Biologics in Psoriasis;
a Step towards Individualized Treatment

Ji Sun van Bezooijen

Layout: Optima Grafische Communicatie, Rotterdam, the Netherlands (www.ogc.nl)
Printed by: Optima Grafische Communicatie, Rotterdam, the Netherlands (www.ogc.nl)

ISBN: 978-94-92683-18-2

Biologics in Psoriasis;
a Step towards Individualized Treatment

Biologics in psoriasis;
een stap richting geïndividualiseerde behandeling

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. H.A.P. Pols
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 24 mei 2017 om 13:30 uur

door

Ji Sun van Bezooijen
geboren te Seoul (Zuid-Korea)

Erasmus University Rotterdam

PROMOTIECOMMISSIE:

Promotoren:
Prof.dr. T. van Gelder
Prof.dr. E.P. Prens

Overige leden:
Prof.dr. T.E.C. Nijsten
Prof. dr. Ph.I. Spuls
Prof.dr. P.M. van Hagen

Copromotoren:
Dr. M.B.A. van Doorn
Dr. M.W.J. Schreurs

This skin is me, I can't get out.
—John Updike

CONTENTS

Chapter 1

General introduction and outline of the thesis

Based on: Ther Drug Monit. 2013;35:588-94.

GENERAL INTRODUCTION

Psoriasis is a common skin disease that affects all age groups and leads to substantial impairment of quality of life. In general, psoriasis is a lifelong disease that not only involves a significant burden for the individual patient but also has a substantial economic impact on society through absenteeism from work, costs of visits to the outpatient clinic, medication and hospitalizations. In the last decade, the treatment of psoriasis has undergone a major revolution. The arrival of biologics has dramatically changed the management, the burden and the outlook for patients with psoriasis. Despite the efficacy, good safety profile and overall treatment satisfaction of these drugs, several difficulties in clinical practice exist, including primary non- response and loss of response over time. Guidelines for the treatment of psoriasis with biologics recommend the same dosing for all patients ('one size fits all') regardless of specific patient characteristics including disease activity, age and treatment history. Off-label treatment with biologics, including different doses and variable dosing intervals is common in clinical practice but lacks sufficient evidence so far. In this introduction, an outline of the current view on psoriasis and issues associated with the treatment of biologics are presented.

CLINICAL FEATURES AND STRATIFICATION OF PSORIASIS

The most common phenotype of psoriasis (90%) is plaque psoriasis, also known as psoriasis vulgaris. Plaque psoriasis is characterized by red sharply demarcated indurated plaques covered with silvery scales (Figure 1). Preferred locations are the extensor sides of the elbow and knees, the scalp and lower back (1). Psoriasis usually affects these predilection areas, but in more severe cases, it can involve the entire surface of the skin. When the latter is the case, hospitalization may be necessary.

Other, less common types of psoriasis include guttate (small drop-like maculae on the body instead of plaques, sometimes triggered by a streptococcal infection), inverse (intertriginous areas or genital areas are affected), pustular (coalescing pustules on specific regions or generalized, in which case it is known as 'Von Zumbusch') and erythrodermic psoriasis. Nail involvement is common in psoriasis, causing nail pitting, onycholysis, discoloration, salmon-pink and oil pots, and also subungual keratosis (2). Importantly, in psoriasis patients the prevalence of arthritis ('psoriatic arthritis'; PsA) is estimated to vary from 6 to 39% (3). Untreated PsA can lead to irreversible damage to the joints.

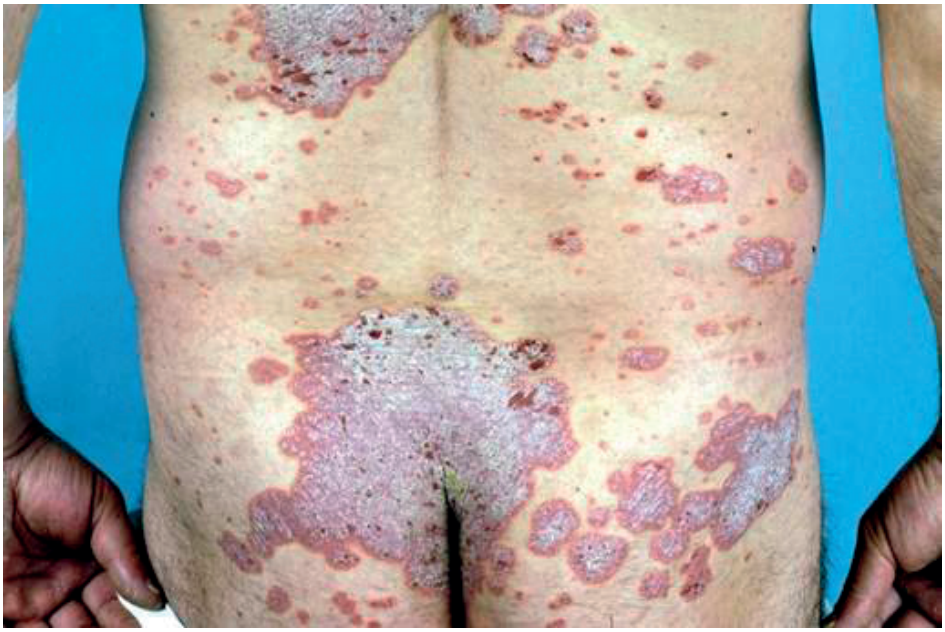

Figure 1. Clinical presentation of psoriasis.

PSORIASIS EPIDEMIOLOGY

The prevalence of psoriasis ranges from 2 to 3% of the Caucasian population in Western countries (4, 5). The exact prevalence and incidence rates vary depending on age, race, geographic region, environmental factors and genetics. The prevalence of psoriasis is generally higher in geographic regions further from the equator (6). In certain ethnic groups, such as the Inuit, Japanese, aboriginal Australians and Indians from South America, the prevalence is lower (7-9). The incidence of psoriasis is higher in adults than in children, showing one peak at the age of 30 years and another peak between 50 to 60 years (10-12). The annual incidence of psoriasis almost doubled between the 1970s and 2000. It is unclear whether this is a true change in incidence or represents changes in the registration of diagnoses (13).

GENETICS

The incidence of psoriasis is significantly higher among relatives of psoriasis patients as compared to the general population (14). The concordance of psoriasis in monozygotic twins is 35-73% and in dizygotic twins 12-20% (11). Currently at least 45 susceptibility loci for psoriasis have been identified (15). Early onset psoriasis has been associated with HLACw6 a variant that lies in the PSOR1 region on chromosome 6 (16). The current view on psoriasis

pathogenesis is that several single-nucleotide polymorphisms (SNP's) lead to dysfunctional molecular pathways that affect the innate and adaptive immune response, epidermal proliferation and the formation of a normal skin barrier (11, 17). Many risk loci of psoriasis are also associated with other immune-mediated diseases including rheumatoid arthritis (RA), ankylosing spondylitis, and Crohn's disease.

PATHOPHYSIOLOGY OF PSORIASIS

Psoriasis is an immune-mediated inflammatory disease (IMID) involving T-cell activation, cytokine production and epidermal hyperplasia (Figure 2). The innate and adaptive immune systems are both involved in the development of psoriasis. The immune activation in psoriasis can be caused by a combination of environmental triggers including stress, specific drugs (e.g. beta-blockers, lithium), physical trauma (Koebner phenomenon), smoking and alcohol.

In psoriatic skin, the keratinocytes show an increased production of antimicrobial peptides (AMP), including LL-37, hBD-2 (β-defensin) and S100A7 (psoriasin) (5). Antimicrobial

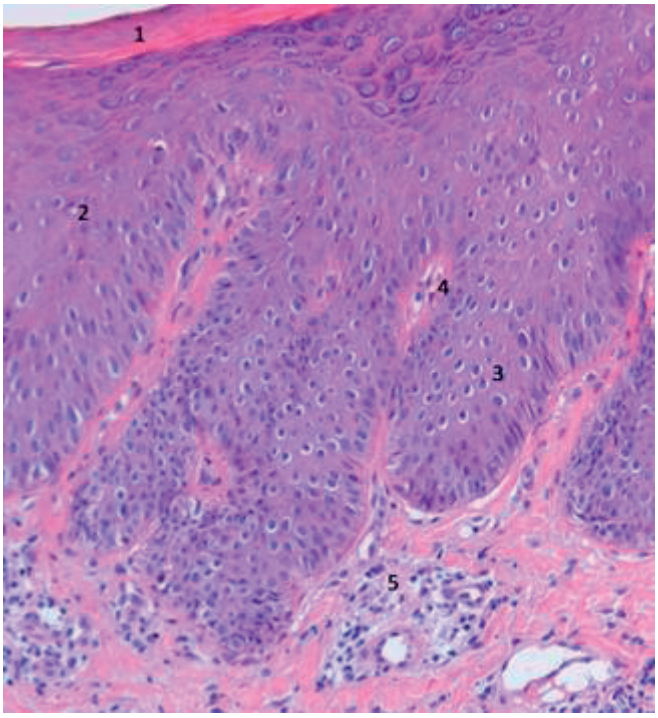

Figure 2. Histopathology of psoriasis. 1) hyperkeratosis and parakeratosis. 2) increased epidermal thickness (acanthosis). 3) epidermal elongations into the dermis (rete ridges). 4) elongated tortuous blood vessels, with neutrophil extravasation. 5) mononuclear infiltrate.

peptide LL-37 has been shown to form complexes with self-DNA and self-RNA that activate toll-like receptors expressed by a.o. plasmacytoid dendritic cells (DC) that in turn produce IFNα (interferon alpha). Psoriatic keratinocytes and inflammatory cells are responsive to antimicrobial peptides and in turn will produce pro-inflammatory cytokines (e.g., Interleukin (IL)-1β, IL-6 and tumor necrosis factor alpha (TNFα) (5). This environment stimulates dermal DCs to become activated and migrate to the draining lymph nodes. These dermal DCs produce IL-12 and IL-23 that subsequently activate the differentiation of T-helper (Th)1 and Th17 cells in the lymph nodes, and in later stages their migration to the skin (18). Two-thirds of patients with moderate-to-severe plaque psoriasis harbour $CD4^+$ and/or $CD8^+$ T cells specific for LL-37, uncovering LL-37 as a T-cell autoantigen. Moreover, $CD8^+$ T cells can recognize ADAMTS-like protein 5 as an autoantigen presented by melanocytes via HLA-CW06:02. Via this autoantigenic activation the psoriasis signature cytokine, IL-17A is induced (19).

Psoriasis was initially considered to be a Th1 mediated condition, characterized by the production of interferon gamma (IFN-γ) and TNFα under the influence of IL-12 (20). IL-12 stimulates natural killer cells and drives the differentiation of naïve $CD4^+$ T cells to Th1 cells. TNFα, mainly produced by IFNγ-activated macrophages, is one of the important cytokines in the development of psoriasis, because TNFα modulates many other cell types involved in psoriasis (1). The mechanism of action of TNFα is the induction of pro-inflammatory cytokines such as IL-1, IL-6, GM-CSF and vascular endothelial growth factor (VEGF) production by other cells. TNFα also activates endothelial cells to express adhesion molecules and increase the endothelial layer permeability, facilitating migration of leukocytes into inflammatory lesions (21).

The understanding of the pathogenesis of psoriasis has advanced by the discovery of the previously mentioned Th17 involvement. Differentiation of naïve T cells into Th17 cells that produce mediators such as IL-17A, IL-17F, and IL-22 are now considered to be the major drivers of the disease (19, 22). Also, other cells like innate lymphoid cells (ILC) are capable of producing IL-22, mast cells and neutrophils can produce IL-17 (23). Mediators such as IL-17A, IL-17F and IL-22 cause hyperproliferation of keratinocytes in the epidermis and further stimulate the production of AMP (LL-37, hBD2 and S100A7). As a result, the vicious cycle of inflammation in psoriasis is maintained (Figure 3) (24).

TREATMENT OF PSORIASIS

Currently, the treatment of psoriasis consists of topical therapies, phototherapy, and a wide variety of systemic medications. Topical therapies are used to treat mild psoriasis or as adjuvant treatment in patients receiving systemic treatment. These therapies comprise corticosteroids, tacrolimus or vitamin D3 derivatives, or a combination of corticosteroids and vitamin D3, and coal tar (25). For moderate to severe psoriasis UVB or PUVA phototherapy is used, which

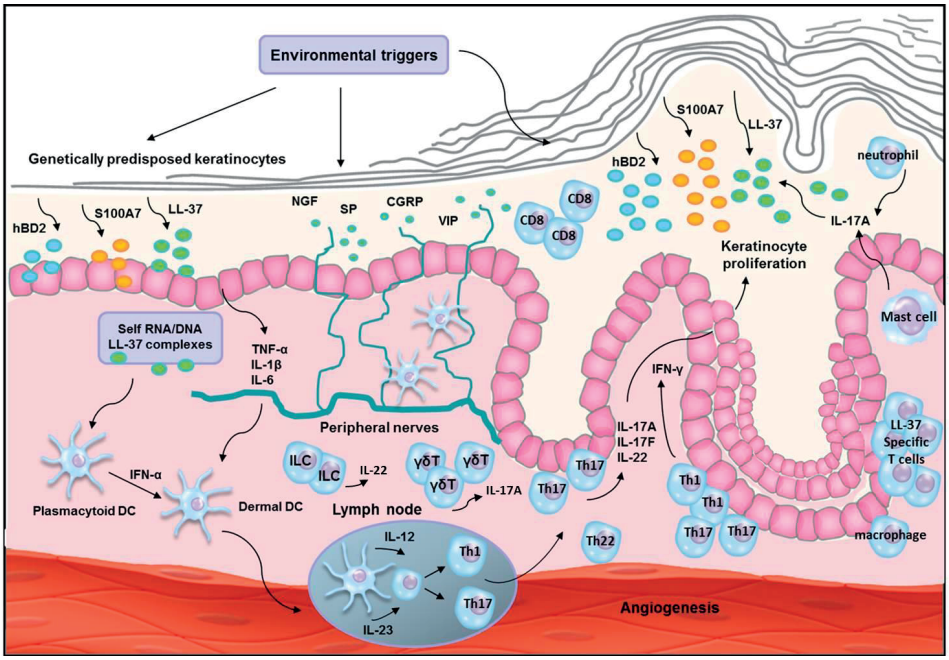

Figure 3. Pathogenesis of psoriasis. Illustrating the interaction between environmental triggers, keratinocytes, T-cells, cytokines, pro-inflammatory mediators and AMP.
Parts of this figure were provided by E.M. Baerveldt.

can also be combined with topical therapies. In case a large body surface area of the skin is affected by psoriasis, oral systemic treatments with methotrexate (MTX), ciclosporin, retinoids, fumarates and apremilast are indicated (26).

Oral systemic treatment

MTX is a folic acid antagonist, which inhibits the dihydrofolate reductase enzyme. This affects cell proliferation by inhibition of thymidylate and purine synthesis required for RNA and DNA synthesis. MTX was originally mainly used for the treatment of RA but was shown to be an effective treatment for psoriasis as well. Low-dose MTX (<15-20mg/week) is an effective therapy for moderate to severe psoriasis. Unfortunately, the use of MTX may be complicated by hepatotoxicity and bone marrow suppression (27).

Ciclosporin is a calcineurine inhibitor that reduces the production of IL-2 and T-cell proliferation. Ciclosporin is generally used on a short-term basis (on average 3-6 months), due to its nephrotoxicity and induction of hypertension. It is one of the most effective and rapidly working systemic drugs for psoriasis. The efficacy of ciclosporin in psoriasis and the occurrence of (sometimes severe) adverse events are dose dependent. In clinical practice the daily dose of ciclosporin generally is between 2.5-5 mg/kg.

Systemic retinoids (derivatives of vitamin A) such as acitretin are particularly effective for pustular psoriasis and erythrodermic psoriasis and psoriasis affecting the hand and feet. Their exact mechanism of action is largely unknown but involves modest anti-inflammatory effects and inhibition of epidermal proliferation and differentiation. The most common side effects are on the liver, musculoskeletal and neurological system. Teratogenicity remains the primary concern in women with childbearing potential.

According to the European guidelines, fumarates (also known as fumaric acid esters) may be used as a first in line oral systemic treatment for moderate to severe plaque psoriasis (26). However fumarates are still not officially licensed for psoriasis and are still not used worldwide, despite their proven efficacy in the treatment of psoriasis in Western European countries. Fumarates inhibit the proliferation of keratinocytes and the production of mediators of inflammation in psoriasis (28, 29). Fumarates are safe with an acceptable tolerability without an increased risk of malignancies (30). However, common and potentially serious side effects of fumarates are leukopenia and/or lymphopenia. Six cases of progressive multifocal leukoencephalopathy (PML) in long-term fumarates users have been reported. It should be mentioned that most of the reported PML cases were lymphopenic for a prolonged period of time (31-33).

Recently, apremilast, a new oral drug for the treatment of psoriasis was introduced. Apremilast is a selective inhibitor of the enzyme phosphodiesterase 4 (PDE4) and inhibits the production of a.o. TNFα, IL-17, and IL-23, and up-regulates the anti-inflammatory cytokine IL-10. Apremilast is well tolerated and associated with generally mild gastrointestinal complaints (34).

Biologics

Treatment with biologic drugs is indicated when traditional treatments such as topical agents, phototherapy and systemic agents have failed or because of patient-specific contraindications. As mentioned before, systemic treatments such as methotrexate and ciclosporin are associated with cumulative, dose-dependent toxicities limiting their safe and long-term uninterrupted use (27, 35). Biologics that are used for the treatment of psoriasis are mostly therapeutic monoclonal antibodies that are produced with recombinant DNA-technology. The six currently available biologics infliximab, adalimumab, etanercept, ustekinumab, secukinumab, and ixekizumab are indicated for the long-term control of psoriasis. Figure 4 illustrates which therapy is indicated; depending on the severity of psoriasis, and which therapy can be used if the other fails.

STRATIFICATION AND BIOMARKERS FOR (PREDICTION OF) TREATMENT RESPONSE

In order to optimize the treatment of psoriasis, it is essential to stratify clinical psoriasis subtypes (Figure 4). Currently, there are no validated biomarkers that predict the responsiveness to a biologic, disease progression or side effects. Pharmacogenomic and immunologic research

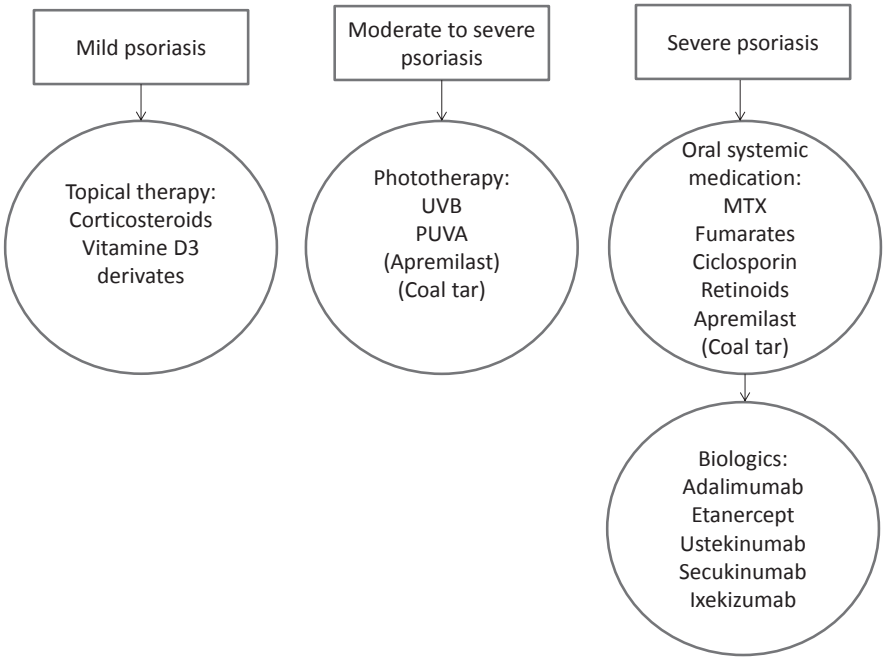

Figure 4. Schematic flow chart of anti-psoriatic therapy use according to current guidelines (25, 26).

is necessary to identify specific genes and or proteins that predict the therapeutic response. Recently, van den Reek et al. showed that patients heterozygous for the CD84 SNP had a better clinical response to etanercept. Patients homozygous for the TNFA IP3 SNP showed a lesser response to ustekinumab (36). There is an urgent need for more biomarkers that predict the treatment response of biologics. If such biomarkers become available in the future, this could result in a more individualized and cost-effective treatment of psoriasis.

