Bayes Conference
Utrecht – October 26th, 2023

Longitudinal Analysis of *In Vivo* mRNA Expression Data Using Bayesian P-Splines

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Vaccine Research Statistics, GSK

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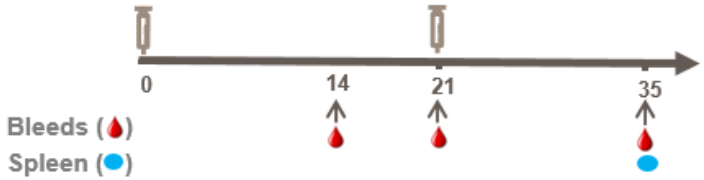
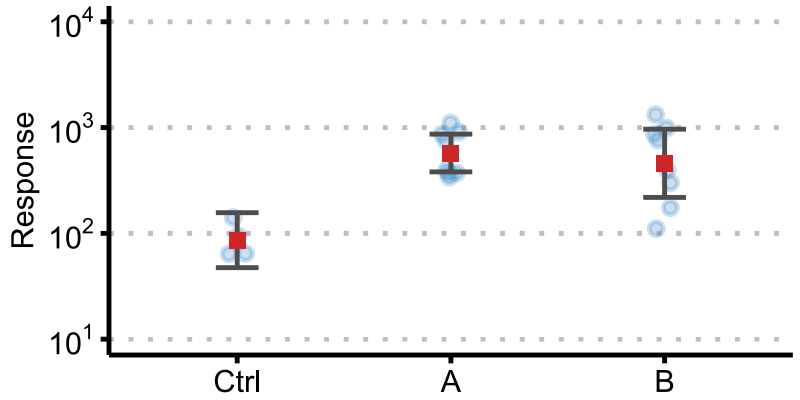
Vaccines in the Preclinical Research Setup

- Vaccine immunogenicity
  = Ability to induce an immune response
- Mainly assessed in small animal models
  on limited sample sizes (≤ 10 / group)
- Inbred mice (Balb/c, C57BL/6)
- Responses: Antigen-specific antibodies in sera, cellular responses (T cells,…) in the spleen, bacterial load…





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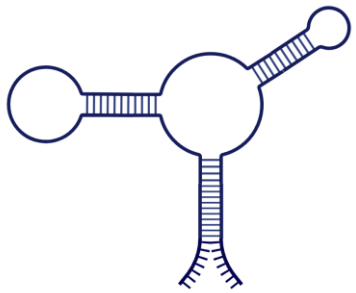
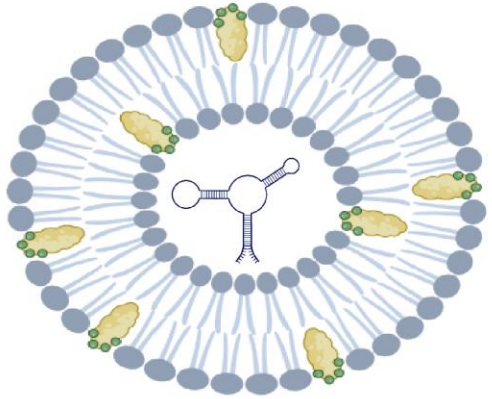
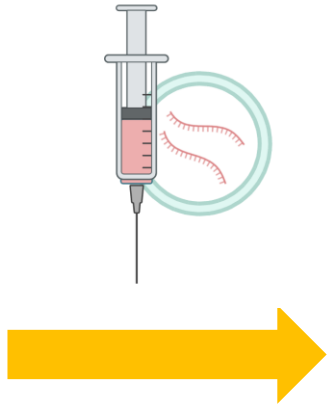
Adapted from C. Taverne (GSK Vaccine)

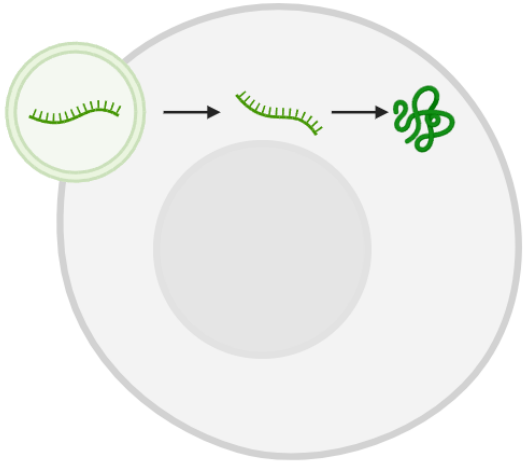
RNA Vaccines: Expression and timing

RNA backbone + Delivery vehicle









Challenges

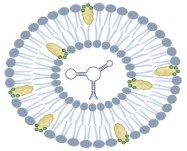
- **Low expression level**
- (Innate immune sensing)

Created with BioRender.com

Pardi N et al. (2018): mRNA vaccines - a new era in vaccinology. Nature reviews. Drug discovery (https://doi.org/10.1038/nrd.2017.243)

In Vivo tracking of particles by BioLuminescent Imaging (BLI)

mRNA coding for bioluminescent Luciferase
+ substrate



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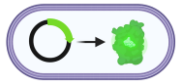
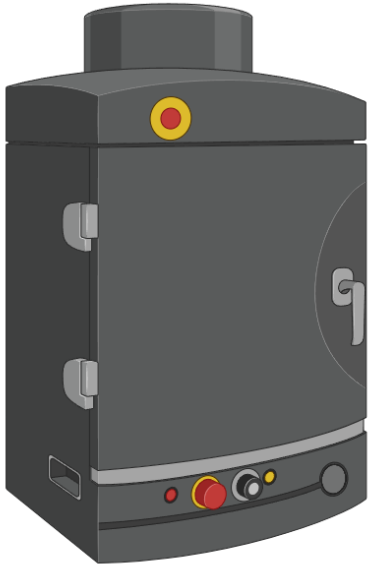


