

IMI1 Final Project Report Public Summary

Project Acronym: U-BIOPRED

Project Title: Unbiased Biomarkers
for the Prediction of Respiratory
Disease Outcomes

Grant Agreement: 115010

Project Duration: 01/10/2009 - 30/09/2015



Innovative Medicines Initiative

IMI PROJECT FINAL REPORT INCLUDING THE PERIODIC REPORT FOR THE LAST PERIOD¹

Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes

U-BIOPRED

115010

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Reporting Period 6

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Description of work – v8 1st July 2014

Submission deadline

¹ See Articles II. 4.1 and II 4.2 of the IMI model Grant Agreement.

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Appendix 1 – Response on IMI questions financial part

Appendix 2 – Response on IMI questions technical part

Declaration of the coordinator

I, the coordinator of this project, declare that,

The final report submitted is in line with the obligations as stated in Article II.2.3 of the Grant Agreement:

The attached report represents an accurate description of the work carried out in this project for the last reporting period as well as for the whole duration of the project;

For the last period, the project (*tick as appropriate*):

has fully achieved its objectives and technical goals; has achieved most of its objectives and technical goals for the period with relatively minor deviations²;

has failed to achieve critical objectives and/or is not at all on schedule³.

For the whole duration of the project, the project (*tick as appropriate*):

has fully achieved its objectives and technical goals;

has achieved most of its objectives and technical goals with relatively minor deviations³;

has failed to achieve critical objectives and/or is not at all on schedule³.

The public project website www.ubiopred.eu³ is up to date.

To my best knowledge, the financial statements which are being submitted as part of this final report are in line with the actual work carried out and are consistent with the report on the resources used for the project (section 7) and if applicable with the certificate on financial statement.

All participants, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes or deviations have been reported under section 6 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

Name of the Coordinator: Prof. Peter Sterk

Date: 30/ 11/ 2015

Signature of the Coordinator:



² If either of these boxes is ticked, the report should reflect these and any remedial actions taken.

³ Please add the address of the public project website. The home page of the website should contain the generic IMI logo which is available in electronic format at the IMI website. The area of activity of the project should also be mentioned.

1. Executive summary

1.1. Project rationale and overall objectives of the project

Context

Asthma is one of the most common chronic diseases, affecting patients from childhood to elderly age. In most patients symptoms can be sufficiently suppressed by currently available medicines. However, the 3-5% of patients with the most severe disease cannot be treated adequately, providing a large personal and societal burden. Development of new treatments for individuals with severe asthma is urgently needed but hampered by lack of validated clinical and biological disease markers, underperforming of pre-clinical models, inadequate sub-phenotyping, and insufficient understanding of disease mechanisms. U-BIOPRED aimed to newly discover molecular networks operative in severe asthma and to link those to the clinical expressions of the disease.

Hypothesis

The use of biomarker profiles comprised of various types of high-dimensional data, integrated with an innovative systems biology approach into distinct *phenotype handprints*, will enable significantly better prediction of therapeutic efficacy in severe asthma than single or even clustered biomarkers of one data type, and will identify novel targets.

Objectives

1. Generating consensus and global standard operating procedures (SOPs) on diagnostic criteria, clinical phenotyping and disease outcome D2.4, D5.3,
2. Creating adult/paediatric cohorts and biobank for cross-sectional and longitudinal studies in well characterized severe asthmatics and controls D3.2, D3.5, D3.6, M8
3. Generating phenotype handprints of severe asthma by an innovative systems biology strategy D4.2,
4. Validating the accuracy of phenotype handprints in identification of newly included patients D8.5
5. Refining phenotype handprints with pre-clinical animal and human exacerbation models D6.3, D6.4
6. Validating the handprints for their predictive efficacy in gold standard and experimental therapeutic intervention D7.3
7. Refining the diagnostic criteria and phenotypes of severe asthma by incorporating the newly established handprints D8.5
8. Establishing a platform for exchange, education and dissemination. D1.4, D10.7

1.2. Overall deliverables of the project

The project objectives were divided into 10 Workpackages (WP) and 69 deliverables (D) and the entire project took 6 years (5 years plus 1 year budget neutral extension). This has allowed to deliver 63 deliverables, whilst 6 deliverables are being finalized during the first year in the post-funding period.

Governance

At the start of the project the Management Office and the Finance Office were installed and made operational. This included a web-based platform for interactive monitoring progress, a week by week schedule of teleconferences based on WPs or specific tasks within the project and face-to-face meetings when needed in addition to the broad annual consortium meetings. This structured management served the project very well. The Finance Office was in direct contact with all partners and assisted in preparing the yearly progress reports. Based on the development of the project, new business cases could be submitted to the Management Board, based on a standard template, in order to optimally facilitate the demands of the Workpackages. Both, the Management Board and Scientific Board took all decisions by consensus.

Consensus and standardisation

The scientific work started by reaching consensus amongst all academic and industrial partners about the definition of severe asthma and the clinical algorithm how to confirm the diagnosis of true severe asthma amongst patients with clinically 'difficult' asthma. This U-BIOPRED consensus was published in 2011. Having those criteria on board allowed the development of Standard Operating Procedures (SOPs) for measuring the clinical parameters of the study. These SOPs were aligned with the ones used by colleagues in the US and subsequently became a standard for several projects in parallel or following U-BIOPRED elsewhere. Eventually, after including all patients (see below) the cohort, the SOPs, the biobank and the database collectively formed the U-BIOPRED registry. Renewed consensus was reached at the end of the funding period, but this will still be revised based on the final results of the project in the first 6 months after the funding period (Q2 2016).

Adult and paediatric cohorts

The major clinical effort was the inclusion of the adult and paediatric patients. Despite the enormous expertise of the clinical centres in the study, this inclusion of the cohort took twice as long as anticipated. This was due to sincere optimism by the centres on their eligible patient numbers, and the very intensive and time consuming procedures related to building the agreed clinical protocol, the eCRFs, and the de-central ethics approval in the various centres and countries. Eventually, the cohorts were delivered adding up to 1025 patients/controls in total. Due to these delays the follow-up period of the severe asthma patients was shortened to 12-18 month, so both the baseline dataset and the longitudinal data could be delivered during the lifespan of the project and are secured in the biobank and the TransSMART database (see below).

Biological samples

Good biological samples were vital to the project. That is why extensive standard operating procedures (SOPs) were developed for ensuring the quality of samples and data from blood, sputum and bronchial brushes and bronchial biopsies. Sputum induction and processing was centrally trained, whilst sputum cell counting was done centrally by a single lab. Similarly, bronchial biopsy specimens from each clinical centre were first checked for quality and only after accreditation a particular study centre could proceed. The biobank was centralized and all the biobanking procedures were according to international recommendations. These QC steps have largely contributed to the quality of the data.

Experimental exacerbation

The clinical study was paralleled by a human experimental study, meant to mimic an asthma exacerbation in the lab. Exacerbations are one of the main unresolved problems in severe asthma and are mostly primed by a rhinovirus infection. That is why U-BIOPRED developed a Good Manufacturing Practice (GMP) rhinovirus and performed experimental rhinovirus infection based on strict SOPs as a model of exacerbations (in moderately severe asthmatics). The dose-escalation study in healthy and asthmatic volunteers provided a safe and effective dose (for cold symptoms) of rhinovirus 16 (RV16) and the asthma biomarker study with RV16 has been accomplished by Dec 31, 2015. The omics platforms and bioinformatics of the RV16 study are currently being run in the post-funding period.

