

QUALITY OF LIFE AND MANAGEMENT OF LIVING RESOURCES PROGRAMME (1998-2002)

FINAL REPORT

Contract number : QLG1-CT-2000-01185

Project acronym : BIOAIR

QoL action line : 1.1.1.-7.2: Research and Technological Development Activities of a Generic Nature; Chronic and Degenerative Diseases, Cancer, Diabetes, Cardiovascular Diseases and Rare Diseases; Evaluation and Therapies through multinational, large scale studies/trials.

Reporting period for the last progress report:

01/01/2003-31/12/2003

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SECTION I: PROJECT IDENTIFICATION

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Acronym of the project: BIOAIR
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QoL action line: 1.1.1.-7.2: Research and Technological Development Activities of a Generic Nature; Chronic and Degenerative Diseases, Cancer, Diabetes, Cardiovascular Diseases and Rare Diseases; Evaluation and Therapies through multinational, large scale studies/trials.
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SECTION IIa: PROJECT PROGRESS REPORT OF THE LAST REPORTING PERIOD (01/01/2003 – 31/12/2003)

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1. OVERVIEW OF PROGRESS DURING THE REPORTING PERIOD

1.1 Main objectives of the project for this reporting period

The main objectives of the current reporting period were for all patients to conclude the study, to finalise the sample collections (including bronchial biopsies), to clean and close the database, and to start with analyses of the clinical data, as well as some analyses of the biomarker samples and the bronchial biopsies. Dissemination activities were to be planned and prepared (publications in scientific journals and presentations at scientific conferences).

1.2 Overview of the scientific progress of the project as a whole in the period

The first patient was included month 15 and the last patient month 37. In total, 266 patients were recruited to the study of which 33 turned out to be screening failures. A total of 233 patients were randomised (76 mild asthmatics, 93 severe asthmatics and 64 COPD patients), of which 43 dropped out during different stages of the study. Table A shows the number of patients that completed Phase I of the study (including steroid trial), and the percentage of those that completed the follow-up year, which was 93.5% of all patients.

Table A. Number of patients that completed different stages of the BIOAIR study.

	Mild	Severe	COPD	Total
Recruited	85	106	75	266
Completed Phase I	66 (77%)	85 (80%)	52 (69%)	203 (76%)
Completed study	65	77	48	190
Follow-up year	98.5%	90.5%	92.3%	93.5%

Bronchoscopy has been carried out in 34 patients (14 mild asthmatics, 14 severe asthmatics and 6 COPD patients). All biopsies were processed locally, and afterwards shipped to Southampton for quality check and analysis. Of GMA processed biopsies 79% of patients leaving biopsies had at least one biopsy of good quality. The number of biopsies taken is lower than projected at the start of the study. There are three main reasons for this: 1/ A proportion of eligible patients did not consent to undergo this procedure. 2/ Several of the patients with severe airway disease did not meet the inclusion/exclusion criteria for bronchoscopy (For safety reasons we adhered to strict internationally recognised guidelines). 3/ Five out of the twelve centres did not perform bronchoscopy because of lack of experience and/or because failure to obtain ethical permission for the procedure. Nonetheless, these biopsies will be valuable and will be used to study one or several

specific scientific questions arising from the analysis of clinical phenotype data and biomarker measurements in other samples.

A total of 203 patients have completed the steroid trial of which 102 patients received prednisolone and 101 placebo. An oral double-blind and placebo-controlled steroid trial comparing asthma and COPD in the same study have previously not been done, and the addition of biomarkers of steroid responsiveness is another novel approach.

Regarding exacerbations, 8 have been recorded for mild asthmatics, 48 for severe asthmatics and 18 for COPD patients. This gives data for the annual number of exacerbations to 0.12, 0.62 and 0.38 for mild asthma, severe asthma and COPD, respectively. Since the statistical analysis of the database is not yet ready, this number is likely to increase. For example, exacerbations might have been missed by the patients initially, but may be detected upon analysis of the daily PEF and diary card recordings, and this might in turn increase the number of exacerbations further.

Spirometry and questionnaire data (St. Georges and Asthma Control Questionnaire) are available for more than 95% of subjects and visits. Also daily lung function measurements (~90% of patients) and diary cards questions (~85% of patients) seem to have been completed very well.

Urine and blood samples are available for more than 95% of subjects and visits. Exhaled NO values are available for 60-70% of patients at the centres that carry out these measurements, nasal aspirate in 50-70% of patients. Regarding sputum, cell counts are available in 50-75% of patients, supernatant is available in 60% of patients and sputum plugs in 15-30% of patients (generally, the lower number in the range concerns COPD patients, whereas the higher number concerns asthma patients). These figures are based on information entered in the eCRF. Since not all data has yet been entered these figures may still increase.

The collected biomarker samples comprise a large and unique biobank for investigating severe asthma, with proper control groups. This biobank has all credentials to give rise to many scientific breakthroughs regarding the diagnosis and understanding of the mechanisms of severe asthma. It will be used to test the hypotheses raised in the BIOAIR study directly, but it will also constitute unique opportunities for future research when new questions might need to be answered. It is therefore extremely important the samples are kept under secure conditions (mainly at -80°C), as well as the information regarding phenotype of the patients. For some sub-studies it may be necessary to anonymise the samples (e.g. genetic studies). It is hoped that integration support from the GA²LEN NoE for asthma and allergy research may aid storage and use of the BIOAIR biobank.

Regarding analyses of the many samples, a start was made during 2004, when MBL (Mannose-Binding Lectin) was measured in Leiden in serum samples taken at visit 1 of all patients, and (SAE-IgE) Staphylococcus enterotoxin IgE antibody as well as total IgE were measured in Gent also in serum samples taken at visit 1 of all patients. The cleaning of the phenotypical and clinical data awaits the comparisons between patient groups.

Performing these analyses created awareness towards the huge task of the shipments of all samples. In order to do the MBL and SAE-IgE analyses, all centres were asked to ship the serum samples of visit 1 to Leiden and Gent. This process took two months, and was next to time-consuming, also cost-consuming, due to the fact the shipments had to be made on dry ice in order to ensure the quality of the samples. Currently, a central storage of all samples is considered, in order to minimise shipment costs (all samples can be shipped to the central location in one shipment) as well as to have better control over the samples. However, national legislations need to be considered, since not all countries allow permanent storage of samples abroad.

In October 2004, a scientific workshop was organised in Modena, Italy, for members of the Work Groups. This meeting was used to actively go through the data available of the first descriptive and cross-sectional phase of the study (including the steroid trial). A start was made with raising queries regarding the available data, and these queries were sent to the centres afterwards. It was decided that data analyses could not start (and would not be correct) until all datasets were completed and all data cleaned. During the months after the Modena meeting it became clear that this would be a time-consuming process. However, progress was being made, and by March 2005 all sputum cell count data until visit 3B was cleaned, as well as most of the lung function data.

In March 2005 80% of the electronic database was completed up until visit 3B. The last patient completed the study in April 2005 and in June 2005 the electronic database was completed. In some cases the patients were not able to use the electronic PEF meters, and they have used manual PEF meters (mini-Wrights) instead. This manual data needs to be entered manually into the eCRF, which is a very time-consuming process, considering it consists of daily data collected during more than one year.

All in all, it is expected the immense amount of data collected will give rise to a large number of high-quality scientific publications. It will probably take several years until this source is exhausted. The results will be available to the general public in this way. It is hoped that in the end, the result of this study will give rise to new or better methods for diagnosis, prediction and treatment of patients suffering from severe asthma.

1.3 Update of tables 1, 2 and 3 from the technical annex

Table 1 BIOAIR Technical Annex: Work Package (WP) list – Updated June 2005

WP No	Description	Responsible	Person months	Start month	End month	Deliverables	Current Status (June 2005)
1	Project management	P1	18	0	36	1	Management structure set-up completed month 2
2	Data acquisition and statistics	Was P6, now P1 and BWG1	12	1	36	2-6	Electronic data acquisition system ready for use month 14
3	Study Protocol	BWG1	7	1	6	7-9	Study Protocol completed month 9, Procedures Handbook completed month 13, Ethical Permissions completed month 20
4	Clinical observations	BWG1	211	6	30	10-13	Patient recruitment started month 15. 265 patients recruited month 37 (patient inclusion was then closed).
5	Bronchoscopy and tissues	BWG2	4	1	36	14-16	Bronchoscopy guidelines completed month 7, workshop conducted month 7. Biopsies available from 34 patients.
6	Biomarkers of airway remodelling	BWG3	17	1	36	17-18	Proposal for set of measurements completed month 11, workshop sputum conducted month 7. The first biomarkers were analysed month 45.
7	Glucocorticosteroid responsiveness	BWG4	11	1	36	19-20	Contributions to protocol and proc. handbook completed month 9 and 11. The first analyses to be done during 2005.
8	Leukotrienes and other mediators	BWG5	9	1	36	21	Contributions to protocol and handbook completed month 9 and 11. The first analyses to be done during 2005.
9	Infections	BWG6	6	1	36	22	Contributions to protocol and handbook completed month 9 and 11. The first analyses to be done during 2005.
10	Genotype	BWG7	3	1	36	23	Contributions to protocol and handbook completed month 9 and 11. The first analyses to be done during 2005.
11	Global outcome and Exploitations	P1 and all partners	23	18	36	24-28	In progress
	TOTAL		321				

Table 2 BIOAIR Technical Annex: List of Milestones (M) – Updated June 2005

M no	Specification	Month	Update June 2005	Involved Partners
1	Management team and operational procedures	4	Completed month 2	P1
2	Protocol, methods, permissions and data handling for clinical investigation	6	Protocol: 9 Methods: 13 Permissions: 15 Data handling: 15	P1-P13
3	100 patients recruited to study; Bronchoscopy and Induced Sputum Workshops completed	9	Workshops completed month 7, Recruitment started month 15. 265 patients recruited month 37.	P1-P13
4	300 patients included in study	15	265 patients by month 37 (recruitment closed)	P1-P13
5	450 patients included in study	18	265 patients by month 37	P1-P13
6	450 patients completed clinical study; 30 in each group having been subjected to repeated bronchoscopy	30	265 patients by month 37. 34 patients subjected to bronchoscopy (14 mild asthma, 14 severe asthma, 6 COPD)	P1-P13
7	Cleaned database and results of clinical study	32	In progress, estimated to be ready end of 2 nd quarter 2005.	BWG1 ¹
8	Cleaned database and results of measurements of biomarkers, including leukotrienes, GCS-axis, viruses, and candidate genes	34	In progress	BWG3 ²
9	Conclusions on global outcome: Molecular and clinical markers of severe chronic airflow disease	36	In progress	P1-P13

¹/ Co-ordinated by BIOAIR Work Group 1: P1, P2, P3, P7, **P8** (chair), P10 & P13, but the work will involve all partners and work groups.

²/ Co-ordinated by BIOAIR Work Group 3: **P1** (chair), P8, P10, P11 & P12, but the work will involve all partners and work groups.

Table 3 BIOAIR Technical Annex: List of deliverables (DL)

DL no	SPECIFICATION	Delivery date	Status June 2005	Nature	Dissemination level
1	Establishment of management team and operational procedures	Month 4	Completed month 2	O	CO
2	Establish methods for data handling	Month 6	Completed month 15	O	CO
3	Cleaned database for results of clinical study	Month 32	Estimated to be ready 2 nd quarter 2005	R	RE
4	Cleaned database for results of measurements of biomarkers	Month 33	In progress	R	RE
5	Preliminary compilation of findings in clinical part of study	Month 33	Estimated to be ready by the end of 2005.	R	RE
6	Overview of findings in analytical part of study	Month 36	Estimated to be ready 2006.	R	RE
7	Study Protocol	Month 6	Completed month 9	R	PU
8	Study Procedures Handbook, Logistic guidelines and Clinical Record Forms (CRFs)	Month 6	Handbook month 13 Logistics 14 CRF 14	R	RE
9	Acquisition of ethical and other permissions	Month 6	Completed month 15	R	PU
10	150 patients recruited to study	Month 9	Recruitment started month 15. 139 patients recruited month 27	P	CO
11	300 patients included in study	Month 15	265 patients recruited month 37 (end of inclusion)	P	CO
12	450 patients included in study	Month 18	265 patients recruited month 37 (end of inclusion)	P	CO
13	450 patients completed study	Month 30	March 2005: all but 5 patients have completed	P	CO
14	Bronchoscopy Guidelines and Procedures	Month 6	Completed month 7	R	RE
15	BAL fluid from all eligible patients	Month 30	During the course of the study it was decided not to take BAL fluid.	O	CO

16	Biopsy specimens from all eligible patients	Month 30	Month 43	O	CO
17	Proposal of primary standard set of measurements of biomarkers	Month 6	Completed month 11	R	CO
18	Completed measurements of biomarkers of remodelling in collected specimens	Month 34	In progress	R	CO
19	Clinical response to oral glucocorticosteroids	Month 30	In progress	R	RE
20	Biochemical and morphological characterisation of steroid responsiveness	Month 34	In progress	R	RE
21	Integrated assessment of leukotriene pathway (levels, enzymes, receptors)	Month 34	In progress	R	RE
22	Integrated assessment of viral infections	Month 34	In progress	R	RE
23	Candidate gene screen	Month 34	In progress	R	RE
24	Internal reports from BWGs to team	Month 35	In progress	R	RE
25	Preliminary conclusions from Team Workshop	Month 35	In progress	R	RE
26	Assessment of global outcome: Molecular and clinical markers of severe chronic airflow disease	Month 36	In progress	R	PU
27	Publications on guidelines for diagnosis and treatment of severe chronic airflow obstruction	Month 36 and thereafter	In progress	R	PU
28	Results on Website and Reports to the Public	Month 36 and thereafter	In progress	R	PU

2 STATUS OF THE INDIVIDUAL WORK PACKAGES

2.1 WP1: Project management

Start date or event: Project start (day 1, month 1)

Completion: Project end (month 36)

Responsible partner: **P1** (The Co-ordinator)

Person months per partner and total: 18 months by P1 (50% project manager)

Objectives: The longitudinal prospective approach of clinical observation of patients with severe airflow limitation requires repeated sampling of the same patients and, as this will in most instances involve repetition of many procedures, strict common protocols will have to be prepared and approved. Also the great number of samples to be collected requires attention to logistics. The number of centres united in this Project is another reason why an effective management structure is required. There are accordingly several reasons to organise the action efficiently. The overall objective will therefore be to create an effective management team and to ensure that contracted deliverables are fulfilled and the stated milestones achieved. For this reporting period the most important objective was to continue the clinical work and to finalise patient recruitment and follow-up, and to start analysing the data and samples.

