Synergy theory: murine Harderian gland tumors and *in vitro* chromosome aberrations induced by exposure to mixed beams with some high-LET components

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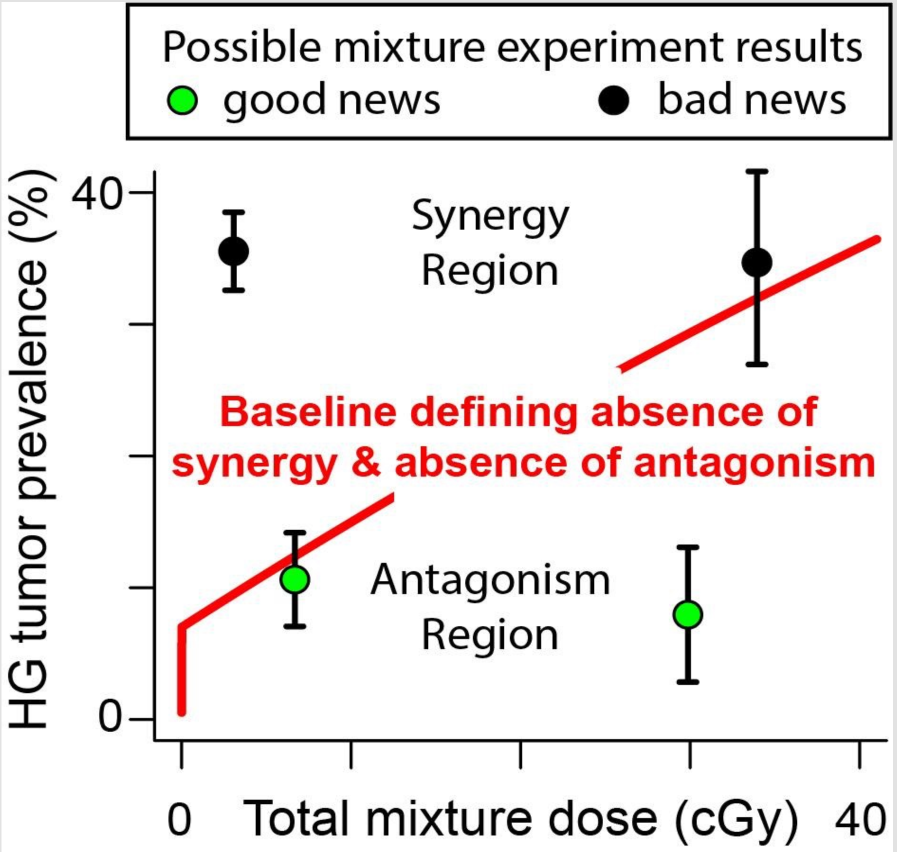


Talk preview and review

Schematic figure: murine Harderian gland (HG) tumor prevalence vs dose

Red curve is the dose effect relation defining the boundary between synergy and antagonism.

The apparent jump up at the lower left is actually a region of very high but finite slope. In addition, there is very large concavity (negative second derivative).

Points show possible but hypothetical mixed field data: black for (damage enhancing) synergy (**bad news**), green for antagonism (**good news**).

