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Establishment of Quality Control Ranges for the Broth Microdilution Susceptibility Testing of Rezafungin against Yeast

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ABSTRACT

Background: Rezafungin (formerly CD101) is a novel echinocandin with a broad spectrum antifungal activity against Candida, Aspergillus, and Pneumocystis.

The high rezafungin exposures and long half-life in plasma and tissues allow for once-weekly administration that could enable reduced length of hospitalization and facilitate outpatient dosing in the treatment and prevention of invasive disease.

The safety and efficacy of once-weekly rezafungin has been demonstrated in a Phase 2 trial (STREVu) for the treatment of candidemia and invasive candidiasis relative to once-daily caspofungin.

Phase 3 trials of rezafungin are scheduled to initiate in 2018.

To allow for controlled susceptibility testing during clinical development, a Tier 2 M2 study was conducted to establish quality control (QC) ranges for the broth microdilution susceptibility testing of rezafungin against yeast QC organisms (per M27 guidelines).

METHODS

• The Tier 2 broth microdilution QC study was conducted in accordance with CLSI guidelines M23.

• Nine participating labs tested per CLSI guidelines CLSI M27-A3 and CLSI M27-A4.

• Rezafungin and micafungin (test as a comparator) were tested over a range of 0.015-32 g/mL.

• M27-A3 guidelines were used at 24 hr and M27-A4 guidelines at 48 hr. Each site tested 10 independent replicates of each test isolate across five QC ranges.

• Recommended QC ranges as determined in a multi-center study were utilized going forward for clinical trial testing and surveillance and, ultimately, for testing in clinical labs and development of automated test methods.

RESULTS

TABLE 1. Rezafungin MICs – C. parapsilosis ATCC 22019, 24 hr

<table>
<thead>
<tr>
<th>Organsms</th>
<th>MIC Range</th>
<th>QC Range</th>
<th>n</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. parapsilosis ATCC 22019</td>
<td>0.015 – 32 g/mL</td>
<td>0.015 – 1 g/mL</td>
<td>270</td>
<td>0.12 ± 0.04</td>
</tr>
</tbody>
</table>

A total of 2393 MIC values were consistent for each QC organism with minimal variation across the five QC ranges.

TABLE 2. Rezafungin MICs – C. krusei ATCC 270, 24 hr

<table>
<thead>
<tr>
<th>Organsms</th>
<th>MIC Range</th>
<th>QC Range</th>
<th>n</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. krusei ATCC 270</td>
<td>0.015 – 32 g/mL</td>
<td>0.015 – 1 g/mL</td>
<td>270</td>
<td>0.06 ± 0.05</td>
</tr>
</tbody>
</table>

MIC values were read at 24 hr (standard incubation time for echinocandins) as well as 48 hr based on an endpoint of 90% inhibition relative to the growth control (standard for echinocandins).

- Results were tabulated for each organism and evaluated overall, by test site, and by medium type.

- QC ranges were determined after analysis by CLSI methods (modified MIC ≤ one-dilution; when there is a significant shoulder >60% of the model) the range is extended in the direction of the shoulder by an additional dilution and by RangeFinder (Turmidge and Boshart AAC 2007,51:2483).

- There was no growth in unincubated negative control wells and growth in no drug positive control across all panels.

- QC values for rezafungin were within the established CLSI QC ranges for both 24 and 48 hr for both QC organisms with the exception of one laboratory where 3 results for C. krusei ATCC 270 were out of QC at 24 hr.

- Removal of rezafungin data from analysis in instances when rezafungin was out of QC had no impact on the recommended QC range.

- Rezafungin tested consistently with minimal interlaboratory and interassay variation and no apparent variation by media lot.

- QC ranges for the rezafungin data (ATCC 6258) were as follows:

  - C. parapsilosis ATCC 22019: 0.50 (0.25-1) g/mL
  - C. krusei ATCC 270: 0.12 (0.06-0.5) g/mL

- Recommended ranges were identical using both CLSI and RangeFinder methods.

- Ranges were approved for use by the CLSI antifungal AST subcommittee in January 2018.

CONCLUSIONS

- The QC ranges as determined in a multi-lab, distributed, Tier 2 study allow for a reliable and consistent way to qualify and test in clinical labs and development of automated test methods post-approval.

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