RxRSI

First and foremost, we would like to thank reviewer #1 for his/her careful assessment of our manuscript. There are a number of very legitimate concerns brought up, all of which, when addressed, will make the manuscript stronger. Upon first reading, we were most concerned by the issues raised surrounding the mathematical soundness. All other issues raised seemed addressable, and indeed would make the article stronger. No changes, however, would matter, if the mathematical analysis was indeed flawed – so we first endeavoured to ascertain where either the error (or the misunderstanding), lay.

Thankfully, the reviewer laid out his/her concerns clearly, and with appropriate references to the text. This allowed us to quickly ascertain that the perceived flaws actually arise from ambiguities in our notation rather than our analysis. Please let us explain, and after consideration of our re-structuring/clarification of the analysis, please consider allowing us a full rebuttal. We will address what we feel are the most concerning statements about the mathematics here, in turn. If allowed a full rebuttal, we would, of course, address the rest of the concerns as well.

"I also have severe problems with the mathematical development in the paper. The equation defining α_g cannot be correct, except when $n = 1$, and $d = 2$, as RSI is defined based on SF2. If $n = 1$ and $d = 2$ then it is clear that RSI is the predicted SF2, and α_g is the resulting alpha parameter to be used in the LQ model, assuming all the variability shows up as variability in α, and none in β. Why not state this?"

This is indeed worth stating, as we did in our Lancet Oncology paper. We have yet to make any attempt at capturing changes in β, and therefore set it as a constant, and equal to the mean for a given disease site pulled from the literature. This will be amended in future versions of the manuscript, and indeed, we are working to determine β as well, but this is beyond the scope of the current manuscript. Further, α_g is absolutely defined with $n = 1$ and $d = 2$ as the reviewer intuits. This is the crux of the analysis: this parameter is derived from the genomics and the LQ-model (as a surrogate for SF2), and then is subsequently used to determine overall effect of repeated doses. Specifically, we define α_g as:

$$\alpha_g = \frac{\ln \mathrm{RSI}}{-nd} - \beta d, \tag{1}$$

which simplifies to

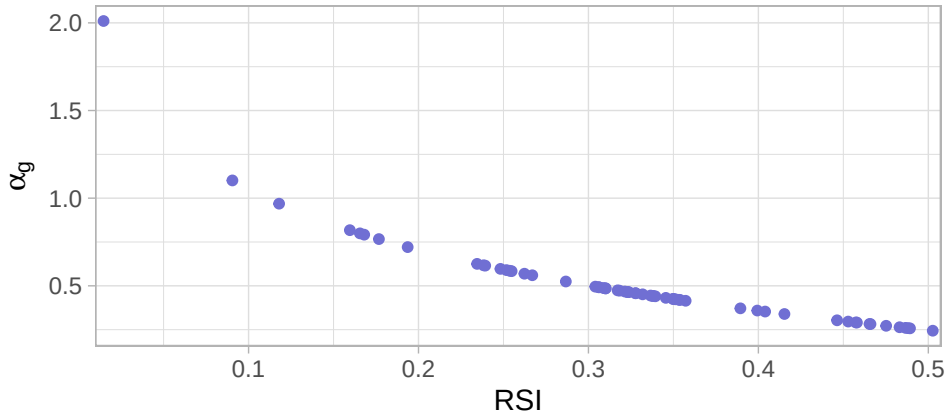
$$\alpha_g = \frac{\ln \mathrm{RSI}}{-(1)2\mathrm{Gy}} - \beta\, 2\mathrm{Gy}. \tag{2}$$

Code Excerpt:

> Here we show a snippet of the code where we calculate α_g for each patient and plot the distribution as a function of RSI. the rich heterogeneity of RSI is preserved, but transformed (Figure 1). The data points represent patients used in the analysis, and this is an excerpt from the code we used for the paper, demonstrating that the original computation, and therefore results, match the clarified exposition.

```
n_e = 1
d_e = 2
beta = 0.05
df <- mutate(df, alpha_g = log(RSI)/(-n_e*d_e) - beta*d_e)
```

Figure 1



"Another statement that simply cannot be true is this one (bottom, p. 3): "It is worth noting that in the case when a patient receives 2Gy fractions, the beta * d terms drop out, and GARD reduces to -ln(RSI)." Yet RSI is a number that only depends on gene expression, and has nothing to do with dose, according to the first, un-numbered equation. Dose can be anything, GARD presumably changes, yet must equal -ln(RSI)...?"

