Project Final Report

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SysmedIBD

Logo: 

Project title: Systems medicine of chronic inflammatory bowel disease

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1.1 Executive summary

The Systems Medicine of Inflammatory Bowel Disease (SysmedIBD) was a multidisciplinary project that developed tools for a better stratification of patients with Inflammatory Bowel disease. Inflammatory Bowel Disease is a chronic inflammation of the gut. Two such diseases are ulcerative colitis and Crohn's disease. In Europe about 10 in 100.000 people suffer from ulcerative colitis and about 5 in 100.000 people from Crohn's disease. The disease can start at an early age. The disease cannot be cured, and lifelong management of the disease is required. A systems medicine approach, including as much data as can be collected and incorporated into mathematical models and cellular pathways, can lead to new discoveries in the understanding of the disease.

SysmedIBD focused on one pathway active in all cells of the human body, the so-called NF-kappaB pathway. This pathway is essential for life and one of its function is to regulate inflammatory processes. The NF-kappaB pathway itself is a key component of an inflammatory response. In SysmedIBD we developed an extensive map of the NF-kappaB pathway based on published data including data from human and mice. Based on gene expression analyses at several time points of an inflammatory response, we mapped the elements of the pathway on a dynamic map. This dynamic map allowed us subsequently to add additional cellular pathways that are linked to the NF-kappaB pathway and in turn regulate the outcome of the NF-kappaB pathway.

The extensive dynamic data related to the NF-kappaB pathway was then used as a template to search for small molecules that can interfere with key components of the pathway in the computer. This computer-based screen then resulted in a list of molecules we then tested in model systems for their ability to alter the outcome of the NF-kappaB pathway.

Based on the dynamic pathway map, SysmedIBD developed in vitro tests that can be used on frozen cells from patients with Inflammatory Bowel Disease and determine dynamic parameters of the NF-kappaB pathway signalling capacity. Using this new in vitro test system, we were able to stratify patients with Inflammatory bowel disease into two distinct groups, one group with a reduced NF-kappaB response compared to healthy controls and the second group with an enhanced NF-kappaB response compared to healthy controls.

The patient samples in SysmedIBD were collected by three clinical partners, hospitals from University Aachen, Maastricht University and University of Liverpool, and using the new stratification of patients we were then able to map clinical parameters onto the patient groups. This new stratification system together with the historic data on the patient cohorts will in the future better allow to find the best treatment for newly diagnosed patients.

The animal models developed in SysmedIBD were used to reproduce the observations seen in in vitro systems. We were able to visualise the dynamics of the NF-kappaB signalling pathway using cells from mice with fluorescent proteins linked to NF-kappaB elements. Using mice transplanted with human fetal gut, SysmedIBD could verify that in the direct comparison of the inflammatory response between mouse and human tissue the gene expression profiles can be very similar if the correct conditions are used. We could also link known risk alleles from human patients to the NF-kappaB pathway using mouse mutants with defined mutations that are known to be linked to an increased risk of the development of Inflammatory bowel disease.

Based on the humanised mouse model we also established a new system that for the first time allows to study fistula formation in an animal model. Fistula formation is one of the very frequent co-morbidities of chronic inflammatory bowel disease that could not be studied previously. Our model will now allow us to better understand the mechanism of fistula formation.

Taken together, the multidisciplinary approach between mathematicians, bioinformaticians, cell biologists, immunologists and clinicians allowed the SysmedIBD consortium to develop a multi-dimensional, dynamic system for inflammatory bowel disease. This system can be now used to study other inflammatory diseases, to compare and contrast mouse models with human diseases and provide a framework to better stratify patients and to select future drugs. One of the outcome of SysmedIBD was the “rediscovery” of a very well-known drug with the potential to be beneficial for a subgroup of IBD patients.

1.2 Summary description of project context and objectives

Background and Aims

SysmedIBD, a systems medicine approach towards improved understanding and better treatment of chronic inflammatory bowel diseases.

IBD is extremely debilitating chronic inflammatory condition, with a number of associated co-morbidities, which result from a systemic spread of inflammatory cues. Although IBD has no clear pathogenic agent and is strongly associated with unclear environmental factors, we have chosen IBD as the disease to study as the inflamed tissue is readily accessible for study, 50% of relapses in IBD are linked to diarrhoeal pathogens pointing to inappropriate prolongation of the inflammatory response, potentially linked to the NF-kappaB pathway.

Current treatment concepts, like the anti TNF therapy, aim to dampen inflammatory signalling pathways such as the NF-kappaB pathway. NF-kappaB, the central regulator of innate immune responses1, consists of several components and it is switched on by the release of a preformed transcription factor from a complex in the cytoplasm, the translocation of the transcription factor to the nucleus and the activation of target genes like proinflammatory cytokines, co-activators and anti-microbial peptides. However, NF-kappaB regulates in addition multiple cellular functions such as cell development and growth. For example, increased NF-kappaB activity drives pro-inflammatory functions in immune cells in the course of IBD. On the other hand, NF-kappaB is a key factor for the survival of intestinal epithelial cells and is therefore required to maintain the intestinal barrier. This example explains that general targeting of the NF-kappaB pathway may have detrimental side effects. In addition, very recent studies have shown that NF-kappaB is not only a static switch on/off pathway but has an oscillating behaviour, similar to biological clocks. This oscillating behaviour seems to have a fundamental role on the outcome of NF-kappaB activation.

SysmedIBD is a consortium consisting of experimental basic scientists, mathematical model developers and clinical groups. One of the members of the consortium, Mike White (Manchester), has shown that a central inflammation pathway, called the NF-kappaB signalling pathway, is a very dynamic signalling process and displays rhythmic behaviour1. The group of David Rand (WARWICK), also a member of the consortium, developed mathematical models describing the rhythmic behaviour of the NF-kappaB signalling pathway. This showed that activated NF-kappaB, a key regulator of inflammation, oscillates in and out of the nucleus in cycles of around 100 minutes. The hypothesis follows that there may be an optimum cycling time for health and that this may be particularly important in organs such as the intestine where there is, even in health, a continual state of low-grade NF-kappaB activation. This pioneering work was performed using cell lines. Further, a context dependent modulation of DNA binding and NF-kappaB-associated transcriptional activity has been described which adds yet another layer of complexity, which will be assessed by Chip- and RNAseq approaches in an unbiased manner. In SysmedIBD we will take these in vitro findings to in vivo and study the NF-kappaB dynamics in the context of chronic inflammatory bowel disease. We believe that the dynamics of the NF-kappaB pathway is key to understanding the pathology of the disease and for the development of novel therapeutic interventions in this difficult to treat chronic disease with multiple co morbidities. Since we hypothesise that there is an optimal dynamic NF-kappaB signalling activity for health treatment rather than just blocking NF-kappaB activity may need to readjust the “healthy” NF-kappaB dynamic state.

The SysmedIBD consortium brings together renowned researchers from across Europe, each with a strong scientific and/or clinical background. Thomas Höfer (03DKFZ), David Rand (04WARWICK) cover the systems biology part in the consortium, supported by two SMEs 09LIFEGLIMMER (Vitor dos Santos) and 10GENEXPLAIN GMBH (Alexander Kel). Mike White and Dean Jackson (01UNIMAN) generated many tools for the visualisation of the NF-kappaB dynamics. Two experimental groups generate data for the modelling group, one specialised on mouse models (Werner Müller, 01UNIMAN, and Nahum Spiegel, 08HUJI) and the other working with patients (Christian Trautwein, 05UNIAACHEN, Marieke Pierik, 07MUMC, Jon Rhodes and Chris Probert, 06UNILIV, and Lynnette Ferguson, 11UOA). The groups of Stefan Schreiber and Philip Rosenstiel (02CAU) provide genomics data from samples provided from animal models and from patient samples and links to major Biobanks with samples from IBD patients. The SME ARTTIC manages the consortium and supports the coordinator.

The aims of SysmedIBD are:

• To elucidate the pathophysiological mechanisms of NF-kappaB signalling dynamics and the underlying regulation of transcriptional networks in inflammatory bowel disease.

• To define better diagnostic marker (sets) based on the modelling of signalling dynamics within the gut tissue and other affected organs in order to detect early and monitor the onset of IBD relapse, to predict complications and co-morbidities, and to support effective and efficient treatment of patients.

• To use small molecules interfering with critical components of the inflammation pathway's oscillating behaviour with the aim of developing novel treatments for IBD.

Work strategy and general description

SysmedIBD set out an ambitious research plan with the ultimate intention of delivering an analytical platform that

will provide a quantitative description of IBD severity with prognostic power. This can only be possible within a

project that synergises the expertise of clinical and research teams in a way that emphasises the critical needs of

the relevant stakeholders – most notably the patient groups.

The major objectives of SysmedIBD are predicated on developing a detailed understanding of NF-kappaB dynamics and its control by immune regulation in IBD. To understand the aetiology of IBD, it is essential to define how risk factors (e.g. environmental, genetic and epigenetic) influence the balance of pro- and anti-inflammatory cues in the gut, which is inappropriately regulated in the tissue niche in IBD.

Signalling through NF-kappaB is a major driver of inflammation in IBD, which we will explore by:

i) – measuring dynamic properties of NF-kappaB signalling with high information content time-lapse confocal microscopy using fluorescent NF-kappaB proteins expressed either from BACs or lentivirus vectors. In: human and mouse cell lines (01UNIMAN); cell lines and primary cells from animal models and patient samples (01UNIMAN, 06UNILIV, 05UNIAACHEN, 07MUMC); and in xenografts of human gut tissue in humanised mice (08HUJI). Assays may incorporate existing inhibitors of signalling or candidate small molecule inhibitors designed in SysmedIBD (10GENEXPLAIN, 09LIFEGLIMMER).

ii) – determining signalling inputs (e.g. cytokines, microbial biomolecules - 01UNIMAN) that activate NFkappa-

B, associated changes (e.g. protein modifications and location - 01UNIMAN) and outputs (e.g. gene expression changes over time using a range of technique - 02CAU). Influence of inhibitors on signalling dynamics and outputs will be assessed (10GENEXPLAIN, 09LIFEGLIMMER).

iii) – mathematical modelling of network topology, providing systems level under-standing of onset and resolution of inflammation (03DKFZ, 04WARWICK). This will also identify similarities and difference in the mouse and human networks based on experimental output (above), data mining and bioinformatic studies (09LIFEGLIMMER). This will be critical for the description of potential biomarkers (01UNIMAN, 02CAU; 09LIFEGLIMMER).

