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Editorial

A very warm welcome to the fifth EuroCareCF newsletter. In this issue, we report on the first EuroCareCF partnering meeting organised jointly by Workpackage 4 (SMEs) and 5 (Novel Therapies). I would like to take this opportunity to thank Andreas Reimann, Massimo Conese and their colleagues for organising this important event.

Partnering meetings aim to build effective working relationships between academic researchers and companies (especially Small and Middle-Sized Enterprises; SMEs) active in the CF area. Close cooperation between researchers, clinicians and companies is imperative for the development of new therapies for CF patients. The effort that participants made to attend the first EuroCareCF partnering meeting demonstrated clearly that EuroCareCF was meeting an unmet need of the community. However, as the meeting progressed, it was also clear that participants had very different reasons for attending the meeting. In general, the academic researchers had novel ideas at an early stage of development, which they desired to see translated into therapies for CF patients. By contrast, the SMEs sought to make contact with clinical researchers experienced in the conduct of clinical trials of new therapies for CF.

In no way should the first EuroCareCF partnering meeting be considered a failure. It proved an important forum for researchers and SMEs to discuss the problems that they face turning laboratory discoveries to effective therapies for CF patients. For many researchers, the difficulty they face is how far do they need to progress a novel therapeutic before a company will licence that therapeutic. With the establishment of specific funding programmes for translation research by CF charities and national funding agencies, this type of research is becoming easier to undertake. For SMEs, there is also the prospect of significant help with the conduct of clinical trials of new therapies for CF. In close cooperation with the ECFS, Kris De Boeck and her colleagues in Workpackage 3 of EuroCareCF are preparing the groundwork for a European Clinical Trials Network for CF. Kris and her colleagues will report on these and other developments from Workpackage 3 in a forthcoming EuroCareCF newsletter.

David Sheppard

EuroCareCF Partnering Meeting

Frankfurt, Germany: 15 May 2007

From Bench to Bedside

– How Academic Science and Companies Can Make It Work

WP5: Novel Therapies

The partnering meeting between WP4 ("Small- and Middle-sized Enterprises") and WP5 ("Novel Therapies") was held in Frankfurt on the 15th of May.

The main aim of this meeting was to foster collaboration between academic centres and SMEs through the reciprocal exchange of knowledge. This reciprocity is based on the advantage that both kind of entities could have from such interplay: the SMEs might have access to basic research, whereas academic centres can take advantage on the resources (human and technical) that cannot be found in a single laboratory. The ultimate goal is to find a way for transferring novel therapies from the bench to the bedside.

Representatives of academic centres were invited to present their research lines about the following subjects:

- Gene and cell therapy (Fiorentina Ascenzioni, Dirk Schindelbauer, Joseph Rosenecker);
- Pharmacological therapies aimed to correct the basic CF defect (Frederic Becq, Olga Zegarra-Moran, David Sheppard, Hugo de Jonge, Jordi Ehrenfeld)
- Antimicrobial therapies (Robert Bals, Giulio Cabrini, Susanne Häußler, Tania Pressler)

All representatives presented their data especially the tools and expertise for their research needs and suitable for collaboration with SMEs:

- New vector formulations for transfer of large DNA molecules
- Cellular models for testing these novel formulations
- Bacterial culture scale up
- Automation of gel purification for DNA production

- High throughput screening (HTS) for identifying and testing novel drugs aimed to correct the basic CF defect ("correctors" and "potentiators")
- Production/collection of analogs of hits
- Medicinal chemistry, preliminary pharmacology: toxicity and ADME analysis in animal models
- Primary cultures of respiratory epithelial cells (CF and non-CF)
- CF mouse models and their availability
- In vivo lung inhalation devices for small molecules and gene therapy agents
- Pharmacology of ion channels potentiators and inhibitors
- Libraries of chemicals to evaluate their anti-inflammatory potential

For SMEs, the necessity of enrolling more patients for Phase II and III clinical trials was highlighted.

The major conclusion we can draw from this meeting is, from the academic centers point of view, that future partnering meetings should involve larger size industries which are interested in developing and exploiting:

- 1) novel DNA formulations;
- 2) new correctors and potentiators coming from HTS;
- 3) chemicals with anti-inflammatory potentials.

