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Parasite-host dynamics throughout antimalarial drug development stages complicate the translation of parasite clearance

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1 **Parasite-host dynamics throughout antimalarial drug**
2 **development stages complicate the translation of parasite**
3 **clearance**

4 Running title: Parasite clearance in antimalarial drug development

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29 **1. Abstract**

30 Ensuring continued success against malaria depends on a pipeline of new antimalarials.
31 Antimalarial drug development utilizes pre-clinical murine and experimental human malaria
32 infection studies to evaluate drug efficacy. A sequential approach is typically adapted, with
33 results from each stage, informing the design of the next stage of development. The validity of
34 this approach depends on confidence that results from murine malarial studies predict the
35 outcome of clinical trials in humans. Parasite clearance rates following treatment are key
36 parameters of drug efficacy. To investigate the validity of forward predictions, we developed a
37 suite of mathematical models to capture parasite growth and drug clearance along the drug
38 development pathway and estimated parasite clearance rates. When comparing the three
39 infection experiments, we identified different relationships of parasite clearance with dose, and
40 different maximum parasite clearance rates: in *P. berghei*-NMRI mouse infections we estimated
41 a maximum parasite clearance rate of 0.2 [1/h]; in *P. falciparum*-SCID mouse infections 0.05
42 [1/h]; while in human volunteer infection studies with *P. falciparum*, we found a maximum
43 parasite clearance rate of 0.12 [1/h] and 0.18 [1/h] after treatment with OZ439 and MMV048,
44 respectively. Sensitivity analysis revealed that host-parasite driven processes account for up to
45 25% of variance in parasite clearance for medium-high doses of antimalarials. Although there are
46 limitations in translating parasite clearance rates across these experiments, they provide insight
47 into characterising key parameters of drug action and dose response, and assist in decision-
48 making regarding dosage for further drug development.

49 **Keywords:** antimalarial drug development, translational medicine, parasite-host interactions,
50 parasite clearance, within-host models

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52

53 **2. Introduction and Background**

54 Recent progress in reducing malaria burden is threatened by emerging resistance against current
55 first-line treatments and by sub-optimal adherence to existing treatment schedules. Development
56 of new antimalarial treatments is therefore more urgent than ever (1). Requirements for new
57 antimalarial treatment regimens are multi-faceted, spanning safety, efficacy, and dose
58 optimization for all populations, including pregnant women, infants and children (2).

59 Before testing compounds *in vivo*, promising compounds are identified *in vitro*, and their
60 parasitocidal efficacy is assessed in whole-cell or target-based assays (3). In pre-clinical stages,
61 drug efficacy is often investigated using murine malarial infection. Historically, infection of
62 normal mice with *P. berghei* was shown to be a useful experiment to measure crude drug
63 efficacy and to select promising candidates (4). However, enzymatic differences between the
64 human malaria parasite *P. falciparum* led to selection bias in candidate selection (5). More
65 recently, the humanized mouse model of NOD^{scidIL-2R^c-/-} (SCID) mice infected with *P.*
66 *falciparum* has been shown to provide insights into efficacy against the human parasite *in vivo*
67 (6–8) and further assists candidate selection. Early human efficacy studies via experimental
68 infection of malaria-naïve individuals, termed volunteer infection studies (VIS)(9) or controlled
69 human malaria infection (CHMI) studies, provide an opportunity to evaluate antimalarial activity
70 in humans with low parasite burden in a controlled setting. These studies avoid the confounding
71 factors of drug efficacy observed in clinical malaria cases such as acquired immunity,
72 concomitant diseases and medication (10–12).

73 Drug efficacy indices, used to summarize drug effect over time, are key measures by which to
74 evaluate the progression of drug candidates from the preclinical to clinical stages of the drug
75 development pipeline. For malaria, pharmacodynamic/efficacy indices include: i) measurements
76 of total or proportional parasite clearance such as the parasite clearance rate or parasite reduction
77 ratio over 48 hours (PRR₄₈) (4); ii) drug exposure typically reported by indicative drug
78 concentrations such as the minimum inhibitory concentration (MIC) (8); and iii) clinical
79 endpoints such as adequate clinical and parasitological response (ACPR) (13). Parasite clearance
80 measures are widely used in antimalarial drug development to guide compound selection (2), and
81 are also reported in clinical studies in endemic areas as a measure of drug efficacy (14).

82 In a previous paper, we developed a suite of mathematical models of parasite growth and drug-
83 parasite dynamics to investigate murine malaria infection and malaria drug experimental tests.
84 Through extensive simulation of these models and comparison with data for several drugs, we
85 found that the experimental systems and differences between the two murine malaria infections
86 had appreciable effects on measured drug efficacy and treatment outcomes. More specifically,
87 we found drug efficacy is influenced by host-parasite dynamics in *P. berghei*-NMRI mouse
88 infection where resource limitation is caused by aggressive parasite growth, namely limitations
89 of red blood cells (RBC)(15). In *P. falciparum*-SCID mouse infection, we found continued

90 injections of human RBCs has a noticeable impact on subsequent clearance patterns of
91 uninfected and infected RBCs. We additionally identified mechanisms of observed
92 recrudescence patterns after non-curative treatment that are not discernable from the
93 experimental data nor captured by current modelling approaches of antimalarial drugs. These
94 unknown mechanisms may include altered parasite maturation or dormancy and affect
95 experimental measures of cure, thus limiting interpretations of curative dose for a particular drug
96 (15).

97 In this study, we examined the ability of the parasite clearance after treatment estimated from
98 murine experiments to translate to estimates in human studies. This analysis used data from
99 studies of two antimalarials, MMV048 and OZ439 (artefenomel), in the *P.berghei* – NMRI
100 mouse, in *P. falciparum* (strain 3D7) –SCID mouse and in *P.falciparum* (strain 3D7) -human
101 infection experiments. Both compounds are part of the Medicines for Malaria Venture portfolio
102 (<https://www.mmv.org/research-development/mmv-supported-projects>). OZ439 is a synthetic
103 peroxide antimalarial candidate that is fast acting against all asexual erythrocytic parasite stages.
104 It is currently being evaluated in combination with Ferroquine in phase II clinical studies(11,
105 16–18). MMV048 is a *Plasmodial* phosphatidylinositol 4-kinase (PI4K) inhibitor efficacious
106 against liver erythrocytic parasite-life cycle stages currently in phase IIa (19–21).

107 We modelled parasite growth and clearance following two new antimalarial treatments in murine
108 and human malarial infections (Figure 1a: model development and calibration). We included
109 relevant and potentially important features of parasite growth, host, and drug dynamics in the
110 models of all three testing systems following a comprehensive examination of their biological
111 and experimental background. Through simulation and global sensitivity analysis of these
112 models we explored and compared the estimates of parasite clearance for a range of dosing
113 regimens, and parasite and host assumptions in all three systems (Fig. 1b). Through this analysis,
114 we demonstrated which of the factors; host, parasite, host-parasite and drug dynamics, primarily
115 determine the relationship between parasite clearance estimates across the antimalarial drug
116 development pathway and assess implications for decision making in drug development.

117 3. Results

118 The design of the studies/experiments used to evaluate the candidate antimalarials (MMV048
119 and OZ439) in normal mice, SCID mice and human volunteers is described in Table 1 and Fig. 2
120 (left side of panels a-c). Mice were infected with around 2×10^7 parasites or greater inocula
121 resulting in progression to severe disease with high parasitemia of up to 60-80 % (*P. berghei*,
122 parasitized mouse RBCs) and 15-20 % (*P. falciparum*, parasitized human RBCs) within a week
123 of inoculation. By design, human volunteers do not progress to high parasitemia to avoid severe
124 clinical illness (9). Volunteers reach parasitemia of around 10^4 p/mL (corresponding to 0.0002%
125 under the assumption of 5×10^9 RBCs/mL in male humans (22)) before treatment.