We thank the reviewer for pointing out this error in our exposition. We intended to define a term "GARD_{2Gy}" here. This is defined as the *effect* of a 2Gy dose of radiation in terms of GARD.

$$\text{GARD}_{2Gy} = nd(\alpha_g + \beta d), \tag{3}$$

$$\text{GARD}_{2Gy} = (1) \cdot 2\text{Gy}(\alpha_g + \beta \cdot 2\text{Gy}), \tag{4}$$

$$\text{GARD}_{2Gy} = 2\text{Gy}\left[\frac{-\ln \text{RSI}}{2\text{Gy}} - \beta\, 2\text{Gy} + \beta\, 2\text{Gy}\right] = -\ln \text{RSI}. \tag{5}$$

For a clinical gard (i.e. the GARD a patient experiences upon repeated doses of radiation, which we will from here on denote GARD_c), one would have to scale this by the number of fractions given, so (assuming 2Gy fractions) this would yield

$$\text{GARD}_c = \frac{n_c}{n_e}(-\ln \text{RSI}), \tag{6}$$

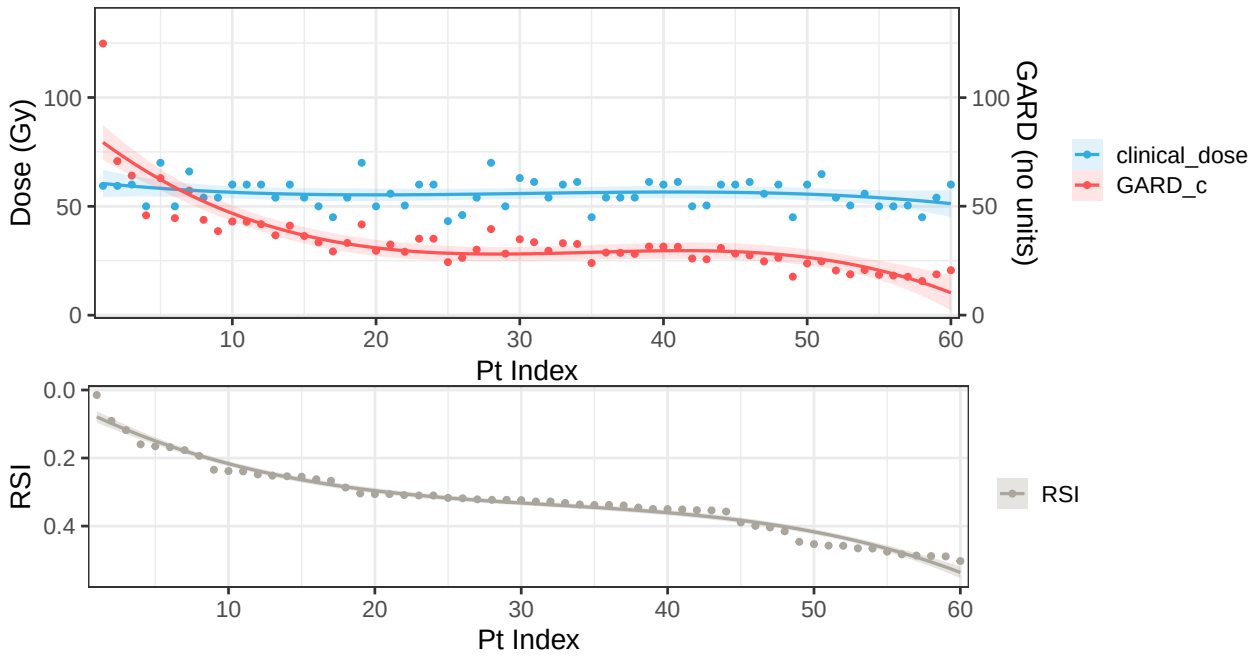
where n_i where $i \in \{c, e\}$ is the number of doses given in the clinical scenario, and the experimental ($n_e = 1$) conditions. By failing to define n_c here, as we intended, we started what turned out to be a confusing chain of notational ambiguities which we believe led to misunderstanding our entire analysis.

