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## International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*



Paul E. Verweij<sup>a,\*</sup>, Michelle Ananda-Rajah<sup>b</sup>, David Andes<sup>c</sup>, Maiken C. Arendrup<sup>d</sup>, Roger J. Brüggemann<sup>e</sup>, Anuradha Chowdhary<sup>f</sup>, Oliver A. Cornely<sup>g</sup>, David W. Denning<sup>h</sup>, Andreas H. Groll<sup>i</sup>, Koichi Izumikawa<sup>j</sup>, Bart Jan Kullberg<sup>k</sup>, Katrien Lagrou<sup>l</sup>, Johan Maertens<sup>m</sup>, Jacques F. Meis<sup>a,n</sup>, Pippa Newton<sup>h</sup>, Iain Page<sup>h</sup>, Seyedmojtaba Seyedmousavi<sup>a</sup>, Donald C. Sheppard<sup>o</sup>, Claudio Viscoli<sup>p</sup>, Adilia Warris<sup>q</sup>, J. Peter Donnelly<sup>r</sup>

<sup>a</sup> Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands

<sup>b</sup> General Medicine and Infectious Diseases Units, Alfred Health, Melbourne, Australia

<sup>c</sup> University of Wisconsin School of Medicine and Public Health, Madison, USA

<sup>d</sup> Unit of Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark

<sup>e</sup> Department of Pharmacy, Radboud University Medical Centre, Nijmegen, The Netherlands

<sup>f</sup> Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

<sup>g</sup> 1st Department of Internal Medicine, Clinical Trials Centre Cologne – BMBF 01KN1106, Centre for Integrated Oncology CIO Köln Bonn, and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Cologne, Germany

<sup>h</sup> National Aspergillosis Centre, University Hospital of South Manchester, The University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom

<sup>i</sup> Infectious Disease Research Program, Center for Bone Marrow Transplantation and Department of Paediatric Haematology/Oncology, University Children's Hospital, Muenster, Germany

<sup>j</sup> Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

<sup>k</sup> Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands

<sup>l</sup> Department of Microbiology and Immunology and National Reference Center for Mycosis, Universitaire Ziekenhuizen Campus Gasthuisberg, KU Leuven, Leuven, Belgium

<sup>m</sup> Department of Haematology, Universitaire Ziekenhuizen Campus Gasthuisberg, KU Leuven, Leuven, Belgium

<sup>n</sup> Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

<sup>o</sup> Departments of Medicine, and Microbiology and Immunology, McGill University, Montreal, Canada

<sup>p</sup> Division of Infectious Diseases, University of Genova (DISSAL), A.O.U. IRCCS San Martino-IST, Genoa, Italy

<sup>q</sup> Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

<sup>r</sup> Department of Haematology, Radboud University Medical Centre, Nijmegen, The Netherlands

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## ABSTRACT

An international expert panel was convened to deliberate the management of azole-resistant aspergillosis. In culture-positive cases, in vitro susceptibility testing should always be performed if antifungal therapy is intended. Different patterns of resistance are seen, with multi-azole and pan-azole resistance more common than resistance to a single triazole. In confirmed invasive pulmonary aspergillosis due to an azole-resistant *Aspergillus*, the experts recommended a switch from voriconazole to liposomal amphotericin B (L-AmB; Ambisome®). In regions with environmental resistance rates of  $\geq 10\%$ , a voriconazole–echinocandin combination or L-AmB were favoured as initial therapy. All experts recommended L-AmB as core therapy for central nervous system aspergillosis suspected to be due to an azole-resistant *Aspergillus*, and considered the addition of a second agent with the majority favouring flucytosine. Intravenous therapy with either micafungin or L-AmB given as either intermittent or continuous therapy was recommended for chronic pulmonary aspergillosis due to a pan-azole-resistant *Aspergillus*. Local and national surveillance with identification of clinical and environmental resistance

\* Corresponding author at: Department of Medical Microbiology, Radboud University Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: +31 24 3614356.

E-mail address: [Paul.Verweij@radboudumc.nl](mailto:Paul.Verweij@radboudumc.nl) (P.E. Verweij).

patterns, rapid diagnostics, better quality clinical outcome data, and a greater understanding of the factors driving or minimising environmental resistance are areas where research is urgently needed, as well as the development of new oral agents outside the azole drug class.

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## 1. Introduction

*Aspergillus* spp. are responsible for over 200,000 cases of invasive aspergillosis (IA) annually, and global estimates suggest that over 1.2 million patients have chronic pulmonary aspergillosis (CPA) and 4.8 million suffer from allergic bronchopulmonary aspergillosis (ABPA) with most infections being caused by *Aspergillus fumigatus* (Denning et al., 2011b, 2013). Potentially fatal IA was first highlighted in 1970 by Young and colleagues who reported a series of 98 patients with IA diagnosed at autopsy (Young et al., 1970). Therapeutic advances in cancer, organ transplantation and inflammatory/autoimmune disorders have inadvertently resulted an ever increasing number of patients who are susceptible to IA (Brown et al., 2012; Patterson, 2005). Furthermore, tuberculosis, especially in developing countries and the high incidence of chronic obstructive pulmonary disease (COPD) contribute to the high rate of CPA, while the enormous worldwide burden of asthma contributes to the rate of occurrence of ABPA (Brown et al., 2012; Denning et al., 2011b). The mortality associated with IA remains significant despite treatment, i.e. 28.5% in a recent population-based study (Bitar et al., 2014).