Work accomplished compared to activities planned:

In order to coordinate the work between the centres and to keep track of patient recruitment and study progress, several Steering Committee (SC) meetings were organised during the reporting period. March 2003 a whole day SC meeting took place at Frankfurt airport. In addition, short SC meetings took place in Seattle USA in May 2003 in conjunction with the annual ATS conference, and in Vienna in September 2003 in conjunction with the annual ERS conference.

In order to increase transatlantic collaboration, an ATS NIH sponsored Severe Asthma workshop was organised in conjunction with the ATS conference in Seattle on 17th May 2003. This meeting was attended by BIOAIR members as well as members of the American Network.

A successful ERS sponsored workshop entitled 'Biomarkers in phenotypic and genomic research on severe airway disease' was organised by the BIOAIR group and in particular Partner 1 (Dr Mina Gaga from the University of Athens) and took place 31st May-1st June 2003 in Athens. The workshop was attended by BIOAIR members and others interested in the topic. The speakers consisted of BIOAIR members.

An important task of the management team during 2003 was to monitor patient recruitment, inclusion and follow-up. Since it proved to be far more difficult than envisaged at the start of the study to locate and recruit suitable patients, the recruitment period was extended several times. The last patient was included in January 2004, and the last patient completed the study in April 2005. The electronic Case Record Form (eCRF) system was used to closely monitor the progress of each centre. In addition, the management team closely monitored completion of data entry in the eCRF.

The Clinical Research Monitor (CRM) continued during 2003 to monitor the study. Several monitoring visits took place at the centres. The monitoring visits consisted of source data verification, check whether the correct methodologies were used and study progress. A monitoring manual was developed in collaboration with an experienced CRM, Mr. Östen Karlsson, from AstraZeneca (unconditional sponsorship), with clear

instructions to the local monitors as to how the monitoring should be carried out. The Central monitor visited all BIOAIR centres once at which time point source data verification took place and the local monitors were instructed.

During the third and fourth year, the data cleaning process was initiated. On a regular basis Excel sheets with all entered results were extracted from the database in order to get a good impression of how well the datasets were completed, as well as to be able to start with the data cleaning process. In October 2004 a workshop was organised in Modena, Italy, which was attended by Work Group members. At that meeting the Work Group members looked at the data in great detail and listed down queries. These queries were subsequently sent on to the centres involved, where they were looked into, and corrected dataset files were returned. This whole process proved to be very time-consuming. Currently (June 2005) complete cleaned data files have been received by all centres for the bronchodilator reversibility test at screening visit, and the sputum cell counts from visit 1 until visit 3B. Many datasets have not yet been completed and therefore it has not been possible to start with the analysis of the data. However, the centres are working hard to complete the eCRF, and the Work Groups will put an effort into checking the data and raising queries to the centres. The whole process of data cleaning is currently estimated to be finished by the end of October 2005. After that, the analysis of the data can commence.

In order to plan for the analyses of the samples, the coordinator together with the Biomarker Work Group organised a meeting in Leiden in June 2004. It was discussed again at the meeting in Modena in October 2004. One of the main issues to deal with first is the logistics of the storage and shipments of the samples. It will be too time-consuming and expensive to have the centres ship individual samples to the analysing centres each time an analysis will take place, and therefore the possibility was discussed to ship all samples to a central and store them until analysis. The final decision still needs to be made since there are financial and ethical issues that need to be resolved.

2.2 WP2: Data acquisition and statistics

Start date or event: Project start (month 1)
 Completion: Project end (month 36)
 Partners responsible: **P1**, all partners will however be involved as the data collection and quality control will be an interactive process.
 Person months per partner and in total: 12 months in total
 8 months for partner 1, for remainder of partners, the work is part of clinical investigation (WP4). Co-ordinator's resources (50% project manager and 50% clinical research monitor [CRM]) will presumably use part of their time for this work package, estimated to amount to 4 months work.

Objective:

It is necessary for a Project of this dimension with strict adherence to a common protocol and methods for efficient collection, storage and analysis of data. A particularly important aim will be to develop methods for computerised transmission of data directly to the data centre, including spirometry results that will be standardised by the use of the same device in each centre, certification of the user and then transmission of data directly to the study data centre. The data acquisition system will

also be used by the CRM for on-line control of data quality. Finally, this work package also includes the final statistical analysis of all study results.

Work accomplished, compared to activities planned:

As described in the Periodic Reports of Year 1 and 2, after withdrawal of Partner 6 from the project the coordinator was assigned the task of organising WP2. It was decided that a company based in Germany (Varigon AG in Lübeck) would build an electronic CRF and database system. In month 15 the first version ready for use in the study could be launched. Entrance to the eCRF is password protected, and only authorised personnel is allowed access to the patients data.

After successfully introducing the eCRF system during Year 2, the usage was intensified during Year 3, since most patients were recruited and included during the second half of Year 2 and during the whole of Year 3. Study nurses, physicians and technicians used to eCRF to enter all data collected at the study visits. The CRM and local monitors used the eCRF to check study progress and source data verification. Varigon and later on its successor Pulmonary Research Network provided the management team with regular overview of data entry progress. Although the eCRF is an effective means to keep track of study data, several centres were often behind with data entry. This made it difficult to keep a good track on patient and study progress, as the eCRF not always was up to date with actual events that had occurred.

In order to prepare for the statistical analyses at an early stage, a meeting was organised in March 2003 including Nils Drews (CEO) and Nikolaus Peterknecht (IT manager) from Varigon AG, and Professor Dahlén, the PM and CRM, in order to discuss possibilities to build a way of statistical analyses in the eCRF. It was agreed that part of the resources intended for a statistician (originally allocated to Partner 6, but after its withdrawal allocated to the Coordinator) would be used to pay Varigon AG as a subcontractor to build in this system in the eCRF. This would ease the statistical analyses later on. In collaboration with Dr. Josephine Hjoberg (part-time appointed researcher/statistician) the details were further discussed and developed during 2003.

In order to start with data cleaning and analysis it was essential all data was included in the eCRF. Before the meeting in Modena October 2004 all centres were therefore urged to complete all datasets until visit 3B (as at that stage all patients had passed the steroid trial which finished at visit 3B, but not all patients had completed the whole study). This was done fairly well, and a start could be made with the data cleaning. The data cleaning process in itself also took a lot of time, since not all centres were able to check and correct the data right away.

The main reason for some of the centres not being able to keep the eCRF up to date was lack of resources. The resources allocated to them via the BIOAIR project were not sufficient to cover all the staff and time needed for the work. The work therefore had to be spread out over a longer period by staff paid by other grants. In addition, some patients (mainly the elderly ones) were not able to use the electronic Peak Flow meters correctly, which meant that they were given manual Peak Flow meters instead. The data from these manual measurements could not be uploaded into the eCRF directly, but had to be entered manually, a very time-consuming process.

The result is that as of yet it has not been possible to start with statistics and analysis of the data. It was however felt, that despite the delays, it was important not to start doing preliminary analysis of the data before it could be sure the data was correct. Doing the analyses too soon could give false impressions of the results. It is currently estimated all data will be entered by the end of June 2005 and all data will be cleaned

by the end of October 2005. Strict deadlines have been given to all centres. After that a start can be made with the analysis.

2.3 WP3: Establishment of study protocol and clinical investigative procedures

Start date or event: Project start-up team meeting

Completion: Month 6

Partners responsible: **P8**, P1, P2, P3, P7, P10 and P13 in BIOAIR work group for clinical investigations (BWG1); all partners will however be involved in the review of protocol proposals. A slight rearrangement took place in the composition of BWG1 during year 1, see study protocol Appendix I for details of the members.

Person months per partner and total: 1 month per work group member, altogether 7 months.

Objective: A general study plan and a desired set of clinical measurements to be performed have been agreed upon among the partners at the time of presenting the proposal. However, it is the subject of this particular work package to refine this protocol and make the adjustments that may be required to meet the overall study objectives. This includes estimations of the power of the study to meet primary and secondary end-points (also to be defined in the process of completing this work package). This work package will be co-ordinated by BWG1, the Clinical Observations Group. This group will be chaired by P8 and include members from P1, P2, P3, P7, P10 and P13, plus the CRM and the project statistician.

Work accomplished: The study protocol was finalised month 9, and an amended study protocol was prepared month 17. The Study Procedures Handbook was finalised month 14, and updated versions were distributed later during Year 2 prepared by the PM in collaboration with BWG1. It should be recognised that the Study Procedure Handbook is a very detailed and up-to-date 150 A4 pages collection of methods and procedures for clinical investigations of airway diseases, probably second to none that have been attempted in the field. All centres had finalised their ethical, drug regulatory and other permissions (if required) by month 20.

2.4 WP4: Clinical observations

Start date or event: Month 6

Completion: Month 36

Partners responsible: **P8**, P1, P2, P3, P7, P10 and P13 in BIOAIR work group for clinical observations (BWG1); All partners in the actual collection and study of patients; Co-ordinator for monitoring and management. A slight rearrangement took place in the composition of BWG1 during year 1, see study protocol Appendix I for details of the members.

Person months per partner: About 211 months in total
14.4 months per centre; 18 months by co-ordinator for monitoring by project manager/clinical research monitor; one month per member of BWG1.

Objective: To conduct the clinical investigation as defined in WP3. Therefore, this work package will also be co-ordinated by BWG1, the Clinical Observations Group. Once the clinical study has been concluded with the targeted number of recruited patients in each group completing the twelve month observation period, this BWG will together with the

Project statisticians have the primary responsibility for the analyses of the physiological and clinical measurements undertaken during the observational part of the clinical study. This information will essentially build the subject characterisation database to be used when searching for associations with markers of inflammation and tissue degeneration as well as for the genotyping (please see WPs 5-10).

Work accomplished: The first patient was included in the study month 15 at the site of Partner 7. The last patient was included January 2004 and the last patient to complete the study had his final visit in April 2005. A total of 266 patients were recruited to the study, of which 33 failed at screening and 43 dropped out during the course of the study (26 dropped out before or during the steroid trial, and 10 after completing the steroid trial, information is currently not available for 7 patients). A total of 190 patients have completed the study, of which 54 are mild asthmatics, 77 severe asthmatics and 48 COPD patients. See also table 4 for an overview of the completed patients per centre and per patient group.

Table 4, overview of completed patients per centre and patient group, status June 2005.

Centre	Total completed	Mild asthmatics	Severe asthmatics	COPD	Drop-outs
1. Stockholm	12	4	5	3	3
2. Athens	30	12	12	6	3
3. Heraklion	10	2	4	4	18
4. Ferrara	22	3	11	8	6
5. Ghent	5	4	1	0	0
7. Grosshansdorf	24	10	11	3	1
8. Leiden	25	9	8	8	5
9. London	10	1	4	5	4
10. Montpellier	26	5	13	8	0
11. Palermo	12	8	2	2	0
12. Southampton	7	6	1	0	3
13. Krakow	7	1	5	1	0
Total	190	65	77	48	43

As already explained in the Period Report of Year 2, it proved to be difficult to recruit the numbers of patients that were envisaged at the start of the project. As a result, some of the inclusion criteria (such as age) were widened, and the deadline for including the last patient was extended on several occasions. The final inclusion stop was finally set at January 2004, which is when the last patient was included. This was decided after mutual agreement by the Working Groups, and after review of study hypotheses and

endpoints it was agreed the number of patients included in the study nevertheless would be sufficient to test the main study hypotheses properly.

In addition to the recruitment difficulties, several patients dropped out during the course of the study (due to different reasons, but main reasons were non-compliance with the study protocol, withdrawal of consent, or illness other than related to the lungs). Some centres had more difficulties with this than others. However, the data of many of the drop-outs can still be used in the cross-sectional data analysis and even in the analysis of the steroid trial (since at least 10 of the drop-outs in fact completed the steroid trial). For an overview of the patients completing the steroid trial see below in the section of WP7.

Table 5. Overview of reported exacerbations in the eCRF.

Centre	Mild asthmatics	Severe asthmatics	COPD	Total
1. Stockholm	2	9*	6*	17
2. Athens	2	8*	0	10
3. Crete	0	1	0	1
4. Ferrara	0	3	0	3
5. Gent	0	0	0	0
7. Grossh	3	8*	1	12
8. Leiden	1	11*	2	14
9. London	0	3	4*	7
10. Montp	0	5*	5*	10
11. Palermo	0	0	0	0
12. South	0	0	0	0
13. Krakow	0	0	0	0
Total	8	48	18	74
Number in group per year:	0.12	0.62	0.38	

* At least one patient with more than one exacerbation.

