Practical statistical considerations for investigating anti-tumor treatments in mice

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Anti-tumor murine experiments

Rationale

1. pre-clinical *in vivo* efficacy, safety
2. dose projections
3. biomarkers, i.e., translational

Statistical considerations

1. small sample designs
2. rudimentary analyses
3. variable and heterogeneous outcomes

Case study drug combination

Design

1. *n* = 10 biological replicates, i.e., tumor implants,
   groups: control, drugs A, B, and A+B
2. *dosed 6 days* after tumor implant, tumors size ≈125mm^3,
   repeat dosing days 9 and 14
3. follow up measure *tumor size* every 3-4 days / 8 weeks

Aims

1. evaluate superior A+B efficacy to both A and B
2. investigate and explore trends in outcomes to treatments

Tumor outcomes combination study

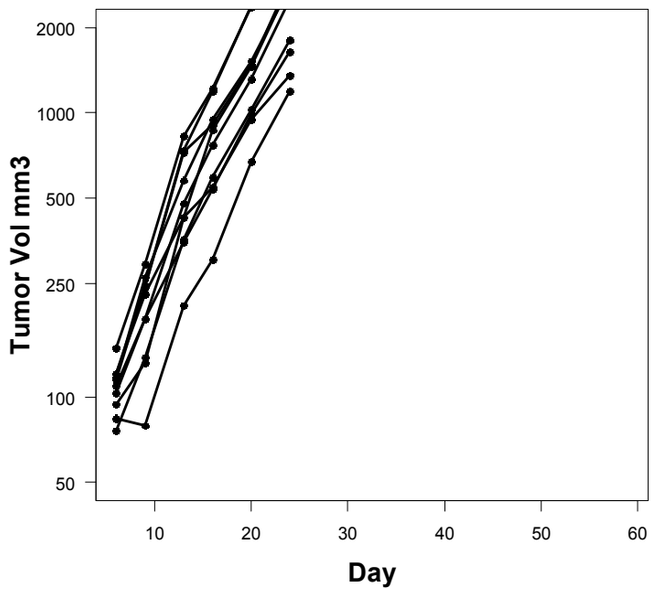
Control group:
log-linear growth

Drug A group:
minimal slowed pace of progression

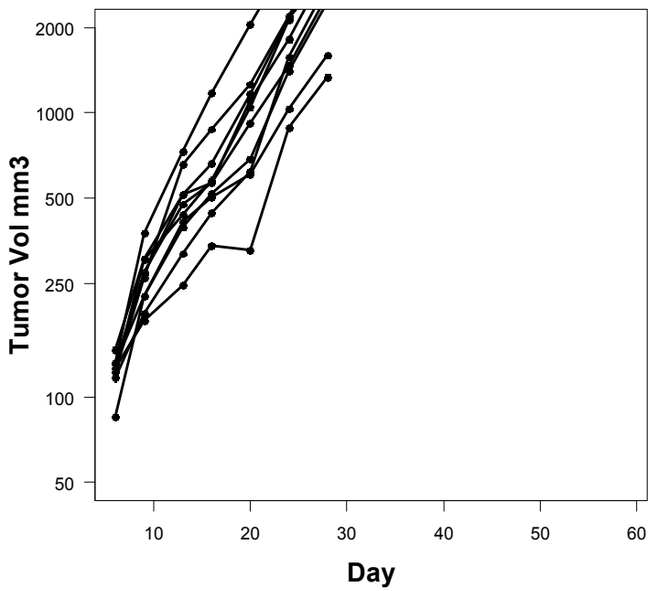
Drug B group:
tumor growth inhibition
slowed pace of progression
1 TF event

Drug combination A+B group:
tumor growth inhibition
slowed pace of progression
3 TF events

