



# NEWSLETTER of the ISHAM Working Group on "Fungal respiratory infections in Cystic Fibrosis"

**Edition 1 August 2009.**

## INTRODUCTION

As most of you are already aware, the inaugural meeting of the ISHAM working group on Fungal Respiratory Infections in Cystic Fibrosis was held in Angers, France in June of this year. This highly successful meeting, which was organised by Jean-Philippe Bouchara, permitted many of the current members of the WG to familiarise themselves with the current research and clinical interests of the WG members and also to propose a number of ways in which the WG activities can move forward. During the general discussion at the end of the inaugural meeting, Jean-Philippe expressed a need for aid in running the working group, and contacted Françoise Symoens, from the Scientific Institute of Public Health in Brussels (Belgium) and myself (Andy Borman, HPA Mycology Reference Laboratory, Bristol, UK) to act as co-convenors.

The planned roles of the convenors will include the preparation of an annual report of the activities of the working group (indicating the collaborative works that have been initiated, the list of publications on the topic by members of the working group, the new members of the working group, and the organization of future meetings or special sessions in large congresses.

We also decided to prepare an official newsletter for the members of the working group. The format for the newsletter will be flexible from one edition to another, and is intended to provide up-to-date information relevant to the scientific, political and social activities of the working group.

This first edition has been compiled predominantly in response to the questionnaires completed by participants of the Angers meeting, and includes a list of the current members of the WG (with full contact details), the clinical/research interests stated by the participants and importantly, also a full list of collaborators/collaborations wanted with (where available) details of materials that the various collaborations require. Future editions will include requests for collaborators and materials, research projects and funding proposals which require partners, and updates on working group activities and progress. Material for inclusion in future newsletters (and remarks/corrections to any errors in the current newsletter) can be sent electronically to [Andy.Borman@uhBristol.nhs.uk](mailto:Andy.Borman@uhBristol.nhs.uk).

Finally, on behalf of all members of the working group, I would like to thank Jean-Philippe Bouchara for his exceptional past and continued efforts in initiating and continuing to organise this working group.

*Andy Borman*

*The following short text presenting our network and objectives has been prepared by Tom Coenye and Christopher Thornton. May we ask you to address this text to your national contact for the European Community. It's only if they are contacted by a large number of teams from several European countries that we will have a chance to see our research topic selected for a next call for proposal.*

**Proposal to include the topic “Fungal respiratory infections in cystic fibrosis : from basic research to improvement of patient management”  
in the next call of the Health theme within FP7**

## **1. Background**

Cystic fibrosis (CF) (also called “mucoviscidosis”) is the most common hereditary disease in Europe. It is an autosomal recessive hereditary disease caused by one or more mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. In Europe, the carrier frequency is appr. 1/25 and the frequency of the disease is appr. 1 in 2000 live births. Patients with CF suffer from a wide range of symptoms, most notably related to the respiratory and gastrointestinal tract. Over recent decades, considerable attention has been paid to (i) improved early diagnosis of CF, (ii) the prevention and treatment of bacterial respiratory infections (most notably these caused by *Pseudomonas aeruginosa*) and (iii) nutritional status of people with CF. Because of this, life expectancy of CF patients has improved dramatically.

## **2. Fungal respiratory tract infections in CF**

Although there have been significant improvements in terms of preventing and treating bacterial respiratory tract infections in people with CF, relatively little progress has been made with regards to respiratory tract infections caused by fungi. Colonization of the airways with fungi, facilitated by the frequent and prolonged use of antibiotics and by the use of corticosteroids, lead to chronic respiratory infections. As life expectancy of CF patients is increasing, due to increased standards of care, fungal infections are increasing in frequency. Historically, attention has been focused on infections with *Aspergillus fumigatus*, but more recent research indicates that a wide range of fungi can colonise the respiratory tract of patients with CF, including the *Scedosporium apiospermum* species complex, *Aspergillus terreus*, *Exophiala dermatitidis* and *Scedosporium prolificans*, and that these infections often lead to a deterioration of lung function and ultimately result in increased morbidity and mortality.

## **3. Proposed areas for future research within the FP7 framework**

Below, we have identified four areas of collaborative research that require urgent attention. We propose that these areas be considered for funding in the next call of the Health theme within FP7.

### *3.1. Improving diagnosis*

Many of the fungal species recovered from respiratory secretions of CF patients are not (or are only rarely) recovered from clinical samples and appropriate tools for their rapid detection and identification are lacking. These tools include appropriate selective media and culture

conditions, novel PCR and antibody-based diagnostic tests and other culture-independent approaches for rapid detection.

### 3.2. Ecology and epidemiology

So far, little is known about the natural environmental reservoirs and the modes of acquisition of fungal pathogens in CF patients. Clearly, more research is needed to determine the source of infective propagules and their modes of transmission (environment-to-patient and potentially also patient-to-patient), to establish evidence-based guidelines for infection control.

### 3.3. Physiopathology of airway colonization

Very little is known about the interaction of fungal cells with the airway epithelium and/or their interaction with other micro-organisms (bacteria, *Candida albicans*). Similarly, much remains to be learned about the virulence factors of species like *Scedosporium apiospermum*/*Pseudallescheria boydii*.

### 3.4. Clinical aspects

Clearly, more (preferably large-scale) studies are required to determine the clinical impact of these infections on CF patients. In addition, systematic studies on antifungal susceptibility (both *in vitro* and *in vivo*) are required to address the question of treatment.

## 4. Established networks within Europe

Recently, the International Society for Human and Animal Mycology (ISHAM) has launched two working groups, one on “Fungal infections in CF” and one on “*Pseudallescheria/Scedosporium* infections”. Within the framework of these working groups, informal networks (mainly composed of European research groups) have evolved, addressing some of the topics mentioned above.

With sufficient funding, a large group of scientists could be assembled in order to address the problems described. To illustrate this point, we have included a list of research groups active in this field (see appendix). This is by no means a complete list and merely serves to show that the topic of “Fungal infections in cystic fibrosis” has attracted a lot of attention in the EU. Other stakeholders include the European Cystic Fibrosis Society (ECFS) and various national patient organisations e.g. Vaincre la mucoviscidose (FR), Cystic Fibrosis Trust (UK), Fondazione per la ricerca sulla fibrosi cistica (IT), Mukoviszidose eV (GE), Belgische Vereniging voor Strijd tegen mucoviscidose (BE) etc.

## 5. Contact

- Prof. Dr. Tom Coenye, Laboratory of Pharmaceutical Microbiology, Ghent University, Gent, Belgium (Tom.Coenye@ugent.be)
- Dr. Jean-Philippe Bouchara, Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Parasitologie-Mycologie, CHU, Angers, France (Jean-Philippe.Bouchara@univ-angers.fr)
- Dr. Christopher Thornton, Hybridoma Laboratory, School of Biosciences, University of Exeter, United Kingdom (C.R.Thornton@exeter.ac.uk)

## Appendix : European research groups active in this field

### Belgium

- Ghent University (Laboratory for Pharmaceutical Microbiology, Tom Coenye)
- Scientific Institute of Public Health (Mycology Section, Francoise Symoens)

- Catholic University of Leuven (UZ Department of Microbiology, Katrien Lagrou)
- Université Catholique de Louvain (Unité de Pneumologie Pédiatrique et Mucoviscidose, Patrick Lebecque)

#### Denmark

- Statens Serum Institut (Department of Bacteriology, Mycology and Parasitology, Maiken Cavling Arendrup)

#### France

- Institut Pasteur (Paris)  
Unité de Défense Innée et Inflammation (Vivianne Balloy)  
Unité des *Aspergillus* (Jean-Paul Latgé, Anne Beauvais)
- Host-Pathogen Interaction Study Group, UPRES-EA 3142, CHU d'Angers (Jean-Philippe Bouchara)
- Ecology of Parasitism, UPRES-EA 3609, CHRU de Lille (Laurence Delhaes)
- Laboratoire de Parasitologie-Mycologie, CHU Rangueil, Toulouse (Sophie Cassaing).
- Laboratoire de Parasitologie-Mycologie, UPRES-EA 4427, CHU de Rennes (Jean-Pierre Gangneux)
- Laboratoire de Parasitologie-Mycologie, AP-HM Timone, Marseille (Stéphane Ranque)