Management structure and procedures

Project Coordinator Prof. Müller from the University Manchester will be the Project Coordinator of SysmedIBD. He is the intermediary between the European Commission and the Consortium as well as the supervisor of the overall progress of the project.
Central Office and Project Office (01UNIMAN and 13ART) of SysmedIBD is established by and based at the Coordinator site in Manchester and Munich respectively. This office is concerned with the coordination of all research activities (scientific management) and with all management tasks relating the coordination of the project.
To facilitate the organization and management, the Scientific Programme of the project is structured in 12 work packages (WP), which together comprise the project. Each Work package will be headed and coordinated by an experienced principal investigator as Work package Lead and a deputy leader. They are responsible for the management of their WPs. The WP Lead supervises and adjusts the process flow. The designated WP Lead has an integrating function and is responsible for engaging and communicating with all partners in the Work package. The WP Lead will report on the progress of the Work package in relation to the deliverables and milestones achieved and any issues causing delays. To ensure and document that this is being achieved the WP Lead will periodically send an internal Interim Report to the Steering Committee (STC) in a structured form and report to the General Assembly. The STC is formed by the WP leaders and is in charge of monitoring all activities towards the objective of the project in order to deliver as promised, in due time and in the budget. The General Assembly consists of one representative of each Participant with authority to vote. All other non-voting researchers working for this project may join the meetings and discussions.

Objectives of SysmedIBD:

The main objectives of the project are:

Objective 1: Determine the dynamics of NF-kappaB pathway in vivo in normal and inflamed tissue in mice, humanised mice and patients.

When SysmedIBD started there was already the observation that the NF-kappaB pathway in cells displays a clock like oscillatory behaviour when activated appropriately in tumor cell lines. In SysmedIBD we could show that this oscillatory behaviour can also be observed in normal cells derived from newly established transgenic mouse lines that express fusion proteins of the NF-kappaB pathway and fluorescence proteins. Subsequently we could show, using lentil viral vectors that oscillation can also be observed in human cells.

Using NF-kappaB specific promotor driven enzyme-based constructs that are able to emit light using the appropriate substrate (luciferase and luciferin) we developed a new cell-based screening system that works with frozen cells from the blood of patients. Based on this assay we are able to stratify patients with Inflammatory bowel disease. We could also use these systems successfully in humanised mice. In these mice we could observe that the inflammation is not uniform but occurs in small patches with areas that are not inflamed. This previously not observed pattern (it is not seen in cell culture models) was then used to model this patch like inflammation.

Objective 2: Modelling of NF-kappaB pathway in normal and inflamed tissue

In humanised mouse models we have seen a patchy inflammatory response. This patchy behaviour was then modelled in the computer. In the future, a very detailed analysis of gene expression dynamics at the border between inflamed and non-inflamed areas will give us insight into regulatory processes that could lead to novel immune regulatory principles to control and treat inflammation.

Based on extensive time series of gene expression profiles from resting and activated cell population, mimicking the inflamed tissue, we could develop a time line of the events that occur after the first inflammatory signal. We could develop an extensive network of pathways downstream of the NF-kappaB pathway that as their output generate molecules with the potential to activate or to suppress further NF-kappaB pathways. We think that these downstream pathways could be key to explan the patchlike NF-kappaB activation observed in the tissue.

Objective 3: Modulation of NF-kappaB pathway dynamics towards new treatment of chronic inflammatory bowel diseases

Using the dynamic measurements of NF-kappaB, we screen many small molecules derived from a computer-based screen using our extended NF-kappaB network developed in SysmedIBD. We found several compounds that interfere with NF-kappaB activation. To our surprise we found in the list of compounds selected by the computer program a class of molecules called macrolides. These are used as antibiotics in the clinic but not in the context of Inflammatory bowel disease. Clinical trials using these molecules were unsuccessful of inconclusive in the past. In SysmedIBD we could verify the biological potential of these molecules to modulate NF-kappaB signals and have observed a protective response in a mouse model for inflammatory bowel disease. We think that in combination with our new stratification protocol for IBD patients based on the NF-kappaB signal response, we may be able to identify a subgroup of patients that may benefit from macrolides once we have established the best criteria underlying the new stratification system.

1.3 Description of the main S&T results/foregrounds of SysmedIBD

WP01 – Visualising NF-kappa-B pathway dynamics in IBD in mice

When SysmedIBD started it has been shown that the NF-kappB pathway has an oscillatory behaviour. Two proteins, the p65 protein and the IkappaBalpha protein form a complex and have been shown to change expression or the cellular localisation, when the NF-kappaB pathway is actived in tumor cell lines in vitro. Upon activation the Ikkbalpha protein, which is complexed with the p65 protein in the cytoplasm of cells, is degraded and the p65 protein, which is not any longer associated with the Ikkbalpha protein, migrates to the nucleus and acts as a transcription factor. One of its target genes is the Ikkbalpha protein. When the p65 protein is in the nucleus, new Ikkbalpha protein is expressed and subsequently the p65 protein is exported from the nucleus to the cytoplasm and a new complex between the p65 protein and the IkkBlalpha protein is formed. Then the process starts again like a clock, within about 100 minutes in a tumour cell line.

Within SysmedIBD we have generated transgenic mouse lines that carry the human p65 protein and the human Ikkbaphla protein coupled to fluorescents proteins and we could show that NF-kappaB oscillation also happens in normal cells upon activation. We could show that the oscillation frequency is different in various cell types and in most cases independent from the signalling molecule.

We generated new transgenic NF-kappaB protein transgenic mouse lines that allow the visualisation of the NF-kappaB cells in mice. One of the transgenic mouse line, the human p65 protein transgenic mouse line, has an increased sensitivity towards inflammation. We made various modification in the ratio of the endogenous p65 gene and the human p65 transgene but were not able to overcome this increased sensitivity. Our current hypothesis is that one particular region in the p65 gene, a proline rich region, is responsible for this increased sensitivity. This question will be followed up in a subsequent project. If this hypothesis is true, we speculate that it is possible that in humans point mutations in the proline rich loupe could also have an increased sensitivity of inflammation and such point mutations have the potential to act in a dominant trait and could contribute to the outcome of inflammatory diseases.

In addition to the NF-kappaB protein transgenic mouse line, we utilised a new mouse line expressing the luciferase gene in a human Tumor Necrosis factor (TNF) transgenic mouse line. Cells from this mouse line allow a very efficient screen of small molecules which is faster compared to the use of cells from the p65/IkappaB transgenic mouse line we established at the beginning of the project. We used these in vitro systems to screen many small molecules. The findings of these screens are reported in the corresponding work packages.

Based on the results of the cells of the transgenic mouse lines, lentiviral vectors were developed that were then be used in cells from patients and in humanised mouse models to visualise NF-kappaB dynamics. The results again are reported in the corresponding work packages.

As part of this work package we also analysed mutations linked to inflammatory bowel disease in mice and could link known mutations not yet linked to the NF-kappaB pathway to the NF-kappaB pathway.

New, better NF-kappaB protein transgenic mouse lines are being developed. The role of the proline rich loop in the p65 protein requires further investigation and its role in inflammatory disease in humans could be potentially very interesting. Also, as we noticed that NF-kappaB oscillations is real and happens in primary cells, we have evidence from our human TNF transgenic mouse line that NF-kappaB signalling per se does not require NF-kappaB oscillation and can occur under triggering condition with low dose not sufficient to induce oscillatory behaviour.

WP02 – Mathematical modelling of NF-kappaB signalling dynamics

At the beginning of the project, it had been well-established by researchers in Manchester, Warwick and others that TNF-induced NF-kappaB signals take the form of oscillations in various cell lines. A number of mathematical models had been developed showing that the NF-kappaB oscillator is driven by a negative transcriptional feedback loop, with active NF-kappaB inducing the expression of its IkappaB family inhibitors. Moreover, experimental data indicated that the frequency of NF-kappaB oscillations controls the expression of NF-kappaB target genes. As NF-kappaB is a key mediator of inflammatory signalling in the human gut, and mutations in components of the NF-kappaB signalling pathway had been implicated in the aetiology of inflammatory bowel disease (IBD), the work in SysmedIBD investigated the hypothesis that oscillatory NF-kappaB signalling might be perturbed in IBD. Thus a central aim of SysmedIBD was to measure NF-kappaB signalling in primary cells – human gut endothelial cells as well as immune cells – and rationalize the underlying molecular mechanisms with the help of mathematical models.
In a first step, we constructed molecular interaction networks for NF-kappaB that served as a basis for the development of dynamic mathematical models. A conspicuous interaction that did not feature in previous mathematical models was positive feedback, mediated by the transcriptional upregulation of NF-kappaB isoforms by active NF-kappaB. Biological oscillators that combine negative and positive feedback, such as cellular calcium oscillators, had previously been shown to serve as frequency encoders that convert the level of an external stimulus into the frequency of intracellular oscillations of signalling molecules. However, we found that the frequency of the NF-kappaB oscillations remained rather constant at different concentrations of typical stimuli, such as TNF-alpha, arguing against frequency encoding of TNF-alpha dose. Consistent with the observed absence of frequency encoding, sensitivity analysis of the mathematical model showed that the oscillation frequency was distributed across a large number of parameters, where each parameter has only a small influence. However, the model made an interesting prediction: When we mimicked the natural variability of protein levels in single cells, we found that submaximal doses of stimuli such as TNF-alpha would result in only a fraction of cells showing NF-kappaB oscillations, while other cells responded with a single spike and the remainder showed no response. This phenomenon could be traced to a scenario for the onset of oscillations that is known as ‘hard excitation’ in mathematics. Experiments on macrophages confirmed this model prediction of pervasive heterogeneity of NF-

kappaB responses in single cells of a population. This led us to rephrase the original hypothesis by asking whether the ratio of cells with oscillatory versus non-oscillatory NF-kappaB signalling was altered in IBD. Indeed, experimental work on cell samples from patients showed two distinct groups, one group with hyporesponsive, strongly attenuated NF-kappaB signalling and another group with hyperresponsive NF-kappaB signalling. Future iterative work between experiments and modelling will aim to elucidate the mechanistic bases for these distinct patterns and thus may also inform the search for treatment approaches that restore normal responsiveness of the NF-kappaB pathway.

