Rezafungin *In Vitro* Activity against Phase 2 STRIVE Part A and Contemporary Clinical Nordic *Candida* Isolates Determined by the EUCAST Reference Method

Marie Helleberg[1,2], Karin Meinike Jørgensen[2], Raluca Datcu[2], Rasmus Krøger Hare[2], Maiken Cavling Arendrup[2,3,4]

[1]CHIP, Dept Infectious Diseases, Rigshospitalet, [2]Unit of Mycology, Statens Serum Institut, [3]Dept Clinical Microbiology, Rigshospitalet, [4]Dept Clinical Medicine, Copenhagen University , Denmark

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Objectives

Rezafungin is a novel echinocandin, which can be dosed once-weekly due to a prolonged half-life and high plasma exposure). Epidemiological cut-off values (ECOFFs) of rezafungin have not yet been established.

We determined the *in vitro* activity of rezafungin and four comparators against contemporary Nordic clinical isolates of *Candida* and other yeasts and established single centre wildtype upper limits (WT-UL). Subsequently, these WT-UL were used to evaluate rezafungin susceptibility for clinical isolates from the completed part A of STRIVE, the phase 2 trial of rezafungin for treatment of candidaemia and invasive candidiasis (NCT02734862).

Material & methods

- 1,293 clinical isolates (19 *Candida,* 13 other yeast species) from Denmark, Norway and Sweden received at the Danish mycology reference laboratory in 2017-18 were identified using CHROMagar, MALDI-TOF and, when needed, internal transcribed spacer sequencing.
- 95 previously speciated *Candida* clinical isolates were included from the rezafungin Phase 2 STRIVE part A trial.
- EUCAST E.Def 7.3.1 susceptibility testing included rezafungin, anidulafungin, micafungin, amphotericin B and fluconazole.
- WT-UL were established following EUCAST principles for visual and statistical ECOFF value setting.
- *FKS* target genes were sequenced for echinocandin non-WT isolates.

Results

Rezafungin MIC distributions for the most common *Candida* species and modal MICs for rezafungin and comparators are shown in Table 1. Rezafungin had species-specific *in vitro* activity similar to that of anidulafungin and micafungin. At a mg/L basis rezafungin was overall less active (modal MICs ≥ 2 two-fold dilutions higher) than anidulafungin and micafungin, but equally or more active than fluconazole and amphotericin B, except against *C. parapsilosis*, other *Candida* (amphotericin B only) and other yeasts.

Rates of rezafungin non-WT isolates among the most common *Candida* species were <3%, only marginally higher than for amphotericin B and lower than for fluconazole, anidulafungin and micafungin (Table 2).

We identified 26 (2.0%) echinocandin non-WT isolates (presented as n, species specific non-WT rate):
C. albicans (11, 1.9%), *C. glabrata* (10, 3.3%), *C. tropicalis* (2, 2.7%), *C. dubliniensis* (2, 2.9%) and *C. krusei* (1, 1.2%).

Alterations in Fks1 and/or Fks2 hot-spots (or within ±3 amino acids) were found in all of the 26 non-WT isolates:
Fks1: *C. albicans*: D648Y (1), S645P (1), P1354P/S (2), P1354S (4), R1361R/S (1), R1361G (2); *C. tropicalis*: F650S (1) and S654P (1); *C. dubliniensis*: S645P (2); *C. krusei*: S659F (1).
Fks2: *C. glabrata*: S663P (2), S663F (4) and F659del (1).
Fks1+Fks2: *C. glabrata*: S663F/L630Q (2) and L664Q + Y658N + premature stop-codon (1). The rezafungin MICs for non-WT isolates were 0.25–2 mg/L.

Rezafungin susceptibility testing of 95 *Candida* blood isolates from the STRIVE trial yielded MIC distributions comparable to those for the Nordic clinical isolates (Table 1). One *C. rugosa* isolate had an MIC of >8 mg/L (Table 3), suggesting intrinsic resistance, and one *C. glabrata* isolate had an MIC of 0.5 mg/L, but no mutations in *FKS* target genes.

