Improving Methods for Analyzing Antimalarial Drug Efficacy Trials: Molecular Correction Based on Length-Polymorphic Markers *msp-1*, *msp-2*, and *glurp*

Sam Jones, Katherine Kay, Eva Maria Hodel, Sophy Chy, Aimable Mbituymuremyi, Aline Uwimana, Didier Ménard , Ingrid Felger , Ian Hastings

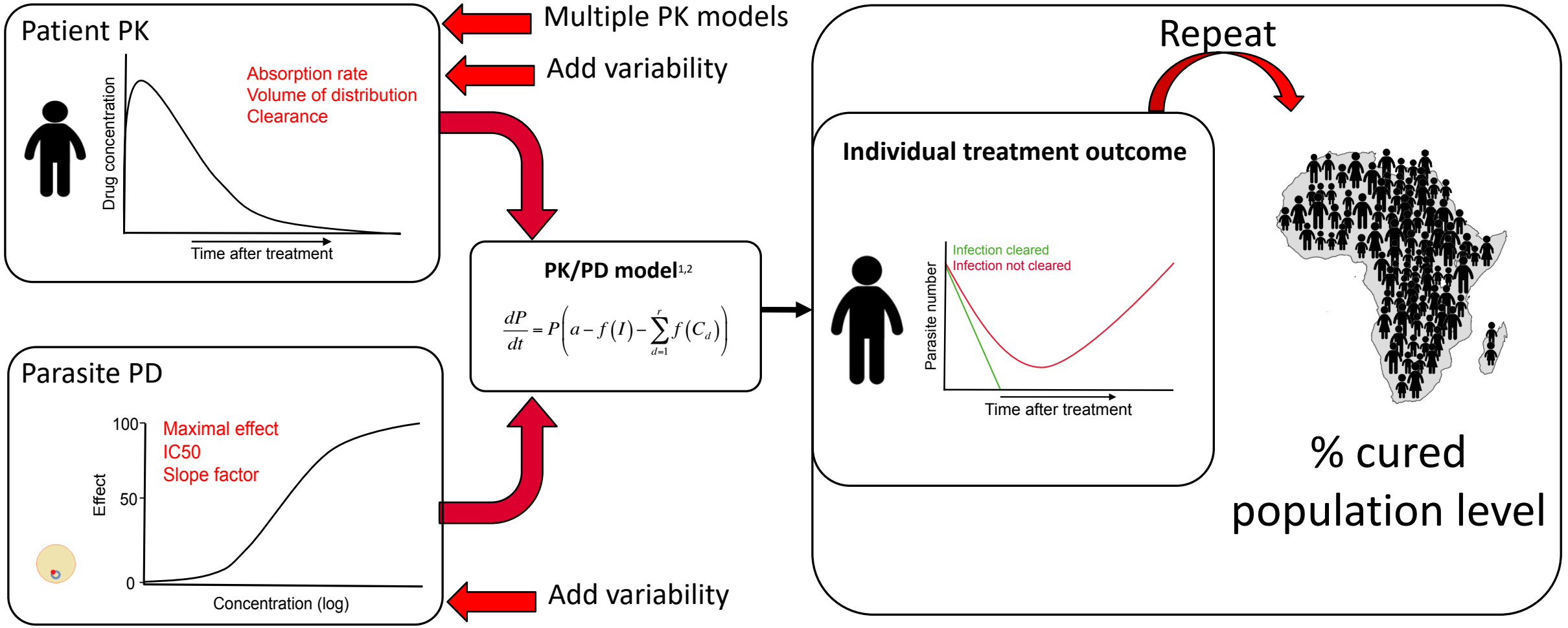
METRUM RESEARCH GROUP

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Malaria

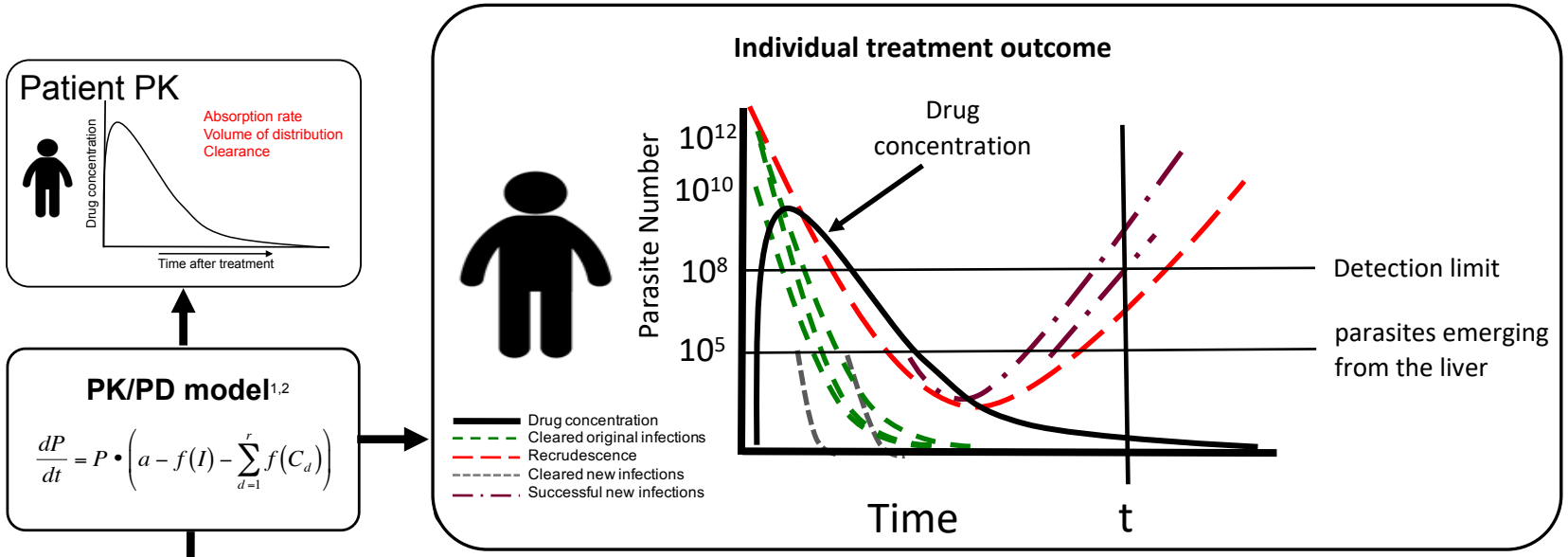
- Endemic in over 100 countries and causes ~400,000 deaths per annum
- Prompt treatment is an essential and effective public-health tool but drug resistance poses a constant threat
- Drug efficacy trials monitor the continued efficacy of front-line drugs against falciparum malaria
  - Over-estimates = countries retaining a failing drug as first-line treatment with associated increases in morbidity and mortality
  - Under-estimates = removal of an effective treatment with substantial practical and economic implications
- Trials are challenging
  - Require long durations of follow-up to detect drug failures
  - Patients are frequently re-infected during follow up

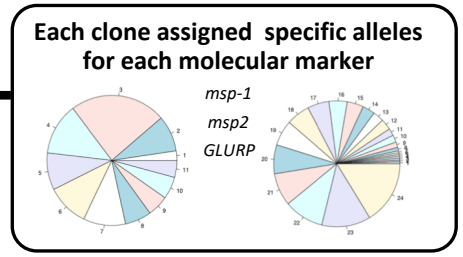
Mechanistic PK-PD model for malaria



Kay, K. and I. M. Hastings (2011). "Development, evaluation, and application of an *in silico* model for antimalarial drug treatment and failure." Antimicrob Agents Chemother **55**(7): 3380-3392

Modifications for clinical trial analysis





Major challenge of efficacy trials

- Distinguishing reason for recurrent infection i.e. recrudescent versus new infection

Advantage of R

- Free, open-source software that is readily available to researchers in developing countries

Modifications

- Multiple clones at the time of treatment
- Reinfections emerge from the liver at a rate reflecting local malaria intensity
- Parasitemia of each clone was tracked and updated each day to reflect
  1. Extent of drug killing based on the PKPD
  2. Growth rate of each clone
- Each clone assigned a genetic profile based on 3 specific molecular markers

Schoepflin et al. 2009. Comparison of Plasmodium falciparum allelic frequency distribution in different endemic settings by high-resolution genotyping. Malar J 8:250.
Messerli et al. 2017. Critical evaluation of molecular monitoring in malaria drug efficacy trials and pitfalls of length-polymorphic markers. Antimicrob Agents Chemother 61:e01500 -16.