126 Parasite-host dynamics and experimental design influence treatment in murine malaria 127 infection

128 In our previous work (15), we developed several mathematical models of parasite and drug
129 dynamics in both *P. berghei*-NMRI and *P. falciparum*-SCID mice (Fig. 1a). The experimental
130 system and mathematical models are described in Fig. 2, panels a and b, respectively. Using the
131 parameter estimates from the original paper (for both parasite growth and PKPD models), we
132 simulated the models of murine infection to compare their results with our VIS simulations
133 below (Figure 1b). Here, we primarily compared parasite clearance after treatment across the
134 three testing systems (Fig. 1b: Comparison of parasite clearance across experimental systems) as
135 estimated from the models for a range of drug doses. We subsequently assessed the influence of
136 experimental design and parasite-host system on parasite clearance throughout the antimalarial
137 development pathway by sampling from the posterior distributions for VIS, and by sampling
138 parameters from a log-normal distribution with 20% standard deviation for the murine models
139 (Fig. 1b: sensitivity analysis).

140 In the two murine systems, several experimental and parasite-host traits were examined by
141 including them in a suite of models (further details in Methods and Table S5) (15). From an
142 experimental perspective, data availability and experimental design differ between the two
143 murine infection systems. For example, in *P. berghei*-NMRI infection, the volume of blood
144 sample needed for analysis and aggressive parasite growth limits the frequency of collection for
145 measurements of parasitaemia. From the host perspective, we investigated and quantified
146 adaptations of the host-system to increasing parasite burden. In *P. berghei*-NMRI infection, we
147 included erythropoiesis (23, 24) or clearance patterns, as well as parasite adaptations such as
148 parasite maturation (25) and target-RBC preferences (23) in our models to capture patterns of
149 RBC availability and the occurrence of anemia (Fig. 2a). In *P. falciparum*-SCID infection
150 experiments, continued injections of human erythrocytes hinder the occurrence of anemia and
151 lead to an increase in the proportion of available human host-cells throughout the experiment
152 (Fig. 2b). The injected human RBC volume of 4.55×10^9 RBCs every two to three days
153 (corresponds to 46% of total mouse RBCs) leads to a high clearance of excess erythrocytes
154 throughout the experiments, thus likely affecting measures of drug efficacy (6). Different

155 hypotheses regarding the influences on RBC clearance mechanisms were formalized in our
156 models of *P. falciparum*-SCID mouse infection (Fig. 2b).

157 Using a maximum likelihood approach, we estimated the parameters of our parasite growth
158 models by fitting them to the pooled experimental control data of untreated parasite growth. The
159 fitted models were then combined with the PD models to analyze the influence of the parasite-
160 host dynamics and experimental design on parasite clearance after treatment.

161 For MMV048, we captured the parasite clearance data after treatment by including a delayed
162 parasite clearance of parasites damaged or killed by the drug, modelled by the clearance rate of
163 dead parasites Cl_Y , in both murine hosts (Table S5- clearance PD model selection: Cl_Y range of
164 0.036 to 0.041 (1/h) in *P. berghei*-NMRI and Cl_Y range of 0.068 to 0.071 (1/h) in *P. falciparum*-
165 SCID infection over all mechanistic parasite growth models). In contrast, for OZ439, we
166 observed a delayed drug effect through a turnover (Table S5- turnover PD model selection: k_R
167 range of 0.013-0.06 (1/h) in *P. berghei*-NMRI and k_R range of 0.013-0.016 (1/h) in *P.*
168 *falciparum*-SCID infection over all mechanistic parasite growth models) (15). In both murine
169 experimental systems, we found that assumptions on parasite-host dynamics result in differing
170 estimates of parasite clearance times therefore influencing the evaluation of new compounds (see
171 Fig. 4 in previous paper) (15).

172 **Human volunteer infection studies exhibit large variation in observed parasite growth** 173 **and clearance dynamics**

174 We modelled parasite growth prior to treatment using data from 177 volunteers in 27 treatment
175 cohorts in VIS conducted at Berghofer QIMR, Brisbane between 2012–2016 (26). These cohorts
176 were part of 14 studies investigating new antimalarials in development and currently available
177 antimalarials. Volunteers were infected with 1800–2800 infected RBCs and treated 7–9 days
178 after inoculation (26).

179 Sampling frequency of human volunteers was much higher compared with murine experiments,
180 however, the number of measurements above the lower limit of quantification (LLOQ, 111
181 p/mL) varied between cohorts and volunteers with a median of five quantifiable measurements
182 (ranging from one to eight). These detailed data allowed us to capture features of the parasite life
183 cycle, such as the characteristic oscillation in parasite densities. This phenomena is caused by
184 periodical sequestration of late stage asexual parasites stages to the deep microvasculature of the
185 host organs (27) and synchronicity in parasite growth determined by the distribution of parasite
186 age in the inoculum (28).

187 The models of asexual parasite growth in in human volunteers are described in Fig 2c. We
188 adapted a discrete time model (29) previously used to investigate antimalarial treatment (28),
189 resistance against artemisinin combination therapy (30), and the impact of new antimalarial
190 combinations (31). A set of difference equations (*model S-parasite stages*, Equation 7) describes
191 the parasite inoculation, capturing its size and age distribution, and the subsequent mechanism of

192 intra-erythrocytic parasite development including ageing and parasite death. Based on analysis
193 by Wockner et al., that quantified parasite growth behavior of *P. falciparum* (3D7) in VIS, the
194 life-cycle length was set to 39 hours. (26) For comparison, we tested a second model that
195 assumes exponential parasite growth (*model i*, Equation 10), summarizing parasite replication
196 and death into one growth parameter. This model is commonly used to capture parasite growth
197 and treatment effects in antimalarial drug development (8, 11).

198 We incorporated the models into a Bayesian hierarchical framework to estimate the posterior
199 distributions of the model parameters from the VIS data. We investigated different levels of
200 parameter variability, distinguishing between parasite and host dependent parameters by varying
201 hierarchical parameter allocation in different parasite growth model specifications (Table S2,
202 *models S1–S3*). *Model S3*, from here on referred to as *model S*, was selected based on the
203 Watanabe-Akaike information criterion (WAIC) (Table S2). The parameters describing
204 distribution of the initial parasite load (mean μ_{ipl} and standard deviation σ_{ipl}) and the intrinsic
205 parasite multiplication factor, r_p , were found to vary on a cohort level whereas the initial parasite
206 load i_{pl} and parasite death rate due to host clearance δ_p vary between individuals (Figure S7). The
207 population posterior predictions of the two parasite growth models (Fig. 3) begin on day 4 after
208 inoculation, when quantifiable parasitemia measurements from the volunteers are first available
209 (Fig. 2). We estimated a parasite multiplication factor *pmf*, of 17.0 (15.3–20.0) over one life
210 cycle of 39 h (Equation 7). Identifiability issues of growth rate r_p were detected by the partial
211 congruency of the marginal prior and posterior distributions (Figure S7). This means that data on
212 parasite growth is not informative enough for estimating this parameter, similar to previous
213 model analysis(32). For exponential growth *model i*, we estimated a *pmf* of 12.61 (11.2–14.2)
214 over one parasite life cycle. Differences in the parasite growth rate r_p between individuals within
215 cohorts, could not be linked to cohort or subject specific parameters for *model i* (Fig. S8).

