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Editorial

It was a great pleasure to meet many friends and colleagues in Régua, Douro, Portugal for the 2008 ECFS Basic Science conference last month. I congratulate Margarida Amaral and Phil Thomas on the excellent programme of symposia, workshops and special group discussions that they arranged, Christine Dubois and Sarah Young for their efficient organisation and Margarida's postdocs and students for their enthusiastic help. The meeting featured two EuroCareCF workshops, one focused on Animal Models and a second on Resources for Basic Research. For further information about these workshops, see the reports in this issue of the Newsletter. I was excited to learn about the work to develop new animal models for CF and astonished at the interim results of the phase IIa clinical trial of the CFTR potentiator VX-770 developed by Vertex Pharmaceuticals (Cambridge, MA, USA). I encourage you strongly to take a few moments to read the press release issued by Vertex Pharmaceuticals, which reports the interim results of the trial (see <http://investors.vrtx.com/releasedetail.cfm?ReleaseID=301749>).

At the same time as the 2008 ECFS Basic Science conference, clinical microbiologists from across Europe were gathering at the Hannover Medical School, Hannover, Germany for the second EuroCareCF "Hands-On" training course on the processing of microbiological specimens from individuals with CF, organised by Burkhard Tümmler and his colleagues. This intensive training course included lectures, demonstrations and practicals. Each attendee of the course also received a EuroCareCF strain parcel from Peter Vandamme to assist them with the identification of uncommon, difficult to identify bacteria. In this issue of the Newsletter, EvaLena Ericson of the Karolinska University Hospital, Stockholm, Sweden writes about her experiences on the Hands-On course.

At the "Hands-On" training course for clinical microbiologists, Burkhard revealed the results of the first European Quality Assurance Trial in CF Microbiology, which he had organised with Michael Hogardt. The correct identification of microbial species from respiratory specimens and the accurate evaluation of their susceptibility to antimicrobial agents are critical to the effective delivery of care to CF patients. However, it is unknown how effectively microbiological laboratories in Europe identify CF pathogens. The experience of European human genetics laboratories identifying CF mutations, argues that quality assurance is vital to maintain a uniform high standard of performance by reference laboratories. I commend strongly Burkhard and Michael for organising this quality assurance trial in CF microbiology. I very much hope that the results of this trial will lead to improvements the identification of microbial pathogens infecting the lungs of CF patients and hence, the care of these individuals.

This issue of the EuroCareCF newsletter also contains reports of activities organised by Workpackages 1 (Patient Care) and 3 (Clinical Research), which aim to improve patient care. Kris De Boeck and Michael Wilschanski report on the joint meeting of the Clinical Diagnosis group of Workpackage 3 and the ECFS Diagnostic Network held in Hannover in February. This meeting featured presentations by four young investigators supported by EuroCareCF. Rita Nobili and Carla Colombo report on a workshop about the application of evidence-based medicine to patient care organised by Workpackage 1 held in Milan, Italy last November. Finally, Fiona Kerr, a research physiotherapist at Belfast City Hospital (Belfast, UK) shares her experiences on the course "Advanced Cystic Fibrosis: Best Research, Best Care" held in Nice, France last March, which was organised by the European Respiratory Society (ERS) and ECFS and supported by EuroCareCF. We look forward to working again with the ERS this summer when Robert Bals and Pieter Hiemstra organise a Research Seminar on Host-Pathogen Interactions in the Lung in Davos, Switzerland.

Finally, I look forward to meeting many of you next month in Prague, Czech Republic at the 31st European CF Conference, where EuroCareCF are organising a rich variety of activities. Whatever your CF specialism, there should be something to interest you!

David Sheppard

Training Grant Reports

When EuroCareCF awards Training Grants, we ask recipients to provide feedback on their experience, and 'trainees' submit reports about the relevance of the training they received and the benefits they gained. Information on two recent training visits is detailed below.

Elena Galfrè's visit to Trieste, Italy

Elena Galfrè, a PhD student with Oscar Moran at the Istituto di Biofisica at Genova, Italy visited Alessandro Vindigni's group at the International Centre for Genetic Engineering and Biotechnology (ICGEB) (Trieste, Italy) to learn new techniques in protein expression.

The aim of our project at the Istituto di Biofisica at Genova, Italy is the study of the biophysical and biochemical properties of the interaction between the nucleotide-binding domains (NBDs) of CFTR and CFTR potentiators. The objective of my visit to Alessandro Vindigni's group at the ICGEB (Trieste, Italy) was to learn new techniques for the production of these two intracellular CFTR domains: NBD1 and NBD2, that our group have proposed as the putative target for these drugs, that are potential candidates for the design of cystic fibrosis pharmacologic therapy.

The drug discovery process can be significantly improved by the identification of the binding site on the target. Based on molecular modelling and site-directed mutagenesis experiments, we have proposed that CFTR potentiators exert their effects by binding to the NBDs. To characterise such interaction we have initiated a physicochemical analysis of such interaction, using recombinant NBDs produced in bacteria. However, the protein produced with this method is denatured, and needs to follow a long renaturation process, that is difficult to optimise. We expect to improve the quality and the quantity of the protein produced using the baculovirus method. Moreover, with the baculovirus method we will be able to produce complete CFTR protein, which is impossible in bacterial systems. Thanks to Dr. Christine Bear (Toronto, Canada) we have received the constructs of CFTR, NBD1 and NBD2 in baculovirus, to be expressed in insect cells.

Expression of proteins by the baculovirus system provides several advantages, as the possibility to obtain the material with post-translational modifications, a high yield of proteins in a relatively short culture time, and the possibility to obtain proteins in native, folded conditions. For these reasons we were interested to set up the expression of proteins in insect cells infected by baculovirus, that will allow us to improve the yield of soluble proteins, like the NBDs. Moreover, with this method, we will be able to obtain folded membrane proteins, like the complete CFTR protein. Hence, we took advantage of the extensive know-how in Dr Alessandro Vindigni's laboratory at the ICGEB, Trieste. Dr Vindigni's group has long experience of the production of recombinant protein using this system.