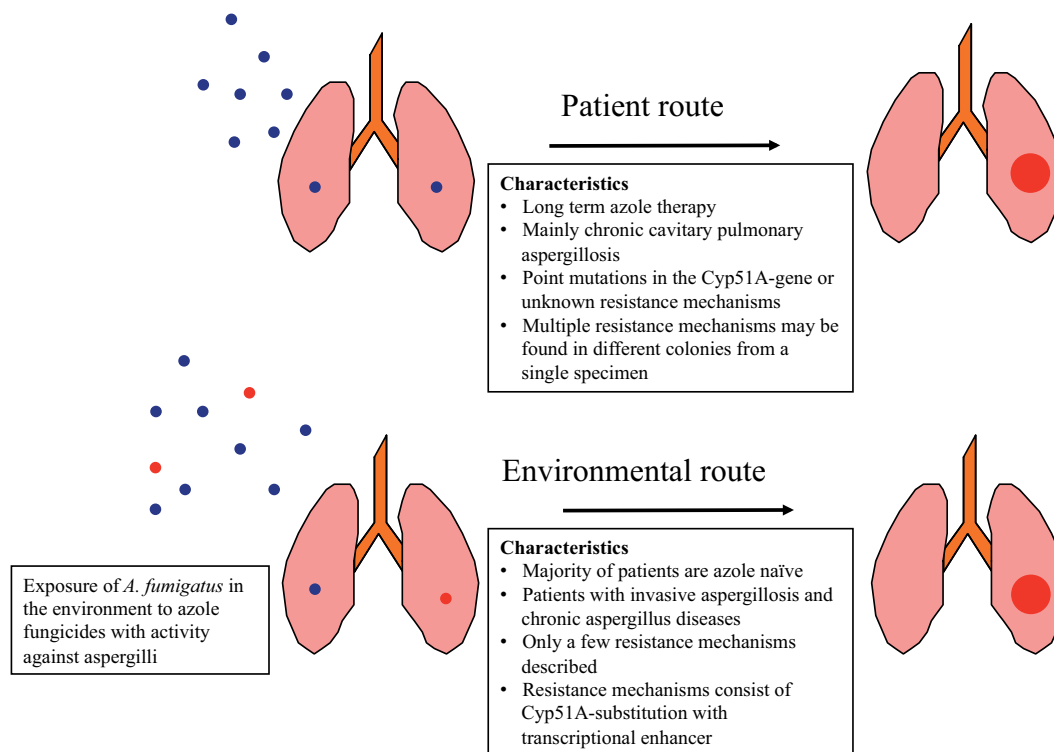
## 2. Clinical background

Since the 1990s, the therapeutic arsenal of antifungal agents has expanded and the azole class of antifungal drugs has become the mainstay for both the treatment and prevention of acute and chronic aspergillosis, especially among immunocompromised hosts (Herbrecht et al., 2002; Walsh et al., 2008; Maertens et al., 2011; Herbrecht et al., 2015). Mould-active azoles are the only systemic agents that can be used both orally and intravenously, and their use is associated with improved survival and quality of life in patients with aspergillosis (Neofytos et al., 2009). However, *A. fumigatus* isolates harbouring mutations to one or more azole drugs, the latter denoting a multi-azole – or pan-azole – resistant phenotype, have emerged among patients receiving chronic azole therapy (Howard et al., 2009; Camps et al., 2012). Azole resistance is commonly due to mutations in the *cyp51A*-gene, which encodes the target enzyme of antifungal azoles, and *A. fumigatus* colonies cultured from patients receiving long-term treatment can represent different azole-resistant genotypes (Fig. 1). By contrast, studies from the Netherlands have shown a limited number of resistance mechanisms to predominate in *A. fumigatus* isolates recovered from azole-naïve patients and from the environment (Fig. 1). The main resistance mechanism elaborated by these isolates consists of a substitution of leucine for histidine at codon 98 of the *cyp51A*-gene, in combination with a 34-bp tandem repeat in the promoter region of this gene (TR<sub>34</sub>/L98H) (Snelders et al., 2008; van der Linden et al., 2011). Moreover, TR<sub>34</sub>/L98H has also been reported in other European countries (van der Linden et al., 2015) and there is a fear that azole resistance could become a global public health threat as fungal spores could disperse widely given their ability to cover thousands of miles in the air (Vermeulen et al., 2013; Chowdhary et al., 2013). This could explain why TR<sub>34</sub>/L98H has also been reported in countries as far afield as China, the Middle East, India, Africa, Australia and most recently Turkey (Lockhart et al., 2011; Chowdhary et al., 2012; Seyedmousavi et al., 2013a; Chowdhary et al., 2014b; Chowdhary et al., 2015; Kidd et al., 2015;

Özmerdiven et al., 2015). Surveillance studies from Europe, India and Africa have also described the emergence of a new combination of mutations (TR<sub>46</sub>/Y121F/T289A) in *A. fumigatus* that confers high level resistance to voriconazole (Vermeulen et al., 2012; van der Linden et al., 2013; Astvad et al., 2014; Chowdhary et al., 2014a; Chowdhary et al., 2014b; Steinmann et al., 2015; van der Linden et al., 2015; Lavergne et al., 2015). Alarming, azole-resistant isolates recovered from clinical specimens, show a voriconazole MIC<sub>50</sub> of 8 mg/l for isolates harbouring the TR<sub>34</sub>/L98H resistance mechanisms and a MIC<sub>50</sub> of >16 mg/l for TR<sub>46</sub>/Y121F/T289A isolates (van Ingen et al., 2015). Even isavuconazole, which has only recently been licensed for use showed reduced in vitro activity (Howard et al., 2013b; Gregson et al., 2013) and reduced in vivo efficacy (Lepak et al., 2013a; Seyedmousavi et al., 2015) in *A. fumigatus* isolates harbouring these resistance mechanisms. Isavuconazole MICs were higher in strains with reduced susceptibilities to other triazoles, mirroring changes in voriconazole susceptibility (Gregson et al., 2013). Furthermore, there is a likelihood of treatment failure and a fatal outcome when voriconazole is given as monotherapy in accordance with the current recommendations to patients with IA due to azole-resistant isolates harbouring one of these resistance mechanisms (Walsh et al., 2008; Maertens et al., 2011; Snelders et al., 2008; van der Linden et al., 2011; van der Linden et al., 2013; Steinmann et al., 2015).