Preclinical models

A vital aspect of the U-BIOPRED study was to line up the various pre-clinical models of (severe) asthma that were used in academia and pharmaceutical industry. The immediate added value of the project was the exchange of such models and their SOPs and results between a large number of laboratories. It appeared that the standard chronic allergen (house-dust mite) mouse model is not matching vital aspects of severe asthma (such as steroid insensitivity). But this was accomplished by adding influenza virus rather than rhinovirus or with CFA as adjuvant. These models provided immunological (Th1, Th2, Th17), inflammatory and gene expression features that could be suppressed by novel treatments (CRID3). In addition to mouse models, human *in vitro* and *ex vivo* models comprising bronchial epithelial cell and airway smooth muscle cell cultures and also precision cut lung slices have delivered the samples for detailed transcriptomic analysis that will be finalized by Q2 2016.

'Omics' platforms

The core activity of U-BIOPRED was to examine high-dimensional molecular profiles as obtained in blood, sputum, bronchial brushes, bronchial biopsies, urine and exhaled air. This was done at the RNA (transcriptomics), protein (proteomics), lipid (lipidomics) and metabolic (metabolomics including breathomics) levels. Microbiome analysis was added by extra (ENSO) funding. The first step was to implement SOPs and quality control standards across the consortium. Subsequently, differentially expressed compounds between severe asthma, mild to moderate asthma and healthy controls were delineated. In parallel, unbiased fingerprints from all these platforms were generated, providing entirely novel subgroups of (severe) asthma based on molecular profiling. For instance, eosinophilic and neutrophilic severe asthma appears to be associated with 3 and 4 different proteomic signatures, respectively, pointing towards different endotypes underlying particular inflammatory profiles. This has direct implications for selecting targets for phenotype-driven, novel treatments and thereby represents a core deliverable of the project. Similar paediatric fingerprints are due in Q2 2016.

Bioinformatics

U-BIOPRED relied on cutting-edge bioinformatics. The chain between raw data and eventual fingerprints and handprints was standardised and developed where necessary. This included a detailed Data Analysis Plan for both 'regular' statistics and high-dimensional analysis (U-BIOPRED

Tool Box) that combined rigorous statistics with topological analysis. The TranSMART knowledge management system was used as starting point and was further tailored for the U-BIOPRED needs. The fingerprint and handprint analysis covered new grounds in data subsetting, feature filtering, omics-based clustering and biomarker identification. Where possible the analysis was done by a priori selection of a training set and a validation set. Platform fingerprints were generated, providing entirely novel phenotypes of (severe) asthma that are associated with, but are also beyond traditional inflammatory phenotyping. The first cross-sectional, multi-scale handprints integrating various 'omics' platforms were generated from sputum (sputum handprint 1, 2, and 3) and blood (blood handprint 1, 2, 3, and 4). These molecular handprints appeared to be associated with clinical symptoms and inflammation. These handprints are used for biomarker discovery using the U-BIOPRED Asthma Map of mechanistic networks, and are now being mirrored to a limited biomarker set (analyte set) for usage in the clinical setting. These very final steps will be delivered in Q2 2016. Delivery of these newly discovered handprints is the major outcome the project.

Dissemination and communication

The project and its results were brought closer to the public by various means. The public website was quickly online and was constantly adapted with material promoting the project and its results (interviews, video's, art contest). Patients participating in U-BIOPRED judged and adjusted scientific material (lay abstracts) in order to bridge the gap between professional and societal communication. This increasingly included social media. The professionals followed and are following a step-wise process, in which abstracts, presentations and scientific papers are being announced, endorsed and monitored by a publication matrix. The open and collective strategies between patients and professionals in the project can be regarded as a large achievement by U-BIOPRED.

Ethics and patient participation

Right from the start U-BIOPRED implemented an Ethics board and Safety Monitoring Board for independent monitoring of protocols, patient burden, severe adverse events (SAEs), sample management, publications and potential crisis situations. The boards required well-defined charters and SOPs of these boards, which are applicable outside U-BIOPRED. The activities of the boards were summarized in annual reports. The experimental rhinovirus study was most closely monitored by weekly involvement of patient representatives. The Patient Input Platform published its U-BIOPRED experience, which can be regarded as a step-change in patient participation in biomedical EU projects.

Conclusion

U-BIOPRED has delivered in response to the IMI call 'Understanding of severe asthma': its cohorts, its 'omics' analysis, its fingerprints and its multi-scale handprints. As planned, these are now input into new intervention studies by pharma industries, in order to select patients for effective treatment. This public-private collaboration has been a step-change in the research of severe asthma and the respiratory field in general.

All deliverables are summarized in the table below.

Deliverables (Grey = complete)					
1.1	Finance office	4.5	Bronchoscopic brushing bio bank	8.2	SOPs, data formats, quality metrics
1.2	Consortium interaction platform	4.6	Bronchoscopic biopsy bio bank	8.3	Analysis of legacy and public data
1.3	Monitoring and planning platform	5.1	GMP rhinovirus inoculum	8.4	Data integration method
1.4	Progress reports	5.2	SOP's viral challenge	8.5	Cross-sectional handprints
1.5	Yearly meetings	5.3	Dataset viral safety study	8.6	Handprints pre-clinical lab models
1.6	Report on possible business models	5.4	Dataset viral challenge study in patients	8.7	Handprints clin. models & interventional model
1.7	Report on initiation of business model	5.5	Standard protocol viral challenges	8.8	Refined handprints longitudinal data
2.1	Consensus meeting	6.1	Meeting on animal models and SOPs	8.9	Analyte, microbiomic, metabolic fingerprint
2.2	Published consensus document	6.3	Dataset HDM model, initial RV infection	9.1	Consortium website
2.3	Globally agreed SOPs	6.2	Dataset ex vivo tissue models	9.2	Short video
2.4	Severe asthma registry	6.4	Meeting to refine models	9.3	Art contest
2.5	Handprint refined consensus	6.5	Dataset initial humanized mice model	9.4	Materials for local meetings
2.6	Refined criteria for phenotypes	6.6	Analysis of pre-clinical models and humanized mouse model	9.5	Framework for writing committee
3.1	Study protocols	6.7	Mechanism validation	9.6	Personnel exchange platform
3.2	eCRFs	7.1	Summary of best available omics tech	9.7	Promotion & comms strategy
3.4	Adult cohort	7.2	SOPs for omics assessments	10.1	Charter for Ethics Board
3.5	Paediatric cohort	7.3	Biomarkers from pre-clinical studies	10.2	Charter for Safety Board
3.6	Baseline data-base and biobank	7.4	Novel individual biomarkers	10.3	SOPs for ethical conduct of science
3.7	Longitudinal data-base and biobank	7.5	Omics dataset for phenotype handprint	10.4	Harmonized informed consent form
3.8	Exacerbation data-base & biobank	7.6	Dataset of analyte results	10.5	SOPs for safety monitoring
4.1	SOPs bronchoscopic & nasal biopsy	7.7	Microbiomics dataset	10.6	SOPs for safety crisis management
4.3	Accreditation adult & paediatric biopsy	7.8	Dataset of Metabolomics results	10.7	Annual safety and ethics reports
4.4	Framework for bio banking	8.1	Knowledge Management structure		

All the deliverables in the table above have an associated deliverable report. Those deliverables in black text show reports that pertain to information that is expected to change as further work, in the legacy period, is completed. In all cases this is due to the continued analysis work on the data.

1.3. Summary of progress versus plan since last period

The final period of U-BIOPRED saw the completion of all of the clinical and laboratory analysis planned within the project period. The analysis of the data generated by U-BIOPRED was and still is a large-scale task and this will provide data for detail analysis for years to come. However, the core initial analysis as defined in the deliverables has been achieved, while work on further handprints

(including handprints related to D8.7 and D8.8) will be needed prior to a renewed position paper (D2.5 and D2.6) is published.

The legacy period has been well prepared for, with the key data generating activities complete and funding secured for maintenance of the core outcomes, including the Rhinovirus storage, biobank, data storage and a core management infrastructure.

