Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

New-generation triazole antifungal drugs: review of the Phase II and III trials

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In this article, the pharmacological, microbiological and clinical development progress from Phase II and III clinical trials with the new generation triazoles albaconazole, isavuconazole, posaconazole, ravuconazole and voriconazole are reviewed. These drugs exhibit a favorable toxicity profile and possess high activity against resistant and emerging fungal pathogens. Pharmacokinetic may be affected by variability in metabolism and/or gastrointestinal absorption. Only voriconazole and posaconazole have been adequately investigated and are now indicated in the treatment and prophylaxis of invasive fungal diseases. Other triazoles; albaconazole, isavuconazole and ravuconazole are under development; therefore, their future use is unknown.

Keywords: albaconazole • antifungal prophylaxis • antifungal therapy • isavuconazole • Phase II and III trials • posaconazole • ravuconazole • triazoles • voriconazole

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Opportunistic invasive fungal diseases (IFDs) are a major cause of morbidity and mortality in immunocompromised patients, particularly those affected by hematological diseases and cancer, and those undergoing transplant procedures or prolonged immunosuppressive therapy. Considerable progress in treating systemic mycoses has been achieved in recent years through better use of old antifungal agents and through development of new drugs in association with more advanced diagnostic procedures. The search for new antifungal strategies has been mainly focused on the reduction of toxicity, enhancement of bioavailability, improvement of the antifungal spectrum and counteraction of resistance. The introduction of the first-generation triazoles; fluconazole and itraconazole, represented a major advance in the treatment of IFDs, and they have been recommended, along with amphotericin B, as a first-line prophylaxis and therapy of IFDs for several years, until the advent of the new antifungal molecules [1,2]. Both drugs continue to be widely used for the prevention and treatment of superficial and deep-seated fungal infections; however, a number of clinically important limitations were demonstrated early in relation to their spectrum of activity, the development of resistance and some toxicity. In order to overcome these limitations, several analogues have been developed. These new generation triazole antifungal drugs, the so-called second-generation triazoles, include albaconazole (Stiefel Laboratories Inc.), isavuconazole (Basilea Pharmaceutica International), posaconazole (Merk Sharp & Dohme), ravuconazole (Bristol-Myers Squibb) and voriconazole (Pfizer Pharmaceuticals). These new drugs are more active against difficult to treat and emerging pathogens compared with fluconazole and itraconazole; they have all been evaluated in *in vitro* preclinical and Phase I studies and some of them have been investigated in Phase II and III clinical trials.

Pharmacokinetic characteristics of the new generation triazole antifungals

Among second-generation triazoles, albaconazole (UR-9825), posaconazole and voriconazole are available as active drugs. Isavuconazole (BAL-4815) is the active metabolite of the water-soluble prodrug isavuconazonium (BAL-8557) [3–6]. The iv. prodrug of ravuconazole (BMS-207147) is ravuconazole di-lysine phosphoester

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(BMS-379224) [7–12]. The chemical structures of albaconazole, isavuconazonium, ravuconazole and voriconazole are similar to that of fluconazole, whereas posaconazole bears chemical resemblance to itraconazole.

The pharmacokinetic characteristics of the second-generation triazoles are summarized in Table 1 [3–21]. All triazoles have been synthesized as oral formulation. All the triazoles except albaconazole are also available as an iv. formulation. An iv. formulation of posaconazole has been more recently synthesized, and a Phase I study in adult leukemic patients is ongoing (NCT01075984). The absorption of itraconazole capsules, posaconazole and ravuconazole is enhanced by co-administration with food or nutritional supplements [8–10,14–18]. On the contrary, co-administration with food decreases the absorption of fluconazole, itraconazole solution and voriconazole. No clinically relevant food effect on isavuconazole oral absorption has been observed [19]. No data are available for albaconazole in humans to our knowledge.

Despite similar mechanisms of action, structural differences of the triazoles result in distinct pharmacokinetic properties including metabolism and elimination. A common characteristic of these antifungal drugs is the variability of concentration in blood among different patients and during the treatment course in the same patient. Triazoles are degraded mainly in the liver, where they undergo glucuronidation, and their metabolism is greatly influenced by the activity of the hepatic enzymes. In particular, voriconazole is subject to variability in blood concentrations for both iv. and p.o. formulations. This variability is not related primarily to absorption, unlike itraconazole and posaconazole [20–22]. Some studies demonstrated that most of the pharmacokinetic variability of voriconazole is due to the ability to metabolize the drug via the CYP2C19 P450 enzyme [20,22]. Polymorphism in the gene encoding this enzyme is common and results in variable rates of voriconazole metabolism. These polymorphisms differ among the various ethnic groups, in fact most Caucasians are homozygous extensive metabolizers and Asians are more frequently homozygous poor metabolizers. The genetic variability of CYP2C19 represents an important issue in the definition of proper dosages and response to treatment of voriconazole. For posaconazole, pharmacokinetic investigation has identified marked interpatient variability related to the erratic absorption of the oral drug more than to the variable metabolism considering that posaconazole metabolism is related only to CYP3A4 [23–25]. Additionally, the more recently developed triazoles; albaconazole, isavuconazole and ravuconazole are metabolized via the P450 enzymes although there is little information available to date.

Reduced absorption and increased or decreased metabolism related to genetic factors or drug interactions may result in insufficient exposure of the fungal pathogen to the treatment, or excess drug concentrations with potential toxicity. The only tool available to determine drug exposure to the patient is monitoring of drug concentration in blood by therapeutic drug monitoring (TDM) [22,26–31]. Validated assays have been developed for each of the commonly used antifungal drugs. These assays most often include either a microbiological or chromatographic assay, but it should be mentioned that microbiological methods may give nonlinear results and are not able to distinguish between antifungal drugs in patients on multiple antifungals. A definitive voriconazole trough concentration goal is likely to be in the range of 2–6 µg/ml [26–31]. The adequate serum levels of posaconazole have not been well defined to date; however, some authors proposed a trough posaconazole goal ranging between 0.5 and 1.5 µg/ml for patients treated for IFDs and levels more than 0.5 µg/ml for prophylaxis [22]. Recent literature increasingly underlines the crucial role of adequate plasma levels of voriconazole and posaconazole in the efficacy of treatment and prevention of IFDs in immunocompromised patients, respectively [22,25–33]. In particular, studies in the pediatric population demonstrate that TDM and individual dose adjustments are recommended for optimal and less toxic voriconazole treatments, especially for <3 year-old children [31,34,35]. However, the available, good-quality, prospectively obtained data in the therapeutic and prophylactic setting are insufficient to justify the routine use of TDM in patients under treatment with voriconazole and posaconazole. In general, the assay should be considered for both voriconazole and posaconazole in the event of poor clinical response, co-administration of potentially interacting drugs and deteriorating hepatic function. For voriconazole, TDM may be indicated in suspected neurologic toxicity related to overdosing. A specific indication for monitoring posaconazole levels is the occurrence of conditions that put the patient at risk of impaired absorption, such as severe mucositis, vomiting, diarrhea, intestinal graft-versus-host disease, and impaired dietary intake. However, only a few centers have a clinical pharmacology laboratory capable of routinely performing TDM of the triazoles. An increase in the number of such laboratories should be encouraged in order to gather more data and better define the indications of TDM of these antifungal drugs in clinical practice.