OUTCOME MEASURES FOR TREATMENT OF PSORIASIS

Clinical trials use many different outcome measures, some of which are disease specific. For this thesis, we used several outcome measures including patient reported outcomes in our clinical trials. For better understanding and interpretation of the results, a short explanation of the outcome measures is provided.

The Psoriasis Activity and Severity Index (PASI) is a validated tool for monitoring the disease activity. The PASI is calculated based on the intensity of redness (erythema), thickness (induration), scaling (desquamation), and the affected surface area for each body part (head, upper extremities, lower extremities and trunk). This leads to a PASI score ranging from 0 (no disease)

to 72 (maximum disease activity). Most patients fall in the range of PASI 0-15 and not in the range of 15-72; a PASI above 40 is highly uncommon, even in severely affected patients (37). The PASI score is the main outcome measure for success of treatment in clinical practice and clinical trials. In most studies, the percentage of improvement compared to baseline is used. A PASI-75 means 75% improvement of PASI score as compared to baseline and is accepted as a clinically significant improvement in the Dermatology.

The 5-point Physician Global Assessment (PGA) scale is a modified and more simplified tool for evaluating plaque psoriasis severity and improvement in clinical trials. It is divided in clear, almost clear, mild, moderate and severe score for the disease activity (38). Global assessments can be done for extensive disease as well as for single plaques. There are two primary forms: a static form, which measures the physician's impression of the disease at a single point, and a dynamic form in which the physician assesses the global improvement from baseline. Because the latter requires the dubious assumption that physicians can remember the severity of psoriasis at baseline over the course of the trial, the static PGA has become the standard. Although the PASI is the gold standard for assessment of severe psoriasis, the PGA is also widely used.

Another important outcome measure used in many clinical trials is the Dermatology Life Quality Index (DLQI). This is a ten-item questionnaire used to measure the impact of a skin disease on the quality of life of an affected person. Each question refers to the impact of the skin disease on the patient's life over the previous week. The DLQI can provide more insight into the impairment of quality of life of psoriasis patients and supports more appropriate clinical decisions (39).

Finally, an increasingly popular outcome measure in the literature is 'drug survival'. Drug survival reflects the 'time on drug' and is thought to summarize the efficacy, safety, patient and doctors preferences (40). The most common reasons for discontinuation of a given drug is ineffectiveness and side effects. It is believed that a longer drug survival for a biologic is associated with a better outcome for the patient.

BIOLOGICS IN PSORIASIS

TNFα-inhibitors

TNFα is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the key cytokines involved in the pathogenesis of psoriasis. TNFα is mainly produced by activated macrophages, but it can also be produced by other cell types including CD4+ T-cells, natural killer cells, eosinophils, mast cells and neurons (41). TNFα binds to TNFα receptors on cells that are involved in the autoimmune/inflammatory response of psoriasis. Adalimumab, infliximab, and etanercept are recombinant TNFα-inhibitors. They reduce the bioavailability of free circulating TNFα to reduce the inflammatory response. The mechanism of action differs between the three anti-TNF drugs.

Adalimumab, a fully human immunoglobulin G (IgG) antibody, blocks the interaction with the p55 and p75 cell surface TNF receptors, interfering with expression of adhesion molecules responsible for leukocyte migration (42, 43). Infliximab is a chimeric murine/human IgG antibody. Infliximab binds with high affinity to both soluble and membrane bound TNFα, a related cytokine that utilizes the same receptors as TNFα. Infliximab inhibits TNFα -induced mitogenesis, IL-6 secretion, activation of neutrophils and stimulation of eosinophils (44). Infliximab reduces several biological activities of TNFα, which may result in lysis of the cells that produce TNFα (45).

Etanercept differs from the other TNFα-inhibitors because it is not an antibody, but a fusion protein comprising the type 2, p75 TNFα-receptor and the Fc chain of the IgG1 antibody. Etanercept binds soluble, non-membrane bound TNFα- and neutralizes its biological activity. Only etanercept, but not adalimumab and inflximab binds and neturalizes lymphotoxin α ($LT\alpha_3$) and lymphotoxin-β ($LT\alpha_2\beta_1$) (46). Etanercept prevents TNFα from mediating signal transduction by preventing cross-linking of its cell surface receptors. The differences between etanercept and the other TNFα-inhibitors are their TNFα binding characteristics (47).

The anti-TNFα-biologics have a good safety profile, most side effects are the result of immunosuppressive properties of the biologic treatment (48). A higher risk of serious infections has been reported for adalimumab and infliximab treatment compared to etanercept and ustekinumab treatment (49). TNFα has a central role in mycobacterial infection and disease and reactivation of hepatitis. As a consequence patients who are treated with TNFα neutralizing biologics have an increased risk of reactivation of tuberculosis (TBC) and hepatitis. However, the exact risk is difficult to estimate. Therefore screening for TBC, hepatitis infection and liver enzymes is mandatory before initiation of treatment (50).

IL- 12 and 23 inhibitor

Ustekinumab is a fully human IgG1 antibody directed against the shared p-40 protein of IL-12 and IL-23, although it was originally designed to target IL-12. Dendritic cells secrete IL-12 and IL-23, cytokines that induce the differentiation of Th1 and Th17 cells in the lymph nodes (51). Ustekinumab binds with high affinity to p-40 and so it inhibits IL-12 and IL-23 binding to the cell surface IL-12Rb1 receptor. Although ustekinumab was not initially designed to inhibit IL-23, neutralization of this cytokine appears part of its mechanism of action (52).

IL-17 inhibitors

Recently two IL-17 inhibitors have been approved for the treatment of psoriasis in The Netherlands (secukinumab and ixekizumab). Secukinumab is a fully human IgG1 antibody that selectively binds to and neutralizes the pro-inflammatory cytokine IL-17A. The IL-17 receptor is expressed on various cell types including keratinocytes. Secukinumab inhibits T cells, keratinocytes and other IL-17-responsive cells to secrete pro-inflammatory cytokines, chemokines, and mediators of inflammation(53). Ixekizumab is a humanized IgG4 antibody that binds with a high affin-

ity to IL-17A and IL-17F. IL-17A stimulates the production of cytokines and chemokines that mobilize neutrophils and memory T-cells to the site of injury or inflammation, thereby maintaining a proinflammatory state. As such, IL-17A appears to play an important role in chronic inflammation and autoimmunity. IL-17A and IL-17F are both involved in the immune reaction against extracellular bacteria and fungi. Patients treated with an IL-17 inhibitor therefore have an increased risk of candida infections and neutropenia may be a concern (53).

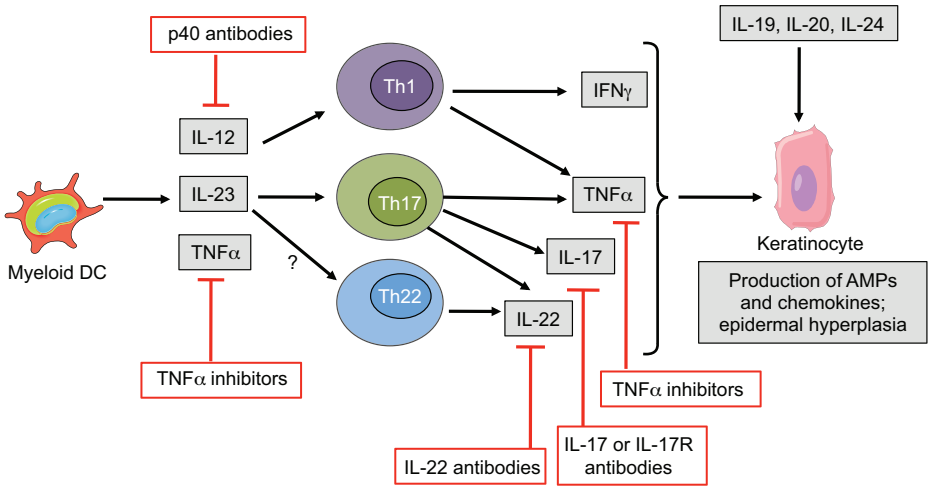

Figure 5. Targeted biologic therapy in relation to the pathogenesis of psoriasis (54). Currently, IL-22 inhibitors are being developed for the treatment of psoriasis because they can inhibit keratinocyte proliferation/differentiation and epidermal alterations (55). Other biologics in the pipeline are the IL-23 inhibitors. If the IL-23 receptor is activated it induces the differentiation of IL-17. This results in production of cytokines such as IL-17A, IL-17F and IL-22 which are all involved in the development of psoriasis (56).

PHARMACOKINETICS OF BIOLOGICS

Bioavailability of (therapeutic) proteins is very limited after oral administration. Therefore, most biologics are administered intravenously or subcutaneously. Systemic exposure from the subcutaneous depot via both lymphatic and blood vessels is slow and peaks after 2-8 days. Common renal and hepatic pathways are not involved in metabolism and elimination of biologics. Rather, fluid-phase endocytosis by numerous cell types followed by lysosomal degradation is the most important route of elimination. Most antigen-specific biologics bind to the neonatal Fc receptor (FcRn) in endosomal compartments, resulting in recycling back to the cell surface and escape from degradation, which can explain the half-lives of 1-3 weeks (Figure 6) (57). In Table 1 an overview is presented of the half-life, maximum concentration (Cmax) and therapeutic target of biologics used in psoriasis.

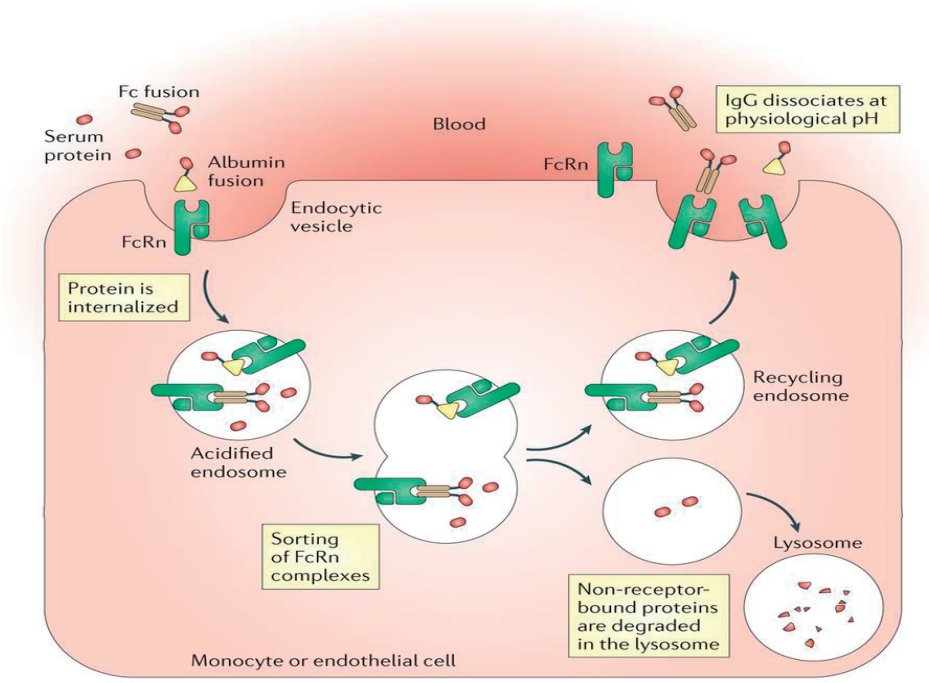

Figure 6. FcRn recycling mechanism (58).

Table 1. The characteristics of the currently approved biologics for psoriasis.

	Infliximab	Adalimumab	Etanercept	Ustekinumab	Secukinumab	Ixekizumab
Therapeutic target	TNFα	TNFα	TNFα p75 TNF receptor	IL-12 and IL-23	IL-17	IL-17
Structure	Chimeric monoclonal	Human monoclonal	Human Immunoglobulin Fc fusion protein	Human monoclonal IgG1 antibody	Human monoclonal IgG1/κ Antibody	Humanized monoclonal IgG4 Antibody
Dose	5mg/kg IV every 8 weeks	40mg sc every 2 weeks	50mg sc every week	45 or 90mg sc every 12 weeks	150 or 300mg sc every 4 weeks	80mg sc every 4 weeks
T1/2	8-9.5 days	±14 days	2.9 days (70hours)	21 (15-32) days	27 (18-46) days	13 days
Cmax	1 hour	5 days	2 days	8.5 days	31-34 days	4-7 days
Occurrence of ADA	Yes	Yes	No	No	Unknown	Unknown

IMMUNOGENICITY (ANTI-DRUG ANTIBODIES)

Immunogenicity is the ability of an antigen or epitope to provoke a humoral and/or cell-mediated immune response. Biologics have the risk of being recognized as foreign by the patient's immune system. The adaptive immune response, characterized by the development of high affinity, highly specific antibodies, and long-lasting memory T lymphocytes are primarily involved in the development of anti-drug antibodies (ADA) against a biologic. Such immune responses are polyclonal and may have either a neutralizing or a non-neutralizing effect on the biologic (59). ADA usually exhibit a high affinity for the biologic and can prevent the biologic to bind to its target. As a result the ADA forms an immune complex with the biologic (Figure 7).

Development of ADA can be reduced by intermittent or continuous use of immunosuppressant drugs as co-medication, such as methotrexate (MTX), prednisone or azathioprine. Especially the role of MTX is interesting. MTX reduces the immune response against a foreign epitope (idiotype of biologic) by blocking the expansion of B-cell and T-cell populations that can produce ADA through plasma cell differentiation (60, 61).

Undesirable effects of immunogenicity are reduced efficacy, anaphylaxis and occasionally autoimmunity. Reduced clinical efficacy of a biologic is a consequence of the blocking of the active site (TNFα can no longer bind to the drug) as well as accelerated clearance of

Figure 7. Formation of immune complex of anti-drug antibodies with a biologic.
This figure is provided by S. Garcês.

Figure 8. Accelerated clearance of drug-antibody complexes.
This figure is provided by and with permission of S. Garcês.

drug-antibody complexes. Figure 8 shows the dynamics between drug and anti-drug antibodies between two drug administrations. Most ADA become detectable between 12 and 24 weeks after initiation of biologic therapy.

TDM OF BIOLOGICS

Monitoring of biologic drug serum trough concentrations may contribute to improving personalized dosing and drug choice, but so far such monitoring is infrequently performed in routine clinical practice. For several biologics, large interindividual variation in serum concentrations has been demonstrated, partly due to the development of ADA. Low trough concentrations caused by the development of ADA are associated with reduced efficacy (62, 63). Two specific arguments may favor the use of therapeutic drug monitoring (TDM) for biologics.

First, many of these drugs are used for a long-term control of IMID diseases with a spontaneous relapsing-remitting pattern, due to which it is very difficult to assess drug efficacy based on clinical outcome alone. Most patients who have reached a remission are treated according to treatment protocols, with a fixed dose administered at fixed time intervals. On the other hand, dosages of several biologics are increased (dose increase or interval decrease) during active disease in clinical practice and remain high upon disease remission (64, 65). When treatments are continued long term this may result in chronic overtreatment in a substantial proportion of patients (66).

Second, nearly all biologics are expensive and chronic treatment is associated with high total drug costs for a certain indication or disease. Safe tapering and personalized dosing without loss of efficacy may reduce costs significantly but is only sporadically done in daily clinical practice. In order to use the concentrations of biologics for TDM and to define a therapeutic window, it is important to measure only trough concentrations. A trough concentration moment is prior to the next administration of the subsequent biologic dose (figure 9).

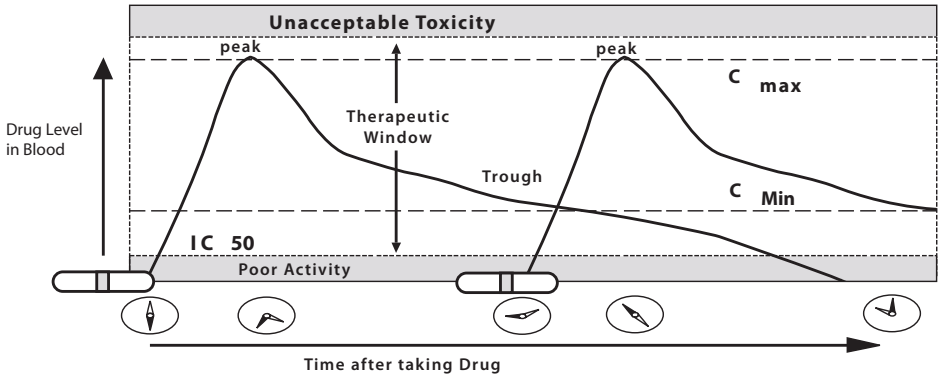

Figure 9. The schematically illustration of the course of a drug concentration.

ASSAYS FOR TDM OF BIOLOGICS INCLUDING ANTI-DRUG ANTIBODIES

The methods mostly used for analysis of small molecule drugs in human plasma or serum, such as high-performance liquid chromatography and liquid chromatography coupled to mass spectrometry are not readily available for protein drugs or have several limitations. Therefore, custom-made immunoassays have been developed successfully. To detect therapeutic biologics in plasma or serum, specific and sensitive enzyme-linked immunosorbent assays (ELISA) (Figure 10) have been described (67-69). The antigen-specific biologic in plasma or serum may be captured on a solid phase by its target protein or an antibody specific for the antigen-binding site (anti-idiotype antibody). Subsequently, the biologic can be detected using again its target protein, an anti-idiotype antibody, or a less specific anti-IgG antibody, coupled to an enzyme that can react with the substrate for detection.

Tetramethylbenzidine (TMB) is a chromogenic substrate used as being a visualizing reagent used in ELISA. The enzyme horseradish peroxidase (HRP), found in the roots of horseradish, has the ability to amplify a weak signal and increase detectability of a target molecule.

Analysis of ADA is more challenging since they can only be detected in the absence of or in presence of very low concentrations of most biologics. Most assays for ADA detection need to be quantified based on an arbitrary reference sample, and false-positive results have been reported. Both ELISAs and radioimmunoassays (RIAs) have been developed using an antigen-specific biologic to capture ADA, and an enzyme- or radio-labelled biologic for detection (70, 71). Alternative assays using e.g. liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) are also under development (72-75). Furthermore, a bioassay has been developed to detect the drug concentration by measuring its neutralizing capacity of

TNFα bioactivity as a result of interference with TNFα binding to its receptor.

According to the current consensus, detection of ADA is less clinically relevant compared to measuring biologic trough concentrations. ADA detection is only useful to provide an explanation why the biologic trough concentration is low or undetectable.

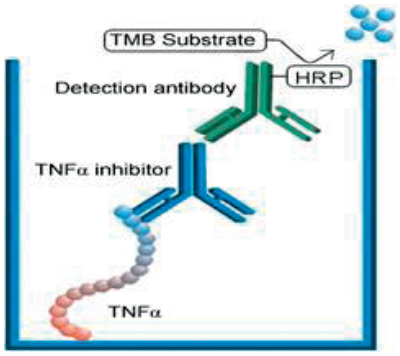

Figure 10. The principle of an ELISA for measurement of TNFα inhibitor concentration.

Preferably, assays should be cross-validated between different laboratories and use the same reference sample to allow direct comparison of the results. To define target ranges for serum concentrations of biologics and compare development and concentrations of ADA, such cross-validation and standardization are of utmost importance to better compare the results of future clinical trials.

SCOPE OF THE THESIS

Biologics are the future. Several antigen-specific biologics have resulted in remarkable improvements in treatment outcome of psoriasis and a dramatic reduction in clinical stays. More biologics are currently being developed and investigated in clinical trials. However, some difficulties with the treatment of psoriasis with biologics are encountered. One problem is that biologic monotherapy appears to loose efficacy over time which results in reduced drug survival. This is caused partly by of the formation of ADA which reduce the efficacy and some patients become refractory to a biologic treatment. Another problem is that a proportion of patients with stable low disease activity on long-term maintenance treatment with biologics may be overtreated. We believe that individualized treatment of biologics is necessary to overcome these problems. In addition, TDM could be an useful tool to optimize individualized treatment of biologics.

