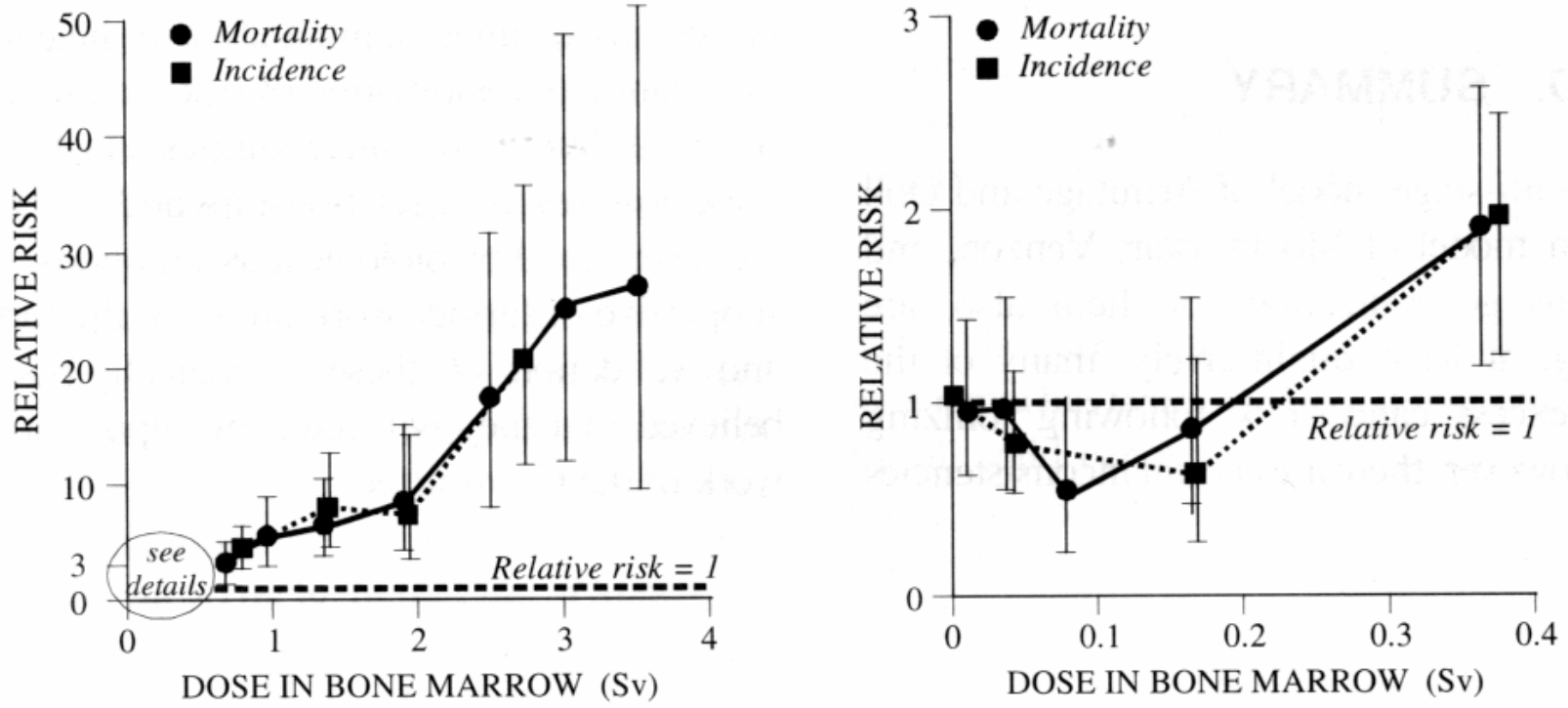
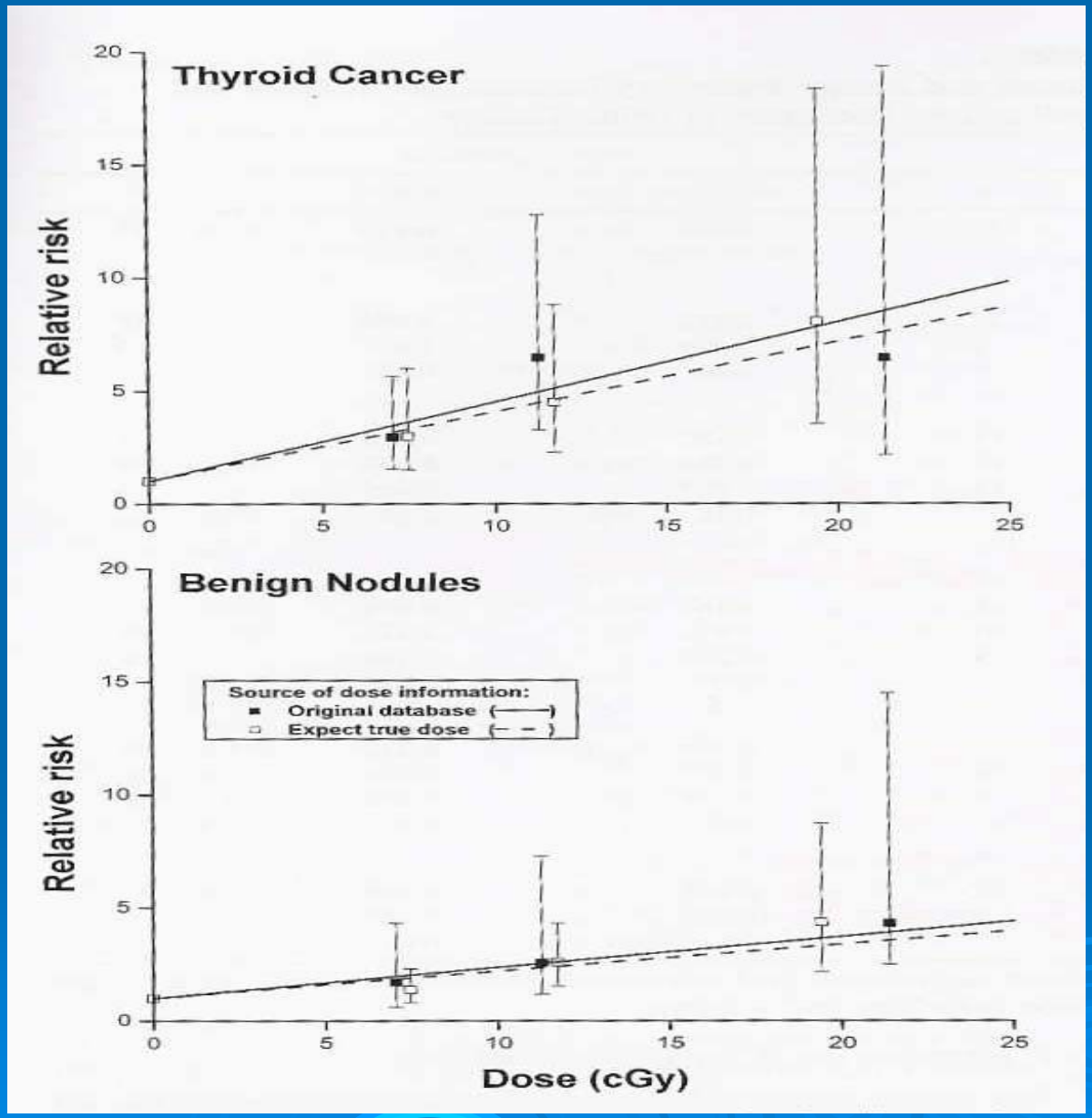


Figure XXVIII. Relative risk of leukaemia in survivors of the atomic bombings [L44].
The diagram on the right shows the low-dose region in detail.

