Chapter 9

General discussion and summary
This thesis is dedicated to studies in psoriatic arthritis (PsA), and focuses on the peripheral arthritis of PsA, the synovial infiltrate, and the effect of therapeutic interventions.

**Background**

With the introduction of novel targeted treatments, the so-called biologicals, the treatment of PsA has dramatically improved for many patients. Blocking tumor necrosis factor alpha (TNFα) by a monoclonal antibody or soluble receptor is effective for 60-70% of patients with PsA, who experienced persistent active arthritis despite treatment with conventional disease-modifying antirheumatic drug (DMARD) therapy (1-4). Since not all patients with PsA benefit from conventional DMARD treatment or treatment with TNFα blocking therapy due to primary lack of response, loss of the initial response, or side effects, there is still a need for new and better treatment options for these patients. New drugs, directed against TNFα or other potential targets, are being developed.

The discovery and development of compounds with potential therapeutic effect includes several phases. The preclinical phase involves identification of a potential target, and in vitro and in vivo testing of a (therapeutic) intervention. After studying in vitro and in vivo effects, using animal models of a disease, the next step is to test the compound in patients with active disease, to study clinical efficacy and safety (adverse effects) of the compound.

The recent rise in new drugs discovered may have consequences for the way these potential novel therapies are tested in patients. This used to be done in relatively large, placebo-controlled proof of concept clinical trials. It is becoming increasingly difficult, however, to include a large number of patients with active disease in these trials, because of the growing number of compounds to be tested, and the fact that effective treatment is available for many patients nowadays. Therefore, in an early stage of drug development it would be favourable to test potential drugs in a short intensive proof-of-principle trial for selection purposes, using a small number of patients in which a large amount of data is collected. If no clinical or biological effect is found in such a trial, the compound appears less likely to be effective in a larger clinical trial of longer duration. If, on the other hand, there is a clear clinical and/or biological effect, the compound may be effective, and could be considered for conventional phase 2 clinical trials to determine whether these effects appear clinically meaningful. The crucial question, of course, is which biological data should be collected in such a small intensive proof-of-principle trial alongside clinical data.
Main findings

In **Chapter 1** a general introduction is given to the clinical entity PsA. An overview is presented on the heterogeneous clinical manifestations, development of classification criteria, pathogenesis and current treatment options.

**Chapter 2** describes a detailed immunohistochemical (IHC) analysis, comparing the synovial tissue (ST) of 19 PsA patients with 24 rheumatoid arthritis (RA) patients, who were matched for disease duration and medication. ST biopsy specimens were obtained by arthroscopy from an inflamed joint. The ST biopsies were analysed using IHC staining to detect T cells, plasma cells, fibroblast-like synoviocytes, macrophages, several pro-inflammatory cytokines, matrix metalloproteinases (MMPs), adhesion molecules and vascular markers, and subsequently evaluated by digital image analysis (DIA). It was shown that the synovial infiltrate of PsA and RA was largely comparable with regard to the cellular infiltrate. Remarkably, only T cell numbers were significantly lower in PsA synovium. The expression of several pro-inflammatory cytokines, including TNFα and interleukin (IL)-6, was as high in PsA as in RA, as was the case for MMPs, adhesion molecules and vascular markers. We conclude that there are mainly similarities in the synovial infiltrate of two well-matched cohorts of patients with RA and PsA, supporting the view that, in addition to TNFα blockade, targeted treatments against other pro-inflammatory cytokines such as IL-6 might perhaps be effective in PsA as well.