#### Greece

- University of Thessaloniki (3rd Dept Pediatrics Hippokration Hospital, Emmanuel Roilides, Maria Simitsopoulou)

#### Germany

- University of Heidelberg (Department of Pediatrics, Frank-Michael Müller)
- Robert Koch-Institut (FG 16 Mykologie, Kathrin Tintelnot)
- University Hospital RWTH Aachen (Institute of Medical Microbiology, Gerhard Haase)
- Federal Institute for Drugs and Medical Devices (Regine Horré)

#### Italy

- University of Genoa (Laboratory for Cystic Fibrosis Microbiology, Grazianno Manno)

#### Spain

- Instituto de Biomedicina de Sevilla (CIBER de Epidemiología y Salud Pública, Enrique J. Calderon Sandubete)
- Universitari la Fe (Unidad de Micología, Javier Péman, Amparo Solé)

#### The Netherlands

- Canisius Wilhelmina Hospital (Department of Medical Microbiology and Infectious Diseases, Corne Klaassen, Jacques Meis)

#### United Kingdom and Northern Ireland

- Health Protection Agency (Mycology Reference Laboratory, Andrew Borman)
- King's College London (Pharmaceutical Science Division, Kenneth Bruce, Geraint Rogers)
- Belfast City Hospital (Adult CF Centre, Stuart Elborn - John Moore)
- University of Exeter (Hybridoma Laboratory, Christopher Thornton)

## **RESULTS of QUESTIONNAIRES COMPLETED AT THE ANGERS MEETING**

### **Research/ Clinical interests of the working group members:**

#### **- Clinicians involved in the follow-up of paediatric patients**

Jacqueline Carrère – Laurent Mély (240 patients)  
Patrick Lebecque (84 patients)  
Jan-Bart Yntema (35 patients)  
Frank-Michael Müller (65 patients)  
Emmanuel Roilides (100 patients)  
Refika Hamutcu Ersu (180 patients)  
Carlos Milla (180 patients)  
Marina Almeida (130 patients)

#### **- Clinicians involved in the follow-up of adult patients**

Jacqueline Carrère – Laurent Mély  
Perrine Parize (230 patients)  
Sanjay Haresh Chotirmall (100 patients)  
Caroline Baxter  
Patrick Lebecque (85 patients)  
Enrique Calderone (100 patients)  
Luiz Maiz Carro (65 patients)  
Amparo Solé (150 patients)  
Carlos Milla (200 patients)  
Laurie Whittaker (70 patients)  
Ilma Paschoal – Gisele Yonezawa (90 patients)

#### **- Clinicians involved in lung transplantation**

Eliane Billaud (pharmacologist – 10 à 12 patients per year)  
Amparo Solé (6 per year – 82 CF patients among a total number of 366 lung transplant recipients)  
Carlos Milla (10 patients per year)

#### **- Mycologists involved in the follow-up of CF patients**

Jean-Philippe Bouchara – Marc Pihet (50 patients)  
Laurence Delhaes – Emilie Fréalle  
Isabelle Durand-Joly (50 patients)  
Loic Favennec (100 patients)  
Françoise Botterel  
André Paugam  
Frédéric Dalle  
Florence Persat  
Claudine Pinel  
Jacqueline Carrère  
Stéphane Ranque  
Sophie Cassaing

Frédéric Gabriel – Isabelle Accocébéry (40 patients)  
Jean-Pierre Gangneux – Sylviane Chevrier  
Katrien Lagrou (200 patients)  
Jacques F. Meis (60 patients)  
Maiken Cavling Arendrup  
Nahid Kondori  
Kathrin Tintelnot  
Regine Horré / Gunter Marklein (about 100 patients)  
Gerhard Haase (100 patients)  
Astrid Mayr  
Emmanuel Roilides (100 patients)  
Graziana Manno (250 patients)  
Gordana Mircevska  
Javier Peman (250 patients)

**- Scientists involved in basic research**

Jean-Philippe Bouchara – Sandrine Giraud – Alphonse Calenda – Sandrine Nail – Gérald Larcher : Ecology, epidemiology, diagnosis, molecular identification, genotype studies, virulence factors of *Scedosporium apiospermum s. l.*  
Isabelle Durand-Joly : *Pneumocystis* detection  
Anne Beauvais : *Aspergillus* biofilm  
Judith Fillaux : Epidemiology, infectious diseases  
Jean-Pierre Gangneux : Immunopathology, therapeutic options  
Andrew Borman : Taxonomy, diagnosis, identification, antifungal susceptibility  
Christopher Thornton : *Aspergillus*, *Pseudallescheria* and *Scedosporium* diagnostic  
Geraint Rogers – Kenneth Bruce : Profiling microbial communities in respiratory disease  
Françoise Symoens : Identification, genotyping, culture collection  
Tom Coenye – Lies Vanhee : Genotype studies, ecology of opportunistic fungi  
Patrick Lebecque : NPD measurements, curative treatment for the F508 mutation in mouse model and CF patients, lung inflammation in the CF mouse (BAL, Histology, IL, ...).  
Corne Klaassen: Molecular epidemiology, detection, identification  
Klaus Leth Mortensen : *Aspergillus* in CF patients – clinical significance and antifungal susceptibility  
Nahid Kondori  
Frank-Michael Müller : *In vitro* antifungal susceptibility, *Candida* and *Aspergillus* biofilms  
Kathrin Tintelnot : Ecology, epidemiology of *Scedosporium/Pseudallescheria* species  
Gerhard Haase : Taxonomy of black yeasts  
Regine Horré / Günter Marklein : detection, identification of *Exophiala* and *Scedosporium/Pseudallescheria* species.  
Krzysztof Ulfig : Ecology of opportunistic fungi  
Vladimir Havlicek : Characterization of *Pseudallescheria* and *Scedosporium* strains based on biomolecular profiles  
Maria Simitsopoulou : Immunopathology  
Enrique Calderon : Epidemiology, detection of *Pneumocystis jirovecii*  
Vicente Friaiza : Identification of microbial communities  
Rachid Zouhair : Ecology of *Scedosporium* species, genotype studies  
Wieland Meyer : Ecology, epidemiology, molecular identification, genotyping of *Scedosporium/Pseudallescheria* species, virulence study

**The following people agreed to be contacted for various projects:**

**For clinical surveillance (± epidemiological studies):**

Jean-Philippe Bouchara – Marc Pihet  
Laurence Delhaes  
Isabelle Durand-Joly  
Loïc Favennec  
André Paugam  
Jacqueline Carrère  
Judith Fillaux  
Sophie Cassaing  
Jean-Pierre Gangneux – Sylviane Chevrier  
Sanjay Chotirmall  
Andrew Borman  
Caroline Baxter  
Geraint Rogers – Kenneth Bruce  
Patrick Lebecque  
Katrien Lagrou  
Jacques Meis  
Jan-Bart Yntema  
Maiken Arendrup – Klaus Leth Mortensen  
Nahid Kondori  
Frank-Michael Müller  
Regine Horre  
Astrid Mayr  
Emmanuel Roilides  
Grazziana Manno  
Amparo Solé  
Enrique Calderon  
Luiz Maiz Carro  
Refika Ersu  
Carlos Milla  
Laurie Whittaker  
Ilma Paschoal – Gisele Yonezawa  
Marina Almeida  
Wieland Meyer

**For determination of genetic risk factors:**

Jean-Philippe Bouchara – Marc Pihet  
Laurence Delhaes  
André Paugam  
Frédéric Dalle  
Stéphane Ranque  
Judith Fillaux  
Andrew Borman  
Geraint Rogers – Kenneth Bruce  
Caroline Baxter

