

Modelling based methods to account for dropouts in xenograft experiments

Emma C. Martin¹, Leon Aarons¹, James W.T. Yates²

1. Centre for Applied Pharmacokinetic Research, Manchester Pharmacy School, The University of Manchester, UK

2. AstraZeneca, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, U.K.

INTRODUCTION

Xenograft studies are commonly used to assess the efficacy of new compounds and characterise their dose response relationships [1].

Analysis often involves comparing the final tumour sizes across dose groups [2] using t-tests or tumour growth inhibition indexes. This can cause bias, as often in xenograft studies a tumour burden limit (TBL) is imposed for ethical reasons [3], leading to the animals with the largest tumours being excluded from the final analysis. This means the average tumour size, particularly in the control group, is underestimated, leading to an underestimate of the treatment effect.

Four modelling based methods are proposed to account for this drop out.

METHODS

A data set, based on paclitaxel treatment, was simulated [4] with a control group and two dose groups (4mg/kg and 8mg/kg daily). Animals were dropped from the study when their tumours reached a size of 2.5g. Each of the proposed methods was applied to the simulated data set. The estimated dose response curve from each method was calculated, where response was assumed to be the predicted size of the tumour at each dose on the final day of the experiment.

Modelling

The same model used to simulate the data was then used to fitted to the data following drop out, meaning all available data was used to estimate the dose response curve, rather than the final measurement only.

Censoring

The drop out data was assumed to be censored using the M3 method [5]. The likelihood for missing values was replaced by the likelihood that the missing value would truly have been above the tumour burden limit, given that the observation was missing due to drop out (Figure 1).