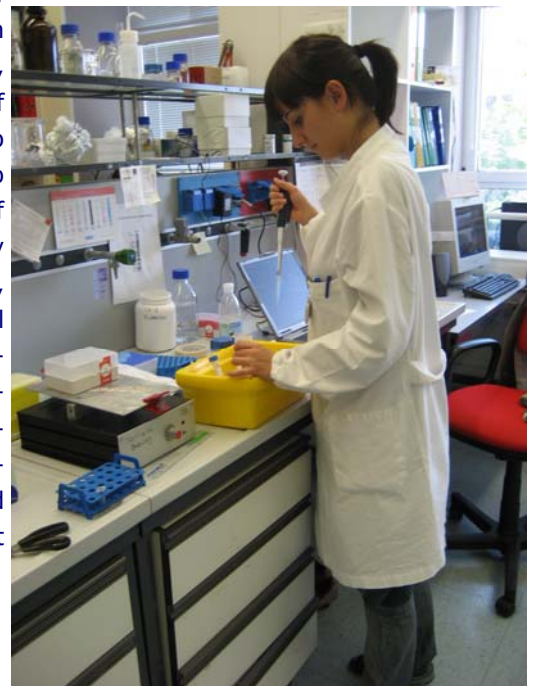
During my stay at Trieste, I was trained in the following tasks:

1. Protocols for growth and maintenance of insect cell lines
2. Testing the titre of viral stocks obtained from infected sf9 cells
3. Amplification procedure of baculoviral stocks
4. Expression and analysis of recombinant proteins, and purification with affinity resins.

The training consisted of how to effectively execute all the tasks listed above, using a sample of NBD1 expressing baculovirus construct, that was kindly provided by Dr. Christine Bear.

I obtained exhaustive explanations on the different aspects of the method of expression. Explanations were extended to a description of baculovirus expression using the Bac-to-Bac system that simplifies and enhances the process of generating recombinant bacmid DNA. I also had the opportunity to discuss the protocols about how to generate a recombinant pFastBac Vector, transformation of purified plasmid into competent *Escherichia coli* cells for transposition into the bacmide, control and analysis of the *E. coli* colonies transformed with recombinant bacmide with a miniprep kit, and the transfection of insect cells with Cellfectin reagent.

In conclusion, I have received most of the information necessary for the production and purification of the proteins expressed in insect cells infected by baculovirus, from the generation of a baculovirus, the production of donor plasmid with the gene of interest, to the infection of insect cells. I also had the chance to execute most of these steps directly in the laboratory, including the small tricks never described in the literature, that are advantageous for setting up the method in our laboratory at Genova.



Elena Galfrè

Sashka Mihaylova-Mikova's visit to Gent

January 2008

Sashka Mihaylova-Mikova is a physician and works as a clinical microbiologist in the hospital laboratory associated with the University of Medicine, Pleven, Bulgaria. Sashka has just completed her Ph.D project focusing on *Burkholderia cepacia*. She applied to undertake advanced training with Peter Vandamme at the European *Burkholderia cepacia* complex Referral Laboratory and Repository, Gent, Belgium.

In Bulgaria there is no referral laboratory for CF specimens. University hospitals are responsible for the treatment of CF patients and their microbiological laboratories carry out the analyses of microbial infections.

The European *Burkholderia cepacia* complex Referral Laboratory and Repository (EBcCL) is one of the leading international laboratories working with a diverse range of isolates from cystic fibrosis patients. EBcCL is developing and implementing methods for the identification and preservation of microorganisms of clinical relevance, particularly genotyping of isolates. The EBcCL specialises in performing taxonomic analyses of the *Burkholderia cepacia* complex, *Burkholderia cepacia*-like bacteria and unusual isolates from CF patients.

Chronic bacterial colonisation of the respiratory tract, leading to exacerbations of pulmonary infection is the major cause of disease and death in CF patients. Determination of the clinical relevance of Gram-negative nonfermenting bacteria other than *Pseudomonas aeruginosa* in that specific patient population is hampered by difficulty in identifying these pathogens by conventional laboratory techniques. Species related to *Burkholderia*, *Ralstonia*, *Pandoraea*, *Stenotrophomonas*, *Achromobacter*, *Inquilinus*, etc., are emerging as significant pathogens in CF patients. The increasing complexity of bacterial taxonomy presents challenges for the clinical microbiologist. The significance of the proposed training is related to the application of rapid, reliable protocols for identifying pathogenic microorganisms from the *Burkholderia cepacia* complex and *Burkholderia cepacia*-like bacteria.

During Sashka's training visit, EuroCareCF reference strains and Bulgarian clinical isolates were processed by DNA extraction and profiling.

The purpose of the training was to enable me to acquire knowledge and skills in the technical aspects of running samples and to learn how to analyse the acquired data.

The main directions of training were the following:

- Methods for DNA preparation, DNA detection and DNA measurement;
- Application of phylogeny-based protocols for identification of bacterial pathogens at the species level by *recA* RFLP and *recA* sequencing analyses;
- Application of protocols for epidemiological monitoring of pathogenic species at the strain level by RAPD- and rep-PCR profiling analyses;
- Analyses of band patterns by visual inspection and computer programs;
- Methods for preservation of reference strains.

The significance of the training is related to the application of rapid, reliable protocols for genotyping bacteria from the target group providing the possibility to choose the best approaches for species identification and epidemiological typing.

Sashka Mihaylova-Mikova

From Evidence Based Medicine to Evidence Based Practice

EuroCareCF workshop—Milan, Italy: 30th November 2007

Carla Colombo and Rita Nobili provide a report on the workshop that Workpackage 1 (Patient Care) organised at the Third Congress of the Italian CF Society in Milan, Italy on 30 November 2007.

The aims of the workshop were to discuss how to use information from clinical research in patient care, review the implementation across Europe of the ECFS standards of care document, and support the use of standard guidelines in daily clinical practice. Instruments to collect data as well as to measure clinical outcomes coming from Evidence Based Practice were also discussed as well as a proposal to monitor adverse drug reactions.

First, Alberto Tozzi examined data mining. There is a need to analyse in depth large quantities of clinical data with user-friendly tools. There is also a need to link different databases containing patient records. Finally, the analysis of data must be relevant to clinical practice. Data mining facilitates the classification of individuals and events according to specific patterns. It can also be used to forecast which events will follow one another. Unlike statistical analysis, data mining does not require assumptions about the distribution and structure of data. However, data mining is not without its problems. Most of the patterns identified are not interesting, while others might be incorrect or spurious. Randomised Clinical Trials (RCTs) are considered the gold standard for scientific investigation. However, clinical trials that demonstrate drug efficacy might include insufficient patients to measure adverse events of the same drugs, even when studied for long periods. It is also unusual for clinical trials to be conducted to study adverse effects. Prof. Tozzi proposed that a potential solution would be to make available all information from clinical trials. He concluded by emphasising the importance of developing new approaches to handle large amounts of information so that the correct information is retrieved at the right time.

Luigi Pagliaro's presentation focused on translating clinical research to clinical practice. He argued that patient care would be improved significantly if knowledge gained from health research were better translated into practice. He identified two roadblocks on the route towards improved public health. First, translating from pre-clinical studies with cell and animal models to testing drugs in humans. Second, translating from clinical trials to clinical practice. Prof. Pagliaro emphasised that while a drug might demonstrate efficacy in an RCT, the same drug might not prove effective in clinical practice. To enhance drug use, RCTs should provide clinicians with full information about how a drug might be used in clinical practice. Prof. Pagliaro argued that by attending to risk without treatment, responsiveness to treatment, vulnerability to adverse effects, and utility for different outcomes, clinical researchers can design RCTs that better characterise who will—and who will not—benefit from medical interventions. Clinicians and policymakers can, in turn, make better use

of the results of such studies.

