Final publishable summary report

Masterswitch: "Mechanisms to Attack Steering Effectors of Rheumatoid Syndromes with Innovated Therapy Choices"

1 Executive summary

Chronic inflammatory diseases include a wide range of conditions that cause suffering and debilitation affecting millions of people in the EU. These conditions include asthma, rheumatic diseases, inflammatory organ-diseases such as inflammatory disease of the gastro-intestinal track like inflammatory bowel disease, as well as inflammatory disease of the brain, endocrine system or skin such as psoriasis.

Scientists initiated the EU-funded project 'Mechanisms to attack steering effectors of rheumatoid syndromes with innovated therapy choices' (MASTERSWITCH) with two goals. The first is to identify the underlying mechanisms or biochemical switches that control induction, progression and resolution of inflammation. The second is, using the above generated information, to identify biological targets for therapies. RA, perhaps the best characterised chronic inflammatory disease, serves as a model disease as RA can be used as prototype disease for many other chronic inflammatory disorders as also evidenced by the first effective use of targeted therapies in RA, followed by their implementation in other disease areas.

From a clinical perspective, studies demonstrated that while early treatment minimised joint damage and enhanced quality of life, in most cases there appeared to be a considerable delay between onset of symptoms and medical assessment. Therefore, several MASTERSWITCH members have now initiated novel "Early Arthritis Recognition Clinics" to minimize delay of treatment thereby improving health care and quality of life by preventing loss of, often irreversible, damage through early and adequate treatment.

From a biological perspective, studies demonstrated that disease development was preceded by an autoantibody response strongly correlated with the subsequent presentation of arthritis. This auto-antibody response increased in magnitude and complexity shortly before the onset of first clinical symptoms. These findings therefore indicate that the immune system deranges before disease and that measurement of the deranged auto-immune response in people at risk for RA-development might provide a "window of opportunity" preventing disease through even earlier treatment initiation. Likewise, the contribution of several immune cells and the mechanisms by which these cells are activated have been investigated in depth revealing several novel and promising targets that could be used for the development of new drugs and treatment options.

Thus, the MASTERSWITCH consortium not only identified several novel, previously unrecognized and potentially drugable pathways and immune responses pivotal in the initiation and perpetuation of chronic inflammatory disease, but also already implemented some of the findings in the clinic through the initiation of "early recognition Clinics".

2 Summary description of project context and main objectives

The objective of the MASTERSWITCH project was to gain a better understanding of the biological and molecular pathways that initiate and drive chronic inflammatory disease to allow the development of novel anti-inflammatory reagents and innovations. Within the project, focus is given to Rheumatoid Arthritis (RA) as RA can be used as prototype disease for many other chronic inflammatory disorders as also evidenced by the first effective use of targeted therapies in RA, followed by their implementation in other disease areas.

Many chronic inflammatory diseases can not be cured, probably because the drivers to which the immune system reacted to cannot be eradicated. In case of diseases like inflammatory bowel disease, this could be the microbial architecture that is always present in the gut whereas in the case of RA, this could be the body's own molecules that are constantly generated. As a consequence, the immune system will keep on being activated resulting in chronic inflammation at the site where the drivers to which the immune system reacts are expressed. Unfortunately, current treatments do not cure disease and can lead to severe side-effects. Moreover, the continuous immune activation present at the affected sites in the body will lead to progressive tissue damage and loss of function. Therefore, novel strategies are needed to treat early in the disease course to prevent damage on organs and inflammatory sites and to optimize the chance on a permanent cure. To allow such strategies, detailed knowledge should be gathered on the principles and pathways that are underlying different phases (i.e. the induction, the progression and the (lack of) resolution) of inflammation. As normally inflammation ceases within weeks, it is likely that also the pathways responsible for persistency and resolution are in place early after the initiation of inflammation. Clearly, in case of chronic inflammatory diseases such as RA, inflammation is persistent.

The main objectives of the project included the identification and validation of the molecular networks that are involved in the processes to establish chronicity, the so-called MASTERSWITCHES (objective 1). Moreover, the better insight into the functional pathways and molecular networks will translate into clinical applications in the form of novel drugs or drug targets and protein therapeutics for chronic inflammation in general and RA in particular. Therefore, the second objective of the project was to identify and validate novel targets for therapeutic interventions.

Thus the overall strategy of the project was centered on the critical switch moments involved in the various processes that drive chronicity of inflammation. By and large these switch moments can be divided as follows:

1) Induction of inflammation
2) Progression of inflammation
3) (Lack of) resolution of inflammation

As it is likely that all three phases are involved in the installation of chronic inflammation, the project was structured in such a way that all three aspects are approached via different perspectives; A) a clinical; B) a molecular and C) a cellular perspective.

To endorse this strategy and to reach these goals of the project, a multidisciplinary consortium was formed that included young biotech companies, immunologists, molecular biologists, bioinformaticians, cell biologists and rheumatologists.

A full and comprehensive overview of the achievements obtained by the MASTERSWITCH consortium, such as an overview of all scientific publications, can be found on the MASTERSWITCH website. Some of the main achievements coming from the different perspectives are summarized below:

A. The clinical perspective

Obviously, the clinical perspective is of most direct relevance for patients suffering from chronic inflammatory disorders such as RA. A main challenge in clinical care of chronic diseases in general and in RA in particular relates to the differentiation among patients with (early) disease who will remit spontaneously or on therapy from those who will develop chronic, destructive disease. This is very important, also because remission can be achieved more easily in case patients are treated early after start of symptoms and sometimes can occur spontaneously. However, the factors that are predictive for remission/chronic disease are ill defined. Therefore, the clinical MASTERSWITCHES predicting early disease course, ranging from early spontaneous remission to development of chronic, progressive disease despite extensive treatment have been studies in depth. These efforts have resulted in important novel insight through the realization that symptom duration before visiting an rheumatologist was among the factors predicting outcome of disease. Importantly, those patients that visited the rheumatologist within 12 weeks of symptom onset had considerably less joint destruction and a higher chance on achieving sustained DMARD-free remission then patients with a longer delay in assessment. These findings indicate that the initiation of an early and adequate treatment is likely lowering the change of entering a chronic, destructive disease phase and thereby increases the quality of life for patients and lowers the costs for society by preventing disability, loss of labour participation and need for more expensive medical care later in life.

Because of this realization, several beneficiaries working in the MASTERSWITCH consortium have started "early arthritis recognition clinics" (e.g. Leiden, Leeds and Vienna) where patients are seen by a trained rheumatologist and, when required, treated early with adequate anti-rheumatic drugs. The early access to the "Early arthritis recognition clinics" is, for example, optimized through notifying General Practitioners that they can send patients for a short consultation on the existence of arthritis. The first evaluation of the initiation of the early arthritis recognition clinic revealed that approximately two times more patients are visiting a rheumatologist within 12 weeks after first onset of symptoms. In more over 40% of these patients, arthritis was detected and an adequate treatment plan could be offered. The longer term effects of the Early Arthritis Recognition clinics are currently being evaluated in subsequent studies that outrun the duration of the MASTERSWITCH project.

B. The molecular perspective

One of the molecules that have received detailed attention by many of the MASTERSWITCH investigators were the auto-antibodies that are associated with rheumatic disease because they are intimately implicated in disease pathogenesis. Especially, antibodies against proteins that have been modified by the body through post-translational modifications have been studies extensively. In this respect, the auto-antibody response against citrullinated proteins have been studied in depth. These studies have revealed that such antibodies are

already present before disease onset, but also that their levels in blood and their "complexity" changes before disease precipitation as both antibody-levels and complexity of the antibody response increases substantially before onset of disease. These findings are important as they indicate that the auto-immune response precedes the clinical symptoms and that onset of clinical symptoms is associated with a "broadening" or maturation of the underlying auto-immune response. These observations are, for example, meaningful in explaining our observation that initiation of adequate treatment early after symptom onset is linked to the prevention of subsequent disease progression.

