PROJECT FINAL REPORT

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4.1 Final publishable summary report

Executive Summary

Primary Ciliary Dyskinesia (PCD) is a rare, genetically heterogeneous disorder resulting from dysfunction of multiple motile cilia. It causes severe, chronic destructive airway disease with progressive loss of lung function. Currently, management of PCD is deduced from observations made in other respiratory disease, as there is a complete lack of evidence-based knowledge. Diagnosing PCD is a difficult process requiring multiple diagnostic tests as well as intensive training of personnel. Hence, PCD is often unrecognized or the diagnosis is established too late.

The main goal of BESTCILIA was to characterize the clinical course and to improve the diagnosis and treatment of PCD patients. This goal of BESTCILIA has been fully achieved:

We have established an international prospective PCD registry (http://www.pcdregistry.eu) for systematic data collection on incidence, clinical presentation, treatments and course of the disease. As of 31/05/2016, 428 individuals with PCD have entered the registry and a manuscript was published to summarize the achievements and to raise awareness of the registry. This registry now represents the crucial basis for clinical and translational research in PCD on European and global level and will be maintained and extended after the BESTCILIA funding period.

Early diagnosis and appropriate treatment strategies are important to prevent further lung damage in PCD. We therefore developed and implemented standardized diagnostic tests for PCD in three European Centers of countries with low health care expenditures (Cyprus, Greece, Poland) as prove of principle to reach patients with delayed or missing diagnosis. The necessary equipment for the performance of the diagnostic tests in Cyprus, Poland and Greece was acquired and installed in a uniform manner. The newly diagnosed PCD cases increased in the three countries by 21.4% demonstrating the success of the BESTCILIA strategy to implement standardized diagnostic tests in countries where access or expertise was limited prior to BESTCILIA. This approach is paradigmatic for other countries with limited diagnostic facilities and will be continued after the BESTCILIA funding period.

We also performed an observational trial for answering pertinent questions on the clinical phenotype of PCD, severity, prognosis and effect of treatments on outcomes. We have performed an individual patient data meta-analysis of all existing cross-sectional and longitudinal datasets, which include data from more than 3000 PCD patients. We initially evaluated growth, weight and lung function as relevant marker for disease progression and confirmed that these criteria have to be included in PCD follow ups for the characterization of disease progression and treatment success. Further analysis of this dataset will give a much more detailed picture of the disease spectrum and will represent the basis for evidence based diagnostics and treatment approaches.

To date, no medications to treat PCD have been approved by regulatory bodies, and a major obstacle to monitor disease progression and evaluate new treatments is the lack of disease-specific outcome measures. Current outcome measures, such as spirometry, chest computed tomography and lung clearance index all have limitations in terms of their sensitivity and feasibility for evaluating new therapies or disease progression. Importantly, these physiological measures do not reflect the impact of the disease on patients' daily symptoms or functioning (e.g., physical, respiratory, social) as required by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

Thus, measures were needed to assess the impact of PCD, from the *patient perspective*, on all domains of daily functioning. Health-related quality of life (HRQoL) measures are valid, reliable and informative indices of symptoms and functioning and sensitive to patient concerns. However, at start of BESTCILIA no validated HRQoL measures were available for patients with PCD. We therefore developed harmonized (North America and Europe) pediatric PCD-specific HRQoL instruments informed by guidance from the FDA and EMA to be used as primary or secondary outcomes in clinical trials. In addition, because patient-reported outcomes measures demonstrate optimal reliability and validity when they are specific to the respondent's developmental stage, we developed three separate age-appropriate versions of this instrument: children (aged 6-12 years); adolescents (aged 13-17 years), and parent proxy (for children aged 6-12 years). This HRQoL has been optimized, translated into different languages and validated and will now be used as a valid outcome measure for clinical trials and longitudinal observational studies in PCD.

In addition, we have performed the first ever multi-centre randomized controlled clinical trial on the use of azithromycin in PCD. This still ongoing trial not only aimed on the determination of efficacy and safety of azithromycin (AZN) in PCD but also successfully served to explore new outcome measures such as multiple breath washout (MBW) and the new health-related quality of life questionnaire HRQoL and to promote evidence-based guidelines for PCD.

Overall, BESTCILIA achieved all the objectives defined at the beginning of the project and raised awareness of PCD on different levels through successful dissemination of BESTCILIA research towards the research community and general public. BESTCILIA influenced the field of PCD Research and clinical care of PCD patients to an incomparable extend. BESTCILIA will continue to influence PCD research and clinical care through international collaborations which have been firmly established during BESTCILIA and which continue within e.g. the framework of the European funded COST Action BEAT-PCD.

Description of project context and objectives:

Primary Ciliary Dyskinesia (PCD) is a rare, genetically heterogeneous disorder that affects approximately 1 in 20,000 individuals. It results from dysfunction of small hair-like organelles (cilia), which are responsible to clean our upper and lower airways. It causes severe, chronic destructive airway disease with progressive loss of lung function. Additional health burden results from chronic ENT problems impairing hearing. In addition, many other organ systems such as the cardiovascular (congenital heart defects) and reproductive system (infertility) can be affected as well. Currently, management of PCD is deduced from observations made in other respiratory disease, as there is a complete lack of evidence-based knowledge. However, this strategy is unsafe as PCD is unique in pathophysiology. PCD is underdiagnosed and patients are being diagnosed too late. Although unproven, clinical experience and observational reports suggest that early diagnosis is important for the preservation of pulmonary function, quality of life and life expectancy; unfortunately, appropriate studies to validate this hypothesis are lacking. Whereas in other rare respiratory diseases such as CF, evidence-based guidelines and treatment strategies have significantly contributed to improved survival and quality of life, PCD individuals have not yet benefited by similar progress. Therefore, there is a critical need for observational trials as well as randomised controlled trials to put evidence-based diagnostic and treatment approaches for PCD into effect. The main goal of BESTCILIA was to characterize the clinical course and improve the diagnosis and treatment of PCD patients. To accomplish this, we worked on 5 S&T Objectives:

S&T Objective 1: To perform an observational trial for answering pertinent questions on clinical phenotype of PCD, severity, prognosis and effect of treatments on outcomes. This includes an individual patient data (IPD) meta-analysis of all existing cross-sectional and longitudinal datasets (WP1).

