Treatment options in severe fungal asthma and allergic bronchopulmonary aspergillosis

Richard B. Moss

Affiliation:

Dept of Pediatrics, Stanford University School of Medicine, Palo Alto, CA, USA.

Correspondence:

R.B. Moss, Center for Excellence in Pulmonary Biology, 770, Welch Road, Suite 350, Palo Alto, CA 94304-5882, USA.

E-mail: rmoss@stanford.edu

ABSTRACT Severe asthma with fungal sensitisation and allergic bronchopulmonary aspergillosis encompass two closely related subgroups of patients with severe allergic asthma. Pulmonary disease is due to pronounced host inflammatory responses to noninvasive subclinical endobronchial infection with filamentous fungi, usually *Aspergillus fumigatus*. These patients usually do not achieve satisfactory disease control with conventional treatment of severe asthma, *i.e.* high-dose inhaled corticosteroids and long-acting bronchodilators. Although prolonged systemic corticosteroids are effective, they carry a substantial toxicity profile. Supplementary or alternative therapies have primarily focused on use of antifungal agents including oral triazoles and inhaled amphotericin B. Immunomodulation with omalizumab, a humanised anti-IgE monoclonal antibody, or "pulse" monthly high-dose intravenous corticosteroid, has also been employed. This article considers the experience with these approaches, with emphasis on recent clinical trials.

@ERSpublications

Treatment of fungal asthma includes glucocorticoids, azoles, amphotericin and anti-IgE. Trial validation is needed. <http://ow.ly/uavHn>

Received: Aug 10 2013 | Accepted after revision: Nov 20 2013 | First published online: Dec 05 2013

Support statement: Work described herein was funded by a research grant from Genentech, Inc. (San Francisco, CA, USA) (grant number Genentech C4-150174), the manufacturer of omalizumab (Xolair).

Conflict of interest: Disclosures can be found alongside the online version of this article at www.erj.ersjournals.com

Copyright ©ERS 2014

Introduction

It is thought that up to 10% of people with asthma have poorly controlled disease with major life impact despite guideline-based combination high-dose inhaled corticosteroid/long-acting bronchodilator therapy, *i.e.* severe asthma. One-third to one-half of these severe asthmatics has atopic sensitisation to filamentous fungi, most prominently to *Aspergillus fumigatus* [1]. Evidence has mounted that fungal sensitisation is associated with a more severe asthma phenotype [2–8]. Thus, an important identifiable subgroup of asthma, termed severe asthma with fungal sensitisation (SAFS), has emerged [4, 9]. Identification of SAFS as a recognisable asthma phenotype appears to carry important therapeutic implications.

It is also becoming clear that many asthmatics with an even more severe form of fungal inflammatory lung disease, usually due to *A. fumigatus* and known as allergic bronchopulmonary aspergillosis (ABPA), are often not properly diagnosed and have significant unmet diagnostic and therapeutic needs [10–13]. ABPA occurs almost exclusively in people with asthma or cystic fibrosis (CF). It results from atopic sensitisation to hyphal antigens of filamentous fungi (*A. fumigatus* in >90% of cases), which provokes a florid innate and adaptive immunoinflammatory response clinically characterised by: wheezy dyspnoea; malaise; and productive cough; very high IgE levels, elevated IgE and IgG antibodies to *A. fumigatus*; pronounced granulocytic (eosinophilic>neutrophilic) endo- and peribronchial pulmonary inflammation, pulmonary infiltrates with mucoid impaction of bronchi; proximal bronchiectasis; and, if left untreated, pulmonary fibrosis with the progressive loss of lung function (table 1) [11, 13, 15]. The pathophysiology of ABPA results from florid T-helper cell (Th)2 innate and adaptive immune responses in susceptible hosts who are unable to efficiently clear the respiratory epithelium of inhaled fungal spores (fig. 1) [15–18].

This article will consider SAFS and ABPA as closely related (and probably overlapping) nosological categories of severe asthma caused by noninvasive fungal airway infection, with emphasis upon recent therapeutic approaches and trials in these patients. With the World Health Organization estimated worldwide asthma prevalence of 300 000 000 cases, the possibility of up to 30 000 000 of these falling into the SAFS category is a very sobering prospect. In addition, deductive epidemiological modelling based upon literature reports suggests that ABPA causes an estimated worldwide illness burden of nearly 5 000 000 adult cases [11, 19].

Oral glucocorticosteroids have been employed as the fundamental therapy of ABPA for several decades, based on what appears to be clear efficacy in widespread empirical experience, despite a lack of randomised placebo-controlled trials [20]. Recently, a randomised open-label controlled trial of two oral prednisolone dose regimens has been completed but the results of this study have not yet been reported (ClinicalTrials.gov identifier NCT00974766). As conventional high-dose inhaled corticosteroid therapy is insufficient to control SAFS and ABPA, and chronic recurrent oral steroid therapy carries a troublesome toxicity profile,

TABLE 1 Criteria for diagnosis of allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitisation

Allergic bronchopulmonary aspergillosis
Predisposing conditions
Asthma or cystic fibrosis
Obligatory criteria (both present)
Total baseline serum IgE >1000 IU·mL ⁻¹ #
Positive immediate hypersensitivity skin test or elevated <i>in vitro</i> specific IgE to <i>Aspergillus fumigatus</i>
Supportive criteria (≥2 present)
Eosinophilia >500 cells·μL ⁻¹ †
Serum precipitating or IgG antibodies to <i>Aspergillus fumigatus</i>
Consistent radiographic opacities ⁺
Severe asthma with fungal sensitisation
Severe asthma [§]
Positive immediate skin test or <i>in vitro</i> specific IgE to ≥1 filamentous fungus
Exclusion of allergic bronchopulmonary aspergillosis

Adapted from [13]. #: if all other criteria are met, IgE <1000 IU·mL⁻¹ may be acceptable; †: steroid naïve or historical; +: transient (consolidation, nodules, tram-track or finger-in-glove) or permanent (bronchiectasis or fibrosis) pulmonary opacities; §: American Thoracic Society definition [14] is need for oral steroids ≥50% of time and need for high-dose inhaled steroid (beclomethasone ≥1200 μg·day⁻¹ or equivalent) and ≥1 other controller (e.g. long-acting bronchodilator, montelukast, etc.) to achieve control at level of mild persistent asthma. A positive respiratory culture or DNA test for *Aspergillus fumigatus* is supportive but not necessary for diagnosis of allergic bronchopulmonary aspergillosis or severe asthma with fungal sensitisation.

FIGURE 1 Pathophysiology of allergic bronchopulmonary aspergillosis. Pathogen-associated molecular pattern (PAMP) structures of *Aspergillus fumigatus* germinating spores and hyphae activate dendritic and respiratory epithelial cells *via* innate recognition receptors, such as Toll-like receptor (TLR)2/4 to produce T-helper cell (Th)2 immune-deviating cytokines; chemokines and costimulatory molecules that include thymic stromal lymphopoietin (TSLP), interleukin (IL)-25, IL-33, OX40 ligand (OX40L) and CCL17 (thymus-activated and -regulated chemokine), which in turn orchestrate differentiation, chemotaxis and activation of CD4⁺ Th2 cells. CCL17 also attracts regulatory T-cells (Treg) capable of suppressing protective Th1 responses and suppressing macrophage activation, thereby impairing fungal killing. Th2 cells produce a suite of cytokines including IL-4 and IL-5 that attract and activate eosinophils and drive differentiation of B-cells to IgE-secreting plasmacytes. IgE antibodies affix to tissue mast cells and circulating basophils to trigger immediate hypersensitivity reactions upon re-exposure to *A. fumigatus* allergens. The resulting granulocytic luminal mucus plugging and bronchocentric mucosal inflammation lead to bronchiectasis that may progress to fibrosis if untreated. The actions of current treatments are shown by red arrows; note the pleiotropic potential of glucocorticosteroids to act on multiple cell types and levels of the disease process. AM: alveolar macrophage; DC: dendritic cell; TNF: tumour necrosis factor. Reproduced and modified with permission from [16]; see also [17] and [18] for detailed discussion.

other therapies have been explored to address the unmet treatment needs for both of these related diseases. These are reviewed herein.

Antifungal therapy in ABPA and SAFS

The most prominent of alternatives to long-term oral steroids is the use of antifungal agents as an add-on or even as primary treatment of ABPA and SAFS.