## 3. Role of the environment

There is mounting evidence that environmental exposure to azole fungicides is driving the emergence of TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A resistance mechanisms (Verweij et al., 2009; Snelders et al., 2012). Isolates harbouring these mechanisms of resistance are widespread in the environment and have been recovered from the environment and in hospitals located in several European countries as well as India, the Middle-East and Africa (Snelders et al., 2009; van der Linden et al., 2011; van der Linden et al., 2013; Badali et al., 2013; Ahmad et al., 2014; Chowdhary et al., 2014a; Chowdhary et al., 2014b; Bader et al., 2015). Crucially, between 64% and 71% of patients with IA due to an azole-resistant *Aspergillus* had never received an azole antifungal and can be considered as azole-naïve (van der Linden et al., 2011; van der Linden et al., 2013). Furthermore, surveillance studies indicate that the TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A resistance mechanisms were responsible for over 80% of aspergillosis due to azole-resistant *Aspergillus* (Vermeulen et al., 2015; Verweij and Leenstra, 2014), and therefore are an important cause of disease in regions where environmental resistance is found. Azole resistance complicates patient management with respect to early detection and treatment, as clinical risk factors for IA due to azole-resistant *Aspergillus* have not been identified and the reported mortality rates are as high as 88% for culture-positive patients (van der Linden et al., 2011; Steinmann et al., 2015). However, there are no consensus guidelines available as yet and only limited clinical evidence is available to guide physicians who will be confronted with managing aspergillosis due to azole-resistant *Aspergillus*. Hence, an international expert meeting was convened to discuss the clinical, diagnostic and therapeutic implications of aspergillosis due to azole-resistant *Aspergillus*.



**Fig. 1.** The two routes of azole resistance development in *A. fumigatus*, and their currently known characteristics (Verweij et al., 2009). Blue indicates azole-susceptible and red azole-resistant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

#### 4. Participants and methods

The panel comprised 21 experts ((paediatric) infectious diseases physicians, pulmonary physicians, medical microbiologists, clinical pharmacologists and haematologists) from 11 countries including Canada, the United States of America, India, Australia, Japan, and six European countries. A straightforward selection procedure was followed to compose the expert panel based on three criteria: professional experience (in the area's of medical mycology, knowledge in general and specifically relating to azole-resistant *Aspergillus* and aspergillosis in general, including mycological diagnosis, in vitro susceptibility testing, antifungal pharmacology, clinical experience in the management of aspergillosis), independency, and ability to work in a group, according to European standards ([http://ec.europa.eu/europeaid/evaluation/methodology/examples/too\\_pan\\_res\\_en.pdf](http://ec.europa.eu/europeaid/evaluation/methodology/examples/too_pan_res_en.pdf)). The panel discussions were chaired by PEV and JPD. Their role was to guide the study panel, propose working arrangements, record findings, encourage contributions, and to facilitate debates. Three clinical scenarios prepared by the chairpersons were presented to the panel (see web extra material for details of the cases). These case discussions generated care pathways that captured critical clinical decision-making nodes (see Figs. 2 and 3). Given the paucity of clinical evidence currently available, the aim was neither to formulate practice guidelines nor to achieve a complete consensus but rather to provide practical recommendations for clinicians pending better quality evidence. Differences in opinion were captured by voting. Finally, areas of research and unmet needs were identified.

#### 5. Invasive pulmonary aspergillosis

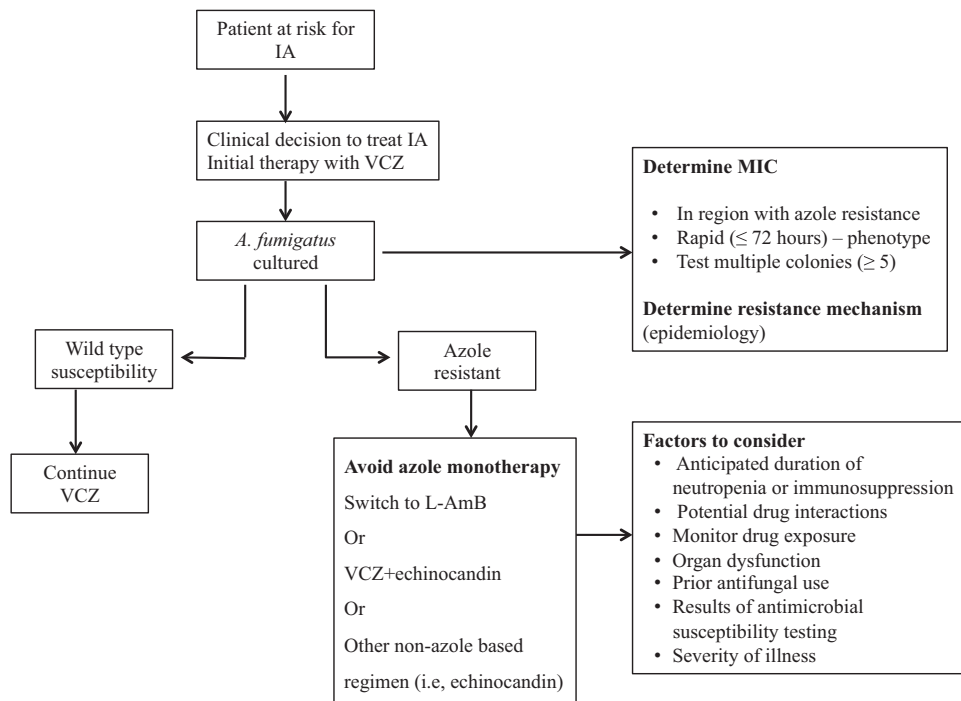
Diagnosis among patients at risk of invasive pulmonary aspergillosis (IPA) is often based solely on the detection of *Aspergillus* galactomannan antigen in clinical specimens, and identification of characteristic abnormalities on computed tomographic

(CT) scan. Unfortunately, conventional culture remains insensitive even though isolation of *Aspergillus* followed by in vitro susceptibility testing is usually required to detect azole resistance. Direct detection of resistance mutations by polymerase chain reaction (PCR) from clinical specimens may expedite the identification of resistance. However, PCR-based assays are not standardised or validated, are currently restricted to reference laboratories and the sensitivity of these tests as well as the spectrum of resistance mechanisms detected are a matter of concern (van der Linden et al., 2010; Denning et al., 2011a; Zhao et al., 2013).