Rationale and goal of this study

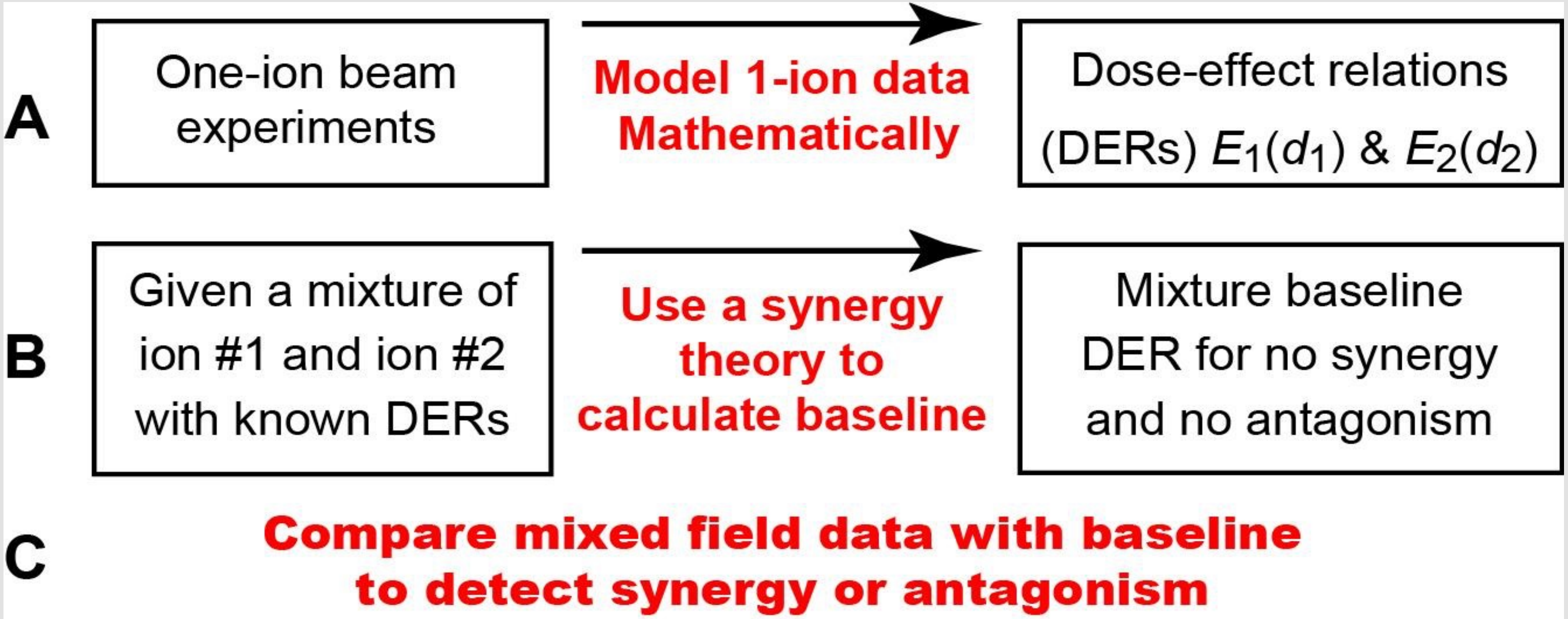
- Whether Galactic Cosmic Ray (GCR) mixed fields will show synergy between their individual components, thereby enhancing risk of astronaut carcinogenesis or other damage, is not currently known.
- The goal of this in silico study was to investigate data on murine tumorigenesis due to whole-body rapid exposure to simulated GCR mixtures (Blakely talk). Our recently introduced approach to synergy theory [1-3] was used.
- Related topics include experimental and theoretical results on chromosome aberrations by Hada and coworkers.

[1] N. Siranart et al. (2016) Radiat Res 186(6): 577-591.

[2] D.W. Ham, et al. (2018) Radiat Res 189(3) 225-237.

[3] E.G. Huang et al. accepted for publication (2019) Radiation and Environmental Biophysics.