Table 5 shows the number of exacerbations as currently reported in the eCRF. This number is somewhat lower than was anticipated at the start, especially considering that one of the inclusion criteria for the severe asthmatics was that they should have experienced at least one exacerbation during the year before they started the study. However, we believe that after analysing the daily PEF measurements, several more exacerbations will be identified. A likely explanation is that many exacerbating patients did not feel bad enough to contact the clinic, alternatively they sought care in a different clinic without always informing the BIOAIR clinic. It is well known that the patients with chronic disease are used to treat themselves and/or have a high tolerance to worsening of their disease. Since at the time of preparing this report the analysis of data

in the eCRF has not been completed, it is thus likely that the number of exacerbations will increase.

Table 6. Patient baseline characteristics (screening visit).

	Mild	severe	COPD
Number	66	78	48
age (y)	43.4±12.8	50.2±12.7	64.1±8.1
Sex ratio female:male	1.5:1	1.4:1	0.4:1
FEV₁ (L)	2.8±0.7	2.1±0.8	1.4±0.6
FEV₁ (%pred)	88.1±18.1	72.3±20.8	48.0±14.9

Values expressed as mean±SD.

Table 6 shows some patient characteristics at screening.

Table 7. Bronchodilator reversibility test at screening visit.

	mild	severe	COPD
FEV₁ post salbutamol (%improvement) all patients	10.6±5.5	9.1±7.8	3.5±3.7
% of pat. requiring ipratropium bromide (<9% impr.)	39.2	50.0	95.2
% of pat. receiving ipratropium	31.1	33.0	69.8
FEV₁ post atrovent (%improvement) only for subjects receiving ipratropium	9.1±5.7	8.5±5.7	4.7±3.4

Values expressed as mean±SD.

Table 7 shows the mean bronchodilator reversibility to a short-acting beta-agonist (salbutamol) in the 3 patient groups. Those patients showing an improvement of < 9% to salbutamol were consequently given an anti-muscarinic (ipratropium bromide). The percentage of patients requiring ipratropium bromide after salbutamol was around 30% in asthma patients and around 70% in COPD patients.

In October 2004, at the Scientific Workshop arranged in Modena for all WP members and Site Leaders, a start was made with the data cleaning process. All lung function, sputum cell count and medication data up until visit 3B (including the steroid trial) was checked by members of the Clinical Observations Work Group, and queries were raised to certain data. After the workshop these queries were sent to the centres, who were given the opportunity to resolve them. This process was time-consuming, and all queries were not resolved until June 2005. All data will be entered in the eCRF by the end of June 2005 and the cleaning of data is projected to be completed by the end of October 2005. Strict deadlines have been given to all centres. After that, the final statistical analysis of clinical data may commence.

2.5 WP5: Bronchoscopy and tissue sections

Start date or event: Month 1/Team start-up meeting

Completion: Month 36

Partners responsible: **P8**, P1, P4, and P12 in BIOAIR work group for bronchoscopy (BWG2); All partners in the actual collection of biopsies, this is included in the 14.4 months per centre for WP4. A slight rearrangement took place in the composition of BWG2 during year 1, see study protocol Appendix I for details of the members.

Person months per partner and total: 4 months in total
1 month per member of BWG2.

Objective: The overall objective is to collect bronchial mucosal biopsies on at least 30 subjects in each subgroup at the beginning of the study. The work group for bronchoscopy (BWG2) will provide guidelines for how to perform the bronchoscopy, including collection of biopsies and bronchial lavages, and define who is eligible for this part of the study. These instructions will comply with previously published international recommendations and current ongoing revisions (where several of the investigators participating in this study in fact are involved).

Work accomplished: During Years 2 and 3 several patients in the 7 centres that carry out bronchoscopy agreed to undergo this procedure and therefore several biopsies were taken, processed and stored according to the instructions in the Bronchoscopy Manual. See table 8 for an overview of all the biopsies that were taken. All biopsies were processed locally, and afterwards shipped to Southampton for quality check and analysis. Of GMA processed biopsies 79% of patients leaving biopsies had at least one biopsy of good quality. Since these monitoring procedures involve regular shipments of biopsies on dry ice for the centres that perform bronchoscopy, an extra budget for courier shipments was allocated to these centres for years 2 and 3.

Table 8. Overview of number of biopsies taken per centre and per patient group.

	I	II	III	Total
Stockholm	2	0	0	2
Ferrara	0	1	0	1
Leiden	5	1	1	7
London	0	1	0	1
Montp	3	10	5	18
Palermo	1	0	0	1
South	3	1	0	4
Total	14	14	6	34
% of eligible patients at bronchoscopy centres	37	35	17	

The number of biopsies taken is lower than envisaged at the start of the study. There are three main reasons for this: 1/ A proportion of eligible patients did not consent to undergo

this procedure. 2/ Several of the patients with severe airway disease did not meet the inclusion/exclusion criteria for bronchoscopy (For safety reasons we adhered to strict internationally recognised guidelines). 3/ Five out of the twelve centres did not perform bronchoscopy because of lack of experience and/or because failure to obtain ethical permission for the procedure. Nonetheless, these biopsies will be valuable and will be used to study one or several specific scientific questions arising from the analysis of clinical phenotype data and biomarker measurements in other samples.

2.6 WP6: Biomarkers of airway remodelling

Start date or event: Month 1/Team start-up meeting

Completion: Month 36

Partners responsible: P1, P8, P10, P11, P12, in BIOAIR work group for biomarkers (BWG3); All partners in the actual collection of material for measurements (included in the 14.4 months per centre for WP4). A slight rearrangement took place in the composition of BWG3 during year 1, see study protocol Appendix I for details of the members.

Person months per partner and total: 17 months

1 month per member of BWG3, and 3 months for analytical work by each of partners 5, 8, 11 and 12.

Objective: To assess degrees of tissue degeneration and repair by measurements of biomarkers in the collected samples and tissue specimens. This part of the Action will be led by the *Biomarker Group* (BWG3). It is chaired by P1, with members from P1, P8, P10, P11 and P12. Particular emphasis will be placed on following markers of remodelling in all collected samples and to integrate the measurements to optimise the use of collected materials and to focus the study. This group will also have a responsibility to provide the Steering Committee with proposals about how to integrate measurements between different WPs by taking into account how much sample is available for analysis and how the overall priorities of the BIOAIR Project best are served.

Work accomplished:

As previously explained, it is essential for a study of this scope to have an organised way of labelling and storing the samples. This will simplify the retrieval of samples enormously at the time of analyses. It was decided after careful consideration that the barcode labelling system from Brady should be acquired, as this would allow a labelling system whereby each sample would receive a unique barcode. Brady offered a significant discount from their listing price for all BIOAIR partners. With this system each sample can be traced at any time with help of a scanner. Through contacts that were established between Brady and Varigon, the Brady system could be incorporated in the eCRF, so that the barcode can be scanned into the eCRF directly. At the time of analyses the details of the samples can then be found by scanning the code into a Search function in the eCRF. The Brady system was acquired month 15. In addition to the Brady system, each centre also acquired a standard set of sample tubes, organised centrally by the PM. This to ensure that each centre will use the right type of tubes. In addition a colour code was developed so that each type of sample is stored in a tube of predefined colour, which will simplify later identification.

June 2004 a meeting was organised in Leiden by the Biomarker Work Group to discuss and plan for the analyses to be done in the study, and a proposal was made regarding the substances to be measured, taking into account the type and number of samples available.

This was again discussed at the meeting organised in Modena October 2004. A final decision will be made when the first analyses of the clinical data are available in the autumn of 2005, as well as when final information regarding the number of available samples is available. Much is dependent on the number of samples available, especially the sputum samples, as many analyses will be done in the sputum aliquots, but it was not always possible to obtain a large number of aliquots from the same sample. Table 9 shows that at visit 1 at least one sputum supernatant aliquot is available in 69% of patients. At least 5 sputum supernatant samples are available for 54% of patients at visit 1, which means that at least 5 different analyses can be done at the same visit for the same patient in 54% of patients. This percentage appears to decrease further in the study, but this is because this particular information was collected at a time when the database had not been updated with all information on visits 4-6. It is therefore with considerable confidence we can conclude at this stage that a unique number of sputum samples are available from the well phenotyped subjects participating in the study.

Table 9. Overview of number of collected sputum supernatant aliquots per sample (before completion of data base for later visits).

Number of aliquots	V1	V2A	V3A	V4	V6	Ex1	Ex2
0	62	62	71	71	53	13	5
1	4	4	1	2	1	0	0
2	7	5	1	3	1	0	0
3	7	6	8	5	1	1	0
4	10	5	4	3	0	1	0
5	16	15	8	4	1	0	0
6	18	16	19	8	4	5	0
>6	71	77	73	63	45	18	4
no entry in eCRF	7	12	17	42	95		
total pat. with samples	133	128	114	89	54	25	4
total patients in study	194	194	194	194	194	55	9
% pat. that have samples (1 aliquot or more)	69	66	59	46	28	45	44

2.7 WP7: Glucocorticosteroid responsiveness

Start date or event: Month 1/Team start-up meeting

Completion: Month 36
Partners responsible: **P8**, P1, P10, P11, in BIOAIR work group for steroid responsiveness (BWG4); All partners in the actual collection of material for measurements (included in the 14.4 months per centre for WP4). A slight rearrangement took place in the composition of BWG4 during year 1, see study protocol Appendix I for details of the members.

Person months per partner and total: 11 months in total
1 month per member of BWG4, and 6 months for analytical work by partner 1.

Objective: To assess clinical and molecular responsiveness to glucocorticosteroids, including relation to levels of sex hormones, and to correlate these results with clinical data and findings in the other WPs. This work package will be co-ordinated by BWG4, and one of their tasks is to decide if these measurements should be done in all patients or in representative subgroups within each cohort. The costs of these measurements may argue for the second approach. The clinical response to a 14 day course of oral prednisone will be recorded during the first quarter of the clinical study period to establish how many of the examined patients that are truly unresponsive to glucocorticosteroids. Another reason for doing this is the possibility that low compliance with therapy otherwise could confuse the remainder of observations.

Work accomplished:

A total of 203 patients have completed the glucocorticosteroid trial of which 102 patients received prednisolone and 101 placebo. Table 10 displays an overview showing the number of patients receiving prednisolone and placebo divided by patient group and study centre. An oral double-blind and placebo-controlled steroid trial comparing asthma and COPD in the same study have previously not been done, and the addition of biomarkers of steroid responsiveness is another novel approach.

A start will be made with the analyses of the clinical data and the samples with regard to the steroid trial once the eCRF is completed and cleaned datasets have been reached, most likely in the autumn of 2005.

Table 10. Overview of number of patients that completed the steroid trial, per patient group and study centre.

	I		II		III		Total		Total
	GCS	P	GCS	P	GCS	P	GCS	P	
Stockholm	1	3	4	2	2	2	7	7	14
Athens	6	6	6	7	4	2	16	15	31
Crete	3	0	3	4	3	3	9	7	16
Ferrara	1	2	5	6	4	4	10	12	22
Gent	2	2	1	0	0	0	3	2	5
Grosshansdorf	4	6	7	4	2	2	13	12	25
Leiden	2	7	7	2	3	5	12	14	26
London	1	0	2	3	2	3	5	6	11
Montpellier	3	2	6	7	4	4	13	13	26
Palermo	5	3	0	2	1	1	6	6	12
Southampton	3	3	1	1	0	0	4	4	8
Krakow	1	0	3	2	0	1	4	3	7
Total	32	34	45	40	25	27	102	101	203

GCS = Glucocorticosteroid; P = Placebo

2.8 WP8: Leukotrienes and other inflammatory mediators

Start date or event: Month 1/Team start-up meeting

Completion: Month 36

Partners responsible: **P1** and P12, in BIOAIR work group for leukotriene (LT) responsiveness (BWG5); All partners in the actual collection of material for measurements (included in the 14.4 months per centre for WP4). A slight rearrangement took place in the composition of BWG5 during year 1, see study protocol Appendix I for details of the members.

Person months per partner and total: 9 in total

1 month per member of BWG4, and 6 months (together) for analytical work by partners 1 and 12.

Objective: To assess how important the leukotrienes are for the symptoms and the disease progression in severe asthma and COPD. The capacity for synthesis or responsiveness to leukotrienes is assessed at different levels and both *in vitro* and *in vivo* by the strategies listed below.

Work accomplished: The samples needed for these analyses have been taken during Year 2 and Year 3, as part of WP4. It is currently estimated that up to 20.000 urine samples have been taken in the study, all to be shipped to and analysed at the site of Partner 1 (Stockholm). At least half of the total number of urine samples taken in the

study have already been shipped to the centre of partner 1. They were all checked upon arrival and were consequently logged in so as to be able to easily retrieve them when the analyses will start. The remaining urine samples will be shipped to Stockholm during 2005, and the primary analyses are started in September 2005.

2.9 WP9: Infections

Start date or event: Month 1/Team start-up meeting

Completion: Month 36

Partners responsible: **P9**, P2 and P4, in BIOAIR work group for infections (BWG6); All partners in the actual collection of material for measurements (included in the 14.4 months per centre for WP4). A slight rearrangement took place in the composition of BWG6 during year 1, see study protocol Appendix I for details of the members.

Person months per partner and total: 6 in total

1 month for partners 9 and 4 and 4 months for analytical work by partner 9.

Objective: To determine (a) whether respiratory virus infection is present in the lower airway as well as in the upper; (b) if these infections are chronic, and if so, for how long they persist; (c) if viruses can be associated with exacerbations of asthma or COPD.

Work accomplished: The samples needed for these analyses have been taken during Year 2, 3 and 4. A final decision regarding the analyses will be made during the third quarter of 2005. All samples will be shipped to and analysed at the centre of Partner 9 (London).

2.10 WP10: Genotype

Start date or event: Month 1/Team start-up meeting

Completion: Month 36

Partners responsible: **P12**, P3 and P6, in BIOAIR work group for genotyping (BWG7); All partners in the actual collection of material for measurements.

Person months per partner and total: 3 months in total

1 per each of P3, P6 and P12.