UNSCEAR, 2000 from Little & Muirhead, 1998.

Low Dose Radiation Carcinogenesis In Vivo

- Little and Muirhead (IJRB 74:47-180, 1998) indicated that the dose-response was curved but with “absence of evidence of threshold”, yet a:
- Fit of a threshold model with a linear dose response above the threshold resulted in a best estimate of a threshold of 0.16 Sv (95% CI 0.05-0.40) with two-sided p=0.001 for departure from a threshold of zero
- Fit of a threshold model with a quadratic term above the threshold resulted in a best estimate of a threshold of 0.09 Sv (95% CI <0.00-0.29) with two-sided p=0.07 for departure from threshold



Lubin et al., Rad. Res. 161:359-368, 2004

Neoplastic Transformation In Vitro

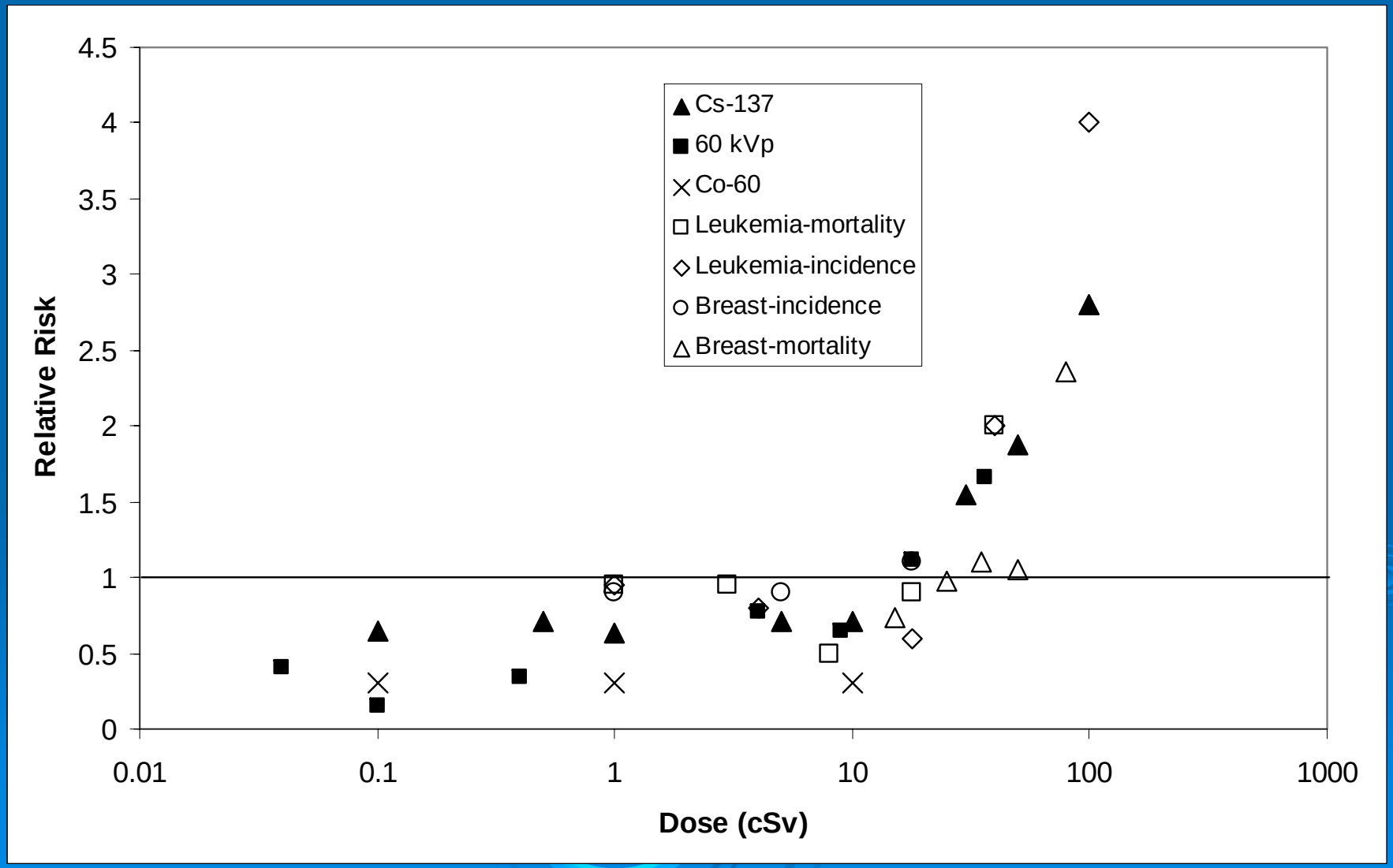
- It is clear that the degree of suppression and threshold dose is going to be target tissue-dependent
- It will also be very dose-rate dependent and there are very little human epidemiological data at low dose-rates
- Animal data at low dose-rate often show apparent thresholds for low LET radiation

Neoplastic Transformation In Vitro

- Can in vitro experiments provide data that can give an idea of the relative risk of cancer induction in humans?
- For a whole lot of reasons alluded to earlier one would say no. However, it is interesting that when compares relative risks from epidemiologic data with those from in vitro transformation data there is surprising agreement, at least for certain tumors.

