Summary

SCOPE OF THE THESIS

Multiple Sclerosis (MS) and Rheumatoid Arthritis (RA) are complex diseases that are heterogeneous in nature. Variability in drug-responsiveness exemplifies this heterogeneity and suggests that for both MS and RA, underlying disease mechanisms may vary among patients. It appeared that differential activity of the type I interferon (IFN) pathway is present in a subset of patients with several autoimmune diseases and is characteristic for the disease heterogeneity. Here we aimed to study the role of IFN activity in relation to IFNβ therapy in MS and B-cell depletion therapy via rituximab (RTX) in RA. Besides the relation of IFN activity to treatment outcome, we also studied the role of type I IFN activity in autoimmunity in relation to pathogenic or clinical characteristics. In RA we studied the relation of *IRF5*-genetics to cardiovascular disease, since it is well known that IFNβ is an important mediator in this process. In patients with Inflammatory Idiopathic Myopathies, the relation between autoantibodies that play an important role in this disease, and type I IFN activity was studied. Increased understanding of the molecular base of drug responsiveness will ultimately enable improved treatment strategies and personalized medicine in these diseases.

MOLECULAR MARKERS FOR TREATMENT RESPONSE IN MULTIPLE SCLEROSIS AND RHEUMATOIDE ARTHRITIS

Given the destructive nature of MS and RA, it is highly desirable to predict in an early stage the most beneficial treatment for the patients at risk. If we rely solely on clinical or (neuro) degenerative manifestations we will probably act to late in order to maximize protection. Unfortunately, criteria to make selections of patients for optimal treatment are currently lacking. Such criteria would be highly beneficial to increase the likelihood of a beneficial response. Ideally, it would be desirable to make predictions on the success before the start of therapy. Ultimately, this may lead to a personalised form of medicine, whereby a specific therapy will be applied that is best suited for an individual patient.

With the aid of genomics technology, we are now in a position to provide sufficient knowledge to determine the pretreatment status and pharmacodynamic outcomes of treatment in complex and heterogeneous diseases such as MS and RA. Pharmacogenomics of

IFNβ and RTX therapy in respectively MS and RA, as described in this thesis, has considerably advanced our understanding of the pharmacological effects these treatment strategies and resulted in promising candidate markers for prediction of treatment response. In **chapter 1.1**, genome wide transcription profiles of MS patients starting on IFNβ treatment were studied. A subgroup of patients revealed a pharmacodynamic increase of transcription levels of type I IFN pathway related genes ("the type I IFN system") after the start of IFNβ treatment. Surprisingly, an increased activity of the type I IFN system was already observed in a subset of patients prior to treatment and it was shown that IFNβ treatment did not lead to a further increase of the IFN system, indicative of a saturated IFN system at baseline. Although we were not able to link the pharmacological response or baseline type I IFN signature to the clinical response outcome in this patient cohort, it was successively demonstrated by others that type I IFN response genes at baseline were predictive for the clinical outcome of IFNβ treatment in RRMS. As anticipated from our pharmacodynamic study, non-responders were characterized by an increased expression of type I IFN response genes at baseline. Additionally, in **chapter 1.2** a genetic explanation for the dysregulated type I IFN signature was described. Genetic variation in components of the upstream type I IFN pathway was studied for a relation with the (dysregulated) type I IFN signature. It was shown that genetic variation in the gene encoding interferon regulatory factor 5 (*IRF5),* a master regulator of the IFN system, was associated with activation of the type I IFN pathway during IFNβ treatment. Furthermore, this *IRF5* genetic variant associates to non-response as determined by several clinical parameters, including MRI-based outcome measures. Altogether, these results are promising but warrant further studies to demonstrate the clinical utility of the IFN signature as possible biomarker to predict the response to IFNβ treatment.

In **chapter 2.1** transcriptomics are used to understand molecular heterogeneity in RA patients receiving RTX treatment. Analysis of transcription profiles during treatment revealed that RTX-associated changes in type I IFN related genes are related to treatment outcome. Patients with increased activation as a consequence of RTX treatment appear to be good responders, whereas patients without (further) increase under RTX treatment are non-responders. In addition, it was observed that baseline transcriptional activity of the type I IFN pathway also relates to treatment outcome, e.g. high transcriptional activity at baseline predicts non-response whereas no or low transcriptional activity predicts good response. In **chapter 2.2**, the predictive and clinical value of the increased baseline type I IFN pathway activity in RTX non-responders was demonstrated and validated. The findings on the correlation between the IFN system and clinical outcome add new information to our understanding of the mechanism that underlie the clinical outcome of rituximab treatment. Furthermore, it provides a basis for the clinical utility of the IFN-system as biomarker for prediction of clinical response to RTX, and eventually effective dosing and timing of treatment towards patient-tailored treatment in RA. Further validation of the clinical utility

of this biomarker is necessary, eventually in combination with other biomarkers and/or clinical variables, in a multicenter setting using prospective studies. Ultimately, these results may provide the basis for a method that can be implemented in clinical practice to meet the unmet need to subscribe the most effective therapy for a particular patient.