Synergy theory methods



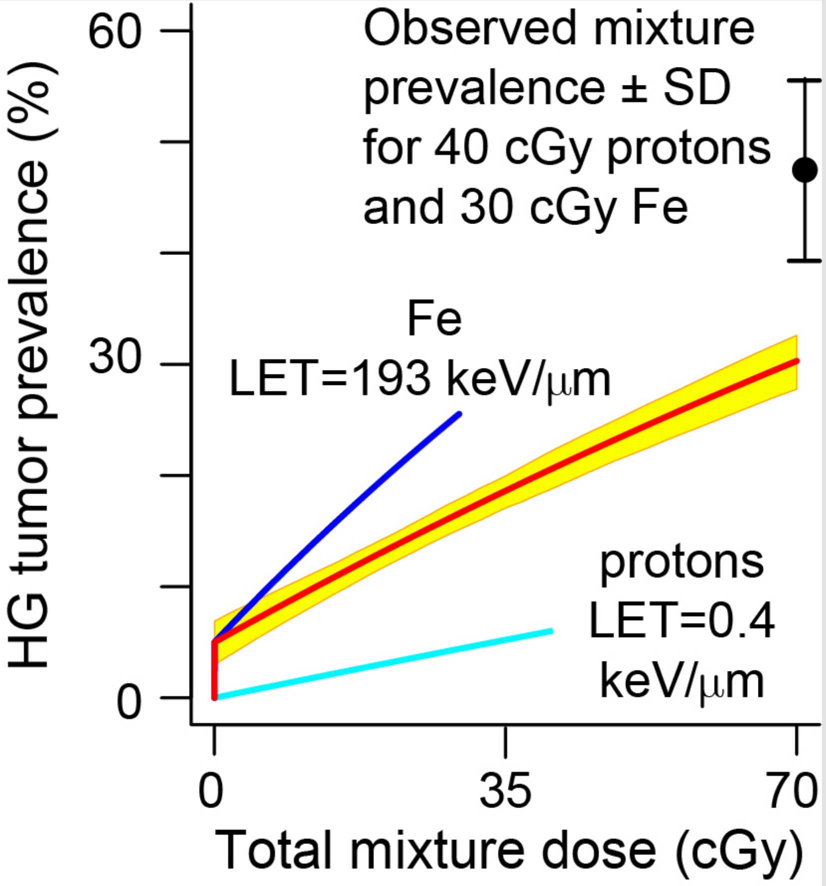
- In row A our main one-ion HZE model has 3 adjustable parameters all of which differ from 0 at the $p < 0.001$ level, indicating parsimony (in the sense of Occam's razor).
- Mathematically, a similar approach can be used for a multi-ion mixture

Simple effect additivity synergy theory

- Uses the sum of the effect contributions from each 1-ion mixture component as the baseline defining absence of synergy and absence of antagonism in a mixed radiation field
- Is usually taken for granted as the obvious synergy theory, but has long been known to be unreliable in various fields of biology, e.g. pharmacology & toxicology. Many replacements are in current use.
- Has trouble when dealing with dose-effect relation (DER) curvilinearity such as the high concavity at low doses in modeled 1-ion DERs that take non-targeted effects into account.
- Was never used in our synergy analyses.

Incremental effect additivity (IEA) synergy theory

- Recently introduced replacement for simple effect additivity used in our calculations of no-synergy/no-antagonism mixture baselines.
- Small increments of effect are determined by the total effect already contributed by all mixture components.
- Satisfies mix-mix principle, unlike simple effect additivity or most of its replacements.
- Uses an autonomous ordinary differential equation initial value problem.
- Has become practical because computers have become efficient at solving nonlinear ordinary differential equations numerically.