Patients with impaired renal function can be treated with the standard dosage of oral voriconazole. iv. voriconazole is not indicated in this setting due to

Table 1. Summary of pharmacokinetic properties of the second-generation triazoles.

Pharmacokinetic property	Voriconazole	Posaconazole	Isavuconazole	Ravuconazole	Albaconazole
Available forms	Oral and intravenous	Oral only	Oral and intravenous	Oral and intravenous	Oral only
Absorption rate: time of maximum plasma concentrations after oral administration (h)	2	5	2–3	4	2–4
Bioavailability. Expressed as ratio of serum level after oral route to those after intravenous route	95%	Not applicable in humans (only oral formulation available) In animals 52–100%	Very high	48–74% in animals	Not applicable in humans (only oral formulation available) In animals 80–100%
Food effect on absorption	Decreased	Increased, particularly by fatty meals	No effect	Increased	No data
Protein binding (%)	58	99	98	98	98
Volume of distribution (l/kg)	4.6	6.5	4.4–7.7	10.8	Very large
Maximum plasma concentrations (mg/l)	3 (dose 3 mg/kg b.i.d. intravenously); 1.89 (dose 200 mg b.i.d. given orally)	0.6 (after a single dose of 400 mg); 1.3 (after 200 mg tid)	2.5 (dose 100 mg)	0.8–1.7 (doses ranging from 400 to 800 mg given orally)	No data
Steady state following oral administration	Achieved after 5–6 days	Achieved after 7–10 days	Achieved after 7–10 days	Achieved after 29 days	No data
Serum levels at the steady state considering the through plasma concentrations (dosage)	5 mg/l (4 mg/kg/12 h)	1 mg/l (400 mg/12 h)	2 mg/l (100 mg/24 h)	6 mg/l (400 mg/24 h)	No data
CNS/CSF penetration (% of serum levels)	>50	Low	Low in CSF, higher in brain	10 in rabbit model	15 in rabbit model
Vitreal penetration (% of serum levels)	38	26	Low	15 in rabbit model	No data
Metabolism	Various hepatic enzymes (CYP3A4, CYP2C19, CYP2C8/9)	Only by CYP3A4 hepatic enzyme	Hepatic enzymes (few data)	Only by CYP3A4 hepatic enzyme	Hepatic enzymes (few data)
Half-life (h)	6–12	16–35	56–104	76–202	30–56
Route of elimination	Renal	Fecal	Fecal	Fecal	Fecal
Urinary elimination in active form (%)	<2	<2	<1	No data	No data
Dose adjustment	Need to adjust dose in hepatic dysfunction; intravenous formulation is contraindicated in patients with creatinine clearance rates <50 ml/min	Not dose adjustment needed for both renal and hepatic dysfunction	Administration to patients with mild or moderate hepatic impairment requires a dose adjustment compared with normal patients. No dose adjustment needed for both renal and hepatic dysfunction	No dose adjustment needed for both renal and hepatic dysfunction	No data

the solvent cyclodextrin accumulates, although its potential nephrotoxicity is uncertain. In patients with moderate hepatic failure (Child–Pugh A and B stage) the voriconazole clearance has been found to be reduced by approximately 50%; therefore, a 50% reduction of the maintenance dose is required. For patients with advanced liver cirrhosis (Child–Pugh C stage) pharmacokinetic data are lacking and the administration of the drug is not indicated [36]. Renal clearance of posaconazole is negligible; therefore, in patients with renal impairment as well as in patients on intermittent hemodialysis, no significant alteration in posaconazole pharmacokinetic was observed and no dose adjustment is required [37]. Administration of isavuconazole to patients with mild or moderate hepatic impairment will require a dose adjustment compared with normal patients [38].

Antimicrobial characteristics of the new-generation triazole antifungals

The phenomenon of resistance to triazoles has been largely described for fluconazole and itraconazole and it continues to be an important issue for second-generation triazoles. While clinically relevant data are available for *Candida* resistance to triazole, the epidemiological and clinical impact of triazole resistance among moulds is less known. Azole antimycotics block the conversion of lanosterol to ergosterol through the inhibition of C14-α demethylase. C14-α demetilase is the product of the *ERG11* gene present in practically all yeasts and moulds. A low activity of triazoles against *Candida* spp can occur by mutations of the *ERG11* gene that modify the demethylase, resulting in a reduced affinity of azoles to the target molecule, or by the overexpression of the *ERG11* gene resulting in an overproduction of the demethylase. Another important resistance mechanism is the overexpression of the efflux pump genes encoded by the *CDR* genes of the ATP-binding cassette and major facilitator class. The resistance mechanism related to mutation or overexpression of target molecule impairs drug binding, whereas overexpression of the efflux pump decreases intracellular drug concentration [39,40].

The phenomenon of resistance to the new triazoles has been recently observed for *Aspergillus* spp. The emergence of triazole resistance in *Aspergillus* isolates, particularly *A. fumigatus*, has been reported in several countries and some studies seem to show that this phenomenon might have a significant impact on the role of azoles in the management of invasive aspergillosis (IA) [39,41]. Several mechanisms of resistance to azoles have been described for *Aspergillus* spp: point mutations of *Cyp51A* (gene encoding 14-α-sterol demethylase) with reduced concentration of intracellular drug, over expression of efflux pumps or reduced drug penetration [39–42]. Recent studies show that the pan-azole cross resistance pattern in *A. fumigatus* strains may develop through exposure to azole compounds in the environment [43].

Currently, there are two independent standards for antifungal susceptibility testing of triazoles against fungi: the broth microdilution method, developed by the Clinical and Laboratory Standards Institute and that of the European Committee on Antimicrobial Susceptibility Testing. To define the susceptibility pattern of fungi the two methods differently determined the clinical breakpoints (CBT) based upon the correlation of *in vitro* data with clinical outcome and the epidemiological cut-off values (ECV) that would discriminate wild type strains from those with acquired resistance mechanisms. The CBT is used to predict the response to treatment based on the level of susceptibility of the pathogen, whereas the ECV could serve as the foundation for the laboratory detection of acquired resistance and be used to monitor resistance development. As detailed in Table 2, both CBT and ECV are available for *Candida* spp, whereas, in the absence of the necessary clinical data, CBTs have not been established for any *Aspergillus*/drug combination and only ECVs have been defined [44–47].

Isavuconazole, ravuconazole and albaconazole have not been assigned an interpretive breakpoint; however, for purpose of comparison and based on pharmacokinetic data, some authors employed the susceptibility/resistance breakpoints of voriconazole also for these drugs. Table 3 details the distribution of the minimal inhibitory concentrations required to inhibit the growth of 90% of treated organisms (MIC_{90}) of fluconazole, itraconazole, voriconazole, posaconazole, ravuconazole, isavuconazole and albaconazole against *Candida* spp and *Cryptococcus neoformans* isolates from large international studies [48–64]. Second-generation triazoles demonstrated potent *in vitro* activity against all species of *Candida* and *C. neoformans*. In particular albaconazole showed the lowest MIC_{90} for all *Candida* spp. One factor behind the development of second-generation triazoles was the rapid appearance of fluconazole-resistant organisms during long-term treatment. Generally, all these drugs are more active than fluconazole and itraconazole against a wide variety of *Candida* spp, such as *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*, whereas their activity continues to be moderate against a significant proportion of *C.glabrata* isolates (Table 4).