Consequently, discussion regarding the management of patients with aspergillosis due to azole-resistant *Aspergillus* focused on culture-based diagnostic and therapeutic aspects, informed by the local epidemiology or clinical and environmental azole resistance.

##### 5.1. Microbiological diagnosis

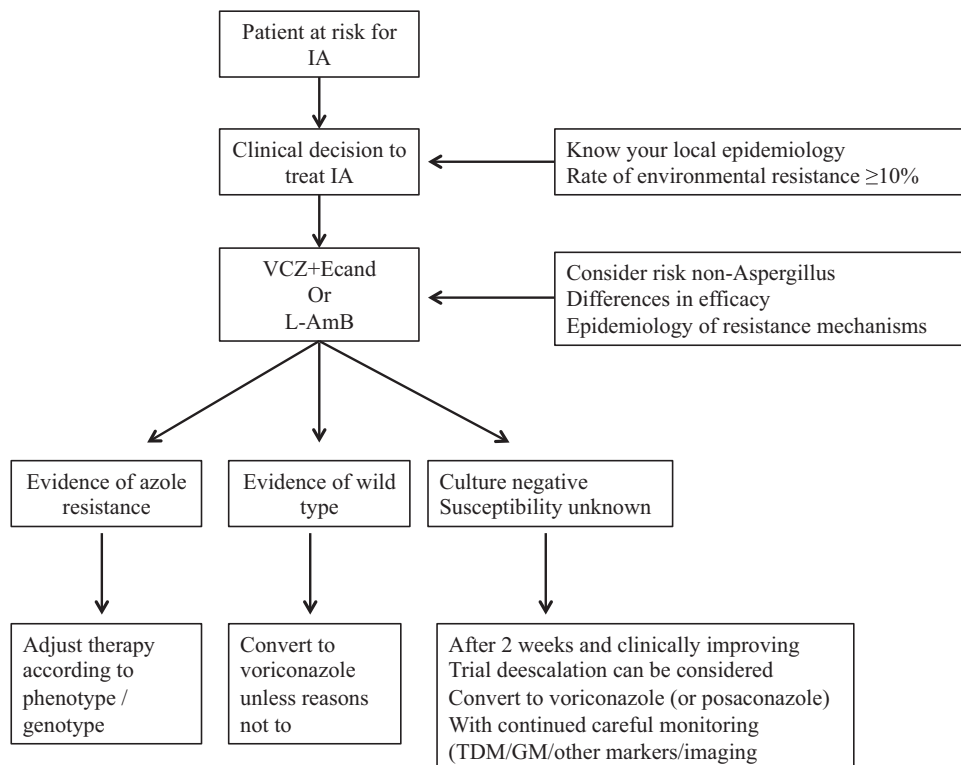
All experts agreed that establishing a microbiological diagnosis was critical to guiding therapy, especially in regions or institutions with high rates of azole resistance. Samples should be obtained prior to therapy and tested using culture-based methods. In practice, this means obtaining appropriate respiratory samples since *Aspergillus* is rarely recovered from blood cultures. All isolates of *Aspergillus* recovered from clinical specimens from patients who will receive antifungal treatment should be identified to species complex level, by the local clinical microbiology laboratory or through referral to a specialist laboratory (Schelenz et al., 2015). Drug susceptibility testing should also be performed, and the results should be reported ideally within 72 h of recovery of *A. fumigatus*. Whenever possible, it was advised to test different, up to five, colonies as different azole susceptibility phenotypes might be present in a single culture. If an azole-resistant *A. fumigatus* is isolated, molecular identification of the resistance mechanism should also be performed for epidemiological reasons although this should not delay MIC determination nor treatment modification.



**Fig. 2.** Management of patients with IPA in regions with no/minimal azole resistance in the environment. IA, invasive aspergillosis; L-AmB, liposomal amphotericin B, VCZ, voriconazole.

To-date, susceptibility testing of azole-resistant *A. fumigatus* isolates has been primarily generated using the CLSI M38-A2 microdilution method and EUCAST microdilution technique (CLSI, 2008; Rodriguez-Tudela et al., 2008; Arendrup et al., 2013). These methods allow azole-resistant phenotypes in *A. fumigatus* to be detected and have enabled epidemiological cut-off

values (ECOFF/ECV) to be established for itraconazole (1 mg/l), voriconazole (1 mg/l) and posaconazole (0.25 mg/l). A preliminary ECOFF/ECV for isavuconazole (CLSI: 1 mg/l; EUCAST 2 mg/l) has also recently been proposed (Espinel-Ingroff et al., 2013; Howard et al., 2013b). Clinical breakpoints for resistance of >2 mg/l have been established for itraconazole and voriconazole and >0.25 mg/l for



**Fig. 3.** Management of patients with clinical suspicion of IPA in regions with environmental resistance of ≥10%. IA, invasive aspergillosis; L-AmB, liposomal amphotericin B, VCZ, voriconazole; Ecand, echinocandin; TDM, therapeutic drug monitoring; GM, galactomannan.

posaconazole (Arendrup et al., 2013). Commercial methods of susceptibility testing are available but currently no studies have fully validated their use for detecting azole resistance in *Aspergillus*. If the appropriate technology is not available in a given centre, the panel recommended that up to five colonies of the isolate should be sent to a reference laboratory for in vitro susceptibility testing. Storage of all the primary isolation plates or subcultures and the results of molecular studies should be considered for future testing, including epidemiological investigations, in order to track the development of acquired resistance in patients on long term azole therapy, or to identify the possible cause of clinical failure in the same patient or in other patients.

