Microbiological Analysis From a Phase 2 Randomized Study in Adults Evaluating Single Oral Doses of Gepotidacin in the Treatment of Uncomplicated Urogenital Gonorrhea Caused by Neisseria gonorrhoeae

Running Title:
Gepotidacin for gonorrhea—microbiological evaluation

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ABSTRACT

We evaluated microbiological correlates of successful treatment of *Neisseria gonorrhoeae* isolates from a Phase 2 study of gepotidacin, a novel triazaacenaphthylene antibacterial, for therapy of uncomplicated urogenital gonorrhea. Culture, susceptibility testing, genotypic characterization, and frequency of resistance (FoR) were performed for selected isolates. Microbiological success was defined as culture-confirmed eradication of *N. gonorrhoeae*. Against 69 baseline urogenital isolates, gepotidacin MICs ranged from ≤0.06 to 1 µg/mL (MIC$_{90}$ = 0.5 µg/mL). For gepotidacin, the ratio of the area under the free-drug concentration-time curve to MIC ($f_{AUC}/MIC$) was associated with therapeutic success. Success was 100% ($61/61$) at $f_{AUC}/MICs ≥48$ and decreased to 63% ($5/8$) for $f_{AUC}/MICs ≤25$. All 3 isolates from microbiological failures were ciprofloxacin-resistant, had a baseline gepotidacin MIC of 1 µg/mL, and carried a pre-existing ParC D86N mutation, a critical residue for gepotidacin binding. At test-of-cure, resistance to gepotidacin emerged in 2 isolates (MICs increased ≥32-fold) with an additional GyrA A92T mutation, also implicated in gepotidacin binding. Test-of-cure isolates had the same sequence type as the corresponding baseline isolates. For 5 selected baseline isolates, all carrying a ParC D86N mutation, *in vitro* FoR to gepotidacin was low ($10^{-9}$ to $10^{-10}$); resistant mutants had the same A92T mutation as the 2 emergence of resistance isolates. Five participants with isolates with a ParC D86N mutation were successes. In summary, $f_{AUC}/MICs ≥48$ predicted 100% microbiological success, including 3 isolates with the ParC D86N mutation ($f_{AUC}/MICs ≥97$). Pharmacokinetic/pharmacodynamic determinations may help to evaluate new therapies for gonorrhea; further study of gepotidacin is warranted.

ClinicalTrials.gov Registry Number: NCT02294682
INTRODUCTION

The prevalence of gonorrhea infections continues to rise while effective treatment options have decreased due to progressive, steadily emerging antimicrobial resistance in Neisseria gonorrhoeae (1–5). While investigational antibacterials have been recently evaluated in the clinic, novel antibacterials and treatment strategies are urgently needed to address the threat of potentially untreatable gonorrhea (6–9). N. gonorrhoeae gene mutations occur frequently and act through a variety of resistance mechanisms, including altering antibiotic influx and efflux, enzymatic inactivation of antibacterials, and modifying antibiotic binding affinity (10, 11).

Gepotidacin (GSK2140944) is a first-in-class triazaacenaphthylene bacterial type II topoisomerase inhibitor in development for gonorrhea treatment. Gepotidacin interacts in a unique way on the GyrA subunit of bacterial DNA gyrase and the ParC subunit of bacterial topoisomerase IV, with activity against most target pathogens resistant to established antibacterials, including fluoroquinolones (12, 13), and activity against drug-resistant N. gonorrhoeae strains (14). A randomized, dose-ranging (1500 or 3000 mg), single oral dose, Phase 2 study demonstrated that gepotidacin was 96% effective in eradicating (culture-confirmed) N. gonorrhoeae from participants with uncomplicated urogenital gonorrhea for the combined dose groups (15). Gepotidacin warrants further clinical evaluation as an alternative treatment option for gonorrhea. Our microbiological evaluation of N. gonorrhoeae isolates from this Phase 2 study is presented herein.