A second major aim of the mathematical modelling was to understand the spatial coordination of NF-kappaB signalling in the gut tissue, including the resident immune cells. Target genes of NF-kappaB include cytokines that stimulate the NF-kappaB pathway, such as tumour necrosis factor alpha (TNF-alpha). These feedback loops raise the possibility that NF-kappaB signals can propagate via paracrine mechanisms in the tissue. However, the spatial range of cytokine signal has been a matter of intense debate. On the one hand, cytokines diffuse in the extracellular space and may therefore support long-ranging signals, which could be harmful by propagating an inflammatory state. On the other hand, experimental observations within SysmedIBD of NF-kappaB signals in human gut in a humanized mouse model show localized hotspots. We have developed mathematical models of intercellular communication of NF-kappaB signals in gut tissue that couple the intracellular biochemical reactions of the NF-kappaB pathway and downstream TNF-alpha expression to the extracellular diffusion of TNF-alpha (reaction-diffusion models). To this end, we have developed computational tools to simulate the dynamics of this system in realistic three-dimensional space, utilizing finite-element methods. Using these tools, we have systematically studied which parameters control the spatial range of signal propagation and found that the TNF-alpha uptake rate by the cells is the most critical parameter. We then implemented a parameterized model of NF-kappaB signalling in macrophages, which are key secretors of TNF-alpha in the gut, and simulated intercellular communication by TNF-alpha diffusion. We found that these signals propagate over a few cell diameters and then stop, which is consistent with the experimentally observed hotspots of NF-kappaB signalling.

A third focus of the modelling was a better mechanistic understanding of how various types of NF-kappaB signals – low-amplitude activation, spikes or sustained oscillations – drive target gene expression programs. To this end, we devised bioinformatics tools and analyses as well as developed a novel mechanistic model of how NF-kappaB and other transcription factors control the transcription rate of their target genes. An overarching finding of the bioinformatics analyses was that NF-kappaB likely interacts with a variety of other transcription factors in a genome-wide context (notably among them several E-box binders). Future work will be devoted to understanding how the dynamic type and amplitude of NF-kappaB signals drives particular target gene programs, probably in part through differential interaction with other factors.

WP03 – Visualising NF-kappaB pathway dynamics in humanized mouse models

The model system and experimental techniques

In the framework of the SysmedIBD project, the Hebrew University (HU) partner extensively collaborating with all other partners, have used and greatly refined an experimental platform of humanized mouse model. Human fetal gut (Nissim-Eliraz et all, Infection & Immunity 2017), when transplanted subcutaneously in mature immunodeficient mice, developed normally with formation of mature epithelial and mesenchymal layers. Moreover, we have demonstrated for the first time, the establishment of human enteric innate and adaptive immune systems and enteric nervous system (ENS) in these xenografts. Next, experimental protocols and techniques were developed to elicit inflammatory response in the human gut and to visualize the spatial and temporal dynamics of inflammation in normal and inflamed human gut. The close collaboration between teams in Manchester University and HU was instrumental for successful development of lenti virus technology that enabled genetic expression of luminescence reporter systems in the human gut transplants.

NF-kappaB activation unmasks the presence of inflammatory hotspots in the gut

The single-epithelial cell layer of the gut mucosa is an essential barrier between the host and luminal microflora. This epithelium plays a key role in the innate immune response to invading pathogens. Although different regions of the gut may exhibit distinct response patterns, a tacit assumption in this field has been that the immune response potency is uniform within a region. SysmedIBD research shows that the response of human gut, developed as xenografts in immune deficient mice, to a general insult by systemic LPS injection consistently activates focal “hotspots” within a region. Remarkably, NF-kappaB, a crucial regulator of inflammation and immunity, is activated in a select subset of “inflammatory epithelial cells” in the human gut mucosa. These unique cells are clustered in the gut into discrete hotspots that are visible in steady state and selectively activated by systemic LPS and TNFα or luminal bacteria. The presence of inflammatory hotspots in the normal and inflamed gut might explain the patchy mucosal lesions characterizing Crohn’s Disease (CD) and thus may have important implications for diagnosis and therapy.

We plan to further validate this observation in mouse gut and to better understand the underlying mechanisms involved in the formation of inflammatory "hotspots" in the gut. The notion of "inflammatory epithelial cells" and inflammatory hotspots" in the gut might completely change current concepts and experimental approaches in preclinical and clinical studies of the inflamed gut. Within SysmedIBD, partner at the DKFZ developed a mathematical model to describe the focused inflammatory hot spots.

Genomic responses in inflamed mouse gut greatly mimic inflamed human gut
Mice are extensively used as a model system in basic and translational research of inflammatory conditions of the gut. The value of these models was debated by contradictory evaluation of genomic responses in mouse models and human inflammatory conditions. SysmedIBD researchers have used the humanized mouse as an experimental platform to compare the genomic response between inflamed human and mouse gut. The genomic response was evaluated in human gut, developed as xenografts, and host mouse gut in response to systemic LPS injection. The gene expression levels in the mouse and human gut showed significant correlations and revealed that numerous inflammatory and immune response pathways are commonly regulated in the mouse and human gut. We conclude that genomic responses in inflamed mouse gut greatly mimic inflamed human gut underscoring the utility of mouse models in IBD research. However, our analysis also unveil important differences in immune response between mouse and human gut. Understanding these differences will help us to avoid misinterpretation of mouse data leading to failures of translational research in the development of drugs for human patients.

Establishment of fistulating gut model in human gut xenografts
Crohn's disease (CD) associated fistula is a frequent complication or comorbidity and the lifetime risk of developing enterocutaneous fistula in CD patients ranges from 20 to 40%. To date, our understanding of the pathophysiology of fistula formation in CD patients is still poor. The current therapeutic outcome is often insufficient to achieve fistula closure and surgical resection is frequently required. Therefore, it is obvious that CD-associated fistulae represent a severe and still unresolved problem in the care of CD patients. IBD research has largely relied on in vivo animal studies and to date there is no reliable and validated animal model of intestinal fistulae available. This lack of in vivo models represents a huge disadvantage in fistula research and the development of new therapeutic approaches for fistula treatment. Based on the human gut xenograft model in SCID mice, SysmedIBD researchers have developed a novel animal model of fistulating human gut. Extensive histochemical analysis of fistulating human gut xenografts revealed striking similarities between the model system and clinical samples.

Several new projects have been developed based on the experimental platform established in SysmedIBD.
• Intestinal xenografts as a tool for studying development and maintenance of the normal enteric nervous system in man.

• Deciphering the impact of the maternal gut microbiota on development and turnover of the human enteric nervous system.

• A novel in vivo platform for the evaluation of new therapies for Crohn's disease fistulae.

• Chronic inflammation alters DNA methylation and promote inflammation in the human gut.

• Linking inflammation, aberrant DNA methylation and tumorigenesis in the human gut.

WP04 – Biomarkers indicative for NF-kappa-B pathway oscillation dynamics

The underpinning hypothesis of SysmedIBD was that dynamic changes of the NF-kappaB signalling network would correlate with altered inflammatory cues in the disease setting. Hence, detailed understanding of NF-kappaB network architecture might reveal novel dynamic biomarkers that could be used to better stratify and personalise treatment programmes for IBD. We began by verifying that the NF-kappaB oscillations seen in cell culture models were also seen in transgenic mouse models, and described a range of discrete tissue-specific signalling patterns. The mouse models were then used to place this signalling data in the scientific literature and support the hypothesis that dynamic changes in signalling through the NF-kappaB network might contribute to the aetiology of inflammatory disease. Expression of pathway components in the disease setting was then performed to identify potential biomarkers, which correlated with changes in the NF-kappaB network architecture in the disease state.
After developing a static representation of the NF-kappaB network architecture for signalling through NF-kappaB, using biological data and bioinformatics techniques, we superimposed data from time series of cell populations upon

