

A Liver Model for Chemoprotection Against Malaria

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Background

Malaria is a deadly parasitic threat and to prevent the disease, it is important to protect people from infection, particularly from *Plasmodium falciparum*, which is the most prevalent human malaria strain in Africa. Chemoprotective drugs are developed for this purpose. These should be taken at regular intervals for as long as a person is at risk. The intervals should be infrequent, ideally monthly. To reach this goal, the drug must kill the parasites not only during blood-stage but also during development in the liver, the first stage of infection after an infectious mosquito bite.

Whilst PKPD models have been developed to understand and describe the blood-stage activity of potential antimalarials, hardly any model has been proposed for liver-stage activity.

In vitro [1] and preclinical *in vivo* [2, 3] experiments have been developed to assess the efficacy of drugs on the liver-stage, as well as a liver-stage controlled human malaria infection model (CHMI). However, what makes it difficult to obtain a PKPD model for liver-stage activity is that unlike for blood-stage, where direct measurement of the infection load is possible, this is technically not feasible for liver-stage parasites.

Objective

To develop a PKPD model to describe the drug killing effect on the liver-stage parasites. DSM265, a plasmodial dihydroorotate dehydrogenase (DHODH) inhibitor, which is currently in development, is chosen as an example to illustrate the approach.

Methods

Data:

- Two liver-stage Controlled Human Malaria Infection (CHMI) studies (Table 1) were conducted to evaluate the prophylaxis efficacy of DSM265.
- A 400mg single dose, or placebo, was administered 1, 3 or 7 days to healthy volunteers before I.V. injection of 3200 sporozoites or 5 bites from infected mosquito.
- DSM265 concentration (Fig. 1A) and blood-stage parasitemia (Fig. 1B) were measured in all participants [4].

Study, Cohort #	# Subjects (Placebo)	Treatment Day	Vehicle	Inoculation
Tübingen #1	8 (2)	-7	I.V.	3200 p.
Tübingen #2	7 (2)	-1	I.V.	3200 p.
Seattle #1	8 (2)	-7	I.V.	3200 p.
Seattle #2	8 (2)	-3	I.V.	3200 p.
Seattle #3	8 (2)	-7	Mosquitoes	5 bites