In this thesis, we have explored three strategies towards individualized treatment of biologics in psoriasis.

- **Combination therapy (part I)**
- **Biologic dosing interval prolongation (part II)**
- **Conditions for therapeutic drug monitoring (TDM) (part III)**

PART I: COMBINATION THERAPY

One possible strategy to improve the efficacy of biologics is to combine a biologic with MTX. MTX has the ability to reduce or even prevent neutralizing ADA formation which could lead to more sustained and adequate drug concentrations. This strategy is already applied in daily clinical practice for patients with rheumatoid arthritis, but is currently not in use during treatment of psoriasis. Therefore, we searched the literature for studies investigating the effects of combined therapy of biologics with MTX in psoriasis. These results are presented and reviewed in **Chapter 2.**

Subsequently, we initiated a multi-centre randomized controlled trial (RCT) to compare combination treatment of adalimumab and methotrexate with adalimumab monotherapy in patients with psoriasis (OPTIMAP study). The primary outcome is adalimumab drug survival

at week 49 in both groups. Secondary outcomes are long-term data on the efficacy and safety of adalimumab combined with MTX compared to adalimumab monotherapy; to assess the impact of concomitant MTX on adalimumab immunogenicity (ADA formation) and serum concentrations; to test appropriate candidate genes and correlate genotypes with trial outcomes. The rationale, pitfalls, and limitations of this RCT are discussed in **Chapter 3.**

We also explored combination therapies in less immunogenic biologics such as etanercept. A few years ago when this study protocol was initiated, etanercept was still widely used and some patients became refractory. Our aim was to improve the efficacy of etanercept and to compare monotherapy etanercept with combination therapy of etanercept with fumarates in a prospective explorative study (FUMBREL study). In **Chapter 4** we followed 33 patients for 48 weeks and we monitored the PASI, PGA, DLQI, tolerability and safety of etanercept and fumarates. We have chosen fumarates as combination therapy because they are widely used in the Netherlands and other Western European countries and they have a relatively good safety profile.

PART II: BIOLOGIC DOSING INTERVAL PROLONGATION

Another problem besides loss of efficacy is overtreatment of biologics in psoriasis patients. Therefore the aim was to investigate the feasibility of dosing interval prolongations in psoriasis patients with stable low disease activity who were on biologic maintenance treatment. In **Chapter 5** we followed a cohort of 59 psoriasis patients treated with adalimumab, etanercept or ustekinumab for at least six months. So far, treatments were largely based on fixed protocols, administering the same dose at the same interval to all patients. In this explorative study (POEMA study) we stepwise prolonged the dosing interval of adalimumab, etanercept, and ustekinumab. The patients were monitored every 12 weeks for the efficacy and quality of life and at these visits blood was drawn to measure the biologic trough concentration. In the literature concentration-effect relationships have been found and the first therapeutic target ranges are being established for adalimumab. Therefore we tried to determine the minimal biologic drug serum trough concentration required to maintain stable disease activity.

PART III: CONDITIONS FOR TDM OF BIOLOGICS

To optimize individualized treatment of biologics, the possibilities for implementing TDM of biologics should be explored. Before TDM can be recommended, a number of conditions need to be fulfilled. One of these conditions is the availability of a reliable and practical assay. In the literature, there is some controversy over the performance of commercially available assays. Assays for the detection of these biologics should be compared using the same reference

material. Our aim was to perform a comparative analysis of the performance of three commercially available assays designed for anti-TNFα drug TDM in routine practice. In **Chapter 6** we compared the two most frequently used commercially available ELISA systems for the detection of infliximab, adalimumab, and etanercept. In addition, we included a bioassay as a third arm, a non-ELISA based comparator.

A high inter-patient variability and a low intra-patient variability (IPV) in the drug concentrations is another condition for making TDM of biologics a useful tool. Previous studies have shown a large between-subject variability in the pharmacokinetics of the various biologics. However, the IPV of the biologic concentrations has not been studied previously. Repetitive serum samples of psoriasis patients on per label etanercept maintenance treatment were collected during the FUMBREL study. In these samples, etanercept trough concentrations were determined and IPV was assessed, in relation to response to treatment. The results of this longitudinal study are presented in **Chapter 7.**

Finally, in **Chapter 8** we discuss the findings of our studies in light of recent literature and suggest options for future research.

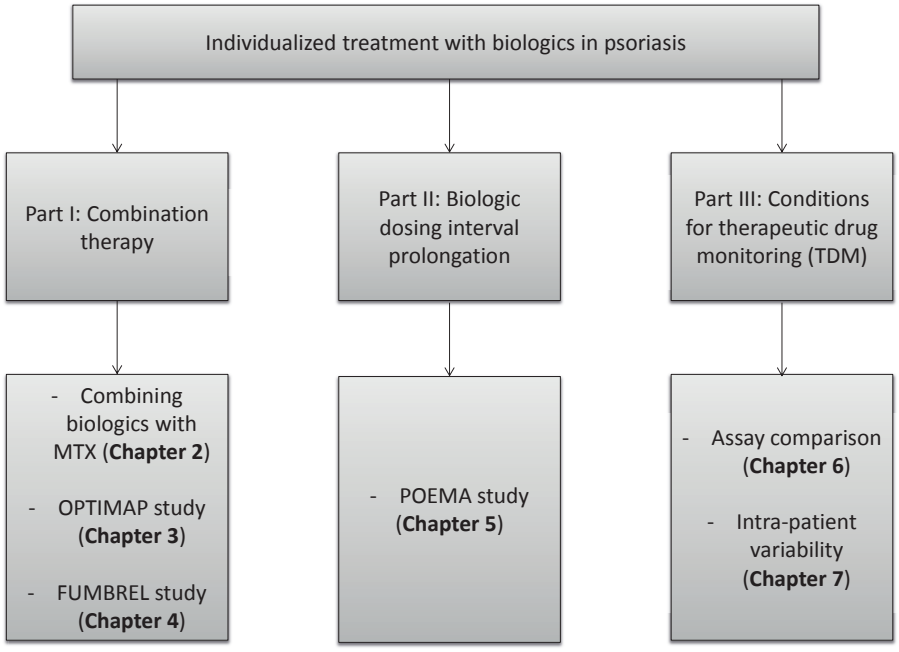

Figure 11. The outline of the thesis with a schematic representation of the three parts and clinical studies.

REFERENCES

1. Boehncke WH, Schon MP. Psoriasis. Lancet. 2015;386(9997):983-94.
2. Klaassen KM, van de Kerkhof PC, Pasch MC. Nail psoriasis: a questionnaire-based survey. Br J Dermatol. 2013;169(2):314-9.
3. Ogdie A, Langan S, Love T, Haynes K, Shin D, Seminara N, et al. Prevalence and treatment patterns of psoriatic arthritis in the UK. Rheumatology (Oxford). 2013;52(3):568-75.
4. Gelfand JM, Weinstein R, Porter SB, Neimann AL, Berlin JA, Margolis DJ. Prevalence and treatment of psoriasis in the United Kingdom: a population-based study. Arch Dermatol. 2005;141(12):1537-41.
5. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med. 2009;361(5):496-509.
6. Parisi R, Symmons DP, Griffiths CE, Ashcroft DM, Identification, Management of P, et al. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. J Invest Dermatol. 2013;133(2):377-85.
7. Green AC. Australian Aborigines and psoriasis. Australas J Dermatol. 1984;25(1):18-24.
8. Farber EM, Nall L. Psoriasis in the tropics. Epidemiologic, genetic, clinical, and therapeutic aspects. Dermatol Clin. 1994;12(4):805-16.
9. Langley RG, Krueger GG, Griffiths CE. Psoriasis: epidemiology, clinical features, and quality of life. Ann Rheum Dis. 2005;64 Suppl 2:ii18-23; discussion ii4-5.
10. Smith AE, Kassab JY, Rowland Payne CM, Beer WE. Bimodality in age of onset of psoriasis, in both patients and their relatives. Dermatology. 1993;186(3):181-6.
11. Perera GK, Di Meglio P, Nestle FO. Psoriasis. Annu Rev Pathol. 2012;7:385-422.
12. Ferrandiz C, Pujol RM, Garcia-Patos V, Bordas X, Smandia JA. Psoriasis of early and late onset: a clinical and epidemiologic study from Spain. J Am Acad Dermatol. 2002;46(6):867-73.
13. Icen M, Crowson CS, McEvoy MT, Dann FJ, Gabriel SE, Maradit Kremers H. Trends in incidence of adult-onset psoriasis over three decades: a population-based study. J Am Acad Dermatol. 2009;60(3):394-401.
14. Farber EM, Nall ML, Watson W. Natural history of psoriasis in 61 twin pairs. Arch Dermatol. 1974;109(2):207-11.
15. Yin X, Low HQ, Wang L, Li Y, Ellinghaus E, Han J, et al. Genome-wide meta-analysis identifies multiple novel associations and ethnic heterogeneity of psoriasis susceptibility. Nat Commun. 2015;6:6916.
16. Gudjonsson JE, Karason A, Antonsdottir AA, Runarsdottir EH, Gulcher JR, Stefansson K, et al. HLA-Cw6-positive and HLA-Cw6-negative patients with Psoriasis vulgaris have distinct clinical features. J Invest Dermatol. 2002;118(2):362-5.
17. Roberson ED, Bowcock AM. Psoriasis genetics: breaking the barrier. Trends Genet. 2010;26(9): 415-23.
18. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007;449(7162):564-9.
19. Kirkham BW, Kavanaugh A, Reich K. Interleukin-17A: a unique pathway in immune-mediated diseases: psoriasis, psoriatic arthritis and rheumatoid arthritis. Immunology. 2014;141(2):133-42.
20. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. Cell Mol Immunol. 2012;9(4):302-9.
21. Feldmann M, Steinman L. Design of effective immunotherapy for human autoimmunity. Nature. 2005;435(7042):612-9.
22. Ward NL, Umetsu DT. A new player on the psoriasis block: IL-17A- and IL-22-producing innate lymphoid cells. J Invest Dermatol. 2014;134(9):2305-7.

23. Teunissen MB, Munneke JM, Bernink JH, Spuls PI, Res PC, Te Velde A, et al. Composition of innate lymphoid cell subsets in the human skin: enrichment of NCR(+) ILC3 in lesional skin and blood of psoriasis patients. J Invest Dermatol. 2014;134(9):2351-60.
24. Fitch E, Harper E, Skorcheva I, Kurtz SE, Blauvelt A. Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. Curr Rheumatol Rep. 2007;9(6):461-7.
25. Menter A, Korman NJ, Elmets CA, Feldman SR, Gelfand JM, Gordon KB, et al. Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 3. Guidelines of care for the management and treatment of psoriasis with topical therapies. J Am Acad Dermatol. 2009;60(4):643-59.
26. Nast A, Gisondi P, Ormerod AD, Saiag P, Smith C, Spuls PI, et al. European S3-Guidelines on the systemic treatment of psoriasis vulgaris--Update 2015--Short version--EDF in cooperation with EADV and IPC. J Eur Acad Dermatol Venereol. 2015;29(12):2277-94.
27. Haustein UF, Rytter M. Methotrexate in psoriasis: 26 years' experience with low-dose long-term treatment. J Eur Acad Dermatol Venereol. 2000;14(5):382-8.
28. Fallah Arani S, Neumann H, Hop WC, Thio HB. Fumarates vs. methotrexate in moderate to severe chronic plaque psoriasis: a multicentre prospective randomized controlled clinical trial. Br J Dermatol. 2011;164(4):855-61.
29. Ghoreschi K, Bruck J, Kellerer C, Deng C, Peng H, Rothfuss O, et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. J Exp Med. 2011;208(11):2291-303.
30. Hoefnagel JJ, Thio HB, Willemze R, Bouwes Bavinck JN. Long-term safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol. 2003;149(2):363-9.
31. Bartsch T, Rempe T, Wrede A, Leypoldt F, Bruck W, Adams O, et al. Progressive neurologic dysfunction in a psoriasis patient treated with dimethyl fumarate. Ann Neurol. 2015;78(4): 501-14.
32. Dammeier N, Schubert V, Hauser TK, Bornemann A, Bischof F. Case report of a patient with progressive multifocal leukoencephalopathy under treatment with dimethyl fumarate. BMC Neurol. 2015;15:108.
33. van Oosten BW, Killestein J, Wattjes MP. Case reports of PML in patients treated for psoriasis. N Engl J Med. 2013;369(11):1081-2.
34. Gooderham M, Papp K. Selective Phosphodiesterase Inhibitors for Psoriasis: Focus on Apremilast. BioDrugs. 2015;29(5):327-39.
35. Maza A, Montaudie H, Sbidian E, Gallini A, Aractingi S, Aubin F, et al. Oral cyclosporin in psoriasis: a systematic review on treatment modalities, risk of kidney toxicity and evidence for use in non-plaque psoriasis. J Eur Acad Dermatol Venereol. 2011;25 Suppl 2:19-27.
36. van den Reek JM, Coenen MJ, van de L'Isle Arias M, Zweegers J, Rodijk-Olthuis D, Schalkwijk J, et al. Polymorphisms in CD84, IL12B and TNFA IP3 are associated with response to biologics in patients with psoriasis. Br J Dermatol. 2016.
37. Feldman SR, Krueger GG. Psoriasis assessment tools in clinical trials. Ann Rheum Dis. 2005;64 Suppl 2:ii65-8; discussion ii9-73.
38. Langley RG, Feldman SR, Nyirady J, van de Kerkhof P, Papavassilis C. The 5-point Investigator's Global Assessment (IGA) Scale: A modified tool for evaluating plaque psoriasis severity in clinical trials. J Dermatolog Treat. 2015;26(1):23-31.
39. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994;19(3):210-6.
40. van den Reek JM, Kievit W, Gniadecki R, Goeman JJ, Zweegers J, van de Kerkhof PC, et al. Drug Survival Studies in Dermatology:Principles, Purposes, and Pitfalls. J Invest Dermatol. 2015;135(7).
41. Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. Pharmacol Ther. 2008;117(2):244-79.

42. Patel T, Gordon KB. Adalimumab: efficacy and safety in psoriasis and rheumatoid arthritis. Dermatol Ther. 2004;17(5):427-31.
43. Calabrese LH. Molecular differences in anticytokine therapies. Clin Exp Rheumatol. 2003;21(2):241-8.
44. Siegel SA, Shealy DJ, Nakada MT, Le J, Woulfe DS, Probert L, et al. The mouse/human chimeric monoclonal antibody cA2 neutralizes TNF in vitro and protects transgenic mice from cachexia and TNF lethality in vivo. Cytokine. 1995;7(1):15-25.
45. Scallon BJ, Moore MA, Trinh H, Knight DM, Ghrayeb J. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. Cytokine. 1995;7(3):251-9.
46. Mitoma H, Horiuchi T, Tsukamoto H, Ueda N. Molecular mechanisms of action of anti-TNF-alpha agents - Comparison among therapeutic TNF-alpha antagonists. Cytokine. 2016.
47. Scallon B, Cai A, Solowski N, Rosenberg A, Song XY, Shealy D, et al. Binding and functional comparisons of two types of tumor necrosis factor antagonists. J Pharmacol Exp Ther. 2002;301(2): 418-26.
48. Mansouri Y, Goldenberg G. Biologic safety in psoriasis: review of long-term safety data. J Clin Aesthet Dermatol. 2015;8(2):30-42.
49. Kalb RE, Fiorentino DF, Lebwohl MG, Toole J, Poulin Y, Cohen AD, et al. Risk of Serious Infection With Biologic and Systemic Treatment of Psoriasis: Results From the Psoriasis Longitudinal Assessment and Registry (PSOLAR). JAMA Dermatol. 2015;151(9):961-9.
50. Gardam MA, Keystone EC, Menzies R, Manners S, Skamene E, Long R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect Dis. 2003;3(3):148-55.
51. Benson JM, Sachs CW, Treacy G, Zhou H, Pendley CE, Brodmerkel CM, et al. Therapeutic targeting of the IL-12/23 pathways: generation and characterization of ustekinumab. Nat Biotechnol. 2011;29(7):615-24.
52. Gaspari AA, Tyring S. New and emerging biologic therapies for moderate-to-severe plaque psoriasis: mechanistic rationales and recent clinical data for IL-17 and IL-23 inhibitors. Dermatol Ther. 2015;28(4):179-93.
53. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, et al. Secukinumab in plaque psoriasis--results of two phase 3 trials. N Engl J Med. 2014;371(4):326-38.
54. Johnson-Huang LM, Lowes MA, Krueger JG. Putting together the psoriasis puzzle: an update on developing targeted therapies. Dis Model Mech. 2012;5(4):423-33.
55. Hao JQ. Targeting interleukin-22 in psoriasis. Inflammation. 2014;37(1):94-9.
56. Kopp T, Riedl E, Bangert C, Bowman EP, Greisenegger E, Horowitz A, et al. Clinical improvement in psoriasis with specific targeting of interleukin-23. 2015;521(7551):222-6.
57. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. MAbs. 2011;3(5):422-30.
58. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. 2014;13(9):655-72.
59. Baker MP, Reynolds HM, Lumicisi B, Bryson CJ. Immunogenicity of protein therapeutics: The key causes, consequences and challenges. Self Nonself. 2010;1(4):314-22.
60. Garcês S, Demengeot J, Benito-Garcia E. The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis. Ann Rheum Dis. 2013;72(12):1947-55.
61. Krieckaert CL, Bartelds GM, Lems WF, Wolbink GJ. The effect of immunomodulators on the immunogenicity of TNF-blocking therapeutic monoclonal antibodies: a review. Arthritis Res Ther. 2010;12(5):217.

62. Vande Casteele N, Gils A, Singh S, Ohrmund L, Hauenstein S, Rutgeerts P, et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. Am J Gastroenterol. 2013;108(6):962-71.
63. Lecluse LL, Driessen RJ, Spuls PI, de Jong EM, Stapel SO, van Doorn MB, et al. Extent and clinical consequences of antibody formation against adalimumab in patients with plaque psoriasis. Arch Dermatol. 2010;146(2):127-32.
64. Janssen Biologics B.V. Summary of Product Characteristics: Remicade 100mg powder for concentrate for solution for infusion. 2 July 2009:www.ema.europa.eu.
65. Abbott Laboratories Ltd. Summary of Product Characteristics: Humira 40mg solution for injection in pre-filled syringe. 8 September 2008:www.ema.europa.eu.
66. van Vollenhoven RF, Brannemark S, Klareskog L. Dose escalation of infliximab in clinical practice: improvements seen may be explained by a regression-like effect. Ann Rheum Dis. 2004;63(4):426-30.
67. Ternant D, Mulleman D, Degenne D, Willot S, Guillaumin JM, Watier H, et al. An enzyme-linked immunosorbent assay for therapeutic drug monitoring of infliximab. Ther Drug Monit. 2006;28(2):169-74.
68. Ceze N, Ternant D, Piller F, Degenne D, Azzopardi N, Dorval E, et al. An enzyme-linked immunosorbent assay for therapeutic drug monitoring of cetuximab. Ther Drug Monit. 2009;31(5):597-601.
69. Damen CW, de Groot ER, Heij M, Boss DS, Schellens JH, Rosing H, et al. Development and validation of an enzyme-linked immunosorbent assay for the quantification of trastuzumab in human serum and plasma. Anal Biochem. 2009;391(2):114-20.
70. Aarden L, Ruuls SR, Wolbink G. Immunogenicity of anti-tumor necrosis factor antibodies-toward improved methods of anti-antibody measurement. Curr Opin Immunol. 2008;20(4):431-5.
71. Hart MH, de Vrieze H, Wouters D, Wolbink GJ, Killestein J, de Groot ER, et al. Differential effect of drug interference in immunogenicity assays. J Immunol Methods. 2011.
72. Damen CW, Schellens JH, Beijnen JH. Bioanalytical methods for the quantification of therapeutic monoclonal antibodies and their application in clinical pharmacokinetic studies. Hum Antibodies. 2009;18(3):47-73.
73. Li H, Ortiz R, Tran L, Hall M, Spahr C, Walker K, et al. General LC-MS/MS method approach to quantify therapeutic monoclonal antibodies using a common whole antibody internal standard with application to preclinical studies. Anal Chem. 2012;84(3):1267-73.
74. Damen CW, Rosing H, Schellens JH, Beijnen JH. Quantitative aspects of the analysis of the monoclonal antibody trastuzumab using high-performance liquid chromatography coupled with electrospray mass spectrometry. J Pharm Biomed Anal. 2008;46(3):449-55.
75. Becher F, Dubois M, Fenaille F, Ezan E. Mass spectrometry protocol for the absolute quantification of a monoclonal antibody in serum with immunopurification. Methods Mol Biol. 2013;988:345-52.