Patrick Lebecque  
Klaus Leth Mortensen  
Frank-Michael Müller  
Emmanuel Roilides  
Luiz Maiz Carro  
Carlos Milla  
Laurie Whittaker  
Wieland Meyer

**For evaluation of procedures for mycological examination of sputum samples:**

Jean-Philippe Bouchara – Marc Pihet  
Laurence Delhaes  
Isabelle Durand-Joly  
Loïc Favennec  
André Paugam  
Françoise Botterel  
Frédéric Dalle  
Claudine Pinel  
Jacqueline Carrère  
Stéphane Ranque  
Sophie Cassaing  
Frédéric Gabriel – Isabelle Accocéberry  
Jean-Pierre Gangneux – Sylviane Chevrier  
Andrew Borman  
Geraint Rogers – Kenneth Bruce  
Caroline Baxter  
Tom Coenye  
Patrick Lebecque  
Katrien Lagrou  
Jacques Meis  
Maiken Arendrup – Klaus Leth Mortensen  
Nahid Kondori  
Frank-Michael Müller  
Kathrin Tintelnot  
Gerhard Haase  
Regine Horré / Günter Marklein  
Astrid Mayr  
Gordana Mircevska  
Emmanuel Roilides  
Grazziana Manno  
Javier Peman  
Carlos Milla  
Laurie Whittaker  
Ilma Paschoal – Gisele Yonezawa  
Wieland Meyer

**For evaluation of molecular detection from clinical samples:**

Jean-Philippe Bouchara – Sandrine Giraud  
Laurence Delhaes  
Isabelle Durand-Joly

Françoise Botterel  
André Paugam  
Sophie Cassaing  
Stéphane Ranque  
Jean-Pierre Gangneux  
Andrew Borman  
Geraint Rogers – Kenneth Bruce  
Caroline Baxter  
Patrick Lebecque  
Katrien Lagrou  
Tom Coenye – Lies Vanhee  
Corne Klaasen  
Jacques Meis  
Maiken Arendrup – Klaus Leth Mortensen  
Frank-Michael Müller  
Gunter Marklein  
Kathrin Tintelnot  
Vladimir Havlicek  
Gordana Mircevska  
Emmanuel Roilides  
Grazziana Manno  
Enrique Calderon – Vicente Friaza  
Carlos Milla  
Laurie Whittaker  
Ilma Paschoal  
Wieland Meyer

**For evaluation of serological tests:**

Jean-Philippe Bouchara – Marc Pihet – Sandrine Nail  
Laurence Delhaes – Emilie Fréal  
Loïc Favennec  
André Paugam  
Florence Persat  
Claudine Pinel  
Sophie Cassaing  
Catherine Kauffmann-Lacroix  
Jean-Pierre Gangneux – Sylviane Chevrier  
Andrew Borman  
Geraint Rogers – Kenneth Bruce  
Caroline Baxter  
Patrick Lebecque  
Katrien Lagrou  
Maiken Arendrup  
Frank-Michael Müller  
Kathrin Tintelnot  
Gordana Mircevska  
Emmanuel Roilides  
Grazziana Manno  
Laurie Whittaker

**For environmental studies:**

Jean-Philippe Bouchara – Sandrine Giraud  
Laurence Delhaes – Emilie Fréalle  
Isabelle Durant-Joly  
André Paugam  
Claudine Pinel  
Judith Fillaux  
Jean-Pierre Gangneux  
Andrew Borman  
Christopher Thornton  
Geraint Rogers – Kenneth Bruce  
Tom Coenye – Lies Vanhee  
Patrick Lebecque  
Jan-Bart Yntema  
Maiken Arendrup – Klaus Leth Mortensen  
Nahid Kondori  
Frank-Michael Müller  
Kathrin Tintelnot  
Regine Horré  
Astrid Mayr  
Krzysztof Ulfig  
Emmanuel Roilides  
Enrique Calderon – Vicente Friaza  
Laurie Whittaker  
Ilma Paschoal – Gisele Yonezawa  
Wieland Meyer

**For genotype studies on fungal isolates:**

Jean-Philippe Bouchara  
Laurence Delhaes  
André Paugam  
Françoise Botterel  
Stéphane Ranque  
Sophie Cassaing  
Frédéric Gabriel – Isabelle Accocéberry  
Andrew Borman  
Geraint Rogers – Kenneth Bruce  
Caroline Baxter  
Tom Coenye – Lies Vanhee  
Patrick Lebecque  
Françoise Symoens  
Jacques Meis – Corne Klaassen  
Klaus Leth Mortensen  
Frank-Michael Müller  
Kathrin Tintelnot  
Luiz Maiz Carro  
Grazziana Manno  
Enrique Calderon – Vicente Friaza  
Laurie Whittaker  
Wieland Meyer

**The following members are searching for collaborators/partners/materials:**

- Vladimir Havlicek : Evaluation of in-house developed ELISA kits for serodiagnosis of Pseudallescheriasis/scedosporiosis (needs sera from patients with proven pseudallescheriasis/scedosporiosis).
  - Christopher Thornton would be interested in collaborating with any member of the group who is interested in detecting *P. boydii/Sc. apiospermum* and *Aspergillus* species in sputum samples using MAb-based diagnostics. He would need sputum samples from CF patients who had colonisation previously proven by plate culture or any other diagnostic technique. Control samples from known un-colonised patients would also be useful. He needs approximately 400 microlitres of fluid, preferably stored at -20°C and with no additional treatment.
  - Enrique Calderon and Gisele Yonezawa would be interested in the evaluation of the prevalence of *P. jirovecii* in CF and the clinical relevance of its detection. ***A detailed research proposal for a multicentre study on Pneumocystis jirovecii colonization in Cystic Fibrosis is included below.***
  - Ilma Paschoal would be interested in similarities and differences in fungal respiratory infections between CF lung disease and non-CF bronchiectasis.
  - Frank-Michael Müller would be interested in the evaluation of new antifungal therapy in CF patients (Pk, PD studies). Similar questions were raised by Jean-Pierre Gangneux and Eliane Billaud (how and when to treat, duration of treatment).
  - Grazziana Manno, Gordana Mircevska and Maria Simitsopoulou would be interested in the evaluation of antifungal susceptibility on clinical isolates, or are searching partners to do this.
  - Klaus Leth Mortensen is searching partners for studies on antifungal resistance mechanisms.
  - Gunter Marklein is interested in rapid and accurate determination of clinical yeasts and moulds by MALDI-TOF-MS
  - Maria Simisopoulou is interested in the host immune response against clinical isolates.
  - Patrick Lebecque with T. Leal have developed mouse models of chronic respiratory infections (in CF mice).
  - Françoise Botterel proposes a study of the interactions between bacteria and fungi on the airway epithelium.
  - Wieland Meyer proposes his help for molecular identification within the *Scedosporium apiospermum* complex.
  - Loïc Favennec is interested to in the evaluation of antifungal susceptibilities of clinical isolates.
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**Project submitted to the ISHAM Working Group on Fungal infections in Cystic Fibrosis :**

**Multicentre study on *Pneumocystis jirovecii* colonization in Cystic Fibrosis**

**Enrique J. Calderon**

Instituto de Biomedicina de Sevilla, and CIBER de Epidemiología y Salud Pública. Servicio de Medicina Interna, Virgen del Rocío University Hospital, Seville, Spain.

Telephone number: +34 955 01 3284,

FAX number: +34 955 01 3292;

e-mail: [ecalderon@ibis-sevilla.es](mailto:ecalderon@ibis-sevilla.es)

**Concept and objectives**

*Pneumocystis jirovecii* (human-derived *Pneumocystis*) is an atypical opportunistic fungus with lung tropism and worldwide distribution that causes pneumonia in immunosuppressed individuals. Basic research on *Pneumocystis* has been hampered by the lack of a reliable in vitro culture system; nevertheless, through the use of molecular techniques and experimental models, progress has been made over the last decades in our understanding of the epidemiological and clinical features of the infection (Calderon, Protist 2002; 153, 303–310).