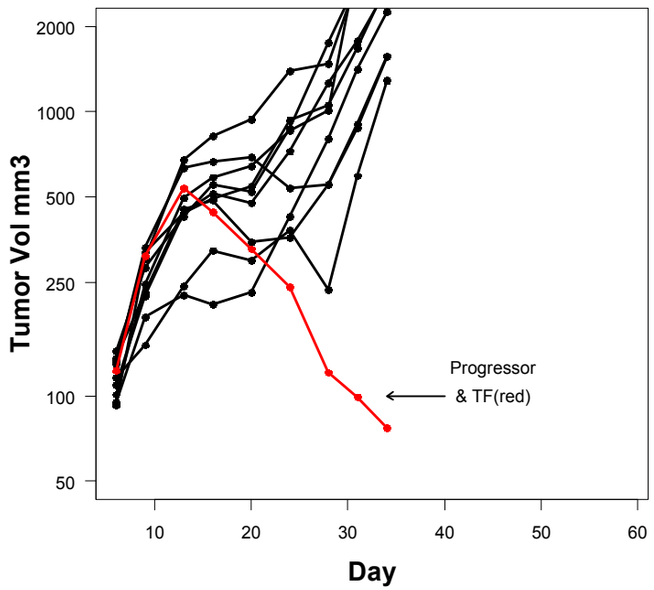
control



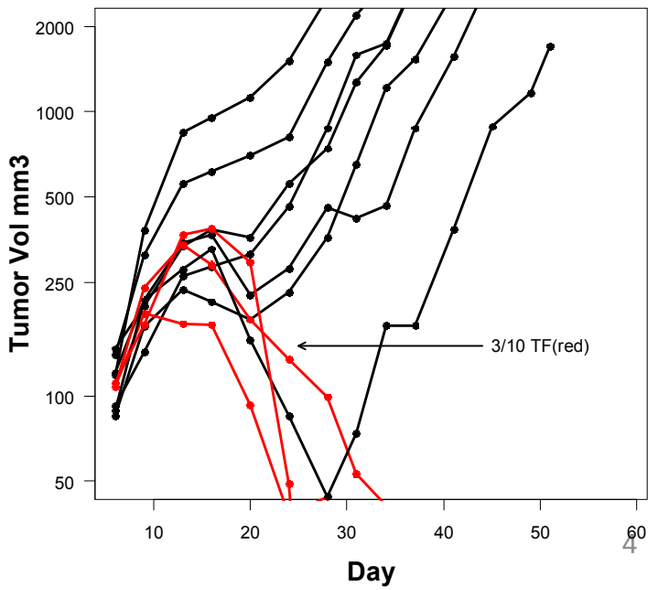
drug A



drug B



drugs A+B

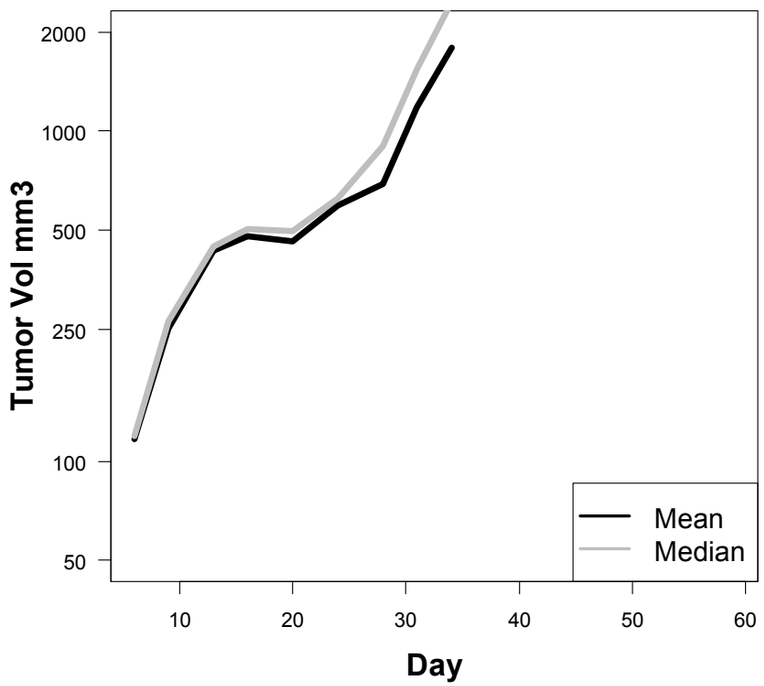


Different analyses, different questions

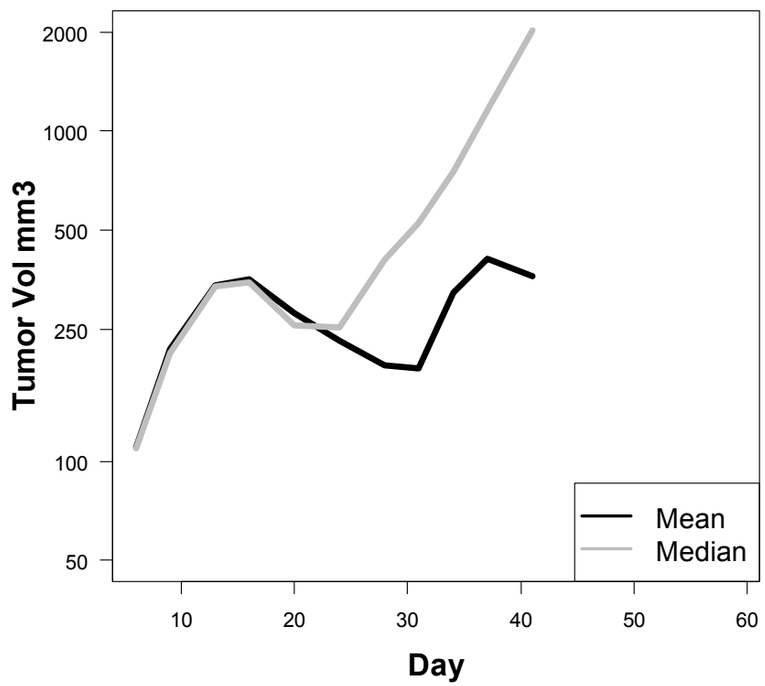
Analysis	Questions	Caveats
1. visual and risk analysis	trends in tumor growth, PFS, and OS	ignores variability/outcomes
2. relative tumor volume (RTV) analysis	compare mean tumor growth	small *n*, power
3. nonlinear mixed effect, non-linear least squares (pooled)	compare tumor growth inhibition, pace of progression	modelling assumptions and diagnostics
4. piece-wise model, two change points	compare tumor growth inhibition, pace of progression	as above and limited time points
5. tumor free (TF), eradication	compare TF frequencies	small *n*, dual end-points