Antibacterial therapeutic success is impacted by both the MIC of a pathogen and its exposure to an antibiotic. As such, pharmacokinetic/pharmacodynamic (PK/PD) evaluations are important to
identify exposures required to maximize efficacy against isolates with different MIC values. Adequate PK exposures may prevent the opportunity for selection of *N. gonorrhoeae* mutations and development of resistance (16–18). PK/PD indices, such as the ratio of the area under the free-drug concentration-time curve to MIC ([fAUC/MIC](#)), may be used to identify optimal therapeutic regimens. Typically, nonclinical models are used to determine the PK/PD index and target that best predict a successful exposure-response, which is then followed by Monte Carlo simulation using PK data to predict how many humans would likely achieve the PK/PD target for a specific dose across a clinically relevant range of MIC values (19). The PK/PD index and target may vary across clinical indications, antibacterial agents, bacterial species, and antibacterial mechanisms of action, which may be time- or concentration-dependent (16–18).

Because there were no validated preclinical models to determine the PK/PD target (PK/PD index and magnitude) predictive of gepotidacin efficacy for urogenital gonorrhea treatment, a nontraditional PK/PD approach was used to select gepotidacin doses for this Phase 2 study. A population PK model was developed using concentration-time data from a Phase 1 oral, dose-ranging study in healthy volunteers (20). Based on nonclinical models for other bacterial species, the PK/PD index used to model theoretical efficacy was [fAUC/MIC](#) (21). Monte Carlo simulation of the 1500-mg and 3000-mg single oral doses was then conducted to predict PK/PD magnitudes over a clinically relevant range of gepotidacin MICs. For *N. gonorrhoeae* isolates with a gepotidacin MIC of 1 µg/mL, the gepotidacin MIC$_{90}$ of *N. gonorrhoeae*, simulation results indicated that 90% of participants would achieve [fAUCs](#) of 10 and 20 µg · h/mL for the 1500-mg and 3000-mg doses, respectively.
We studied microbiological data from the oral gepotidacin Phase 2 urogenital gonorrhea study, including antibacterial susceptibility, quinolone resistance-determining region (QRDR) genotyping of GyrA and ParC, sequence typing, frequency of resistance (FoR), and efficacy assessments based on PK/PD magnitudes.

**MATERIALS AND METHODS**

**Study Design**

The Phase 2, randomized, multicenter, open-label, dose-ranging, single oral dose study of gepotidacin (1500 mg or 3000 mg in a 1:1 ratio stratified by gender) for the treatment of urogenital gonorrhea has been previously described (15). Briefly, a single oral dose of gepotidacin was administered at baseline followed by test-of-cure (TOC) 3 to 7 days after dosing. Pretreatment and TOC urogenital swab specimens were obtained; rectal and pharyngeal specimens were also collected. The microbiologically evaluable population consisted of 69 randomly assigned participants (67 male and 2 female) with culture-confirmed urogenital gonorrhea at baseline who received gepotidacin and returned for TOC (15). Of the 69 microbiologically evaluable participants, 2 also had culture-confirmed pharyngeal gonorrhea and 3 also had culture-confirmed rectal gonorrhea (15). Microbiological success was defined as culture-confirmed eradication of *N. gonorrhoeae* at TOC. Microbiological failure was defined as culture-confirmed bacterial persistence of *N. gonorrhoeae* at TOC or the inability to determine the response (e.g., lost sample) of the baseline pathogen at TOC.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice, and applicable country-specific requirements were...
followed, including Institutional Review Board approval at each study site. All participants provided signed informed consent.

Microbiological Evaluation

Specimens were processed for culture at local laboratories according to accepted microbiological procedures (22). All presumptively identified *N. gonorrhoeae* isolates from local laboratories were sent to the central laboratory for confirmatory identification testing (University of Alabama at Birmingham, Birmingham, Alabama). Agar dilution antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) and Gonococcal Isolate Surveillance Program methods at the central laboratory (23–25). MICs were determined for gepotidacin, azithromycin, cefixime, ceftriaxone, ciprofloxacin, penicillin G, spectinomycin, and tetracycline and, where available, CLSI breakpoints were applied. For azithromycin, CLSI epidemiological cutoff values were applied (24).

