Project Periodic Report

Grant Agreement number:	**305564**
Project acronym:	**SysmedIBD**
Project title:	**Systems medicine of chronic inflammatory bowel disease**
Funding Scheme:	**FP 7, Collaborative Project**
Date of latest version of Annex I against which the assessment will be made:	**21-07-2016**
Periodic report:	**1st ☐ 2nd ☐ 3rd ☐ 4th ☒ 5th ☐**
Period covered:	**From 01-12-2015 to 30-11-2016**

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Publishable summary

SysmedIBD

Logo: 

Project title: Systems medicine of chronic inflammatory bowel disease

Website: www.sysmedibd.eu

Contractors involved (SysmedIBD consortium):

Prof Werner Müller - University of Manchester

Other partners and team leaders:

Beneficiary No.	Beneficiary Name	Acronym	Team leader
01a	The University of Manchester, UK	UNIMAN	Prof. Werner Müller
01b	The University of Manchester, UK	UNIMAN	Prof. Dean Jackson
01c	The University of Manchester, UK	UNIMAN	Prof. Mike White
02	Christian-Albrechts-Universität zu Kiel, Germany	CAU	Prof. Philip Rosenstiel
03	Deutsches Krebsforschungszentrum Heidelberg, Germany	DKFZ	Prof. Thomas Höfer
04	The University of Warwick, United Kingdom	WARWICK	Prof. David Rand
05	Universitätsklinikum Aachen, Germany	UNIAACHEN	Prof. Christian Trautwein
06	The University of Liverpool, United Kingdom	UNILIV	Prof. Chris Probert
07	Universiteit Maastricht, The Netherlands	MUMC	Dr. Marieke Pierik
08	The Hebrew University of Jerusalem, Israel	HUJI	Dr. Nahum Shpigel
09	LifeGlimmer GmBH, Germany	LIFEGLIMMER	Prof Vitor dos Santos Dr. Madeleine Kittner
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1.1 Summary description of project context and objectives

SysmedIBD stands for Systems Medicine for Inflammatory bowel disease. Inflammatory bowel disease or IBD in short is a disease of people that suffer from chronic inflammation of the gut. IBD can be divided in two major forms, Ulcerative colitis, effecting in Europe about 10 in 100.000 people and Crohn's disease effecting about 5 in 100.000 people. The disease can start at an early stage and once started requires life time disease management. If treatments by medication stops to work, surgery to remove the inflamed tissue is used to at least temporary remove the symptoms.

SysmedIBD is focusing on one central pathway of cell signalling that is altered during inflammation, called the NF-kappa-B pathway. NF-kappa-B signalling is always active in the cells to various degrees. The pathway is not just a simple switch on/off mechanism but consists of a very dynamic process with a build in oscillator. This oscillator can be visualised in cells by fluorescently labelling components of the pathway and then follow individual cells using fluorescence microscopy. The big step forward in the project was to refine the way we are able determine NF-kappa-B signalling dynamics. We are now able to measure NF-kappa-B signalling dynamics in primary cells from human patients based on our work with animal models. After the generation of mouse models with fluorescence proteins linked to NF-kappa-B proteins, SysmedIBD determined the oscillation frequencies in a number of cell types that are located in the gut tissue like macrophages and gut epithelia cells as well as fibroblasts. From the measurements it became apparent that individual cell types have a unique oscillation frequency. Also SysmedIBD determined which signals can trigger NF-kappa-B oscillation in these cell types and found that selective mediators of inflammation will induce NF-kappa-B oscillation for a given cell types, information that is now used in mathematical models to describe the complex events during acute and chronic inflammation. After the determination of the oscillation frequencies of the NF-kappa-B signalling pathway in primary cell types, SysmedIBD performed extensive analysis of gene transcription of these cell types in time series, comparing mouse and human cells as well as different NF-kappa-B activators. SysmedIBD was able to identify groups of genes that have similar dynamics in terms of gene transcription dynamics in different cell types and the comparisons of cell types allowed SysmedIBD to identify a minimal gene set that is required the dynamics behaviour of the NF-kappa-B signalling pathway. Comparing mouse and human NF-kappa-B pathways we have now identified a small subset of master regulators of the NF-kappa-B pathway, one of which is unique to the human pathway.

In the SysmedIBD a very well characterised patient group is being followed both historically and prospectively to find better criteria to diagnose and to treat patients with IBD. We have computerised historic data for about 3000 patients In order to best integrate the work in the basic mechanism of NF-kappa-B pathway dynamics in chronic inflammation and the application for patient groups, SysmedIBD measured NF-kappa-B dynamics and dynamic gene expression profiles in a subset of patients from the patient cohorts within SysmedIBD, utilising the technology developed within SysmedIBD during the first three years of the project This work has already led to a better understanding of a potential mechanism of the role of smoking for the disease severity.

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1.2 Work performed since the beginning of the project and the main results achieved so far

SysmedIBD has so far generated a transgenic mouse model with fluorescence protein labelled proteins of the NF-kappa-B pathway which allows the determination of NF-kappa-B oscillation in various primary cell types. This animal model allowed us to demonstrate that NF-kappa-B oscillation, initially observed in tumour cell lines, can also be observed in primary cells and therefore does not represent an artefact of a particular tumour cell line but rather is a physiological feature of the NF-kappa-B pathway. It has further refined the mouse models and characterised a new mouse line expressing a luciferase transgene under the control of the Tumor necrosis factor gene regulation. This system allows very efficient screening of small molecules for regulation of NF-kappa-B signalling dynamics.