Relative Risk vs. Dose

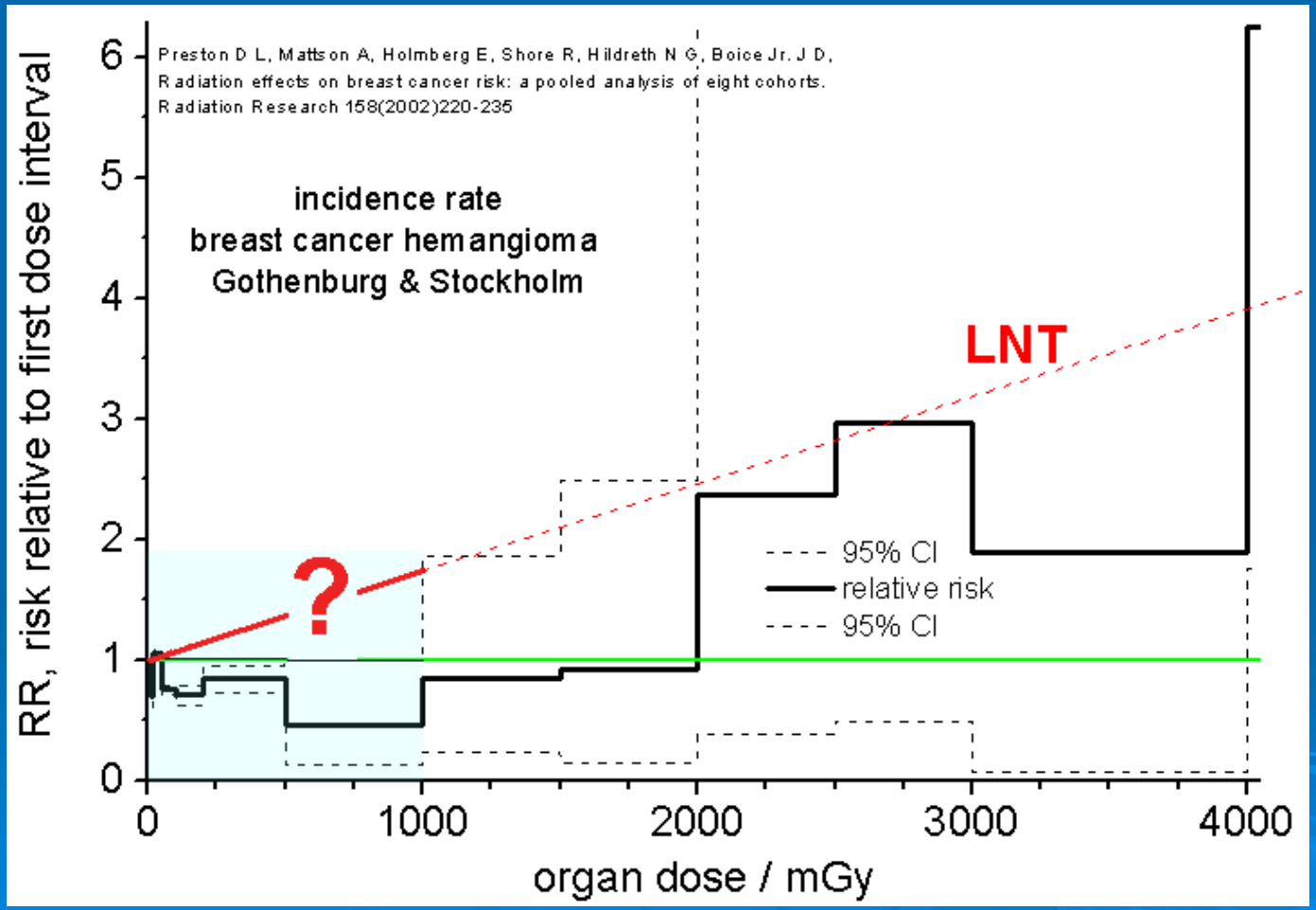
Comparison of In Vitro and Epidemiologic Data



Neoplastic Transformation In Vitro

- What about a comparison of risk estimations (epi vs. in vitro) for low dose-rate radiation?
- Very little epi data for chronic exposures), but there is a study from Sweden (Lundell et. al., Rad. Res. 151:626-632, 1999) for breast cancer as a result of low dose-rate (median dose rate to breast of 0.4 mGy/min) radium plaque treatments for hemangiomas.
- Data from this study is included in a paper on radiation-induced breast cancer by Preston et al., Rad. Res. 158:220-235, 2002).
- Both of these papers concluded that the data were consistent with the LNT model BUT Lundell did indicate that only at doses >1 Gy was there a positive association with breast cancer risk.

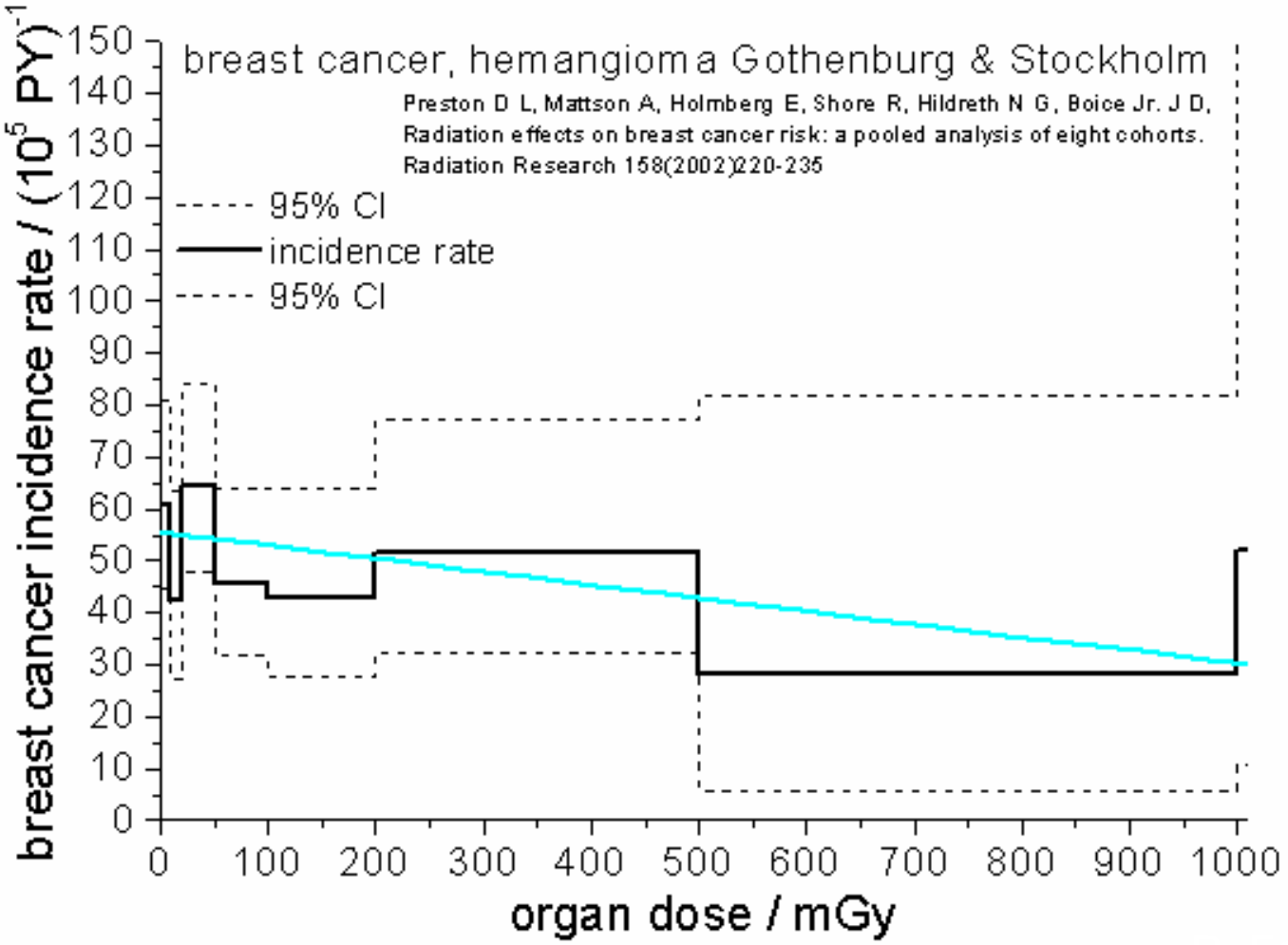
Relative Risk vs. Dose
Breast cancer following LDR treatment for hemangioma