QRDR genotyping of GyrA and ParC was performed for all *N. gonorrhoeae* isolates by GlaxoSmithKline (Collegeville, Pennsylvania). *N. gonorrhoeae* was subcultured from frozen stock onto Chocolate II Agar plates and incubated at 35°C for 18 h with 5% carbon dioxide. A loopful (1 µL loop) of cells from a fresh plat was transferred into 50 µL Tris-EDTA buffer pH 8 and boiled for 10 min. The tube was then put on ice for 2 min and then centrifuged at 14,000 rpm in an Eppendorf 5415C for 1 min. Supernatant (2 µL) was used as template for PCR. PCR primers for amplification of *N. gonorrhoeae* gyrA and parC encoding gyrase subunit A and topoisomerase IV subunit C, respectively, were described previously by Vernel-Pauillac et al. (26). PCR was carried out using GeneAmp® PCR System 9700, under the following
conditions: 5 min at 95°C; 35 cycles of 30 s at 95°C, 45 s at 48°C, 1 min at 72°C; 7 min at 72°C

for 1 cycle, then 4°C. Invitrogen™ PCR SuperMix High Fidelity (Invitrogen 10790-020) was
used. PCR products were separated, visualized, and sized by electrophoresis on a 1% agarose gel
containing ethidium bromide, and purified following manufacturer’s instructions using a
QIAquick PCR Purification Kit (Qiagen 28104). PCR products were sequenced using BigDye®
Terminator v3.1 Cycle Sequencing Kit followed by analysis using a 3730xl DNA Analyzer; all
equipment was from Applied Biosystems (Foster City, California). Isolate sequences were
compared to parent and reference sequence FA1090 obtained from The National Center for
(DYNASTAR, Inc., Madison, Wisconsin) was used to identify nucleotide changes resulting in
amino acid residue substitutions.

Whole genome sequencing was conducted for the baseline and TOC isolates from all
participants who were microbiological failures. FASTA sequences for each isolate were entered
into the following online sequence typing databases: Multi Locus Sequence Typing (MLST;
www.mlst.net), N. gonorrhoeae Sequence Typing for Antimicrobial Resistance (NG-STAR;
https://ngstar.canada.ca), and N. gonorrhoeae Multi Antigen Sequence Typing (NG-MAST;
www.ng-mast.net).

To assess FoR, 2 separate studies were performed by GlaxoSmithKline (Collegeville,
Pennsylvania) using Oxoid GC agar base containing BB BBL™ IsovitaleX Enrichment in
study 1 and Remel agar base plus hemoglobin and BB BBL™ IsovitaleX Enrichment in study 2.
A select set of N. gonorrhoeae isolates recovered from participants, including microbiological
successes and failures, from the Phase 2 clinical trial having the GyrA (S91F and D95A/G) and ParC (D86N) genotype were tested. This study was performed to determine the FoR to gepotidacin in urogenital baseline isolates with the genotype associated with microbiological failure.

Gepotidacin was added to the appropriate medium-containing molten agar to yield 20 mL of agar at the correct multiple of the MIC for each organism. Plates containing gepotidacin at 4× MIC or 10× MIC were poured and left to cool and to solidify. Plates containing no compound were also prepared to be used for viable counts and to serve as a growth control. Cultures were prepared by direct colony suspension in saline solutions to a turbidity equivalent to a 4 McFarland prepared from colonies grown on a chocolate plate after overnight incubation at 35°C in 5% carbon dioxide. To determine the number of CFU present in the initial test inoculum, each organism suspension was serially diluted 1:10, and three 20-µL drops from each dilution were plated on agar and incubated overnight at 35°C in 5% carbon dioxide. Counts were performed at the dilution that provided distinguishable colonies, and an average of the 3 samples was used to calculate the number of CFU in the original suspension. In addition, 100 µL of each cell suspension was spread onto the surface of plates containing the appropriate multiple of the MIC concentration of compound and a control agar plate containing no compound. Plates were incubated at 35°C in 5% carbon dioxide. After 48 h incubation, single colonies that grew on the FoR plates were streaked onto new plates containing identical drug concentrations and incubated at 35°C in 5% carbon dioxide (23, 24). These purified resistant colonies were then isolated to plain chocolate agar plates and frozen in broth media containing glycerol. In order to confirm
their resistance phenotype, the susceptibility of these isolates to gepotidacin was tested by agar
dilution methodology. PCR and sequencing of the QRDR was performed on all resistant isolates.

**PK**

The AUC of gepotidacin achieved over 24 h for each gepotidacin dose was estimated from
healthy volunteer data using a previously described population PK 2-compartmental model with
an absorption lag-time and zero-order input for oral absorption (20). The model had a low
coefficient of variation for all model parameter estimates of ≤2.2 and a residual variability of
39%. Variance was not inflated as PK variability was expected to be similar between participants
with gonorrhea and healthy volunteers. Mean gepotidacin 2-h postdose plasma concentrations in
participants with gonorrhea were 2.89 and 6.35 µg/mL for the 1500-mg and 3000-mg doses,
respectively (15).