Table 1: Summary of the CHMI data for DSM265

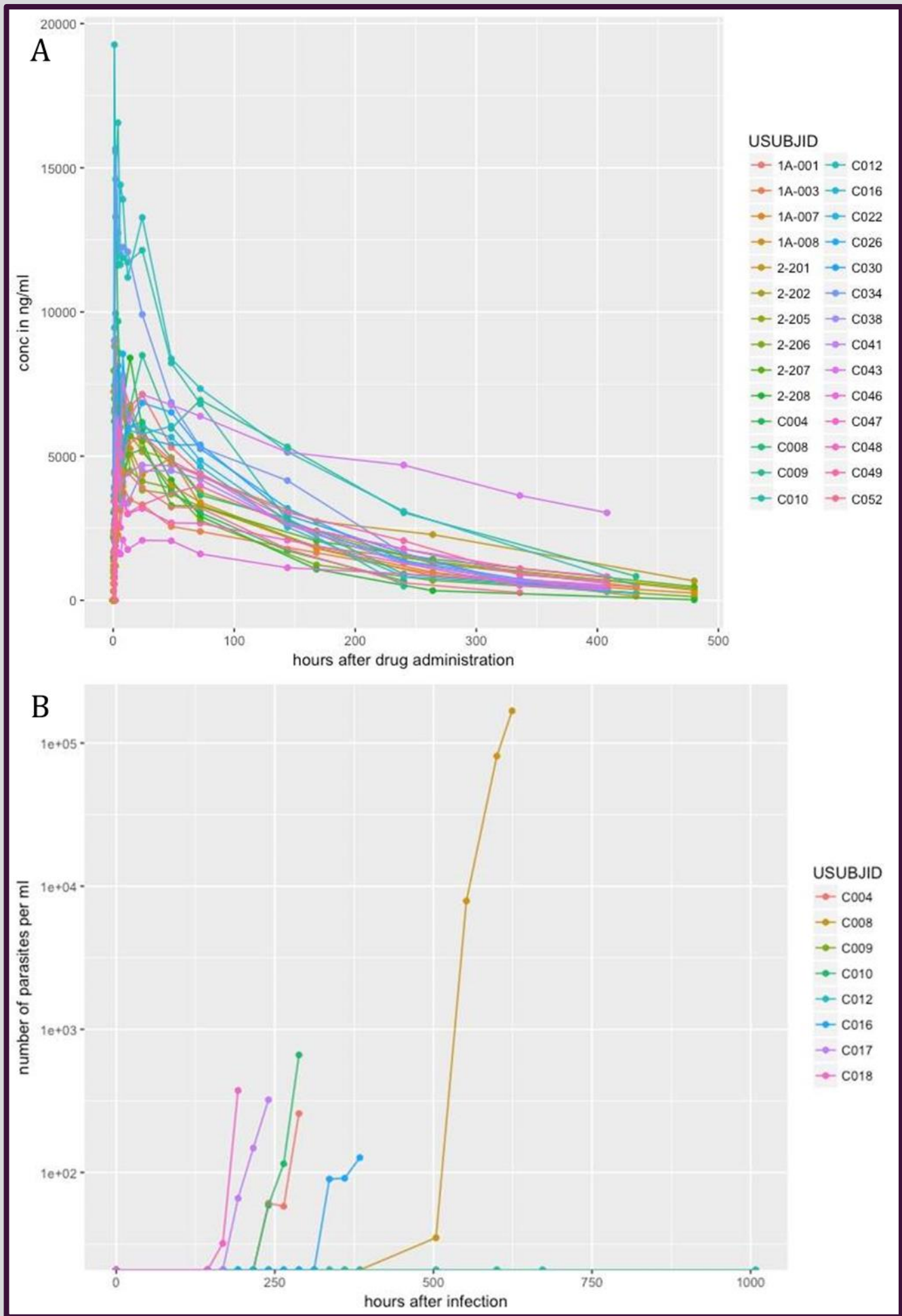


Figure 1: Example of CHMI data; (A) PK data after a 400mg single dose of DSM265, (B) Parasitemia data of volunteers who received DSM265 or placebo 3 days prior sporozoite inoculation.