*Pneumocystis* colonization (that is, detection of the organism or its DNA, without signs or symptoms of pneumonia) has recently been described, and accumulating evidence suggests that it may be an important clinical phenomenon (Morris, J Infect Dis 2008; 197:10-7). Because of the absence of *in vitro* culture system, sensitive molecular techniques such as polymerase chain reaction need to be used to identify *Pneumocystis* colonization. Low levels of *Pneumocystis* in the lungs may stimulate pulmonary inflammation and may play a role in the development of lung diseases such as chronic obstructive pulmonary disease. In this sense, one study in human has showed an association between *Pneumocystis* colonization and severity of airflow obstruction in smokers and other recent study shows that patients colonized by *Pneumocystis* have higher pro-inflammatory cytokines levels than non-colonized patients (Morris, Am J Respir Crit Care Med 2004; 170: 408-413; Calderon, Clin Infec Dis 2007; 45: e17-9).

Only a few studies carried out in Europe have evaluated the prevalence of *Pneumocystis* colonization in patients with Cystic Fibrosis (CF), reporting ranges from 7.4% to 22% (Sing, J. Clin. Microbiol.2001; 39: 2717-2718; Respaldiza, Clin. Microbiol. Infect 2005;11:1012-1015), but there are not data about the clinical significance of *Pneumocystis* colonization in Cystic Fibrosis patients. Until recently, the high prevalence of *Pneumocystis jirovecii* colonization among CF patients was unknown. However, it has been suggested that *P. jirovecii* could be involved in the progression of CF by means of its capacity to induce alveolar macrophage activation, proinflammatory interleukin elevation, changes in pulmonary surfactant during very early stages of the infection and possible interactions with other microorganisms.

### General objective:

The main goal of this project is to provide new knowledge on the epidemiology and the clinical impact of *P. jirovecii* colonization in Cystic Fibrosis patients.

### Goals of the research

- 1) To improve our knowledge of the epidemiology of colonization by *P.jirovecii* in CF patients.
- 2) To explore potential correlation between prevalence rates of *P. jirovecii* colonization and geographic, meteorological or other environmental factors.
- 3) To determine if there is a correlation between prevalence of *P. jirovecii* colonization and CF severity
- 4) To determine if prevalence of the *Pneumocystis* colonization is conditioned by host genetic factors
- 5) To improve our knowledge on the potential interactions between *P. jirovecii* and pathogenic microorganisms in the ecological niche of CF patients

### **Brief study design**

This project is the first international multicentre study on *Pneumocystis jirovecii* colonization in CF patients, in which basic and clinical researches will be combined in order to assess its importance for pathogenesis of this disease. It is designed so that following an initial epidemiological study in which all the groups participate, providing information on the real dimensions of the problem, a series of studies may be developed for an in-depth analysis of specific aspects of the colonization in CF subjects. Ideally, each group should include between 30 and 60 cases.

### Study population

#### *Inclusion Criteria*

- Male or female aged > 5 years
- Legally effective consent available
- Well-established diagnosis of Cystic fibrosis

Cystic fibrosis is diagnosed if a patient has a sweat chloride concentration > 60 mEq/l and chronic pulmonary disease of an appropriate character (obstructive pulmonary disease with bacterial infection) or pancreatic insufficiency or both, or a history of CF in a sibling or first cousin

All the subjects included in the study (or their parents) will be informed of its content and asked to give their informed consent. All of them will provide epidemiological and clinical data, as well as biological samples (sputum and serum) which will be properly preserved for the later development of specific studies.

The epidemiological and clinical data of the patients included in the study will be recorded on previously prepared Case report form (CRF) as well as in a local computerized register. All the epidemiological and clinical data of the subjects included in the study will be handled at all times confidentially.

The following samples will be obtained from each subject included in the study: two sputum samples and 10 ml of serum sample. The samples will be handled according to all universally recommended safety norms. In each case, one sample of sputum will be used for the local microbiological diagnosis and the rest of the samples properly preserved for the subsequent specific studies. Serum samples and DNA extracted from sputum samples will be send to Instituto de Biomedicina de Sevilla for studying *P. jirovecii* colonization and specific anti-*Pneumocystis* antibodies.

*P. jirovecii* colonization will be detected by a nested-PCR assay that amplifies a portion of the gene encoding the mitochondrial large-subunit (mtLSU) rRNA. Genotyping of isolates will be done at two independent gen loci: mtLSU rRNA fragment (assessed by direct sequencing) and dihydropteroate synthase (assessed by restriction fragment-length polymorphism). Serum samples will be examined for antibodies against *Pneumocystis* by immunoblotting.

#### Methods for Extraction of DNA

Divide each sputum sample into aliquots, each of approximately 500 µl volume.

Label each sample with patient code, date of collection of sample and aliquot number.

Use disposable plastic-ware throughout the procedure. Use sterile racked hydrophobic filter tips for micropipettes (for example Laser, catalogue number LATF-20-R-S). Take all necessary precautions to avoid cross contamination between samples.

Stock solutions of reagent (EDTA, SDS, proteinase K, H<sub>2</sub>O) should be divided into small volumes in microfuge tubes. For each experiment, a new tube should be used and then discarded.

1. Thaw 1 aliquot of sample to be tested, approximate volume 500 µl.
2. Add EDTA (ethylenediaminetetraacetic acid), pH8.0, to a final concentration of 10mM (for example: 10 µl of 0.5M EDTA, pH8.0, to 500 µl sample).  
EDTA: Sigma, catalogue number E7889, 0.5M solution, store at room temperature.
3. Add SDS (sodium dodecyl sulphate) to a final concentration of 0.5% (v/v) (for example: 25µl of 10% SDS to 500 µl sample).  
SDS: Sigma, catalogue number L4522, 10% solution, store at room temperature.
4. Add proteinase K to a final concentration of 1mg/ml (for example add 20 µl of proteinase K (14mg/ml) to 500 µl sample).  
Proteinase K: Roche, catalogue number 1-373-196, stock solution at 14-22 mg/ml. Store at 4°C.
5. Incubate sample at 56°C. until complete lysis is obtained (at least 4 h). Vortex occasionally during incubation or use a shaking incubator. (Samples can be incubated overnight as well).  
minimum : 4 hours (the sample must be completely fluid and homogeneous)  
maximum: overnight incubation

After PK treatment, DNA extraction can be done (preferable) or sample can be frozen at -20°C up to DNA extraction

DNA extraction can be done according to the protocol from Macherey -Nagel (GMBH & Co, Cat Number 740 952):

Briefly,

1. Add 200ul Buffer B3
2. Add 210ul Ethanol
3. For each sample, place one column into a collection tube.
4. Apply 750 ml of treated sample, and centrifuge for 1 min at 11000 g.

5. Discard flow-through and place the column back into the collection tube.
6. Wash silica membrane with 500 ml BW. and centrifuge for 1 min at 11000 g.
7. Discard flow-through and place the column back into the collection tube.
8. Wash silica membrane with 600 ml Buffer 5, and centrifuge for 1 min at 11000 g.
9. Discard flow-through and place the column into a new the collection tube.
10. Dry silica membrane: Centrifuge the colum 2 min at 11000 g.
11. To elute pure DNA: Place the column into a clean 1.5 ml eppendorf and add 55 ml highly pure water.
12. Incubate at room temperature for at least 1 min. Centrifuge 1 min at 11000 g.
13. The DNA must be stored at  $-20^{\circ}\text{C}$ .

