EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Posaconazole and *Aspergillus* spp.	**Rationale for the EUCAST clinical breakpoints, V. 1.0**	17th January 2012

Foreword

EUCAST
The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is organised by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the European Centre for Disease Prevention and Control (ECDC), and the active national antimicrobial breakpoint committees in Europe. EUCAST was established by ESCMID in 1997, was restructured in 2001-2002 and has been in operation in its current form since 2002. The current remit of EUCAST is to harmonise clinical breakpoints for existing drugs in Europe, to determine clinical breakpoints for new drugs, to set epidemiological (microbiological) breakpoints, to revise breakpoints as required, to harmonise methodology for antimicrobial susceptibility testing, to develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms and to liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance.

Information on EUCAST and EUCAST breakpoints is available on the EUCAST website at http://www.EUCAST.org.

EUCAST rationale documents
EUCAST rationale documents summarise the information on which the EUCAST clinical breakpoints are based.

Availability of EUCAST document
All EUCAST documents are freely available from the EUCAST website at http://www.EUCAST.org.

Citation of EUCAST documents
This rationale document should be cited as: "European Committee on Antimicrobial Susceptibility Testing. Posaconazole and *Aspergillus* spp.: Rationale for the clinical breakpoints, version 1.0, 2012. http://www.eucast.org.

Introduction

Posaconazole is a triazole antifungal agent that has in vitro activity against *Aspergillus, Candida* and *Cryptococcus* spp., as well other less common medically important yeasts and moulds. The agent is approved for the following indications:

i) Refractory or second line invasive fungal diseases: invasive aspergillosis, fusariosis, chromoblastomycosis, coccidioidomycosis and mycetoma.
ii) First-line therapy for the treatment of oropharyngeal candidiasis of patients who have severe disease or who are immunocompromised, for whom a response to topical therapy is expected to be poor. The Diagnostic and Management Guideline for *Candida* Diseases, 2011, by the European Society of Clinical Microbiology and Infectious Disease (ESCMID) recommends posaconazole should not be used as first-line therapy, although it may be used for refractory OPC in cases of fluconazole resistance. It may be also considered for secondary prophylaxis of mucosal candidiasis and/or oesophagitis.
iii) Posaconazole is indicated for prophylaxis of invasive fungal infections in the following patients:
    a. Patients receiving remission-induction chemotherapy for acute myelogenous leukemia (AML) or myelodysplastic syndromes (MDS) expected to result in prolonged neutropenia and who are at high risk of developing invasive fungal infections.
    b. Hematopoietic stem cell transplant (HSCT) recipients who are undergoing high-dose immunosuppressive therapy for graft versus host disease and who are at high risk of developing invasive fungal infections.

The mould species most frequently causing human infections include *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus* and *Aspergillus niger*. The in vitro activity of posaconazole against these species of *Aspergillus* is reasonably uniform, but acquired resistance has been reported, even among isolates obtained from triazole naïve patients (hence routine susceptibility testing is of utmost importance). It should be noted that *Aspergillus* spp. are complexes that comprise rarer sibling species that exhibit differences in their susceptibility to antifungal agents.

The European Committee on Antimicrobial susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has determined breakpoints for posaconazole against *Aspergillus* spp. These breakpoints are tentative and will be revised after two years.

1. Dosage	
	Austria, Denmark, Belgium, Estonia, Finland, France, Germany, Greece, Italy, The Netherlands, Norway, Russia, Spain, Sweden, Switzerland, Turkey, UK
Most common dosage (mg/day)	Refractory invasive fungal infections: In adults, 400 mg twice a day. In patients who cannot tolerate a meal or a nutritional supplement, posaconazole should be administered at a dose of 200 mg four times a day. Duration of therapy should be based on the severity of the underlying disease, recovery from immunosuppression, and clinical response. More fractioned regimens may result in higher systemic drug exposure. Oropharyngeal candidiasis: Loading dose of 200 mg once a day on the first day, then 100 mg once a day for 13 days. Each dose of posaconazole should be administered with a meal, or with a nutritional supplement in patients who cannot tolerate food to enhance the oral absorption and to ensure adequate exposure. Prophylaxis of invasive fungal infections: 200 mg three times a day. Each dose of posaconazole should be administered with a meal, or with a nutritional supplement in patients who cannot tolerate food to enhance the oral absorption and to ensure adequate exposure. The duration of therapy is based on recovery from neutropenia or immunosuppression. For patients with acute myelogenous leukemia or myelodysplastic syndromes, prophylaxis with posaconazole should start several days before the anticipated onset of neutropenia and continue for seven days after the neutrophil count rises above 500 cells per mm^3.
Maximum dosage (mg/day)	400 mg twice a day or 200 mg four times a day. Higher dosages have been used, but may not provide a dose proportional increase in AUC; instead, a decrease in exposure has been observed in pharmacokinetic studies in healthy adult volunteers.
Available formulations	Oral solution.