216 Given our calibrated VIS parasite growth models, we incorporated PK models and modelled
217 drug effects (pharmacodynamics) (Fig. 1a) to analyze single-dose treatment of 20 volunteers
218 with MMV048 and 24 volunteers with OZ439 (Table S1). Drug concentrations after treatment
219 were predicted using pharmacokinetic (PK) models (Equation S1 and S2) and individual PK
220 parameters specified (Supplementary File 1). We simulated individual PK profiles to exclude
221 large variation in drug-concentration as an influencing factor in the following analysis. Drug
222 concentration over time was described by a two-compartment model with zero-order absorption
223 for MMV048 and a 2-compartment model with linear absorption for OZ439. In the following
224 section, we explain the results for OZ439. The analysis of MMV048 is detailed in the
225 Supplement, and any deviations are highlighted and discussed here.

226 We incorporated a direct effect of drug concentration on parasite death (Equation 8) and
227 additionally tested for drug concentration induced retarded parasite growth (Equation 9) due to
228 the reduced parasite growth observed after non-curative dosing with OZ439 and the shift in
229 oscillation patterns to a longer period. This phenomenon was also previously described for

230 artemisinin derivatives (33). A comparison of drug action model based on WAIC and additional
231 observations is included in Table S3.

232 We detected drug-concentration dependent prolongation of parasite stages after treatment with
233 MMV048 and OZ439, meaning that at sufficiently high drug concentrations each life cycle stage
234 can be prolonged by 26 min or 65 min respectively (Equation 13). Through description of
235 individual treatment effects via subject-specific parameters of drug efficacy (Fig. S9-S12) we
236 could capture individual parasite clearance and recrudescence curves after treatment with
237 MMV048 (Figure S3 and S4) and OZ439 (Figure S5 and Figure S6) for all subjects.

238 Although the posterior predictions of recrudescence (Fig. 4) fit the data well, we note that
239 informed mechanistic models, and inclusion of experimental recrudescence outcomes and cure
240 are hindered by limited data on cure probability since each cohort only consisted of eight
241 subjects. Additionally, insights into minimum parasite concentrations for cure are missing.

242 **Parasite clearance is unlikely comparable across the preclinical and clinical** 243 **development pathway**

244 The parasite clearance after treatment was estimated from our model simulations for murine and
245 human malaria infection (Fig. 1b) over a wide range of single doses of each drug. The range of
246 doses and models facilitated comparison across preclinical and clinical stages. We chose seven
247 doses per experimental system that capture realistic testing dose ranges. We simulated 1000 trials
248 for each murine experiment with parameter estimates published in (15) (see Table S5 for an
249 overview) and variability added between trials, with parameters fixed within one trial over all
250 doses. In human-*P. falciparum* infections we simulated 100 trials with 20 subjects per dose and
251 varied the parasite-dependent parameters between trials and the host-dependent parameters
252 between subjects within a trial. The parameters of the PK models were fixed to population values
253 for all model simulations to allow for adequate characterization of host-parasite dependent
254 dynamics and variability. The simulation set-up is detailed in Table S4.

255 Fig. 5 compares parasite clearance calculated from simulation output after single dose treatment
256 of OZ439 over the two murine and experimental human infection experiments. We find varying
257 relationships of estimated parasite clearance with doses across the experimental systems. Median
258 estimates of parasite clearance rates plateau around 0.2 [1/h] (\log_{10} PRR₄₈: 4.17) in *P. berghei*-
259 NMRI infection and 0.05 [1/h] (\log_{10} PRR₄₈: 1.04) for *P. falciparum*-SCID infection. Analysis
260 for MMV048 shows similar maximum clearance rates in murine infection (Fig. S13) and varying
261 relationships with dose. In experimental *P. falciparum*-human infection the parasite clearance
262 rate for OZ439 plateaus with *model S* around 0.12 [1/h] (\log_{10} PRR₄₈: 2.50). The ranges of the
263 predicted clearance time of *P. falciparum*-human infection between *model i* and *model S* are also
264 reflected in the wider posterior predictive intervals of the former after treatment with OZ439
265 (Fig. 4).

266 Parasite clearance is influenced by varying parasite-host dynamics throughout the analyzed
267 experiments of murine and human malaria infection. Despite the poor comparability across
268 experimental systems, the parasite clearance predicted from mechanistic models within an
269 experimental system are comparable, but with different levels of variation. Via a global
270 sensitivity analysis, we thus identified which host-, parasite-, and drug-dynamics cause the
271 highest variability in parasite clearance after treatment with MMV048 and OZ439. We first
272 partitioned variability by classifying parameters by their influence and dependency on parasite-,
273 host- or drug-concentrations. For example, the parameters of infection induced RBC clearance γ
274 in murine malaria models are classified as host-parasite parameters since they are induced by
275 parasite concentration and influence overall RBC concentration (Table 2). We then undertook a
276 global sensitivity analysis of the models on simulated parasite clearance rates reducing
277 computational time by using emulators and sobol-analysis, which calculates first order indices
278 and total effect indices to evaluate individual and combined parameter contributions to the
279 variance of parasite clearance rates (see Methods).

280 In general, we find in all experimental systems, that parasite clearance after treatment is most
281 sensitive to drug action parameters, see Table 2. This is expected as we are measuring the effect
282 of the drug in these systems. We also find that interactions of the drug action parameters
283 (increased total effect over first-order effect) occur over all doses but decrease with increasing
284 dose, evident in the convergence of first order and total effect values. However, in both the
285 murine (Fig. 6b) and human *P. falciparum* (Fig. 6c) infections, we find at lower doses an
286 increasing influence of both the host or parasite, or host-parasite parameters. This indicates a
287 greater influence of the experimental system on measured parasite clearance. Even in medium-
288 high dose ranges, drug unrelated parameters and their interactions account for up to 25% of
289 variance in parasite clearance. This is mainly caused by sensitivity towards maximum infection
290 induced RBC clearance γ_{max} and parameter ϕ , which capture splenic/liver clearance and
291 maximum infection induced RBC clearance γ_{max} . The exponential growth *model i* shows
292 diminishing dependence on parasite replication rate r_p with increasing doses in *P. falciparum* –
293 SCID infection.

294 Estimated treatment effects of experimental *P. falciparum*-human infection are sensitive to the
295 maximum drug action parameter E_{max} , resulting in a large range of parasite clearance rate
296 prediction throughout all doses. In the lower dose ranges of OZ439 treatment, the total effects of
297 the age distribution parameters μ_{ipl} and σ_{ipl} of *model S* account for up to 50% of all variance (c).
298 In contrast, throughout all regimens, parasite parameters have negligible influence on clearance
299 for MMV048 (Fig. S14). The full set of individual parameter contributions in all experimental
300 systems can be found in Supplementary File 2. The complete set of results for both drugs are
301 shown in Fig. S14 and Fig. S15.

302 4. Discussion

303 Our analysis of data from three unique experimental systems of drug action in *Plasmodium*
304 infection: *P. berghei*-NMRI mouse infection, *P. falciparum*-SCID mouse infection and the
305 human VIS, demonstrates a discrepancy in influencing parasite-, host- and drug-dynamics on
306 parasite clearance between the pre-clinical and clinical antimalarial testing stages. This
307 complicates the translation of results between these different experimental systems, which aim to
308 collectively inform drug development. We initially intended to identify antimalarial drug
309 efficacy indices that were reliable for translation. Although our analysis did not result in a
310 unifying output, our insights into parasite-host dynamics across the preclinical murine and early
311 clinical experimental infections in humans via the mathematical models provide a pathway to
312 facilitate inference from the data. By describing the different experimental testing systems with
313 data-calibrated mechanistic models, we used model predictions coupled with sensitivity analysis
314 to identify factors significantly influencing experiments aimed to characterise drug efficacy. In
315 addition to the drug and dose being tested, we identified differing magnitudes of host-parasite
316 dynamics between the experimental systems as a driver of estimated parasite clearance rate.