WP1 aimed to identify all existing cross-sectional and longitudinal datasets of PCD patients from clinical databases, national or regional registries and research projects in Europe and the US. It described the information contained in these datasets and pooled them in an individual patient data (IPD) meta-analysis to answer pertinent questions on clinical phenotype, disease severity, prognosis and effect of treatments on PCD outcomes. We used this pooled dataset to (1) Describe the spectrum of clinical phenotypes and disease severity in PCD patients by age-group, sex and time period of diagnosis in a cross-sectional analysis; (2) Describe short-term and long-term prognosis of PCD, looking at important outcomes such as death, lung function, bacterial colonisation, hearing loss, fertility and quality of life; and (3) Identify predictors of poor outcomes, such as age at diagnosis, current age, year of diagnosis, clinical phenotype, genotype, treatments, frequency of clinical follow-up, size of treatment centre and specialists involved in PCD clinics.

S&T Objective 2: To establish an international prospective PCD registry for systematic data collection on incidence, clinical presentation, treatments and course of the disease. This allowed to monitor trends in incidence, management and outcomes and to recruit patients for

studies (WP2).

WP 2 aimed to establish an international prospective PCD registry for systematic data collection on incidence, clinical presentation, treatment and course of the disease. This registry is an instrument to monitor trends in clinical presentation, diagnostic accuracy and applied treatments, monitor changes in long-term outcomes and recruit patients for multicentre clinical studies. This work package interacted with other work packages: WP3 (introduction of diagnostic testing in European countries): newly identified patients of WP3 have been entered in the registry and thus profit from participation in future trials. WP1 has collected data retrospectively providing essential initial information. However, the available data in WP1 are heterogeneous and the common dataset for the meta-analysis has limitations. In contrast, the registry established in WP2 is a tool for an international data collection in a systematic and prospective manner, which will continue after the BESTCILIA funding period. This allows monitoring international trends in diagnosis, management and outcomes and shows how efforts to improve diagnosis and treatments will change clinical practice and long-term outcomes.

S&T Objective 3: To improve clinical practice in PCD by introducing standardized diagnostic testing for PCD in European countries, where this is currently not available (WP3).

WP3 aimed to improve equity of access to specialist PCD services and enhance diagnostic accuracy by the establishment of PCD diagnostic centres in areas of Europe where PCD centres were unable to meet ERS diagnostic guidelines. This approach included the development of standardized operational procedures (SOPs) for PCD diagnostic testing. This work package also aimed to assess changes in diagnosing PCD, before and after the introduction of SOPs for diagnostic testing, to compare nasal Nitric Oxide (nNO) measurements using a cheaper electrochemical method with the more expensive chemiluminescence gold standard. Greece, Cyprus and Poland were selected as model countries to demonstrate how PCD diagnostic services meeting the European consensus criteria can be established because they fulfilled the following criteria:

- (Pediatric) pulmonology services are available and can be used as BESTCILIA study centres.
- In all three countries, there are national representatives acting as active members in the European PCD Task Group ensuring a high motivation in participating in the project.
- These countries show differences in PCD epidemiology: Cyprus has the highest PCD prevalence in Europe whereas in Greece, there are virtually no PCD cases diagnosed. In Poland, the PCD prevalence is not known but estimated to be in the normal European average. Thus, it will be possible to describe the effects of establishing PCD diagnostic services in low-incidence (Greece), medium-incidence (Poland) and high-incidence (Cyprus) countries.

S&T Objective 4: To establish PCD-specific Health-Related Quality of Life Questionnaires (HRQOLQ) as an outcome measure (WP4).

The development and cross-sectional and longitudinal validation of age-appropriate Health Related Quality of Life (HRQoL) questionnaires for patients with PCD. The HRQoLQs will be developed and validated using the gold standard methods described by Guyatt et al. (item generation, item reduction, internal and test-re-test reliability, construct validity and content validity) in English, before translating and performing cross-cultural validation in other European languages. Procedures will comply with EC, FDA and WHO Guidelines for development of health surveys. Partners 7 and 8 and their collaborators have extensive experience of HRQoLQ development, validation and use in Europe and North America.

S&T Objective 5: To perform a randomized controlled clinical trial on the use of azithromycin in PCD (WP5).

The aim of WP5 was to perform a randomized controlled trial (RCT) to determine the efficacy and safety of azithromycin (AZN) in PCD in order to provide future evidence-based treatment of PCD airway disease and concurrently explore usefulness of new outcome measures such as PCD specific HRQoL and Lunge Clearance Index (LCI).

Description of the main S&T results/foregrounds:

In BESTCILIA **WP1** has successfully identified available international cross-sectional and longitudinal datasets of PCD patients. Anonymous data from eligible studies have been obtained, evaluated, standardised and pooled into a retrospective international PCD cohort (iPCD Cohort). The iPCD Cohort dataset is continuously being updated, and its further maintenance and development is supported by the EU COST Action Beat-PCD. New centres are still joining and some participating centres are still adding patients, or more data on registered patients. By May 2016, data from 21 single centres or Consortia from 18 countries had been contributed. The pooled dataset contained data on 3013 patients. First analyses on cross-sectional and longitudinal data focus on growth and lung function. Analyses on different topics using the iPCD Cohort dataset are on-going or planned. A manuscript related to the data identification process, titled "Clinical manifestations in Primary Ciliary Dyskinesia: systematic review and meta-analysis" (Goutaki et al) has been accepted for publication by the European Respiratory Journal (in press).