SysmedIBD has performed extensive RNAseq analysis of NF-kappa-B dynamics in cell types like monocytes, macrophages and gut epithelia cells, all of which have relevance for the development of inflammatory bowel disease. In these datasets we could observe gene expression patterns of induced or suppressed genes to be clustered according to the time of gene expression after activation and we could define a common set of early induced genes can be found in all cell types studied so far. When comparing the different cell types, we can also identify genes expressed only in specific cell types. For example, cells of the immune cells express more immune modularly gene products compared to non-immune cells.

SysmedIBD has developed a series of gene delivery systems that encode for fluorescence labelled NF-kappa-B proteins. These can be used to transfect primary cells and NF-kappa-B oscillation can be measured upon activation just like it was the observed in the NF-kappa-B protein transgenic mice. With this technology we can now study blood derived primary cells, blood or bone marrow derived macrophages and also more complex 3D organ cultures like gut organoids.

SysmedIBD has developed methods to measure the dynamics of NF-kappa-B activation in blood cells from patients using either enzyme dependent or fluorescence based systems. These studies link closely to gene expression analyses in time series as described above.

SysmedIBD has digitalised most of the historic data (20 years) of the South Limburg Patient cohorts with inflammatory bowel disease (IBDSL). For this stand-alone computer programs have been developed that can be used to translate data into an anonymous, digital format for further analyses starting with records in various formats.

SysmedIBD has developed pathway maps for the NF-kappa-B pathway in mice and human and is using these maps to compute communalities and differences between mouse and human cells, to map gene expression profile measurements have been mapped onto the NF-kappa-B pathway map and to compute potential biomarkers. Comparison of mouse and humans pathways we identified at least one master regulator in humans not observed in the mouse model.

SysmedIBD has analysed so far about 1 million natural compounds and small molecules by a computer program predicting potential interaction of these compounds and small molecules with the pathway maps developed and about 35 natural compounds have been identified so far, a subset of these will be tested in the in vitro systems developed by SysmedIBD.

SysmedIBD has developed methods to measure NF-kappa-B activation in cells from patients

- Dynamic measurements using NF-kappa-B controlled luciferase expression profiles
- Dynamic measurements using NF-kappa-B protein encoded viruses to determine NF-kappa-B dynamics
- Dynamic measurements using flow cytometry and anti NF-kappa-B protein antibodies
- Static measurements using RNAseq and gene arrays from patient cells and faecal samples

SysmedIBD has digitalised most of the historic data (20 years) of the South Limburg Patient cohorts with inflammatory bowel disease (IBDSL) and the following achievements have been made so far

- Computer programs have been developed that can be used in the clinics directly to deanomynise and to digitalise patient records for further analyses starting with paper records in various formats
- The key statistical parameters of the IBDSL were calculated

SysmedIBD has developed pathway maps for the NF-kappa-B pathway in mice and human and using these maps the following achievements have been made so far

- Differences and communalities between mouse and human has been determined
- A set of master regulators have been computed.
- Gene expression profile measurements have been mapped onto the NF-kappa-B pathway map
- Gene products feeding into or are derived from other signalling pathways have been identified
- Potential biomarkers were mapped onto the NF-kappa-B pathway maps

SysmedIBD has analysed so far about 1 million natural compounds and small molecules by a computer program predicting potential interaction of these compounds and small molecules with the pathway maps developed and about 37 natural compounds have been identified so far, a subset of these will be tested in in vitro systems developed by SysmedIBD.

SysmedIBD has established a clear pipeline of dataflow between different activities within the consortium, with clear assignments of data production, processing and analyses between the partners. The data is maintained by Warwick for all partners and analyses pipelines are maintained by 10GeneXplain on their computing platform. Primary data is held at the genome centre by 02CAU and only processed data is moved to the server in 04Warwick for further data processing.

1.3 The expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project so far)

SysmedIBD is combining high content microscopy, in vitro systems, mouse models, modern genetic tools, extensive biological pathway maps, mathematical models, computer based screening of natural compounds with well characterised patients to better stratify patients and subsequently to suggest the most successful treatments based on the knowledge build during the course of the SysmedIBD project. We expect that at the end of the project that SysmedIBD

- has visualised the dynamics of NF-kappa-B signalling pathway during gut inflammation
- has used fluorescence marked NF-kappa-B proteins to map the role of genetic mutations leading to increased susceptibility of IBD onto the NF-kappa-B pathway
- has produced a comprehensive NF-kappa-B pathway map joint between mouse and human in order to map experimental data from both species on a common pathway map indicating both similarities and differences between both species
- has established visualisation of the NF-kappa-B pathway dynamics in mouse and human 3D gut organoid cultures
- has established and verified protocols for measuring NF-kappa-B dynamics in cells derived from patients
- will computed a list of biomarker candidates based on extensive NF-kappa-B network analyses
- will have produced a list of natural compounds computed from our model that should be tested in in vitro systems developed by the consortium
- will have fully analysed the patient records of the IBDSL patient records and define clusters of patients within the IBDSL patient cohort leading to better stratifications of new patients
- may be able to define new NF-kappa-B related biomarkers with predictive value for patient stratification and for therapy efficiency predictions
- will suggest a new view on the role of smoking on the pathology of IBD.