**Those interested in participating in this joint project could contact Enrique by e-mail ([ecalderon@ibis-sevilla.es](mailto:ecalderon@ibis-sevilla.es)) for further details**

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## Current members of the working group

- \* *Dr. Isabelle Accocéberry* (UMR - CNRS 5162, Université Victor Ségalen Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux Cedex. Phone: 33-(0)5-56 79 58 37; Télécopie : 33-(0)5-56 79 58 79; E-mail: [isabelle.accoceberry@chu-bordeaux.fr](mailto:isabelle.accoceberry@chu-bordeaux.fr)) - Clinical surveillance, biological diagnosis.
- \* *Pr. Marina Almeida* (Faculdade de Medicina da Universidade de São Paulo (FMUSP), Rua Martim Francisco, 748 casa 2, CEP 01226-000, São Paulo, SP, Brazil. Phone: 55-11-3666 4678; Fax: 55-11-3032 5226; E-mail: [m.buarque@terra.com.br](mailto:m.buarque@terra.com.br)) – Clinical surveillance.
- \* *Pr. Maiken Cawling Arendrup* (Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Building 43/117, DK-2300 Copenhagen, Denmark. Phone: 45-32-68 32 23; Fax: 45-32-68 81 80; E-mail: [mad@ssi.dk](mailto:mad@ssi.dk)) - Epidemiology, Biological diagnosis, environmental studies.
- \* *Dr. Viviane Balloy* (Unité de Défense Innée et Inflammation, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France. Phone: 33-(0)1-40 61 32 02; Fax: 33-(0)1-45 68 87 03; E-mail: [vballoy@pasteur.fr](mailto:vballoy@pasteur.fr)) - Physiopathology.
- \* *Dr. Caroline Baxter* (Manchester Adult Cystic Fibrosis Unit, Wythenshawe Hospital, Southmoor Road, Manchester, M23 9LT, United Kingdom. Phone: 44-161-291 2046; Fax: 44-161-291 2080; E-mail: [cbaxter@doctors.org.uk](mailto:cbaxter@doctors.org.uk)) - Genotype studies.
- \* *Dr. Anne Beauvais* (Unité des *Aspergillus*, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France. Phone: 33-(0)1-45 68 82 25 / 33-(0)1-40 61 34 51; Fax: 33-(0)1-40 61 34 19; E-mail: [abeauvai@pasteur.fr](mailto:abeauvai@pasteur.fr)) - Physiopathology.
- \* *Pr. Eliane Billaud* (Laboratoire de Pharmacologie-Toxicologie, HEGP, 20 rue Leblanc, 75908 Paris Cedex 15, France. Phone: 33-(0)1-56 09 39 45; Fax: 33-(0)1-56 09 39 92; E-mail: [eliane.billaud@egp.ap-hop-paris.fr](mailto:eliane.billaud@egp.ap-hop-paris.fr)) - Pharmacology.
- \* *Dr. Andrew M. Borman* (Mycology Reference Laboratory, Health Protection Agency, South-West regional Laboratory, Myrtle Road, Bristol BS2 8EL, United Kingdom. Phone: 44-117-926 8683; Fax: 44-117-922 6611; E-mail: [Andy.Borman@uhBristol.nhs.uk](mailto:Andy.Borman@uhBristol.nhs.uk)) - Clinical surveillance.
- \* *Dr. Françoise Botterel* (Laboratoire de Parasitologie-Mycologie, CHU Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil cedex, France . Phone: 33 (0) 149 81 35 91; Fax: 33 (0) 149 81 36 01; E-mail: [botterel@univ-paris12.fr](mailto:botterel@univ-paris12.fr)) - Physiopathology.
- \* *Dr. Jean-Philippe Bouchara* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Parasitologie-Mycologie, CHU, 4 rue Larrey, F-49933 Angers cedex 9, France. Phone: 33-(0)2-41 35 34 72; Fax: 33-(0)2-41 35 36 16; E-mail: [Jean-Philippe.Bouchara@univ-angers.fr](mailto:Jean-Philippe.Bouchara@univ-angers.fr)) - Clinical surveillance, biological diagnosis, physiopathology, epidemiological and environmental studies.
- \* *Dr. Kenneth Bruce* (King's College London, Pharmaceutical Science Division, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NH, UK. Phone: 44-(0)207 848 4670; Fax: 44-(0)207 848 4500; E-mail: [kenneth.bruce@kcl.ac.uk](mailto:kenneth.bruce@kcl.ac.uk)) – Clinical surveillance.

- \* *Pr. Enrique J. Calderon* (Instituto de Biomedicina de Sevilla (IBIS). CIBER de Epidemiología y Salud Pública (CIBERESP). Servicio de Medicina Interna. Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot s/n, 41013 Seville, Spain. Phone: 34-955 01 3284; Fax: 34-955 01 3292; E-mail: [ecalderon@ibis-sevilla.es](mailto:ecalderon@ibis-sevilla.es)) – Biological diagnosis, physiopathology.
- \* *Pr. Alphonse Calenda* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, UFR Sciences Pharmaceutiques et Ingénierie de la Santé, 16 Bd Daviers, F-49045 Angers cedex, France. Phone: 33-(0)2-41 22 67 22; E-mail: [Alphonse.Calenda@univ-angers.fr](mailto:Alphonse.Calenda@univ-angers.fr)) - Physiopathology and treatment, environmental studies.
- \* *Dr. Jacqueline Carrère* (Laboratoire de Biologie, Hôpital Renée Sabran, Giens. Phone: 33-(0)4-94 38 17 80; Fax: 33-(0)4-94 38 17 72; E-mail: [jacqueline.carrere@chu-lyon.fr](mailto:jacqueline.carrere@chu-lyon.fr)) - Clinical surveillance.
- \* *Pr. Luiz Maiz Carro* (Unidad de Fibrosis Quística, Hospital Ramón y Cajal, 28034 Madrid, Spain. Phone: 34-91-336 80 90; Fax: 34-91-336 84 17; E-mail: [lmaiz.hrc@salud.madrid.org](mailto:lmaiz.hrc@salud.madrid.org)) - Clinical surveillance, genetic risk factors, genotype studies.
- \* *Dr. Sophie Cassaing* (Laboratoire de Parasitologie-Mycologie, CHU Rangueil, 1 Avenue Jean Poulhès, TSA 50032, 31095 Toulouse cedex. Phone: 33-(0)5-61 32 32 09; Fax: 33-(0)5-61 32 20 96; E-mail: [cassaing.s@chu-toulouse.fr](mailto:cassaing.s@chu-toulouse.fr)) - Clinical surveillance, physiopathology.
- \* *Pr. Dominique Chabasse* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Parasitologie-Mycologie, CHU, 4 rue Larrey, F-49933 Angers cedex 9, France. Phone: 33-(0)2-41 35 34 72; Fax: 33-(0)2-41 35 36 16; E-mail: [DoChabasse@chu-angers.fr](mailto:DoChabasse@chu-angers.fr)) - Clinical surveillance, biological diagnosis.
- \* *Pr. Tsung Chain Chang* (Department of Medical Laboratory Science and Biotechnology, School of Medicine, National Cheng Kung University, 1 University Road, Tainan 701, Taiwan, ROC. Phone: 886-6-2353535 ext. 5790; Fax: 886-6-2363956, E-mail: [tsungcha@mail.ncku.edu.tw](mailto:tsungcha@mail.ncku.edu.tw)) – Biological diagnosis.
- \* *Dr. Sanjay Haresh Chotirmall* (Respiratory Research Division, Education & Research Centre, Beaumont Hospital, Beaumont Road, Dublin 9, Republic of Ireland. E-mail: [schotirmall@rcsi.ie](mailto:schotirmall@rcsi.ie)) - Clinical surveillance.
- \* *Dr. Sylviane Chevrier* (Laboratoire de Parasitologie-Mycologie, Faculté de Médecine, 2 rue du Pr Léon Bernard, 35043 Rennes Cedex, France. Phone: 33-(0)2-23 23 44 90; Fax: 33-(0)2-23 23 46 29; E-mail: [sylviane.chevrier@chu-rennes.fr](mailto:sylviane.chevrier@chu-rennes.fr)) - Clinical surveillance, biological diagnosis, treatment.
- \* *Dr. Bernard Cimon* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Parasitologie-Mycologie, CHU, 4 rue Larrey, F-49933 Angers cedex 9, France. Phone: 33-(0)2-41 35 34 72; Fax: 33-(0)2-41 35 36 16; E-mail: [BeCimon@univ-angers.fr](mailto:BeCimon@univ-angers.fr)) - Clinical surveillance, biological diagnosis.
- \* *Pr. Tom Coenye* (Laboratorium voor Farmaceutische Microbiologie, Universiteit Ghent, Harelbekestraat 72, 9000 Gent, Belgium. Phone: 32-9-2648141; Fax: 32-9-26458091; E-mail: [tom.coenye@ugent.be](mailto:tom.coenye@ugent.be)) - Genotype studies.
- \* *Dr. Frédéric Dalle* (UPRES-EA 562 - Laboratoire de Parasitologie-Mycologie, Laboratoire de Microbiologie Médicale et Moléculaire, Hôpital du Bocage, BP 77908, 21079 Dijon Cedex. Phone: 33-(0)3-80 29 50 14; Fax: 33-(0)3-80 29 32 80; E-mail: [frederic.dalle@chu-dijon.fr](mailto:frederic.dalle@chu-dijon.fr)) - Clinical surveillance, biological diagnosis.

