



Developing collaborative works for faster progress on fungal respiratory infections in cystic fibrosis

Carsten Schwarz, Patrick Vandeputte, Amandine Rougeron, Sandrine Giraud, Thomas Dugé de Bernonville, Ludovic Duvaux, Amandine Gastebois, Ana Alastruey-Izquierdo, Maria Teresa Martin-Gomez, Estrella Martin Mazuelos, et al.

► To cite this version:

Carsten Schwarz, Patrick Vandeputte, Amandine Rougeron, Sandrine Giraud, Thomas Dugé de Bernonville, et al.. Developing collaborative works for faster progress on fungal respiratory infections in cystic fibrosis. *Medical Mycology*, 2018, 56 (suppl_1), pp.S42-S59. 10.1093/mmy/myx106 . hal-01789211

HAL Id: hal-01789211

<https://hal.science/hal-01789211>

Submitted on 15 Nov 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License



Review Article

Developing collaborative works for faster progress on fungal respiratory infections in cystic fibrosis

Carsten Schwarz¹, Patrick Vandeputte^{2,3}, Amandine Rougeron⁴, Sandrine Giraud², Thomas Dugé de Bernonville⁵, Ludovic Duvaux^{2,6}, Amandine Gastebois², Ana Alastruey-Izquierdo⁷, Maria Teresa Martín-Gomez⁸, Estrella Martín Mazuelos⁹, Amparo Sole¹⁰, Josep Cano¹¹, Javier Pemán¹², Guillermo Quindos¹³, Françoise Botterel¹⁴, Marie-Elisabeth Bougnoux¹⁵, Sharon Chen¹⁶, Laurence Delhaès¹⁷, Loïc Favennec¹⁸, Stéphane Ranque¹⁹, Ludwig Sedlacek²⁰, Joerg Steinmann²¹, Jose Vazquez²², Craig Williams²³, Wieland Meyer²⁴, Solène Le Gal^{25,26}, Gilles Nevez^{25,26}, Maxime Fleury², Nicolas Papon², Françoise Symoens², Jean-Philippe Bouchara^{2,3,*} and the ECMM/ISHAM working group
Fungal respiratory infections in Cystic Fibrosis (Fri-CF)[†]

¹Department of Pediatric Pneumology and Immunology, Cystic Fibrosis Center Berlin/Charité - Universitätsmedizin Berlin, Berlin, Germany, ²Host-Pathogen Interaction Study Group (EA 3142), UNIV Angers, UNIV Brest, Université Bretagne-Loire, Angers, France, ³Laboratoire de Parasitologie-Mycologie, CHU, Angers, France, ⁴Université de Bordeaux, Microbiologie Fondamentale et Pathogénicité UMR 5234, Bordeaux, France; CNRS, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France; Laboratoire de Parasitologie-Mycologie, CHU, Bordeaux, France, ⁵Biomolécules et Biotechnologies Végétales (EA 2106), Département de Biologie et Physiologie Végétales, UFR Sciences et Techniques, Université François Rabelais, Tours, ⁶Institut de Recherche en Horticulture et Semences (IRHS), UMR INRA 1345, Beaucouzé, France, ⁷Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain, ⁸Respiratory Bacteriology Unit & Clinical Mycology Unit, Department of Microbiology, Vall D'Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain, ⁹Departamento de Microbiología, Hospital Universitario de Valme, Sevilla, España, ¹⁰Unidad de Trasplante Pulmonar y Fibrosis Quística, Hospital Universitari la Fe, Valencia, Spain, ¹¹Mycology Unit, Medical School/Oenology School, Universitat Rovira i Virgili, Reus, Spain, ¹²Unidad de Micología, Servicio de Microbiología, Universitari la Fe, Valencia, Spain, ¹³Laboratorio de Micología Médica, Departamento de Inmunología, Microbiología y Parasitología, Facultad de Medicina y Enfermería, Universidad del País Vasco, Bilbao, Spain, ¹⁴Laboratoire de Parasitologie-Mycologie, CHU Henri Mondor, Créteil, France, ¹⁵Laboratoire de Microbiologie, Hôpital Necker Enfants Malades, Paris, France, ¹⁶Centre for Infectious Diseases and Microbiology Laboratory Services, ICPMR – Pathology West, Westmead Hospital, Westmead, New South Wales, Australia, ¹⁷Center for Cardiothoracic Research of Bordeaux, Inserm U1045, Bordeaux, France, ¹⁸Laboratoire de Parasitologie-Mycologie, EA 3800, CHU Charles Nicolle and Université de Rouen, Rouen, France, ¹⁹Laboratoire de Parasitologie-Mycologie, AP-HM Timone,

Marseille, France, ²⁰Institute of Medical Microbiology and Hospital Epidemiology, Medical School Hannover, Hannover, Germany, ²¹Institute of Medical Microbiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany, ²²Division of Infectious Diseases, Department of Medicine, Georgia Regents University, Augusta, GA, USA, ²³University of the West of Scotland, Institute of Healthcare Associated Infection, University Hospital Crosshouse, Kilmarnock, United Kingdom, ²⁴Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School, Westmead Clinical School, Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Westmead Hospital, Westmead Institute for Medical Research, Westmead, NSW, Australia, ²⁵Host-Pathogen Interaction Study Group (EA 3142), UNIV Angers, UNIV Brest, Université Bretagne-Loire, Brest, France and ²⁶Laboratoire de Parasitologie-Mycologie, CHU, Brest, France

*To whom correspondence should be addressed. Pr. Jean-Philippe Bouchara, Host-Pathogen Interaction Study Group (EA 3142), Institut de Biologie en Santé-IRIS, CHU, 4 rue Larrey, 49933 Angers cedex 9, France.

E-mail: jean-philippe.bouchara@univ-angers.fr

†Other members of the ECMM/ISHAM working group *Fungal respiratory infections in Cystic Fibrosis (Fri-CF)* are listed in the appendix.

Received 31 May 2017; Revised 7 September 2017; Accepted 6 October 2017; Editorial Decision 17 September 2017

Abstract

Cystic fibrosis (CF) is the major genetic inherited disease in Caucasian populations. The respiratory tract of CF patients displays a sticky viscous mucus, which allows for the entrapment of airborne bacteria and fungal spores and provides a suitable environment for growth of microorganisms, including numerous yeast and filamentous fungal species. As a consequence, respiratory infections are the major cause of morbidity and mortality in this clinical context. Although bacteria remain the most common agents of these infections, fungal respiratory infections have emerged as an important cause of disease. Therefore, the International Society for Human and Animal Mycology (ISHAM) has launched a working group on *Fungal respiratory infections in Cystic Fibrosis (Fri-CF)* in October 2006, which was subsequently approved by the European Confederation of Medical Mycology (ECMM). Meetings of this working group, comprising both clinicians and mycologists involved in the follow-up of CF patients, as well as basic scientists interested in the fungal species involved, provided the opportunity to initiate collaborative works aimed to improve our knowledge on these infections to assist clinicians in patient management. The current review highlights the outcomes of some of these collaborative works in clinical surveillance, pathogenesis and treatment, giving special emphasis to standardization of culture procedures, improvement of species identification methods including the development of nonculture-based diagnostic methods, microbiome studies and identification of new biological markers, and the description of genotyping studies aiming to differentiate transient carriage and chronic colonization of the airways. The review also reports on the breakthrough in sequencing the genomes of the main *Scedosporium* species as basis for a better understanding of the pathogenic mechanisms of these fungi, and discusses treatment options of infections caused by multidrug resistant microorganisms, such as *Scedosporium* and *Lomentospora* species and members of the *Rasamsonia argillacea* species complex.

Key words: cystic fibrosis, fungal respiratory infections, biological diagnosis, pathogenic mechanisms, treatment, *Scedosporium* species.

Introduction

With a frequency of approximately 1 in 2500 live births, cystic fibrosis (CF) is the major genetic inherited disease in Caucasian populations. The disease is due to mutations in the gene *CFTR* (Cystic Fibrosis Transmembrane conductance Regulator), which encodes a chloride channel involved in electrolyte exchanges at the apical membrane of numerous epithelial cell types.^{1,2} Although several organs are affected prognosis hinges on the severity of the lesions of the lungs. In the respiratory tract, *CFTR* gene mutations lead to a defect in the efflux of chloride and bicarbonate anions, and therefore to the dehydration of the bronchial mucus, resulting in a sticky viscous mucus which allows the entrapment of airborne bacteria and fungi.^{3,4} The respiratory tract therefore is often colonized by various microorganisms, including yeasts and filamentous fungi. For a review on cystic fibrosis and its clinical manifestations, see Castellani and Assael 2017.⁵

Over the past decades, considerable attention has been paid to improve the treatment and prevention of bacterial respiratory infections, which leads to a marked increase in life expectancy.^{6–8} However, microorganisms other than bacteria, including fungal species, also colonize the respiratory tract of CF patient, sometimes causing respiratory infections of which the frequency of these infections regularly increased along with the increase in life expectancy.^{9–13}

Among fungi, *Aspergillus fumigatus* remains by far the most common agent of airway colonization, but other fungal species are increasingly reported, such as *Scedosporium* species, *A. terreus*, *Exophiala dermatitidis*, *Lomentospora prolificans* (formerly *Scedosporium prolificans*), and *Rasamsonia* species.⁹ The prevalence of these fungi in the CF context is certainly underestimated due to the lack of standardization for mycological examination of clinical samples, and the limited knowledge of some of these fungi.¹⁴ Apart from *A. fumigatus*, which is well known by clinicians and microbiologists involved in the follow-up of CF patients, the clinical significance of the recovery of fungi from respiratory secretions still remains to be defined. Additionally, some of these fungal species present an innate resistance or limited susceptibility to current antifungals, thus leading to major therapeutic problems in case of disseminated infections in immunosuppressed individuals.

Numerous questions therefore remain regarding fungal colonization of CF airways. Studies aiming to improve the diagnosis of these infections or their treatment, as well as basic research on the ecology of these fungi, their biochemistry, their pathogenic mechanisms or their antifungal resistance mechanisms should be promoted. To address these

questions, the International Society for Human and Animal Mycology (ISHAM) launched a working group on *Fungal respiratory infections in Cystic Fibrosis (Fri-CF)* in October 2006, which was approved by the European Confederation of Medical Mycology (ECMM) in 2009. Since then, four meetings have been held, together with dedicated sessions in the ISHAM congresses in Berlin (2012) and Melbourne (2015). These meetings provided the opportunity to initiate collaborative works that will be highlighted in this review, especially in three areas: (i) the improvement of the biological diagnosis of fungal colonization of the airways/respiratory infections, (ii) the pathogenesis of fungal infections coupled with the genome sequencing project in *Scedosporium* species, and (iii) treatment of these infections.

Improving the biological diagnosis of fungal respiratory infections in CF

Recurrent respiratory infections leading to a significant inflammation of the respiratory tract are the major cause of morbidity and mortality in patients with CF.^{15–17} *Pseudomonas aeruginosa* plays the most important role regarding acute and chronic infections of the respiratory system in CF.^{17,18} However, fungi have recently moved into the focus because of increasing awareness as airway colonizers in CF.¹⁹ Whether this is due to improvement of the detection methods or to more frequent use of antibacterial and anti-inflammatory treatment in an aging patient cohort is subject to an ongoing discussion. Filamentous fungi are more common in patients with CF with advanced lung disease.^{20,21}

Our knowledge on predisposing factors for a fungal colonization of the airways or a fungal respiratory infection is limited, especially regarding possible genetic factors of susceptibility. Several single nucleotide polymorphisms (SNPs) have been identified as risk factors for invasive aspergillosis or allergic broncho-pulmonary aspergillosis (ABPA).^{22,23} However, these SNPs have been investigated in patients with hematological malignancies or in asthmatic patients, and only few studies focused on the genetic risk factors for a fungal colonization of the airways or respiratory infection in CF. It has been suggested that susceptibility or resistance to ABPA in CF could be associated with particular HLA antigens, HLA-DRB1, and HLA-DQB1, respectively.^{24,25} Polymorphisms in the promoter region of the gene encoding interleukin (IL) 10 may also influence the host response to *A. fumigatus* in the context of CF.²⁶ Some SNPs in the gene encoding the IL-4 receptor alpha chain, principally ile75val, appear to be a genetic risk for the development of ABPA.²⁷

Even if *A. fumigatus* remains the most frequent filamentous fungus colonizing the airways of CF patients, several studies have established that a large variety of molds may be recovered from CF respiratory secretions.^{9,28–33} Nevertheless, discrepancies in their respective frequency may be seen from one study to another, depending on the geographic origin of the patients, variations in the demographic characteristics of the CF population studied or differences in the methodologies employed for detection or identification, making it difficult to compare data across studies.

