# Non-Targeted Effects and the Dose Response for Heavy Ion Tumorigenesis

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## Abstract

BACKGROUND

There is no human epidemiology data available to estimate the heavy ion cancer risks experienced by astronauts in space. Studies of tumor induction in mice are a necessary step to estimate risks to astronauts. Previous experimental data can be better utilized to model dose response for heavy ion tumorigenesis and plan future low dose studies.

DOSE RESPONSE MODELS

The Harderian Gland data of Alpen et al.[1-3] was re-analyzed [4] using non-linear least square regression. The data set measured the induction of Harderian gland tumors in mice by high-energy protons, helium, neon, iron, niobium and lanthanum with LET's ranging from 0.4 to 950 keV/micron. We were able to strengthen the individual ion models by combining data for all ions into a model that relates both radiation dose and LET for the ion to tumor prevalence. We compared models based on Targeted Effects (TE) to one motivated by Non-targeted Effects (NTE) that included a bystander term that increased tumor induction at low doses non-linearly. When comparing fitted models to the experimental data, we considered the adjusted $R^2$, the Akaike Information Criteria (AIC), and the Bayesian Information Criteria (BIC) to test for Goodness of fit. In the adjusted $R^2$ test, the model with the highest $R^2$ values provides a better fit to the available data. In the AIC and BIC tests, the model with the smaller values of the summary value provides the better fit. The non-linear NTE models fit the combined data better than the TE models that are linear at low doses. We evaluated the differences in the relative biological effectiveness (RBE) and found the NTE model provides a higher RBE at low dose compared to the TE model.

POWER ANALYSIS

The final NTE model estimates were used to simulate example data to consider the design of new experiments to detect NTE at low dose for validation. Power and sample sizes were calculated for a variety of radiation qualities including some not considered in the Harderian Gland data set and with different background tumor incidences. We considered different experimental designs with varying number of doses and varying low doses dependant on the LET of the radiation. The optimal design to detect a NTE for an individual ion had 4 doses equally spaced below a maximal dose where "bending" due to cell sterilization was < 2%. For example at 100 keV/micron we would irradiate at 0.03 Gy, 0.065 Gy, 0.13 Gy, and 0.26 Gy and require 850 mice including a control dose for a sensitivity to detect NTE with 80% power. Sample sizes could be improved by combining ions similar to the methods used with the Harderian Gland data.

## Color Categories

Uncertainty Reduction/Risk Mitigation Category Color = Green

Mechanistic/Descriptive Category Color = Light Yellow

## Blue Bullets Progress

- Applying a NTE model motivated by the dose response observed for bystander effects and genomic instability in cell culture, we show that the NTE model provides a superior fit to the dose response for tumors in mice based on several model ranking tests. These results add important empirical evidence in support of the NTE model based on *in vivo* data for tumor responses.
- We were able to fit models for the LET dependence of tumors over a broad range from protons to heavy ions and describe a dose dependent RBE applicable to low dose exposures.
- Power analysis based on the Harderian gland data suggest the optimal number of mice to be studied for future dose response experiments and suggest sample size reductions will occur when results for several radiation qualities are combined into a single model.

## Red Bullets Gaps in Progress and Knowledge

- The relative contribution to cancer risks from targeted effects and non-targeted effects remains elusive with too few experiments designed to test the shape of the dose response at low doses (<0.3 Gy) applicable to space missions.
- Only a few murine model tumors have been studied with only a few ion types. The paucity of data limits the building and testing of models of cancer risk from space radiation.
- The usage of human cell culture models in 2D or 3D is needed to support the applicability of murine models to human risk prediction, however much work remains in making the necessary connections. Of importance is the need for more expansive data set on radiation quality at a variety of low doses to understand the shape of the dose response for cancer processes induced by heavy ions.