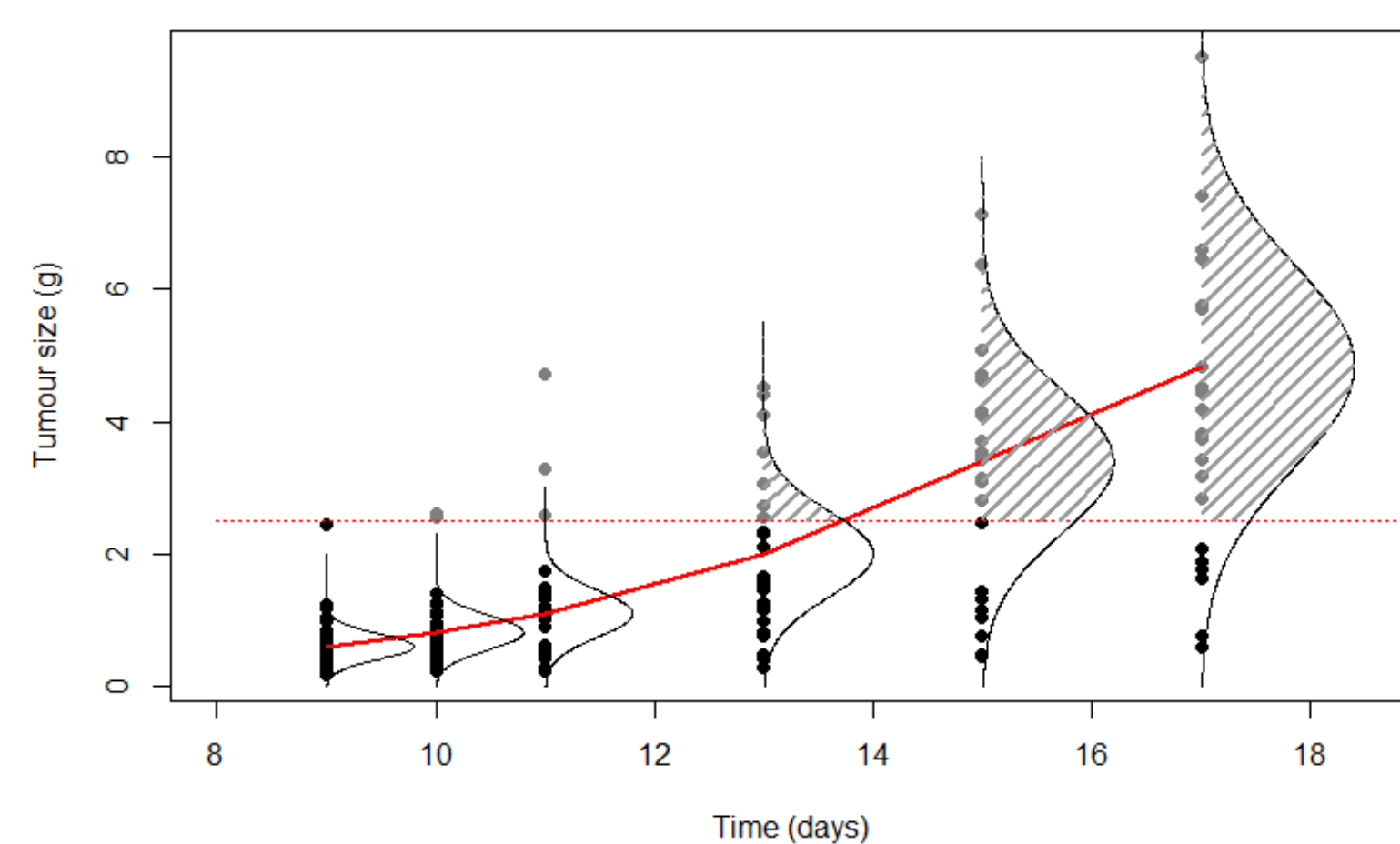


Figure 1: Diagram explaining the use of the M3 method in the control group, with observed tumour sizes (black) and missing observations (grey), the population model (solid line), with the normal distribution around each predicted point [6]

Pattern mixture modelling

Missing data was imputed 5 times using models fitted to data that followed similar drop out patterns, the parameter estimates from each of these 5 data sets were then averaged. Several patterns were collapsed to reduce the overall number, and the available case missing value (ACMV) method was then used as described in Yeun *et al.*, 2014 [7].