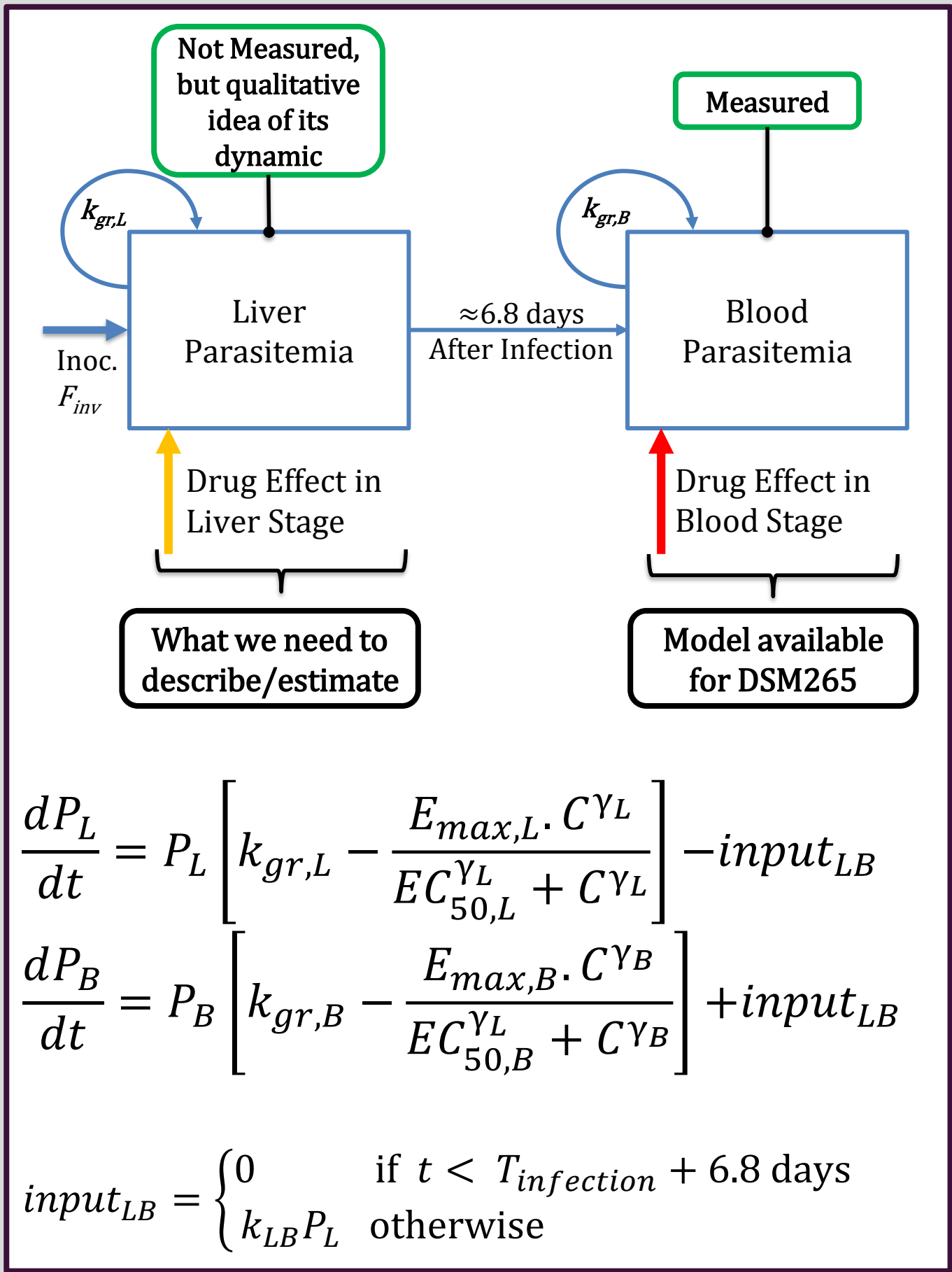


Figure 2: Schematic representation of the PKPD model for chemoprotection, and its equations. P_L and P_B being the parasitemia in the Liver and the Blood, respectively. The effect of DSM265 on the liver-stage and in the blood-stage is assumed to be driven by the blood concentration.

Mathematical Model:

- Only a fraction F_{inv} of the injected sporozoites are assumed infectious.
- At 6.8 day after infection the parasites are transferred from liver to blood [5].
- Assuming exponential growth and every sporozoite leads to 30,000 merozoites in 6.8 days, the parasite growth rate in the liver was estimated to be $k_{gr,L} = 0.0632 \text{ hour}^{-1}$.
- Blood activity parameters were fixed from a previous blood-stage CHMI study (Table 2).

Parameter	Units	Value
$k_{gr,B}$	1/h	0.068
$E_{max,B}$	1/h	0.202
$EC_{50,B}$	ng/mL	1660
γ_B	-	2

Table 2: PD parameters of DSM265 for blood-stage. Results from a previous blood-stage CHMI study

Results

- $E_{max,L}$ was not estimated due to a high correlation between $E_{max,L}$ and $EC_{50,L}$ (not shown), but fixed to the blood-stage value.
- F_{inv} was estimated to be 0.17%, which translates to 5-6 hepatocytes that have been infected (Table 3).
- No False-Negative are predicted (Fig. 3 & Table 4)

Definitions:

- True Positive:** Blood parasitemia observed and predicted
- False Positive:** Blood parasitemia predicted but not observed
- True Negative:** Blood parasitemia neither observed nor predicted
- False Negative:** Blood Parasitemia observed but not predicted

