Necessity of Bumped Kinase Inhibitor Gastrointestinal Exposure in Treating Cryptosporidium Infection

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There is a substantial need for novel therapeutics to combat the widespread impact caused by Cryptosporidium infection. However, there is a lack of knowledge as to which drug pharmacokinetic (PK) characteristics are key to generate an in vivo response, specifically whether systemic drug exposure is crucial for in vivo efficacy. To identify which PK properties are correlated with in vivo efficacy, we generated physiologically based PK models to simulate systemic and gastrointestinal drug concentrations for a series of bumped kinase inhibitors (BKIs) that have nearly identical in vitro potency against Cryptosporidium but display divergent PK properties. When BKI concentrations were used to predict in vivo efficacy with a neonatal model of Cryptosporidium infection, these concentrations in the large intestine were the sole predictors of the observed in vivo efficacy. The significance of large intestinal BKI exposure for predicting in vivo efficacy was further supported with an adult mouse model of Cryptosporidium infection. This study suggests that drug exposure in the large intestine is essential for generating a superior in vivo response, and that physiologically based PK models can assist in the prioritization of leading preclinical drug candidates for in vivo testing.

Keywords. Cryptosporidium; physiologically based pharmacokinetic model; gastrointestinal; drug development.

Cryptosporidium, a genus of protozoan parasites distributed worldwide, causes severe diarrhea disproportionately affecting those with poor nutrition and/or immunodeficiency. The Global Enteric Multicenter Study (GEMS) found Cryptosporidium to be the second leading cause of moderate to severe diarrheal disease and the leading pathogen associated with death in 6–18-month-old children at sites in both Africa and Asia [1–3]. Furthermore, children who survive cryptosporidiosis have associated growth and development stunting known to contribute to increased all-cause mortality rates and developmental issues [4]. The sole drug for cryptosporidiosis, nitazoxanide, has only a 30% response rate in malnourished children and no efficacy in human immunodeficiency virus–infected individuals [5]. Improved therapeutics are the greatest hope in the near term for addressing death and disability from Cryptosporidium infection. Species and strain antigenic differences and a lack of cross-protection impedes the development of a broadly protective vaccine [6].

There are unique challenges in drug development for cryptosporidiosis in that Cryptosporidium is a protozoan parasite and is localized to the gastrointestinal (GI) tract. As a protozoan parasite, Cryptosporidium is not susceptible to antibiotics which target processes specific to bacteria. In addition, Cryptosporidium is more closely related to humans than to bacteria or viruses, and this increases the risk for potential drug toxicity. We have reduced host toxicity by developing bumped kinase inhibitors (BKIs) that selectively target calcium-dependent protein kinase 1 (CpCDPK1) compared with host kinases. A subset of the BKIs have shown inhibitor constant (Ki) values <1 nmol/L against CpCDPK1 and inhibit the growth of Cryptosporidium in vitro with EC50 values <5 μmol/L against the parasite itself while demonstrating little toxicity to human cells or in preclinical animal models [7].

Although we and others have made progress in the development of selective inhibitors of Cryptosporidium growth, the pharmacokinetic (PK) parameters that predict efficacy are unknown for compounds addressing its distinct localization. PK/pharmacodynamic (PD) indices use PK parameters such as the maximal and average unbound drug concentration (Cmax, Cavg) and area under the drug concentration–time curve (AUC), along with in vitro–derived efficacy parameters, such as the 50% minimum inhibitory concentration or EC50, to predict the in vivo efficacy of drug candidates [8]. In the antimicrobial field, these indices have been well established and are used to guide the selection of dosing regimens for drug classes such as
\[ \text{Inhibition of C. Parvum Growth}(\%) = \frac{E_{\text{max}} \cdot f_u[I]}{f_u[I] + EC_{50}} \] (1)

where \( E_{\text{max}} \) is the maximum observed inhibition in vitro, \( [I] \) is the BKI concentration (\( C_{\text{max}} \) or \( C_{\text{avg}} \)), \( f_u \) is the fraction unbound to proteins, and the \( EC_{50} \) is the BKI concentration that inhibited 50\% of \( C. parvum \) growth in vitro. For all BKIs, the maximum observed inhibition in vitro was 100\%. To predict the efficacy of BKI with simulated GI concentrations, the \( C_{\text{max}} \) and \( C_{\text{avg}} \) were determined for each section of the GI tract and used as the value for \( [I] \). The \( f_u \) value was set to 1 for predictions for BKI GI concentrations.