PART I

Combination therapy

Chapter 2

Combining biologics with methotrexate in psoriasis

J.S. van Bezooijen, E.P. Prens, M.S. Pradeepti,
R.A. Atiqi, M.W.J. Schreurs, B.C.P. Koch,
T. van Gelder, M.B.A. van Doorn

Based on: Br J Dermatol. 2015;172(6):1676-80.

ABSTRACT

Background

Biologics are highly effective drugs that can induce rapid and impressive clinical improvement in patients with psoriasis. In clinical practice biologics may lose efficacy over time, which makes switching to another drug necessary. Loss of efficacy can be partly attributed to anti-drug antibody formation resulting in neutralization of pharmacological action. Combined therapy of biologics and methotrexate (MTX) has been shown to improve both efficacy and drug survival in a variety of immune-mediated inflammatory diseases such as rheumatoid arthritis. Combination treatment is currently not recommended for moderate-to-severe psoriasis.

Objective

To conduct a review on combination therapies of biologics and MTX in psoriasis, and to discuss the potential implications for current treatment guidelines.

Methods

Titles and abstracts of articles in relevant public databases were screened for combination therapy of a biologic and MTX and appropriate articles were selected.

Results

Eight publications fulfilled the criteria for inclusion in this review. Limited evidence suggests that combination therapy of a biologic and MTX results in higher efficacy and good tolerability.

Conclusion

The evidence is insufficient to draw firm conclusions about the impact of combined treatment on efficacy, safety or drug survival in psoriasis. Most studies primarily investigated the short-term clinical efficacy of combination therapy, whereas (long-term) maintenance of clinical improvement or 'drug survival' was not evaluated. Adequately powered long-term randomized clinical trials comparing MTX combination therapy versus biologic monotherapy in moderate to severe plaque psoriasis are warranted.

BACKGROUND

Psoriasis is a chronic, inflammatory skin disease affecting 2–3% of the Caucasian population in Western countries (1). Biologic drugs, further referred to as 'biologics', have revolutionized the treatment of patients with extensive and therapy resistant disease (2). Their use is indicated when topical agents, phototherapy and traditional immunosuppressive systemic agents have failed, or when patient-specific contraindications are present. Currently, four biologics (anti-TNFα: adalimumab, infliximab and etanercept and anti-p40(IL-12 and IL-23): ustekinumab) are available for the treatment of moderate to severe chronic plaque psoriasis in Europe (3).

Biologics are effective drugs which can induce rapid and impressive clinical improvement; e.g. PASI-75 improvement between 68% and 88% in patients with infliximab and PASI 90 in 41,6% of patients for ustekinumab after 12 weeks of treatment in clinical trials (4, 5). Unfortunately, in clinical practice not all patients reach these impressive efficacy results. Even more importantly, all biologics appear to lose efficacy over time and many patients are switched to another biologic eventually (6, 7). This loss of response to a biologic occurs primarily within one year after starting the drug and steadily increases in the years thereafter (8).

For the monoclonal antibody based (MAb) anti-TNFα drugs this loss of efficacy has been partly attributed to immunogenicity. Neutralizing anti-drug antibody (ADA) formation against the biologic drug leads to inhibition of function and formation of drug-antibody complexes resulting in accelerated clearance from the circulation (8).

To overcome these short- and longer term efficacy problems, feasibility of off-label combination therapies of biologics and traditional immunosuppressive systemic agents are currently being explored. At present, there is insufficient evidence to support a significant role for ciclosporin, acitretin or azathioprine in the prevention of immunogenicity (9, 10).

In contrast, combined treatment with MTX may improve the short-term clinical efficacy and especially drug survival. The latter effect may be achieved by decreasing neutralizing ADA formation and thus maintaining adequate exposure to MAb-based treatments. A growing body of evidence has shown that in a variety of immune-mediated-inflammatory diseases (IMID), combination therapy of a biologic and MTX is more effective than biologic monotherapy (11-20).

At present, the European League against Rheumatism (EULAR) recommends combination therapy of TNFα inhibitors (TNFi) and MTX for the treatment of rheumatoid arthritis (RA) (21). However, European S3 guidelines on the systemic treatment of psoriasis do not recommend this combination therapy (22). In this review the combined therapy of biologics and MTX in psoriasis is reviewed, and the potential implications for current treatment recommendations are discussed.

METHODS

The electronic databases EMBASE (Embase and Medline), MEDLINE (OvidSP), Cochrane Central Register of Controlled Trials, PubMed and Web of Science were searched up to October 27th 2014 to identify studies on the combination therapy of MTX and four biologics with marketing approval for the treatment of psoriasis in Europe. The following terms were translated into a search strategy together with an information specialist: "methotrexate" with "adalimumab", "infliximab", "etanercept", "ustekinumab", "combination therapy" and "psoriasis". The searches were limited to English language articles. The preliminary selection for eligible trials based on title and abstract was performed by two independent investigators, subsequently a second selection based on the full text was performed. The flow chart presented in Figure 1. illustrates which inclusion and exclusion criteria were used to screen titles, abstracts and full text. Any discrepancies were resolved by discussion or by referral to a third investigator.

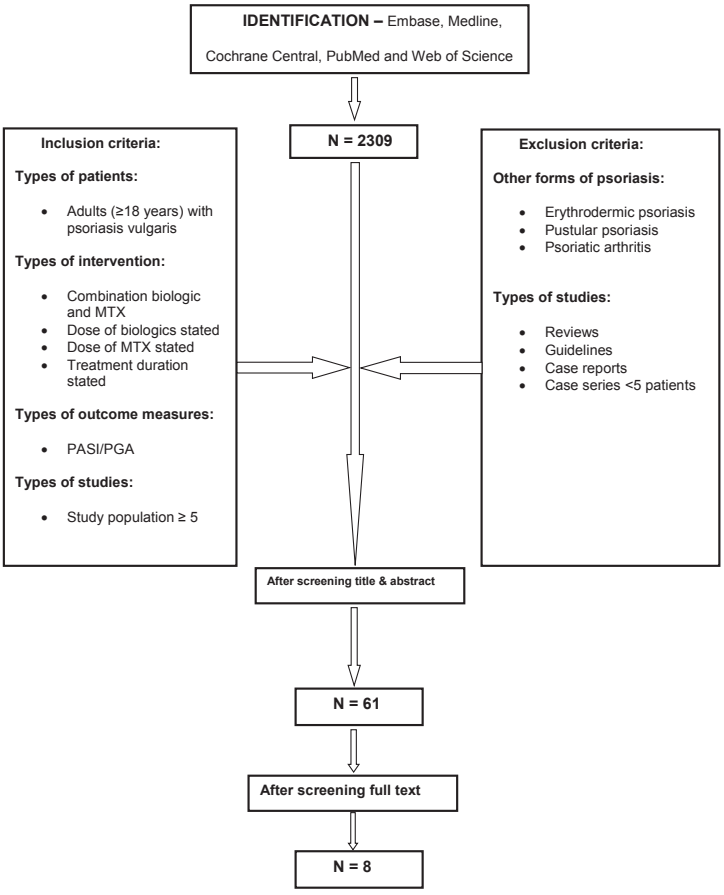

Figure 1. Flowchart of the search and selection process

RESULTS

Eight studies were selected and reviewed (see Table 1). These studies generally showed that combination therapy of a biologic and MTX had higher efficacy than biologic monotherapy. Combination therapy was well tolerated and not associated with higher rates of clinically relevant adverse events.

Combination therapy of adalimumab and MTX

Lopez-Ferrer et al. retrospectively collected data from 119 consecutive patients with moderate to severe plaque psoriasis, they were treated with adalimumab between January 2008 till March 2013 (23). The objective was to analyze the efficacy and safety of adalimumab in a large number of patients with a long follow-up and to assess the possible influence of several variables, including previous exposure to biological agents, on response rates and drug survival. Combination treatment with MTX at a dose ranging from 5 to 12.5mg per week was used in 26 patients. MTX was introduced at the time of loss of response to adalimumab monotherapy. Combination treatment significantly increased PASI-75 (74% vs. 44%) and PASI 90 (68% vs. 35%) response after 6 months of treatment compared to adalimumab monotherapy. The achievement of PASI-90 after 6 months was the only independent predictor for drug survival after 5 years of treatment. Combination therapy with MTX might provide an explanation for the relatively high rate of excellent responders and consequent increase in drug survival. Occurrence of adverse events (AEs) was not reported separately for the combination treatment group.

Philipp et al. performed a retrospective study in 39 patients from six dermatology departments in Germany (24). The objective was to evaluate the effectiveness and safety of the combination of adalimumab and other systemic drugs (MTX, acitretin and ciclosporin) in patients with psoriasis. The group with adalimumab and MTX co-treatment was the largest (n=32), followed by acitretin (n=4) and ciclosporin (n=3). Most patients received a standard dosing regimen of adalimumab and the average MTX dose was 12.4 ± 4.5mg with a median of 15mg per week. Patients were co-treated with MTX for 10.8 ± 11.2 months (range 0.5- 43 months). MTX and adalimumab were started concomitantly or adalimumab was added to ongoing MTX therapy in 20 patients. In 17 out of these 20 patients (85%) a good or a very good efficacy was reported, defined as a PASI score reduction between 50% and 75%. In 12 patients MTX was added because of an insufficient clinical response to adalimumab monotherapy. Combined treatment then led to a good or a very good clinical response, reflected by a PASI reduction between 50% and 75% in eight of 12 patients (67%). The combination therapy showed good overall safety and tolerability. There were 24 AEs in 18 patients; none were severe and/or required hospitalization.

Van den Reek et al. selected a group of 11 psoriasis patients with an insufficient response to adalimumab monotherapy (25). The decision whether a response was considered “insufficient” was made by the treating physician to his/her own discretion . All patients had been treated

Table 1. Selected publications on combination therapy of biologics with MTX in psoriasis

Reference	Type study	No. of patients with biologic and MTX	Mean treat ment duration in weeks	Biologic and MTX average dose	Timing of MTX	Efficacy	Tolerability
Lopez-Ferrer et al. *Br J Dermatol* 2013; **169**: 1141-7	Retro spective	26	24	Adalimumab 40mg eow 5 –12.5mg MTX/week[2]	Add-on MTX when insufficient response to adalimumab	**After 24 weeks** **Combination group** 73.5% PASI-75 67.5% PASI-90 **Monotherapy group** 43.5% PASI-75 34.8% PASI-90	AEs not specified for each group
Philipp et al. *J Dtsch Dermatol Ges* 2012; **10**: 821-37	Retro spective	32	*43*	Adalimumab 40mg eow 12.4±*4.5mg MTX/week*	20 patients received MTX concomitantly	85% PASI-50-75[1]	No serious AEs No treatment-related AEs
					12 patients received add-on MTX when insufficient response to adalimumab	67% PASI-50-75 [1]	
Van den Reek et al. *J Dermatolog Treat* 2013	Prospec tive	11	24	Adalimumab 40mg eow 9.5±*3.2mg MTX/week*	Add-on MTX when insufficient response to adalimumab	**After 12 weeks** 9% PASI-50 **After 24 weeks** 18% PASI-50	No serious AEs No treatment-related AEs
Dalaker et al. *J Eur Acad Dermatol Venereol* 2009; **23**: 277-82	Retro pective	18	106	Infliximab 3-5mg/kg 11.66mg MTX/week[2]	MTX started concomitantly	**After 14 weeks** 91.3% PASI-50 69.6% PASI-75 39.1% PASI-90 **After 1 year** 80% PASI-50 60% PASI-75 33.3% PASI-90	No serious AEs No treatment-related AEs

Table 1. Selected publications on combination therapy of biologics with MTX in psoriasis (continued)

Reference	Type study	No. of patients with biologic and MTX	Mean treat ment duration in weeks	Biologic and MTX average dose	Timing of MTX	Efficacy	Tolerability
Driessen et al. *Br J Dermatol* 2008; **159**: 460-3	Prospec tive	14	40	Etanercept 50mg twice weekly the first 12 weeks, than 25mg twice weekly 12.5mg MTX/week[2]	8 patients started with MTX and received add-on etanercept	Discontinuation of MTX in 6 of these patients resulted in a decrease in clinical efficacy in 5 patients [3]	No serious AEs 8 possible treatment-related AEs to MTX (gastro-intestinal complaints and liver enzyme elevation)
					6 patients received add-on MTX when insufficient response to etanercept	67% improvement efficacy [3]	
Zachariae et al. *Acta Derm Venereol* 2008; **88**: 495-501	Prospec tive	31	24	Etanercept 50mg twice weekly the first 12 weeks, than 25mg twice weekly 13.4mg [2] MTX/week	Add-on etanercept when insufficient response to MTX	**After 24 weeks** **Combination group** 76.4% PASI-75	50 AEs 2 serious treatment-related AEs (infection and vomiting) were considered treatment-related. (infection and
					Etanercept with MTX tapered treatment	**Tapered MTX group** 51.3% PASI-75	51 AEs 5 serious treatment-related AEs (infection, pustular psoriasis, heart failure and atrial fibrillation)
Antoniou et al. *J Eur Acad Dermatol Venereol* 2010; **24**: 1413-20	Retro spective	11	24	Etanercept 50mg twice weekly the first 12 weeks, than 25mg twice weekly 15mg MTX/week[2]	MTX started concomitantly	**After 24 weeks** **Combination group** 36.4% PASI-75 27.2% PASI-50 **Monotherapy group** 41.7% PASI-75 8.3% PASI-50	AEs not specified for each group

Table 1. Selected publications on combination therapy of biologics with MTX in psoriasis (continued)

Reference	Type study	No. of patients with biologic and MTX	Mean treat ment duration in weeks	Biologic and MTX average dose	Timing of MTX	Efficacy	Tolerability
Gottlieb et al. *Br J Dermatol* 2012; **167**: 649-57	Prospec tive	239	24	Etanercept 50mg twice weekly the first 12 weeks, than 50mg once weekly MTX ranging 7.5-15mg/ week [2]	MTX started concomitantly	**After 12 weeks** **Combination group** 70.2% PASI-75 **Monotherapy group** 54.3% PASI-75 **After 24 weeks** **Combination group** 77.3% PASI-75 **Monotherapy group** 60.3% PASI-75	**Combination group** 179 AE 3 serious AEs (lumbar spinal stenosis, synovial cyst and bacterial pneumonia) 7 treatment-related AEs (liver enzyme elevation) **Monotherapy group** 143 AE 3 serious AEs (asthma, cholecystitis and myocardial infarction) 4 treatment-related AEs (liver enzyme elevation)

eow, every other week; PASI, Psoriasis Activity Severity Index; AE; Adverse Events

[1]The average time period of achieving PASI 50-75 is not mentioned in the original paper.

[2] Standard deviation not reported.

[3] Specific PASI score were not mentioned

with adalimumab 40mg every other week. Subsequently, patients were assigned based on the decision by the treating physician, to either adalimumab dose escalation to 40mg per week, or addition of MTX to adalimumab every other week, or to both interventions. In eleven patients an average dose of 9.5mg MTX per week was added to the adalimumab treatment. From these patients, 9% and 18% achieved PASI-50 after 12 and 24 weeks, respectively. In the adalimumab dose escalation group 25% and 35% of the patients achieved PASI-50 after 12 and 24 weeks, respectively. The PASI improvements were measured upon initiation of the treatment intervention. Two SAE (psoriasis exacerbation with hospitalization and death due to bleeding of esophageal varices) were reported after adalimumab dose escalation and were considered "probably unrelated". Tolerability of the combination treatment was not reported.

Combination therapy of infliximab and MTX

Dalaker et al. retrospectively investigated the medical charts of 23 infliximab treated psoriasis patients (26). Eighteen of 23 patients received infliximab 3 - 5mg/kg in combination with an average dose of 11.7mg MTX per week (range 7.5-15mg). The other 5 patients received infliximab 5mg/kg in combination with azathioprine (AZA) 50mg/ day. After 14 weeks of treatment 21 of 23 patients (91,3%) achieved PASI 50, 16 of 23 (70%) achieved PASI-75, and 9 of 23 (39%) achieved PASI-90. After 1 year 12 of 15 patients (80%) retained a PASI-50 improvement, 9 of 15 patients (60%) a PASI-75 and 5 of 15 (33%) a PASI-90 improvement. The results were not described separately for AZA and MTX treatment. Moreover, the number of patients was too small to compare the efficacy of AZA vs. MTX as a concomitant immunosuppressive agent to infliximab therapy. Combination regimens of infliximab with methotrexate or azathioprine were well tolerated, and only one patient discontinued therapy because of an adverse event (lung embolism) after two infusions with infliximab.

Combination therapy of etanercept and MTX

Driessen et al. extracted data from a Dutch database of psoriasis patients that were treated with biologics (27). A group of 14 patients used etanercept 50mg twice weekly the first 12 weeks, than 25mg twice weekly and MTX with average dose of 12.5mg per week (2.5- 35mg weekly) simultaneously. The mean duration of the combination therapy was 40.8 weeks. In six of 14 patients, MTX was introduced during etanercept maintenance therapy to avoid further loss of clinical response, which resulted in an improvement of the clinical efficacy (expressed in PASI) in 4 of the 6 patients (67%). Specific PASI scores were not mentioned. In eight of 14 patients MTX monotherapy was initiated prior to start of etanercept. Discontinuation of MTX in 6 of these 14 patients resulted in a decrease in clinical efficacy in five patients, but again specific PASI scores were not mentioned . Etanercept combined with MTX was well tolerated, and only mild AEs were reported.

Zachariae et al., evaluated in a randomized, open-label, 24-week pilot study, the effect of adding etanercept in cases where MTX monotherapy had failed or had insufficient effect (28). This study randomized patients with plaque psoriasis to either etanercept with an average dose

of 14mg MTX per week (ranging 7.5-25.0mg) that was tapered and discontinued (n=28) or etanercept with continuous average dose of 13.4 MTX per week (ranging 7.5-25.0mg) (n=31). Results for PASI 75 at the end of the study (24 weeks) were significantly better for continuous combination treatment than for etanercept with MTX taper treatment even when adjusted for gender differences (76.4 vs. 51.3%, respectively; P=0.019; adjusted for gender p=0.021). Reported total AEs were similar for both groups, with 51 AEs in the etanercept/MTX taper group and 50 AEs in the combination treatment group.

Antoniou et al. reported a study in which they evaluated the effectiveness and safety of etanercept with or without combination with MTX or ciclosporin as a sequential treatment in 35 patients previously treated with efalizumab(29). All patients that were switched from efalizumab received combination therapy of etanercept and MTX (15mg/week) or ciclosporin (3mg/kg) where the initial etanercept regimen was 50mg twice a week for the first 12 weeks, followed by 25mg twice a week. Baseline PASI (before transition to etanercept) ranged from 1.5 to 34.8. Eleven patients received combination therapy of etanercept and MTX for a period of 2-4 months. During this period the MTX dose was gradually tapered. At 24 weeks 36% patients of the combination therapy vs 42% patients of the monotherapy group reached PASI-75 and 27% patients of the combination therapy vs 8% patients of the monotherapy group reached PASI-50. AEs were not reported separately for the MTX combination therapy group.

Gottlieb et al. conducted a randomized, double-blind, placebo-controlled, multicentre, phase IIIb study of 24 weeks duration (30). This study evaluated the combination therapy of etanercept with MTX vs. etanercept monotherapy in patients with moderate to severe plaque psoriasis who had not failed prior MTX or TNFi therapy. Patients were excluded if they had received an anti-TNFα therapy or other biologics within 3 months or IL-12 / IL-23 inhibitors within 6 months prior to the study. Prior use of MTX or etanercept did not exclude enrolment. Patients received the standard dose of etanercept 50mg twice weekly for 12 weeks followed by 50mg once weekly for 12 weeks and were randomized 1:1 to receive MTX (7.5-15mgweekly) or placebo. The MTX dose was titrated from 7.5mg in week 1 and 2, 10mg in week 3 and 4, finally to a maximum of 15mg from week 5 until week 24. Two hundred thirty-nine (239) patients were enrolled in each arm. The combination therapy group had a significantly higher proportion of patients with PASI-75 improvement at week 24 compared with the monotherapy group (77% vs. 60%; P<0.0001). The PASI improvement scores at week 12 were also significantly higher in the combination group than in the monotherapy group (70% vs. 54%; P=0.01). There were significantly more AEs reported in the combination arm (179 AE) than in the monotherapy arm (143 AE), but most AEs were mild or moderate in severity.