Visual trends: drug B versus combo A+B

drug B

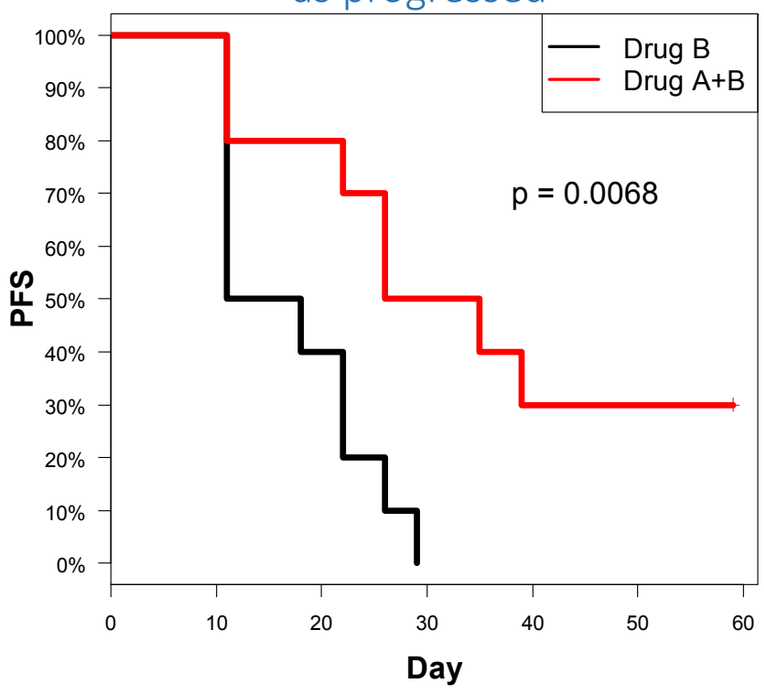


combination A+B

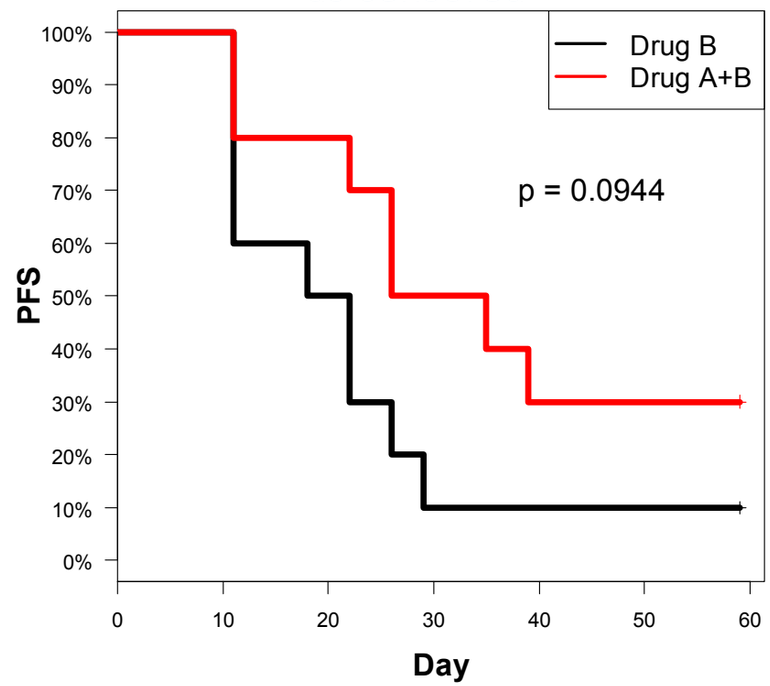


Progression: twice doubling of tumor size

count TF responder to drug B:
as progressed



count TF responder to drug B:
as censored

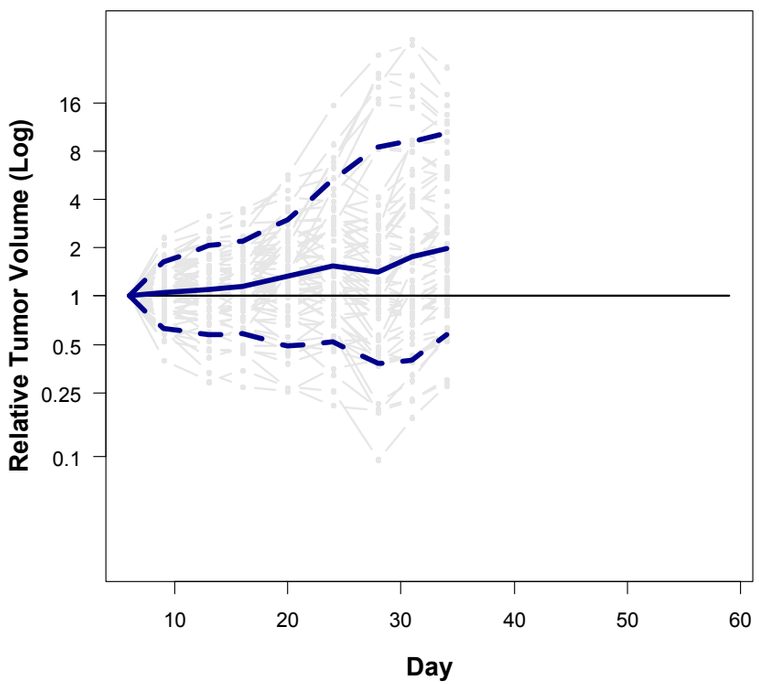