## Description of Alpen data.

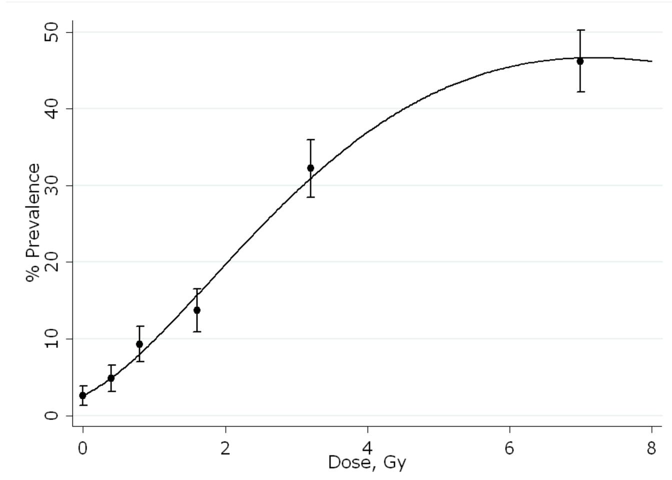
| Ion | Energy (MeV/u) | LET (keV/µm) |
|---|---|---|
| Hydrogen | 250 | 0.4 |
| Helium | 228 | 1.6 |
| Neon | 670 | 25 |
| Iron | 600 | 193 |
| Iron | 350 | 253 |
| Niobium | 600 | 464 |
| Lanthanum | 593 | 953 |

- Measured Harderian gland tumors in female $B6CF_1$/Anl mice.
- A variety of heavy ions were accelerated at the Lawrence Berkeley National Lab for the exposures.
- Mice were irradiated at 100 to 120 days.
- Tumor prevalence was determined 16 months after irradiation.
- Tumor appearance was accelerated using pituitary implants
  - Reduced the effects of competing tumor risks and reduced costs of experiments (sacrifice at 600 d compared to lifespan of ~900 d)
- Fe nuclei showed same tumor response with or without the pituitary implants.
- γ-rays were used as a low-LET reference radiation

## γ-ray TE model relating dose to tumor prevalence

$$P_{\mathrm{TE}} = P_0 + [\underbrace{\alpha D + \beta D^2}_{\text{Dose induction term}}]\underbrace{e^{-\lambda D}}_{\text{Cell sterilization term}}$$

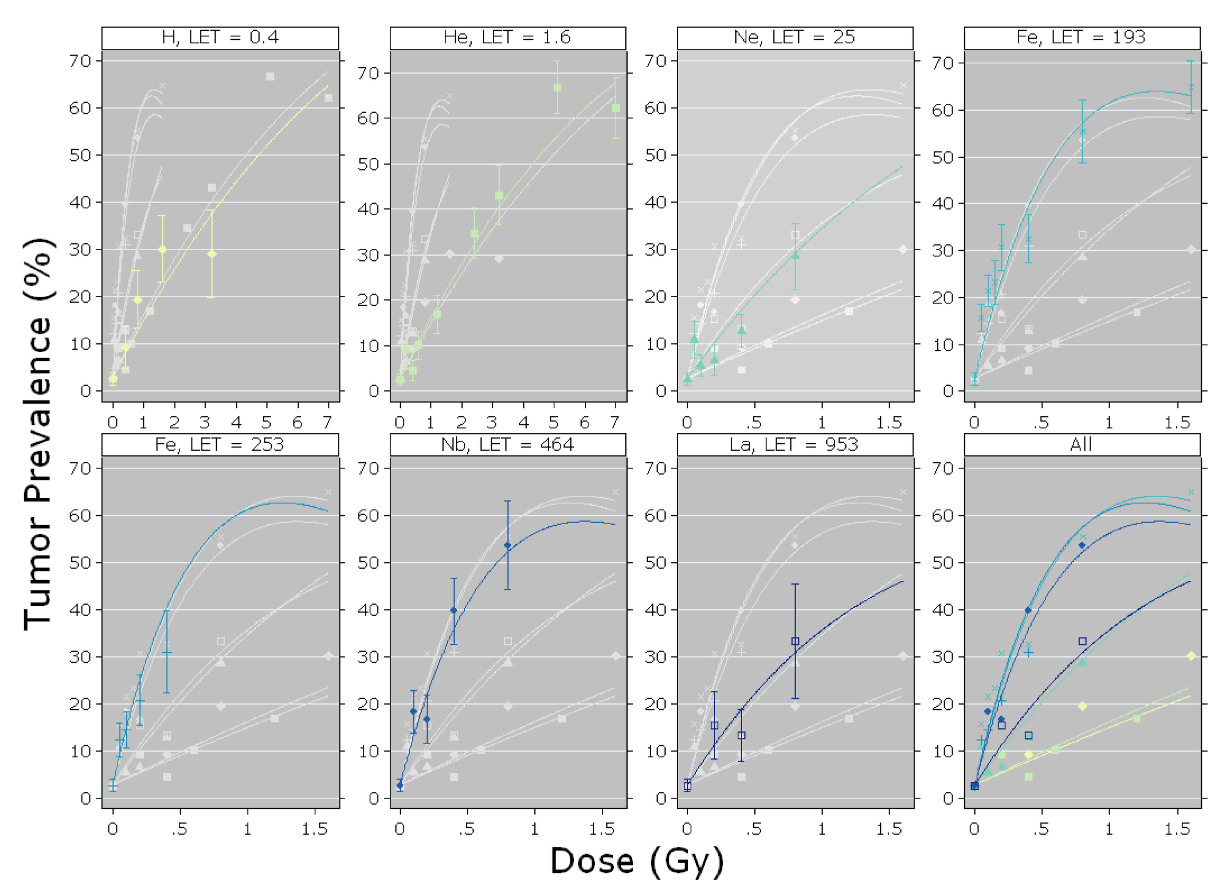
| | Estimate | Standard Error |
|---|---|---|
| $P_0$ | 2.64 | 0.77 |
| α | 4.31 | 3.09 |
| β | 5.08 | 2.25 |
| λ | 0.264 | 0.055 |