Code Excerpt:

Here GARD_c is calculated computationally as a function of treatment dose and individual α_g, with each point along the x-axis representing an individual patient. RSI for each patient (matched vertically between the plots) is plotted underneath in order to demonstrate the relationship between between the two parameters.

```
df <- mutate(df, GARD_c = n_c*d*(alpha_g + beta*d))
```

Figure 2



"For other reasons as well, the definition of GARD itself is confusing. First of all, it does not have units of dose. As the authors note that GARD has been presented before, I looked up that reference and got different definition of GARD: "The calculation for GARD is similar to biologically effective dose, except that patient-specific alpha is derived by substituting the radiosensitivity index for survival (S) in equation 1, where dose (d) is 2 Gy, n=1, and β is a constant ($0.05/\text{Gy}^2$)."""

The reviewer is again correct, GARD is unitless. We apologize for any confusion about the defintion of GARD. In our previous paper (Scott et al. Lancet Oncology, 2016), from where the quote is derived, we hoped to capture the spirit of GARD, which is indeed *similar* to BED, but not mathematically equivalent, in order to appeal to the understanding of clinicians. However, in the appendices, which we reference in the paragraph from where that quote is derived, the mathematical definition of GARD takes the same form we use here, which is the same form as *biological Effect*: $E = nd(\alpha + \beta d)$, and subsequently $\text{GARD} = n_c d(\alpha_g + \beta d)$. We do NOT use the formalism for BED, and, as the reviewer points out in the subsequent paragraph, this would yield 'a very different kind of GARD'. So, utilizing our new, hopefully clearer, notation:

$$\alpha_g = \frac{-\ln RSI}{n_e d} + \beta d \quad \text{and,} \tag{7}$$

$$\text{GARD}_c = n_c d(\alpha_g + \beta d). \tag{8}$$

"Confusion extends to RxRSI, which is repeatedly called a biologically optimized dose, yet the

definition in the paper of RxRSI is given as GARD / (alpha + beta * d), which by definition apparently always reduces to n *d. This equation obviously does not involve biology."

The reviewer has caught the final, and most important error in our exposition. As written, the reviewer is entirely correct, we have essentially 'cancelled out' any biology from the formalism. Luckily, our mathematical exposition here does not match our computation, which does NOT cancel the biology, and the solution to the exposition is as simple as introducing some new notation to clear up an ambiguity. The crux of the matter is that the GARD in the equation for RxRSI is a static number. Please let us explain (of course, if allowed a full rebuttal/revision, this clarified exposition would become part of the manuscript, or at least the supplements).

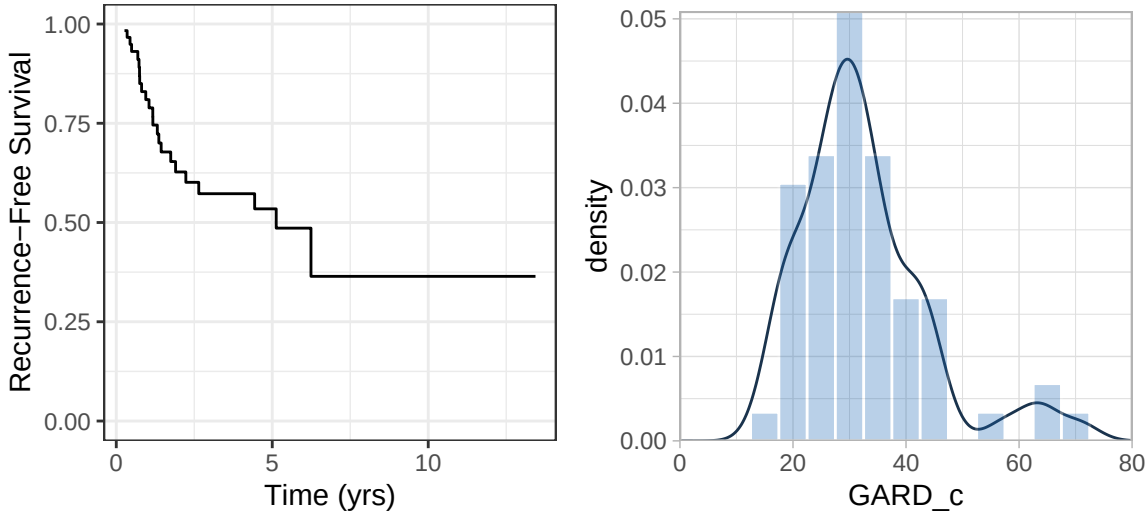
Once we derived GARD_c for every individual patient in the cohort (i.e. using each individually derived α_g and the actual dose of radiation received: $n_c d$), we created a distribution.