## 6. Choice of antifungal therapy in regions with no/minimal azole resistance

In regions, institutions or departments with reliable data showing minimal (<5%) or no azole resistance, all experts agreed that national or international therapy guidelines should be followed; these currently recommend voriconazole as initial therapy for almost all patients with suspected IA (Table 1) (Herbrecht et al., 2002; Walsh et al., 2008; Maertens et al., 2011; Lortholary et al., 2011). All experts but one agreed that voriconazole monotherapy should be discontinued if a voriconazole-resistant *A. fumigatus* was subsequently identified. The dissenting expert argued that the decision should also be based on the patient's clinical conditions, with a patient who was otherwise improving possibly staying on voriconazole monotherapy; this opinion was also based on the absence of clinical data demonstrating a clear-cut correlation between in vitro resistance and clinical outcome. The merit of using voriconazole combination therapy or switching to an alternative agent was also debated. Combined therapy with voriconazole plus anidulafungin showed a trend towards improved outcome compared with voriconazole monotherapy for patients with IA (Marr et al., 2015), but in vitro susceptibility testing results of culture-positive patients were not reported and azole resistance most probably accounted for only a minority of patients, if any. Although preclinical data suggest that the additive action of an azole-echinocandin combination might render an azole-resistant *A. fumigatus* strain more susceptible (Jeans et al., 2012; Seyedmousavi et al., 2013b; Lepak et al., 2013b), no clinical evidence exists to support this combination in IA due to azole-resistant *Aspergillus*. Echinocandin monotherapy might be an alternative, as the in vitro activity of echinocandins against *A. fumigatus* appear unaffected by the presence of an azole resistance mechanism (van Ingen et al., 2015). Caspofungin has been shown to be efficacious for patients with IA (Viscoli et al., 2009; Herbrecht et al., 2010; Cornely et al., 2011), but the success rates were disappointing with outcomes of 50% or less. One experimental study has shown comparable effectiveness of L-AmB against IA due to wild-type and azole-resistant *Aspergillus*, indicating that azole resistance does not diminish the efficacy of L-AmB (Seyedmousavi et al., 2013c). Thus, for azole-resistant strains, there was unanimous agreement that treatment should be switched to L-AmB, depending on the patient and on institutional variables such as cost (Fig. 2).

## 7. Choice of antifungal therapy in regions with epidemiological evidence of azole resistance in environmental isolates

Although azole resistance is increasingly being recognised, resistance rates vary considerably between regions and institutions (Vermeulen et al., 2013; Chowdhary et al., 2013). All experts considered that the most current epidemiological data from national and local surveillance programs should be used to guide the choice

of initial therapy. Levels of environmental azole resistance of 5% to <10% and  $\geq 10\%$  were chosen as the basis for panel discussion regarding the choice of initial antifungal therapy prior to susceptibility results or in the absence of culture.

### 7.1. Azole resistance rate due to environmental mechanisms of >10%

A >10% level of azole resistance was considered by the majority of experts to represent a “high level of resistance” which should prompt a re-evaluation of voriconazole monotherapy for primary treatment. The panel advocated either a combination of voriconazole plus an echinocandin or L-AmB as initial empiric therapy in regions, institutions or departments with this level of resistance pending susceptibility data (Fig. 3). Subsequent therapy would then be dependent upon the results of susceptibility testing, drug monitoring results and clinical considerations, as follows:

- IA due to azole-resistant *Aspergillus* confirmed: ongoing therapy should be with an agent to which the organism is susceptible.
- IA due to azole-susceptible *Aspergillus* confirmed: all experts would continue with voriconazole monotherapy. A minority of experts who chose L-AmB as initial therapy advocated a period of overlap, i.e. continuing with L-AmB until therapeutic levels of voriconazole were achieved. Similar views were expressed by those who chose the voriconazole-echinocandin combination as initial therapy, with discontinuation of the echinocandin once voriconazole levels were adequate. Posaconazole was considered a reasonable alternative when voriconazole was unsuitable.
- Unknown susceptibility (due to culture negativity): the majority of experts who chose L-AmB as initial therapy supported a cautious de-escalation to voriconazole or posaconazole monotherapy after 2 weeks of L-AmB provided the patient was responding clinically. This approach may appear to contradict the rationale for using L-AmB as initial empiric therapy but it was regarded as a pragmatic course of action for a patient who had survived the acute phase of the infection and had achieved clinical stability. This would also allow the use of oral maintenance therapy and discharge to the outpatient setting. Even in the high-level resistance setting, there would still be a high probability that the isolate was azole susceptible. All experts advocated close clinical follow-up, with therapeutic drug monitoring (TDM), relevant biomarkers, and imaging to monitor progress.

### 7.2. Resistance rate due to environmental mechanisms of 5% to <10%

In contrast to the previous scenario, opinions were more divided regarding choice of initial therapy in regions with resistance prevalence rates of 5% to <10%. Approximately half the panel advocated voriconazole monotherapy and the remainder favoured a combination of voriconazole and an echinocandin or L-AmB monotherapy.

Discussion focussed on survival rates shown in studies using voriconazole, L-AmB and caspofungin (all given as monotherapy), or the voriconazole-anidulafungin combination. Although voriconazole was shown to be more effective than conventional AmB, patients starting with this drug could switch to therapy with other licensed agents, including lipid-formulations of AmB, as dictated by the occurrence of toxic effects or a lack of response (Herbrecht et al., 2002). As no head-to-head comparative clinical study between L-AmB and voriconazole has been performed, some experts referred to numerous post-marketing observational reports that have indicated that the effectiveness of voriconazole in the treatment of IA is 15–20% higher than for all amphotericin

**Table 1**Recommendations from national and international guidelines on anti-*Aspergillus* first line drugs (B.E. de Pauw and J.P. Donnelly, unpublished).

Drugs	IDSA	ECIL	UK	Italy	Germany	Australia
Conventional AmB	D	D	D	D	D	AII
L-AmB	AI	BI	AI	BI	AII	BI
ABLC		BII		BII	BII	BII
ABCD		D		BIII	D	BIII
Itraconazole		CIII			BII	
Voriconazole	AI	AI	AI	AI	AI	AI
Posaconazole					D	
Caspofungin		CII	AI	BII	BII	BII
Micafungin					BIII	

AmB, amphotericin B; L-AmB, liposomal amphotericin B; ABLC, amphotericin B lipid complex; ABCD, amphotericin B colloidal dispersion.