Combination therapy ustekinumab and MTX

No published data are available on the combination therapy of ustekinumab with MTX in psoriasis.

DISCUSSION

The reviewed studies in general show favourable results with regard to efficacy for the combination therapy of a biologic and MTX compared to biologic monotherapy. In addition, combination therapy was well tolerated and did not appear to be associated with higher rates of clinically relevant adverse events. This is consistent with previous findings in other immune mediated inflammatory diseases like rheumatoid arthritis.

However, the following limitations of the studies reviewed should be taken into account. Firstly, most studies were performed with relatively small numbers of patients (range 11-32). The only exception was the randomized controlled trial (RCT) by Gottlieb et al., which prospectively investigated the efficacy and safety of etanercept monotherapy versus MTX combination treatment in a relatively large number (n=239) of patients (30). Secondly, most treatment durations were short (24 weeks) and had a retrospective design, with the inherent disadvantage that factors such as the dosing regimen and duration of treatment were not the same for individual patients.

In contrast with our results some studies have reported that concomitant use of MTX does not improve the drug survival (23, 31-33). However, these studies were excluded in the selection process of this systematic review since pivotal efficacy data such as PASI or PGA score and or dose of MTX were not reported. Without these pivotal data, it is unclear how the authors of these studies came to their conclusions and can in our opinion not be regarded as true contrasting results.

However, large prospective studies with a long follow-up have been performed in rheumatoid arthritis (RA). These studies have shown that, when compared with biologic monotherapy, combination therapy of biologics (e.g. etanercept, adalimumab and infliximab) and MTX yields better clinical efficacy and has a comparable tolerability and safety profile (12, 19, 20). Consequently, combination regimens with MTX are included in the EULAR recommendations for the treatment of RA (21).

It was previously shown that in RA and psoriasis patients treated with adalimumab that good responders had significantly higher serum drug concentrations than non-responders and moderate responders (34, 35). The serum adalimumab concentration time profile depends on different factors such as absorption rate from subcutaneous tissue, distribution and clearance of the drug. These pharmacokinetics are influenced by many factors such as weight, age and disease activity (36). The production of neutralizing anti-drug antibodies (ADA) leads to direct inhibition of the pharmacological action and the formation of drug–ADA immune complexes resulting in enhanced clearance of the drug. Consequently, patients producing higher titers of ADA will have lower or undetectable concentrations of adalimumab that can still bind to TNFα, resulting in a higher disease activity (36). Recent evidence from rheumatology shows that one of the main factors influencing the pharmacokinetics of adalimumab was the concomitant use of MTX (36). On average, patients with adalimumab monotherapy had an

adalimumab concentration of 4.1 μg/mL, whereas patients concomitantly treated with MTX had a median concentration of 7.4 μg/mL (36). It is hypothesized that MTX reduces the immune response against a foreign epitope (idiotype of biologic) by blocking the expansion of B-cell populations that can produce ADA through plasma cell differentiation (10). Since ADA bind to the idiotype of adalimumab (37), functional drug concentrations are higher in patients taking concomitant MTX.

Hypothetically, in some patients combination treatment may not only improve the pharmacokinetics, but will also allow dose reductions of the biologic (e.g. prolonging of the dosing interval) without losing clinical response. This idea is supported by observations in RA patients treated with adalimumab by Pouw et al. who observed that concentrations exceeding 8 μg/mL compared with 5–8 μg/mL resulted in no additional improvement of clinical response (36). However, the effects of dose tapering or prolonging of dosing intervals in this "overtreated" group still need to be investigated in clinical studies.

From a theoretical point of view, the timing of MTX co-treatment initiation might be a factor that influences the success of combination therapy. However, there is insufficient evidence from the literature reviewed to support this concept (Table 1). There is also no consensus in the current literature on the most effective and safe dose of MTX for combination treatment. Although the long-term safety results of MTX combination therapy in RA patients are reassuring, this may not be the same for psoriasis patients because of an inherent higher risk of non-alcoholic fatty liver disease (38, 39). In view of the results in Table 1, a MTX dose ranging from 5-15mg/week may be considered for improving efficacy and drug survival while limiting the risk of hepatotoxicity (24,30,33).

CONCLUSION

We conclude that the available evidence on combination treatment of biologics and MTX in psoriasis is currently not sufficient to propose an amendment of the current treatment guidelines. However, our findings do support the initiation of adequately powered RCTs to compare biologic monotherapy versus MTX combination therapy in psoriasis. Based on the RA studies, we propose a trial of 12-24 weeks to assess the short-term clinical outcomes, and have a follow-up of at least five years to assess long-term safety and drug survival.

REFERENCES

1. Rapp SR, Feldman SR, Exum ML, Fleischer AB, Jr., Reboussin DM. Psoriasis causes as much disability as other major medical diseases. J Am Acad Dermatol. 1999;41(3 Pt 1):401-7.
2. Vincent FB, Morand EF, Murphy K, Mackay F, Mariette X, Marcelli C. Antidrug antibodies (ADAb) to tumour necrosis factor (TNF)-specific neutralising agents in chronic inflammatory diseases: A real issue, a clinical perspective. Ann Rheum Dis. 2013;72(2):165-78.
3. Brezinski EA, Armstrong AW. Off-label biologic regimens in psoriasis: a systematic review of efficacy and safety of dose escalation, reduction, and interrupted biologic therapy. PLoS One. 2012;7(4):e33486.
4. Schmitt J, Rosumeck S, Thomaschewski G, Sporbeck B, Haufe E, Nast A. Efficacy and safety of systemic treatments for moderate-to-severe psoriasis: meta-analysis of randomized controlled trials. Br J Dermatol. 2014 Feb;170(2):274-303.
5. Leonardi CL, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). Lancet. 2008;371(9625):1665-74.
6. Mrowietz U, de Jong EM, Kragballe K, Langley R, Nast A, Puig L, et al. A consensus report on appropriate treatment optimization and transitioning in the management of moderate-to-severe plaque psoriasis. J Eur Acad Dermatol Venereol. 2014 Apr;28(4):438-53.
7. Zandvliet ML, van Bezooijen JS, Bos MA, Prens EP, van Doorn M, Bijen I, et al. Monitoring antigen-specific biologics: current knowledge and future prospects. Ther Drug Monit. 2013;35(5):588-94.
8. Carrascosa JM, van Doorn MB, Lahfa M, Nestle FO, Jullien D, Prinz JC. Clinical relevance of immunogenicity of biologics in psoriasis: Implications for treatment strategies. J Eur Acad Dermatol Venereol. 2014Nov;28(11):1424-30.
9. Jani M, Barton A, Warren RB, Griffiths CE, Chinoy H. The role of DMARDs in reducing the immunogenicity of TNF inhibitors in chronic inflammatory diseases. Rheumatology (Oxford). 2014 Feb;53(2):213-22.
10. Krieckaert CL, Nurmohamed MT, Wolbink GJ. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. Ann Rheum Dis. 2012;71(11):1914-5.
11. Emery P, Breedveld FC, Hall S, Durez P, Chang DJ, Robertson D, et al. Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis (COMET): a randomised, double-blind, parallel treatment trial. Lancet. 2008;372(9636):375-82.
12. Klareskog L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet. 2004;363(9410):675-81.
13. van der Heijde D, Klareskog L, Rodriguez-Valverde V, Codreanu C, Bolosiu H, Melo-Gomes J, et al. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. Arthritis Rheum. 2006;54(4):1063-74.
14. van Riel PL, Taggart AJ, Sany J, Gaubitz M, Nab HW, Pedersen R, et al. Efficacy and safety of combination etanercept and methotrexate versus etanercept alone in patients with rheumatoid arthritis with an inadequate response to methotrexate: the ADORE study. Ann Rheum Dis. 2006;65(11):1478-83.
15. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med. 1999;340(4):253-9.

16. Weisman MH, Moreland LW, Furst DE, Weinblatt ME, Keystone EC, Paulus HE, et al. Efficacy, pharmacokinetic, and safety assessment of adalimumab, a fully human anti-tumor necrosis factor-alpha monoclonal antibody, in adults with rheumatoid arthritis receiving concomitant methotrexate: a pilot study. Clin Ther. 2003;25(6):1700-21.
17. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum. 2003;48(1):35-45.
18. Bang LM, Keating GM. Adalimumab: a review of its use in rheumatoid arthritis. BioDrugs. 2004;18(2):121-39.
19. Keystone EC, van der Heijde D, Kavanaugh A, Kupper H, Liu S, Guerette B, et al. Clinical, Functional, and Radiographic Benefits of Longterm Adalimumab Plus Methotrexate: Final 10-year Data in Long-standing Rheumatoid Arthritis. J Rheumatol. 2013 Sep;40(9):1487-97.
20. St Clair EW, van der Heijde DM, Smolen JS, Maini RN, Bathon JM, Emery P, et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum. 2004;50(11):3432-43.
21. Smolen JS, Landewe R, Breedveld FC, Buch M, Burmester G, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis. 2014;73(3):492-509.
22. Pathirana D, Ormerod AD, Saiag P, Smith C, Spuls PI, Nast A, et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol. 2009;23 Suppl 2:1-70.
23. Lopez-Ferrer A, Vilarrasa E, Gich IJ, Puig L. Adalimumab for the treatment of psoriasis in real life: A retrospective cohort of 119 patients at a single Spanish centre. Br J Dermatol. 2013;169(5):1141-7.
24. Philipp S, Wilsmann-Theis D, Weyergraf A, Rotterdam S, Frambach Y, Gerdes S, et al. Combination of adalimumab with traditional systemic antipsoriatic drugs - a report of 39 cases. J Dtsch Dermatol Ges. 2012;10(11):821-37.
25. van den Reek JM, van Lumig PP, Kievit W, Zweegers J, van de Kerkhof PC, Seyger MM, et al. Effectiveness of adalimumab dose escalation, combination therapy of adalimumab with methotrexate, or both in patients with psoriasis in daily practice. J Dermatolog Treat. 2013 Oct;24(5):361-8.
26. Dalaker M, Bonesronning JH. Long-term maintenance treatment of moderate-to-severe plaque psoriasis with infliximab in combination with methotrexate or azathioprine in a retrospective cohort. J Eur Acad Dermatol Venereol. 2009;23(3):277-82.
27. Driessen RJ, van de Kerkhof PC, de Jong EM. Etanercept combined with methotrexate for high-need psoriasis. Br J Dermatol. 2008;159(2):460-3.
28. Zachariae C, Mork NJ, Reunala T, Lorentzen H, Falk E, Karvonen SL, et al. The combination of etanercept and methotrexate increases the effectiveness of treatment in active psoriasis despite inadequate effect of methotrexate therapy. Acta Derm Venereol. 2008;88(5):495-501.
29. Antoniou C, Dessinioti C, Vergou T, Stratigos AJ, Avgerinou G, Kostaki M, et al. Sequential treatment with biologics: Switching from efalizumab to etanercept in 35 patients with high-need psoriasis. J Eur Acad Dermatol Venereol. 2010;24(12):1413-20.
30. Gottlieb AB, Langley RG, Strober BE, Papp KA, Klekotka P, Creamer K, et al. A randomized, double-blind, placebo-controlled study to evaluate the addition of methotrexate to etanercept in patients with moderate to severe plaque psoriasis. Br J Dermatol. 2012;167(3):649-57.
31. van den Reek JM, van Lumig PP, Driessen RJ, van de Kerkhof PC, Seyger MM, Kievit W, et al. Determinants of drug survival for etanercept in a long-term daily practice cohort of patients with psoriasis. Br J Dermatol. 2014;170(2):415-24.

32. Gniadecki R, Kragballe K, Dam TN, Skov L. Comparison of drug survival rates for adalimumab, etanercept and infliximab in patients with psoriasis vulgaris. Br J Dermatol. 2011;164(5):1091-6.
33. Antoniou C, Stefanaki I, Stratigos A, Moustou E, Vergou T, Stavropoulos P, et al. Infliximab for the treatment of psoriasis in Greece: 4 years of clinical experience at a single centre. Br J Dermatol. 2010;162(5):1117-23.
34. Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JW, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA. 2011;305(14):1460-8.
35. Lecluse LL, Driessen RJ, Spuls PI, de Jong EM, Stapel SO, van Doorn MB, et al. Extent and clinical consequences of antibody formation against adalimumab in patients with plaque psoriasis. Arch Dermatol. 2010;146(2):127-32.
36. Pouw MF, Krieckaert CL, Nurmohamed MT, van der Kleij D, Aarden L, Rispens T, et al. Key findings towards optimising adalimumab treatment: the concentration-effect curve. Ann Rheum Dis. 2015 Mar;74(3):513-8.
37. van Schouwenburg PA, van de Stadt LA, de Jong RN, van Buren EE, Kruithof S, de Groot E, et al. Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation. Ann Rheum Dis. 2013;72(1):104-9.
38. Gisondi P, Targher G, Zoppini G, Girolomoni G. Non-alcoholic fatty liver disease in patients with chronic plaque psoriasis. J Hepatol. 2009;51(4):758-64.
39. van der Voort EA, Koehler EM, Dowlatshahi EA, Hofman A, Stricker BH, Janssen HL, et al. Psoriasis is independently associated with nonalcoholic fatty liver disease in patients 55 years old or older: Results from a population-based study. J Am Acad Dermatol. 2014;70(3):517-24.

Chapter 3

Optimising adalimumab treatment in psoriasis with concomitant methotrexate (OPTIMAP): study protocol of a pragmatic, single-blinded, investigator-initiated randomized controlled trial

C.I.M. Busard, S.P. Menting*,
J.S. van Bezooijen*, J.M. van den Reek,
E.P. Prens, B.A. Hutten, E.M. de Jong,
M.B.A. van Doorn, P.h. I Spuls

**these authors share second autorship*

Trials. 2017 Feb 2;18(1):52

ABSTRACT

Background

The introduction of anti-TNFs has revolutionized the treatment of psoriasis with achievement of treatment goals (PASI-75, remission) that are not usually met with conventional systemics. Nevertheless, some patients continue to experience persistent disease activity or treatment failure over time. Strategies to optimize treatment outcomes include the use of concomitant methotrexate, which has demonstrated beneficial effects on pharmacokinetics and treatment efficacy in psoriasis and other inflammatory diseases.

Methods

This is an investigator-initiated, multicenter randomized controlled trial (RCT) designed to compare combination treatment of adalimumab and methotrexate with adalimumab monotherapy in patients with psoriasis. Primary outcome is adalimumab drug survival at week 49. Other outcomes include improvement in disease severity and quality of life, tolerability and safety. Moreover, anti-adalimumab antibodies and adalimumab serum concentrations will be measured and correlations between genotypes and clinical outcomes will be assessed. Patient recruitment started in March 2014. Up to now, 36 patients have been randomized. Many more patients have been (pre)screened. A total of 93 patients is desired to meet an adequate sample size. In our experience the main limitation for recruitment is prior adalimumab therapy and intolerability or toxicity for methotrexate in the past.

Discussion

OPTIMAP is the first RCT to examine combination therapy with adalimumab and methotrexate in a psoriasis population. With data derived from this study we expect to provide valuable clinical data on long-term treatment outcomes. These data will be supported by assessment of the impact of concomitant methotrexate on adalimumab pharmacokinetics. Furthermore, the influence of several single nucleotide polymorphisms on adalimumab response will be analysed in order to support the development of a more personalized approach for this targeted therapy.

BACKGROUND

Adalimumab has shown to be highly valued by psoriasis patients due to profound improvements on disease severity and a favorable safety profile (1, 2). Although its introduction (together with other anti-TNFs) has majorly advanced psoriasis care, some patients experience persistent disease activity (primary non-responders), treatment failure over time (secondary non-responders) or side-effects (3-5). Several factors have been identified to play a role in primary and secondary non-response to anti-TNFs, including pharmacokinetic factors such as the formation of anti-drug antibodies (immunogenicity) and inter-individual variation in serum drug concentrations as well as pharmacogenetic factors such as the absence or presence of certain single nucleotide polymorphisms (SNPs) affecting drug metabolization (6, 7).

When anti-drug antibodies are formed in patients treated with an anti-TNFα, clearance of the biologic can, to a certain extent, be accelerated depending on the concentration of the anti-drug antibodies (12). Moreover, antidrug antibodies can be functionally neutralizing, thereby directly affecting treatment efficacy (13). Multiple studies observed an association between the formation of anti-adalimumab antibodies, reduced serum concentrations and diminished clinical response (3, 8-11). In other inflammatory diseases, such as rheumatoid arthritis (RA) and crohn's disease, concomitant use of methotrexate (MTX) during treatment with certain TNFα inhibitors (adalimumab, infliximab and golimumab) has demonstrated to decrease immunogenicity and significantly reduce clearance resulting in higher systemic exposure and enhanced clinical efficacy (9, 14-17).

Therefore, the use of combination therapy may be beneficial for successful long-term adalimumab treatment. In addition, combination therapy may enable dose reductions of individual agents, thereby decreasing toxicity and improving tolerability and compliance (18). Moreover, by targeting unregulated increased cytokine levels associated with inflammatory comorbid conditions, it is hypothesized that combination therapy may also provide a broader benefit to the patient by reducing the risk of, for example, cardiovascular events (19). On the other hand, combination therapy may theoretically convey an increased risk for serious infections and malignancies.

Currently available evidence on anti-TNFα combination therapy with MTX in psoriasis is limited to two randomized controlled trials (RCTs) on etanercept with MTX (22, 23) and few observational studies and case series on the other anti-TNFα agents (18). The two RCTs on etanercept and MTX provided promising results with superior efficacy of etanercept with MTX compared to etanercept monotherapy. RCTs investigating combined treatment with adalimumab and MTX are lacking (18, 24).

In order to investigate whether adalimumab treatment can be optimized by using concomitant MTX, long-term clinical and pharmacokinetic data on the use of adalimumab in combination with MTX are desired. .

Additionally, as several polymorphisms have been identified as potential predictors for anti-TNF therapy in psoriasis (e.g. TNFR1B, TNFAIP3, IL12B/IL23R) (6, 27) and other chronic inflammatory diseases (e.g. FcGR and ATG16L1) (28, 29) it will be valuable to detect genetic factors associated with response to adalimumab in order to support personalized care.

Aims & objectives

- To gain long-term RCT data on the efficacy and safety of adalimumab combined with MTX compared to adalimumab monotherapy
- To assess the impact of concomitant MTX on adalimumab immunogenicity and serum concentrations
- To test appropriate candidate genes and correlate genotypes with trial outcomes

METHODS

The trial was granted ethics approval by the Academic Medical Center research ethics committee (METC 2013_346). The trial is registered at the Netherlands national Trial Register (Trial Number: NTR4499). All participants will sign informed consent before participation. The study is being conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other relevant guidelines, regulations and acts.

Participants

Patients will be recruited from the outpatient clinics of the Departments of Dermatology of the Academic Medical Center (AMC) Amsterdam, Erasmus University Medical Center (EMC) Rotterdam and the Radboud University Medical Center (RUMC) Nijmegen. Moreover, other dermatologists will be contacted to recruit and refer eligible patients to the participating centers. Participants must meet the inclusion criteria and none of the exclusion criteria (Table 1) in order to participate. These will be assessed at the screening visit. Potential participants who are deemed ineligible at screening will be allowed a second screening visit if the reason for ineligibility is a temporary status (e.g. latent tuberculosis).