To predict the efficacy in the IFN-\( \gamma \) KO mouse model, the natural growth of \( C. parvum \) and the inhibition by BKI were both incorporated into the model. The natural growth rate of \( C. parvum \) was determined by natural log-transforming the relative luminescence units (RLUs) observed in the vehicle-treated mice after infection and fitting the data with a linear regression. The slope of the line was used as a rate constant to simulate the growth of \( C. parvum \) infection with a Malthusian growth model. For 5 days after administration of BKI, the RLU (oocysts shedding) was predicted with Equation 2:

\[ \text{Predicted RLU value} = Ae^{kt} \cdot \left[ \frac{E_{\text{max}} \cdot f_u[I]}{f_u[I] + EC_{50}} \right] \] (2)

where \( A \) is the amount of RLUs measured the previous day; \( e \) is the mathematical constant Euler’s number; \( k \), the growth rate constant; and \( t \), the time in days. The predicted RLU values were log-transformed and fit with a linear regression. The slope of the predicted RLU values was compared with that observed in the BKI-treated mice by analysis of covariance, using GraphPad Prism software, and if the slopes differed significantly \( (P \leq .05) \) the prediction was considered inaccurate.

**RESULTS**

**BKI Pharmacokinetics and Predicted Efficacy in a Neonatal Mouse Model of Cryptosporidium Infection**

Although the 8 BKIs used in this study share the same pyrazolo[2,3-d]pyrimidine scaffold, differing chemical side chains generate variances in plasma PK after an oral dose. When a single oral dose of each BKI was administered to mice, 30- to 60-fold differences between the \( C_{\text{max}} \) and AUC values were observed, respectively (Table 1). Given the lack of knowledge on the PK/PD relationship for BKI anti-\( Cryptosporidium \) efficacy, the single-dose BKI PK parameters and their respective \( EC_{50} \) values were obtained and used to predict the in vivo efficacy of BKI against \( C. parvum \) infection.

**METHODS AND MATERIALS**

Information regarding the \( C. parvum \) oocysts, generation of BKIs, CpcDPK1 enzyme inhibition, \( C. parvum \) growth inhibition by BKIs, plasma protein binding, and neonatal mouse model of \( C. parvum \) infection has been described in detail elsewhere [7]. Information on BKI solubility and permeability, Gastroplus modeling, the interferon (IFN) \( \gamma \) knockout (KO) mouse model, and measurement of BKI levels in the GI tract of mice is described in the Supplementary Material. All animal experiments were approved by the Institutional Animal Care and Use Committees at the participating universities (University of Washington, University of Georgia, and University of Arizona). The single-dose BKI PK parameters and their respective \( EC_{50} \) values were obtained and used to predict the in vivo efficacy of BKI against \( C. parvum \) infection.
values do not provide the information necessary for prioritizing compounds to test in preclinical animal models. Therefore, the BKIs were tested in a neonatal model of *C. parvum* infection to determine whether any systemic PK parameters are associated with in vivo efficacy.

Although each of the BKIs generated a significant reduction in *C. parvum* infection when tested in a neonatal model of cryptosporidiosis across the set, the in vivo efficacy had a large range of 33%-70%. Surprisingly, BKI 1553 displayed the highest *C*<sub>max</sub> and largest AUC after a single oral dose, yet it had the least efficacy in clearing parasite infection, other than 1557 (Table 1). The BKI 1369 had the most efficacy in clearing parasite infection, although its *C*<sub>max</sub> was the second lowest of the 8 BKIs, and its *EC<sub>50</sub>* value for in vitro parasite growth inhibition was nearly identical to that of the other BKIs tested.

The single-dose *C*<sub>max</sub> and AUC values were dose normalized and used to determine whether in vivo efficacy could have been predicted with BKI systemic PK values (Figure 1A), with the assumption that the BKI have linear PK. No significant association was observed between the efficacy and the dose-normalized BKI unbound *C*<sub>max</sub>/*EC<sub>50</sub>* value, suggesting that the dose-normalized unbound *C*<sub>max</sub> is not a predictor of in vivo efficacy. A comparable result was obtained when the total dose-normalized *C*<sub>max</sub>/*EC<sub>50</sub>* was used to predict efficacy (data not shown). Similar to the *C*<sub>max</sub> data, the dose-normalized AUC values did not predict the in vivo efficacy (Figure 1B). Finally, a sigmoidal *E*<sub>max</sub> model that incorporated the dose-normalized *C*<sub>max</sub> and the *EC<sub>50</sub>* of each BKI was evaluated for its ability to predict the in vivo efficacy of each compound [19]. However, neither the unbound nor total dose-normalized *C*<sub>max</sub> values predicted the in vivo efficacy (Figure 1C).