B and echinocandin agents (Nivoix et al., 2008; Upton et al., 2007; Pagano et al., 2010; Baddley et al., 2010; Perkhofer et al., 2010; Lortholary et al., 2011). Concern was expressed that a shift away from voriconazole therapy due to increasing azole resistance may come at the expense of lower global efficacy.

## 8. CNS invasive aspergillosis

Central nervous system (CNS) IA, as with other extrapulmonary forms of IA, is uncommon but it is the most feared complication owing to the high mortality rate (Kourkoumpetis et al., 2012). The CNS and the eyes are considered sanctuary sites, because achieving adequate drug concentrations is challenging. Although there are limited data on the treatment of these infections, international guidelines recommend voriconazole as first-line therapy (Walsh et al., 2008) given its favourable pharmacokinetic properties in the brain and greatly improved survival rates (Kethireddy and Andes, 2007; Schwartz et al., 2005). When indicated, neurosurgical intervention may also improve prognosis (Coleman et al., 1995; Kourkoumpetis et al., 2012).

In the context of appreciable rates of environmental azole resistance in *A. fumigatus*, all experts agreed that biopsy samples should be rapidly obtained for microbiological diagnosis, including the use of non-culture-based techniques such as PCR, by a reference laboratory (Kourkoumpetis et al., 2012; Reinwald et al., 2013), rather than relying on characteristic imaging and non-culture-based tests.

Discussion focused on the choice of antifungal therapy for a patient with presumed CNS aspergillosis caused by an azole-resistant *A. fumigatus* isolated from a non-sterile site such as the respiratory tract. Treatment with voriconazole of CNS aspergillosis due to an azole-susceptible *Aspergillus*, has been shown to confer the most favourable clinical response (Schwartz et al., 2005), with other agents such as the polyenes being considered less efficacious (Schwartz et al., 2007). Voriconazole achieves the highest CSF drug levels of any of the azoles with anti-aspergillus activity since itraconazole and posaconazole are virtually undetectable in CSF (Kethireddy and Andes, 2007), although brain tissue concentrations with all these agents are adequate for successful therapy, if systematic exposure is in the accepted range (Felton et al., 2014). The same is true for conventional and lipid-formulations of AmB and for the echinocandins, thus severely limiting the treatment options in patients when the use of voriconazole is precluded. Rabbit models of infection indicated that the absolute concentrations of L-AmB in the brain were significantly higher, 3.6- to 5.2-fold, than for conventional AmB, amphotericin B lipid complex (ABLC) and amphotericin B colloidal dispersion (ABCD) (Lee et al., 1994; Groll et al., 2000). These differences in brain parenchymal concentrations favour the L-AmB formulation and were associated with enhanced therapeutic efficacy in this model. Therefore, based on these limited pharmacokinetic and pharmacodynamic animal data, most experts preferred L-AmB over conventional AmB, ABLC and ABCD for treatment of CNS infection.

There are few reported cases of CNS aspergillosis due to azole-resistant *Aspergillus* and most patients received combination antifungal therapy (van der Linden et al., 2009; Howard et al., 2009; van der Linden et al., 2010; van der Linden et al., 2011; van der Linden et al., 2013). Survival was uniformly poor, with the exception of a single patient who received a combination of L-AmB, caspofungin and posaconazole and survived (van der Linden et al., 2010).

All experts indicated that they would use L-AmB as core therapy in cases of CNS aspergillosis suspected or confirmed to be due to an azole-resistant *Aspergillus*, with L-AmB being administered at a high daily dose (i.e., 5 mg/kg) in accordance with current international guidelines (Walsh et al., 2008). There was unanimous agreement in favour of adding a second drug, with the majority supporting flucytosine (5-FC) and the remaining experts supporting either voriconazole or an echinocandin. Most experts discouraged the use of intrathecal or intralesional antifungal therapy for the treatment of CNS aspergillosis, except in rare circumstances.

The combination of AmB and 5-FC was used for the management of CNS aspergillosis before voriconazole became available in 2002 (Denning and Stevens, 1990). 5-FC achieves good CSF-levels (Block and Bennett, 1972), and experimental models of IA have shown efficacy of 5-FC alone and in combination with AmB (Arroyo et al., 1977; Verweij et al., 2008). Although 5FC has generally shown no in vitro activity against *A. fumigatus* when tested at pH 7.0 the majority of isolates showed low MIC values when tested at pH 5.0 (Verweij et al., 2008). A murine model of disseminated IA showed low MICs of 5-FC at a pH of 5.0 correlated with improved survival (Verweij et al., 2008). These experiments were performed with wild type *A. fumigatus* isolates and, to-date, no in vitro susceptibility data are available for 5-FC against azole-resistant *A. fumigatus* isolates highlighting an area for future research. The use of 5-FC for the management of IA is controversial due to the paucity of clinical studies supporting its use in combination therapy (Burch et al., 1987; Verweij et al., 2008). However, the combination of 5-FC with AmB has been considered to have some role in difficult *Aspergillus* infections (Denning and Stevens, 1990).

Although the in vitro activity of echinocandins against azole-resistant *A. fumigatus* isolates is comparable to that of wild type isolates (van Ingen et al., 2015), echinocandins only attain very low drug levels in the CSF of adult patients and are not recommended for CNS IA (Denning, 2003; Jans et al., 2013), despite the fact that occasional patients have responded to therapy (Maertens et al., 2004).

