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Developing Therapeutics to Reduce *Cryptosporidium* Morbidity and Mortality Among Children in Low-Resource Settings

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Introduction

- *Cryptosporidium* is an intestinal protozoan parasite that is a major cause of diarrheal disease among young children in low-resource settings.[1,2]
- Beyond diarrheal disease, cryptosporidiosis is associated with other chronic conditions, including growth faltering, environmental enteric dysfunction, and possibly impaired cognitive development.[3]
- Current therapeutic options are limited, with only one drug, nitazoxanide, approved by the United States Food and Drug Administration. Nitazoxanide is not approved for children under 1 year of age and has limited efficacy in malnourished children.[4]
- There is only one drug in clinical trials against *Cryptosporidium*: clofazimine, a repurposed leprosy drug developed more than three decades ago. There are no vaccines for *Cryptosporidium* approved or in clinical development.

PATH *Cryptosporidium* Portfolio

PATH actively engages with academic and industry partners to advance anti-*Cryptosporidium* drug development projects. Some of our current projects include:

1. **Calcium-dependent protein kinase 1** (CDPK1) "bumped kinase inhibitors," with the University of Washington (Van Voorhis lab).
2. **Methionyl-tRNA synthetase** (MetRS inhibitors), with the University of Washington (Fan and Buckner labs), the University of Vermont (Huston lab), and Takeda Pharmaceutical Company Limited.
3. **Celgene phenotypic screen**, with Celgene Global Health, the University of Washington (Van Voorhis lab), and the University of Vermont (Huston lab).
4. **Symposium on Innovative Therapeutics for *Cryptosporidium***, a biannual gathering of Crypto researchers from around the world.

Crypto Target Product Profile

Parameter	Ideal	Minimum essential	Nitazoxanide (NTZ)
Indication	Cryptosporidiosis, diarrhea-associated or asymptomatic	Cryptosporidiosis resulting in diarrhea (acute or persistent)	Diarrhea due to *C. parvum* (or *Giardia*)
Target age	Children ≥2 months and adults	Children ≥6 months and adults	Children ≥12 months and adults
Target population	Malnourished, immunocompromised, and/or HIV-positive	Malnourished	Immunocompetent
Regimen	Single dose	BID x3 days	BID x3 days
Clinical efficacy (Cessation of diarrhea)	≥90% of patients in 2 days	Superior to NTZ in malnourished children Non-inferior to NTZ in immunocompetent adults	Malnourished children: ~50% in 7–10 days Immunocompetent adults: ~90% in 7–10 days
Microbiological efficacy (Cessation of shedding)	≥90% of patients in 2 days	Non-inferior to NTZ	~50–90% of patients in 7–10 days
Safety	Safe for syndromic treatment of diarrhea in patients ≥2 months	Safe in patients ≥6 months	Safe in patients ≥12 months
Cost	US$1.00	US$2.00	US$3.00 (generic)

Table 1. Target Product Profile (TPP) for a new treatment for cryptosporidiosis. This TPP is the consensus result of discussions among the members of the Bill & Melinda Gates Foundation *Cryptosporidium* Drug Accelerator.

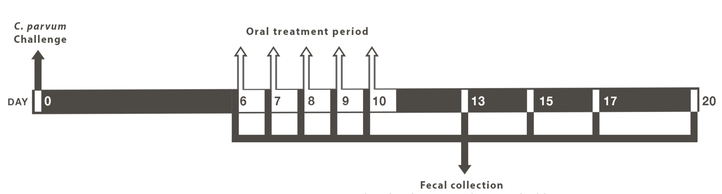

Figure 1. Schematic of interferon gamma knockout mouse model (IFNγ-KO) with Nluc-expressing *C. parvum*.[5,6] Mice (N=3 per treatment group) are infected with 1,000 oocysts on day 0 and then dosed with test compounds by oral gavage on days 6–10. Fecal samples are collected daily during the dosing period, and then every 2-3 days until day 20. Parasite levels are determined by relative luciferase units (RLU) normalized to mass of the stool samples.