In the context of the studies focussing on auto-antibodies against post-translationally modified antigens, the MASTERSWITCH-consortium has also discovered a novel auto-antibody response that is detectable in approximately half of RA-patients. These auto-antibodies recognize carbamylated proteins and their presence is associated with a more severe clinical progression in time. More importantly, they can also be present in part of RA-patients that were previously considered to be auto-antibody negative. Therefore, the detection of these novel auto-antibodies could be of significant diagnostic value as measuring their presence could aid in diagnosing patients with early inflammatory joint complaints that are tested negative by current auto-antibody testing. Approximately, 40% of early RA-patients are currently tested negative for auto-antibodies and can pose a diagnositic challenge for rheumatologists. For these reasons, a diagnostic test is currently under development, together with a diagnostic company, to allow routine clinical testing for this novel auto-antibody system.

C. The cellular perspective

Many studies have been performed addressing the immune- and synovial cells that participate in the induction, perpetuation and resolution of arthritis, both in animal studies for disease as well as in the test tube using cells from patients. Also these studies have revealed important novel insight into the mechanisms involved in the inductions and (lack of) resolution of inflammation.

RA pathogenesis is characterized by a hyperplastic synovial membrane caused by synovial cell proliferation and infiltration by inflammatory cells. Among the many different cell types present in the inflamed synovial tissue, synovial fibroblasts are one of the most dominant cell types that are under the influence of the inflammatory milieu created by the unwanted auto-immune response that is mediated by immune cells. For this reason, synovial fibroblasts have been actively studied to find out how they contribute to disease and disease chronicity. One of the intriguing new findings was that the synovial fibroblast express significant quantities of an enzyme, autotaxin, that can create a lysophosphatidic acid (LPA), a lipid-like molecule that can stimulate cell division and cell activation and that has been linked to cancer. We now have shown that expression of autotaxin by synovial fibroblasts was induced by pro-inflammatory cytokines known to be involved in the pathogenesis of RA (TNF). Likewise, it was shown that the LPA created by autotaxin enhanced the activation of synovial cells and several deleterious effector functions in concert with TNF. Using a mouse model, it was also shown that autotaxin expression by synovial fibroblasts was crucial to the inflammation observed in arthritis as ablation of autotaxin expression resulted in disease attenuation in animal models of arthritis. Because several inhibitors of autotaxin have been developed in the context of cancer, these findings not only establish the autotaxin-LPA axis as a novel player in chronic inflammation and the pathogenesis of arthritis, but also identified autotaxin as a promising therapeutic target.

Thus, together, the MASTERSWITCH consortium has made significant progress, both with respect to gain mechanistic insight into the processes underlying chronic inflammatory diseases in general and RA in particula, as well as with respect to the development of diagnostic tools or the identification of novel targets as well as with respect to the improvement of patient care. Such achievements would not have been possible without the support of the EU that allowed the formation of a broad consortium from top-european institutes that were specialized a variety of disciplines in clinical science, patient care, translational science, basic science and bio-informatics.

3 Description of the main S&T results/foregrounds

The objective of the MASTERSWITCH consortium was to delineate the biological and molecular pathways that initiate and drive chronic inflammatory disease to allow the development of novel anti-inflammatory reagents and interventions. Focus was given to Rheumatoid Arthritis (RA) because of:

- The huge clinical and socio-economic impact of this frequent disease.
- Good access to extravascular sites allow hypothesis testing at the "sites of action", the joint.
- Good animal models that are amendable to genetic testing and of which we have proven
  that they are instrumental for the development of novel therapies

- Inflammatory rheumatic diseases can be rapidly modified by therapeutic intervention
- RA serves as prototype disease for other inflammatory disorders as also evidenced by the fact that success of anti-TNF Therapy was first demonstrated in RA and was subsequently shown to be effective in other diseases as well.

The MASTERSWITCH program had two main goals:

1. The identification of the early processes and molecular networks involved in the establishment of chronic inflammatory disease such as RA
2. The identification and validation of novel targets for therapeutic interventions or diagnostic purposes

General strategy taken

The general strategy for the project was to enable parallel studies that are focussed on critical switch moments in the various processes that drive chronicity of inflammation.

By and large, these switch moments were divided into three processes:

- Induction of inflammation
- Progression of inflammation
- (Lack of) resolution of arthritis

As inflammation normally ceases within a few weeks, it is likely that all three phases, although distinct, are in place already early after being exposed to the inflammatory precipitating event.

This strategy was underpinned by clinical observation of the sequence of events leading to the development of RA. It has been shown that immune tolerance to certain antigens that are targeted by the immune system of RA-patients can be broken years before arthritis becomes manifest. Likewise, deregulated production of inflammatory markers has been observed years before patients present themselves with arthritis. Apparently, only after an, as yet unidentified event, the switch that leads to the development of arthritis is turned on. Most early arthritis patients do not present themselves with RA. Intriguingly, approcimatelyh 50% of these patients with 'Undifferentiated Arthritis" will progress to RA, whereas the other half will remit. Thus, also in this phase, events occur that leads to a switch from Undifferentiated Arthritis to full-blown RA, respectively, resolution of inflammation.
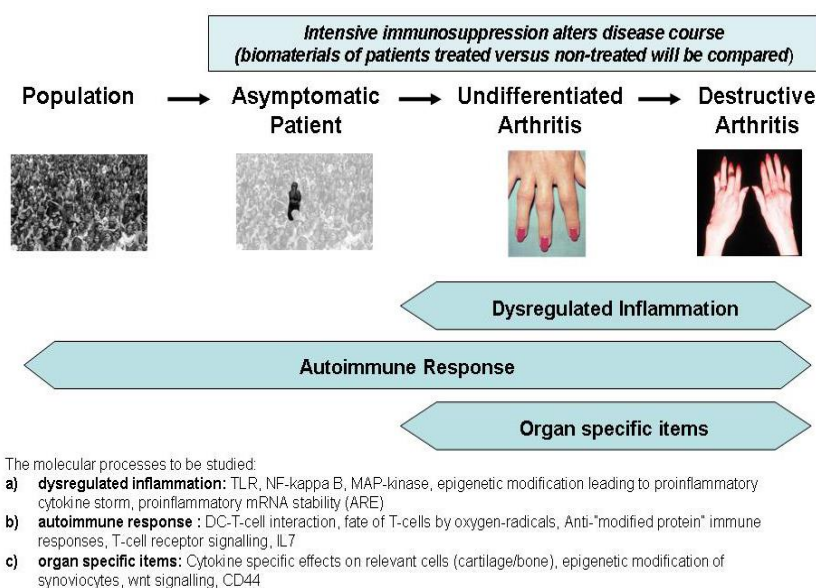

Fig. 1: Putative molecular networks tested in different phases of development of RA

The "MASTERSWITCH" program investigated a number of fundamental mechanisms (figure 1) involved in the emergence of chronic, persistent inflammation and linked these mechanisms, when possible, to clinical findings. Clinical studies indicated that after RA has been diagnosed, intensive therapies may still be effective in inducing long-term drug-free remission. Thus, even after breakdown of immunological tolerance and the emergence of chronic inflammation mechanisms can still be activated to terminate inflammatory responses.