Main achievements of WP1 include

- Data from over 3000 PCD patients collected.
- Data contributed from 18 countries (21 singles centres or Consortia) and more potential data contributors identified and contacted.
- A manuscript related to the data identification process, titled "Clinical manifestations in Primary Ciliary Dyskinesia: systematic review and meta-analysis" (Goutaki et al) has been accepted for publication by the European Respiratory Journal (in press)
- A manuscript titled "The International Primary Ciliary Dyskinesia (iPCD) Cohort: methods and first results" (Goutaki et al) that describes the methods of the iPCD Cohort, the collected dataset and on-going and planned analyses has been submitted (June 2016) to the European Respiratory Journal.
- WP1 progress and results of analyses of cross-sectional data have been presented in national and international congresses (including the ERS conferences 2014 and 2015) and meetings with patients' organisations, physicians and collaborators.
- Two abstracts will be presented at the ERS conference 2016 in London presenting analyses of longitudinal data on growth and lung function.

<u>iPCD Cohort dataset</u>

By April 2016, we have received cross-sectional data from 18 countries (AR, AUS, BE, CA, CH, CY, D, DK, FR, IL, IT, NL, NO, PL, RS, TR, UK, USA). The iPCD Cohort contains now data on 3013 patients. Patients' age ranges from 0 to 77 years; 50% are male.

Not all sub-cohorts contain information on all variables. General information, results of diagnostic tests, and baseline characteristics are available for all 3013 patients. Data on growth are currently available for about 1500 patients and on lung function for almost 1000 patients. Additional data such as microbiology or perinatal history have been delivered by

several groups. Data from 2581 patients have already been cleaned and brought into the standardized format.

Results of iPCD Cohort data analysis

a. Poor growth caused by PCD

Poor growth is a common problem in children with severe chronic respiratory disease, but little is known for PCD. We assessed height and body mass index (BMI) in the multinational iPCD Cohort dataset, compared to WHO reference values as well as to published national growth reference values.

We analysed 5967 measurements of height and weight from 1498 paediatric and adult PCD patients from 19 centres internationally. We used both World Health Organization (WHO) growth reference data and national reference values to calculate z-scores for height and BMI. To account for repeated measurements we used a multilevel model, adjusting for age, sex and study centre.

The mean age of patients at the time of measurement was 19.3 years (range 0-75). We found a lower height compared to the WHO references (z-score: -0.13, $p<0.005$, 95% CI: -0.19 to -0.07). Compared to national references the difference in height was even larger (z-score: -0.29, $p<0.005$, 95% CI: -0.35 to -0.22). We found a higher BMI compared to WHO references (z-score: 0.21, $p<0.005$, 95% CI: 0.15 to 0.27) but the difference was smaller compared to the national references (z-score: 0.08, $p=0.013$, 95% CI: 0.02 to 0.15). Compared to their healthy peers (national references) both sexes and all age groups had a lower height. Results differed by country, but very few centres presented no difference in height z-scores between the PCD patient population and the norm.

Currently, we are determining patient characteristics associated with growth, especially level of lung function, level of diagnostic certainty, age of diagnosis and time passed since diagnosis. A manuscript reporting the results of this analysis is in preparation, to be submitted later in 2016.

Region H, SOTON, UNC, RHB, CUT and VUMC have contributed data for this analysis, provided feedback to the submitted abstracts and contribute to the preparation of the manuscript.

b. Lung function in PCD

Data on lung function in patients with PCD are few and contradictory. We compared lung function (FEV1) of patients with PCD to the Global lung function initiative (GLI) 2012 reference values and to published data from UK patients with Cystic Fibrosis (CF; Goss et al. Thorax 2015).

We calculated z-scores and % predicted values for FEV1 using the GLI reference, and used a multilevel linear regression model, adjusted for age, sex and study centre, to account for repeated measurements.

We obtained 4683 FEV1 measurements of 648 PCD patients from 15 centres. The median age was 16 years (range 6-70), 295 (46%) were females. In the multilevel model FEV1 z-scores (95% CI) were significantly lower than GLI values (FEV1 -1.5 (-1.6 to -1.4); p<0.005). Both sexes and all age groups were affected, with a smaller difference for children aged <10 years (FEV1 z score -1.18 (-1.3 to -1.05).

Compared to published data for CF, FEV1 % predicted (95% CI) was similar in children (e.g. age 6-9: PCD: 87% (86 - 89%); CF: 90% (88 - 91%)); but better in adult PCD patients (age 18-21: PCD: 76% (73 - 80%); CF: 66% (65 - 68%)).

This is the largest study ever conducted on spirometry in patients with PCD. FEV1 was significantly reduced compared to normal reference values for all age groups and both sexes. Young children with PCD and CF both had similarly low FEV1; in older patients, PCD patients had a better lung function than CF patients; although significantly lower than the normal population.

To date, **longitudinal data** have been contributed for 542 patients from 10 countries, with a follow-up period ranging from 2 to 20 years. First analyses using longitudinal data focus on growth and lung function.

a) Changes in height and BMI in children and adolescents with PCD during the growth period (preliminary results)

In the analysis of cross-sectional of growth data, we had found significantly lower height and slightly lower BMI in PCD patients younger than 20 years of age compared to national reference data. We now proceeded to assess changes in height and BMI over time during the growth period.

For this, we used longitudinal measurements of height and BMI from PCD patients of the iPCD Cohort from 10 countries. We calculated height and BMI z-scores using national references and used generalized estimating equations (GEE) to identify changes over time, as a function of sex.

