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In Vivo Efficacy of Dual-Action Molecule TNP-2092 in Mouse *H. pylori* Infection Model: Dose Relationship and Impact of Proton Pump Inhibitor

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ABSTRACT

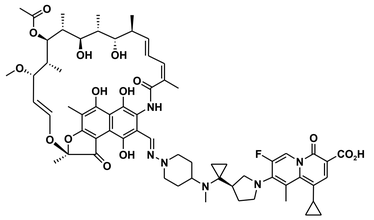
Background: TNP-2092 is a dual-action molecule in development for the treatment of digestive diseases associated with GI tract infections. Previous studies indicated that TNP-2092 is highly active against *H. pylori* clinical isolates, including multidrug resistant strains. It is efficacious as a monotherapy in a mouse *H. pylori* infection model. TNP-2092 has high and prolonged exposure in the gastric mucosal layer following oral administration. Current studies further evaluated the dose relationship of TNP-2092 and impact of a proton pump inhibitor in the mouse *H. pylori* infection model.
Methods: C57/BL6 mice were infected by *H. pylori* SS1 (CagA+, VacA+) and treatment initiated 7 days later. Mice were euthanized and their stomachs isolated, homogenized, serially diluted and plated for colony counts under microaerophilic conditions at 37°C. In the first experiment, TNP-2092 was administered orally at 5, 15 and 45 mg/kg, BID for 7 days. In the second experiment, TNP-2092 was administered orally or subcutaneously, with or without omeprazole, once or twice per day and continued for 7 or 14 days.
Results: In the first experiment, administration of TNP-2092 at 45 mg/kg reduced stomach bacterial titers to 2.56 $\log_{10}$ CFU after 7 days of treatment. The 15 mg/kg, 5 mg/kg and vehicle control groups resulted in 4.67, 4.85 and 5.05 $\log_{10}$ CFU stomach titers, respectively. In the second experiment, TNP-2092 (45 mg/kg, PO) alone reduced bacterial titers to 4.27 and 4.01 $\log_{10}$ CFU after 7 and 14 days of treatment as compared to 6.51 and 6.49 $\log_{10}$ CFU for the vehicle control group, respectively. The bacterial titers for TNP-2092 when administered together with 1 mg/kg omeprazole at 7 and 14 days were 4.48 and 3.51 $\log_{10}$ CFU. TNP-2092 administered subcutaneously at 45 mg/kg did not show efficacy as compared to the untreated controls on Day 7.
Conclusions: The effective dose for TNP-2092 in mouse *H. pylori* infection model was 45 mg/kg. Oral administration was more efficacious than subcutaneous administration and twice per day dosing was more efficacious than once per day administration. However, extension of treatment duration from 7 to 14 days and addition of omeprazole did not significantly improve overall efficacy.

INTRODUCTION

TNP-2092 (CBR-2092) is a non-cleavable, hybrid antibiotic comprised of a rifamycin and a quinolizinone pharmacophore. Previous studies indicated that TNP-2092 is a potent and balanced inhibitor of bacterial RNA polymerase, DNA gyrase and topoisomerase IV (1). TNP-2092 is characterized by having potent activity against persistent bacterial infections in various in vitro models and a low propensity for resistance development in *Staphylococcus aureus* (2-3). TNP-2092 was efficacious in vivo against acute, chronic, and severe biofilm-associated *S. aureus* infections (4). When given orally, the compound remains stable in the gastrointestinal (GI) tract with excellent distribution into the gastric mucosal layer. A fast disintegrating oral formulation of TNP-2092 has been developed and is currently in clinical development for the treatment of digestive diseases associated with *Helicobacter pylori* and other GI tract infections.

H. pylori is a microaerophilic Gram-negative bacteria, which colonizes the human stomach, and is associated with an increased risk of developing gastric cancer and peptic ulcer disease. The most recommended treatment for the eradication of *H. pylori* is triple therapy, using the combination of two antibiotics (clarithromycin plus amoxicillin or metronidazole) and a proton pump inhibitor (PPI) for at least 7 days Unfortunately, eradication rates for the standard triple treatment are declining rapidly due to the development of drug resistance. The current study was performed to determine the effectiveness of TNP-2092 vs. triple therapy in a mouse model of *H. pylori* disease.

TNP-2092 STRUCTURE



Description: **TNP-2092** is a rifamycin-quinolone hybrid agent that combines the pharmacophores of rifamycin SV and a 4*H*-4-oxo-quinolizine via a chiral linking group

Chemical Name: *R*-3-[(4-{1-[I-(3-Carboxy-1-cyclopropyl-7-fluoro-9-methyl-4-oxo-4*H*-quinolizine-8-yl)-pyrrolidin-3-yl-cyclopropyl]- methylamino} piperidin-1-ylimino)-methylenyl]-rifamycin SV

Formula: $C_{65}\ H_{81}\ F\ N_6\ O_{15}$

MW: 1205.38 Daltons

MATERIALS & METHODS

Animals: Female C57/BL6 mice, 5 - 6 weeks of age and 18 - 22 grams in weight.

Bacterial Strains: *H. pylori* SS1 (CagA+, VacA+) & ATCC 43504 (MIC reference strain).

Minimum Inhibitory Concentration: MICs were determined on Mueller-Hinton agar + 5% sheep blood in accordance with CLSI recommendations following incubation at 37°C under microaerophilic conditions for 72 hrs.

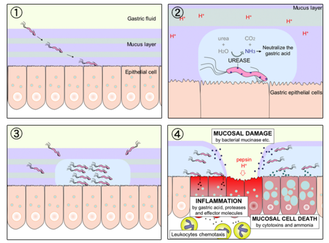
Infection: All animals were fasted prior to infection with 10e6 -10e7 CFU of the *H. pylori* SS1culture 3 times at 48 hr intervals.