Roberto Buzzetti provided insight into the type of scientific information that can be extracted from patient registries using the Comparative Analysis International Registries Overviewed (CAIRO) project as an example. This project considers general epidemiology (CF incidence/prevalence, survival); registries comparison; genetics (genotype/phenotype correlation, different ethnic groups, twins/brothers); neonatal screening and its consequences; growth and nutrition; microbiology; pregnancy and parenthood; complications and transplantation. Prof. Buzzetti's presentation generated much discussion, some focused on the results of the study and others highlighting concern about the management of patient registries.

Nino Cartabellotta discussed interactions between Evidence-based Medicine and Evidence-based Health Care leading to Clinical Governance. She explained the different types of indicators (e.g. structural, process and outcome) used to evaluate different aspects of Health-care delivery, emphasising the importance of clinical audit. Using DoCDATA (Directory of Clinical Database) as an example, Prof. Cartabellotta explained how high quality clinical databases could bring research closer to clinical practice and audit. DoCDATA is characterized by the establishment of criteria for inclusion of clinical databases, development of a checklist for assessing the quality of clinical databases, empirical testing of the checklist and design of a website. In closing, Prof. Cartabellotta emphasised the importance of a collaborative, multi-disciplinary approach to clinical governance, distinguished by international methodological standards.

Prof. Caputi reviewed adverse drug reactions (ADRs). He discussed how to predict whether a drug might cause an ADR and their diagnosis, paying special attention to off-label and unlicensed drugs and paediatric patients. Prof. Caputi emphasised that reporting is the best way to prevent ADRs highlighting the important role that networks of clinicians and healthcare organisations can play.

The title of Dr Jim Littlewood's presentation was "Evidenced based medicine – one aid in providing good CF care". Evidenced-based medicine is defined as "*the conscientious explicit and judicious use of current best evidence in making decisions about care of individual patients*" and used to "*clarify those parts of practice that are in principle subject to scientific methods*" and "*justifying treatment by randomized controlled trials*".

Dr Jim Littlewood considered the valuable contribution evidenced based medicine has made to the management of CF patients but emphasised that the Cochrane Systematic Reviews are but one of the many sources of valuable information available to the clinician. To ignore the many other sources would, and has in the past, re-

From Evidence Based Medicine to Evidence Based Practice

EuroCareCF workshop—Milan, Italy: 30th November 2007 (continued)

sulted in patients receiving suboptimal treatment or delay the introduction of an important treatment policy such as neonatal screening. Fortunately, consensus groups, of which there are now many, go some way to preventing this.

To ensure that CF patients receive the best, most effective and least intrusive patient-friendly care, it is mandatory that the multidisciplinary CF team must be familiar with the most reliable up-to-date information on treatment. Dr Littlewood explained that there are now over 40 Systematic Cochrane Reviews of different aspects of CF patient care. Although these Systematic Reviews are valuable sources of published data on particular aspects of CF care, many will reject a considerable amount of valuable published information which does not reach the Reviewers' high standards, and therefore some of the conclusions are less useful for inexperienced clinicians. Dr Littlewood emphasised the value of the European Standards of Care Consensus document prepared by the ECFS and published in 2005 in the *Journal of Cystic Fibrosis*. This consensus document is based on the best of all the information available using not only Cochrane Reviews but also common sense, clinical experience of the co-authors, conference reports, publications, clinical trials etc.

Dr Littlewood emphasised that the sheer volume of information at their disposal should not overwhelm clinicians. Their aim should be to integrate the best external evidence with their individual clinical expertise and the patients' wishes. Even with extensive guidelines and reviews clinicians require the experience of treating many patients over a prolonged time interval before they become experts. To achieve this, most experts work in large CF centres.

Dr Littlewood considered that CF neonatal screening is essential to diagnose the disease before organ function is damaged. He argued that there is still significant room for improvement of patient treatment, which will gradually result in improved health and survival of CF patients for many years to come. To support his arguments, Dr Littlewood highlighted the steady impressive improvements that have occurred in the life expectancy of CF patients, since the disease was first described in the late 1930s. He was optimistic that if the present trend continues, children with CF born in 2000 in the UK will have a predicted survival of well over 50 years, with the prospect of new treatments further improving their survival.

Carla Colombo & Rita Nobili



David Sheppard, Carla Colombo, Dorota Sands and participants of the From Evidence Based Medicine to Evidence Based Practice Workshop

ECFS Diagnostic Network Meeting

15th-16th February 2008 Hannover, Germany

The ECFS Diagnostic Network (ECFSDN) held its 4th annual conference in Hannover, Germany on 15-16th February 2008, hosted by Manfred Ballman and Burkhard Tümmler.

The Network was honoured with exceptional guest speakers. Prof Sabina Gallati (Berne, Switzerland) provided fascinating insight into new genetic methods for CF diagnosis and she included some novel information about the mutation 3905insT seen only in the Swiss and the American Amish populations.

Prof Schillers (Münster, Germany) presented a new diagnostic test based on CFTR expression in red blood cells (RBCs). The principle behind this is the hemolysis of RBCs induced by Gadolinium (Gd^{3+}) ions. RBCs from CF patients are more resistant to Gd^{3+} -induced hemolysis than RBCs from healthy donors. In another approach, he demonstrated the different Zn^{2+} sensitivity of Gd^{3+} -induced hemolysis of non-CF and CF RBCs. This may be of additional help to diagnose CF.

Dr Frauke Stanke (Hannover, Germany) presented research from the European Twin Study. CF twins and siblings were recruited from 158 CF centers from 14 European countries. She presented new data on two proposed CF modifier genes, TNF and ENaC. The picture is complicated by the fact that with improvement in survival, false-positive findings may be found when patients from different birth cohorts are compared.

Maarten Sinaasappel (Rotterdam, The Netherlands) presented diagnostic evaluations of patients with CF-like symptoms leading to a debate between Chris Taylor and Manfred Baumann as to whether intestinal current measurement is a better tool than the nasal potential difference (NPD) assay to diagnose CF.

Inez Bronsfeld presented methodological differences in European electrophysiological tests. Based on this evaluation of different tests, the Diagnostic Network proposes to work towards the development of a single operating procedure for electrophysiological tests. Inez is coordinating this effort for the Diagnostic Network.

Kris de Boeck (K.U. Leuven) reviewed the activities of EuroCareCF including her role as leader of Workpackage 3 which includes the Diagnostic Network.

Harry Cuppens (K.U. Leuven) gave an overview about current knowledge of ENaC in relation to CFTR function.