Bioluminescent engineered bacteria

In Vivo Imaging system

(acquisition of light signal)



Data

Created with BioRender.com

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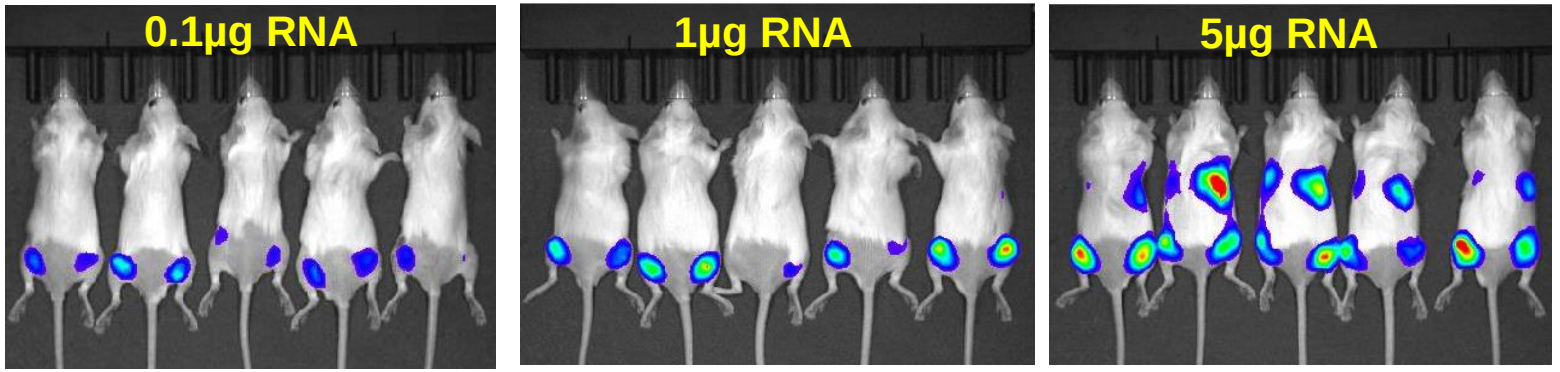
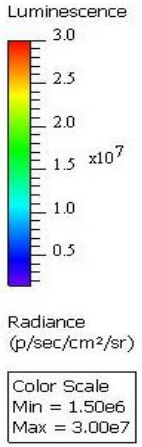
Mezzanotte et al. (2017): In Vivo Molecular Bioluminescence Imaging: New Tools and Applications Trends in Biotechnology. https://doi.org/10.1016/j.tibtech.2017.03.012

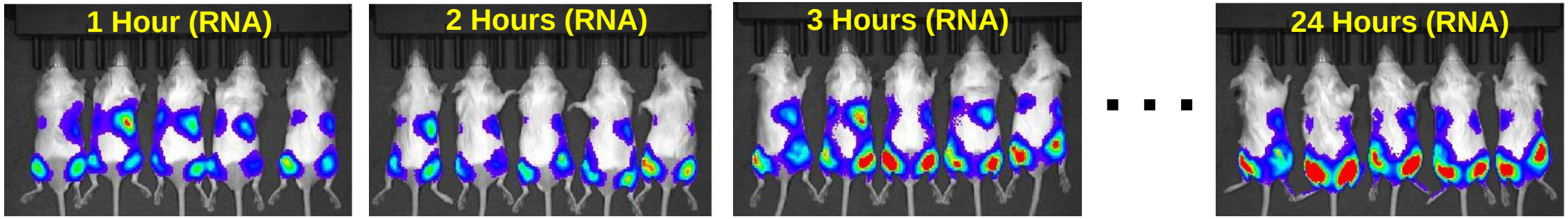
In Vivo BioLuminescent Imaging (BLI)

Proportionality of response signal to “treatment”





