Genetic Evidence That Gepotidacin Shows Well-balanced Dual Targeting Against DNA Gyrase and Topoisomerase IV in *Neisseria gonorrhoeae*

Pan Chan,[1] Karen Ingraham,[1] Sharon Min,[1] Nicole E. Scangarella-Oman,[1] Steve Rittenhouse,[1] and Jianzhong Huang[1]

[1]GlaxoSmithKline, Collegeville, PA, USA

Session: Novel Agents; Poster No. 1249
Presented at the IDWeek Virtual Meeting
21–25 October 2020

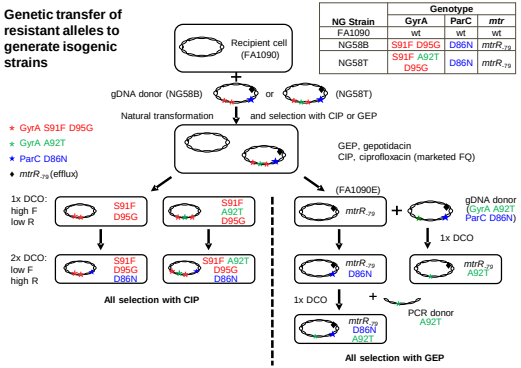
Introduction

- Gepotidacin (GEP) is a novel bacterial topoisomerase type II inhibitor with antibacterial activity against drug-resistant strains of *Neisseria gonorrhoeae* (NG), [1–4] and which exhibits a low frequency of *in vitro* resistance in NG[4–6]
- In a Phase II trial,[5] clinical resistance to GEP was observed in a fluoroquinolone-resistant strain of urogenital NG with a pre-existing ParC D86N mutation and an acquired GyrA A92T mutation. Both residues are important for GEP binding to topoisomerase IV and DNA gyrase
- In this study, we determined the contributory roles of the above resistance alleles to the antibacterial activity of GEP to help overcome potential resistance development in clinical NG isolates

Methods and Results

- We isolated genomic DNA from NG cells, or generated PCR DNA carrying resistant alleles, and naturally transformed these into NG cells[7] (**Figure 1**)

Figure 1. Generation of isogenic GEP and CIP target mutants



NG Strain	Genotype		
	GyrA	ParC	*mtr*
FA1090	wt	wt	wt
NG58B	S91F D95G	D86N	$mtrR_{79}$
NG58T	S91F A92T D95G	D86N	$mtrR_{79}$

DCO, double crossover; F, frequency; FQ, fluoroquinolone; gDNA, genomic DNA; PCR, polymerase chain reaction; R, resistance

- The novel, first-in-class, triazaacenaphthylene bacterial topoisomerase type II inhibitor **gepotidacin** suppresses clinically relevant resistance development in *Neisseria gonorrhoeae* by **well-balanced dual targeting** of DNA gyrase and topoisomerase IV
- Our genetic findings reveal mutations in ParC D86N or GyrA A92T had minimal effect on **gepotidacin** susceptibility alone but led to resistance when in combination, thus supporting a well-balanced dual targeting antibacterial mechanism of action by **gepotidacin** in *Neisseria gonorrhoeae*
- This provides mechanistic insights for appropriate clinical dose selection of **gepotidacin**, with potential to reduce resistance development in subsets of *Neisseria gonorrhoeae* clinical isolates

Please find the online version of this poster, accompanying audio, and summary slides by scanning the QR code or via http://tago.ca/IDWeek11