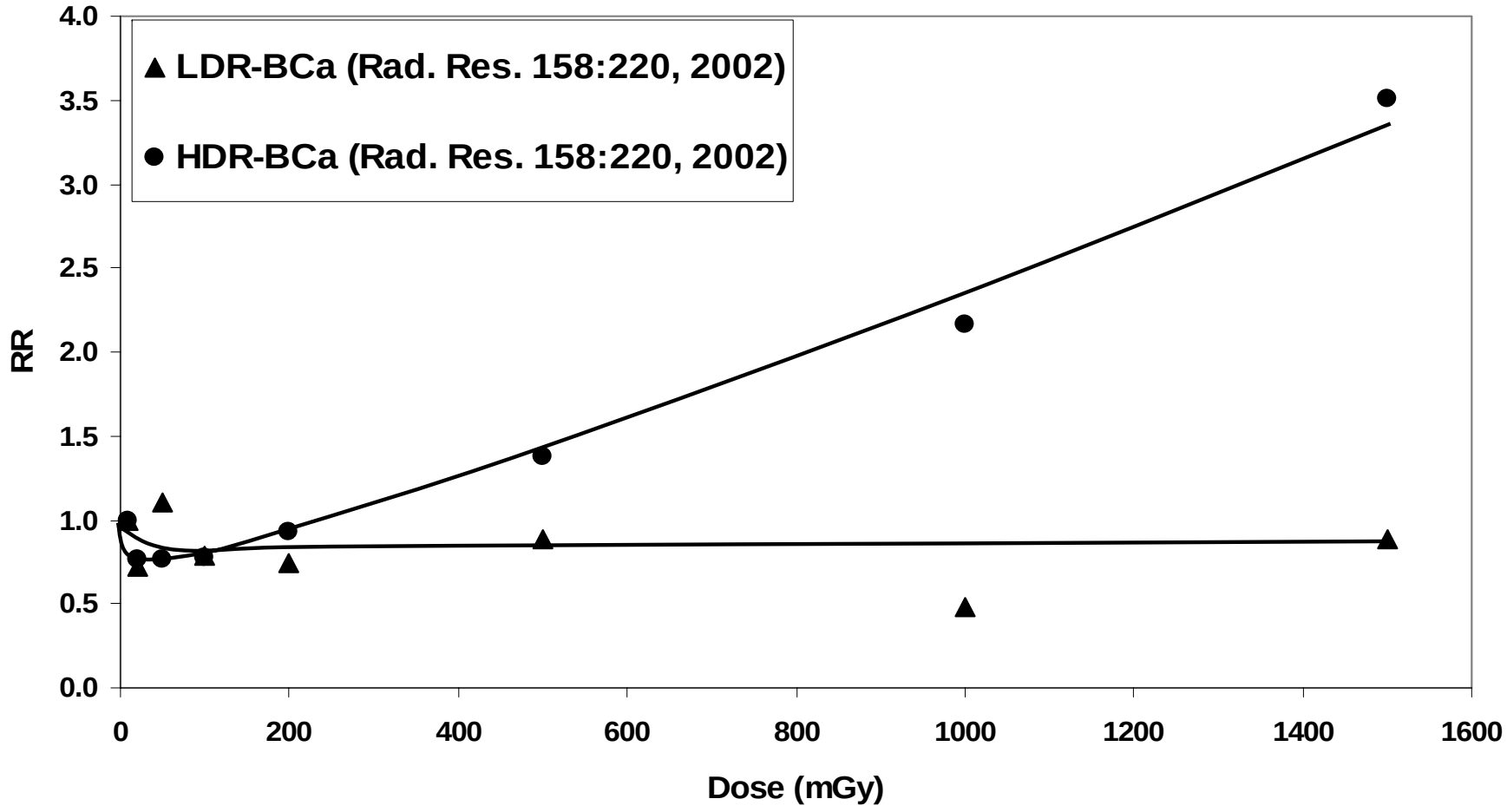
G. Stanford

Breast Cancer Incidence Rate vs. Dose

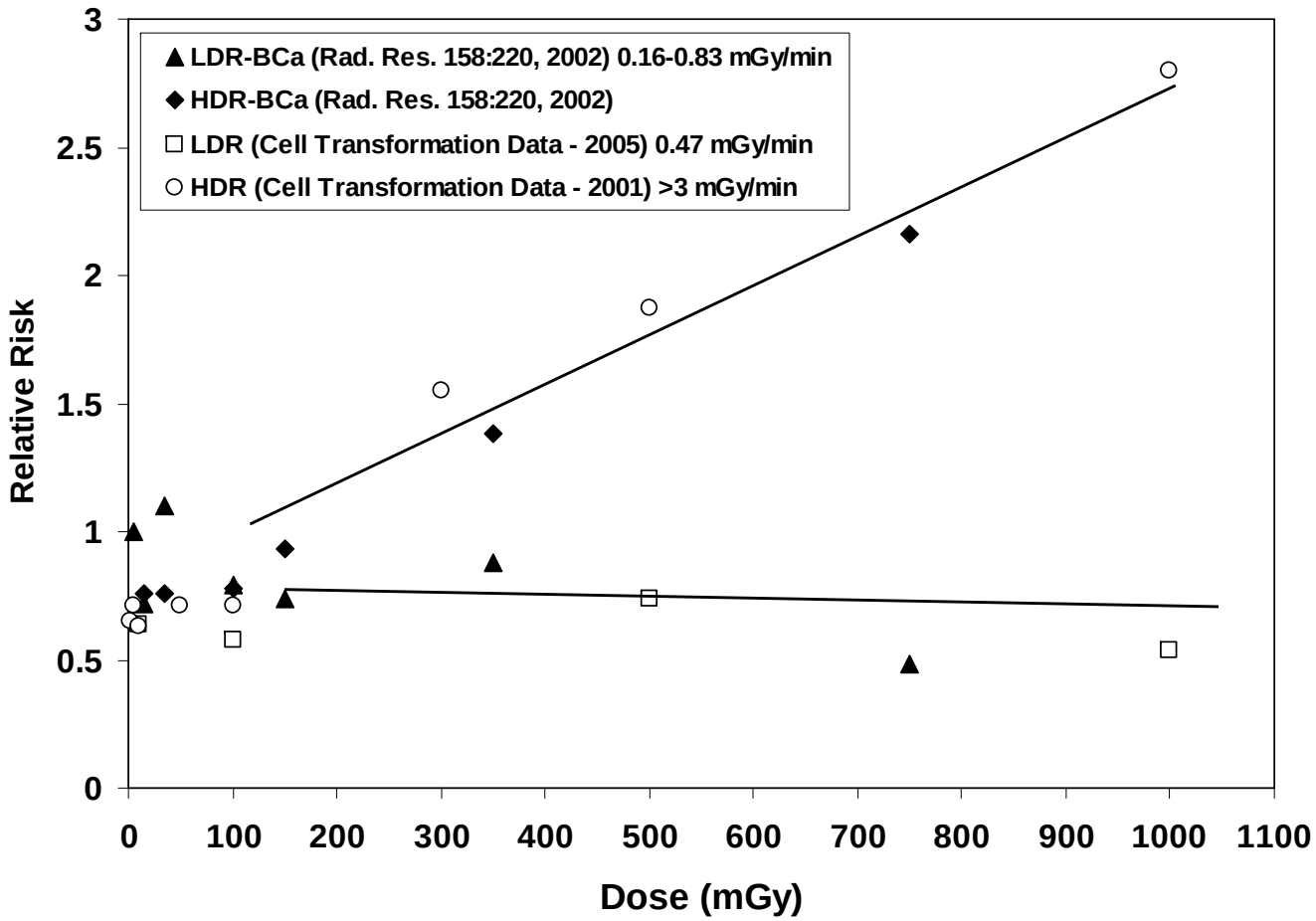
Breast cancer following LDR treatment for hemangioma



Relative Risk vs. Dose for Breast Cancer Comparison of HDR and LDR Epidemiologic Data



Comparison of Relative Risk for Transformation with Breast Cancer Induction at High and Low Dose-Rates



Neoplastic Transformation *In Vitro*, Hormesis and Risk Assessment