An important characteristic of the second-generation triazoles is their activity potentially extended not only to *Aspergillus* spp but also to other less common filamentous fungi such as zygomycetes, *Fusarium* spp and *Scedosporium* spp [65–76]. To date no interpretive breakpoint for susceptibility of moulds to antifungal drugs has been standardized; however,

Table 2. Susceptibility breakpoints according to the Clinical and laboratory standards institute and European committee on antimicrobial susceptibility testing for *Candida* and *Aspergillus* species.

Pathogen	Clinical breakpoints (mg/l)		Epidemiological cut-off value (mg/l)	
	CLSI	EUCAST	CLSI	EUCAST
Candida				
Fluconazole	≤8[†]	≤2[‡]	≤0.5–≤64[§]	≤1–<128[¶]
Itraconazole	≤0.125[†]	NA	NA	NA
Voriconazole	≤1	NA	≤0.03–≤0.5[#]	≤0.06–≤1[††]
Posaconazole	NA	NA	≤0.06–≤2[‡‡]	NA
Aspergillus				
Itraconazole	NA	NA	≤1–≤2[§§]	≤1
Voriconazole	NA	NA	≤1–≤2[¶¶]	≤1
Ravuconazole	NA	NA	NA	≤1
Posaconazole	NA	NA	≤0.25–≤1[##]	≤0.25

[†]Applied to all species of *Candida* except for *C. krusei*.
[‡]Applied to *C. albicans*, *C. parapsilosis* and *C. tropicalis*.
[§]≤0.5 for *C. albicans*; ≤2 for *C. parapsilosis*, *C. tropicalis* and *C. lusitaniae*; ≤8 for *C. guilliermondii*; ≤32 for *C. glabrata* and ≤64 for *C. krusei*.
[¶]≤1 for *C. albicans*; ≤2 for *C. parapsilosis* and *C. tropicalis*; ≤32 for *C. glabrata* and ≤128 for *C. krusei*.
[#]≤0.03 for *C. albicans* and *C. lusitaniae*; ≤0.06 for *C. tropicalis*; ≤0.125 for *C. parapsilosis*; ≤0.25 for *C. guilliermondii* and ≤0.5 for *C. glabrata* and *C. krusei*.
[††]≤0.06 for *C. lusitaniae*; ≤0.125 for *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. paratropicalis*; ≤0.25 for *C. parapsilosis*; ≤5 for *C. guilliermondii* and *C. krusei* and ≤2 for *C. glabrata*.
[‡‡]≤0.06 for *C. albicans*; ≤0.125 for *C. tropicalis* and *C. lusitaniae*; ≤0.25 for *C. guilliermondii* and ≤1 for *C. glabrata* and *C. krusei*.
[§§]≤1 for *A. fumigatus*, *A. flavus*, *A. terreus* and *A. nidulans* and ≤2 for *A. niger* and *A. versicolor*.
[¶¶]≤1 for *A. fumigatus*, A. flavus and *A. terreus*, and ≤2 for *A. niger*, *A. nidulans* and *A. versicolor*.
[##]≤0.25 for *A. flavus*; ≤0.5 for *A. fumigatus*, *A. niger* and *A. terreus* and ≤1 for *A. nidulans* and *A. versicolor*.
CLSI: Clinical and laboratory standards institute; EUCAST: European committee on antimicrobial susceptibility testing; NA: Not available.
Data taken from [43–46,201].

some authors considered a MIC >2 µg/ml as the provisional resistance breakpoint for itraconazole, voriconazole and posaconazole [65]. The definition of these breakpoints is not based on careful correlations of *in vitro* with *in vivo* response to therapy, even though itraconazole and voriconazole clinical failures in aspergillosis have been correlated with MICs >2 µg/ml. We can hypothesize that this resistance breakpoint may be extended to all broad spectrum triazoles including ravuconazole, isavuconazole and albaconazole. Table 5 details the distribution of MIC_{90} of itraconazole, voriconazole, posaconazole, ravuconazole, isavuconazole and albaconazole against *Aspergillus* spp and other filamentous fungi from large international studies [52,53,64,66,67,69,71–76]. All second-generation triazoles are highly active against *Aspergillus* spp including *A. terreus* and *A. flavus,* which may have an intrinsically reduced susceptibility to amphotericin B. Posaconazole is the only triazole active against all species of zygomycetes, and itraconazole showed *in vitro* activity against all zygomycetes except *Mucor* spp. As previously observed, recent literature underlines the emerging phenomenon of azole resistance in *Aspergillus* spp in some European countries, although azole resistance prevalence in *Aspergillus* spp seems uncommon according to multinational studies [38–42,69]. An *in vitro* survey of triazole cross-resistance performed among more than 700 clinical isolates of *Aspergillus* spp, collected from 2000 to 2006 as part of a global antifungal surveillance program, showed a pattern of resistance (MIC >2 µg/ml) for itraconazole in approximately 2% of isolates, and in less than 1% of the isolates for voriconazole, posaconazole or ravuconazole [69].

Phase II & III clinical trials

■ Voriconazole

Clinical trials of voriconazole antifungal therapy

Voriconazole has been evaluated in large comparative trials in the empiric antifungal therapy of febrile neutropenia, the treatment of superficial and deep-seated candidiasis, the primary therapy of IA and the treatment of ocular fungal infections [77–82]. Noncomparative studies on infections caused by rare fungal pathogens, such as *Fusarium* spp and *Scedosporium* spp evaluated the therapeutic role of voriconazole [83,84].

Table 3. *In vitro* **activities of first- and second-generation triazoles against** *Candida* **spp and** *Cryptococcus neoformans.*

Species, reference (no. of isolates)	Range of MIC_{90}[†]						
	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Ravuconazole	Isavuconazole	Albaconazole
C. albicans	0.5–4	0.12–0.25	0.015–0.25	0.06	0.03–0.25	<0.015	<0.0002
C. glabrata	16–64	1–2	1–2	2	1–2	0.5	0.12
C. krusei	64–128	0.25–2	0.5	1	0.5	0.5	0.06
C. parapsilosis	1–2	0.06–0.5	0.06–0.12	0.12	0.06–0.12	0.03	≤0.0002
C. tropicalis	1–8	0.03–0.5	0.06–0.12	0.12	0.12	0.06	0.03
C. guilliermondii	4–16	1	0.12–0.25	0.5	0.25	N/A	N/A
C. dubliniensis	0.25–16	0.25	0.03	0.06	0.03–1	N/A	N/A
C. neoformans	4–32	0.25–0.5	0.125–0.5	0.016–0.5	0.25–0.5	0.016–0.06	0.156

[†]The MIC end point was defined as the lowest concentration that produced 50% inhibition of growth.
MIC: Minimal inhibitory concentration.
Data taken from [48–53,55–58,60–62].

Empirical antifungal therapy

In a randomized, international, multicenter trial, voriconazole was compared with liposomal amphotericin B for empirical antifungal therapy of neutropenic patients with persistent fever [77].