Results

- Minimum inhibitory concentration (MIC) of GEP and ciprofloxacin (CIP, a fluoroquinolone antibiotic) was determined against each isogenic strain by an agar dilution (AD) method according to Clinical and Laboratory Standards Institute[8] and Centers for Disease Control and Prevention[9] standards, and by a broth microdilution (BD) method[6] using Fastidious broth (Remel) and a 48h endpoint, and showed good (all MICs ≤4-fold) correlation between both methods for the specific strains tested in this study
- Overall, GyrA A92T and ParC D86N mutations did not confer a significant (all ≤4-fold) increase in GEP MIC alone, but gave ≥16-fold increases in GEP MIC when together
- Importantly, quinolone target mutations (GyrA S91F D95G and ParC D86N) together showed no significant effect on GEP MIC, but gave a >1000-fold increase in CIP MIC
- As expected, GyrA A92T and ParC D86N mutations alone or together in a wt GyrA background had no significant effect on CIP susceptibility (**Table 1**)
- The ParC D86N mutation is a potential risk marker for clinical resistance development in a subset of NG isolates. This has been further explored by pharmacokinetic and pharmacodynamic studies for informing GEP dose selection to mitigate potential resistance development[6]

Table 1. Antibiotic susceptibility of isogenic NG strains

NG strain	Mutation in			MIC (μg/mL)				Fold change from parents			
	GyrA	ParC	*mtr*	GEPOTIDACIN		CIPROFLOXACIN		GEPOTIDACIN		CIPROFLOXACIN	
				AD	BD	AD	BD	AD	BD	AD	BD
FA1090 (parent)	wt	wt	wt	0.125	0.06	0.004	0.004	-	-	-	-
FA1090-1	S91F D95G	wt	wt	0.125	0.06	0.25	0.5	1	1	64	128
FA1090-3	S91F A92T D95G	wt	wt	0.5	0.125	0.25	0.5	4	2	64	128
FA1090-2	S91F D95G	D86N	wt	0.25	0.125	4	4	2	2	1024	1024
FA1090-4	S91F A92T D95G	D86N	wt	16	8	2	4	128	128	512	1024
FA1090E (parent)*	wt	wt	$mtrR_{79}$	0.5	0.25	0.008	0.004	-	-	-	-
FA1090E-1	A92T	wt	$mtrR_{79}$	2	0.5	0.004	0.002	4	2	0.5	0.5
FA1090E-2	wt	D86N	$mtrR_{79}$	0.5	0.25	0.004	0.004	1	1	0.5	1
FA1090E-3	A92T	D86N	$mtrR_{79}$	8	4	0.004	0.002	16	16	0.5	0.5

>4-fold differences in MIC compared to parents are highlighted in yellow; *FA1090E parent strain has acquired an $mtrR_{79}$ efflux allele compared with FA1090 (ATCC 700825); wt, wild type

References

1. Biedenbach DJ, et al. *Antimicrob Agents Chemother* 2016;60:1918–23;
2. Bax BD, et al. *Nature* 2010;466:935–40;
3. Gibson E, et al. *ACS Infect Dis.* 2019;5:570–81;
4. Farrell DJ, et al. *Antimicrob Agents Chemother* 2017;61:e02047–16;
5. Scangarella-Oman NE, et al. *Antimicrob Agents Chemother* 2018;62:e01221–18;
6. VanScoy BD, et al. *Antimicrob Agents Chemother* 2020;64:e00521–20;
7. Hamilton HL and Dillard JP. *Mol Microbiol* 2006;59:376–85;
8. CLSI M07: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 11th Edition;
9. CDC Gonorrhea Laboratory Information Agar Dilution Antimicrobial Susceptibility Testing. https://www.cdc.gov/std/gonorrhea/lab/agar.htm; Last Accessed 07/10/2020).

Disclosures

This study was funded by GlaxoSmithKline plc. This project was funded in whole or in part with Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced. Research and Development Authority (BARDA), under OTA agreement No. HHSO100201300011C. PFC, KI, SM, NESO, SR, and JH are current employees of GlaxoSmithKline plc. Medical writing support (including editing of content and grammar and collation of author comments) for the poster and accompanying audio recording was provided by Joanna Wilson, PhD, of Gardiner–Caldwell Communications, Ashfield Healthcare (Glasgow, UK), and was funded by GlaxoSmithKline plc.