## Heavy ion TE model relating dose and LET to tumor prevalence

$$P_{\mathrm{TE}} = P_0 + [\alpha(L)D]e^{-\lambda(L)D}$$
$$\alpha(L) = a_0 + a_1 L \exp(-a_2 L)$$
$$\lambda(L) = \lambda_0 + \lambda_1 L \exp(-\lambda_2 L)$$

| | Estimate | Standard Error |
|---|---|---|
| $P_0$ | 2.8 | 0.48 |
| $a_0$ | 12.39 | 2.2 |
| $a_1$ | 1.17 | 0.21 |
| $a_2$ | 0.00369 | 0.00064 |
| $\lambda_0$ | 0.0504 | 0.0298 |
| $\lambda_1$ | 0.0068 | 0.0029 |
| $\lambda_2$ | 0.0033 | 0.002 |




Adjusted $r^2$ = 0.9296, AIC = 210, BIC = 222.1

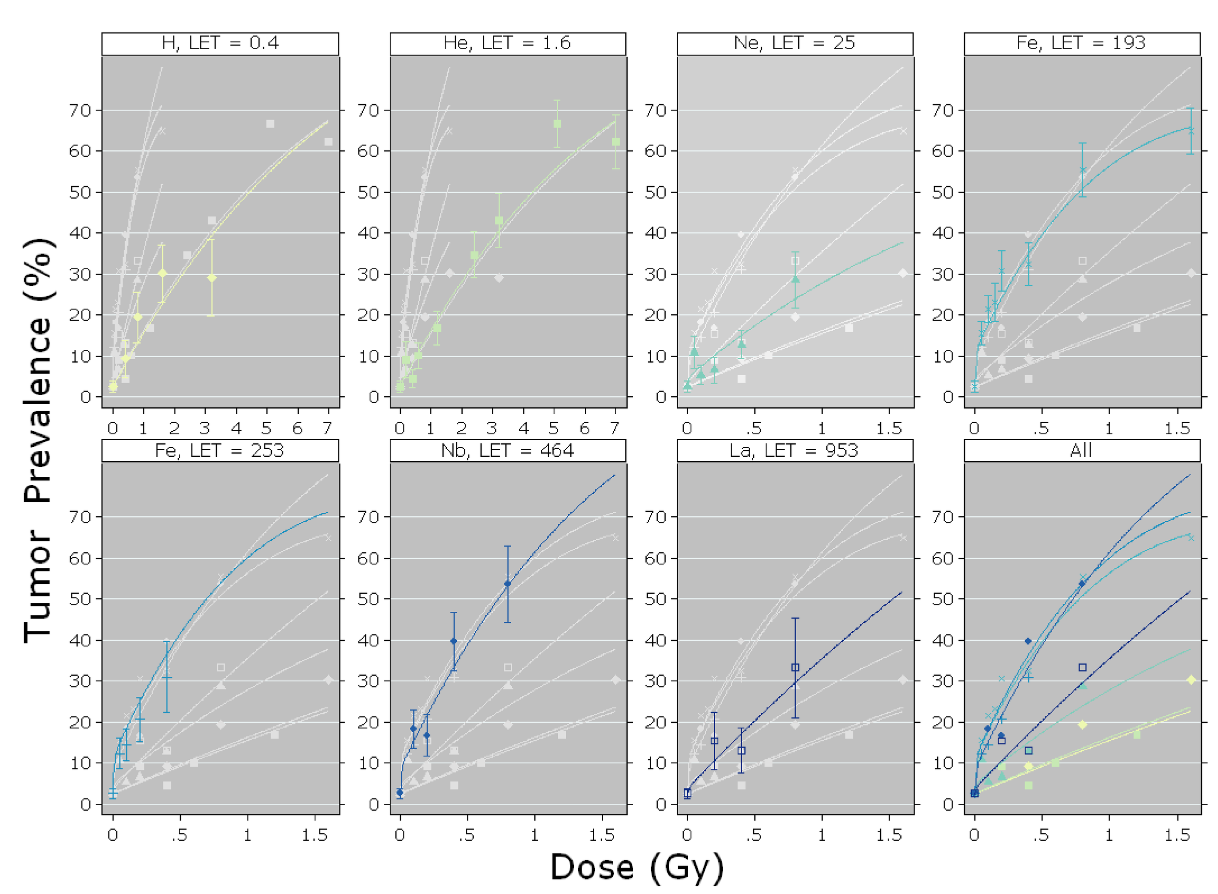
