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In Vivo Efficacy of Dual-Action Molecule TNP-2092 in Mouse *H. pylori* Infection Model as Compared to Triple Therapies and Distribution within the Gastric Mucosal Layer

TenNor Therapeutics Ltd
218 Xinghu Street
Suzhou Industrial Park
China 215123
www.tennorx.com
Contact: xiaomei.wang@tennorx.com

W. J. Weiss,[1] M. Pulse,[1] P. Nguyen,[1] Z. Ma[2]

[1]UNT Health Science Center, Fort Worth, TX; [2]TenNor Therapeutics Ltd, Suzhou, China

ABSTRACT

Background: TNP-2092 is a dual-action molecule in development for the treatment of diseases associated with GI tract infections. Previous studies indicated that TNP-2092 is highly active against *H. pylori* clinical isolates, including multidrug resistant strains. TNP-2092 is locally active in the GI tract after oral administration. Current studies sought to evaluate the *in vivo* efficacy of TNP-2092 in a mouse *H. pylori* infection model as compared to PrevPac and Helidac and study its distribution into gastric mucosal layer.

Methods: C57/BL6 mice were infected by *H. pylori* SS1 (CagA+, VacA+) and treated with TNP-2092 and comparators orally, BID for 7 days. Mice were euthanized and their stomachs isolated, homogenized, serially diluted and plated for colony counts after incubation under microaerophilic conditions at 37°C. For PK studies, mice were euthanized at selected time points on Days 1 and 7 for collection of blood and stomach mucosal samples.

Results: Low plasma exposure was observed for TNP-2092 with C_{max} of 1.2-1.5 ug/mL and $AUC_{0\text{-}inf}$ of 2.8-3.4 ug•hr/mL for Day 1 and Day 7. Exposure in the gastric mucin was high and prolonged with peak levels of 82.9-85.5 ug/g and exposures of 466.4-540.5 ug•hr/g for Days 1 and 7. At 30 mg/kg, TNP-2092 treatment resulted in stomach bacterial titers of 3.41 log_{10} CFU at 24 hours after the last dose. PrevPac (omeprazole + clarithromycin + amoxicillin) exhibited comparable efficacy with bacterial titers of 3.47 log_{10} CFU. Helidac (Bismuth salicylate + metronidazole + tetracycline) was less active with 4.42 log_{10} CFU. Neither rifampin nor rifalazil demonstrated efficacy. At 45 mg/kg, TNP-2092 reduced stomach bacterial titers to below the detection limit (< 2.35 log_{10} CFU).

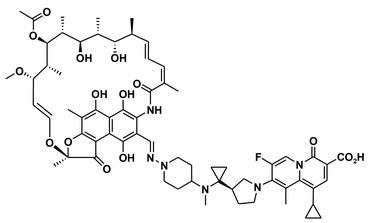
Conclusions: TNP-2092 exhibited high exposures in the gastric mucosal layer with drug levels in excess of its MIC against *H. pylori* 24-36 hours after oral administration at 45 mg/kg. This high exposure resulted in the reduction of the *H. pylori* bacterial titers to below the limit of detection after 7 days of treatment. TNP-2092 as a monotherapy appeared to be equally efficacious as PrevPac and superior to Helidac in mouse *H. pylori* infection model.

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 diseases. The most recommended treatment for the eradication of *H. pylori* is standard 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 therapy 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* infection.

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-[l-(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 Strain: *H. pylori* SS1 (CagA+, VacA+)
Infection: All animals were fasted prior to infection with 10^6-10^7 CFU of the *H. pylori* SS1culture 3 times at 48 hr intervals.
Test Articles: TNP-2092; PrevPac (Omeprazole 1 mg/kg + Clarithromycin 10 mg/kg + Amoxicillin 20 mg/kg); Helidac (Bismuth salicylate 20 mg/kg + Metronidazole 10 mg/kg + Tetracycline 20 mg/kg), Clarithromycin (10 mg/kg), Rifampin (30 mg/kg), Rifalazil (30 mg/kg).
Treatment: Initiated 1 week after the third infection. Treatments were administered twice (b.i.d.) per day via oral gavage and continued for 7 days. The control groups were administered the vehicle alone.
Pharmacokinetics: Mice were euthanized at selected time points on both Day 1 and Day 7 (after 1st dose) for the collection of both blood samples via cardiac puncture (Na-heparin tubes) and stomach mucosal material. The stomach was aseptically isolated and removed. An incision was generated along the long axis of the greater curvature and pinned in place. Using a glass microscope slide, the interior surface of the stomach was gently scraped and the mucin sample obtained transferred into labeled tubes on ice. All samples were stored at -80ºC until analysis.
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.