Massimo Conese

WP4: Small and Middle-Sized Enterprises

The CF-community is fortunate to have highly committed and qualified groups working on potential new treatments at both sides of the Atlantic. While, of course, many findings from basic research may not lead instantly to new formal developing projects, there is exciting science that may make a difference to patients once applied properly. As new scientific concepts emerge, the need to bring them to the patients as quickly as possible is obvious. However, academic scientists are often lacking both experience and resources to translate their brilliant findings into a successful pharmaceutical development. Vice versa, in particular small and middle-sized companies may neither have the competencies nor the financial resources to sustain basic research. It makes therefore perfect sense to bring these parties together. A first joint meeting of work packages 5 (novel therapies) and 4 (small and middle-sized enterprises) trying to achieve exactly that, was held May 15th, 2007 at Frankfurt/Main airport. Leading scientists from 13 academic groups covering both

projects aiming at correcting the basic defect in CF and microbiological therapy presented potential areas of co-operation with industry. 10 companies currently developing products for CF presented their current CF-franchise and projects under development. They pointed out their future areas of interest and their development capabilities and expertise highlighting opportunities for co-operation with academic centres. Participants agreed that this was a useful meeting to find out potential opportunities and hurdles. However, while the academic centres focused very much on projects in a very early development phase, SME's were mostly interested in speeding up clinical development of late-stage projects. There is therefore still an important gap to close. EuroCareCF will help to better address that gap.

Andreas Reimann

Hands-on Training Course on the Processing of Microbiological Specimens from Individuals with CF

Hannover, Germany 3 - 6 May, 2007

Organiser: Prof. Dr. Burkhard Tümmler,
Hosts: Helga Riehn-Kopp, Burkhard Tümmler
Medizinische Hochschule Hannover (MHH)

The 'Hands-on course on the processing of microbiological specimens from individuals with cystic fibrosis' took place at MHH from May 3 – May 6, 2007. Twenty clinical microbiologists from 13 EU countries took part in the course. Each participant provides service in CF microbiological diagnostics for large CF centers. The programme consisted of lectures, demonstration activities and hands-on practical work at the bench.

All participants processed freshly obtained microbiological specimens from CF patients of different age and disease severity and determined the species and the antimicrobial susceptibility of the isolates. Uncommon and emerging pathogens were demonstrated on plates. The demonstration activities dealt with CF specific features of pathogens such as morphotype and hypermutability and more general issues of the characterization of isolates from CF airways such as transcriptome, proteome, genotyping, molecular taxonomy and population biology.

The lectures covered;

- CF pathophysiology,
- pathoadaptive mutations of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in CF airways,
- susceptibility testing, multidrug resistance and hypermutation,
- infection epidemiology and hygienic measures, and
- the ecology, taxonomy and physiology of Burkholderia spp. and emerging pathogens in CF.

One of the major objectives of this course was to establish an 'European Network for Quality Control of the Processing of Microbiological Specimens from Individuals with CF'. Els Dequeker from Leuven runs an European Network for Quality Control of Genetic Diagnostics of CF and she reported on a more than 10-year experience of how much *CFTR* genetic analysis has gained from the annual trials performed by the most proficient human genetics laboratories in Europe. By the end of the meeting the participants agreed to start quality assurance trials among CF microbiology laboratories and to prepare consensus guidelines for CF microbiology diagnostics within the next 18 months.

Burkhard Tümmler

Abstract from a demonstration activity of the Hannover workshop:

Colony morphology variations of *Pseudomonas aeruginosa*

Elza Rakhimova, Medizinische Hochschule Hannover

Once cystic fibrosis (CF) patients become colonized by *P. aeruginosa*, there is a subsequent gradual deterioration in lung function, which determines the course and prognosis in most CF patients. Despite the fact that chronically infected CF patients harbour only one or few *P. aeruginosa* genotypes¹, there is significant phenotypic variation in *P. aeruginosa* isolates from the CF lung, known as dissociative behaviour². The chronically infected CF lung is thought to provide a habitat where *P. aeruginosa* in high cell densities faces a multiplicity of environmental challenges that lead to morphological diversification and the establishment of niche specialists³. In the context of chronic *P. aeruginosa* CF lung infection, attention has long focused on the appearance of the mucoid *P. aeruginosa* phenotype⁴ (Fig.1.a). Other phenotypes, however, including autoaggregative morphology (Fig.1.b) small colony variants (SCVs) (Fig.1.c (black arrow)), colonies with visible autolysis (Fig.1.d) and others were described^{5,6,7,8}. The clinical SCV isolates from different patients were identified to have increased fitness, better biofilm-forming capabilities and a high adherence to a pneumocytic cell line⁹. Even autolysis, which might seem unambiguously detrimental to a unicellular organism, is an adaptive behaviour of *P. aeruginosa* mediated by over production of PQS being an extracellular signal increasing the stringent response and formation of protective biofilm by released DNA after the cells lysis¹⁰. Moreover, c-di-GMP levels regulate the differentiation of the *P. aeruginosa* population into macroscopic cell aggregates and planctonic cells¹¹. Thus, the

appearance of divergent morphology is the result of adaptive genetic diversification resulting in increased chances of bacterial survival under the harsh environmental conditions present in the lung.

¹Breitenstein, S. et al (1997). Direct sputum analysis of *P.aeruginosa* macrorestriction fragment genotypes in patients with cystic fibrosis. *Med Microbiol Immunol.* 186, 93–99.

