U-BIOPRED is an IMI funded project, receiving the support of the EU and EFPIA.
Introducing U-BIOPRED

- Understanding severe asthma

U-BIOPRED aims to bring about a better understanding of the different types of severe asthma, with the long-term goal of developing more effective treatments for people with the condition. People with severe asthma are difficult to treat. Despite taking the highest acceptable doses of oral and/or inhaled anti-inflammatory and bronchodilation therapy, they are still symptomatic and experience a poor quality of life.

It is therefore extremely important to gain a greater understanding of severe asthma. By understanding the individual characteristics of the forms of severe asthma we can develop new medicines which can specifically control the symptoms of people living with this form of asthma. This personalisation of treatments could have a dramatic impact on the quality of life of people living with severe asthma.

- What is U-BIOPRED?

U-BIOPRED (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes) is a European wide research project investigating severe asthma. It is formed by a consortium of scientists from universities, research institutes, the pharmaceutical industry and big and small companies.

U-BIOPRED is made up of several elements, including laboratory research, animal research and a clinical trial. The aim of the clinical trial is to track patients disease experiences and responses to treatment over time, so a highly detailed picture is built up of treatment responses aligned to the patients specific condition.

This research is mostly non-invasive, involving a baseline visit with an assessment of current health status, atopy, pulmonary function, non-invasive measurement of inflammation and genomic studies. Some patients will be asked to undergo a bronchoscopy and to have a CT-scan for a more detailed analysis of their airways.

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The U-BIOPRED art contest

As part of the project’s outreach to the public and patients, an art contest was held last year. The contest was open to people of all ages with asthma to help bridge the gap between doctors’ understanding of the condition and a person’s experience of it. Any type of art was accepted, from poetry to sculpture, music to paintings.

Project lead, Professor Peter Sterk, project lead, says:

“The U-BIOPRED project aims to understand more about asthma and severe asthma. One important way we can do this is to hear from patients directly. This contest enabled patients to express, through art, what the condition is like and how it impacts upon their life”.

1st place: Marije Kootstra - The Netherlands

“My asthma plays an important role in my life. If the weather is bad, I can hardly cycle to school. Because of this, I miss many lessons. I have more than 10 different medicines that I have to take almost every day. I have already had two sessions of respiratory therapy, a nose operation and I have an appointment almost every month with the lung specialist in order to keep my asthma under control. Unfortunately, this doesn’t work completely and I have been admitted to hospital a couple of times because of a serious asthma attack. Still, I hope that with the right medicines I can again get my asthma back under control.”

Runners up:

Yvette Moerdijk
Maria Rubin
Joke Schoneveld

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ERS Congress abstracts
Three abstracts from the U-BIOPRED project were presented at the European Respiratory Society’s annual Congress 2012.

Presenting Author: David Balgoma, Karolinska Institute, Sweden
Session: Session 257: Monday, 03 September 2012, 12:50-14:40 in Halle A-19
Title: Application of a new mass-spectrometric platform for determination of lipid mediators in urine reveals increased oxidative stress during allergen bronchoprovocation of subjects with atopic asthma

Body: Traditionally, mechanisms in allergen-induced bronchoconstriction have been investigated by measurement of a few metabolites of lipid mediators (LMs) in urine. Mass spectrometry enables quantification of a wider panel of compounds per analysis. We applied a new platform that included isoprostanes (IPs), markers of oxidative stress.

Aim: To assess the levels of IPs in urine before and after allergen provocation in asthma patients.

Methods: Eighteen subjects with mild atopic asthma and airway hyperresponsiveness to methacholine were challenged with allergen to produce at least a 20% drop in FEV1. Urine was collected before and after the provocation. Metabolites were extracted and analyzed by a new mass spectrometry platform. Data were normalized per mmol of creatinine.

Results: As expected, metabolites of prostaglandin D2, thromboxane A2, and leukotriene E4 increased after the provocation. However, two IPs (2,3-dinor-8-isoPGF2α and 8,12-ipF2α-VI) also increased significantly. Multivariate analysis showed gender differences in basal levels, potentially due to higher excretion of creatinine by males.