NF-kappaB activation. These dynamic maps then allowed us to link the core NF-kappaB pathway to other signalling networks, which are activated at a later time points during the inflammatory response. In this way, mediators of pro- and anti-inflammatory signalling were monitored, allowing us to identify modulators of the NF-kappaB signalling process and at the same time deliver an extended list of potential biomarkers.
We then proceeded to test various biomarkers based on a range of measurements. One such system is based on the determination of microRNA molecules in the faeces of patients and controls that are changed upon NF-kappaB activation. We identified two potential candidates that could relate to the disease state; miR-223 and miR-1246 were shown to be present at high levels in feces and associated with active Crohn's disease.
Based on the initial work on mouse models we developed lentiviral vector systems that can be used to measure NF-kappaB dynamics at two levels: one at the level of NF-kappaB oscillation in single cells, using live cell imaging and one based on a population analysis of light producing enzyme linked to one of the output signals of the NF-kappaB signalling pathway, namely TNF. After many rounds of developing this approach, we now have a robust screening system that uses a standardised protocol from frozen cells after their isolation from the peripheral blood of patients. This approach provides consistent monitoring as the lab-based analysis can be performed under highly standardised conditions using clinical samples, including samples from biobanks. Cells from the peripheral blood are differentiated into macrophages, after being infected with the light producing vector. In culture, NF-kappaB signalling is then activated, and the light output – as a quantitative reporter of NF-kappaB signalling - is measured. Using this system we were able to identify three discrete patient cohorts with distinct patterns of signalling through NF-kappaB. The majority of IBD patients appear to have strongly impaired signalling through NF-kappaB, normal control groups show a robust response and a subset of IBD patients shows a strongly enhanced response. Using this system we can now subgroup IBD patients into two groups, hypo-responsive and hyper-responsive.
Using these differences as a starting point, we attempted to find candidate biomarkers that could be analysed using high-throughput technologies. Focussing on NF-kappaB activation, we have identified one cytokine in the serum of patients that shows a strong correlation to the clusters identified using the cell culture-based screen. These measurements have been used on samples from the Maastricht Patient cohorts and will in future be used to relate the two clusters to clinical and lifestyle parameters.
Our present understanding of NF-kappaB signalling in IBD patient cohorts leads us to propose that what we call the cluster 3 patients may be more responsive to treatments that suppress NF-kappaB activation and that this knowledge is allowing us to stratify patient cohorts and personalise treatment programmes.

In WP04 we:
• Prepared a list of potential NF-kappaB pathway related biomarkers based on extensive pathway constructrions
• Developed a simple test to screen for microRNA linked to the NF-kappaB pathway activity
• Developed a dynamic NF-kappaB biomarker based on cell culture systems
• Translated the dynamic biomarker to cytokine measurements in the serum
• And were able to group IBD patients into two clusters, cluster 1 and 3, which can be used to stratify patient cohorts and personalise different treatment regime.

In the future, our findings will have to be verified in much larger and independent patient samples. Historic treatment outcomes should be correlated with the patient groups – clusters - that we have now identified in order to confirm the value of different treatment outcome in long term treatment. New clinical trials should include as one parameter the cluster definition we have now developed in SysmedIBD to potentially narrow down success of treatment to specific patient cohorts. It will be important to see if similar cluster definitions can be made in patient groups of other inflammatory diseases.

WP05 – Identifying small molecules to interfere with NF-kappa-B oscillation dynamics

The goals of this workpackage were: (1) to identify potential targets from a predictive regulatory NF-kappa-B network, constructed from literature and new experimental data as well as from upstream analysis of these data; (2) to use the obtained targets to identify small molecules potentially interfering with NF-kappa-B pathways; and (3) to do experimental validation of the effects of these chemical compounds in in-vitro and in-vivo systems.

In order to achieve these goals four partners of the consortium, 01 UNIMAN, 08 HUJI, 09 LIFEGLIMMER and 10 GENEXPLAIN combined their efforts and obtained the following major results.
1) First of all, the "upstream analysis" algorithm for analysis of gene expression data and for prediction of protein targets was developed. This methodology includes search for transcription factor (TF) binding sites in the promoters of differentially expressed genes followed by network analysis upstream of the revealed TFs. The upstream analysis

algorithm was included into the geneXplain platform that allows to apply this algorithm to all available gene expression data generated by the consortium and to the data obtained from the literature.
2) The causative data analysis was performed towards identification of protein targets within the NF-kappaB network based on the experimental data produced by the consortium partners. For this analysis, the geneXplain platform has been used, and the results are stored there and are made available to all partners. The following data sets were analysed:
- Analysis of mouse macrophages data was performed using combined data of the TRANSPATH® database (TNF-alpha and signalling LPS pathways) and protein-protein interactions obtained using the biomedical text mining tool PESCADOR. A shortest path was constructed in this network between the targeted protein IL-1beta and the two stimulators (lipid A, TNFa) that provide good candidates as IBD targets, since the mechanism connecting macrophage stimulation with overexpression of IL-1beta is considered as one of the important cornerstone of development of inflammation in IBD.
- A pathway analysis of early genes across various cell types was done using clustering by shortest path method of geneXplain platform. We found that in the mouse gut organoids at early timepoint gene network cluster BCL-3 gene is up-regulated in the control but not in monocytes from patients with Crohn's disease, whilst relA (a subunit of NfkB) is up-regulated in Chron's but down-regulated in control monocytes (in response to LPS at 45 minutes).
- A detailed pathway analysis of mouse gut organoids was performed applying the "upstream analysis" methodology in 4 time points. Composite modules (identified by CMA algorithm) in the promoters of DEG genes in each time point were identified. Potential master-regulators were revealed such as IL-17A, iNOS and IP-10 that are able to maintain their activation by new positive feedback loops.
- Enrichment analysis of differentially expressed genes in mouse xenograft data was performed through the gene modules experimentally associated with immune processes. Results of this analysis was compared between human and mouse gene expression in gut xenografts. Although the response between mouse and human is highly correlated there were some categories that were more enriched in human, for example antiviral interferon signature, viral sensing and immunity and type 1 interferon response.
- A detailed gene ontology and pathway analysis of mouse xenograft data was performed. Using upstream analysis approach we identified composite modules that regulate expression of genes up-regulated in the xenografts after stimulation by high doses of LPS (as a model of IBD). We found NF-kappaB sites clustered together with sites for such TFs as STAT1 and E2F. We identified clusters of TF binding sites in the promoter of TNFAIP3 gene that encode A20 protein – an important element of NF-kappaB pathway, that is highly upregulated upon LPS treatment. We also identified master regulators such as IL-22, IL-8 and TNF-alpha.
3) As the result of the data analysis a comprehensive set of potential drug targets was constructed. This list includes known targets as well as new targets, like IL32 proposed in this project for further analysis. We combined this list with the list of known IBD targets obtained from HumanPSD (Protein Survey Database) database. As a result a list of 159 potential IBD targets related to the NF-kappaB network was constructed ranked according to the number of evidences obtained from the literature and on the basis of data analysis performed in our project.
4) On the next step, chemoinformatics tools were prepared for the identification of small molecules potentially interfering with NF-kappa-B pathway. PASS (Prediction of Activity Spectra of Sunstances) tool was prepared for the analysis. During the project the training set for PASS of ligands for the selected targets related to IBD was extended significantly. The number of compounds used for the training of SAR models of activity "IBD treatment" increased from 1272 to 2982, which is increase of more then 100% .
5) The PASS tool was used for the computer search in several libraries of small molecules for the compounds potentially interfering with NF-kappa-B pathway dynamics in the context of IBD. The search leads to the following results:
- The list of 24 IBD candidate novel compounds was generated by virtual screening of the iResearch library of about 36 millions of chemical structures.
- The list of 17 promising compounds was identified by screening of the library of human metabolites and 37 promising compounds in the library of natural compounds. Two compounds were identified in the library Top200 drugs, among them one of the most interesting was "Diclofenac".
- The screening of the library of 2840 macrolides leads to identification of 24 promising compounds, among them the most interesting were known antibiotics Azithromycin and Clarithromycin.
6) In-vitro experimental testing of the candidate chemical compounds using TNF reporter luciferase assay was performed. As a result a sensitive cell culture test was created and experimental validation of the compounds identified in the project as potentially impacting NF-kappaB dynamic was performed. Several compounds were validated. Among them there are Diclofenac, the antibiotics Azithromycin and Clarithromycin from the macrolide family and several compounds provided by Merck company. These compounds are very interesting for further in-vivo

validation as potential novel drugs to treat IBD.
9) In-vivo experimental testing of the candidate compound was performed, namely the macrolide antibiotic Clarithromycin (CLA). In the first experiment we demonstrated that LPS stimulated NF-kappaB DNA binding is significantly suppressed in mice treated with the CLA (p=.002). In the second experiment of mice with chemically induced IBD we also demonstrated that IBD mice treated with clarithromycin lost significant less weight (p=.04), and had less histological evidence of colitis than vehicle treated mice (p=.004). These experiments demonstrated the potential use of CLA as an active agent for treatment of experimental IBD.
The results obtained in this workpackage during the SysMedIBD project are very promising. The computational prediction and experimental validation of Clarithromycin as a potential anti-IBD agent opens a very good perspective for further clinical trials. Several other interesting small molecules found in this project may attract attention for their further experimental validation. The discovered NF-kappaB related signal transduction and gene regulatory network involved in the IBD model systems analysed in this WP lay a basis for further investigation of molecular mechanisms of IBD.