The three phases, induction, progression and (lack of) resolution of inflammation, which are involved in the emergence of chronic inflammatory diseases, were studied in a multidisciplinary manner to identify molecular networks involved in establishment and persistence of chronic inflammatory responses. To this end, a unique consortium has been established in which the leading translational groups, as well as the leading immunology, cell biology and molecular biology groups, have joined forces to identify the underlying biological processes of the respective MASTERSWITCHES.

The different processes of inflammation were studied from three different perspectives throughout the duration of the MASTERSWITCH project:

- The clinical level
- The molecular level as well as
- At the level of cells and the entire organism

The distinct master-switch-moments determining outcome of arthritis persistency were studied (level 1) by employing available and newly established biobanks and clinical information in early arthritis patients that either remit, or progress, combined with clinical trials performed in patients with recent onset arthritis, treated with a specific immunosuppressant, such as steroids or anti-TNF. This provided clinically relevant input to the studies performed at "level 2", which addressed the relevant molecular networks pivotal to the three MASTERSWITCHES of chronic inflammation described above. The analyses of biobanks and clinical information obtained from (ex) patients were, vice versa, also dictated by the information obtained within the context of the studies performed at level 2 and 3 as also exemplified by the studies addressing the outcome of disease in relation to newly defined biomarkers.

In this way, we an optimal integration and cross-hybridization between the different levels, allowing productive collaborations between various disciplines such as physicians, immunologists, cell biologist & molecular biologists was obtained.

Starting point

The starting point of the study consisted of resources form several well-characterized clinical- and pre-clinical cohorts allowing us to have the largest sample size collected to date of RA- patients (>10.000) and (still) healthy individuals at risk to develop chronic arthritis. These cohorts had the advantage of already being collected. Furthermore many mutant mice strains for intracellular communication, conditional gene-expression and genetic permutation as well as rodent models for inflammatory diseases were already available at the start of the project. These assets, combined with world-leading expertise in in vitro and in vivo studies enabled us to unravel several molecular mechanisms that were underlying chronic inflammation. By amassing a range of expertises MASTERSWITCH created the critical mass required to surmount the clinical and scientific hurdles. All beneficiaries labs have shown numerous times the scientific prowess required to tackle the complex problem of unravelling the molecular basis of inflammation and chronic inflammatory disease and translating it into drug discovery and diagnostic development.

Main results obtained

Overall, the MASTERSWITCH program has resulted in scientific results that were communicated to the scientific community in over 400 publications. Obviously, it is not feasible to provide a complete overview of all results obtained in this summary, therefore, for a comprehensive overview of all results obtained, we refer to the publications that have resulted from the MASTERSWITCH program. Likewise, several findings made in the context of the MASTERSWITCH program has, most likely, resulted in societal valorisation through the initiation of "Early Arthritis Recognition Clinics" by several MASTERSWITCH beneficiaries. However, such societal valorisation still needs to be confirmed through ongoing studies that are still ongoing after the end of the MASTERSWITCH program.

Below, the main results and findings are summarized grouped according the three different perspectives taken by the MASTERSWITCH investigators.

A. The clinical level

a. From pre-disease to disease

One of the goals of the MASTERSWITCH program was to identify the early processes and molecular networks involved in the establishment of chronic inflammatory diseases such as RA. In order to gain insight into these early processes of human disease, it is crucial to have access to preferably pre-disease samples and (clinical) information from individuals at risk to develop arthritis. For this reason, the MASTERSWITCH consortium has put great effort in creating a respiratory of samples and information of people at risk to develop arthritis on the basis of the presence of auto-antibodies that are associated with arthritic disease. For example, beneficiary 6 (Vienna) in cooperation with the City of Vienna Department for Health and other centers engaged in prospective health examinations. These efforts have resulted in the collection of serum samples from more than 4000 healthy subjects which were tested for the presence of RF and ACPA. Subjects with autoantibodies as well as matched controls negative for autoantibodies were referred to the clinic for further workup. Among the 4061 subjects tested, 21 (0.5%) were positive for Anti-Citrullinated Protein Antibodies (ACPA) and 90 were positive for Rheumatoid Factor (2.2%), another auto-antibody associated with RA. However and in contrast to patients with RA, only 12 subjects had both ACPA and RF. Overall, a total of 99 patients (2.4% of the total population) were positive for at least one of the two autoantibodies but so far only one of these subjects developed signs and symptoms of RA fulfilling the 2010 EULAR/ACR classification criteria.

Thus, in summary, 2.4% of a healthy, working age population carry autoantibodies characteristic of RA. Among the positive individuals (and also autoantibody negative controls), one developed RA during a follow-up period of up to 2 years. In line with this finding, the proportion of individuals with elevated CRP (a marker for inflammation) was similar among autoantibody positive and negative individuals.

It is evident that healthy subjects with autoantibodies, and especially those with high levels, will have to be followed for longer term to learn if they might develop RA to fully appreciate the value of these findings. These studies will be conducted in the future as follow-up of the MASTERSWITCH program.

Likewise, beneficiary 8 (Stockholm) has utilized a subset of the Swedish twin registry, which includes 12,594 monozygotic (MZ) and dizygotic (DZ) twins born 1958 or earlier to analyse the presence of RA-associated auto-antibodies in relation to genetic risk associated with RA-development. All blood samples were analyzed for ACPA. RA or other rheumatic joint diseases were verified by data from register linkage to national care registers or by reviewing medical records.

387 out of 12,594 tested individuals (3.1%) were positive for ACPA. Smokers had an increased risk of developing ACPA as compared with non-smokers (OR 1.33, 95% CI 1.08-1.63) and the risk was highest among those smoking more than 10 pack years (OR 1.49, 95% CI 1.18-1.88). Among the ACPA positive subjects, 312 twin complete pairs were available, 7 concordant and 305 ACPA discordant pairs. In this large population-based cohort of middle-aged twins a low concordance rate was found for ACPA in both MZ and DZ twins. The results indicate that environment, life style and stochastic factors may be more important than

genetics in determining which individuals will develop ACPA, whereas genetic factors may have a larger impact in determining which ACPA-positive individuals that will ultimately develop arthritis. These findings are very important as they indicate that the formation of the auto-immune response, that can be present already years prior to clinical onset, results from an environmental insult, whereas the further development of this response and subsequent development of disease depends on a second, HLA-mediated, hit that could potentially be identified by follow-up of ACPA-positive, (still) healthy, individuals.

The identification of this second hit could pave the way for prevention of disease and is therefore the focus of subsequent studies coming from the MASTERSWITCH program.

One of the most relevant challenges for consortia like the MASTERSWITCH consortium is to prevent disease precipitation in people at risk for disease development. Therefore, we also studied the characteristics of people coming to the rheumatologist with a relatively short duration of joint complaints. These characteristics were subsequently matched to the requirement for a therapeutic intervention and outcome of disease later in time. These studies aiming to further the understanding of the transition to chronic/self resolving arthritis showed that a long referral time to a rheumatologist is associated with a worse clinical outcome. Because patient delay from the general practitioner to a rheumatologist may cause suboptimal use of the therapeutic window in RA, we next investigated the reason for medical help-seeking behaviour of patients with recent-onset arthralgia/arthritis. These studies revealed that a prolonged delay in seeking help was associated with a gradual onset of symptoms and the perception that symptoms would not be serious or would go away. Arthralgia patients who promptly sought medical help more often had an acute onset of symptoms and more frequently reported impairments at work or in daily functioning than patients who postponed seeking help. Patients with and without arthritis generally had similar reasons for seeking help. The proportion of patients who had a prolonged patient delay was comparable between male and female subjects and between age categories. Particularly younger patients postponed seeking help because they thought their symptoms would disappear spontaneously.