Toward standardization of culture procedures

A detailed qualitative and quantitative microbiological analysis of sputa recovered from CF patients is as a prerequisite for the management of these patients.²⁸ However, the prevalence of fungal colonization in CF patients greatly varies from one study to another. The lack of standardized procedures for mycological examination of CF sputum samples at least partly explains these discrepancies.¹⁴ For instance, great variations can be seen in routine procedures used in clinical laboratories, such as the absence of prior digestion of the samples or the use of different mucolytic agents and different durations or temperatures for the digestion step, as well as in the dilution of the digested sputum, in the volume of the sample analyzed, in the number and nature of the culture media used or in the incubation time and duration of incubation.

Homogenization of CF sputum with a mucolytic agent (such as dithiothreitol or dihydroxy-1,4-dithiolbutane) clearly increases the sensitivity of fungal detection, compared to culture of undigested sample.²⁹ Conflicting results, however, were reported by Pashley et al.³⁰ since inoculation of neat sputum plugs conferred better results than the recommended procedure, that is, culture of homogenized and diluted sample (plugs plus saliva).

Mycological culture media, such as Sabouraud glucose agar (containing mycological peptone, glucose, and agar) or yeast extract-peptone-dextrose-agar (YPDA), both supplemented with antibiotics (mix of chloramphenicol and gentamicin for example) have to be included in the routine microbiological analysis of polymicrobial respiratory secretions from CF patients, as fast-growing bacteria can inhibit growth of fungi when inoculated on standard bacteriological media.³¹ Combination of antibiotics succeeds in inhibiting most bacteria including *P. aeruginosa*.³² For instance, utilization of culture media supplemented with antibiotics increased the number of CF patients who were culture-positive for fungi from 18% to 78%.³³

In addition to the inhibition of bacterial growth it is also recommended to suppress the growth of rapidly grow-

ing fungi, for example, *A. fumigatus*, to facilitate the detection of *Scedosporium/Lomentospora* species. *Scedosporium* species usually rank the second among the filamentous fungi colonizing the CF airways, after *A. fumigatus*, and are often associated with this fungus.³⁴ In 2015, the first recommendations for microbiological examination of sputum samples from CF patients were published by the French Society for Microbiology.³⁵ They include a systematic search for fungi in parallel to bacteriological examination, by prior digestion of the sample with a mucolytic agent, followed by a 1/10 dilution of the digested sample and a final inoculation of a defined volume in parallel on a chromogenic medium for detection of yeasts, two Sabouraud dextrose agar or YPDA plates supplemented with chloramphenicol and gentamicin and also with 0.5 g/l cycloheximide for one of them.³⁵ To inhibit growth of the aspergilli and facilitate detection of *Scedosporium* species, inoculation of respiratory secretions onto dichloran-rose Bengal-chloramphenicol-agar supplemented with benomyl (DRBC-benomyl), which has been developed for microbiological analysis of food or soil samples,^{36–40} may lead to an increase in the number of *Scedosporium* culture-positive samples.¹⁴ Likewise, a new semi-selective medium, called SceSel+, based on modified Leonian's agar and supplemented with antibiotics (ciprofloxacin, chloramphenicol, streptomycin), dichloran and benomyl, initially developed to improve the detection of *Scedosporium* species from environmental samples (water, sediment and soil)⁴¹ has been reported to have benefit in the detection of these fungi from CF sputum. In a monocentric German study, 5.3% of CF respiratory samples were culture-positive for *Scedosporium* species using the SceSel+ medium compared with 2% using standard microbiological procedures.^{42,43} This was confirmed in a prospective multicenter study encompassing 11 CF centers in the same country where the benefit of SceSel+ culture medium over standard media was documented on 5472 samples.⁴⁴ In Australia, *Scedosporium* species were recovered on SceSel+ and Sabouraud glucose agar from 90.6% and 46.9% of the specimens tested, respectively.⁴⁵ Recently, two multicenter studies have been performed in France and in different European countries plus Australia, respectively. Results from these studies will lead to the implementation of guidelines for mycological examination of respiratory secretions in CF.

Improvement of the identification methods

Correct identification of yeasts and moulds recovered from respiratory secretions from CF patients is required, not only to improve our knowledge on the epidemiology of fungal infections in this clinical context but also for early detec-

tion of difficult to treat species capable of chronic colonization of the airways and of hematogenous dissemination in case of lung transplantation. Until recently, yeasts were identified according to their carbohydrate assimilation pattern and moulds by their macroscopic and microscopic features according to standard descriptions. Alternatively, species identification can be obtained by sequencing short variable genetic regions and subsequent comparison with reference sequences stored in appropriate databases. Sequencing the internal transcribed spacer (ITS) 1 and 2 regions of ribosomal DNA, the official fungal DNA barcode, is most widely used,⁴⁶ but sequences of other genes including the β -tubulin or calmodulin genes may be more relevant for precise identification within the *Scedosporium* genus or the *R. argillacea* species complex.^{47–49}

Matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS) is now increasingly used for routine identification of yeasts.^{50,51} This technology is also about to revolutionize the identification of moulds as it could replace the traditional examination of macroscopic and microscopic morphology requiring highly skilled mycologists.⁵² Indeed, several mycological research teams have demonstrated that MALDI-TOF/MS could be used successfully for identification of filamentous fungi belonging to the genera *Aspergillus*, *Fusarium*, *Penicillium*, and *Scedosporium* or to the order Mucorales.^{53–57} Common prerequisites are the previous establishment of adequate reference spectrum libraries and the optimization of the initial extraction procedure. A first-developed relevant database including 146 strains belonging to only 63 species and 33 different genera allowed the identification at the species level of 87% of the isolates identified in the routine activity of a clinical laboratory for 5 months.⁵⁸ A second-developed relevant library comprising 294 individual isolates representing 152 species and 76 genera allowed the identification of 88.9% of blinded clinical isolates at the species level, 4.3% additional isolates at the genus level, the remaining strains corresponding to Basidiomycetes or *Penicillium* that were not represented in the database.⁵⁹ Nevertheless, improvement of the databases is still needed since cryptic species have not been identified to date using all MALDI-TOF equipments. Results appear system-specific, and for instance only some of the available MALDI-TOF instruments include in their supplied databases reference spectra allowing precise identification of *Scedosporium* species, and differentiation within the *R. argillacea* species complex is currently not feasible.

In addition, one may be cautious when interpreting the results of mycological examination of sputum cultures because of their possible contamination by environmental molds with the prolonged incubation time, and of possi-

ble transient carriage of inhaled fungal spores that were entrapped into the sticky bronchial mucus at time of sampling. Apart from the recovery of well-known CF pathogens (listed in the Introduction section), analysis of repeated sputum samples is required before to consider the isolated fungus as responsible for an airway colonization or respiratory infection, particularly for non-thermotolerant species.

Genotyping

Typing is very useful to study fungal colonization or infection occurring in a single patient, especially in the CF context. Genotyping methods permit the differentiation between transient carriage of always distinct genotypes unable to establish within the respiratory tract and without clinical significance, and a chronic colonization of the airways by one or two genotypes conserved over time, which may contribute to the inflammatory reaction and therefore to the progressive deterioration of the lung function.⁶⁰ This was illustrated in some collaborative studies performed within the working group.

Regarding pathogenic *Scedosporium* species, a multi-locus sequence typing (MLST) scheme was developed and is accessible at <http://mlst.mycologylab.org>, allowing for large epidemiological studies due to the fact that the highly reproducible genotyping results obtained for the different loci investigated are comparable worldwide.^{61–63} Recently, a panel of sequential and multiple isolates collected from nine CF patients was analyzed using this MLST scheme and the automated Diversilab system based on polymerase chain reaction amplification of repetitive sequences (rep-PCR).⁶⁴ Both methods gave similar results, confirming previous results obtained on the same set of isolates by random amplification of polymorphic DNA (RAPD). Some of the patients were colonized by a single genotype conserved over time, while the others exhibited a predominant genotype associated with one or two genetically closely related genotypes found occasionally.^{64,65}

Microsatellite-based typing was used to show that several distinct genotypes of *A. fumigatus* could be frequently isolated in the same sample, as multiple genotypes were identified in 88.3% (53/60) of all samples with multiple isolates.⁶⁶ This co-colonization concerned the eight patients included. Most of the genotypes differed at multiple loci, suggesting that several distinct strains were able to colonize the patients. This method also demonstrated that some strains of *A. fumigatus* are capable of persistent colonization, while others are only found transiently. All patients but one were colonized by at least one persistent strain.⁶⁶

Similar results were also reported for other fungal species associated with CF. For example, 115 clinical isolates of

A. terreus collected from respiratory secretions of five CF patients were analyzed by microsatellite-based typing.⁶⁷ Seventeen genotypes were identified, corresponding to three distinct colonization patterns: (i) a chronic colonization by one dominant genotype associated with few other genotypes found only incidentally; (ii) a prolonged colonization by two distinct genotypes detected simultaneously; and (iii) multiple different genotypes that were present only transiently.⁶⁷ Likewise, rep-PCR typing was useful for strain delineation within the *R. argillacea* complex. In one study, 116 isolates of this species complex from 26 CF patients were analyzed. Of 29 genotypes, seven were shared between different patients, while 22 were patient specific. In each clinical sample, most isolates exhibited an identical genotype. Some genotypes of *R. argillacea* and *R. aegroticola* were found persisting for months or years in sequentially sampled CF patients, suggesting that these species chronically colonize the airways of CF patients.⁶⁸

DNA-based detection methods

Several methods have been proposed allowing detection of a specific fungal species or a species complex. For example, a PCR-based assay was developed recently for specific detection of some *Rasamsonia* species from respiratory secretions from CF patients.⁶⁹ This assay reliably detected the three species of the *R. argillacea* complex that are known in CF (*R. argillacea*, *R. piperina*, and *R. aegroticola*), but not the fourth species of this complex, *R. eburnea*, which has never been reported from CF.⁴⁹

Nagano et al.³³ demonstrated on sputa from CF patients (77 adults) the superiority of molecular methods compared to conventional cultures even using YPDA supplemented with antibiotics (cotrimoxazole, chloramphenicol, ceftazidime, and colistin). In addition, some species, such as *Saccharomyces cerevisiae*, *Malassezia* spp., *Fusco- poria ferrea*, *Fusarium culmorum*, *Acremonium strictum*, *Thanatephorus cucumeris*, and *Cladosporium* spp. could only be identified by DNA extraction followed by PCR amplification of the ITS2 region of the rDNA gene cluster and sequencing of the obtained amplicons. However, even if direct DNA detection from sputa usually gave a high degree of sensitivity and speed for the detection of fungi, some fungi were detected only by cultures (for example, four of the patients were found to be colonized by *A. fumigatus* by cultures whereas the fungus was never detected by PCR). Thus, molecular methods should be considered as a complementary approach rather than an alternative to mycological cultures.²⁸

More attractive methods have been proposed allowing both direct detection and identification of the different

species that may be encountered in sputum samples from CF patients. In this field, a pioneering study was based on an oligonucleotide array.⁷⁰ Using species-specific oligonucleotide probes designed from the ITS regions of the ribosomal RNA operon, this method was able to identify 20 fungal species. A total of 57 sputum samples collected from 39 CF patients was analyzed by this method, in parallel to mycological examination using five different agar plates, that is, ChromAgar Candida (Becton Dickinson, Paris, France), Sabouraud dextrose agar supplemented with chloramphenicol and gentamicin (Becton Dickinson), Sabouraud dextrose agar supplemented with chloramphenicol and cycloheximide, Dichloran-Rose Bengal agar supplemented with benomyl, and erythritol-chloramphenicol agar, which were incubated at two different incubation temperatures (37°C and 25°C). For 16 sputum samples (28%), concordant results were obtained between the array and culture. For 33 sputum samples (58%), the array detected more fungal species than culture did, while the reverse was found for eight samples (14%).⁷⁰

Denaturing high-performance liquid chromatography (DHPLC) which is based on the chromatographic separation of multiple fragments of double-stranded DNA in denaturing buffer, was investigated for the analysis of the fungal biota of sputa collected from four CF patients.⁷¹ As even fungi that are difficult to cultivate, such as *Malassezia* spp., could be detected by molecular methods, fungal microbiota of CF samples were found to be more diverse using DHPLC than the culture-dependent method. Twelve fungal species including *M. globosa* and *M. restricta* were found by DHPLC whereas they were only three by culture.⁷¹ A closely related method called single-strand conformation polymorphism (SSCP) was applied to the analysis of the bacterial and fungal microbiome of the airways in 56 adult patients with CF from Germany.⁷² By this method, which is based on the electrophoretic separation of the amplicons on polyacrylamide gels as a result of differences in their nucleotide sequence and therefore in their conformational state, sixty fungal species were detected from sputum samples, *versus* 38 bacterial species. Strikingly, up to 44% of the samples were positive for *Candida* species, 4.1% for *S. apiospermum* and 4.1% for *E. dermatitidis*, whereas they were only 2.7% for *A. fumigatus*.⁷² Of note, cultures were not performed in this study; additionally, comparison of the bacterial and fungal communities in successive samples (collected at different time points from the same patients) revealed relatively constant numbers of bacterial species, but great variations in the number of fungal species, suggesting stable bacterial communities with real colonizers but transient carriage of some fungal species which may have hampered the detection of real fungal pathogens.⁷² These

methods, however, are now being superseded by deep sequencing approaches applied to whole genome sequencing (WGS) analysis.