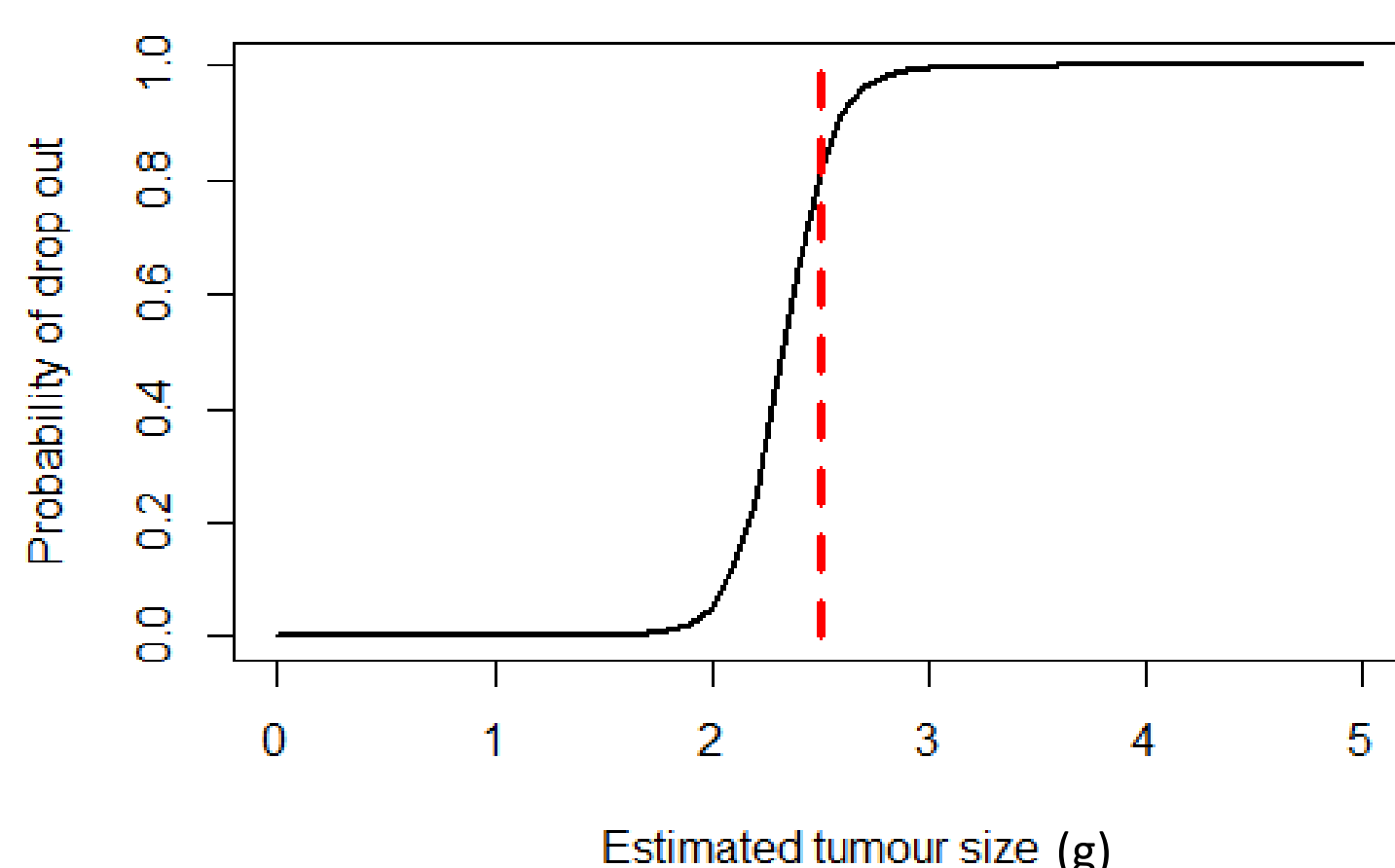


Figure 2: Logistic model for probability of drop out, with estimated tumour size as predictor

Joint modelling

The tumour growth and drop out were jointly modelled through shared random effects. Probability of drop out was modelled with a logistic model, using estimated tumour size from the tumour growth model as a predictor (Figure 2).

SIMULATED DATA

Day 17 was taken to be the final day of the study, to ensure sufficient animals remained in the control group for comparison.

In total 19% of the simulated data had to be deleted due to the drop out limit, rising to 30% of data in the control group.

Following drop out, it appears that the highest dose only causes a reduction in tumour size of less than 1g, but in reality the highest dose reduces tumour size by over 4g.