Objective: To assess candidate genes (isotypes of β_2 -adrenoceptor, TNF α , IL-4 receptor and IgE) proposed to be associated with one or more features of severe asthma. In addition, the activities in the other WPs, where biomarkers and mediators are measured, will be backed up by assessment of candidate genes for relevant compounds whenever available (e.g., α_1 -antitryptase, MMPs, collagen genes, leukotriene pathway enzymes and selective receptors). The potential for new important discoveries in this WP is illustrated by the fact that two of the partners during the course of the BIOAIR study have published on new asthma genes (P12: ADAM33 [Van Eerdewegh et al *Nature* 418:426–430, 2002.] and P1: GPRA[Laitinen et al *Science* 304:300-304, 2004.]) that will be investigated in the collected samples.

Work accomplished: The samples needed for these analyses have been taken during Years 2, 3 and 4, as part of WP4. The centres have currently received ethical permission for anonymous genetic analyses of the samples, this means that it will not be possible to trace the identity of the samples once they have been analysed. BWG7 is currently

discussing an additional protocol to the BIOAIR study which would allow for connecting the genetic data to phenotypic data of each patient. This would be done via a code kept by the study coordinator. This procedure will require additional ethical review board approvals in most centres. According to a survey among the centres, it is likely that such applications will be approved in most of the countries.

2.11 WP11: Outcomes and Exploitations

Start date or event: Month 30

Completion: Month 36

Partners responsible: **P1**; All partners in the actual discussion of findings and in work in the different BWGs.

Person months per partner and total: 23 months in total,
12 per P1; one per all other partners.

Objectives: To integrate the study results and produce the different projected outcomes. This includes distribution of findings in the scientific world, to disseminate the results into clinical practise and to take all possible actions that benefit the European Society and the public. Should the study discover new biomarkers or patterns of changes that may predict disease severity or progression, this phase will obviously involve interaction with industry or other partners that may be interested in the development for example of kits for measurements of molecular markers. One major outcome is obviously the scientific papers that will be published as a result of the BIOAIR study. It can be projected that each of the seven scientific work packages will produce at least one original paper and in addition contribute to review and guideline publications.

Work accomplished: The infrastructure needed to deliver this work package has been laid during the first year. A study protocol has been defined, procedures developed and the clinical part of the study has been concluded. A publication policy has been established and is documented in the consortium agreement. In addition, the awareness of the problem of severe asthma has increased world-wide and the efforts of the BIOAIR group have been instrumental in that process for example by, as reported elsewhere, participation of GA²LEN members in international (ATS, ERS) and national symposia on severe asthma.

3 CONTRIBUTION OF THE PARTICIPANTS

3.1 Participant 1: Karolinska Institutet, Stockholm, Sweden

Site Leader: Professor S-E. Dahlén

Site Coordinator Huddinge Hospital: Assoc. Professor Dr. B. Dahlén

Scientific Team Members: Drs M. Skedinger, M. Kumlin, P. Gyllfors, A. Kallner

At this centre, the last patient completed the study in March 2005. A total of 15 patients (5 mild asthmatics, 6 severe asthmatics and 4 COPD patients) were included at this centre, of which 12 completed the study (3 drop-outs). The data entry in the eCRF was completed in June 2005.

Mrs. Ingrid Delin carried out several of the duties of the research nurse during Year 3, and was responsible for handling all urine samples both collected locally and those shipped to Stockholm from the other BIOAIR centres. In addition, she also prepared and carried out preparations for the analysis of all urine samples in the whole BIOAIR study which is to take place in Stockholm.

Mrs. Agneta Gülich is the research nurse at Karolinska University Hospital in Huddinge, who during Year 3 was responsible for the patient visits, carrying out the investigations and taking care of all the collected samples.

Mrs. Ingrid Martling (CRM) was responsible for the local monitoring work at the venue of Partner 1 and paid several monitoring visits to the Huddinge Hospital in Year 3 (as well as Years 4 and 5).

Several BIOAIR work group meetings took place during Year 3 at which Stockholm was represented by Professor Sven-Erik Dahlén (genetics Work Group and bronchoscopy Work Group meetings in Southampton, and glucocorticoid Work Group and biomarkers Work Group meetings in Leiden).

Steering Committee (Site Leader) meetings were attended on behalf of Stockholm by Dr. Roelinde Middelveld, Mrs. Ingrid Martling, Mrs. Lurdes Magneli (who replaced Dr. Middelveld during her maternity leave) and Professor Sven-Erik Dahlén. On one occasion, Mrs. Martling combined a Steering Committee meeting in Frankfurt with a monitoring visit to the BIOAIR centre in Krakow (together with Dr. Mariusz Duplaga), in order to save travelling costs.

During Year 3 approval was given by Dr. Nimmegern to transfer part of the resources from personnel to subcontracting, in order to pay the subcontractor Varigon AG for implementing a statistical analyses system in the electronic database. Originally these resources had been intended to pay for a statistician, however, rather than paying a person it was considered part of it was used better in aiding the statistical analyses by implementing this in the database system.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Stockholm.

3.2 Participant 2: Athens University, School of Medicine, Greece

Site Leader: Dr. Mina Gaga

Scientific Team Members: E. Oikonomidou, E. Zervas, N. Papageorgiou, K. Saharidou, N. Papadopoulos, N. Koulouris, K. Kostikas, V Kehagia.

At this centre, the last patient completed the study in March 2005. A total of 33 patients (13 mild asthmatics, 14 severe asthmatics and 6 COPD patients) were included at this centre, of which 30 completed the study (3 drop-outs). The data entry in the eCRF was completed in June 2005.

Apart from the Site Leader Dr. Mina Gaga, two research physicians were actively involved in patient recruitment and inclusion during Year 3. Dr. Erasmia Oikonomidou has been working for the study since the beginning of Year 2, whereas Dr. Lefteris Zervas was employed month 21. Drs. Oikonomidou and Zervas form a well-functioning and enthusiastic team, which was the main contributing factor to the fact this centre included and completed the largest number of patients in the study.

The local monitor, Mr. Alexandros Karantzas, was replaced by Ms. Zoe Garagani during Year 3 (sponsored by Novartis SA, Greece) and she carried out several monitoring visits.

Steering Committee meetings were attended by Dr. Gaga, and Drs. Oikonomidou and Zervas attended the BIOAIR meetings that took place during the ERS conference in Vienna September 2003.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Athens.

3.3 Participant 3: University of Crete, Heraklion, Greece

Site Leader: Professor N.M. Siafakas

Site Co-ordinator: Dr. E. Tzortzaki

Scientific Team Members: M. Tsoumakidou, M. Plataki, M. Zervou, K. Samara, E. Paraskakis, E. Stamataki.

At this centre, the last patient completed the study in March 2005. A total of 27 patients (6 mild asthmatics, 9 severe asthmatics and 12 COPD patients) were included at the site of Partner 3, of which 16 completed the cortisol trial and 10 completed the entire study (17 drop-outs). The data entry in the eCRF was completed in June 2005.

The clinical work at the site of Partner 3 is coordinated by Dr. Eleni Tzortzaki. Ms. Maria Zervou (biologist) and Evaggelia Stamataki (research nurse) was employed during year 2 and 3 to collect and manage the clinical data as well as to carry out all laboratory procedures and methods for the collected BIOAIR samples. Maria Tsoumakidou (research physician), Maria Plataki (research physician), Aikaterini Samara (research physician), Emmanouel Paraskakis (research physician) were responsible for the patients' follow-up and the completion of the clinical visits during Year 2 and 3.

Ms. Maria Kandilogiannaki was recruited Year 2 to act as the local monitor at the site of Partner 3. Her monitoring work for the BIOAIR study was sponsored by Glaxo-SmithKline. Ms. Kandilogiannaki has carried out several monitoring visits to ensure the quality of the collected data at the site of Partner 3.

Steering Committee meetings were attended by both Professor Siafakas and Dr. Tzortzaki during Year 3. In addition, they both attended the Scientific Workshop in Modena October 2004.

3.4 Participant 4: Universita degli Studi di Ferrara, Italy

Site Leader: Professor A. Papi

Scientific Team Members: M. Saetta, M. Romagnoli, C. Bellettato, L. Corbetta, G. Turato, L. Fabbri, F. Braccioni, M. Contoli, G. Caramori, G. Casoni.

At this centre, the last patient completed the study in March 2005. A total of 28 patients (4 mild asthmatics, 13 severe asthmatics and 11 COPD patients) were included in

Ferrara and Modena, of which 22 completed the study (6 drop-outs). The data entry in the eCRF will be completed the end of June 2005.

During Year 3, as well as Year 2, due to organisational reasons it was necessary to use the resources for personnel to employ a physician only, instead of both a physician and a nurse. Dr. Gaetano Carmori, the employed physician, is carrying out the duties of both the physician and the nurse in the BIOAIR study. In addition, Dr. Marco Contoli was also appointed as research physician and he worked together with Dr. Carmori to take care of the patients, investigations and samples.

At the subcontracting centre (University of Modena) 3 physicians were employed during Year 2 and 3. The clinical work at that centre is coordinated by Dr. Micaela Romagnoli, and she was assisted by Dr. Giovanni Ferrara and Dr. Monica Losi. The total costs for the work done at the University of Modena were charged to the project after the completion of Year 3.

The local monitoring was taken over by Ms. Elisa Paolucci during Year 3.

Professor Alberto Papi represented Partner 4 at the Steering Committee meetings during Year 3, and he attended the BIOAIR/ERS workshop in Athens May/June 2003. Dr. Romagnoli attended the BIOAIR meetings taken place during the ERS in Vienna September 2003.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Ferrara and Modena.

3.5 Participant 5: Gent University School of Medicine, Belgium

Site Leader: Professor G. Joos

Scientific Team Members: G. Brusselle, V. Schelfhout, V. Collart, A. Neesen, I. De Borle and G. Van Severen

The original Site Leader Professor Romain A. Pauwels was due to severe disease unable to have but a limited involvement in the project, and he was therefore gradually replaced as Site Leader by Professor Guy Joos. Professor Pauwels' untimely death occurred late in 2004.

At this centre, the last patient completed the study in January 2005. A total of 5 patients (4 mild asthmatics, 1 severe asthmatic) were included in Gent, and all of them completed the study. The data entry in the eCRF was completed in June 2005.

Dr. Vanessa Schelfhout (research physician), Ms. Margareta van Severen (research nurse) and Ms. Ann Neessen (technician) collaborated during Year 3 with the patient visits, investigations, sample collection and handling and data entry in the eCRF. In addition, Ms. Van Severen acted as local monitor, a task which was taken over by Ms. Isabelle de Rudder later on. During the later half of Year 3, Dr. Schelfhout was replaced with Dr. Annelore de Hoorne as research physician.

Professor Joos attended, next to the Steering Committee meetings during Year 3, the ERS/BIOAIR workshop in Athens, and the Scientific workshop in Modena in October 2004. In addition, Professor Guy Brusselle became involved with the Biomarker work group and attended a meeting with this group in Leiden June 2004, as well as the scientific workshop in Modena October 2004.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Gent.

3.6 Participant 6: The University of Groningen, The Netherlands

Unfortunately, partner 6 had to withdraw from the BIOAIR project month 1.

3.7 Participant 7: Hospital Grosshansdorf, Germany

Site Leader: Professor H. Magnussen

Site Coordinators at Hospital Grosshansdorf: Dr. K. Richter, Dr. F. Kanniess

At this centre, the last patient completed the study in December 2004. A total of 25 patients (10 mild asthmatics, 11 severe asthmatics and 4 COPD patients) were included in Grosshansdorf, of which 24 completed the study (1 drop-out). The data entry in the eCRF was completed in May 2005.

As during Years 1 and 2, Ms. Daisy Gerding was employed to carry out the duties of the research nurse. The coordinating and responsible physician is Dr. Frank Kanniess who also acts as Site Leader. Ms. Isabel Elena Zuehlke took over the responsibility as local monitor during Year 3.

Partner 7 took responsibility for keeping the eCRF running when Varigon AG went bankrupt during the course of Year 3, and employed Mr. Karsten Finger to take charge of the eCRF.

Steering Committee meetings were attended by both Dr. Kanniess and Dr. Kai Richter on behalf of Partner 7.

3.8 Participant 8: The University of Leiden, The Netherlands

Site Leader: Professor K.F. Rabe

Site Coordinator: Dr. E. Bel

Scientific Team Members: P. Sterk, A. Roldaan, P. Hiemstra, I. van Veen, S. Gauw

At this centre, the last patient completed the study in January 2005. A total of 30 patients (11 mild asthmatics, 10 severe asthmatics and 9 COPD patients) were included in Leiden, of which 25 completed the study (5 drop-outs). The data entry in the eCRF was completed in June 2005.

The clinical work is coordinated by Dr. Elisabeth Bel, who is also chair of BWG1 (Clinical Observations). The work is mainly carried out by Ms. Stefanie Gauw (research nurse) and Dr. Ilonka van Veen (research physician). Due to the fact the BIOAIR study is very time-consuming, it was found necessary to employ Ms. Gauw full-time whereas Dr. Van Veen was working for the project but paid from other sources.

As in year 2, Ms. Nelia Hellenga took charge of the local monitoring in Leiden. She paid several visits to the site of Partner 8. In addition, the CRM visited Partner 8 for a monitoring visit during month 28.

Steering Committee meetings were attended by Professor Klaus Rabe and Dr. Bel. Dr. Pieter Hiemstra, Ms. Stefanie Gauw and Dr. Elisabeth Bel took part in BIOAIR meetings taking place during the ERS conference in Vienna September 2003. In addition, Ms. Gauw attended the ERS/BIOAIR workshop in Athens May/June 2003.