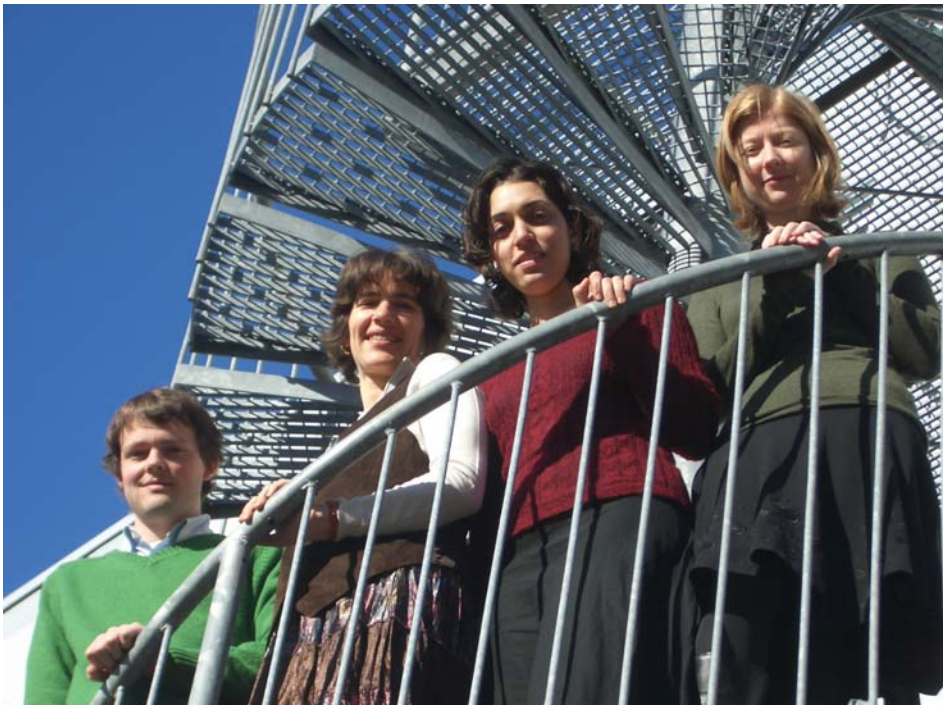
Christophe Goubau and Kris de Boeck presented an update on the Algorithm Validation paper, which will soon be submitted by the Diagnostic Network as a follow up to the original algorithm study.

There was a lecture from Peter Middleton on his experiences doing NPD in Sydney, Australia. Burkhard Tümmler gave a talk on a rare mutation F508del-R553Q, which rescued CFTR function *in vitro*, yet in CF patients did not appear to confer a milder clinical phenotype.

We were delighted that EuroCareCF provided travel grants for 4 Young Investigators to present their research at the meeting.

Paolo Melotti from Verona presented a new diagnostic test based on peripheral monocytes which function differently in CF patients. She also presented new data on a rare mutation S977F. Fiona Healy from Dublin presented an interesting diagnostic problem in a patient with recurrent pancreatitis. Yasmin Yaakov from Jerusalem presented long term follow-up data on the use of NPD in CF diagnosis. Christophe Goubau from Leuven presented phenotypic characterization of patients with intermediate sweat chloride values using the Diagnostic Algorithm.

Inez Bronsfeld and Nicho Derichs presented plans for the Diagnostic Network website.



Young investigators supported by EuroCareCF from left to right: Christophe Dubeau (Leuven), Paolo Melotti (Verona), Yasmin Yaakov (Jerusalem) and Fiona Healy (Dublin)

The next meeting of the ECFSDN will be held in Prague on the day of the Opening Ceremony, 11th June from 14:30 until 16:30 when there will be an opportunity for all members of the ECFS to submit interesting diagnostic cases. Details are available, on request, from the ECFS office.

Kris de Boeck and Michael Wilschanski

ERS School Course on: "Advanced Cystic Fibrosis: Best Research, Best Care"

Fiona Kerr, a Research Physiotherapist at the Adult CF Unit, Belfast City Hospital attended the ERS School Course "Advanced Cystic Fibrosis: Best Research, Best Care" in March 2008 supported by a Training Grant. We asked her to write her thoughts on the ERS course.

When I first saw a flyer for this course I thought – Fantastic! The topics looked interesting and the speakers were some of the most well known names in CF research. I couldn't wait to meet one of the speakers in particular, Dr Chris Goss—two of his papers had really helped me with the development of my research project "Defining a pulmonary exacerbation in adults with Cystic Fibrosis". There was a full programme of presentations and interactive workshops planned for each day:

Day 1:

Dr Jane Davies talked about CFTR structure, function and also the consequences of CFTR dysfunction. Prof. John Govan spoke about the complex organisms *Pseudomonas aeruginosa* and *Burkholderia cepacia* and the inflammatory response they cause in CF lungs. Prof Stuart Elborn put forward the key points about airway inflammation in CF and anti-inflammatory treatment in CF. Unfortunately, Dr Kris De Boeck had lost her voice. However, Dr Eitan Kerem kindly presented her talk on the diagnosis of typical and atypical CF and the signs, symptoms and diagnostic tests for CF.

In the afternoon there were 3 parallel workshops. We divided into 3 groups. The first workshop I attended discussed the strengths and weaknesses of databases and registries such as PortCF. We also discussed the pulmonary exacerbation score developed by the Akron Pulmonary Exacerbation Project. This was particularly useful for my research. The second workshop focused on the design of clinical trials. We discussed the research projects of group members and their specific questions relating to clinical trials. The third workshop on endpoints in clinical trials sparked a lot of discussion about the use of patient reported outcome measures and pulmonary exacerbations in CF as endpoints in clinical trials.

Day 2:

Prof. Rosalind Smyth talked through a variety of clinical trial designs including multicentre trial designs, sample size, randomisation techniques, blinding and outcome measures. Her second talk later that morning highlighted the importance of evidence base practice for the care of CF patients, systematic reviews and the Cochrane library. Dr Chris Goss also gave a talk about up and coming drugs which are currently in pre-clinical development and discussed the processes that drugs must go through before being available for prescription to patients.

Following these lectures, there was a highly interactive panel discussion related to CF research involving all the faculty. Statements were presented on the screen and we were asked whether we agreed or disagreed with them, and for justification of our thoughts, which initiated a lot of debate and discussion.

We then divided into our groups again and critically appraised some journal articles, which were selected by the panel. This was useful as we were able to work through this as a group and could extract both the positive and negative aspects of the papers.

Many course attendees had research projects which they wanted to discuss. We were divided into groups based on our project area. I was given the opportunity to present my research project to Dr Harm Tiddens and Dr Eitan Kerem and other members of the group. This was extremely useful as I was able to take on board some very constructive feedback which I can feed back to my supervisors who are supporting me with my project. It was also useful to see other people's research projects and to contribute to their development.