²Zierdt, C. H. & Schmidt, P. J. (1964). Dissociation in *P. aeruginosa*. *J Bacteriol.* 87, 1003–1010.

³Oliver, A. et al (2000). High frequency of hypermutable *P. aeruginosa* in cystic fibrosis lung infection. *Science* 288, 1251–1254.

⁴Govan, J. R. W. & Deretic, V. (1996). Microbial pathogenesis in cystic fibrosis: mucoid *P. aeruginosa* and *Burkholderia cepacia*. *Microbiol. Rev.* 60, 539–574.

⁵Mary, J. K. et al (2005). Characterization of colony morphology variants isolated from *P. aeruginosa* biofilms. *Appl. Environ. Microbiol.* 71, 4809–4821.

⁶Sriramulu, D. D. et al (2005). Microcolony formation: a novel biofilm model of *P. aeruginosa* for the cystic fibrosis lung. *J. Med. Microbiol.* 54, 667–676.

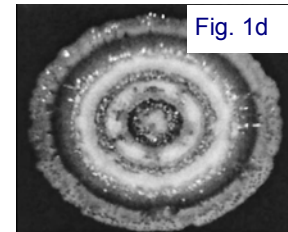
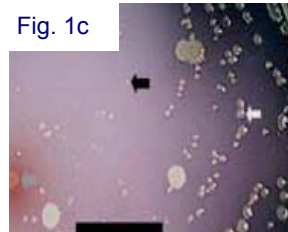
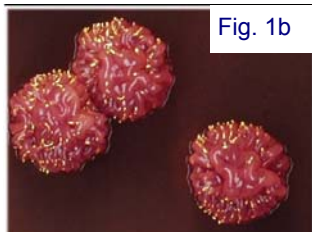
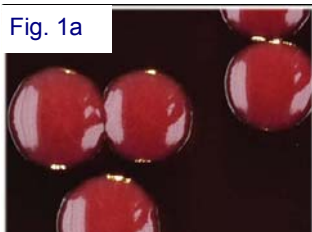
⁷Friedman, L. & Kolter, R. (2004). Two genetic loci produce distinct carbohydrate-rich structural components of the *P. aeruginosa* biofilm matrix. *J. Bacteriol.* 186, 4457–4465.

⁸Drenkard, E. & Ausubel, F. M. (2002). *Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature* 416, 740–743.

⁹Hausler, S. et al (2003). Highly adherent small-colony variants of *P. aeruginosa* in cystic fibrosis lung infection. *J. Med. Microbiol.* 52, 295–301.

¹⁰D'Argenio, D. A. et al (2002). Autolysis and autoaggregation in *P. aeruginosa* colony morphology mutants. *J. Bacteriol.* 184, 6481–6489.

¹¹Klebensberger, J. et al (2007) Detergent-induced cell aggregation in subpopulations of *Pseudomonas aeruginosa* as a pre-adaptive survival strategy. *Environ. Microbiol.* 9, in press.



Hannover Medical School



Hannover Medical School (Medizinische Hochschule Hannover, MHH) is the only medical faculty in Germany that operates as an independent university with about 50,000 inpatients and 150,000 outpatients per year. The MHH is internationally most renowned for transplant medicine. Research focuses on infection, immunity and inflammation, transplants and stem cell research and biomedical technology and implants. There are two CF clinics on the campus that provide care for about 500 individuals with CF. The clinic for adults is run by the Department of Pneumology and that for children and adolescents by the Department of Pediatric Pneu-

mology and Neonatology. About 75% of all CF lung transplants in Germany are performed at the MHH campus.

Pictures of Workshop on Epithelial Biology 2–6 July



Leo from Brazil discusses CF clinical problems with Karl Kunzelmann

Workshop on Epithelial Biology was successfully organised by WP7. A detailed report will appear in a subsequent Newsletter and EuroCareCF website.



Harriet from Sweden is fascinated with the Port-a-Patch technique



Happiness is written on everyone's face

- participants and organisers in front of Faculty of Sciences, Lisbon University after the last section of the course



David's passion for channel gating

EuroCareCF Workshops at ECFS Basic Science Conference

25 – 29 April 2007 Tavira, Algarve, Portugal

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

WP7 Integration of Fundamental Research

Margarida Amaral (University of Lisboa), the leader of workpackage 7 (WP7) introduced the EuroCareCF project to conference delegates. She highlighted the specific aims of WP7, its resources for basic research (e.g. CFTR antibodies) and the opportunities for expert training provided by the workpackage.

Scott Randell (University of North Carolina; UNC) discussed the use of well-differentiated primary cultures of human airway epithelia in CF research. He revealed the number of lung transplants performed globally in 2006 and highlighted how little the CF community exploited this invaluable resource. Randell reviewed the activities of the UNC CF tissue procurement and cell culture core including tissue procurement, cell isolation, training and quality control. To demonstrate the quality of the primary cultures generated by the core, Randell showed movies of mucus hurricanes formed by the movement of mucus over the surface of normal airway epithelia.