- In conclusion, neoplastic transformation *in vitro* has demonstrated strong evidence for hormetic effects of low doses of low-LET radiation at both high and low dose-rates.
- Neoplastic transformation *in vitro* has proven capable of making relative risk estimates of cancer incidence that compare well with those seen epidemiologically for breast cancer and leukemia.

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GRAY CANCER INST.
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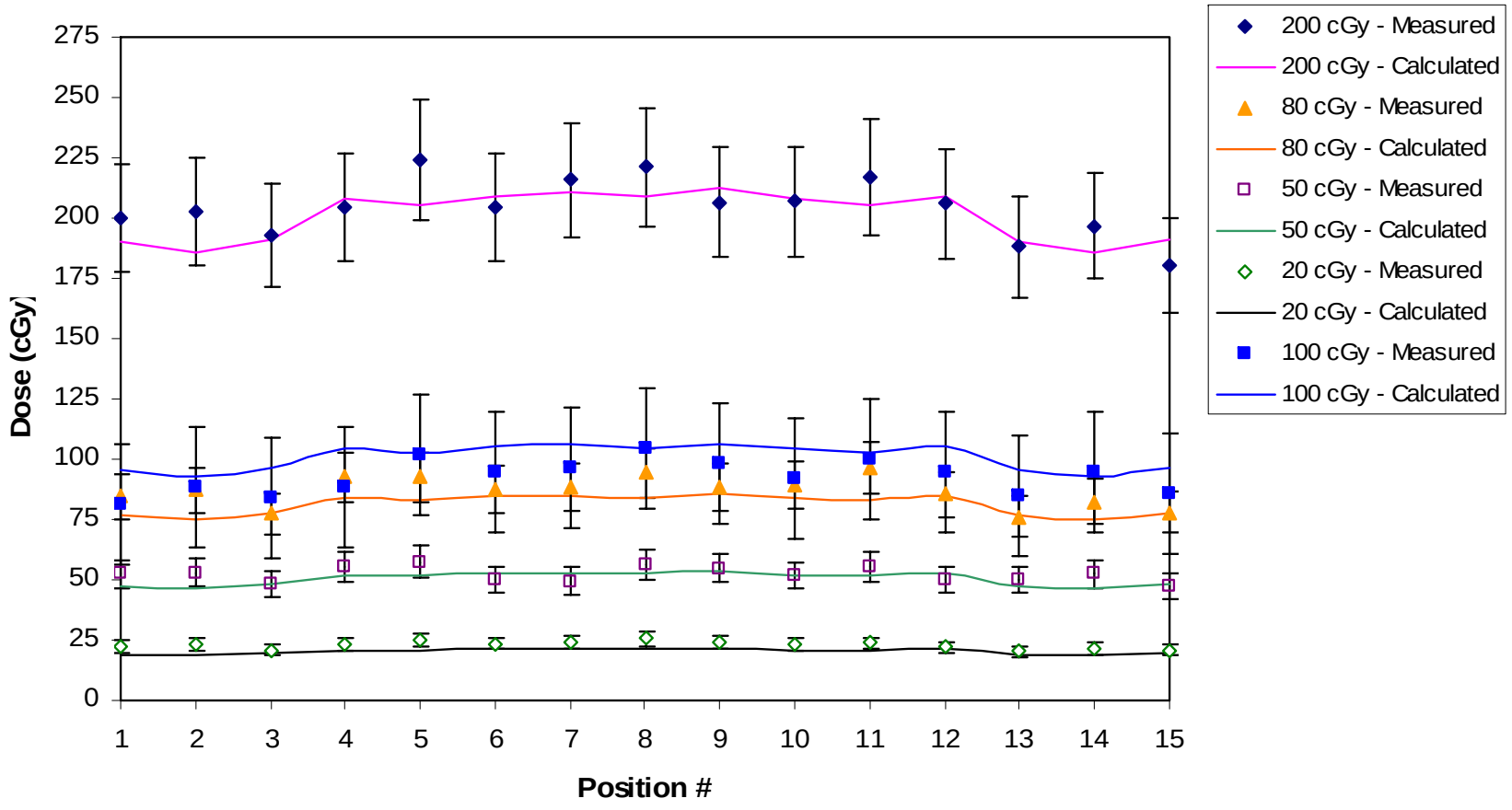
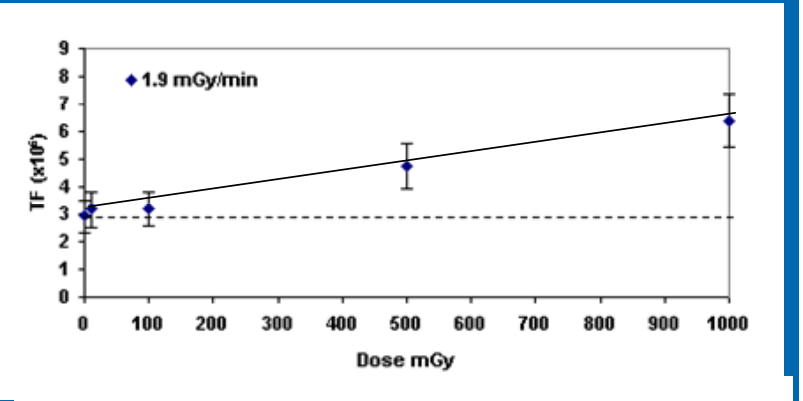
Michael Woodcock