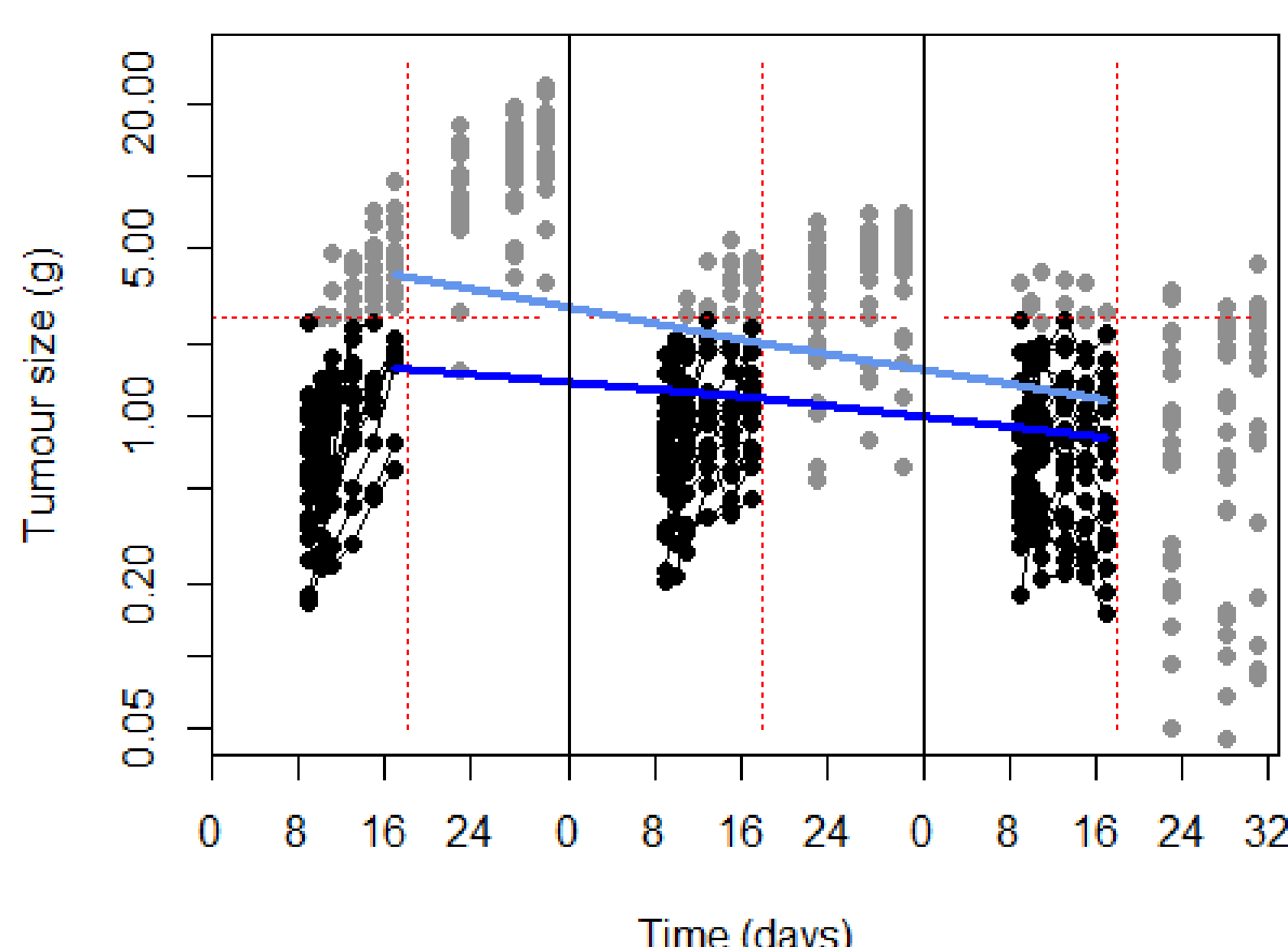


Figure 3: Simulated data by dose group, missing data due to drop out in grey, with estimated dose response curves overlaid before/after drop out in light/dark blue

RESULTS

Both the modelling and pattern mixture methods under-predict the final tumour size in the control group, performing only slightly better than using the average of the observed data.

The censoring and joint modelling methods perform well in terms of estimating the dose response curves, with the joint modelling method outperforming the censoring method in terms of the uncertainty (Figure 4).