Code Excerpt:

The left plot below shows the Kaplan-Meier plot of the 60 patient cohort. The right side shows the kernel density estimate of the calculated GARD_c. These are the same values shown in Figure 2 (top panel).

```
km <- survfit(Surv(time,event) ~ 1, data = df)
```

Figure 3



This distribution was then used to find an optimal GARD threshold (GARD_T), which minimizes the $p-$value of the Kaplan-Meier statistic using the clinical outcomes.

Code Excerpt:

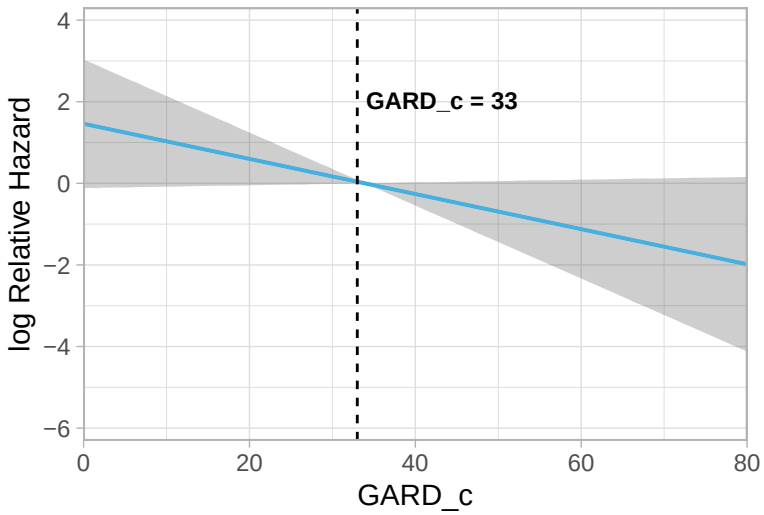
Cox proportional hazard models are shown below in order to briefly illustrate the relationship between GARD_c and outcomes, first as a linear variate (table 1) and then with the derived threshold value (table 2).

```
fit <- cph(Surv(time,event) ~ GARD_c, data = df,x = T, y= T )
```

Table 1

coef	exp(coef)	se	z	chi-sq	p
-0.04	0.96	0.02	-1.82	3.31	0.07

Figure 4




```
fit <- cph(Surv(time,event) ~ GARD_c<33, data = df,x = T, y= T )
```

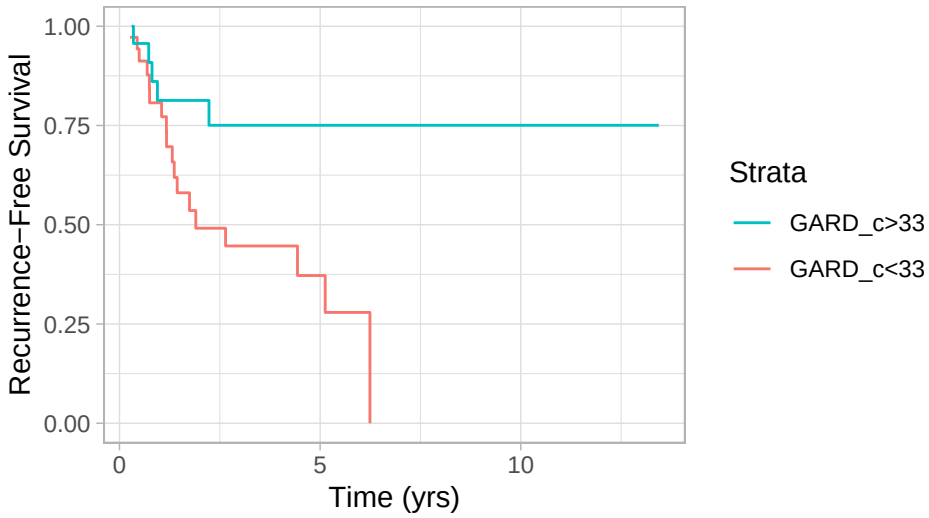
Table 2

coef	exp(coef)	se	z	chi-sq	p
1.23	3.44	0.51	2.41	5.80	0.02

The Kaplan-Meier curves below demonstrate the above described stratification with GARD_T as applied to this patient cohort.

```
km <- survfit(Surv(time,event) ~ GARD_c<33, data = df)
```