WP1: Governance

The governance of the project continued by its 'management by consensus' process. Proposals by U-BIOPRED partners for new, relatively small activities that were not anticipated at the grant writing stage (business cases) were judged by the Management Board.

- The financial aspects of the project have required some adjustments in terms of partners delivering the planned work. These have been implemented with the approval of the Management Board and within the rules for re-allocation of funding by the IMI.
- In addition and in relation to this, the business case process for re-distributing budget within the IMI rules resulted in a further 3 cases being awarded by the Management Board. These cases summarised specific work, including additional allergy and immuno-histo-chemistry tests, and part of the funding related to financial residue related to late contributions from EFPIA partners whose original purpose had been resolved using additional beneficiary contributions. The proposal process was managed by the Management Board.

WP2: Consensus

This Workpackage had its top activities at the very beginning and at the very end of the project.

- After reaching its first refinement of the consensus, WP2 continues to iterate its core outputs as the proposed refined consensus document and refined criteria will be updated as new analysis results emerge during Q2 2016.

WP3: Clinical cohorts

The quality control (QC) of the clinical data of the longitudinal visit has required prolonged efforts, based on double checks between source data and TransSMART data. This has also led to definitively tie up of residual details in the baseline data set. The data cleaning team will release the longitudinal QC data by end of May 2016.

- WP3 has delivered the paediatric and adult cohorts with the planned number of recruited subjects in total (n=1025).
- The cross-sectional baseline data have been quality controlled, and the closed database is being used in TransSMART by the consortium partners.
- The final step of WP3 is to deliver the quality-controlled databases for the longitudinal and exacerbation visits. This will be accomplished by May 2016.

WP4: Biological samples

The quality control and laboratory procedures of all samples were finalized. The biobanked samples were secured, also for the post-funding period.

- All bronchoscopy work was completed according to the description of work.
- The sputum and biopsy material has been quality controlled and immunocytochemistry/immunohistochemistry has been performed.
- Data are now being presented in draft publications.

WP5: Experimental exacerbation

The final year of the project allowed accomplishing the experimental rhinovirus infection study in normal and asthmatic volunteers. In order to do so, the invasive procedures (outcome measurements obtained by repeated bronchoscopies) were omitted. Last patient out was December 31, 2015.

- Part 1 of rhinovirus 16 (RV16) challenge study (Safety/Dose escalation component - D5.3) has been completed. The viral dose of the newly produced GMP RV16, which safely caused viral symptoms and viral replication in the nasal lavage, was selected and defined as the challenge dose for part 2 of the challenge study (main virus challenge biomarker study – D5.4). Generation of the report of part 1 of the study is under way. 100TCID₅₀ was determined to be the correct dose level as it was seen to be effective in over 80% of participants. The previous dose level of 50TCID₅₀ was seen to have a lower infection rate than required.
- Problems with the portable, home-lung function device occurred in part 1. Approximately 30% of the data were not reliable. Therefore, it was necessary to replace the PIKO lung function device in part 2 of the virus challenge study via an amendment 2 of the study protocol. A new Carefusion Microdiary device was deployed as a replacement, which performed better. The home spirometry data obtained with this device were eventually quality checked by a panel of investigators during two teleconferences in March 2016.
- The necessity of the amendment caused a delay of the study start of part 2. Since the timeline of the study was already severely delayed and recruitment in part 2 was felt to be even more difficult compared to part 1, it was decided that the bronchoscopies in part 2 were omitted from the protocol to speed up recruitment. This change was included in the amendment 2. Without the bronchoscopy data, the rhinovirus-induced perturbation of the asthma fingerprints will be less extensive. However, for future application the available and less invasive blood fingerprint and handprint will be much more relevant.
- The new site, MEU (Medicines Evaluation Unit) Manchester, had been contracted by the sponsor (MSD) and was activated before part 2 of the study started. This meant that Amsterdam (AMST), Hannover (HANN) and MEU recruited all participants for part 2 of the study. A CRO in Groningen, the Netherlands (QPS) supported AMST with referring one patient for the study.
- By December 31, 2015 the study was closed when 23 out of 25 subjects were challenged with rhinovirus. Biosamples (nasal brushings, blood for lipidomics and proteomics, etc.), were collected and will be shipped to the Manchester Biobank. In addition, adsorbed breath samples for breathomics were sent to Amsterdam and PBMCs (peripheral blood mononuclear cell) will be sent to MSD for genomics.

- After finishing the study (D5.4.) at the end of December 2015, clinical data and ‘omics’ platforms are being analysed by MSD in close collaboration with the ‘omics’ labs that have generated the omics data in U-BIOPRED and the data will be imported into TranSMART for further analysis. D5.4 will be delivered by May 2016.
- Biosamples sent to the biobank will be available for further analysis by U-BIOPRED partners pending supplementary funding from U-BIOPRED partners themselves, including MSD.
- As planned, the RV16 GMP material is subject of further analysis over time (stability data, non-infectivity data), which are currently determined at Bioreliance. These data are requested by regulators (BfArM) before the material can be used as GMP material in clinical trials. These data will be added to the available IMPD (**Investigational Medicinal Product Dossier**). Note: The requirement for formal safety testing of the virus for future post-project usage became evident during the project, but the funding is only now being processed (the work is ongoing) and therefore it is not included within the project period.
- When the IMPD has been completed with the stability data, the study protocol of part 2 together with the IMPD will be ready for final delivery as D5.5 (Standard protocol viral challenges). D5.5 will be delivered by May 2016.

WP6: Pre-clinical models

The final year was used for aligning various mouse models and for comparison of outcomes in relation to human severe asthma.

- In studies using the CFA/HDM model (Complete Freund’s Adjuvant/House Dust Mite) we were able to show that Penh measurements correlated well with lung resistance (RL) and acetylcholine (Ach) responsiveness ($-\log PC_{200}$) using whole body plethysmography. Penh was subsequently used to reduce numbers of animals in all longitudinal animal studies, because Penh can be measured very reproducibly.
- We developed and validated a humanised mouse model of severe asthma. We demonstrated that it is possible to model interactions between a respiratory virus infection in the lung at very low infectious dose (100pfu – plaque forming units) and development of asthma.
- A robust mouse model of severe asthma exacerbations following infection with low dose influenza virus A/X31 H3N2 was developed.
- Proof of concept for novel model of severe asthma was achieved. Using a mouse model of severe asthma based on chronic allergen (HDM) and adjuvant (CFA) exposure, which is relatively steroid insensitive, we were able to demonstrate that targeting a novel pathway, the NLRP3 inflammasome (described in human severe asthma), is highly effective in reducing airway hyperresponsiveness and inflammation.
- In preliminary studies we were able to show that it is possible to map mouse ‘paw prints’ to human fingerprints using TDA (Topological Data Analysis) on GSVA (Gene Set Variation Analysis) signatures derived from mouse models of asthma. This is a critical outcome,

since it cross-checked measures from animal models against those from patients with severe asthma, thereby validating (parts of) these models for severe asthma research.

- We have begun to map transcriptomic data from mouse models of severe asthma unto human severe asthma transcriptomic data from sputum using a combination of TDA and GSVA approaches either alone or in combination.

WP7: 'Omics' platforms

The final year was used to deliver the 'omics' platforms. This required activities in various academic and industrial labs, which was eventually very successful.