To overcome the inability to predict in vivo efficacy with systemic levels of BKI, the in vivo pharmacokinetics and physicochemical properties of each BKI were incorporated into a physiologically based PK model to simulate the BKI GI concentrations. BKI concentrations were simulated using the dosing protocol of the neonatal study, and the GI concentrations reported refer to the segments of the Gastroplus advanced compartmental absorption and transit simulation model. The mouse advanced compartmental absorption and transit model was adjusted as described in Supplemental Table 1 to represent more appropriately the reported physiology of a neonatal mouse [20]. For each GI segment, simulated

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**Table 1.** BKI Single-Dose Pharmacokinetics and Their Efficacy in the Neonatal Mouse Model

| BKI  | Dose, mg/kg | *C*<sub>max</sub>, μmol/L | Plasma Protein Binding, % | *EC<sub>50</sub>* for Inhibition of *C. parvum* Proliferation | Reduction in Neonatal Mouse *C. parvum* Infection, % |
|------|-------------|-----------------------------|---------------------------|-------------------------------------------------|--------------------------------------------------|
| 1294 | 10          | 0.8                         | 84                        | 2.7                                             | 56                                               |
| 1553 | 10          | 12.8                        | 90                        | 1.6                                             | 36                                               |
| 1318 | 10          | 6.5                         | 55                        | 3.2                                             | 65                                               |
| 1369 | 10          | 0.3                         | 37                        | 2.3                                             | 70                                               |
| 1534 | 10          | 0.2                         | 99                        | 2.2                                             | 61                                               |
| 1649 | 10          | 4.9                         | 90                        | 2.3                                             | 56                                               |
| 1556 | 25          | 7.0                         | 86                        | 3.2                                             | 61                                               |
| 1557 | 10          | 0.9                         | 84                        | 5.5                                             | 33                                               |

Abbreviations: AUC, area under the curve; BKI, bumped kinase inhibitor; *C. parvum*, *Cryptosporidium parvum*; *C*<sub>max</sub>, maximal concentration; *EC<sub>50</sub>* BKI concentration that inhibits 50% of *Cryptosporidium* growth in vitro.

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**Figure 1.** Predicting in vivo efficacy with dose-normalized bumped kinase inhibitor (BKI) maximal concentration (*C*<sub>max</sub>) and area under the curve (AUC). A, Observed reduction in a neonatal mouse model of *Cryptosporidium parvum* infection was plotted as a function of the dose-normalized *C*<sub>max</sub>/*EC<sub>50</sub>* ratio for each BKI where *EC<sub>50</sub>* is the BKI concentration that inhibits 50% of *Cryptosporidium* growth in vitro. There was not a significant association between the 2 values. B, Similar to the dose-normalized *C*<sub>max</sub>/*EC<sub>50</sub>* ratio, the dose-normalized BKI was not a predictor of in vivo efficacy. C, Finally, sigmoidal *E*<sub>max</sub> model was used to predict the in vivo efficacy, but neither the total nor the unbound dose-normalized *C*<sub>max</sub> predicted the in vivo efficacy.

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**GI Drug Exposure in Treating Cryptosporidium Infection • JID 2017:216 (1 July) • 57**
concentrations of BKI in the lumen and the enterocytes were used to determine their respective $C_{\text{max}}$ and $C_{\text{avg}}$ values over the course of the neonatal efficacy study (Supplemental Table 2).

Representative GI concentration versus time profiles for the BKIs are shown in Figure 2 for BKIs 1294 and 1553. The $C_{\text{max}}$ for BKI 1553 is predicted to be greater than for BKI 1294 in plasma and a majority of the GI tract, whereas the $C_{\text{max}}$ for BKI 1553 in the cecum and ascending colon are predicted to be less than for BKI 1294. In addition, although BKI 1553 is predicted to have brief increases in concentration after each dose, BKI 1294 is predicted to remain in the cecum and ascending colon for an extended period. Therefore, the predicted $C_{\text{avg}}$ values for BKI 1294 in these GI sections are much higher than those for BKI 1553.

