HHS Public Access

Author manuscript

Vet Parasitol. Author manuscript; available in PMC 2022 January 01.

Published in final edited form as:

Vet Parasitol. 2021 January ; 289: 109336. doi:10.1016/j.vetpar.2020.109336.

One Health Therapeutics: Target-Based Drug Development for Cryptosporidiosis and Other Apicomplexa Diseases

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Conflict of Interest Statement: Wes Van Voorhis and Tom Kennedy are co-owners of ParaTheraTech Inc., a company that is developing bumped-kinase inhibitors for animal therapy. Otherwise the authors have nothing to report.

Credit Author Statement Vetpar-D-20-14753
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Abstract

This is a review of the development of bumped-kinase inhibitors (BKIs) for the therapy of One Health parasitic apicomplexan diseases. Many apicomplexan infections are shared between humans and livestock, such as cryptosporidiosis and toxoplasmosis, as well as livestock only diseases such as neosporosis. We have demonstrated proof-of-concept for BKI therapy in livestock models of cryptosporidiosis (newborn calves infected with *Cryptosporidium parvum*), toxoplasmosis (pregnant sheep infected with *Toxoplasma gondii*), and neosporosis (pregnant sheep infected with *Neospora caninum*). We discuss the potential uses of BKIs for the treatment of diseases caused by apicomplexan parasites in animals and humans, and the improvements that need to be made to further develop BKIs.

Introduction

Apicomplexa afflict humans and livestock causing morbidity, mortality, and reproductive loss. This review discusses the apicomplexan parasites most likely to respond to a class of compounds called bumped-kinase inhibitors (BKIs), namely *Cryptosporidium parvum*, *C. hominis, Toxoplasma gondii, Neospora caninum, Sarcocystis neurona, Cystoisospora suis*, and *Besnoitia besnoiti*.

Cryptosporidiosis annually afflicts millions of children, less than 2 years-old, with diarrhea in resource limited countries (Khalil et al., 2018). Cryptosporidiosis is responsible for about 25% of moderate-to-severe diarrhea (Kotloff et al., 2013). Cryptosporidiosis is associated with a more than 2-fold increased risk of death compared to children without *Cryptosporidium* with moderate-to-severe diarrhea; these children with moderate-to-severe diarrhea already have a more than 8-fold increased risk of dying compared to non-ill controls (Kotloff et al., 2013). Furthermore, cryptosporidiosis in children in resource-limited countries is highly associated with stunting, and stunting itself is associated with increased mortality and decreased neurodevelopment (Checkley et al., 1998; Checkley et al., 2015; Delahoy et al., 2018; Khalil et al., 2018; Korpe et al., 2018). *Cryptosporidium* is also a severe and sometimes fatal infection in immunocompromised patients, such as those with severe HIV or those immunosuppressed for transplantation (Silverlås et al., 2009; Trotz-Williams et al., 2011; White, 2020). For humans, only nitazoxanide is approved for cryptosporidiosis therapy. However, nitazoxanide is only minimally efficacious for

malnourished children and immunocompromised individuals, those who need therapy the most (Amadi et al., 2002; Amadi et al., 2009; Checkley et al., 2015). In animal health, cryptosporidiosis also takes a severe toll on newborn calves, and is associated with a 34 kg weight gain failure (Shaw, 2017; Shaw et al., 2020). For treatment of livestock, the only licensed product is halofuginone, which reduces the incidence of cryptosporidiosis about 40% if given metaphylactically. However, halofuginone is not effective as a therapeutic once cryptosporidiosis diarrhea is established (Silverlås et al., 2009; Trotz-Williams et al., 2011).

Toxoplasma gondii infection in pregnancy is associated with fetal loss and fetal and newborn abnormalities in both humans and livestock, such as sheep, goats, and equids (Bigna et al., 2020; Dubey, 2009a, b; Dubey et al., 2020a; Dubey et al., 2020b; Rostami et al., 2019; Stelzer et al., 2019). *T. gondii* causes infections in the eye (retina) and brain, necessitating drugs that cross the placenta, brain, and eye barriers for penetration. In addition, acute toxoplasmosis can be caused by (partial) immunosuppression and reactivation of parasites from normally dormant tissue cyst stage bradyzoites. Available drugs in humans include pyrimethamine/sulfadiazine combination, clindamycin, spiramycin, and atovaquone. However, none of these drugs are very active and up to 40% of patients with moderate to severe toxoplasmosis have to stop therapy due to drug toxicity (Neville et al., 2015; Sanchez-Sanchez et al., 2018b).

