WP1:

- U-BIOPRED was co-ordinated by the academic lead (Amsterdam) with support, whenever needed, by the EFPIA lead (Novartis).
- Day to day steering was done by very close and very smooth interaction between BioSci and AMST.
- WP1 had led the co-ordination of the 42 partners and 10 Work Package without internal friction or conflicts. The collaboration was very strong through the 6 years, which has resulted in delivery of the project.
- With a peak of 34 conference calls/TCs a month approximately 1700 calls have been facilitated by WP1 over the 6 years period. U-BIOPRED saw BioSci Consulting develop conference calls as the core organisational feature of large multi-partner research projects.
- 6 annual meetings have been organised and facilitated with an average of over 100 attendees.
- U-BIOPRED has also held meetings at 4 ATS Congress and 6 ERS Congress events.
- Additionally, 11 cross consortia analysis meetings with between 21 and 40 attendees have taken place in the final 3 periods ofthe project.
- 8 amendments to the description of work have been managed by WP1, led by AMST.
- The creation of the Project Agreement, Collaboration Agreement and management of the Grant Agreement with amendments for the latter 2 contracts has been managed by WP1.
- The adding of three additional EFPIA partners (Janssen, Amgen, Merck) and the exit of one EFPIA (Pfizer) partner has been managed, with the associate changes in Annex 1 and financial adjustments and negotiations.
- 5 periodic reports and this final report have been compiled by WP1.
- Relations with the supportive IMI office have been managed and resulted in good mutual contact and understanding.
- WP1 has also managed general queries to the project and approaches from potential collaborators.
- An application to the EMA for advice is being prepared. 5 questions have been identified, on which advice will be requested, and each question has a group working on the background document and other session input

WP2:

- WP2 has organized a consensus meeting at the start of the project and a consensus meeting at the final consortium meeting (Leuven June 2015). The initial consensus has been published in a peer review journal (Bel *et al*. Thorax 2011). The second consensus is available in draft format, but will be subject to further iteration based on final U-BIOPRED data from WP8 (expected April 2016).
- WP2 has produced two very detailed clinical protocols for the clinical cohort studies of WP3 in children and adults. Ethics approval was eventually reached in all clinical centres

- Subsequently, WP2 has built detailed Standard Operation Procedures (SOPs) for all clinical and laboratory procedures by following international quality criteria (Good Clinical Practices: GCP). These SOPs have subsequently been shared with other consortia (COREA, AirPROM, ProAR, Atlantis, RASP UK, SOMOSA) based on mutually signed Memorandums of Understanding (MOU). This mechanism is also available for other researchers who wish to access U-BIOPRED SOPs, allowing for standardization of procedures which gives comparable data and also improved quality approaches in the field.
- The registry of severe asthma patients across Europe has been built based on the paediatric and adults cohorts. The registry is linked to the anonymized central database in TranSMART. In case of future recruitment of patients from the registry, local clinical centres will be the only ones being able to link anonymized U-BIOPRED patients' numbers to patient's personal identification in the hospital databases. The registry is comprised of the study participants from all clinical sites, often building on existing site cohorts and includes 726 Adult and 299 Paediatric participants. This is a valuable resource for consulting for data investigation purposes and for building new research proposals and clinical investigations, allowing the longitudinal aspect of asthma research to be studied.

WP3:

- WP3 has created and published its paediatric cohort (Fleming *et al*. Eur Respir J 2015). This includes 282 children: 99 school-aged severe asthma and 49 controls with mild asthma, 81 pre-schoolers with severe respiratory wheeze and 53 controls with mild to moderate wheeze.
- WP3 has created and published it adult cohort (Shaw *et al*. Eur Respir J 2015). This includes 421 severe asthmatics (311 non-smoking, 110 smoking severe asthmatics), and 81 mild-moderate asthmatics and 101 healthy subjects as controls.
- All baseline and longitudinal visits (12-18 months later) have been performed and 36 adult patients were captured for measurements during an exacerbation as defined by ATS/ERS criteria.
- Data has been provided as per protocol to the investigators, following the quality control process. The samples collected are outlined in WP4.
- A final intervention study with a novel anti-asthma drug was not done during the U- BIOPRED funding period. However, individual U-BIOPRED pharma partners have embarked on intervention studies with their new novel biological drugs and have taken the U-BIOPRED SOPs and fingerprints/handprints on board at their own funding. It was more or less to be expected that this objective of WP3 did not fit into the pre-competitive collaboration under the IMI grant agreement and that application of novel drugs would be driven by individual companies.