Of the 22 platforms or Workflows managed within WP7, 20 have been delivered whilst 2 are delayed and delivery will occur after this report is due. The workflow groups were formed around concrete pieces of work. Sometimes this was a single platform, sometimes this related to a number of platforms. The workflows reflected the type of work and the group of people involved and was created through WP7 and WP8 discussions. Clear progress has been made with analysis already underway and a clear plan for completion in Q1 2016 is in place. These are the following:

- Biomarkers from pre-clinical human challenge models: The primary model in U-BIOPRED consisted of an in vivo study involving a rhinovirus challenge model in which asthmatic participants were exposed to human rhinovirus produced to GMP standards. As reported by WP5, there was significant delay in starting the in vivo study and at the time of writing of this report collection of all the samples has not be completed. The patient numbers (n=25) and thereby the sample numbers are very limited as compared to the samples from the U-BIOPRED clinical cohort. Therefore, the leads of all the 'omics platforms have provided information about the costs and timelines to complete the analyses and there is a commitment to finalize March 2016. This will require additional funding from U-BIOPRED pharma partners or some other source of funding. Therefore, MSD 'omics' labs have been offered as feasible alternative. Delivery will be in May 2016.
- The delivery of the urine metabolomics fingerprints, derived from non-targeted metabolomics, for which funding outside the original U-BIOPRED application was obtained through an ENSO grant, has been delayed by *force majeure*. First, the post-doctoral researcher recruited to perform the analysis left for an industrial position after 1 month into the position. It was therefore necessary to recruit another post-doctoral researcher that took an additional 4 months. Second, a major technical problem occurred with the mass spectrometer purchased to perform the metabolomics analyses. The mass spectrometer was installed in February 2015; however, it did not pass the installation specs until September 2015. Accordingly, urine metabolomics analyses could not be initiated until October 2015. Data acquisition has now begun and preliminary data is shown in the metabolomics deliverables section. The data have become available in March 2016. Full (complete) analysis will be available by end of April 2016.

WP8: Bioinformatics

The key delivery of U-BIOPRED is providing the fingerprints and handprints of severe asthma. Using the available 'omics' data the U-BIOPRED analysis toolbox was run. This delivered several fingerprints (from transcriptomics to breathomics) and the sputum- and blood handprints.

- Initial cross-sectional handprints (D8.5) have been regenerated with inclusion of the amended baseline clinical dataset.
- The other planned handprints (related to deliverables D8.6, D8.7 and D8.8) are ready to be produced. The underlying data to generate these are close to be available and quality controlled. Thereafter, the statistical scripts can be run so that D8.6, D8.7 and D8.8 will definitely be delivered by Q2 2016.

WP9

Both the exploitation side of WP9 and the dissemination work saw major achievements in the final period.

For dissemination U-BIOPRED presented a dedicated symposium at the ERS Congress 2015, with over 1000 people in attendance. 33 Conference abstracts were submitted and all accepted for either poster, symposium or oral presentations. Baseline cohort description papers for the Adult and Paediatric cohorts were published in the European Respiratory Journal and a further 10 papers have been drafted and are in the process of being submitted for publication

- Presence at international conferences has ensured a high profile for the project in the field. U-BIOPRED featured in the World Village at ERS and a booklet of U-BIOPRED activities at Congress was produced.
- Communications approach is outlined on final WP deliverable D9.7
- The website and twitter/LinkedIn platforms continue to be updated
- A new tailor-made video clearly explaining the concept and delivery of U-BIOPRED to lay persons has been uploaded to the website

For exploitation, maintenance level funding was secured from the ERS, supporting management, communications, biobank, data storage and rhinovirus storage and publications support. Updates for the website and social media platforms secured into the sustainability period.

WP10

The three boards of this Workpackage finished and published their work, even though ongoing patient participation is still required and secured.

- The WP10 committees completed their work in the final period. Of the four groups, the Patient Input Platform (PIP) is continuing activity through to the sustainability period. And the group has produced a unique booklet on the patient input experience with support for the printing is from IMI.

- The Ethics Board continued to advise on developments in the RV16 clinical trial and in the storage of data. The Ethics process for the duration of the project has been recorded, with patient input and completed with all issues resolved.
- The Safety Monitoring Board finalised its work and carried out a review of all severe Adverse Events forms produced in the project in order to double check there were no outstanding follow up issues. This was primarily for the WP3 core clinical study as no SAE's were reported in the challenge study. No issues were identified.
- The impact of patient involvement was published in a peer review journal: 'From tokenism to meaningful engagement: best practices in patient involvement in an EU project. <http://www.researchinvolvement.com/content/1/1/5>'

1.4. Significant achievements since last report

WP1

- The knowledge portal has seen final developments in terms of features to download and store data and a large amount of data has been uploaded to the U-BIOPRED instance of transSMART hosted at partner 4 (LOIC).
- The Project management team has initiated and driven the legacy planning process, including developing a legal framework for continuing the collaboration among partners, together with engaging the European Respiratory Society (ERS) to ensure the core outputs/assets of the project (biobank, database, viral storage, consortium activities). Partners BioSci Consulting and AMST have managed the contractual aspects associated to both pieces of work.
- The reporting process has been steered and overseen by WP1, with intense efforts in terms of discussion, channelling scientific objectives and results and forming manuscript writing teams in the past year – and continuing into the legacy period.
- Financial management of the consortium has continued, led by the AMST with support from BioSci Consulting. Clear instructions for reporting and support of the CFS/From D process have been carried out.
- WP1 has supervised the upload of all baseline clinical data and omics datasets to the U-BIOPRED instance of transSMART. This has included extensive efforts to validate the data prior to upload.
- Agreement was obtained from U-BIOPRED members to continue working together developing the results and key outputs/assets, initially on the basis of an amended Project Agreement.
- Engagement of the ERS for the U-BIOPRED legacy was consolidated with a formal contract signed in order to facilitate financial support for the sustainability period. This is a critical post-project achievement to ensure the legacy of U-BIOPRED.
- U-BIOPRED core facilitator systems were carried through into the sustainability period, including the project management platforms, biobank, database management, conference call facility. Explicit management systems (boards, teleconferences) were planned and installed for the sustainability period.

- A business plan and approach to sustainability was agreed and outlined in D1.6 and D1.7. Based on this, the European Respiratory Society has agreed to fund the maintenance of the biobank and database, whilst also covering the storage of the GMP rhinovirus stock.
- Collaboration has been instigated with members of a 1500 subject asthma cohort in Brazil (ProAR), which will enable comparison and cross-validation of findings between the two cohorts

WP2

- Meeting on consensus positions and revised phenotypes was held, leading to the first iteration of the revised consensus statement and the proposed criteria for phenotypes.
- The revised U-BIOPRED consensus from Q3 2016 extends the original one published in Thorax 2011, by stating that sub-classification of asthma based on cluster analysis of clinical characteristics alone is not accurate enough. During the past 5 years the following two major steps have been taken to improve this.
 - 5 domains of chronic airways diseases (including severe asthma) should always be considered: variable airflow obstruction, fixed airflow obstruction, inflammation, infection and remodeling.
 - Biomarker profiles rather than single or even clustered biomarkers of any type are enabling better prediction of therapeutic efficacy. These profiles include 'omics' fingerprints and handprints. The preliminary sputum handprints (SH1-3) and blood handprints (BH1-4) of severe asthma, presented at the ERS Amsterdam September 2015 (figure 2.4.11), are indeed demonstrating differential clinical expression profiles. The definitive handprints will therefore lead to the 3rd and final iteration of the U-BIOPRED consensus in Q3 2016.

WP3

- Delivery and closure of the quality controlled clinical database of the paediatric and adult cohorts in TranSMART. This required multiple queries for the individual site, which were handled by WP3 with the CRO CROM Source.
- The clinical characteristics have been published.
- Clustering of severe asthma using clinical parameters has been done (see 2.4).

WP4

- Bronchoscopy sample collection was completed with delivery of bronchial biopsies, bronchial brushings and nasal brushings from all 4 U-BIOPRED adult cohorts.
- Immunohistochemical biopsy analysis has been performed.
- Immunocytochemistry performed on quality controlled cytopins.
- Quality controlled bronchial biopsy, bronchial brushing and nasal brushing samples have become available for transcriptomic analysis, which has already been done successfully and linked to the airway histology (see 2.4)
- Bronchoscopy samples (biopsies, bronchial brushings and bronchoalveolar lavage) have been delivered for the WP6 programme.
- The role of bronchoscopic samples as a "gold standard" of asthma phenotypes have been evaluated.