Kinetic of response signal



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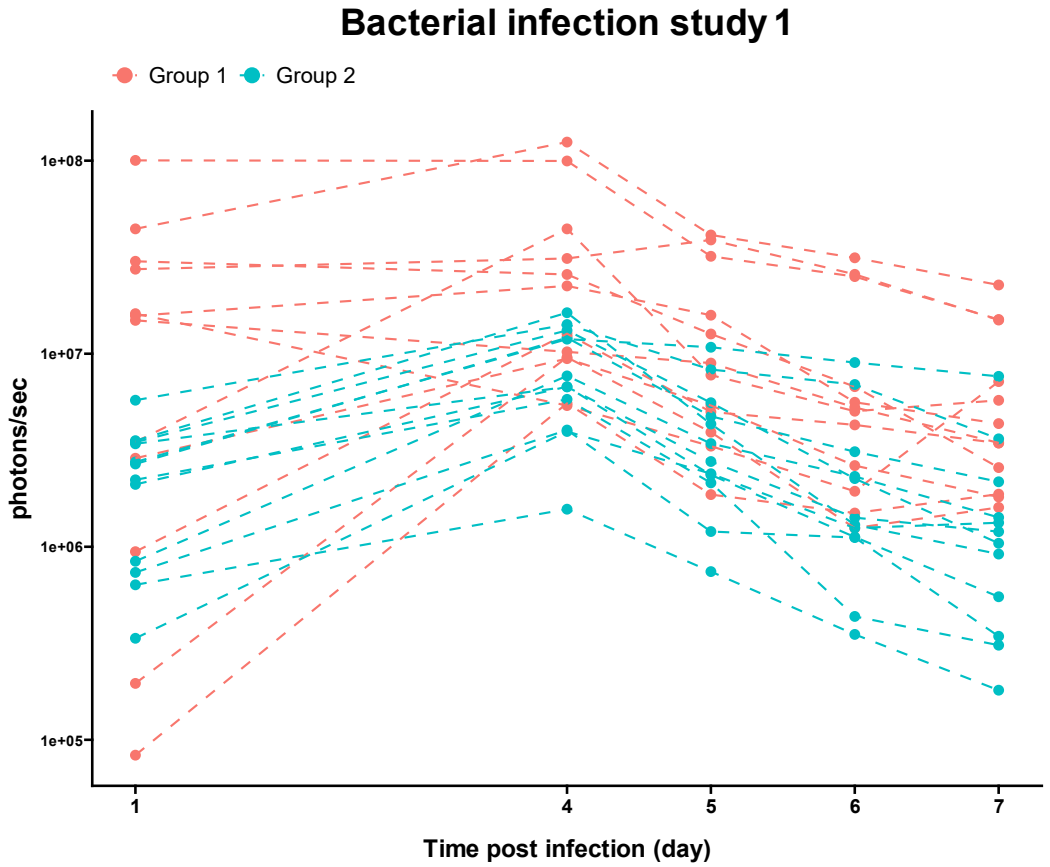
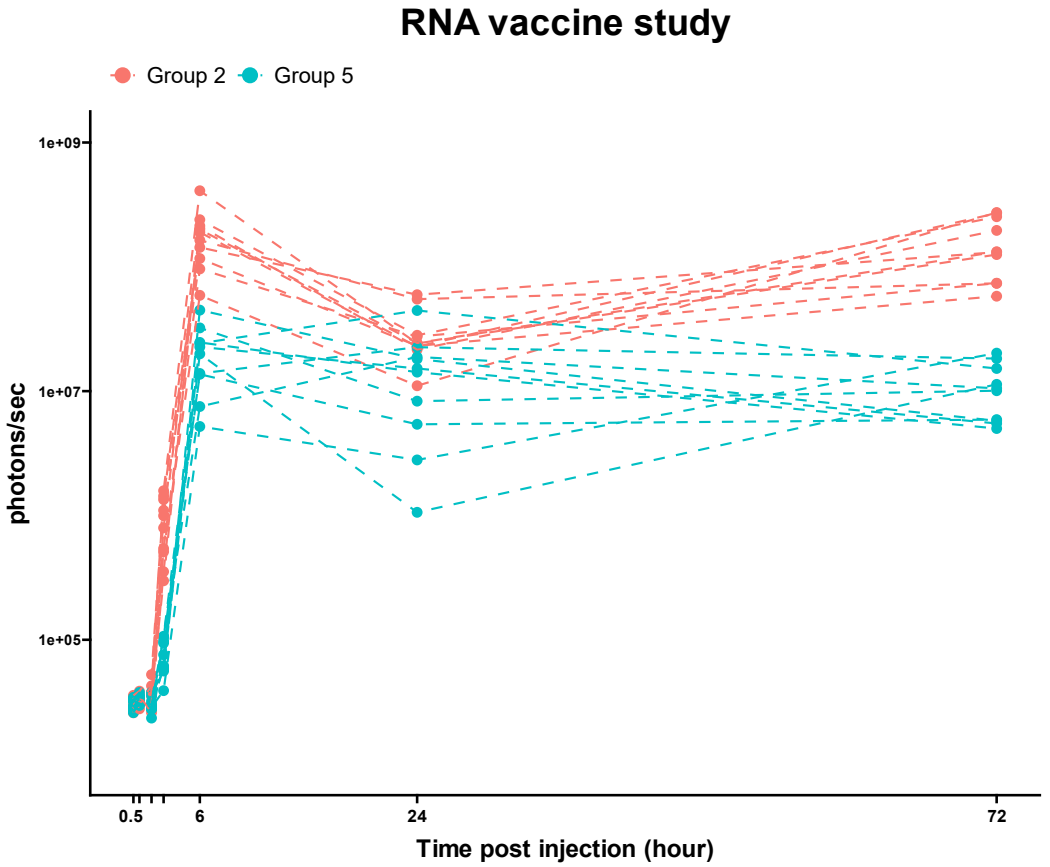
All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee at GSK.