WP06 – Comparison between mouse and human

Extended disease-specific pathway with text-mining
09LIFEGLIMMER has implemented a text-mining pipeline to extract interactions related to IBD and NF-kappaB specific for Homo sapiens and Mus musculus. 124 protein-protein interactions have been extracted for Homo sapiens and 105 interactions for Mus musculus. To overcome the limitation of indirect interactions 09LIFEGLIMMER has used the most updated protein-protein interaction database TRANSPATH® to populate signalling pathways. 09LIFEGLIMMER built species-specific signalling pathways using the TRANSPATH® database and the GeneXplain platform. First, the core pathway was built by merging the TNF-alpha, LPS and IL-1ß pathways. The core pathway was then updated with protein-protein interactions retrieved by text-mining, proteins extracted from GeneCards and Ingenuity (input from 01UNIMAN), and co-operating transcription factors (input from 10GENEXPLAIN). The IBD- and species-specific pathways populated are up-to-date due to our combined approach of text-mining and the TRANSPATH® database.
Pipeline for transcriptomics analysis and visualisation
09LIFEGLIMMER has implemented a modelling framework for comparing NF-kappaB signalling in mouse and human. It has been used for all the transcriptomics data produced within the SysmedIBD project. The R-package DESeq2 was selected to perform the differential expression analysis comparing 4 others tools for his robustness at excluding data from genes with low expression levels or significant dispersion. Several visualisations were created specifically for time-series RNA-seq data, such as heatmaps. The time-series datasets of gene expression measurements of cells had a multi-factorial experimental design. A list of orthologous genes between the two genomes was obtained through the Ensembl biomart database. A comparison between species was done by comparing the orthologous regulated genes and the respective enriched gene ontologies in similar condition between both. Genome normalisation issues hindered a direct comparison with differential gene expression. Gene set enrichment analysis with Gene Ontology of mouse gut compared with human xenograft showed that whilst most immune and inflammation pathways are similarly enriched in both species, there are interesting differences between the species in necroptosis, anti-viral response, autophagy and apoptotic process.
Framework for automatic retrieval and comparison of SysmedIBD transcriptomics data
09LIFEGLIMMER set out to merge findings from human and mouse collected during the SysmedIBD project into a framework from which one can compare and compute data for both species. It contains four main layers: user interface, database, translational analysis and visualization layers. All project data and metadata are available to export or to perform differential expression analysis to make comparisons across cell types, diseases (Crohn's disease and Ulcerative Colitis), treatments and timepoints. The framework can be extended for store and perform analysis on any kind of RNA-seq samples as it was implemented in a flexible database model, the Resource Description Framework model. The results can be visualized on the IBD- and species-specific pathways built with text-mining approach and a combination of databases.
Digitalization, de-indentifion and text-mining of health medical record
Documents were retrieved from 3 hospitals within the population-based IBD-SL cohort which is lead by 07MUMC. Documents were available in different format and were converted to text (conversion rate higher than 99%). Protected health information was removed by the software in the clinics using pattern matching rules. In an iterative process with 07MUMC, appropriate patterns were defined and implemented in a shell script for each hospital separately. All the documents checked that were processed by the pipeline have been correctly de-identified, they represent between 88% to 95% of all documents depending of the hospitals.
09LIFEGLIMMER implemented specific algorithms for Dutch medical language to pre-process and analyse the

documents. They comprised correction of typographical errors using available and newly-created dictionaries and the treatment of negation and mitigation in complex sentences. A Proof-of-Principle analysis was initiated by 07MUMC and done by 09LIFEGLIMMER in order to validate the predictive power of the text-mining pipeline. The recall and precision were already both 75% with still some improvements to be made on the pipeline. The output data from the pipeline was subsequently used to predict new patients cluster based on their disease course.

WP07 – Feeding patient's findings back to basic research and vice versa

Patient stratification

Analyses involving human subjects (i.e. data, bio sample collection and analyses) have been conducted according to local and national guidelines and in accordance with the EU directives. Subjects provided written informed consent prior to participation and subject confidentiality and data security was guaranteed throughout the project. Only coded samples and data were exchanged for analyses between partners. Health records of the IBDSL cohort were 'de-identified'' by partner 07MUMC by running scripts provided by 09LIFEGLIMMER and only thereafter information was exchanged for text mining (WP4).

The Inflammatory Bowel Disease South Limburg (IBDSL) cohort is a population based, prospective inception cohort, representing over 93% of the adult IBD population in South Limburg. Since 1991, all new cases have been enrolled with complete prospective follow-up (incl. phenotype, extra-intestinal manifestations, complications, treatments, surgery, pathology-, endoscopy- and radiology reports) and scaled op with a cross-sectional biobank in 2011. IBDSL comprises 2837 patients (1162 CD and 1675 UC) with at least 5-year follow-up.

• Together with the clinical partners and in line with international guidelines, relevant variables for patient phenotyping and stratification were defined (Del. 7.1).

• Data on e.g. diagnostic reports, medication use and cancers developed after IBD diagnosis were completed within SysmedIBD and detailed analyses were performed on the IBDSL cohort, showing amongst others:

- population-based cohorts to differ from hospital-based cohorts with less phenotypes associated with more severe disease course (i.e. perianal involvement in CD and extensive colitis in UC).
- an increasing incidence of CD and UC over time and a change in phenotype presentation, including a significant rise in elderly onset IBD.
- time trends in medication use and disease course (e.g. increased use of immunomodulators and biologicals in UC and CD; decrease of early but not late colectomy and hospitalisation rates in UC; decrease in hospitalisation and surgery rates in CD but no change in progression to complication disease; a decrease in cumulative days of corticosteroid use in CD).
- increased risk of colorectal cancer in CD with colon involvement, but not in the total CD or UC population; an increased risk of haematological, skin and overall cancer in CD, mainly attributed to thiopurine use.

At present accurate predictors for IBD disease course are not available to aid clinical decision making and information on IBD disease course is merely based on survey data. Real life data of IBDSL were used to define disease activity per 3-month period per CD patient and subsequently 6 groups were identified with varying disease course (see also Del 7.9, collaboration with 09LIFEGLIMMER). Interestingly, 29.6% of CD patients had a quiescent disease course (10 years after diagnosis), which was confirmed by PLS regression analyses (AUROC 0.98 for quiescent versus most severe disease course and AUROC 0.95 (both $p<0.001$) for quiescent disease versus all other patient groups). Combination of the Montreal classification with baseline and clinical data in the first 6 months after diagnosis resulted in an AUROC 0.77, indicating that additional markers are needed to improve the prediction of relevant patient groups to enable patient stratification for targeted therapeutic strategies. Therefore, genotype data have been generated of 992 patients of the IBDSL cohort. In addition, all health records of IBDSL (endoscopy, radiology and pathology reports; >16.000) were digitalised and anonymised. The procedure for text mining has been established by 09LIFEGLIMMER and currently a proof of principle study is being performed to show the feasibility of using data of test mining to identify patient and/or disease characteristics. (see WP4).

Future perspectives:

- adding information from text mining, genetic analyses and markers of NF-KAPPAB activation to improve prediction of relevant patient strata for CD
- to contact subgroups of patients with quiescent versus severe disease course for extra bio sample collection for analyses with regard to NF-KAPPAB dynamics
- to identify patient groups with varying disease course and predictors thereof for UC
- to validate the identified patient disease groups in other population based cohorts in Europe (in collaboration with the Epidemiological Committee (EpiCom) of the European Crohn and Colitis Organisatio)
- to identify responders and non-responders to anti-TNF in the real life IBDSL cohort and to compare clinical and genotype data between groups

- To add information on detailed an automated histopathology analyses of diagnostic biopsies and to study their association with differential disease course groups in both UC and CD.
- To use the IBDSL biobank for further validation of relevant markers

The identification of patient groups with varying disease course (especially the substantial group of those with quiescent disease that should not be over-treated (with drugs with a high risk of side effects), may change future IBD practice. Acceptance of the abstract as oral presentation at the European Crohn and Colitis congress (ECCO, Feb 2018), demonstrates the interest in the field and enables new collaborations and grant applications.

• Biomaterial and clinical data collection for task 7.4-7.6 has been completed by (05UNIAACHEN, 06UNILIV and 07MUMC).

• Paraffin sections from all the 23 CRC cases identified within IBDSL were sent to 02CAU for further analyses as, further described in WP2.

Analysing of NF-kappa-B dynamics in blood derived immune cells and tissue biopsies

To improve stratification and optimize treatment strategies for individuals with inflammatory bowel diseases (IBD) new precision medicine approaches based on innovative biomarkers are needed. Most current biomarkers are collected at fixed time points, while biological processes are intrinsically dynamic.

In a first approach single time point measurements were performed by 02CAU in mucosal biopsies from Crohn's disease and patients with UC, disease controls and healthy individuals (n=63). The samples were subjected to microbiome, transcriptome and splicing analysis, employing next generation sequencing. The three data levels were integrated by different bioinformatic approaches, including systems biology-inspired network and pathway analysis. Both factors point towards a substantial disease-related alteration of metabolic processes. We also observed a strong enrichment of splicing events in inflamed tissues, accompanied by an alteration of the mucosa-attached bacterial taxa. Finally, we noted a striking uncoupling of the three molecular entities when moving from healthy individuals via disease controls to patients with IBD. Using global scaling methods, most of the variation in expression level was explained by tissue type, followed by inflammation status. In contrast, clinical diagnosis did not discriminate well between expression profiles. However, transcription factor analysis inferred signal transducer and activator of transcription 1 (STAT1) and nuclear factor-κB (NF-kappaB) as major inflammation-associated sites, regardless of diagnosis. These results show that NF-kappaB is one of the most important signalling pathways that differentiate inflamed and non-inflamed sites indicating that NF-kappaB is a major signalling pathway driving intestinal inflammation in IBD.

In a next step, two distinct and complementary methods developed by 01UNIMAN and 05UNIAACHEN have been used to screen NF-kappa-B dynamics in blood-derived immune cells from more than 100 controls and IBD patients. The screening was performed on frozen PBMCs collected by clinical partners 05UNIAACHEN, 06UNILIV and 07MUMC. NF-kappaB activation (phosphorylation of p65 and degradation of IκBα) and transcriptional activity of NF-kappaB were assessed by flow cytometry in PBMC-derived immune cells stimulated by TNF, LPS and MDP by 05UNIAACHEN. Independently, PBMC-derived macrophages were transduced with a lentiviral construct encoding an NF-kappaB luciferase reporter by 01UNIMAN. Luciferase activity in response to lipid A, the biological active part of LPS, was quantified. NF-kappaB dynamic responses were analysed using the area under the curve (AUC), the peak intensity and time of the response. On average, IBD patients displayed lower NF-kappaB activation as compared to controls in T cells and monocyte-derived macrophages. However, IBD patients exhibited a very heterogeneous response pattern with high a percentage of patients with either hyperactive or suppressed NF-kappaB activities. The intensity of NF-kappa-B activity in isolated immune cells upon stimulation correlated with the in vitro production of pro-inflammatory cytokines as well as serum levels of NF-kappa-B-dependent cytokine in the respective patients. These findings suggest that the dynamic in vitro measurement correlates well with the immune cell functions in vivo. Our results show that the NF-kappaB dynamic response in immune cells is highly variable in different IBD patients and suggest that NF-kappaB dynamic response may provide a new method for stratification of IBD patients.