As these studies generated important insight into the reasons why adequate treatment for patients with symptoms is delayed, they formed the basis for several implementation that have been initiated by MASTERSWITCH consortium members. These are outlined in a subsequent section.

b. Established disease

A main challenge in clinical care of chronic diseases in general and in RA in particular relates to the differentiation among patients with (early) disease who will remit spontaneously or on therapy from those who will develop chronic, destructive disease. In general, remission can be achieved more easily in case patients are treated early after start of symptoms. Sometimes remission can even occur spontaneously. However, the factors that are predictive for remission/chronic disease are ill defined. Therefore, the MASTERSWITCH consortium dedicated a considerable amount of work to gain more insight into the factors that govern the realization of remission in early- and established arthritis, either as induced by treatment or, alternatively, as consequence of natural disease course.

The MASTERSWITCH consortium investigated the prevalence of and prognostic factors for sustained DMARD-free remission using several patient cohorts. In order to investigate remission as a definitive disease outcome, we often used a stringent definition of remission: the sustained absence of synovitis for at least 1 year after the discontinuation of therapy with

DMARDs. This definition approximates a definitive cure of the disease and, as such, is close to the meaning of remission as used for other diseases such as malignancies. This definition was also used as it is the ambition of the MASTERSWITCH consortium to come to a permanent cure for chronic inflammatory diseases like RA. Such ambition can best be realized when the factors contributing to "permanent cure" are identified. Likewise, investigating remission as a definitive disease outcome, resembling cure, is very important from a pathophysiologic point of view. Knowledge of which clinical and/or immunologic characteristics of the patient are associated with remission could fuel new hypotheses about the mechanistic pathways involved in disease persistence and resolution and would increase our understanding of the disease course of RA.

Several studies investigated the prevalence of and predictive factors for sustained DMARD-free remission in RA patients treated with conventional therapy. The long-term follow-up data available from cohorts from the Netherlands and the UK revealed that sustained DMARD-free remission occurs in 9–15% of RA patients. Furthermore, sustained DMARD-free remission can be predicted by several clinical variables at first presentation that are routinely assessed in outpatient clinics. Six factors were associated with sustained DMARD-free remission that could be replicated in different cohorts: acute onset, short symptom duration before inclusion, not smoking, little radiographic damage at baseline, absence of IgM rheumatoid factor (IgM-RF), and absence of HLA shared epitope alleles. Likewise, multivariate analyses revealed that symptom duration and the absence of auto-antibodies as independent predictors for the achievement of DMARD-free remission in established disease.

Moreover, in order to establish the difference in remission rate between early and advanced disease we performed a systematic literate search including a meta-analysis which revealed that an increased symptom duration at treatment initiation is associated with a decreased hazard on achieving DMARD-free sustained remission, which is a proxy of cure. This effect of time was independent of other predictors for DMARD-free sustained remission such as age, gender, the level of inflammation and the presence of auto-antibodies related to RA.

Our results also revealed that very early therapy of RA with DMARDs is associated with lower levels of joint destruction and a higher chance of achieving remission. Having symptoms for >12 weeks at treatment initiation is a strong and independent risk factor for a persistent disease course. These observations have led to the concept of the 'window of opportunity'. This hypothesis presumes that underlying disease processes are not fully matured in the very early stage of arthritis, making modulation more successful. However, putative biological mechanisms are ill defined. As auto-antibody status is predictive for a worse disease outcome, as also indicated above, but at the same time, does not exclude the possibility to obtained drug-free remission upon adequate treatment, we examined whether patients who were assessed within 12 weeks of symptom onset had a less broadened ACPA response than patients with longer symptom duration. However, these investigations into the constitution of the ACPA-response could not reveal a difference in the characteristics of these auto-antibodies between ACPA-positive RA patients with symptoms <12 weeks and patients with >12 weeks. Nonetheless, ACPA-positive RA patients with symptoms <12 weeks have less progressive disease than patients with a longer symptom duration. Therefore, the broadness of the ACPA response is not different between these groups, indicating that maturation of the autoantibody response occurs even earlier.

c. Implementation of findings

As mentioned above, our efforts have revealed that there can be a substantial lag-phase between onset of symptoms and a visit to a rheumatologist that can start adequate treatment. As we also found that early treatment prevents joint damage and enhances the quality of life of patients, we have investigated the reasons of the patient delay in seeking medical help. These investigations showed that a prolonged delay in seeking help was associated with a gradual onset of symptoms and the perception that symptoms would not be serious or would go away. Although no difference was observed between male and female subjects, particularly younger patients postponed seeking help because they thought their symptoms would disappear spontaneously. This information is now used to better target/inform those patients at risk to develop chronic destructive arthritis that display a "delayed help-seeking behaviour".

Likewise, the clinical characteristics and the optimal evaluation of patients that enter remission have been studied in details. These studies showed that, although it remains difficult to predict whether an individual patient enters remission in the future, the clinical means to define remission are sufficient and that more sophisticated (and more expensive) imaging techniques do not convey an additional benefit.

Probably the most prominent implementation of the findings we made within the context of the MASTERSWITCH program is the initiation of the "Early Arthritis Recognition Clinics" by several partners of the MASTERSWITCH consortium. These findings prompted the initiation of such clinics because they indicated that delayed initiation of therapy after onset of symptoms is correlated with worse outcome later in disease. Importantly, those patients that visited the rheumatologist within 12 weeks of symptom onset had considerably less joint destruction and a higher chance on achieving sustained DMARD-free remission then patients with a longer delay in assessment. These findings indicate that the initiation of an early and adequate treatment is likely lowering the change of entering a chronic, destructive disease phase and thereby increases the quality of life for patients and lowers the costs for society by preventing disability, loss of labour participation and need for more expensive medical care later in life. One of the most prominent and preventable factors explaining the delayed initiation of treatment was found in a delayed referral from the general practitioner to the rheumatologist. Therefore, referral time is now shortened through notifying General Practitioners that they can send patients for a short consultation on the existence of arthritis to the "early arthritis recognition clinics". The efficacy of this health-care intervention is now evaluated as further follow-up of the MASTERSWITCH program.

B. The molecular level

The MASTERSWITCH consortium has studied in intimate detail the characteristics and contribution of several molecular pathways at several levels, ranging from genetics, epigenetics, RNA-processing, signal transduction to the interplay between auto-antibodies with defined cell types involved in inflammation. In this section only a brief overview is given of most relevant studies that aided the further understanding of chronic inflammatory disease and RA.

a. Genetics and epigenetics

In the 5 years enormous progress has been made in the identification of genetic risk-markers that predispose to the development of chronic inflammatory diseases in general and RA in particular. Although, it was these studies were not the main focus of the MASTERSWITCH

consortium, several partners has contributed to progress in this field through sharing and exchange of materials and information that has been collected in the past decade. These collaborative efforts have resulted in significant breakthroughs in the identification of genes and genetic risk factors that contribute to RA and other chronic inflammatory disorders. This is best exemplified by a recent large collaborative study involving groups from Japan, North-America and Europe, including MASTERSWITCH partners, that was performed in an "open science" setting. This international team of researchers have performed the largest genetic study ever carried out, involving nearly 30,000 patients and 75,000 controls and has found more than 40 new areas in DNA that increase the risk of rheumatoid arthritis. bringing the total of RA-risk loci to 101. Subsequently, using bio-informatics, and knowledge obtained from various different sources including knockout mouse phenotypes 98 biological candidate genes at these 101 risk loci were identified. Moreover, it was demonstrate that these genes are the targets of approved therapies for RA, and it was further suggested that drugs approved for other indications may be repurposed for the treatment of RA. Thus, this comprehensive genetic study in which the MASTERSWITCH consortium was represented have sheds light on fundamental genes, pathways and cell types that contribute to RA pathogenesis, and provided important information for drug discovery.