Partner 7 will play an important role in the data analysis, since Dr. Hiemstra, Dr. Bel, Professor Rabe and Professor Sterk have active roles in several of the BIOAIR work groups.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Leiden.

3.9 Participant 9: The Imperial College of Science, Technology and Medicine, London, UK

Site Leader and Site Co-ordinator St. Mary's Hospital: Professor S.L. Johnston

Site Coordinator Royal Brompton Hospital: Dr. D. Robinson

Scientific Team Members: P. Mallia, D. Campbell

At this centre, the last patient completed the study in April 2005. A total of 14 patients (1 mild asthmatic, 6 severe asthmatics and 7 COPD patients) were included in London, of which 10 completed the study (4 drop-outs). The data entry in the eCRF was completed in June 2005.

As during the first year and second year, also during the third year Dr. Patrick Mallia was employed at St. Mary's Hospital to do the clinical work, including the work that normally would have been done by the research nurse.

Ms. Ashley Owers and Ms. Alison Adderkin are responsible for the local monitoring at the site of Partner 9.

Professor Sebastian Johnston attended the Steering Committee meetings on behalf of Partner 9.

Partner 9 will be in charge of analysing all nasal aspirate samples, as well as some of the sputum samples, during Years 4 and 5.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in London.

3.10 Participant 10: University of Montpellier, France

Site Leader: Professor P. Chanez

Scientific Team Members: I. Vachier, L. Halimi, H. Meziane, C. Devautour, A. Bourdin, C. Chavis, P. Godard, J. Bousquet

At this centre, the last patient completed the study in January 2005. A total of 26 patients (5 mild asthmatics, 13 severe asthmatics and 8 COPD patients) were included in Montpellier, and 23 of them completed the study. The data entry in the eCRF was completed in June 2005.

The clinical work at the site of Partner 10 is coordinated by both Professor Pascal Chanez and Dr. Isabelle Vachier, whereas Ms. Catherine Devautour is the research nurse and, during Year 3, Mr. Houari Meziane the research physician taking care of the patients and all practical duties in the study. Due to administrative reasons at the University of Montpellier, no personnel costs could be charged to the BIOAIR project during Year 2. However, the personnel working for the project was paid from other sources. The resources allocated to personnel were used during Year 3.

Professor Chanez, Dr. Vachier and Ms. Devautour attended the Steering Committee meetings, as well as the ERS/BIOAIR workshop in Athens May/June 2003.

Ms. Agnès Mouraret was appointed as the local monitor and has paid several monitoring visits to the site of Partner 10, whereas also the CRM and PM have visited this centre for a monitoring visit in month 26.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Montpellier.

3.11 Participant 11: Istituto di Fisiopatologia Respiratoria (now named “Istituto di Biomedicina e Immunologia Molecolare”), (Consiglio Nazionale Delle Ricerche; CNR) Palermo, Italy.

Site Leader: Dr. M. Gjomarkaj

Scientific Team Members: E. Pace MD, F. Mirabella MD, R. Gagliardo PhD, G. Chiappara PhD, M. Profita PhD, L. Riccobono PhD.

The original Site Leader of Partner 11, Professor A. M. Vignola, contracted leukaemia in 2003 and unfortunately died at the age of forty in December 2004. His duties were taken over by Dr. M. Gjomarkaj, who had been the physician in charge of the clinical work in the BIOAIR study at this centre from the start. This change does not have any contractual implications, because the change took place after the project officially closed.

At this centre, the last patient completed the study in February 2005. A total of 12 patients (8 mild asthmatics, 2 severe asthmatics and 2 COPD patients) were included in Palermo, and all of them completed the study. The data entry in the eCRF was completed in June 2005.

As indicated, Dr. Mark Gjomarkaj is coordinating the clinical work at the site of Partner 11 and he is the main research physician seeing the patients. In addition, Dr. Elisabetta Pace MD and Dr. F. Mirabella MD (research physicians) are also involved with the clinical work. Dr. Guiseppina Chiappara PhD is taking care of processing the bronchial biopsies taken for the study, and Drs Mirella Profita PhD, R. Gagliardo PhD and L. Riccobono PhD are responsible for processing and analysing the BIOAIR samples. All appointed research physicians are sharing the work that would normally have been done by the research nurse, but in the organisation of Partner 11 it is not common to employ a research nurse. So instead the above mentioned physicians were employed for the BIOAIR project in order to ensure the quality of the samples and biopsies.

Dr. Salvatore Battaglia has been appointed as the local monitor and has made several monitoring visits. In addition, both the CRM and PM paid a monitoring visit to the site of Partner 11 month 26, resulting in a positive monitoring report.

Both Professor Maurizio Vignola and Dr. Gjomarkaj attended Steering Committee meetings and the ERS/BIOAIR workshop on behalf of Partner 11.

Regarding the costs put in ‘adjustments to costs previously reported’, these are the costs claimed on the cost statement of Year 2, but which the European Commission had never received and therefore not approved.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Palermo.

3.12 Participant 12: Faculty of Medicine at Southampton University, UK

Site Leader: Dr. P.H. Howarth

Scientific Team Members: J. Holloway, S. Wilson, A. Sampson, T. Shaw, L. Hewitt, L. Lau, S. Holgate.

At this centre, the last patient completed the study in August 2004. A total of 10 patients (6 mild asthmatics, 2 severe asthmatics and 2 COPD patients) were included in Southampton, and 7 of them completed the study (3 drop-outs). The data entry in the eCRF was completed in June 2005.

Dr. Peter Howarth coordinates the scientific and clinical work of the BIOAIR study at Southampton University. Ms. Lorraine Hewitt is the research nurse involved and carries out much of the practical work. In addition, during Year 3 Ms. Helen Rigden was appointed as technician to assist Dr. Wilson to take care of all bronchial biopsies which were shipped to Southampton from all BIOAIR centres. Ms. Rigden carried out the sectioning of the biopsies and the first preliminary analyses (described in more detail in section 2.5 of this report).

Ms. Catherine Spencer has been appointed as local monitor but is currently on maternity leave. The CRM visited the site for a monitoring visit during month 29.

Steering Committee meetings were attended by Dr. Howarth and Dr. Wilson, as well as the ERS/BIOAIR workshop in Athens May/June 2003.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Southampton.

3.13 Participant 13: The Jagellonian University School of Medicine, Krakow, Poland

Site Leader: Professor A. Szczeklik

Scientific Team Members: M. Duplaga, A. Kania, E. Nizankowska, E. Figiel, E. Karpata, M. Kopyto, M. Gluszek, A. Gawlewicz-Mroczka, M. Koza, I. Gross, B. Ziolkowska-Graca, M. Sanak, K. Sladek, G. Bochenek, L. Mastalerz, G. Pinis.

At this centre, the last patient completed the study in May 2004. A total of 7 patients (1 mild asthmatic, 5 severe asthmatics and 1 COPD patient) were included in Krakow, and all of them completed the study.

The data entry in the eCRF was completed in June 2005. Ms. Figiel, Ms. Karpala, Ms. Marzena and Ms. Malgorzata are the research nurses involved in the study, whereas Ms. Gawlewicz-Mroczka, Ms. Gross, Mr. Kania and Ms. Ziolkowska-Graca are the responsible research physicians taking care of the clinical work.

Ms. Koza is, next to being a research physician, also responsible for the local monitoring. The CRM paid a monitoring visit to this centre during month 27, resulting in a positive monitoring report.

Professor Ewa Nizankowska and Dr. Mariusz Duplaga each attended one of the Steering Committee meetings. In addition, Drs. Kania, Mroczka-Gawlewicz and Ziolkowska-Graca attended the ERS/BIOAIR workshop in Athens May/June 2003.

Please see above for an updated list of Scientific Team Members involved with BIOAIR in Krakow.

4. PROJECT MANAGEMENT AND CO-ORDINATION

4.1 Major project co-ordination activities during the period

Since project co-ordination is part of WP 1, 2 and 4, this has previously been described in sections 2.1, 2.2 and 2.4.

In order to coordinate the work between the centres and to keep track of patient recruitment and study progress, several Steering Committee (SC) meetings were organised during the reporting period. March 2003 a whole day SC meeting took place at Frankfurt airport. In addition, short SC meetings took place in Seattle USA in May 2003 in conjunction with the annual ATS conference, and in Vienna in September 2003 in conjunction with the annual ERS conference.

In order to increase transatlantic collaboration, an ATS NIH sponsored Severe Asthma workshop was organised in conjunction with the ATS conference in Seattle on 17th May 2003. This meeting was attended by BIOAIR members as well as members of the American Network.

A successful ERS sponsored workshop entitled 'Biomarkers in phenotypic and genomic research on severe airway disease' was organised by the BIOAIR group and in particular Partner 1 (Dr Mina Gaga from the University of Athens) and took place 31st May-1st June 2003 in Athens. The workshop was attended by BIOAIR members and others interested in the topic. The speakers consisted of BIOAIR members.

An important task of the management team during 2003 was to monitor patient recruitment, inclusion and follow-up. Since it proved to be far more difficult than envisaged at the start of the study to locate and recruit suitable patients, the recruitment period was extended several times. The last patient was included in January 2004, and the last patient completed the study in April 2005. The electronic Case Record Form (eCRF) system was used to closely monitor the progress of each centre. In addition, the management team closely monitored completion of data entry in the eCRF.

The Clinical Research Monitor continued during 2003 to monitor the study. Several monitoring visits took place at the centres. The monitoring visits consisted of source data verification, check whether the correct methodologies were used and study progress. A monitoring manual was developed in collaboration with an experienced CRM, Mr. Östen Karlsson, from AstraZeneca (unconditional sponsorship), with clear instructions to the local monitors as to how the monitoring should be carried out. The Central monitor visited all BIOAIR centres once at which time point source data verification took place and the local monitors were instructed.

During the third and fourth year the data cleaning process was initiated. On a regular basis Excel sheets with all entered results were extracted from the database in order to get a good impression of how well the datasets were completed, as well as to be able to start with the data cleaning process. In October 2004 a workshop was organised in Modena, Italy, which was attended by Work Group members. At that meeting the Work Group members looked at the data in great detail and listed down queries. These queries were subsequently sent on to the centres involved, where they were looked into, and corrected dataset files were returned. This whole process proved to be very time-consuming. Currently (June 2005) complete cleaned data files have been received by all centres for the bronchodilator reversibility test at screening visit, and the sputum cell counts from visit 1 until visit 3B. Many datasets have not yet been completed and therefore it has not been possible to start with the analysis of the data. However, the

centres are working hard to complete the eCRF, and the Work Groups will put an effort into checking the data and raising queries to the centres. The whole process of data cleaning is currently estimated to be finished by the end of October 2005. After that, the statistical analysis of the data can commence.

In order to plan for the analyses of the samples, the coordinator together with the Biomarker Work Group organised a meeting in Leiden in June 2004. It was discussed again at the meeting in Modena in October 2004. One of the main issues to deal with first is the logistics of the storage and shipments of the samples. It will be too time-consuming and expensive to have the centres ship individual samples to the analysing centres each time an analysis will take place, and therefore the possibility was discussed to ship all samples to a central and store them until analysis. The final decision still needs to be made since there are financial and ethical issues that need to be resolved.

As described in the Periodic Reports of Year 1 and 2, after withdrawal of Partner 6 from the project the coordinator was assigned the task of organising WP2. It was decided that a company based in Germany (Varigon AG in Lübeck) would build an electronic CRF and database system. In month 15 the first version ready for use in the study could be launched. Entrance to the eCRF is password protected, and only authorised personnel is allowed access to the patients data.

After successfully introducing the eCRF system during Year 2, the usage was intensified during Year 3, since most patients were recruited and included during the second half of Year 2 and during the whole of Year 3. Study nurses, physicians and technicians used to eCRF to enter all data collected at the study visits. The CRM and local monitors used the eCRF to check study progress and source data verification. Varigon and later on its successor Pulmonary Research Network provided the management team with regular overview of data entry progress. Unfortunately, the centres performed in varying degrees regarding data entry in the eCRF. Some centres were able to update the eCRF within a week after each patient visit, others took much longer time. This made it difficult to keep a good track on patient and study progress, as the eCRF was not always up to date.

In order to prepare for the statistical analyses at an early stage, a meeting was organised in March 2003 including Nils Drews (CEO) and Nikolaus Peterknecht (IT manager) from Varigon AG, and Professor Dahlén, the PM and CRM, in order to discuss possibilities to build a way of statistical analyses in the eCRF. It was agreed that part of the resources intended for a statistician (originally allocated to Partner 6, but after its withdrawal allocated to the Coordinator) would be used to pay Varigon AG as a subcontractor to build in this system in the eCRF. This would ease the statistical analyses later on. In collaboration with Dr. Josephine Hjoberg (part-time appointed researcher/statistician) the details were further discussed and developed during 2003.

Attempts were initiated already in 2004 to start the process of data cleaning and focus was on getting all data included in the eCRF. Before the meeting in Modena October 2004, all centres were therefore urged to complete all datasets until visit 3B (as at that stage all patients had passed the steroid trial which finished at visit 3B, but not all patients had completed the whole study). This was done fairly well, and a start could be made with the data cleaning. The data cleaning process in itself however took a lot of time, since not all centres were able to check and correct the data right away.

The main reason for some of the centres not being able to keep the eCRF up to date was lack of resources. The resources allocated to them via the BIOAIR project were not sufficient to cover all the staff and time needed for the work. The work therefore had to be spread out over a longer period by staff already paid from other grants. In addition,

some patients (mainly the elderly ones) were not able to use the electronic Peak Flow meters correctly, which meant that they were given manual Peak Flow meters instead. The data from these manual measurements could not be uploaded into the eCRF directly, but had to be entered manually, a very time-consuming process for data collected over a year of observation.