To further understand the mechanisms and consequences of these different NF-kappaB dynamic responses 05UNIAACHEN collected peripheral blood monocytes from healthy controls (n = 12) and Crohn's disease patients (n = 8) and stimulated them the same day by the NF-kappa-B agonists TNF, LPS and MDP during 45 min, 120 min and 360 min. RNA-seq has been performed by 02CAU and analyses have been performed by 04WARWICK, 10GENEXPLAIN, 09LIFEGLIMMER and 05UNIAACHEN. The majority of regulated genes show either a transient up- or downregulation at 45 or 120 min or belong to a pattern with exclusively late (360mn) regulation. Our analyses revealed further that expression profiles of monocytes from controls and Crohn's disease patients are different in the unstimulated condition and in all stimulated conditions. We hypothesize that the late response gene may show the most important variations between different individuals and between IBD patients and healthy controls. Final analyses of this part of the project are still ongoing and are suggested to be finished within a couple of month. Furthermore, a larger cohort of patients pre- and post-treatment with steroids and anti-TNF, and a group of newly

diagnosed patients, has been recruited to measure NF-kappaB dynamics in PBMCs in combination with transcriptome and methylation profiling in order to screen for biomarkers predicting response to therapy.

WP08 – Genomics

The dynamics and interactions of several different nucleotide sequence layers represent key elements of the heterogeneity of inflammatory responses across tissues and between health and disease. In cells many different classes of RNA are produced encoded by the DNA, including mRNA, the template for proteins, ncRNA, RNA that does not code for proteins and miRNA, molecules that interfere with the control of coding RNA. The heterogeneity of the RNA level is the most important determinant of NF-kappaB dependent effector mechanisms (e.g. regulation of mRNA, ncRNA, miRNA), but signalling itself is at the same time affected by changes if the genomic sequence (modifying binding of the TF) and DNA methylation/chromatin accessibility. The project hence has been a central analytical hub for generation and first interpretation of the genomic, transcriptomic and epigenomic data of the consortium. It has orchestrated its approach in a collaborative manner with essentially all experimental and clinical work packages of the consortium.
The main goals have been a stable access to state-of-the-art molecular genetic and an adoption of standardized procedures for the different sample input types (cell line work, patients' mouse models) A main focus has been the in depth characterization of transcriptomal signatures of NF-kappaB activation in accordance to the community standards developed in large authoritative sequencing standard consortia (ICGC/IHEC). Approximately 550 RNA, 200 DNA methylation, 100 ATAC and 120 Genome/Exome data sets have been produced. The project was involved in the genomic and functional characterization of several proprietary mouse models which together described a new role for autophagy in fine-tuning NF-kappaB signalling in the context of chronic and acute intestinal inflammation and better defined the relevance of mouse models for studying human diseases.

Selected key findings comprise:
• Analysis of 63 mucosal biopsy specimen from different anatomical sites of the intestine (colon/ileum) from IBD patients, as well as healthy and hospitalized non-IBD controls (bacterial/viral infection). Using a number of tools, we have defined shared and unique patterns of inflammation between IBD subentities, inferred transcription factor activation from gene expression modules and described the interplay between the residing (altered) microbiota and transcriptomal programs. We have demonstrated clear gene sets and splicing events which are associated with IBD in general, but also with each of the respective subentities. A clear involvement of NF-kappaB and NF-kappaB cofactors (HSF1, NF-AT1) was found. Importantly, we could demonstrate a gradual loss of stable co-abundance between these components when moving from healthy individuals over disease controls to inflammatory bowel disease patients. Taken together, the result highlights the close interaction between the microbiome, the host transcriptome and emphasize the importance of NF-kappaB-dependent expression modules for intestinal inflammation (Häsler et al., Gut 2016).
• Defining a new role for autophagy in the context of NF-kappaB dependent inflammation. A coding variant in the human ATG16L1 gene has been associated with an increased risk for developing Crohn´s disease. During the course of the project we have analyzed a conditional mouse model which lacks Atg16l1 expression in different tissues (gut, monocyte/myeloid cells). All models suffer from spontaneous auto-inflammatory reactions related to unrestrained NF-kappaB signaling. Myeloid deficiency of Atg16l1 leads to systemic inflammation and prolonged responses to stimulation with LPS. The activation is tightly linked to Ser311 phosphorylation via atypical PKCs, which are pathologically stabilized by non-functioning autophagy. The results provide new insights into the role of autophagy in controlling inflammation and suggest p62 as a potential therapeutic target modulating NF-kappaB within the context of inflammatory diseases. The revised manuscript will be resubmitted in January 2017. For the ATG16L1 villin-cre animals (deficient in epithelium), we had described a spontaneous Crohn´s like phenotype at the beginning of SysMEDIBD. In this work, we had outlined the importance of amplified TNF signals, however the exact mechanisms remained unexplained (Adolph et al., 2013). In the second half of the project we delineated the involvement of STING-mediated signaling in this context. We showed that ATG16L1 and a partner protein from the ER stress pathway XBP1 are essential to orchestrate beneficial IL-22 signaling in intestinal epithelium. Autophagy defects lead to IL-22 elicited type I IFN signaling modulation of mucosal NF-KAPPAB levels thereby causing epithelial TNFα production and subsequent necroptotic cell death. Measurable outcome: Adolph et al., Nature 2013 and 3 manuscripts submitted and currently in revision
• Several genomic data sets have been produced and led to interesting findings, which are submitted or currently prepared into publications. Contributions of the workpackage were made to stimulation experiments comparing NF-kappaB dynamics in mice vs. human macrophages, transcriptomal signatures of human xenografts in immunocompromiced mice, analysis of NF-kappaB deficient and transgenic mice and the analysis of NF-kappaB

pathway of colitis-associated carcinoma, one of the Co-morbidities of IBD.
• An important focus was on the delineation of transcriptomal alterations related to therapy response in IBD patients. Anti-TNF is one of the most widely used targeted therapies in IBD. Apart from its obvious biological effect of blocking soluble TNF, other mechanisms of action have been proposed, which include reverse signalling via membrane TNF. While effective in a fraction of patients, a considerable number suffers from primary and/or secondary non-response (up to 50% after 1 year of treatment). A number of other therapies (e.g. blocking of integrins by vedolizumab) have been recently approved during the course of the project. Main aim of the work was the delineation of transcriptomal patterns associated with biological therapy in IBD in order to elucidate potential mechanisms of action related to NF-kappaB signalling. A first set of analyses showed the unified mechanisms of response to anti-TNF and a related biological (vedolizumab, an antibody against a4b7 integrin) and find NF-kappaB related mechanisms and innate immunity as central pathways involved (Manuscript submitted). In a last large experiment, combining all resources developed in the consortium has described the differential RNA and DNA methylation signatures of different cell types (CD4, CD8, CD14) before and after anti-TNF therapy and compared these cellular programs to standard (untargeted) steroid therapy.
Future perspectives:
- To extract further genomics-based markers of NF-kappaB activation to improve prediction of individual disease courses
- To validate data obtained in mouse models in the human situation, e.g. non-functioning autophagy (allelic variants in ATG16L1) and the occurrence of a IFN/NF-kappaB hyperinflammatory signature
- To further refine cell-specific signatures from the human therapy experiments and use the insight to develop rationale targeting of immune cell subsets in pre-clinical models
- To develop single cell RNA sequencing into a clinical tool for prediction
- To use phenome-wide approaches (i.e. extraction of all clinical information) and relate the information to hypothesis-free molecular clusters of patients in order to extract meaningful marker sets for complications and co-morbidities
- To develop an understanding of chronic inflammation and molecular processes shared and unique between inflammatory diseases
NB: The SysmedIBD approach was crucial to develop a collaborative European network for systems medicine across 3 inflammatory disorders (SYSCID, funded under #733100 H2020), which aims at the development of individualized prediction of disease course and prediction of therapy response using large patient cohorts.

WP09 – Data storage, management, utilisation and sharing

The role of Workpackage 9 was to support SysmedIBD by developing and administering the data management platforms and by coordinating the data analysis tasks of the project developing new tools, pipelines and workflows as needed. In particular, it had three key tasks, namely to
• provide the environment and tools for data management. sharing and utilisation;
• facilitate the analysis of genomic and imaging data arising from the other WPs; and,
• facilitate the linking of models and data and the use of models for experimental design and hypothesis generation
In our application we proposed a simple structure for data management based on four core components, namely (1) a wiki as a flexible tool for the exchange of information within the consortium, (2) a database platform for storage, exchange, and dissemination of data and models, (3) a publicly accessible database for high throughput and other relevant data, and (4) the geneXplain platform, an online toolbox and workflow management system for a broad range of bioinformatics and systems biology applications to be integrated with the other components. It was found that the users preferred to use the ELGG wiki described below for (1) and (2) and ArrayExpress as the publicly accessible database (3) for our sequencing data. Extensive use was also made of the geneXplain platform.
A private social networking platform at Warwick, based on the Open Source Social Networking engine ELGG was used to facilitate group working between Centres. This provides a robust framework on which to build all kinds of social environments. It is widely used in industry. It provides a powerful data model making the creation of different entities simple, yet flexible, is highly configurable using plugins, powerfully handles user management and relationship requirements, and allows all objects to have an access control level applied. It is secure and significantly more user-friendly than a wiki and has a structure familiar to many users because of their use of other social media. The geneXplain platform was intensively used for the analysis of project-specific as well as relevant public data and has been applied to support analyses throughout the entire project as described in respective reports. Consortium members predominantly used it to apply tools and workflows of the platform for the analysis of transcription factors and genomic binding sites, molecular networks and pathways as well as disease biomarkers.
Genomic Data Analysis

09LifeGlimmer, 10geneXplain and Warwick were all involved in genomic data analysis. They interacted strongly, were highly collaborative and planned activity together. They were strongly integrated with the groups producing the data. The results are reported in the reports of the other workpackages. Analysis of transcriptome data used a broad array of tools and novel approaches as reported in Deliverable 9.3 with regular use of the GeneXplain platform for downstream analysis. An example, where novel analysis methods were needed, was the work on transcriptome data in the mouse gut and human xenograft because of the need to separate mouse and human RNA-seq reads. This has led to a potentially high impact paper which clarifies greatly the current debate about the value of the mouse as a model for human physiology and showing much more clearly than before the high degree of concord when the experiments are well-controlled and ask the right questions. In the final year results and papers in progress were also shared and discussed by 09LifeGlimmer, 04Warwick and 10geneXplain on Slack (https://slack.com/).