The simulated concentrations for BKIs 1294 and 1553 in the plasma and the GI tract were used to predict efficacy in the neonatal mouse model. As expected, both the plasma $C_{\text{max}}$ and the $C_{\text{avg}}$ were not predictors of BKI in vivo efficacy, and accounting for plasma protein binding did not improve the prediction (Figure 3A and 3B).

The predicted BKI efficacy in each section of the GI tract was compared with the observed reduction in neonatal $C.~parvum$ infection (Supplemental Figure 2 and Figure 3). Similar to the efficacy predicted with systemic concentrations, BKI efficacy was not predicted using simulated concentrations in the duodenum, jejunum, or ileum (Supplemental Figure 2). However, when BKI concentrations in the enterocytes of the ascending colon were used to predict the in vivo efficacy, there was a significant ($P < .05$) positive association between the predicted and the observed efficacy (Figure 3C). Furthermore, there was a nonsignificant trend between the predicted and observed efficacy when ascending colon BKI concentrations in the lumen were used for the predictions (Figure 3D). In addition, when BKI concentrations in the lumen of the cecum were used to predict the in vivo efficacy, there was a significant ($P < .05$) positive association between the predicted and observed efficacy (Supplemental Figure 2D).

**Adult Mouse Model of Cryptosporidium parvum Infection**

The ability to predict in vivo efficacy with BKI ascending colon concentrations was further evaluated in an adult model of $C.~parvum$ infection. Female IFN-γ KO mice were infected with transgenic $C.~parvum$ strain UGA1 expressing nanoluciferase (Nluc), and the infection was allowed to proceed for 3–5 days [21]. Infected mice were treated with oral doses of BKI 1294 (60 mg/kg), BKI 1553 (10 mg/kg), or vehicle control.
daily for 5 days, and oocyst shedding was measured by quantifying parasite-derived luminescence in the feces. To account for daily growth of parasites over the course of the study, the observed RLUs in the vehicle-treated mice were used to simulate the growth of the parasite over the course of the experiment (Figure 4A). On each day of the study, the simulated parasite level was based on both the growth of the parasite and the predicted inhibition by BKI \[22–24\].

The BKIs 1553 and 1294 \( \text{C}_{\text{max}} \) and \( \text{C}_{\text{avg}} \) in the lumen and the enterocytes of the ascending colon were used to predict the daily in vivo efficacy of the BKI over the duration of the study. These parameters were calculated for each day of the study using the simulated BKI concentration versus time profile over the 5 days of treatment (Supplemental Table 3). The observed and predicted luminescence was log-transformed, and linear regression was used to fit the data for the 5 days after treatment was initiated. The predicted efficacy was accepted as accurate if the predictions approximated what was observed and if there was not a significant difference \((P > .05)\) between the slopes of the predicted and observed change in luminescence. When the simulated ascending colon luminal \( \text{C}_{\text{max}} \) \((R = 0.60)\) or \( \text{C}_{\text{avg}} \) \((R = 0.47)\) levels were used with the sigmoidal \( E_{\text{max}} \) model. D, The ascending colon lumen concentration of BKI did not predict the in vivo efficacy.

Figure 3. Simulated systemic and gastrointestinal (GI) concentrations of bumped kinase inhibitor (BKI) were used to predict the neonatal in vivo efficacy. A, B, As expected from the dose-normalized predictions, the model described with Equation 1 did not predict the in vivo efficacy when simulated plasma maximal concentration \( \text{C}_{\text{max}} \) \((A)\) or average concentration \( \text{C}_{\text{avg}} \) \((B)\) was used for the predictions. C, However, a significant \((P < .05)\) association between predicted and observed efficacy was obtained when the ascending colon enterocyte \( \text{C}_{\text{avg}} \) \((R = 0.60)\) or \( \text{C}_{\text{max}} \) \((R = 0.47)\) levels were used with the sigmoidal \( E_{\text{max}} \) model. D, The ascending colon lumen concentration of BKI did not predict the in vivo efficacy.
Figure 4. Simulating Cryptosporidium parvum growth in vivo and predicting in vivo efficacy with an adult mouse model of cryptosporidiosis. Adult interferon γ knockout mice were infected with nanoluciferase-expressing C. parvum oocytes. Therefore, oocyst excretion can be quantified by measuring luminescence after lysis of the fecal content. A, In vivo growth rate of C. parvum was simulated by log-transforming the observed relative luminescence in the feces of 3 vehicle-treated mice and fitting the data with a linear regression. B, C. The resulting growth rate was incorporated into the in vivo efficacy studies with bumped kinase inhibitor (BKI) 1553 (8) and 1294 (C). Ascending colon lumen or enterocyte levels were used to predict the inhibition of C. parvum growth with 5 daily oral doses of BKI 1553 (10 mg/kg) (B) or BKI 1294 (60 mg/kg) (C). The slope of the predicted relative luminescence unit (RLU) values was compared with that observed in the mice treated with BKI by analysis of covariance, and if the slopes differed significantly (P ≤ .05), the prediction was considered inaccurate. In vivo efficacy was accurately predicted with BKI 1534 enterocyte values for the average and maximal (C max), but the lumen values overpredicted the in vivo efficacy (B). Similarly, the BKI 1294 efficacy was accurately predicted with the enterocyte C max and C avg but the lumen BKI 1294 levels over predicted the in vivo efficacy (C). For each study, 3 mice were used in both the BKI-treated and the vehicle-treated groups. Open circles represent results generated from the pooled feces of the 3 mice.