**RESULTS**

*In Vitro Susceptibility Testing*

*Gepotidacin* was active against the 69 baseline urogenital *N. gonorrhoeae* isolates tested with
MIC values between ≤0.06 to 1 µg/mL (Fig. 1) and MIC₅₀ and MIC₉₀ values of 0.12 and
0.5 µg/mL, respectively (Table 1). Resistance to ciprofloxacin, penicillin, and tetracycline was
33%, 28%, and 20%, respectively, with no resistance observed for ceftriaxone, cefixime, or
spectinomycin. An elevated azithromycin MIC of 2 µg/mL was observed for 2 baseline urogenital isolates.

Gepotidacin MICs ranged from ≤0.06 to 0.12 µg/mL and 0.12 to 0.25 µg/mL against the 2 pharyngeal *N. gonorrhoeae* isolates and the 3 rectal *N. gonorrhoeae* isolates recovered. Resistance patterns to the tested comparator antimicrobials for these isolates were similar to those seen for the urogenital isolates.

Microbiological Response

*Microbiological Response by PK/PD Magnitude for Urogenital Isolates*

Overall microbiological success was 96% (66/69) (Table 2). PK/PD analysis showed 100% (61/61) microbiological success when the fAUC/MIC was ≥48, irrespective of the gepotidacin MICs of the baseline urogenital *N. gonorrhoeae* isolates. Among baseline urogenital isolates with higher gepotidacin MICs, microbiological success decreased to 63% (5/8) when the fAUC/MIC was ≤25. All 3 urogenital microbiological failures had fAUC/MICs ≤24.

*Microbiological Response by Antimicrobial Agent Susceptibility for Urogenital Isolates*

For participants with baseline urogenital *N. gonorrhoeae* isolates with gepotidacin MICs ≤0.5 µg/mL, microbiological success was 100% (Table 3). Of the 5 participants with baseline urogenital *N. gonorrhoeae* isolates with the highest gepotidacin MIC of 1 µg/mL, 2 were microbiological successes and 3 were microbiological failures. Of the 2 participants with baseline pharyngeal *N. gonorrhoeae* isolates, 1 was a microbiological success (gepotidacin MIC of ≤0.06 µg/mL) and 1 was a microbiological failure (gepotidacin MIC of 0.12 µg/mL). All...
Three participants classified as urogenital microbiological failures each had an isolate with a gepotidacin MIC of 1 µg/mL that was also resistant to tetracycline, ciprofloxacin, and penicillin, with the exception of 1 isolate with intermediate susceptibility to penicillin. Four additional participants with baseline urogenital *N. gonorrhoeae* isolates that were also resistant to tetracycline and ciprofloxacin and nonsusceptible to penicillin were microbiological successes (data not shown). The 2 additional participants with baseline urogenital *N. gonorrhoeae* isolates with a gepotidacin MIC of 1 µg/mL that were only resistant to ciprofloxacin were microbiological successes (data not shown). No trends in microbiological outcome were observed for the few pharyngeal or rectal *N. gonorrhoeae* isolates with regard to baseline antimicrobial agent susceptibility.

**QRDR Genotyping of *N. gonorrhoeae* Isolates from Microbiological Failures**

All 3 participants who were urogenital microbiological failures had baseline *N. gonorrhoeae* isolates that were ciprofloxacin-resistant with a baseline gepotidacin MIC of 1 µg/mL and a pre-existing D86N substitution due to a mutation in the *parC* gene, a critical residue in gepotidacin binding (Table 4) (12). One microbiological failure was treated with gepotidacin 1500 mg and had a fAUC/MIC of 12; no change from the baseline gepotidacin MIC of 1 µg/mL was noted for the *N. gonorrhoeae* isolate recovered at TOC. The other 2 microbiological failures were treated with gepotidacin 3000 mg and had fAUC/MICs of 24; both of these *N. gonorrhoeae* isolates demonstrated resistance emergence to gepotidacin at TOC with gepotidacin MICs that...
increased ≥32-fold plus a new, additional A92T substitution due to a mutation in the gyrA gene, which is also implicated in the gepotidacin binding pocket (12).