The result is that as of yet it has not been possible to start with statistics and analysis of the data. It was however felt, that despite the delays, it was important not to start doing preliminary analysis of the data before it could be sure the data was correct. Doing the analyses too soon could give false impressions of the results. It is currently estimated all data will be entered by the end of June 2005 and all data will be cleaned by the end of October 2005. Strict deadlines have been given to all centres. After that a start can be made with the analysis.

4.2 Actual and planned allocation of human resources to the project

Table 11. Actual and planned allocation of human resources by work package, including person months allocated to BWG members

WP No	Description	Person months allocated (3 year period), including months allocated to BWG members	Person months used after 3 years, including months allocated to BWG members
1	Project management	18	30.5
2	Data acquisition and statistics	12	12
3	Study Protocol	7	7
4	Clinical observations	211	229
5	Bronchoscopy and tissues	4	4
6	Biomarkers of airway remodelling	17	15
7	Glucocorticosteroid responsiveness	11	11
8	Leukotrienes and other mediators	9	8
9	Infections	6	5
10	Genotype	3	2
11	Global outcome and Exploitations	23	5
	TOTAL	321	328.5

Note: please observe that at the end of the project the work had not been completed, so all Work Packages will put in a total number of person months which is much larger than what is stated in the above table, once all analyses have been carried out.

Table 12. Actual and planned allocation of human resources by participant, excluding person months allocated to BWG members

Participant	Person months allocated 3 year period	Person months used after 3 years
1. Stockholm Co-ordinator	44*	50.4
Stockholm Partner 1	26,4	30.6
2. Athens	14,4	14.4
3. Heraklion	14,4	14.4
4. Ferrara	14,8	15
5. Gent	17,4	17.5
6. Groningen	0	0
7. Grosshansdorf	14,4	16.5
8. Leiden	17,4	26.7
9. London	18,4	18.4
10. Montpellier	14,4	16.7
11. Palermo	33,0	33.0
12. Southampton	19,4	19.1
13. Krakow	14,4	17.1
TOTAL	262,8	289.8

*Including 8 months for a scientist/statistician (previously allocated to partner 6 Groningen).

5. EXPLOITATION AND DISSEMINATION ACTIVITIES

5.1 Progress towards exploitation of the project results

A consortium agreement has been signed between participants describing how ownership of results will be dealt with, as well as patents resulting from the study findings. So far, no patent activity has taken place. When analyses of study results has taken place, these will be published in major scientific journals in the respiratory field.

5.2 Major dissemination activities during the period

In order to increase transatlantic collaboration, an ATS NIH sponsored Severe Asthma workshop was organised in conjunction with the ATS conference in Seattle on 17th May 2003. This meeting was attended by BIOAIR members as well as members of the American Network.

A successful ERS sponsored workshop entitled 'Biomarkers in phenotypic and genomic research on severe airway disease' was organised by the BIOAIR group and in particular Partner 1 (Dr Mina Gaga from the University of Athens) and took place 31st May-1st June 2003 in Athens. The workshop was attended by BIOAIR members and others interested in the topic. The speakers consisted of BIOAIR members.

Most Partners have engaged in presentations of the BIOAIR project at local or national meetings during the year, in part because of the need to raise additional funding.

In October 2004 a large Italian national meeting for pulmonologists was organised on 'Mechanisms of Lung Inflammation' in Modena. This meeting included both national and international speakers, among which several BIOAIR members (see enclosed copy of programme). This meeting was attended by several 100 people, and a BIOAIR workshop was organised in conjunction with the meeting.

At the ATS conference in San Diego May 2005, a Scientific Symposium was organised regarding 'Severe Asthma: Advancing our understanding of a distinct phenotype', with speakers from both the NIH network on Severe Asthma and the BIOAIR network. Among others, Professor S-E Dahlén was one of the speakers with the topic 'Severe Asthma: the European perspective and insights' during which he presented the BIOAIR project. This symposium received a lot of attention and the room was overcrowded with about 500 attendees. A copy of the programme is enclosed. Also at the same ATS Conference, one whole day post-graduate course was dedicated to severe asthma and several BIOAIR team members contributed importantly. Professor Pascal Chanez from Montpellier lectured on quality of life in severe asthma and Dr Elisabeth Bel from Leiden presented on the role of infectious diseases in severe asthma.

At the World Allergy Conference in Munich June 2005, Dr Peter Howarth from Southampton and Dr Elisabeth Bel from Leiden used experiences from the BIOAIR study when presenting in a seminar on how to treat severe asthma. Professor Pascal Chanez from Montpellier lectured on how to collect sputum from patients with severe asthma and Professor Klaus Rabe of Leiden gave a major plenary lecture on near fatal severe asthma.

5.3 Publications

The results of the previous study initiated by the ENFUMOSA (European Network for Understanding Mechanisms of Severe Asthma, funded by the European Union's 4th Framework Programme, Contract number BMH4-96-1471)) group were published in the European Respiratory Journal (ERJ 2003, 22: 470-477) (reprint enclosed). This cross-sectional observational study showed that severe asthma is a predominantly

female disease and characterised by neutrophilic infiltration, rather than eosinophilic infiltration as is the case in mild controlled asthma. These findings prompted for longitudinal studies, which resulted in the ENFUMOSA group instigating the BIOAIR study.

In the same issue as the above mentioned publication, an editorial was published by Wenzel S.E. 'A different disease, many diseases or mild asthma gone bad? Challenges of severe asthma'. (ERJ 2003: 22: 397-398) (copy enclosed). This editorial was published regarding the publication of the ENFUMOSA study, and it describes BIOAIR as the next step forward in unravelling severe asthma.

A paper entitled 'Risk factors and characteristics associated with severe and difficult to treat asthma phenotype. An analysis of the ENFUMOSA group of patients based on the ECRHS questionnaire' by Mina Gaga, Niki Papageorgiou, Georgia Yiourgioti, Panagiota, Karydi, Adamantia Liapikou, Hari Bitsakou, Eleftherios Zervas, Nikos G Koulouris and Stephen T Holgate on behalf of the ENFUMOSA study group, has recently been accepted for publication in *Clinical and Experimental Allergy*. The BIOAIR study will build further on these results. Several manuscripts are currently prepared on topics that were first observed in the ENFUMOSA study and then built upon in the BIOAIR project.

One major projected outcome is obviously the scientific papers that will be published as a result of the BIOAIR study. It can be estimated that each of the seven scientific work packages will produce at least one original paper and in addition contribute to review and guideline publications.

In addition to the above publications, the purpose and organisation of the BIOAIR project have been reported in some international newsletters and promotional activities targeting physicians in respiratory medicine.

6. ETHICAL ASPECTS AND SAFETY PROVISIONS

A sample bank has been created by the samples that have been taken and not immediately analysed. All patients have given their approval of storage of these samples for later analysis. It was decided during Year 1 that genetic analysis will only be done at the population level, so it will not be possible to trace back those samples to the patient and this is what the centres have currently received ethical permission for. However, during Year 2 BWG7 discussed whether an additional protocol to the BIOAIR study could be developed which would allow for connecting the genetic data to phenotypic data of each patient. This would be done via a code kept by the study coordinator. This would require new ethical approvals by all centres, and in some cases new approvals by the patients, but it is likely that in most countries this will be approved under certain conditions.

During Year 4 it was discussed whether it would be feasible to store all samples in one central storage location (possibly in the newly purpose-built facilities at the site of Partner 1) in order to ensure all samples are stored properly, and can be retrieved easily when needed. However, it was found out this might not be possible due to different legislations in the different countries. Some countries do not allow permanent storage of patient samples in a different country. This could however be overcome by making this a temporary arrangement. A final decision will be made once it has been decided exactly which analyses will be done at which centre, and how the financial issues related to central storage can be resolved.

SECTION IIb: PROJECT FINAL REPORT

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1. OVERVIEW OF PROGRESS DURING THE LIFETIME OF THE PROJECT

1.1 Main objectives of the project

Asthma and Chronic Obstructive Pulmonary Disease (COPD) are major respiratory disorders that currently affect about 15% of adults in Europe and are increasing in prevalence. Effective treatment of COPD remains an unmet medical need whereas most asthma patients can be adequately treated with inhaled medications. There exists, however, a subgroup of about 10% of asthma patients that respond less well to therapy, including high doses of inhaled or oral glucocorticosteroids. These patients are identified as suffering from severe asthma and their disease has a profound effect on their quality of life. No completely adequate treatment has yet been established for severe asthma. Patients with severe asthma therefore impose a severe burden on health care utilisation through unscheduled visits to general practitioners, emergency visits, hospitalisations, days off work, and a requirement for extensive use of asthma medications. Among patients with asthma, those with severe disease are considered to be at greatest risk of premature death, and it has been estimated that in comparison with patients having mild to moderate asthma, the severe asthmatics are 15 times more likely to use emergency services and 20 times more likely to be admitted to hospital. There are also data supporting that the 10% of the most severe asthmatics make up more than 50% of direct and indirect health care costs. However, the mechanisms that distinguish severe asthma remain undefined.

The overall objective of this Project is to identify mechanisms that make asthma severe. The establishment of such new understanding will provide more accurate methods for diagnosis and assessment of prognosis, and new tools for the detection of high risk populations. Since adequate treatment of patients with severe asthma and COPD is a major unmet medical need, the Project is designed to explain the shortcomings of current treatment strategies as well as to identify new targets for therapeutic interventions.

The Project aimed at accomplishing the first large scale standardised longitudinal observational study of the clinical course in severe persistent asthma, and to compare it with COPD and moderate to mild asthma. This has the potential to provide novel information on the molecular biomarkers and the clinical course in severe asthma. The Project combines cutting edge basic scientific technology with modern clinical methodology to elucidate mechanisms which result in the progressive development of severe persistent airflow limitation in asthma and COPD. The aim is to define markers and mediators which can be measured to predict and follow the course of these diseases and, also, to define novel targets to prevent the development of debilitating forms of these disorders. The design with repeated measurements in well-characterised cohorts defines the phenotype of severe asthma with a temporal precision which will give the analysis of biomarkers of inflammation and tissue remodelling exceptional and unprecedented strength. The study included a double-blind placebo-controlled investigation of the effects of a two week oral glucocorticosteroid treatment where clinical end-points and biomarkers will be assessed. It is hoped to integrate the findings into a comprehensive diagnostic profile for severe asthma, including the establishment of objective morphological or biochemical criteria for identification of individuals at risk to develop severe asthma and factors which are of prognostic importance. The Project has the potential also to identify mechanisms that may become future targets for intervention.

1.2 Scientific progress of the project as a whole

The first main task was to develop a detailed study protocol in order to create the platform for testing the large number of hypotheses. To this purpose all Work Group members attended the Protocol Definition meeting in Leiden, the Netherlands, in month 4 of the project. The protocol was finalised during the months after the Protocol Definition meeting (further discussions via e-mail and telephone conferences).

In short, three groups of patients (mild asthmatics, severe asthmatics and COPD patients) were recruited according to specific inclusion and exclusion criteria. They were well-characterised at the first visit after which they entered a period of 4 weeks during which their medical treatment was optimised. After this optimisation period they participated in a placebo-controlled steroid intervention trial in order to examine their responsiveness to glucocorticoids. They were then followed-up during one year, where they returned to the clinic at 4-month intervals. At all visits to the clinic, among other things, lung function and exhaled nitric oxide (NO) were measured, the patients filled in questionnaires, and induced sputum, blood, nasal aspirate and urine samples were taken. On a daily basis at home, the patients measured their lung function with an electronic Peak Flow meter (PEF) meter and answer diary card questions. Patients came to the clinic on extra visits when they had an exacerbation. Bronchial biopsies were taken in a sub-group of patients.

Collection of as large a number of data as in this study required a well-controlled data collection and storage system. The German company Varigon AG was responsible for the development of an electronic Case Record Form (eCRF) system. This system is Internet-based, so that each centre can access it directly via the Internet and enter data (of course password-protected and access only for those involved with the study). The data is securely stored at a server in Hamburg, Germany.

In order to ensure overall high quality of the same methodology carried out at all centres, several workshops were organised during the first year regarding sputum induction, nasal aspirates, bronchial biopsies, as well as using the eCRF, measuring exhaled NO, using the electronic PEF meters and the Brady barcode system. These workshops were attended by members of all participating centres. In addition, a detailed Study Procedures Handbook was written and distributed to all centres. The Study Procedure Handbook is a very detailed and up-to-date 150 A4 pages collection of methods and procedures for clinical investigations of airway diseases, probably second to none that have been attempted in the field. The team members have been able during the course of the study to download the Study Procedure Handbook from the eCRF data server.

A system for monitoring the quality of the collected data was also developed, both via visits to the centres by the Clinical Research Monitor and via raising monitoring queries in the eCRF.

The first patient was included month 15 and the last patient month 37. In total, 266 patients were recruited to the study of which 33 turned out to be screening failures. A total of 233 patients were randomised (76 mild asthmatics, 93 severe asthmatics and 64 COPD patients), of which 43 dropped out during different stages of the study. Table A shows the number of patients that completed Phase I of the study (cross-sectional characterisation and steroid trial), and the percentage of those that completed the follow-up year, which was 93.5% of all patients. This latter figure is very important as that means that one of the major study outcomes can be properly evaluated, i.e. will the

baseline characteristics of individuals during Phase I and the response to the steroid trial predict the clinical course during the follow-up year.

Table 13. Number of patients completing different stages of the BIOAIR study.