Figure 5. Simulated enterocyte bumped kinase inhibitor (BKI) concentrations in the ascending colon accurately predicted an in vivo dose response. Based on the accuracy of predicted efficacy with ascending colon enterocyte levels of BKI 1294 and 1553, the enterocyte maximal and average concentrations (C max and C avg) for BKI 1554 were used to predict in vivo efficacy with 5 daily oral doses of BKI 1534 at 6 (A), 20 (B), or 60 (C) mg/kg. Predicted efficacy was compared with observed efficacy in an experiment where dosing was initiated on day 3 after infection (open circles). The slope of the predicted relative luminescence unit (RLU) values was compared with that observed in the BKI-treated mice by analysis of covariance, and if the slopes differed significantly (P ≤ .05) the prediction was considered inaccurate. Although the BKI 1534 enterocyte C max and C avg predicted the in vivo efficacy with daily dosing of 6 mg/kg (A), only the C max predicted the in vivo efficacy at daily doses of 20 mg/kg (B) or 60 mg/kg (C). For each study, there were 3 mice in both the BKI-treated and the vehicle-treated groups. Open circles represent results generated from the pooled feces of the 3 mice.

In vivo BKI Exposure Along the GI Tract
To experimentally confirm that plasma concentrations of BKI are indeed not representative of their concentrations in the GI tract, BKI 1294 (60 mg/kg) and BKI 1553 (10 mg/kg) were each given as a single oral dose to 9 mice. The average plasma concentration of BKI 1553 was approximately 10 µmol/L by 0.5 hours, significantly (P < .01) higher than that for BKI 1294 at each time point (Figure 6A and 6B), consistent with findings of the initial single-dose PK study. Although the systemic levels of BKI 1553 were higher than those for BKI 1294, GI concentrations of the latter were higher over the course of the study.

were again used to simulate parasite growth. Three days after infection, mice were treated once daily with a vehicle control or with BKI 1534 at doses of 6, 20, or 60 mg/kg (Figure 5). The ascending colon enterocyte C max and C avg values were calculated from the simulated concentration versus time profiles and used to predict the in vivo efficacy. For the 6-mg/kg study, both the enterocyte C max and C avg accurately predicted the lack of BKI efficacy. However, though the simulated enterocyte C max accurately predicted the in vivo efficacy after doses of 20 or 60 mg/kg, the efficacy predicted with the enterocyte C avg did not match the observed inhibition of parasite growth (Figure 5B and 5C).
Because BKI efficacy was associated with the ascending colon levels of BKI, the concentrations of BKI 1294 and 1553 in the ascending colon were compared using Student t test. At 4 hours, the average BKI 1294 concentration was significantly higher than that of BKI 1553 (46 vs 9 µmol/L, respectively; P < .01).

**DISCUSSION**

The pathogen localization within its host’s organs and tissues is of utmost importance for the development of antimicrobial therapy. Simple models based on serum exposure alone are insufficient to predict cure, and knowledge of the specific site at which drug exposure is necessary is crucial to the development of novel therapeutics. As one of many examples, this is frequently discussed in the context of the blood-brain barrier and its impact on the treatment of different stages of African trypanosomiasis [25]. Information to build a reliable PK/PD index is not currently available for any class of anti-*Cryptosporidium* drugs; thus, we selected 8 BKIs with similar in vitro efficacy but divergent PK properties to identify PK characteristics associated with in vivo efficacy.