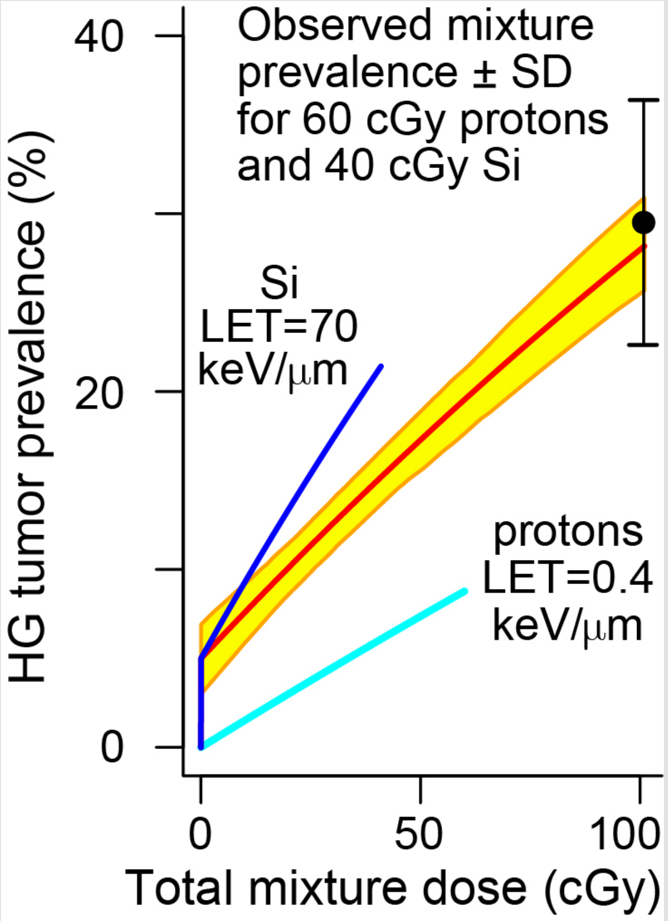
Bad news

This slide adds 95% CI (yellow ribbon) to Blakely's "bad news on synergy" slide.

Blue lines come from 1-ion data and models. They show effects if 1 ion contributed all the dose instead of only its 3/7 or 4/7 proportion Red line, nicely nested between blue lines, is IEA baseline no-synergy/no-antagonism dose effect relation, calculated from 1-ion results.

Vertical intervals on yellow ribbon specify 95% CI, calculated by Monte Carlo sampling.

The error bar does not intersect the yellow ribbon so the observed synergy is significant.



Somewhat reassuring news

No significant synergy observed in a different experiment.

Figure is similar to previous figure but here the data point lies within the yellow ribbon and the red line intersects the error bar.

Importantly, the 95% CI yellow ribbons were calculated taking adjustable parameter correlations into account. This gives a substantially narrower ribbon than incorrectly neglecting correlations.

The narrow ribbons help pinpoint synergy.

Three key challenges

1. Screen for possible major synergy among specific GCR-simulating components

Continue to use IEA synergy theory with newly scored and older HG tumorigenesis data. We anticipate that no systematic major synergy will be found.

2. See if non-targeted effects are important at low fluxes

One should apply IEA synergy theory to NSRL mixed-field data for other endpoints, such as chromosome aberration endpoints

3. Compare two alternatives for NSRL mixed field experiments.

(a) Use one standardized mixture and one standardized dosing protocol for almost all biological endpoints and for investigating almost any question.

(b) Allow for unique features of each endpoint – target size, radio-sensitivity, relaxation times, confounding factors, etc. – in preference to standardizing.

Acknowledgements

NASA NNJ16HP22I under the auspices of the U.S. Department of Energy Office of Science (BER) under Contract No. DE-AC02-05CH11231.

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Questions

One-HZE-ion Model That Assumes Non-Targeted Effects (NTE)

The model equation is

$$P = 1 - \exp[-H(d)],\ \text{where } H(d) = Y_0 + \alpha d L \exp(-\lambda L) + \eta[1 - \exp(-d/d_0)].$$

Here: P = HG tumor prevalence; d is dose (cGy); $H(d)$ is a convenient auxiliary function introduced by Cucinotta and coworkers; $Y_0 = -ln(1\text{-background prevalence})$; L=LET (keV/μm); and $d_0 \leq 0.001$ cGy is a nominal dose where NTE saturate near η.

	Significance Levels			Correlations		
	t-value	p(>|t|)	level	α	λ	η
α	7.06	$4.46\text{x}10^{-8}$	p<0.001	1	0.77	-0.50
λ	8.07	$2.62\ \text{x}10^{-9}$	p<0.001	0.77	1	-0.13
η	4.89	$2.54\ \text{x}10^{-5}$	p<0.001	-0.50	-0.13	1

$\{\alpha, \lambda, \eta\}$ are 3 adjustable parameters with respective dimension {μm cG^{-1} keV^{-1}, μm keV^{-1}, none}. Calibration is by non-linear inverse-variance-weighted least-squares regression from HZE data at doses $\neq 0$.