Antifungal treatment of ABPA, and more recently SAFS, rests upon an assumption that allergic inflammatory responses arise in part from noninvasive airway fungal infection. Evidence for this

FIGURE 2 Endobronchial fungal infection in a young adult with cystic fibrosis and chronic *Aspergillus fumigatus* in sputum cultures. A spontaneously expectorated sputum plug was stored overnight at 4°C. Without processing, the plug was flattened between a coverslip and slide and viewed with an upright Nikon Eclipse E600FN Series microscope (Nikon, Tokyo, Japan) equipped for differential interference contrast microscopy. Digital imaging was via a Retiga-1300, cooled, 12-Bit, colour-Bayer Mosaic CCD camera with RGB Liquid Crystal Color Filter Module (QImaging, Surrey, Canada). Images were made with a $\times 40$ (aperture 0.8, 2-mm working distance) water immersion lens (drop of water added on top of the cover slip, not touching the plug) producing an optical slice of $\sim 1\ \mu\text{m}$. a) Hyphal mat in sputum plug, possibly organising into biofilm (more parallel packed hyphal organisation). Older hyphae suggested by thicker walls and slight pigmentation. Granulocytes and exfoliated ciliated columnar epithelial cells can be seen in lower left quadrant and inset. b) Active endobronchial growth of younger thin-walled hyphae shown by tip extension and septation. Photograph courtesy of J.J. Wine (Cystic Fibrosis Research Laboratory, Stanford University, Stanford, CA, USA). a) Scale bars=20 μm ; b) scale bar=50 μm .

assumption arises from several lines of investigation. First, serological studies in patients with ABPA and SAFS demonstrate IgE responses to fungal products derived from *in vivo* germination of inhaled conidia into hyphae, as these antibodies exist as a response to fungal exoproducts, hyphal cell wall components expressed during growth phase and cytoplasmic antigens [21, 22]. Secondly, there is mounting evidence that fungal hyphal products, including β -glucan and proteases, activate epithelial cells to secrete pro-inflammatory and Th2-polarising cytokines and chemokines directly *via* Toll-like receptor-induced signalling, *via* other innate sensors or *via* protease-activated receptors [23–26]. Thirdly, fungal asthma has been linked to host innate immune responses to chitin, a major fungal cell wall component, as chitinase promoter polymorphisms and associated alterations in chitinase activity and elevated chitinase-like protein YKL-40 levels have been found in SAFS in adults and children [27–33]. Finally, despite variably reported rates of fungal recovery from conventional respiratory cultures taken from patients with SAFS and ABPA, the use of PCR- or deep sequencing-based nonculture methods reveals that the vast majority of these patients have fungal DNA in these *ex vivo* samples. In addition, these patients usually have substantial levels of the hyphal cell wall component, galactomannan, in their sputum samples, often at levels consistent with invasive disease when measured in the blood [34, 35]. Direct *ex vivo* microscopy of such cases clearly demonstrates active hyphal growth in sputum plugs (fig. 2).

To date, antifungal therapy for SAFS and ABPA has been directed against the main fungal pathogen, *A. fumigatus* (table 2). Initial trials of the imidazole ketoconazole and the polyene natamycin (antifungal agents lacking high activity against *Aspergillus* spp.) in patients with ABPA were disappointing [36, 37]. However, case reports and uncontrolled series over several decades have reported treatment success upon addition of the *Aspergillus*-active oral triazole agent itraconazole to steroids for ABPA in asthma patients [38–41]. PACHECO *et al.* [39] showed that itraconazole reduced specific IgG titres in a patient. GERMAUD and TUCHAIS [40] showed effectiveness in 11 out of 12 treated patients, including prevention of exacerbations in six patients weaned off oral steroids. Using a before–after methodology, SALEZ *et al.* [41] showed reduction in exacerbations and oral steroid doses when patients were started on itraconazole.

The effectiveness of itraconazole in ABPA was demonstrated in two randomised, double-blind, placebo-controlled trials in patients with asthma (both trials excluded patients with CF) [42, 43]. A multicentre trial in 55 patients in the United States by STEVENS *et al.* [42] found more responders in those randomised to

TABLE 2 Antifungal therapy of allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS)

AUTHOR [ref.]	Year	Drug dosage	Study design	Population	Treatment/follow-up	Outcome/comments
SHALE [36]	1987	Ketoconazole 400 mg per day by mouth	RDBPCT	7 ABPA, 3 aspergilloma	12 months 6 keto, 4 placebo	Decrease in Af-IgG and symptom score
CURRIE [37]	1990	Natamycin 5 mg nebuliser twice daily	RDBPCT	25 ABPA (13 nata, 12 placebo (intent to treat; 5 withdrew))	12 months	20 completers, no effect on steroids or disease activity
DENNING [38]	1991	ITZ 200 mg per day	Open	6 ABPA (3 CF)	4 months	Decrease in steroids and IgE; increase in PFT
PACHECO [39]	1993	ITZ 200 mg per day	Open	1 ABPA	4 months/6 months	Decrease in steroids and Af-IgG; off rx follow-up return to baseline
GERMAUD [40]	1995	ITZ 200 mg per day	Open	12 ABPA	12 months	11 out of 12 showed major improvement (steroid rx, relapse and serologies)
SALEZ [41]	1999	ITZ 200 mg per day	Open, before-after	14 ABPA	≥12 months	All improved, 7 out of 14 weaned off steroids; increase in PFT and serologies
STEVENS [42]	2000	ITZ 200 mg twice daily	RDBPCT	55 ABPA (28 ITZ, 27 placebo)	16 weeks RCT, 16 weeks open extension	Composite response rate 46% ITZ versus 19% placebo (p=0.04); 36% RCT nonresponders improved on extension
WARK [43]	2003	ITZ 400 mg per day	RDBPCT	29 ABPA (15 ITZ, 14 placebo)	16 weeks	Decrease in relapses, eosinophil count, ECP and tlgE on ITZ (p≤0.03)
MANNES [44]	1993	ITZ 200 mg per day	Open	2 CF-ABPA	To 9 months	Decrease in tlgE, Af-IgG and steroids; increase in PFT and weight
NEPOMUCENO [45]	1999	ITZ 200–400 mg per day	Open, before-after	16 CF-ABPA	12 months	Decrease in steroid dose in 47% (p=0.05), relapses in 55% (p<0.001)
SKOV [46]	2002	ITZ 200–600 mg per day	Open	21 CF-ABPA	To 5 years	Decrease in tlgE and Af-IgG/E; increase in PFT
CASAULTA [47]	2005	ITZ 100 mg per day	Open	9 CF-ABPA	To 32 months	Stable PFT
HILLIARD [48]	2005	VCZ 100–200 mg twice daily	Open	13 CF-ABPA	1–50 weeks	Increase in PFT; decrease in tlgE; adverse events in 33%
GLACKIN [49]	2009	VCZ 100–200 mg twice daily	Open	10 CF-ABPA	NA	Decrease in steroids and tlgE; stable PFT
CHISHIMBA [50]	2012	VCZ 3–6 mg per day POS 800 mg per day	Open	20 ABPA, 5 SAFS	12 months	Selected ITZ failures; clinical responses in VCZ (75%) and POS (78%); 26% of VCZ having an adverse event lead to discontinuation
DENNING [51]	2009	ITZ 200 mg twice daily	RDBPCT	58 SAFS	32 weeks/16 weeks	Increase in symptom score (p=0.01) and PF; decrease in tlgE (p=0.001)
PASQUALOTTO [52]	2009	ITZ, VCZ	Open	11 ABPA, 22 SAFS	12 months	Decrease in tlgE, Af-IgE, eosinophil count and steroids; increase in PFT
VICENCIO [30]	2010	ITZ 100–200 mg twice daily	Open	3 paediatric SAFS	12 months	Decrease in symptoms and steroids
VICENCIO [53]	2010	ITZ 100 mg twice daily	Open	1 paediatric SAFS	6 months 3 months	Decrease in symptoms and steroids; increase in PFT; relapsed off ITZ
CASEY [54]	2002	nAMBd 16–40 mg per day	Open	1 CF-ABPA	4 months	Lung transplant pre-ABPA; decrease in steroids; increase in PFT
LAUDI [55]	2008	nAMB 5 mg twice daily	Open	3 CF-ABPA	>6 months	Symptoms controlled; increase in PFT; decrease in eosinophil count, tlgE and Af-IgE/G
PROESMANS [56]	2010	nAMBd 20 mg thrice weekly, nABLC 50 mb twice weekly	Open	7 CF-ABPA	12 months	6 out of 7 off steroids without relapse; 5 out of 6 showed increase in PFT
HAYES [57]	2010	nAMBd 10 mg twice daily	Open	1 CF-ABPA	9 months	Off steroids, no relapse, decrease in tlgE