Neospora caninum infection in cattle and sheep leads to repeated abortions and an estimated economic loss of 1.3 billion USD per year (Gonzalez-Warleta et al., 2018; Gonzalez-Warleta et al., 2014; Reichel et al., 2013). Current experimental therapies for neosporosis include ponazuril, toltrazuril, decoquinate, and monensin, but none have been shown to be significantly effective in ruminant pregnancy models (Sanchez-Sanchez et al., 2018b).

Calcium-dependent protein kinase 1 and bumped-kinase inhibitors

Calcium-dependent protein kinase 1 (CDPK1) was first recognized to be important in *Toxoplasma gondii* gliding motility, exocytosis, and cell entry and exit (Kieschnick et al., 2001; Nagamune and Sibley, 2006). When the crystal structures of *T. gondii* and *Cryptosporidium parvum* CDPK1s were solved, it was discovered that the gatekeeper of this protein kinase was glycine (Murphy et al., 2010; Ojo et al., 2010; Wernimont et al., 2010). This allowed selective targeting of these kinases with a class of molecules called “bumped-kinase inhibitors” or BKIs, that take advantage of a hydrophobic pocket that is opened up next to the gatekeeper when small sidechain gatekeepers, such as glycine, are present (Bishop et al., 1998; Bishop and Shokat, 1999) (Figure 1, left). BKIs provide increased potency by inhibiting CDPK1 through the hydrophobic “bump” that fills the pocket next to the glycine gatekeeper. But, more importantly, BKIs are excluded from almost all mammalian protein kinases in that they almost uniformly have gatekeeper side-chain residues larger than the hydrogen of glycine (Figure 1, right). Apicomplexan parasites of human and veterinary public health importance, with a CDPK1 known to have a glycine gatekeeper and thus highly sensitive to inhibition by BKIs, include: *C. parvum, C. hominis, T. gondii, Neospora caninum, Sarcocystis neurona, Besnoitia besnoiti*, and *Cystoisospora suis* (Jimenez-Melendez et al., 2017; Murphy et al., 2010; Ojo et al., 2016; Ojo et al., 2010; Ojo et al., 2014; Shrestha et al., 2019).

The most effective BKIs are made on two scaffolds, the pyrazolo[2,3-*d*]pyrimidine (PP) and the 5-aminopyrazole-4-carboxamide (AC) scaffolds (Figure 2) (Huang et al., 2017; Huang et al., 2015; Johnson et al., 2012; Murphy et al., 2010; Zhang et al., 2014). To date, we have made over 750 BKIs optimized for potency against *T. gondii* and *C. parvum* CDPK1 and anti-apicomplexan parasite activity. BKI safety parameters, including limiting mammalian protein kinase activity, pharmacokinetics (PK), including absorption, distribution, metabolism, and excretion properties (ADME), have been investigated.

BKIs for Cryptosporidiosis

Our lead for anti-cryptosporidiosis therapy is a PP compound, BKI-1369 (Figure 2). The characteristics of BKI-1369 are summarized in Table 1. It inhibits 50% of the *C. parvum* CDPK1 (*Cp*CDPK1) recombinant enzyme phosphorylation activity (IC_{50}) at 0.9 nM, but has no detectable inhibition at 10 μM of mammalian SRC protein kinase activity, which we use as a counterscreen for kinase specificity since SRC has one of the smallest mammalian gatekeepers, threonine (Hulverson et al., 2017b). BKI-1369 inhibits *C. parvum* nanoluciferase-expressing (Nluc)-UGA strain by 50% (EC_{50}) at 2.4 μM, yet doesn't inhibit mammalian cell line proliferation up to the limit of solubility, 80 μM (Hulverson et al., 2017a). It does not demonstrate genotoxic properties in the modified AMES assay, has reasonable solubility properties at both pH 2 and pH 6.5, and demonstrates only moderate plasma protein binding in the four species of plasma tested (Arnold et al., 2017). One issue with BKI-1369 is that it blocks the human Ether-à-go-go-Related Gene (hERG) receptor at 2 μM concentration, which is associated with human cardiotoxicity and this may preclude its development for human use. Additionally, BKI-1369 also actively inhibits other strains of *C. parvum* and *C. hominis*, the most common species of *Cryptosporidium* found in humans in the developing world (Table 2) (Hulverson et al., 2017a).