In **Chapter 3** the expression of TNF like weak inducer of apoptosis (TWEAK) and its receptor Fn14 is studied in ST of patients with inflammatory arthritis. The rationale for this was presented by two independent groups that showed that blocking TWEAK using a monoclonal antibody has a beneficial effect on arthritis in an animal model. For our study, ST biopsies were obtained from RA and PsA patients, who were matched for disease duration and treatment. Serial ST samples were obtained from a separate cohort of 13 RA patients before and after infliximab treatment. TWEAK and Fn14 were clearly expressed in ST of PsA and RA patients, although TWEAK expression was significantly higher in RA synovial sublining and intimal lining layer compared to PsA. Fn14 expression was comparable in both diseases. Double immunofluorescence showed TWEAK and Fn14 expression on fibroblast-like synoviocytes and macrophages, but not T cells. There was no change in the expression of TWEAK and Fn14 after TNFα blocking therapy. It was concluded that TWEAK and Fn14 are abundantly expressed in the inflamed synovium of RA and PsA. This indicates that there is a possibility that blocking TWEAK/Fn14 signalling might be a potential therapeutic target in human inflammatory arthritis.
In Chapter 4 we present the results of a randomised controlled clinical trial, evaluating the clinical effect of combining 2 classical DMARDs: the safety and efficacy of adding ciclosporin A (CSA) to the treatment of patients with PsA and an incomplete response to methotrexate (MTX). In this 12 month, randomised, double blind, placebo controlled trial, conducted at five centres in three countries, 72 patients with active PsA were randomised to receive either CSA (n = 38) or placebo (n = 34). In addition to clinical assessments, high resolution ultrasound (HRUS) was performed at one centre (Leeds). Significant improvements were noted at 12 months in the active treatment group, but also in the placebo group, with both groups achieving the primary endpoint (improvement of the modified Ritchie articular index). However, in the active but not the placebo arm there were significant improvements in swollen joint count, and C reactive protein as compared with baseline. The Psoriasis Area and Severity Index (PASI), although in the low ranges, improved in the active group as compared with placebo (p<0.001), and synovitis detected by HRUS (33 patients, 285 joints) was reduced by 33% in the active group compared with 6% in the placebo group (p<0.05). Fewer patients in the MTX/CSA group completed the study than in the MTX/placebo group and there were more drug related adverse events in the MTX/CSA group than in the MTX/placebo group. We concluded that combining CSA and MTX treatment in patients with active PsA and a partial response to MTX, improves the signs of inflammation, and may be a valid therapeutic option in patients that tolerate the combination.

As discussed before, it is increasingly difficult to include large numbers of patients with active disease into placebo-controlled trials, because of the growing number of compounds to be tested, and the fact that effective treatment is available for many patients nowadays. Since synovial inflammation is one of the key manifestations of PsA, we designed a study to identify synovial biomarkers associated with effective treatment. Sensitive synovial biomarkers could then be used as predictors for clinical effect in small proof-of-principle trials. This study is presented in Chapter 5, for which adalimumab was chosen as a proven effective therapy. A total of 24 patients with active PsA were randomised to receive either adalimumab (n = 12) or placebo (n = 12) for 4 weeks, followed by an open-label extension phase in which all patients received adalimumab. Serial ST biopsies were obtained before and after 4 weeks of treatment. IHC analysis was performed to characterise the cell infiltrate, expression of cytokines, MMPs and vascularity, and statistical analysis was performed using covariance analysis. The mean Disease Activity Score in 28 joints (DAS28) after 4 weeks was 1.92 units lower (95% confidence interval (CI) 1.07 to 2.77) after adalimumab therapy compared with placebo. Paired pre and post treatment ST samples were available from 19 patients. Many cell types were reduced after adalimumab treatment compared to placebo. After applying a ranked analysis of
covariance (ANCOVA) model, which corrects for baseline imbalances, a significant effect of treatment was observed on CD3-positive cells (T cells), and on the expression of MMP13; both were significantly reduced after active treatment. This suggests that these parameters, a reduction in synovial expression of CD3 and MMP13 following treatment, could be used as synovial biomarkers that are sensitive to change after active treatment in PsA. Other studies have confirmed the changes in synovial T cells after infliximab and etanercept treatment, respectively (5;6). This finding should be confirmed in other trials in PsA patients, using drugs with another mechanism of action to determine whether this is related to interference with a common final pathway.