2. MIC distributions and epidemiological cut-off (ECOFF) values (mg/L)

	0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	ECOFF
Aspergillus flavus	0	0	0	0	5	25	108	90	29	3	2	0	0	1	0	0	0	0	0	0.50
Aspergillus fumigatus	0	0	3	13	213	680	630	282	131	96	26	4	16	15	6	2	0	0	0	0.25
Aspergillus nidulans	0	0	0	4	12	26	24	21	10	1	1	1	1	2	1	0	0	0	0	0.50
Aspergillus niger	0	0	0	1	3	11	78	92	29	7	0	0	0	1	0	0	0	0	0	0.50
Aspergillus terreus	0	0	0	5	25	118	87	21	12	5	3	1	1	0	0	0	0	0	0	0.25
Aspergillus versicolor	0	0	0	0	0	4	7	6	7	1	0	1	0	2	0	0	0	0	0	ND
Aspergillus sydowii	0	0	0	0	4	5	8	18	13	1	0	0	0	0	0	0	0	0	0	ND

The table includes MIC distributions available at the time breakpoints were set. They represent combined distributions from multiple sources and time periods. The distributions are used to define the epidemiological cut-offs (ECOFF) and give an indication of the MICs for organisms with acquired or mutational resistance mechanisms. They should not be used to infer resistance rates. If there are insufficient data no epidemiological cut-off value has been determined (ND).

3. Breakpoints prior to harmonisation (mg/L) S≤ / R>		
	European breakpoints	**CLSI**
General breakpoints:		
	NA	NA
Species specific breakpoints:		
	NA	NA

NA = Not available

4. Pharmacokinetics

	Neutropenic patients receiving cytotoxic chemotherapy for AML or MDS (n=215)	Febrile neutropenic patients or patients with refractory invasive fungal diseases (n=23)	
Dosage (mg)	200 mg x 3	400 mg x 2	200 mg x 4
Cmax (mg/L)			
Cmin (mg/L)			
Cav (mg/L); Mean (CV%) [Range]	0.58 (65) [0.09-2.20]	0.72 (86) [0.01- 2.26]	0.45 (84)
Total body clearance/F (L/h); Mean (CV%) [Range]	51.2 (65) [10.7 – 146]	76.1 (78) [14.9 – 256]	
T ½ (h); Mean (CV%) [Range]	37.2 (39) [19.1 – 148]	31.7 (42) [12.4 – 67.3]	
AUC24h (ng.h/mL) total drug; Mean (CV%) [Range]	15900 (62) [4100 – 56100]	9093 (80) [1564 – 26794]	10628 (83)
Fraction unbound (%)	2	2	
Volume of distribution/F (L); Mean (CV%) [Range]	2425 (39) [828 – 5702]	3088 (84) [407 – 13140]	
Comments	• Posaconazole serum concentrations vary widely, and may be insufficient in some patients receiving therapy. For example, at an American centre 40.6% of the drug level determinations were <0.5 mg/L (http://www.miravistalabs.com). • Posaconazole absorption is affected by gastric pH, prandial state and the timing of dose administration relative to the time of a meal. Strategies to maximize posaconazole exposure include administration with or after a high-fat meal, with any meal or nutritional supplement with an acid beverage or in divided doses. The administration of proton pump inhibitors must be avoided when possible. • Due to the highly variable absorption of the drug, and to prevent therapeutic failure, therapeutic drug monitoring (TDM) may be helpful either in prophylaxis or for treatment of established disease.		
References	• Posaconazole product information (http://www.spfiles.com/pinoxafil.pdf). • Krishna et al. Antimicrob Agents Chemother 2009.; 53: 958-966. • Howard et al. J Infect Dis 2011; 203:1324-32. • Eiden et al. Eur J Clin Microbiol Infect Dis 2011 May 26. PMID: 21611869. • Lebeaux et al. Antimicrob Agents Chemother 2009; 53: 5224-9. • Ullman et al Antimicrob Agents Chemother 2006; 50: 658-666		