WP4:

- Development of standard operating procedures (SOPs) for sample processing, biobanking and transport of samples.
- Creation of a biobanking framework and establishment of a centralised biobank.
- Establishing and conduct a bronchoscopy accreditation process of the academic centres.
- Training and ongoing feedback, which helped facilitate successful sample collection.
- Completion of bronchoscopy sample collection with delivery of bronchial biopsies, bronchial brushings and nasal brushings from all 4 U-BIOPRED cohorts.
- Establishing and running the QC process for quality assessment of bronchial biopsies
- Immunohistochemical biopsy analysis.
- Provision of good quality bronchial biopsy, bronchial brushing and nasal brushing samples for successful transcriptomic analysis.

- Provision of bronchoscopy samples (biopsies, bronchial brushings and bronchoalveolar lavage) for the WP6 programme.
- Provision of samples to 'omics' platforms for core deliverables, enablement of the ENSO and additional research questions for workflows.
- Immunohistochemical staining of the endobronchial biopsies embedded in GMA resin and their analysis was completed for 139 subjects. Two subjects were subsequently excluded as protocol violators. Immunostaining was undertaken for cells (eosinophils, neutrophils, mast cells and CD3/CD4/CD8/CD25 positive lymphocytes), counted separately in the submucosa, epithelium and smooth muscle, as well as for structural changes (lamina reticularis thickness and smooth muscle volume). Vital staining of the paraffin embedded biopsies was undertaken for other structural assessments (mucus glands, elastin fibres and sub-mucosal collagen). Between group analysis was undertaken to seek for cohort differences.

WP5:

- WP5 Virus Inoculum
- Rhinovirus Study
- Viral challenge SOP for the WP5 study
- Human hRV challenge safety dataset
- Viral challenge biology dataset in asthma patients
- Viral challenge SOP for dissemination

WP6:

- U-BIOPRED has validated various pre-clinical models, in a close and unique academic- industrial collaboration. A major output was the Review of existing models (in vivo and ex vivo) and the publication of a review article on these. As a result of this analysis we were able to conclude that the standard chronic HDM model in mice is not representative of severe asthma due it being extremely sensitivity to corticosteroids and is not robustly exacerbated with RV challenge. However, 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 may be much better models of disease. In WP6 we were able to demonstrate that influenza viral challenge may represent a better in vivo model of exacerbation particularly as it is steroid insensitive but responds to anti-IL-5 therapy mimicking the situation in severe asthma. In contrast, we were unable to demonstrate a robust enhancement of the chronic HDM model using human RV challenge despite its publication in very impact Journals and this model was dropped.
- The development of the CFA/HDM model is another such approach to developing a model of severe asthma. This model using CFA as an adjuvant provides a steroid insensitive mixed T-cell model of severe asthma with the increased expression of eosinophils and neutrophils and increased numbers of Th1, Th2 and Th17 cells. The model has been reproduced at two Pharma companies (UCB and Almirall) as well as in an academic environment (LOIC) with almost complete overlap of responses. Another company (Charles River) has also reproduced the model based on data from a U- BIOPRED poster presentation which reflects the robustness of the model. 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. Novel inflammatory features described in severe asthma patients such as inflammasome activation in non-infected severe asthmatic patients with neutrophilic asthma were recapitulated in this model. Importantly, CRID3, a novel anti-NRLP3 inflammasome targeted drug, was able to completely reverse airways hyperresponsiveness and both neutrophilic and eosinophilic inflammation in this model.

- The virus used aimed to mimic the effect of viral infection in asthmatics by exacerbating the disease. Initial studies using rhinovirus failed to infect the airways of mice and exacerbate the symptoms and inflammation despite publication by others in Nature Medicine. The influenza challenge model developed by U-BIOPRED demonstrates clinically relevant features of asthma exacerbations and can be prevented by systemic steroids and anti-IL-5 but not by inhaled steroids reproducing their effects in severe asthma exacerbations.

