Estimation of In-vitro Dose Response Curves in the Presence of Drug Resistant Viral Mutations.

D. Abainenamar, T. Jacobs, H. Ceulemans, O.Lenz, G. Molenberghs, T. Verbinnen

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OUTLINE

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- Statistical Method and Simulation
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- Conclusion

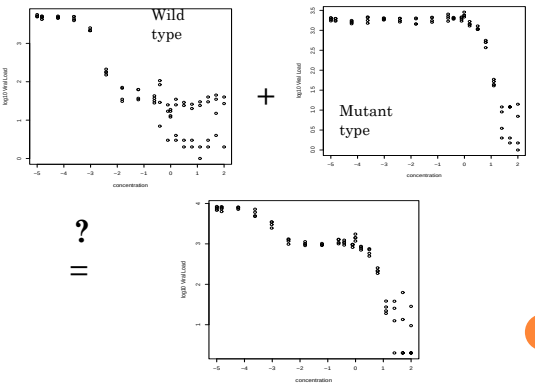
Introduction

- Inhibition of viral replication with direct acting antiviral agents can result in selection of viral variants.
- Difficulty: viral strains become 'smart' and mutate.
  - Creates resistance
  - High doses of antiviral agents needed
- Patients can have both a mutant strain and a wild type.
  - In combination, does the dose response curve change?

Question?

Can we predict the exposure-response for a combination of wild type and mutant virus types, based on the individual exposure response curves?

In-vitro Viral Combinations



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Study Design

- Experiment design
  - The effect of drug exposure is tested in-vitro after 48hrs incubation.
  - Partitions were ranging such that:
    - 100% wild type: 0% mutant strain
    - .
    - .
    - .
    - 0% wild type : 100% mutant strain
- Four wells each containing combinations of wild type and mutant viral strains.

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Methodology

- Statistical model per viral strain:

$$Y_{ij} \sim LN(\mu_{ij}, \sigma^2)$$

$$\mu_{ij} = E_{0j}\left(1 - \frac{E_{\max j}}{1 + 10^{(\log_{10} EC50_j - \log c_p)^{\gamma_j}}}\right)$$

$$E_{\max j} = \frac{\exp(I_{\max j})}{1 + \exp(I_{\max j})}$$

- Estimate the combinatory wells as a mixture of the individual profiles:

$$\mu_{i,comb} = \log_{10}(\pi_{WT} 10^{\mu_{i,WT}} + \pi_{MT} 10^{\mu_{i,MT}})$$

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Methodology

- Set of combinations; one function to fit it.
  - Monotone increasing function constrained between 0 and 1.
    - Mimic the extremes 100%: 0%
  - Flexibility for the points within.
- CDF of a Beta Distribution
  - Re-parametrized based on Ferrari Cribari-Neto
    - the estimators of the proportions are changed such that:

$$\pi_{WTorMT} = F(x; A, B)$$
$$A = \mu\varphi$$
$$B = (1 - \mu)\varphi$$

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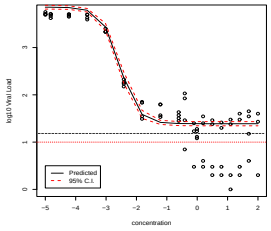
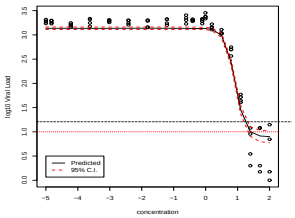
Final Model Fits



100% Wild Type with 95% C.I



100% Mutant with 95% C.I

- Model fit at the 'edges' of the viral strains.
- Wild type appears completely suppressed at lower concentrations than the mutant type.
- Quantification Limit is at $\log_{10}$ (Viral Load)=1

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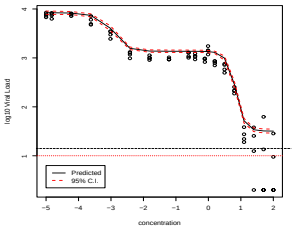
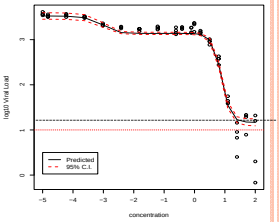
Model Results



50% wild type : 50% mutant



10% wild type: 90% mutant

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Model Results

Table 1: Parameter Estimates from the Final Model

		Wild Type		Mutant Type	
Effect	Parameter	Estimate	95% C.I	Estimate	95% C.I.
Initial Estimate	E_0	2.47	2.41; 2.53	2.24	2.11; 2.37
Hill coefficient	η_j	1.45	1.23; 1.68	2.74	2.36; 3.12
½ Max effective conc.	Log_{10} EC50	-2.54	-2.60; -2.49	0.92	0.89; 0.95
Max. estimate	Em	24.49	21.99; 26.97	7.91	5.59; 10.23
Growth factor	μ	0.18	0.16; 0.21	0.20	0.19; 0.21
Sigma	σ	0.16	0.17; 0.15	0.16	0.17; 0.15

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Simulation for Optimization

- Replica of the study design
  - Scenario mimics experiment with different setting
    - Scenario: 6 partitionings and 6 concentration profiles
  - Approximately 1970 data sets or studies analyzed.
  - Goal: how close do we come to the model's EC50 i.e. the theoretical EC50?

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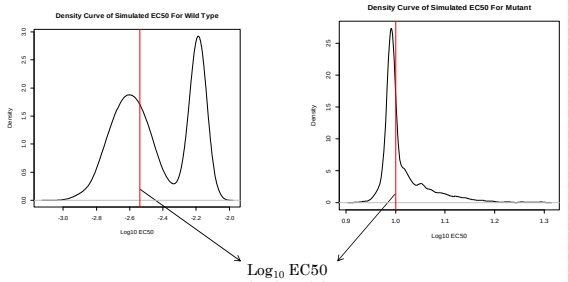
Simulation Results



Log_{10} EC50 final model

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Other Applications

- Patients may carry one or both strains of the virus.
- Possible to not only carry this out but also to find the estimates of the growth rates for a patients using the same model.
- Scenario
  - Blood sample taken and ex-vivo the dose response.

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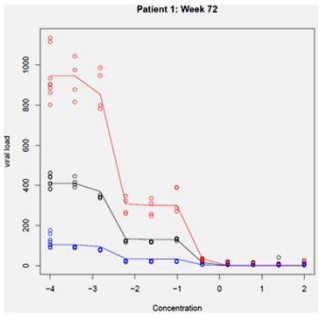
Other Applications



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Conclusion

- Modeling combinatory experiments gives insight to growth rates when both mutant and wild type strains are present.
  - Possible to see at which concentrations when in combinations that viral suppression is achieved.
- Close to theoretical or model EC50 when experiment is optimized.
- Model useful in patient information.
  - Able to tell at which concentrations suppression is achieved regardless of the viral strains present.

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THANK YOU.

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