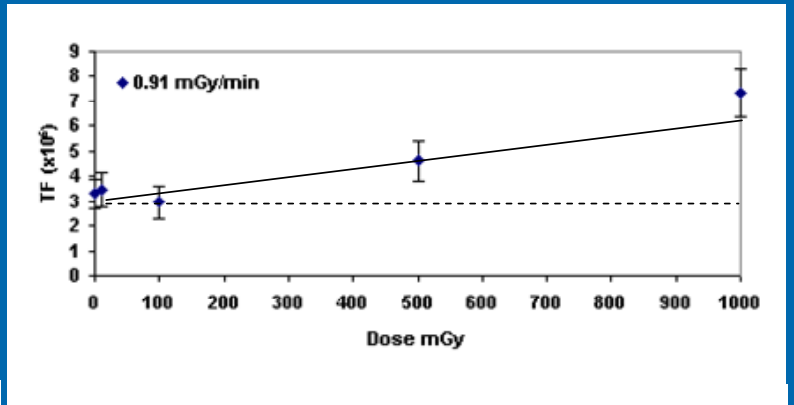
UTSW
Yi-Ching Lio
David Chen

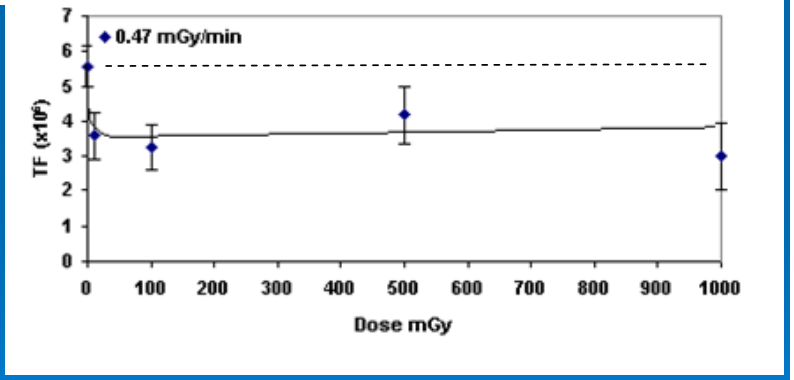
Dose Distribution

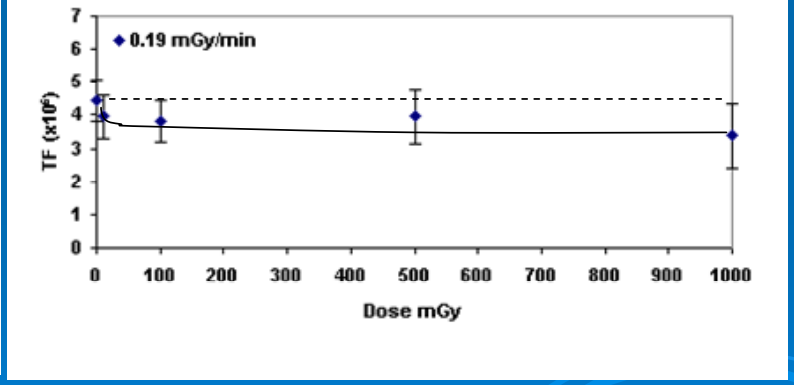


Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates



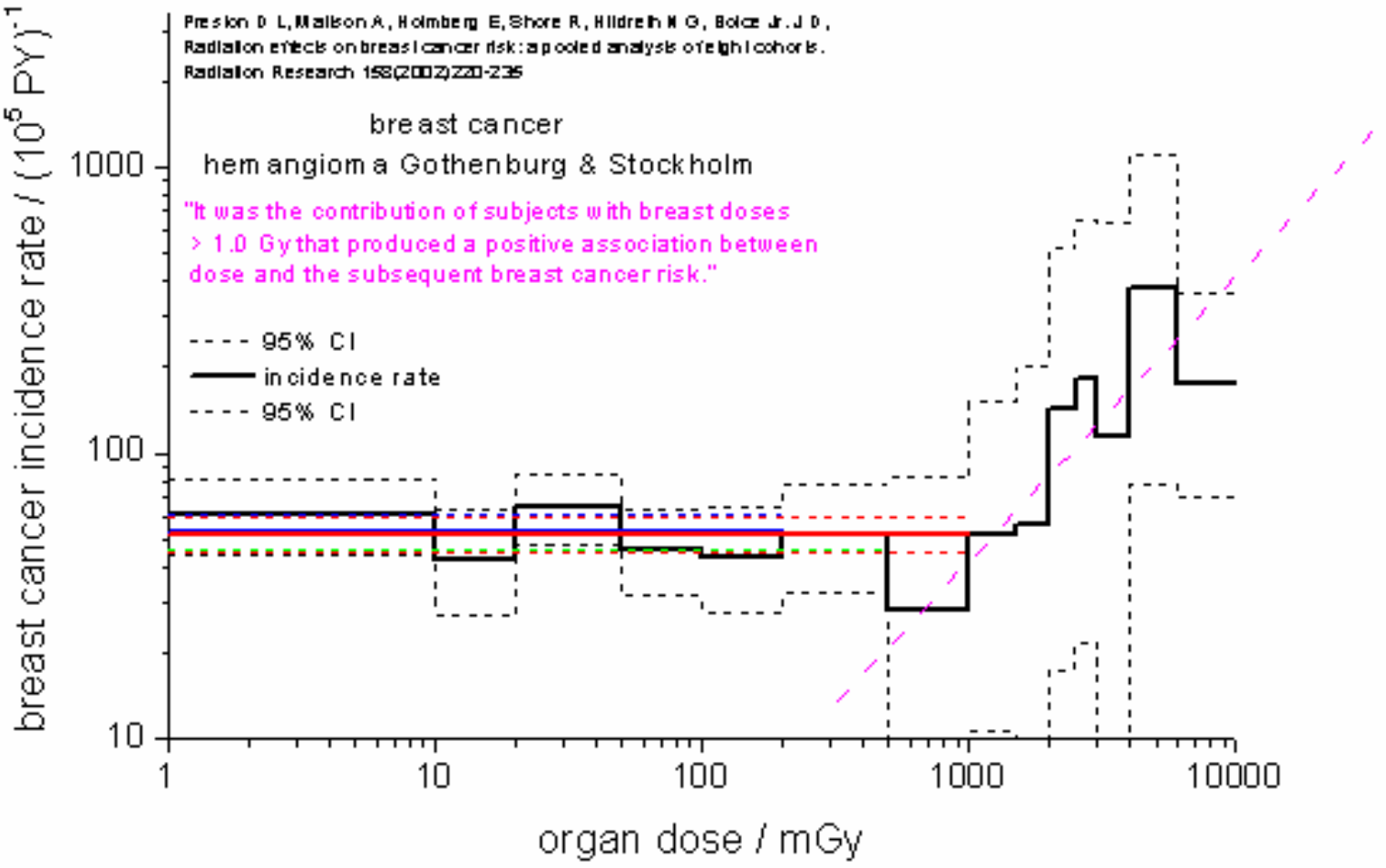




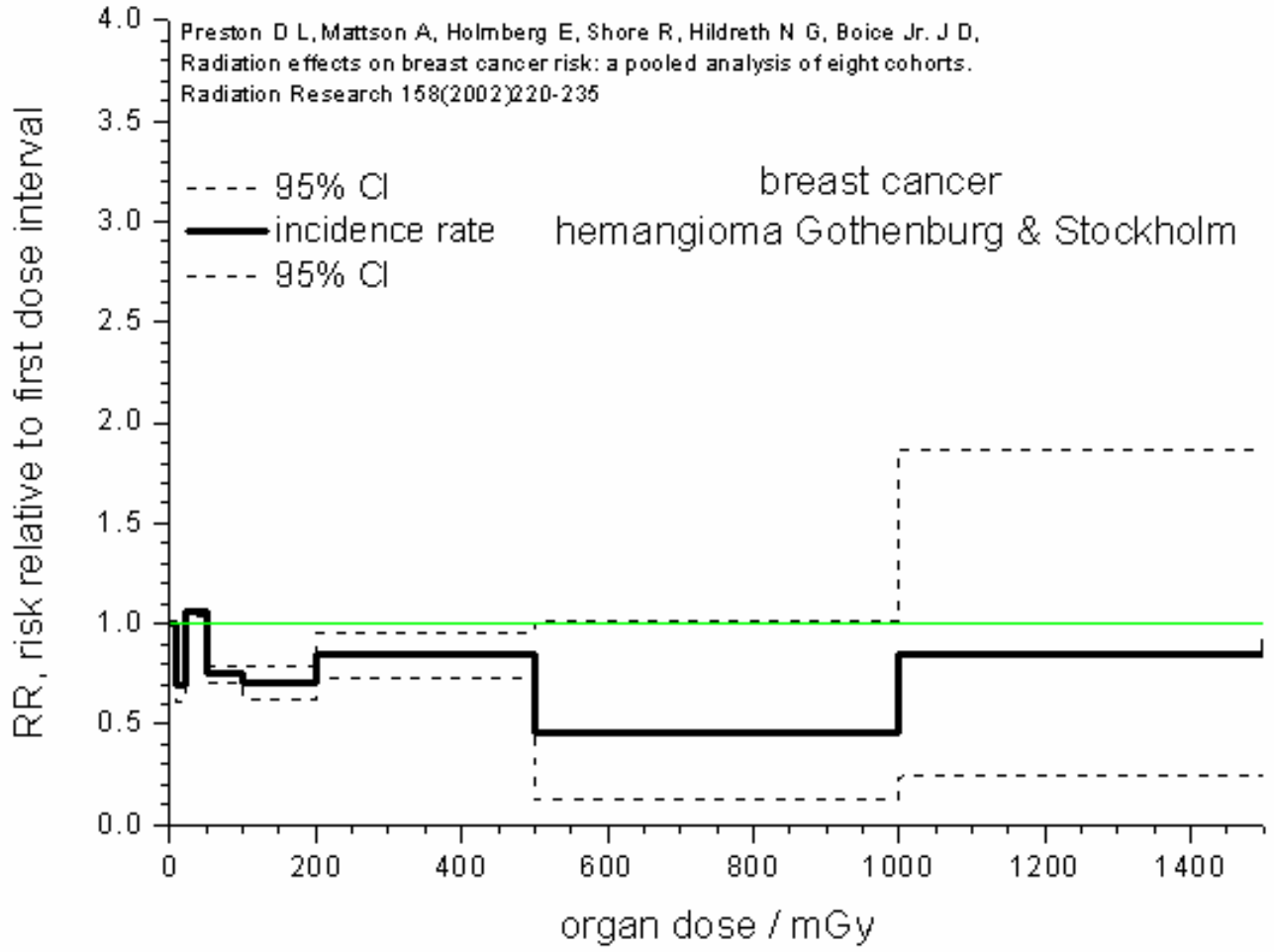


Breast Cancer Incidence Rate vs. Dose

Breast cancer following LDR treatment for hemangioma



Relative Risk vs. Dose
Breast cancer following LDR treatment for hemangioma



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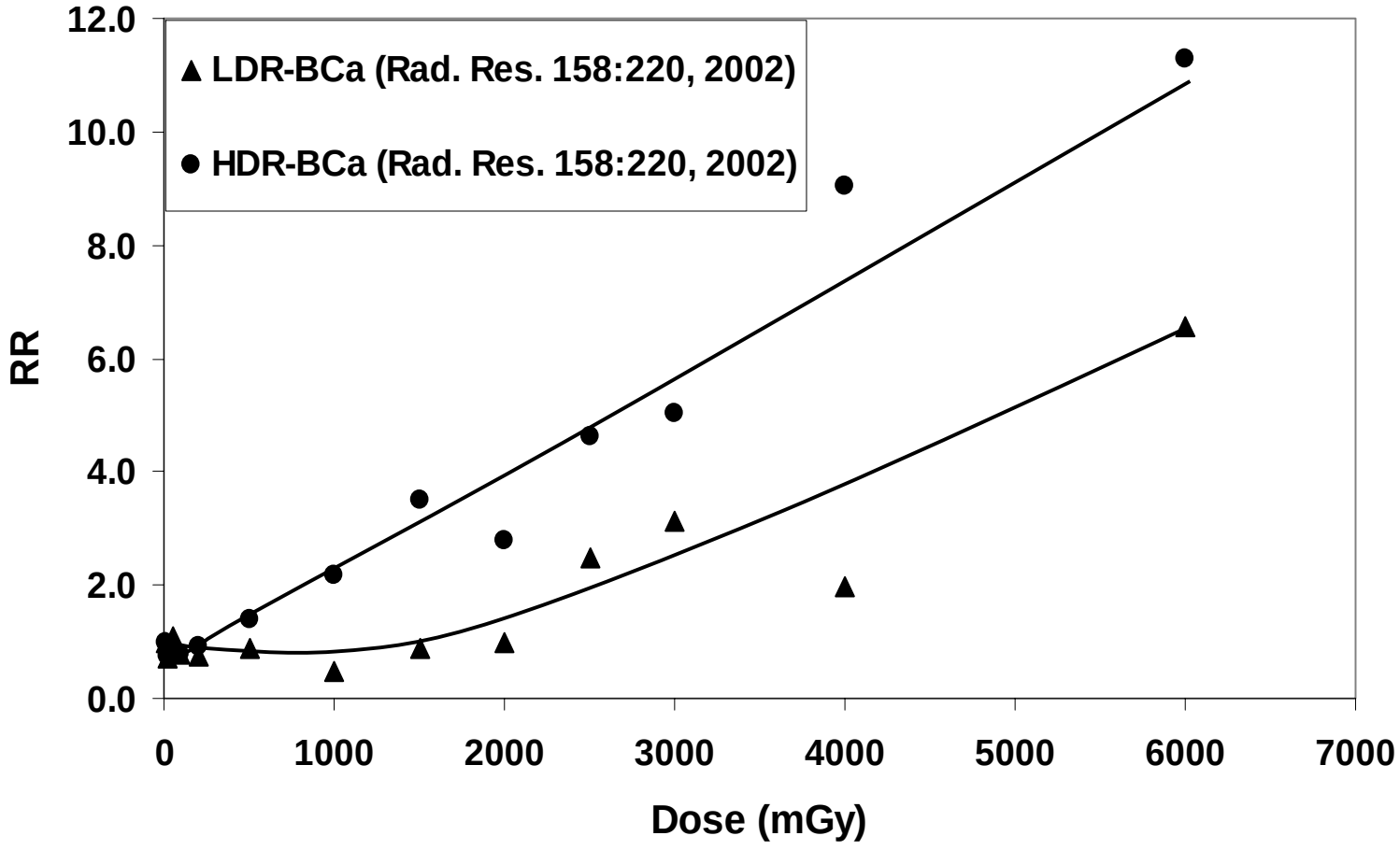