Visual trend and risk analysis summary

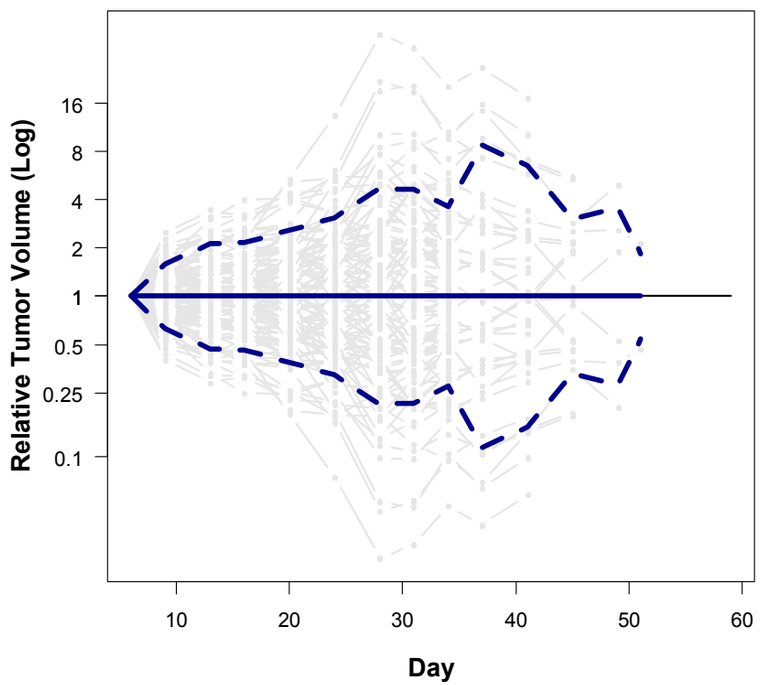
- insufficient to draw confident conclusions objectively from figures
- trends mask variability and qualitatively different outcomes
- K-M analysis may be incompatible with study purpose
- small *n*, influential observations