Conclusively, these chapters illustrate that it indeed is feasible to extract prognostic markers for treatment outcome from genomic profiles.

TYPE I IFN SYSTEM IN RELATION TO DISEASE PARAMETERS IN RA

In **chapter 1.2** genetic variation in *IRF5* was shown to be associated to the activation of the type I IFN pathway during IFNβ treatment. Furthermore, this *IRF5* genetic variant associates to non-response as determined by several clinical parameters, including MRI-based outcome measures. Besides of being an attractive, easy to determine candidate as response marker for IFNβ treatment in MS, the association between *IRF5* genetics and the type I IFN activity in SLE and MS inspired us to use it as a surrogate marker to study type I IFN activation in patient cohort where only DNA, and no RNA, samples were available. In RA inflammatory processes appear to be important in the increased cardiovascular morbidity and mortality although the exact mechanism remains unknown. Since IFNβ is known for its vascular protective role, the relation between *IRF5* genetics and carotid intima media thickness, (cIMT), a marker for atherosclerosis, was studied in **chapter 3.1** It was shown that genetic variation in *IRF5* was related to decreased cIMT in RA patients, possibly via increased expression of IFNβ. Although the precise mechanism on how *IRF5* and possibly IFNβ attenuates CV risk in RA needs to be further delineated, these results are a first step towards better understanding of the type I IFN system in CD in RA.

THE PATHOGENIC ROLE OF TYPE I IFN ACTIVITY IN AUTOIMMUNE DISEASES

The heterogeneous gene expression profiles, especially with respect to type I IFN activity, of patients with clinically defined similar diseases are an exponent of the different intrinsic modes of immune status that may underlie these diseases. It also makes more evident the complexity of the diseases and the relation to therapy responsiveness. To better understand the possible role of type I IFNs in the pathology of autoimmune diseases, systemic lupus erythematosus (SLE) can be considered as an example. Pathogenesis of SLE is partly driven by IFNα. The production of IFNα seems to be the result of a continuous activation of plasmacytoid dendritic cells (pDCs) by immune complexes (ICs), consisting of autoantibodies directed against nucleic acids such as anti-ds/ssDNA and anti-RNA, and nucleic acids binding proteins such anti- snRNP in combination with RNA-containing autoantigens. These interferogenic ICs are internalized via the FCγRIIa expressed on pDCs, reach the endosomes and stimulate toll-like receptor (TLR) 7 or 9, which subsequently leads to IFNα gene transcription. Additionally, genetics of, among others, *IRF5* are related to development and

severity of SLE and might be partly responsible for the differential activation of type I IFNs.

The type I IFN signature was also described in a subset of patients with idiopathic inflammatory myopathies (IIM) and there are some similarities to SLE, e.g. the correlation between IFN signature and disease activity and the presence of autoantibodies directed against RNA-binding protein complexes. In **chapter 3.2** we elaborated on these parallels between SLE and IIM by studying the relation between autoantibody profiles in IIM and the type I IFN signature. We showed that there is indeed a relation between presence of autoantibodies directed against RNA-containing proteins and activation of the type I IFN pathway in IIM. Furthermore, we showed that the serum of a subset of IIM patients activated the type I IFN pathway in healthy PBMCs and that this effect could be blocked by adding anti-IFNα to the bioassay, suggesting a role for IFNα in IIM. This adds to the list of similarities between IIM and SLE with respect to the role of type I IFNs and suggest similar underlying pathogenic mechanisms. Furthermore, it is in line with the heterogeneity in IIM, which is observed at several levels such as autoantibody profiles and clinical and histopathological features, and suggest that different disease mechanisms are in play in different subgroups of myositis. Subclassification based on type I IFN gene expression profiles may lead to improved understanding of these patterns of variation and underlying disease mechanisms.