Relative Risk vs. Dose for Breast Cancer Comparison of HDR and LDR Epidemiologic Data



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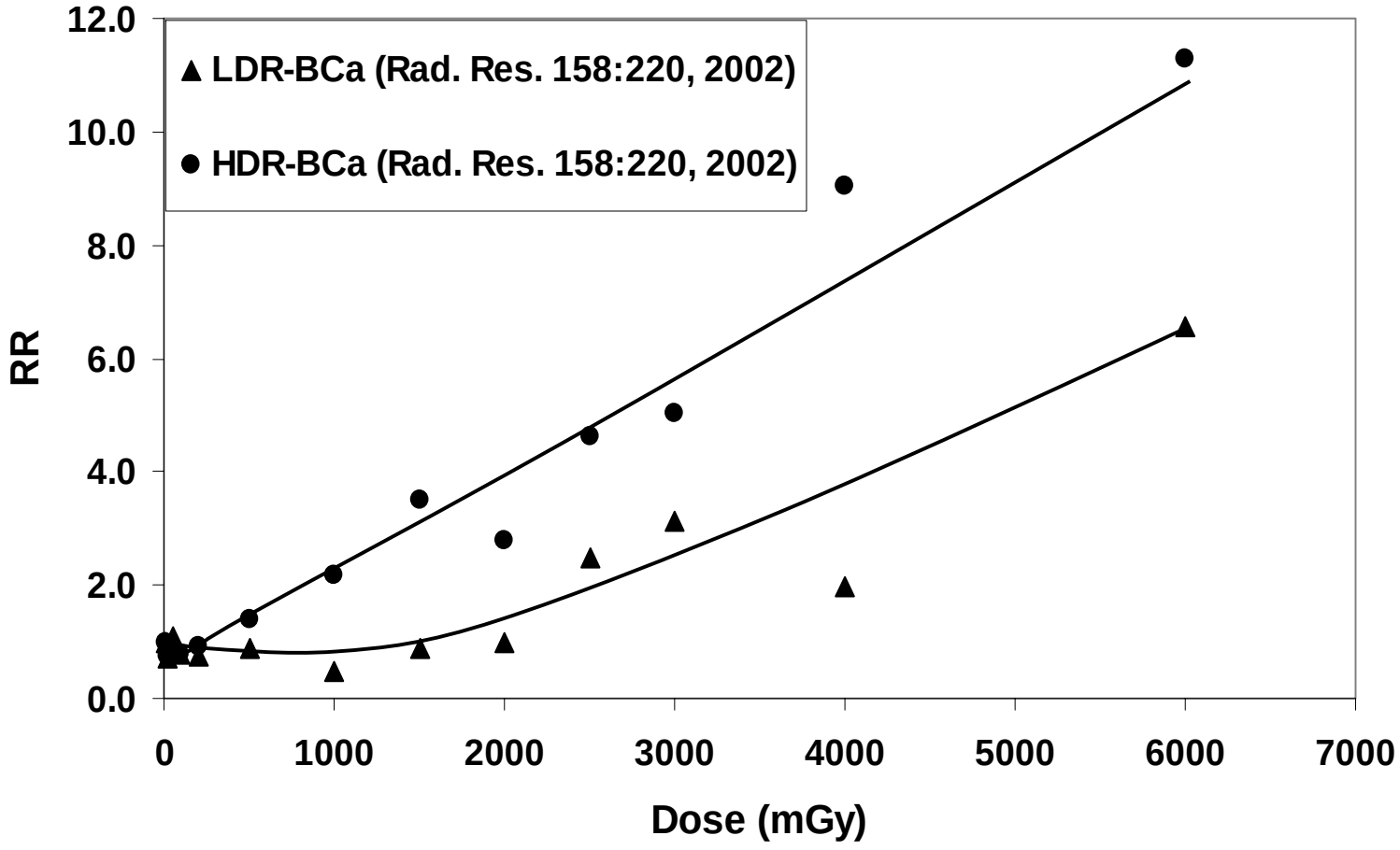

The shape of the dose response curve for radiation-induction of cancer by low LET radiation – Two official positions

- BEIR VII Phase 2 report from the U.S. National Academy of Sciences supports the LNT model down to zero dose.
- The French Academy of Sciences and National Academy of Medicine does not support the LNT model at doses <100 mSv.
- The French committee was of the opinion that the evidence from laboratory, and some epidemiology studies, was sufficiently strong to indicate that the LNT model will likely overestimate risk at doses <100 mSv, and almost certainly at doses <10 mSv.

Neoplastic Transformation *In Vitro*, Hormesis and Risk Assessment

- In conclusion, neoplastic transformation *in vitro* has been demonstrated to describe dose-response curve shapes that are consistent with those seen in epidemiologic studies of radiation-induced breast cancer and leukemia.