Table 1. Eligibility criteria

Inclusion	Exclusion
► ≥18 years ► Diagnosis of moderate to severe plaque psoriasis (PASI ≥8) ► Adalimumab naïve ► Candidate for biologic therapy ► Willing and able to use adequate contraceptives during the study	► History of significant MTX toxicity, intolerability or contraindication ► Known liver or kidney malfunction ► Alcohol abuse ► Bone marrow hypoplasia, leukocytopenia, thrombocytopenia or significant anaemia ► Known severe or chronic infections like tuberculosis or HIV ► Ulcers in the oral cavity or known active ulcers in digestive tract ► Pregnant or nursing women ► Need for live vaccinations ► Use of other immunosuppressive medication (e.g. prednisone, mycophenolatemofetyl (Cellcept), ciclosporine (Neoral), sirolimus (Rapamune), systemic tacrolimus (Prograft))

Interventions

All patients receive adalimumab 40mg subcutaneously every other week starting one week after a loading dose of 80mg and will be randomized 1:1 to receive either oral MTX 10mg weekly (combination group) or no addition of MTX(monotherapy group). MTX therapy will be initiated two weeks prior to baseline and administration will be followed by folic acid 5mg 24 hours after MTX intake (see flowchart; Figure 1).

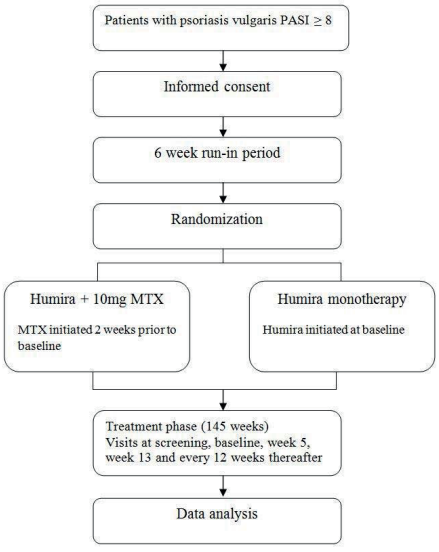

Figure 1. Optimizing adalimumab treatment in plaque psoriasis with concomitant methotrexate; ('OPTIMAP') study flow diagram.

In case of MTX toxicity (e.g. liver toxicity or leukopenia) or intolerability, titration can be paused (for a maximum of two weeks up to four times during the entire study) or the dose can be adjusted to 7.5mg. Moreover, patients are allowed to switch from oral to subcutaneous administration. In case of adalimumab toxicity or intolerability, titration can be paused (for a maximum of 2 weeks up to 4 times during each study year).

Throughout the study, no systemic anti-psoriatic drugs are allowed for treatment other than study medication (Table 2/ Table 3). If medically necessary, (i.e. to control intolerable psoriasis activity), rescue treatment with topical corticosteroids, vitamin D derivates (calcipotriol/ betamethasone or calcitriol) or calcineurin inhibitors may be provided to study patients at the discretion of the investigator after baseline and through week 145 (end of study).

Table 2. Wash out-periods

Therapy	Wash-out period
Topical therapy	2 weeks
Phototherapy	2 weeks
Conventional systemic therapy / etanercept	4 weeks
Infliximab/ ustekinumab	6 weeks

Table 3. Allowed escape medication

Scalp/palms/soles	Low or high potency corticosteroids, calcitriol/ calcipotriol or a combination
Face and body	Low potency corticosteroids, calcitriol/ calcipotriol or topical tacrolimus 0.1% or 0.03%
Psoriasis inverse component	Topical tacrolimus 0.1% or 0.03%

Randomization and blinding

Consecutive patients will be prospectively enrolled and randomly assigned if eligible to either the intervention (adalimumab with MTX) or control (adalimumab monotherapy) group after obtaining informed consent. Each consecutive patient will be assigned a randomization number according to a computer-generated randomization list (ALEA) using random block sizes of 2, 4, 6, and 8 to ensure allocation concealment. Randomization is stratified for TNFα-blocker exposure status to achieve balance with regard to prior TNFα-blocker exposure in the study population.

This is an observer-blinded study. The observer (outcome assessor) will perform clinical outcome assessments of disease severity (PASI and investigator global assessment (IGA)) at each study visit. The clinician performs all other study procedures and is not blinded. Both clinician and participant know the treatment allocation, as such no special measures are required to allow for breaking of treatment codes. However, treatment allocation will not be revealed to the recruiting physician until participants' details and key stratification variables have been irrevocably entered onto the web-based randomization site.

Endpoints

Primary outcomes:

– Adalimumab drug survival (number of patients still on adalimumab treatment) at week 49

Secondary outcomes:

– Adalimumab drug survival (number of patients still on adalimumab treatment) at week 145
– Proportion of patients that reach treatment goals* at week 13, week 25, week 49 and week 145
– Proportion of patients achieving PASI-75 at weeks 49 and 145
– Proportion of patients achieving IGA clear or almost clear at weeks 49 and 145
– Mean improvement in PASI at weeks 49 and 145
– Proportion of patients with PGA clear or almost clear at weeks 49 and 145
– Mean improvement in DLQI and Skindex at weeks 49 and 145
– Proportion of patients with (serious) adverse events at weeks 49 and 145
– Proportion of patients with changes in laboratory assessments at weeks 49 and 145
– Proportion of patients with (no, low or high) levels of antibodies at weeks 49 and 145
– Median adalimumab trough concentrations (mg/L) at week 49 and 145
– Correlation between genetic polymorphisms and adalimumab response

*Treatment goals will be achieved if patients reach PASI ≥75 or PASI ≥ 50 in combination with DLQI≤5. Treatment goals will not be achieved in case PASI<50 or PASI ≥50<75 in combination with DLQI≥5. (25)

Procedures and Assessments

Patients will visit the outpatient clinic at screening, baseline week 5, week 13 and every 12 weeks thereafter until study completion (weeks 25, 37, 49, 61, 73, 85, 97, 109, 121, 133, 145) (Figure 1).

A variety of parameters will be collected during each visit to assess efficacy, including physician (PASI/ IGA (static; scale 0-4 (26)) and patient reported (patient reported global assessment (PGA static; scale 0-4)) outcomes. Quality of life assessment will be performed using Skindex and DLQI questionnaires. Safety will be assessed by evaluating the incidence of (serious) adverse events, obtaining a detailed medical history, thorough physical examination, vital signs, clinical laboratory testing and urinalysis (including pregnancy tests females of childbearing potential at screening). Concomitant medication and medical procedures will be collected from obtainment of informed consent up to end of study. Patients will receive a diary in which they will register the administration dates of adalimumab (and MTX in the intervention group), any changes in their health status and/or changes in concomitant medication used. The local investigator reviews the diary to determine drug adherence and the incidence

and type of adverse events. An independent Data Safety Monitoring Board (DSMB) has been established to review efficacy and safety data periodically in an unblinded fashion.

Laboratory testing

Blood samples will be collected at each visit (serum samples are collected just before administration of adalimumab to ensure accurate determination of serum through concentrations) to monitor drug safety, to determine immunogenicity against adalimumab and to measure adalimumab serum through concentrations. Samples for serum preparation are kept at room temperature during 1-2 hours for coagulation, followed by centrifugation at 3000 RPM for 15 minutes at room temperature. Supernatant is collected, aliquoted and stored at -20°C until further use. Adalimumab serum through levels will be determined using a non-commercial ELISA (Sanquin, The Netherlands). Detection of anti-adalimumab antibodies will be performed through a radioimmunoassay (Sanquin, The Netherlands). The antibody test will be considered positive when the antibody concentration exceeds 12 AU/mL. Concentrations between 12 and 100 AU/mL will be considered low antibody titers and those above 100 AU/mL will be considered high antibody titers.

Additionally, a single blood sample will be collected at screening from which DNA will be collected and stored at -80 Celsius. As scientific interest in this field is currently increasing, DNA analysis will be performed based upon accumulating data acquired from (ongoing) pharmacokinetic studies.

Justification of sample size

A total of 84 patients (randomised 1:1 to concomitant MTX or no MTX) will give the study at least 80% power at a 0.05 two-sided significance level using a two-sample Chi-square test to detect a difference of 28% in drug survival at week 49. We aim to enroll 93 patients to allow for an approximate 10% loss to follow-up. These calculations were performed using Nquery 6.0.2. The expected clinically relevant difference in drug survival between both treatment groups was hypothesized based on studies performed in RA patients due to the lack of data in a psoriasis population. The prevalence of (clinically relevant) anti-drug antibody formation is estimated to be 45% in patients on adalimumab monotherapy (a similar percentage is found in psoriasis patients (30)) and around 17% in patients on adalimumab with low dose (5-10mg) MTX after 49 weeks (31). A clear correlation between antibody formation and treatment failure (with subsequent treatment discontinuation) in patients on adalimumab has been demonstrated (30). Based on these data, drug survival is estimated to be 83% (100 minus 17) for the experimental group and 55% (100 minus 45) for the control group after 49 weeks of follow up.

Statistical analysis

The primary analysis will be conducted on the intention-to-treat population, including all randomized participants in the groups to which they were randomized. A per protocol popula-

tion (excluding major protocol violations) will be used to check the robustness of the primary analyses. The safety population will consist of all patients receiving at least one dose of the study drug.

Adverse events will be coded according to the Med-DRA adverse event dictionary. The overall incidence of serious adverse events and adverse events and number and proportion of patients reporting such events will be summarized by treatment group.

Differences in dichotomous outcomes among the two study groups will be analyzed using the chi-square test or Fisher's exact test when the expected cell frequencies fall below five. We will express differences in drug survival as absolute differences and relative risks, with associated 95% confidence intervals, with the group on adalimumab monotherapy as the reference. In case patients will be lost to follow-up during the study period, we will analyze these data by means of survival analysis. We will construct cumulative survival curves (Kaplan-Meier method) for the treatment groups and these curves will be compared using the log-rank test. .

One-way analysis-of-variance statistics will be calculated to compare continuous outcome measures between groups.

There are no formal planned interim analyses, but progress reports on all data issues are presented to the Data Safety Monitoring Board (DSMB).

TRIAL STATUS

Patient recruitment started in March 2014 and is currently ongoing. Based on our experience so far, recruitment is limited by two main factors; prior use of adalimumab and intolerability or toxicity for MTX in the past.

Moreover, disease activity in patients that are transitioned from another biologic is often suppressed (< PASI 8). To enlarge the geographical area in which patients can participate to the study and to enhance patient recruitment three additional hospitals have been activated for patient recruitment; Amphia Hospital Breda and Bravis Hospital Bergen op Zoom (The Netherlands) and Ghent University Hospital (Belgium).

DISCUSSION

Although combination treatment with anti-TNFs and MTX are being prescribed for psoriasis in clinical practice, available evidence and guidance on the use of combination treatment is limited. No consensus about certain treatments aspect such as timing of initiation of MTX (prior to anti-TNF or during anti-TNF therapy) and MTX dosing exists. Therefore, besides the rationale for our primary endpoints, we would like to emphasize the choice of dosing and initiation of comedication for the current RCT.

Primary endpoint

In this study, drug survival after 49 weeks of treatment is chosen as primary endpoint. Based on currently available evidence on other inflammatory diseases response rates to anti-TNFs in patients with and without concomitant MTX may remain similar, however, drug survival is often superior in patients receiving co-medication compared to monotherapy and this difference tends to be more prominent than differences in response rates. Moreover, by categorizing reasons for treatment discontinuation (lack of efficacy, safety concerns), several important treatment aspects are being combined.

Initiation of MTX prior to adalimumab therapy

Concomitant use of MTX has demonstrated to significantly reduce the clearance of adalimumab, resulting in higher adalimumab trough levels in patients with RA (14, 32, 33). However, it takes time for MTX to exert a full effect on the pharmacokinetics of adalimumab (33). The slow onset of drug action of MTX can be attributed to an intracellular accumulation process (34, 35). After MTX uptake into cells, it is converted to MTX-polyglutamates, active metabolites which are believed to exert the anti-inflammatory actions of MTX. The current product label for adalimumab indicates that methotrexate decreases the apparent clearance of adalimumab after single and multiple doses by 29% and 44%, respectively (33). In order to ensure maximal potential for MTX to exert a beneficial effect on adalimumab pharmacokinetics from the start on, MTX therapy is initiated two weeks before administration of adalimumab (at baseline) in the intervention group.

Choice of MTX dosing

The dose of MTX as monotherapy can range from 7.5 to 25mg/week, depending on national guidelines and patient / physician's preference. A systematic literature review of MTX monotherapy has recommended initial treatment with 10–15mg orally with dose increases to 20mg/week if needed and tolerated (36). Available evidence suggests that MTX toxicity is dose-dependent and low dose MTX monotherapy treatment can be effective. However, no RCTs have explored the minimally effective dose of MTX in a group of patients when used in combination with an TNFα inhibitor. This dose may differ from minimally effective monotherapy doses.

In a prospective cohort study (n=272) in RA patients a substantial decrease in immunogenicity against adalimumab was demonstrated with low-dose MTX (5-10mg/week) (31). These data are confirmed in a recently conducted RCT in 395 patients with RA. Results indicate an (non-significant) increase in adalimumab serum concentrations with higher doses of MTX (10-20mg) compared to low-dose MTX (2.5-5mg). However, a dose of 5mg of concomitant MTX seems sufficient to maintain serum concentrations within the therapeutic range.

In the treatment of psoriasis, methotrexate 10mg per week is an accepted dose for treating psoriasis according to (inter)national guidelines (32). In order to avoid an increased risk of side

effects like hepatotoxicity and subsequent early study termination a dosage of 10mg MTX/ week is chosen in this RCT over a higher dose.

With this RCT we aim to improve the body of evidence on efficacy and safety of adalimumab and MTX combination treatment in order to investigate whether MTX can optimize adalimumab treatment. Moreover, with the analysis of pharmacogenetic data, we hope to support personalized medicine and more accurate prediction of treatment response.

Study strengths and limitations

This study represents the first RCT on combined treatment with adalimumab and MTX. Data will be extracted and analyzed independent of industry. It is an observer-blinded study with concealment of allocation. Both clinical, pharmacokinetic and pharmacogenetic outcomes will be assessed on short and long-term.

However, some limitations apply. Due to the pragmatic study design, trial conduction is not double-blind. Moreover, the sample size limits assessment of correlations between genetic polymorphisms and clinical or pharmacokinetic outcomes. Optimal dosing and timing of methotrexate comedication are not evaluated in this study and will have to be investigated in future research.

Trial status

This RTC is ongoing.

REFERENCES

1. Saurat JH, Stingl G, Dubertret L, Papp K, Langley RG, Ortonne JP, et al. Efficacy and safety results from the randomized controlled comparative study of adalimumab vs. methotrexate vs. placebo in patients with psoriasis (CHAMPION). Br J Dermatol. 2008;158(3):558-66.
2. van den Reek JM, van Luumig PP, Otero ME, Zweegers J, van de Kerkhof PC, Ossenkoppele PM, et al. Satisfaction of treatment with biologics is high in psoriasis: results from the Bio-CAPTURE network. Br J Dermatol. 2014;170(5):1158-65.
3. Menting SP, van Lumig PP, de Vries AC, van den Reek JM, van der Kleij D, de Jong EM, et al. Extent and consequences of antibody formation against adalimumab in patients with psoriasis: one-year follow-up. JAMA Dermatol. 2014;150(2):130-6.
4. Warren RB, Smith CH, Yiu ZZ, Ashcroft DM, Barker JN, Burden AD, et al. Differential Drug Survival of Biologic Therapies for the Treatment of Psoriasis: A Prospective Observational Cohort Study from the British Association of Dermatologists Biologic Interventions Register (BADBIR). J Invest Dermatol. 2015;135(11):2632-40.
5. Bito T, Nishikawa R, Hatakeyama M, Kikusawa A, Kanki H, Nagai H, et al. Influence of neutralizing antibodies to adalimumab and infliximab on the treatment of psoriasis. Br J Dermatol. 2014;170(4):922-9.
6. Prieto-Perez R, Cabaleiro T, Dauden E, Abad-Santos F. Gene polymorphisms that can predict response to anti-TNF therapy in patients with psoriasis and related autoimmune diseases. Pharmacogenomics J. 2013;13(4):297-305.
7. Coto-Segura P, Batalla A, Gonzalez-Fernandez D, Gomez J, Santos-Juanes J, Queiro R, et al. CD-KAL1 gene variants affect the anti-TNF response among Psoriasis patients. Int Immunopharmacol. 2015;29(2):947-9.
8. Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JW, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA. 2011;305(14):1460-8.
9. Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, et al. Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. Ann Rheum Dis. 2007;66(7):921-6.
10. Radstake TR, Svenson M, Eijsbouts AM, van den Hoogen FH, Enevold C, van Riel PL, et al. Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. Ann Rheum Dis. 2009;68(11):1739-45.
11. West RL, Zelinkova Z, Wolbink GJ, Kuipers EJ, Stokkers PC, van der Woude CJ. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. Aliment Pharmacol Ther. 2008;28(9):1122-6.
12. Xu Z, Davis HM, Zhou H. Clinical impact of concomitant immunomodulators on biologic therapy: Pharmacokinetics, immunogenicity, efficacy and safety. J Clin Pharmacol. 2015;55 Suppl 3:S60-74.
13. van Schouwenburg PA, van de Stadt LA, de Jong RN, van Buren EE, Kruithof S, de Groot E, et al. Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation. Ann Rheum Dis. 2013;72(1):104-9.
14. Zhuang Y, Xu Z, Frederick B, de Vries DE, Ford JA, Keen M, et al. Golimumab pharmacokinetics after repeated subcutaneous and intravenous administrations in patients with rheumatoid arthritis and the effect of concomitant methotrexate: an open-label, randomized study. Clin Ther. 2012;34(1):77-90.
15. Baert F, Noman M, Vermeire S, Van Assche G, G DH, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med. 2003;348(7):601-8.

16. Pouw MF, Krieckaert CL, Nurmohamed MT, van der Kleij D, Aarden L, Rispens T, et al. Key findings towards optimising adalimumab treatment: the concentration-effect curve. Ann Rheum Dis. 2015;74(3):513-8.
17. Kuriya B, Arkema EV, Bykerk VP, Keystone EC. Efficacy of initial methotrexate monotherapy versus combination therapy with a biological agent in early rheumatoid arthritis: a meta-analysis of clinical and radiographic remission. Ann Rheum Dis. 2010;69(7):1298-304.
18. Busard C, Zweegers J, Limpens J, Langendam M, Spuls PI. Combined use of systemic agents for psoriasis: a systematic review. JAMA Dermatol. 2014;150(11):1213-20.
19. Hugh J, Van Voorhees AS, Nijhawan RI, Bagel J, Lebwohl M, Blauvelt A, et al. From the Medical Board of the National Psoriasis Foundation: The risk of cardiovascular disease in individuals with psoriasis and the potential impact of current therapies. J Am Acad Dermatol. 2014;70(1):168-77.
20. Weisman MH, Moreland LW, Furst DE, Weinblatt ME, Keystone EC, Paulus HE, et al. Efficacy, pharmacokinetic, and safety assessment of adalimumab, a fully human anti-tumor necrosis factor-alpha monoclonal antibody, in adults with rheumatoid arthritis receiving concomitant methotrexate: a pilot study. Clin Ther. 2003;25(6):1700-21.
21. Lorenzetti R, Zullo A, Ridola L, Diamanti AP, Lagana B, Gatta L, et al. Higher risk of tuberculosis reactivation when anti-TNF is combined with immunosuppressive agents: a systematic review of randomized controlled trials. Ann Med. 2014;46(7):547-54.
22. Gottlieb AB, Langley RG, Strober BE, Papp KA, Klekotka P, Creamer K, et al. A randomized, double-blind, placebo-controlled study to evaluate the addition of methotrexate to etanercept in patients with moderate to severe plaque psoriasis. Br J Dermatol. 2012;167(3):649-57.
23. Zachariae C, Mork NJ, Reunala T, Lorentzen H, Falk E, Karvonen SL, et al. The combination of etanercept and methotrexate increases the effectiveness of treatment in active psoriasis despite inadequate effect of methotrexate therapy. Acta Derm Venereol. 2008;88(5):495-501.
24. van Bezooijen JS, Prens EP, Pradeepti MS, Atiqi R, Schreurs MW, Koch BC, et al. Combining biologics with methotrexate in psoriasis: a systematic review. Br J Dermatol. 2015;172(6):1676-80.
25. Mrowietz U, Kragballe K, Reich K, Spuls P, Griffiths CE, Nast A, et al. Definition of treatment goals for moderate to severe psoriasis: a European consensus. Arch Dermatol Res. 2011;303(1):1-10.
26. Langley RG, Feldman SR, Nyirady J, van de Kerkhof P, Papavassilis C. The 5-point Investigator's Global Assessment (IGA) Scale: A modified tool for evaluating plaque psoriasis severity in clinical trials. J Dermatolog Treat. 2015;26(1):23-31.
27. Gallo E, Cabaleiro T, Roman M, Solano-Lopez G, Abad-Santos F, Garcia-Diez A, et al. The relationship between tumour necrosis factor (TNF)-alpha promoter and IL12B/IL-23R genes polymorphisms and the efficacy of anti-TNF-alpha therapy in psoriasis: a case-control study. Br J Dermatol. 2013;169(4):819-29.
28. Koder S, Repnik K, Ferkolj I, Pernat C, Skok P, Weersma RK, et al. Genetic polymorphism in ATG16L1 gene influences the response to adalimumab in Crohn's disease patients. Pharmacogenomics. 2015;16(3):191-204.
29. Davila-Fajardo CL, van der Straaten T, Baak-Pablo R, Medarde Caballero C, Cabeza Barrera J, Huizinga TW, et al. FcGR genetic polymorphisms and the response to adalimumab in patients with rheumatoid arthritis. Pharmacogenomics. 2015;16(4):373-81.
30. Menting SP, van den Reek JM, Baerveldt EM, de Jong EM, Prens EP, Lecluse LL, et al. The correlation of clinical efficacy, serum trough levels and antidrug antibodies in ustekinumab-treated patients with psoriasis in a clinical-practice setting. Br J Dermatol. 2015;173(3):855-7.