In **Chapter 6** a similar approach was used to detect early changes in serum levels of several candidate biomarkers involved in cartilage and bone metabolism. Serum samples were obtained from the 24 active PsA patients (described in Chapter 5) at baseline and after 4 and 12 weeks of treatment. The concentration of the following markers was determined: CPII and PINP (synthesis of type II and type I procollagen), melanoma inhibitory activity (MIA) (chondrocyte anabolism), MMP-3, C2C and cartilage oligomeric matrix protein (COMP) (type II collagen degradation), osteocalcin (OC) (bone formation), NTX-I and ICTP (both type I collagen degradation). After 4 weeks of treatment a significant decrease in serum MMP-3, and a significant increase in serum MIA levels was noted in adalimumab treated patients, while no change was observed in the placebo group. After 12 weeks there was a marked reduction in serum MMP-3 in all adalimumab treated patients. Other biomarkers were unaltered compared to baseline. This suggests that MMP-3 and MIA, being soluble biomarkers associated with cartilage metabolism, may assist in distinguishing between effective and ineffective therapy in small, proof-of-principle studies of short duration in PsA.

In **Chapter 7** the model of using synovial biomarkers to predict clinical effect is applied in patients with PsA using open-label alefacept (a fusion protein that blocks a costimulatory signal for T cell activation at the LFA-3/CD2 interaction). Eleven patients with active PsA were treated with iv alefacept for 12 weeks in this open-label and explorative study. Serial ST biopsies from an inflamed index joint were obtained by arthroscopy at baseline and after 4 and 12 weeks of treatment. At the completion of treatment, 6 of 11 patients (55%) fulfilled the Disease Activity Score (DAS) response criteria. There was a statistically significant reduction in synovial CD4+ and CD8+ T lymphocytes after 4 and 12 weeks, and a significant reduction of CD68+ macrophages in the ST after 12 weeks of treatment. The ST and peripheral blood of those patients fulfilling the DAS response criteria contained more CD45RO+ cells at baseline and displayed a significant reduction in these cells compared with non responding patients. The changes in ST after treatment
with alefacept, together with the improvement in clinical scores, support the hypothesis that alefacept could be an effective treatment in PsA, and that T cell activation plays an important role in PsA. Furthermore, since alefacept, a T cell-specific agent, led to reduced macrophage infiltration after 12 weeks of treatment, the data indicate that memory T cells seem to be involved in organizing the synovial inflammation in PsA. The efficacy of alefacept was more recently confirmed in a conventional phase 3 study (7).

As indicated before, most patients with PsA respond well to treatment with TNFα blocking therapies, e.g. most of the 24 patients studied in Chapters 5 and 6 demonstrated a swift clinical improvement after initiation of adalimumab. However, some of the patients do not respond well or lose their initial good response. An explanation for the latter phenomenon could be a lower adalimumab concentration caused by the development of anti-adalimumab antibodies, which has been reported in RA. In Chapter 8 the incidence of anti-adalimumab antibodies in PsA was studied, as well as the relationship with serum adalimumab concentration and clinical response. Serum samples were collected just before the next injection with adalimumab in 22 patients with PsA who started adalimumab treatment. The DAS28 was chosen to monitor clinical disease activity, and EULAR response criteria were applied. At 3 months there were 12 moderate and 10 good responders. At 12 months there were 4 non-responders, 8 moderate and 10 good responders. In 4 patients (18%) anti-adalimumab antibodies were detected at any time point. Patients with anti-adalimumab antibodies had significantly lower median adalimumab concentrations than those without. Adalimumab concentration was undetectable in the 2 patients with a high titre of antibodies (>100 AE/ml) at 12 months. In PsA patients treated with adalimumab, anti-adalimumab antibodies seem to develop in a minority of PsA patients, but they are associated with lower serum levels of adalimumab and a diminished clinical response to treatment.