RDBPCT: randomised double-blind placebo-controlled trial; Af-IgG/E: *Aspergillus fumigatus*-specific IgG and/or IgE antibody; nata: natamycin; ITZ: itraconazole; CF: cystic fibrosis; rx: treatment; PFT: spirometric pulmonary functions; RCT: randomised clinical trial; ECP: eosinophil cationic protein; tlgE: total IgE; VCZ: voriconazole; NA: not available; POS: posaconazole; PF: peak expiratory flow rate; nAMB: nebulised amphotericin B; nAMBd: nAMB deoxycholate; nABLC: nAMB lipid complex.

receive itraconazole (n=28) for 16 weeks as compared with placebo (n=27). The primary efficacy end-point was a composite measure consisting of at least a 50% reduction in oral steroid dose, at least a 25% reduction in total IgE and at least a 25% improvement in exercise tolerance or resolution of pulmonary infiltrates. Using these criteria, 46% of patients receiving itraconazole responded compared with 19% of those receiving placebo (p=0.04). Additionally, a third of the nonresponders in the placebo-controlled portion of the trial then responded during a subsequent 16-week open-label extension. No relapses occurred in patients receiving itraconazole during the study. In a second trial, conducted at a single centre in the UK, WARK *et al.* [43] extended these observations in stable asthma patients with ABPA. This trial randomised patients to itraconazole (n=15) or placebo (n=14) for 16 weeks. The study population here was different from that of the study by STEVENS *et al.* [42] in that only one-third of these patients were receiving oral steroids during the trial; all were on inhaled steroids on an average dose of 2000 µg daily, and half were also receiving a daily leukotriene receptor antagonist. In the study by WARK *et al.* [43], the main outcome indicators evaluated were immunological biomarkers. Patients receiving itraconazole showed normalisation of sputum eosinophilia and eosinophil cationic protein level, and a decrease in serum total IgE level and *Aspergillus*-specific IgG level. With regard to clinical outcomes, fewer itraconazole-treated patients had exacerbations than patients receiving placebo. These results suggest an anti-inflammatory benefit of itraconazole in ABPA in asthma patients, which may be due to a reduction in fungal burden or perhaps other nonantimicrobial mechanisms. Overall, pooled data from the placebo-controlled trials indicate that

itraconazole is effective in ~60% of asthma-ABPA patients (number needed to treat=3.58; personal communication: D. Denning, National Aspergillus Centre, University of Manchester, Manchester, UK). The use of azoles for ABPA in asthma patients was reviewed and recommended by the Cochrane collaboration [58, 59].

ABPA occurs in ~8% of CF patients (meta-analysis 95% confidence interval 6–10%) [60]. Extended courses of oral corticosteroids are considered first-line treatment for ABPA in asthma, and this is also the case in patients with CF [61–65]. As in asthma, itraconazole add-on therapy has been reported to be clinically beneficial in several uncontrolled studies of ABPA in CF patients [38, 44–47]. In these studies, reductions in oral steroid dose and stabilisation of lung function have been found. NEPOMUCENO *et al.* [45] also reported a significant decrease in exacerbations compared with a control period [46]. The Cystic Fibrosis Foundation Consensus Conference on ABPA in patients with CF recommended the use of itraconazole as an add-on therapy to oral steroids in patients with slow or poor response to oral steroids, relapse, steroid toxicity or steroid-dependence [65]. A 2000 Cochrane Collaboration review of the use of itraconazole for ABPA in patients with CF cautioned that use was “experimental” in the absence of controlled trials, but the 2012 update concluded that azole therapy is “potentially useful” in CF while in need of further trials with clear outcome measures [66, 67]. Some reports have suggested that itraconazole monotherapy may be a viable alternative to azole add-on treatment after oral glucocorticosteroids, but as yet there is no data from a controlled trial comparing azoles with steroids as monotherapy for ABPA [46, 47, 68–71]. However, recently, a randomised, open-label 3-month trial with a 3-month follow-up in 50 adolescent and adult asthma patients with ABPA comparing itraconazole with oral prednisolone monotherapy ([ClinicalTrials.gov](#) identifier NCT01321827), and a similar 50 patient trial comparing voriconazole with prednisolone ([ClinicalTrials.gov](#) identifier NCT01621321) have been initiated.

Use of itraconazole is limited by issues of poor absorption and bioavailability, pharmacogenetic variability in cytochrome P450 enzyme-mediated hepatic metabolism and toxicities [72]. Therefore, therapeutic drug monitoring has been recommended [73]. These problems are exaggerated in patients with CF as compared with asthma and, therefore, higher doses of itraconazole capsule or use of the cyclodextrin liquid formulation have been recommended for CF patients [74–76]. It may be difficult to achieve optimal efficacy with itraconazole in CF patients. This is due to its poor bioavailability, but also due to the aggravated absorption defects associated with pancreatic insufficiency, concomitant hepatobiliary disease and small bowel involvement, as well as the requirement for acidic gastric pH to ensure optimal itraconazole absorption being hindered by widespread use of gastric acid-suppressing agents. As itraconazole is highly lipophilic, a suspension in cyclodextrin is 20–50% more bioavailable than the capsule formulation. In order to ameliorate these problems, monitoring of blood levels is recommended in CF whenever therapeutic response is disappointing or there is concern about toxicity [65]. The recommended therapeutic steady-state itraconazole level, based on typical *Aspergillus* minimal inhibitory concentrations, and clinical studies in a variety of disease states, is 1–5 $\mu\text{g}\cdot\text{mL}^{-1}$, as measured by the most commonly used method, *i.e.* high-pressure liquid chromatography [73]. It should be noted that recommendations for target dosing for therapeutic efficacy based on monitoring drug levels in the blood have been derived from studies of invasive aspergillosis (where subtherapeutic trough levels have been associated with treatment failure) rather than direct evidence from treating ABPA [77]. Toxicities reported in $\geq 4\%$ of patients (peripheral neuropathy, fluid retention, gastrointestinal intolerance, elevated hepatic transaminases, rash, headache, tremor and sleep disturbance) have been found with high steady-state triazole levels in patients with chronic pulmonary aspergillosis [72, 78]. In addition, an important drug–drug interaction exists between itraconazole and several corticosteroids, including oral or intravenous methylprednisolone and inhaled budesonide and fluticasone; the azole impairs metabolism of these exogenous glucocorticosteroids resulting in potential adrenal suppression, including overt Cushing syndrome [79–87]. It is, therefore, safer to use oral prednisone or prednisolone (neither of which has these interactions), or perhaps inhaled beclomethasone (which to date has not been shown to have an azole interaction but has also not been systematically studied in this regard), or ciclesonide (a prodrug with topical respiratory metabolism) [88], if using itraconazole or other triazoles in treating ABPA or SAFS.

Newer oral triazoles with excellent anti-*Aspergillus* activity (voriconazole and posaconazole) have also been reported as beneficial in the treatment of ABPA, particularly in patients with CF [48, 49, 89–91]. In one study, voriconazole was used as monotherapy in 13 CF patients with ABPA; significant and sustained improvements in clinical status, lung function and serologies occurred with prolonged treatment, although nine patients required oral steroids [48]. In another study, 10 out of 11 steroid-dependent patients were able to reduce oral steroid needs while having a marked drop in IgE levels [49]. Voriconazole has the advantage of excellent oral bioavailability. However, voriconazole has strong inhibitory effects on hepatic cytochrome P450 enzymes CYP3A4, CYP2C19 and CYP2C9; making for notoriously unpredictable steady-state levels and drug–drug interactions. This complex metabolism results in much greater inter-individual variability in

steady-state voriconazole levels when compared with itraconazole (up to 100-fold range for voriconazole *versus* 15-fold range for itraconazole), making therapeutic drug monitoring highly advisable [73, 92, 93]. In addition, there appears to be both greater incidence and severity of toxicities with voriconazole when compared with itraconazole [91, 94]. Voriconazole levels of $>6 \mu\text{g}\cdot\text{mL}^{-1}$ are predictive of increased toxicity, including: hepatic; ophthalmological and photosensitive dermatological adverse reactions; and rare, but more serious, cardiac (Torsades de pointes) and neurological events. Voriconazole is also much more expensive. Most recently, posaconazole, a triazole with higher activity against *Aspergillus* spp. and fewer side-effects than voriconazole, has also been reported to have benefits in treatment of ABPA as well as chronic pulmonary aspergillosis, but it is even more expensive than voriconazole and therapeutic drug monitoring is also advised [50, 95, 96].