- \* *Dr. Laurence Delhaes* (Ecology of Parasitism, UPRES-EA 3609 - Laboratoire de Parasitologie-Mycologie, Centre de Biologie Pathologie, CHRU de Lille, Boulevard du Pr. J. Leclercq, F-59037 Lille Cedex, France. Phone: 33-(0)3-20 44 59 62; Fax : 33-(0)3-20 44 42 64; E-mail: [l-delhaes@chru-lille.fr](mailto:l-delhaes@chru-lille.fr)) - Clinical surveillance, biological diagnosis, physiopathology and treatment, environmental studies.
- \* *Dr. Isabelle Durand-Joly* (Ecology of Parasitism, UPRES-EA 3609, Centre Hospitalier de Dunkerque, Service d'Hygiène Hospitalière et Département de Microbiologie, France. Phone: 33-(0)3-28 28 59 17; Fax: 33-(0)3-28 28 54 41; E-mail: [isabelle.joly@ch-dunkerque.fr](mailto:isabelle.joly@ch-dunkerque.fr)) - Clinical surveillance, biological diagnosis, physiopathology and treatment, environmental studies.
- \* *Pr. Stuart Elborn* (Belfast City Hospital, Adult CF Centre, Ground Floor, Lisburn Rd, Belfast BT9 7AB, Northern Ireland, UK. Phone: 44-28-9026 3683; Fax: 44-28-9026 3546; E-mail: [stuart.elborn@belfasttrust.hscni.net](mailto:stuart.elborn@belfasttrust.hscni.net)) – Clinical surveillance, biological diagnosis.
- \* *Pr. Loïc Favennec* (Laboratoire de Parasitologie Mycologie, ADEN-EA 3234, CHU Charles Nicolle, 76031 Rouen. Phone: 33-(0)2-32 88 66 39; Fax: 33-(0)2-32 88 68 75; E-mail: [Loic.Favennec@chu-rouen.fr](mailto:Loic.Favennec@chu-rouen.fr)) - Clinical surveillance, biological diagnosis.
- \* *Dr. Judith Fillaux* (Laboratoire de Parasitologie-Mycologie, CHU Rangueil, 1 Avenue Jean Poulhès, TSA 50032, 31095 Toulouse cedex. Phone: 33-(0)5-61 32 32 05; Fax: 33-(0)5-61 32 20 96; E-mail: [fillaux@cict.fr](mailto:fillaux@cict.fr)) - Clinical surveillance, physiopathology.
- \* *Dr. Emilie Fréalle* (Ecology of Parasitism, UPRES-EA 3609 - Laboratoire de Parasitologie-Mycologie, Centre de Biologie Pathologie, CHRU de Lille, Boulevard du Pr. J. Leclercq, F-59037 Lille Cedex, France. Phone: 33-(0)3-20 44 59 62; Fax : 33-(0)3-20 44 42 64; E-mail: [emilie.frealle@pasteur-lille.fr](mailto:emilie.frealle@pasteur-lille.fr)) - Clinical surveillance, biological diagnosis, physiopathology and treatment, environmental studies.
- \* *Dr. Vicente Friaiza* (Instituto de Biomedicina de Sevilla (IBIS), CIBER de Epidemiología y Salud Pública (CIBERESP), Servicio de Medicina Interna, Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot s/n, 41013 Seville, Spain. E-mail: [vfriaiza@hotmail.com](mailto:vfriaiza@hotmail.com)) – Clinical surveillance, biological diagnosis.
- \* *Dr. Frédéric Gabriel* (Laboratoire de Parasitologie-Mycologie, CHU de Bordeaux - Hôpital Saint André, 1 rue Jean Burguet, 33076 Bordeaux Cedex. Phone: 33-(0)5-56 79 58 37 / 58 39; Fax : 33-(0)5-56 79 58 79; E-mail: [fredgab@yahoo.fr](mailto:fredgab@yahoo.fr)) - Clinical surveillance, genotype studies.
- \* *Pr. Jean-Pierre Gangneux* (Laboratoire de Parasitologie-Mycologie, UPRES-EA 4427, Faculté de Médecine, 2 rue du Pr Léon Bernard, 35043 Rennes Cedex, France. Phone: 33-(0)2-23 23 44 90; Fax: 33-(0)2-23 23 46 29; E-mail: [jean-pierre.gangneux@univ-rennes1.fr](mailto:jean-pierre.gangneux@univ-rennes1.fr) or [jean-pierre.gangneux@chu-rennes.fr](mailto:jean-pierre.gangneux@chu-rennes.fr)) – Clinical surveillance, biological diagnosis, treatment.
- \* *Dr. Sandrine Giraud* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Batiment Montclair, CHU, 4 rue Larrey, F-49933 Angers cedex 9, France. Phone: 33-(0)2-41 53 36 37; E-mail: [Sandrine.giraud@univ-angers.fr](mailto:Sandrine.giraud@univ-angers.fr)) - Physiopathology and treatment.
- \* *Pr. Gerhard Haase* (Institute of Medical Microbiology, University Hospital, RWTH Aachen, Pauwelsstr. 30, 52074 Aachen, Germany. Phone: 49-241 8089515 or 49-241 8089510; Fax: 49-241 80 33 89515; E-mail: [ghaase@ukaachen.de](mailto:ghaase@ukaachen.de)). Clinical surveillance, biological diagnosis.