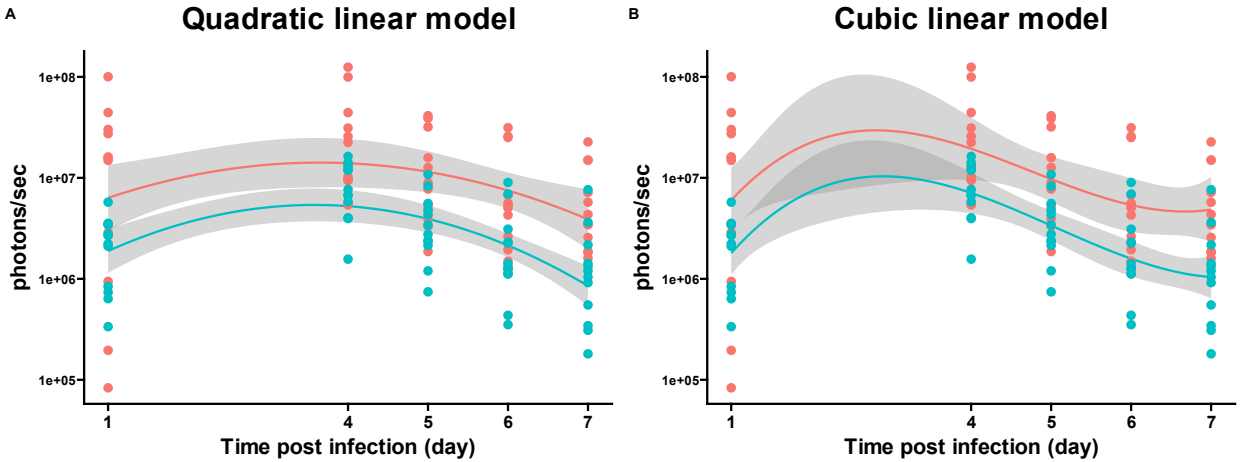
Diversity of response profiles



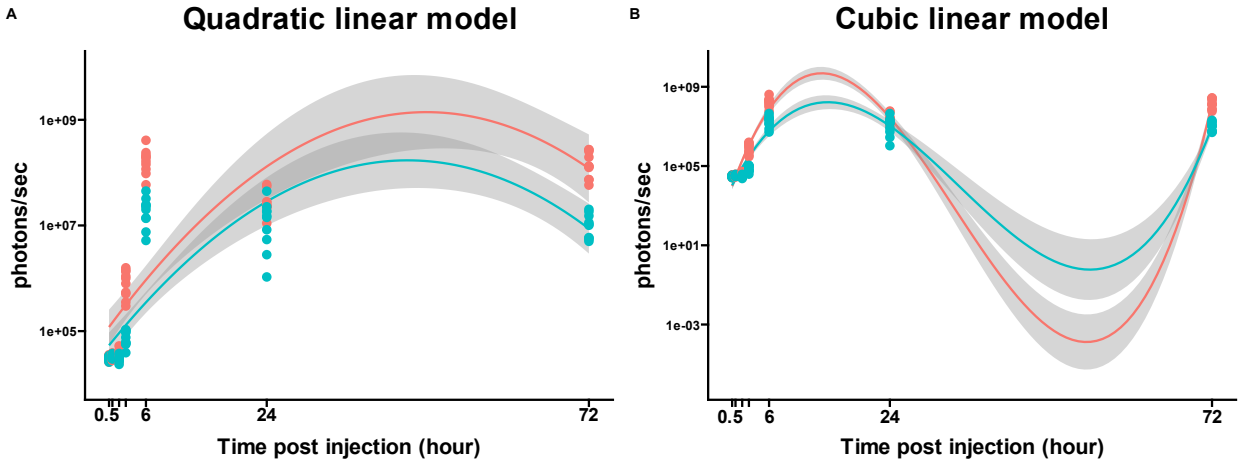


Fitting Models

Bacterial infection study 1



RNA vaccine study



Increase polynomial order? $y_i = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_2 x^3 + \cdots + \beta_k x^k + \epsilon_i$

Devise a mathematical model for each response profile?

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Generalized Additive Models (GAM)

smooth functions

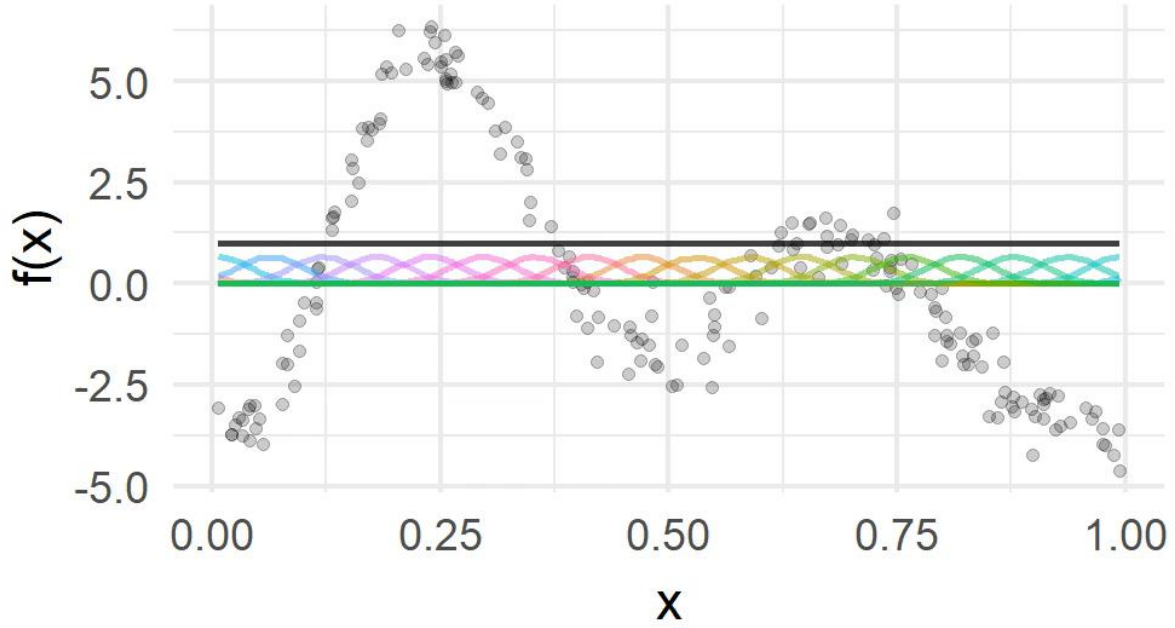
$$g(y_i) = \beta_0 + \sum_j s_j(x_{ji}) + \epsilon_i$$

"weighting"

base functions

$$s(x) = \sum_k^K \beta_k b_k(x)$$

with $\epsilon_i \sim N(0, \sigma^2)$



Adapted from: Introduction to Generalized Additive Models with R and mgcv – YouTube channel Bottom of the Heap by Gavin Simpson, University of Regina - Canada