317 By employing mechanistic models of parasite growth and drug effects in all three testing systems
318 we found that several important dynamics affect translation of parasite clearance after treatment
319 and likely other parasitological outcome measures between preclinical and clinical systems.

320 Firstly, as expected, our sensitivity analysis of parasite clearance revealed a high sensitivity to
321 parameters of drug action in higher dose ranges in all three experimental systems. In contrast, we
322 found a variation in overall sensitivity of parasite clearance to parasite-host dynamics in lower-
323 medium dose ranges between the three experimental systems. In murine and human *P.*
324 *falciparum* infection, parameters of parasite growth influenced parasite clearance after treatment
325 with lower doses. Additionally, the occurrence of clearance mechanisms of excess RBCs in *P.*
326 *falciparum*-SCID infection, could place additional constraints on parasite growth. Overall, the
327 increased susceptibility to the experimental set-up of the SCID system limits direct translation
328 and comparison of results between laboratories and highlights the need for strict experimental
329 protocols (15, 34). Thus, to predict human equivalent doses by exploiting the value of the *P.*
330 *falciparum*-SCID mouse system, additional information is required concerning the interplay of
331 disease dynamics and the experimental background which could be quantified with our
332 modelling approach. For *P. berghei*-NMRI infection data, estimation of the parasite clearance
333 was less sensitive to parasite-host dynamics. However, the aggressive parasite growth in this
334 experimental system might lead to host-cell limitations and therefore different growth dynamics
335 compared to *P. falciparum* infection, challenging the translatability of drug efficacy measures.
336 Although not investigated here, the uncertainties regarding clearance mechanisms may also
337 hinder the investigation of drug-drug interactions. The testing of combination therapies relies on
338 the ability to allocate contribution of each drug to effects on parasite clearance and to estimate
339 potential drug interactions. *In vitro* experiments could prove to be valuable alternatives in

340 establishing formal descriptions of these interactions, which could thus be incorporated into
341 mathematical models to predict the efficacy of combination (31).

342 Secondly, the sensitivity of parasite clearance measured at lower drug doses due to factors other
343 than the drug raises questions for current approaches to determine concentration-effect
344 relationships (Equation 12) that rely on information gained in lower-medium dose ranges to
345 define the concentration with half-maximum effect (EC_{50}). Additionally, other common drug
346 efficacy indices such as the minimum inhibitory drug concentration (MIC) are influenced by
347 EC_{50} measures (Equation 16). We therefore recommend that inferences based on data gathered at
348 low–medium doses across the preclinical and clinical stages of drug development are assessed by
349 considering factors connected to experimental and host-parasite pairing. For example, in *P.*
350 *falciparum*-SCID and *P. falciparum*-human infection experiments, the sensitivity of the dose-
351 response relationship of data obtained at lower dose ranges should be investigated for each drug
352 candidate to avoid bias in decision making. In experiments with a new compound where drug
353 efficacy analysis reveals a substantial influence of parasite-host dynamics in lower dose ranges,
354 these data should not be used to characterize the MIC for decision making.

355 Thirdly, our earlier work indicated that recrudescence and thus estimates of MIC are potentially
356 influenced by additional parasite mechanisms including retarded parasite growth in *P.*
357 *falciparum*-SCID experiments(15). Experiments explicitly aimed at observing recrudescence are
358 considered highly informative in understanding the dose-response relationships in these
359 experimental systems. However, utilizing these experiments for modelling proved that estimation
360 or prediction of cure might be hindered by insufficient data on cure rates and curative doses in
361 both murine and human malaria infection (Table 1). Thus, any analysis we could undertake on
362 cure or recrudescence dynamics is driven by model assumptions and was therefore excluded
363 from this analysis. We further excluded drug-concentration-dependent indices such as maximum
364 concentration, time above a threshold concentration, and the area under the concentration time
365 curve (AUC) because information on PK variability was missing, especially in murine
366 experiments.

367 How well the parasite clearance rate could be quantified varied between the murine and human
368 volunteer experimental infection data. In the murine malaria experiments, there is limited
369 repeated blood sampling of each mouse and evaluating progression of infection was limited to
370 measuring percentage of infected RBCs (Fig. 2). Additionally, the evidence for delayed
371 clearance of dead parasites after treatment with MMV048 for both murine models (15) indicates
372 that not only drug properties but also the host's ability to clear damaged/dead parasites are
373 factors that are determining maximum clearance rate. In the VIS studies, drug treatment is
374 typically administered at an earlier timepoint at a lower parasitemia, when volunteers are
375 typically asymptomatic. Thus, data from these studies are collected at parasitemia levels up to
376 five logs below those studied in endemic settings, a factor that may confound analysis of
377 maximal parasite clearance.

378 We were able to compare drug efficacy as parasite clearance across the murine and human
379 infection experiments, thereby measuring drug efficacy throughout antimalarial drug
380 development, because the models formally considered experimental background (i.e. parasite-
381 host combination and experimental procedures) as well as parasite dynamics. Across the
382 mathematical models of parasite growth and treatment, we found that different levels of detail
383 between the preclinical and clinical infection experiments were required to capture parasite
384 growth and thus drug effect. In the mechanistic models of murine malaria infection, high-
385 parasitemia and RBC clearance mechanisms necessitate inclusion of host-cell dynamics in the
386 models. Parasite-host dynamics were influenced by either rapidly increasing parasite load (in the
387 case of *P. berghei*-NMRI infection) or the experimental set-up including the continued human
388 erythrocyte injections (in the case of *P. falciparum*-SCID infection). However, in VIS, explicit
389 description of host-cell dynamics is not required due to lower parasite load and relatively short
390 infection follow-up. This means, as patients are treated due to safety reasons when parasitaemia
391 reaches a certain level, only few parasitaemia measurements during the initial growth phase are
392 available and any effect of immune response on parasite dynamics is likely to be minimal, thus
393 obviating the need to consider host responses in this model. Furthermore, due to the significant
394 variability in parasite growth observed in the murine experiments, the parasite-growth models
395 required calibrated parasite fitness parameters to capture differences between laboratories, as
396 well as unmeasured differences in virulence of parasites transferred from donor mice in each
397 experiment (ability of the parasite to infect new RBCs). Similarly, in VIS, parasite growth and
398 synchronicity were dependent on the distribution of viable parasite life-cycle stages at
399 inoculation, and models required estimates of these age distribution to recover observed
400 oscillations.

401 For drug dynamics, we evaluated different pharmacodynamic models to capture the observed
402 parasite clearance in murine and human infection. In murine experiments, delayed clearance of
403 parasites damaged/killed by the drug and drug action through a turnover model were identified.
404 Although *in vitro* experiments reported slight stage-specificity of OZ439(16), with an increased
405 action against trophozoites and schizonts, data were too sparse in the VIS studies to provide a
406 detailed analysis. We did not find evidence of delayed clearance of damaged or dead parasites
407 (27) for the two compounds analyzed. However, this could change for other antimalarials
408 depending on the mode of action, parasite load and data resolution.