- Extensive quality control has been conducted on 159 bronchoscopy related samples as below.
- The first histology paper has been written and is close to submission to a peer review journal.
- Bronchoscopy related samples:
 - Group A – 53
 - Group B – 20
 - Group C – 40
 - Group D - 46

SAMPLE	Number taken	Pass quality control	Action
Bronchial Biopsy (GMA)	159	139 (87.4%)	Immuno- histochemical analysis (inflammation)
Bronchial Biopsy (Paraffin)	159	97 (61.8%)	Histochemical analysis (remodelling)
Bronchial brushing	159	147 (92.5%)	Gene array
Bronchial biopsy (mRNA)	116	107 (92.2%)	Gene array
Nasal brushing	100	89 (89%)	Gene array

WP5

- Viral challenge study Part 1 (safety & dose escalation) was completed. The viral challenge dose for part 2 was selected (100 TCID50) and defined.
- The new site, MEU Manchester, has been contracted by the sponsor, MSD, and was activated before part 2 of the study started.
- In the viral challenge study Part 2 (main virus challenge biomarker study), was finished by challenging 23 of the target 25 study patients (mild/moderate asthmatics not taking LABAs) with GMP RV16. Final patient out was reached on December 31, 2015. Biosamples (nasal lavage, induced sputum, nasal brush, plasma for lipidomics, serum for proteomics, blood for RNA (PAXgene), urine for lipidomics) were collected and have been shipped to the Manchester Biobank. In addition, breath samples on adsorption

tubes for breathomics were sent to Amsterdam and PBMC will be sent to MSD for 'omics' analysis.

- RV16 GMP storage at Fraunhofer ITEM – Braunschweig is secured for another 12 months
- RV16 GMP ownership agreement between Amsterdam and Fraunhofer has been almost finalized (access document for RV16 GMP use by other parties is in the final review process)

WP6

Updates – In Vivo

AMC:

- HDM model exacerbation with influenza and steroid response profiling complete
- Epithelial cell brushing pilot study in microarray did not yield sufficient RNA; RNA prepared for another microarray study.

Almirall:

- CFA/HDM exacerbation (polyIC) complete.
- Influenza exacerbation model complete.
- Study with Ghent (humanized mice model) finalizing.
- Second set of lung samples mRNA prepared and being analysed by microarray.

Fraunhofer/Hannover:

- Precision cut lung slices (PCLS) model characterization in mouse and human with HRV1b complete.
- Initial microarray study complete.
- Increasing replicates is being finalized.

ICL

- FCA/HDM model with various anti-Th2 cytokine therapeutics complete.
- NLRP3 inhibitor study completed and analysed.

Updates - In Vitro

ICL

- Cultured epithelial cells for microarray done.
- Samples awaiting microarray analysis.
- Validation of genes in biopsies and sputum – RT-qPCR and IHC, ELISA/WB completed.
- Sample profiling (transcriptomics/proteomics) is behind schedule as human airway brushing/blood samples were prioritized: delivery end of April 2016.

Table of models (*in vivo* and *ex-vivo* utilized by U-BIOPRED WP6. The lack of an adequate model hampers mechanistic insight and the development of new therapeutics.

Model	Purpose	Stage of development/validation	Context for use	Further work
In vivo				
CFA/HDM steroid resistant model	Develop a mixed Th1/2/17 model of steroid-resistant asthma	<p>Published results showing steroid insensitive and can be exacerbated with poly(I:C).</p> <p>Full description of model is being written, including extensive time course of immune responses and effect of anti-IL-4/13 intervention</p>	<p>Rapid, robust screening model for possible drugs and novel target assessment.</p> <p>Described inflammasome target in subset of severe asthma patients, which was prevented by CRID3, an anti-inflammasome agent in this model.</p>	Will form part of submission to EMA.
Standard chronic HDM exacerbation model	Validate previously published data showing exacerbation of model with RV infection.	Unable to show any exacerbation of model with RV and no effect on steroid responsiveness as reported in man.	Screen drugs for anti-exacerbation activity in context of chronic asthmatic inflammation.	Not robust or reproducible – no future work planned.
Chronic HDM/influenza infection	To develop a robust model of severe asthma exacerbations based on chronic HDM and infection with low dose influenza virus A/X31 H3N2.	<p>The model led to prolonged deterioration of lung function, aggravated mucus production, peri-vascular, peri-bronchial and allergic inflammation that was unresponsive to inhaled corticosteroids, but responsive to oral corticosteroids. This reflects the clinical situation in severe asthma.</p> <p>The exacerbation was preceded by a marked innate,</p>	<p>Robust screen for new drugs in ICS-insensitive viral-induced exacerbations.</p> <p>Anti-IL-5 prevented the exacerbations in this model recapitulating its effects on severe asthma exacerbations.</p>	<p>Submitted for publication.</p> <p>Will be part of the package for EMA evaluation.</p>

		but not T2 response and enhanced eosinophilia.	Also led to new ideas as to potential innate immune targets for exacerbations of human severe asthma.	
Ex vivo				
Bronchial Biopsies	Wanted to develop a robust model that reflects complexity of cells within the airway to examine whether reflects susceptibility of asthmatic airways to exacerbation.	Possible to detect presence of subsets of cells within Biopsies using FACs and of some differences in mediator release following steroid treatment. However, were not able to exacerbate biopsies with RV and only minimally with influenza. RNA quality not good.	Better 3D model of asthma/severe asthma involving cell-cell interactions.	Not being taken further.
Bronchial brushings	These are often used as a model and the epithelium was considered the driver of the down-stream immune defects in asthma.	Possible to get good RNA and protein expression data. Few differences seen at baseline after culture but clear differences in innate immune responses with increasing severity of asthma when cells are stimulated.	Model reflects innate immune differences in severe asthma following stimulation. May be particularly useful for testing innate immune-directed drugs	Gene arrays to be completed and link with brushings obtained directly from patients at bronchoscopy.
Airway smooth muscle cells	Responsible for airway contraction and AHR, major features of severe asthma. Model used to determine whether differences in cell function reflect human responses.	Possible to get good RNA and protein expression data. Few differences seen at baseline after culture but clear differences in innate immune responses with increasing severity of asthma when cells are stimulated.	Model reflects some aspect of severe disease and data indicated association with cough.	Papers to be submitted. Model will be continued.
Precision cut lung slices	An alternative 3D model of asthma but retaining all structural cells in comparison with biopsy model. Can show contraction of airways with this			

	model.			
Peripheral blood cells	Readily accessible cell type that has previously been shown to reflect some aspects of severe asthma such as steroid responsiveness.			

WP7

- All the laboratory analyses in all the ‘omics platforms defined in the grant application have been completed (except the delayed urine metabolomics, see 1.3 and below) and the majority of them have produced sets of ‘omics fingerprints, i.e. sets of phenotypes/endotypes based on clusters for each of the ‘omics technologies.
- The delivery of urine metabolomic fingerprints, funded by the ENSO additional grant, are in production, with committed funding and accomplished delivery by March 2016.
- The ENSO-funded Analyte Set has been delivered as detailed in Deliverable report 7.6 and is now being evaluated to identify biomarkers of asthma phenotypes, either alone or in combination with other U-BIOPRED data.
- Based on the ENSO, microbial profiling has delivered a number of operational taxonomic units (OUT) that reflect individual microbial species which were differentially abundant; clustering of these is ongoing and will be completed by April 2016.