"Smoothing" parameters estimation

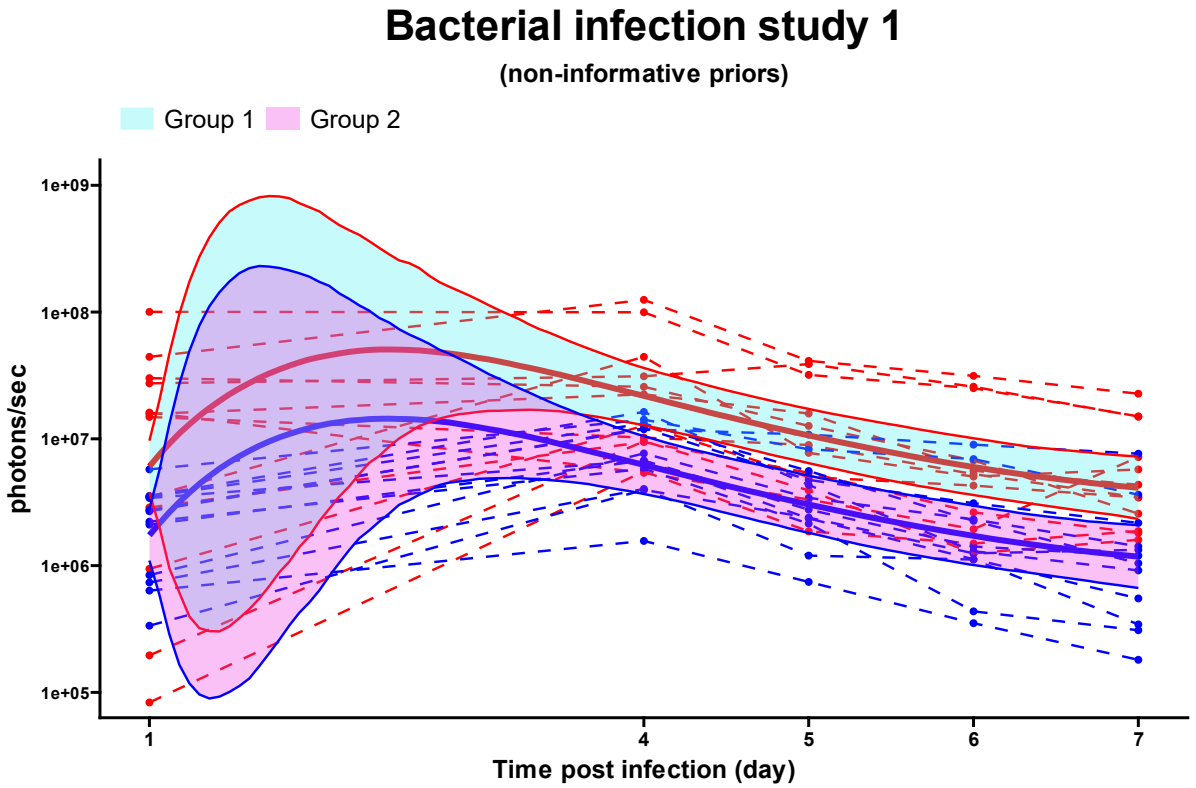
$$\mathcal{L}_p(\beta, \lambda) = \mathcal{L}(\beta) exp(-\beta \boldsymbol{S}_\lambda \beta)$$

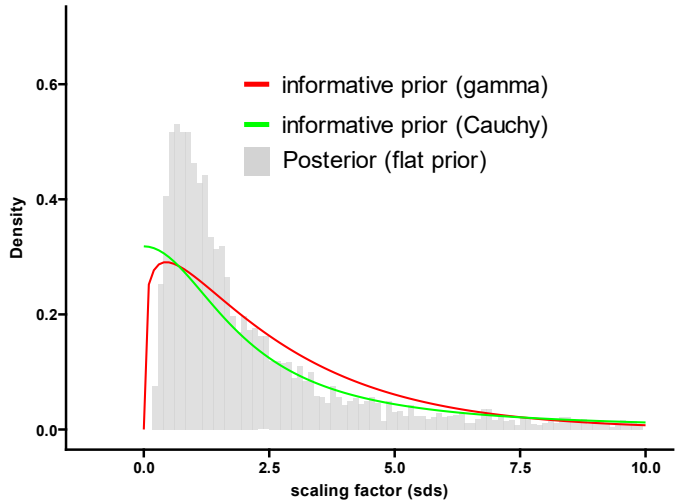
penalty

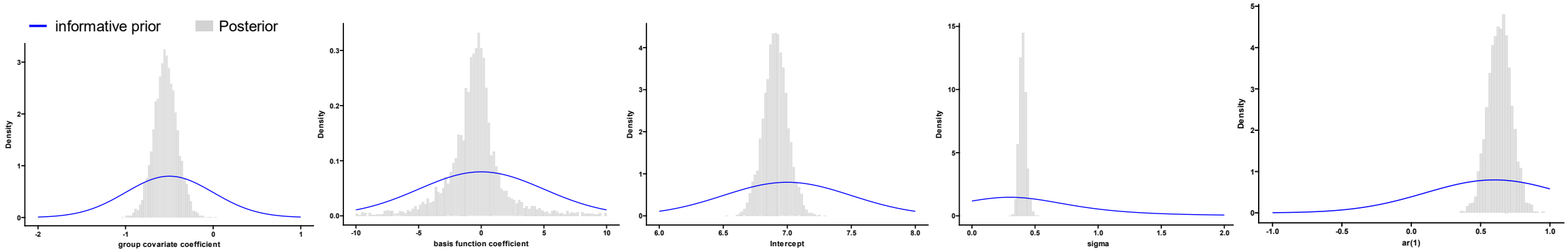
wiggliness

Hastie, T.J. (1990). Generalized Additive Models (1st ed.). Routledge. https://doi.org/10.1201/9780203753781