Day 3:

There was a very striking talk on ethics by Dr Suzanne van der Vathorst. She used shocking statements and movie clips to stimulate thought about why ethics is so important and why it is important that we adhere to ethical policies. Dr Chris Goss also talked about important aspects for any successful research centre. I picked up a lot of tips from this session which I can feed back to my own centre

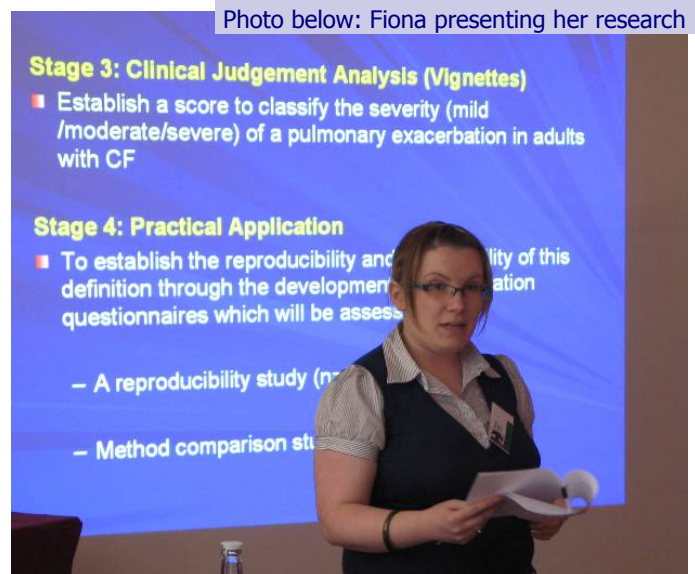


Photo below: Fiona presenting her research

and implement in practice particularly regarding recruitment of patients into clinical trials. Prof. Stuart Elborn then spoke about applying for financial funding and about the peer review process and gave some advice on getting your work published and what the journals want to see included in your paper.

My thoughts...

The course was fantastic. I got to meet and have discussions with some of the world's leading CF researchers. I absorbed lots of information which I will be able to apply both to my research project and in my future career as a researcher. I will also be able to feedback to the research team I work in to help enhance the running of our research centre. I also got to meet a lot of interesting people who are all enthusiastic about CF research and who knows maybe this is the next generation of expert CF researchers!

Fiona Kerr

EuroCareCF Workshops at the ECFS Basic Science Conference

9 – 13 April 2008 Régua, Douro, Portugal

Two EuroCareCF workpackages organised workshops at this year's ECFS Basic Science Conference held at Régua, Portugal in April.

WP6 Animal Models workshop

This workshop organised by Bob Scholte discussed lung pathophysiology in CFTR knockout mice and introduced new animal models for CF research.

Bob Scholte (Erasmus Medical Center, Rotterdam) focused on chronic activation of inflammatory pathways in CF mutant mice and the possible relationship between injury and excessive tissue remodeling in CF lung disease. He presented evidence that several genes involved in inflammation and tissue remodeling are upregulated in F508del mutant mice. The pattern of gene expression observed in unchallenged F508del mice is consistent with chronic inflammation with higher production of IL-6, IL-1B and other cytokines. Very interestingly, morphogens that are involved in tissue injury/repair and morphogenesis are also upregulated in the CF mice. This is accompanied by a sustained inflammatory and extracellular matrix remodelling response after airway injury. Bob Scholte concluded that the CF mouse lung is affected by inflammation, even in the absence of pathogens, which chronically activates pro-fibrotic pathways. If the same holds for the human CF lung, this suggests that anti-inflammatory agents or inhibitors of specific morphogens could help prevent irreversible and eventually fatal tissue remodeling.

Jacky Jacquot (Hôpital St-Antoine, Paris) highlighted the inflammatory responses of CF mutant mice challenged by oxidative stress. Following exposure to reactive oxidative species (ROS), CF lung epithelial cell lines (IB3-1 and CFBE41o-) failed to activate caspase-3 and NF- κ B suggesting CF-specific regulation of NF- κ B/ κ B- α signaling. Using CFTR deficient (cfr^{-/-}) mice, Jacky and his colleagues observed that upon exposure to hyperoxia-mediated oxidative stress, the CF mice lung exhibited significantly higher proteasomal activity and that this was accompanied by reduced caspase-3 immunoreactivity and an absence of the degradation of the NF- κ B inhibitor I κ B- α . These effects were confirmed by blocking CFTR Cl⁻ channel activity in the normal human bronchial cell line 16HBE14o- with the specific CFTR inhibitor CFTR_{inh}-172. In summary, Jacky and his colleagues demonstrated that the CFTR Cl⁻ channel plays a crucial role in regulating lung proteasomal degradation, caspase-3 activity and NF- κ B-dependent transcriptional activity under oxidative stress conditions.

John F. Engelhardt (Carver College of Medicine, University of Iowa) discussed the development of new animal models for studies of CF pathophysiology and therapy development. He began by comparing and contrasting the anatomy, cell biology and epithelial ion transport of human, mouse, pig and ferret airways. Among the

species differences that John discussed was the processing and trafficking of F508del-CFTR, the most common CF mutation. In contrast to the human mutant protein, mouse and pig F508del-CFTR are partially processed, generating some mature protein. This suggests that the cell surface expression of mouse F508del-CFTR protein might explain, in part, differences in disease phenotype between humans and mice. John then discussed the extensive efforts of his own laboratory and that of Michael Welsh to develop ferret and pig models of CF. For further information about the development of these models, see Sun X *et al. J Clin Invest.* 2008; **118**:1578-1583 and Rogers CS *et al. J Clin Invest.* 2008; **118**:1571-1577. John finished his presentation by reviewing Geoffrey McLennan's work at the University of Iowa to investigate lung function in humans, pigs and ferrets. Using computed tomography (CT) imaging, Geoffrey has developed three-dimensional models of pig and ferret respiratory airways and used them to investigate airway clearance in normal pigs and ferrets in preparation for studies using knockout animals. John's presentation highlighted the exciting work to develop large animal models of CF and the potential use of these animals.

WP7 Resources for Basic Research workshop

This workshop organized by Margarida Amaral (University of Lisboa, Portugal) consisted of two sessions one focused on "Endpoints for CFTR Rescuing Therapies" and a second, on "Cellular Systems for CF Research".

Session I: Endpoints of CFTR Rescuing Therapies:

Karl Kunzelmann (University of Regensburg, Germany) gave a presentation on behalf of Martin Hug (University Hospital Freiburg, Germany) who regrettably was unable to attend the Conference. Karl compared and contrasted the different techniques used to evaluate CFTR function in human subjects, including the sweat test, the nasal potential difference assay and rectal potential difference measurements. He then discussed *in vitro* and *ex vivo* assays of CFTR activity including halide efflux and intestinal current and voltage measurements (termed ICM and V_{TE}M). Karl explained how ICM and V_{TE}M were applied to rectal biopsies studied in micro-Ussing chambers. He highlighted the pros and cons of these techniques, including high reproducibility, ability to detect small changes and their application to diagnose CF and test CFTR modulators, particularly CFTR potentiators.