WGS approaches have recently been utilized to explore the respiratory mycobiome in CF patients. In a French study, sputum samples collected from four CF patients were analyzed by pyrosequencing and cultures.⁷³ WGS approach permitted the detection of a huge diversity of species or genera (24 vs. only four by cultures) and demonstrated a reduced diversity and richness of the microbiome in patients with decreased lung function and poor clinical status.⁷³ A large diversity of fungal species was also reported from the analysis of sputum samples collected from six CF patients upon admission to hospital for pulmonary exacerbation.⁷⁴ *Candida* yeasts and *Malassezia* species accounted for a large part of the fungal species detected (from 13 to 55 species per patient) without significant change in their relative abundance after two weeks of antibacterial treatment.⁷⁴

Other noncultural detection methods

Although there is limited information in this field, analytical methods revealing fungal biomarkers, and in some cases fungal species-specific markers, may assist the rapid diagnosis of a fungal colonization of the airways. For example, *A. fumigatus* and other fungi including *A. flavus*, *A. niger*, and *Scedosporium* species, but not the Mucoromycetes, are able to produce *in vitro* the small volatile molecule, 2-pentylfuran (2-PF) when cultured on diverse media.⁷⁵ As this molecule is not known to be produced in normal mammalian metabolism, and has not been found in surveys of human breath, 2-PF has been proposed as a marker for detection of *A. fumigatus* in the respiratory tract of CF patients. In a preliminary study, 2-PF was detectable in the breath of a small number of CF patients colonized by *Aspergillus* species.⁷⁵ When compared with recurrent isolation of *Aspergillus* from sputum or bronchoalveolar lavage (BAL) fluid over two months, sensitivity and specificity values of 77% and 78%, respectively, were seen for the 2-PF detection test indicating potential usefulness of this patented biomarker.⁷⁶ Detection of a combination of some volatile organic compounds in exhaled breath has also been recently proposed as a noninvasive method to detect respiratory infections in CF patients, but larger scale clinical validation remains to be done.⁷⁷

Siderophores are small, high-affinity, iron-chelating compounds secreted by microorganisms for uptake of the extracellular iron. Two distinct siderophores, dimeric acid and *N*^α-methyl coprogen B, were identified from cultures of *S. apiospermum* under iron-restricted conditions.⁷⁸ Among them *N*^α-methyl coprogen B which seems to be

specific for *Scedosporium* species and which was detected from three out of five sputum samples from CF patients that were culture-positive for these fungi, could be considered as a marker of the airway colonization by *Scedosporium* species.⁷⁹ Nevertheless, secondary metabolism of the fungal species associated with CF should be investigated further. For example, recent studies demonstrated that high performance liquid chromatography (HPLC)-MS/MS detection of gliotoxin, another secondary metabolite produced by nonribosomal peptide synthesis,⁸⁰ or better its more stable derivative bis(methylthio)-gliotoxin,^{81,82} from serum samples may be useful for rapid diagnosis of an invasive *Aspergillus* infection.

Serodiagnosis as a mean to differentiate airway colonization from respiratory infection

In the CF context, detection of serum-specific antibodies may be a valuable means of discriminating between fungal colonization of the airways and true infection. Several commercial kits are available for detection of total and specific serum IgE or IgG against *A. fumigatus*. A novel classification for designating the types of *Aspergillus* disease in CF patients (ABPA, sensitization, bronchitis, or colonization), which includes the use of these serological tests, as well as sputum analysis by culture and *Aspergillus* PCR and galactomannan (GM) detection has been recently proposed.⁸³ Four groups of patients with positive sputum cultures were defined: (i) *A. fumigatus*-colonized patients with or without positive PCR from sputum, but without sputum GM and humoral response (nondiseased patients); (ii) patients with bronchitis which are characterized by positive GM and DNA detection from sputum, and presence of serum specific IgG without IgE; (iii) *A. fumigatus*-sensitized patients with or without *A. fumigatus* DNA in sputum but no GM, but more importantly with the presence of serum specific IgE without specific IgG; and (iv) patients with ABPA which differed from sensitized patients by the presence of GM in sputum, together with *A. fumigatus* DNA in sputum and serum specific IgG.⁸³ While corticosteroids ± an anti-IgE therapy are widely used for treatment of ABPA,⁸⁴ triazole antifungals are required for treatment of an *Aspergillus* bronchitis. Other immunological markers have been investigated in the past to improve early detection of ABPA and monitoring in CF patients (for a review, see Delhaes et al.⁸⁵), including the thymus- and activation-regulated chemokine (TARC)⁸⁶ or serum specific IgE directed toward recombinant antigens derived from a 30-kDa protein (rAsp f 4) or a manganese superoxide dismutase (rAsp f 6) from *A. fumigatus*.^{87,88} Nevertheless, these tests do not seem to be in use today. By contrast, recent studies showed some interest for the

basophil activation test for diagnosis of *A. fumigatus*-induced hypersensitivity reactions in CF patients (ABPA and *Aspergillus* sensitization).^{89,90} Likewise, although a further evaluation is needed, the recently commercialized enzyme-linked immunosorbent assay (ELISA) kit combining both somatic and metabolic antigens of *A. fumigatus* with two recombinant proteins, namely, the ribonuclease mitogillin (rAsp f 1) and dipeptidylpeptidase V, might be useful for diagnosis of ABPA in CF patients.^{91,92}

Unlike *A. fumigatus*, there are no standardized commercial methods to date to detect antibodies or antigens to *Scedosporium/Lomentospora* species, with serodiagnosis performed only in a few specialized laboratories by counter-immunoelectrophoresis (CIE) using homemade crude antigenic extracts. As numerous proteins and cell wall polysaccharides are shared by pathogenic fungi, cross-reactivity with other filamentous fungi, such as *A. fumigatus* may occur, leading to false-positive results.³⁴ Nonetheless, an ELISA assay using a crude *Scedosporium* somatic extract was used to assess the *Scedosporium* seroprevalence in a large monocentric cohort of CF patients (373), showing serum antibodies in 9.4% of the patients;⁹³ possible cross-reactions with other fungi, however, cannot be excluded.

Recently, the potential value of a *S. boydii* antigen, mycelial catalase A1, in serodiagnosis of a *Scedosporium* infection was investigated by ELISA, using sera from CF patients.^{94,95} Whatever the *Scedosporium* species involved (including *S. boydii*, *S. apiospermum* and *S. aurantiacum*), sera from *Scedosporium*-infected patients were clearly differentiated from sera from *A. fumigatus*-infected CF patients. The assay exhibited a 100% sensitivity and a very high specificity (97.44%), with discordant results obtained for only one patient.^{94,95} Other candidate antigens include the heat shock proteins (Hsp) 70 and 90 or enolase which have been revealed to be immunodominant antigens in *L. prolificans* in some immunoproteomic studies.⁹⁶

The *Scedosporium* genome sequencing project

Early research aiming at identifying fungal specific factors associated with the pathogenesis of *Scedosporium* infections was initially based on biochemical and molecular approaches including chromatography-based purification of proteins and cloning of corresponding genes. This strategy allowed for the identification and characterization of a range of *Scedosporium* enzymes potentially involved in host tissue invasion or in evasion of the fungus from the host immune defenses.^{94,95,97–100} In addition, sequence-based gene homolog approaches led to the cloning of two *Scedosporium* genes encoding enzymes of the dihydroxynaphthalene-melanin biosynthetic pathway.¹⁰¹ This was a significant

milestone as for a long time, the lack of genomic resources has seriously hampered the identification of pathogenic factors. Therefore, the ECMM/ISHAM working group on fungal respiratory infections in cystic fibrosis launched in 2011 a global program aiming at sequencing the genome of a representative strain from each of the pathogenic *Scedosporium* species.

Pioneering genomic resource in *Scedosporium* species: the *S. apiospermum* genome

The first sequenced genome was that of *S. apiospermum sensu stricto*, more precisely strain IHEM 14462 isolated in 1998 from a sputum sample from a CF patient.¹⁰² Two distinct Illumina high-throughput sequencing technologies (a paired-end run and a mate-pairs run) together with an additional single-molecule real-time sequencing on a PacBio RSII instrument revealed a genome size of 43.4 Mbp. A total of 10,919 coding sequences (CDS) was predicted and putative functions were attributed to more than 80% of these CDS. A refined functional annotation was performed recently using Interproscan and allowed the identification of roughly 1,000 supplemental predicted proteins, which was confirmed by the analysis of transcriptomic data using the programs BRAKER/Augustus (unpublished results). As observed in other filamentous fungi, *S. apiospermum* genome encodes a large number of transporter proteins (particularly ATP-Binding Cassette (ABC) and Major Facilitator (MF) transporters), aspartyl proteases and transcription factors (with zinc finger C₂H₂ or Zn(2)-C₆ fungal-type DNA-binding domains) (Fig. 1). Several two-component system (TCS) elements as well as G-protein coupled receptors were also detected and which be used for sensing of and adaptation to environmental variations. *Aspergillus fumigatus* and *S. apiospermum* exhibit high similarities in the number of genes for the main functional groups, reflecting the conservation of core components between the two species (Fig. 1). However, one unexpected feature was that compared with the *A. fumigatus* genome, the *S. apiospermum* genome comprises more than twice as much ankyrin repeat-bearing proteins (Fig. 1). Although the ankyrin motif is considered to be one of the most common protein-protein interaction motifs in nature, it is described to be involved in host-pathogen interactions.¹⁰³ It is thus possible that the expanded *S. apiospermum* ankyrin motif-containing protein battery could participate in the establishment of the fungus within the respiratory tract of CF patients. Similarly, a greater number of genes encoding putative methyltransferases and oxidoreductases were found in the genome sequence of *S. apiospermum*. Whether this larger magnitude of activities allows the fungus to use a wider range of