U-BIOPRED is an IMI funded project, receiving the support of the EU and EFPIA
**Presenting Author:** Paul Brinkman, University of Amsterdam, The Netherlands

**Session:** Session 441: Tuesday, 04 September 2012, 14:45-16:45 in Room C2

**Title:** Calibration of a (semi)-automatic measurement and control platform for centralized, simultaneous electronic nose (eNose) analyses in multi-centre trials

**Body:** Breath analysis by electronic nose (eNose) technology represents a promising diagnostic tool in lung disease. A critical step in making this technology suitable for multi-centre trials, such as the U-BIOPRED Study, is to facilitate centralized measurements on multiple eNoses simultaneously. This can be accomplished with a (semi)-automatic measurement and control platform.

**Aim:** To calibrate and analyze repeatability of multiple sensors in an eNose platform (5 eNoses, 4 brands).

**Methods:** Ethanol was chosen as one of the calibration gases. Different concentrations (500 ppb-8 ppm) were generated by a permeation system. Measurements at all concentrations were done in duplicate. Total number of sensors in the platform was 81. The obtained data were processed by averaging duplicate measurements after normalisation (scale 0-1).

**Results:** The platform (not all individual sensors) was sensitive to ethanol at used concentrations (fig). The difference in normalized sensor deflections between duplicate measurements at 2 ppm was (mean[SD], range): 0.09 [0.1], 0.55-0.0004. The eNose platform is capable of detecting ethanol at concentrations from 500 ppb to 8 ppm level with acceptable repeatability. This method of platform calibration with standard gases is feasible and mandatory for quality control of eNose assessments in a multi-centre setting.
**Presenting Author:** Jorge De Alba, Almirall R&D, Spain

**Session:** Session 257: Monday, 03 September 2012, 12:50-14:40 in Halle A-19

**Title:** Poly:IC causes exacerbation in a murine allergic inflammation model driven by house dust mite in Freund’s complete adjuvant

**Body:** RNA viruses are major causes of respiratory infections and known to exacerbate asthma and other respiratory diseases.

**Aim:** The objective of the study was to use poly I:C, a synthetic analogue dsRNA, to elicit exacerbation in a model of allergic inflammation driven by house dust mite (HDM) in Freund’s Complete Adjuvant (FCA). This model, developed in partnership with UCB as part of UBIOPRED WP6, is characterized by airway hyperresponsiveness (AHR) and a mixed T-helper phenotype (1).

**Methods:** BALB/c mice were sensitised subcutaneously on day 0 with HDM (100µg) in FCA as previously described (1). On day 14, mice were exposed to saline or HDM (25µg) via intranasal instillation (i.n.). Poly I:C (30 µg) was administered i.n. 24hrs before (-24hr), at the same time (0hrs) or after (+6hours,+24hours) HDM challenge. 24 hours post-challenge, non-invasive whole-body plethysmography was used to assess AHR stimulated by aerosolised methacholine (MCh, 0-16mg/ml). 48 hours after HDM challenge, the bronchoalveolar lavage fluid (BALF) was collected to measure inflammatory cells.

**Results:** Poly I:C exacerbated BALF neutrophils (-24, 0,+6), macrophages (-24, 0,+6) and lymphocytes (-24, 0) in the HDM challenged animals. At -24hrs or +6hrs, the AHR associated to MCh was also significantly exacerbated. Poly I:C exacerbates the inflammation and AHR in a murine model that mimics certain aspects of persistent asthma. This model could be used to investigate new mechanisms of action underlying viral exacerbation in persistent asthma and for the assessment and evaluation of novel therapies for such condition.(1)Nasra J. et al. Am J Respir Crit Care Med 181;2010:A2842

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ATS Congress abstracts
Three abstracts from the U-BIOPRED project were presented at the American Thoracic Society’s annual Congress 2012.

Presenting Author: K. Daham, Karolinska Institutet, Stockholm
Session: Sunday, 20 May 2012, 08:15- 16:30
Title: Safety of the COX-2 inhibitor etoricoxib in allergen-challenged asthmatics

Body: Prostaglandins (PG) that constrict and relax airways are biosynthesised in reactions catalysed by either cyclooxygenase isoenzymes 1 or 2 (COX-1 and COX-2). In mice models of asthma, COX-2 inhibition aggravates airway hyperresponsiveness (AHR). It is unclear if inhibition of COX-2 in subjects with asthma selectively removes PGs that make asthmatic responses better or worse.