Relative tumor volume B divided by A+B

all possible pairwise differences B to A+B

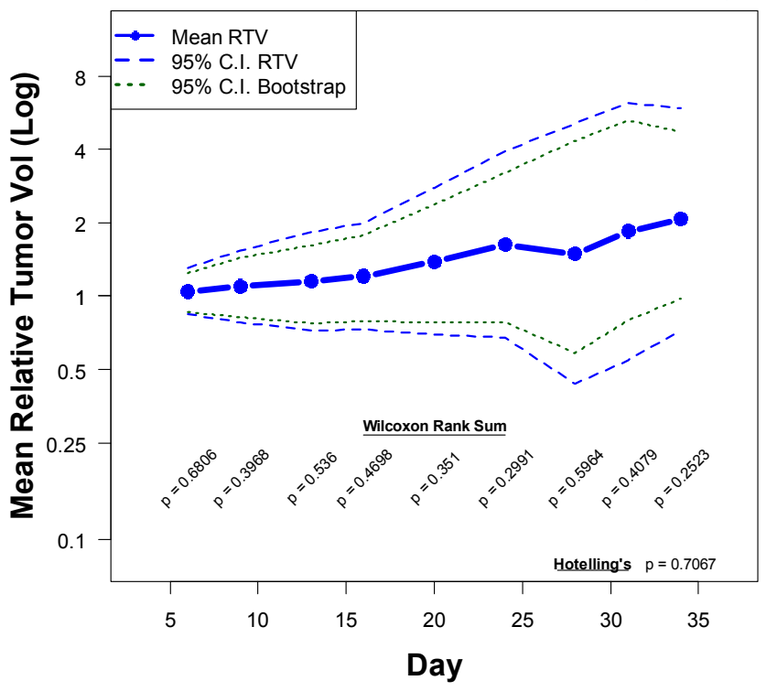


all possible pairwise differences scrambled

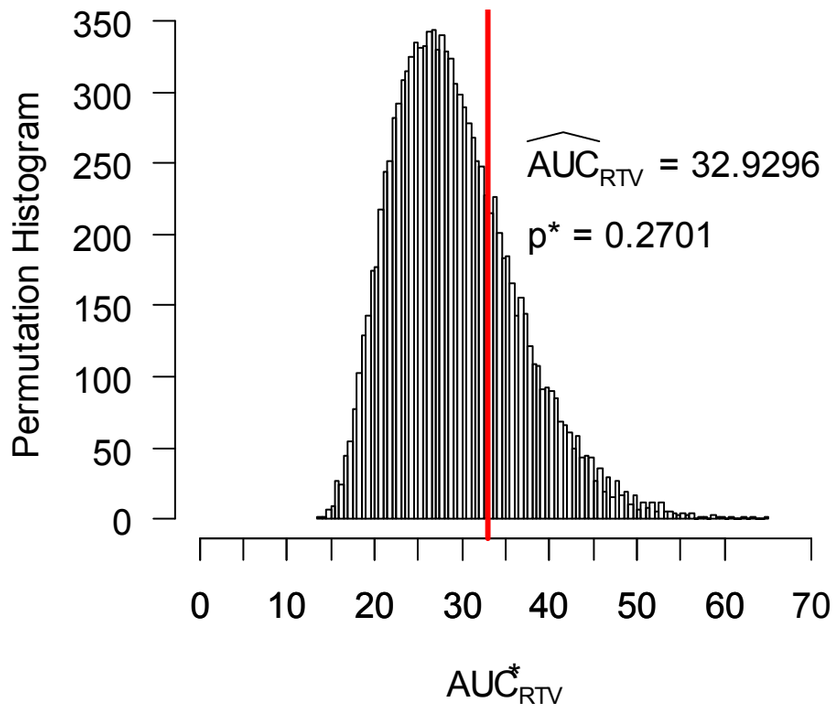


Relative tumor volume B divided by A+B

relative tumor volume (RTV)



permutation test area under RTV curve



Relative tumor volume analysis summary

- differences not detected between groups B versus A+B
- small *n*, variable longitudinal outcomes
- power lacking for statistical inference

Three-phase nonlinear model

log tumor volume *y*, time *t*, mouse *j*=1,…, n_i by treatment arm *i*, $\varepsilon_{tij} \sim N(0, \sigma)$.

$$y_{\mathrm{tij}} = \beta_{0\mathrm{ij}} + \beta_{1\mathrm{ij}} t - \beta_{2\mathrm{ij}} \cdot \frac{1}{1 + \exp\left(-\left(t - \theta_{\mathrm{ij}}\right)/\eta_{\mathrm{ij}}\right)} + \varepsilon_{\mathrm{tij}} \qquad (1)$$

