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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 (RZF; formerly CD101) is a novel echinocandin with a long plasma half-life and enhanced stability that allows for once-weekly IV dosing. RZF has potent in vitro activity against *Candida* spp. with minimal inhibitory concentration (MIC) values similar to those of anidulafungin, micafungin, and caspofungin. It is currently in clinical development for the treatment of candidemia and invasive candidiasis (NCT02734862). A multi-laboratory study conducted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines has been conducted to establish quality control (QC) ranges for the broth microdilution (BMD) susceptibility testing of RZF against yeast.

Methods: A tier 2 QC study was conducted at 9 test laboratories in accordance with CLSI M23 guidelines. BMD testing of RZF alongside the control agent micafungin was conducted under standard conditions (CLSI M27-A3, M27-S4) using frozen commercial panels (Thermo Fisher, Cleveland, OH). ATCC QC strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were tested. Each laboratory tested 10 independent inocula of each QC strain across 3 lots of RPMI medium from separate manufacturers. MIC values were recorded after 24 and 48 hr of incubation based on 50% inhibition relative to the growth control (standard reporting for echinocandins is based on 50% inhibition at 24 hr).

Results: RZF MIC values were consistent for each QC organism with minimal variation across testing laboratories and no apparent variation by media lot. MIC values of micafungin were within the established CLSI QC range at both 24 and 48 hr for both QC organisms with the exception of one laboratory where 13 results for *C. krusei* ATCC 6258 were out of QC at 24 hr. The recommended RZF QC ranges as determined by both CLSI M23 guidelines and RangeFinder analysis methods were identical. Removal of RZF data from analysis for instances where micafungin was out of QC for *C. krusei* ATCC 6258 at 24 hr had no impact on the recommended range. Recommended QC ranges for the testing of RZF based on the compiled data are as follows:

Organism	Incubation Time	Recommended QC Range (µg/mL)	N	n (%) in Range
C. parapsilosis ATCC 22019	24 hr	0.25 – 1	270	267 (98.9)
	48 hr	0.25 – 2	270	270 (100)
C. krusei ATCC 6258	24 hr	0.015 – 0.12	270	270 (100)
	48 hr	0.015 – 0.12	270	270 (100)

Conclusions: The QC ranges recommended for RZF and the QC organisms above provide a reliable means for the quality control of RZF during BMD susceptibility testing against *Candida* spp. going forward.

INTRODUCTION

- Rezafungin (formerly CD101) is a novel echinocandin with 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 (STRIVE) 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 M23 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 guideline M23.
- Nine participating labs tested per CLSI guidelines CLSI M27-A3 and CLSI M27-S4:

Investigator	Laboratory
Gerald A. Denys	Indiana University School of Medicine, Indianapolis, IA
Dwight J. Hardy	University of Rochester Medical Center, Rochester, NY
Dan Diekema	University of Iowa, Iowa City, IA
Maria M. Traczewski	The Clinical Microbiology Institute, Wilsonville, OR
Cynthia C. Knapp	Thermo Fisher Scientific, Inc., Cleveland, OH
Chris Pillar	Micromyx, LLC, Kalamazoo, MI
Robert Rennie	University of Alberta Hospital, Edmonton, Alberta, Canada
Mahmoud Ghannoum	Case Western Reserve University, Cleveland, OH
Michael Huband	JMI Laboratories, North Liberty, IA

- Frozen broth microdilution panels were manufactured by Thermo Fisher Scientific (Cleveland, OH) and were distributed to the sites for testing.
- Each panel contained three different lots of RPMI from three separate manufacturers (Hyclone, Irvine Scientific, and Sigma).
- Rezafungin and micafungin (tested as a comparator/control) were both tested over a range of 0.002 – 4 µg/mL.
- Test isolates: *C. parapsilosis* ATCC 22019 & *C. krusei* ATCC 6258
- Each site tested 10 independent replicates of each test isolate (270 MIC values overall per isolate, 30 per site [10 inocula across three lots]).
- Each site determined the concentration of viable yeast inoculated for a minimum of 5 replicates for each organism during the course of the study:

Organism	Counts (N)	Range (CFU/mL)	Mean ± SD (CFU/mL)
C. parapsilosis ATCC 22019	58	$1.60 \times 10^2 – 2.32 \times 10^3$	$1.01 \times 10^3 \pm 4.70 \times 10^2$
C. krusei ATCC 6258	59	$1.40 \times 10^2 – 2.06 \times 10^3$	$7.78 \times 10^2 \pm 3.75 \times 10^2$

Standard inoculum density = $5 \times 10^2 – 2.5 \times 10^3$ CFU/mL

- MIC values were read at 24 hr (standard incubation time for echinocandins) as well as 48 hr based on an endpoint of 50% inhibition relative to the growth control well (standard for echinocandins).
- Results were tabulated for each organism and evaluated overall, by test site, and by medium lot.
- QC ranges were determined after analysis by CLSI methods (modal MIC ± one-dilution; when there is a significant shoulder [>60% of the mode] the range is extended in the direction of the shoulder by an additional dilution) and by RangeFinder (Turnidge and Bordash AAC 2007;51:2483).

RESULTS

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

MIC (µg/mL)	Media Lot A	Media Lot B	Media Lot C	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Total
0.06													
0.12	1	1	1							3			3
0.25	5	4	4	7						3		3	13
0.5	59	59	53	17	30	30	10	30	2	21	4	27	171
1	25	26	32	6			20		28	3	26		83
2													
4													
Total	90	90	90	30	30	30	30	30	30	30	30	30	270
GEOMEAN	0.574	0.583	0.611	0.489	0.500	0.500	0.794	0.500	0.955	0.434	0.912	0.467	0.589
MODE	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	1	0.5	1	0.5	0.5
MIN	0.12	0.12	0.12	0.25	0.5	0.5	0.5	0.5	0.5	0.12	0.5	0.25	0.12
MAX	1	1	1	1	0.5	0.5	1	0.5	1	1	1	0.5	1
RANGE	4	4	4	3	1	1	2	1	2	4	2	2	4

TABLE 2. Rezafungin MICs – *C. parapsilosis* ATCC 22019, 48 hr

MIC (µg/mL)	Media Lot A	Media Lot B	Media Lot C	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Total
0.06													
0.12													
0.25													
0.5	40	39	22		27	22		18		4		30	101
1	23	41	62	25	3	8	22	12	11	26	19		126
2	27	10	6	5			8		19		11		43
4													
Total	90	90	90	30	30	30	30	30	30	30	30	30	270
GEOMEAN	0.905	0.800	0.884	1.122	0.536	0.602	1.203	0.660	1.551	0.912	1.289	0.500	0.862
MODE	0.5	1	1	1	0.5	0.5	1	0.5	2	1	1	0.5	1
MIN	0.5	0.5	0.5	1	0.5	0.5	1	0.5	1	0.5	1	0.5	0.5
MAX	2	2	2	2	1	1	2	1	2	1	2	0.5	2
RANGE	3	3	3	2	2	2	2	2	2	2	2	1	3

TABLE 3. Rezafungin MICs – *C. krusei* ATCC 6258, 24 hr

MIC (µg/mL)	Media Lot A	Media Lot B	Media Lot C	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Total
0.004													
0.008													
0.015	2	21	15	5		9		3		2		19	38
0.03	43	41	49	9	23	17	7	26	2	16	22	11	133
0.06	34	25	23	16	4	4	22	1	15	12	8		82
0.12	4				3		1						4
0.25													
Total	83	87	87	30	30	30	30	30	17	30	30	30	257
GEOMEAN	0.042	0.031	0.032	0.039	0.038	0.027	0.052	0.029	0.055	0.038	0.036	0.019	0.035
MODE	0.03	0.03	0.03	0.06	0.03	0.03	0.06	0.03	0.06	0.03	0.03	0.015	0.03
MIN	0.015	0.015	0.015	0.015	0.03	0.015	0.03	0.015	0.03	0.015	0.03	0.015	0.015
MAX	0.12	0.06	0.06	0.06	0.12	0.06	0.12	0.06	0.06	0.06	0.06	0.03	0.12
RANGE	4	3	3	3	3	3	3	3	2	3	2	2	4