Primary cultures of airway epithelial cells can be passaged no more than 5 – 9 times before senescence develops. To circumvent this problem, airway epithelial cells can be immortalised with sv40ER or hTERT. However, Randell warned that immortalised cells do not last indefinitely. Instead, they frequently become unstable, losing their ability to form polarised epithelia before undergoing senescence. Randell explained that senescence was caused by induction of the cyclin-dependent kinase inhibitor p16, but that a transcription repressor of p16, the oncogene Bmi-1 had been identified. Randell reported that his group had simultaneously infected six cell lines (3 normal and 3 CF) with lentiviruses expressing Bmi-1 and hTERT. These cell lines had grown normally for up to 40 passages, whereas without Bmi-1, cells senesced prior to passage 20. Using chamber experiments with the Bmi-1 / hTERT transformed cells at passage 15 revealed that these cell lines had the same functional properties as primary cultures, suggesting that they were likely to be a valuable resource for the CF community. Randell closed his presentation by calling for improved worldwide procurement of lung tissue, urging that use of this valuable resource should be the rule, not the exception.

Melissa Ashlock (CF Foundation; CFF) briefly reviewed the activities of CFF especially those aimed at providing resources to the CF community. She emphasized that CFF had a deep focus on the provision of resources and indicated that CFF fund a number of groups to provide core facilities. These cores supply cell lines, antibodies, small molecules and microbiological reference material. Ashlock requested feedback from CF researchers to guide the development of further tools for the community. She highlighted the CFF's efforts to develop guidelines for translation research and the standardisation of outcome measures (e.g. nasal potential difference and sweat chloride concentration assays). Finally, Ashlock announced that CFF had revamped its website and encouraged researchers to use the site (www.cff.org).

Robert Dormer (University of Cardiff) discussed endpoints for the assessment of therapies aimed at the CFTR defect. He began by defining endpoints, arguing that changes in clinical endpoints were



often slow and difficult to follow. Instead, simple, rapid, non-invasive bioassays were required that permitted repeated sampling, ideally with patients serving as their own controls. Dormer considered which CFTR functions to assay and the assays that could be used *in vivo* and *ex vivo*. Importantly, Dormer raised the concern about whether a single positive result in a single assay was sufficient to merit the progression of a candidate therapy from the laboratory to a clinical trial. But equally, are positive results in a battery of tests required before a candidate drug is allowed to progress? Perhaps instead, the answer lies somewhere between these extremes. Dormer discussed the endpoints currently used in clinical trials in the UK and USA highlighting the difficulty of finding endpoints that effectively predict efficacy. He speculated about the value of biomarkers and whether they might be tested with healthy volunteers or would be specific for CF patients. In concluding, Dormer was optimistic that efficacious therapies would be easy to identify.

In summary, the workshop proved a valuable discussion of the provision of resources for CF research and endpoints for therapy evaluation. Each presentation was followed by a lively discussion, with the audience contributing valuable comments and suggestions.

WP6 Animal Models

This workshop discussed CFTR knockout and ENaC overexpressing mice as models for CF lung disease and alveolar fluid clearance.

Bob Scholte (Erasmus Medical Center Rotterdam) reviewed the variety of mouse cells and tissues affected by CFTR mutation as well as some of the different measurements that can be made with CF mice. He then considered two myths about CF mice. First, the idea that CF mice have no lung phenotype. Scholte reminded the audience that CF mice develop "spontaneous" lung inflammation and tissue remodelling and when challenged with bacteria demonstrate reduced clearance and increased inflammation. The question is whether this phenotype can be compared to what we observe in humans. Scholte argued strongly that the problem was not with mice, but with man! There was a crucial lack of knowledge about the early stages of CF lung pathology in humans. This lack of knowledge contributed significantly to the myth that CF mice have no lung disease. Second myth: CF is caused by hyperabsorption of Na⁺. Scholte reminded those present that a large amiloride-sensitive short circuit current did not necessarily equate to a large

Continues on page 3

Na⁺ and concurrent water flux under open circuit (*in vivo*) conditions, and that great care was required when interpreting the effects of amiloride on airway epithelia. Direct measurement of all relevant flux parameters and resistances under physiological conditions is still virtually impossible in animals as well as in differentiated cell culture systems. This results in an explanatory model that necessarily relies on assumptions. Here too, we cannot answer the question of what actually happens in human lungs for lack of methods and material. A CF pig model, that is being developed by Mike Welsh et al (Iowa, USA) may help to answer some of these questions.