In addition to analysis of the genetic code as indicated above, in recent years more and more studies have concentrated on changes in the epigenetic code; the code underlying the heritable changes in gene activity that are not caused by changes in the DNA-sequence. Epigenetic mechanisms determine which genes in a cell are transcribed and thus contribute to the phenotype of a cell. The epigenetic code can be changed by environmental influences, which allows cells to adapt to longstanding changes in the environment. In chronic inflammatory diseases epigenetic changes were found to correlate with disease severity and progression. Knowledge about these epigenetic changes might therefore help that epigenetic modifications can be used in the future as biomarkers, prognostic factors and therapeutic targets, but understanding these changes is likely also contributing to the understanding of the mechanisms underlying chronic inflammatory diseases.

Indeed, several groups from the MASTERSWITCH consortium have studied the epigenetic modifications present in various cells types and found to that they are likely contributing to the development of RA by affecting diverse aspects of the disease and modifying gene expression levels and behavior of several cell types, first and foremost joint resident synovial fibroblasts. RA synovial fibroblasts are the most common cell type at the site of invasion in the inflamed joint. Owing to their aggressive, intrinsically activated phenotype, RA synovial fibroblasts are active contributors in joint damage. RA synovial fibroblasts are characterized by their ability to secrete cytokines, chemokines and joint-damaging enzymes. Furthermore, these cells are resistant to apoptosis, leading to hyperplasia of the synovium. In addition, RA synovial fibroblasts have invasive and migratory properties that could lead to spreading of the disease to unaffected joints. Epigenetic modifications, including DNA methylation and post-translational histone modifications, such as histone (de)acetylation, histone methylation and histone sumoylation were identified as regulatory mechanisms in controlling aggressive cell activation *in vitro* and in disease outcome in animal models *in vivo*. For example, the MASTERSWITCH consortium has shown that blocking enzymes involved in histone de-acetylation alters the behaviour of RA synovial fibroblasts by promoting their cell arrest and reducing their pro-inflammatory potential. Likewise, also the role of other epigenetic mechanisms have been studied in detail in RA synovial fibroblasts all indicating that epigenetics contribute to the aggressive, intrinsic activated phenotype of RA synovial

fibroblasts. Therefore, the data generated raise the hope that, in future, detailed knowledge of epigenetic regulatory mechanisms in RA will help to develop new treatment strategies or will predict treatment efficacy and treatment outcome.

b. RNA-processing

Induction, regulation, and resolution of the immune response involves a wide spectrum of inflammatory reactions. If inflammatory triggers cannot cease and be eliminated, or if the pro-inflammatory state persists, inflammation converts to a chronic response, which contributes to tissue damage and loss of tissue homeostasis.

The efficacy of the (innate) immune response relies on the production and posttranscriptional regulation of mRNAs encoding effector proteins. Such mRNAs are often labile and contain adenylate uridylate–rich elements (AREs) in their untranslated termini. Specific ribonucleoprotein (RNP) complexes are targeted by inflammatory signals to control the intracellular transport, translation, and decay of the inflammatory mRNAs. The importance of the negative, ARE-mediated control is underlined by the development of inflammatory pathologies in transgenic mice with dysfunctional AREs or ARE-binding proteins. In the context of the MASTERSWITCH program the exact role of several proteins involved in RNA decay for the induction and progression of inflammation in relation to their cellular expression pattern has been elucidated. For example, we have found that the RNA-binding protein HuR maintains inflammatory homeostasis by controlling macrophage plasticity and migration. Mice lacking HuR in myeloid-lineage cells, which include many of the cells of the innate immune system, displayed enhanced sensitivity to rapid progression of chemical-induced colitis. The myeloid cell–specific HuR-deficient mice displayed an exacerbated inflammatory cytokine profile and showed enhanced macrophage chemotaxis. At the molecular level, activated macrophages from these mice showed enhancements in the use of inflammatory mRNAs due to a lack of inhibitory effects on their inducible translation and/or stability. Conversely, myeloid overexpression of HuR induced posttranscriptional silencing, reduced inflammatory profiles, and protected mice from colitis. These results, therefore, highlight the role of HuR as a homeostatic coordinator of mRNAs that encode molecules that guide innate inflammatory effects and demonstrate the potential of harnessing the effects of HuR for clinical benefit against pathologic inflammation.

Intriguingly, however, mice deficient in HuR in the myeloid lineage were not more susceptible for the development of arthritis, contrary to what was observed for acute and intestinal models. However, the synovial fibroblast-restricted deletion of HuR provided significant susceptibility to TNF-mediated arthritis correlating with changes in synovial fibroblast proliferation, death and evasiveness. Zooming in on the effects of HuR-deficiency revealed 4 distinct features of FLS targeted by HuR: (a) growth and proliferation relating to archetypical/developmental growth signals; (b) a key oxidative stress response; (c) a capacity to modulate immune recruitment via selective chemokine secretion; and (d) selective expression of metaloproteinases for tissue remodeling. In addition the data pointed towards the direct interaction of HuR to cytoskeletal components which relates to the apparent phenotypic changes of arthritic synovial fibroblasts.

Likewise, specific targeting o HuR in T-cells showed that mice became refractory to the induction of autoimmunity as modelled by Collagen-Induced Arthritis. HuR-deficient T-cells maintained the capacity to home to most inflammatory sites and differentiate further to effector (Th1, Th17) and regulatory (T-reg) subsets; however they showed compromised priming responses and increased thresholds of TCR-activation. In conclusion, this research

has highlighted the importance of the RNA-binding proteins in inflammation control and indicated that one RNA-binding protein, HuR, can be exploited for the management and therapy of several inflammatory conditions.

c. Signal-transduction and cell activation

While the immunological response in the arthritic joint has been extensively characterized, the early events triggering the initiation of joint inflammation that culminates in chronic disease have not been extensively investigated and remain very poorly understood. One of the aims of the MASTERSWITCH consortium was to characterize the role of specific molecular players in the early events contributing to the induction of inflammation during the initiation of the pathogenesis of arthritis. These studies focused particularly on the NF-κB and TLR-signalling pathways, which are major regulators of immune and inflammatory responses and contribute to the pathogenesis of chronic inflammatory diseases. To comprehensively address the role of NF-κB and TLR signalling in arthritis initiation, studies of both human synovial tissue and immune cells as well as functional studies in genetic mouse models were included.

In the analysis of the role of TLR signaling in the inflammatory response in arthritic joints, we could show that already at an early stage of the disease the expression of TLRs is elevated in synovial tissues of RA patients. Specific analysis of synovial fibroblasts revealed that activation of TLRs and other molecules that can sense "danger" leads to the secretion of pro-inflammatory cytokines and matrix-degrading enzymes in these cell. Most importantly, we found that TLRs synergize with other innate immune receptor molecules in the activation of the inflammatory response, so that activation of one innate immune receptor leads to increased expression and synergistic activation of the pathways of the other pattern recognition receptors on synovial fibroblasts. Thereby the presence of any pattern-recognition ligand in the joints of RA patients increases the sensitivity of synovial fibroblast towards other damage- and/or pathogen-associated molecular pattern molecules intensifying the innate immune response. Moreover, in a mouse model for arthritis, it was shown that targeting TLR7 resulted in a highly protective effect on paw swelling and joint destruction. These data highlight the potential role for endosomal TLRs in the maintenance of inflammation in RA and support the concept of a role for TLR7 in experimental arthritis models. Together, these studies illustrates the potential benefit that may be afforded by therapeutically inhibiting the endosomal TLRs in RA.