Testing of BKI-1369 in animal models of *C. parvum* and *C. hominis* demonstrated efficacy leading to profound reductions in parasite shedding, and in clinical models in reduction of diarrhea and better health outcomes. For instance, in the Nluc-*C. parvum* gamma-interferon knockout mouse model, 60 mg/kg and 30 mg/kg, administered once a day on days 6–10 after infection, led to maximal clearing of infection, but even 5 mg/kg and 15 mg/kg were effective at reducing parasite excretion (Figure 3) (Hulverson et al., 2017a). In the newborn calf model of *C. parvum* infection, twelve two day-old calves were infected with 5×10^7 *C. parvum* Iowa strain oocysts (Cryptosporidium Production Lab, University of Arizona), and on day 2 P.I., randomized for treatment with BKI-1369 (5 mg/kg twice a day for 5 days) or vehicle control(Heine et al., 1984; Riggs and Schaefer, 2020). Calves that were treated with BKI-1369 had a 30-fold reduction in total oocyst excretion on days 3–10 P.I., and had almost immediate resolution of diarrhea, with a significant reduction of fecal volume on days 4–8 P.I.. The treated group had a 4.5% weight gain whereas placebo treated animals had a slight weight loss by 10 days P.I. (Hulverson et al., 2017a) (Figure 4). In the gnotobiotic piglet model, eighteen piglets were infected with 10^6 *C. hominis* oocysts orally two days after birth, then randomized to treatment or vehicle only, twice a day for five days, and then followed for 10 days after therapy. In this *C. hominis* model, BKI-1369 administered at 10 mg/kg twice a day was superior to vehicle control in total oocyst excretion (~10-fold reduction of oocysts in feces) (Figure 5). *C. hominis* DNA excreted in stool, and diarrhea

score were also significantly better in BKI-1369 group on days 1–3 post treatment (Lee et al., 2018).

For human cryptosporidiosis as mentioned above, avoiding hERG inhibition has led us to search for therapeutic alternatives. We have found active compounds on both the PP and AC scaffolds and these are under active investigation for the right balance of safety and efficacy parameters (Huang et al., 2017; Hulverson et al., 2019; Vidadala et al., 2016).

BKIs for Toxoplasmosis

The PK properties for BKIs that drive efficacy for cryptosporidiosis, where drug needs to be delivered to infected gut tissue, are very likely different than the PK properties of BKIs for systemic infections such as toxoplasmosis, where penetration of the blood brain barrier, placenta, and the eye are necessary for optimal therapy (Arnold et al., 2017). BKI-1369 does not reach very high plasma and CNS levels during efficacious therapy for cryptosporidiosis and high exposure in blood or brain is likely to decrease the safety unnecessarily for *Cryptosporidium* therapy. However, BKIs such as BKI-1294 and BKI-1553 have shown proof-of-concept in small and large animal models of systemic apicomplexan infections, such as toxoplasmosis (Figure 2).

BKI-1294 has a 3 nM IC_{50} when inhibiting *T. gondii* CDPK1 (*Tg*CDPK1) and inhibits *T. gondii* proliferation in vitro at an EC_{50} of 0.137 μM. BKI-1294 was shown to primarily target *Tg*CDPK1 by demonstration of an 11-fold resistance to BKI-1294 generated by expression of a mutant *Tg*CDPK1 where the gatekeeper changed from glycine to methionine (Johnson et al., 2012). It shares many of the good safety aspects of BKI-1369, such as no measurable mammalian cell cytotoxicity and safety in pregnancy in mice and sheep (Johnson et al., 2012; Sánchez-Sánchez et al., 2019; Winzer et al., 2015). BKI-1294 was shown to be effective in reducing the intraperitoneal *T. gondii* RH strain by 93% at 30 mg/kg given orally once a day for five days and more than 99% by 100 mg/kg dosed the same way (Doggett et al., 2014). In a mouse model of congenital toxoplasmosis based on oral infection of pregnant mice with 20 oocysts of the *T. gondii* ME49 strain, only 4 of 55 (7%) of surviving pups treated with BKI-1294 at 50 mg/kg orally once a day for five days had detectable *T. gondii* in their brains, whereas 67 of 80 (84%) pups treated with placebo were *T. gondii* infected (Muller et al., 2017b). Thus, in two mouse models, including one involving transplacental and blood brain barrier passage, BKI-1294 appears to be efficacious and safe.

We have gone on to show that BKI-1294 is safe and effective in a sheep pregnancy model (Sánchez-Sánchez et al., 2019). In this model, pregnant sheep (Rasa Aragonesa breed) were infected with 1000 *T. gondii* sporulated oocysts of the *Tg*ShSp1 strain at 90 days gestation. Sheep were treated orally with 100 mg/kg BKI-1294 every other day for 5 treatments or with vehicle alone. An uninfected pregnant control group was treated with BKI-1294 as well. There was 100% fetal loss, in 8 out of 8 infected pregnant ewes by 9 days P.I., in the vehicle treated group. However, in the infected BKI-1294 treated pregnant ewes, fetal mortality was only detected in 2 of 7 ewes (71% reduction in fetal mortality) and upon examination of remaining lambs, there was a 53% reduction of *T. gondii* vertical transmission (Sánchez-Sánchez et al., 2019).