TABLE 4. Rezafungin MICs – *C. krusei* ATCC 6258, 48 hr

MIC (µg/mL)	Media Lot A	Media Lot B	Media Lot C	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Total
0.004													
0.008													
0.015	1	22	13	5		8		3				20	36
0.03	41	40	53	9	23	18	8	25	4	18	19	10	134
0.06	45	28	24	16	4	4	22	2	26	12	11		97
0.12	3				3								3
0.25													
Total	90	90	90	30	30	30	30	30	30	30	30	30	270
GEOMEAN	0.044	0.031	0.033	0.039	0.038	0.027	0.050	0.029	0.055	0.040	0.039	0.019	0.036
MODE	0.06	0.03	0.03	0.06	0.03	0.03	0.06	0.03	0.06	0.03	0.03	0.015	0.03
MIN	0.015	0.015	0.015	0.015	0.03	0.015	0.03	0.015	0.03	0.03	0.03	0.015	0.015
MAX	0.12	0.06	0.06	0.06	0.12	0.06	0.06	0.06	0.06	0.06	0.06	0.03	0.12
RANGE	4	3	3	3	3	3	2	3	2	2	2	2	4

NOTE: Dashed lines indicate recommended QC range

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

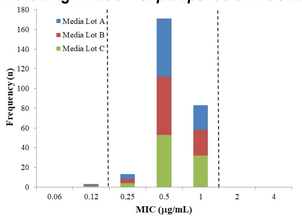


FIGURE 2. Rezafungin MICs – *C. parapsilosis* ATCC 22019, 48 hr

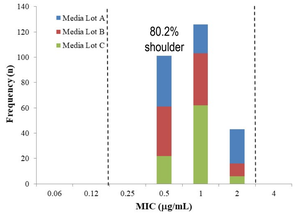


FIGURE 3. Rezafungin MICs – *C. krusei* ATCC 6258, 24 hr

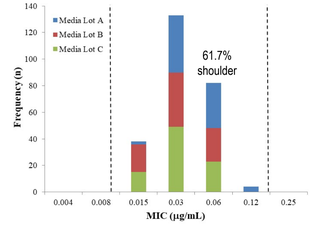
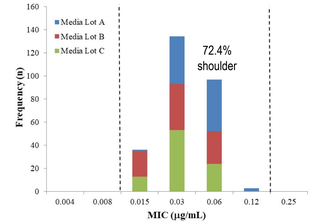


FIGURE 4. Rezafungin MICs – *C. krusei* ATCC 6258, 48 hr



- There was no growth in uninoculated negative control wells and growth in no drug positive control wells across all panels.
- MIC values for micafungin were within the established CLSI QC range at both 24 and 48 hr for both QC organisms with the exception of one laboratory where 13 results for *C. krusei* ATCC 6258 were out of QC at 24 hr.
- Removal of rezafungin data from analysis in instances where micafungin was out of QC had no impact on the recommended QC range.
- Rezafungin tested consistently with minimal interlaboratory and intralaboratory variation and no apparent variation by media lot at both 24 and 48 hr for each of the evaluated organisms (**Tables 1-4; Figures 1-4**).
- The recommended QC ranges for rezafungin were as follows:

Organism - incubation time	Requested QC Range (µg/mL)	Total N	n (%) in Range
C. parapsilosis ATCC 22019 – 24 hr	0.25 – 1	270	267 (98.9)
C. parapsilosis ATCC 22019 – 48 hr	0.25 – 2	270	270 (100)
C. krusei ATCC 6258 – 24 hr	0.015 – 0.12	270	270 (100)
C. krusei ATCC 6258 – 24 hr	0.015 – 0.12	257[1]	257 (100)
C. krusei ATCC 6258 – 48 hr	0.015 – 0.12	270	270 (100)

[1] 13 rezafungin MIC values removed due to out of QC results with micafungin at Lab 6

- 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 provide a reliable way to quality control rezafungin broth microdilution susceptibility testing of *Candida* spp.
- These ranges should be utilized going forward for clinical trial testing and surveillance and, ultimately, for testing in clinical labs and development of automated test methods post-approval.

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