- \* *Pr. Vladimír Havlicek* (Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic. Phone: 42-02-41062786; Fax: 42-02-41062749; E-mail: [vlhavlic@biomed.cas.cz](mailto:vlhavlic@biomed.cas.cz) ; <http://ms.biomed.cas.cz>) - Biological diagnosis, physiopathology and treatment.
- \* *Dr Francisca Hernandez* (Laboratorio de Micología Médica, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, C.P. 04510, México, D. F., México. e-mail : [micoher@hotmail.com](mailto:micoher@hotmail.com)) – Diagnosis.
- \* *Dr. Regine Horré* (Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger-Allee 3, D-53175 Bonn, Germany. Phone: 49-228-207 3267; E-mail: [regine.horre@gmx.de](mailto:regine.horre@gmx.de))– Clinical surveillance, Diagnosis.
- \* *Dr. Corne H.W. Klaassen* (Department of Medical Microbiology and Infectious Diseases, C70 Canisius Wilhelmina Hospital, Weg door Jonkerbos 100, 6532 SZ Nijmegen, The Netherlands. Phone: 31-24-3658677; Fax: 31-24-3657516; E-mail: [c.klaassen@cwz.nl](mailto:c.klaassen@cwz.nl)) – Genotype studies.
- \* *Dr. Nahid Kondori* (Sahlgrenska University Hospital, Department of Clinical Bacteriology, Box 7193 402 34 Göteborg, Sweden. Phone: 46-31-342 4226/ 342 4650; Fax: +46-31-342 4975; E-mail: [nahid.kondori@microbio.gu.se](mailto:nahid.kondori@microbio.gu.se)) - Clinical surveillance, biological studies, environmental studies.
- \* *Pr. Katrien Lagrou* (University Hospital, Department of Microbiology, UZ Gasthuisberg, Dienst Laboratoriumgeneeskunde, Herestraat 49, Leuven 3000, Belgium. Phone: 32-16-34 70 98; Fax: 32-16-34 79 31; E-mail: [katrien.lagrou@uz.kuleuven.ac.be](mailto:katrien.lagrou@uz.kuleuven.ac.be)) - Clinical surveillance, biological diagnosis.
- \* *Dr. Gérald Larcher* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Biochimie, UFR Sciences Pharmaceutiques et Ingénierie de la Santé, 16 Bd Daviers, F-49045 Angers cedex, France. Phone: 33-(0)2-41 22 66 00 / 67 41; Fax: 33-(0)2-41 48 67 33; E-mail: [Gerald.Larcher@univ-angers.fr](mailto:Gerald.Larcher@univ-angers.fr)) - Physiopathology and treatment, environmental studies.
- \* *Pr. Patrick Lebecque* (Unité de Pneumologie Pédiatrique et mucoviscidose, Cliniques Universitaires Saint-Luc - Université Catholique de Louvain, 10 Avenue Hippocrate, 1200 Woluwe, Brussels, Belgium. Phone: 32-2-764 1939; Fax: 32-8-764 8906; E-mail: [patrick\\_lebecque@hotmail.com](mailto:patrick_lebecque@hotmail.com) or [Patrick.Lebecque@uclouvain.be](mailto:Patrick.Lebecque@uclouvain.be)) – Clinical surveillance, physiopathology.
- \* *Dr. Graziana Manno* (Laboratory for Cystic Fibrosis Microbiology, Department of Pediatrics, University of Genova, G. Gaslini Children's Hospital, Largo G. Gaslini 516147 Genova, Italy. Phone: 39-0105636290; Fax: 39-0103773210; E-mail: [Graziana@unige.it](mailto:Graziana@unige.it) or [graziana.manno@alice.it](mailto:graziana.manno@alice.it)) - Clinical surveillance, biological diagnosis.
- \* *Dr. Günter Marklein* (Institute for Medical Microbiology, Immunology and Parasitology, University of Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany. Phone: 49-228-2871 5536; Fax: 49-228-2871 9185; E-mail: [marklein@mibi03.meb.uni-bonn.de](mailto:marklein@mibi03.meb.uni-bonn.de)) - Biological diagnosis.
- \* *Dr. Astrid Mayr* (Department for Hygiene, Microbiology and Social Medicine, Hospital Hygiene/Mycology, Schöpfstraße 41, Medical university of Innsbruck, A-6020 Innsbruck. Phone: 43-(0)512-9003/70727; Fax: 43-(0)512-9003/73700; E-mail: [astrid.mayr@i-med.ac.at](mailto:astrid.mayr@i-med.ac.at)) – Clinical surveillance, biological diagnosis.

- \* *Pr. Jacques Meis* (Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, PO Box 9015, 6500 GS Nijmegen, The Netherlands. Phone: 31-24 365 7514; Fax: 31-24 365 7516; E-mail: [j.meis@cwz.nl](mailto:j.meis@cwz.nl)) - Genotype studies.
- \* *Pr. Wieland Meyer* (Western Clinical School, University of Sydney, Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, ICPMR, Level 3, Room 3114A, Darcy Road, Westmead Hospital Westmead, NSW 2145, Australia. Phone: 61-2-9845 6895; Fax: 61-2-9891 5317, E-mail: [w.meyer@usyd.edu.au](mailto:w.meyer@usyd.edu.au)) - Clinical surveillance, epidemiological studies.
- \* *Dr. Carlos E. Milla* (Center for Excellence in Pulmonary Biology, Department of Pediatrics, Stanford University, 770 Welch Rd.; Suite 350, MC 5882, Palo Alto, CA 94304, USA. Phone: 650-723-5191; Fax: 650-723-5201; E-Mail: [cmilla@stanford.edu](mailto:cmilla@stanford.edu)) – Clinical surveillance.
- \* *Dr. Gordana Mircevska* (Sole Stojcev 2 I/13, 1000 Skopje, Republic of Macedonia. Phone/fax : 38970221902; E-mail: [gordmir@yahoo.com](mailto:gordmir@yahoo.com)) - Biological diagnosis.
- \* *Dr. Klaus Leth Mortensen* (Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Mycology Unit Building 43/116, Artillerivej 5, DK-2300 Copenhagen S, Denmark. Phone: 45-32-68 85 22; Fax: 45-32-68 38 73; E-mail: [klm@ssi.dk](mailto:klm@ssi.dk)) - Epidemiology, Biological diagnosis.
- \* *Pr. Frank-Michael Müller* (Zentrum für Kinder- u. Jugendmedizin III, Päd. Pneumologie, Allergologie, Mukoviszidose & spez. Infektiologie, Im Neuenheimer Feld 430, D-69120 Heidelberg, Germany. Phone: 49-6221-56-8345 (or 49-6221-56-7273); Fax: 49-6221-56-33853; E-mail: [Frank-Michael\\_Mueller@med.uni-heidelberg.de](mailto:Frank-Michael_Mueller@med.uni-heidelberg.de)) - clinical surveillance.
- \* *Dr. Sandrine Nail-Billaud* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Parasitologie-Mycologie, UFR Sciences Pharmaceutiques et Ingénierie de la Santé, 16 Bd Daviers, F-49045 Angers cedex, France. Phone: 33-(0)2-41 22 66 75; Fax: 33-(0)2-41 22 37 33; E-mail: [sandrine.nail@univ-angers.fr](mailto:sandrine.nail@univ-angers.fr)) - Biological diagnosis.
- \* *Perrine Parize* (Centre de Ressources et de Compétence de la mucoviscidose, Service de médecine interne et pathologie vasculaire, Groupement hospitalier Sud, 69495, Pierre-Bénite cedex, France. Phone: 33-(0)4 78 56 90 49; E-mail: [pparize@hotmail.com](mailto:pparize@hotmail.com)) - Clinical surveillance, biological diagnosis.
- \* *Pr. Ilma Aparecida Paschoal* (Hospital de Clínicas da Unicamp- Setor de Procedimentos Especializados, Rua Vital Brasil, 250, sala C2-309, 2o andar, Cidade universitária Zeferino Vaz, Subdistrito de Barão Geraldo, CEP 13083-888, Campinas, São Paulo, Brasil. Phone/Fax: 55-19-32 516 244; E-mail: [ilma@mpc.com.br](mailto:ilma@mpc.com.br) or [ilma@fcm.unicamp.br](mailto:ilma@fcm.unicamp.br)) - Clinical surveillance.
- \* *Dr. André Paugam* (Parasitologie-Mycologie, CHU Cochin, 27 rue du Faubourg Saint-Jacques, 75014 Paris, France. Tél : 33-(0)1-58 41 22 51; Fax : 33-(0)1-58 41 22 45; E-mail: [andre.paugam@cch.aphp.fr](mailto:andre.paugam@cch.aphp.fr)) – Clinical surveillance, biological diagnosis.
- \* *Dr. Javier Pemán* (Unidad de Micología, Servicio de Microbiología, Universitari la Fe, Avda Campanar 21, 46009 Valencia, Spain. Phone: 34-96-197 3333; fax: 34-96-197 3177; E-mail: [peman\\_jav@gva.es](mailto:peman_jav@gva.es)) - Clinical surveillance, biological diagnosis, physiopathology and treatment.
- \* *Dr. Florence Persat* (Service Paludisme, Parasites du Sang et Mycologie Médicale, Groupement Hospitalier Nord, 103 Grande Rue de la Croix-Rousse, 69 317 Lyon Cedex 04, France. Phone : 33-(0)4 72 00 15 16; E-mail: [florence.persat@chu-lyon.fr](mailto:florence.persat@chu-lyon.fr)) - Clinical surveillance.