The use of azoles for treatment of ABPA has recently been extended to patients with SAFS. Severe asthma inadequately controlled despite combination inhaled corticosteroid/long-acting bronchodilator therapy may be found in up to 20% of asthmatics, and up to half of this large group of difficult patients have atopic fungal sensitivities, most commonly to *Aspergillus* species but often to multiple fungi. SAFS patients do not meet the necessary constellation of clinical, serological and radiological criteria for a diagnosis of ABPA, usually because total IgE levels are $<1000 \text{ IU}\cdot\text{mL}^{-1}$ and/or key radiographic findings, such as mucoid impaction or bronchiectasis, are lacking. As noted previously, the link between fungal sensitisation and severe asthma has been increasingly recognised as a significant piece of the larger asthma puzzle [1–9]. Recently this association between fungal sensitisation and the severe asthma phenotype has been further supported by significant correlations between indoor *Aspergillus* spore air sample concentrations, recovery of fungi (most commonly but not solely *A. fumigatus*) from respiratory tract cultures and more severe clinical asthma [97, 98]. In one recent paediatric study, 59% of children with severe persistent asthma were found to have fungal sensitisation [99].

After anecdotal experience in adults with SAFS suggested antifungal therapy with itraconazole may be beneficial with reduced hospital admissions and steroid courses, DENNING *et al.* [52] conducted a randomised, double-blind, placebo-controlled trial of itraconazole in 58 patients with SAFS, $>40\%$ of whom had been hospitalised within the previous year. The treatment effect on the primary end-point and a validated asthma quality-of-life score was significant. In addition, the rhinitis score, morning peak-flow rates and serum IgE levels were also significantly improved. However, it is important to note that side-effects leading to discontinuation occurred in five out of 29 treated patients and drug–drug interactions resulting in suppression of cortisol levels in half the treated subjects were reported. Similarly to ABPA, $\sim 60\%$ of SAFS patients were responders to itraconazole (number needed to treat=3.22). In a real-life effectiveness study, outcomes in 22 SAFS patients, as well as 11 asthmatic ABPA patients, treated with open-label itraconazole for at least 6 months were followed [52]. Lung function was improved, while dosage and courses of oral steroids were decreased, and 40% of patients were weaned off oral steroids after 6 months of therapy. Serological measures (total and *Aspergillus*-specific IgE) and eosinophils were decreased in patients treated for 6–12 months. Recently, similar success in treating SAFS in children with itraconazole has also been reported [30, 53].

While oral azoles, thus far, appear to be an effective component in successful management of ABPA and SAFS, several important caveats exist. These include inter-individual variability in absorption and metabolism, toxicity, drug–drug interactions and cost. It has not yet been clearly demonstrated that the beneficial effects of azoles in ABPA and SAFS are due to their antifungal activity as opposed to alterations in concomitantly administered glucocorticosteroid metabolism or independent anti-inflammatory azole effects [43, 51]. Most troubling, however, is the emerging evidence that increased azole usage for various medical conditions and (at least in some geographical regions) agricultural applications is leading to a higher prevalence of azole resistance in clinical *A. fumigatus* isolates, most commonly due to point mutations in the *cyp51A* gene [100–108]. The *Aspergillus cyp51A* gene encodes cytochrome P450 sterol 14 α -demethylase and is the target for azole drugs. Between 5% and 20% of CF patients exposed to recent itraconazole courses were found to be either colonised or infected with azole-resistant *A. fumigatus* in recent studies, while, in another study, 4% of *A. fumigatus* respiratory isolates from a variety of different patient groups (including CF, chronic obstructive pulmonary disease, intensive care unit cases and ABPA) were itraconazole-resistant [104, 105, 108]. In some instances, azole cross-resistance has also been documented. Resistance to both itraconazole and voriconazole has been found in patients with ABPA [102, 109].

Alternatives to azoles

In part due to the potential problems of metabolism, tolerance and resistance associated with azole therapy of ABPA and SAFS, further alternative approaches have been investigated utilising both anti-infective and anti-inflammatory target modalities.

Amphotericin B

Anti-infective alternatives to azole therapy for SAFS and ABPA have, thus far, been limited to the use of inhaled formulations of amphotericin B, as topical delivery avoids most systemic toxicity issues. Amphotericin deoxycholate has been used by inhalation to treat pulmonary fungal infection for over half a century, primarily in the settings of cancer treatment or lung transplantation [110]. Unfortunately the literature on inhaled amphotericin is muddled by a plethora of unstandardised and often poorly validated delivery systems and dose regimes; by the availability of multiple formulations (approved for *i.v.* use) including water-soluble lyophilised amphotericin deoxycholate and three commercially available lipid preparations; and, finally, by a substantial diversity of the diseases for which these agents have been nebulised. A variety of nebulisation devices can deliver amphotericin B particles with good tolerability to the lower respiratory tract in doses capable of exceeding typical *Aspergillus* minimal inhibitory concentrations in the epithelial lining fluid [111]. Systemic levels are low, reducing the risk of renal and other toxicities. Thus, inhaled amphotericin is a plausible therapy for the chronic or recurrent noninvasive *Aspergillus* respiratory infection seen in SAFS and ABPA.

However, clinical results for ABPA, while positive, are available for scrutiny in only a few case reports and one small open-labelled series in CF patients [54–57, 112]. Two recent reports utilised amphotericin deoxycholate or liposomal amphotericin in aerosol doses of 10 mg twice daily or 20 mg thrice weekly, respectively, with success [56, 57]. There have been no published reports of inhaled amphotericin use in SAFS. Based on the available literature, it is unclear what the optimal amphotericin formulation, dose, schedule and delivery system should be. Care should be taken to initiate inhaled amphotericin B therapy under observation as cough and bronchospasm may occur, especially with low baseline lung function [113]. An interesting future prospect is the development of a dry powder inhalational formulation and rapid portable delivery system for amphotericin B [114].

Alternative anti-inflammatory approaches to use of oral corticosteroids in ABPA have included the use of high-dose inhaled glucocorticosteroids, *i.v.* monthly “pulse” high-dose glucocorticosteroids and immunomodulation of the allergic response with omalizumab (humanised monoclonal anti-IgE). Inhaled steroids are already the basic treatment of all severe asthma phenotypes, and none of the other modalities have been reported as yet in SAFS. Leukotriene antagonists have not been evaluated in the treatment of SAFS or ABPA, but would not be expected to provide much benefit given their recommended use for milder asthma phenotypes [115].

Alternative corticosteroid regimes

Inhaled corticosteroids, while useful for concomitant asthma management in patients with ABPA, do not control the pathophysiology or clinical manifestations of ABPA [116–120]. In contrast, “pulse” steroid therapy (10–20 mg·kg⁻¹·day⁻¹ *i.v.* methylprednisolone infused on three consecutive days every 3–4 weeks) was generally safe and effective in two open-labelled series of 13 steroid-dependent ABPA CF patients selected for this treatment because they were either not well controlled or had severe corticosteroid side-effects on conventional oral prednisone treatment [121, 122]. In most cases, pulse *i.v.* steroid therapy was well tolerated, with disease control allowing discontinuation of pulse therapy after 6–12 months. However, long-term follow-up data is not available and this published experience is uncontrolled and sparse.

Anti-IgE

Omalizumab, a monoclonal antibody to IgE that prevents allergen-induced IgE-mediated signalling of the classic allergic inflammatory cascade, is licensed in many countries for use in patients with severe allergic asthma [123]. It is increasingly utilised in the treatment of ABPA. While SAFS patients have undoubtedly been included in the many large clinical trials and effectiveness studies of omalizumab that have focused on the approved indication of patients with severe allergic asthma, SAFS is not identifiable as a distinct subgroup for analysis in these studies as selection criteria in most trials, and in subgroup analyses when reported, focused on allergic sensitisation to perennial indoor allergens, *i.e.* dust mite, cockroach and cat or dog dander [124, 125]. The rationale and pharmacodynamic issues involved in the use of omalizumab for ABPA are reviewed elsewhere [126]. Omalizumab-treated ABPA patients reported in the literature have generally responded well, with reduced exacerbation rates, decreased oral steroid exposure and decreased steroid toxicity being the three major observed benefits of therapy [127–142]. For example, two open-labelled series from Spain and France (34 subjects when pooled, including two with CF-ABPA) showed significant reductions in exacerbations and oral steroid doses [137, 138]. However, a recent multicentre open-labelled retrospective series from France found variable results over an average 21-month observation period in 32 CF-ABPA patients on omalizumab, with a reduction in steroid need but no change in lung function or use of *i.v.* antibiotics [143].