Scott Randell (University of North Carolina; UNC) provided an update on the mouse strain that over expresses the β ENaC subunit (*Scnn1b*) in airway cells. This mouse displays a lung phenotype characterised by reduced periciliary liquid height, increased solid content of mucus, mucus plugging, goblet cell hyperplasia and reduced bacterial clearance (*Nat Med* 10(5) 487-93,2004). He reported that the CF Research Center at UNC had begun backcrossing β ENaC mice onto different genetic backgrounds. The data indicate that β ENaC mice survive best on a Black 6 background (available from Jackson), intermediate on a Balb C background and worse on a C3H background. Interestingly, however, lung pathology appeared similar on the different genetic backgrounds. Randell did not know the identity of the modifier gene(s) responsible for the difference in survival of β ENaC mice, but indicated that he and his colleagues were actively seeking to identify the modifier gene(s). Data suggest that the IL4 receptor, which is involved in abundance of mucous secreting cells in neonatal mice is not responsible for goblet cell hyperplasia in *Scnn1b* mice. Mucus stasis and pre-existing inflammation in *Scnn1b* mice affect sensitivity to viral infections (Sendai). This model is useful to understand the pathogenesis of diseases such as CF characterised by impaired mucus clearance.



Carole Planès (INSERM U773, Paris) then discussed the role of ENaC in alveolar fluid clearance (AFC) studied with the β -Liddle strain of mouse harbouring a gain-of-function mutation within the *Scnn1b* gene. She began by showing that at the level of histology, the lungs of the ENaC L/L mice were no different from those of wild-type (+/+) mice, in contrast to the

model overexpressing *Scnn1b* from a CC10 promoter. The expression of the α and γ ENaC subunits were normal, but expression of the β subunit was depressed. Planès reported several interesting differences between ENaC L/L and wild-type (+/+) mice. First, the ENaC L/L mice had a three-fold increased level of baseline AFC compared to wild-type (+/+) mice. Second, the cAMP agonist terbutaline stimulated AFC in wild-type (+/+) mice, but not ENaC L/L mice.

Third, volume-overload caused alveolar oedema in wild-type (+/+) mice, but not ENaC L/L mice. Because cardiac function was similar in wild-type (+/+) and ENaC L/L mice, Planès interpreted her data to suggest that ENaC plays a crucial role in transepithelial alveolar Na⁺ transport and AFC in the mouse. She also speculated that therapies, which enhance ENaC activity might hasten the resolution of hydrostatic pulmonary oedema. In discussion it was pointed out that a study by Lindert et al in CF mutant mice has provided evidence that the basal state of the alveolar epithelium is secretory, which is at least in part Cfr dependent. This appears to contrast with the finding in AFC experiments, which clearly shows ENaC dependent alveolar resorption upon challenge with an instilled fluid volume. This suggests that this tissue, like other secretory epithelia is able to shift gear from secretion to resorption.

Jörn Karhausen (University of Tübingen) discussed the possible relationship between the (inflammatory) tissue response to hypoxia and the apparent pro-inflammatory phenotype in CF patients and mouse models. Interestingly, Karhausen speculated that CFTR dysfunction may affect the cytokine signalling pathways activated by hypoxia, which may contribute to the inflammation observed in CF. He discussed experiments to test the hypothesis that the hypoxia response would be affected in CF mouse models.

Teresinha Leal (Université Catholique de Louvain, Brussels) reported that primary cultures of murine peritoneal and alveolar macrophages display different patterns of cytokine expression in response to lipopolysaccharide. Further, she demonstrated an inflammatory imbalance in peritoneal macrophages from CF mice characterised by pro-inflammatory cytokine (TNF- α) upregulation and anti-inflammatory cytokine (IL-10) downregulation. Leal also found that the proinflammatory IL-1 β was upregulated in CF alveolar macrophages, whereas TNF- α and IL-10 levels were comparable to normal. The anti-inflammatory macrolide azithromycin reduced this upregulation to normal levels, but was without effect on peritoneal macrophages. Leal concluded first that the CF mouse model is characterised by a 'spontaneous' state of inflammation not only in the lungs but also in the peritoneum, that may at least in part be the result of abnormal behaviour of resident macrophages/monocytes. Second, the anti-inflammatory effects of azithromycin previously shown in LPS challenge experiments with CF mutant mice (Leal 2006) are mediated, at least in part, by the drug's action on alveolar macrophages.

Taken together, the presentations highlighted the value of CFTR knockout and ENaC overexpressing mice in understanding lung physiology, the pathogenesis of CF and therapy development.