Methods: Fifteen subjects with mild atopic asthma (Table 1), AHR to methacholine (MCh) and demonstrated allergen-induced early phase bronchoconstriction, were challenged with rising doses of allergen and MCh to determine the provocative dose causing 20% drop in FEV1 (PD20) during a control session and following 10-12 days of treatment with the COX-2 inhibitor etoricoxib (90 mg once daily). Induced sputum was collected before and after the allergen challenges. The effect of the treatment was validated biochemically by measurements of thromboxane generation in clotted blood for COX-1 and LPS-induced formation of PGE2 in leukocytes for COX-2, respectively. Urine was also collected before and after allergen provocations for establishment of treatment effects on excretion of prostaglandin metabolites.

Results: Etoricoxib treatment did not change pre-challenge baseline lung function (mean ± SEM FEV1 3.78 ± 0.20 and 3.82 ± 0.20 after drug and control, respectively), AHR to MCh (Fig 1) nor the sensitivity (PD20) to allergen (Fig.1). Neither was the magnitude of the fall in FEV1 for the PD20 dose different after treatment with etoricoxib (mean ± SEM % drop in FEV130.4±1.3 and 25.3± 3.6 after drug and control, respectively), nor the allergen-induced increase in sputum eosinophils post challenge. The biochemical assays confirmed the effectiveness and COX-2 selectivity of the treatment. This first study of a COX-2 inhibitor in the allergen-challenge setting found no negative effects of etoricoxib on airflow obstruction and sputum eosinophils induced by the challenge, basal lung function or MCh responsiveness in subjects with atopic asthma. Taken together with previous observations on the safety of COX-2 inhibitors in aspirin-intolerant asthma, it appears that short-term use of COX-2 inhibitors may be acceptable in asthmatics. Mechanistically, the data support that bronchoprotective PGE2 is derived from COX-1.

U-BIOPRED is an IMI funded project, receiving the support of the EU and EFPIA
**Presenting Author:** D. Balgoma, Karolinska Institutet, Stockholm  
**Session:** Tuesday, May 22, 08:15 - 16:30  
**Title:** Gender differences in 5- and 12/15-lipoxygenases products in bronchoalveolar lavage fluid from healthy never-smokers, smoker and COPD patients

**Body:** Chronic obstructive pulmonary disease (COPD) is a leading cause of mortality and morbidity worldwide, which is increasing particularly among females. Smoking represents the main risk factor for developing COPD; however, the chronic inflammation persists following smoking cessation. A plethora of inflammatory mediators are likely to orchestrate this persistent inflammation with resulting tissue remodeling and decline in lung function. We have investigated the effects of smoking, in relation to disease, on oxylipins as key lipid mediators in the inflammatory response in the lower airways.

**Methods:** Bronchoalveolar lavage fluid (BALF) was obtained from healthy never-smokers, non-symptomatic smokers, and COPD patients of GOLD stage I-II (smokers and ex-smokers) of both genders (20 per group, n=120). Eighty-five different lipid mediators derived from the cytochrome P450 (CYP), lipoxygenase (LOX) and cyclooxygenase (COX) pathways were simultaneously analyzed by offline solid phase extraction followed by ultraperformance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

**Results:** Thirty-five lipid mediators were detected in >50% of the samples. Data were analyzed by principal component analysis (PCA, R2=0.608 Q2=0.498), with partial separation observed between smokers and non-smokers (Figure 1.B). Products of 12/15-LOX (n=9) and 5-LOX (n=5) clustered in the loadings plot (Figure 1.A). Therefore, all quantified products of theses enzymes were summed for further statistical comparisons. The sum of 12/15-LOX products showed gender-specific behavior in the disease path, but only two comparisons were significant: male vs. female healthy non-smokers and non-smoker vs. smoker healthy males. A different trend was observed for 5-LOX products with male and female healthy non-smokers showing lower levels of 5-LOX products compared, respectively, to male and female healthy smokers. There were no difference in 5-LOX levels between smokers and smoking COPD patients. However, in COPD ex-smokers the levels of 5-LOX products were decreased compared to COPD smokers.(significant for females, but not for males; posteriori P 0.11). Lipoxygenase activity in BALF shows gender-specific regulation in relation to both smoking and disease. The observed shifts were 5-LOX- and 15-LOX-specific, with smoking in both genders driving changes in 5-LOX activity whereas 12/15-LOX showed gender-specific regulation in COPD. These findings suggest that distinct lipid mediator biosynthetic pathways evidence unique responses to both disease and smoking. The observed gender-specific component provides potential insight into the gender imbalance in COPD cases and warrants further investigation.