To address the functional role of down-stream events following triggering of molecules like TLRs NF-κB signalling in the initiation of joint inflammation we employed conditional targeting of IKK2, the kinase mediating canonical NF-κB activation, in synovial fibroblasts in the well characterized hTNF-Tg genetic mouse model of TNF-driven rheumatoid arthritis. Fibroblast specific IKK2 deficiency efficiently inhibited NF-κB activation and considerably delayed the progression of, demonstrating that activation of NF-κB signalling in synovial fibroblasts is critical for the pathogenesis of destructive arthritis in response to increased TNF expression. In contrast, fibroblast specific knockout of Cyld, a negative regulator of NF-κB signalling, led to the upregulation of several inflammatory cytokines and MMPs in synovial fibroblasts and aggravated the pathogenesis of arthritis. Together, these studies show that activation of NF-κB signalling in synovial fibroblasts contributes to the inflammatory response causing the pathogenesis of chronic destructive arthritis.

In a different study, we addressed the role of A20 (also known as TNFAIP3 and one of the genetic risk factors predisposing to RA) in macrophages in the pathogenesis of arthritis. We could show that specific ablation of *A20* in myeloid cells resulted in spontaneous development of a severe destructive polyarthritis with many features of rheumatoid arthritis. This destructive polyarthritis in myeloid A20 knockout mice was dependent on TLR4 and MyD88, showing that A20-dependent negative regulation of TLR4/MyD88 signalling has an essential physiological role in preventing arthritis development. These observation are also compatible with the well-established role of A20 in negatively regulating NF-κB signalling downstream of TLRs.

Thus, this work significantly contributed to our better understanding of how TLR- and NF-κB-mediated signalling events orchestrate the molecular networks regulating the induction of inflammation during the different stages of disease initiation. It also provided additional support for the targeting of molecules participating in these signalling events as options for the development of new treatments.

d. Auto-antibodies

Auto-antibodies, especially Anti-Citrullinated Auto-antibodies (ACPA) and Rheumatoid factor (RF), are a hallmark of RA and thought to be involved in the pathogenesis of RA by recruiting various immune activation mechanism and triggering of immune cells. Therefore, the MASTERSWITCH consortium has studied the presence and composition of the auto-antibody response in relation to clinical phenotype, genetic background, disease outcome and response to therapy in great detail. These results can be summarized as follows:

- ACPA use all isotypes. Isotype usage expands before onset of RA after which it stabilizes. Interestingly, the broader the isotype usage, the higher the risk to develop more severe RA as measured by radiological progression. However, we were not able to link radiological progression to one specific antibody isotype.
- ACPA-positive and negative RA are different with respect to underlying genetic predisposition, inflammatory synovial infiltrate, outcome to therapy and chance to remission.
- Anti-CCP-antibodies are ACPA as anti-CCP-antibodies recognize a variety of citrullinated proteins. ACPA are highly cross-reactive at the citrullinated protein level as ACPA isolated through interaction with one protein will also recognize other citrullinated proteins. However, at the single epitope level using specific citrullinated antigens, non-cross-reactive antibodies are present next to the presence of cross-reactive antibodies. The citrullinated epitope repertoire of ACPA expands before disease onset after which it stabilizes. In contrast to isotype usage, we were unable to detect an association between the epitope recognition and radiological joint damage over time. Likewise, we were unable to define specific clinical subsets or characteristics in association with the ACPA-recognition profile. However, a strong association was observed between ACPA levels and the number of citrullinated epitopes recognized by ACPA.
- ACPA are, by and large, of low avidity and do not display a strong avidity-maturation over time. If avidity-maturation of the ACPA response takes plays in a certain individual, it takes place before disease onset after which no further avidity maturation is observed.
- ACPA can activate both the alternative as well as the classical pathway of complement activation.

- ACPA show an aberrant Fc-glycosylation with a strongly increased presence of N-linked glycans lacking terminal sialic acid and galactose residues ("G0-glycans"). Aberrant Fc-glycosylation is relatively ACPA-specific as ACPA display considerably more "G0-glycans" as compared to IgG-molecules obtained from the same patients. Aberrant ACPA-Fc glycosylation is strongly enhanced in the synovial compartment (i.e. synovial fluid) and associated with the presence of rheumatoid factors. Our findings suggest a link between ACPA "G0-glycans" and rheumatoid factors.

- A strong and specific association between ACPA and serum markers for osteoclast-mediated bone resorption in RA patients was found. Moreover, human osteoclasts expressed enzymes eliciting protein citrullination, and specific N-terminal citrullination of vimentin was induced during osteoclast differentiation. ACPA not only bound to osteoclast surfaces, but also led to robust induction of osteoclastogenesis and bone-resorptive activity. Adoptive transfer of ACPA into mice induced osteopenia and increased osteoclastogenesis. This effect was based on the inducible release of TNF-α from osteoclast precursors and the subsequent increase of osteoclast precursor cell numbers with enhanced expression of activation and growth factor receptors. Therefore, these data suggest that autoantibody formation in response to citrullinated proteins can directly induce bone loss, providing a link between the adaptive immune system and bone.

Citrulline highly resembles homocitrulline, another posttranslationally modified amino acid. Homocitrulline is one methylene group longer, but similar in structure. Homocitrulline is generated from a lysine residue following a reaction of cyanate, which is present in the body in equilibrium with urea. Under physiological conditions the urea concentration may be too low to allow extensive carbamylation but the conversion process leading to the formation of homocitrulline from lysine in proteins does occur in vivo. It has been shown recently that homocitrulline-containing proteins are present in the RA joint and that they may affect T-cell triggering and possibly autoantibody formation in rodents. Although highly similar, carbamylation differs from citrullination as, next to their structural difference, lysine is modified instead of arginine. Therefore, homocitrulline will, by definition, be located at other positions in proteins than citrulline. Because of the similarity between citrulline and homocitrulline, we set out to analyze whether autoantibodies against carbamylated proteins are present in RA and whether these antibodies differ from ACPA with respect to antigen binding and clinical associations. In doing so, we discovered that the presence of a novel autoantibody system that discriminates between citrulline- and homocitrulline-containing antigens in the sera of RA-patients. IgG antibodies recognizing carbamylated (homocitrulline-containing) antigens were present in sera of over 45% of RA-patients. ACPA and anti-Carbamylated Protein (CarP) antibodies are distinct autoantibodies because, in selected double-positive patients, the anti-CarP antibody binding to carbamylated antigens could be inhibited by carbamylated antigens, but not by control or citrullinated antigens. In line with this observation, 20-30% of ACPA-negative RA-patients harbored anti-CarP antibodies. The presence of anti-CarP antibodies was predictive for a more severe disease course in ACPA-negative patients as measured by radiological progression. Taken together, these data show the presence of a unique autoantibody system recognizing carbamylated, but not citrullinated, protein antigens. These antibodies are predictive for a more severe clinical course in ACPA-negative RA-patients, indicating that anti-CarP antibodies are a unique and relevant serological marker for ACPA-negative RA.

C. The level of cells and the entire organism

The MASTERSWITCH consortium has investigated the contribution and biology of several different cell types in the induction, perpetuation and resolution of arthritis, both using cells from humans ex vivo as well as in vivo using appropriate animal models. These cell types included the synovial fibroblast as well as a wide variety of immune cells such as B-cells, T-cells, macrophages and monocytes, osteoclasts, granulatocytes, mast cells as well as dendritic cells. Again, it is not feasible to mention all results obtained in the past 5 years by the MASTERSWITCH consortium. Therefore, we refer to the MASTERSWITCH publications for more detailed information on the findings obtained and highlight a few these findings below.