Hongyu Li and Bob Scholte

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Forthcoming Events

EuroCareCF Pro-Con Debate on the Treatment of the Na⁺ Hyperabsorption Defect in Cystic Fibrosis

Apex City Quay Hotel & Spa, Dundee, UK: 5 September 2007

The focus of the novel therapies workpackage for EuroCareCF is to design schemes for the development of novel therapies for CF. Taking advantage of the gathering in Dundee of experts on Na⁺ transport in airway epithelia, we are organising a Pro-Con Debate on the value of therapeutic strategies that target Na⁺ hyperabsorption in CF airways. The debate will address two motions pertinent to the development of rational new therapies for CF:

Motion 1: Inhibition of Na⁺ hyperabsorption in CF airways has therapeutic potential for the treatment of CF lung disease

For the motion: Marcus Mall (University of Heidelberg, Heidelberg, Germany)

Against the motion: Stephen T. Ballard (University of South Alabama, Mobile, USA)

Motion 2: Restoration of function to CF mutants will correct Na⁺ hyperabsorption in CF airways

For the motion: Chris Poll (Novartis, Horsham, UK)

Against the motion: Bob J. Scholte (Erasmus University, Rotterdam, The Netherlands)

After the presentations for and against each motion, there will be a general discussion. Based on notes taken at the debate a consensus report will be drafted and circulated to debate participants for approval.

For further information about the EuroCareCF Pro-Con Debate, please contact Sheila Krawczyk (e-mail: s.krawczyk@dundee.ac.uk)

If you are unable to attend the debate, but would like to contribute to the consensus report, please contact Hongyu Li (e-mail: H.Li@bristol.ac.uk)

Cell Signalling and Lung Development Meetings

Apex City Quay Hotel & Spa, Dundee, UK: 5 September 2007

Dundee, Scotland will be the venue for a series of meetings in September focused on cell signalling and lung development organised by Anil Mehta and colleagues.

7th International Congress of the NDP Kinase/NM23/awd Family

Apex City Quay Hotel & Spa, Dundee, UK: 2-5 September 2007

The multifunctional gene family known as NDP kinases, Nm23 and *Drosophila* awd have important roles in cell physiology, the pathogenesis of genetic diseases (e.g. cystic fibrosis) and cancer metastasis. This conference will provide an in-depth review of the field and present new, mechanistic data of both basic and translational importance.

Special Lung Development Symposium in honour of Professor RE Olver, University of Dundee

Apex City Quay Hotel & Spa, Dundee, UK: 5-6 September 2007

This symposium will celebrate Professor Richard Olver's research on fetal lung development. It will include presentations on lung and tracheal development, ENaC and CFTR channel regulation, ion transport by surface and gland epithelial cells, mouse models of lung disease and rational approaches to therapy for CF.

For further information about the NDP kinase conference and the Lung Development symposium, please see the link below:

<http://www.dundee.ac.uk/mchs/ndpk/>

2007 North American CF Conference

Anaheim Convention Center, Anaheim, California: 3-6 October 2007

EuroCareCF will be organising several workshops as satellite meetings to this year's NACF conference, including workshops on orphan drugs and mouse models of CF. Please see the EuroCareCF website for further information: eurocarecf.eu

3rd Scientific Meeting of the Italian Society for CF (SIFC)

Milano, Italy: 29 November-1 December 2007

Details to follow. Please see the EuroCareCF website for further information: eurocarecf.eu

Training Grant Opportunities in Patient Care 'Managing the care of children and adults with CF' and European Respiratory Society 'CF School'

To provide specialist training in patient care for all members of the multidisciplinary team, EuroCareCF training grants may be used to cover the cost of attending the following patient care training courses:

Managing the care of children and adults with CF

Organiser: Su Madge

Venue: Royal Brompton Hospital, London

Date: 5-day course run twice each year in April and September

European Respiratory Society 'CF School'

Provisional Date: March 2008

Further information to follow; covers many aspects of CF including

lung disease, gastrointestinal disease, diagnosis, terminal care, complications of CF, psychosocial aspects, microbiology etc

These training courses are open to all members of the multidisciplinary CF team.

For further information about both courses, please contact Su Madge (e-mail: s.madge@rbht.nhs.uk)

If you would like to apply for a EuroCareCF training grant to attend either of these courses, please complete the application form (available from the EuroCareCF website) and return it to David Sheppard (e-mail: D.N.Sheppard@bristol.ac.uk).

Notices

Applications are invited for a post-doctoral position in the UK to study CFTR

Based in the Institute for Cell and Molecular Biosciences, University of Newcastle, Medical School, Newcastle upon Tyne, UK (<http://www.ncl.ac.uk/camb/>, 5/5* RAE 2001), you will join a laboratory studying the role of CFTR in epithelial anion transport. Our recent research (Wright *et al.* 2004 *J. Biol. Chem.* 279:41658) has shown that CFTR activity is controlled by a novel mechanism involving changes in the Cl⁻ concentration of the extracellular (luminal) fluid. Such regulation is important for epithelial bicarbonate secretion and the control of luminal surface pH in CF-affected epithelia. The major goal of this project is to delineate the molecular mechanism underlying this Cl⁻ dependent control mechanism, as well as identify the Cl⁻ binding site on the CFTR molecule. This will be achieved by combining macroscopic and microscopic analysis of current records from normal and mutant forms of CFTR, as well as studying the enzymatic properties of the purified protein, in collaboration with scientists from the US and Canada.