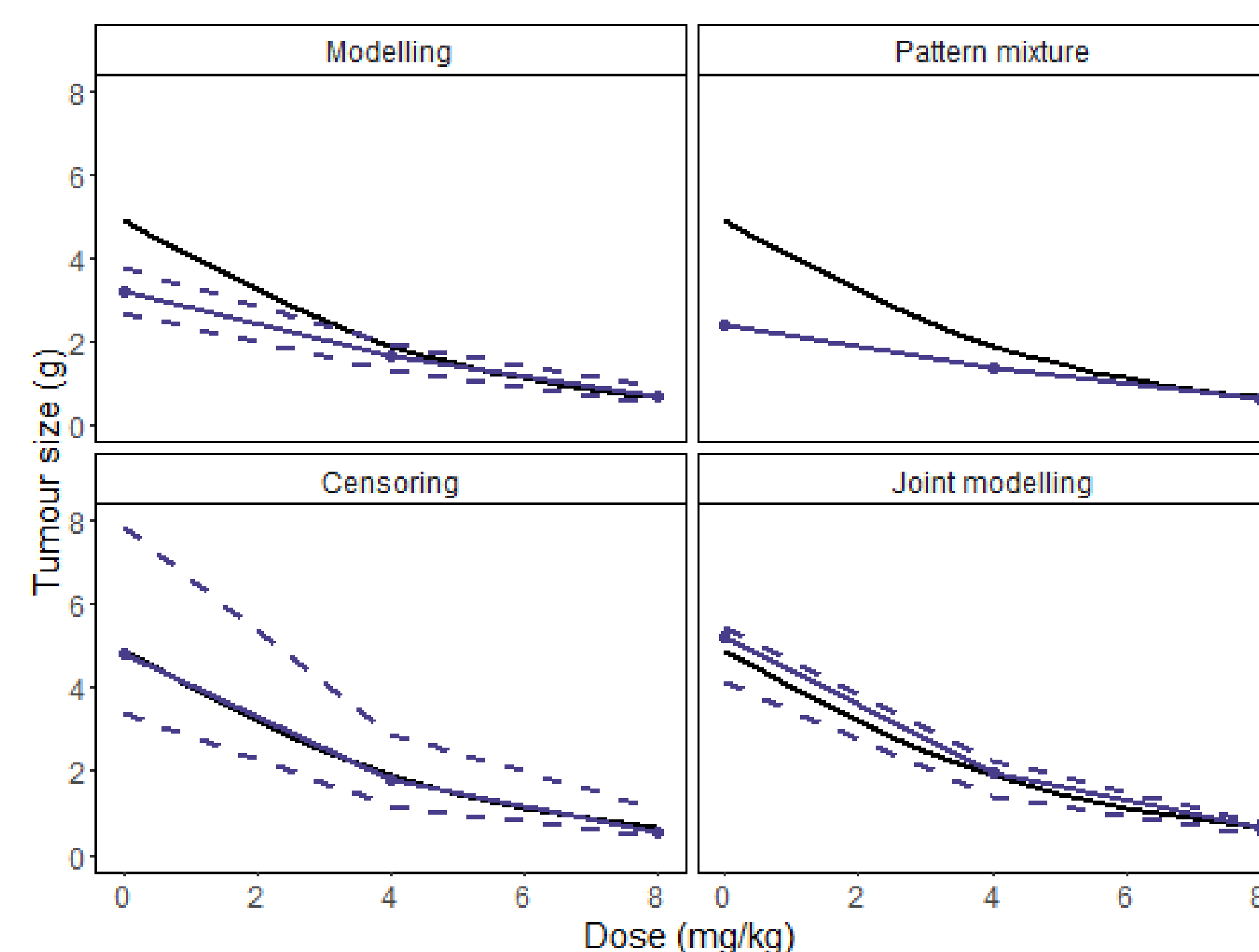


Figure 4: True dose response curve (solid black line) with estimated dose response curve from each method (solid blue line) with 95% confidence intervals from bootstrapping (dashed blue line)

All models took similar lengths of time to converge (Table 1), with the pattern mixture modelling method taking longest overall as multiple models needed to be fitted.

Method	Time (mins)
Modelling	27
Pattern mixture	~128*
Censoring	28
Joint modelling	17

Table 1: Time taken for each of the methods, * shows time taken for model fitting only, not including imputation time

Method	Estimated tumour size on day 17 (95% CI)		
	Control group	4mg/kg group	8mg/kg group
True values	4.90	1.91	0.68
Means	1.56 (1.00 – 1.88)	1.18 (0.93 – 1.49)	0.80 (0.60 – 1.11)
Modelling	3.19 (2.70 – 3.77)	1.68 (1.30 – 1.97)	0.68 (0.52 – 0.89)
Pattern mixture	2.40	1.36	0.62
Censoring	4.84 (3.38 – 7.84)	1.79 (1.19 – 2.91)	0.56 (0.43 – 1.12)
Joint modelling	5.22 (4.16 – 5.47)	1.99 (1.39 – 2.27)	0.64 (0.46 – 0.76)

Table 2: Estimated tumour size on the final day of the study with 95% confidence intervals from bootstrapping [8]

DISCUSSION

The joint modelling method performs the best based on ability to estimate the dose response relationship, and confidence interval around the estimate. Both the censoring and joint modelling methods characterise the dose relationships well and are relatively easy to implement, and quick to run.

In some cases, where drop out may be obscuring the dose response relationship, using one of these methods may provide a method to recover the relationship.

Whilst the Simeoni model of tumour growth has been used for both simulation and modelling, it would be possible to use any model of tumour growth with these methods to account for drop out.

REFERENCES

- [1] Tan, M., et al., Repeated-measures models with constrained parameters for incomplete data in tumour xenograft experiments. *Stat Med*, 2005. **24**(1): p. 109-19.
- [2] Hather, G., et al., Growth rate analysis and efficient experimental design for tumor xenograft studies. *Cancer Inform*, 2014. **13**(Suppl 4): p. 65-72.
- [3] Workman, P., et al., Guidelines for the welfare and use of animals in cancer research. *Br J Cancer*, 2010. **102**(11): p. 1555-77.
- [4] Simeoni, M., et al., *Predictive pharmacokinetic-pharmacodynamic modeling of tumor growth kinetics in xenograft models after administration of anticancer agents*. *Cancer Res*, 2004. **64**(3): p. 1094-101.
- [5] Beal, S.L., Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn*, 2001. **28**(5): p. 481-504.
- [6] Bonate PL (2005) *Pharmacokinetic-pharmacodynamic modeling and simulation*. Springer, New York
- [7] Yuen, E., I. Gueorguieva, and L. Aarons, *Handling missing data in a duloxetine population pharmacokinetic/pharmacodynamic model - imputation methods and selection models*. *Pharm Res*, 2014. **31**(10): p. 2829-43.
- [8] Martin EC, Aarons L, Yates JW (2016) Accounting for dropout in xenografted tumour efficacy studies: integrated endpoint analysis, reduced bias and better use of animals. *Cancer chemotherapy and pharmacology*. doi:10.1007/s00280-016-3059-x