As yet, no placebo-controlled trials of omalizumab in ABPA or SAFS have been completed, leading to a call by the Cochrane Collaboration for completion of a randomised controlled trial [144]. A multicentre, randomised, double-blind, placebo-controlled, 6-month trial with a 6-month open-labelled extension in CF-ABPA patients aged ≥ 12 years concomitantly treated with prednisone (on a prescribed tapering regime) and itraconazole 400 mg twice daily was initiated in Europe in 2008. It had rescue oral steroid use as the primary outcome (ClinicalTrials.gov identifier NCT00787917). The study was terminated by the sponsor, Novartis Pharmaceuticals, in 2011 after enrolment of only 14 subjects (mean $\pm$ SD age 23 ± 7 years). Both enrolment and dropouts apparently were impacted by an arduous study design that included daily subcutaneous injections of omalizumab at doses of up to 600 mg or placebo. Of nine subjects randomised to omalizumab, only four completed the 6-month placebo-controlled trial; discontinuations were attributed to an adverse event in one, lack of efficacy in one and “administrative problems” in three, and two out of five subjects randomised to placebo also dropped out due to “administrative problems.” Of the seven subjects going on to the 6-month open-labelled extension, only three completed it, with dropouts attributed to unsatisfactory therapeutic effect ($n=1$) and “administrative problems” ($n=3$). Crucially, this failed trial utilised omalizumab in a much more intensive and intrusive regime than the way it is marketed and clinically used for allergic asthma (*i.e.* maximum dose 375 mg every 2 weeks), leaving open the question of whether a similar “real world” design (as in the published off-labelled case reports and series) might not be a more feasible and potentially successful way to examine efficacy in a controlled trial.

The package insert dosing table for omalizumab treatment of asthma, which caps recommended maximal dosing at 375 mg every 2 weeks, encompasses a baseline (free) serum total IgE range of 30–700 IU·mL⁻¹ and a bodyweight range of 20–150 kg. The dosing table is based on clinical trial doses targeted to reduce free IgE levels in blood to <25 IU·mL⁻¹ in $\geq 95\%$ of recipients meeting the baseline IgE level range requirements [145]. However, the dosing table recommendations generally correspond well to a published formula of $0.016 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{IgE}^{-1}$ (in IU·mL⁻¹) monthly that is based on calculations of dose required to bind $>90\%$ free IgE *in vitro* [146, 147]. As patients with ABPA by definition have baseline IgE levels exceeding the dosing table upper limit (>1000 versus 700 IU·mL⁻¹, respectively), and many SAFS patients may also have IgE levels above the dosing table range, the apparent clinical efficacy in the literature, generally using doses at or only modestly greater than the dosing table, suggests that dosing for ABPA and SAFS is not substantially greater than that currently recommended for patients with lower baseline IgE levels and may suffice for clinical benefit. Recently, two CF-ABPA patients with baseline IgE levels of 1039 and 1782 IU·mL⁻¹ were treated on the basis of the formula, resulting in omalizumab regimes of 450 mg monthly in the first patient and 450 mg every 2 weeks in the second. They had reductions in free IgE of 88% and 96% after 6 and 3 months treatment, respectively, with corresponding marked clinical improvement [148]. Altogether the clinical experience suggests that omalizumab treatment is rational in patients with SAFS or ABPA, despite IgE >700 IU, especially if the formula, rather than dosing table, is utilised to calculate an optimal dose and interval adjusted for tolerability. In a recent alternative strategy of interest, a recent case report of omalizumab therapy in a patient with SAFS suggested that using the immunological effect of azole therapy to reduce the baseline IgE value may help establish an omalizumab regime within the dosing table [43, 149].

An important difficulty in properly evaluating any emerging therapy for ABPA is the potential differential response to therapeutic interventions in patients with different degrees of structural lung damage. Only one published trial, stratified or otherwise, distinguished ABPA patients without bronchiectasis (“ABPA-serologic”) from those with bronchiectasis [120, 150]; those with hyperattenuating mucoid impaction have not been compared with those without impaction, despite apparent differences in severity of immunopathology and prognosis [13, 151].

In conclusion, active noninvasive endobronchial fungal infection is likely to play an important role in a significant segment of asthma and CF patients with more severe pulmonary pathology and illness. Therapies aimed at lowering the fungal burden and at down-regulating host allergic immune response show indications of efficacy, which are supported by the improved understanding of SAFS and ABPA pathophysiology, distinct nosological entities along a spectrum of asthma that is induced by fungal infection and Th2-biased immune response. Their role in the overall management of these patients remains to be determined, hopefully by controlled trials, where such evidence is lacking, and by comparative effectiveness studies comparing conventional with alternative treatments and alternative treatments with each other.

Acknowledgements

The author thanks J. Wine (Cystic Fibrosis Research Laboratory, Stanford University, Stanford, CA, USA) for kindly providing the photomicrograph shown in the figure 2.

References

- 1 Knutsen AP, Bush RK, Demain JG, *et al.* Fungi and allergic lower respiratory tract disease. *J Allergy Clin Immunol* 2012; 129: 280–291.
- 2 Zureik M, Nuekirch C, Leynaert B, *et al.* Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ* 2002; 325: 411–414.
- 3 O'Driscoll RB, Hopkinson L, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pulm Med* 2005; 5: 4.
- 4 Denning DW, O'Driscoll BR, Hogaboam CM, *et al.* The link between fungi and asthma: a summary of the evidence. *Eur Respir J* 2006; 27: 615–626.
- 5 Fairs A, Agbetile J, Hargadon B, *et al.* IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma. *Am J Respir Crit Care Med* 2010; 182: 1362–1368.
- 6 Agarwal R, Gupta D. Severe asthma and fungi: current evidence. *Med Mycol* 2011; 49: Suppl. 1, S150–S157.
- 7 Menzies D, Holmes L, McCumesky G, *et al.* *Aspergillus* sensitization is associated with airflow limitation and bronchiectasis in severe asthma. *Allergy* 2011; 66: 679–685.
- 8 Kennedy JL, Heymann PW, Platts-Mills TAE. The role of allergy in severe asthma. *Clin Exp Allergy* 2012; 42: 659–669.
- 9 Agarwal R. Severe asthma with fungal sensitization. *Curr Allergy Asthma Rep* 2011; 11: 403–413.
- 10 Agarwal R, Aggarwal AN, Gupta D, *et al.* *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2009; 13: 936–944.
- 11 Hogan C, Denning DW. Allergic bronchopulmonary aspergillosis and related allergic syndromes. *Semin Respir Crit Care Med* 2011; 32: 682–692.
- 12 Gernez Y, Dunn CE, Everson C, *et al.* Blood basophils from cystic fibrosis patients with allergic bronchopulmonary aspergillosis are primed and hyper-responsive to stimulation by *Aspergillus* allergens. *J Cyst Fibros* 2012; 11: 502–510.
- 13 Agarwal R, Chakrabarti A, Shah A, *et al.* Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013; 43: 850–873.
- 14 Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med* 2000; 162: 2341–2351.
- 15 Moss RB. Allergic bronchopulmonary aspergillosis. In: Latge JP, Steinbach WJ, eds. *Aspergillus fumigatus* and Aspergillosis. Washington DC, American Society of Microbiology Press, 2009; pp. 333–350.
- 16 Hartl D, Buckland KF, Hogaboam CM. Chemokines in allergic aspergillosis – from animal models to human lung diseases. *Inflamm Allergy Drug Targets* 2006; 5: 219–228.
- 17 Kreindler JL, Steele C, Nguyen N, *et al.* Vitamin D3 attenuates Th2 responses to *Aspergillus fumigatus* mounted by CD4+ T cells from cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J Clin Invest* 2010; 120: 3242–3254.
- 18 Knutsen AP, Slavin RG. Allergic bronchopulmonary aspergillosis in asthma and cystic fibrosis. *Clin Devel Immunol* 2011; 2011: 843763.
- 19 Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med Mycol* 2013; 51: 361–370.
- 20 Moss RB. Critique of trials in allergic bronchopulmonary aspergillosis and fungal allergy. *Med Mycol* 2006; 44: Suppl. 1, 269–272.
- 21 Kurup VP, Knutsen AP, Moss RB, *et al.* Specific antibodies to recombinant allergens of *Aspergillus fumigatus* in cystic fibrosis patients with ABPA. *Clin Mol Allergy* 2006; 4: 11.
- 22 Fricker-Hidalgo H, Coltey B, Llerena C, *et al.* Recombinant allergens combined with biological markers in the diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis patients. *Clin Vaccine Immunol* 2010; 17: 1330–1336.
- 23 Kheradmand F, Kiss A, Xu J, *et al.* A protease-activated pathway underlying Th cell type 2 activation and allergic lung disease. *J Immunol* 2002; 169: 5904–5911.
- 24 Porter P, Susarla SC, Polikepahad S, *et al.* Link between allergic asthma and airway mucosal infection suggested by proteinase-secreting household fungi. *Mucosal Immunol* 2009; 2: 504–517.
- 25 Neveu WA, Bernardo E, Allard JL, *et al.* Fungal allergen β -glucans trigger p38 mitogen-activated protein kinase-mediated IL-6 translation in lung epithelial cells. *Am J Respir Cell Mol Biol* 2011; 45: 1133–1141.
- 26 Porter PC, Onger V, Luong A, *et al.* Seeking common pathophysiology in asthma, atopy and sinusitis. *Trends Immunol* 2011; 32: 43–49.
- 27 Elias JA, Homer RJ, Hamid Q, *et al.* Chitinases and chitinase-like proteins in T(H)2 inflammation and asthma. *J Allergy Clin Immunol* 2005; 116: 497–500.
- 28 Chupp GL, Lee CG, Jarjour N, *et al.* A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007; 357: 2016–2027.
- 29 Wu AC, Lasky-Su J, Rogers CA, *et al.* Fungal exposure modulates the effect of polymorphisms of chitinases on emergency department visits and hospitalizations. *Am J Respir Crit Care Med* 2010; 182: 884–889.
- 30 Vicencio AG, Chupp GL, Tsirilakis K, *et al.* CHIT1 mutations: genetic risk factor for severe asthma with fungal sensitization? *Pediatrics* 2010; 126: e982–e985.
- 31 Van Dyken SJ, Garcia D, Porter P, *et al.* Fungal chitin from asthma-associated home environments induces eosinophilic lung infiltration. *J Immunol* 2011; 187: 2261–2267.
- 32 Goldman DL, Li X, Tsirilakis K, *et al.* Increased chitinase expression and fungal-specific antibodies in the bronchoalveolar lavage fluid of asthmatic children. *Clin Exp Allergy* 2012; 42: 523–530.
- 33 Goldman DL, Vicencio AG. The chitin connection. *MBio* 2012; 3: e00056–12.
- 34 Denning DW, Park S, Lass-Flörl C, *et al.* High-frequency triazole resistance found in unculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clin Infect Dis* 2011; 52: 1123–1129.
- 35 Baxter CG, Dunn G, Jones AM, *et al.* Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol* 2013; 132: 560–566.
- 36 Shale DJ, Faux JA, Lane DJ. Trial of ketoconazole in non-invasive pulmonary aspergillosis. *Thorax* 1987; 42: 26–31.