- A number of in *vitro/ex vivo* models were also examined including analysis of PBMCs, airway smooth muscle cells (ASM), airway epithelial cells (AEC), bronchial biopsies and precision cut lung slices (PCLS). Transcriptomic data from most of these samples was collected although that from biopsies was not of sufficient quality for gene array analysis. The biopsy, ASM and AEC models provided evidence for altered innate immune responses at baseline in the asthma versus healthy subjects. This was amplified in the stimulated ASMs and AEC model.
- We were unable to obtain a heightened inflammatory response or any evidence of infection with RV in the biopsy model although infection was obtained with influenza virus. In contrast, human RV was able to infect the PCLS model and generate clear evidence for an interferon response using unbiased analysis of microarray data. Overall, it is likely that human tissue cell models such as PCLS and primary cells in 3D culture provides 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, is almost accomplished by April 2016 and is essential to confirm the rationale for the favoured use of primary human cells from patients with severe asthma.
- We were able to demonstrate, using GSVA, that the CFA/HDM model expressed elevated levels of inflammasome genes similar to those expressed in human severe asthma sputum. We therefore, examined the effect of CRID3 on lung function and inflammation in this steroid insensitive model of severe asthma. Treatment with CRID3 (200mg/kg i.p.) had no effect on RL or AHR in baseline HDM- sensitised but unchallenged mice. However, CRID3 significantly reduced maximal RL and AHR back to baseline. The increase in total cell counts with HDM challenge was completely abrogated by CRID3 treatment and this was also reflected in differential cell counts. CRID3 also completely abrogated the CFA/HDM-induced expression of Th2 cytokines, IL-17, chemokines and several growth factors.
- As mentioned above rhinovirus failed to mimic the effect of viral infection in asthmatics by exacerbating the disease. The influenza challenge model developed by U-BIOPRED demonstrates clinically relevant features of asthma exacerbations and can be prevented by systemic steroids and anti-IL-5 but not by inhaled steroids reproducing their effects in severe asthma exacerbations.

WP7:

- WP7 has successfully completed this reporting period, with an estimated 90% delivery of all the committed outcomes. The main deliverables were:

1) individual biomarkers identified by 'omics technologies that differentiate between asthma and health and are related to asthma severity,

2) a set of fingerprints composed of individual biomarkers (integrated by systems biology methods) that enable unbiased classification of asthma, on the basis of underlying pathobiology, into phenotypes/endotypes,

3) biomarkers predictive of relevant clinical parameters (e.g. disease severity) and predictive for the identified fingerprints.

- The fingerprints data were passed on to WP8 for further integration, thereby to generate handprints of asthma – the ultimate deliverable of U-BIOPRED. All of these deliverables were planned to use samples from the cross-sectional phase of U-BIOPRED. The largest set of samples was to come from the adult asthmatics (severe or mild to moderate) and non- asthmatic control participants. A smaller set was to be generated from paediatric asthmatics (see WP3 report), restricting the analyses to samples that can be acquired in children (blood, urine and, in some patients only, induced sputum).
- Further samples were acquired in a subset of severe asthmatics as part of the longitudinal study 12 to 18 months after the baseline assessment and a smaller group of asthmatics was also assessed during acute asthma exacerbations.
- In respect of adult participant samples from the cross-sectional study, all the laboratory analyses specified in the application have been completed by all the 'omics platforms; a total of 12 'omics datasets have been produced and uploaded into the central knowledge management platform, tranSMART. The analysis of the paediatric cohort samples are ongoing and should all be completed by April 2016 using internal resources of the partners (for more detail on paediatric analyses, please see below). Analysis of longitudinal samples are partially complete. For example, two EFPIA partners (Janssen and Amgen), committed to transcriptomics analysis, have analysed by micro-array 262 adult blood samples and 113 paediatric samples. Although these transcriptomics data have not been analysed statistically, plans are in place to do this by sprint 2016.
- The datasets used to create the core U-BIOPRED adult asthma fingerprints were delivered using the following 'omics platforms provided by a combination of academic and pharma laboratories: 1) transcriptomics was delivered by a combined effort of pharma companies Janssen/Johnson&Johnson, Amgen and Merck and applied to nasal brushings, epithelial brushing, bronchial biopsies, whole blood, sputum cell pellets 2) proteomics (Southampton), applied to serum and induced sputum supernatant 3) unbiased lipidomics (Southampton) applied to plasma and induced sputum supernatant, 4) biased (eicosanoid) urine lipidomics (Karolinska Institute, Stockholm), 5) biased (eicosanoid) sputum lipidomics (Krakow), 6) breathomics using eNose technology (Amsterdam) and 7) GC-MS (Phillips).
- The unbiased omics outputs of U-BIOPRED, together with network analysis and prior knowledge, started to identify a range of specific analytes that may associate with particular asthma phenotypes/ clusters/ handprints during 2014/ 2015. Whilst they could be followed up and replicated using the original omics methods, these analyte sets are more cost- effective and more easily translatable into diagnostics/ prognostics for clinical practice. Since the original U-BIOPRED application was made several novel technologies have become commercially available for this purpose:

a) Sensitive multiplex immunoassay panels of the analytes of interest such as the Mesoscale Discovery and the Luminex Xmap platforms, which can typically measure a 10-20-plexes on small volumes of sample;
b) Ultra-sensitive assays such as Singulex Erenna that bring more analytes (especially cytokines and chemokines) into the detectable range in human plasma or serum;
c) Very highly multiplexable assays, particularly SomaLogic's SomaScan platform which can currently assay 1129 analytes and the Human Proteome Array which can measure>160 proteins in single samples; and

d) Clinically validated biomarker assays developed by U-BIOPRED partners as part of the analyte set work, analyte set, validated against fingerprints and handprints.

- A selected combination of these has been applied to the U-BIOPRED adult cohort blood samples to seek clinically-accessible biomarkers of handprints to guide treatment, funded by the ENSO extension. This work has used expertise and resources at the Karolinska Institute, Genentech, Boehringer Ingelheim and Janssen to deliver a substantial database of Analyte concentrations in the blood of the adult participants in U-BIOPRED both at baseline and at one year follow-up, which is now available to investigate as biomarkers of asthma phenotypes.
- Additional data from the ENSO component of U-BIOPRED were provided by 1) urine and blood metabolomics (Karolinska Institute, Stockholm) and 2 sputum microbial profiling (Janssen/Johnson&Johnson, with commissioned service from the company, Second Genome).
- The platforms have delivered an extremely rich set of 'omics technology-derived biomarkers for adult asthma. A large number of these were seen to be differentially expressed (for transcript) or abundant (for the other 'omics biomarkers) when comparing severe asthmatics (SA) and healthy participants (HP) or when comparison SA and mild to moderate (MMA) asthmatics. Nevertheless, 19 paediatric baseline and 5 paediatric longitudinal samples have been analysed by the proteomics platform while many more samples will be analysed for lipidomics biomarkers by the end of January 2016 (baseline n=250, and longitudinal follow up n=113). As already stated, blood samples from the paediatric cohort have been analysed by microarray technology but the full analysis remains to be completed.
- Microbiome analysis of the paediatric throat swabs has been undertaken by Professor Hans Bisgaard from The University of Copenhagen Paediatric Asthma Centre (at no cost to U-BIOPRED) and the preliminary analysis has been done.
- As well as the planned skin prick testing and specific IgE assessment, the allergic status of the cohort has been assessed in greater detail with a state of the art approach using the ISAC chip. Rather than assaying specific IgE to whole allergen, the chip assesses the presence of specific IgE to the various major component of whole allergens. Only some of these components are related to clinical disease so this approach allows a much more detailed understanding of the allergic phenotype. The laboratory analysis for the assessment of allergic status using this ISAC chip technology has been completed and the data will be analysed by May 2016.
- The data for most of the above deliverables have been communicated in preliminary form to the scientific community at the American Thoracic Society Conference in May 2015 and the European Respiratory Society Conference in September 2015. Several papers are being written, with the expectation that they will be published in 2016.
- A further, major achievement has been the development of a spin-out project with Novartis (Study of Mechanisms of Action of Omalizumab in Severe Asthma [SoMOSA]) which was launched in October 2015. The objective of the project is to apply all the 'omics platforms used in U-BIOPRED to improve understanding of the mechanisms of action of the anti-IgE monoclonal antibody, Omalizumab (Xolair^{R}) and to identify biomarkers that are predictive of efficacy of this drug for severe asthma. Enabling stratification and improving the understanding of severe asthma for new asthma drugs has been, and remains, the major objective of U-BIOPRED so this collaboration with Novartis is a major dissemination and demonstrates the value of IMI support for U- BIOPRED, without which this project with Novartis would not have been possible.
- A further achievement is the award of a large MRC Stratified Medicine Grant award to a number of the UK's members of U-BIOPRED. This project will be stratifying asthma according to adherence

to corticosteroids and then into T2-high and T2 low phenotypes. Samples will also be taken for application using U-BIOPRED platforms, although funding for this has not yet been secured.