409 Our estimated value of 17.0 (95% credible interval: 15.3-20.0) for the multiplication factor of the
410 parasite after every 39-hour life cycle in *P. falciparum* (3D7) VIS is comparable to previous
411 estimates of 16.4 (95% confidence interval: 15.1–17.8) (26) and 21.8 (95% credible
412 interval:17.6–26.9, at a life cycle length of 42 h)(32) in VIS studies. The growth of parasitemia
413 has been modelled using a variety of methods e.g. testing statistical models for quantification
414 (26), assays for *in vivo* determination (35) and linking it to disease severity(36). However, we
415 found a potential issue of statistical parameter identifiability for this rate where the intrinsic
416 parasite multiplication rate exhibits weak identifiability and strong correlation with the parasite

417 death rate. Nevertheless, we decided to maintain both dynamics, an intrinsic growth rate and
418 parasite death rate in the model instead of a net growth rate to capture differences between
419 cohorts and individuals, and to highlight gaps in knowledge. In theory, the synchronous parasite
420 growth in VIS could inform these two parameters if information on parasite age-distributions
421 throughout the parasite life cycle were collected (37). However, this information was not
422 available in the cohorts analyzed and in practice may be difficult to obtain. As parasite numbers
423 after treatment are very low in VIS, the role of immunity for successful treatment is unknown
424 from these studies. However, further analysis and modelling could combine information on
425 parasite growth in clinical field trials in pre-exposed patients (38–40). Thus, potentially estimate
426 the effect of the immune system on parasite growth to inform dosing recommendation.

427 The necessity for timely decisions regarding the progression of antimalarial compounds to the
428 next experimental stages and design of future experiments might not allow for extensive analysis
429 of data through mechanistic models for each drug. However, the mechanistic models developed
430 and validated in the context of this study could serve as a tool to assess potential influences
431 stemming from parasite-host dynamics and help to improve the experimental design of future
432 experiments providing more depth than the analysis of experimental data alone.

433 Although drug efficacy data from these three discussed experimental systems allow the
434 comparison of compounds within one *in vivo* experimental system, we are currently not able to
435 translate parasite clearance measures between the systems. Furthermore, as assumptions on the
436 mode of drug action potentially influence clearance patterns, either by data analysis or as
437 predicted by the models, the comparison of compounds with different modes of action is
438 complicated. Our analysis shows that there are different underlying parasite-host dynamics in
439 each system that influence experimental analysis and thus drug efficacy evaluation (either by
440 extrapolation or by models). Therefore, a reliable strategy to translate efficacy measures and
441 produce insights from drug-parasite-host dynamics between the development stages has not yet
442 been identified. Additionally, preclinical and early clinical testing of new compounds is
443 expensive and time-consuming. This is especially true for antimalarials, as only combination
444 therapies will be considered for Phase 3 clinical trials and future implementation. To identify
445 appropriate combination therapies, combinations are currently tested in both, *P. falciparum*-
446 SCID experiments (41) and *P. falciparum*-human infection in VIS (42). Therefore, we suggest
447 that the value of current and new approaches in *in vitro* drug efficacy experiments should be
448 revisited in antimalarial development.

449 *In vitro* experiments allow the possible identification of mode of drug action, stage specificity,
450 and evaluation of parasite killing in dependence on the unbound drug-concentration without the
451 influence of parasite-host dynamics. This can be achieved with current *in vitro* experiments (43,
452 44) or by developing new *in vitro* approaches.

453

454

455 Modelling and simulation strategies could thus be informed by more appropriate *in vitro* data
456 unlike today, where large assumptions need to be made on parasite-host dynamics *in vivo*. The *in*
457 *vitro* dose-response relationship could then be translated to predict human drug efficacy by
458 taking into account parasite growth and clearance mechanisms in human VIS using mechanistic
459 within-host parasite growth models. If validated, this approach could accelerate drug selection in
460 antimalarial drug development by allowing a streamlined prediction of drug efficacy from *in*
461 *vitro* experiments to humans, therefore reducing the need for substantial or complicated murine
462 efficacy experiments. An investment in setting up new and appropriate *in vitro* experiments and
463 analysis and/or model frameworks could therefore support the cost-efficient translation between
464 experimental systems through efficient candidate selection and ability to inform early clinical
465 dosing in real-life settings.

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473 **5. Materials and Methods**474 **Data, parasite growth and PKPD models in murine experiments**

475 Models of murine malaria infection (Fig. 2) were calibrated to extensive parasite growth data and
 476 subsequent treatment with multiple antimalarial drugs (Table 1). Here, we briefly present the
 477 base structure (*model a*) of the ordinary differential equations for the murine malaria infection
 478 model as specified in (15). Parasite growth within the host is described by ordinary differential
 479 equations capturing dynamics of uninfected murine host RBCs ($X_{m(\text{murine})}$), infected RBCs (Y_{X_m})
 480 and merozoites (M). RBC dynamics are incorporated with constant production v [cells/h] and
 481 decay rate μ [1/h] and become infected through invasion with merozoites dependent on the
 482 infectivity parameter β [cells/mL h].

$$483 \quad \frac{dX_m}{dt} = v - \mu_{X_m} X_m - \beta X_m M \quad (1)$$

484 Infected RBCs (Y) burst after $1/\alpha$ hours to release r new Merozoites that die with rate δ [1/h].
 485 The aging of the parasite throughout the parasite-life cycle is incorporated via n transit
 486 compartments.

$$487 \quad \frac{dY_{X_m,1}}{dt} = \beta X_m M - \alpha Y_{X_m,1} \quad (2 \text{ a})$$

$$488 \quad \frac{dY_{X_m,i}}{dt} = \alpha Y_{X_m,i-1} - \alpha Y_{X_m,i}, \quad i = 2, \dots, n \quad (2 \text{ b})$$

$$489 \quad \frac{dM}{dt} = -\beta(X_m + Y_{X_m})M + \alpha r Y_{X_m,n} - \delta M \quad (3)$$

490 The parasitemia, as percentage of infected RBCs, is calculated by summing over the parasite
 491 age-stages and dividing by the total number of RBCs:

$$492 \quad Y_{X_m} = \sum_{i=1}^n Y_{X_m,i} \quad (4)$$

$$493 \quad P = \frac{Y_{X_m}}{X_m + Y_{X_m}} 100 \quad (5)$$

494 The initial number of infected RBCs is informed by the inoculum size, its viability ω and the
 495 mouse blood volume V_n .

$$496 \quad Y_{X_m,0,i} = \omega \frac{\text{inoculum}}{V_n} \quad (6)$$

497 For reference, population models for murine parasite growth and drug treatment were estimated
 498 via a maximum likelihood approach on trust region optimization.

499

500 **Data, parasite growth and PKPD models in human VIS experiments**

501

502 **Data in VIS**

503 Parameters for the parasite growth models were estimated using previously published (26)
504 parasite growth data from 177 malaria-naïve healthy volunteers enrolled in 27 cohorts as part of
505 14 VIS studies. Briefly, volunteers were inoculated intravenously on Day 0 with human
506 erythrocytes infected with *P. falciparum* (3D7 strain). Treatment commenced on day 7-9 after
507 infection. Parasite growth was monitored using a quantitative PCR assay (*P. falciparum* 18S
508 rRNA gene). Specifics of parasite growth monitoring and data processing can be found in (26).

509 Parameters for drug efficacy were estimated from parasite clearance data (Table S1) collected
510 from volunteers administered single doses of OZ439 (artefenomel)(11) (Cohorts 4, 5, and 6) and
511 MMV048 (Cohorts 15, 16, and 27) (20). In the MMV048 cohorts, gametocyte concentration data
512 were also available, where parasite measurements were discarded if the gametocyte count
513 exceeded 10% of the total parasite count. Further details of the data and clinical trial identifiers
514 are given in Table S1. All data was previously published (11, 21). As previously reported, all
515 studies were approved by the QIMR-B Human Research Ethics Committee and all subjects
516 provided informed consent (26).