Methionyl-tRNA Synthetase

- Amino acyl-tRNA synthetases have recently emerged as promising targets for treating a range of bacterial and protozoan pathogens.
- This target-based, structure-guided approach has identified potent inhibitors of *Cp*MetRS with activity in vitro and in vivo (**Table 2, Figures 2 and 3**).
- Current efforts are focused on improving selectivity against *Cp*MetRS in comparison to the homologous human mitochondrial MetRS, since inhibition of this enzyme is associated with bone marrow suppression toxicity. A short treatment duration and minimized systemic exposure may help mitigate this risk.

Cpd ID	*C. parvum* MetRS K_i nM	*C. parvum* EC_{50} µM	*C. hominis* EC_{50} µM	HepG2 CC_{50} µM	MTCO1 EC_{50} µM	Selectivity MTCO1 EC_{50} / *C. parvum* EC_{50}	Mouse PK C_{max} µM	Mouse PK AUC min*µM	Mouse PK feces µM
1312	0.64	18.6	n/d	>20	2.43	0.1	0.73	110	n/d
1962	1.33	>20	n/d	>50	>25	n/a	5.2	1,600	n/d
2093	0.0009	0.036	0.104	>50	0.039	1.1	5.8	1,863	31.1
2114	0.0023	0.053	0.147	>50	0.075	1.4	7.5	1,854	11.4
2259	0.0038	0.134	0.132	>50	0.164	1.2	21.6	4,724	25.8
2261	n/d	0.451	n/d	>50	1.600	3.5	n/d	n/d	n/d
2169	n/d	0.170	n/d	>50	0.560	3.3	11.3	2,004	114
2230	n/d	0.204	n/d	30	0.821	4.0	14.5	2,996	29.5

Table 2. Summary of efficacy, cytotoxicity, and DMPK properties of MetRS inhibitors.

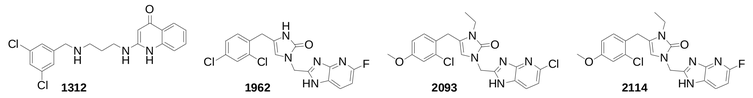

Figure 2. Structures of representative MetRS inhibitor compounds.

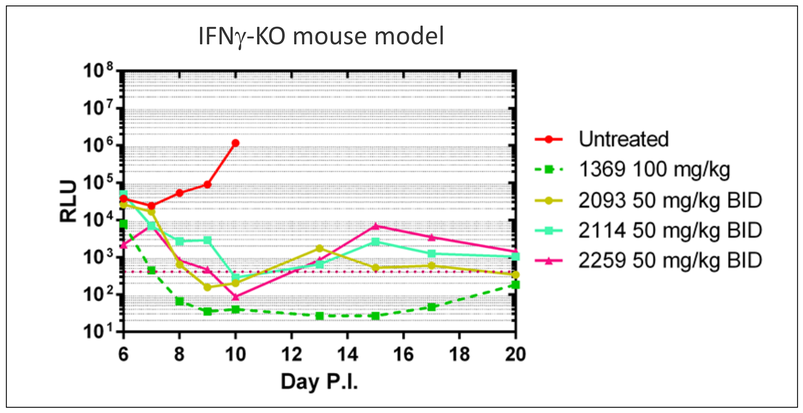

Figure 3. *Cp*MetRS inhibitors 2093, 2114, and 2259 are active in the IFNγ-KO mouse model. Y-axis: relative luciferase units in pooled fecal samples collected from mice infected with Nluc-expressing *C. parvum*. Untreated control mice were euthanized on day 10 post-infection (PI) due to morbidity. 1369 is a CDPK1 bumped kinase inhibitor used as a positive control.