We analysed 1833 repeated measurements of height and BMI from 343 patients aged 0-20 years. We found a significantly lower height (z-score: -0.16, 95%CI: -0.30 to -0.02, p=0.03) and BMI (-0.15, -0.31 to -0.03, p=0.05) in PCD patients compared to reference. We found no significant change in height z-scores (p=0.93) but a small increase in BMI z-scores (0.02, 0.01 to 0.03, p<0.001) over time compared to normal values. Height (p=0.30) and BMI (p=0.13) z-scores did not differ by sex.

Height z-scores were significantly reduced in children with PCD compared to reference values, but they did not change over time. This might suggest that PCD affects growth already at an early stage of life but there is no catch-up until the age of 20. In the on-going study, we will look at birth weights, stratify by levels of diagnostic certainty and determine patient characteristics associated with growth, such as lung function, microbiology and clinical characteristics.

Eventually, this work will contribute to discover preventable risk factors for poor growth, and help to design interventions studies.

b) Lung growth in children and young adults with PCD (preliminary results)

In the analysis of cross-sectional lung function data, we had found significantly lower lung function (FEV1) in a multinational cross-sectional dataset of patients with PCD compared to the Global lung function initiative (GLI) 2012 reference values. We now proceeded to assess changes in FEV1 over time in children and adolescents with PCD (i.e. lung growth).

In our analysis we included longitudinal measurements of FEV1 from PCD patients from 11 countries using the iPCD cohort. We calculated FEV1 z-scores using the GLI reference and used generalised estimating equation (GEE) models to identify changes of lung function over time, as a function of sex.

We analysed 1431 repeated measurements of FEV1 from 276 patients aged 6-20 years. We found a significant decline in FEV1 z-scores over time (per year: -0.07, 95% CI: -0.08 to -0.06, $p<0.001$) compared to reference values. We did not find differences in FEV1 z-scores between sexes ($p=0.79$).

FEV1 z-scores of PCD children and adolescents declined significantly over time compared to normal reference, which suggests that lung growth of PCD patients is already impaired from early childhood. In the on-going study, we will determine patient characteristics associated with changes in lung function, for example BMI, level of diagnostic certainty, microbiology and available clinical characteristics.

Eventually, this work will contribute to discover preventable risk factors for poor lung function, and help to design intervention studies.

In **WP2**, we have established a prospective international PCD registry for systematic data collection on incidence, clinical presentation, treatment and course of the disease. Thus, an instrument to monitor clinical presentation of PCD, accuracy of diagnostic tests, applied treatments, changes in long-term outcomes as well as recruitment of patients for multicentre clinical studies has been made available. The registry is accessible via the Internet - http://www.pcdregistry.eu. Generation of registry items has been completed by 31.05.2013. Registry items include: age at diagnosis, family history (consanguinity), associated malformation/diseases, laterality defects, clinical manifestation (otitis media, rhinosinusitis, pneumonia, bronchiectasis, neonatal respiratory distress syndrome), microbiological results, diagnostic findings (video microscopy, electron microscopy, nasal NO, immunofluorescence, radiological findings), therapeutic measures (inhalation, antibiotics, oxygen, ventilation, ENT surgery, lung surgery), and quality of life. Meanwhile, all partners have obtained ethical approvals to enter data and have started entering data. As of 31/05/2016, 428 individuals with PCD have entered the registry. A manuscript was published to summarize the achievements and to raise awareness of the registry (Werner C, Lablans M, Ataian M, Raidt J, Wallmeier J, Große-Onnebrink J, Kuehni CE, Haarman EG, Leigh MW, Quittner AL, Lucas JS, Hogg C, Witt M, Priftis KN, Yiallouros P, Nielsen KG, Santamaria F, Ückert F, Omran H. An

international registry for primary ciliary dyskinesia. Eur. Respir. J. 2015; ERJ – 00776–02015.).
As a feedback to diverse dissemination activities, PCD centres have approached us from Austria, Belgium, Germany, Israel and Slovakia who wish to join the registry consortium as soon as they have completed all necessary legal and ethical steps. Beyond BESTCILIA partners, several new registry centres now have started recruiting patients in Germany, Austria and Italy.
The above-mentioned manuscript also comprised a cross-sectional data analysis. Until July 2015, 201 individuals had been enrolled. Gender distribution was 110 (54.7 %) females and 91 (45.3%) males. Out of the 201 individuals, 98 (48.8 %) were under 18 years old and 103 (51.3 %) were adults. The median age of participants was 18 years with a range from 1 month to 76 years.

Using linear regression analysis, we were able to determine a mean annual decline of FEV1% predicted of 0,59% in our cohort.

We also evaluated, which methods were used to diagnose PCD. Diagnostic tests used for PCD work-up included nasal nitric oxide measurement (nNO), high speed video microscopy (HVMA), transmission electron microscopy (TEM), immunofluorescence microscopy (IF), and genetic analysis. HVMA was most commonly used (144 individuals, 70%). It was abnormal in 140 individuals. Among 142 cases with nNO testing, 10 individuals (5%) exhibited normal results. TEM showed normal results in 30 out of 119 tested individuals (25.2%) with definite PCD. IF was performed in 69 individuals. In 48 patients, IF showed clearly abnormal findings, whereas in 21 individuals, IF staining was normal. Genetic results were available in a subset of 40 individuals, 30 of which displayed biallelic disease causing mutations. The majority of individuals had two (n=50; 25%) or three (n=61; 30%) tests. Results from all five tests were available only in a subset of individuals. In most cases, findings from two to three tests were available.

In sum, we have established an international PCD registry as described in objective 2. Both BESTCILIA WP2 partners and additional non-BESTCILIA centres still actively recruit patients and contribute to the collection of data.