The role of various molecular and cellular players involved in the induction as well as clearance of inflammation has been studied intensely. Important and novel insight has been obtained into the role and mechanisms of several molecules involved in dampening inflammatory reactions. For example, our efforts have further unravelled the reasons how synovial fibroblasts are involved in disease pathogenesis of RA. Under the influence of the proinflammatory milieu, synovial fibroblasts become activated and hyperplastic, releasing proinflammatory factors and tissue-remodeling enzymes. We have now shown by analysing that synovial fibroblasts from human patients and animal models express significant quantities of autotaxin, a lysophospholipase D that catalyzes the conversion of lysophosphatidylcholine to lysophosphatidic acid (LPA). Autotaxin expression from synovial fibroblasts was induced by the pro-inflammatory cytokine TNF, and LPA induced synovial fibrobalst activation and effector functions in synergy with TNF. Conditional genetic ablation of autotaxin in mesenchymal cells, including synovial fibroblasts, resulted in disease attenuation in animal models of arthritis, establishing the autotaxin/LPA axis as a novel player in chronic inflammation and the pathogenesis of arthritis and a promising therapeutic target.

Furthermore, our investigations have shown identified the enzyme 12/15-lipoxygenase (12/15-LO) as a central factor governing the sorting of apoptotic cells into differentially activated monocyte subpopulations. The clearance of apoptotic cells is crucial to maintain self-tolerance and thereby in the prevention of auto-immunity. 12/15-LO was shown to be a central factor governing the sorting of apoptotic cells into differentially activated monocyte subpopulations. During inflammation, uptake of apoptotic cells was confined to a population of 12/15-LO-expressing, alternatively activated resident macrophages, which blocked uptake of apoptotic cells into freshly recruited inflammatory monocytes. Loss of 12/15-LO activity resulted in an aberrant phagocytosis of apoptotic cells by inflammatory monocytes, subsequent antigen presentation of apoptotic cells -derived antigens, and a lupus-like autoimmune disease. Therefore, these data reveal an unexpected key role for enzymatic lipid oxidation during the maintenance of self-tolerance.

Dendritic cells (DC) are considered the orchestrators of the immune system as they initiate and direct both the nature and magnitude of many cellular immune responses, including auto-immune responses. Therefore, they are likely to provide an essential MASTERSWITCH for the initiation and perpetuation of autoimmune responses. Until now, it has been difficult to attribute a specific function to distinct DC subsets present in specific anatomical sites in response to pathogens or in peripheral tolerance mechanisms avoiding autoimmune responses. By using innovative transgenic animal system, important insight has been obtained

in the functional properties of distinct dendritic cell subsets. Likewise, we have identified specific markers visualizing lymphoid-tissue resident and migratory dendritic cells. These markers are used in a consistent manner across all mammalian species, including humans and may allow the modulation function in specific DC-subsets. Moreover, our results revealed a high degree of functional specialization among the mononuclear phagocytes in the tissues where some were specialized in taking up- and presenting antigens to T-cells, whereas others excelled in scavenging and phagocytic activities.

This basic knowledge generated on the phenotype, origin and functional capabilities of different mononuclear phagocytes is important for the design of optimal protocols to modulate the outcome of chronic autoimmune disease.

4 The expected final results and their potential impact and use

The objective of the MASTERSWITCH project is to delineate the biological and molecular pathways that initiate and drive chronic inflammatory disease to allow the development of novel reagents and innovations.

To achieve these aims the overall strategy of the project is centered on critical switch moments involved in the various processes that drive chronicity of inflammation. We achieved to get a holistic approach by putting the MASTERSWITCH processes in a clinical, molecular and cellular perspective.

These complementary perspectives in one project opened up a novel multidisciplinary avenue within research on inflammatory disorders. Within the MASTERSWITCH network, 12 leading European expert groups and 5 biotech companies have joined forces. Within this consortium, both groups with a clear fundamental scientific focus, as well as clinically orientated groups have been collaborating.

The MASTERSWITCH program has resulted in over 400 scientific publications in peer-reveiwed journals of which a considerable number have been published in journals with a high-impact. Likewise, MASTERSWITCH researchers have communicated the outcome of the research on numerous meetings, both scientific meetings as well as meetings for the lay public.

The clinical perspective was fuelled by the lead role that Masterswitch Centers played in the development of new (2010) criteria for RA (first author from Vienna). These criteria were especially developed to allow classification of patients with recent-onset arthritis to treat as soon as they fulfill the new classification criteria with the motivation that the putative Masterswitches that drive chronicity will be prevented from being turned on. The clinical partners within the Masterswitch consortium were able to rapidly replicate and validate the new criteria because due to the Masterswitch consortium the infrastructure of pan-European cohorts was established. This pan-European replication of the new criteria paved the way for rapid implementation within European rheumatology (wider societal impact of the project).

Next to the establishment of new criteria the perspective of clinical intervention to prevent the critical switch moments in induction of inflammation, progression of inflammation and lack of resolution of inflammation were studied in the so called "IMPROVED" study in which a treatment strategy steered at remission was studied. This treatment strategy was rapid

induction of remission and then followed if possible by withdrawing of drugs to achieve drug-free remission.

Within the molecular perspective much attention has been paid to the composition of the immune response to citrullinated proteins with respect to isotype usage, epitope recognition and avidity maturation. Due to the unique consortium we were able to get enough samples from pre-disease, arthralgia, recent onset artritis, RA and longstanding RA. The wider societal impact of these studies was that the medical community became aware that the antibody response to citrullinated proteins is highly specific for RA development and many doctors use the results of this test to guide there referral decisions. The existence of this antibody response is used a marker for persistence of disease with data that RA patients that have such antibodies have a more persistence disease course.

Within the above mentioned IMPROVED study it has been demonstrated that early referral and rapid induction of remission leads to drug-free remission in equal proportions of anti-citrullinated antibody positive as well as anti-citrullinated antibody negative patients, therebye indirectly suggesting that early rapid intervention with anti-inflammatory drugs is indeed able to prevent chronicity driving Masterswitch switches to be turned on.

Apart from this broad impact on society the Masterswitch concept also led to the realization that health care strategies needs to be evaluated. It appeared that often treatment was started late because patients were not promptly referred by the general practitioner to the rheumatologist. Within the MASTERSWITCH consortium it was shown that delay in referral was associated with bad prognosis. This notion had broad impact a nice example is a study in the USA where it was shown that there is an astonishing unequal distribution of rheumatologists through the USA which such a low level of rheumatology care in certain States that waiting and travel times are unexpectedly long.

Within the MASTERSWITCH consortium attempts to facilitate a shorter referral time were made. General practitioners were informed about the initiation of early arthritis recognition clinics where patients with early symptoms that are suspect for arthritis can be seen for a quick scan by a trained rheumatologist. This approach has resulted in a considerable overall shortening of the delay time that prevented the start of adequate treatment. It is expected that this initiative will not only enhance the quality of life of patients but also that it will reduce costs of health care because permanent remission are expected to be induced more often. Likewise, costs for society are likely reduced further through a higher maintenance of working ability and participation of patients.

The research performed by the MASTERSWITCH consortium has also been translated into research aiming to evaluate the adaptation of health care strategies by the initiation of "Early Arthritis Recognition Clinics". These clinics were opened by several MASTERSWITCH partners in different European countries because it was observed that the start of early and adequate treatment of patients early after onset of symptoms prevents joint damage and loss of function later in life.

As stated above the discovery that anti-citrullinated antibodies predict development of RA has had broad impact for those patients to be referred in time by general practitioners, however 50% of patients with new onset RA donot have such antibodies. Within the Masterswitch consortium a new auto-antibody system was discovered (anti-carbamylated antigen antibodies= anti-CARB). It turned out that these autoantibodies are also present in

those patients that do not harbor anti-citrullinated antibodies). The use of these antibodies for clinical testing has been patented and currently in collaboration with an industrial partner this will be further developed thereby creating jobs.

