#1052

Genetic relationship and mechanisms contributing to reduced gepotidacin susceptibility in isolates from EAGLE-2/EAGLE-3 clinical trials for uncomplicated urinary tract infections

Treatment-emergent reduced gepotidacin susceptibility is rare in *E. coli* and *K. pneumoniae* uropathogens.

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Digital poster

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Introduction

- Gepotidacin is a novel, bactericidal, first-in-class triazaacenaphthylene antibiotic that inhibits bacterial DNA replication by a unique mechanism of action, distinct binding site, and well-balanced inhibition (for most uncomplicated urinary tract infections [uUTI] uropathogens and *Neisseria gonorrhoeae*) of two different Type II topoisomerase enzymes.
- Gepotidacin has *in vitro* activity against most isolates of target pathogens, such as *Escherichia coli*, *Staphylococcus saprophyticus*, and *N. gonorrhoeae*, including those resistant to current antibiotics.
- Results from two phase 3 clinical trials demonstrated the efficacy of gepotidacin for the treatment of uUTIs. More recently, gepotidacin met its primary efficacy endpoint of non-inferiority in a phase 3 trial comparing gepotidacin with intramuscular ceftriaxone plus oral azithromycin combination therapy for the treatment of urogenital gonorrhea.[6]
- This study reports on the further molecular characterization of isolates from the gepotidacin phase 3 uUTI clinical trials [EAGLE-2 (NCT04020341) and EAGLE-3 (NCT04187144)], where post-baseline isolates displayed gepotidacin MIC results ≥4-fold higher than the respective baseline isolate.

Methods

Susceptibility testing, genome sequencing and analysis

- Gepotidacin MIC values were determined by triplicate broth microdilution susceptibility testing following CLSI procedures.[1]
- Short-read Illumina sequencing data were utilized for multi-locus sequence typing to define high-level genetic relatedness between baseline and post-baseline isolates.
- Long-read Oxford nanopore sequencing followed by hybrid assembly of short and long read data was used to perform single nucleotide polymorphism (SNP) and insertion-deletion (indel) analysis.
- Post-baseline isolates were considered to have originated from the respective baseline isolates when ≤1 SNP/indel were observed within 5 days; or ≤2 SNP/indel were observed within 6-14 days; or ≤5 SNP/indel were observed within 15-35 days.[2-5]

Inclusion Criteria

- Post-baseline isolates with reproducible gepotidacin MIC results ≥4-fold higher than the respective baseline isolate and showing the same multi-locus sequence type (MLST) were included in this study.

Results

- Thirty-one isolates, including 17 *E. coli* from 7 participants and 14 *Klebsiella pneumoniae* from 6 participants met the inclusion criteria across both treatment arms.
- Among EAGLE-2/EAGLE-3 uUTI clinical trial participants with a uropathogen and across both treatment arms, 0.63% (13/2056) had post-baseline isolates with reproducible gepotidacin MIC values ≥4-fold higher than the respective baseline isolate and the same MLST (Tables 1 and 2).
  - Gepotidacin treatment arm: 0.67% (7/1045)
  - Nitrofurantoin treatment arm: 0.59% (6/1011)

Analysis from participants (*n*=7) with post-baseline *E. coli* isolates that met inclusion criteria.

Post-baseline isolates were collected from participants #1, #2, #3, #5 and #7 within 4–30 days of their respective baseline isolates.

- 6–12,492 SNP/indels were observed in these post-baseline isolates compared to baseline isolates. These results indicated that isolates collected post-baseline did not originate from respective baseline isolates.
- Post-baseline isolates from participants #1, #5, and #7 carried variations in the efflux pump component AcrB (n=2) or MdfA (n=1). The gepotidacin MIC values of these post-baseline isolates were 4- to 8-fold higher than the MICs of the respective baseline isolates.
- Post-baseline isolates from participants #2 and #3 carried *qnrB19* and *qnrS1* genes, respectively. Neither were observed in the isolates collected at the baseline visits. Gepotidacin MICs of these post-baseline isolates were 8- to 16-fold higher than the MICs of the respective baseline isolates.

Two post-baseline isolates were cultured at the test-of-cure (TOC; day 12) and follow-up (FU; day 47) visits from participant #4.

- An IS6/IS26-family transposase was inserted within *qnrS1* in the baseline isolate, which introduced a premature stop codon within this gene.
- Both post-baseline isolates possessed an intact *qnrS1* and had a gepotidacin MIC value 4-fold higher than the baseline isolate.
- The absence of IS-induced excision sequences in the *qnrS1* gene of post-baseline isolates suggests these isolates did not originate from the baseline isolates.