The project is funded by the Wellcome Trust for up to 3 years and

technical support specific to the project will also be available. The ideal candidate will have experience in patch clamp electrophysiology, and preferably in single channel recordings.

Further information about the project can be obtained by contacting Dr. Mike Gray (m.a.gray@ncl.ac.uk).

Closing date: Friday 20 July 2007.

Applicants should submit a full CV and covering letter with the names and addresses of two referees to Mrs. Joanna Keith, Institute Manager, Institute for Cell & Molecular Biosciences, University of Newcastle, Medical School, Newcastle upon Tyne NE2 4HH. E-mail: Joanna.Keith@ncl.ac.uk to arrive no later than **20 July 2007**. **It is essential that you also return a completed Employment Record form.** The Employment Record form may be downloaded from the University web page: <http://www.ncl.ac.uk/vacancies/employ.rtf>

Training Grant News

Stojka Naceva— Fustik's training visit to Carla Colombo in Milan, Italy

Stojka Fustik (pictured right) is Head of the CF Centre at Skopje, Macedonia. She recently visited Carla Colombo in Milan to learn about the management and treatment of CF liver disease.

The EuroCareCF training program enabled me to spend two weeks at the Cystic Fibrosis Center in Milan. The main goal of my training visit was to learn about the management and treatment of chronic liver disease in CF patients. Our CF team has insufficient experience in the management of complications of chronic liver disease, especially portal hypertension. Therefore, I visited Professor Carla Colombo, a leading expert in CF liver disease, so that I can provide better care to CF patients in Macedonia.

During my two week training visit, I received comprehensive training in the management and treatment of liver disease in CF. I was acquainted with all diagnostic procedures for identification of liver disease and estimation of disease severity, including clinical examination, liver function tests, ultrasonography, hepatobiliary scintigraphy, liver biopsy and magnetic resonance imaging. Moreover, I attended several echographic examinations of the upper abdomen. I saw many patients with liver disease at inpatient and outpatient clinics and discussed each specific case with Carla and her colleagues. On the first day of my visit to the CF Center in Milan, there was an emergency admission of a patient with portal hypertension and varical bleeding. The patient had a transjugular portosystemic shunt (TIPS) inserted 4 years ago. To stop the bleeding, an endoscopy variceal ligation was performed.

CF patients with more advanced liver disease, severe portal hypertension and hypersplenism are the most serious problems encountered in the clinic. I studied the literature and discussed with Professor Colombo the therapeutic interventions in patients with complications from portal hypertension. Prophylactic variceal ligation or sclerotherapy is not recommended because it is not effective in reducing subsequent bleeding. CF patients with portal hypertension and hy-

persplenism may remain in a stable condition for years.

Once a varical haemorrhage has occurred, sclerotherapy or banding of varices is the immediate therapeutic procedure, together with resuscitation with plasma, blood transfusion and medical treatment. If bleeding is recurrent, surgical portosystemic shunting may be considered in a selective group of patients. TIPS has been reported as a good alternative method for portal decompression. The advantages of TIPS over surgical shunts are that there is a lower risk of encephalopathy, the procedure can be performed without general anaesthesia, and complications like shunt stenosis or occlusion are rare with the new models of TIPS stents. Therefore, TIPS is the preferred method in the Milan Clinic as the bridge to liver transplantation in patients with limited liver function. I attended the procedure to insert TIPS in one adult patient. Finally, I also visited the liver transplant centres in Milan (Ospedale Maggiore) and Bergamo. I discussed the experiences of transplant surgeons, especially the indications and optimal timing for liver transplants for CF patients. The centres prefer to perform liver transplantation prior to the development of end-stage liver disease and critical deterioration of lung function.

My training visit to the CF Centre in Milan was a great experience for me and has opened up possibilities of establishing contacts with colleagues from specialist centres and of initiating collaborations with them. I would like to express my gratitude to Professor Carla Colombo and her team for their hospitality and efforts to make my study visit in Milan a beneficial and fruitful experience.



Stojka Naceva - Fustik

Marta Palma's training visit to California Pacific Medical Centre Research Institute (CPMCRI)

To continue her training in the production of novel cell lines from normal and CF airway epithelia, Marta Palma from Lisboa visited the laboratory of Dieter Gruenert in San Francisco to learn about the immortalisation of primary cultures

The main goal of my training visit to the California Pacific Medical Centre Research Institute (CPMCRI) was to learn the techniques to transform and immortalise primary cultures of human epithelial cells to produce novel cell lines.

The development of immortalised cell lines has been of significant benefit to the study of human diseases. This has been particularly relevant to the analysis of inherited diseases such as cystic fibrosis (CF) where the paucity of material provided by tissue and primary cell systems limits studies of the CF defect, including biochemical and metabolic correlations with disease pathology.