Concluding remarks

Taken together, there have been major advances in understanding the pathophysiology of PsA and improvement in treatment of PsA patients over the last decade. In addition to an expansion of treatment options, the development of new targeted biologic drugs has led to an acceleration of clinical and preclinical research, as well as an increased interest in PsA and the field of rheumatology as a whole. The success of TNFα blocking therapy has resulted in better treatment and provided proof that targeted therapies may change the life of patients in a very positive way. This has led to research aimed at discovery of an array of new possible therapeutic targets.
The detailed analysis of RA and PsA synovium presented in this thesis, showed mainly similarities: the synovial infiltrate was largely comparable with regard to the cellular infiltrate, the expression of several pro-inflammatory cytokines, MMPs, adhesion molecules and vascular markers. Although there are clear clinical, and probably also pathophysiological, differences between both diseases, this finding suggests that synovitis in advanced inflammatory diseases may follow a common final pathway. This may also have clinical implications for the treatment of our patients, usually with advanced disease. As a potential new target for treatment, we demonstrated that TWEAK and its receptor Fn14 are expressed in RA and PsA synovitis, and that this expression is unaltered after TNFα blocking therapy. Whether blocking the TWEAK/Fn14 pathway will be as effective in human inflammatory joint disease as was demonstrated in a murine model, will have to be studied.

It has long been recognized that T cell targeted therapy, e.g. ciclosporin A (CSA), is very effective in the treatment of psoriasis. In this thesis 2 chapters are dedicated to the effects of T cell directed therapy in PsA. Although the first study was hampered by a lack of power, and a high percentage of patients achieving primary endpoint in the placebo group, it was shown that addition of CSA to treatment with MTX in patients with PsA improves the signs of inflammation (swollen joint count, CRP and synovitis detected by ultrasound), and that CSA, if tolerated, may be a valid therapeutic option in patients with an incomplete response to MTX. The effects of another T cell targeted therapy, alefacept, were studied on clinical symptoms and the synovial infiltrate of PsA. Alongside clinical improvement, we found a significant reduction of synovial T cells after 4 weeks of treatment, followed by a reduction in synovial macrophages after 12 weeks. These findings underline the important role of T cell activation in PsA, and because treatment with a T cell-specific agent led to a subsequent reduced macrophage infiltration, it also indicates that (memory) T cells seem to be involved in organizing the synovial inflammation in PsA. Interestingly, we also found that a reduction in synovial CD3+ T cells correlated best with clinical improvement after TNFα blocking therapy, indicating that the reduction in T cells, observed after different types of therapy, may be related to interference with a common final pathway (4;5). This underlines the potential of this marker, a reduction of synovial T cells after therapy, to act as a candidate biomarker for efficacy on the group level.

The rise in new drugs discovered faces us with new challenges. As it turns out, not all patients respond to the new therapies available now, or lose their initial good response. As demonstrated in chapter 8 the latter phenomenon may partly be explained by the development of antibodies against these therapeutic proteins, a new problem that we will have to deal with. Obviously, there is still an urge to find better drugs to treat the patients that are not responding to traditional DMARD or present biological therapies. It also raises
the need to reconsider the way these potential novel therapies are often tested in clinical trials. It will be increasingly difficult to include hundreds of patients with active disease into large placebo-controlled trials for all these potential novel drugs. As a selection mechanism in an early stage of human drug development, intensive trials with a small number of patients in which a large amount of data is collected to study the effects of the compound tested, could be a solution to screen for possible efficacy. This approach could be used to discontinue development when there is no robust change in biological and/or clinical parameters in a small proof-of-principle study. The identification of biomarkers that could be used in this context is of pivotal importance. In these proof-of-principle studies three types of data are obtained: 1. clinical data, 2. biomarker data related to the mechanism of action of the compound tested (obtained from ST, serum or skin in PsA and psoriasis), 3. biomarker data unrelated to the direct mechanism of action, but related to common final pathways associated with clinical improvement (e.g. reduction of CRP in serum). In this thesis, an attempt was made to identify potential biomarkers in ST and serum that could be used to facilitate early go/no-go decisions. Synovial T cells and MMP13 appear candidate synovial biomarkers that could be used for this purpose in PsA. In addition, serum MMP3 and MIA might be relevant candidate biomarkers related to cartilage metabolism. While the need for sensitive biomarkers is clear, future studies in other cohorts of PsA patients should be conducted to confirm whether these biomarkers will be the most suitable for the prediction of potential efficacy.
Reference List