## 9. Other types of aspergillosis

Patients with CPA, aspergillus bronchitis, ABPA, and severe asthma with fungal sensitisation (SAFS) often respond to antifungal therapy. While discussion mainly focussed on patients with CPA, the experts considered that the same management principles also applied to other conditions. CPA is associated with progressive lung

destruction and a 5-year mortality rate of 50–85% (Nakamoto et al., 2013; Ohba et al., 2012). A recent longitudinal study showed that long-term antifungal therapy in CPA improved the quality of life and prevented progression (Al-Shair et al., 2013; Agarwal et al., 2013). Oral therapy with itraconazole or voriconazole is recommended for patients with CPA due to azole-susceptible *Aspergillus* (Walsh et al., 2008). However, patients receiving long-term azole therapy are at risk for the selection of azole-resistant *A. fumigatus*, especially for difficult-to-treat diseases such as chronic cavitary pulmonary aspergillosis, and when an aspergilloma is present (Verweij et al., 2009; Camps et al., 2012; Howard et al., 2013a). Azole resistance is common in this patient group, the mechanisms of resistance are extremely diverse and they are primarily caused by non-environmental mutations (Howard et al., 2009). In light of this information, the panel discussed strategies by which the management of patients with CPA could be improved.

### 9.1. Diagnostic issues

Azole resistance is frequently under-recognised or inadequately diagnosed in CPA (Denning et al., 2011b). One study using an ultra-sensitive real-time PCR assay detected resistance mutations in 55% of culture-negative, *Aspergillus* PCR positive respiratory samples (Denning et al., 2011a). Although this was not confirmed in a subsequent study using the same methodology and a similar patient cohort (Zhao et al., 2013), the panel agreed that diagnosis could be improved by: (a) periodic culture and susceptibility testing of all isolates from patients on long-term azole therapy; (b) the standard use of high volume sputum culture (Fraczek et al., 2014), with early screening of resistance using azole-containing agar plates possibly being useful; (c) testing of multiple colonies from sputum cultures; and using primary plates to detect resistance, and (d) ensuring that identified isolates are sent to a reference centre for confirmation of the resistance mechanism.

### 9.2. Treatment issues

Evidence shows that suboptimal azole exposure in patients on long-term therapy is a powerful predictor for the emergence of resistance (Howard et al., 2009; Escribano et al., 2012). Suboptimal exposure may result from inadequate dosing, poor adherence, or pharmacokinetic drug–drug interactions, and may be more likely if the burden of infection is high, for example when an aspergilloma is involved (Howard et al., 2009). Thus, the panel recommended the following measures: (a) TDM to ensure sufficient azole exposure; and (b) patient education, emphasising the importance of administration issues and adherence.

CPA requires long-term oral treatment. As azoles are the only oral agent available, they should remain first line therapy regardless of environmental resistance rates. If an individual patient develops resistance to one or two azoles, the panel agreed that therapy should be switched to an alternative azole agent to which the organism is susceptible. In the event of pan-azole resistance development (i.e. to itraconazole, voriconazole, posaconazole), switch to intravenous therapy with a non-azole agent was strongly recommended. Randomised controlled trials in Japan have shown that intravenous micafungin is associated with similar short-term clinical efficacy to voriconazole and to caspofungin in the treatment of CPA (Kohno et al., 2010; Kohno et al., 2011; Kohno et al., 2013). Micafungin is most intensively studied in CPA with over 180 patients reported in the literature (Kohno et al., 2004; Izumikawa et al., 2007; Yasuda et al., 2009; Kohno et al., 2010; Kohno et al., 2011; Kohno et al., 2013), compared to less than 50 CPA patients treated with caspofungin (Kohno et al., 2013; Keir et al., 2014). The clinical response rates were between 42.4% and 78%. A duration of 2 weeks therapy is the minimum demonstrated to be effective and

many patients have been treated for up to 90 days, with benefit. Evidence that supports the use of anidulafungin for the treatment of CPA is currently lacking.

Limited data suggest that L-AmB may be considered as an alternative to azole therapy (Newton et al., 2013). Surgical resection with intravenous antifungal perioperative cover was considered an option for patients with localised azole-resistant disease (Farid et al., 2013). Since CPA generally requires long-term therapy, patients should be carefully monitored for adverse events, including drug toxicities or intolerance.

Non-azole oral agents and other therapeutic approaches were also discussed. Limited data regarding the use of terbinafine are conflicting, and further research is needed to establish the utility of 5-FC. Nebulised AmB may be suitable for some patients with ABPA and SAFS but its use is limited by tolerability, a contraindication for patients with poor pulmonary function, and practical considerations (Chishimba et al., 2014). Intracavitary instillation of AmB may be of benefit for some patients but this treatment should only be done by an expert.

## 10. Research agenda and unmet medical needs

The experts identified a number of areas that require further research and/or represent unmet medical needs.

### 10.1. Epidemiology and diagnosis

Further research is required to establish the environmental prevalence and clinical incidence of azole resistance in *A. fumigatus*. These efforts would be facilitated by local and multi-national surveillance programs, and by active reporting of azole-resistant aspergillosis to public health authorities. There is an urgent need for outcome data documenting clinical success, as well as failure, through European and international registries, pending clinical trial initiatives. In addition, there is a need for a better understanding of the factors driving environmental resistance, together with identification of the practices that can minimise the emergence and persistence of resistance. From a laboratory perspective, validation of commercial methods that enable the rapid and accurate detection of resistance are urgently required, including primary screening agar plates for in vitro susceptibility testing and MIC determination, and methods for phenotypic and genotypic identification. Recently, a commercial PCR assay became available, which enables the detection of two azole resistance mechanisms (TR<sub>34</sub>/L98H and TR<sub>46</sub>/Y121F/T289A) in BAL-fluid and serum (AsperGenius, PathoNostics, The Netherlands). The performance of the PCR-assay is promising in BAL-fluid as it detects and differentiates wild-type from resistant strains, even if BAL-fluid cultures remain negative (Chong et al., 2015). The sensitivity of the assay to detect resistance mutations in serum is suboptimal, as the *Cyp51A*-gene is a single copy gene (White et al., 2015).

### 10.2. Treatment

Investment in drug development for oral alternatives to the azole class of antifungals is needed since we are at risk of losing these important drugs (Denning and Bromley, 2015). Clinical trials are also required to fill gaps in our knowledge regarding existing antifungal agents such as trials to determine the comparative efficacy of azoles and L-AmB, or azoles in combination with L-AmB. Improved in vivo and in vitro models designed to evaluate pharmacokinetic/pharmacodynamic profiles, especially of new drug formulations (e.g. posaconazole), would provide data to further validate clinical breakpoints and guide therapy. The argument for funding these research proposals would be strengthened by

analysing the health and economic consequences of azole resistance.