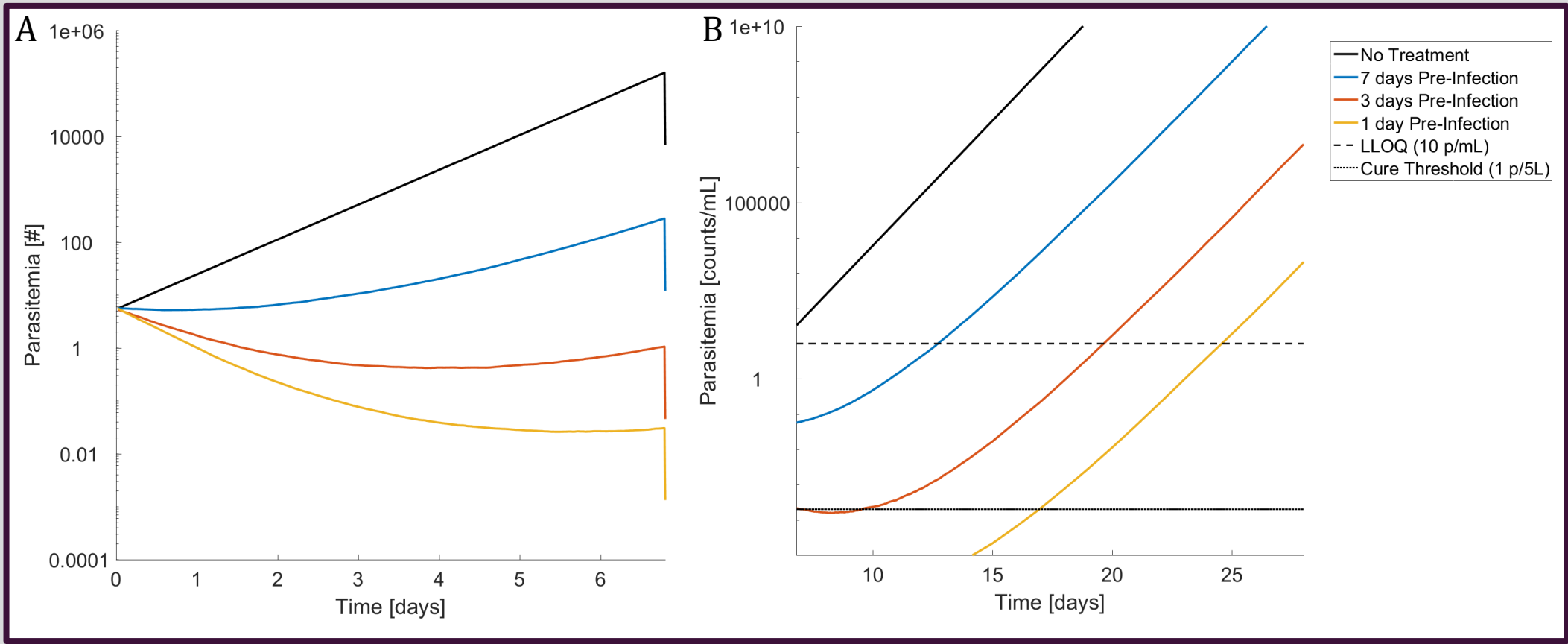


Figure 4: Population prediction of the effect of DSM265 on liver parasitemia (A) and blood parasitemia (B) for different day of administration prior sporozoite injection.