In **WP3**, standardized diagnostic tests for PCD have been developed and implemented in three European centres of countries with low health care expenditures (Cyprus, Greece, Poland) to reach patients with delayed or missing diagnosis; these centres are now able to continuously recruit patients for PCD-work-up. The necessary equipment for the performance of the diagnostic tests in Cyprus, Poland and Greece was acquired and installed in a uniform manner. In parallel, awareness campaigns in each country with the support of newly established patient organizations managed to develop referral networks to detect and direct for testing new suspect PCD cases. Diagnostic testing of new suspect cases and known ambiguous cases started in April 2014. The newly diagnosed cases by month 24 were 15 and by month 42 (end of the project) 51, demonstrating an increase in diagnosed patients in the three countries by 21.4%.

In addition, meta-analyses of published evidence on the diagnostic tests (nNO, TEM, HSVM) for PCD have been performed (Kouis P, Papatheodorou SI, Yiallouros PK. Diagnostic accuracy of nasal nitric oxide for establishing diagnosis of primary ciliary dyskinesia: a meta-analysis. BMC Pulm Med. 2015 Dec 3;15:153. doi: 10.1186/s12890-015-0147-3; Kouis P, Yiallouros PK, Middleton N, Evans JS, Kyriacou K, Papatheodorou SI. Prevalence of Primary Ciliary Dyskinesia in consecutive referrals of suspect cases and the Transmission Electron Microscopy detection rate: A systematic review and meta-analysis. Submitted to Pediatric Pulmonology. A description of the baseline picture in Cyprus with regards to PCD diagnosed cases has been published (Yiallouros PK, Kouis P, Middleton N, Nearchou M, Adamidi T, Georgiou A, Eleftheriou A, Ioannou P, Hadjisavvas A, Kyriacou K. Clinical features of primary ciliary dyskinesia in Cyprus with emphasis on lobectomized patients. Respir Med 2015; 109(3): 347-356).

From April 2014 to May 2016 serial measurements of nasal NO production rate with the Ecomedics chemiluminescence analyser and the NIOX MINO electrochemical analyser were performed in the new referred suspect cases/ the retested known ambiguous cases/ the known patients with missing diagnostic tests.

In total 186 patients (in whom their PCD/non-PCD status was elicited) had measurement of nNO with Ecomedics analyser and 112 with the NIOX MINO. In summary, both analysers were able to discriminate between PCD and non-PCD patients in almost all cases. The nNO production rate values for PCD patients with the Ecomedics analyser was median 22.44 nl/min (Inter Quartile Range: 12.0-55.4) and for non-PCD patients: median 149.0 nl/min (IQR: 103.4-226.0).

The distribution of nNO production rates for NIOX MINO was 13.2 nl/min (IQR:6.4-25.7) for PCD patients and 84. nl/min 4 (IQR:53.6-119.7) for non-PCD patients. The comparison of the diagnostic accuracy of the two methods for measuring nNO is under way and a manuscript is expected to be submitted for publication by early 2017.

WP4 has successfully developed, validated and translated age-appropriate quality of life questionnaires for patients with PCD (QOL-PCD). QOL-PCD is available in seven languages; translations into 6 additional languages are in progress. The QOL-PCD has been rapidly adopted as an outcome measure by the research community, not only in the BESTCILIA clinical trial (WP5) but also for a forthcoming commercial trial (Parion/ Vertex) and a number of investigator-led studies. It is also contributing to clinical care providing longitudinal monitoring of patients in a number of countries. Dissemination of our success include peer reviewed manuscripts, and symposium talks & abstract presentations at major international conferences.

Noteworthy achievements include:

- QOL-PCD was developed for adults, children (6-12 years), adolescents (13-17 years), and parent respondents initially in the English language, according to FDA and EMA guidelines. The developments have been presented in peer reviewed publications and in abstracts at international meetings, e.g.Lucas JS, Behan L, Dunn Galvin A, Alpern A, Morris

AM, Carroll MP, Knowles MR, Leigh MW, Quittner AL. A quality-of life measure for adults with primary ciliary dyskinesia: QOL-PCD. Eur Respir J. 2015 Aug; 46(2):375-83.

- Psychometric validation of the English versions of QOL-PCD has taken place in UK, Ireland, USA and Canada. We have demonstrated that the adults' version of QOL-PCD is reliable, valid and responsive to change; validations for the children's versions are nearing completion. The manuscript using data from the adult version has been submitted to the American Journal of Respiratory Critical Care Medicine; the data has also been submitted as a 'late breaking abstract' to European Respiratory Society Congress. Data for the children's versions will be submitted by October 2016.

- English, Danish, Dutch and German languages versions of QOL-PCD are being used as a secondary outcome measure for the randomised clinical trial (RCT) in WP5

- The children's electronic version of QOL-PCD is available in different languages as a 'talking version' in which the instructions, questions and response options are read to the child. This allows children to answer independently even in settings were a researcher is not available to administer the questionnaire. There are also electronic adult, teen and parent-proxy versions of QOL-PCD. Electronic versions have been made available also for use in German, Danish and Dutch. Pen-and paper versions are available to ensure the measures can be used in a variety of settings.

- In addition to conducting translations for the BESTCILIA RCT, QOL-PCD has been translated and validated (through comprehensive cognitive interviews) in Greek and French. Translations and cognitive validations are underway in Turkish, Arabic, Spanish, Italian and Spanish (Latin America).

WP5 is conducting the very first multi-centre RCT on pharmacotherapy in PCD. This trial will facilitate future RCTs on pharmacotherapy in PCD by exploring new outcome measures such as multiple breath washout (MBW) and the new health-related quality of life questionnaire (developed in WP4) as exploratory secondary outcome measures (WP5) and promote evidence-based guidelines for PCD.