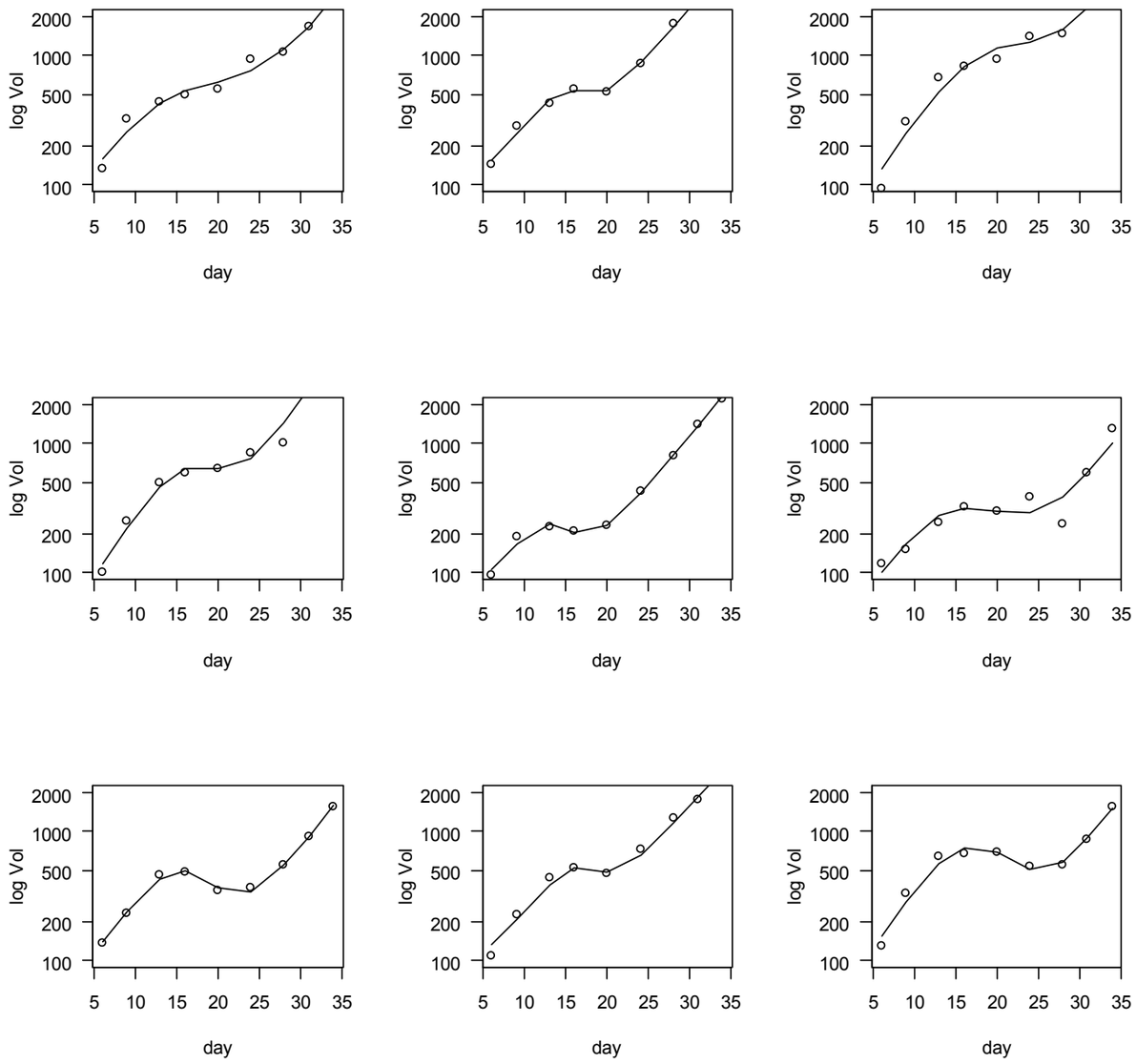
log-linear growth

logistic decay

inferences on i. pace of progression through the parameter $\beta_{1\mathrm{ij}}$

ii. tumor growth inhibition through the parameter $\beta_{2\mathrm{ij}}$

Drug B group, fitted nonlinear least squares



Nonlinear mixed effects

Output **nlme** function in **R**

Treatment	Parameter	Est.	S.E.
Control	β_0	3.75	0.10
	β_1	0.17	0.01
A	-	-	-
B	-	-	-
	β_0	4.23	0.18
	β_1	0.12	0.01
A+B	β_2	1.37	0.61
	θ	16.76	0.91
	η	2.38	0.35

Starting values explored in multiple ways

convergence failed

high autocovariances!