WP8

- All analyses between WP7 and WP8 had been specified in so-called Work Flows (WF), with predefined research questions.
- All the acquired data were uploaded on to transSMART and fingerprints constructed. Fingerprints have been delivered for transcriptomics in blood, sputum pellets, nasal brushings, epithelial brushings and bronchial biopsies, urine eicosanoids; proteomics in induced sputum fluid phase (airway secretions) and serum and sputum eicosanoids.
- Full validation has been achieved for some of these fingerprints and further validation of those fingerprints on additional cohorts has been scheduled. Breathomics fingerprints created by eNose and GC-MS technology have also been generated and eNose fingerprints have successfully been replicated using data from the longitudinal study visit (see 2.4).
- WP8 has successfully produced two major handprints on the data from the adult cohorts: a ‘blood handprint’ on 227 asthma participants and a ‘sputum handprint’ on 72 asthma participants (see 2.4). The difference in numbers of patient samples

available was due to the numbers of samples available in the respective omics platforms. These handprints that are combining various 'omics' platforms have been presented at various symposia at the ERS meeting in Amsterdam (September 2015) and are representing the core, innovative output of U-BIOPRED.

- The blood handprint analysis produced 5 separate blood handprints (BH), identifying 5 types of asthma patients. Each of the 5 handprints can be seen as a cluster and used as an initial phenotypical definition, with the different variables and characteristics of each group presented. The table below shows the clustering of data around a number of measurements producing the handprint grouping. The data could also adequately be captured by using 4 blood handprints.
- The sputum handprint produced 3 clusters on the lower numbers involved. Summary tables were presented at the ERS Congress 2015.

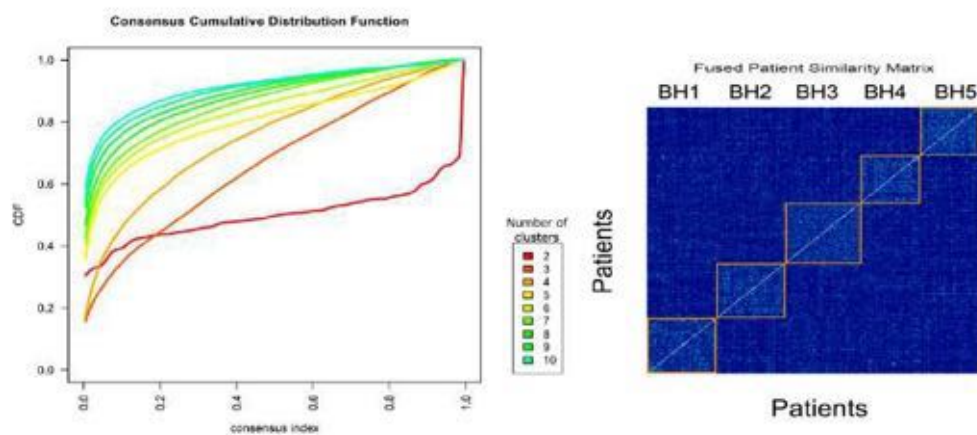


Figure 1.4.1. Blood handprint validation. *Left:* The cumulative distribution functions (CDF) for various number of clusters in the handprint. *Right:* Stability assessment of the similarity network fusion (SNF) analysis. The data are showing that 4 to 5 handprint clusters are stable.

WP9

- U-BIOPRED again presented a dedicated symposium at the ERS Congress 2015, with over 1000 people in attendance. This was a valuable opportunity to present on the project and focused on proteomics data in relation to inflammation and clinical characteristics and on presenting the first U-BIOPRED handprint: the blood handprint.
- 33 Conference abstracts were submitted and all accepted for either poster, symposium or oral presentations.
- Dissemination efforts continued, with publications on the Adult and Paediatric cohorts in the European Respiratory Journal and an editorial focusing on U-BIOPRED and SARP (US consortium) in The Lancet Respiratory Medicine journal
- U-BIOPRED featured in the World Village at ERS and a booklet of U-BIOPRED activities at Congress was produced.

- A further 10 papers have been drafted and are in the process of being submitted for publication
- Updates for the website and social media platforms secured into the sustainability period.
- Presence at international conferences has ensured a high profile for the project in the field
- Communications approach outlined on final WP deliverable D9.7
- The website and twitter/LinkedIn platforms have been kept updated with news, a process which will continue into the legacy period.
- A new tailor-made video clearly explaining the concept and delivery of U-BIOPRED to lay persons has been uploaded to the website.

Website Statistics: 1 October 2014 – 30 September 2015

- Total sessions: 5,631.
- Total users: 3,693.
- Total page views: 23,555.
- Averages pages per session: 4.18.
- Average session duration: 3.44 minutes.
- Most popular countries: UK (28%), USA (11.5%), Belgium (8.7%), Netherlands (6.8%), Italy (4.8%).
- Site found in 59% of the cases by organic search, in 17% by directly entering the address and in 14% of the cases by referral.

WP10

- Patient Input Platform (PIP) continued activity in Period 6 will be continuing through to the sustainability period.
- A booklet on the patient input experience has been presented, being led by ELF with very active patient input and authorship. Support for the printing is expected from IMI.
- Ethics process for the duration of the project has been recorded, with patient input and completed with all issues resolved.
- The Ethics Board has continued to advise on developments in the RV16 clinical trial and in the storage of data.
- The Patient Input Platform (PIP) has seen its activity increase in this last period, with monthly conference calls and three face to face meetings occurring in the period. The PIP group are continuing to work in the legacy period and are involved in reviewing publications and developing lay abstracts as well as assisting in dissemination work.
- The Safety Monitoring Board finalised its work and carried out a review of all severe adverse Events forms in the core clinical study as an assurance measure.
- The impact of patient involvement was published in a peer review journal: 'From tokenism to meaningful engagement: best practices in patient involvement in an EU project. <http://www.researchinvolvement.com/content/1/1/5>'

ENSO funded work – summary supplement

Sustainability

D1.6 (Report on possible business models) and D1.7 (Report on initiation of business model) have been delivered. The team was led by BioSci Consulting (Thierry Nicloux/Scott Wagers) and included Anthony Rowe (Janssen), Chris Compton (GSK), David Myles (GSK), Ratko Djukanovic (Southampton), Fan Chung (LOIC), Ian Adcock (LOIC), Jason Hannon (Roche), Jorge Belata (Almirall), Julie Corfield (AZ), Kathrin Riemann (BI), Leon Carayannopoulos (MSD), Norbert Krug (Fraunhofer) and Peter Sterk (AMST).

Funding has been secured from ERS for a maintenance level of basic project outputs (biobank/data base and data management, consortium activities) for 6 months with a forthcoming budget review for a further 12 months. A contract has been signed and a new set of Deliverables and Milestones developed to track progress in this legacy period. Contacts with a range of external people continue to feed into the planning process for future work, and the EPFIA group continues to meet, which should lead to proposals being put forward for support by others in the group and eventual endorsement. U-BIOPRED partners agreed in a General Assembly vote to continue their collaboration under the terms of the existing Project Agreement. Amsterdam provides daily representation of U-BIOPRED as also agreed during the General Assembly meeting in June 2015.

Fingerprint ENSO

The analyte set has completed an extensive process of analyte nominations and subsequent sample analysis planning. The related deliverable reports, D7.6 and D8.9 have been completed. A broad range of people from across the project have been involved in the process. Anna James (BI) and Sven-Erik Dahlen (KI) lead their respective organisations work.

Microbiome: Southampton and Janssen have taken forward this work and all samples have been analysed, completing D7.7. The leaders are Peter Howarth (SOH) and Frederic Baribaud (Janssen).

Metabolomics: KI have successfully re-established the mass spectrometer machine and samples have been analysed, as reported in D7.8. Craig Wheelock (KI) is leading this work. Delivery accomplished by March 2016.