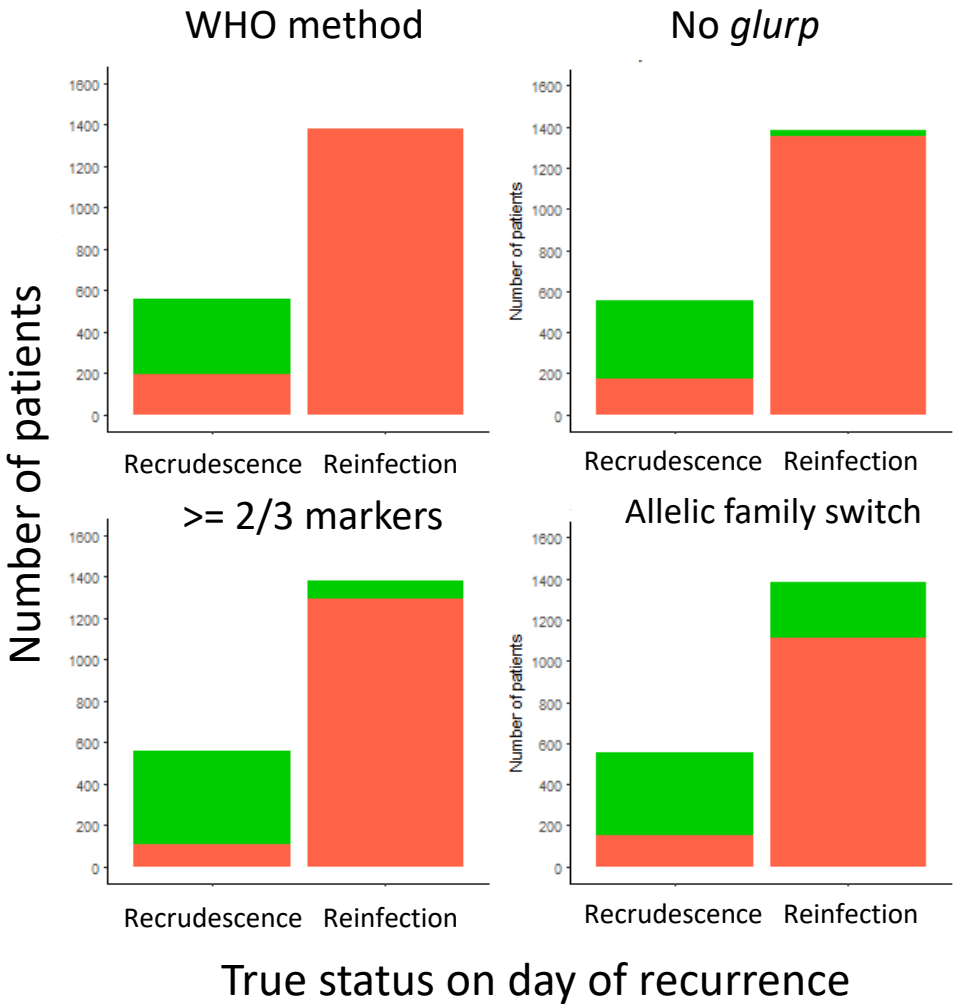
Clinical trial simulations

PK-PD Model: tracks the genotype of all existing and new malaria clones over time and allows us to determine the true drug failure rate

Reality: not all genetic signals will be observed in every blood sample, detectability varies based on relative frequency, allelic length and family

Simulations: incorporated these problems to generate the genetic signals that would be observed in samples and investigated the predictive capability of four molecular correction algorithms

Results – comparing algorithms



Patient classified as
- Recrudescence
- Reinfection

- WHO algorithm correctly classifies nearly all reinfections, but misclassifies around one-third of recrudescences
- No-*glurp* algorithm is similar to the WHO algorithm
- >= 2/3 markers algorithm had fewer misclassifications and was also more balanced
- Allelic family switch algorithm correctly classifies a large proportion of recrudescences but misclassifies a large number of reinfections.

Results – re-analysis of clinical trial data

Country	Drug	Molecular assignment	Number of infections classified by algorithm			
			WHO	No glurp	≥ 2/3 markers	Allelic family switch
Rwanda	Artemether - Lumefantrine	Recrudescence	17	27	36	59
		New infections	93	83	73	51
	DHA - Piperquine	Recrudescence	3	6	8	18
		New infections	40	37	35	25

- Model predictions using the WHO method were highly consistent with existing in vivo data
- Predictions with the newly proposed "≥ 2/3 markers" algorithm suggest the WHO-method under-estimates true treatment failure rates

Jones et al. (2019) Improving Methods for Analyzing Antimalarial Drug Efficacy Trials: Molecular Correction Based on Length-Polymorphic Markers *msp-1*, *msp-2*, and *glurp*. AAC, 63 (9) e00590-19; **DOI:** 10.1128/AAC.00590-19

Summary

- Model was applied to multiple drugs across a range of transmission settings and was able to quantify the accuracy of failure rate estimates in therapeutic efficacy studies
- Accurately predicting failure estimates is clinically important
  - Molecular correction is essential to avoid substantial over-estimates of failure rates.
  - Current WHO-recommended algorithm consistently under-estimates the true failure rate.
  - A newly-proposed algorithm (“≥ 2/3 markers”) produces accurate failure rate estimates, robust at all levels of transmission intensity.
  - Long durations of patient follow-up may be counterproductive; large numbers of new infections accumulate and may be misclassified, over-estimating drug failure rate.

Summary

- Implemented in R - mainly base R, with *dplyr*, *ggplot*, *survival and survminer* packages for calculating drug efficacy
- Open source software particularly attractive for tropical diseases as they typically occur in countries with limited resources and paying for licenses can be an unnecessary barrier to progress
- Future
  - Developing a Bayesian algorithm to more accurately predict failure rates
  - Developing an R package that could be used to process user sample data and return failure rate estimates based on all the algorithms

Acknowledgements

Liverpool School of Tropical Medicine

- Sam Jones
- Ian Hastings
- Eva Maria Hodel

Institut Pasteur

- Didier Ménard

Swiss Tropical and Public Health Institute

- Ingrid Felger

Institut Pasteur in Cambodia

- Sophy Chy

Rwanda Biomedical Center

- Aimable Mbituymuremyi
- Aline Uwimana

Grant: 1032350

Grant: G1100522 & MR/L022508/1

Grant: UWSC9757