*Cp*MetRS Target Candidate Profile

Category	Parameter	2093
Efficacy	In vitro EC_{50} <500 nM vs. *C. parvum* and *C. hominis*	33 nM (*C. parvum*) 104 nM (*C. hominis*)
	IFNγ-KO mouse: >3 log reduction in stool parasites	Yes
	Neonatal calf: >50% reduction in duration of diarrhea	TBD
Safety	Cytotoxicity: CC_{50}/EC_{50} >100	Yes
	Host mitochondrial protein synthesis: MTCO1 EC_{50}/*C. parvum* EC_{50} >3	No (1.0)
	Ames and in vitro micronucleus: negative	Yes
	hERG IC_{50} > 30x C_{max} (plasma free fraction)	Yes
	Receptor/channel/transporter profiling: no binding or inhibition preventing development	Alpha-2a adrenergic IC_{50} 0.9 µM
	CYP450 inhibition (panel of 5 CYPs): IC_{50} >10 µM	2C8: 87%@10 µM
	No unmanageable signals in exploratory rodent tox; TI (AUC @NOAEL)/AUC @MED*) >3	TBD
DMPK	Human dose prediction: <30 mg/kg/day QD or BID for 1-3 days	TBD
CMC	Straightforward synthesis, not more than 1 chiral center	Yes
	Formulation compatible with oral delivery	TBD

*MED = Minimum efficacious dose giving >3-log reduction in stool parasites.

Table 3. Target Candidate Profile for *Cp*MetRS inhibitors and comparison with lead compound **2093**.

Celgene Global Health Project

- Celgene's Diversity Compound Set of 416 compounds was screened and several promising series were identified with EC_{50} against *C. parvum* ranging from ~50–500 nM.
- Representatives from three series were active in the IFNγ-KO model with Nluc-expressing *C. parvum* (**Figure 4**).
- The three series have a diverse range of physiochemical and pharmacokinetic properties and offer ample prospects for further optimization. Series 1 has high permeability and systemic exposure, whereas Series 2 has comparatively lower permeability and exposure (**Table 4**). The impact of these parameters on in vivo efficacy is under further investigation.

Cpd ID	Series #	*C. parvum* EC_{50} µM	*C. hominis* EC_{50} µM	HepG2 Cytotox CC_{50} µM	IFNγ-KO 60 mg/kg QD (day 13 log reduction)	PK @30 mg/kg AUC µM-hr	PK @30 mg/kg C_{max} µM	Permeability MDR1-MDCK A to B P_{app} (10^{-6} cm/s)	Solubility pH 6.5 (µM)
CELG-001	1	0.281	0.697	>40	5.6	34.0	7.0	42	9.6
CELG-002	2	0.242	0.551	19.5	6.1	4.2	0.79	8.7	>50
CELG-003	3	0.365	0.756	>40	2.4	17.6	10.6	5.8	49.5

Table 4. Summary of efficacy, cytotoxicity, and DMPK properties of Celgene lead compounds.

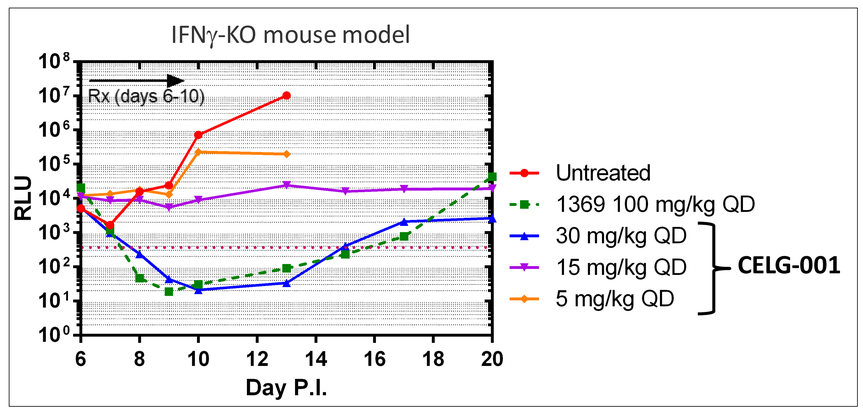

Figure 4. CELG-001 is active in the IFNγ-KO mouse model. Y-axis: relative luciferase units in pooled fecal samples collected from mice infected with Nluc-expressing *C. parvum*. Untreated control mice and mice treated with CELG-001 at 5 mg/kg QD were euthanized on day 13 post-infection (PI) due to morbidity. 1369 is a CDPK1 bumped kinase inhibitor used as a positive control.

Conclusions

PATH and collaborators have developed a promising portfolio of lead optimization stage drug candidates against *Cryptosporidium* with potential to meet Target Candidate Profile and Target Product Profile criteria.

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