Sherif Gabriel (University of North Carolina, Chapel Hill, NC, USA) reviewed ongoing work in the USA to establish and standardise intestinal outcome measures for multicentre CF clinical trials. This work involves setting up rectal biopsy tissue assessment for CFTR function based on the methods already in place in Europe and discussed in the preceding presentation by Karl Kunzelmann.

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EuroCareCF Workshops at the ECFS Basic Science Conference (Continued)



John Engelhardt discussing animal models in Portugal

Michael Barker (Helios-Klinikum E.v. bel, Berlin, Germany) introduced a completely different perspective with potential applicability to the diagnosis of CF lung disease – the measurement of trace gases in exhaled breath. Michael began by explaining that the detection of nitric oxide in breath is used as a marker of inflammation in atopic asthma and that carbon monoxide is detected in the breath of active smokers. He emphasized the vast spectrum of exhaled gaseous compounds and the critical question, which compound was the best marker. Michael then reviewed the methods used to analyse compounds in exhaled air as well as the technical challenges involved. Because real-time detection is not yet possible, these measurements depend on collecting exhaled air in special containers. Then, highly sensitive analytical methods (e.g. gas chromatography) are used to measure molecular concentrations in the order of parts per trillion. Michael joked that the atmospheric chemists who developed this technology boast that they can detect bad breath at 10 km. Michael reviewed some published studies about exhaled breath analysis of CF patients. For example, with colleagues in Aachen (Germany), he had investigated volatile organic compounds (e.g. ethane, propane and n-pentane) in the exhaled breath of 20 healthy subjects and 20 CF patients. This study demonstrated that the exhaled breath of CF patients exhibited higher n-pentane and lower dimethyl sulphide levels than the breath of the healthy subjects; ten other compounds tested did not differ between the healthy controls and CF patients (for further information, see Barker et al. *Eur Respir J.* 2006; 27:929-36). Michael further highlighted the capacity of this technique to monitor bacterial infection of CF airways. As an example, he explained that cyanide is measured in the air above bacterial cultures and that the compound is detected in the sputum of CF patients chronically colonised with *Pseudomonas*. Michael speculated that in the future hand-held devices (similar to those currently used by the police to assess alcohol levels in drivers) might be used to diagnose CF, identify infectious agents, monitor treatment adherence and identify smokers. He concluded by proposing that biochemical breath analysis is both feasible and informative. However, for the reliable usage of this methodology analytical technologies and target compound identification still required further development and validation.

Session II: Cellular Systems for CF Research

Garry Cutting (Johns Hopkins University School of Medicine, Baltimore, MD, USA) introduced the Clinical and Functional Translation

of CFTR project (acronym: CFTR2), which aims to functionally characterise all CFTR mutations so far reported at the CFTR Mutation Database. The rationale for the project is the increased use of genetic information namely, CFTR mutations for diagnosis (e.g. neonatal screening) and therapy (e.g. "mutation-specific" approaches). However, Garry argued that functional characterisation of rare mutations would also provide valuable mechanistic insight into CFTR biogenesis and function. The CFTR2 database would contain all CFTR mutations known to cause CF together with data on the respective cellular and/or functional defect. It would be hosted at Johns Hopkins University and linked to the current CFTR Mutation Database in Toronto. Garry highlighted the ambitious goal of the project to perform biochemical and functional studies of all CF

mutations in a uniform manner and in the same cell line. To develop stable cell lines, the project planned to use recombinase-mediated stable transfection. As an example, Garry showed that expression data for R1070P, a CF mutation located in the fourth intracellular loop, obtained with this approach agreed well with the published literature. Garry concluded his presentation by discussing some of the issues that the CFTR2 project is currently grappling with, including variation in patient phenotype, and the choice of the most suitable cell lines and functional assays.

Malcolm Brodrie (Freeman Hospital and University of Newcastle, Newcastle, UK) shared his experience of isolating and culturing primary bronchial epithelial cells from the lungs of CF patients undergoing lung transplant. Malcolm explained that the Freeman Hospital is the largest lung transplant centre in the UK with 20 people with CF transplanted annually. To date, Malcolm and his colleagues have successfully isolated and cultured primary bronchial epithelial cells from three out of four CF patients. Malcolm reviewed the procedure for cell isolation and culture and highlighted the technical challenges faced by his group, chief of which is dealing in cell culture with the significant bacterial infection present in the CF lungs. In the discussion that followed Malcolm's presentation Olga Zegarra-Moran (Genoa, Italy) shared her experience of treating bacterial infections in primary cultures from CF patients, while Anil Mehta (Dundee, UK) questioned why Malcolm's pathologist required a large portion of the removed tissue, precluding the harvesting of further cells from this precious resource.

Hongyu Li (University of Bristol, Bristol, UK) reported the results of the EuroCareCF questionnaire for lung transplant centers in Europe. This on-line survey provided information about the numbers of CF lung transplants and the use of the removed tissue. It also revealed that there is general interest in participating in a "Hands-On" training course to learn about isolating and culturing primary airway epithelial cells for CF related research. For further information about the results of the questionnaire, see the separate article by Hongyu in this issue of the Newsletter (page 10).

Melissa Ashlock (Cystic Fibrosis Foundation Therapeutics (CFFT); Bethesda, MD, USA) reviewed the efforts of CFFT to support drug discovery and development. Melissa discussed in turn the different resources CFFT has developed or is in the process of developing to promote drug discovery. These include "control" chemicals (i.e. small molecules that act as CFTR trafficking correctors and channel potentiators or inhibitors), primary and secondary assays for high-

EuroCareCF Workshops at the ECFS Basic Science Conference (continued)

throughput screening, anti-CFTR antibodies, primary cultures of airway epithelial cells and cell lines, assays to identify drug targets and the mechanisms of action of drugs, protein structures and bioinformatic models. Some of these resources, including CFTR modulators and antibodies, are distributed by CFFT to the CF scientific community for free or at nominal cost. Other resources, such as primary and secondary assays, are performed by companies or academic groups on a fee-for-service basis.