5. Pharmacodynamics

Total drug AUC:MIC for half-maximum antifungal effect	167-498			
Total drug AUC associated with a 75% response rate in patients receiving posaconazole as salvage therapy for invasive aspergillosis	Approximately 30 mg.h/L			
Comments	• The AUC:MIC may be the pharmacodynamic index that best links drug exposure with the observed outcome, but this has not been formally demonstrated for *Aspergillus* spp. • An average concentration of 1.25 mg/L is associated with a higher probability of a clinical response for patients with invasive aspergillosis receiving posaconazole as salvage therapy [Walsh et al]. This is equivalent to an AUC of approximately30 mg.h/L. • The posaconazole MIC for *Aspergillus fumigatus* is an important determinant of exposure-response relationships and outcome in preclinical models of invasive aspergillosis [Howard et al]. • An AUC:MIC of 167 is associated with half-maximal effect in a murine neutropenic inhalational model of IPA using galactomannan as a biomarker [Howard et al]. • An AUC:MIC of 321 and 498 are associated with 50% survival in a non-neutropenic model of disseminated aspergillosis using CLSI and EUCAST methodology, respectively [Mavridou et al].			
References	• Posaconazole product information (http://www.spfiles.com/pinoxafil.pdf). • Walsh et al. Clin Infect Dis 2007; 44 :2-12. • Mavridou et al. Antimicrob Agents Chemother 2010; 54: 860-65. • Howard et al. J Infect Dis 2011; 203: 1324-32.			

6. Monte Carlo simulations and Pk/Pd breakpoints

When posaconazole is used as salvage therapy for invasive aspergillosis, the mean AUC following a dosage of 800 mg/day is approximately 13 mg.h/L, which is considerably lower than associated with near maximal antifungal activity.

A population PK model [Abu Tarif et al] fitted to data from patients receiving posaconazole 200 mg three times a day for prophylaxis was used in the Monte Carlo simulations. An assumption was made that there is a dose proportional increase in AUC (i.e. the AUC increases in a linear manner from a dosage of 200 mg three times a day to 200 mg four times a day). A Pk/Pd target from the study of Howard et al (see section 5) was used. The proportion of patients infected with an isolate with the following MICs and receiving 800 mg/day (200 mg four times a day) who achieve a pharmacodynamic target ≥AUC:MIC of 167 is as follows:

MIC (mg/L)	% Target attainment
0.015	99.9%
0.03	96%
0.06	68%
0.125	15.3%
0.25	0.6%

Thus, with fixed dosing of 800 mg/day (200 mg four times a day), drug exposures may not be high enough to cover the entire wild-type distribution reliably. For isolates that are true wild type with an MIC of 0.12 mg/L, a posaconazole trough concentration of 0.9 mg/L is required to achieve an AUC:MIC of 167. For isolates with an MIC of 0.06 mg/L, a trough concentration if 0.4 mg/L is required to achieve an AUC:MIC of 167.

Walsh et al. Clin Infect Dis 2007; 44: 2-12
AbuTarif et al. Curr Med Res Opin 2010;26:397-405
Howard et al. J Infect Dis 2011; 203:1324-32.

7. Clinical data

Correlation of in vitro MIC data with clinical outcome has not been possible as such data sets are not available for EUCAST MICs.