## Heavy ion NTE model relating dose and LET to tumor prevalence

$$P_{\mathrm{NTE}} = P_0 + [\alpha(L)D]e^{-\lambda(L)D} + [\kappa_1 L \exp(-\kappa_2 L)]\Theta(D_{th})e^{-\lambda(L)D}$$

$\Theta(D_{th})$ represents a step function with threshold dose ($D_{th} = 0.01$Gy) for the bystander effect.

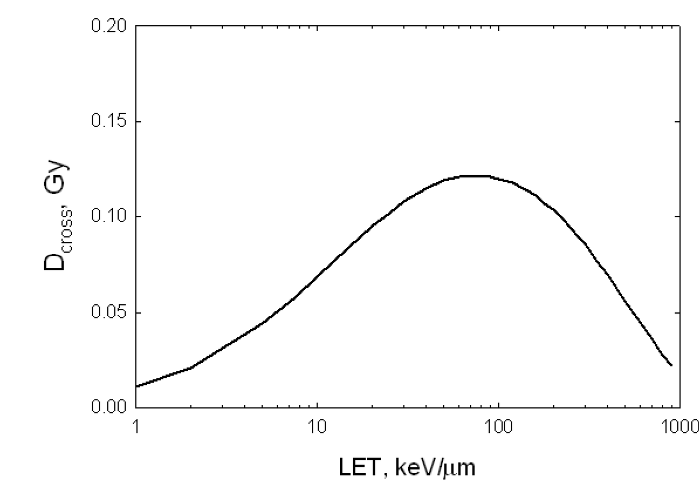
| | Estimate | Standard Error |
|---|---|---|
| $P_0$ | 2.44 | 0.41 |
| $a_0$ | 13.60 | 1.87 |
| $a_1$ | 0.64 | 0.21 |
| $a_2$ | 0.00353 | 0.00081 |
| $\lambda_0$ | 0.055 | 0.025 |
| $\lambda_1$ | 0.0059 | 0.0055 |
| $\lambda_2$ | 0.0055 | 0.0048 |
| $\kappa_1$* | 0.107 | 0.054 |
| $\kappa_2$* | 0.0045 | 0.0020 |

*P-values are 0.056 and 0.03 respectively.