Unfortunately, BKI-1294 also has an issue with inhibition of hERG at efficacious levels, and thus potential cardiotoxicity. We continue to actively search for a compound without this issue for toxoplasmosis therapy. However, the proof-of-concept experiment with BKI-1294 to block congenital transmission in the sheep model demonstrates unprecedented protection of fetuses during acute toxoplasmosis infection, spurring us to find the ideal BKI for this indication for both human and animal therapy.

BKI-1553 (Figure 2, Compound 32 in the reference) has a 1 nM IC_{50} when inhibiting *T. gondii* CDPK1 (*Tg*CDPK1) and inhibits *T. gondii* proliferation in vitro at an EC_{50} of 0.060 μM and did not inhibit hERG up to 10 μM (Vidadala et al., 2016). BKI-1553 was very effective at greatly reducing *T. gondii* in acute mouse infection (Vidadala et al., 2014). In experiments where mice were infected for 5 weeks with ME49 strain *T. gondii*, to allow establishment of bradyzoites in brain, treatment with BKI-1553 for 2 weeks led to 89% reduction in brain bradyzoites (Vidadala et al., 2016). This demonstrates that BKI therapy can treat the latent form of toxoplasmosis.

BKIs for Neosporosis

BKI-1294 and BKI-1553 (Figure 2) are both active against *N. caninum* CDPK1 (3 nM and 1 nM IC_{50}s, respectively) and *N. caninum* growth in vitro (360 nM and 180 nM EC_{50}s, respectively) (Müller et al., 2017; Ojo et al., 2014). In a non-pregnant mouse model of *N. caninum* infection, treatment with BKI-1294 was found to reduce the brain load of parasites by over 80%, whether 50 mg/kg daily treatment was applied for either 5 or 10 days after infection (Ojo et al., 2014). In a pregnant mouse model using infection with *N. caninum* Nc-Liv or Nc-Spain7 strains, 50 mg/kg × 5 days demonstrated an 87% reduction in brain infection with both strains; 100% of mice given vehicle alone had brain infection (Winzer et al., 2015). BKI-1553 was also used in a pregnant *N. caninum* Nc-Spain7 infection mouse model. BKI-1553 led to about 33% increased neonatal mortality at 20 mg/kg daily for 5 days, probably representing an adverse effect of the high levels of BKI-1553 achieved, but surviving BKI-1553 treated fetuses had 44% reduced frequency of *N. caninum* in the brain compared to 100% infection of the control group (Müller et al., 2017).

Finally, BKI-1553 has demonstrated partial efficacy in a pregnant sheep model of *N. caninum* infection (Sánchez-Sánchez et al., 2018). In this model, pregnant ewes at day 90 of gestation were infected intravenously with 10^6 *N. caninum* tachyzoites of the Nc-Spain7 strain. They were treated at 48 hr. P.I. with either of two dose regimens (#1: 35 mg/kg injected subcutaneously (SQ) at the first dose and 10 mg/kg SQ on a subsequent dose 7 days later; #2: 10 mg/kg administered every other day for 7 total doses). The BKI-1553 dose regimen #1 resulted in a 37% reduction in fetal loss (vehicle control and *N. caninum* infection led to 100% fetal loss) while the BKI-1553 dose regimen #2 led to a 50% reduction in fetal loss. Parasite detection in fetal brain tissue decreased from 94% in the infected/vehicle treated control group to about 70% in the two treated groups (Sánchez-Sánchez et al., 2018). Though this experiment demonstrated BKI-1553 was not optimal for treating *N. caninum* in sheep pregnancy, it did demonstrate that partial efficacy could be obtained. Thus, a BKI with better transplacental and brain penetration, and with better pregnancy safety characteristics might be expected to effectively treat *N. caninum* associated pregnancy loss.

Discussion and Conclusion

BKIs show great promise as a one-health therapeutic for cryptosporidiosis, toxoplasmosis, and neosporosis, as reviewed above (Table 3). In addition to these indications, BKIs show great promise in the therapy of the livestock diseases sarcocystiosis or equine protozoal myeloencephalitis (EPM), cystoisosporosis or epidemic diarrhea in piglets, and besnoitiosis in livestock (Table 3). BKIs have low nanomolar activity against CDPK1 in the parasites that cause these diseases and have nanomolar activity against the parasites in cell culture (Jimenez-Melendez et al., 2017; Ojo et al., 2016; Shrestha et al., 2019). In a mouse model of sarcocystiosis, infection with *Sarcocystis neurona* was greatly reduced or eliminated with BKI treatment (Ojo et al., 2016). Two studies in the piglet model of cystoisosporosis, where diarrhea was induced with *Cystoisospora suis*, demonstrated efficacy in abrogating diarrhea and eliminating parasite shedding, and in the most recent study, only two doses of BKI-1369 were required for efficacy (Shrestha et al., 2019; Shrestha et al., 2020). Further optimization of efficacy and safety is likely required for the systemic apicomplexan diseases, especially to provide transplacental and CNS coverage.