The trial protocol has been adjusted to be acceptable to PCD management and treatment as well as national regulations in five European countries and has passed a general scientific assessment for clinical trials in EU member states, the so-called 'Voluntary Harmonization Procedure' (VHP). To date, 86 patients have been randomized in the trial and more patients have been screened. Recruitment of trial cases is still going on at all centres.

An amendment to the trial protocol was prepared in spring 2015. The amendment contained an increase in age range, less strict withdrawal criteria, and a prolongation of the trial duration. This amendment has been approved by competent authorities and ethics committees in the five participating countries. A second batch of blinded trial medication has been produced and delivered to the trial sites. Recruitment for the trial is still on-going. In conclusion, WP5 is conducting the first-ever multi-centre RCT on pharmacotherapy in PCD.

This trial will facilitate future RCTs on pharmacotherapy in PCD by exploring new outcome measures and promote evidence-based guidelines for PCD.

A manuscript on the study protocol, rationale and recruitment in the RCT has recently been accepted for publication in an open access journal.

Main achievements of WP5 include:

- Completion of remaining regulatory tasks required before initiation of the RCT could take place at each of the trial sites.
- Implementation of equipment for Multiple Breath Washout measurement (new outcome measure) at the remaining trial sites and continuous training in performing and analysing the measurements.
- Final parts of translation of QOL-PCD (new outcome measure) into German/Swiss German and Dutch; conducting cognitive interviews in the respective languages; and preparation of the speaking questionnaire for children.
- Setup and initiation of the European multi-centre trial at all six trial sites.
- Recruitment, screening and randomization of patients at all trial sites. In total, 86 patients have passed screening and have been randomized.
- Conduction of the RCT, in which participation lasts 7 months for each patient and includes at least five trial visits. Continuous data capture and monitoring.
- Preparation of an amendment to the trial protocol and approval of this amendment by competent authorities and/or ethics committees in all participating countries.
- Prolongation of clinical trial insurances due to the prolonged trial duration.
- Preparation and distribution of a second batch of blinded trial medication (azithromycin tablets and identical placebo tablets) by Dutch hospital pharmacy.
- Continuous maintenance of electronic Case Report Form for the trial, which has been prepared by an external consultancy in Denmark.
- Preparation of a manuscript on the study protocol, rationale and recruitment in the RCT, which has been accepted for publication in an open access journal.

The potential impact and the main dissemination activities and exploitation of results:

In Europe, there are marked discrepancies between countries in the availability of PCD diagnostic services. Thus, in order to fulfil the aim to improve clinical practice in PCD care, we have generated standard operation procedures (SOPs) and introduced standardized diagnostic testing in countries where this has not been available before. This approach is paradigmatic for other countries with limited diagnostic facilities. Little is known about PCD epidemiology and clinical presentation. We have collected the largest dataset worldwide on available cross-sectional data about PCD. Analysis of this dataset will give a much more detailed picture of the disease spectrum. The international PCD registry is a long-term project, it will provide information on epidemiology, clinical presentation of PCD, accuracy of diagnostic tests, effect of treatments, and changes in long-term outcome of PCD.

We have generated age-appropriate quality of life questionnaires for PCD patients that will be available to the public as outcome parameter of clinical studies. We have conducted the first ever multi-centre RCT on pharmacotherapy in PCD. This trial will facilitate future RCTs. This trial, together with the international PCD registry represents the first step towards evidence-based guidelines for PCD.

Knowledge of PCD diagnostics and management has been provided to caretakers via both internet (http://bestcilia.eu/) and several workshops and scientific meetings **(WP7)**. We have not only targeted patients, their families and family support groups, but also broader public, specifically medical caregivers, who are key targets of our dissemination activities as the nexus for building trust with and awareness to PCD patients. A webpage and brochure articulating BESTCILIA has been made publicly available (http://bestcilia.eu/) providing both scientific information and easy to understand information on PCD in different languages (http://bestcilia.eu/what-is-pcd/). So far, BESTCILIA members have published 38 scientific articles, 30 conference proceedings, and 2 book chapters, presented 139 oral presentations and 29 posters, and organized 25 meetings, courses or workshops, including 2 external workshops for clinicians and 1 workshop for PCD patients.

The project website has been operating since April 2013 and has been regularly updated by IIMCB with information on project work package (WP) progress, BESTCILIA publications and BESTCILIA-related dissemination activities and events. After the relatively few notes published in the first period of the project (**16**), the number of notes published in the second reporting period rapidly increased, reaching total of 68 notes at the end of the project (31st of May 2016).

The project website has also been a place of dissemination for information materials on PCD in 6 languages, which have been published on the project website from 09-2013 to 02-2014 (Task 7.2, see Deliverable Report 7.2) and are still available there. In Cyprus, the materials were used during the recruitment process for WP3 (Task 3.2). In Poland, the Polish version of the materials was used during the workshop for Polish PCD patients organized by IIMCB in September 2015. Materials have also been published on the website of Polish Ciliary

Dyskinesia Society, improving the knowledge on PCD management and treatment not only between PCD patients but also to the public.

After having organized two workshops during the first period of the project, BESTCILIA partners have organized two additional workshops on PCD diagnostics, adjacent to the regular project meetings (in Poland and Cyprus, in October 2015 and May 2016, respectively). Each workshop included presentations of external speakers, members of the Advisory Board, and representatives from American Thoracic Society PCD Initiative and the ERS PCD Task Force, as planned in Annex I.

The same as in reporting period 1, information about the meetings has been announced through previously developed communication channels: BESTCILIA website, PCD mailing list at the University of Bern (pcd_info@unibe.ch) and personal communication of BESTCILIA project participants. Thanks to the efficient communication as well as the open-to everyone and free-of-charge policies, the number of participants attending each of these meetings reached considerable numbers (Cracow, Poland: 65 attendees from 10 countries; Limassol, Cyprus: 65 attendees from 15 countries). The number of participants attending the PCD diagnostic workshops organized by BESTCILIA participants underlined the need for organizing such workshops, especially in countries with low health expenditures (Greece, Cyprus, Poland), where knowledge on PCD is still scarce.