- 37 Currie DC, Lueck C, Milburn HJ, *et al.* Controlled trial of natamycin in the treatment of allergic bronchopulmonary aspergillosis. *Thorax* 1990; 45: 447–450.
- 38 Denning DW, Van Wye JE, Lewiston NJ, *et al.* Adjunctive therapy of allergic bronchopulmonary aspergillosis with itraconazole. *Chest* 1991; 100: 813–819.
- 39 Pacheco A, Martin JA, Cuevas M. Serologic response to itraconazole in allergic bronchopulmonary aspergillosis. *Chest* 1993; 103: 980–981.
- 40 Germaud P, Tuchais E. Allergic bronchopulmonary aspergillosis treated with itraconazole. *Chest* 1995; 107: 883.
- 41 Salez F, Brichet A, Desurmont S, *et al.* Effects of itraconazole therapy in allergic bronchopulmonary aspergillosis. *Chest* 1999; 116: 1665–1668.
- 42 Stevens DA, Schwartz HJ, Lee JY, *et al.* A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 2000; 342: 756–762.
- 43 Wark PA, Hensley MJ, Saltos N, *et al.* Anti-inflammatory effect of itraconazole in stable allergic bronchopulmonary aspergillosis: a randomized controlled trial. *J Allergy Clin Immunol* 2003; 111: 952–957.
- 44 Mannes GP, van der Heide S, van Aalderen WMC, *et al.* Itraconazole and allergic bronchopulmonary aspergillosis in twin brothers with cystic fibrosis. *Lancet* 1993; 341: 492.
- 45 Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis: role of atopy and response to itraconazole. *Chest* 1999; 115: 364–370.
- 46 Skov M, Hoiby N, Koch C. Itraconazole treatment of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Allergy* 2002; 57: 723–728.
- 47 Casaulta C, Flückinger S, Cramer R, *et al.* Time course of antibody response to recombinant *Aspergillus fumigatus* antigens in cystic fibrosis with and without ABPA. *Pediatr Allergy Immunol* 2005; 16: 217–225.
- 48 Hilliard T, Edwards S, Buchdahl R, *et al.* Voriconazole therapy in children with cystic fibrosis. *J Cyst Fibros* 2005; 4: 215–220.
- 49 Glackin L, Leen G, Elnazir B, *et al.* Voriconazole in the treatment of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Ir Med J* 2009; 102: 29.
- 50 Chishimba L, Niven RM, Cooley J, *et al.* Voriconazole and posaconazole improve asthma severity in allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitization. *J Asthma* 2012; 49: 423–433.
- 51 Denning DW, O'Driscoll BR, Powell G, *et al.* Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitization: the Fungal Asthma Sensitization Trial (FAST) study. *Am J Respir Crit Care Med* 2009; 179: 11–18.
- 52 Pasqualotto AC, Powell G, Niven R, *et al.* The effects of antifungal therapy on severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis. *Respirology* 2009; 14: 1121–1127.
- 53 Vicencio AG, Muzumdar H, Tsirilakis K, *et al.* Severe asthma with fungal sensitization in a child: response to itraconazole therapy. *Pediatrics* 2010; 125: e1255–e1258.
- 54 Casey P, Garrett J, Eaton T. Allergic bronchopulmonary aspergillosis in a lung transplant patient successfully treated with nebulized amphotericin. *J Heart Lung Transplant* 2002; 21: 1237–1241.
- 55 Laoudi Y, Paolini JB, Grimfed A, *et al.* Nebulized corticosteroid and amphotericin B: an alternative treatment for ABPA? *Eur Respir J* 2008; 31: 908–909.
- 56 Proesmans M, Vermeulen F, Vreys M, *et al.* Use of nebulized amphotericin B in the treatment of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Int J Pediatr* 2010; 2010: 376287.
- 57 Hayes D Jr, Murphy BS, Lynch JE, *et al.* Aerosolized amphotericin for the treatment of allergic bronchopulmonary aspergillosis. *Pediatr Pulmonol* 2010; 45: 1145–1148.
- 58 Wark P. Pathogenesis of allergic bronchopulmonary aspergillosis and an evidence-based review of azoles in treatment. *Respir Med* 2004; 98: 915–923.
- 59 Wark PA, Gibson PG, Wilson AJ. Azoles for allergic bronchopulmonary aspergillosis associated with asthma. *Cochrane Database Syst Rev* 2004; 3: CD001108.
- 60 Agarwal R. Allergic bronchopulmonary aspergillosis. *Chest* 2009; 135: 805–826.
- 61 Imbeau SA, Nichols D, Flaherty D, *et al.* Relationships between prednisone therapy, disease activity and the total serum IgE level in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1978; 62: 91–95.
- 62 Wang JL, Patterson R, Roberts M, *et al.* The management of allergic bronchopulmonary aspergillosis. *Am Rev Respir Dis* 1979; 120: 87–92.
- 63 Capewell S, Chapman BJ, Alexander F, *et al.* Corticosteroid treatment and prognosis in pulmonary eosinophilia. *Thorax* 1989; 44: 925–929.
- 64 Greenberger PA. Allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 2002; 110: 685–692.
- 65 Stevens DA, Moss RB, Kurup VP, *et al.* Participants in the Cystic Fibrosis Foundation Consensus Conference. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *Clin Infect Dis* 2003; 37: Suppl. 3, S225–S264.
- 66 Elphick H, Southern K. Antifungal therapies for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database Syst Rev* 2000; 4: CD002204.
- 67 Elphick H, Southern K. Antifungal therapies for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database Syst Rev* 2012; 6: CD002204.
- 68 Fujimori Y, Tada S, Kataoka M, *et al.* [Allergic bronchopulmonary aspergillosis effectively treated with itraconazole]. *Nihon Kokyuki Gakkai Zasshi* 1998; 36: 781–786.
- 69 Matsuzaki Y, Jimi T, Tao Y, *et al.* [Allergic bronchopulmonary aspergillosis successfully treated with itraconazole]. *Nihon Kyobu Shikkan Gakkai Zasshi* 1997; 35: 352–356.
- 70 Uji M, Sugama Y, Nishida K, *et al.* A case of effective readministration of itraconazole for recurrent allergic bronchopulmonary aspergillosis. *Nihon Kokyuki Gakkai Zasshi* 2005; 43: 412–416.
- 71 Isobe Z, Suga T, Hamaguchi S, *et al.* [Case of allergic bronchopulmonary aspergillosis successfully treated with itraconazole]. *Arerugi* 2007; 56: 1390–1396.
- 72 Lestner JM, Roberts SA, Moore CB, *et al.* Toxicodynamics of itraconazole: implications for therapeutic drug monitoring. *Clin Infect Dis* 2009; 49: 928–930.
- 73 Hope WW, Billaud EM, Lestner J, *et al.* Therapeutic drug monitoring for triazoles. *Curr Opin Infect Dis* 2008; 21: 580–586.
- 74 Conway SP, Etherington C, Peckham DG, *et al.* Pharmacokinetics and safety of itraconazole in patients with cystic fibrosis. *J Antimicrob Chemother* 2004; 53: 841–847.