Melissa then provided a brief update on the very promising results from an ongoing clinical trial with the CFTR potentiator VX-770 (Vertex Pharmaceuticals, Cambridge, MA, USA). After reviewing the history of the development of this compound starting from high-throughput screening of compound libraries, Melissa indicated that interim results of the first part of the phase IIa clinical trial of VX-770 in 20 CF patients with the G551D mutation who took this drug orally were quite encouraging. She pointed out that the detailed information she would summarize had recently been made public by Vertex in a press release (<http://investors.vrtx.com/releasedetail.cfm?ReleaseID=301749>). Melissa explained that the performance of VX-770 in this clinical trial had exceeded all expectations and showed improvements in sweat

chloride, nasal potential difference and lung function. She stressed the exciting findings followed from a great foundation of basic research from the CF scientific community and a large team effort in an 8 year long collaboration between CFFT and Vertex. The clinical results were also a consequence of hard work and coordination by Vertex, the principle investigator, Frank Accurso, and by the clinical centers and their staff and patients. Melissa concluded her presentation by indicating plans are underway for further clinical testing of VX-770, and that the Phase I clinical trial with the CFTR corrector VX-809, is underway.

To summarize, the EuroCareCF workshops at the ECFS Basic Science conference proved an excellent opportunity to learn the latest news about the development of new animal models, review endpoints for clinical assessment of therapies that correct the basic defects in CF, discuss cellular systems for CF research and be updated on the testing of CFTR potentiators in the clinic. The workshops were distinguished by high quality presentations of excellent science and stimulating discussion.

Hongyu Li

EuroCareCF Questionnaire for Lung Transplant Centres

To assess potential interest in the production of primary cultures and novel cell lines for cystic fibrosis research, EuroCareCF conducted an online survey in February and March 2008.

We sent out a letter to 42 lung transplant centres in Europe inviting them to complete a questionnaire. 14 lung transplant centres responded. The survey shows that every year, there are **250** lung transplants in these **14** transplant centres; among them, **94** are cystic fibrosis patients. The tissues removed at lung transplant are used for research, teaching and other purposes. However, 41.70% of the tissues are wasted (Figure 1).

7 lung transplant centres collaborate with basic scientists in areas of CF related diseases, cell and molecular biology and immunology. However, none of these centres collaborated with either pharmacologists or physiologists (Figure 2).

6 lung transplant centres have no active collaborations with basic scientists, because there are none close by.

2 centres are already involved in the production of primary non-polarised cultures of epithelial cells from CF (and/or non-CF) lungs. Another **9** centres are interested in generating primary cultures of which **8** centres are interested in attending a "Hands-On" training workshop. (In a previous survey conducted by the ECFS, another 5 centres expressed their interest in attending a training course).

In summary, there is significant potential for obtaining CF lung tissue from lung transplant centres and using this tissue to generate CF cell lines.

Based on the interest in attending a "Hands-On" training course about the production and immortalization of primary cultures of airway epithelial cells, EuroCareCF is actively exploring the possibility of organizing a further training course. Further information, will be made available via the EuroCareCF website and newsletter.

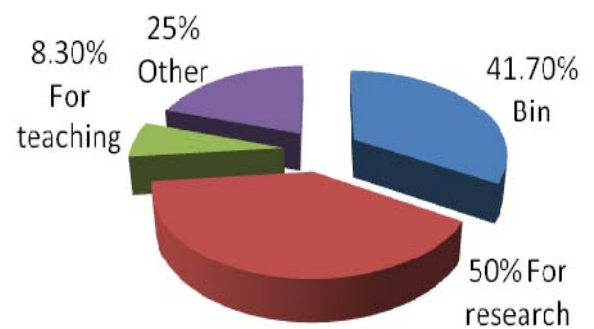


Figure 1: Usage of tissue removed at lung transplant

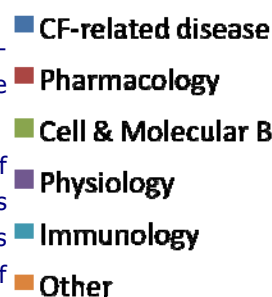


Figure 2: Areas of collaboration with basic scientists

Hongyu Li

EuroCareCF Hands-on Training Course on the Processing of Microbiological Specimens from Individuals with Cystic Fibrosis

Dr EvaLena Ericson from Karolinska University Hospital, Stockholm, Sweden, recently attended the Second Hands-On Training Course for Clinical Microbiologists organised by Burkhard Tümmler and colleagues in Hannover, Germany. We asked EvaLena for her thoughts about the course.

The EuroCareCF Hands-On Training Course on the *Processing of Microbiological Specimens from Individuals with Cystic Fibrosis* was held at Hannover Medical School between 10-13 April 2008. Burkhard Tümmler and Helga Riehn-Kopp of the Clinical Research Group at the Children's Clinic organized the course. Seventeen attendees from 13 European countries (Turkey included) participated. I, as the only participant from Sweden, represented the Karolinska University Laboratory.

Our laboratory serves the Stockholm Centre of Cystic Fibrosis, which is one of four CF-centres in Sweden. This centre takes care of approximately 220 patients out of the 580 nationwide. Because I have medical responsibility for specimens from the respiratory tract in our laboratory, including CF-sputa, I was very keen to attend this Hands-On course. Also, I was very curious about how other microbiological laboratories had performed in the first European Quality Assurance Trial of CF Microbiology, which our laboratory had participated in during November and December 2007. Our own results were unfortunately not very encouraging. Which methods did the others use?

The lectures comprised several topics, including diversity of colony morphologies of several species, hypermutators, multi-drug resistance and emerging gram-negative pathogens. In addition, there were many presentations that informed us about new technologies for identification, susceptibility testing and epidemiology studies. We also learned about interactions between *Staphylococcus aureus*

and *Pseudomonas aeruginosa*, and how *P. aeruginosa* adapts to the CF-lung. As almost two weeks have now passed, I think I have forgotten something...

Between the theoretical lectures and the practical exercises there were opportunities to socialise and, as it was my first meeting in the CF-field, I made many useful contacts. I found it very interesting to compare our methods with that of other colleagues'. It was encouraging to find that, in general, our laboratory uses similar procedures as elsewhere. In some aspects I find our routines compared with the other laboratories a little over-cautious, while other routines could do with some improvements. For example, we do not find any SCV (small colony variants), neither of *S. aureus* nor of *P. aeruginosa*. To be able to identify another new acquaintance, *Inquilinus limosus*, and even other emerging gram-negative pathogens, we must keep our *B. cepacia* selective agar plate for more than 48 hours!

When the review from the European Quality Assurance Trials was presented on Sunday morning, I realised that our lab was in good company. Due to shortage of time (we received the samples the same day the template should be returned) we were not able to use sequencing, which was necessary to identify three of the strains. So next trial...

Finally, I would like to thank Burkhard, Helga and EuroCareCF for organizing this substantial and intensive course, and for arranging pleasant social activities, which were highly appreciated.

Dr EvaLena Ericson, M.D



Course lecturers and participants



Elza Rakhimova (MHH, Hannover) giving explanations to participants

EuroCareCF events at the 31st ECFS Annual Conference 11-15 June 2008 - Prague, Czech Republic

Please check the [website](#) of the 31st ECFS Congress for up-to-date information.

