Scope of the thesis
Rheumatoid arthritis (RA) is a challenging disease: due to its heterogeneous nature, there is large variability in disease severity, symptoms and response to therapy. Insight into the activated pathways in RA subgroups and/or predictors of therapy response is of vital importance to unravel its pathology and ultimately enable personalized treatment strategies for RA patients. Type I interferon (IFN) activity has proven to play an important role in rheumatoid arthritis and other autoimmune diseases. Approximately 50% of RA patients display a type I IFN signature, which has shown to be associated to a poor response to rituximab treatment. The research presented in this thesis was aimed to gain more insight into the clinical utility of the type I IFN signature in RA, as well as into the potential mechanisms behind the type I IFN signature in RA and other autoimmune diseases.

Clinical characterization of the type I IFN signature in rheumatoid arthritis
The first part of this thesis, which was described in Chapter 2, was focused on the potential clinical applicability of the IFN signature in RA.

One question that has been lingering since the IFN signature was described in RA for the first time in 2007, is whether it would be related to clinical parameters, as it appeared to represent a subgroup of patients. In Chapter 2.1, we investigated the association of the IFN signature with disease activity, inflammation parameters, erosive disease and antibody positivity, which did not reveal any significant associations. We did, however, observe downregulation of the signature upon treatment with hydroxychloroquine (HCQ), sulphasalazine (SSZ) and prednisone (PREDN).

Chapters 2.2 and 2.3 were aimed to optimize the previously described type I IFN-based prediction of the response to rituximab treatment in RA patients. In Chapter 2.2, we studied the effect of prednisone use on this prediction. Interference between glucocorticoids (GCs), such as prednisone, and type I IFN signaling has previously been demonstrated in vitro, and since the use and dose of oral GCs is highly variable among RA patients prior to the start of treatment with rituximab, we determined what the effect of GC use was on the type I IFN response gene (IRG) expression in relation to the clinical response to rituximab.

We demonstrated that expression of the 8 predictive IRGs was suppressed in RA patients who used prednisone at the time of blood collection. This suppression was dependent on prednisone use and most prominent in patients using ≥10mg/day prednisone. Consequently, the predictive performance of the IRG profile was lower in prednisone users (PREDN+) compared to RA patients without prednisone use (PREDN-); receiver operating characteristics (ROC) analysis in PREDN+ patients resulted in an area under the curve (AUC) of 0.80, whereas an excellent AUC of 0.98 was reached for PREDN- patients. Using an IRG expression cutoff that would result in correct classification of all responders, 92% of the PREDN+ patients were correctly classified, compared to only 63% of the PREDN- patients. In conclusion, these findings indicate that the IFN-signature-based rituximab response prediction could be optimized by stratification of prednisone use.
Chapter 2.3 was focused on the identification of clinical parameters that could predict the non-response to rituximab therapy in addition to the type I IFN response gene profile. This study resulted in a multi-parameter model containing 3 parameters – baseline disease activity score (DAS28), positivity for IgM-rheumatoid factor (IgM-RF) and antibodies against citrullinated proteins (ACPA) and the type I IFN response gene profile –, which displayed a good performance (AUC 0.84). The combination of predictive parameters greatly improved the prediction compared to prediction based on each parameter alone. However, upon validation in an independent cohort, we observed that prednisone again appeared a disturbing factor in the IFN-related response prediction. The validation cohort only contained PREDN+ patients whose dose had been stable for at least 4 weeks, whereas this was not the case in the test cohort, implying that these patients might have reached higher cumulative doses of prednisone than the patients in the test cohort. We hypothesize that the effect on the IFN signature would depend on cumulative dosing, i.e. the dose combined with the duration of treatment.

Altogether, presence of the IFN signature does not seem to reflect a certain disease subtype or state based on clinical parameters. However, it is related to a poor response to rituximab, which might be clinically applicable to predict non-responders prior to start of treatment. In order to achieve this, combination with other predictive (clinical) parameters and exact definition of the suppression by prednisone are highly important.

Molecular characterization of the type I IFN signature in rheumatic diseases

Besides clinical applicability of the type I IFN signature in rheumatoid arthritis, the exact mechanism behind this signature is largely unknown. More insight into the mechanism of type I IFN activity in RA could provide information about its role in RA pathology and subsequently the relation to response to rituximab treatment. The three chapters of this part approached this issue from different perspectives, ranging from up- to downstream IFN signaling; whereas in Chapter 3.1 the upstream activation of the IFN signaling pathway was studied, Chapter 3.2 was focused on the identification of the cell type(s) in which this signaling would take place, and in Chapter 3.3 we investigated the composition of the IFN response gene program itself.