Imaging Data Analysis

An early analysis of data managements requirements identified the need for improve the CellTracker software for cell tracking and image segmentation as key because this was the bottleneck in the imaging pipeline. In particular, it was felt that improvement of the user interface would be particularly beneficial. A new repository, based on the software control system Subversion, facilitated collaborative code development, easier maintenance of specific releases, and secure backups. An extensive range of improvements were made and feedback from users was used to prioritise future development. In particular, new methods for single and multiple cell segmentation of nuclei and cytoplasm were introduced that were more user-friendly and increased both accuracy and speed and were applicable in both 2d and 3d. A huge amount of effort also went into improving the user interface and making it significantly more powerful. The updated cell profiler software is available from the University of Warwick web page (https://warwick.ac.uk/fac/sci/dcs/people/till_bretschneider/celltracker/).

Linking of models and data

A substantial range of both deterministic and stochastic models were built. For example, new deterministic models were constructed to, for example, reconcile previous models to low-dose data, study refractory states controlling NF-kappaB dynamics and understand temperature dependence of the NF-kappaB system. Current models of NF-kappaB have high-dimensional phase spaces and many parameters. Modelling and analysing them is therefore a challenge, particularly if one wants to both take account of stochasticity so as to model cell-to-cell variability and also to develop an analytical approach enabling quantification of various aspects of the system in a more controlled way than is possible by simulation alone. This creates real challenges in linking models to data. To overcome this we introduced a new approach, the pcLNA, that enables faster simulation and a more analytical approach (Minas & Rand PLoS Comp. Bio. (2017) 13(7):e1005676).

A broad range of tools were used for model analysis many of which were developed by us. PeTTSy is a MATLAB package developed at Warwick (Domijan et al. BMC Bioinformatics 2016 17:124 DOI: 10.1186/s12859-016-0972-2). As such it is well-documented and will run on any platform that MATLAB supports. PeTTSy does the most important calculations that underlie the perturbation theory of dynamical systems. It provides tools to enable this perturbation theory to be used for the analysis, adjustment, optimisation and design of models including complex models with large numbers of parameters and variables. It allows one to probe the model dynamics and to understand their behaviour under parameter changes. These changes can mimic perturbations to some rates, pulse experiments, or can even mimic the creation of specific mutations such as gene knock-outs or knock-downs. A new approach to model fitting and experimental optimisation using constraints was also developed (Domijan & Rand, J. R. Soc. Interface (2015) 12(104):20141303). The constraints are functions which characterise the properties that the model is required to satisfy, and the theory provides a detailed analysis of the region in parameter space that satisfy these constraints. The approach provides a method for experimental optimisation which is far simpler to apply than current approaches when dealing with large models of the sort considered here. Both PeTTSy and the constraints approach were developed for deterministic systems but extensions to stochastic systems have now been done. The classical approach to experimental optimisation uses the Fisher information matrix. However, methods have not been available to calculate this for the larger systems we are interested in. The pcLNA provides the first practical methods of doing this for large stochastic models. A MATLAB software toolbox called ReTrOS (Reconstructing Transcription Open Software) was used. This provided tools for several approaches for processing and analysing either gene or protein expression time series data sets, with an easy-to-use graphical interface for user interaction (Minas et al. BMC Bioinformatics (2017) 18:316). Other tools to facilitate modelling and linking data were developed including conversion tools which allow us to convert models from one format to another.

Future use and development.

Perspectives for the future are potentially substantial because the tools should be of very broad use across the biological and biomedical sciences. They mainly relate to future use and development of the tools in this workpackage to analyse genomic and imaging data and fit models to this data.

WP10 – Ethics

SysmedIBD followed established protocols for compliance with appropriate ethical and regulatory processes at institutional, national and European levels. Regulatory changes to these processes were monitored and reviews of existing ethics protocols within the project undertaken in response to relevant revisions to the regulations. All ethics and oversight procedures were tracked via Work Package 10 and developments reported in annual WP reports and periodic deliverables reports D10.1-D10.9.

Throughout SysmedIBD, because of the complexity of the disease aetiology, a multi-disciplinary 'system medicine' approach was used. The complexity of the systems medicine approach involves the following project specific ethical issues:

• Animal models for IBD including mouse human tissue xenografts, mice with humanised immune systems and transgenic animals used for monitoring inflammatory signalling dynamics in cells and tissues.

• Patient samples from IBD and control healthy patients were collected for sample analysis and longitudinal sample collection for tissues and lifestyle data recorded.

• Secure data archiving and protection of patient identity and personal information security was maintained throughout the project.

All relevant EU regulations and directives and appropriate local, national and international laws and guidelines were followed. Hence, the role of the ethics work package was to define the legal ethical framework that was in place throughout SysmedIBD and ensure that all monitoring and reporting was in place. This was managed by the project Ethics Advisory Board (EAB). The remit of the EAB was to: Oversees full compliance of SysmedIBD will all relevant Ethical regulations and guidelines. The EAB ensured that relevant ethical permissions were communicated to the Commission. The EAB met at regular intervals to ensure full compliance with Ethics regulation. Relevant members of the EAB provided expertise necessary to evaluate specialised experimental and clinical procedures and processes and deliver relevant advice on Ethical legislation and guidelines to SysmedIBD partners directly involved in the work.

In addition to the legal ethical principles that were in place across the project, guidance on ethical best practice was followed throughout the project. This included: 'The European code of conduct for research integrity' (www.allea.org March 2011) and the 'Singapore Statement on Research Integrity' (www.singaporestatement.org).

In addition to working with the existing legal ethical guidelines, the EAB was also responsible for monitoring relevant ethical developments as they might be applied to SysmedIBD:

Systems medicine research, 'Big data' and data regulation: The methods and approaches applied in systems medicine raise particular ethical issues that are connected to big data research and data governance. The project is data-intensive, involving the collection, use and collation of patient data from multiple sources. Appropriate data security measures were followed and no specific related issues arose during the project, but a note on the broader ethical concerns around the use of big data in biomedical and health research is warranted. While the current technological landscape enables the aggregation and analysis of large quantities of varied medical and health data with the potential for new approaches to biomedical research and treatment, personal health information is widely regarded as private and sensitive. These include issues around ownership, access and use of these data. However, the usefulness of much medical and health data depends on or is increased by the ability to connect it with other relevant information such as demographic or genomic factors, which then increase the possibility of re-identification.

In April 2016, the EU parliament approved the General Data Protection Regulation (GDPR), which will come into force in May 2018, and has the aim of strengthening digital privacy rights and harmonising data privacy laws across Europe. While the GDPR has no direct impact on the project, becoming active in 2018, the policy will carry implications for related and similar research projects in the future. These include requirements for data minimisation and restrictions on the transfer of data and samples outside the EU, as well as tightened consent requirements for active and explicit consent (although broad consent for scientific research will still be allowed). The GDPR exemplifies the importance of big data in relation to future directions and the focus around biomedical research ethics and regulatory policy.

In the bioethical as well as biomedical literature, it has been argued that systems medicine has the potential to transform medicine and healthcare, because the wide adoption of systems approaches will enable a shift in focus towards prevention, prediction, and the optimisation of health and wellness rather than treatment and cure of disease. One ethical concern is the resuting responsibilisation of health, where individuals take greater responsibility over their own health by keeping track of their health data and modifying lifestyle in response to personalised and preventative medical information.

Use of animals and research involving human participants: Ethical issues and considerations around the use of animals in research as well as around research involving human participants are well-established and there exist extensive national and international guidance and regulations around both areas. Discussion and debate around research involving human participants is focused on informed consent, the meaning and scope of the concepts of

'informed' and 'consent', and the related concepts of privacy and autonomy. Data-intensive or 'big data' research, the use of biomaterials removed from the body, and the use of digital technologies have extended the relevant ethical debate in relation to data ownership, appropriate use, protection, security, and sharing as noted above.
The creation and use of human-animal chimeric organisms in research is an ethically contentious area. The project involved the use of chimeric mice with human xenografted tissue. While work within the SysmedIBD project was governed by appropriate ethics procedures and many related ethical issues are not of immediate concern in relation to this project, the bioethical discussions and policy developments in this area are worthy of note. Issues discussed and debated in the bioethical literature around chimera research have focused on concerns around human exceptionalism, the humanisation of chimeric organisms, the moral status of such organisms, and animal welfare as well as on the breakdown of the human-animal binary and inter- or cross-species reproduction. These have been contrasted against the empirical realities of scientific practice focused on the development of animal models with human tissue for research on human development, disease, and organ transplantation.
Future perspectives: Fully engaging with ethical issues in biomedical research implies a commitment to extending bioethical as well as scientific understanding. To further develop understanding and knowledge of the ethical dimensions of systems medicine as well as to produce an ethical framework for future systems medicine research, we have built on the foundations for inquiry established by Work Package 10 to develop independent research on the ethical and social dimensions of systems medicine.
In collaboration with SysmedIBD Partners, the project external ethics advisor obtained funding on the theme of "Patienthood and Participation in the Digital Era" (WT 201652/Z/16/Z), which includes a pilot case study examining the ethical challenges of combining data-intensive research and patient care by taking the SysmedIBD project as the focus. This ongoing study explores new ethical challenges that are raised by the use of big data in medical research, the reconceptualization of health and disease enabled by systems approaches, and the changing role of patients and research participants as active and engaged in relation to systems medicine. Through this work, we aim to produce an ethical framework for systems medicine research that can be applied to projects beyond SysmedIBD. We will also contribute to the literature around systems medicine, big data, patienthood and research participation through publication and we will prepare a policy briefing to be disseminated to policymakers to achieve wider impact from this work.