The aim of the study was to demonstrate noninferiority of voriconazole predefined as a difference in success rates (considering a composite end point) between the two drugs of no more than 10 percentage points. A total of 415 patients were assigned to voriconazole and 422 to liposomal amphotericin B. The overall success rates were 26.0% with voriconazole and 30.6% with liposomal amphotericin B (95% CI for the difference, -10.6 to 1.6%). According to this result voriconazole did not reach the end point of non-inferiority, compared with liposomal amphotericin B. However, patients treated with voriconazole experienced fewer breakthrough IFDs (1.9 vs 5.0%; $p = 0.02$), fewer cases of severe infusion-related reactions ($p < 0.01$) and of nephrotoxicity ($p < 0.001$) than those treated with liposomal amphotericin B. The incidence of hepatotoxicity was similar in the two groups. Patients receiving voriconazole suffered from more episodes of transient visual disturbances than those receiving liposomal amphotericin B (22 vs 1%; $p < 0.001$) and more hallucinations (4.3 vs 0.5%; $p < 0.001$). Based on the results of this study, voriconazole did not receive the approval for empiric antifungal therapy in patients with febrile neutropenia by regulatory agencies.

Invasive candidiasis

The efficacy, safety, and tolerability of voriconazole and fluconazole were compared in 391 immunocompromised patients with mycology- and biopsy-proven esophageal candidiasis [78]. Primary efficacy analysis (256 patients) of esophageal treatment, as assessed by esophagoscopy, revealed success rates of 98.3% with voriconazole and 95.1% with fluconazole. The overall safety and tolerability of both antifungals were acceptable. The most frequent adverse events (23%) with voriconazole were mild, transient visual disturbances.

A multicenter, randomized, noninferiority study compared voriconazole (283 patients) with a regimen of amphotericin B followed by fluconazole

Table 4. *In vitro* **activities of itraconazole and second-generation triazoles against** *Candida* **spp isolates resistant to fluconazole.**

Species, reference (no. of isolates)	MIC_{90} (% susceptible according to CLSI clinical breakpoints)[†]					
	Itraconazole	Voriconazole	Posaconazole	Ravuconazole	Isavuconazole[‡]	Albaconazole[‡]
C. albicans	>8 (0)	>8 (57)	1 (98)	>8 (73)	1	0.03
C. glabrata	>8 (0)	8 (13)	>8 (5)	8 (11)	1	1
C. krusei	1 (2)	1 (98)	1 (98)	1 (98)	0.06	0.06

[†]The MIC end point was defined as the lowest concentration that produced 50% inhibition of growth.
[‡]The few data available for isavuconazole and albaconazole do not allow to calculate the percentage of susceptibility.
MIC: Minimal inhibitory concentrations.
Data taken from [48,55,57,63,64].

Table 5. *In vitro* activities of first- and second-generation triazoles against *Aspergillus* spp and other moulds.

Species	Range of MIC_{90}[†]					
	Itraconazole	Voriconazole	Posaconazole	Ravuconazole	Isavuconazole	Albaconazole
A. fumigatus	0.5–>8	0.5–1	0.5	0.5	1–2	0.125
A. flavus	0.25–1	0.5–1	0.5	1	1–2	0.25
A. niger	0.5–>8	0.5–2	0.5–1	2–4	2–4	0.5
A. terreus	0.25–0.5	0.5–2	0.25	0.5	0.5–2	N/A
Mucor spp	>16	>16	2	8	16	N/A
Rhyzopus spp	4	>16	1	8	16	N/A
Absidia spp	1	>16	0.25	N/A	N/A	N/A
Cunninghamella spp	4	>8	1	N/A	N/A	N/A
S. apiospermum	1–>16	0.25–1	0.25–1	0.12	N/A	1
S. prolificans	>16	4–>16	16	16	N/A	2
Fusarium spp	16	4–16	16	8–16	>16	>16
Penicillium spp	2	1	1	1	N/A	N/A

[†]In most of studies the MIC end point was defined as the lowest concentration that produced complete inhibition of growth, but in some studies a 50% inhibition of growth was considered.
MIC: Minimal inhibitory concentrations.
Data taken from [52,53,64,66,67,69,71–76].

(139 patients) for the treatment of candidemia in non-neutropenic patients [79]. Voriconazole was non-inferior to amphotericin B/fluconazole in the primary efficacy analysis, with successful outcomes in 41% of patients in both treatment groups. At the last evaluable assessment, outcome was successful in 65% of patients assigned to voriconazole and in 71% of those assigned to amphotericin B/fluconazole (p = 0.25). In the voriconazole group, treatment discontinuation due to all-cause adverse events was more frequent, but serious adverse events and cases of renal toxicity were significantly fewer than in the amphotericin B/fluconazole group.

IA

Voriconazole has become the drug of choice for the treatment of IA. The change from amphotericin B to voriconazole for primary therapy of aspergillosis followed the publication of an open noncomparative multicenter study and a Phase III large multinational randomized trial [80,81]. In the first study, 141 immunocompromised patients with IA were enrolled to be treated with iv. voriconazole 6 mg/kg b.i.d. iv. twice and then 3 mg/kg b.i.d. for 6–27 days, followed by 200 mg b.i.d. p.o. for up to 24 weeks [80]. Out of 116 assessable patients, complete or partial responses were seen in 48% of cases. Good responses were seen in 60% of those with pulmonary or tracheobronchial IA, 16% with cerebral IA, 58% with hematologic disorders and 26% of allogeneic stem cell transplant recipients. In the second study a total of 144 and 133 patients randomly received voriconazole or deoxycholate amphotericin B, respectively [81]. Most of the patients were affected by acute leukemia, or other hematologic diseases undergoing intensive chemotherapy or allogeneic hematopoietic-cell transplantation (HSCT). At week 12, response was observed in 52.8% of the patients in the voriconazole group (complete responses in 20.8% and partial responses in 31.9%) and 31.6% of those in the amphotericin B group (complete responses in 16.5% and partial responses in 15.0%). The survival rate at 12 weeks was 70.8% in the voriconazole group and 57.9% in the amphotericin B group. Voriconazole-treated patients had significantly fewer severe drug-related adverse events, but transient visual disturbances were common with voriconazole (occurring in 44.8% of patients).

Fungal keratitis

Voriconazole was compared with natamycin as a topical treatment in a multicenter, double-masked, clinical trial that included 120 patients with fungal keratitis [82]. The primary outcome was best spectacle-corrected visual acuity (BSCVA) at 3 months. Other outcomes included scar size, perforations and a subanalysis of BSCVA at 3 months in patients with an enrollment visual acuity of 20/40 to 20/400. Compared with those who received natamycin, voriconazole-treated patients had an approximately 1-line improvement in BSCVA at 3 months in a multivariate regression model, but the difference was not statistically significant (p = 0.29). Scar size and corneal perforations at 3 months was not

significantly different in the two treatment groups. Patients with baseline BSCVA of 20/40 to 20/400 demonstrated a trend toward a 2-line improvement in visual acuity with voriconazole (p = 0.07).

Invasive fusariosis

The spectrum of antifungal activity of voriconazole includes *Fusarium* spp and its efficacy in the treatment of this frequently fatal infection has been evaluated in retrospective studies [83,84]. In a recently published international retrospective analysis of 73 cases of invasive fusariosis, treated with voriconazole the 90 day survival was 42% [84]. The outcome differed according to site of infection, underlying condition and *Fusarium* species. The authors conclude that voriconazole is a therapeutic option for invasive fusariosis.