WP8:

- Work package 8 had the goal of generating various handprints generated from various 'omics' platforms ('omics' data sets are described in the section from WP7). The overall goal of this work package was to enable a new and thorough characterisation of severe asthma patient groups to align it with the most efficient existing or upcoming therapeutic intervention.
- The agreed Data Analysis Plan of WP8 has formed the basis of all analyses.
- Research questions were prioritized and monitored based on the agreed Work Flows between WP7 and WP8.
- While to date over 95% of the 'omics' data sets have been generated and delivered, the complete execution of the various handprints has been delayed due to the incomplete availability of the all 'omics' data sets which prevented execution of the various handprints.
- However, all fingerprints based on the available data have been generated. More importantly the first two handprints, one from blood and one from sputum (figure 2.4.11) have also been generated based on the article in preparation by De Meulder *et al* 'A Bioinformatics and statistical framework for the generation of molecular fingerprints and phenotypic handprints of complex diseases from multiple data sources'. These analyses are in line with the expected deliverables for work package
  8. Once the outstanding data are available the remaining fingerprints will be generated which will enable the generation of a new set of handprints.
  - Based on the Data Analysis Plan, WP8 has generated an entirely novel Toolbox of analyses and validation steps for producing fingerprints and handprints

WP9:

- WP9 has been able to deliver on all proposed deliverables and all aspects of the description of work over the course of the project. The dissemination efforts are continuing into the legacy period. As the dissemination WP, the key outputs are described and listed in the table in section 2.1.
- The main outputs fall into four categories: Strategic communication supporting sustainability efforts; Scientific presentations and publications; Dissemination to patients and the public and; Online Dissemination.

WP10:

The main achievements of WP10 have revolved around the three boards set up to advise and monitor the project; The U-BIOPRED Ethics Board (EB) and Safety Monitoring Board (SMB) and The Patient Input Platform (PIP).

- Ethics Board (EB)

The Ethics Board (EB) functioned as an academic and patient-driven advisory group for evaluation and guidance on all ethics and scientific conduct issues within U-BIOPRED. The EB acted in a collaborative and inclusive way according to the Declaration of Helsinki on human rights to provide ethical guidance and balanced opinion on the research carried out in U-BIOPRED.

The Ethics Board played a major role in advising and monitoring the WP3 and the WP5 clinical studies, and in advising on the use of data – also related to the eTRIKS project support of U-BIOPRED. The initial focus was on the development of SOP's and protocol and advises included reducing the need to reduce the burden on the patient, to provide full and consistent information and to look at the finer detail of each procedural document. Guarantying use of collected samples was a question raised numerous times. During the trial themselves, decisions relating to bronchoscopies and amendments were considered. A member of the ethics board or PIP regularly attended WP3 and WP5 conference calls.

- Safety Monitoring Board (SMB)

  Throughout U-BIOPRED, the Safety Monitoring Board (SMB) monitored patient safety, made decisions on safety issues, coordinated crisis management and evaluates the efficacy of any interventions. The SMB also acted in a collaborative and inclusive way to provide safety guidance and balanced opinion. Members of the SMB included health and research professionals, and patients or patient representatives.

The SMB reviewed information on 89 adult and 64 paediatric counts of hospitalisation for the entire study period, as part of a review exercise ahead of the compilation of the Adult and Paediatric cohort description papers. Three of the adult SAEs requiring hospitalization (having occurred prior to October 2013) were related ("bronchospasm during bronchoscopy", "asthma exacerbation induced by a study procedure", "exacerbation of asthma"), one of the paediatric SAEs requiring hospitalization occurring Dec 2013 was related to the study ("asthma exacerbation"). All patients recovered. None of the life-threatening events were judged related to the study.

- The Patient Input Platform (PIP).

  The level and impact of patient involvement in U-BIOPRED has been one of the successes of the project. Patients have also been involved in the EB and SMB, but the focus for activity has been the PIP group. This group of 14 active participants has met regularly over the course of the project, attending annual meetings and through their activity winning attendance rights to the ERS Congress. All patient input platforms members were affiliated to a U-BIOPRED partner, either as a member or voluntary governance member. None were employees. The U-BIOPRED PIP is being seen as an exemplar of patient participation in EU research projects and the IMI have supported a 'how to' booklet to advise future groups on the set up and maintenance of patient involvement.