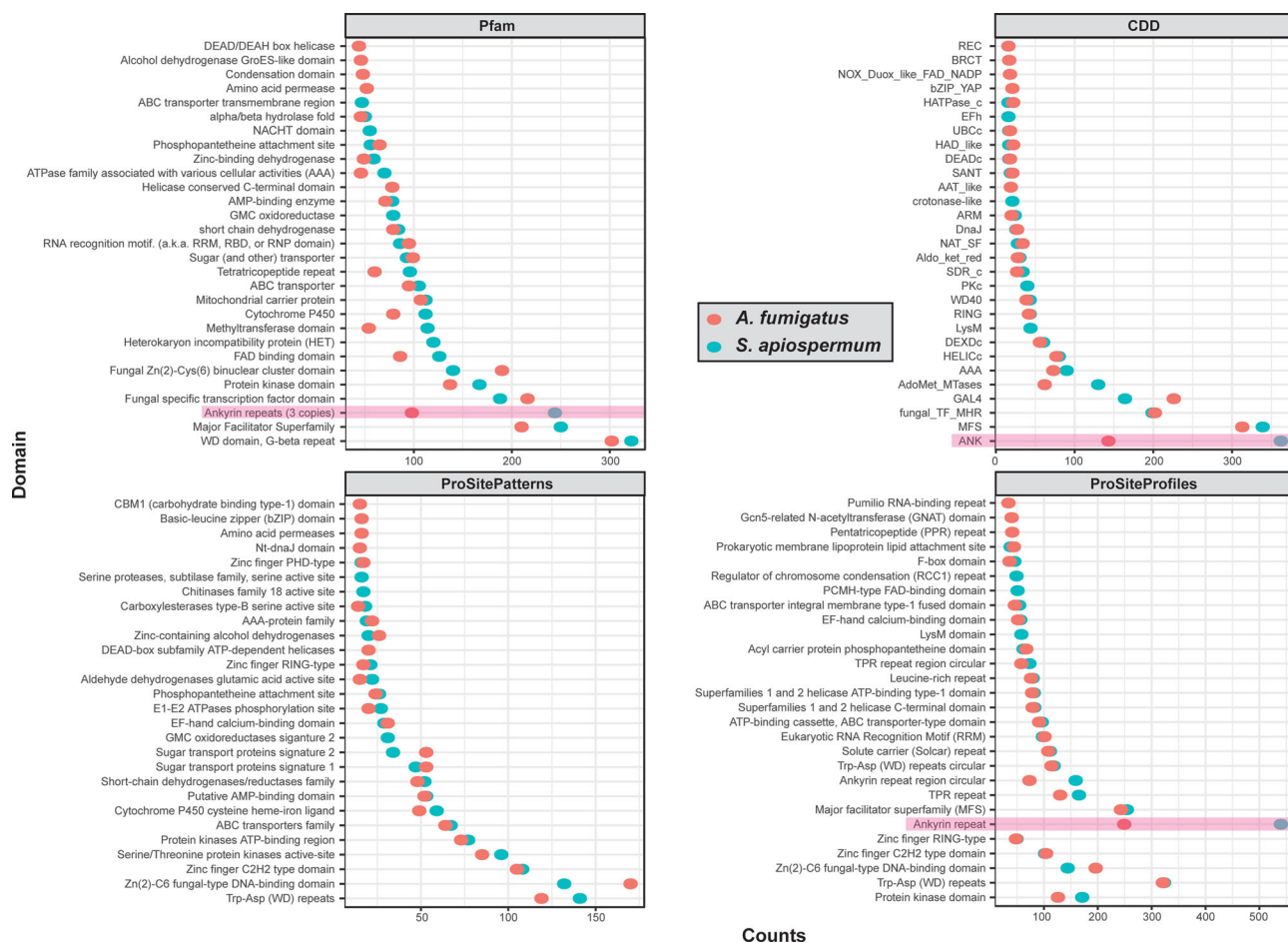


Figure 1. Comparison of most represented functional domains in *A. fumigatus* and *S. apiospermum*. The 25 most represented terms of Pfam, CDD, ProSitePatterns, and ProSiteProfiles are depicted. *Aspergillus fumigatus* and *S. apiospermum* predicted peptides (including 3607 genes initially annotated as pseudogenes in the draft genome of *S. apiospermum*, i.e., $8375 + 3607 = 11,982$ predicted peptides) were subjected to Interproscan (v5.23-62.0) analysis.¹⁷² The predominance of ankyrin motif-containing proteins is highlighted in pink. This Figure is reproduced in color in the online version of *Medical Mycology*.

nutritive substrates or to be able to synthesize specialized metabolites remains to be elucidated.

The *Scedosporium* genome project, a cornerstone for all “omics” approaches

Importantly, availability of the genome sequence of *S. apiospermum sensu stricto* likely will support in a near future the development of “omics” technologies that would help in identifying a series of candidate genes potentially involved in the adaptation and pathogenicity of this fungus, but also it will inform genomic research in the other *Scedosporium* species.

This was elegantly illustrated by the recent genomic assembly of a second species of the genus *Scedosporium*, *S. aurantiacum*, and more recently by the assembly and annotation of the genome of a third species, *S. boydii*.^{104,105} The genome of the sequenced strain WM 09.24, isolated from

the environment at Circular Quay in Sydney (Australia) displayed a reduced size (39.9 Mbp) as well as a slightly reduced number of coding sequences (10,525) compared to those of *S. apiospermum* IHEM 14462 or *S. boydii* IHEM 23826 (11,694), isolated from respiratory secretions of a CF patient at the University Hospital in Angers, France.

Further efforts within the working group are needed to obtain a representative genome sequence for the other *Scedosporium* species. Such important genomic resources will enable powerful comparative genomic approaches for the identification of genes associated with specific epidemiological or physiological traits of each species. For instance, this strategy may provide the opportunity to identify genes that are present in the genome of the four species that have been identified as capable to chronically colonize the CF lungs but absent in *S. dehoogii*, which is common in the environment but unable to establish chronically in the respiratory tract of CF patients.

It can be also expected that the availability of the genome sequence of *Scedosporium* species will facilitate the development of other “omics” approaches. An illustration to this end is given by the recent publication of studies demonstrating that proteomics progressively emerges as a powerful strategy to identify promising candidates for developing innovative diagnostic and therapeutic tools for *Scedosporium/Lomentospora* infections in CF patients.^{96,106} Likewise, another proteomic study focused on the biochemical changes associated with germination in *S. boydii* and the identification of conidial- or hyphal wall-specific glycosylphosphatidyl inositol (GPI)-anchored proteins became feasible from these genomic resources.¹⁰⁷ In the same way, it is highly likely that *Scedosporium* transcriptomic data will increasingly accumulate in dedicated databases. For instance, transcriptomic analyses are currently underway to unravel the mechanisms developed by the fungus to establish within the respiratory tract of CF patients. These would certainly shed light shortly on the genes differentially expressed upon *S. apiospermum* exposure to the physicochemical conditions encountered in the respiratory tract of CF patients (including hypercapnia, hypoxia and acid pH), and in turn help in deciphering the crucial mechanisms allowing these opportunistic pathogens to specifically adapt to the CF bronchial mucus.

Despite the availability of the genome sequence of some *Scedosporium* species, further efforts are needed in order to access efficient genetic strategies for exploring the role of selected genes in pathogenesis of scedosporiosis. The first step in developing functional genomics tools for *Scedosporium* species would consist in the identification of selection markers suitable for these fungi to elaborate a convenient genetic transformation protocol,¹⁰⁸ as successfully done recently in *Lomentospora prolificans* with the disruption of three of the genes involved in the melanin biosynthesis pathway.¹⁰⁹ Looking further ahead, additional genetic tools should be developed such as recyclable marker modules, reporter genes, a nonhomologous end-joining pathway deficient strain, and conditional gene expression systems.^{110–113} This represents an essential prerequisite for expediting gene manipulation opportunities and for validating gene function in *Scedosporium* species.

Treatment of infections caused by multidrug resistant fungal pathogens

Progress has been made in the past few years for treatment of *Aspergillus* infections in CF patients, with the availability of some new antifungal drugs (isavuconazole and the echinocandins, caspofungin, micafungin, and anidulafungin). Recently, the Cystic Fibrosis Foundation Therapeu-

tics awarded the Pulmatrix company (Lexington, MA) for the development of a new formulation combining itraconazole with the dry powder iSPERSE™ technology which allows delivering by inhalation high doses of drug directly in the lungs while minimizing side effects. This formulation called PUR1900 has been designated by the US Food and Drug Administration a Qualified Infectious Disease Product (QIDP) as a potential treatment of ABPA in patients with CF. However, treatment of fungal respiratory infections in CF remains challenging when they are caused by multiresistant microorganisms like *Scedosporium* or *Lomentospora* species or the emerging *Rasamsonia* species. *In vitro* studies showed that these fungi exhibit a total resistance or a low susceptibility to most available antifungal drugs. Nevertheless, several bodies of work performed in the past few years highlight the potential for antifungal combination therapy for of *Scedosporium/Lomentospora* infections, some of which include the use of the echinocandins, particularly micafungin, for example for *Rasamsonia* infections.

In vitro susceptibility of *Scedosporium/Lomentospora* species

Several studies have been conducted over the past two decades on *in vitro* susceptibility of *Scedosporium* species and *L. prolificans* species to systemic antifungals. They revealed the lack of activity of flucytosine, as well as amphotericin B and the triazole drugs, fluconazole, itraconazole, and isavuconazole.^{113–119} Besides, the first cases of airway colonization by *Scedosporium* species in CF patients were detected after eradication of *A. fumigatus* with itraconazole therapy in patients diagnosed retrospectively as co-colonized.¹²⁰ *In vitro*, the best results were obtained for voriconazole and to a lesser extent for posaconazole, thus confirming voriconazole as the recommended treatment for scedosporiosis and its higher efficacy against *Scedosporium* species compared to *L. prolificans*.¹²¹ In addition, although not licensed for these infections, the echinocandins exhibit *in vitro* some activity against *Scedosporium* species, as well as terbinafine against *L. prolificans*.¹²²

At the species level, *S. apiospermum* and *S. boydii* share highly similar *in vitro* antifungal susceptibility patterns, whereas distinct antifungal susceptibility profiles were found for *S. aurantiacum* or *L. prolificans*.^{117,119} Additionally, in a recently published epidemiological study, 161 isolates from 73 patients were tested.⁴⁴ The results revealed a wide range of minimum inhibitory concentrations (MICs), particularly for *S. apiospermum* and *S. boydii*. Therefore, precise species identification and *in vitro* susceptibility testing are crucial at the beginning of—or even

better before initiating—an antifungal treatment for a *Scedosporium/Lomentospora* infection. Discussing antifungal treatment in case of such infections, the logical consequence is to implement a broad treatment with a combination of two or even three drugs for an efficient clinical response and to prevent acquired resistance. This strategy is supported by recent *in vitro* studies testing the efficacy of double combinations of antifungal agents against *Scedosporium* spp. Thirty-five combinations of antifungals were tested against *Scedosporium* species by a checkerboard microdilution method, with determination of the fractional concentration index.¹²³ This study revealed that combinations of voriconazole with an echinocandin or with terbinafine may have a therapeutic advantage in case of *Scedosporium* or *L. prolificans* infections, respectively.¹²³ Other studies also showed a synergistic effect of combinations of voriconazole or amphotericin B with echinocandins against both *S. apiospermum* and *L. prolificans*,¹²⁴ as well as terbinafine plus itraconazole, miconazole or voriconazole against *L. prolificans*.^{125–127} So although there is insufficient evidence to make any recommendation, combination therapy could have clinical efficacy against *Scedosporium/Lomentospora* infections, as illustrated in the next section of this review.