The pharyngeal microbiological failure occurred for a participant with a N. gonorrhoeae isolate that had a lower gepotidacin MIC of 0.12 µg/mL and no observed mutations in the QRDR of ParC or GyrA.

QRDR Genotyping of All N. gonorrhoeae Isolates

Of all the urogenital, pharyngeal, and rectal isolates available for sequencing, 12% (8/69) of baseline urethral isolates had a ParC D86N mutation and none of the pharyngeal or rectal isolates tested harbored a ParC D86N mutation.

All 8 isolates with the ParC D86N mutation were ciprofloxacin-resistant and had 2 mutations in GyrA (Table 5). The ParC D86N mutation was observed for all 5 baseline urogenital isolates with a gepotidacin MIC of 1 µg/mL; however, this mutation was also observed for 3 isolates with lower gepotidacin MICs of ≤0.06 (1 isolate) and 0.25 µg/mL (2 isolates).

As described above, N. gonorrhoeae isolates from all participants who were urogenital microbiological failures had gepotidacin MICs of 1 µg/mL and the ParC D86N mutation, whereas the remaining 5 isolates with the ParC D86N mutation had gepotidacin MICs of ≤0.25 µg/mL (3 isolates) and 1 µg/mL (2 isolates) and were from participants who were microbiological successes.
Sequence Types of *N. gonorrhoeae* Isolates from Microbiological Failures

Using data from whole genome sequencing analysis, with the primary objective being to ascertain whether a participant had failed treatment due to reinfection by a different strain, sequence types of the baseline and TOC isolates from participants who were microbiological failures were determined by MLST, NG-STAR, and NG-MAST methods. With the exception of NG-STAR for participant 4 and 6 (due to the GyrA A92T mutation present in their posttreatment isolates), results from all 3 sequence type methods indicated that the baseline isolates from participants who were microbiological failures were the same strain as those recovered at TOC; therefore, reinfection by a different strain was unlikely to be the cause of gepotidacin treatment failure. None of the sequence typing methods alone or in combination were predictive of gepotidacin treatment failure or emergence of resistance. Tabular results are provided in Table S1.

Spontaneous FoR of Selected *N. gonorrhoeae* Isolates

The 5 baseline urogenital isolates evaluated for spontaneous FoR against gepotidacin were fluoroquinolone-resistant with mutations in GyrA (S91F, D95A/G) and ParC (D86N), the same genotype seen in the baseline isolates from the 3 participants who were urogenital microbiological failures. From both FoR studies, at 4× and 10× MIC, the spontaneous FoR to gepotidacin was low, <9.1 × 10⁹, <3.4 × 10⁹, <3.8 × 10⁹, ≤2.9 × 10⁹, and <6.7 × 10⁹ for the isolates from participants 2, 4, 5, 7, and 8, respectively. With the exception of the isolate from participant 7, no resistant mutants were isolated at 4× and 10× MIC. The urogenital baseline isolate from participant 7 had 1 resistant mutant isolated in the first study and 2 resistant mutants isolated in the second study, both at 4× the MIC of gepotidacin. These 3 isolates were
genotypically characterized and found to carry a GyrA A92T mutation, the same mutation identified in the 2 isolates from participants who were urogenital failures and demonstrated resistance emergence to gepotidacin at TOC, in addition to the pre-existing GyrA S91F and D95A and ParC D86N mutations. The gepotidacin MIC increased 16-fold compared with the parent strain.

**DISCUSSION**

Emerging *N. gonorrhoeae* antimicrobial resistance is an urgent public health threat. Gepotidacin, with its novel mechanism of action, warrants further study as a potential oral treatment option for gonorrhea infections. Our evaluation of microbiological data from a previously reported Phase 2 study of oral gepotidacin in uncomplicated gonorrhea (15) provides insights for further evaluation, including antimicrobial susceptibility, QRDR mutations, sequence types, FoR, and efficacy assessments based on $f$AUC/MIC magnitudes. Our evaluation of $f$AUC/MIC magnitudes and their association with treatment outcomes represents a novel approach for predicting gonorrhea therapy outcomes.