The workshop in Cracow, Poland took place on 20-21th of October 2014 and was organized alongside with a large international conference on PCD. The event gathered 65 participants from Europe and the US, including 35 clinicians from Poland. During the meeting, most recent advances in the molecular genetics of PCD, cellular, clinical and diagnostic aspects of PCD were addressed and presented, in combination with a hands-on workshop on nasal brushing, HSVM, nNO measurement and analysis of cilia by TEM (lead by WWU). Additionally, the workshop also included a special training session on the diagnosis of difficult PCD cases (lead by RBH). During that session, PCD experts trained less experienced clinicians on the diagnosis of difficult PCD cases, both by presenting already diagnosed difficult cases and advising on problematic cases presented by other participating clinicians.

The last in the series of the workshops organized by BESTCILIA was a large international workshop held in Cyprus - a country with low health expenditures and limited knowledge of PCD. The workshop (led by WWU) took place in Limassol, Cyprus, on the 20-21st of May 2016 and 65 attendees from 15 countries participated in this event. Like before, the workshop was conjunct with a large international conference on PCD (organized by CUT) and included both theoretical lectures on PCD and practical hands-on training on PCD diagnostic techniques.

In addition to the large international workshops organized by BESTCILIA, collaborators from IIMCB shared their knowledge on PCD diagnostics acquired during the project duration, by organizing local trainings for collaborating clinicians (e.g. local paediatric clinician training in June 2014 in Poznan, Poland) or PCD patients (PCD patient workshop in Sept 2015 in Warsaw, Poland).

Dissemination of project results – discourse with the scientific community:

The required goal of 5 publications during the project duration has been reached already during the first 18 months of the project (12 scientific in peer-reviewed publications and 5 conference/workshop proceedings were published at that time). During the second period of the project, the publishing activity of the BESTCILIA partners increased further, reaching the total of 38 scientific articles and 30 conference proceedings, which acknowledged BESTCILIA (period 2 alone: 29 articles, 25 proceedings and 2 book chapters). Further three scientific papers, two conference proceedings and one book chapter are in press.

When it comes to participation in scientific or clinical conferences, the initial obligation of BESTCILIA project (36 meetings/conferences during the whole project duration) has been clearly fulfilled (50 talks and 5 posters already in period 1).

During the second reporting period, the dissemination activity continued, in a form of 85 talks, 22 posters and 17 organized workshops & conferences, yielding during the whole project an impressive number of 139 talks, 29 posters and 25 workshops (see final D7.4 report for details). These activities took place during meetings on the international or national level, and were directed to different types of auditoria (patients or lay public/medical and scientific professionals).

The quality of BESTCILIA research presented during large international conferences has been well recognized by fellow scientists. In 2014, Myrofora Goutaki (UBERN) has received an ERS Grant for the Best Abstract in Paediatric Respiratory Epidemiology, for "Growth in patients with primary ciliary dyskinesia (PCD): A multinational study" (BESTCILIA WP1) presented during the ERS Annual Meeting in Munich. One year later, the same prize has been received by Panayiotis Kouis (CUT), during the ERS Annual Meeting 2015 in Amsterdam, for: "Diagnostic accuracy of nasal nitric oxide measurement for establishing diagnosis of primary ciliary dyskinesia: A systematic review and meta-analysis" (BESTCILIA WP3). During the same meeting, Claire Jackson (SOTON team) has received the BLF/BPRS Award for the presentation entitled "Seasonal variation in success and ciliation of airway epithelial cells cultured at air-liquid interface; patients samples referred for primary ciliary dyskinesia testing". In addition, just recently, at the Joint conference of the Swiss Societies of Cardiology and Pulmonology 2016, Florian Halbeisen (UBERN team) has received a prize for one of the best abstracts in Pulmonology, for his work on "Lung function in patients with PCD: a multinational study" (BESTCILIA", which is a part of the WP1 work).

In addition to the active participation in scientific and clinical meetings, BESTCILIA partners were also involved in organisation of international scientific and clinical conferences related to PCD and lung care. On these occasions, other members of BESTCILIA consortium have been invited to participate and disseminate information about the project and its results. Jane Lucas (SOTON) as a member of European Respiratory Society, helped to organize the ERS task force meeting during the ERS Congress in Munich in September 2014 which was hosted by Heymut Omran (WWU). Claire Hogg from RBH co-organised the Cilia 2014 conference in Paris in November 2014. Alexandra Quittner (UM) has co-organized the 28th Annual North American Cystic Fibrosis Conference, Atlanta USA, 9-11 Oct 2014. Young scientists from UBERN, SOTON and REGION H, were also the initiators and co-organizers of the First Meeting of Young Researchers in PCD, which took place on 12-13th of March

2015 in Bern, Switzerland. This 2-day meeting gathered over 40 attendees, including 15 presenters from multidisciplinary backgrounds, including epidemiologists, clinicians, basic scientists, diagnosticians, engineers, allied health professionals and social scientists.

BESTCILIA consortium members were also actively involved in work on the recommendations for pulmonologists and medical doctors, regarding PCD diagnostics. Jane Lucas (SOTON), Claudia Kuehni (UBERN), Andrew Bush and Angelo Barbato (BESTCILIA Advisory Board members) have successfully applied for the ERS Task Force funding to develop guidelines for the diagnosis of PCD (ERS Task Force "Diagnosis of primary ciliary dyskinesia (PCD) in a molecular age: A practice guideline for diagnosing patients with PCD"). The first meeting of the Task Force took place in Sept 2014 and the guidelines manuscript was recently submitted for publication.