517 **Pharmacokinetic models of OZ439 and MMV048 in VIS**

518 Human Population pharmacokinetic (PK) modelling of the OZ439 and MMV048 concentration
519 versus time profiles was performed using Monolix (Version Monolix 2018R1). A 2-compartment
520 PK model with zero order absorption (Equation S1) best described MMV048 concentrations and
521 a 2-compartment PK model with first order absorption described the OZ439 concentrations
522 (Equation S2). Structural PK model specifications and individual parameter can be found in
523 Supplementary File 1.

524 **Mathematical models of within-host parasite growth and post-treatment parasite clearance**
525 **in VIS**

526 *P. falciparum*-human infection is described via difference equations able to capture the changing
527 age-structure of the parasitemia over time. The difference equations for *model S* (Equations 7-9)
528 and *model i* (Equations 10 and 11), with incorporated drug action as specified in the Results
529 section are given below (Equations 7-11. We estimated parasite growth and treatment effects in a
530 two-step sequential process. Firstly, parasite growth parameters were estimated from parasite
531 growth data before treatment. Secondly, we fixed the individual posterior median of parasite
532 growth parameters and individual PK parameters to estimate parameters of drug efficacy (Fig.
533 1a). Treatment effect E incorporates parasite death through treatment as an increase in parasite
534 death rate δ_p . The evaluated treatment models include a direct drug effect model and additional
535 drug induced growth retardation causing a lengthening of the parasite life cycle length α_i . Model

536 output of *model S* is the number of circulating parasites P_{circ} (Equation 14). The parasite
537 multiplication factor *pmf* can be calculated for *model S* using the intrinsic parasite multiplication
538 rate r_p and death rate δ_p . It constitutes an estimation of the average number of merozoites that
539 emerge from one infected RBC after a reproduction cycle within an RBC has been finished and
540 successfully invade new RBCs at parasite age $a=1$ has completed (Eq. 7). Details of the
541 parameters are provided in Table 3.

542 *Model S*, mechanistic asexual parasite growth model incorporating parasite stages and drug
543 effect:

544 Our mechanistic model of growth of parasite density P , over the life cycle of length $a_L=39$ hours,
545 at age a and time t is given by:

$$546 \quad P(a, t) = \begin{cases} P(a-1, t-1)e^{-\delta_p}, & a = 2, 3, \dots, a_L \\ r_p P(a_L, t-1)e^{-\delta_p}, & a = 1 \end{cases} \quad (7)$$

547 The direct drug effect on this model is incorporated via E (Equation 12) representing the drug-
548 concentration dependent increase of parasite death rate δ_p .

$$549 \quad P(a, t) = \begin{cases} P(a-1, t-1)e^{-\delta_p-E}, & a = 2, 3, \dots, a_L \\ r_p P(a_L, t-1)e^{-\delta_p-E}, & a = 1 \end{cases} \quad (8)$$

550 The direct drug effect model is extended to include drug concentration dependent growth
551 retardation.

$$552 \quad P(a, t) = \begin{cases} P(a, t-1)k_{\text{ret}} \frac{(1-e^{-\delta_p-E-k_{\text{ret}}})}{\delta_p+E+k_{\text{ret}}} + P(a-1, t-1)e^{-\delta_p-E-k_{\text{ret}}}, & a = 2, 3, \dots, a_L \\ P(a, t-1)k_{\text{ret}} \frac{(1-e^{-\delta_p-E-k_{\text{ret}}})}{\delta_p+E+k_{\text{ret}}} + r_p P(a_L, t-1)e^{-\delta_p-E-k_{\text{ret}}}, & a = 1 \end{cases} \quad (9)$$

553 *Model i*, exponential parasite growth model incorporating drug effect:

554 Parasite growth for the exponential *model i* is modelled on the logarithmic scale with the initial
555 parasite concentration P_0 and parasite growth rate p_{gr} .

$$556 \quad \ln(P(t)) = P_0 + p_{gr} t. \quad (10)$$

557 Treatment is included by decreasing parasite growth rate:

$$558 \quad \ln(P(t)) = P_0 + \int_0^t (p_{gr} - E). \quad (11)$$

559 *Additional equations:*

560 In both models, drug effect is given by E ;

561
$$E = \frac{E_{max}C^{\gamma}}{EC50^{\gamma} + C^{\gamma}}. \quad (12)$$

562 and growth retardation in mechanistic *model S* by k_{ret} ;

563
$$k_{ret} = \frac{k_{ret,max}C^{\gamma}}{EC50^{\gamma} + C^{\gamma}}. \quad (13)$$

564 The maximum possible parasite age stage prolongation is therefore given by $((1/1 - k_{ret,max} [h^{-1}]) -$
565 $1 [h]) \times 60$, where one hour is the original length of the age stage and 60 converts hours into
566 minutes.

567 In *model S*, the number of circulating parasites is given by summing parasite concentration up to
568 the parasite-age stage α_s after which parasites sequester

569
$$P_{circ}(t) = \sum_{a=1}^{\alpha_s} P(a, t). \quad (14)$$

570 The parasite multiplication factor over one parasite life cycle with length a_l for *model S* is given
571 by

572
$$pmf = r_p e^{-\delta_p a_l}. \quad (15)$$

573 The parasite growth rate p_{gr} of exponential growth *model i* results in a parasite multiplication
574 factor of:

575
$$pmf = \exp(p_{gr} a_l). \quad (15)$$

576 Under the assumption of exponential parasite growth (*model i*), the minimum inhibitory
577 concentration, where parasite replication equals zero can be calculated with

578
$$MIC = EC50 \left(\frac{p_{gr}}{E_{max} + p_{gr}} \right)^{\frac{1}{\gamma}}. \quad (16)$$

579 **Parameter estimation**

580 Parasite growth and pharmacodynamic parameters for humans were estimated in R (Version
581 3.6.0) with package RStan (Version 2.18.2(45)) using a Bayesian hierarchical modeling
582 approach. In brief, this means that subject and/or trial dependent parameters were defined as a
583 second hierarchy level in addition to the population parameters. Parameters were estimated using
584 a non-centered parameterization approach (46) and then transformed using inverse logit
585 transformation within pre-specified lower and upper bounds(47) based on biological background
586 information (Table 3). Prior distributions for the population mean parameters were given by
587 standard normal distributions before the logit transformation. Priors for the inter-individual
588 variability were defined by the Cholesky factors of the correlation matrices using a Cholesky
589 LKJ correlation distribution with shape parameter of 2 for efficiency and computational

590 stability(47). We ran five chains with 4000 iterations each of which 2000 were used as a burn-in.
591 The posterior samples were cumulated over all chains to illustrate the joint and marginal
592 posterior distributions in Fig. S7-S12. The 95% credible interval of each parameter is given by
593 the 2.5% and 97.5% quantiles of the posterior distribution (Fig. S3-S6). The M3-method was
594 used for dealing with parasite measurements below the lower limit of quantification of 111
595 p/mL.

596 Models were evaluated using the R-package bayesplot (Version 1.6.0) and loo (Version 2.1.0),
597 and model selection was based on the Watanabe-Akaike information criterion (WAIC, (48)), and
598 the effective sample size n_{eff} , an estimate of the number of independent draws from the posterior
599 distribution. n_{eff} was required to be over 0.1 for all parameters. Additionally, divergences in any
600 of the chains was evaluated visually and the convergence criteria \hat{R} was calculated (potential
601 scale reduction statistic should be less than 1.05). Posterior predictive checks were performed to
602 visually assess how well the model fits the parasitological data.

603 **Model simulation and analysis: Parasite clearance**

604 We used the well calibrated models to estimate efficacy index parasite clearance rate and \log_{10}
605 PRR_{48} for a range of drug doses and regimes in both murine and human testing systems (Fig. 1b:
606 Parasite clearance analysis).