Aspergillosis
Posaconazole has not been investigated as a first-line agent for the treatment of invasive aspergillosis. A clinical trial investigated the efficacy and safety of posaconazole oral suspension (800 mg/day in divided doses) as monotherapy in an open-label, multicenter cohort study in patients with invasive aspergillosis and other mycoses who were refractory to or intolerant of conventional antifungal therapy (i.e., amphotericin B or itraconazole). A total of 107 posaconazole recipients and 86 external control subjects (modified intent-to-treat population) were included. The overall success was 42% for posaconazole recipients and 26% for control subjects (odds ratio, 4.06; 95% confidence interval, 1.50-11.04; P=0.006). The overall success increased from 53% up to 75% in case of mean posaconazole concentrations at 0.719 and 1.250 mg/L, respectively (Walsh et al).

Posaconazole is licensed for the prophylaxis of invasive fungal disease, including aspergillosis, of patients receiving remission-induction chemotherapy for acute myelogenous leukemia or myelodysplastic syndromes as well as for hematopoietic stem cell transplant recipients with Graft versus Host Disease (GvHD). In patients undergoing chemotherapy for acute myelogenous leukemia or the myelodysplastic syndrome, posaconazole prevented the occurrence of invasive fungal infections more effectively than did either fluconazole or itraconazole and improved overall survival (Cornely et al).

Walsh et al. Clin Infect Dis 2007 ; 44: 2-12
Cornely et al. New Eng J Med 2007 ; 356: 348-359
Ullmann et al. New Eng J Med 2007 ; 356: 335-347

Acquired Resistance
The frequency of posaconazole resistance is largely undefined, as many centres do not routinely test the susceptibility of *Aspergillus* isolates. Itraconazole resistance and cross resistance to other triazoles have currently been reported in Belgium, Canada, China, Denmark, France, Norway, Spain, Sweden, The Netherlands, the UK and the USA. Most commonly, resistance is linked to point mutations in the target gene *cyp51A.* However, at some centres a significant proportion of the isolates with elevated posaconazole MICs lack such mutations, suggesting that other mechanisms including upregulated efflux pumps or up-regulation of target production may also play a role. Importantly, in some areas, *A. fumigatus* isolates with acquired resistance mechanisms have been increasingly found in the environment and also found in triazole naive patients failing therapy. This is probably related to agricultural azole pesticide use. The EUCAST MICs for such isolates vary according to the underlying mechanism but are ≥0.25 mg/L for the most commonly identified mutants.

Isolates with defined hot spot *CYP51A* mutations associated with itraconazole resistance may, depending on the target alteration, be cross resistant to posaconazole and/or voriconazole. The posaconazole MICs for such isolates separate less well from the wild type population than with itraconazole (posaconazole peak MIC of 0.5 mg/L versus itraconazole peak MIC of >16 mg/L), making separation of such isolates from the wild type population difficult. Infections caused by such isolates should not be treated with posaconazole as long as clinical evidence is not available that infections involving such isolates respond to treatment as well as infections caused by wild type organisms.

Howard et al. Emerg Infect Dis 2009; 15: 1068-1076.
Snelders et al. PLoS Med 2008; 5: e219.
Rodriguez-Tudela et al. Antimicrob Agents Chemother 2008; 52: 2468-72.

Arendrup et al. PLoS One 2010; 5: e10080.
Snelders et al. Appl Environ Microbiol 2009; 75: 4053-4057.
Mortensen et al. Antimicrob Agents Chemother 2010; 54: 4545-9.
Howard et al. Med Mycol 2011; 49 Suppl 1: S90-5.
Mortensen et al. J Clin Microbiol 2011; 49: 2243-2251.
Chryssanthou. Scan J Infect Dis 1997; 29: 509-12.
Verweij et al. Drug Resistance Updates 2009; 12: 141–147.
Mellado E et al. Antimicrob Agents Chemother 2007; 51: 1897-904.
Mellado E et al. Antimicrob Agents Chemother 2005; 49: 2536-8.
Mellado E et al. Antimicrob Agents Chemother 2004; 48: 2747-50.
Diaz-Guerra TM et al. Antimicrob Agents Chemother. 2003; 47: 1120-4. Erratum in: Antimicrob Agents Chemother 2004; 48:1071.