Regarding triple combinations of antifungals, very few studies have been conducted. Two triple combinations (amphotericin B plus voriconazole plus anidulafungin or micafungin) have been evaluated. *In vitro* testing showed they were synergistic against *L. prolificans*,¹²⁴ but this was not confirmed in a murine model: double combinations of micafungin plus amphotericin B or voriconazole were able to reduce fungal burden in the brain and kidneys conversely to that observed when the triple combination was used.¹²⁸

Treatment of *Scedosporium* species/*Lomentospora prolificans* infections

Although *Scedosporium* species and *L. prolificans* are often isolated as asymptomatic colonizers of the respiratory tract,^{9–11} patients with CF whose decreased lung function is associated with the isolation of *Scedosporium* species as the only potential pathogen have been described.^{129–132} Regarding the entities of fungal diseases, these fungi may cause in CF allergic broncho-pulmonary mycosis (ABPM) similar to the well-known ABPA.³⁴ The occurrence of other pulmonary infections in patients with CF is not yet well evaluated, but invasive mycosis are common in immunocompromised patients, such as solid organ transplant recipients.^{133–135} *Scedosporium* infections are described in one study in 3.5% of patients after lung transplantation. The 3-month all-cause mortality in this cohort was 21.7%, and

the time to onset of scedosporiosis was 12 months post-transplantation.¹³⁶ Particular attention therefore should be paid before lung transplantation in patients already colonized by multidrug resistant molds. Although voriconazole is the recommended treatment of a *Scedosporium* or *Lomentospora* infection, limited success has been reported so far with single therapy.^{135,137,138}

Four cases of treatment for a suspected pulmonary scedosporiosis in CF patients without organ transplantation have been reported since 2013. The first case described an adolescent with CF and a *S. apiospermum* infection who was treated successfully with systemic amphotericin B and voriconazole in addition to inhaled voriconazole.¹²⁹ The second case was a 35-year-old female with pneumonia due to *S. apiospermum*, which was isolated from BAL fluid. In this case the severe infection could only be treated successfully by the use of systemic caspofungin and voriconazole together with inhaled amphotericin B.¹³⁰ In a third case, an endobronchial acute manifestation of a *S. apiospermum* infection was described. Treatment with voriconazole showed no significant clinical response. Only a bronchoscopy, which was initiated, showing an obstruction by mucus plugs and bronchial cast, could improve the patient's clinical status by removal of the mucus plugs.¹³¹ The fourth case described a 24-year-old female with CF who developed an acute infection caused by *S. apiospermum*. Treatment was initiated with posaconazole orally, which allowed no clinical benefits. Implementing the treatment with a combination of oral voriconazole and terbinafine stabilized the clinical status of the patient awaiting lung transplantation. Post-transplantation, a bronchoscopy was routinely performed revealing negative direct examination and culture for fungi from the BAL fluid.¹³⁹ In addition to severe pulmonary infections, osteomyelitis as well is described in lung transplanted CF patients¹⁴⁰ but may also occur in less severely immunocompromised patients as it has been reported a case of spondylodiscitis in a young CF adult 1 year after diagnosis of pancreatic insufficiency and cortico-steroid induced diabetes.¹⁴¹

Therefore, to sum up, one should be cautious with monotherapy with voriconazole, since breakthrough infections can occur and have been reported in the literature.^{142,143} If the highly resistant *L. prolificans* is detected, a brought combined antifungal therapy including voriconazole should be considered,^{144,145} associated for example with an echinocandin as described in a successfully treated patient with osteomyelitis in the United States.¹⁴⁵ However, although a variable outcome has been reported, the combination of voriconazole with terbinafine or liposomal amphotericin B may also show some efficacy for treatment of a *L. prolificans* infection.^{146–154}

Interestingly, combinations of antifungals with miltefosine, antipsychotic drugs, or cysteine derivatives might be promising therapeutic strategies and have been already tested for the treatment of scedosporiosis.^{155–157} Additionally, new antifungal drugs targeting the acylation step in the glycosylinositol pathway of GPI-anchored cell wall proteins or the dihydroorotate dehydrogenase should be evaluated in animal models since they exhibit potent *in vitro* activities against *Scedosporium* species and *Lomentospora prolificans*.^{158,159}

Rasamsonia argillacea species complex

Rasamsonia species, formerly considered as *Geosmithia* species, are morphologically very similar to *Penicillium* and *Paecilomyces* species. *Rasamsonia argillacea*, which is now considered a four-species complex,⁴⁹ has been described to colonize the airways of CF patients.^{160–162} Using rep-PCR, the first molecular evidence of chronic airway colonization by these fungi in CF patients was demonstrated.⁶⁸

The impact of these fungi on the clinical course in CF appears to be small. No decline in lung function or reduction in body mass index is evident to date. In addition, severe pulmonary infections due to *Rasamsonia* species in non-transplanted CF patients have not been reported, although single case reports of clinical deterioration and lung function decline have been recently described.⁶⁹ Significant broncho-pulmonary exacerbations have been reported in one patient. Several antifungal drugs were used successively, but only with micafungin the therapeutic intervention could influence the exacerbation in a significant manner.¹⁶³

Nevertheless, virulence of these fungi has been demonstrated in two case series covering nine patients with chronic granulomatous disease^{164,165} or in a case report from the United States describing a pulmonary and aortic graft infection by *R. argillacea* in an immunocompetent patient.¹⁶⁶ In this case the fungus was first misdiagnosed as *Penicillium* sp. Re-identification of the fungus as *R. argillacea* and subsequent treatment with posaconazole and micafungin together with surgery reached sufficient clinical response. Most recently, a case report on a CF patient after lung transplantation demonstrated the pathogenicity in immunosuppressed patients. A 21-year-old patient with CF died after transplantation without diagnosing the underlying respiratory disease, but the autopsy demonstrated disseminated angioinvasive fungal infection involving lungs, heart, thyroid, large bowel, small bowel, kidneys, right eye, skin, diaphragm, and skeletal muscle. Gross examination demonstrated innumerable tan-yellow nodules involving the pericardium, endocardium, myocardium, lungs, and pleura. The fungus was confirmed as *R. aegroticola*

based on sequencing the ITS regions of rDNA.¹⁶⁷ Interestingly, this patient was colonized two years before lung transplantation by a fungus initially considered morphologically as *Paecilomyces* sp. After death of the patient, pre-transplant isolates were reidentified by molecular methods as *R. aegroticola* and RAPD analysis demonstrated that, as described by Symoens et al.¹³⁵ for a fatal disseminated infection following lung transplantation in a 26-year-old female with CF, pre- and post-transplant available isolates belonged to the same genotype.¹⁶⁷ Therefore, in single cases it might be essential to discuss the impact of *R. argillacea* species on an individual basis.

Although there are no epidemiological cutoff values for species of the *R. argillacea* complex, most studies revealed for these fungi a high level of resistance to voriconazole (MIC ≥ 16 μ g/ml) and more variable results for itraconazole (0.5 to 16), posaconazole (0.25 to 16) and amphotericin B (1 to 8).^{49,162} Likewise, it has been recently shown that the *in vitro* isavuconazole MICs for species of the *R. argillacea* complex are high, suggesting no meaningful activity for this new triazole.¹⁶⁸ By contrast, all isolates were highly susceptible to the echinocandins, caspofungin or micafungin,^{49,161,162,168} and as aforementioned micafungin was already used successfully for eradication in a CF patient with severe pulmonary infection.¹⁶³ Administration of an echinocandin therefore seems to be the best therapeutic option for a *Rasamsonia* infection, and in cases of disseminated infections, a double combination of micafungin or caspofungin with posaconazole should be discussed.

Future directions

Although some progress has been made recently in the detection of fungal colonization of the airways/respiratory infections in CF, and in the treatment of the severe disseminated infections these fungi may cause in case of immunodeficiency, much remains to be done. Continuous attention in the clinical surveillance is required to detect the emergence of new fungal pathogens. Consensus guidelines for mycological examination of respiratory secretions from CF patients are urgently needed for accurate determination of the prevalence of the different fungal species associated with CF and of its variations according to *CFTR* mutations, or geographical origin and age of the patients. Likewise, collaborative studies should be conducted to define the value of some recently described molecular methods and markers, including antibodies and other immunological markers, to distinguish respiratory infections from an airway colonization, or to determine the clinical significance of a chronic colonization of the airways and the possible genetic risk factors for fungal airway colonization/respiratory

infections. Another important question, which needs to be answered, deals with the early treatment of airway colonization, independently of a respiratory infection. Likewise, given the paucity of treatment options for fungal infections in patients with CF, clinical studies should be conducted on the new antifungal drugs or formulations that are in the pipeline.^{169–171} Finally, with the recent availability of genomic resources on *Scedosporium* species, basic research on these pathogens should be conducted in order to improve our understanding of the pathogenesis of fungal respiratory infections in CF and to define new antifungal targets.

Acknowledgments

The genomic studies have been supported by a National Health and Medical Research Council (NH&MRC) grant from Australia APP1031943 to WM for *S. aurantiacum*, by the French association Vaincre la Mucoviscidose (RF20120600725/1/1/110) to JPB for *S. apiospermum* and by Angers University (Appel à projets internes 2014) to AG for *S. boydii*. Some of the works performed regarding improvement of the biological diagnosis of fungal respiratory infections in CF were supported by grants from Vaincre la Mucoviscidose to JPB (2010/IC1003), MEB (RC20120600707/1/2/110) or MF (RF20130500840).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

- Hamutcu R, Rowland JM, Horn MV et al. Clinical findings and lung pathology in children with cystic fibrosis. *Am J Respir Crit Care Med*. 2002; 165: 1172–1175.
- Morrissey BM, Schock BC, Marelich GP, Cross CE. Cystic fibrosis in adults: current and future management strategies. *Clin Rev Allergy Immunol*. 2003; 25: 275–287.
- Riordan JR. CFTR function and prospects for therapy. *Annu Rev Biochem*. 2008; 77: 701–726.
- Moran O. The biophysics, biochemistry and physiology of CFTR. *Cell Mol Life Sci*. 2017; 74: 1–2.
- Castellani C, Assael BM. Cystic fibrosis: a clinical view. *Cell Mol Life Sci*. 2017; 74: 129–140.
- George AM, Jones PM, Middleton PG. Cystic fibrosis infections: treatment strategies and prospects. *FEMS Microbiol Lett*. 2009; 300: 153–164.
- Cohen-Cymbarknoh M, Shoseyov D, Kerem E. Managing cystic fibrosis: strategies that increase life expectancy and improve quality of life. *Am J Respir Crit Care Med*. 2011; 183: 1463–1471.
- Langan KM, Kotsimbos T, Peleg AY. Managing *Pseudomonas aeruginosa* respiratory infections in cystic fibrosis. *Curr Opin Infect Dis*. 2015; 28: 547–556.
- Pihet M, Carrère J, Cimon B et al. Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis—a review. *Med Mycol*. 2009; 47: 387–397.
- Müller FM, Seidler M. Characteristics of pathogenic fungi and antifungal therapy in cystic fibrosis. *Expert Rev Anti Infect Ther*. 2010; 8: 957–964.
- Middleton PG, Chen SC, Meyer W. Fungal infections and treatment in cystic fibrosis. *Curr Opin Pulm Med*. 2013; 19: 670–675.
- Chotirmall SH, McElvaney NG. Fungi in the cystic fibrosis lung: bystanders or pathogens? *Int J Biochem Cell Biol*. 2014; 52: 161–173.
- Williams C, Ranjendran R, Ramage G. Pathogenesis of fungal infections in cystic fibrosis. *Curr Fungal Infect Rep*. 2016; 10: 163–169.
- Borman AM, Palmer MD, Delhaes L et al. Lack of standardization in the procedures for mycological examination of sputum samples from CF patients: a possible cause for variations in the prevalence of filamentous fungi. *Med Mycol*. 2010; 48: S88–S97.
- Accurso FJ. Update in cystic fibrosis 2005. *Am J Respir Crit Care Med*. 2006; 173: 944–947.
- Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. *Chest*. 2008; 133: 489–495.
- Burns JL, Gibson RL, McNamara S et al. Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *J Infect Dis*. 2001; 183: 444–452.
- Döring G, Flume P, Heijerman H, Elborn JS, Group Consensus Study. Treatment of lung infection in patients with cystic fibrosis: current and future strategies. *J Cyst Fibros*. 2012; 11: 461–479.
- Horré R, Symoens F, Delhaes L, Bouchara JP. Fungal respiratory infections in cystic fibrosis: a growing problem. *Med Mycol*. 2010; 48: S1–S3.
- Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med*. 2003; 168: 918–951.
- Sudfeld CR, Dasenbrook EC, Merz WG, Carroll KC, Boyle MP. Prevalence and risk factors for recovery of filamentous fungi in individuals with cystic fibrosis. *J Cyst Fibros*. 2010; 9: 110–116.
- Maskarinec SA, Johnson MD, Perfect JR. Genetic susceptibility to fungal infections: what is in the genes? *Curr Clin Microbiol Rep*. 2016; 3: 81–91.
- Overton NL, Denning DW, Bowyer P, Simpson A. Genetic susceptibility to allergic bronchopulmonary aspergillosis in asthma: a genetic association study. *Allergy Asthma Clin Immunol*. 2016; 12: 47.
- Chauhan B, Santiago L, Hutcheson PS et al. Evidence for the involvement of two different MHC class II regions in susceptibility or protection in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol*. 2000; 106: 723–729.
- Muro M, Mondejar-López P, Moya-Quiles MR et al. HLA-DRB1 and HLA-DQB1 genes on susceptibility to and protection from allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Microbiol Immunol*. 2013; 57: 193–197.
- Brouard J, Knauer N, Boelle PY et al. Influence of interleukin-10 on *Aspergillus fumigatus* infection in patients with cystic fibrosis. *J Infect Dis*. 2005; 191: 1988–1991.
- Knutsen AP, Kariuki B, Consolino JD, Warriar MR. IL-4 alpha chain receptor (IL-4Ralpha) polymorphisms in allergic bronchopulmonary aspergillosis. *Clin Mol Allergy*. 2006; 4: 3.
- Bauernfeind A, Bertele RM, Harms K et al. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. *Infection*. 1987; 15: 270–277.
- Masoud-Landgraf L, Badura A, Eber E, Feierl G, Marth E, Buzina W. Modified culture method detects a high diversity of fungal species in cystic fibrosis patients. *Med Mycol*. 2014; 52: 179–186.
- Pashley CH, Fairs A, Morley JP et al. Routine processing procedures for isolating filamentous fungi from respiratory sputum samples may underestimate fungal prevalence. *Med Mycol*. 2012; 50: 433–438.
- Bakare N, Rickerts V, Bargon J, Just-Nübling G. Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. *Mycoses*. 2003; 46: 19–23.
- Nagano Y, Millar BC, Goldsmith CE et al. Development of selective media for the isolation of yeasts and filamentous fungi from the sputum of adult patients with cystic fibrosis (CF). *J Cyst Fibros*. 2008; 7: 566–572.
- Nagano Y, Elborn JS, Millar BC et al. Comparison of techniques to examine the diversity of fungi in adult patients with cystic fibrosis. *Med Mycol*. 2010; 48: 166–176.