- 75 Hennig S, Waterhouse TH, Bell SC, *et al.* A D-optimal designed population pharmacokinetic study of oral itraconazole in adult cystic fibrosis patients. *Br J Clin Pharmacol* 2007; 63: 438–450.
- 76 Sermet-Gaudelus I, Lesne-Hulin A, Lenoir G, *et al.* Sputum itraconazole concentrations in cystic fibrosis patients. *Antimicrob Agents Chemother* 2001; 45: 1937–1938.
- 77 Batista MV, Costa SF, Shikanai-Yasuda M, *et al.* Current treatment options for invasive aspergillosis. *Drugs Today (Barc)* 2013; 49: 213–226.
- 78 Baxter CG, Marshall A, Roberts M, *et al.* Peripheral neuropathy in patients on long-term triazole antifungal therapy. *J Antimicrob Chemother* 2011; 66: 2136–2139.
- 79 Varis T, Kivistö KT, Backman JT, *et al.* Itraconazole decreases the clearance and enhances the effects of intravenously administered methylprednisolone in healthy volunteers. *Pharmacol Toxicol* 1999; 85: 29–32.
- 80 Parmar JS, Howell T, Kelly J, *et al.* Profound adrenal suppression secondary to treatment with low dose inhaled steroids and itraconazole in allergic bronchopulmonary aspergillosis in cystic fibrosis. *Thorax* 2002; 57: 749–750.
- 81 Main KM, Skov M, Sillesen IB, *et al.* Cushing's syndrome due to pharmacological interaction in a cystic fibrosis patient. *Acta Paediatr* 2002; 91: 1008–1011.
- 82 Skov M, Main KM, Sillesen IB, *et al.* Iatrogenic adrenal insufficiency as a side-effect of combined treatment of itraconazole and budesonide. *Eur Respir J* 2002; 20: 127–133.
- 83 De Wachter E, Vanbesien J, De Schutter I, *et al.* Rapidly developing Cushing syndrome in a 4-year-old patient during combined treatment with itraconazole and inhaled budesonide. *Eur J Pediatr* 2003; 162: 488–489.
- 84 De Wachter E, Malfroot A, De Schutter I, *et al.* Inhaled budesonide induced Cushing's syndrome in cystic fibrosis patients, due to drug inhibition of cytochrome P450. *J Cyst Fibros* 2003; 2: 72–75.
- 85 Bolland MJ, Bagg W, Thomas MG, *et al.* Cushing's syndrome due to interaction between inhaled corticosteroids and itraconazole. *Ann Pharmacother* 2004; 38: 46–49.
- 86 Naef R, Schmid C, Hofe M, *et al.* Itraconazole comedication increases systemic levels of inhaled fluticasone in lung transplant recipients. *Respiration* 2007; 74: 418–422.
- 87 Gilchrist FJ, Cox KJ, Rowe R, *et al.* Itraconazole and inhaled fluticasone causing hypothalamic-pituitary-adrenal axis suppression in adults with cystic fibrosis. *J Cyst Fibros* 2013; 12: 399–402.
- 88 Böhmer GM, Drollman A, Gleiter CH, *et al.* Effect of coadministered ketoconazole, a strong cytochrome P450 3A4 enzyme inhibitor, on the pharmacokinetics of ciclesonide and its active metabolite, desisobutyl-ciclesonide. *Clin Pharmacokinet* 2008; 47: 343–349.
- 89 Mulliez P, Croxo C, Roy-Saint Georges F, *et al.* Aspergillose broncho-pulmonaire allergique traitée par voriconazole [Allergic broncho-pulmonary aspergillosis treated with voriconazole]. *Rev Mal Respir* 2006; 23: 93–94.
- 90 Clifton IJ, Whitaker P, Metcalfe R, *et al.* Pharmacokinetics of oral voriconazole in patients with cystic fibrosis. *J Antimicrob Chemother* 2011; 66: 2438–2440.
- 91 Markantonis SL, Katelari A, Pappa E, *et al.* Voriconazole pharmacokinetics and photosensitivity in children with cystic fibrosis. *J Cyst Fibros* 2012; 11: 246–252.
- 92 Pasqualotto AC, Shah M, Wynn R, *et al.* Voriconazole plasma monitoring. *Arch Dis Child* 2008; 93: 578–581.
- 93 Berge M, Guillemain R, Boussaud V, *et al.* Voriconazole pharmacokinetic variability in cystic fibrosis lung transplant patients. *Transpl Infect Dis* 2009; 11: 211–219.
- 94 Jain LR, Denning DW. The efficacy and tolerability of voriconazole in the treatment of chronic cavitary pulmonary aspergillosis. *J Infect* 2006; 52: e133–e137.
- 95 Felton TW, Baxter C, Moore CB, *et al.* Efficacy and safety of posaconazole for chronic pulmonary aspergillosis. *Clin Infect Dis* 2010; 51: 1383–1391.
- 96 Howard SJ, Felton TW, Gomez-Lopez A, *et al.* Posaconazole: the case for therapeutic drug monitoring. *Ther Drug Monit* 2012; 34: 72–77.
- 97 Agbetele J, Fairs A, Desai D, *et al.* Isolation of filamentous fungi from sputum in asthma is associated with reduced post-bronchodilator FEV1. *Clin Exp Allergy* 2012; 42: 782–791.
- 98 Fairs A, Agbetele J, Bourne M, *et al.* Isolation of *Aspergillus fumigatus* from sputum is associated with elevated airborne levels in home of patients with asthma. *Indoor Air* 2013; 23: 275–284.
- 99 Vicencio AG, Santiago MT, Tsirilakis K, *et al.* Fungal sensitization in childhood persistent asthma is associated with disease severity. *Pediatr Pulmonol* 2014; 49: 8–14.
- 100 Verweij PE, Howard SJ, Melchers WJ, *et al.* Azole-resistance in *Aspergillus*: proposed nomenclature and breakpoints. *Drug Resist Updat* 2009; 12: 141–147.
- 101 Bueid A, Howard SJ, Moore CB, *et al.* Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. *J Antimicrob Chemother* 2010; 65: 2116–2118.
- 102 Howard SJ, Pasqualotto AC, Denning DW. Azole resistance in allergic bronchopulmonary aspergillosis and *Aspergillus* bronchitis. *Clin Microbiol Infect* 2010; 16: 683–688.
- 103 Escribano P, Recio S, Peláez T, *et al.* *Aspergillus fumigatus* strains with mutations in the cyp51A gene do not always show phenotypic resistance to itraconazole, voriconazole, or posaconazole. *Antimicrob Agents Chemother* 2011; 55: 2460–2462.
- 104 Mortensen KL, Jensen RH, Johansen HK, *et al.* *Aspergillus* species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. *J Clin Microbiol* 2011; 49: 2243–2251.
- 105 Mortensen KL, Johansen HK, Fuursted K, *et al.* A prospective survey of *Aspergillus* spp. in respiratory tract samples: prevalence, clinical impact and antifungal susceptibility. *Eur J Clin Microbiol Infect Dis* 2011; 309: 1355–1363.
- 106 Bowyer P, Moore CB, Rautemaa R, *et al.* Azole antifungal resistance today: focus on *Aspergillus*. *Curr Infect Dis Rep* 2011; 13: 485–491.
- 107 Denning DW, Perlin DS. Azole resistance in *Aspergillus*: a growing public health menace. *Future Microbiol* 2011; 6: 1229–1232.
- 108 Burgel PR, Baixench MT, Amsellem M, *et al.* High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole. *Antimicrob Agents Chemother* 2012; 56: 869–874.
- 109 Howard SJ, Cerar D, Anderson MJ, *et al.* Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009; 15: 1068–1076.