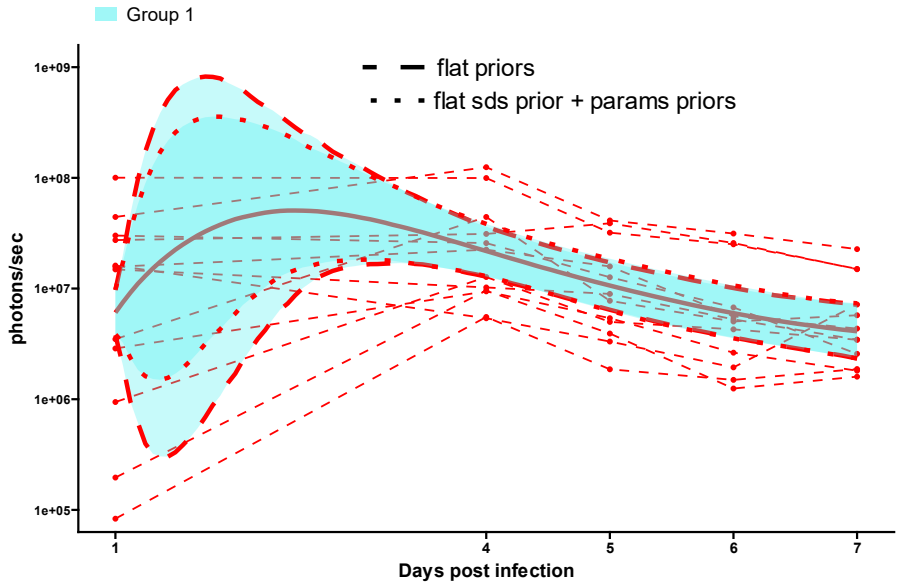
Building the priors for future studies

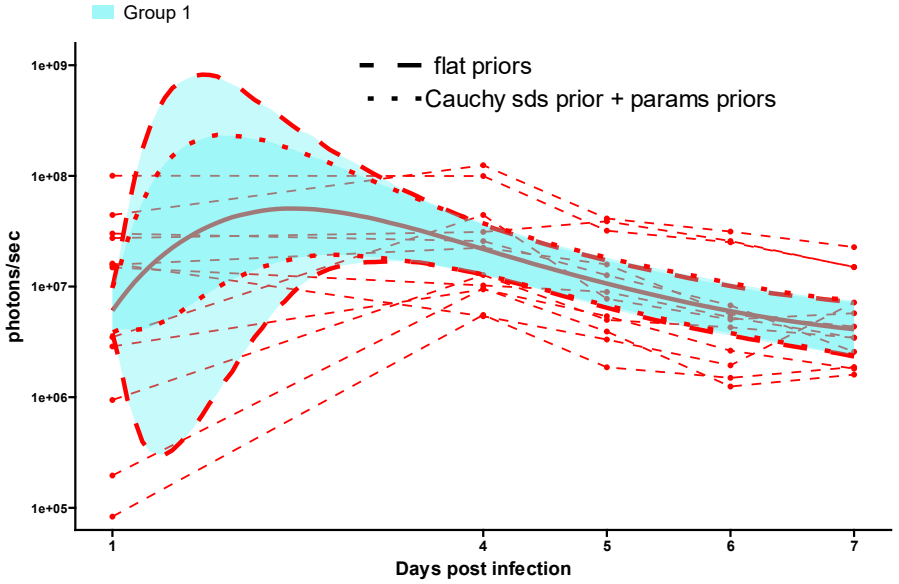


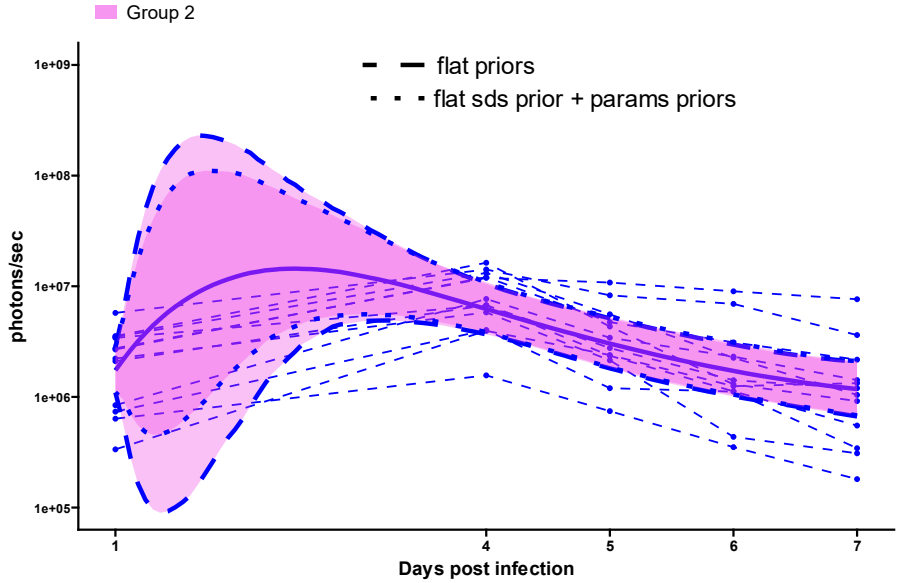


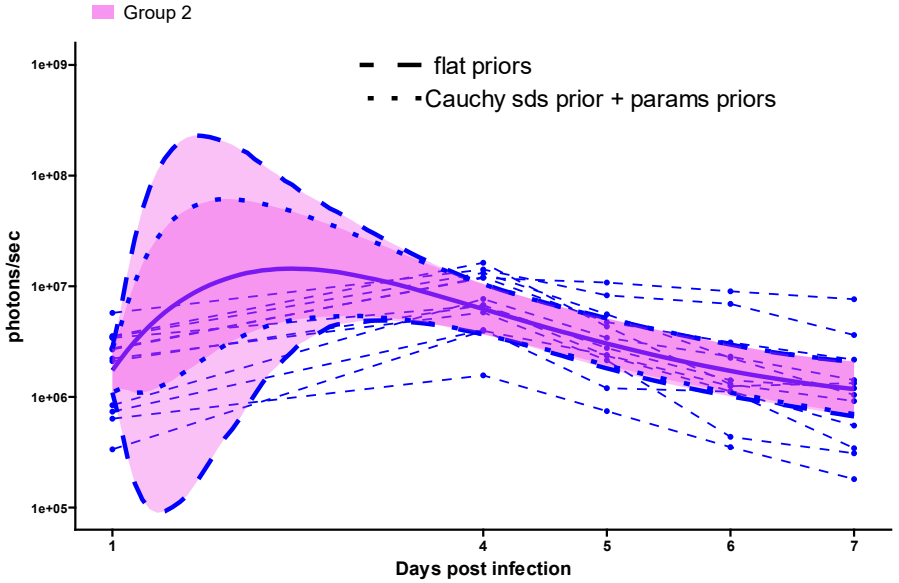