	Mild	Severe	COPD	Total
Recruited	85	106	75	266
Completed Phase I (% of recruited)	66 (77%)	85 (80%)	52 (69%)	203 (76%)
Completed study (% of completed phase I)	65 (98.5%)	77 (90.5%)	48 (92.3%)	190 (93.5%)

Bronchoscopy has been carried out in 34 patients (14 mild asthmatics, 14 severe asthmatics and 6 COPD patients). All biopsies were processed locally, and afterwards shipped to Southampton for quality check and analysis. Of GMA processed biopsies 79% of patients leaving biopsies had at least one biopsy of good quality. The number of biopsies taken is lower than projected at the start of the study. There are three main reasons for this: 1/ A proportion of eligible patients did not consent to undergo this procedure. 2/ Several of the patients with severe airway disease did not meet the inclusion/exclusion criteria for bronchoscopy (For safety reasons we adhered to strict internationally recognised guidelines). 3/ Five out of the twelve centres did not perform bronchoscopy because of lack of experience and/or because failure to obtain ethical permission for the procedure. Nonetheless, these biopsies will be valuable and will be used to study one or several specific scientific questions arising from the analysis of clinical phenotype data and biomarker measurements in other samples.

A total of 203 patients have completed the steroid trial of which 102 patients received prednisolone and 101 placebo. An oral double-blind and placebo-controlled steroid trial comparing asthma and COPD in the same study have previously not been done, and the addition of molecular biomarkers of steroid responsiveness is another novel approach.

Regarding exacerbations, 8 have been recorded for mild asthmatics, 48 for severe asthmatics and 18h for COPD patients. Since the completion of the database is not yet ready, this number is likely to increase. In addition, exacerbations might have been missed by the patients initially, but may be detected upon analysis of the daily PEF results, and this might in turn increase the number of exacerbations further. Spirometry and questionnaires data (St. Georges and Asthma Control Questionnaire) are available for more than 95% of subjects and visits. Also daily lung function measurements (~90% of patients) and diary cards questions (~85% of patients) seem to have been completed very well.

Urine and blood samples are available for more than 95% of subjects and visits. Exhaled NO values are available for 60-70% of patients at the centres that carry out these measurements, nasal aspirate in 50-70% of patients. Regarding sputum, cell counts are available in 50-75% of patients, supernatant is available in 60% of patients and sputum plugs in 15-30% of patients (generally, the lower number in the range

concerns COPD patients, whereas the higher number concerns asthma patients). These figures are based on information entered in the eCRF. Since not all data has yet been entered these figures may still increase.

Once all samples have been collected they will comprise a large and unique biobank for investigating severe asthma. This biobank has all credentials to give rise to many scientific breakthroughs regarding the diagnosis and understanding of the mechanisms of severe asthma. It will be used to test the hypotheses raised in the BIOAIR study directly, but it will also constitute unique opportunities for future research when new questions might need to be answered. It is therefore extremely important the samples are kept under secure conditions (mainly at -80°C), as well as the information regarding phenotype of the patients. For some sub-studies it may be necessary to anonymise the samples (e.g. genetic studies).

Regarding analyses of the many samples, a start was made during 2004, when MBL (Mannose-Binding Lectin) was measured in Leiden in serum samples taken at visit 1 of all patients, and (SAE-IgE) Staphylococcus enterotoxin IgE antibody as well as total IgE were measured in Gent also in serum samples taken at visit 1 of all patients. The cleaning of the phenotypical and clinical data awaits the comparisons between patient groups.

Performing these analyses created awareness towards the huge task of the shipments of all samples. In order to do the MBL and SAE-IgE analyses, all centres were asked to ship the serum samples of visit 1 to Leiden and Gent. This process took two months, and was next to time-consuming, also cost-consuming, due to the fact the shipments had to be made on dry ice in order to ensure the quality of the samples. Currently, a central storage of all samples is considered, in order to minimise shipment costs (all samples can be shipped to the central location in one shipment) as well as to have better control over the samples. However, national legislations need to be considered, since not all countries may allow permanent storage of samples abroad.

In October 2004, a scientific workshop was organised in Modena, Italy, for members of the Work Groups. This meeting was used to actively go through the data available of the first descriptive and cross-sectional phase of the study (including the steroid trial). A start was made with raising queries regarding the available data, and these queries were sent to the centres afterwards. It was decided that data analyses could not start (and would not be correct) until all datasets were completed and all data cleaned. During the months after the Modena meeting it became clear that this would be a time-consuming process. However, progress was being made, and by March 2005 all sputum cell count data until visit 3B was cleaned, as well as most of the lung function data.

In March 2005 80% of the electronic database was completed up until visit 3B. The last patient completed the study in April 2005 and in June 2005 the electronic database was completed and the main data cleaning process will take place in the next three months.

All in all, it is expected the immense amount of data collected will give rise to a large number of high-quality scientific publications. The results will be available to the general public in this way. It is hoped that in the end, the result of this study will give rise to new or better treatments for patients suffering from severe asthma.

1.3 Update of tables 1, 2 and 3 from the technical annex

Table 1 BIOAIR Technical Annex: Work Package (WP) list – Updated June 2005

WP No	Description	Responsible	Person months	Start month	End month	Deliverables	Current Status (June 2005)
1	Project management	P1	18	0	36	1	Management structure set-up completed month 2
2	Data acquisition and statistics	Was P6, now P1 and BWG1	12	1	36	2-6	Electronic data acquisition system ready for use month 14
3	Study Protocol	BWG1	7	1	6	7-9	Study Protocol completed month 9, Procedures Handbook completed month 13, Ethical Permissions completed month 20
4	Clinical observations	BWG1	211	6	30	10-13	Patient recruitment started month 15. 265 patients recruited month 37 (patient inclusion was then closed).
5	Bronchoscopy and tissues	BWG2	4	1	36	14-16	Bronchoscopy guidelines completed month 7, workshop conducted month 7. Biopsies available from 34 patients.
6	Biomarkers of airway remodelling	BWG3	17	1	36	17-18	Proposal for set of measurements completed month 11, workshop sputum conducted month 7. The first biomarkers were analysed month 45.
7	Glucocorticosteroid responsiveness	BWG4	11	1	36	19-20	Contributions to protocol and proc. handbook completed month 9 and 11. The first analyses to be done during 2005.
8	Leukotrienes and other mediators	BWG5	9	1	36	21	Contributions to protocol and handbook completed month 9 and 11. The first analyses to be done during 2005.
9	Infections	BWG6	6	1	36	22	Contributions to protocol and handbook completed month 9 and 11. The first analyses to be done during 2005.
10	Genotype	BWG7	3	1	36	23	Contributions to protocol and handbook completed month 9 and 11. The first analyses to be done during 2005.
11	Global outcome and Exploitations	P1 and all partners	23	18	36	24-28	In progress
	TOTAL		321				

Table 2 BIOAIR Technical Annex: List of Milestones (M) – Updated June 2005

M no	Specification	Month	Update June 2005	Involved Partners
1	Management team and operational procedures	4	Completed month 2	P1
2	Protocol, methods, permissions and data handling for clinical investigation	6	Protocol: 9 Methods: 13 Permissions: 15 Data handling: 15	P1-P13
3	100 patients recruited to study; Bronchoscopy and Induced Sputum Workshops completed	9	Workshops completed month 7, Recruitment started month 15. 265 patients recruited month 37.	P1-P13
4	300 patients included in study	15	265 patients by month 37 (recruitment closed)	P1-P13
5	450 patients included in study	18	265 patients by month 37	P1-P13
6	450 patients completed clinical study; 30 in each group having been subjected to repeated bronchoscopy	30	265 patients by month 37. 34 patients subjected to bronchoscopy (14 mild asthma, 14 severe asthma, 6 COPD)	P1-P13
7	Cleaned database and results of clinical study	32	In progress, estimated to be ready end of 3rd quarter 2005.	BWG1 ¹
8	Cleaned database and results of measurements of biomarkers, including leukotrienes, GCS-axis, viruses, and candidate genes	34	In progress	BWG3 ²
9	Conclusions on global outcome: Molecular and clinical markers of severe chronic airflow disease	36	In progress	P1-P13

¹/ Co-ordinated by BIOAIR Work Group 1: P1, P2, P3, P7, **P8** (chair), P10 & P13, but the work will involve all partners and work groups.

²/ Co-ordinated by BIOAIR Work Group 3: **P1** (chair), P8, P10, P11 & P12, but the work will involve all partners and work groups.

Table 3 BIOAIR Technical Annex: List of deliverables (DL)

DL no	SPECIFICATION	Delivery date	Status June 2005	Nature	Dissemination level
1	Establishment of management team and operational procedures	Month 4	Completed month 2	O	CO
2	Establish methods for data handling	Month 6	Completed month 15	O	CO
3	Cleaned database for results of clinical study	Month 32	Estimated to be ready 3rd quarter 2005	R	RE
4	Cleaned database for results of measurements of biomarkers	Month 33	In progress	R	RE
5	Preliminary compilation of findings in clinical part of study	Month 33	Estimated to be ready by the end of 2005.	R	RE
6	Overview of findings in analytical part of study	Month 36	Estimated to be ready 2006.	R	RE
7	Study Protocol	Month 6	Completed month 9	R	PU
8	Study Procedures Handbook, Logistic guidelines and Clinical Record Forms (CRFs)	Month 6	Handbook month 13 Logistics 14 CRF 14	R	RE
9	Acquisition of ethical and other permissions	Month 6	Completed month 15	R	PU
10	150 patients recruited to study	Month 9	Recruitment started month 15. 139 patients recruited month 27	P	CO
11	300 patients included in study	Month 15	265 patients recruited month 37 (end of inclusion)	P	CO
12	450 patients included in study	Month 18	265 patients recruited month 37 (end of inclusion)	P	CO
13	450 patients completed study	Month 30	June 2005: all patients have completed	P	CO
14	Bronchoscopy Guidelines and Procedures	Month 6	Completed month 7	R	RE
15	BAL fluid from all eligible patients	Month 30	During the course of the study it was decided not to take BAL fluid.	O	CO

16	Biopsy specimens from all eligible patients	Month 30	Month 43	O	CO
17	Proposal of primary standard set of measurements of biomarkers	Month 6	Completed month 11	R	CO
18	Completed measurements of biomarkers of remodelling in collected specimens	Month 34	In progress	R	CO
19	Clinical response to oral glucocorticosteroids	Month 30	In progress	R	RE
20	Biochemical and morphological characterisation of steroid responsiveness	Month 34	In progress	R	RE
21	Integrated assessment of leukotriene pathway (levels, enzymes, receptors)	Month 34	In progress	R	RE
22	Integrated assessment of viral infections	Month 34	In progress	R	RE
23	Candidate gene screen	Month 34	In progress	R	RE
24	Internal reports from BWGs to team	Month 35	In progress	R	RE
25	Preliminary conclusions from Team Workshop	Month 35	In progress	R	RE
26	Assessment of global outcome: Molecular and clinical markers of severe chronic airflow disease	Month 36	In progress	R	PU
27	Publications on guidelines for diagnosis and treatment of severe chronic airflow obstruction	Month 36 and thereafter	In progress	R	PU
28	Results on Website and Reports to the Public	Month 36 and thereafter	In progress	R	PU

2. EXPLOITATION AND DISSEMINATION ACTIVITIES

2.1 Results achieved towards exploitation

A consortium agreement has been signed between participants describing how ownership of results will be dealt with, as well as patents resulting from the study findings. So far, no patent activity has taken place. When analyses of study results have taken place, these will be published in major scientific journals in the respiratory field.

2.2 Major dissemination activities during the period

A project website was launched month 10 (<http://www.bioair.org/>). In addition, the BIOAIR eCRF portal (<https://bioair.varignon.de/>) also provided the Team members with information about the project.

In collaboration with Vitalograph and Aerocrine two press releases were organised in year 1, indicating the collaboration between both companies and BIOAIR.

Workshops and Team Information meetings were organised during year 1. Month 4 the Protocol Definition workshop and meeting took place. Month 5 a BIOAIR Team Information meeting was organised at the ATS annual conference in San Francisco, USA. A workshop on induced sputum/bronchoscopy/nasal aspirate methodology was organised in Southampton in month 7. This workshop was aimed to inform Team members about this methodology. Month 9 a study Start-up meeting for Team members was organised for Team members, at which time-point also workshops were organised on the use of the eCRF, Vitalograph Peak Flow meters and Aerocrine's exhaled NO analysing machine NIOX. Month 9 also a Team Information meeting took place in Berlin, Germany, during the ERS annual conference.

At the annual ATS congress in Atlanta USA during Year 2 (2002) a joint symposium of the European Respiratory Society and American Thoracic Society was organised, called 'Approach to tackling severe refractory asthma: the major issues' (a copy of the programme is enclosed). At this symposium speakers representing the American network on Severe Asthma (sponsored by the NHLBI) and the European BIOAIR network (sponsored by the European Union) presented their latest research results. A concluding presentation was held by Professor Dahlén describing the BIOAIR project. This symposium was well-attended and improved the awareness among the Americans about European research into severe asthma by the BIOAIR group.

In addition, a closed meeting was organised with the American network on Severe Asthma during the same ATS congress, in order to inform each other about their research and discuss possible collaborations.

A BIOAIR Team meeting was also organised during the ATS congress in Atlanta. This meeting was attended by members of most centres.

In order to establish better relations between the BIOAIR centres and to increase the possibilities for collaboration between the centres in this network, a one-day BIOAIR Scientific Workshop was organised in conjunction with the ERS congress and the Mid-Term Review meeting in Stockholm month 21. This successful workshop was attended by members of all centres. Next to providing a global overview of the status of the BIOAIR study, and providing the opportunity to discuss practical study-related issues and problems, this was also an opportunity for each Partner to present its centre and research. This successful day was concluded with a Team dinner.

The co-ordinator and one of the Partners (Dr. E.H.D. Bel, P8: University of Leiden) each contributed with a 30 min presentation at the annual autumn meeting of the Swedish Society for Allergology where a whole day meeting was dedicated to discuss the problem

of severe asthma.