Figure 5



At this point, GARD_T is a **set, static number: a target biological Effect to achieve through physical dosing**, it no longer has the α_g term in it. Therefore, when we define RxRSI, using GARD_T

$$\text{RxRSI} = \frac{\text{GARD}_T}{\alpha_g + \beta d}, \tag{9}$$

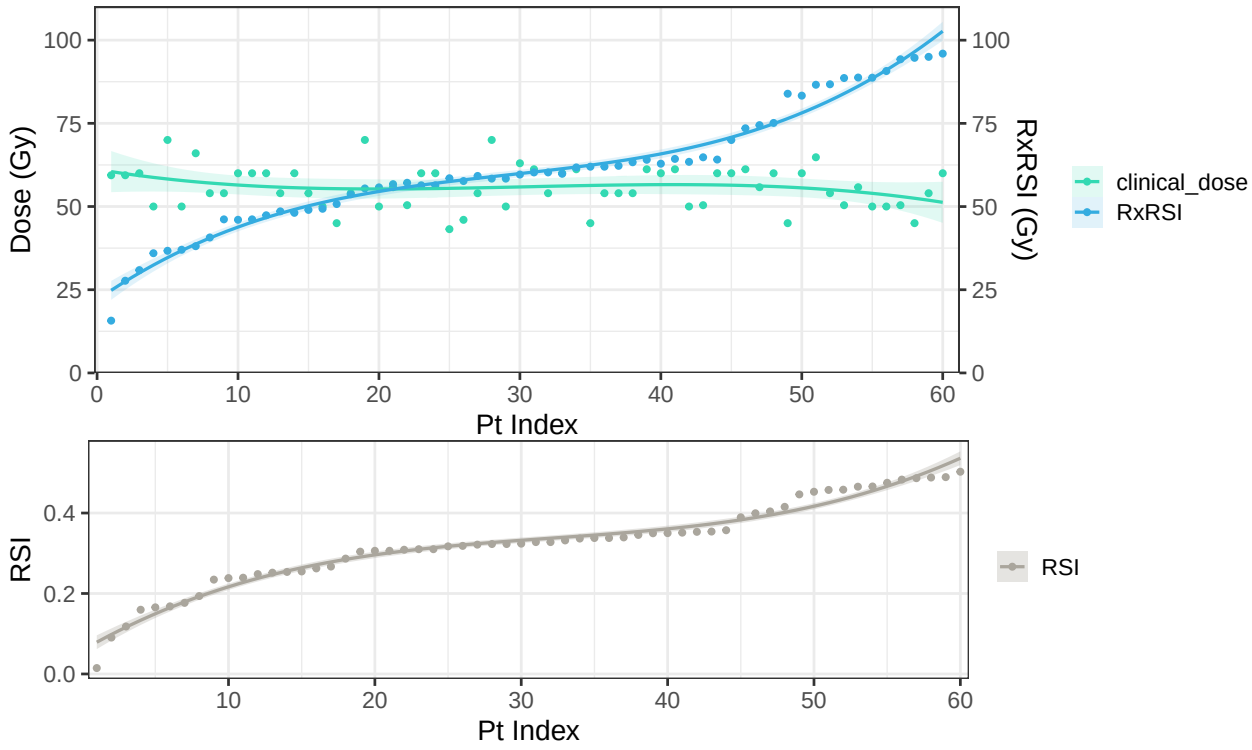
we are NOT cancelling out the biology, but actually reinserting it (note here also that the units of RxRSI are now Gy). For each patient, we ask what physical dose (nd), modulated by their biology (through α_g) is needed to reach this **target effect ($\mathbf{GARD}_T = 33$)**?

$$\text{RxRSI} = \frac{33}{\alpha_g + \beta d}. \tag{10}$$

Code Excerpt:

```
GARD_T = 33
df <- mutate(df, RxRSI = GARD_T/(alpha_g + beta*d))
```

Figure 6



In summary, we immensely the appreciate the careful and detailed reading of our manuscript, and apologize for the confusion surrounding our mathematical analysis. We contend, however, that the issues lay only in exposition, not in computation or conception. We hope that our clarifications here give you the confidence to allow us to perform a full revision. We are excited to help move the field toward true personalization, and realize that this can not be done by any one entity, but instead by a community of scientists standing on one anothers shoulders, and helping to advance one another's work.