With respect to the human apicomplexan diseases, cryptosporidiosis and toxoplasmosis, further optimization is necessary to remove hERG activity, which is indicative of potential cardiotoxicity in humans. The BKI AC scaffold compounds tend to lack hERG activity and are highly efficacious against *C. parvum* and *T. gondii* (Figure 2) (Castellanos-Gonzalez et al., 2016; Huang et al., 2017; Huang et al., 2019; Huang et al., 2015; Hulverson et al., 2019; Schaefer et al., 2016). In addition, transplacental, ocular, and CNS penetration, and balancing efficacy and safety are needed for successful therapy of human toxoplasmosis, and we are conducting new synthesis and testing of BKI AC compounds to find the correct balance of these properties.

Acknowledgments and any additional information concerning research grants, etc.

The authors would like to acknowledge Drs. Wim Hol, Eric T. Larson, and Ethan Merritt for their structural expertise in solving the CDPK1 structure, scientists at AbbVie Inc. who helped with characterizing safety and PK of BKIs including Drs. Dale J. Kempf, Kennan Marsh, James J. Lynch, and Wayne R. Buck, scientists at PATH who have advised us on drug discovery and particularly Dr. Robert Choy, Dr. Anja Joachim and her group at the University of Vienna, Austria, for their work on *C. suis*, and Dr. Gema Alvarez-Garcia and her group for the work on besnoitiosis. Funding for these studies was provided by National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant numbers R21AI123690, R01AI089441, R01AI111341, R01A1112427, and R01HD080670), the US Department of Agriculture, National Institute of Food and Agriculture (grant numbers 2014-06183 and 2019-07512; Project #ARZT-5704210-A50-133), and the Swiss National Science Foundation (grant number 310030_1846629), the Bill & Melinda Gates Foundation under award number [OPP ID: OPP1160955], and PATH award # [DFI.1850-02-01291794-SUB; and DFI 1850-02-405099]. J. Stone Doggett received funding from VA Merit Review Award BX004522 from the U.S. Department of Veterans Affairs Biomedical Laboratory Research and Development. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation.

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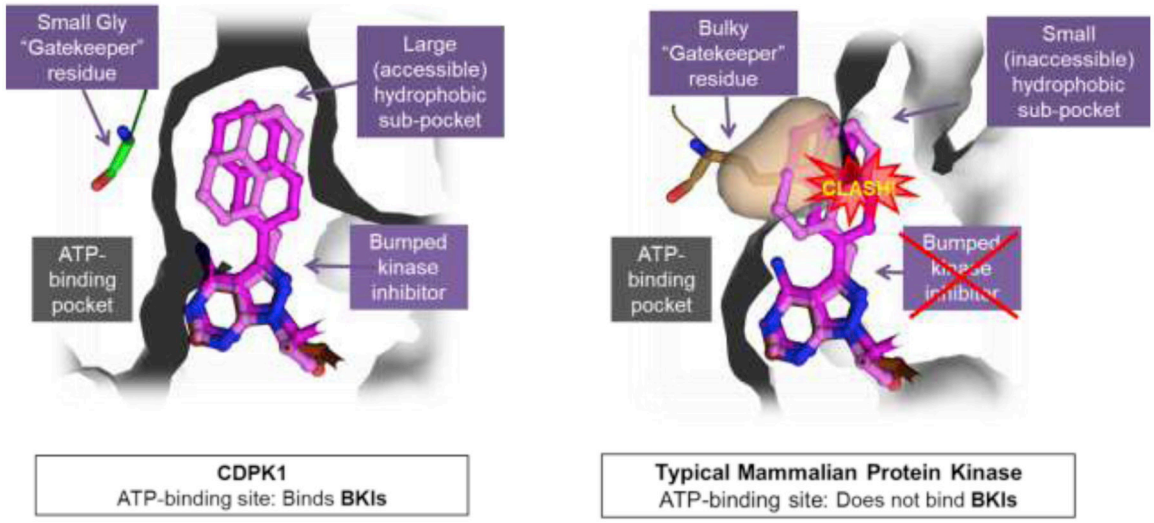

Figure 1: Bumped Kinase Inhibitors (BKIs) exploit the small gatekeeper structural differences to achieve both potency and specificity.