- \* *Dr. Marc Pihet* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Parasitologie-Mycologie, CHU, 4 rue Larrey, F-49933 Angers cedex 9, France. Phone: 33-(0)2-41 35 34 72; Fax: 33-(0)2-41 35 36 16; E-mail: [mapihet@chu-angers.fr](mailto:mapihet@chu-angers.fr)) - Clinical surveillance, biological diagnosis, physiopathology and treatment, environmental studies.
- \* *Dr. Claudine Pinel* (Département des Agents infectieux, Service de Parasitologie Mycologie, CHU Albert Michallon, BP 217, 38053 Grenoble cedex 1. Phone: 33-(0)4-76 76 54 90; Fax: 33-(0)4-76 76 56 60; E-mail: [CPinel@chu-grenoble.fr](mailto:CPinel@chu-grenoble.fr)) – Clinical surveillance, biological diagnosis.
- \* *Dr Stéphane Ranque* (Laboratoire de Parasitologie-Mycologie, AP-HM Timone, F-13385 Marseille cedex 05, France. Phone: 33-(0)4-91 38 60 90; Fax: 33-(0)4-91 38 49 58; E-mail: [Stephane.Ranque@mail.ap-hm.fr](mailto:Stephane.Ranque@mail.ap-hm.fr)) – Clinical surveillance, biological diagnosis.
- \* *Dr. Refika Hamutcu Ersu* (Marmara University, Department of Pediatrics, Division of Pediatric Pulmonology, Tophanelioglu caddesi 13/15, 81190 Altunizade-Uskudar, Istanbul, Turkey. Phone: 90-532-615 9939; E-mail: [rersu@yahoo.com](mailto:rersu@yahoo.com)) - clinical surveillance.
- \* *Pr. Raymond Robert* (Groupe d'Etude des Interactions Hôte-Pathogène, UPRES-EA 3142, Laboratoire de Parasitologie-Mycologie, UFR Sciences Pharmaceutiques et Ingénierie de la Santé, 16 Bd Daviers, F-49045 Angers cedex, France. Phone: 33-(0)2-41 22 66 62; Fax: 33-(0)2-41 48 67 33; E-mail: [Raymond.Robert@univ-angers.fr](mailto:Raymond.Robert@univ-angers.fr)) – Biological diagnosis.
- \* *Dr. Geraint Rogers* (King's College London, Pharmaceutical Science Division, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NH, UK. Tel: 44-(0)207 848 4467; Fax: 44-(0)207 848 4500; E-mail: [geraint.b.rogers@gmail.com](mailto:geraint.b.rogers@gmail.com)) - Clinical surveillance.
- \* *Pr. Emmanuel Roilides* (3rd Dept Pediatrics, University of Thessaloniki, Hippokration Hospital, Konstantinoupoleos 49, GR-54642 Thessaloniki, Greece; Phone: 30-2310-892444; Fax: 30-2310-992983; E-mail: [roilidee@mail.nih.gov](mailto:roilidee@mail.nih.gov)) - Clinical surveillance, physiopathology and treatment, environmental studies.
- \* *Marc Seidler* (Zentrum für Kinder- u. Jugendmedizin III, Päd. Pneumologie, Allergologie, Mukoviszidose & spez. Infektiologie, Im Neuenheimer Feld 430, D-69120 Heidelberg/Germany. Phone: 49-6221-56 8214; fax: 49-6221-56 4580; E-mail: [Marc.Seidler@med.uni-heidelberg.de](mailto:Marc.Seidler@med.uni-heidelberg.de)) – Physiopathology.
- \* *Dr. Maria Simitsopoulou* (3rd Dept Pediatrics, Univ of Thessaloniki, Hippokration Hospital, Konstantinoupoleos 49, GR-54642 Thessaloniki, Greece. Phone: 30-2310-892447; Fax: 30-2310-992983; E-mail: [simitsop@med.auth.gr](mailto:simitsop@med.auth.gr)) - Biological diagnosis, physiopathology.
- \* *Dr. Amparo Solé* (Unidad de Trasplante Pulmonar y Fibrosis Quística, Hospital Universitari la Fe, Avda Campanar 21, 46009 Valencia, Spain. Phone: 34-96-386 2700 ext 440459; Fax: 34-96-197 3007; E-mail: [sole\\_amp@gva.es](mailto:sole_amp@gva.es)) - Clinical surveillance and treatment.
- \* *Dr. Françoise Symoens* (Scientific Institute of Public Health, Mycology Section, Juliette Wytsmanstraat 14, B-1050 Brussels, Belgium. Phone: 32-2-642 56 30; Fax: 32-2-642 55 19; E-mail: [f.symoens@iph.fgov.be](mailto:f.symoens@iph.fgov.be)) - Preservation of clinical and environmental isolates, genotype studies.
- \* *Dr. Christopher Thornton* (Hybridoma Laboratory, School of Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter, EX4 4QD, United Kingdom. Phone: 44 (0)1392 264653; Fax: 44 (0)1392 263434; E-mail: [C.R.Thornton@exeter.ac.uk](mailto:C.R.Thornton@exeter.ac.uk)) – Production of Hybridoma, Environmental studies.

- \* *Dr. Kathrin Tintelnot* (Robert Koch-Institut, FG 16 Mykologie, Nordufer 20, 13353 Berlin, Germany. Phone: 33-2-51 44 62 12; fax: 33-2-51 44 62 94; E-mail: [tintelnotK@rki.de](mailto:tintelnotK@rki.de)) – Clinical surveillance, environmental studies.
- \* *Krzysztof Ulfig* (Polymer Institute, Technical University of Szczecin, Pułaskiego St. 10, 70-322 Szczecin, Poland. Phone: 091 449 4285; Fax: 091 449 4566; E-mail: [k\\_ulfig@zut.edu.pl](mailto:k_ulfig@zut.edu.pl)) – Environmental studies.
- \* *Lies Vanhee* (Laboratorium voor Farmaceutische Microbiologie, Universiteit Ghent, Harelbekestraat 72, 9000 Gent, Belgium. Phone: 32-9-264 8142; Fax: 32-9-264 8195; E-mail: [lies.vanhee@ugent.be](mailto:lies.vanhee@ugent.be)) - Genotype studies.
- \* *Dr. Laurie A. Whittaker* (Adult CF Program Director, HSRF 222, 149 Beaumont Avenue, Burlington, VT 05405, USA. Phone: 802-656-9400; fax: 802-656-8926; E-mail: [Laurie.Whittaker@uvm.edu](mailto:Laurie.Whittaker@uvm.edu)) – Clinical surveillance, biological diagnosis, genetic risk factors, genotype studies, environmental studies.
- \* *Dr. Gisele Yonezawa* (Hospital de Clínicas da Unicamp- Setor de Procedimentos Especializados, Rua Vital Brasil, 250, sala C2-309, 2o andar, Cidade universitária Zeferino Vaz, Subdistrito de Barão Geraldo, CEP 13083-888, Campinas, São Paulo, Brasil. Phone: 55-19-32 546 595; Fax: 55-19-95 217 907; E-mail: [yonezawa@unicamp.br](mailto:yonezawa@unicamp.br)) - Clinical surveillance.
- \* *Dr. Jan-Bart Yntema* (Department of Pediatric Pulmonology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands. E-mail: [J.Yntema@cukz.umcn.nl](mailto:J.Yntema@cukz.umcn.nl)) – Clinical surveillance.
- \* *Dr. Laila Zougaghi* (Laboratoire de Parasitologie-Mycologie, Faculté de Médecine et de Pharmacie de Marrakech, BP 7010, Sidi Abbad, 40000 Marrakech, Maroc. Phone: 212-24 43 73 46; Fax: 212-37 67 12 41; E-mail:[laila.zougaghi@menara.ma](mailto:laila.zougaghi@menara.ma)).
- \* *Dr. Rachid Zouhair* (Département de Biologie, Faculté des Sciences, Université de Meknès, Meknès, Morocco. Phone : 212 670 88 59 60; E-mail: [zouhair\\_rachid@hotmail.com](mailto:zouhair_rachid@hotmail.com)) - Genotype studies.