34. Cimon B, Carrère J, Vinatier JF, Chazalotte JP, Chabasse D, Bouchara JP. Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis*. 2000; 19: 53–56.
35. Plésiat P, Herrmann JL, Ferroni A et al. Infections broncho-pulmonaires et mucoviscidose. Rémic, Référentiel en microbiologie médicale, 5ème édition 2015. *Société Française de Microbiologie*. 2015; 5.1: 193–200.
36. Downes FP, Ito K. *Compendium of Methods for the Microbiological Examination of Foods*. Fourth edition. Washington, DC: American Public Health Association, 2001.
37. Hocking AD, Pitt JI. Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. *Appl Environ Microbiol*. 1980; 39: 488–492.
38. Jarvis B. Comparison of an improved rose bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. *J Appl Bacteriol*. 1973; 36: 723–727.
39. King AD, Hocking AD, Pitt JI. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. *Appl Environ Microbiol*. 1979; 37: 959–964.
40. Summerbell RC. The benomyl test as a fundamental diagnostic method for medical mycology. *J Clin Microbiol*. 1993; 31: 572–577.
41. Rainer J, Kaltseis J, de Hoog SG, Summerbell RC. Efficacy of a selective isolation procedure for members of the *Pseudallescheria boydii* complex. *Antonie Van Leeuwenhoek*. 2008; 93: 315–322.
42. Horrè R, Marklein G, Siekmeier R, Nidermajer S, Reiffert SM. Selective isolation of *Pseudallescheria* and *Scedosporium* species from respiratory tract specimens of cystic fibrosis patients. *Respiration*. 2009; 77: 320–324.
43. Horrè R, Marklein G, Siekmeier R, Reiffert SM. Detection of hyphomycetes in the upper respiratory tract of patients with cystic fibrosis. *Mycoses*. 2011; 54: 514–522.
44. Sedlacek L, Graf B, Schwarz C et al. Prevalence of *Scedosporium* species and *Lomentospora prolificans* in patients with cystic fibrosis in a multicenter trial by use of a selective medium. *J Cyst Fibros*. 2015; 14: 237–241.
45. Blyth CC, Harun A, Middleton PG et al. Detection of occult *Scedosporium* species in respiratory tract specimens from patients with cystic fibrosis by use of selective media. *J Clin Microbiol*. 2010; 48: 314–316.
46. Schoch CL, Seifert KA, Huhndorf S et al. The nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci U S A*. 2012; 109: 6241–6246.
47. Alastruey-Izquierdo A, Mellado E, Peláez T et al. Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). *Antimicrob Agents Chemother*. 2013; 57: 3380–3387.
48. Gilgado F, Cano J, Gené J, Guarro J. Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. *J Clin Microbiol*. 2005; 43: 4930–4942.
49. Houbraken J, Giraud S, Meijer M et al. Taxonomy and antifungal susceptibility of clinically important *Rasamsonia* species. *J Clin Microbiol*. 2013; 51: 22–30.
50. Croxatto A, Prod'homme G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol Rev*. 2012; 36: 380–407.
51. Ferroni A, Suarez S, Beretti JL et al. Real-time identification of bacteria and *Candida* species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol*. 2010; 48: 1542–1548.
52. Posteraro B, De Carolis E Vella A, Sanguinetti M. MALDI-TOF mass spectrometry in the clinical mycology laboratory: identification of fungi and beyond. *Expert Rev Proteomics*. 2013; 10: 151–164.
53. Hettick JM, Green BJ, Buskirk AD et al. Discrimination of *Penicillium* isolates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry fingerprinting. *Rapid Commun Mass Spectrom*. 2008; 22: 2555–2560.
54. De Carolis E, Posteraro B, Lass-Flörl C et al. Species identification of *Aspergillus*, *Fusarium* and Mucorales with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Microbiol Infect*. 2012; 18: 475–484.
55. Sitterlé E, Giraud S, Leto J et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and accurate identification of *Pseudallescheria/Scedosporium* species. *Clin Microbiol Infect*. 2014; 20: 929–935.
56. Triest D, Stubbe D, De Cremer K et al. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of molds of the *Fusarium* genus. *J Clin Microbiol*. 2015; 53: 465–476.
57. Sleiman S, Halliday CL, Chapman B et al. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of *Aspergillus*, *Scedosporium*, and *Fusarium* spp. in the Australian clinical setting. *J Clin Microbiol*. 2016; 54: 2182–2186.
58. Cassagne C, Ranque S, Normand AC et al. Mould routine identification in the clinical laboratory by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *PloS One*. 2011; 6: e28425.
59. Lau AF, Drake SK, Calhoun LB, Henderson CM, Zelazny AM. Development of a clinically comprehensive database and a simple procedure for identification of molds from solid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol*. 2013; 51: 828–834.
60. Vanhee LME, Nelis HJ, Coenye T. What can be learned from genotyping of fungi? *Med Mycol*. 2010; 48: S60–S69.
61. Harun A, Perdomo H, Gilgado F et al. Genotyping of *Scedosporium* species: a review of molecular approaches. *Med Mycol*. 2009; 47: 406–414.
62. Kaur J, Duan SY, Vaas LA et al. Phenotypic profiling of *Scedosporium aurantiacum*, an opportunistic pathogen colonizing human lungs. *PLoS One*. 2015; 10: e0122354.
63. Bernhardt A, Sedlacek L, Wagner S, Schwarz C, Würstl B, Tintelnot K. Multilocus sequence typing of *Scedosporium apiospermum* and *Pseudallescheria boydii* isolates from cystic fibrosis patients. *J Cyst Fibros*. 2013; 12: 592–598.
64. Matray O, Mouhajir A, Giraud S et al. Semi-automated repetitive sequence-based PCR amplification for species of the *Scedosporium apiospermum* complex. *Med Mycol*. 2016; 54: 409–419.
65. Defontaine A, Zouhair R, Cimon B et al. Genotyping study of *Scedosporium apiospermum* isolates from patients with cystic fibrosis. *J Clin Microbiol*. 2002; 40: 2108–2114.
66. Vanhee LME, Symoens F, Bouchara JP, Nelis HJ, Coenye T. High-resolution genotyping of *Aspergillus fumigatus* isolates recovered from chronically colonised patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis*. 2008; 27: 1005–1007.
67. Rougeron A, Giraud S, Razafimandimby B, Meis JF, Bouchara JP, Klaassen CHW. Different colonization patterns of *Aspergillus terreus* in patients with cystic fibrosis. *Clin Microbiol Infect*. 2014; 20: 327–333.
68. Mouhajir A, Matray O, Giraud S et al. Long-term *Rasamsonia argillacea* complex species colonization revealed by PCR amplification of repetitive DNA sequences in cystic fibrosis patients. *J Clin Microbiol*. 2016; 54: 2804–2812.
69. Steinmann J, Giraud S, Schmidt D et al. Validation of a novel real-time PCR for detecting *Rasamsonia argillacea* species complex in respiratory secretions from cystic fibrosis patients. *New Microbes New Infect*. 2014; 2: 72–78.
70. Bouchara JP, Hsieh HY, Croquefer S et al. Development of an oligonucleotide array for direct detection of fungi in sputum samples from patients with cystic fibrosis. *J Clin Microbiol*. 2009; 47: 142–152.
71. Mounier J, Gouëlo A, Keravec M et al. Use of denaturing high-performance liquid chromatography (DHPLC) to characterize the bacterial and fungal airway microbiota of cystic fibrosis patients. *J Microbiol*. 2014; 52: 307–314.
72. Kramer R, Sauer-Heilborn A, Welte T, Guzman CA, Abraham WR, Höfle MG. Cohort study of airway mycobiome in adult cystic fibrosis patients: differences in community structure between fungi and bacteria reveal predominance of transient fungal elements. *J Clin Microbiol*. 2015; 53: 2900–2907.