Participant #6 had post-baseline *E. coli* isolates collected at days 1, 9 and 33, in which 2, 4 and 4 SNPs were identified relative to the baseline isolate (Table 1). The isolate recovered at day 33 was collected outside of the FU visit window.

- Gepotidacin MIC values of the post-baseline isolates were >4-fold higher than the MICs of the baseline isolate.
- All 3 post-baseline isolates had a D79G variation in the ParC subunit of topoisomerase IV and a P372S variation in a gene encoding a putative major facilitator superfamily protein.
- Post-baseline isolates cultured at TOC and day 33 also had unique variations in the AcrB efflux pump component (G288A and A279T, respectively).

Analysis from participants (*n*=6) with post-baseline *K. pneumoniae* isolates that met inclusion criteria.

- Based on the number of SNP/indels observed, no post-baseline *K. pneumoniae* originated from the respective baseline isolate (Table 2).
- Three post-baseline isolates carried QnrS1-encoding genes. Gepotidacin MICs of these isolates were 4- to >8-fold higher than MICs of the respective baseline isolates.
- Six post-baseline isolates, including 1 with *qnrS1*, had variations in the *oqxAB* efflux system repressor, *oqxR*.[7] Gepotidacin MICs of the post-baseline isolates were 4- to >8-fold higher compared to the respective baseline isolates.

Table 1 Characterization of *E. coli* isolates from participants (*n*=7) in the EAGLE-2/EAGLE-3 clinical trials who had post-baseline isolates with reproducible gepotidacin MIC results ≥4-fold higher than the respective baseline isolate and showing the same MLST.

					Fluoroquinolone resistance mechanism		SNP/indel Analysis	
Participant	Treatment Arm	Visit	Days post-baseline	GEP MIC	QRDR[a]	Other	Number	Relevant variations[b]
1	GEP	Baseline	N/A	8	S83L, D87N/S80I/WT	QnrS1		
		OT	4	64	S83L, D87N/S80I/WT	QnrS1	13	$AcrB^{S288G\ G570C}$
2	NTF	Baseline	N/A	0.25	WT/WT/WT			
		FU	23	4	WT/WT/WT	QnrB19	7,037	QnrB19
3	GEP	Baseline	N/A	4	WT/WT/WT	QnrB19		
		FU	29	32	WT/WT/WT	QnrS1	12,492	QnrS1
4[c]	NTF	Baseline	N/A	2	WT/WT/WT	QnrS1		QnrS1 (disrupted)
		TOC	12	8	WT/WT/WT	QnrS1	1	QnrS1
		FU	47	8	WT/WT/WT	QnrS1	0	QnrS1
5	GEP	Baseline	N/A	1	S83L, D87N/S80I/L416F			
		TOC[d]	22	4	S83L, D87N/S80I/L416F		6	$AcrB^{G288}$
6	GEP	Baseline	N/A	16	WT/WT/WT	QnrS1/QnrB19		
		OT	1	>64	WT/D79G/WT	QnrS1/QnrB19	2	$ParC^{D79G}$ MFS^{P372S}
		TOC	9	>64	WT/D79G/WT	QnrS1/QnrB19	4	$ParC^{D79G}$ MFS^{P372S} $AcrB^{G288A}$
		FU[d]	33	>64	WT/D79G/WT	QnrS1/QnrB19	4	$ParC^{D79G}$ MFS^{P372S} $AcrB^{A279T}$
7	GEP	Baseline	N/A	0.12	S83L, D87N/S80I/L416F			
		FU	30	0.5	S83L, D87N/S80I/L416F		18	$MdfA^{G10R}$

Abbreviations: GEP, gepotidacin; NTF, nitrofurantoin; QRDR, quinolone resistance determining region; minimum inhibitory concentration in mg/L; SNP, single nucleotide polymorphism; OT, on-therapy; TOC, test-of-cure; FU, follow-up; WT, wild-type; N/A = not applicable.
[a] Presented as GyrA/ParC/ParE; variations were not detected in GyrB.
[b] Relevant variations possibly associated with lower susceptibility to gepotidacin.
[c] SNP observed at TOC was synonymous (acyl-CoA dehydrogenase) and observed differences in QnrS1 (intact/disrupted) between baseline and post-baseline indicate absence of relatedness
[d] Isolates were collected outside of TOC/FU collection windows

Table 2 Characterization of *K. pneumoniae* isolates from participants (*n*=6) in the EAGLE-2/EAGLE-3 clinical trials who had post-baseline isolates with reproducible gepotidacin MIC results ≥4-fold higher than the respective baseline isolate and showing the same MLST.