- 110 Rjinders BJ, Cornelissen JJ, Slobe L, *et al.* Aerosolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: a randomized, placebo-controlled trial. *Clin Infect Dis* 2008; 46: 1401–1408.
- 111 Kuiper L, Ruijgrok EJ. A review on the clinical use of inhaled amphotericin B. *J Aerosol Med Pulm Drug Deliv* 2009; 22: 213–227.
- 112 Godet C, Meurice JC, Roblot F, *et al.* Efficacy of nebulized liposomal amphotericin B in the attack and maintenance treatment of ABPA. *Eur Respir J* 2012; 39: 1261–1263.
- 113 Slobbe L, Boersma E, Rjinders BJ. Tolerability of prophylactic aerosolized liposomal amphotericin-B and impact on pulmonary function: data from a randomized placebo-controlled trial. *Pulm Pharmacol Ther* 2008; 21: 855–859.
- 114 Kirkpatrick WR, Najvar LK, Vallor AC, *et al.* Prophylactic efficacy of single dose pulmonary administration of amphotericin B inhalation powder in a guinea pig model of invasive pulmonary aspergillosis. *J Antimicrob Chemother* 2012; 67: 970–976.
- 115 Scott JP, Peters-Golden M. Antileukotriene agents for treatment of lung disease. *Am J Respir Crit Care Med* 2013; 188: 538–544.
- 116 Inhaled beclomethasone dipropionate in allergic bronchopulmonary aspergillosis. Report to the Research Committee of the British Thoracic Association. *Br J Dis Chest* 1979; 73: 349–356.
- 117 Heinig JH, Weeke ER, Groth S, *et al.* High-dose local steroid treatment in bronchopulmonary aspergillosis. A pilot study. *Allergy* 1988; 43: 24–31.
- 118 Imbeault B, Cormier Y. Usefulness of inhaled high-dose corticosteroids in allergic bronchopulmonary aspergillosis. *Chest* 1993; 103: 1614–1617.
- 119 Seaton A, Seaton RA, Wightman AJ. Management of allergic bronchopulmonary aspergillosis without maintenance oral corticosteroids: a fifteen-year follow-up. *QJM* 1994; 87: 529–537.
- 120 Agarwal R, Khan A, Aggarwal AN, *et al.* Role of inhaled corticosteroids in the management of serological allergic bronchopulmonary aspergillosis (ABPA). *Intern Med* 2011; 50: 855–860.
- 121 Thomson JM, Wesley A, Byrnes CA, *et al.* Pulse intravenous methylprednisolone for resistant allergic bronchopulmonary aspergillosis in cystic fibrosis. *Pediatr Pulmonol* 2006; 41: 164–170.
- 122 Cohen-Cymberek M, Blau H, Shoseyov D, *et al.* Intravenous monthly pulse methylprednisolone treatment for ABPA in patients with cystic fibrosis. *J Cyst Fibros* 2009; 8: 253–257.
- 123 Holgate S, Casale T, Wenzel S, *et al.* The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* 2005; 115: 459–465.
- 124 Busse WW, Morgan WJ, Gergen PJ, *et al.* Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med* 2011; 364: 1005–1015.
- 125 Kuhl K, Hanania NA. Targeting IgE in asthma. *Curr Opin Pulm Med* 2012; 18: 1–5.
- 126 Moss RB. The use of biological agents for the treatment of fungal asthma and allergic bronchopulmonary aspergillosis. *Ann N Y Acad Sci* 2012; 1272: 49–57.
- 127 van der Ent CK, Hoekstra H, Rijkers GT. Successful treatment of allergic bronchopulmonary aspergillosis with recombinant anti-IgE antibody. *Thorax* 2007; 62: 276–277.
- 128 Zirbes JM, Milla CE. Steroid-sparing effect of omalizumab for allergic bronchopulmonary aspergillosis and cystic fibrosis. *Pediatr Pulmonol* 2008; 43: 607–610.
- 129 Kanu A, Patel K. Treatment of allergic bronchopulmonary aspergillosis (ABPA) in CF with anti-IgE antibody (omalizumab). *Pediatr Pulmonol* 2008; 43: 1249–1251.
- 130 Lebecque P, Leonard A, Pilette C. Omalizumab for treatment of ABPA exacerbations in CF patients. *Pediatr Pulmonol* 2009; 44: 516.
- 131 Brinkmann F, Schwerk N, Hansen G, *et al.* Steroid dependency despite omalizumab treatment of ABPA in cystic fibrosis. *Allergy* 2010; 65: 134–135.
- 132 Thomas MF. Life-threatening allergic bronchopulmonary aspergillosis treated with methylprednisolone and anti-IgE monoclonal antibody. *J R Soc Med* 2009; 102: 49–53.
- 133 Randhawa I, Chin T, Nussbaum E. Resolution of corticosteroid-induced diabetes in allergic bronchopulmonary aspergillosis with omalizumab therapy: a novel approach. *J Asthma* 2009; 46: 445–447.
- 134 Quintás Vázquez LM, Ortiz Piquer M, Pérez de Llano LA. Tratamiento efectivo con anticuerpo antiinmunoglobulina E en un paciente con aspergilosis broncopulmonar alérgica [Effective anti-immunoglobulin-E antibody treatment of a patient with allergic bronchopulmonary aspergillosis]. *Arch Bronconeumol* 2009; 45: 207.
- 135 Lin RY, Sethi S, Bhargava GA. Measured immunoglobulin E in allergic bronchopulmonary aspergillosis treated with omalizumab. *J Asthma* 2010; 47: 942–945.
- 136 Schulze J, Zissler U, Christmann M, *et al.* Allergic bronchopulmonary aspergillosis (ABPA) an IgE mediated disease? *Respir Med CME* 2011; 4: 33–34.
- 137 Pérez-de-Llano LA, Vennema MC, Parra A, *et al.* Effects of omalizumab in *Aspergillus*-associated airway disease. *Thorax* 2011; 66: 539–540.
- 138 Tillie-Leblond I, Germaud P, Leroyer C, *et al.* Allergic bronchopulmonary aspergillosis and omalizumab. *Allergy* 2011; 66: 1254–1256.
- 139 Genentech, Inc. Xolair – use in the treatment of allergic bronchopulmonary aspergillosis. US-XOL-S040, version 13.0. August 16, 2011.
- 140 Collins J, Devos G, Hudes G, *et al.* Allergic bronchopulmonary aspergillosis treated successfully for one year with omalizumab. *J Asthma Allergy* 2012; 5: 65–70.
- 141 Sastre I, Blanco J, Mata H, *et al.* A case of allergic bronchopulmonary aspergillosis treated with omalizumab. *J Investig Allergol Clin Immunol* 2012; 22: 145–147.
- 142 Wong R, Wong M, Robinson PD, *et al.* Omalizumab in the management of steroid dependent allergic bronchopulmonary aspergillosis (ABPA) complicating cystic fibrosis. *Paediatr Respir Rev* 2013; 14: 22–24.
- 143 Grard S, Auzou L, Nove-Josserand R, *et al.* Omalizumab (Xolair[®]) in the treatment of ABPA in 32 patients with cystic fibrosis. *J Cystic Fibros* 2013; 12: Suppl. 1, S96.
- 144 Jat KR, Walia DK, Khairwa A. Anti-IgE therapy for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database Syst Rev* 2013; 9: CD010288.
- 145 Jaffe JS, Massarnari M. In response to dosing omalizumab in allergic asthma. *J Allergy Clin Immunol* 2007; 119: 255–256.

- 146 Rambasek T, Kavuru MS. Omalizumab dosing *via* the recommended card *versus* use of the published formula. *J Allergy Clin Immunol* 2006; 117: 708–109.
- 147 Lowe PJ, Tannenbaum S, Gautier A, *et al.* Relationship between omalizumab pharmacokinetics, IgE pharmacodynamics and symptoms in patients with severe persistent allergic (IgE-mediated) asthma. *Br J Clin Pharmacol* 2009; 68: 61–76.
- 148 Elmallah MK, Hendeles L, Hamilton RG, *et al.* Management of patients with cystic fibrosis and allergic bronchopulmonary aspergillosis using anti-immunoglobulin E therapy (omalizumab). *J Pediatr Pharmacol Ther* 2012; 17: 88–92.
- 149 Pizzimenti S, Bussolino C, Badiu I, *et al.* Itraconazole as “bridge therapy” to anti-IgE in a patient with severe asthma with fungal sensitization. *BMJ Case Rep*, 2013; pii, bcr2012008462.
- 150 Greenberger PA, Miller TP, Roberts M, *et al.* Allergic bronchopulmonary aspergillosis in patients with and without evidence of bronchiectasis. *Ann Allergy* 1993; 70: 333–338.
- 151 Agarwal R, Khan A, Gupta D, *et al.* An alternate method of classifying allergic bronchopulmonary aspergillosis based on high-attenuation mucus. *PLoS One* 2010; 5: e15346.