When doses were selected for this clinical study, gepotidacin had been evaluated using an *in vivo* mouse gonorrhea vaginal colonization model, which provided potential efficacy information, but was not validated for PK/PD characterization (27). Therefore, a nonclinical model conducted for other bacterial species was adapted to determine that gepotidacin efficacy is concentration-dependent, and thus, by extension, $f$AUC/MIC may be the most appropriate PK/PD index predictive of gepotidacin efficacy (21). A population PK model using Phase 1 data and Monte Carlo simulation was used for exposure predictions. While the optimum PK/PD index
and target for gepotidacin efficacy against *N. gonorrhoeae* were not known, the simulations predicted that 90% of participants would achieve fAUCs of 10 and 20 µg · h/mL for the 1500- and 3000-mg doses, respectively. The PK predictions for dose selection were close to the actual study results, where fAUCs were approximately 12 and 24 µg · h/mL for the low and high doses, respectively. In addition, the distribution of gepotidacin MICs for *N. gonorrhoeae* in this study were similar to a prior surveillance study, where approximately 7% of *N. gonorrhoeae* isolates had a gepotidacin MICs of 1 µg/mL (28). Progress continues to be made in mouse models for gonorrhea infection, which will also continue to advance preclinical research efforts (29).

**Microbiological** success was achieved for all participants with fAUC/MICs $\geq$48, including success for 3 participants with urogenital *N. gonorrhoeae* isolates with a ParC D86N mutation and fAUC/MICs $\geq$97. All 3 participants who were classified as microbiological urogenital failures had fAUC/MICs $\leq$24.

*N. gonorrhoeae* isolates from participants who were urogenital failures had common features. All were ciprofloxacin-resistant, had baseline gepotidacin MICs of 1 µg/mL, and harbored a pre-existing ParC D86N mutation, which has a prevalence of up to 30% in fluoroquinolone-resistant *N. gonorrhoeae* (4, 30). In addition, 2 of these isolates demonstrated resistance emergence (≥32-fold increase in the gepotidacin MIC from baseline to TOC) and had an additional GyrA A92T mutation in the TOC isolate. Three sequence typing methods indicated that the baseline isolates from participants who were microbiological failures were the same strain as those recovered at TOC, therefore reinfection by a different strain was unlikely the
cause of treatment failure. Both ParC D86 and GyrA A92 are critical to gepotidacin’s interaction with bacterial type IIA topoisomerases (12). As gepotidacin works by inhibiting both DNA gyrase and topoisomerase IV, the pre-existing ParC D86N mutation in these treatment-failure isolates reduced the dual-targeting activity of gepotidacin to a single target and increased the resistance emergence potential. The pre-existing ParC D86N mutation appeared to be recessive and did not significantly affect the initial gepotidacin MIC, but likely increased the potential for resistance development and contributed to the large gepotidacin MIC increase seen at TOC upon acquisition of the additional target mutation, which was observed for 2 N. gonorrhoeae isolates from participants who were microbiological failures. This is further supported by subsequent whole genome sequencing of a subset of N. gonorrhoeae isolates, which did not identify target mutations in GyrA/GryB, ParC/ParE, or mutations in other genes associated with gepotidacin failure or resistance emergence (data not shown). These data suggest that future doses of gepotidacin need to achieve a higher PK/PD magnitude to support optimum gepotidacin efficacy in gonorrhea treatment, especially in global regions where N. gonorrhoeae isolates with higher gepotidacin MICs and fluoroquinolone resistance rates may be observed (5). The highest daily oral dose of gepotidacin that has been studied in the clinic is 6000 mg (31, 32).

The FoR to gepotidacin at 4× and 10× MIC for a selected subset of isolates with the same genotype as the baseline isolates from participants who were urogenital microbiological failures, was low (10⁹ to 10¹⁰). The 3 resistant mutants recovered in the FoR studies were found to carry an additional GyrA A92T mutation, the same additional mutation found in 2 urogenital TOC isolates with resistance emergence. The increase in gepotidacin MIC (16-fold) seen for the FoR resistant mutants was also similar to the gepotidacin MIC increase (≥32-fold) of the 2 urogenital
TOC isolates with resistance emergence. These results suggest that these clinical isolates containing mutations in GyrA (S91F, D95A/G) and ParC (D86N) show a low in vitro frequency of spontaneous resistance to gepotidacin and that although mutants with an additional GyrA A92T mutation were isolated, the in vitro frequency of this occurrence was also low. The rationale for why these low in vitro FoR results are discordant with the amount of resistance emergence seen in the clinical trial (2 out of 69 participants) is unknown.