Shown at left is the binding site of *T. gondii* CDPK1 with a small glycine (Gly) gatekeeper residue, forming a large hydrophobic sub-pocket that allows the BKI to bind at the ATP-binding pocket of CDPK1. On the right is the binding pocket of a typical mammalian kinase with a bulky gatekeeper residue, in this case methionine, demonstrating a clash takes place with the "bump" of BKIs, such that BKIs are excluded from the ATP binding pocket, granting BKIs specificity over almost all mammalian protein kinases.

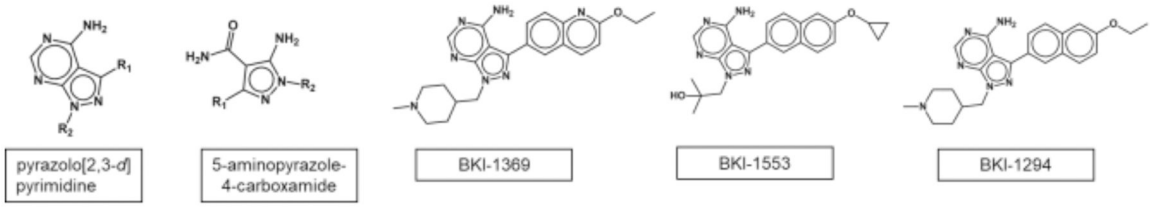

Figure 2:
Pyrazolo-pyrimidine and aminopyrazole-carboxamide scaffolds, compounds described in this paper

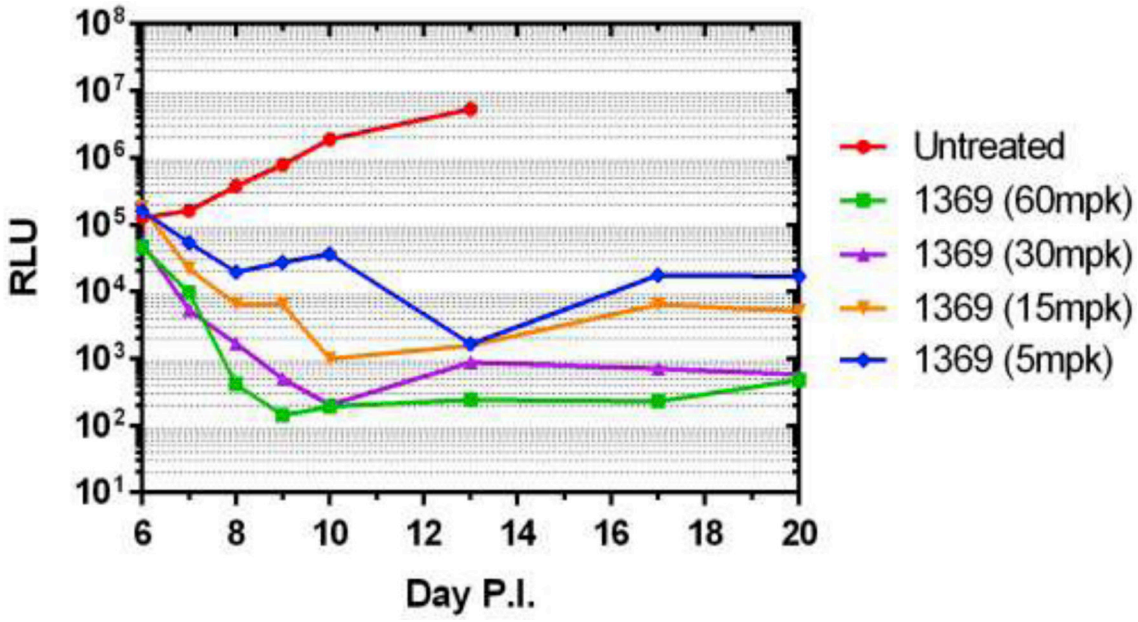

Figure 3: BKI-1369 is effective at reducing *nanoluciferase*-tagged *C. parvum* from gamma-interferon knock out mice:

BKI-1369 was administered orally at the doses shown (mpk = milligrams per kg), on day 6–10 post infection (P.I.) with 10,000 oocysts of Nluc-UGA1 *C. parvum*. The stool was isolated and relative luminescence units (RLU) are shown on the days P.I. Data from that published in (Hulverson et al., 2017a).