However, a major disadvantage of primary epithelial cell cultures is

their limited lifespan. Although airway epithelial cell lines can provide good model systems for investigating biochemical mechanisms underlying airway epithelial cell function, limitations are often encountered because the cells senesce. One approach to overcome this difficulty is to transform airway epithelial cells. Transformed cells can either be directly obtained from airway carcinomas or they can be generated by exposing airway epithelia to a variety of agents including chemicals, physical stress or viruses. The transforming agents used to induce immortalisation of airway epithelial cells include: SV40(pSvori-), HPV, Adeno-SV40, or the hERT/oncogenic plasmid. Once transformed, epithelial cells have an enhanced or

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Marta Palma's training visit to CPMCRI (continued)

even unlimited lifespan.

The transformation protocol that I learnt during my training visit consisted of:

1. Isolating primary airway epithelial cells
2. Transfecting/infecting cells with the transforming agent of choice
3. Growing cells to confluence
4. Waiting for the untransformed cells to die
5. Isolating individual clones or a mixed population of clones

("pool") and

6. Characterising cells in terms of desired phenotypic features (morphology, immunocytochemistry for given markers, electrophysiology, etc)

For further information about the protocol, please see the EuroCareCF website (www.eurocarecf.eu).

I will now employ this protocol to generate novel epithelial cell lines at the University of Lisboa.

Marta Palma

Eric Alton's laboratory is a popular place to learn about the assessment of novel therapies for CF lung disease. Below, Elena Copreni from Milano and Nico Derichs from Hannover report on their training visits to Eric Alton's laboratory

Elena Copreni's training visit to National Heart & Lung Institute, UK

Eric Alton's group at the National Heart and Lung Institute, Imperial College, UK focuses on the development of gene therapy for CF. To determine the efficacy of gene transfer to airway epithelia, they have developed a series of new end points to evaluate gene transfer. My training visit enabled me to learn about these novel therapeutic end points for validation of gene transfer in preclinical models of airway epithelia. They include air surface liquid (ASL) depth analysis, nasal potential difference measurement and tissue processing for immunochemistry in the mouse nasal epithelium. Another goal of my training visit was to encourage collaboration between European CF groups.

During my training visit, I learnt several techniques—mouse nose dissection and tissue processing for ASL depth analysis, tissue processing for immunochemistry and protein detection in the mouse nasal epithelium. I aim to put these techniques into practice in Massimo Conese's lab at the Fondazione Centro San Raffaele del Monte Tabor in Milan and use them as standard methods to validate gene transfer into the airway epithelium. My intention is to use

these end points to study the efficacy of a lentivirus-based RNAi strategy to down-regulate the epithelial sodium channel (ENaC) in CF and β ENaC transgenic mice. The shRNA-lentiviral vector expressing a cassette for the inhibition of alpha ENaC subunit expression and the marker gene NGFR (Nerve Growth Factor Receptor) will be administered through intranasal instillation in mice and the efficiency of gene transfer will be evaluated by the NGFR gene expression performed by immunohistochemistry on nasal epithelia tissues. The activity of the shRNA-lentiviral vector will be investigated by ASL depth analysis and nasal potential difference measurement in lentiviral-transduced and control mice.

My training with Eric Alton and his colleagues allowed me to greatly improve my skills and knowledge in gene transfer approaches for the treatment of CF-related diseases. I presented my research project at a seminar and had the opportunity to discuss with Eric Alton's group and to start up collaborative programmes.

Elena Copreni

Nico Derichs' training visit to National Heart & Lung Institute, UK

During my stay in London, I intended to learn more about the preparation of respiratory epithelia from sheep and mice as a model for ion transport studies of human airway epithelia. In particular, I wished to learn about the mounting of airway tissue in Ussing chambers and the measurement of short-circuit current to evaluate transepithelial ion transport.

This training is important and directly relevant to the work that I undertake at the Medizinische Hochschule Hannover. The transplant centre at the Medizinische Hochschule Hannover performs many lung transplants (adult and pediatric) and we are keen to examine transepithelial ion transport by respiratory tissue from CF lung explants in our Ion Transport Laboratory. The aim of our work will be ex vivo preclinical testing of CFTR pharmacotherapeutics both in our established setup of intestinal current measurement (ICM) on rectal biopsies and in the new setup of lung explants from CF patients with different *CFTR* mutations.

During my 3-day visit, I received very useful hands-on training in the preparation of freshly obtained sheep tracheal/bronchial tissue and its adequate preservation for mounting into Ussing chambers. I also learnt about the preparation of mouse nose tissue and its subsequent electrophysiological characterisation in Ussing chambers. The training included information about the basal respiratory tissue properties and the responses of the epithelium to agonists.



Nico Derichs