Previous FoR studies with *N. gonorrhoeae* isolates also showed a low FoR ($10^{-9}$ to $10^{-10}$) to gepotidacin at 4x and 8x MIC for fluoroquinolone-susceptible and -resistant isolates and no resistant mutants were recovered (14), suggesting that gepotidacin has a well-balanced dual targeting against both DNA gyrase and topoisomerase IV. However, none of the isolates tested in this prior study had the pre-existing ParC D86N mutation (14). The microbiological data from the current clinical trial are largely consistent with those previous in vitro FoR results (14) in that no resistance development to gepotidacin was observed in *N. gonorrhoeae* isolates lacking the pre-existing ParC D86N mutation.

There were several limitations of this microbiological evaluation. Only a few *N. gonorrhoeae* isolates were from extragenital body sites. Because antimicrobial concentrations may vary at these mucosal sites, future studies should seek to include participants with extragenital *N. gonorrhoeae* infections. The *N. gonorrhoeae* isolates from this study only reflect US epidemiology and different resistance patterns may be observed in other geographic regions. However, in a recent publication, gepotidacin had an MIC$_{90}$ value of 1 µg/mL against a global collection of 252 *N. gonorrhoeae* isolates, which was similar to the gepotidacin MIC$_{90}$ value of
0.5 µg/mL in this clinical study (33). Potential studies for future consideration are whole genome sequencing of additional *N. gonorrhoeae* isolates recovered from the study and experiments to understand the potential for transmission of ParC D86N and GyrA A92T mutations to susceptible gonococcal strains.

**Our** data demonstrated that microbiological success was achieved for all participants with urogenital *N. gonorrhoeae* isolates when the $fAUC/MIC$ was ≥48. Microbiological success decreased to 63% (5/8) when $fAUC/MICs$ were ≤25, resulting in urogenital microbiological failure for 3 participants with ciprofloxacin-resistant *N. gonorrhoeae* isolates, which had baseline gepotidacin MICs of 1 µg/mL and harbored a pre-existing ParC D86N mutation, thereby reducing activity of gepotidacin for 1 of the 2 bacterial targets. However, microbiological success was achieved for 5 participants with isolates that harbored a pre-existing ParC D86N mutation, 2 with $fAUC/MICs$ ≤24 and 3 at higher $fAUC/MICs$ of ≥97. These results indicate that further evaluation of gepotidacin in the treatment of gonorrhea is warranted, including demonstration that higher exposures increase efficacy and suppress resistance in key isolate subsets.
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REFERENCES


FIGURE LEGENDS

FIG 1 Frequency distribution of gepotidacin MICs against baseline urogenital *N. gonorrhoeae* isolates (N = 69).
<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>MIC (μg/mL)</th>
<th>Interpretation (n [%])$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gepotidacin</td>
<td>0.12 0.5</td>
<td>≤0.06 to 1  <em>...</em></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.004 8</td>
<td>≤0.001 to 16 46 (67) 0 (0) 23 (33)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.008 0.03</td>
<td>≤0.001 to 0.06 69 (100) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Cefixime</td>
<td>0.015 0.06</td>
<td>≤0.001 to 0.06 69 (100) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.25 0.5</td>
<td>≤0.008 to 2  <em>...</em></td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.5 4</td>
<td>≤0.03 to &gt;64 1 (1) 49 (71) 19 (28)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1 2</td>
<td>0.12 to 32 11 (16) 44 (64) 14 (20)</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>16 32</td>
<td>≤4 to 32 69 (100) 0 (0) 0 (0)</td>
</tr>
</tbody>
</table>

$^a$Susceptibility interpretations were per M100-S27 Clinical and Laboratory Standards Institute breakpoints.