## 11. Summary

Our current understanding of the emergence of azole resistance in *A. fumigatus* is still limited, as is our clinical experience of treating cases with aspergillosis due to azole-resistant *Aspergillus*. Nevertheless, emerging resistance associated with very poor clinical outcomes was the impetus for these practical recommendations, which, of necessity, were based on the best available evidence supplemented with expert opinion informed by clinical experience and the results of preclinical research. Opinions differed in some instances, but the care pathways proposed may provide some guidance to those confronted with aspergillosis due to azole resistant *Aspergillus*.

## Contributors

The expert meeting was proposed by PEV on behalf of the Dutch Society for Medical Mycology (Nederlandse Vereniging voor Medische Mycologie, NvMy). The meeting took place on October 14th and 15th 2013 in Copenhagen, Denmark. All authors contributed to the discussion, data collection, analysis and writing of the manuscript. Patricia Ingram minuted the expert meeting and assisted with drafting and revising the manuscript, for which she received financial support.

## Conflicts of interest

PEV has received research grants and/or consulted for Pfizer Inc., Merck Sharpe and Dohme Corp., Astellas Pharma Inc., Basilea, F2G Ltd, and Gilead Sciences. MAR has received lecture honoraria from Schering Plough, Gilead Sciences, Merck Sharpe and Dohme Corp. and has been an advisor to Merck Sharpe and Dohme Corp. DA has received research grants and/or consulted for Pfizer Inc., Merck Sharpe and Dohme Corp., Astellas Pharma Inc., Scynexis, and Viamet. MCA has received research grants and acted as a speaker for Astellas Pharma Inc., Gilead Sciences, Merck Sharpe and Dohme Corp. and Pfizer Inc., and has been a consultant for Gilead Sciences, Merck Sharpe and Dohme Corp. and Pcovery. RB has served as a consultant to and has received unrestricted research grants from Astellas Pharma Inc., Gilead Sciences, Merck Sharpe and Dohme Corp., and Pfizer Inc. AC has no conflicts of interests to declare. OAC is supported by the German Federal Ministry of Research and Education (BMBF grant 01KN1106) and the European Commission, and has received research grants from, is an advisor to, or has received lecture honoraria from 3M, Actelion, Astellas Pharma Inc., Basilea, Bayer, Celgene, Cubist, F2G Ltd, Genzyme, Gilead Sciences, GlaxoSmithKline, Merck Sharpe and Dohme Corp., Miltenyi, Optimer, Pfizer Inc., Quintiles, Sanofi Pasteur, Summit/Vifor, Viropharma. DWD holds Founder shares in F2G Ltd a University of Manchester spin-out antifungal discovery company, in Novocyt which markets the Myconostica real-time molecular assays and has current grant support from the National Institute of Allergy and Infectious Diseases, National Institute of Health Research, NorthWest Lung Centre Charity, Medical Research Council, Astellas Pharma Inc. and the Fungal Infection Trust. He acts as a consultant to Trinity group, T2 Biosystems, GSK, Sigma Tau, Oxon Epidemiology and Pulmircort. In the last 3 years, he has been paid for talks on behalf of Astellas Pharma Inc., Gilead Sciences, Merck Sharpe and Dohme Corp. and Pfizer Inc. AHG has served on the speaker's bureau and as a consultant to Astellas Pharma Inc., Cephalon, Gilead Sciences, Merck Sharpe and Dohme Corp., Pfizer Inc., Schering-Plough, and Vicuron Pharmaceuticals. He has received research grants from

Gilead Sciences, Merck Sharpe and Dohme Corp., and Pfizer Inc. KI has received honorarium from Astellas Pharma Inc., Merck Sharpe and Dohme Corp., Pfizer Inc., and Dainippon Sumitomo Pharma Co., Ltd (distributor of L-AMB in Japan). BJK has received lecture honoraria from Pfizer Inc. and has served as an advisor to Astellas Pharma Inc. and Cidara. KL has received research grants from Gilead Sciences, Merck Sharpe and Dohme Corp and Pfizer Inc., received travel support from Merck Sharpe and Dohme Corp., Pfizer Inc. and Gilead Sciences and received lecture honoraria from Gilead Sciences, Merck Sharpe and Dohme Corp., and Pfizer Inc. JM has received grants, personal fees and non-financial support from Pfizer Inc. and Merck Sharpe and Dohme Corp., and personal fees and non-financial support from Gilead Sciences and Astellas Pharma Inc. JFM received grants from Astellas Pharma Inc., Basilea, and Merck Sharpe and Dohme Corp. He has been a consultant to Astellas Pharma Inc., Basilea, and Merck Sharpe and Dohme Corp. and received speaker's fees from Merck Sharpe and Dohme Corp. and Gilead Sciences. PN has received travel support from Merck Sharpe and Dohme Corp., Gilead Sciences, Bristol-Myers Squibb, Johnson & Johnson, and Janssen Pharmaceuticals Inc. IP has received research grants and other donations from Astellas Pharma Inc., Siemens, Serion, Omega, Dynamiker, Associates of Cape Cod and OLM diagnostics. SS has received travel grants from Astellas Pharma Inc. and Gilead Sciences, and a research grant from Astellas Pharma Inc. DCS has received lecture honoraria from Pfizer Inc., Astellas Pharma Inc. and Merck Sharpe and Dohme Corp. and research grants from Merck Sharpe and Dohme Corp. CV has no conflicts of interests to declare. AW has received institutional educational research grants from Gilead Sciences and Pfizer Inc. JPD is a member of the advisory board for Gilead Sciences, and Pfizer Inc., and has been on a speaker's bureau for Gilead Sciences, Merck Sharpe and Dohme Corp. and Pfizer Inc.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.drug.2015.08.001>

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