Plasma BKI levels do not predict in vivo efficacy, suggesting that systemic distribution of BKI is not required for anti-*Cryptosporidium* efficacy in mice. The lack of association between systemic exposure and in vivo efficacy is probably due to the GI localization of the parasite along the entire GI tract in both IFN-γ KO [26] and SCID/beige mice [27]. Dose-normalized PK values for the BKIs were used along with in vitro derived EC$_{50}$ values to predict their respective efficacy in a neonatal model of *C. parvum* infection, but no associations were identified between any of the PK/PD indices and in vivo efficacy. Previous work in our laboratory identified BKIs lacking systemic exposure that generated an in vivo reduction in *C. parvum* infection [7], and a similar lack of association between systemic exposure and efficacy has been reported for the treatment of bacterial diarrhea by oral bicozamycin [28] and aztreonam [29]. Owing to the poor efficacy predictions generated with systemic levels of BKI and a correlation previously reported between high fecal levels of BKI and in vivo efficacy [7], we chose to investigate the importance of GI drug exposure for in vivo BKI efficacy.

Simulated large intestinal BKI levels accurately predicted the efficacy of the BKIs. Based on the parasite localization throughout the GI tract, it is likely that exposure of BKI in both the small and large intestines is crucial for efficacy, because BKIs with low exposure in the large intestine fail to clear the infection. It is likely that permeable compounds are quickly absorbed in the small intestine (eg, BKI 1553) and less permeable compounds are carried further along the GI tract (eg, BKI 1294), allowing...
them to maintain anti-Cryptosporidium activity. Although these initial BKI observations provided encouraging results, we tested the model with an IFN-γ KO adult mouse model of C. parvum infection to confirm the ability to predict in vivo efficacy with ascending colon BKI levels.

In agreement with findings in the neonatal mouse model, simulated ascending colon BKI concentrations accurately predicted the in vivo response to BKI treatment in the IFN-γ KO adult mouse model of Cryptosporidium infection. Although the neonatal mouse model provides only a single end point for assessing BKI efficacy, IFN-γ KO mice infected with Nluc-expressing C. parvum allow for daily measurement of C. parvum infection. This enabled development of a dynamic BKI efficacy model based on previous PK/PD modeling efforts for antibacterials and antitumor drugs [12, 22–24]. This model can account for differences in proliferation rates between Cryptosporidium strains or even in batches of oocysts.

We confirmed that simulated levels of BKI in the enterocytes of the ascending colon accurately predicted the in vivo efficacy of 3 BKIs. However, the importance of the C\textsubscript{max} and/or the C\textsubscript{avg} in the efficacy prediction was not consistent across the data set, so it is unclear whether the pharmacodynamics of the BKIs are time or concentration dependent. The GI BKI values measured in vivo were in agreement with the predicted Gastroplus values. The average maximum BKI 1294 and 1553 concentrations measured in the ascending colon were slightly higher than the values predicted for the enterocytes. This elevation could be due to residual BKI remaining after the lumen was flushed with phosphate-buffered saline and BKI in cells other than enterocytes. Although the measured ascending colon concentrations were higher than those simulated, the BKI 1553 values were significantly less than those measured with BKI 1294, correlating to the efficacy underperformance of BKI 1553 compared with BKI 1294.

Taken together, the results presented here provide strong support for optimizing drug exposure along the entire GI tract when treating Cryptosporidium infection. Therefore, adjusting the formulation to achieve the desired GI exposure profile will be essential for developing anti-Cryptosporidium therapies. Formulation strategies for such treatments may be especially challenging, because it is unclear how disease and altered GI transit times will affect drug dissolution and absorption. Mice do not develop clinical diarrhea with C. parvum infection, but studies in infected calves that exhibit diarrheal symptoms of cryptosporidiosis suggest that BKI exposure is not significantly altered by infection [30]. Malnourished children are an important focus of the current target product profile for Cryptosporidium drugs [31, 32], but it is unclear whether malnourishment will affect BKI pharmacokinetics, because previous studies have observed significant changes in antibiotic exposure in children with severe acute malnutrition [33–40].

In conclusion, to our knowledge this is the first study to investigate the importance of GI drug exposure for in vivo anti-Cryptosporidium efficacy. The results of this work have the potential to provide fundamental support for drug discovery programs in prioritizing lead drug candidates. In addition, these predictions can be tested in other GI infections and disorders in which large intestine epithelial cell delivery may be important.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

**Disclaimer.** The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Institute of Food and Agriculture, US Department of Agriculture or the positions or policies of the Bill & Melinda Gates Foundation.

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