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U-BIOPRED at the half way point

As U-BIOPRED enters the second half of its project period, good progress continues to be made. Although this ambitious project is not without its challenges, all Work Packages have advanced to a point where the key outputs are in sight and the steps that need to be taken to achieve the original objectives are clear.

The two key areas of work in the past year have been the clinical trial and the preparation of the systems to collate and analyse the data. In the clinical trial recruitment is now half way (circa 400 Adults and 120 Children included each with four cohorts; August 2012).

The key challenge is getting patients included, which is something we are now tackling with the help of partner sites. Patient organisations and dissemination through local networks are helping the process. Development of standard SOPs for sputum, blood, exhaled air, CT scans and bronchoscopies have seen a process where change and best practice has needed to be included in an iterative fashion.

The time allowed for Ethics approval did also lead to delays in the start of recruitment. Sample collection training and monitoring of sample quality has seen collaborative management as a crucial factor in keeping the widely spread work on track and of a good quality. These are some of lessons which U-BIOPRED has learnt and which, with the help of IMI, we are beginning to disseminate to other IMI projects.

The other key challenge is in the preparation of the databases and of the systems needed to facilitate a systems biology approach to the data analysis. U-BIOPRED is developing a handprint composed of a number of statistical models (fingerprints). The work on creating the fingerprint models is well underway and they have been tested with the first sample data.

The next step is to feed more data into the analyses and to move to integrate the models into a handprint. The aim is to see if the handprint can serve as a means of identifying sub-phenotypes of severe asthma. In this way drug development can become more focused and specific achieving both a more rapid development timeline and a degree of therapeutic personalisation.
A substantial achievement has been the completion of an interim analysis early in the data collection cycle. This has identified challenges in the complex data/sample handling processes that are now being addressed as opposed to waiting for the end of the study.

This is more evidence that if not anything, UBIOPRED is developing infrastructure for conducting complex translational research studies that are needed to embrace the richness of the datasets being generated with new high throughput techniques.

The effort underway in the clinical trial is being complemented by the work on a viral challenge model as well as laboratory models. A major hurdle for the viral challenge studies was the production of a viral inoculum that was produced under a high quality standard (GMP). This will allow for recognition and acceptance of the viral challenge studies done with this virus by regulatory authorities. Such a viral inoculum is now in production with delivery expected in the fall of 2012.

Work is now focused on establishing the protocol and procedures for the viral challenge studies themselves. Laboratory model work is progressing in tackling a major challenge – producing laboratory models that reflect severe asthma and are accepted by multiple laboratories. To date everyone tends to have their own protocol.

UBIOPRED has been successful in getting multiple groups to operate under the same protocol and is using that approach to test different approaches to making models that reflect severe asthma. While definitive models have yet to be defined, there has been a significant amount collaborative data generation that has at least defined a number of models that do not work.

The challenges are not yet over, but there are already significant successes to encourage us on our way. A positive review from IMI has confirmed this. This review has also helped us develop our plans in the key areas where mitigation is needed and where dissemination of our experiences will be valuable to others. Our website is updated with the latest news from the project, so to see how we are getting on go to http://www.ubiopred.european-lung-foundation.org/
The U-BIOPRED project has also fed into a new Research Criteria Booklet, compiled by the European Innovative Medicines Initiative (IMI) project.

The booklet is a practical guide for lay people and patients who would like to get more involved in scientific research. The criteria in the booklet will help readers to evaluate guidelines and research proposals to see if they are relevant to a patient.

The booklet advises patients to ask a set 25 questions about the research to determine their opinion on the topic and evaluate the document. These questions are structured around themes that include: relevance to patients, quality of life, quality of care, ethics and safety, information and communication and participation.

In addition to the U-BIOPRED model of patient input being used as a basis for this document, patients within the project can now also use the Research Criteria Booklet to help inform their decision-making within U-BIOPRED.

Further information

U-BIOPRED (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes) is a research project funded by the Innovative Medicines Initiative (IMI), to understand more about severe asthma that involves universities, research institutes, the pharmaceutical industry, small companies and patient organisations.

More information over U-BIOPRED can be found at:
www.ubiopred.european-lung.foundation.org

More information over IMI and the IMI U-BIOPRED factsheet can be found at:
www.imi-europe.org
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