It is seen that: (a) all 3 parameters differ from zero significantly (t-test p << 0.001), indicating model parsimony; and (b), there are strong linear correlations between α and λ, which need to be taken into account.

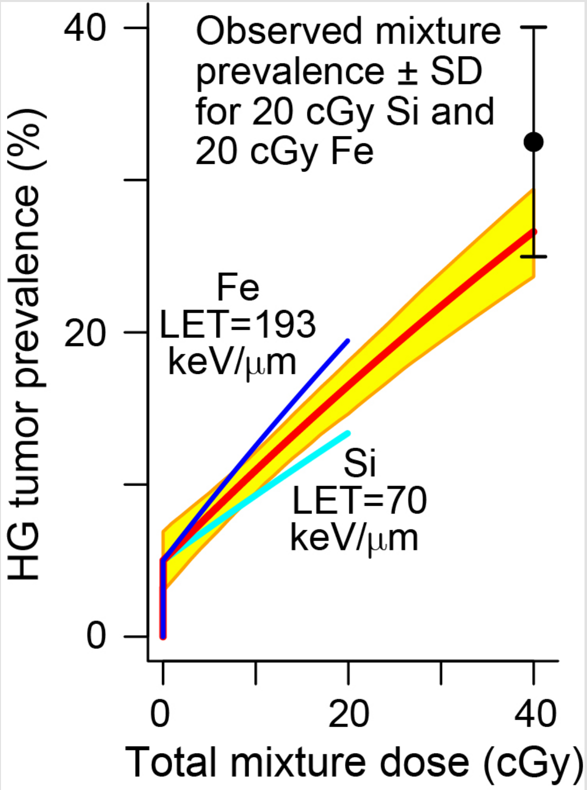
The Incremental Effect Additivity (IEA) Baseline Equation

$$[1]\ \ (\mathrm{A})\ \ d_j = r_j d;\ (\mathrm{B})\ \ r_j > 0;\ (\mathrm{C})\ \ \sum_{j=1}^{N} r_j = 1;\ (\mathrm{D})\ \ \sum_{j=1}^{N} d_j = d.$$

$$[2]\ \ (\mathrm{A})\ \ \mathrm{d}I / \mathrm{d}d = \sum_{j=1}^{N} r_j \left[\mathrm{d}E_j / \mathrm{d}d_j\right]_{d_j = D_j(I)};\ (\mathrm{B})\ \ d = 0 \Leftrightarrow I = 0.$$

For a mixed beam of N ≥ 2 components with 1-ion DERs $E_j(d_j)$ the dose proportions r_j that the different components contribute to total mixture dose d obey Eq. [1]. Eq. [2] is the initial value problem for the IEA no-synergy/no-antagonism baseline DER I(d). In Eq. [2A] the slope dI/dd is expressed as a weighted average of 1-ion DER slopes, evaluated at $d_j=D_j(I)$, where D_j is the compositional inverse function for the function E_j. In the present context the initial condition [2B] insures there is a unique solution for I(d). Key ideas include:

- Because slopes describe a linear relation between incremental effect and incremental dose, IEA synergy theory circumvents the non-linearities that plague simple effect additivity synergy theory.
- Using $d_j=D_j(I)$, means using effect I, a system variable, as independent variable instead of d. This leads to each component appropriately adjusting its incremental effect to the effect all the components acting jointly have already induced.
- The fact that Eq. [2A] involves a sum leads to IEA synergy theory obeying the mix-mix principle, unlike almost all other current synergy theories.



Somewhat Interesting News: synergy but not significant synergy observed in a different experiment

Here the yellow ribbon, and even the red line intersect the error bar.

To review:

In all, three 2-ion mixtures were recently scored. One showed significant synergy. This figure indicates synergy but not significant synergy. The remaining experiment showed absence of synergy and absence of antagonism.

SEA versus IEA in a hypothetical 4 HZE mixture