607 Simulation and subsequent analysis of murine *P. berghei*-NMRI and *P. falciparum*-SCID
608 infection was executed in a pooled manner: one set of parameters was drawn and simulated over
609 all doses for each experiment. Population parameter distributions are defined as a log-normal
610 distribution $LN(\mu, 0.2\mu)$ with the previously estimated mean μ (see Table S5 for a summary)(15).
611 The experimental parameters infectivity parameter β and initial percentage of human RBCs H_0
612 were drawn from the pool of estimated parameter values. Simulations were performed using R-
613 package IQRtools (Version 0.9.999).

614 *P. falciparum* – human infection was simulated with parameter variability implemented on a trial
615 and subject level. Within one trial subjects were allocated the subject-specific parameters i_{pl} and
616 δ_p and shared the parasite related parameters μ_{ipl} , σ_{ipl} and r_p . Parameters were drawn from the
617 estimated variance-covariance within the bounds specified for estimation (Table 3). Simulations
618 were performed in R. Human PK parameters were fixed to population level parameters.
619 Additional information on the simulations can be found in Table S4. Parameters not previously
620 estimated were fixed to values previously reported (15, 26). Simulation results were processed to
621 extract the parasite clearance rate as described in (49). Potential lag phases after treatment at the
622 beginning of the clearance curve and tail phases at the end of the clearance curve are excluded
623 from analysis as described in (49). The clearance rate is extracted from the clearance curve on
624 the log scale using linear regression and corresponds to the slope of the parasite clearance curve.

625 **Model simulation and analysis: sensitivity of parasite clearance to host-parasite and**
626 **drug dynamics**

627 A global sensitivity analysis was performed to assess the sensitivity of parasite clearance after
628 treatment to the parameters describing drug action and parasite growth. We performed a global
629 sensitivity analysis by decomposition of the variance of model output (in this case parasite
630 clearance rate) via sobol analysis (50). Calculation of the first order indices and total effect
631 indices for all model parameters allows assessment of individual and combined parameter
632 contributions to the variance of parasite clearance rate across the whole parameter space (51). As
633 sobol analysis is computationally intensive due to the required number of points across the input
634 parameter space (n=200,000) and bootstrap replicates (n=1000), we reduced computational time
635 needed to simulate the parasite-PK-PD models by training emulators of the original models. We
636 thus trained a Gaussian process model on simulation output for each of the parasite-growth
637 models and doses analyzed using R-packages *hetGP* (Version 1.1.2). We normalized input
638 parameters and output to be between 0 and 1 due to the large differences in scales. The criterion
639 for acceptance of our trained model was out of sample prediction with a predictive accuracy of R^2
640 >0.97 . The sensitivity analysis was performed using the function *soboljansen* in the R-packages
641 *sensitivity* (Version 1.16.2) on the trained Gaussian-process model. Parameters contributing
642 under 1% were excluded from further analysis. Remaining parameters were summarized into
643 parameters of host, parasite, host-parasite, and drug dynamics for the visualization of results
644 according to Table 2.

645 **Data availability**

646 The datasets analysed during the current study are available from the corresponding author on
647 request and with permission of Medicines for Malaria Venture

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816

817 **Figure 1: Standardized workflow for the systematic investigation of parasite-host-drug dynamics throughout the (pre)-**
818 **clinical antimalarial development process.** (a) Mechanistic models of parasite growth are calibrated to extensive undisturbed
819 parasite growth (control) data in murine and human infection experiments on a population (mouse) or individual (human) level.
820 Combined with models of drug-concentration (PK) over time, they are used to calibrate to treatment data over multiple doses and
821 drugs. Models were selected for further analysis based on appropriate goodness of fit measures and assessment of biological
822 plausibility. (b) Model simulation over multiple drugs and doses facilitates the comparison of the parasite reduction rate over all
823 experimental systems. Subsequent sensitivity analysis allows the identification of parasite-, host-, or drug-dynamics as the drivers
824 of experimental outcomes.

825 **Figure 2: Experimental sampling design and investigated parasite-host dynamics in preclinical and clinical antimalarial**
826 **drug development.** Parasitemia of a typical subject in each experimental system is shown on the left side. Subjects are
827 inoculated on day 0 (black syringe) and treatment (blue syringe, oral dose) commences on the same day or up to four days
828 afterwards in murine malaria infection (a, b) and after seven to nine days in human infection (c). Each dot represents one
829 measurement, with black dots indicating untreated growth/growth before treatment and blue dots representing parasitemia after
830 treatment. Separate control groups were measured in murine experiments. The murine infections are measured in percent of
831 infected RBCs P[%] (a, b), and human infections are measured in infected RBCs per mL P[p/mL] (c) (Table 1). For illustrative
832 purposes, we added a conversion of P[p/mL] to P[%] in VIS (c) based on the assumption of 5×10^9 RBCs/mL in male humans
833 (22). The schematics of mathematical models used to describe parasite growth in the respective system are shown on the right
834 side. In murine malaria infection (a, b), capturing uninfected (X) and infected (Y) RBCs dynamics is crucial to understand
835 implications of resource limitation (*P. berghei*-NMRI infection) and resource replenishment (*P. falciparum*-SCID infection) (15).
836 Details on murine model structure can be found in the Material and Methods, Equations 1-6. In contrast, low total numbers of
837 parasites P(t) in *P. falciparum*-human infection and increased number of measurements shift modelling focus on dynamics of
838 intra-erythrocytic parasite-stages over time (c). The exponential growth *model i*, can only be used to capture parasite growth of *P.*
839 *falciparum* in SCID mouse and human as data are not informative enough in *P. berghei* – NMRI infection.

840 **Figure 3: Population parasite growth prediction in VIS.** Median parasite densities (black dots) with their 90-percentiles over
841 the time starting four days after inoculation for the 177 subjects in VIS(26). Model predictions show the median (red) and 90th
842 percentile (blue) with credible intervals over 100 trials with 20 subjects. (a) The mechanistic growth *model S* captures parasite
843 growth trends well over time with discrepancies between data and prediction being centered around parasitemia under the lower
844 limit of quantification (LLOQ) of 111 p/mL and lower limit of detection of 10 p/mL (LLOD). We found a population posterior
845 median (credible interval) of the initial parasite load (four days after infection) of 2.59 (2.44-2.74) [log(p/mL)], a median parasite
846 age μ_{pi} of 14.0 h (12.1 – 15.6) with a standard deviation σ_{pi} of 4.32 h (3.83-4.90). The intrinsic parasite multiplication rate r_p of
847 55.2 (46.3 – 68.5) and death rate δ_p of 0.0302 (0.0263-0.0353) [1/h] describe the intra-erythrocytic replication dynamics of the
848 parasite. (b) The exponential parasite growth in *model i* leads to linear growth behavior on the log-scale, so does not capture the
849 oscillating parasite growth behavior. We estimated a growth rate r_p of 0.0649 [1/h] (0.0620-0.0678). The posterior predictive
850 checks are illustrated in Fig. S7 (*model S*) and Fig. S8 (*model i*).

851

852 **Figure 4: Population prediction after treatment with OZ439 in *P. falciparum*-human infection for mechanistic growth**
853 ***model S* (a-c) and exponential *model i* (d-f).** The simulated median (red) and 90th percentile (blue) with credible intervals over
854 100 trials with 20 subjects are compared to data of individual parasite densities (black dots) in the respective treatment group. For
855 each treatment group, parasite clearance of a typical subject (Subject 1, 15 and 19) immediately after treatment is illustrated with
856 individual predictions in the inset figures (for all subjects see Fig. S12 and Fig. S13). Immediately after treatment with 100 mg
857 OZ439 (a, d) an increase in parasite densities, and transient decelerated parasite growth was observed which is captured by *model*
858 *S* through the lengthening of the parasite life cycle. After treatment with higher doses (b-c, e-f), this effect is less influential, with
859 more prominent parasite killing by the drug. After treatment with 500 mg (c, f) only four out of eight volunteers exhibited
860 parasite recrudescence (see individual data in Fig. S5 and Fig. S6 and posterior distributions in Fig. S11 and S12). Vertical line (-
861 --) indicates time of treatment and the horizontal lines the LLOQ of 111 p/mL and LLOD of 10 p/mL.