In order to increase transatlantic collaboration, an ATS NIH sponsored Severe Asthma workshop was organised in conjunction with the ATS conference in Seattle on 17th May 2003. This meeting was attended by BIOAIR members as well as members of the American Network.

A successful ERS sponsored workshop entitled 'Biomarkers in phenotypic and genomic research on severe airway disease' was organised by the BIOAIR group and in particular Partner 1 (Dr Mina Gaga from the University of Athens) and took place 31st May-1st June 2003 in Athens. The workshop was attended by BIOAIR members and others interested in the topic. The speakers consisted of BIOAIR members. There was also at this occasions presentations by BIOAIR members for local Greek physicians on research and treatment of severe asthma. Dr Gaga also raised significant sponsorship from local sources.

In October 2004 a large Italian national meeting for pulmonologists was organised on 'Mechanisms of Lung Inflammation' in Modena. This meeting included both national and international speakers, among which several BIOAIR members (see enclosed copy of programme). This meeting was attended by several 100 people, and a BIOAIR workshop was organised in conjunction with the meeting. The meeting was possible to hold with the aid of significant sponsorship raised by Professor Leo Fabbri in Modena (P4)

At the ATS conference in San Diego 2005, a Scientific Symposium was organised regarding 'Severe Asthma: Advancing our understanding of a distinct phenotype', with speakers from both the NIH network on Severe Asthma and the BIOAIR network. Among others, Professor S-E Dahlén was one of the speakers with the topic 'Severe Asthma: the European perspective and insights' during which he presented the BIOAIR project. This symposium was well-visited, and a copy of the programme is enclosed. Also at the same ATS Conference, one whole day post-graduate course was dedicated to severe asthma and several BIOAIR team members contributed importantly. Professor Pascal Chanez from Montpellier lectured on quality of life in severe asthma and Dr Elisabeth Bel from Leiden presented on the role of infectious diseases in severe asthma.

At the World Allergy Conference in Munich June 2005, Dr Peter Howarth from Southampton and Dr Elisabeth Bel from Leiden used experiences from the BIOAIR study when presenting in a seminar on how to treat severe asthma. Professor Pascal Chanez from Montpellier lectured on how to collect sputum from patients with severe asthma and Professor Klaus Rabe of Leiden gave a major plenary lecture on near fatal severe asthma.

Most Partners have engaged in presentations of the BIOAIR project at local or national meetings during the course of the project, in part because of the need to raise additional funding. It is fair to conclude that the BIOAIR group during the course of the project has acquired significant international recognition for the study protocol and for the expertise in severe asthma that it possesses.

2.3 Publications directly emanating from the project

It is expected a large number of publications directly emanating from the project will appear in a number of scientific respiratory journals, during the years to come. The large amount of collected clinical data, as well as the large number of samples still to be analysed will contain an immense amount of previously unavailable data.

3. ETHICAL ASPECTS AND SAFETY PROVISIONS

Several ethical issues arose during the development of the protocol. Since a large part of the patients have severely decreased lung function, these issues needed to be considered especially carefully, in particular the invasive investigations. Before inclusion in the study all patients were given an information sheet containing information about the study and the investigations. Each patient signed an informed consent form before inclusion. The following ethical considerations can be made:

At each visit venous blood samples will be taken (on two occasions also arterial blood gas measurements will be done). The amount of blood taken is however limited (a maximum of 100 ml on two occasions and 40-60 ml at the other visits). The blood gas samples, in addition, give important basic information about the patients and need therefore to be taken.

Nasal aspirate samples will be taken during 4 visits. This involves the rinsing the nose with saline, and is not painful or uncomfortable. In some occasions if not sufficient mucus is obtained, a histamine-containing spray will be used, which may cause a slight itching in the nose. This effect is however short-lasting.

With regard to the induced sputum and bronchoscopy procedures, these are obviously the most invasive investigations and could potentially be associated with a risk of adverse events, especially for those that have a decreased lung function. Therefore, care has been taken to develop criteria to only include patients in these procedures. These criteria are internationally accepted and standardised and several of the investigators participating in BIOAIR were in fact involved in the development of these international criteria. The criteria are such that according to strict guidelines, patients that have a severely decreased lung function will not undergo these investigations. The safety of the patient must always go first. The procedures will only be carried out by experienced nurses and physicians, and the patients will constantly be monitored during and after the procedure.

The patients will undergo a two-week oral trial of prednisolone tablets or capsules. The treatment with prednisolone is standard at a worsening of asthma or COPD, and should if anything improve the patients' condition, also those with mild asthma. Many of the patients are already treated chronically with this medication, whereas for the others it is a too short time-period to give rise to chronic steroid side effects.

All participating centres have received approval from their local ethical review boards for conducting this study. Patient information sheets and informed consent forms have been translated into the local languages and have been prepared according to national regulations.

A sample bank has been created by the samples that have been taken and not immediately analysed. All patients have given their approval of storage of these samples for later analysis. It was decided during Year 1 that genetic analysis will only be done at the population level, so it will not be possible to trace back those samples to the patient and this is what the centres have currently received ethical permission for. However, during Year 2 BWG7 discussed whether an additional protocol to the BIOAIR study could be developed which would allow for connecting the genetic data to phenotypic data of each patient. This would be done via a code kept by the study coordinator. This would require new ethical approvals by all centres, and in some cases new approvals by the patients, but it is likely that in most countries this will be approved under certain conditions.

During Year 4 it was discussed whether it would be feasible to store all samples in

one central storage location (possibly in the newly purpose-built facilities at the site of Partner 1) in order to ensure all samples are stored properly, and can be retrieved easily when needed. However, it was found out this might not be possible due to different legislations in the different countries. Some countries do not allow permanent storage of patient samples in a different country. This could however be overcome by making this a temporary arrangement. A final decision will be made once it has been decided exactly which analyses will be done at which centre, and how the financial issues related to central storage can be resolved.

4. CONCLUSIONS

Severe asthma has been estimated to affect less than 15% of adult asthma patients in Europe, but to account for more than 50% of the overall society costs for asthma. In addition to increased quality of life for the European population suffering from this condition, there would also certainly be an economical benefit if more effective diagnosis, treatment and prevention of severe asthma could be found. Even if a new treatment in itself might be more expensive than the current treatments used for severe asthma, there would be overall savings for the European society in terms of reduced secondary costs (hospitalisation, absence from work, etc.). Since the mechanisms underlying severe asthma remain at large, the main intention of this project was to conduct a study that will aid to define mechanisms of relevance to severe asthma, thereby possibly contributing to the overall goals of developing better methods for diagnosis, prevention and treatment.

The results of the previous study initiated by the ENFUMOSA (European Network for Understanding Mechanisms of Severe Asthma, funded by the European Union's 4th Framework Programme, Contract number BMH4-96-1471) group were published in the European Respiratory Journal (ERJ 2003, 22: 470-477). This cross-sectional observational study showed that severe asthma is a predominantly female disease and characterised by neutrophilic infiltration, rather than eosinophilic infiltration as is the case in mild controlled asthma. These findings prompted for longitudinal studies, which resulted in the ENFUMOSA group developing the BIOAIR study protocol and conducting the clinical part of the trial during the past three years.

The initial analysis of the BIOAIR study results will focus on the clinical data. A better definition of the clinical phenotype(s) in severe asthma is the fundamental basis on which discovery of new molecular mechanisms can take place. The main aim will be to determine whether there are any specific clinical characteristics that can predict asthma outcome. The patients' baseline values have been determined at the start of the project and after a treatment optimisation period of 4 weeks. These values will then be compared with the values after undergoing the oral steroid intervention trial. This will give indications as to the patients' responsiveness to glucocorticoids, both with regard to clinical outcomes and molecular biomarkers. During the follow-up period of one year succeeding the steroid trial, the patients have been observed during continued optimised treatment. This has included measurements of lung function and diary card questions about symptoms and medication use on a daily basis.

One of the main endpoints is exacerbations and it is hoped to find specific biomarker indications for the onset and severity of an exacerbation. From the preliminary assessment of data, it seems that the two patient groups with severe airways disease (COPD and severe asthma) have had less exacerbations during the study year than in the preceding year. This presumably illustrates the importance of regular contacts with the health care provider to aid the patient optimise the treatment.

In the next phase of our work, focus will be on analysing the many biomarker samples collected in the study. Analysing is hoped to give new clues for specific biomarkers associated with severe asthma, as well as more information regarding tissue remodelling, the leukotriene pathway, the role of nitric oxide, the influence of viral infections, responsiveness to glucocorticoids, and genetics. Following the primary analysis of the clinical outcomes, there will therefore be a scientific meeting where the BIOAIR WPs interact to discuss findings and decide upon the priorities for measurement of biomarker molecules.

The co-ordinator of the BIOAIR project has in addition applied to the executive committee of the GA²LEN EC NoE to obtain support for holding a scientific meeting where the members of GA²LEN may benefit from reports on the results of the BIOAIR study. We have in fact proposed that GA²LEN forms a severe asthma network. Eight of the BIOAIR partners are already members of GA²LEN, and by expanding into a network comprised of 26 Universities, it is likely that the knowledge of the BIOAIR group could be more effectively disseminated into both research and clinical care in Europe. The integration of new studies within GA²LEN could certainly benefit from access to the methodology, experiences and biobanks gained by the ENFUMOSA group during the BIOAIR study

One major lesson learned by the BIOAIR project is how difficult it is for academic partners to conduct a major clinical trial without funding for the full study cost. The BIOAIR study would not have been possible to start without the important support of the European Commission, but at this time in the project, it is felt appropriate also to evaluate what could have been done differently in terms of financial support.

First, budgeting before the study start is not optimal. It would have been a great advantage for the coordinator to have had substantial parts of the funding undistributed from the beginning. This in order to use a performance related reimbursement system to stimulate recruitment of patients and completion of other deliverables.

Second, there should be mechanisms for successful studies such as the BIOAIR study to obtain supplemental funding from the Commission in order to better utilise the data collected and the investments already made in the study by the Commission. Perhaps a mechanism for re-negotiation of the study budget at the time of the mid-term review could be considered? In our case, at the time of the Mid-term review, it was clear that the total study costs were met by the partners to more than 80% (6708 k€ out of total cost 8200 k€), far above the shared cost level. It should be recognised that this is the first study ever of this particular kind that has been conducted in severe asthma. It is therefore unfortunate that the full future use of the findings and the comprehensive exploration of samples in the biobank created by BIOAIR to some extent must await new funding.

We are aware that the Sixth Framework has implemented more flexible methods for the use of funding, and we hope this is continued even more when the important support of European clinical research is decided for the Seventh Framework Programme. It should in the future be a primary objective of the Commission to provide significant support for corporate-independent European clinical research in respiratory medicine.

In conclusion, the BIOAIR study is an unprecedented and unique mechanistic study conducted in a very severe group of subjects. The extensive preparations before the start of the study and the professional and accurate conductance of the BIOAIR study, as well as outstanding quality control of the data, have created an excellent database and biomarker bank that now will be analysed in great detail in the next few years. It is hoped that this platform will lead to opportunities for the definition of novel mechanisms of importance in severe asthma. This will hopefully result in a series of important scientific discoveries that lead to improved quality of life for severe asthma patients as well as reducing costs for the European Community. It is estimated that up to 1.0% of the European population suffers from severe asthma.

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SECTION III: SCHEMATIC DESCRIPTION OF THE PROJECT

Overall objectives of the project:

Around 10% of asthma patients suffer from severe asthma, a condition that remains poorly defined but carries a severe burden to both patients and national health care systems. The main objective of this project was to clarify the biological mechanisms that explain why certain patients have severe asthma, which cannot be adequately treated. It was hoped to find objective morphological or biochemical criteria ('biomarkers') that can help to identify patients that are at risk of developing severe asthma, or that can predict asthma exacerbations. The overall goal was to find more effective strategies for diagnosis, prevention and treatment of this disease. A main objective is also to establish differences and similarities between severe asthma and Chronic Obstructive Pulmonary Disease (COPD).

Experimental approach and working method:

Twelve leading European pulmonary research clinics recruited patients to the BIOAIR study. The patients were divided into three groups according to strict inclusion criteria: mild asthmatics, severe asthmatics and COPD patients. The study had two phases. During the first phase all patients visited their clinics frequently (4 to 5 visits within a 10 week period), during which time a thorough cross-sectional characterisation of the patients was conducted. This included collection of clinical and laboratory data, and the documentation of the response to a two week oral glucocorticosteroid treatment. The second phase consisted of a one year follow-up during which the patients visited their clinics every three months. During the follow-up, a standard set of investigations were made at each visit, including clinical and physiological assessments and collection of blood, urine, sputum, nasal secretions and bronchial biopsies for future measurement of key biomarkers. Moreover, daily home recordings were made of lung function, respiratory symptoms and the use of asthma medications. Similar and specific measurements were made when the patients had exacerbations of their disease. For each group of studied patients, the data from the first cross-sectional phase of the study will be related to the clinical outcome in the same group during the one-year follow-up. The design also allows for comparisons between the three study groups.

Achievements and results:

In total, 266 patients were recruited to the study, and 233 patients were randomised (76 mild asthmatics, 93 severe asthmatics and 64 COPD patients), of which 43 dropped out during different stages of the study. Of those patients that completed the cross-sectional phase, 93.5% also completed the follow-up year. A large clinical database has been collected, as well as a large number of biomarker samples. The last patient completed the study in April 2005, the electronic database was completed in June 2005, and it is currently estimated the data cleaning will be completed in October 2005. After that the analyses of the data and samples will commence and scientific meetings will be held to interpret and disseminate the results.

The five most relevant publications emanating from the project:

It is expected a large number of publications directly emanating from the project will appear in a number of scientific respiratory journals, during the years to come. This large amount of clinical data and biomarker samples have never before been collected in this particular group of patients with severe airway disease.