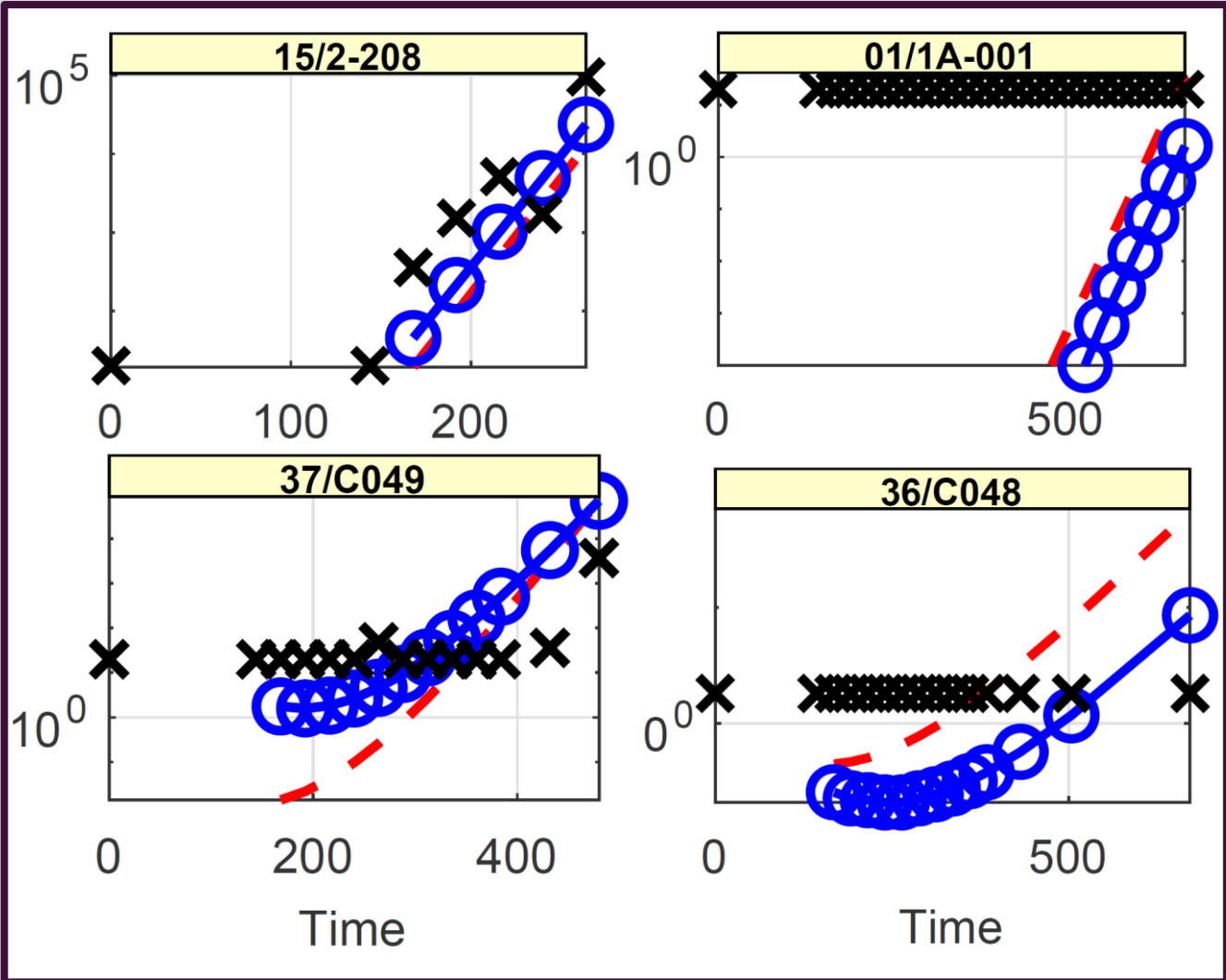


Figure 3: Individual fit examples of parasitemia data; The red and blue lines represent the population and individual predictions, respectively. The two plots on the left are true positives, the top right a true negative and the bottom right a false positive.

Parameter	Units	Value
$k_{gr,L}$	1/h	0.063 (Fixed)
$E_{max,L}$	1/h	0.202 ($=E_{max,B}$)
$EC_{50,L}$	ng/mL	2950
γ_L	-	2 ($=\gamma_B$)
F_{inv}	%	0.17

Table 3: Estimation results using Monolix v4.3.3.

	True	False	Total
Positive	51.7%	20.7%	72.4%
Negative	27.6%	0%	27.6%
Total	79.3	20.7%	100%

Table 4: Summary of the individual prediction.