31. Krieckaert CL, Nurmohamed MT, Wolbink GJ. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. Ann Rheum Dis. 2012;71(11):1914-5.
32. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, et al. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum. 1998;41(9):1552-63.
33. AbbVie Inc. Humira US Prescribing Information 2013 [cited 2015 23 December]. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125057s327lbl.pdf.
34. Nielsen OH, Bjerrum JT, Herfarth H, Rogler G. Recent advances using immunomodulators for inflammatory bowel disease. J Clin Pharmacol. 2013;53(6):575-88.
35. Shen DD, Azarnoff DL. Clinical pharmacokinetics of methotrexate. Clin Pharmacokinet. 1978;3(1):1-13.
36. Menting SP, Dekker PM, Limpens J, Hooft L, Spuls PI. Methotrexate Dosing Regimen for Plaque-type Psoriasis: A Systematic Review of the Use of Test-dose, Start-dose, Dosing Scheme, Dose Adjustments, Maximum Dose and Folic Acid Supplementation. Acta Derm Venereol. 2015.

Chapter 4

Combination therapy of etanercept and fumarates versus etanercept monotherapy in psoriasis: a randomized exploratory study

J.S. van Bezooijen, D.M.W. Balak, M.B.A. van Doorn, C.N. Looman, M.W.J. Schreurs, B.C.P. Koch, T. van Gelder, E.P. Prens

Dermatology 2016;232:407-414

ABSTRACT

Background

Biologics are a safe and efficacious therapy for psoriasis. The drug survival of biologics may be disappointing, primarily due to loss of efficacy. Therefore, safe combination treatments are sought to improve their clinical response.

Objective

To assess the efficacy, safety and tolerability of the combination therapy of etanercept with fumarates versus etanercept monotherapy.

Methods

Thirty-three patients with psoriasis were randomized 1:1 to receive etanercept combined with fumarates or etanercept monotherapy. The primary outcome measure was the difference in PASI-75 response after 24 weeks, additionally a longitudinal analysis was performed. An important secondary outcome measure was the proportion of patients with a PGA clear or almost clear. Adverse events were collected throughout the study.

Results

In the combination therapy group 78% (14 out of 18 patients) reached PASI-75 at week 24 vs. 57% (8 out of 14 patients)in the monotherapy group, P=0.27. The longitudinal analysis showed a PASI reduction of 5.97% per week for the combination therapy group and 4.76% for the monotherapy group (P=0.11). In the combination therapy group 94% (17 out of 18 patients) of patients had PGA of clear/almost clear vs. 64% (9 out of 14 patients) in the monotherapy group, P=0.064. The incidence of mild gastro-intestinal complaints was higher in the combination group than in the monotherapy group.

Conclusion

Using the PGA, combination therapy showed a trend towards faster improvement in the first 24 weeks. The difference in PASI score between the two groups was not statistically significant. Addition of fumarates to etanercept for 48 weeks appeared safe with an acceptable tolerability.

BACKGROUND

Psoriasis is a chronic, immune-mediated inflammatory skin disease affecting approximately 1 to 3% of the Caucasian population (1). In most patients, psoriasis has a relapsing course that considerably impairs their quality of life (2, 3). Biologics are effective drugs that are capable of inducing a rapid and meaningful clinical improvement. However, the drug survival of anti-tumor necrosis factor-alpha (TNFα) biologics in clinical practice appears disappointing, mainly due to a gradual loss of efficacy over time (4, 5). In a prospective Danish registry, etanercept had a limited drug survival with a median survival of 30 months after a 10-year follow up. Loss of efficacy was the primary reason for 67% of discontinuations for all biologics (6-8).

Etanercept binds and neutralizes TNFα via a recombinant soluble p75 TNFα receptor fused to an IgG1 constant chain, and combines a satisfactory efficacy with a favourable safety profile (9). Despite the increasing use of IL-12 and IL-23 and IL-17 inhibitors in the treatment of psoriasis, etanercept is still widely used in clinical practice. Several studies have shown that after 12 weeks of treatment with the recommended induction dose of 2 x 50mg per week, approximately 50% of the patients achieve a 75% or greater improvement in their psoriasis area and severity index (PASI-75 response) (9-12). After the induction phase of 2 x 50mg weekly, current guidelines and the label recommend reducing the dose of etanercept to 1 x 50mg weekly from week 12 onwards (13). However, at this once weekly dosage, clinical response deteriorates in many patients. Van den Reek et al. showed that 33.7% of patients discontinued etanercept because of deterioration of their psoriasis at this dosage (7).

An option to counteract this loss of efficacy is to combine etanercept with other systemic agents (14-17). The ester derivatives of fumaric acid are mainly used in the Netherlands and Germany as a first line systemic drug for moderate to severe psoriasis. Fumaric acid esters, or fumarates, are in use for the treatment of psoriasis for over four decades and are considered safe and effective as long as the treatment guidelines are followed (18-22). The fumaric acid ester derivative dimethylfumarate (DMF), is metabolized in the body to monomethylfumarate (MMF), which is regarded as the most bioactive metabolite. In daily clinical practice, we noticed in some patients that the addition of oral fumarates to etanercept 50mg once weekly improved the clinical response and drug survival (personal unpublished observation). At present, evidence supporting the safety of the combination therapy of etanercept and fumarates in psoriasis is virtually lacking. Therefore, it is not recognized as a feasible treatment option among dermatologists. In this exploratory study the key objectives were to evaluate the efficacy, safety, tolerability, of the combination therapy of etanercept with fumarates in psoriasis.

METHODS

Study design

This was an investigator-initiated, single center, randomized, assessor-blinded, study conducted at the department of Dermatology in the Erasmus Medical Center, Rotterdam, the Netherlands between July 2013 and June 2015. This study was approved by the Institutional Review Board of the Erasmus University Medical Center Rotterdam (MEC-2011-500) and the national medical authority (The Central Committee on Research Involving Human Subjects (CCMO)). All patients provided written informed consent. The study was conducted according to the guidelines of Good Clinical Practice. The trial is registered in the European Clinical Trials Database (EudraCT) under EudraCT number 2011-005685-38. This investigator-initiated study was supported by a grant of Pfizer Pharmaceuticals. Pfizer was not involved in any study procedure, Pfizer was granted the right to read, but not to edit the manuscript prior to submission for publication. Provision and reimbursement of etanercept medication was executed via the Dutch health insurance.

Patients

All included patients were 18 years or older, had stable, moderate to severe plaque-psoriasis for more than 6 months, affecting more than 10% body surface area (BSA), had a PASI greater than 10 at screening and at baseline, and were candidates for biologic treatment according to the approved product labeling and to Dutch guidelines.

Patients were recruited from the dermatology outpatient clinic from our hospital. Patients were excluded if they had any other sub-type of psoriasis or previous treatment failure on etanercept or fumarates or had a clinically significant adverse event (AE) with prior use of both drugs. Pregnant or lactating women were not eligible.

Patients with severe recalcitrant psoriasis who experienced lack of efficacy during prior use of other biologics were also eligible, in order to represent real-life daily practice. The washout period for a TNF-blocking agent or any other biologic was three months and for other systemic treatments (including fumarates) or UV therapy was four weeks. All patients were screened for hepatitis B and C, HIV, and (latent) tuberculosis according to the Dutch psoriasis treatment guidelines.

Study objectives

The primary objective of this study was to compare the clinical efficacy of the combination therapy of etanercept and fumarates with etanercept monotherapy per label after 24 weeks. The clinical efficacy was expressed as the proportion of patients achieving at least 75% reduction in their PASI after treatment. Additionally a longitudinal analysis was performed to assess the PASI reduction per week for each group.

Secondary objectives were to evaluate the efficacy at week 12 and 48, the proportion of patients with a PGA clear or almost clear, the change in DLQI score, and treatment satisfaction (visual analog scale) scores after 12, 24 and 48 weeks. Drug survival after one year was assessed by a post hoc analysis and was defined as the proportion of patients who were still on the treatment they were originally randomized to and who also achieved at least 75% reduction in their PASI. Adverse events were collected through the entire study period.

Study procedures

Using a computer-generated randomization list, patients were randomized at baseline to a 1:1 ratio to receive either etanercept combined with oral fumarates (combination group) or etanercept only (monotherapy group). Patients and the study physicians were not blinded for the allocated treatment group. The independent PASI assessor (EP) was blinded to treatment throughout the course of the study.

All patients received etanercept 50mg subcutaneously twice weekly for 12 weeks followed by 50mg once weekly for an additional 12 weeks. Subjects randomized to the combination group were treated with additional fumarates, of which the daily dose was gradually increased in 4 weeks from 215mg once daily up to a maximum of 215mg four times a day. A large batch of enteric-coated tablets containing a total of 215mg fumaric acid esters (120mg dimethylfumarate and 95mg calcium-monoethylfumarate) was specifically manufactured for this trial by Fagron, in a GMP certified facility (Capelle aan den IJssel, the Netherlands).

Patients in the monotherapy group who did not achieve a PASI-75% response after 24 weeks were switched to the combination therapy (Figure 1).

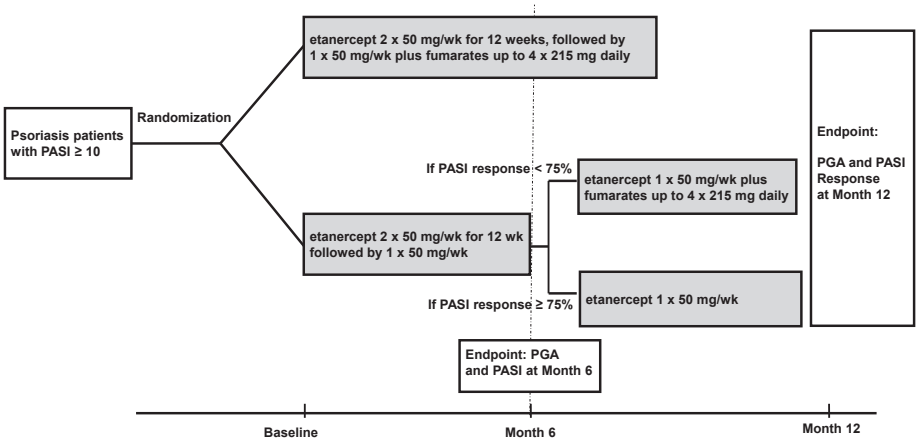

Figure 1. A schematic flowchart representing the study methods for the exploratory study comparing etanercept monotherapy versus combination therapy with fumarates.

Patient visits were scheduled at week 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 48. At each study visit, data were collected on PASI and PGA scores, tolerability, adverse events, and laboratory testing (full blood count, aspartate aminotransferase, alanine aminotransferase, bilirubin, gamma-GT, serum creatinine, sedimentation rate, C-reactive protein, and urine analysis). Patients were asked to fill in the DLQI questionnaire and a visual analogue scale (VAS) treatment satisfaction on a monthly basis. Patient data were collected using the computer programme "Open Clinica".

Statistical analysis

The proportion of patients achieving at least a 75% PASI reduction after 12, 24 (primary objective) and 48 weeks of treatment was analyzed using a Chi-square or Fisher exact-test. The Chi-square or Fischer exact-test was used for the outcomes of the PGA and for the proportion of patients achieving drug survival. Patients who switched to combination therapy after 24 weeks were considered as failures in the monotherapy group. Patients lost to follow-up were not included for the PASI-75 response and PGA score analyses. For the longitudinal analysis a linear mixed model analysis was used to calculate the reduction in PASI score per week up to 48 weeks. We used the lme4 package in R (https://cran.r-project.org/web/packages/lme4/lme4.pdf). Time and group, and the interaction, were predictors. We used log-transformed PASI in the regression model to achieve changes to be relative.

We used unpaired t-test for comparing changes in DLQI and VAS score between the monotherapy and combination therapy at 12, 24 and 48 weeks. If the residuals were not normally distributed we used the bootstrap option in SPSS. We used descriptive statistics by presenting the PASI score per patient in a graph.

RESULTS

Patients

In total 33 patients were enrolled: 15 patients were randomized to etanercept monotherapy and 18 patients to combination therapy with etanercept and fumarates.

Patient demographics and baseline disease characteristics are shown in Table 1. At baseline only the BMI and previous use of biologics differed significantly between the combination and monotherapy group ($P<0.05$).

The flow chart in Figure 2, shows the number of patients that were enrolled and dropped out the study together with the reasons for discontinuation. Twenty two out of 33 patients (67%) finished the entire study. In the monotherapy group, 9 of 15 patients (60%) completed the study, and in the combination therapy group 13 of 18 patients (72%) $P=0.71$.

Table 1 Baseline patient characteristics according to treatment arm.

Patient characteristics	**Monotherapy etanercept (n=15)**		**Combination therapy etanercept with fumarates (n=18)**	
Gender, n (%)				
Males	8	(53)	14	(78)
Females	7	(47)	4	(22)
Age mean (SD), y	45	(16)	43	(17)
Height mean (SD), m	1.72	(0.13)	1.77	(0.11)
BMI, mean (SD)	30	(6)*	26	(6)*
PGA score, n (%)				
moderate	6	(40)	13	(72)
severe	9	(60)	5	(28)
PASI score, Median (Q1,Q3)	14	(11, 21)	12	(10,16)
DLQI score, Median (Q1,Q3)	9	(5, 20)	8	(3, 13)
Duration of psoriasis, mean (SD), y	19	(10)	22	(10)
History of psoriatic arthritis, n (%)	1	(7)	5	(28)
Prior therapy, n (%)				
UVB/PUVA)	15	(93)	17	(94)
Fumarates	13	(87)	11	(61)
Methotrexate	13	(87)	13	(72)
Ciclosporin	4	(27)	6	(33)
Acitretin	6	(40)	10	(56)
Biologic	3	(20)*	10	(56)*

*Statistically significant different between monotherapy and combination group.

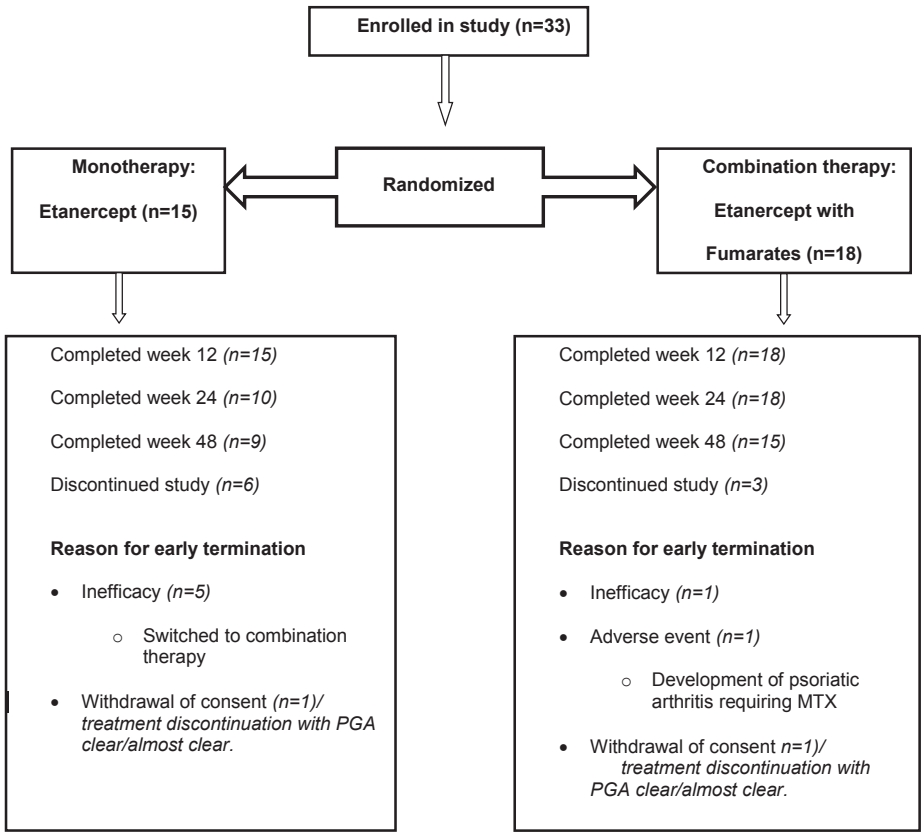

Figure 2. Schematic representation of included, randomized and evaluable patients for the combination therapy and for the monotherapy group.

CLINICAL EFFICACY

The PASI-75 response is presented in Table 2 for both groups. The difference between the two groups was not statistically significant at all time points. In both groups all patients showed a clear improvement in their PASI score from baseline.

Table 2. Statistical analysis of PASI-75 response and PGA score.

	Monotherapy etanercept (n=15) Mean (95 CI %)	Absolute numbers	Combination therapy etanercept with fumarates (n=18) Mean (95 CI %)	Absolute numbers	P-value*
PASI-75 at week 12	64% (39-89)	9 out of 14	67% (42-91)	12 out of 18	1.00[a]
PASI-75 at week 24	57% (31-83)	8 out of 14	78% (59-97)	14 out of 18	.27[b]
PASI-75 at week 48	64% (42-86)	9 out of 14	87% (70-104)	13 out of 15	.22[b]
PGA (clear/ almost clear) at week 12	57% (31-83)	8 out of 14	89% (74-103)	16 out of 18	.096[b]
PGA (clear/ almost clear) at week 24	64% (39-89)	9 out of 14	94% (84-105)	17 out of 18	.064[b]
PGA (clear/ almost clear) at week 48	64% (39-89)	9 out of 14	87% (70-104)	13 out of 15	.22[b]

[a]Chi-Square test, [b]Fisher's exact test

Missing data: one patient in monotherapy group had missing visits at week 12 and 24. One patient was lost to follow-up in monotherapy at week 48. Three patients were lost to follow-up in combination therapy at week 48. As a consequence, these patients were not included in the statistical analysis of the PASI-75 and PGA score .

The longitudinal analysis demonstrating the reduction of the PASI score for the combination therapy and monotherapy group from baseline up to week 48. All observation were aggregated in a 4 weeks period separated in two groups, the median per group per time are shown in the graph (Figure 3a).The reduction in PASI score per week for the combination therapy was 5.97%, 95% CI [5.08 ; 6.85] and in the monotherapy group 4.76%, 95% CI [3.57 ; 5.93], P=0.11.

