EUCAST Susceptibility Testing of APX001A:

MIC Data for Contemporary Clinical Blood Stream Isolates

STATENS SERUM INSTITUT

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Objectives

The new antifungal APX001 is a first-in-class small molecule drug candidate and the methyl phosphate prodrug of the active moiety APX001A. It targets and inhibits the conserved fungal inositol acyltransferase enzyme Gwt1, thereby preventing GPI-anchored protein maturation and compromising fungal growth. APX001 is in the reporting phase of Phase 1 clinical trials (oral and IV). APX001A was included in our EUCAST AFST on blood stream isolates referred for identification and susceptibility determination as part of the nationwide fungaemia surveillance programme in Denmark to generate contemporary MIC data for this compound and for future epidemiologic cut off (ECOFF) and clinical breakpoint setting

Material/methods

The MICs of APX001A and 7 other antifungal agents were determined against 425 clinical blood stream isolates (obtained Oct 2016 to Aug 2017). The ECOFFfinder programme was used for determining a statistical APX001A ECOFF for species represented by more than 50 isolates. Methods in brief:

1. EUCAST E.Def 7.3 (yeast) and EUCAST E.Def 9.3 (mould) using Cell-culture treated Nunc plates
2. Plates prepared by serial two-fold dilution and stored at –80 °C for ≥24 h prior to inoculation
3. MICs determined spectrophotometrically as 50% inhibition endpoints
4. Antimycotics and concentration ranges for presented data: APX001A; 0.5-0.0005 mg/L, amphotericin B, anidulafungin and micafungin; 4-0.004 mg/L and fluconazole; 32-0.03 mg/L

In total, 3400 MICs were generated.

Results

Isolates

Over the 11-month period, 425 clinical blood stream isolates were investigated. They comprised 19 different species from four different genera (Table 1).

Candida

APX001A displayed potent activity across most species including: *C. albicans* MIC_{50} 0.008 mg/L (range 0.001-0.03 mg/L), *C. dubliniensis* MIC_{50} 0.004 mg/L (range 0.002-0.03 mg/L), *C. parapsilosis* MIC_{50} 0.03 mg/L (range 0.008-0.03 mg/L) and *C. tropicalis* MIC_{50} 0.008 (range 0.004-0.125 mg/L), as well as against the more rare species such as *C. fermentati, C. guilliermondii, C. lusitaniae, C. orthopsilosis,* and *C. pelliculosa* for which the MICs were all ≤0.03 mg/L. The APX001A MICs were slightly higher against *C. glabrata* (MIC_{50} 0.06 mg/L; range 0.008-0.25 mg/L) whereas they were clearly elevated against *C. krusei* (≥0.5 mg/L) which constituted 5.4% (23/425) of the blood stream isolates. Tentative single centre statistical ECOFFs were determined for *C. albicans*: 0.03 mg/L and *C. glabrata*: 0.125 mg/L.

Non-*Candida*

Six blood stream isolates were non-*Candida* species. The MICs against *C. neoformans* and *Magnusiomyces capitatus* were 0.125-0.5 mg/L and the MEC against the *Fusarium dimerum* 0.06 mg/L.

Table 1. APX001A EUCAST MICs against 425 blood stream isolates received between Oct 2016 and Aug 2017.

		MIC (mg/L)											
Species	**N**	**≤0.0005**	**0.001**	**0.002**	**0.004**	**0.008**	**0.016**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**>0.5**
Candida													
C. albicans	171		1	8	18	81	58	5					
C. dubliniensis	24			5	10	8		1					
C. fermentati	2				1	1							
C. glabrata	136					1	6	36	76	16	1		
C. guilliermondii	6				2	3		1					
C. kefyr	2									1	1		
C. krusei	23											1	22
C. lusitaniae	5				1	2		2					
C. metapsilosis	1					1							
C. nivariensis	1							1					
C. norvegensis	1												1
C. orthopsilosis	1							1					
C. parapsilosis	15					2	5	8					
C. pelliculosa	1	1											
C. tropicalis	29				9	10	8		1	1			
C. utilis	1					1							
Non-*Candida*													
C. neoformans	4										3	1	
*F. dimerum**	1								1				
M. capitatus	1									1			
In total	425	1	1	13	41	110	77	55	78	19	5	2	23

*MEC (minimum effective concentration)

Table 2. MIC_{50} and ranges (mg/L) of APX001A and four of the comparator antifungals.

		MIC_{50} (mg/L) (range) (mg/L)				
	No.	**APX001A**	**Amphotericin B**	**Anidulafungin**	**Micafungin**	**Fluconazole**
Candida						
C. albicans	171	0.008 (0.001 – 0.03)	0.25 (0.06 – 0.5)	≤0.004 (≤0.004 – 0.016)	0.008 (≤0.004 – 0.03)	0.25 (0.06 - 1)
C. dubliniensis	24	0.004 (0.002 - 0.03)	0.06 (0.03 - 0.125)	0.008 (≤0.004 – 0.016)	0.016 (≤0.004 – 0.03)	0.25 (0.06 - 0.5)
C. glabrata	136	0.06 (0.008 – 0.25)	0.5 (0.06 - 0.5)	0.016 (0.008 - 0.25)	0.016 (≤0.004 – 0.125)	4 (0.5 - >32)
C. krusei	23	>0.5 (0.5 - >0.5)	1 (0.5 - 1)	0.03 (0.016 - 0.06)	0.125 (0.06 - 0.5)	32 (16 - >32)
C. parapsilosis	15	0.03 (0.008 – 0.03)	0.5 (0.25 - 1)	1 (0.5 - 2)	2 (0.5 - 2)	1 (0.5 - 2)
C. tropicalis	29	0.008 (0.004 – 0.125)	0.5 (0.25 - 0.5)	0.016 (≤0.004 – 0.03)	0.03 (≤0.004 – 0.06)	0.5 (0.25 - >32)
Other*	21	0.008 (≤0.0005 - >0.5)	0.25 (0.125 - 1)	0.125 (≤0.004 - 2)	0.125 (0.016 - 0.5)	2 (0.25 - 32)
Non-*Candida*						
C. neoformans	4	0.25 (0.25 - 0.5)	0.25 (0.25 - 0.5)	>4 (>4)	>4 (>4)	8 (2 - 16)
*F. dimerum***	1	- (0.06)	- (1)	- (>4)	- (>4)	- (>32)
M.capitatus	1	- (0.125)	- (1)	- (4)	- (>4)	- (16)
In total	**425**					

*The 10 least frequent *Candida* species included in table 1 are grouped together here. **MEC (minimum effective concentration)

Conclusion

APX001A displayed uniform and potent activity against most blood stream isolates with MIC_{50}s ≤0.06 mg/L for 88% of the isolates including a range of species such as *C. glabrata*, *C. fermentati, C. guilliermondii, C. lusitaniae, C. parapsilosis* and *C. orthopsilosis* that are characterised by intrinsic reduced susceptibility to at least one of the currently available three antifungal drug classes. *C. glabrata* was slightly less susceptible but still characterised by a single centre ECOFF of 0.125 mg/L. Ongoing Phase 1 studies will provide important data regarding achievable drug exposure and thus whether this MIC range is sufficiently covered during standard dosing. *C. krusei*, which is an uncommon human pathogen, was the only *Candida* species for which MICs were consistently at 0.5 mg/L or greater. Thus, APX001A exhibited promising *in vitro* activity against contemporary blood stream isolates in this ongoing nationwide surveillance programme.

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