Table 1: MIC ranges (mg/L) for rezafungin and modal MICs (mg/L) for rezafungin and comparators against clinical isolates of *Candida* and other yeasts

Species	Nordic Isolates							STRIVE isolates		
		RZF		ANF	MFG	AMB	FLC	RZF		
	n	MIC range	Modal MIC	Modal MIC	Modal MIC	Modal MIC	Modal MIC	n	MIC range	Modal MIC
C. albicans	569	0.016-1	0.06	0.004	0.016	0.25	0.125	43	0.016-0.03	0.03
C. dubliniensis	68	0.03 -2	0.125	0.008	0.03	0.06	0.125	3	0.03 - 0.06	ND
C. glabrata	328	0.03 -2	0.125	0.03	0.016	0.25	4	17	0.06 - 0.5	0.06
C. krusei	82	0.03-1	0.125	0.03	0.125	0.5	16	1	1	ND
C. lusitaniae	20	0.125-0.4	0.125	0.03	0.06	0.125	0.5	0		
C. parapsilosis	61	1-4	2	1	2	0.5	0.5	13	2 - 4	4
C. tropicalis	73	0.03-2	0.125	0.03	0.03	0.25	0.5	15	0.03-0.125	0.06
S. cerevisiae	15	0.125-0.5	0.25	0.06	0.125	0.25	8	0		
Other *Candida*[1]	41	0.03- >4	1	0.25	0.25	0.25	4	3	0.125- >4	ND
Other yeast	36	0.03- >4	>4	>4	>4	0.5	0.25	0		
Total	1,293	0.016->4	0.06	0.004	0.016	0.25	0.125	95	0.03 - >4	0.06

[1]Specified in Table 3

Table 2: Rates of non-WT isolates for rezafungin and comparators

Species	Nordic isolates					STRIVE
	% >WT-UL	% >ECOFF				% >WT-UL
	RZF	ANF	MFG	AMB	FLC	RZF
C. albicans	1.9	1.1	6.2	0	5.6	0
C. dubliniensis	2.9	2.9[1]	2.9[1]	0[1]	6.3[1]	0
C. glabrata	3.0	3.3	4.9	0	8.2	5.9
C. krusei	1.2	2.4	3.7	0	ND	0
C. lusitaniae	0	0[1]	5.0[1]	5.0[1]	10.0[1]	ND
C. parapsilosis	0	0	0	0	4.9	0
C. tropicalis	2.7	2.7	2.7	0	8.2	0
S. cerevisiae	0	0[1]	0[1]	0[1]	0[1]	ND

[1]% >WT-UL based on contemporary Nordic clinical isolates.
AMB: amphotericin B, ANF: anidulafungin, FLC: fluconazole, MFG: micafungin, RZF: rezafungin.

Table 3: Rezafungin MICs (mg/L) for Other *Candida* species

Susceptibility	Species	n	MIC Range
High	*C. inconspicua*	3	0.06
	C. intermedia	1	0.125
	C. nivariensis	2	0.06-0.125
	C. norvegensis	1	0.125
	C. pelliculosa	1	0.06
	C. utilis	3	0.03-0.06
Mixed	*Candida* spp. (new)[1]	4	0.06->8
Low	*C. doubushaemulonis*	1	0.5
	C. fermentati	3	1-2
	C. guilliermondii	14	0.25-2
	C. metapsilosis	5	0.5-1
	C. orthopsilosis	5	0.5-2
	C. rugosa	1	>8

41 Nordic isolates and 3 isolates from STRIVE
[1]Related to *C. blankii,* increase in rezafungin MIC over time with exposure to echinocandin

Conclusion

- Rezafungin displayed broad *in vitro* activity with a species-specific susceptibility pattern similar to that of other echinocandins.
- WT-UL were suggested for the most common species
- Few non-WT strains with alterations in *FKS* target genes or reduced susceptibility to rezafungin were identified among clinical *Candida* isolates from Nordic surveillance and the STRIVE trial.

Contact: marie.helleberg@regionh.dk or MACA@ssi.dk

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