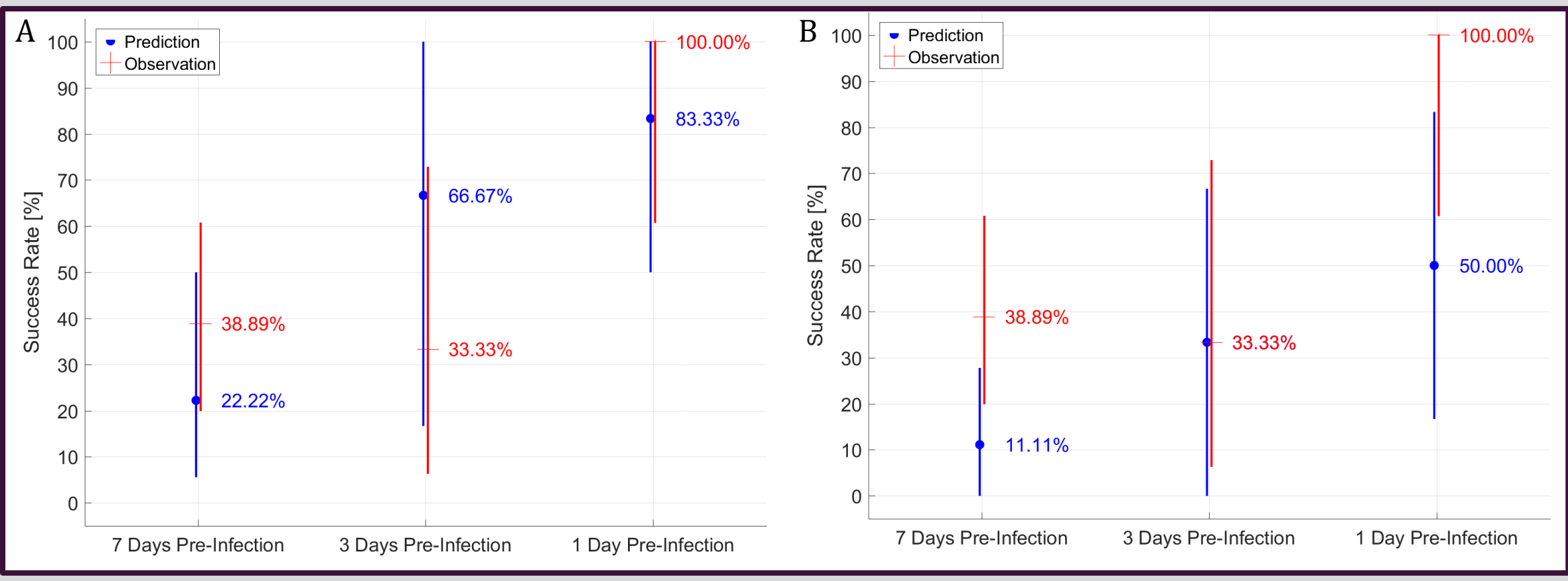


Figure 5: Comparison between the observed and predicted success for each day of administration: (A) With and (B) without a cure threshold applied for blood parasitemia. Success defined as no parasitemia observed at day 28 (i.e. Parasitemia < LLOQ), whereas having a cure threshold means that if the parasitemia goes under this value, the parasites stop growing.

Conclusions

Findings:

- By combining blood-stage and liver-stage CHMI, the PKPD relation was relatively well described (Fig. 5A).
- Including a cure threshold during the blood-stage improved the prediction

What's Next?

- Can the cure threshold be better defined? Should one be defined for the liver-stage?
- Test the effect of the assumptions in the liver growth; e.g. sensitivity to $k_{gr,L}$ or time to release in the blood stream.
- Incorporate *in vitro/in vivo* data to enrich the model.
- Develop a more mechanistic model to describe liver-stage growth.

References

[1] March, S. *et al.*: A Microscale Human Liver Platform that Supports the Hepatic Stages of Plasmodium falciparum and vivax. Cell Host Microbe. 14, 104–115 (2013). [2] Vaughan, A.M. *et al.*: Complete Plasmodium falciparum liver-stage development in liver-chimeric mice. J. Clin. Invest. 122, (2012). [3] Vaughan, A.M. *et al.*: Plasmodium falciparum genetic crosses in a humanized mouse model. Nat. Methods. 12, 631–633 (2015). [4] Sulyok, M. *et al.*: DSM265 for Plasmodium falciparum chemoprophylaxis: a randomised, double blinded, phase 1 trial with controlled human malaria infection. Lancet Infect. Dis. 17, 636–644 (2017). [5] Coffeng L. E. *et al.*: The Power of Malaria Vaccine Trials Using Controlled Human Malaria Infection. PLoS Computational Biology. 13(1) (2017).