$^b$Ellipses indicate that no breakpoint was available.
TABLE 2 Microbiological success by $f$AUC/MIC against urogenital *N. gonorrhoeae* at baseline$^a$

<table>
<thead>
<tr>
<th>$f$AUC/MIC (range)</th>
<th>n/N</th>
<th>Microbiological success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥198</td>
<td>27/27</td>
<td>100</td>
</tr>
<tr>
<td>95 to 103</td>
<td>25/25</td>
<td>100</td>
</tr>
<tr>
<td>48 to 49</td>
<td>9/9</td>
<td>100</td>
</tr>
<tr>
<td>24 to 25</td>
<td>4/6</td>
<td>67</td>
</tr>
<tr>
<td>12</td>
<td>1/2</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>66/69</td>
<td>96</td>
</tr>
</tbody>
</table>

$^a$fAUC/MIC, ratio of the area under the free-drug concentration-time curve to MIC.
TABLE 3 Microbiological response by gepotidacin MIC against N. gonorrhoeae at baseline

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Gepotidacin 1500 mg (N = 30)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gepotidacin 3000 mg (N = 39)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/mL)</td>
<td>n</td>
</tr>
<tr>
<td>Urogenital</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤0.06</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pharyngeal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤0.06</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>Rectal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0</td>
</tr>
</tbody>
</table>
MF, microbiological failure; MS, microbiological success.

Participants were only required to have a urogenital *N. gonorrhoeae* isolate at baseline to qualify for the microbiologically evaluable population. Pharyngeal or rectal *N. gonorrhoeae* isolates at baseline were not required for this population. Therefore, the $n$ values for the pharyngeal and rectal data are each a subset of the full population (i.e., of the overall 69 participants in this population, 2 had a baseline pharyngeal *N. gonorrhoeae* isolate and 3 had a baseline rectal *N. gonorrhoeae* isolate).
<table>
<thead>
<tr>
<th>Participant number (Gender)</th>
<th>GEP dose (mg)</th>
<th>Visit</th>
<th>Microbiological response</th>
<th>Mutation</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (Male) 3000 Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>S91F D86N</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D95G</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOC Failure</td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>S91F D86N</td>
<td>&gt;32</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>A92T D95G</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>CIP</td>
<td>0.06</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>CRO</td>
<td>8</td>
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<tr>
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<td></td>
<td>SPT</td>
<td>4</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>PEN</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AZI</td>
<td>2</td>
</tr>
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<td>TET</td>
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<td></td>
<td></td>
<td></td>
<td>CFM</td>
<td></td>
</tr>
<tr>
<td>6 (Male) 3000 Baseline</td>
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<td>S91F D86N</td>
<td>4</td>
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<td></td>
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<td></td>
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<td>D95G</td>
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<td></td>
<td></td>
<td>TOC Failure</td>
<td></td>
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</tr>
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<td></td>
<td>CFM</td>
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<td>7 (Male) 1500 Baseline</td>
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<td>Failure</td>
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<td>D86N</td>
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<td>0.03</td>
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</tbody>
</table>

*AZI, azithromycin; CFM, cefixime; CIP, ciprofloxacin; CLSI, Clinical and Laboratory Standards Institute; CRO, ceftriaxone; GEP, gepotidacin; PEN, penicillin; QRDR, quinolone-resistance determining region; SPT, spectinomycin; TET, tetracycline; TOC, test of cure.

*Dark shading = resistance by M100-S27 CLSI breakpoints. For AZI, the CLSI non-wildtype epidemiological cutoff value was applied.

Light shading = intermediate by M100-S27 CLSI breakpoints.

No shading = susceptible by M100-S27 CLSI breakpoints. For AZI, the CLSI wildtype epidemiological cutoff value was applied.

*Sequencing of QRDR only.

*No breakpoints are currently available for GEP.
### TABLE 5 Baseline urogenital N. gonorrhoeae isolates with a ParC D86N mutation

<table>
<thead>
<tr>
<th>Participant number</th>
<th>Mutation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MIC (µg/mL)</th>
<th>GEP</th>
<th>Microbiological response</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>GyrA</td>
<td>ParC</td>
<td>GEP</td>
<td>CRO</td>
</tr>
<tr>
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<td>≤0.06</td>
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<td>D86N</td>
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<tr>
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<td></td>
<td>0.008</td>
</tr>
</tbody>
</table>

*AZI, azithromycin; CFM, cefixime; CIP, ciprofloxacin; CRO, ceftriaxone; AUC/MIC, ratio of the area under the free-drug concentration-time curve to MIC; GEP, gepotidacin; PEN, penicillin; QRDR, quinolone resistance-determining region; SPT, spectinomycin; TET, tetracycline.

b Sequencing of QRDR only.

c No breakpoints are currently available for GEP.