Building the studies: Bacterial infection study 2









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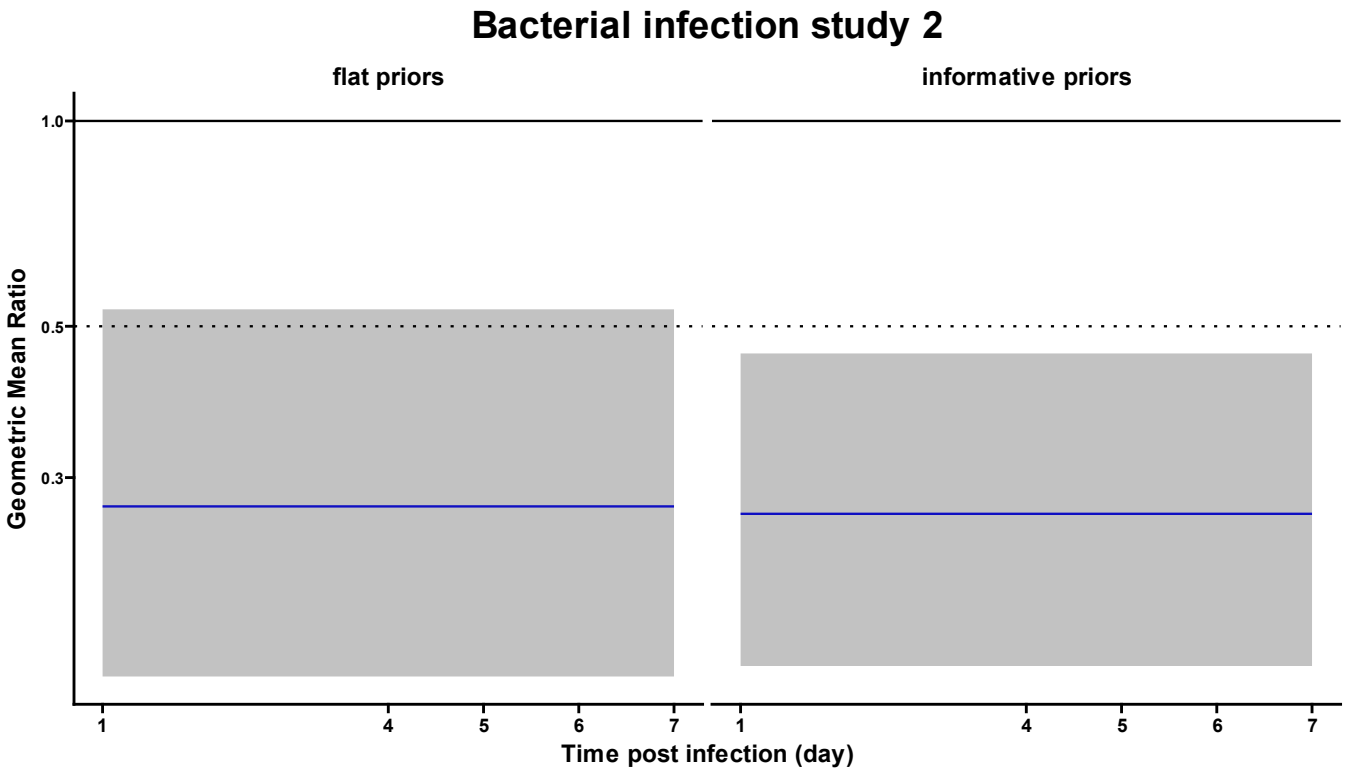
Informative priors impacted the model variability