The Phase II and III clinical trials of therapy with voriconazole conducted in the last decade allowed to be defined the position of the triazole in the treatment armamentarium of severe invasive mycoses, and highlighted by the international guidelines [85–87]. Available literature data strongly support the primary role of voriconazole in the front-line therapy of IA, on the contrary, its use in the management of invasive candidiasis is questionable compared with other antifungal drugs, such as echinocandins and lipid formulations of amphotericin B, due to the possible reduced activity against certain *Candida* isolates. Voriconazole may be used in the treatment of some severe, unusual fungal infections, such as fusariosis; however, it has not been compared with other antifungal drugs, such as lipid formulations of amphotericin B; therefore, the indication of voriconazole as a first choice treatment of such infections has not been established.

■ Clinical trials of voriconazole antifungal prophylaxis

Two controlled studies of primary prophylaxis and one noncomparative study of secondary prophylaxis of IFDs with voriconazole have been conducted in allogeneic stem cell transplant patients [88–90].

Primary prophylaxis

In the first study of primary prophylaxis, conducted in 35 centers participating in the Bone and Marrow Transplant Clinical Trials Network, voriconazole was compared with fluconazole (295 vs 305 patients) in a randomized, double-blind trial [88]. This study was characterized by a predefined, structured fungal screening program based on serum galactomannan detection triggering mandatory evaluation of IFD. Patients undergoing myeloablative allogeneic HSCT were randomized before transplant to receive study drugs for 100 days, or for 180 days in higher risk patients. The primary end point was freedom from IFD or death, fungal-free survival at 180 days. Despite trends to fewer IFDs (7.3 vs 11.2%; p = 0.12), *Aspergillus* infections (9 vs 17; p = 0.09), and less frequent empiric antifungal therapy (24.1 vs 30.2%; p = 0.11) with voriconazole, fungal-free survival rates (75 vs 78%; p = 0.49) at 180 days were similar with fluconazole and voriconazole, respectively. Relapse-free and overall survival and the incidence of severe adverse events were also similar. This study demonstrates comparable efficacy of fluconazole or voriconazole prophylaxis in allogeneic stem cell transplant patients; however, a careful interpretation of the results is required. The study population considered in this trial was at low risk of IFD. Indeed, approximately 90% of patients had a standard disease risk status, over half of transplants were matched related, the HLA match was 6/6 in 96% of cases, half of patients did not develop acute or chronic graft-versus-host disease (GVHD) and the incidence of disease relapse/progression was only approximately 10%. One would be interested to evaluate voriconazole's performance in a higher risk population. This consideration is even more valid when looking at the results among patients with acute myeloid leukemia, a population at higher risk for IFD and with a poorer fungal-free survival. Interestingly, in this patient population voriconazole reduced IFDs (8.5 vs 21%; p = 0.04) and improved fungal-free-survival (78% vs 61%; p = 0.04) compared with fluconazole [91].

The second study of primary prophylaxis compared voriconazole (200 mg b.i.d.) versus itraconazole (200 mg b.i.d.) in 489 patients receiving allogeneic HSCT for at least 100 days, and up to 180 days from conditioning [89]. The primary objective was assessed on a composite end point, including survival at 180 days after transplant and no proven or probable breakthrough IFD and no discontinuation of the study drug for more than 14 days during the 100 day prophylactic period. The voriconazole arm met the criteria for superiority in the primary end point when compared with the itraconazole arm (49.1 vs 34.5%; p = 0.0004). The median duration of voriconazole prophylaxis was longer (97 days) than that of itraconazole (68 days), probably due to significantly more gastrointestinal adverse events (nausea, vomiting and diarrhea) in the itraconazole group. However, the main concern with this study was the low rate of proven or probable IFDs (three in the voriconazole arm and six in the itraconazole arm) and the fact that superiority of voriconazole was related to the tolerability more than to the efficacy.

Overall, the two clinical trials demonstrated an uncertain role of voriconazole in the primary antifungal prophylaxis of patients at high risk for IFDs, although the ECIL guidelines, pending the publication

of the full papers, provisionally graded voriconazole as AI drug in the primary antifungal prophylaxis of allogeneic HSCT patients [87]. As commented above, the design of the studies may have had a role in the uncertain results.

Secondary prophylaxis

Voriconazole (4 mg/kg/12 h iv. or 200 mg/12 h p.o.) was evaluated in a prospective, open-label, multicenter trial as a secondary antifungal prophylaxis in 45 allogeneic HSCT recipients with previous proven or probable IFD [90]. Previous IFD was a proven or probable IA in most cases (n = 31). The primary end point of the study was the incidence of proven or probable recurrance of new IFD after transplant. The median duration of voriconazole prophylaxis was 94 days. Eleven patients (24%) died within 12 months of transplantation, but only one due to an IFD. The 1 year cumulative incidence of IFD was 6.7 ± 3.6%. Two relapses of infection (one candidemia and one fatal scedosporiosis) and one new breakthrough zygomycosis in a patient with a previous IA occurred post-transplantation. None of the 31 patients with a previous proven or probable IA experienced recurrence of their infection. Voriconazole was discontinued in only two patients owing to treatment-related hepatotoxicity. This is the first prospective evidence of the efficacy and safety of secondary antifungal prophylaxis in protecting allogeneic HSCT recipients from recurring IFD. However, it should be considering that most patients in this series were in complete clinical and radiological remission of their IFD at the time of transplant, which may itself be an important determinant of the favorable outcomes observed in this high risk patient population. Conversely, the role of voriconazole, and of other antifungal drugs, in the control of infection reactivation in patients with a residual or active IFD at the time of transplant deserves further investigation [92].

An important problem raised by some authors after the first clinical experiences of voriconazole treatment was represented by the emergence of breakthrough infections by zygomycetes, fungi intrinsically not susceptible to the drug [93,94]. However, it was unclear if this was a casual phenomenon related to local epidemiology or if the use of voriconazole represented an independent risk factor for the development of such severe infections. The multicenter, prospective studies of voriconazole primary and secondary prophylaxis did not show any increased incidence of zygomycoses, despite prolonged administration of the drug in high risk patients.

Ongoing clinical trials with voriconazole

Voriconazole is under investigation in the following Phase II and III clinical trials: association therapy for aspergillosis, treatment of *Candida* infections, timing of empirical antifungal therapy in neutropenic patients, pharmacologic optimization, pharmacokinetics and safety in children, adolescents and in obese subjects, comparison with other antifungal drugs in the treatment and prevention of fungal infections, kinetics of 1,3 β-d-glucan assay in patients with hematologic malignancies receiving voriconazole (NCT00001940, NCT00059878, NCT00075803, NCT00174473, NCT00150319, NCT00150345, NCT00159822, NCT00289991, NCT00418951, NCT00556998, NCT00739934, NCT00893555, NCT00836875, NCT00904995, NCT01030653, NCT01092832 and NCT01207128).

Posaconazole

■ Clinical trials of posaconazole antifungal therapy

Clinical trials with posaconazole investigated the safety and efficacy of the triazole in the treatment of oropharyngeal candidiasis (OPC), esophageal candidiasis, and salvage therapy of IFDs refractory to previous antifungal treatments. No trial has been conducted in the primary therapy of IFDs.