11 June 2008

14:00-17:00 Workpackages 4 & 5—Drug development for CF: The European Experience (North Hall)

Chairs: David Sheppard and Andreas Reimann

14:00-14:10 Welcome and Introduction - David Sheppard

Part I - drug development in CF - the academic experience

Chair: David Sheppard

14:10-14:30 What happens when a compound hits: Partners and patenting facilities in French academic labs - Frédéric Becq

14:30-14:50 Discovering and characterizing NCEs - how to partner with industry - Louis Galietta

14:50-15:00 Discussion

15:00-15:15 Coffee Break

Part II: drug development in CF: the industry perspective

Chair: Andreas Reimann

15:15-15:35 CF and its role in the development portfolio in "big pharma" - the Novartis case - Jens Grueger (tbc)

15:35-15:55 CF as a main focus of a company: Galapagos (tbd)

Part III: drug development in CF - the role of patient organisations and regulatory authorities

Chair: Andreas Reimann

15:55-16:15 The role of European CF-organisations in CF-drug development - Franck Dufour

16:15-16:35 How can national and European regulatory authorities support CF-drug development - (tbc)

16:35-16:55 Discussion on Parts II and III

16:55-17:00 Concluding remarks

14:00-17:00 Work Package 3 Diagnostic Group/ECFS Diagnostic Group Meeting (Meeting Hall 1)

CF, Atypical CF or no CF - That is the question. Chair: M. Wilschanski

14:00-14:20 A new American consensus on CF diagnosis-C. Castellani

14:20-14:40 Polymorphisms and the diagnosis of CF-M. Schwartz

14:40-15:00 CF Diagnostic dilemmas from down under-P. Middleton

15:00-15:20 CFTR immunofluorescence: an aid for the diagnosis? - I. Sermet

15:20-15:40 Break

Nasal Potential Difference Measurements

Chair: K. De Boeck

15:40-16:15 Different methods, different results - I. Bronsveld

16:15-17:00 Nasal PD standardisation efforts: general discussion

12 June 2008

12:00-14:30 EuroCareCF/ECFS CF Registry Steering Committee (North Hall)

18:30-21:30: **EuroCareCF WP5/WP6 Partnering Meeting**—Will new animal models and new strategies achieve and efficacious gene therapy for the treatment of CF patients? (Panorama Hall)

Motion 1: Other animal models than cystic fibrosis mice can be more predictive of the efficacy of CFTR gene transfer

For the motion: Chris Boyd

Against the motion: Bob Scholte

Motion 2: In utero gene therapy has therapeutic potential for the treatment of CF lung disease

For the motion: Charles Coutelle

Against the motion: Joseph Rosenecker

18:15-19:15 EuroCareCF/ECFS CF Registry Meeting (North Hall)

18:30-21:00 **Workpackage 1 Clinical Care: CF treatment focused on adult patients issues** (Forum Hall)

Chairpersons: Carla Colombo and Dorota Sands

18:30-18:40 Introduction-Carla Colombo

18:40-19:00 Liver disease in adults-Dominique Debray

19:00-19:20 Airway glucose as a biomarker and flexible insulin treatment of CFRD-Khin Gyi

19:20-19:35 Discussion

19:35-19:55 Bone mineral density in CF: are there consequences in the adult age?-Isabelle Sermet

19:55-20:20 Chronic pulmonary insufficiency. Home oxygen therapy in Poland-Wojtek Skorupa

20:20-20:40 Recurrent and difficult pneumothorax and major haemoptysis-Khin Gyi

20:40-21:00 Discussion

15-16 June 2008

2nd EuroCareCF Workshop on CFTR-related Diseases (Kaiserstejn Palace)

15 June 2008

09:00 Welcome: M. Macek; D.N. Sheppard, P.F. Pignatti

Introduction to the meeting: P. F. Pignatti, C. Ferec

Objective: Classification of diseases as "CFTR related"

09:30–11:00 A/ Clinical point of view:

- "Classical" CF - J. Dodge, D. Bilton
- "CFTR-RDs" – C. Castellani, P. F. Pignatti
- Andrologist's view: J. Zielenski

EuroCareCF events at the 31st ECFS Annual Conference (Continued) 11-15 June 2008 - Prague, Czech Republic

- Pneumologist's view: S. Elborn, E. Kerem
 - Gastroenterologists view: P. Durie, A. Munck
- 11:00-11:30 Break
- 11:30-13:00 B/ Functional point of view: I. Sermet, G. R. Cutting
- Sweat test: P. M. Farrell
 - NPD: M. Wilshanski
 - *In vitro* tests: D. N. Sheppard, G.R. Cutting
- 13:00-14:00 Lunch
- 14:00-15:00 C/ Genetics of CFTR-RDs: J. Zielenski, B-S. Kerem
- CBAVD: M. Stuhmann, M. Claustres
 - Chronic pancreatitis: C. Ferec, M. Tzetis
 - Disseminated bronchiectases: C. Bombieri, P.F. Pignatti
 - Complex allele R117H -5/7T : A. Munck, H. Cuppens
 - Complex allele R117H -5/7T- relevance to NBS: K. Southern
- 15:00- 15:30 Break
- 15.30 – 16.00 D/ Diagnostic algorithms for CF and CFTR-RDs
European perspective: E. Girodon
US perspective: G.R. Cutting
- 16:00 – 18:00 General discussion: C. Ferec, P. F. Pignatti, C. Castellani

19:30-23:00 Dinner at Restaurant Nebozizek: www.nebozizek.cz

Monday 16 June 2008

- 09:00 – 10:00 E/ Towards an European CFTR-RD database: C. Bareil, M. Claustres
- for CBAVD
 - Chronic pancreatitis
- F/ Coordination of contributions to the European CFTR-RD database:
How to organize it ? How to contribute ? How to finance it ?
- 10.00 – 10.30 Break
- 10:30 – 12:30 Synthesis of both days: P.F. Pignatti, C. Ferec, D.N. Sheppard
- Working group to write a final report, including recommendations for the Classification of diseases as CFTR-related
- Project for the future, including publication of the report in a scientific journal
- 12:30 Lunch and Conference Adjourns

Forthcoming events

ERS Research Seminar: Host-pathogen interactions in the lung: Implications for treatment of respiratory infections and inflammatory lung diseases, Hochgebirgsklinik—Davos, Switzerland: **20-22 June 2008**. For further information e-mail Robert Bals (bals@staff.uni-marburg.de)

WP6 Animal Models—Workshop:

Animal models of lung disease, Rotterdam. For further information, please e-mail Bob Scholte (b.scholte@erasmusmc.nl)

The BA Festival of Science, Liverpool: **6-11 September 2008**.

For further information, please visit www.the-ba.net

EuroCareCF is organising an educational event with the Physiological Society to highlight the latest advances in CF research. Further information about this event will be published later on the EuroCareCF website

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