1.4 The potential impact

Socio-economic impact and the wider societal implications of the project

Contribution to Community and social objectives

SysmedIBD has contributed to the general community firstly by public engagement events, specifically by the partner 07MUMC who is regularly updating Dutch patient organisations about the current research in the field of IBD and progress towards better diagnosis, patient stratification and treatment. On the German side, Aachen has interacted constantly with patients sufferers of IBD informing about efforts done in the project for better stratification and personalized medicine in order to respond better and faster to the patient needs. In UK, University of Liverpool has participated to festivals, NHS meetings and other events keeping the general public up to date with the intensive efforts for mildewing the symptoms in IBD and developing new drug and new strategies to cure the disease.

For the general public and also for clinicians, SysmedIBD group has published regularly videos explaining inflammatory bowel disease and what the group hopes, systems medicine will bring to its treatment in the future. One further activity, still ongoing after the SysmedIBD project has closed, is to look at the ethical frameworks of systems medicine approaches in general, using SysmedIBD as an example. This work is being performed by the University of Edinburgh.

Based on the experience within SysmedIBD consortium, with examples of three European health systems, it appears difficult to provide one general suggestion for a systems medicine framework suitable for all EU countries. Therefore, one of the tasks to make systems medicine approaches a success in Europe, is to define a general health framework that focuses on a personalised and systems medicine approaches in a way that it not only treats but also prevents in particular, chronic and difficult to treat lifelong diseases in a personalised way.

Main dissemination activities and exploitation of results

The SysmedIBD project lies in basic and biomedical sciences, with important implications for applied research and

clinical practices. The work done in SysmedIBD is of interest not only to cell biologists and physiologists, but also to clinicians and mathematical biologists and other theoreticians with an interest in biological systems.
The Dissemination work package in cooperation with the Project Management Office and under leading from UNIMAN and ARTTIC coordinated and worked towards comprehensive internal and external dissemination of project results and knowledge.
Internal dissemination had the aim at sharing information; building team spirit; strengthening complementarities of the partners and developing mutual specialization; supporting management; and offering training.
External dissemination was involving targeting the scientific community, but also the general public, patient groups, scientific societies and professional boards, students.

Over the five years, the SysmedIBD Consortium members participated to major international congresses showing results generated in the project to the scientific community, Industry, Civil society and Policy makers and establishing new connections and collaborations with scientists and medical doctors in the field of IBD and Systems Medicine.

This research work has generated 38 publications in high ranked journals, the most important ones mentioned below: In 5 years SysmedIBD project, the Consortium has published 38 articles in high ranked journals and has on the pipeline several manuscripts which most probably will be online after end of November 2017. Below you can see the Publication list which is also on SESAM and on project website https://www.sysmedibd.eu/publications/.

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In 2016 and 2017 training activities for young scientist took place in Venice and Kiel respectively.
The first SysmedIBD Spring School took place in March 2016, at the premises of University of Warwick, at Palazzo Pesaro Papafava in Venice, Italy. This event was open to the public and we had both younger members of SysmedIBD and non SysmedIBD participants at the event.
The Spring School in Kiel in March 2017 had about 15 young trainees, scientists and medical doctors, who were interested in Systems Medicine and Inflammatory Bowel Disease. They were mostly impressed by the approaches in systems medicine, expertise from group leaders involved in SysmedIBD, Animal Models + clinical aspects of IBD, NF-kappaB modelling (in silico) animal models, RNA sequencing and geneXplain platform and practical exercise. More details and impressions can be seen here: https://www.sysmedibd.eu/press/
The SysmedIBD Consortium has organized over time several Symposia:

- SysmedIBD Satellite Symposium ´Get to know SysmedIBD´ at European Congress of Immunology (ECI) in Vienna on 6 September 2015
- SysmedIBD Satellite Symposium at the first European congress on Systems Medicine, 26th October 2016, Berlin, Germany
- SysmedIBD Outreach Community Meeting, 24th November 2017, Brussels

presenting the research data to the large scientific community and stakeholders.
SysmedIBD scientists participated over the 5 years to different major international Congresses like European IBD congres of European Corhns and Colitis Organisation (ECCO), Digestive Disease Week, International congress of mucosal immunology (ICMI) talking about the major achievements in the research focused on IBD, NF-kappaB pathway, personalized medicine, biomarkers and gene platforms.
From the beginning, the SysmedIBD project was announced to the scientific community in the field of Systems Medicine by different articles describing briefly the project and the Consortium but also by interviews from Prof. Werner Müller on systemsmedicine.net.

Interaction with relevant commercial and industrial stakeholders is crucial to the success of biomedical endeavour in the present research climate.
From a commercial perspective, a major ethos of the FP7 programme is to stimulate economic development through engaging SMEs and it is important that the 3 SMEs partners within SysmedIBD are able to develop tools, resources and associated intellectual property that will drive their continued success.
For this purpose, SysmedIBD has used a variety of tools like information material in the form of white paper, project Flyer, Project portofolio, project presentations, stakeholder meetings, and meetings focused on commercial perspectives.
First meeting with stakeholder organised by 01UNIMAN and 12GABO:mi took place at the SysmedIBD Satellite Symposium ´Get to know SysmedIBD´ at ECI in Vienna on 6 September 2015 (http://www.eci-vienna2015.org/index.php/scientific-satellite-meetings) and was a real success. Partners 01UNIMAN, 02CAU, 09Lifeglimmer, and 10geneXplain have talked about SysmedIBD and their involvement and contribution to the project.
SME Partners 10geneXplain and 09LifeGlimmer participated at FitForHealth 2.0 partnering event organized by the EU in 2015, 29th Mammalian Genome Conference, Yokohama in 2015 and other events to meet with potential commercial collaboration partners.

SysmedIBD group was always in strong contact with IBD patient groups and representatives of patient's organisation like Crohn's & Colitis UK but also in Maastricht and Aachen. 07MUMC's division organises every year, in collaboration with the two other regional hospitals an information event for the IBD patients in the South of Limburg. 07MUMC represented by Marieke Pierik and Daisy Jonkers had recently a talk at the Annual Patient Day in

Zuyderland Medical Center Sittard, NL, 23 September 2017 (for about 200 patients) and acknowledged SysmedIBD. The German patients organization DCCV (Deutsche Morbus Crohn/Colitis ulcerosa Vereinigung) provides information and specific support to patients with inflammatory bowel diseases. At 05UNIAACHEN the recommendation is given to patients to get in contact with the DCCV. Together with the DCCV, 05UNIAACHEN organized for the first time in Aachen an IBD symposium for patients at November 29, 2014.
Katie Lloyd from 06UNILIV on 17th May 2017 promoted the research of the SysmedIBD project performed at the University of Liverpool by a talk at the pint of science festival in Liverpool with an audience about 60 people.
Mike Burkitt from 06UNILIV participated in the Royal Liverpool and Broadgreen University Hospitals NHS Trust Grand Round on October 6th, 2017. The audience was made up of clinical staff from the hospitals trust and included approximately 50 people.

The SysmedIBD homepage is up to date and contains public information about the project meetings, publications, video interviews. So, the public-accessible area includes information for specialists (academic and industrial researchers), as well as information for the press and for the lay public. The project web site https://www.sysmedibd.eu/ was created by 12GABOmi and later on maintained by 13ARTTIC under the direct supervision of the Coordinator or other designated scientists. The website was regularly updated with news from the project and Consortium, publications, events, video interviews https://www.youtube.com/watch?v=UfQAY05O4oI and animation video. The animation video https://www.youtube.com/watch?v=vfobRasGuJE is explaining to the general public the development and risk factors for inflammatory bowel disease but is also showing the efforts done by SysmedIBD researchers with the EU funding on understanding the disease mechanism and working towards personalized medicine future.

Outlook and future research

SysmedIBD has established a new stratification parameter for patients with inflammatory bowel disease, based on the NF-kappaB activation response in blood derived macrophages. Based on this stratification, one serum marker has been identified that follows this stratification parameter. In the future this serum marker should be developed to a new biomarker for the stratification of patients with inflammatory bowel disease.

During the screen of compounds that can modulate the NF-kappaB pathway we identified one drug that is used for the treatment of other diseases that could be beneficial for a subset of patients with inflammatory bowel disease. So far, we have positive evidence from in vitro culture systems and in one mouse model of inflammatory bowel disease. This should be followed up in further studies.

Based on the scientific literature and our data collected in SysmedIBD, we have developed a more comprehensive NF-kappaB pathway model that also includes downstream signalling pathways with the potential to modulate the NF-kappaB pathway. This model is available through interactions with the two SME GeneXplain and Lifeglimmer.

Finally, the humanised mouse model based on engraftment of human fetal gut provides a very good way to study inflammation in human tissue directly. This model also now provides for the first time an insight into the mechanism of fistula formation, one of the major co-morbidities in inflammatory bowel disease.

The SysmedIBD web page (www.sysmedibd.eu) will be available after the end of the project and will follow the output of the project and will link to projects that follow on the work that has been done in this project.