Oropharyngeal & esophageal candidiasis

The clinical efficacy of posaconazole and fluconazole in the treatment of OPC in HIV-infected patients was compared in a randomized, controlled, evaluator-blinded study [95]. Patients received either posaconazole 200 mg (178 cases) or fluconazole 200 mg (172 cases) oral suspension on day 1, followed by 100 mg/day of the same drug for the next 13 days. The rate of clinical success at the 14 day primary end point was similar in the posaconazole and fluconazole arms (91.7 and 92.5%, respectively; 95% CI; -6.61–5.04). Additionally, mycologic success was comparable at this time point (68% in both arms). However, at day 42 follow-up, fewer relapses and greated mycologic success occurred in the posaconazole arm compared with fluconazole arm (40.6 vs 26.4%; p = 0.038).

A multicenter, Phase III, open-label study investigated the use of posaconazole in 199 HIV-infected patients with either OPC or esophageal candidiasis refractory to previous fluconazole of itraconazole therapy [96]. Clinical and mycological responses were obtained in 75.0 and 36.5% of patients, respectively. In particular, clinical responses occurred in 73% of subjects with isolates resistant to fluconazole, 74% of subjects with isolates resistant to itraconazole and 74% of subjects with isolates resistant to both. Out of 132 clinical responders, 80 patients were assessed at a 4-week follow-up. The overall clinical relapse rate in these subjects was 74%. Eight subjects (4%) discontinued therapy as a result of a treatment-related adverse event.

IA

Posaconazole was used as salvage therapy in patients with IA or other mycoses refractory or intolerant to conventional antifungals [97–100]. In a *post hoc* analysis, 107 patients with IA treated with posaconazole (800 mg/day in divided doses) and 86 retrospective controls were compared [97]. Controls received the best available standard of care for salvage therapy, in accordance with the clinical practice at each center (mainly amphotericin B, but also itraconazole voriconazole, echinocandins or a combination of drugs). Conventional or lipid formulation of amphotericin B and itraconazole were the drugs mainly used in the prior antifungal therapy in both groups. The median duration of posaconazole therapy was 56 days; the control group received salvage therapy for a median of 22 days. The overall success rate was 42% for posaconazole recipients and 26% for control subjects (odds ratio: 4.06; 95% CI: 1.50–11.04; p = 0.006). The response to posaconazole was greater in patients with extrapulmonary rather than pulmonary aspergillosis (53 vs 39% responders, respectively).

Zygomycosis & fusariosis

Other retrospective studies evaluated the efficacy of posaconazole as a salvage therapy of invasive fusariosis and zygomycosis [98–100]. In a study including 91 patients with invasive zygomycosis who had infection that was refractory to prior antifungal treatment (n = 81) or were intolerant of such treatment (n = 10) the rate of success at 12 weeks after treatment initiation was 60%, and 21% of patients had stable disease [98]. The overall high success and survival rates in this experience provide encouraging data regarding posaconazole as an alternative therapy for zygomycosis. A retrospective analysis of 21 patients with invasive fusariosis from three open-label clinical trials who had disease refractory, to or who were intolerant of standard antifungal therapy, showed a successful outcome in 48% of cases. For patients who recovered from myelosuppression, the success rate was 67%, compared with 20% for those with persistent neutropenia [100]. Taken together, these data indicate the efficacy of posaconazole as salvage therapy for infections with a variety of different fungal pathogens. The role of this agent in primary therapy remains to be defined.

■ Clinical trials of posaconazole antifungal prophylaxis

Two Phase III clinical studies indicated that posaconazole at a dose of 200 mg p.o. t.i.d. was at least noninferior to a standard-of-care azole antifungal agent for preventing IFDs in leukemia and HSCT patients [101,102]. The first study was a multicenter, randomized, open-label trial that compared posaconazole with fluconazole (400 mg/day) or itraconazole (200 mg/b.i.d.) for the prophylaxis of IFDs in 602 patients (aged >13 years) at high risk for neutropenia after receiving standard induction chemotherapy for a new diagnosis or first relapse of acute myelogenous leukemia or myelodysplastic syndrome [101]. Study drugs were administered at the start of each cycle of chemotherapy and were continued for a maximum of 12 weeks (84 days) or until recovery from neutropenia and complete remission, or occurrence of an IFD or adverse reaction to the study drug. The primary efficacy end point was an intent-to-treat analysis of treatment failure, defined as the occurrence of proven or probable IFDs from randomization to the end of the oral treatment phase (last dose of study drug plus 7 days). Secondary end points included the incidence of IA during the oral treatment phase and at 100 days after the end of the randomization period, death from any cause and time to death. Significantly fewer patients in the posaconazole arm compared with the fluconazole/itraconazole arm developed an IFD during the oral treatment phase (>2 vs >8%, respectively; absolute reduction in the posaconazole group, -6% [95% CI: -9.7 to -2.5]; p = 0.001). Although there was only a minor difference in the frequency of infections caused by *Candida* spp between groups during the oral treatment phase, significantly fewer patients in the posaconazole group had IA (2 [1%] vs 20 [7%]; p < 0.001).

Survival was significantly longer among recipients of posaconazole than among recipients of fluconazole or itraconazole (p = 0.04). Serious adverse events possibly, or probably, related to treatment were reported in 6 and 2% of patients in the posaconazole and fluconazole or itraconazole group, respectively (p = 0.01).

The second study was a randomized, double-blind, multicenter trial in which posaconazole was compared with fluconazole (400 mg/day) for the prophylaxis of IFDs in 600 allogeneic HSCT recipients (aged >13 years) with GVHD on high-dose immunosuppressive therapy [102]. Treatment was continued for 112 days or until the occurrence of a proven or probable IFD. The primary efficacy end point was the incidence of proven or probable IFDs during the period from randomization to day 112. Other end points were the incidence of proven or probable IA during the treatment period, the incidence of breakthrough proven or probable IFDs while patients were receiving study medications (exposure period), the time to the occurrence of an IFD, the overall mortality in the intention-to-treat population, and mortality attributable to fungal infection in the intention-to-treat population. Discontinuation of study drug for >5 days was considered treatment failure for the purposes of the intent-to-treat analysis at the 112 days end point. The mean duration of posaconazole and fluconazole therapy was 80 days and 77 days, respectively. At the end of the

fixed 112 day treatment period, the overall rates of IFD did not differ significantly between the two drugs (5.3% in posaconazole group vs 9.0% in fluconazole group; odds ratio: 0.56; 95% CI: 0.30–1.07; p = 0.07). Although the incidence of infections caused by *Candida* spp was similar in both groups (<1% of patients in either arm), posaconazole was superior to fluconazole in preventing proven or probable IA (2.3 vs 7.0%; odds ratio: 0.31; 95% CI: 0.13–0.75; p = 0.006). During exposure period, in the posaconazole group, as compared with the fluconazole group, there were fewer breakthrough IFDs (2.4 v. 7.6%; p = 0.004), particularly IA (1.0 vs 5.9%; p = 0.001). Overall mortality was similar in the two groups, but the number of deaths from IFDs was lower in the posaconazole group (1 vs 4%; p = 0.046). The incidence of treatment-related adverse events was similar in the two groups (36% in the posaconazole group and 38% in the fluconazole group), and the rates of treatment related serious adverse events were 13 and 10%, respectively.