1.5. Scientific and technical results/foregrounds of the project

- The U-BIOPRED project has accomplished a public-private collaboration and exchange in the field of medical research, on the topic of understanding severe asthma, in order to subsequently allow EFPIA companies to develop novel interventions targeting previously unknown networks of biological mechanisms that are driving this difficult-to-treat disease. This has resulted in injecting EFPIA expertise into academic centres in order to deliver a cohort/registry of severe asthma patients in amongst countries in Europe that meets the highest standards (GCP) in terms of quality control in terms of measurement procedures (SOPs), biobanking and database management. Alternatively, this public-private collaboration has allowed academic laboratory and biomarker expertise to be shared with EFPIA laboratories based on shared protocols. This particularly relates to pre-clinical animal and in vitro models, which similarly have for the first time been exchanged and optimised between EFPIA companies. This would not have been accomplished without this IMI project.

- The adult and paediatric cohorts have already been published in terms of their clinical characteristics (Shaw *et al.* ERJ 2015, Fleming *et al.* ERJ 2015), which definitely shows that severe asthma in Europe does not entirely match severe asthma in e.g. the US. The discrepancy clinical characteristics of severe asthma between Europe and US is important and is related to differences in the health care systems between EU and US. In Europe we dealing with true severe asthma, as defined by U-BIOPRED by Bel *et al.* Thorax2011.
- The U-BIOPRED definition of severe asthma and the algorithm to clinically diagnose true severe asthma amongst difficult-to-treat asthma has been published by U-BIOPRED (Bel *et al.* Thorax 2011) and has meant a step-change in clinical decision making.
- Just by using clinical data, the U-BIOPRED cluster analysis of the data amongst the asthma cohorts has produced 4 clusters that can be distinguished by routine assessment in clinical care. Notably, these clinical clusters were significantly different with regard to the sputum transcriptomic and proteomic networks. This shows that clinical and biological phenotyping provides separate groups of (severe) asthma patients, which definitely required (partially) distinct therapeutic approaches. This is a main accomplishment of the project and shows that severe asthma has 4 distinct groups which require different treatment approaches.
- The details regarding the U-BIOPRED clinic-physiological clusters for the adult cohorts are the following. Four reproducible and stable clusters of asthmatics were identified. The training set cluster T1 consists of well-controlled moderate-to-severe asthmatics, while cluster T2 is a group of late-onset severe asthmatics with history of smoking and chronic airflow obstruction. Cluster T3 is similar to cluster T2 in terms of chronic airflow obstruction but is composed of non-smokers. Cluster T4 is predominantly composed of obese female uncontrolled severe asthmatics with increased exacerbations, but with normal lung function. The validation set exhibited similar clusters, demonstrating reproducibility of the classification. There were significant differences in sputum proteomics and transcriptomics between the clusters. The severe asthma clusters, T2, T3 and T4, had higher sputum eosinophilia than T1 with no differences in sputum neutrophil counts, exhaled nitric oxide and serum IgE levels. Taken together, this U-BIOPRED cluster analysis in the adults cohort shows that clinico-physiological parameters yield 4 stable and reproducible clusters that associate with different pathobiological pathways. This paves the way to critical mechanisms and thereby potential targets for therapy in relation to clinical phenotypes of the disease. The manuscript is in revision in the J Allergy Clin Immunol (impact 12).
- U-BIOPRED has succeeded in sampling blood, urine, sputum and bronchial biopsies from the same clinically well-characterized patients.
 - Due to a number of factors the overlap from one data type to another data type, derived from analysing the various samples, is variable. The reasons for this are patient decisions, including opting out of procedures such as bronchoscopies and CT scanning, the success rate of some patient-demanding procedures such as sputum induction and the sample data removed during the quality control process. These factors are inevitable, and this has not hampered integrative analysis as shown by the first cluster and handprint analyses. The first draft paper on linking ‘omics platforms to histological

outcomes has been written, showing the complementary value of the U-BIOPRED sampling procedures.

- During the course of the project it became evident that the ‘omics’ platforms, required for unbiased biological phenotyping, needed comprehensive analysis of the available technologies and validation. This has been done and published (Wheelock *et al.* ERJ 2013). This included the extensive requirements for adequately applying and quality control of transcriptomics, proteomics, lipidomics, metabolomics and breathomics. The integrative assessment of the strengths and limitations of these technologies had never been done, and is an accomplishment of U-BIOPRED. This is also applicable outside the respiratory field.
- ‘Omics’ analysis in medicine requires stringent recommendation regarding validation, as recently published by experts outside U-BIOPRED (McShane *et al.* Nature 2013). When the ‘omics’ data became available in U-BIOPRED, a stepwise Data Analysis Plan (DAP) has followed these recommendations. This has led to the usage of so-called Training- and Validation Sets of data as obtained by: a) a priori split half procedures, and b) temporal validation (using the longitudinal visit for replication of data as obtained at baseline. This has ensured the quality output by U-BIOPRED.
- ‘Omics’ U-BIOPRED fingerprints have thus been obtained and presented during the ERS congress in September 2015. The main message of these fingerprint analysis is that traditional clinical/inflammatory phenotyping of patients with severe asthma that has been done so far, does not suffice in capturing the relevant underlying biology of the various disease phenotypes. A main paradigm in asthma during the past years has been that eosinophilic inflammation represents a particular phenotype that requires separate therapeutic strategies with existing and novel drugs. U-BIOPRED now shows by proteomic analysis that the biology behind eosinophilic inflammation in severe asthma can be separated into distinct networks. Unbiased proteomics shows 4 separate molecular clusters underlying eosinophilia. This is what U-BIOPRED was heading for: discovery of previously unrecognized molecular phenotypes underlying groups that are clinically similar (Figure 2.4.8. More recent transcriptomics analysis in sputum confirms and validates this finding by showing different networks underlying eosinophilia in severe asthma. This is a major result, for the first time allowing access to differing fundamental mechanisms associated with inflammatory profiles. This kind of result is meeting the expectations for EFPIA partners when actually joining U-BIOPRED years ago.
- The handprints represent the highest level output of U-BIOPRED. Handprints are derived from multi-scale analysis using various ‘omics platforms and clinical data in order to establish comprehensive phenotypes of severe asthma.
 - The first two handprints have been delivered and presented (ERS 2015) as derived from the baseline data sets: the blood handprint and the sputum handprint. This has generated novel severe asthma phenotypes, using all the information available, by an entirely unbiased procedure.
 - These handprints are awaiting replication on the longitudinal datasets and will be published in 2016. These handprints have been derived by following cutting-edge analyses, which will be separately published, because U-BIOPRED is the first to do so in the medical field.

Several handprints have already been produced (on blood and sputum related samples), which will be refined when additional baseline datasets will be made available.

Moreover, these handprints have been produced in a completely unbiased manner; more focused handprints will also be produced with a feature reduction step (based on correlation values for example). Longitudinal data, pending availability of the omics platform results, will be used to assess stability over time of the clusters of patients identified in the baseline handprints. Finally, handprints based on fingerprints will be produced once all required fingerprints are available.

- Predictive models based on few informative features for all handprints have/will be built, which will help doctors assess in which cluster their patients belong to, depending on which type of data they have available for their patients. The list of features included in the models can then be used in clinical practice using simpler, focused measurements.
- U-BIOPRED has validated various pre-clinical models, in close academic-industrial collaboration. The main outcome is that the standard chronic HDM model in mice is not representative of severe asthma due to its extreme sensitivity to corticosteroids and is not robustly exacerbated with RV challenge. Influenza challenge appears to represent a better *in vivo* model of exacerbation. The similarity between the genes over-expressed in this model and those reported in severe asthma suggest that related models that are made steroid insensitive are better models of disease.
 - The development of the CFA/HDM model is one such approach as this provides a steroid insensitive mixed T-cell model of severe asthma. The limitation of this model is the acute nature of the model in comparison to the chronic nature of human asthma. This is off-set by the relatively long inflammatory window seen in this model which allows therapeutic dosing.
 - It is likely that human tissue cell models such as precision cut lung slices (PCLS) and primary cells in 3D culture may provide the best disease model as they are able to be exacerbated by RV and reflect many aspects of severe asthma including abnormal innate immune responses. Further analysis of these models, including bioinformatics comparison across models and primary samples has been scheduled, because this is the final step to establish the rationale for the favoured use of primary human cells from patients with severe asthma.