The underlying cellular processes are studied both in isolated cells from patients as well as in animal models. A highlight is the discovery of overexpression of an enzyme lysophospholipase D in the cells that make the synovial fluid in the joint, the synovial like fibroblasts. Specific inhibition of this enzyme by genetic ablation attenuates arthritis in animal models, thereby providing a hope that influencing the expression or function of this enzyme may be a new target to treat RA.

The MASTERSWITCH consortium took the initiative for consensus meetings on the use and formulation of guidelines for animal experiments for chronic diseases together with the European League against Rheumatism (EULAR). EULAR is the organisation which represents the patient, health professional and scientific societies of rheumatology of all the European nations. EULAR endeavours to stimulate, promote, and support the research, prevention, treatment and rehabilitation of rheumatic diseases. These efforts have resulted in recommendations for future use of animal models for rheumatic disease. This has broad societal impact because these guidelines can help to balance concerns in all groups of society to arrive at a wise use of laboratory animals to enhance understanding of a chronic destructive disease as RA.

Critical masterswitches in an immune-mediated disease is the orchestration of the immune system by dendritic cells that initiate and direct nature and magnitude of many cellular responses. Until now, it has been difficult to attribute a specific function to distinct DC subsets in specific anatomical sites or in peripheral tolerance mechanisms avoiding autoimmune diseases. By using innovative transgenic animal models it has now became more clear which distinct dendritic cell population can guide tolerance decisions. This will have broad impact for a number of autoimmune diseases as well as organ transplantation.

The MASTERSWITCH consortium has led to several patents and patent applications and to a further expansion the participating companies. For example, Redoxis (ben. 19) used several different routes for dissemination. In collaboration with significant academic partners for research publications have with partner Karolinska Institutet (ben. 8) published an article that further have deciphered the genetic regulation of the NOX2 enzyme and production of reactive oxygen species (ROS). As the NOX2 enzyme is the major drug target of Redoxis small molecule program, involvement in characterisation of this target and description of the immunological mechanism of the function is absolutely pivotal for further preclinical development of Redoxis small molecules for treatment of severe autoimmunity.

Also, Redoxis (ben. 19) continued during Masterswitch to complete and strengthen the IP portfolio and has extended the patent applications by national application and national dividends for our patent portfolio. Redoxis has a patent portfolio that consist of i) Target and method patent application and ii) Compositional matter applications that covers our lead compound classes and main intellectual property. All these IPR matters have been further improved and strengthened during Masterswitch.

As an SME working with preclinical development of drugs it is essential to identify industrial partners and collaborators for clinical development and regulatory studies of Redoxis new drugs. Redoxis is currently in preclinical phase, but will with coming years initiate regulatory

toxicology and clinical studies. These studies require partnership where Redoxis have met great interest for our project. To meet and join with possible future collaborator for candidate drug characterization and clinical trial, Redoxis participates in a multitude of international biopartnering events and forums for preclinical drug development.

5 Additional details

Contact: T.W.J.Huizinga@lumc.nl

The website is http://www.masterswitchproject.eu/

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List of beneficiaries

1	Leiden University Medical Center	Prof.dr. T.W.J. Huizinga
	Dept. of Rheumatology; C1-R	Head dept. of Rheumatology
	P.O. Box 9600	Prof.dr. R.E.M. Toes
	2300 RC Leiden	Head of lab.
	The Netherlands	Mrs. J.J.A. Kuijper
		Project coordinator
2	BSRC	Dr. G. Kollias
	Al. Fleming Street 34	President & Scientific Director
	Vari-Athens 16672	Dr. P. Papadaki, PhD
	Greece	Project Implementation Manager
3	Imperial College London	Prof. M. Feldmann
	The Charing Cross Hospital Campus	
	Arthritis Research Campaign Building	Beneficiary until 01.08.2011;
	65 Aspenlea Road	(see beneficiary 20)
	London W6 8LH	
	United Kingdom	
4	Centre d'Immunologie de Marseille-Luminy	Dr. B. Malissen
	(CIML)	Team leader
	Parc Scientifique de Luminy	
	Case 906	
	Marseille, 13288/9	
	France	
5	F.-A. Universität Erlangen	Prof.dr. med. G. Schett
	Universitätsklinikum	
	Medizinische Klinik 3	
	Ulmenweg 18	
	91054 Erlangen	
	Deutschland	
6	Medizinische Universität Wien	Prof. J. Smolen
	Internal Medicine III; Rheum.	Head of Department
	Waehringer Guertel 18	Dr. G. Steiner
	Vienna A 1090	Associate Professor
	Austria	
7	University of Leeds	Prof. P. Emery
	Musculoskeletal Disease	Head of Department
	Chapel Allerton Hospital	Dr. F. Ponchel
	Chapeltown	Senior Academic Research
	Leeds LS7 4SA	Fellow
	United Kingdom	LIMM, section musculoskeletal
		disease

8	Karolinska Institutet Med. Inflammation Research Dept. of Medical Biochemistry Scheeles Väg 2 Stockholm 17177 Sweden	Prof. R. Holmdahl Prof. L. Klareskog
9	Universität zu Zürich Center of Experimental Rheumatology and WHO Collaborating Center Gloriastrasse 25 Zürich 8091 Switzerland	Prof. S. Gay Director Dr. R. Gay
10	Universität zu Köln Institut for Genetics Zülpicher Strasse 47 Köln D-50674 Deutschland	Prof. M. Pasparakis
11	University of Glasgow Biomedical Research Centre Centre for Rheumatic Diseases University Palace 120 Glasgow G12 8TA United Kingdom	Prof. I.B. McInnes
12	Dept. of Immunology and Oncology Centro Nacional de Biotecnologica C/Darwin, 3 UAM-Camus de Cantoblanco E-28049 Madrid Spain	Mr. M. Mellado, PhD
13	Biomedcode SA Alexander Fleming Street 34 Vari (Athens) 16672 Greece	Dr. M. Denis
14	Hybrigenics SA 3-5, Impasse Reille Paris 75014 France	Dr. E. Formstecher Director Scientific Projects
15	Maimonidex RA Ltd./Maimonidex (UK) Ltd Swansea University Institute of Life Sciences Singleton Park Swansea SA2 8PP	Dr. I. Golan CEO

United Kingdom

16	Cranfield University Translational Medicine Cranfield Health, Vincent Bldg. 52, Cranfield University Bedfordshire, MK43 4DT United Kingdom	Prof. R. Aspinall Beneficiary, starting date 01.12.2009
17	King's College London Department of Rheumatology Room 1.26, New Hunts House Great Maze Pond London, SE1 1UL United Kingdom	Prof. A. Cope Beneficiary, starting date 01.12.2009
18	University of Sussex Trafford Centre Brighton and Sussex Medical School University of Sussex Falmer BN1 9RY Brighton United Kingdom	Dr. S. Sacre Beneficiary starting date 1.07.2010
19	Redoxis AB Biotech Building – Plan 7 Arvid Wallgrens Backe 20 SE-413 46 Göteborg Sweden	P. Olofsson, PhD, MBA CEO Beneficiary starting date 01.12.2010
20	Kennedy Institute of Rheumatology Division University of Oxford NDORMS, Nuffield Orthopaedic Centre Windmill Road Oxford, OX3 7LC United Kingdom	Prof. M. Feldmann. Dr. R. O'Williams Beneficiary starting date 01.08.2011 (see beneficiary 3)