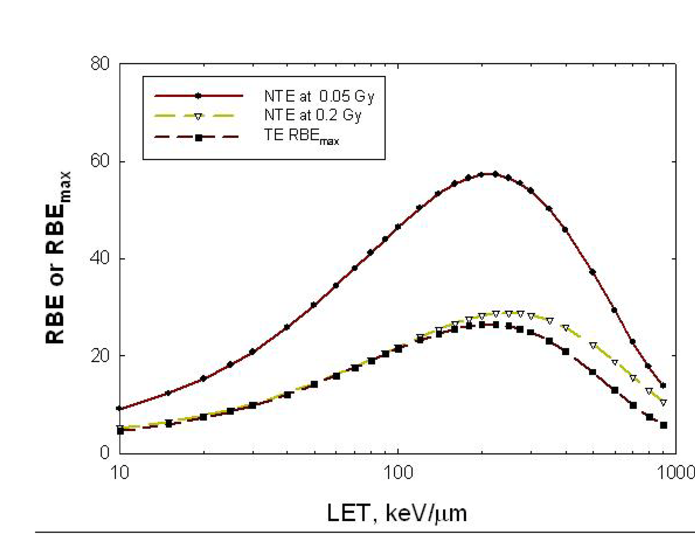



Adjusted $r^2$ = 0.9514, AIC = 195.9, BIC = 211.6

## Applications of Models for Risk Estimation




Cross-over dose is defined as the dose where TE = NTE




A dose dependent relative biological effectiveness (RBE) is found in the NTE model

## Power Analysis Specifications

- The study focuses on low doses where the cell sterilization term can be ignored.
- Parameter estimates from the NTE model were used to generate simulations for each ion individually.
- Simulations were analyzed using generalized linear models with binomial errors following the low dose model.
- Power was determined by the ability to detect if NT Effects ($\kappa$) are significant at low dose
- Doses were chosen at 0Gy and 3 or 4 irradiation doses chosen as seen in the figure with sample sizes such that all doses have equal binomial variances.
- Dosing Schemes for irradiated mice:
  1. Three radiation doses (D1, D2, and D3 from figure) were considered and d ≈ 2%.
  2. Three radiation doses were considered and d ≈ 1%.
  3. Four radiation doses (D1, D2, D3, and D4 from figure) were considered and d ≈ 2%.

**Equation: The individual NTE Model**

$$P_{ion} = P_0 + [\alpha D + \kappa\Theta(D_{th})]e^{-\lambda D}$$

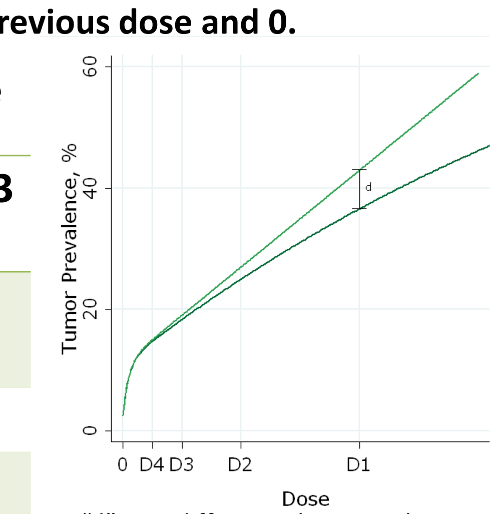
**Equation: The linear low dose Model**

$$P_{ion} = P_0 + \alpha D + \kappa\Theta(D_{th})$$

**Table: The LET values chosen for the study and parameter estimates from the NTE model**

| | α | λ | κ |
|---|---|---|---|
| LET = 70 | 48.59 | 0.317 | 5.47 |
| LET = 100 | 58.56 | 0.372 | 6.82 |
| Fe, LET = 193 | 76.01 | 0.449 | 8.62 |

**Figure: Representation of how doses were chosen. Each additional dose is half way between the previous dose and 0.**




"d" is %-difference between linear low dose model (light green) and the individual NTE models (dark green)

**Table: Sensitivity of sample size to detect NTE with 80% power to the scheme choice and background %-Prevalence ($P_0$).**