862 **Figure 5: Parasite clearance across the murine and human experiments after single dose treatment with OZ439. Within**
863 **one experimental system the predicted clearance rates are fairly consistent.** (a) *Model c* (*comp. erythr.*) of *P. berghei*-NMRI
864 infection estimated a lower maximum drug effect E_{max} resulting in lower clearance rates (Table S5). (b) Compared to panel a and
865 c, *P. falciparum*-SCID infection shows less variability. (c) The wide clearance range observed in model predictions of the
866 exponential growth *model i* for *P. falciparum*-human infection stems from the wide posterior distribution of the maximum drug
867 effect E_{max} . Parasite clearance rates were calculated from simulation output using the methodology provided in (49). For
868 comparability a conversion to $\log_{10} PRR_{48}$ was added as secondary y-axis where $\log_{10} PRR_{48} = \log_{10}(e^{\text{parasite clearance rate} \times 48})$.

869 **Figure 6: Exemplary results of the sensitivity analysis of parasite clearance after OZ439 treatment towards parameters of**
870 **host, parasite, host-parasite, and drug dynamics for (a) *P. berghei* – NMRI, (b) *P. falciparum* – SCID, and (c) *P.***
871 ***falciparum*- human infection.** Using sobol sensitivity analysis, first order effects measure individual parameter contributions and
872 the total effect indices summarize individual and interactive parameter contributions to the outcome variance (see Material and
873 Methods).

874

875 **Table 1: Overview of the data and experimental outcomes in our analysis of murine and human malaria infection.**
 876 Untreated parasite growth behavior was informed by a separate control group in murine experiments, and by parasite growth data
 877 before treatment commences in human infection. Experimental outcomes evolve over the preclinical and clinical stages with
 878 increasing data richness per subject over time from crude efficacy measures such as activity and parasite reduction to more
 879 detailed concentration effect relationships. Measures of parasite reduction (e.g. parasite clearance rate or proportional
 880 antimalarial activity) are frequently used to evaluate compounds throughout the clinical development stages (2). SD: single dose,
 881 DD: double dose, TD: triple dose, QD: quadruple dose.

	<i>P. berghei</i> ANKA in NMRI mice	<i>P. falciparum</i> (3D7) in SCID mice	<i>P. falciparum</i> (3D7) in human
no. subjects			
Control (Separate)	215 (Y)	132 (Y)	177 (N)
MMV390048	65	50	20
OZ439	200	48	24
no. subjects/dose	3-10	1-2	6-8
no. dose levels			
MMV390048	7 SD, 5 QD	2 DD, 6 QD	3 SD
OZ439	14 SD, 3 TD	10 SD, 1 DD	3 SD
Min exp. length [d]	3-4 (up to 30)	8 (up to 31)	8 (up to 30)
Inoculum [iRBCs]	2×10^7	3.5×10^7	1800-2800 (viable parasites)
Total cure (no. mice)			
MMV048	4x3 mg/kg (3)	4x20 mg/kg (2)	1x80 mg (8)
OZ439	1x30 mg/kg (30)	1x100 mg/kg (2)	-
Outcomes	- Activity - Cure (Survival) (Parasite clearance) - Cure	- PKPD relationship - Parasite clearance - Cure	- PKPD relationship - Parasite clearance - Cure
Parasitemia output	Percentage of infected RBCs	Percentage of infected RBCs	Concentration of infected RBCs per mL

882

883 **Table 2: Classification of model parameters into host, parasite, host-parasite and drug parameters.** Parameters were
 884 classified based on their dependency on host and parasite variables states and induction through those variables. Detailed
 885 description of parameters for murine models of *P. berghei* – NMRI and *P. falciparum* – SCID infection can be found in (15).
 886 Details on parameters of *P. falciparum* – human infection can be found in Fig. 2 and Table 3.

887

	<i>P. berghei</i> – NMRI	<i>P. falciparum</i> – SCID	<i>P. falciparum</i> – human
Host		<ul style="list-style-type: none"> • Initial percentage of human RBCs H_0 • Base death rate of all RBCs λ • Parameters of RBC density dependent RBC clearance χ_{\max} and $k\chi_{50}$ 	<ul style="list-style-type: none"> • Parasite death rate at each time stage δ_p
Parasite	<ul style="list-style-type: none"> • Infectivity parameter β • Number of merozoites per inf. RBC r • Attraction of parasite to reticulocyte ε • Parasite inoculum viability ω 	<ul style="list-style-type: none"> • Infectivity parameter β • Number of merozoites per inf. RBC r • Parasite inoculum viability ω • Exp. parasite growth rate r_p • Initial parasitemia P_0 	<ul style="list-style-type: none"> • Intrinsic parasite multiplication rate r_p • Distribution parameters of the initial parasite density μ_{ipl} and σ_{ipl}
Host- Parasite	<ul style="list-style-type: none"> • Parameters of infection induced RBC clearance γ_{\max} and $k\gamma_{50}$ • Conc. of inf. Mouse RBCs achieving 0.5 growth retardation effect $k_{l,50}$ 	<ul style="list-style-type: none"> • Parameters of infection induced RBC clearance γ_{\max} and $k\gamma_{50}$ • Base clearance of inf. RBCs φ 	<ul style="list-style-type: none"> • Initial parasite density i_{pl}
Drug	<ul style="list-style-type: none"> • Parameters of concentration-effect relationship E_{\max}, EC_{50} • Clearance rate for damaged parasites Cl_Y • First order rate constant for biological intermediate k_R 	<ul style="list-style-type: none"> • Parameters of concentration-effect relationship E_{\max}, EC_{50} • Clearance rate for damaged parasites Cl_Y • First order rate constant for biological intermediate k_R 	<ul style="list-style-type: none"> • Parameters of concentration-effect relationship E_{\max}, EC_{50} • Growth retardation parameter k_{ret}

888

889 **Table 3: Parameters of the parasite growth and treatment models.** The bounds for parameter estimation were set to include
890 all plausible values based on previously published models (28, 30–32).

Parameter	Unit	Description	Bounds [lower, upper]
i_{pl}	[p/mL]	Parasite density four days after inoculation (log transformed)	[1, 15]
μ_{ipl}	[h]	Mean of the initial parasite age distribution	[5, 10]
σ_{ipl}	[h]	SD of the initial parasite age distribution	[2, 20]
δ_p	[1/h]	Drug independent parasite death rate	[0.001, 1]
r_p	[-]	Parasite replication rate	[40, 80]
α_l	[h]	Length of the parasite life cycle	fixed to 39 h
α_s	[h]	Sequestration age of asexual parasites	fixed to 25 h
pmf	[]	Parasite multiplication factor	-
EC_{50}	[mg/mL]	Drug concentration causing 50% of E_{max}	MMV048: [0.001, 0.8] OZ439: [1E-6, 0.1]
E_{max}	[1/h]	Maximum effect of the drug	[0.0001, 1]
γ	[]	Hill-coefficient, steepness of the C-E curve	-
k_{ret}	[1/h]	growth retardation due to drug treatment	-
$k_{ret,max}$	[1/h]	Maximum growth retardation	[0.0001, 1]

891