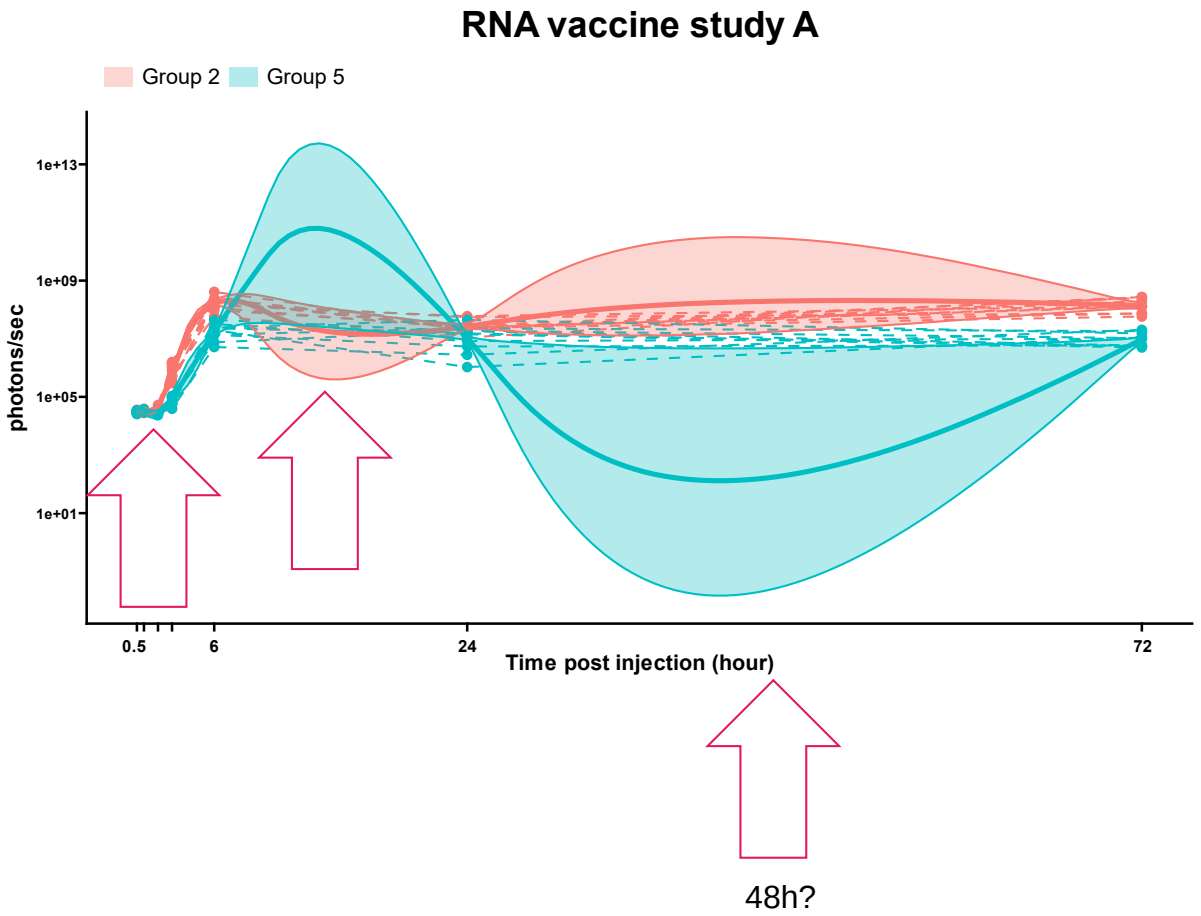
Building the studies (2)

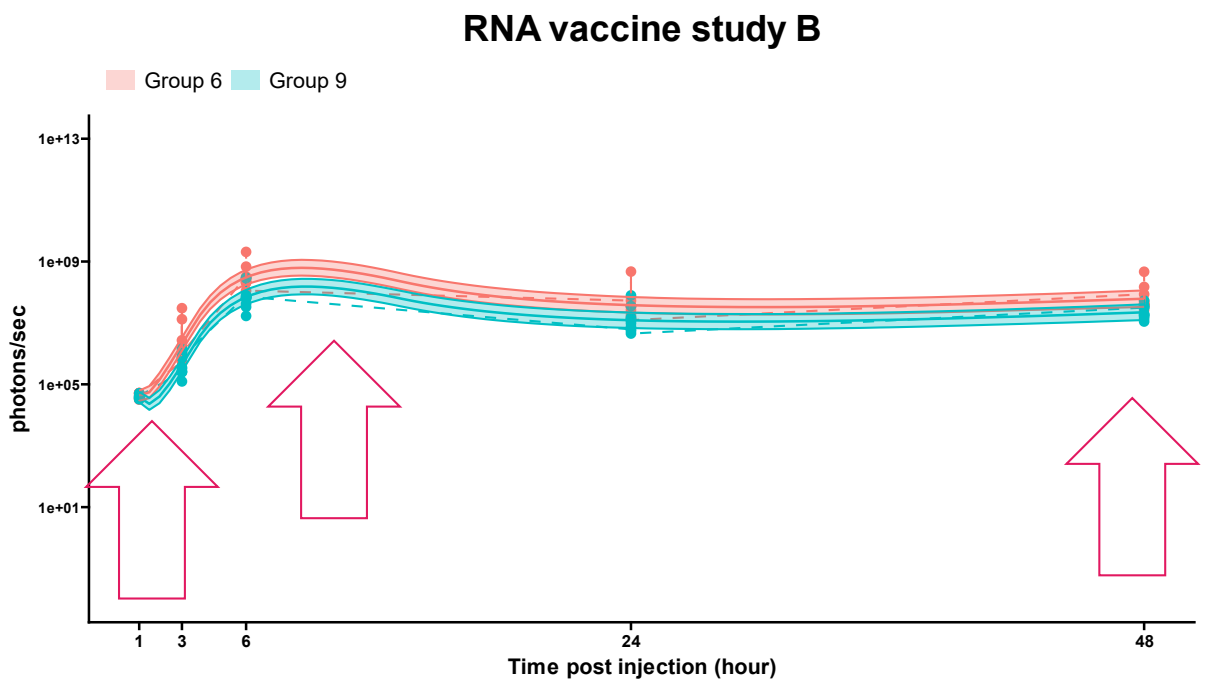


Success criteria

Conclusion impacted !

Impact of the study design





Appropriate time point measurements still critical

Concluding remarks

Advantages

- **Interpretability**
- **Flexibility and Automation**
- **Regularization**
- **Reduction of sample size (3Rs)**

Outstanding issues

- **Time points (study design) must be selected appropriately**
- **In-build autocorrelation specification not always available**

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Thank you for your attention!

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Disclaimer

All authors are employees of the GSK group of companies.
This work was sponsored by GlaxoSmithKline Biologicals SA.

3Rs statement - GSK is committed to the replacement, reduction, and refinement of animal studies (3Rs). Non-animal models and alternative technologies are part of our strategy and employed where possible. When animals are required, application of robust study design principles and peer review minimizes animal use, reduces harm, and improves benefit in studies.

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