					Fluoroquinolone resistance mechanism		SNP/indel Analysis	
Participant	Treatment Arm	Visit	Days post-baseline	GEP MIC	QRDR[a]	Other	Number	Relevant variations[b]
8	GEP	Baseline	N/A	8	WT/WT/WT	$OqxA^{G26D}$/OqxB14		
		TOC	10	32	WT/WT/WT	QnrS1/$OqxA^{G26D}$/OqxB14	3	QnrS1
9	GEP	Baseline	N/A	4	WT/WT/WT	OqxA/$OqxB19^{A551V\ V562M}$		
		OT	3	32	WT/WT/WT	OqxA/$OqxB19^{A551V\ V562M}$	2	$OqxR^{\Delta Y109}$
10	NTF	Baseline	N/A	8	WT/WT/WT	OqxA/$OqxB5^{A1050T}$		
		FU	28	64	D87G/WT/WT	QnrS1/ OqxA/$OqxB5^{A1050T}$	31	QnrS1
		FU	28	>64	WT/WT/WT	QnrS1/ OqxA/$OqxB5^{A1050T}$	11	QnrS1; $OqxR^{G68-R69insRNGSIHLG}$
11	NTF	Baseline	N/A	4	WT/WT/WT	OqxA/OqxB25		
		OT	3	32	WT/WT/WT	OqxA/OqxB25	2	IS*Kpn*26 insertion in OqxR
		TOC	11	32	WT/WT/WT	OqxA/OqxB25	3	$OqxR^{R69H}$
12	NTF	Baseline	N/A	4	WT/WT/WT	OqxA/OqxB14		
		OT	1	16	WT/WT/WT	OqxA/OqxB14	5	$OqxR^{E20X}$
13	NTF	Baseline	N/A	4	WT/WT/WT	OqxA10/OqxB19		
		OT	2	32	WT/WT/WT	OqxA10/OqxB19	5	$OqxR^{R25fs-L49X}$

Abbreviations: GEP, gepotidacin; NTF, nitrofurantoin; QRDR, quinolone resistance determining region; minimum inhibitory concentration in mg/L; SNP, single nucleotide polymorphism; OT, on-therapy; TOC, test-of-cure; FU, follow-up; WT, wild-type; N/A = not applicable.
[a] Presented as GyrA/ParC/ParE; variations were not detected in GyrB.
[b] Relevant variations possibly associated with lower susceptibility to gepotidacin.

Conclusions

- Among 13 participants evaluated, post-baseline isolates from one [0.096% (1/1045)] (gepotidacin treatment arm, participant #6) may have developed reduced susceptibility to gepotidacin, although it is unclear if these isolates originated from the baseline isolate as the number of observed SNPs/indels exceeded that expected for the defined timeframe.
  - Participant #6 had post-baseline isolates with 2-4 variations relative to the baseline isolate.
  - Elevated gepotidacin MICs were associated with variations in ParC, MFS and/or AcrAB-TolC.
  - While the OT and TOC isolates from patient #6 did not meet SNP/indel criteria to be considered definitively to have originated from the baseline isolate, given the target specific and efflux specific variations identified, GSK has conservatively considered the possibility that the post-baseline isolates may have originated from the baseline isolate.
- Post-baseline *E. coli* isolates from the remaining 6 participants (#1-5, #7) are considered not to have originated from the respective baseline isolates. Lower gepotidacin susceptibility in post-baseline *E. coli* was potentially associated with Qnr and efflux-pumps.
- All post-baseline *K. pneumoniae* isolates from the 6 participants (#8-13) are considered not to have originated from the respective baseline isolates. Lower gepotidacin susceptibility in post-baseline *K. pneumoniae* was potentially associated with Qnr and OqxAB activity.
- Twelve participants (12/13, 92.3%) included in this study had post-baseline isolates that are considered not to have originated from the respective baseline isolate. These participants may represent cases of reinfection with isolates less susceptible to gepotidacin, potentially from other anatomical compartments (e.g. gut, vaginal canal, perineum, or bladder). However, further studies are necessary to better understand the origin of these post-baseline isolates.

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Abbreviations

uUTI, uncomplicated urinary tract infection
MIC, minimum inhibitory concentration
CLSI, Clinical and Laboratory Standards Institute
MLST; multi-locus sequence type
SNP, single nucleotide polymorphism
Indel, insertion/deletion
TOC, test-of cure
FU, follow-up
OT, on-therapy
GEP, gepotidacin
NTF, nitrofurantoin
CIP, ciprofloxacin
LEV, levofloxacin
QRDR, quinolone resistance determining region
WT, wildtype

Disclosures

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•Conflict of Interest: JHK, MC and REM are employees of JMI Laboratories; CAW, JB, DB, JW, NES-O, and AS are employees of and shareholders in GSK.

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