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Multicenter Investigation of Gepotidacin (GSK2140944) Agar Dilution Quality Control Determinations for *Neisseria gonorrhoeae* ATCC 49226

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Gepotidacin, a novel triazaacenaphthylene antibacterial agent, is the first in a new class of type IIA topoisomerase inhibitors with activity against many biothreat and conventional pathogens, including *Neisseria gonorrhoeae*. To assist ongoing clinical studies of gepotidacin to treat gonorrhea, a multilaboratory quality assurance investigation determined the reference organism (*N. gonorrhoeae* ATCC 49226) quality control MIC range to be 0.25 to 1 μg/ml (88.8% of gepotidacin MIC results at the 0.5 μg/ml mode).

Antimicrobial resistance (AMR) among *Neisseria gonorrhoeae* isolates continues to be reported worldwide; most recently, reports included high-level AMR to the fluoroquinolones, extended-spectrum β-lactams, and azithromycin (1–4). To address this public health concern, new antimicrobial agents, drug combinations, and compounds having novel modes of action are being studied (5–11).

Gepotidacin (GSK2140944) is a novel triazaacenaphthylene antibacterial agent, the first in a new class of bacterial type IIA topoisomerase inhibitors. This novel agent does not share cross-resistance with the fluoroquinolones due to a different binding mode and exhibits *in vitro* activity against levofloxacin-resistant *Staphylococcus aureus* (MIC_{90}, 0.5 μg/ml) and *Streptococcus pneumoniae* (MIC_{90}, 0.5 μg/ml) (12, 13). One potential focus of clinical use would be the treatment of gonorrhea, especially in geographic areas where AMR has become highly prevalent (2). To facilitate gepotidacin development and to expand the standardized susceptibility testing to this agent for sexually transmitted diseases, a Clinical and Laboratory Standards Institute (CLSI) M23-style quality control (QC) study (14) was conducted to establish the agar dilution MIC QC range for testing *N. gonorrhoeae* ATCC 49226 by the CLSI M07-A10 method (15). These results could be used during ongoing clinical investigations along with previously published QC investigations by our group (16).

The study protocol, with eight participating laboratories, was organized and monitored by JMI Laboratories (North Liberty, IA, USA). The participant laboratories (and site director) were the Infectious Diseases Research Laboratory, Detroit, MI (M. Zervos); ThermoFisher Scientific, Cleveland, OH (C. Knapp); University of Rochester, Rochester, NY (D. Hardy); University of Alberta, Edmonton, Canada (R. Rennie); Cleveland Clinic Foundation, Cleveland, OH (G. Procop); JMI Laboratories, North Liberty, IA (R. Jones); Methodist Hospital, Indianapolis, IN (G. Denys); and Tufts University Medical Center, Boston, MA (D. Snydman).

The site investigators applied the CLSI M07-A10 agar dilution method (15) for testing the *N. gonorrhoeae* ATCC 49226 QC organism. Each site was provided with 3 lots of GC agar base deeps (BD, Difco, and Oxoid) with 1% defined growth supplement to test gepotidacin (GlaxoSmithKline; lot 132377141) and the control agent ciprofloxacin (Sigma; lot BCBM7969V) across 7 doubling dilution steps (0.06 to 4 and 0.0005 to 0.03 μg/ml, respectively). Ten replicates of each drug and medium lot (only one lot for ciprofloxacin) were performed over at least 2 testing days with a maximum of 5 replicates per day. This design generated 30 MIC results at each laboratory site, or 240 total gepotidacin MIC values (80 for ciprofloxacin). Colony counts to monitor inoculum density were determined at each location. The average inoculum count across all sites and experiments was 1.6×10^5 CFU/spot (range, 7.4×10^4 to 5.0×10^5 CFU/spot).

Gepotidacin MIC results for *N. gonorrhoeae* ATCC 49226 were very reproducible at each laboratory. All 8 participant sites had a modal value of 0.5 μg/ml, and the majority of gepotidacin MIC results (57 to 79 of 80 replicate MICs) were at the mode with each GC agar base lot. The geometric mean MIC results only varied from 0.40 to 0.50 μg/ml among the 8 participant sites and ranged from 0.41 to 0.51 μg/ml for the three tested medium lots.

Figure 1 shows the gepotidacin MIC distribution for *N. gonorrhoeae* ATCC 49226 (240 values). A total of 213 MIC results (88.8%) were at the 0.5 μg/ml mode, and all MIC results were within the proposed 3-$\log_2$ dilution QC range of 0.25 to 1 μg/ml, as calculated from the Gavan method (14). The Range Finder statistical method (17) also suggested an identical MIC QC range. All ciprofloxacin control MIC values were within the CLSI-published range of 0.001 to 0.008 μg/ml (18). The control QC strain MIC for gepotidacin was consistent with reported clinical *N. gonorrhoeae* strains (23), having MIC_{50} and MIC_{90} results of 0.12 and 0.25 μg/ml, respectively.

Gepotidacin, with a unique mode of action, provides an opportunity to further study a novel agent for the treatment of uncomplicated gonorrhea, thus potentially limiting the probability of cross-resistance or coresistance (12, 19). The *in vitro* activity of

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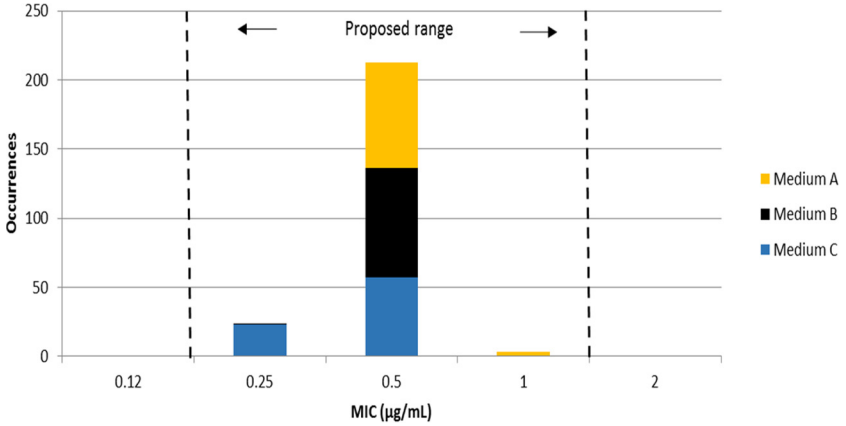

FIG 1 Gepotidacin (GSK2140944) reference agar dilution MIC results from an 8-laboratory investigation to determine QC ranges for *N. gonorrhoeae* ATCC 49226. All results are within the proposed MIC range (0.25 to 1 μg/ml).

gepotidacin (MIC mode, 0.5 μg/ml) against the *N. gonorrhoeae* QC strain (ATCC 49226), having nonsusceptible characteristics to penicillin and tetracycline, appears promising when combined with favorable findings from safety and pharmacokinetic/pharmacodynamic studies (20, 21). As AMR among *N. gonorrhoeae* continues to evolve in numerous countries, gepotidacin warrants further study (as listed at ClinicalTrials.gov) (22) supported by reference-quality susceptibility tests (15, 18) and guided by the MIC QC guidance published here.

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