Pooled nonlinear least squares

- unconstrained versus constrained model fitting
  i. $\beta_{1ij} < 0.22$
  ii. $0 < \beta_{2ij} < 10$
  iii. $1 < \eta_{ij} < 5$

- pooled estimates and standard errors
  within: bootstrap model simulation
  between: bootstrap resampling

Pooled nonlinear least squares

Pace of progression

		unconstrained		constrained	
Treatment	Parameter	Est.	S.E.	Est.	S.E.
A	β_1	0.35	2.55	0.20	0.24
B	β_1	0.33	1.10	0.20	0.17
A+B	β_1	0.17	0.62	0.14	0.07

Growth inhibition

		unconstrained		constrained	
Treatment	Parameter	Est.	S.E.	Est.	S.E.
A	β_2	7.82	154.97	1.46	8.07
B	β_2	8.29	61.57	2.68	5.90
A+B	β_2	3.83	47.86	1.91	2.84

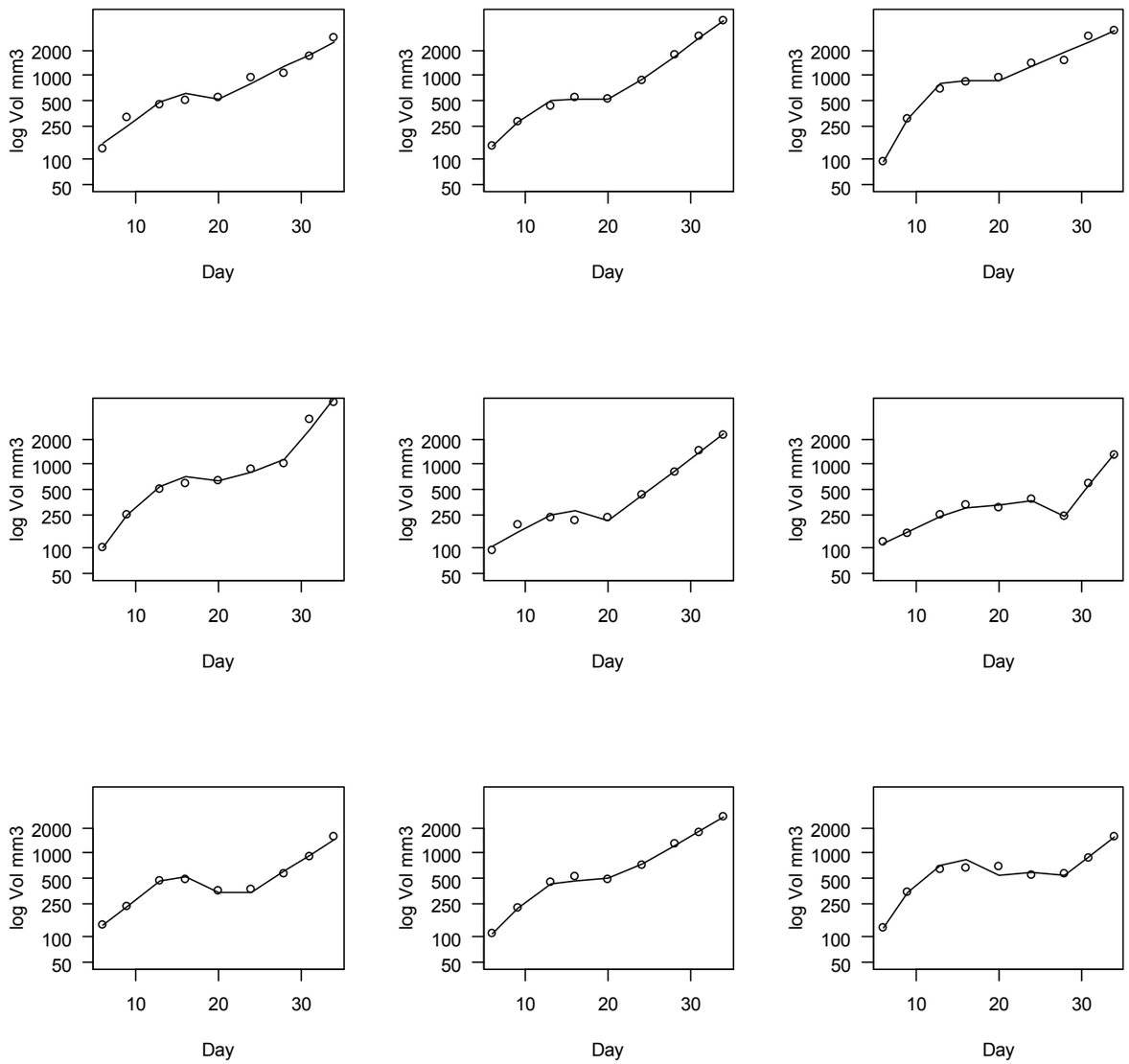
*Fit by **nls** function in R, pooling by bootstrap simulation and resampling with replacement