1.6. Potential impact and main dissemination activities and exploitation of results

- The impact of U-BIOPRED on socio-economic benefits for European citizens lies in its spin-off. Severe asthma forms 3-8% of the asthma population (Hekking and Bel *et al.* J Allergy Clin Immunol 2015), and actually represents those patients who are consuming most of the health budget related to asthma. Because of providing new and separate categories of severe asthma patients, the project will allow selective usage of newly developed and targeted medicines. Up until now biological therapies can hardly be used selectively because of the absence of validated markers for selecting the right patient for the right drug. The

socio-economic benefit of the fingerprints and handprints of U-BIOPRED will be the avoidance of unnecessary treatments to patients who will not benefit from novel therapies.

- Thereby, the project will contribute to health in Europe. So far, severe asthma cannot be adequately treated. These patients represent the largest part of the burden of asthma in Europe. U-BIOPRED has now discovered new subgroups of patients that are driven by different disease mechanisms. This will not only allow selecting the right drug for the right patients but will also fuel pharmaceutical research in to finding new targets for treatment in patients who do not respond to available (biological) drugs. This will definitely benefit health of European citizens.
 - These outputs have concrete implications for current scientific and industrial developments. First, scientifically, apart from publishing the data for wide dissemination, the U-BIOPRED fingerprints have already been input into at least two novel H2020 applications and various national governmental and industrial grants by April 2016. Second, the industrial exploitation has also been taken up. Already 3 EFPIA partners from U-BIOPRED are now using the project's clinical and 'omics' fingerprints as entry characteristic for the prediction of treatment efficacy in new phase 2 and 3 clinical trials with biological interventions.
 - Even though various fingerprints have already shown to be very effective in the discovery of novel phenotypes of severe asthma (e.g. 4 types of eosinophilic asthma based on proteomics: figure 2.4.8), it is envisaged that particularly the integration of fingerprints and clinical data will get us the closest to real Precision Medicine. For instance, the sputum handprints S1, S2 and S3 appear to represent groups of patients that are significantly different in lung function (FEV1 and FEV1/FVC), sputum inflammatory profile (percentage eosinophils, neutrophils and macrophages) and circulatory biomarkers (periostin and IL-13). This indicates that handprints are a powerful tool to find groups that represent biological as well as clinical differences that are meaningful for intervention strategies.
 - The U-BIOPRED fingerprints and handprints can be regarded as novel research tools allowing in depth phenotyping for academia and industry. At present this is being pushed towards the clinic by selecting a 'simplified' set of biomarkers. This includes: a) rapid 'omics' at point-of-care (such as breathomics) and b) the selection of the definitive U-BIOPRED Analyte Set mirroring the major fingerprints and handprints (end of Q2 2016). The Analyte Set is based on the optimal composite of more easily accessible individual biomarkers in blood and urine in the clinical setting. These two disseminations of U-BIOPRED will represent direct exploitation of the project's results.
- The U-BIOPRED project has boosted industrial collaboration amongst 12 pharma industries and their interaction with academic centres in Europe. The shared pre-competitive new knowledge from the project will speed up the process of drug development because of newly discovered biological networks in the patients of interest and because of the its validation of new, composite biomarkers for the disease phenotypes. The latter are being presented to EMA for further development. Therefore, this IMI project with public-private collaboration will contribute to European competitiveness in general and of pharma industry in particular, since the US has lagged behind in such structured pre-competitive, academic industrial collaboration. The fact that U-BIOPRED has delivered shows that this format represents a successful European signature.

1.7. Lessons learned and further opportunities for research

- As indicated above, the public private partnership (PPP) of U-BIOPRED has been critical to its success. This is based on the following modes of interaction:
 - Collaborative writing of the full project application and its key objectives.
 - Collaborative writing of the clinical protocols, integrating academic experience with rigour from pharma.
 - Jointly establishing Standard Operating Procedures (SOPs) for U-BIOPRED and future usage.
 - Sharing and collaboratively optimizing pre-clinical models, not only between academic and pharma, but also amongst pharma companies.
 - Jointly developing and optimizing Data Analysis Plans.
 - Generating shared appreciation for inclusion of the patient perspective in all layers of the project.
 - The agreed Consortium Agreement, in which academia and pharma reached consensus on using background and sharing foreground.
 - Joint Management Board and Scientific Boards, with synergism in the monitoring and steering of the project.
 - Weekly joint progress conferences, with intensive mutual exchange.
- Based on the U-BIOPRED experience, Public Private Partnerships (PPP) may even be reinforced by:
 - More strongly settling the partnership before the project starts. Under the current IMI structure, private partners after having agreed to join the consortium can leave as they wish, without commitment up to the level of the original agreement. This has affected U-BIOPRED, causing a sudden deficit and serious delays and unnecessary person months in ensuring delivery.
 - Avoiding the need for one to one agreements and contracts between individual EFPIA companies and individual beneficiaries within the consortium. Ensuring the scope of the project agreement and grant agreement affords a wider basis for collaboration and avoids the very lengthy processes where additional contracts have to be put in place. A centralized EFPIA budget for the project will be far more efficient as opposed to multiple individual company budgets and decentralized local company decisions on those.
- The opportunities for new research after U-BIOPRED are enormous. In short, this includes the following
 - A further longitudinal assessment would throw more light on both the stability of the phenotypes identified and also on the movement of individual patients between phenotypes.
 - The planned comparison of findings across other asthma cohorts (such a ProAR in Brazil) offers the opportunity to both validate the phenotypes in different patient populations, as well as the opportunity to identify possible differences, perhaps due to different environmental and ethnic factors.
 - Similarly, combination and mutual validation of 'omics' datasets across allergic and immunological diseases is very timely. In fact, previous partners from U-BIOPRED and

other consortia have applied for a H2020 grant regarding this in April 2016 (SIMBA project).

- Testing new biological interventions in phenotypes of severe asthma as derived from U-BIOPRED. Some of these studies have already been started and will carry the deliverables of the project towards concrete targeting of drugs for patients.
- Even though U-BIOPRED has already started doing so in one of its ENSOs (analyte set), research on clinical applicable diagnostics that adequately captures the complex U-BIOPRED phenotypes is mandatory. Simple blood and breath tests should be further validated and optimized in order to allow clinical application of U-BIOPRED findings in first and second line care.
- Expanding the U-BIOPRED concept into a broader spectrum of airways disease. It is now acknowledged that asthma and COPD cannot and should not be distinguished when it comes to the potential efficacy of new drugs. The phenotypic markers eventually determine eventually whether a new biological will be effective. Therefore, asthma and COPD are now collectively looked when it comes to define treatable traits. The U-BIOPRED approach is therefore highly needed in a broad group of patients also including COPD. This will definitely benefit drug discovery and tailored therapy.
- Similarly, the U-BIOPRED concept is equally applicable in complex morbidities in respiratory medicine, such as interstitial lung disease, respiratory infection and even beyond respiratory medicine. This needs to be taken up. Also in orphan diseases that mostly have not been scrutinized with respect to the relationship between clinical expression and underlying biological networks.
- Starting from the U-BIOPRED handprints, pre-clinical models can now be further optimized for rapid drug assessment. Not only for drug development but even for selecting drugs for individual patients e.g. by using organoids (a three-dimensional organ-bud grown in vitro) or other in vitro systems validated against U-BIOPRED handprints.
- With the knowledge from U-BIOPRED early phenotyping of infants and children can be improved, in order to come to real secondary or even primary prevention strategies.