PHARMACOKINETICS OF TNP-2092

in *H. pylori* infected C57/Bl6 mice following oral administration

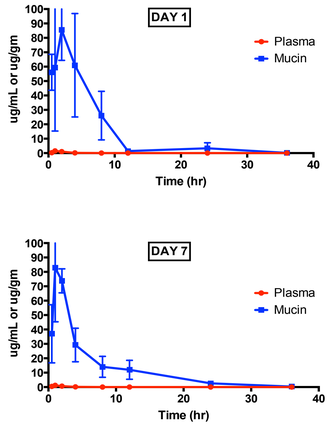
	Plasma Concentration (ug/mL)				Mucin Concentration (ug/gm)			
	Day 1		Day 7		Day 1		Day 7	
Time (hr)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5	0.29	0.13	0.48	0.03	56.1	12.49	36.99	20.2
1	1.51	0.22	1.22	0.12	59.28	43.97	82.88	37.54
2	1.05	0.62	0.69	0.42	85.52	21.17	73.76	8.36
4	0.14	0.09	0.14	0.09	60.98	35.93	29.2	11.68
8	0.02	0.01	0.02	0.01	26.01	16.85	14.04	7.3
12	0.01		0.01		1.43	0.57	11.99	6.6
24					3.35	3.87	2.61	1.71
36					0.13	0.08	0.32	0.35
C_{max} (ug/mL)	1.51		1.2		85.5		82.9	
T_{max} (hr)	1		1		2		1	
AUC_{0-t} (ug-hr/mL)	3.4		2.8		540.2		464.1	
AUC_{0-inf} (ug-hr/mL)	3.4		2.8		540.5		466.4	
Half-life (hr)	1.76		1.78		1.75		5.07	

n=3 mice per time point

- Plasma and gastric mucosal samples were taken at selected time points following the 1st orally administered dose of TNP-2092 (45 mg/kg) on Days 1 and 7 (6 days prior, b.i.d.).
- Minimal plasma exposure was observed for TNP-2092 following oral administration indicating very low bioavailability.
- Exposure in the gastric mucin, which is the colonization site of *H. pylori*, was high and prolonged with peak levels of 82.9-85.5 ug/g on Days 1 and 7 of dosing.

PHARMACOKINETICS OF TNP-2092

in *H. pylori* infected C57/Bl6 mice following oral administration



EFFICACY OF TNP-2092

– Mean *H. pylori* Stomach Titers

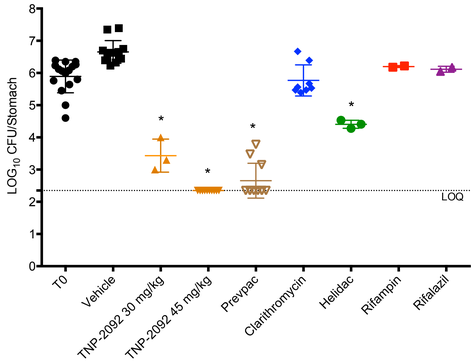
Test Article	Dose (mg/kg)	Log_{10} CFU	SD	Log_{10} CFU reduction vs Vehicle	p-value vs Vehicle
T0	na	5.89	0.51	na	na
Vehicle	na	6.58	0.52	na	na
TNP-2092	30	3.43	0.51	-3.15	< 0.0001
	45	< 2.35	0.00	-4.23	< 0.0001
Prevpac	31	2.66	0.54	-3.92	< 0.0001
Clarithromycin	10	5.77	0.48	-0.81	0.002
Helidac	50	4.42	0.13	-2.16	< 0.0001
Rifampin	30	6.20	0.03	-0.38	0.335
Rifalazil	30	6.11	0.09	-0.47	0.245

n = 3 - 8 mice per treatment

- TNP-2092 treatment at 30 mg/kg for 7 days (b.i.d.) resulted in 3.43 mean log_{10} CFU/stomach at 24 hours after the last dose.
- At 45 mg/kg, TNP-2092 reduced stomach *H. pylori* titers to below the detection limit (< 2.35 log_{10} CFU).
- PrevPac (Clarithromycin + Amoxicillin + Omeprazole) treatment resulted in 2.66 mean log_{10} stomach CFU, while Clarithromycin alone exhibited minimal efficacy.
- Helidac (Metronidazole + Tetracycline + bismuth salicylate) was less active with 4.42 log_{10} CFU at the end of treatment.
- Neither Rifampin nor Rifalazil demonstrated efficacy.

EFFICACY OF TNP-2092

– *H. pylori* Stomach Titers



* Mean value is significantly different (p<0.0001) vs. vehicle controls (one-way ANOVA)

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 *in vitro* activity against *H. pylori* isolates.**
- **TNP-2092 exhibits low plasma exposure, but has an elevated concentration within gastric mucosal layers that is in excess of its MIC against *H. pylori* for 24-36 hours following oral administration.**
- **In efficacy studies with the mouse model of *H. pylori* infection, TNP-2092 significantly reduced *H. pylori* bacterial titers in the gastric mucin and appeared to be more efficacious than the two standard of care triple therapy regimens.**
- **TNP-2092, as a single agent, represents an excellent alternative to currently approved regimens consisting of three or more agents for the treatment of diseases associated with *H. pylori* infections.**

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