73. Delhaes L, Monchy S, Fréalle E et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community—implications for therapeutic management. *PLoS One*. 2012; 7: e36313.
74. Willger SD, Grim SL, Dolben EL et al. Characterization and quantification of the fungal microbiome in serial samples from individuals with cystic fibrosis. *Microbiome*. 2014; 2: 40.
75. Syhre M, Scotter JM, Chambers ST. Investigation into the production of 2-pentylfuran by *Aspergillus fumigatus* and other respiratory pathogens *in vitro* and human breath samples. *Med Mycol*. 2008; 46: 209–215.
76. Chambers ST, Syhre M, Murdoch DR, McCartin F, Epton MJ. Detection of 2-pentylfuran in the breath of patients with *Aspergillus fumigatus*. *Med Mycol*. 2009; 47: 468–476.
77. Neerinx AH, Geurts BP, Habets MF et al. Identification of *Pseudomonas aeruginosa* and *Aspergillus fumigatus* mono- and co-cultures based on volatile biomarker combinations. *J Breath Res*. 2016; 10: 016002.
78. Bertrand S, Larcher G, Landreau A, Richomme P, Duval O, Bouchara JP. Hydroxamate siderophores of *Scedosporium apiospermum*. *Biometals*. 2009; 22: 1019–1029.
79. Bertrand S, Bouchara JP, Venier MC, Richomme P, Duval O, Larcher G. N(α)-methyl coprophen B, a potential marker of the airway colonization by *Scedosporium apiospermum* in patients with cystic fibrosis. *Med Mycol* 2010; 48: S98–S107.
80. Cerqueira LB, de Francisco TM, Gasparetto JC, Campos FR, Pontarolo R. Development and validation of an HPLC-MS/MS method for the early diagnosis of aspergillosis. *PLoS One*. 2014; 9: e92851.
81. Domingo MP, Colmenarejo C, Martínez-Lostao L et al. Bis(methyl)gliotoxin proves to be a more stable and reliable marker for invasive aspergillosis than gliotoxin and suitable for use in diagnosis. *Diagn Microbiol Infect Dis*. 2012; 73: 57–64.
82. Vidal-García M, Domingo MP, De Rueda B et al. Clinical validity of bis(methylthio)gliotoxin for the diagnosis of invasive aspergillosis. *Appl Microbiol Biotechnol*. 2016; 100: 2327–2334.
83. 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.
84. Jat KR, Walia DK, Khairwa A. Anti-IgE therapy for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database Syst Rev*. 2013; CD010288.
85. Delhaes L, Frealle E, Pinel C. Serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis: state of the art and further challenges. *Med Mycol*. 2010; 48: S77–S87.
86. Latzin P, Hartl D, Regamey N, Frey U, Schoeni MH, Casaulta C. Comparison of serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur Respir J*. 2008; 31: 36–42.
87. Casaulta C, Flückiger S, Cramer R, Blaser K, Schoeni MH. Time course of antibody response to recombinant *Aspergillus fumigatus* antigens in cystic fibrosis with and without ABPA. *Pediatr Allergy Immunol*. 2005; 16: 217–225.
88. 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.
89. Gernez Y, Walters J, Mirković B et al. Blood basophil activation is a reliable biomarker of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur Respir J*. 2016; 47: 177–185.
90. Katelari A, Tzanoudaki M, Noni M et al. The role of basophil activation test in allergic bronchopulmonary aspergillosis and *Aspergillus fumigatus* sensitization in cystic fibrosis patients. *J Cyst Fibros*. 2016; 15: 587–596.
91. Barrera C, Richaud-Thiriez B, Rocchi S et al. New commercially available IgG kits and Time-Resolved Fluorometric IgE assay for diagnosis of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Clin Vaccine Immunol*. 2015; 23: 196–203.
92. Dumollard C, Bailly S, Perriot S et al. Prospective evaluation of a new *Aspergillus* IgG enzyme immunoassay kit for diagnosis of chronic and allergic pulmonary aspergillosis. *J Clin Microbiol*. 2016; 54: 1236–1242.
93. Parize P, Billaud S, Bienvenu AL et al. Impact of *Scedosporium apiospermum* complex seroprevalence in patients with cystic fibrosis. *J Cyst Fibros*. 2014; 13: 667–673.
94. Mina S, Marot-Leblond A, Cimon B et al. Purification and characterization of a mycelial catalase from *Scedosporium boydii*, a useful tool for specific antibody detection in patients with cystic fibrosis. *Clin Vaccine Immunol*. 2015; 22: 37–45.
95. Mina S, Staerck C, d'Almeida SM et al. Identification of *Scedosporium boydii* catalase A1 gene, a reactive oxygen species detoxification factor highly expressed in response to oxidative stress and phagocytic cells. *Fungal Biol*. 2015; 119: 1322–1333.
96. Pellon A, Ramirez-Garcia A, Buldain I, Antoran A, Rementeria A, Hernandez FL. Immunoproteomics-based analysis of the immunocompetent serological response to *Lomentospora prolificans*. *J Proteome Res*. 2016; 15: 595–607.
97. Larcher G, Cimon B, Symoens F, Tronchin G, Chabasse D, Bouchara JP. A 33 kDa serine proteinase from *Scedosporium apiospermum*. *Biochem J*. 1996; 315:119–126.
98. Silva BA, Pinto MR, Soares RM, Barreto-Bergter E, Santos AL. *Pseudallescheria boydii* releases metalloproteinases capable of cleaving several proteinaceous compounds. *Res Microbiol*. 2006; 157: 425–432.
99. Pereira MM, Silva BA, Pinto MR, Barreto-Bergter E, dos Santos AL. Proteins and peptidases from conidia and mycelia of *Scedosporium apiospermum* strain HLPB. *Mycopathologia*. 2009; 167: 25–30.
100. Lima OC, Larcher G, Vandeputte P et al. Molecular cloning and biochemical characterization of a Cu,Zn-superoxide dismutase from *Scedosporium apiospermum*. *Microbes Infect*. 2007; 9: 558–565.
101. Ghamrawi S, Rénier G, Saulnier P et al. Cell wall modifications during conidial maturation of the human pathogenic fungus *Pseudallescheria boydii*. *PLoS One*. 2014; 9: e100290.
102. Vandeputte P, Ghamrawi S, Rechenmann M et al. Draft genome sequence of the pathogenic fungus *Scedosporium apiospermum*. *Genome Announc*. 2014; 2: pii: e00988–14.
103. Al-Khodori S, Price CT, Kalia A, Abu Kwaik Y. Functional diversity of ankyrin repeats in microbial proteins. *Trends Microbiol*. 2010; 18: 132–129.
104. Á Pérez-Bercoff, A Papanicolaou, Ramsperger M et al. Draft genome of Australian environmental strain WM 09.24 of the opportunistic human pathogen *Scedosporium aurantiacum*. *Genome Announc*. 2015; 3: pii: e01526–14.
105. Duvaux L, Shiller J, Vandeputte P et al. Draft genome sequence of the human pathogenic fungus *Scedosporium boydii*. *Genome Announc*. 2017 (accepted for publication).
106. Ramirez-Garcia A, Pellon A, Buldain I et al. Proteomics as a tool to identify new targets *Aspergillus* and *Scedosporium* in the context of cystic fibrosis. *Mycopathologia*. 2017 May 8. doi: 10.1007/s11046-017-0139-3.
107. Ghamrawi S, Gastebois A, Zykwinska A et al. A multifaceted study of *Scedosporium boydii* cell wall changes during germination and identification of GPI-anchored proteins. *PLoS One*. 2015; 10: e0128680.
108. Cairns TC, Studholme DJ, Talbot NJ, Haynes K. New and improved techniques for the study of pathogenic fungi. *Trends Microbiol*. 2016; 24: 35–50.
109. Al-Laeiby A, Kershaw MJ, Penn TJ, Thornton CR. Targeted disruption of melanin biosynthesis genes in the human pathogenic fungus *Lomentospora prolificans* and its consequences for pathogen survival. *Int J Mol Sci*. 2016; 17: 444.
110. Krappmann S. Genetic surgery in fungi: employing site-specific recombinases for genome manipulation. *Appl Microbiol Biotechnol*. 2014; 98: 1971–1982.
111. Papon N, Courdavault V, Lanoue A, Clastre M, Brock M. Illuminating fungal infections with bioluminescence. *PLoS Pathog*. 2014; 10: e1004179.
112. Kegel A, Martinez P, Carter SD, Aström SU. Genome wide distribution of illegitimate recombination events in *Kluyveromyces lactis*. *Nucleic Acids Res*. 2006; 34: 1633–1645.

113. Wanka F, Cairns T, Boecker S et al. Tet-on, or Tet-off, that is the question: Advanced conditional gene expression in *Aspergillus*. *Fungal Genet Biol*. 2016; 89: 72–83.
114. Cuenca-Estrella M, Ruiz-Díez B, Martínez-Suárez JV, Monzón A, Rodríguez-Tudela JL. Comparative *in-vitro* activity of voriconazole (UK-109,496) and six other antifungal agents against clinical isolates of *Scedosporium prolificans* and *Scedosporium apiospermum*. *J Antimicrob Chemother*. 1999; 43: 149–151.
115. Carrillo AJ, Guarro J. *In vitro* activities of four novel triazoles against *Scedosporium* spp. *Antimicrob Agents Chemother*. 2001; 45: 2151–2153.
116. Meletiadis J, Meis JF, Mouton JW, Rodríguez-Tudela JL, Donnelly JP, Verweij PE; EUROFUNG Network. *In vitro* activities of new and conventional antifungal agents against clinical *Scedosporium* isolates. *Antimicrob Agents Chemother*. 2002; 46: 62–68.
117. Gilgado F, Serena C, Cano J, Gené J, Guarro J. Antifungal susceptibilities of the species of the *Pseudallescheria boydii* complex. *Antimicrob Agents Chemother*. 2006; 50: 4211–4213.
118. Guinea J, Peláez T, Recio S, Torres-Narbona M, Bouza E. *In vitro* antifungal activities of isavuconazole (BAL4815), voriconazole, and fluconazole against 1,007 isolates of zygomycete, *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* species. *Antimicrob Agents Chemother*. 2008; 52: 1396–1400.
119. Lackner M, de Hoog GS, Verweij PE et al. Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. *Antimicrob Agents Chemother*. 2012; 56: 2635–2642.
120. Chabasse D, Bouchara JP, Chazalotte JP et al. Mucoviscidose et colonisation fongique à *Scedosporium apiospermum*. A propos de trois observations. *J Mycol Méd*. 1991; 1: 152–155.
121. Tortorano AM, Richardson M, Roilides E et al.; European Society of Clinical Microbiology and Infectious Diseases Fungal Infection Study Group; European Confederation of Medical Mycology. ESCMID and EMM joint guidelines on diagnosis and management of hyalohyphomycosis: *Fusarium* spp., *Scedosporium* spp. and others. *Clin Microbiol Infect*. 2014; 20: 27–46.
122. Lackner M, Rezusta A, Villuendas MC, Palacian MP, Meis JF, Klaassen CH. Infection and colonisation due to *Scedosporium* in Northern Spain. An *in vitro* antifungal susceptibility and molecular epidemiology study of 60 isolates. *Mycoses*. 2011; 54: 12–21.
123. Cuenca-Estrella M, Alastruey-Izquierdo A, Alcazar-Fuoli L et al. *In vitro* activities of 35 double combinations of antifungal agents against *Scedosporium apiospermum* and *Scedosporium prolificans*. *Antimicrob Agents Chemother*. 2008; 52: 1136–1139.
124. Martín-Vicente A, Guarro J, Capilla J. Does a triple combination have better activity than double combinations against multiresistant fungi? Experimental *in vitro* evaluation. *Int J Antimicrob Agents*. 2017; 49: 422–426.
125. Cortez KJ, Roilides E, Quiroz-Telles F et al. Infections caused by *Scedosporium* spp. *Clin Microbiol Rev*. 2008; 21: 157–197.
126. Meletiadis J, Mouton JW, Meis JF, Verweij PE. Combination chemotherapy for the treatment of invasive infections by *Scedosporium prolificans*. *Clin Microbiol Infect*. 2000; 6: 336–337.
127. Meletiadis J, Mouton JW, Meis JF, Verweij PE. *In vitro* drug interaction modeling of combinations of azoles with terbinafine against clinical *Scedosporium prolificans* isolates. *Antimicrob Agents Chemother*. 2003; 47: 106–117.
128. Rodríguez MM, Calvo E, Serena C, Maríné M, Pastor FJ, Guarro J. Effects of double and triple combinations of antifungal drugs in a murine model of disseminated infection by *Scedosporium prolificans*. *Antimicrob Agents Chemother*. 2009; 53: 2153–2155.
129. Holle J, Leichsenring M, Meissner PE. Nebulized voriconazole in infections with *Scedosporium apiospermum*—case report and review of the literature. *J Cyst Fibros*. 2014; 13: 400–402.
130. Schwarz C, Thronick C, Staab D, Tintelnot K. *Scedosporium apiospermum*: a fungal pathogen causing pneumonia in a patient with cystic fibrosis. *JMM Case Rep*. 2015; 2: doi: 10.1099/jmmcr.0.000061.
131. Padoan R, Poli P, Colombrita D, Borghi E, Timpano S, Berlucchi M. Acute *Scedosporium apiospermum* endobronchial infection in cystic fibrosis. *Pediatr Infect Dis J*. 2016; 35: 701–702.
132. Schwarz C, Brandt C, Antweiler E et al. Prospective multicenter German study on pulmonary colonization with *Scedosporium* (*Lomentospora*) species in cystic fibrosis: Epidemiology and new association factors. *PLoS One*. 2017; 12: e0171485.
133. Vagefi MR, Kim ET, Alvarado RG, Duncan JL, Howes EL, Crawford JB. Bilateral endogenous *Scedosporium prolificans* endophthalmitis after lung transplantation. *Am J Ophthalmol*. 2005; 139: 370–373.
134. Balandin B, Aguilar M, Sánchez I et al. *Scedosporium apiospermum* and *S. prolificans* mixed disseminated infection in a lung transplant recipient: An unusual case of long-term survival with combined systemic and local antifungal therapy in intensive care unit. *Med Mycol Case Rep*. 2016; 11: 53–56.
135. Symoens F, Knoop C, Schrooyen M et al. Disseminated *Scedosporium apiospermum* infection in a cystic fibrosis patient after double-lung transplantation. *J Heart Lung Transplant*. 2006; 25: 603–607.
136. Doligalski CT, Benedict K, Cleveland AA et al. Epidemiology of invasive mold infections in lung transplant recipients. *Am J Transplant*. 2014; 14: 1328–1333.
137. Borghi E, Iatta R, Manca A, Montagna MT, Morace G. Chronic airway colonization by *Scedosporium apiospermum* with a fatal outcome in a patient with cystic fibrosis. *Med Mycol*. 2010; 48: S108–S113.
138. Lujik B, Ekkeleenkamp MB, DeJong PA et al. Effective prolonged therapy with voriconazole in a lung transplant recipient with spondylodiscitis induced by *Scedosporium apiospermum*. *Case Rep Infect Dis*. 2011; 2011: 460313.
139. Rolfe NE, Haddad TJ, Wills TS. Management of *Scedosporium apiospermum* in a pre- and post-lung transplant patient with cystic fibrosis. *Med Mycol Case Rep*. 2013; 2: 37–39.
140. Denton EJ, Smibert O, Gooi J et al. Invasive *Scedosporium* sternal osteomyelitis following lung transplant: Cured. *Med Mycol Case Rep*. 2016; 12: 14–16.
141. Guignard S, Hubert D, Dupont B et al. Multifocal *Scedosporium apiospermum* spondylitis in a cystic fibrosis patient. *J Cyst Fibros*. 2008; 7: 89–91.
142. Ananda-Rajah MR, Grigg A, Slavin MA. Breakthrough disseminated *Scedosporium prolificans* infection in a patient with relapsed leukemia on prolonged voriconazole followed by posaconazole prophylaxis. *Mycopathologia*. 2008; 166: 83–86.
143. Ohashi R, Kato M, Katsura Y et al. Breakthrough lung *Scedosporium prolificans* infection with multiple cavity lesions in a patient receiving voriconazole for probable invasive aspergillosis associated with monoclonal gammopathy of undetermined significance (MGUS). *Med Mycol J*. 2011; 52: 33–38.
144. Kesson AM, Bellemore MC, O'Mara TJ, Ellis DH, Sorrell TC. *Scedosporium prolificans* osteomyelitis in an immunocompetent child treated with a novel agent, hexadecylphosphocholine (miltefosine), in combination with terbinafine and voriconazole: A case report. *Clin Infect Dis*. 2009; 48: 1257–1261.
145. Steinbach WJ, Schell WA, Miller JL, Perfect JR. *Scedosporium prolificans* osteomyelitis in an immunocompetent child treated with voriconazole and caspofungin, as well as locally applied polyhexamethylene biguanide. *J Clin Microbiol*. 2003; 41: 3981–3985.
146. Ochi Y, Hiramoto N, Takegawa H et al. Infective endocarditis caused by *Scedosporium prolificans* infection in a patient with acute myeloid leukemia undergoing induction chemotherapy. *Int J Hematol*. 2015; 101: 620–625.
147. Uno K, Kasahara K, Kutsuna S et al. Infective endocarditis and meningitis due to *Scedosporium prolificans* in a renal transplant recipient. *J Infect Chemother*. 2014; 20: 131–133.
148. Nishimori M, Takahashi T, Suzuki E et al. Fatal fungemia with *Scedosporium prolificans* in a patient with acute myeloid leukemia. *Med Mycol J*. 2014; 55: E63–70.