Piecewise nonlinear model

$$\mu_{tij} = \begin{cases} \beta_{10ij} + \beta_{11\mathrm{ij}} t & t \le c_1 \\ \beta_{10ij} + \beta_{11\mathrm{ij}} t - \beta_{2\mathrm{ij}} \cdot \dfrac{1}{1 + \exp\left(-\left(t - \theta_{\mathrm{ij}}\right)/\eta_{\mathrm{ij}}\right)} & t \epsilon\, (c_1, c_2] \\ \beta_{30ij} + \beta_{31ij} t & t > c_2 \end{cases} \quad (2)$$

i. log-linear growth $t \le c_1$,
ii. growth inhibition $(c_1, c_2]$
iii. log-linear progression $t > c_2$

Drug B group, fitted piecewise linear model



Piecewise nonlinear model

Output in R, selected parameters

	A		B		A+B	
Parameter	Est.	S.E.	Est.	S.E.	Est.	S.E.
β_2	1.39	0.23	2.49	0.49	2.67	0.58
β_{31}	0.13	0.01	0.17	0.02	0.12	0.01

Drug combination A+B

i. superior tumor growth inhibition versus A, $p = 0.04$,
ii. slowed post-treatment tumor progression versus B, $p = 0.025$.

Nonlinear modeling summary

- mixed effects computational model fitting challenges, high autocovariances!
- constrained nonlinear least squares improved model fitting
- piecewise model appeared best supported by case study data,
  slower post-dosing pace of progression

Tumor Free (TF) Counts

- compare TF event rate B v. A+B

  $TF_B = 1$ v. $TF_{A+B} = 3$, Fisher's Test $p = 0.58$

- sequential testing: TF at $\alpha=0.04$ and then RTV permutation test at $\alpha=0.01$

  significance not achieved

- Fisher's composite *p*-value: dual endpoints TF and RTV permutation test

  significance not achieved

Recommendations

1. Mouse anti-tumor studies exhibit variable and qualitatively distinct/complex outcomes, we recommend careful considerations of design, analysis, and reproducibility.

2. Visual and risk analysis can be insufficient alone to conclude efficacy or treatment superiority, and should be accompanied by additional and appropriate statistical analysis and validation.

3. Relative tumor volume analysis should include appropriate error control, measures of confidence, and adjustments for longitudinal correlation.

4. Nonlinear modeling appears promising, but sensitive to assumptions, and we recommend qualitative and empirical model validation/selection to accompany the reported findings of mouse tumor outcomes.

Recommendations con't

5. Mouse anti-tumor experiments are typically not powered to compare TF outcomes, and if desired, should report full uncertainty in statistical conclusions and or consider combining with other endpoints.

6. Scientific rationales in support of FIH drug trials should be based on real science, including adequate and sufficient data, analysis, and interpretation to support the scientific conclusions claimed in the study protocol.

7. Recommend for our case study: well designed study to confirm that the combination treatment A+B offers combined benefits of (i) slowed post-treatment tumor progression associated with drug A, and (ii) the superior tumor growth inhibition that was associated with drug B.

Concluding remarks

Faster delivery of safer and more effective medicines through collective strategies

Smart translational applications, better decisions sooner

Our statistical findings should not be interpreted as questioning the validity or predictability of mouse tumor models

Back-Up

Pooled nonlinear least squares

		Unconstrained		Constrained	
Treatment	Parameter	Est.	S.E.	Est.	S.E.
A	β_0	4.06	29.83	3.74	0.62
	β_1	0.35	2.55	0.20	0.24
	β_2	7.82	154.97	1.46	8.07
	θ	17.58	2.32	17.69	2.65
	η	5.82	49.31	2.93	10.66
B	β_0	3.78	9.16	3.74	0.54
	β_1	0.33	1.10	0.20	0.17
	β_2	8.29	61.57	2.68	5.90
	θ	19.11	0.78	19.14	1.04
	η	4.82	14.11	2.85	4.24
A+B	β_0	4.19	7.71	4.06	0.35
	β_1	0.17	0.62	0.14	0.07
	β_2	3.83	47.86	1.91	2.84
	θ	19.61	1.53	19.52	1.62
	η	3.07	14.23	2.12	4.26

Piecewise nonlinear model

	A		B		A+B	
Parameter	Est.	S.E.	Est.	S.E.	Est.	S.E.
β_{10}	3.36	0.18	3.43	0.25	3.45	0.27
β_{11}	0.24	0.03	0.23	0.03	0.21	0.04
c_1	9.49	0.46	11.11	0.96	10.93	0.98
c_2	17.42	0.65	21.5	0.74	22	1.07
β_2	1.39	0.23	2.49	0.49	2.67	0.58
β_{30}	4.22	0.21	2.03	0.79	3.02	0.70
β_{31}	0.13	0.01	0.17	0.02	0.12	0.01