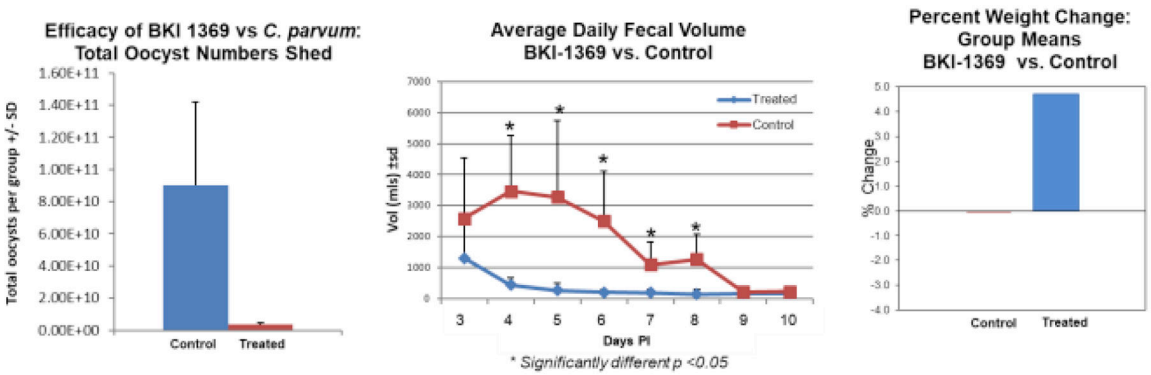

Figure 4: *C. parvum* neonatal calf model demonstrates efficacy of BKI-1369 via reduction in total oocyst excretion, diminished fecal output, and favorable weight gain compared with control infected calves.

Two-day old calves were infected orally with 5×10^7 *C. parvum* (Iowa strain, *Cryptosporidium* Production Laboratory, Univ. AZ) and two days later begun on BKI-1369 treatment or vehicle alone. Shown, left to right, are (1) the total number of oocysts excreted over the 5 days of treatment and 3 days after treatment in each group of control (vehicle alone treated on days 3–7 post infection (P.I.)) or treated (BKI-1369 5 mg/kg administered twice a day orally); (2) the total daily fecal volume excreted by the calves in each group; and, (3) weight gain or loss in each group over the 10-day observation period. Data replotted from (Hulverson et al., 2017a).

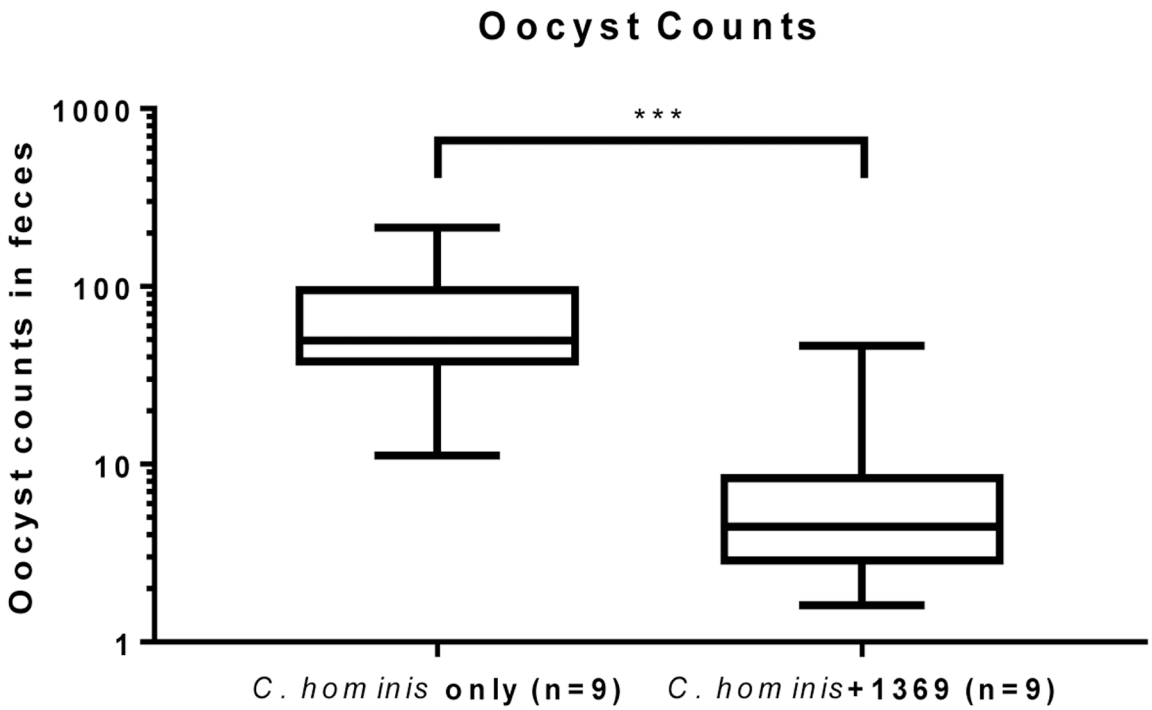

Figure 5: Gnotobiotic piglet *C. hominis* infection shows a significant reduction in oocysts excreted during and after therapy with BKI-1369.