In Chapter 3.1, we studied the ability of RA patient serum to induce a type I IFN response in healthy control peripheral blood mononuclear cells (PBMCs) and compared this to the well-characterized IRG-inducing ability of serum from SLE patients. This information could provide more insight into the source of induction of the IFN response gene program in RA.

The exposure of healthy control PBMCs to patient sera revealed essential differences in IRG induction by RA and SLE serum. As previously described, serum from the majority of SLE patients caused a rapid IRG induction that appeared directly mediated by IFNa. Serum from RA patients, on the other hand, displayed IRG induction that was considerably slower (after 8 hours of incubation instead of 4 hours) which did not require IFNa activity but rather seemed to occur via another, indirect signaling route. This indicates that the source of the type I IFN response in RA is different from SLE. As expected, we observed that the IFN response induced by SLE serum
was associated to positivity for antinuclear antibodies. However, virtually all RA patients were negative for these antibodies, and their IRG induction was not correlated to positivity or titers of RA-related antibodies, such as rheumatoid factor or antibodies against citrullinated proteins either. Conclusively, we have demonstrated that serum from a part of the RA patients contains an IRG-inducing factor. Up until now, we know that the mechanism of IRG induction differs from that in SLE patients, but more detailed characterization requires follow-up experiments.

In Chapter 3.2, we investigated the contribution of the major peripheral blood leukocyte subsets to the type I IFN signature in peripheral blood from RA patients. This revealed that the signature is present in all subsets, but that it was most pronounced in the polymorphonuclear leukocyte, or granulocyte (PMN) fraction. This fraction displayed higher induction than CD4⁺ T cells, CD8⁺ T cells, monocytes and CD19⁺ B cells, which could not be solely explained by the high abundance of PMNs in blood. Moreover, we observed elevated expression of the type I IFN receptors IFNAR1 and IFNAR2 in PMNs isolated from RA patients compared to PMNs isolated from healthy controls, whereas this was not the case for the RA PBMCs compared to healthy control PBMCs.

Altogether, these data indicate that RA PMNs are the main contributors to the IFN signature, which appeared to be caused by an increased sensitivity towards type I IFN signaling compared to other leukocyte subsets.

In Chapter 3.3, we studied the gene composition of type I IFN signatures in several autoimmune diseases. In systemic lupus erythematosus (SLE), it is known that the type I IFN signature is the result of predominant IFNα activity. Comparison of the IFNα-induced response gene profiles of SLE patients to those of IFNβ-treated MS patients resulted in the identification of gene profiles that reflect IFNα-dominant signatures (gene cluster (GC-) A) and IFNβ-dominant signatures (gene cluster (GC-) B). Using the log-ratio between these two profiles resulted in excellent separation of SLE patients and IFNβ-treated MS patients. These analyses were extended to myositis patients, IFNβ-naive MS patients and RA patients, which revealed that these diseases also displayed differences in the IFNα- and/or IFNβ dominance. All myositis patients showed a type I IFN response profile similar to the SLE patients, indicating IFNα dominance. MS patients, on the other hand, displayed large inter-individual variability; some patients displayed slight IFNα dominance, whereas others showed IFNβ dominance, or the GC-A/GC-B log-ratio approached zero, indicating similar contributions of both IFNα and IFNβ. RA patients displayed slight IFNα dominance in most patients, though less pronounced than SLE and myositis patients. The GC-A/GC-B log-ratios were closer to zero, which suggests involvement of both IFNα and IFNβ in RA. In conclusion, this study demonstrated that the IFN signatures display distinct differences between autoimmune diseases. Considering the pro-inflammatory nature of IFNα in SLE and the anti-inflammatory role of IFNβ in MS, specification of the type I IFN response in autoimmune diseases might give new insights into its role in pathology and/or its therapeutic potential.

In summary, the IFN signature is a biomarker for RA that shows potential clinical applicability provided that the interference by suppressive treatment is taken into account. Furthermore, the exact mechanism behind the IFN signature in RA remains to be unraveled, but we established that it appears different from SLE and myositis and mainly originated from peripheral blood granulocytes.