Test Article: TNP-2092; Omeprazole

Treatment: Initiated 1 week after the third infection. Treatments were administered once (q.d.) or twice (b.i.d.) daily via oral gavage or subcutaneous injection and continued for 7 or 14 days. The control groups were administered the vehicle alone.

Sampling: Food was removed 18 hours prior to sampling. Mice were euthanized approximately 18-20 hrs after the last administered dose. Stomachs were removed by cutting the esophagus away from the superior aspect of the stomach and the duodenum away from the pyloric region, rinsed in sterile PBS, homogenized and diluted in PBS then spot plated onto Columbia agar with 7% laked horse blood ± the DENT selective antibiotic supplement. Plates were incubated microaerophilically at 37°C and CFU counts determined after 6-7 days of incubation. Limit of quantitation (LOQ) <2.35 $\log_{10}$ CFU per stomach.

H. pylori Pathogenesis



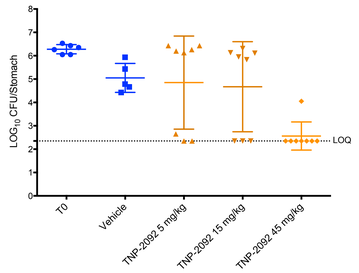
In Vitro Activity of TNP-2092

	MIC (ug/mL)	
Test Article	SS1 Strain	ATCC43504
Amoxicillin	≤ 0.008	≤ 0.008
Clarithromycin	≤ 0.008	≤ 0.008
Omeprazole	1	2
Tetracycline	0.016	0.06
Metronidazole	8	8
TNP-2092	0.06	≤ 0.008

Efficacy of TNP-2092 – Mean *H. pylori* Stomach Titers after 7 Days of Treatment

Test Article	Dose (mg/kg)	Mean $\log_{10}$ CFU	SD	$\log_{10}$ CFU reduction vs Vehicle	p-value vs vehicle*
Start of Treatment	na	6.28	0.20	na	na
Vehicle	na	5.05	0.62	na	na
	5	4.85	1.99	-0.2	0.831
TNP-2092	15	4.67	1.93	-0.38	0.681
	45	2.56	0.60	-2.49	0.00002

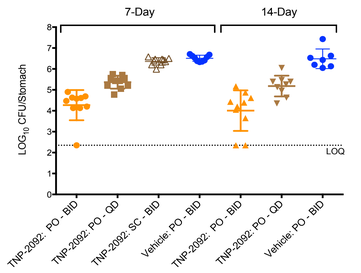
*One-way ANOVA w/ Tukey post-hoc (95% CI); n= 5 – 8 mice per treatment



Dosing Route and Regimen Effect on TNP-2092 Efficacy

			7-Day Treatment Regimen			14-Day Treatment Regimen		
Test Article	Dose (mg/kg)	Route	$\log_{10}$ CFU	SD	$\log_{10}$ CFU reduction vs Vehicle	$\log_{10}$ CFU	SD	$\log_{10}$ CFU reduction vs Vehicle
Vehicle	na	PO	6.51	0.15	na	6.49	0.47	na
	45 BID	PO	4.27	0.72	-2.24*	4.01	0.97	-2.48*
TNP-2092	45 QD	PO	5.37	0.31	-1.14*	4.90	1.01	-1.59*
	45 BID	SC	6.36	0.18	-0.15	na	na	na

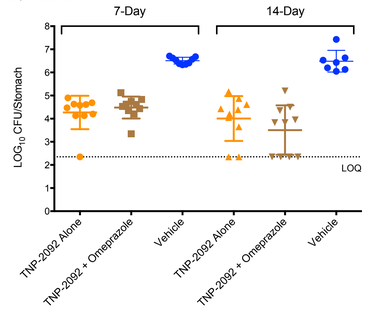
* p<0.0001: One-way ANOVA w/ Tukey post-hoc (95% CI); n= 10 mice per treatment



Effect of PPI on TNP-2092 Efficacy

			7-Day Treatment Regimen		14-Day Treatment Regimen	
Test Article	Dose (mg/kg)	Route	$\log_{10}$ CFU	SD	$\log_{10}$ CFU	SD
TNP-2092	45	PO	4.27	0.72	4.01	0.97
TNP-2092 + Omeprazole	45 + 1	PO	4.48	0.47	3.51	1.07
Vehicle	na	PO	6.51	0.15	6.49	0.47

n= 10 mice per treatment



SUMMARY & CONCLUSIONS

- ***H. pylori* colonizes the stomach in about 50% of all humans, which can generate gastric lining inflammation and result in disease manifestations of simple gastritis to gastric carcinoma.**
- **TNP-2092, a dual-acting antibiotic comprised of a rifamycin and a quinolizinone pharmacophore connected by a stable linker has demonstrated potent *in vitro* activity against *H. pylori* isolates.**
- **The effective dose for TNP-2092 in mouse *H. pylori* infection model was 45 mg/kg.**
- **Oral administration of TNP-2092 was more efficacious than subcutaneous administration and administration of two oral doses of TNP-2092 per day resulted in greater efficacy than a single dose in the mouse model of *H. pylori* infection.**
- **Extending the duration of treatment from 7 to 14 days or the addition of omeprazole to TNP-2092 therapy did not significantly improve overall efficacy.**
- **TNP-2092 monotherapy represents an excellent alternative to currently approved regimens consisting of three or more agents for the treatment of *H. pylori* associated disease.**

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