In view of the results of these large, multicenter, controlled studies international guidelines graded posaconazole as AI drug in the primary antifungal prophylaxis of allogeneic HSCT patients with GVHD and of acute myelogeous leukemia patients undergoing induction chemotherapy [85,87]. However, a major problem with oral posaconazole is represented by the unpredictable gastrointestinal absorption particularly in patients with intestinal GVHD and with mucositis, a complication frequently encountered after intensive chemotherapy. While waiting for the iv. formulation of posaconazole, the use of TDM may be required in the clinical practice.

Ongoing clinical trials with posaconazole

Posaconazole is under investigation in Phase II and III clinical trials studies on salvage therapy in refractory IFDs, treatment of toenail onychomycosis, prophylaxis of IFDs, pharmacokinetics of posaconazole prophylaxis in patients with acute leukemia and chronic granulomatous disease, and prevention of IFDs in comparison with other antifungal drugs (NCT00750737, NCT00491764, NCT00550732, NCT00811642 NCT00936117, NCT01200355, NCT00750737, NCT00799071, NCT00811928, NCT00686621).

Ravuconazole, isavuconazole & albaconazole

Most data on the *in vivo* use of the new triazoles ravuconazole, isavuconazole and albaconazole are limited to animal studies and few Phase I and II studies have been performed in humans. Therefore, very little information on clinical efficacy is available for ravuconazole, isavuconazole and albaconazole.

Animal studies

In a study published in 1996 oral ravuconazole was compared with itraconazole and fluconazole in a murine neutropenic model of pulmonary aspergillosis [103]. Ravuconazole reduced the tissue burden in the lungs significantly, compared with that of the controls. Ravuconazole was more effective at 40 mg/kg or as effective at lowed dosages than itraconazole against pulmonary aspergillosis. In a more recent experimental study in a neutropenic guinea pig model of IA, ravuconazole was more active than itraconazole at low dosages, whereas high dosages (10 mg/kg) of the two drugs were comparable in terms of survival and reduction of the tissue burden of *Aspergillus* [104]. Both experiences seem to show a significant activity of ravuconazole against *Aspergillus* also at low tissue levels of the drug. The efficacy of ravuconazole was compared with that of the echinocandin LY-303366 in a neutropenic rabbit model of IA. Ravuconazole eliminated mortality, cleared *Aspergillus* antigen from the serum, and eliminated *A. fumigatus* organisms from tissues of animals with invasive infection. On the contrary the echinocandin prolonged survival and reduced antigenemia, but it did not eliminate *Aspergillus* organisms from tissues [105]. In experimental models of pulmonary candidosis and intracranial cryptococcosis, ravuconazole was more effective than itraconazole and was as effective as fluconazole [103]. Ravuconazole was also effective against pulmonary candidiasis caused by fluconazole-resistant *C. albicans* [103].

The activity of isavuconazole was compared with that of p.o. itraconazole, p.o. voriconazole and iv. caspofungin in a neutropenic murine model of disseminated *A. flavus* infection. The same reduction in the tissue burden was observed with the different treatments [106,107]. The dose-response of isavuconazole, voriconazole and fluconazole was assessed in neutropenic mice with disseminated *C. tropicalis* and *C. krusei* infections [108]. Isavuconazole significantly reduced kidney burden in mice infected with *C. tropicalis* and both kidney and brain burden in mice infected with *C. krusei*. Isavuconazole was as effective as voriconazole and much more effective than fluconazole at reducing brain burden.

Albaconazole was tested in a steroid-immunosuppressed rat model of disseminated aspergillosis and compared with amphotericin B [109]. Albaconazole, as well as amphotericin B, prevented infection giving 100% protection. In a murine model of systemic candidosis, albaconazole was as effective as fluconazole [110]. In a rabbit model of cryptococcal meningitis the efficacy of albaconazole was similar to that of fluconazole at all doses tested (from 5 to 80 mg/kg). However, the drug was detected in cerebrospinal fluid only at higher dosages [62].

Albaconazole was evaluated in an immunocompetent rabbit model of *S. prolificans* systemic infection. Only at high dosages (25–50 mg/kg daily) animals survived. Rabbits showed 100% survival when they were treated with 50 mg/kg of albaconazole, and only this dosage was able to reduce tissue burden significantly in spleen, kidney, liver, lungs and brain [111].

Clinical trials

By 1999, ravuconazole was undergoing Phase I/II clinical trials in the US. In a Phase II trial, a comparative evaluation of the efficacy of ravuconazole (400 mg once daily) and fluconazole (200 mg once daily) against esophageal candidiasis in HIV patients revealed that a better success rate was achieved with ravuconazole (86%) than with fluconazole (78%) [112]. Ravuconazole cure improved to 93% if individuals taking rifampicin were excluded, since rifampicin reduced ravuconazole levels by 50%. Another Phase I/II trial evaluated the effect of three 12 week dosing-regimens of ravuconazole in patients with onychomycosis (200 mg daily or 100 mg weekly and 400 mg weekly). Effective cure was obtained only in patients who received 200 mg daily of ravuconazole [11]. A Phase I–II prophylactic trial was conducted in allogeneic stem cell transplant patients who received ravuconazole at three different dosages (400, 600 or 800 mg daily) during the engraftment, pancytopenic period. The trial is ongoing (NCT00064311) and partial results of this study offered only pharmacokinetic data [12]. No Phase III clinical trials with ravuconazole have been conducted so far.

Isavuconazole has been used in humans in studies aimed at the definition of the pharmacokinetic properties of the drug [3,4]. In a Phase II study, isavuconazole at various dosages was compared with fluconazole (100 mg daily) in the treatment of esophageal candidiasis [113]. Patients received treatment for 14–21 days and all groups received a loading dose on day 1. A clinical cure was obtained in 95–98% patients treated with isavuconazole and in 95% of those treated with fluconazole. Co-administration of rifampicin and isavuconazole resulted in a 35-fold increase in systemic clearance of the drug. Isavuconazole is under investigation in Phase II and III clinical trials for treatment of *Candida*, *Aspergillus* and rare fungi infections and prophylaxis of IFDs in leukemia patients, (NCT00413218, NCT00634049, NCT00444366, NCT00412893, NCT00413439).

In a Phase II study the efficacy of a single dose of albaconazole at various dosages, ranging from 10 to 320 mg was compared with that of fluconazole in 64 women affected by *Candida vulvovaginitis* [114]. A single dose of albaconazole ≥40 mg was more efficacious than fluconazole at 150 mg. Albaconazole is under investigation in a Phase II clinical trial in subjects with distal subungual onychomycosis (NCT00730405). No Phase III studies on albaconazole are yet available.

Future perspective

In light of the Phase II and III clinical trials conducted up to now, voriconazole and posaconazole are the only second-generation triazoles approved for clinical use. Voriconazole has been approved by both the US FDA and the European Medicines Agency for the primary treatment of IA and candidemia in non-neutropenic patients and for salvage therapy for infrequent but serious IFDs caused by *S. apiospermum* and *Fusarium* spp. Posaconazole has been approved by both FDA and European Medicines Agency for prophylaxis against invasive *Aspergillus* and *Candida* infections in patients with acute myeloid leukemia and in allogeneic HSCT with GVHD and for treatment of oropharyngeal candidiasis in patients who have severe disease or are immunocompromised. The indication of the use of posaconazole in the treatment of aspergillosis, fusariosis, chromoblastomycosis and coccidioidomycosis in patients that are refractory or intolerant to other treatments has been approved only by the European Medicines Agency. The treatment of zygomycosis is a further indication of posaconazole not approved by regulatory agencies. The other second-generation triazoles ravuconazole, isavuconazole and albaconazole have not been approved for clinical use due to the lack of information based on proper clinical trials.