8. Clinical breakpoints

Non-species-related breakpoints	There is insufficient evidence to set non-species-related breakpoints.
Species-related breakpoints	Breakpoints were based on pharmacokinetic data, microbiological data and clinical experience. There are no clinical data regarding the use of posaconazole as primary therapy for invasive aspergillosis. Clinical data suggest that the wild type population of *A. fumigatus* is susceptible to posaconazole providing adequate serum drug exposure is achieved. Optimal antifungal efficacy in the setting of salvage therapy of invasive aspergillosis requires mean plasma concentrations of approximately 1.25 mg/L. The modal MIC for isolates with mutations in the target gene is 0.5 mg/L, raising concerns that wild type and mutant populations are either poorly separated or overlap. Hence, the breakpoints for *A. fumigatus* are established by classifying the right tail of the wild type distribution as intermediate in order to notify the microbiologist and/or clinician that the isolate may possess acquired resistance mechanisms that may not be an appropriate target for posaconazole. Such isolates should be retested and if necessary referred for confirmatory investigations such as DNA sequencing. MICs of itraconazole and voriconazole should also be determined for these isolates. Isolates classified as being posaconazole "intermediate", and which are not susceptible to itraconazole, probably should not be treated with posaconazole until more clinical information is available. No clinical studies have examined the clinical outcome for a significant number of infections caused by species other than *A. fumigatus*. ***Aspergillus fumigatus*: S ≤0.12, R >0.25 mg/L.** Provided adequate drug exposure has been confirmed using TDM.[1] [1]There remains some uncertainty regarding cut-off values for posaconazole concentrations that separate patients with a high probability of clinical success from those with a low probability of clinical success. In some circumstances (e.g. patients with persistent and profound neutropenia, large lesions, or those with other features associated with a poor clinical outcome) a relatively high trough concentration should be sought. Preclinical and clinical data suggest this value should be >1 mg/L at steady state. For other patient groups a lower trough concentration may be acceptable. For prophylaxis a target concentration of >0.7 mg/L has been suggested. Jang et al. Clin Pharmacol Ther. 2010; 88: 115-9. Andes et al. AAC 2009; 53: 24-34. Rochat et al. AAC 2010; 54: 5074-5081 Decosterd et al. AAC 2010; 54: 5303-5315 Center for Drug Evaluation and Research. Application number 22-003. FDA 2006 Howard et al. Therapeutic Drug Monitoring, *in press* Hope et al. Curr Opin Infect Dis. 2008 Dec; 21(6):580-6

Species without breakpoints	There is inadequate clinical information on the clinical outcome for patients infected with wild type strains of *A. terreus*. The MIC distributions are similar to those for *A. fumigatus*. If the posaconazole exposure-response relationships for *A. terreus* are similar to those for *A. fumigatus* the breakpoints for *A. fumigatus* could be applied to *A. terreus*. However, EUCAST has refrained from setting breakpoints for *A. terreus*, until more data are available. The MIC values for isolates of *A. flavus, A. niger* and *A. nidulans* are in general one dilution step higher than those for *A. fumigatus*. Whether this translates into a poorer clinical response is unknown.
Clinical qualifications	The EUCAST AFST considers posaconazole appropriate therapy for the following *Aspergillus* infections when caused by wild type isolates • Salvage treatment for invasive aspergillosis • Prophylaxis of invasive aspergillosis
Dosage	Breakpoints apply to licensed dosing: Oral dose, 200 mg x 4 or 400 mg x 2 for salvage treatment for invasive aspergillosis. Oral dose, 200 mg x 3 for prophylaxis of invasive aspergillosis.
Additional comments	Posaconazole TDM is recommended to ensure optimal drug exposure. Breakpoints for posaconazole will be reviewed when more data are available for *Aspergillus* species which were not assigned breakpoints during the present review, when there are clinical data for isolates with MIC values outside the wild type distribution or when there are further data related to optimal drug exposures for posaconazole. Effective quality control is essential to avoid loss of potency of posaconazole in susceptibility testing and thereby avoid the risk of susceptible isolates being misclassified as intermediate or resistant.

9. EUCAST clinical MIC breakpoints

All EUCAST breakpoints can be found at **http://www.eucast.org**.

10. Exceptions noted for individual national committees

None.