Shown are cumulative oocyst counts from daily rectal swabs taken on days 1–10 post-initiation of BKI-1369 ('*C. hominis* +1369', 10 mg/kg administered orally twice a day for the first 5 days) or vehicle alone ('*C. hominis* only'). Data replotted from (Lee et al., 2018).

Table 1:

Properties of BKI-1369

Compound	*C. parvum* CDPK1 IC_{50} (μM)	*Human* SRC IC_{50} (μM)	*NIuc-C. parvum* EC_{50} (μM)	*Mammalian cytotox* CC_{50} (μM)	*Cardiotox* hERG IC_{50} (μM)	MUTAGEN/ GENOTOX	*Aqueous* SOLUBILITY (μM)	% PLASMA PROTEIN BINDING	
BKI-1369	0.0009	>10	2.5	>80	1.5	(−)	100	77 Human	40 Dog
				>80			54	40 Mouse	76 Rat

IC_{50}: Concentration in micromolar (μM) that gives 50% inhibition of enzyme activity; Human SRC: Proto-oncogene tyrosine-protein kinase SRC; CC_{50}: concentration that yields 50% growth inhibition (cytotoxicity) of mammalian CRL-8155 or HEPG2 cell lines; hERG: human ether-a-go-go related gene, a potassium channel found in heart tissue; Modified AMES test is the AMES test that includes liver microsome metabolized compound; Aqueous endpoint solubility performed at 2 pHs shown; and plasma protein binding shows the bound percentage of compound when incubated with plasma from species shown. Adapted from data in (Hulverson et al., 2017a; Hulverson et al., 2017b)

Table 2:

In vitro potency of BKI-1369 against various *Cryptosporidium* isolates

***Cryptosporidium* Isolate**	**EC_{50} (μM)**
Cryptosporidium Production Lab Iowa *C. parvum*[1]	0.8
Bunch Grass Farm *C. parvum*[1]	1.1
C. hominis[1]	0.3

[1]Assayed using high-content imaging for *Cryptosporidium* proliferation: Melissa Love and Case McNamara, CALIBR/Scripps, La Jolla, CA, adapted from data in (Hulverson et al., 2017a)

Table 3

BKI potential uses: Animal Health

Treatment of sick calves: cryptosporidiosis/scours
Moderate/severe crypto → sustained growth detriment, 34 kg average (Hannah Shaw, Moredun Univ) (Shaw, 2017)

- *Proof-of-Concept (POC) calves, Michael Riggs (Univ. of AZ)* (Hulverson et al., 2017b; Schaefer et al., 2016)

***Cryptosporidium* metaprophylaxis: prevent disease on contaminated farms**
Get calves to milk/market earlier & establish immunity

- *POC calves, Jennifer Zambriski (Virginia Tech) (Unpublished)*

Therapy or prophylaxis of *Neospora caninum:* epidemic abortion in cattle
Eliminate need for culling

- *POC pregnant mice, Andrew Hemphill (Univ. of Bern)* (Muller et al., 2017a; Winzer et al., 2015)
- *POC pregnant sheep, Luis Ortega-Mora (UCM)* (Sanchez-Sanchez et al., 2018a; Sánchez-Sánchez et al., 2018)

Active low nM: *Toxoplasma gondii:* a frequent cause of sheep/goat abortions

- *POC pregnant sheep, Luis Ortega-Mora (UCM)* (Sánchez-Sánchez et al., 2019)

Prophylactic treatment of felines for *T. gondii* to prevent feline-derived *T. gondii* infection?

Active low nM on *Sarcocystis neurona:* Equine protozoal myeloencephalitis (EPM) in the Americas

- *POC in mice, J.P. Dubey/Dan Howe (Univ of KY)*(Ojo et al., 2016)

Active low nM: *Cystoisospora suis:* epidemic diarrhea in piglets

- *POC in infected piglets, Anja Joachim (Vetmeduni, Vienna)* (Shrestha et al., 2019; Shrestha et al., 2020)

Active low nM on *Besnoiti besnoiti:* cattle disease in Europe/Central Asia, skin and systemic damage

- *POC in vitro, Gema Álvarez-García (UCM)* (Jimenez-Melendez et al., 2017)