In order to continue and expand the collaborations initiated during the project, BESTCILIA participants have also joined to apply for funding within the frame of EU-funded COST Action. The project entitled "Translational research in primary ciliary dyskinesia - bench, bedside, and population perspectives (BEAT-PCD)" chaired by Jane Lucas (SOTON), has received funding from EU and started operating since November 2015. Focused solely on PCD-related research, the project aims to integrate the growing group of professionals working on PCD research (both scientific and medical), contributes to the sharing of resources and expertise and to the development of new experimental projects. The grant collaborates with BESTCILIA and held a joint meeting at ERS Amsterdam 2015.

Other contacts with medical professionals:
BESTCILIA members have been in direct contacts with medical professionals (GPs, paediatricians, pulmonologists, paediatric pulmonologists, ENT specialists and other medical doctors), to raise the awareness of PCD and information on BESTCILIA project in the medical community.
Claudia Kuehni and Myrofora Goutaki (UBERN) met Swiss paediatric pulmonologists in Bern and Basel to present BESTCILIA and WP1 activities. In Cyprus, Greece and Poland, contacts with the medical community also served as a part of the patient recruitment campaign (Task 3.2 of WP3). In addition, to increase the awareness of BESTCILIA and other projects integrating the community of researchers and medical professionals working on PCD (including the Beat-PCD project), BESTCILIA project participants have organized a PCD day during the annual meeting of the European Respiratory Society (Amsterdam, September 2015).
Other initiatives allowing discourse and raising awareness of BESTCILIA research in the clinical and scientific community were the external workshops organized within BESTCILIA (see Deliverable Report 7.4). During the reporting period 2, two workshops were organized (Poland and Cyprus), which collectively allowed approx. 130 medical professionals to learn about the PCD diagnostics and BESTCILIA initiatives.

Discourse with lay public and patient groups:
Awareness of the BESTCILIA project and its activities has also been raised among the general public. After three press releases published in the first 18 months of the project, in the

second part of the project, BESTCILIA consortium has published four releases on international press release portals, two on BESTCILIA partner's institution website (UBERN and WWU) and one on a specialized press release portal (ERS). The press releases on international portals have yielded between 1100 – 4000 views (on AlphaGalileo and EurekAlert, respectively). Some of the releases have been later re-published/cited by other portals, including the well-known websites such as European Lung Foundation (www.europeanlung.org), High Beam Research (www.highbeam.com), Lung Disease News (www.lungdiseasenews.com), Medical News Today (www. medicalnewstoday.com), or Science Daily (www.sciencedaily.com), resulting in significantly increased visibility of BESTCILIA project.

Collaboration with PCD patients:
The collaboration with PCD patients is an integral part of the BESTCILIA activities. The whole project was designed in close collaboration with PCD patient organizations from the UK, the U.S., Germany, Switzerland and Poland. Representatives of the patients were always invited to participate in each project meeting and workshop. During the second period of the project, BESTCILIA partners have further worked on these long-standing collaborations, especially focusing on collaborations with regional/national PCD groups.

Representatives of WWU, Heymut Omran and Claudius Werner participated in the general meetings of the German PCD support groups (2014, 2015). Florian Halbeisen and Elisabeth Maurer (UBERN) presented the results of BESTCILIA workpackages 1, 2, 4 and 5 to the Swiss patient organization during their yearly meetings (e.g. in March 2016). SOTON members (Laura Behan and Jane Lucas) shared the results of BESTCILIA research to the PCD patients in the UK. In addition, Claire Hogg (RBH) was a member of the organizing committee of the international Cilia 2014 conference, which was organized by four large European cilia-related networks (the Ciliopathy Alliance, the Groupement de Recherche CIL, the Nordic Cilia and Centrosome Network and the EU FP7 programme SYSCILIA).

The contacts with the PCD community served also to support the patient recruitment campaigns to refer them to the PCD diagnostic centers developed within BESTCILIA WP3 (in Cyprus, Greece and Poland). One of the consequences of the recruitment campaign in Cyprus was the establishment of the Cyprus PCD patients' organization ("Breathing Together") during the second half of the project. Through direct contacts with patients and families, the organization significantly contributed to the PCD awareness campaign and recruitment of PCD patients in Cyprus. In Greece, an informational leaflet has been prepared, which has been distributed between parents of paediatric PCD patients.

In Poland, where the PCD patient organization ("Polish Ciliary Dyskinesia Society") has been established already previously, Michal Witt (IIMCB) and the collaborating physicians from ITLD (H. Mazurek, A. Pogorzelski) co-organized a workshop for Polish PCD patients (September 2015, Warsaw). During the event, Polish PCD patients met medical doctors from ITLD, received training on management and every day-life with PCD and learned about initiatives pursued by the Polish Ciliary Dyskinesia Society. Furthermore, the Polish version

of the PCD information materials/PCD brochure developed by BESTCILIA (Task 7.2) was presented to the attendees. The event was extremely successful, as the number of participating patients and their family members reached to 120, which is a significant part of all PCD patients in Poland. In addition, IIMCB and ITLD have also assisted in designing the website for the Polish Ciliary Dyskinesia Society. As a supplement for the meeting and the source of information for other people interested in PCD, BESTCILIA PCD information brochure has also been published there.

Overall, BESTCILIA raised awareness of PCD on different levels through successful dissemination of BESTCILIA research towards the research community and general public. BESTCILIA influenced the field of PCD Research and clinical care of PCD patients to an incomparable extend. BESTCILIA will continue to influence PCD research and clinical care through international collaborations, which have been firmly established during BESTCILIA, and which continue within e.g. the framework of the European funded COST Action BEAT-PCD.

Project public website:

http://www.bestcilia.eu