149. Bhat SV, Paterson DL, Rinaldi MG, Veldkamp PJ. *Scedosporium prolificans* brain abscess in a patient with chronic granulomatous disease: successful combination therapy with voriconazole and terbinafine. *Scand J Infect Dis*. 2007; 39: 87–90.
150. Gosbell IB, Toumasatos V, Yong J, Kuo RS, Ellis DH, Perrie RC. Cure of orthopaedic infection with *Scedosporium prolificans*, using voriconazole plus terbinafine, without the need for radical surgery. *Mycoses*. 2003; 46: 233–236.
151. Howden BP, Slavin MA, Schwarzer AP, Mijch AM. Successful control of disseminated *Scedosporium prolificans* infection with a combination of voriconazole and terbinafine. *Eur J Clin Microbiol Infect Dis*. 2003; 22: 111–113.
152. Li JY, Yong TY, Grove DI, Coates PT. Successful control of *Scedosporium prolificans* septic arthritis and probable osteomyelitis without radical surgery in a long-term renal transplant recipient. *Transpl Infect Dis*. 2008; 10: 63–65.
153. Studahl M, Backteman T, Stålhammar F, Chryssanthou E, Petrini B. Bone and joint infection after traumatic implantation of *Scedosporium prolificans* treated with voriconazole and surgery. *Acta Paediatr*. 2003; 92: 980–982.
154. Tong SY, Peleg AY, Yoong J, Handke R, Szer J, Slavin M. Breakthrough *Scedosporium prolificans* infection while receiving voriconazole prophylaxis in an allogeneic stem cell transplant recipient. *Transpl Infect Dis*. 2007; 9: 241–243.
155. Homa M, Galgóczy L, Tóth E, et al. *In vitro* antifungal activity of antipsychotic drugs and their combinations with conventional antifungals against *Scedosporium* and *Pseudallescheria* isolates. *Med Mycol*. 2015; 53: 890–895.
156. Homa M, Galgóczy L, Tóth E et al. *In vitro* susceptibility of *Scedosporium* isolates to N-acetyl-L-cysteine alone and in combination with conventional antifungal agents. *Med Mycol*. 2016; 54: 776–779.
157. Biswas C, Sorrell TC, Djordjevic JT, Zuo X, Jolliffe KA, Chen SC. *In vitro* activity of miltefosine as a single agent and in combination with voriconazole or posaconazole against uncommon filamentous fungal pathogens. *J Antimicrob Chemother*. 2013; 68: 2842–2846.
158. Castanheira M, Duncanson FP, Diekema DJ, Guarro J, Jones RN, Pfaller MA. Activities of E1210 and comparator agents tested by CLSI and EUCAST broth microdilution methods against *Fusarium* and *Scedosporium* species identified using molecular methods. *Antimicrob Agents Chemother*. 2012; 56: 352–357.
159. Wiederhold NP, Law D, Birch M. Dihydroorotate dehydrogenase inhibitor F901318 has potent *in vitro* activity against *Scedosporium* species and *Lomentospora prolificans*. *J Antimicrob Chemother*. 2017 Mar 15. doi: 10.1093/jac/dkx065. [Epub ahead of print].
160. Cimon B, Carrère J, Chazalotte JP, Vinatier F, Chabasse D, Bouchara JP. Chronic airway colonization by *Penicillium emersonii* in a patient with cystic fibrosis. *Med Mycol*. 1999; 37: 291–293.
161. Barton RC, Borman AM, Johnson EM et al. Isolation of the fungus *Geosmithia argillacea* in sputum of people with cystic fibrosis. *J Clin Microbiol*. 2010; 48: 2615–2617.
162. Giraud S, Pihet M, Razafimandimby B et al. *Geosmithia argillacea*: an emerging pathogen in patients with cystic fibrosis. *J Clin Microbiol*. 2010; 48: 2381–2386.
163. Marguet C, Favennec L, Matray O et al. Clinical and microbiological efficacy of micafungin on *Geosmithia argillacea* infection in a cystic fibrosis patient. *Med Mycol Case Rep*. 2012; 1: 79–81.
164. De Ravin SS, Challipalli M, Anderson V et al. *Geosmithia argillacea*: an emerging cause of invasive mycosis in human chronic granulomatous disease. *Clin Infect Dis*. 2011; 52: e136–e143.
165. Machouart M, Garcia-Hermoso D, Rivier A et al. Emergence of disseminated infections due to *Geosmithia argillacea* in patients with chronic granulomatous disease receiving long-term azole antifungal prophylaxis. *J Clin Microbiol*. 2011; 49: 1681–1683.
166. Doyon JB, Sutton DA, Theodore P et al. *Rasamsonia argillacea* pulmonary and aortic graft infection in an immune-competent patient. *J Clin Microbiol*. 2013; 51: 719–722.
167. Hong G, White M, Lechtzin N et al. Fatal disseminated *Rasamsonia* infection in cystic fibrosis post-lung transplantation. *J Cyst Fibros*. 2017; 16: e3–e7.
168. Steinmann J, Dittmer S, Houbraeken J, Buer J, Rath PM. *In vitro* activity of isavuconazole against *Rasamsonia* species. *Antimicrob Agents Chemother*. 2016; 60: 6890–6891.
169. Denning DW, Bromley MJ. Infectious disease : how to bolster the antifungal pipeline. *Science*. 2015; 347: 1414–1416.
170. Osheroov N, Kontoyiannis DP. The anti-*Aspergillus* drug pipeline: Is the glass half full or empty? *Med Mycol*. 2017; 55: 118–124.
171. Perfect JR. The antifungal pipeline: a reality check. *Nat Rev Drug Discov*. 2017; 16: 603–616.
172. Jones P, Binns D, Chang HY et al. InterProScan 5: genome-scale protein function classification. *Bioinformatics*. 2014; 30: 1236–40.

Appendix

List of the other members of the ECMM/ISHAM working group on *Fungal respiratory infections in Cystic Fibrosis (Fri-CF)*: Isabelle Accocéberry (Bordeaux, France), Marina Almeida (São Paulo, Brazil), Maiken Cawling Arendrup (Copenhagen, Denmark), Darius Armstrong-James (London, UK), Viviane Balloy (Paris, France), Caroline Baxter (Manchester, UK), Anne Beauvais (Paris, France), Eliane Billaud (Paris, France), Elisa Borghi (Milan, Italy), Andrew M. Borman (Bristol, UK), Kenneth Bruce (London, UK), Enrique J. Calderon (Seville, Spain), Alphonse Calenda (Angers, France), Rafael Cantón (Madrid, Spain), Emilie Cardot-Martin (Suresnes, France), Luiz Maiz Carro (Madrid, Spain), Sophie Cassaing (Toulouse, France), Sanjay Haresh Chotirmall (Singapore), Sylviane Chevrier (Rennes, France), Vanda Chrenkova (Prague, Czech Republic), Frédéric Dalle (Dijon, France), Eric Dannaoui (Paris, France), Isabelle Durand-Joly (Lille, France), Stuart Elborn (Belfast, UK), Odile Eloy (Le Chesnay, France), Zayre Erturan (Istanbul, Turkey), Judith Fillaux (Toulouse, France), Ersilia Vita Fiscarelli (Rome, Italy), Emilie Fréal (Lille, France), Vicente Friaza (Seville, Spain), Frédéric Gabriel (Bordeaux, France), Karianne Wiger Gammelsrud (Oslo, Norway), Jean-Pierre Gangneux (Rennes, France), Frédéric Grenouillet (Besançon, France), Nina Gunde-Cimerman (Ljubljana, Slovenia), Özge Güngör (Istanbul, Turkey), Gerhard Haase (Aachen, Germany), Axel Hamprecht (Cologne, Germany), Vladimir Havlicek (Prague, Czech Republic), Patrick Hickey (Bethesda, USA), Virginie Jubin (Bron, France), Nahid Kondori (Gothenburg, Sweden), Uroš Krivec (Ljubljana, Slovenia), Michaela Lackner (Innsbruck, Austria), Katrien Lagrou (Leuven, Belgium), Gérald Larcher (Angers, France), Yohann Le Govic (Angers, France), Patrick Lebecque (Brussels, Belgium), Marie Machouart (Vandoeuvre les Nancy, France), Graziana Manno (Genova, Italy), Agnès Marot (Angers, France), Tadeja Matos

(Ljubljana, Slovenia), Astrid Mayr (Innsbruck, Austria), Jacques Meis (Nijmegen, The Netherlands), Carlos E. Milla (Palo Alto, USA), Gordana Mircevska (Skopje, Republic of Macedonia), Maria Teresa Montagna (Bari, Italy), Florent Morio (Nantes, France), Klaus Leth Mortensen (Aarhus, Denmark), Frank-Michael Müller (Heidelberg, Germany), Perrine Parize (Pierre-Bénite, France), Ilma Aparecida Paschoal (São Paulo, Brasil), André Paugam (Paris, France), Elia Gomez G. de la Pedrosa (Madrid, Spain), Florence Persat (Lyon, France), Marc Pihet (Angers, France), Refika Hamutcu Ersu (Istanbul, Turkey), Gabriella

Ricciotti (Rome, Italy), Geraint Rogers (Adelaide, Australia), Emmanuel Roilides (Thessaloniki, Greece), Ewa Romanowska (Warsaw, Poland), Maria Simitsopoulou (Thessaloniki, Greece), Nicholas Simmonds (London, United Kingdom), Christopher Thornton (Exeter, UK), Kathrin Tintelnot (Berlin, Germany), Dominique Toubas (Reims, France), Krzysztof Ulfig (Szczecin, Poland), Laurie A. Whitaker (Burlington, USA), Gisele Yonezawa (São Paulo, Brasil), Jan-Bart Yntema (Nijmegen, The Netherlands), Sean Zhang (Baltimore, USA), Rachid Zouhair (Meknès, Morocco).