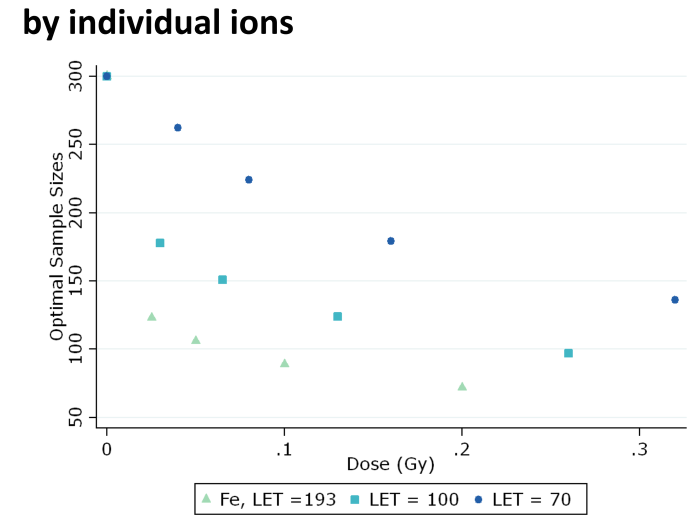
| | Scheme 1 $P_0$ = 2.44 | Scheme 2 $P_0$ = 2.44 | Scheme 3 $P_0$ = 2.44 | Scheme 3 $P_0$ = 5 | Scheme 3 $P_0$ = 10 |
|---|---|---|---|---|---|
| Fe, LET = 193 | 900 | 1031 | 690 | 810 | 1069 |
| LET = 100 | 1200 | 1180 | 850 | 1061 | 1810 |
| LET = 70 | 1620 | 1620 | 1101 | 1600 | >2100* |
| Total** | 3120 | 3231 | 2041 | 2871 | >4379 |

*Power = 68.27% **Assuming controls same across the 3 ions

## Power Analysis Conclusions

- Dosing Scheme 3 was optimal.
- More mice are needed as the value of $P_0$ increases.
- The sample size estimates to test for NTE for single ions will over-estimate sample size requirements when results from several ions are combined into a model that describes the LET dependence of the tumor response.

**Figure: Doses and sample sizes for Scheme 3 by individual ions**




**Source of tumor data for heavy ions**

[1] Fry RJM, Powers-Risius P, Alpen EL, Ainsworth EJ. High LET radiation carcinogenesis. *Radiat. Res.* ***104: S188–195 (1985).***

[2] Alpen EL, Powers-Risius P, Curtis SB, DeGuzman R: Tumorigenic potential of high-Z, high-LET charged particle radiations. *Radiat. Res.* ***88: 132–143 (1993).***

[3] Alpen EL, Powers-Risius P, Curtis SB, DeGuzman R, Fry RJM. Fluence-based relative biological effectiveness for charged particle carcinogenesis in mouse Harderian gland. *Adv. Space Res.* ***14: 573–581 (1994).***

**Related Literature**

[4] Cucinotta FA, and Chappell LJ, Non-targeted Effects and the Dose Response for Heavy Ion Tumor Induction. *Mut. Res.* ***687: 49-53 (2010).***

[5] Weil MM, Bedford JS, Bielefeldt-Ohmann H, Ray AF, Gernik PC, Ehrhart EJ, Falgren CM, Hailu F, Battaglia CLR, Charles C, Callan MA, and Ullrich RL. Incidence of acute myeloid leukemia and hepatocellular carcinoma in mice irradiated with 1 GeV/nucleon $^{56}Fe$ ions. *Radiat. Res.* ***172: 213-219 (2009).***

[6] Dicello JF, Cucinotta, FA, Gridley, D, Mann J, Moyers MF, Novak GR, Piantadosi S, Ricart-Arbona R, Simonson DM, Williams, JR, Zhang Y, Zhou H, and Huso D *In vivo* mammary tumorigenesis in the sprague-dawley rat and microdosimetric correlates. *Phys. Med. Biol.* ***49: 3817–3830 (2004).***

[7] Cucinotta FA and Wilson JW. Initiation-promotion model for tumor prevalence from high energy and charge radiation. *Phys. Med. Biol.* ***39: 1811-1831 (1994).***