To define the future perspective of the therapeutic role of voriconazole and posaconazole, some specific antimicrobial and pharmacological characteristics of the drugs and new clinical settings of application should be considered.

Firstly, all second-generation triazoles exhibit an improved *in vitro* activity compared with fluconazole and itraconazole. However, if both drugs overcame the problem of the intrinsical fluconazole resistance of *C. krusei*, several strains of *C. glabrata* continue to show low susceptibility to the second-generation triazoles. This phenomenon represents an important problem in clinical practice. The recent IDSA guidelines for the treatment of candidiasis underline that voriconazole can be used in the treatment of candidiasis but with little advantage over fluconazole, and recommend it as stepdown oral therapy for selected cases of candidiasis due to *C. krusei* or voriconazole-susceptible *C. glabrata.* Furthermore, azoles should not be used for empirical therapy of patients with suspected candidiasis who have received an azole for prophylaxis [86]. The phenomenon of triazole resistance should also be carefully considered

for *Aspergillus* in the future. The recently reported triazole resistance of *A. fumigatus,* related to exposure to fungicidal compounds in the environment, is an uncommon and localized phenomenon that does not seem to represent a relevant problem in the current clinical practice. However, azoles are abundantly and widely used in the environment and the resistance of *A. fumigatus* to medical triazoles may be a major challenge in the future due to the possibility of worldwide spread of resistant isolates. National and local epidemiological surveys of triazole resistance for both yeasts and filamentous fungi seems to be of crucial importance in the near future.

Secondly, the variability in the absorption and/or metabolism of the triazoles may determine an unpredictable exposure of the pathogens to the antifungal treatments. Recent literature demonstrates that TDM may be crucial for the proper management of severe IFDs. However, the pharmacoeconomic impact of antifungal TDM has not been investigated, no guidelines are available in this setting and there are a limited number of specialized clinical laboratories that perform TDM assays. Consequently, triazoles continue to be used in the majority of centers without awareness of the real blood levels and antimicrobial efficacy. Considering the frequently unfavorable outcome of IFDs in immunocompromised patients, it may be difficult to define if a therapeutic failure is related to the clinical resistance of the infection, or to the inadequate and under-dosed pharmacological treatment. A major challenge for the future will be the definition and extension of TDM strategies in order to conduct optimal prevention and treatment of IFDs with triazoles.

Thirdly, the efficacy and safety of voriconazole and posaconazole probably deserve further evaluation in specific clinical settings. In particular, their use in the prophylaxis of IFDs has been studied in patients with acute myeloid leukemia after remission-induction chemotherapy or allogeneic HSCT recipients. The possible indication of primary antifungal prophylaxis with second-generation triazoles should be evaluated in other acute leukemia settings, such as during consolidation chemotherapy for acute myeloid leukemia or in adult patients with acute lymphoid leukemia being treated with acute myeloid leukemia-like chemotherapy schedules. Secondary antifungal prophylaxis is widely used in clinical practice but

Executive summary

- The second-generation triazoles voriconazole, posaconazole, ravuconazole, isavuconazole and albaconazole, are an important class of antifungal drugs in the management of superficial and invasive fungal infections. This derives from their favorable antimicrobial spectrum, pharmacokinetic properties and toxicity profile. All these drugs are available in oral formulation and only voriconazole, ravuconazole and isavuconazole are also available in the iv. formulation. They exhibit an improved *in vitro* activity compared with fluconazole and itraconazole, extending to some fluconazole resistant Candida strains and filamentous fungi.
- Only voriconazole and posaconazole have been properly investigated in Phase II and III clinical trials.
- Voriconazole is considered the first-line treatment of invasive aspergillosis and less common invasive fungal diseases (IFDs), such as fusariosis and scedosporiosis. Voriconazole is also indicated in the treatment of superficial and invasive candidiasis but with little advantage over fluconazole. It did not offer significant advantages compared with fluconazole and itraconazole in the primary prophylaxis of IFDs in the allogeneic hematopoietic stem cell transplantation setting.
- Posaconazole may be indicated in the treatment of oropharyngeal candidiasis and esophageal candidiasis in immunocompromised patients. To date, it is not indicated as primary therapy of IFDs but proved to be effective as salvage therapy of IFDs refractory or intolerant to previous antifungal treatments. Posaconazole is considered the golden standard in the primary prophylaxis of IFDs in neutropenic acute myeloid leukemia patients and in allogeneic HSCT recipients with graft-versus-host disease.
- The other second-generation triazoles albaconazole, isavuconazole and ravuconazole are under development; therefore, their future employ is unknown.
- In view of the well known resistance pattern of some *Candida* species and the emerging phenomenon of *Aspergillus fumigatus* triazole panresistance, epidemiological survey of triazoles resistance for both yeasts and filamentous fungi seems to be of crucial importance in the near future.
- Clinicians should be aware of the possibility of a wide interpatient metabolic and absorption variability for voriconazole and posaconazole, respectively. An insufficient exposure of the fungal pathogen to the treatment or excess drug concentrations with potential toxicity should be considered in clinical practice. The availability of therapeutic drug monitoring may be required in order to conduct optimal treatment of severe and frequently fatal IFDs with these triazoles. A major challenge for the future will be the definition and extension of therapeutic drug monitoring strategies in order to conduct optimal prevention and treatment of IFDs with triazoles.
- The possible indication of antifungal prophylaxis with second-generation triazoles should be evaluated in clinical settings other than acute myeloid leukemia and graft-versus-host disease in allogeneic HSCT. The role of posaconazole as primary therapy of IFDs should be further evaluated if iv. formulation of the drug are available in the future.

there is no consensus regarding the indications, the duration of treatment and the drug of choice. Suppressive voriconazole therapy in patients with IA in microbiological, clinical and radiological remission proved to prevent reactivation of the disease, despite prolonged neutropenia or profound immunosuppression post-allografting. However, the efficacy of secondary antifungal prophylaxis in patients with active fungal infection or with persistent radiological abnormalities remains unclear. Additional well-designed studies of primary and secondary antifungal prophylaxis are needed, not only to confirm or re-evaluate the efficacy of voriconazole and posaconazole, but also to define risk stratification criteria and tailored prevention strategies in the different clinical settings. With regard to the treatment of proven-probable IFDs the role of voriconazole has been adequately investigated. The triazole is considered the first choice in IA and other uncommon IFDs and a second choice in invasive candidiasis. On the contrary, the role of posaconazole in the first-line treatment of IFDs has not been clearly defined, probably due to the drug being available only as oral route. The lack of an iv. formulation represents a crucial limit for this drug, particularly in the aggressive first-line therapy of severe IFDs or in patients with gastrointestinal diseases and possibly reduced absorption. If iv. posaconazole will be available in the future, indications of its use will likely be extended in first line therapy.

In vitro and preliminary *in vivo* studies on albaconazole, isavuconazole and ravuconazole show interesting antimicrobial and safety characteristics of these second-generation triazoles; however, considering that few important clinical trials have been conducted or are ongoing with these drugs, the real possibilities of these agents as competitors with posaconazole and voriconazole for the treatment and prevention of IFDs in the clinical setting are still unknown.

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