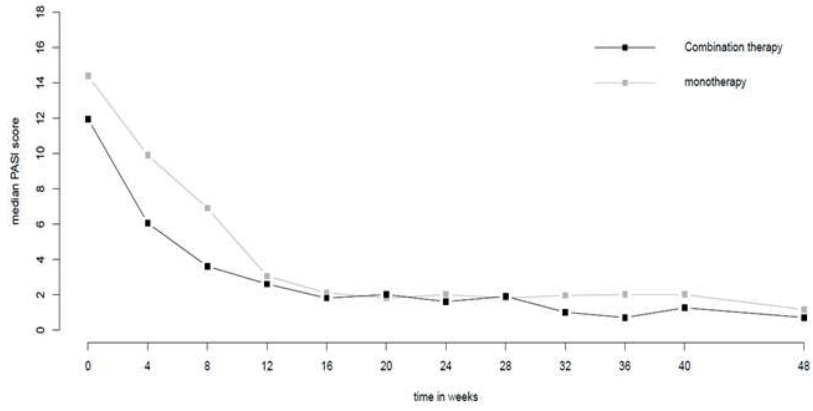

Figure 3a. The results of a longitudinal analysis using a linear mixed model demonstrating the reduction of the PASI score for the combination therapy and monotherapy group from baseline up to week 48. All observation were aggregated in a 4 weeks period separated in two groups, the median per group per time are shown in the graph. We used log-transformed PASI in the regression model to achieve changes to be relative. This is consistent with presenting medians at the original scale.

Figures 3b and 3c show the changes in PASI score per patient. Five patients who did not respond sufficiently to monotherapy were switched to combination therapy at week 24 according to the protocol. Only one out of 5 switchers achieved a PASI-75 improvement after 24 weeks of combination treatment.

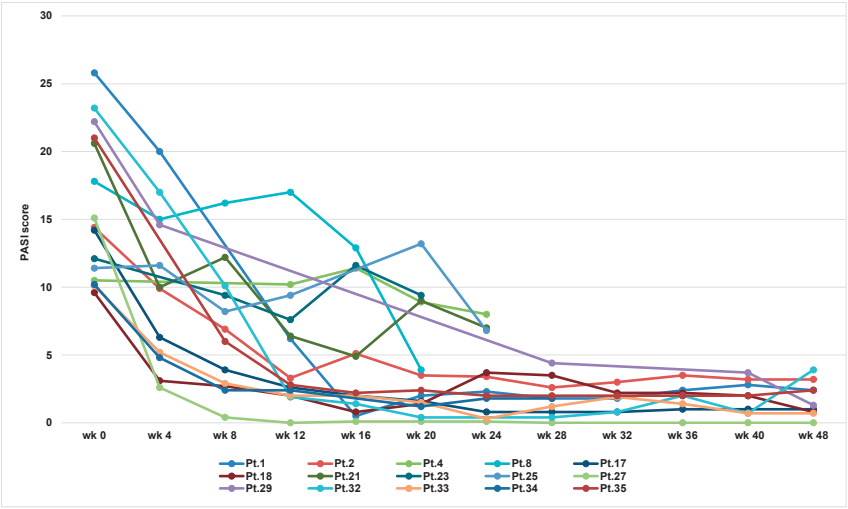

Figure 3b. PASI score per patient on etanercept alone (monotherapy) from baseline to week 48. Five patients switched from monotherapy to combination therapy at week 20 and 24, these PASI scores are not further presented in the graph.

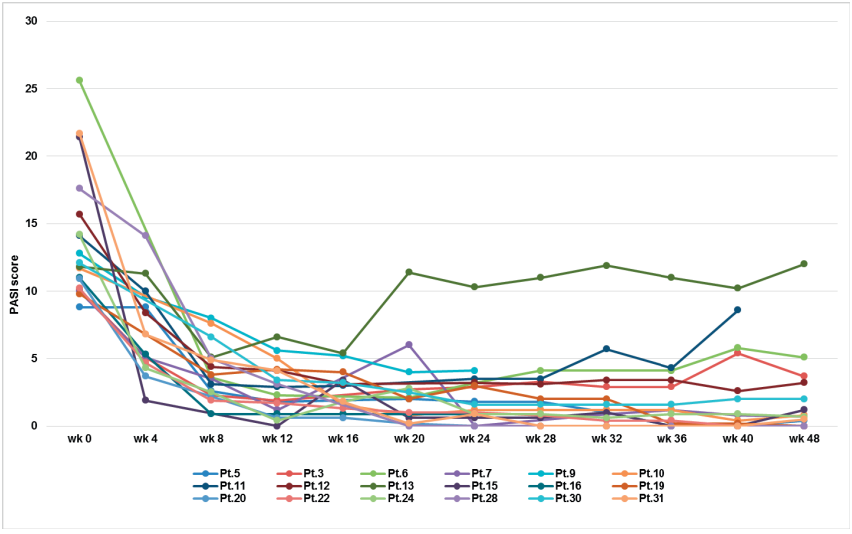

Figure 3c. PASI score per patient on etanercept with fumarates (combination therapy) from baseline to week 48.

The proportion of patients with a PGA score clear or almost clear is presented in Table 2.

The drug survival in the monotherapy group was 60% after 48 weeks. Nine out of 15 patients (60%) remained on etanercept therapy after 48 weeks. The drug survival in the combination group was 72%. Thirteen out of 18 patients (72%) remained on etanercept with fumarates treatment after 48 weeks. The drug survival was not significantly different for the combination therapy group compared with the monotherapy group 72%, 95% CI [51 ; 93] vs. 60%, 95% CI [35 ; 85], P=0.71.

The time to the onset of action was 9 weeks for both groups.

QUALITY OF LIFE (DLQI SCORE) AND TREATMENT SATISFACTION (VAS SCORE)

The results of the median change in DLQI score are shown in Table 3. In both groups DLQI scores decreased significantly over time. The difference between the two groups at 24 and 48 weeks was not statistically significant.

Table 3. Dermatology Life Quality Index (DLQI) during treatment

	Change in DLQI monotherapy etanercept Mean (SD)	Change in DLQI in combination therapy etanercept with fumarates Mean (SD)	P-value*
Week 12	5.5 (6.8)	7.1 (6.9)	.53
Week 24	5.4 (7.9)	7.1 (6.4)	.56
Week 48	9.4 (8.6)	7.3 (6.2)	.51

*Unpaired t-test

The results of the VAS scores on treatment satisfaction are shown in Table 4. Similar to the DLQI scores, the differences between the two groups did not reach statistical significance at either 24 or 48 weeks. The five switchers were excluded from the analysis.

Table 4. Treatment satisfaction measured by visual analogue scale (VAS) from 0 to 10.

	VAS monotherapy etanercept Mean (SD)	VAS combination therapy etanercept and fumarates Mean (SD)	P-value*
Week 12	8.0 (1.47)	8.1 (1.55)	.92
Week 24	8.1 (1.45)	8.1 (1.43)	.95
Week 48	8.9 (1.17)	8.2 (1.70)	.30

*Unpaired t-test

ADVERSE EVENT (AE)

The most frequently reported AE in the monotherapy group was flu-like symptoms, present in 9 vs. 14 AEs) in the combination therapy group. Gastro-intestinal complaints (38), consisting of diarrhea and abdominal cramps, were most frequently observed in the combination therapy group compared to the monotherapy group (1). One patient developed iron deficiency anemia, which was caused by a carcinoma of the rectum. This was diagnosed after the patient had finished the study, and was considered not to be related to the study medication. None of the (severe) AEs led to discontinuation of the study. No leukopenia and/or lymphopenia was observed in either treatment group. All adverse events are listed in Table 5.

Table 5. Adverse events.

Minor adverse events	**Monotherapy etanercept**	**Patients (n)**	**Combination therapy etanercept with fumarates**	**Patients (n)**
Gastro-intestinal complaints	1	1	38	11
Flushing	1	1	4	4
Elevation liver enzymes			1	1
Influenza-flu like symptoms	9	7	14	10
Headache			1	1
Fatigue	2	1	1	1
Pruritus	2	1	2	2
Injection site reactions	1	1	1	1
Operation	1 (tonsillectomy)	1	1 (meniscus surgery)	1
Other	1 (minor trauma)	1	2 (1bact. Infection + 1 bladder infection)	2
Major adverse events				
Serious adverse event	1 iron deficiency anemia	1		
Severe adverse event	1(admission to the hospital)	1	2 (admission to the hospital)	1

DISCUSSION

In this exploratory randomized study we prospectively compared the clinical efficacy, safety, tolerability of etanercept with oral fumarates combination therapy with etanercept monotherapy per label up to 48 weeks of treatment. The assumption was that addition of fumarates would be a safe and low cost option to increase the clinical efficacy and drug survival of etanercept. The primary outcome of this study was that the combination treatment led to a numerically higher efficacy compared to etanercept monotherapy (78% vs. 57% PASI-75) at week 24. However the numerical differences in efficacy were not statistically significant. Also

the longitudinal analysis using a linear mixed model analysis yielded no significant differences in efficacy between the two treatment groups.

Using the PGA as a secondary outcome measure, the combination therapy with fumarates resulted in a trend towards better efficacy during the first 24 weeks compared to etanercept monotherapy. The 94% of patients with a PGA of clear/almost clear in de combination group was remarkably high and the difference with the monotherapy group approached almost statistical significance. Furthermore, the DQLI and VAS score did not differ between the two groups, suggesting that concomitant use of fumarates (and related side effects) did not negatively affect the quality of life and treatment satisfaction in our patients.

The efficacy rates observed in our study are comparable with those of Gottlieb et al. whereby 239 patients were randomized to etanercept monotherapy or etanercept with MTX. After 24 weeks the PASI-75 was significantly higher in the etanercept with MTX group than in the etanercept monotherapy group (77·3% vs. 60·3%; $P < 0{\cdot}0001$).

In daily practice methotrexate (MTX) is more frequently combined with biologics than fumarates, because it is assumed that (low dose) MTX increases the clinical efficacy of biologics by reducing the development of anti-drug antibodies (23, 24). Anti-etanercept antibodies have only sporadically been observed in clinical studies, indicating that loss of clinical efficacy for etanercept is probably caused by other, yet unidentified factors (25). We argued that for improvement of the clinical efficacy of etancercept, inhibition of anti-drug antibodies was less important, and that combination with oral fumarates could have an additive clinical effect.

Wilsmann-Theis et al. performed a retrospective study on combination therapies in which they concluded that fumarates could be safely combined with biologics in an off-label real life setting (26). Although a similar fumarate dose of 4 times 215mg a day as in our study was used, their study comprised only four patients with fumarates in combination with etanercept. In two cases, fumarates were added to etanercept treatment, while the other two patients had started on fumarates and subsequently were treated additionally with etanercept. Only two of the four cases showed a good clinical response to the combination treatment after six months and 2 years (26). Fumarates have also been used in combination with other systemic agents such as ciclosporin, acitretin, hydroxyurea and MTX (27). However, no prospective randomized clinical trials were performed to evaluate the efficacy and safety of these combinations (28).

This is the first prospective randomized trial to show that combination therapy with fumarates appears to be relatively safe and have an acceptable tolerability up to 48 weeks. Since higher doses of fumarates are known to be associated with increased incidence of side effects, an adjusted dose of up to 215mg 4 times a day was used instead of the commonly used maximum dose of 6 tablets a day. We believe that therefore no patients had to discontinue treatment with fumarates because of side effects. A common and potential serious side effect of fumarates, leukopenia and/or lymphopenia was also not observed in any of our patients during the one-year course of the study. This is particularly of interest because of the recently reported cases

of progressive multifocal leukoencephalopathy (PML) in long-term fumaric acid users. It has to be noted that most of the reported PML cases were lymphopenic for a prolonged period.

It is remarkable that the occurrence of gastro-intestinal side effects in the combination therapy group did not lead to a significantly altered quality of life, as expressed by the DLQI, or less treatment satisfaction. When a higher dose had been used, we potentially could have achieved a higher efficacy and statistically significant differences between the two groups. However this could also have resulted in more drop-outs because of fumarate related side-effects.

This study was performed in a real life clinical setting and was therefore confronted with some practical clinical limitations from daily practice. First of all, we had issues with patient compliance. Three patients (one in the monotherapy and two in the combination therapy group) decided, for personal reasons and despite a clear/almost clear clinical response at week 24, to withdraw consent and to discontinue the study. Secondly, the combination therapy group contained significantly more patients who had previously used and failed one or more biologics, namely 56% against 20% in the monotherapy. Several studies have shown that patients with prior use of biologics have a shorter drug survival in comparison with biologic naïve patients (7, 29, 30). However, in daily clinical practice non-naïve patients also require treatment. Furthermore, patients were not blinded to treatment as we did not use placebo fumarate tablets in the etanercept monotherapy arm. However, the 'blinding effects' of placebo tablets would have been minimal due to the high frequency of gastrointestinal complaints typically associated with fumarates. Finally, in the etanercept monotherapy group, five patients were switched to the combination therapy because of inefficacy. However, only one of these five patients showed a clear improvement after the addition of fumarates to etanercept, the other four had persistent brittle psoriasis and did not achieve a PASI-75 response and had to be switched to other biologics after week 48. These four patients were notorious therapy-resistant patients who had failed several other anti-psoriatic therapies including biologics.

In conclusion, in this study, using the PGA, the combination therapy showed a trend towards a faster rate of improvement in the first 24 weeks in. The difference in PASI score between the two groups was not statistically significant. Addition of fumarates to etanercept for 48 weeks appeared safe with an acceptable tolerability

REFERENCES

1. Raychaudhuri SP, Farber EM. The prevalence of psoriasis in the world. J Eur Acad Dermatol Venereol. 2001;15(1):16-7.
2. Rapp SR, Feldman SR, Exum ML, Fleischer AB, Jr., Reboussin DM. Psoriasis causes as much disability as other major medical diseases. J Am Acad Dermatol. 1999;41:401-7.
3. Stern RS, Nijsten T, Feldman SR, Margolis DJ, Rolstad T. Psoriasis is common, carries a substantial burden even when not extensive, and is associated with widespread treatment dissatisfaction. J Investig Dermatol Symp Proc. 2004;9(2):136-9.
4. Mrowietz U, de Jong EM, Kragballe K, Langley R, Nast A, Puig L, et al. A consensus report on appropriate treatment optimization and transitioning in the management of moderate-to-severe plaque psoriasis. J Eur Acad Dermatol Venereol. 2014;28(4):438-53.
5. Zandvliet ML, van Bezooijen JS, Bos MA, Prens EP, van Doorn M, Bijen I, et al. Monitoring antigen-specific biologics: current knowledge and future prospects. Ther Drug Monit. 2013;35(5):588-94.
6. Gniadecki R, Kragballe K, Dam TN, Skov L. Comparison of drug survival rates for adalimumab, etanercept and infliximab in patients with psoriasis vulgaris. Br J Dermatol. 2011;164(5):1091-6.
7. van den Reek JM, van Lumig PP, Driessen RJ, van de Kerkhof PC, Seyger MM, Kievit W, et al. Determinants of drug survival for etanercept in a long-term daily practice cohort of patients with psoriasis. Br J Dermatol. 2014;170(2):415-24.
8. Gniadecki R, Bang B, Bryld LE, Iversen L, Lasthein S, Skov L. Comparison of long-term drug survival and safety of biologic agents in patients with psoriasis vulgaris. Br J Dermatol. 2015;172(1):244-52.
9. Leonardi CL, Powers JL, Matheson RT, Goffe BS, Zitnik R, Wang A, et al. Etanercept as monotherapy in patients with psoriasis. N Engl J Med. 2003;349(21):2014-22.
10. Cassano N, Loconsole F, Galluccio A, Miracapillo A, Pezza M, Vena GA. Once-weekly administration of high-dosage Etanercept in patients with plaque psoriasis: results of a pilot experience (power study). Int J Immunopathol Pharmacol. 2006;19(1):225-9.
11. Papp KA, Tyring S, Lahfa M, Prinz J, Griffiths CE, Nakanishi AM, et al. A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. Br J Dermatol. 2005;152(6):1304-12.
12. Tyring S, Gottlieb A, Papp K, Gordon K, Leonardi C, Wang A, et al. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. Lancet. 2006;367(9504):29-35.
13. Nast A, Boehncke WH, Mrowietz U, Ockenfels HM, Philipp S, Reich K, et al. S3 - Guidelines on the treatment of psoriasis vulgaris (English version). Update. J Dtsch Dermatol Ges. 2012;10 Suppl 2:S1-95.
14. Foley PA, Quirk C, Sullivan JR, Dolianitis C, Hack SP, Thirunavukkarasu K, et al. Combining etanercept with traditional agents in the treatment of psoriasis: a review of the clinical evidence. J Eur Acad Dermatol Venereol. 2010;24(10):1135-43.
15. Heffernan MP. Combining traditional systemic and biologic therapies for psoriasis. Semin Cutan Med Surg. 2010;29(1):67-9.
16. Wilsmann-Theis D, Frambach Y, Philipp S, Weyergraf AJ, Jacobi A, Mossner R, et al. Systemic Antipsoriatic Combination Therapy with Fumaric Acid Esters for Plaque-Type Psoriasis: Report on 17 Cases. Dermatology. 2015;230(2):119-27.
17. Calzavara-Pinton PG, Sala R, Arisi M, Rossi MT, Venturini M, Ortel B. Synergism between narrowband ultraviolet B phototherapy and etanercept for the treatment of plaque-type psoriasis. Br J Dermatol. 2013;169(1):130-6.

18. Warren RB, Brown BC, Lavery D, Ashcroft DM, Griffiths CE. Biologic therapies for psoriasis: practical experience in a U.K. tertiary referral centre. Br J Dermatol. 2009;160(1):162-9.
19. Reich K, Thaci D, Mrowietz U, Kamps A, Neureither M, Luger T. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis--a retrospective study (FUTURE). J Dtsch Dermatol Ges. 2009;7(7):603-11.
20. Hoefnagel JJ, Thio HB, Willemze R, Bouwes Bavinck JN. Long-term safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol. 2003;149(2):363-9.
21. Wain EM, Darling MI, Pleass RD, Barker JN, Smith CH. Treatment of severe, recalcitrant, chronic plaque psoriasis with fumaric acid esters: a prospective study. Br J Dermatol. 2010;162(2):427-34.
22. Fallah Arani S, Neumann H, Hop WC, Thio HB. Fumarates vs. methotrexate in moderate to severe chronic plaque psoriasis: a multicentre prospective randomized controlled clinical trial. Br J Dermatol. 2011;164(4):855-61.
23. van Bezooijen JS, Prens EP, Pradeepti MS, Atiqi R, Schreurs MW, Koch BC, et al. Combining biologics with methotrexate in psoriasis: a systematic review. Br J Dermatol. 2015;172(6):1676-80.
24. Armstrong AW, Bagel J, Van Voorhees AS, Robertson AD, Yamauchi PS. Combining biologic therapies with other systemic treatments in psoriasis: evidence-based, best-practice recommendations from the Medical Board of the National Psoriasis Foundation. JAMA Dermatol. 2015;151(4):432-8.
25. Krieckaert CL, Jamnitski A, Nurmohamed MT, Kostense PJ, Boers M, Wolbink G. Comparison of long-term clinical outcome with etanercept treatment and adalimumab treatment of rheumatoid arthritis with respect to immunogenicity. Arthritis Rheum. 2012;64(12):3850-5.
26. Wilsmann-Theis D, Frambach Y, Philipp S, Weyergraf AJ, Jacobi A, Mossner R, et al. Systemic antipsoriatic combination therapy with fumaric acid esters for plaque-type psoriasis: report on 17 cases. Dermatology. 2015;230(2):119-27.
27. Balasubramaniam P, Stevenson O, Berth-Jones J. Fumaric acid esters in severe psoriasis, including experience of use in combination with other systemic modalities. Br J Dermatol. 2004;150(4):741-6.
28. Busard C, Zweegers J, Limpens J, Langendam M, Spuls PI. Combined use of systemic agents for psoriasis: a systematic review. JAMA Dermatol. 2014;150(11):1213-20.
29. Lopez-Ferrer A, Vilarrasa E, Gich IJ, Puig L. Adalimumab for the treatment of psoriasis in real life: a retrospective cohort of 119 patients at a single Spanish centre. Br J Dermatol. 2013;169(5):1141-7.
30. Piaserico S, Cazzaniga S, Chimenti S, Giannetti A, Maccarone M, Picardo M, et al. Efficacy of switching between tumor necrosis factor-alfa inhibitors in psoriasis: results from the Italian Psocare registry. J Am Acad Dermatol. 2014;70(2):257-62 .

PART II

Biologic dosing interval prolongation

Chapter 5

Prolongation of biologic dosing intervals in patients with stable psoriasis; a feasibility study

J.S. van Bezooijen, M.B.A. van Doorn, M.W.J. Schreurs, B.C.P. Koch, H. te Velthuis, E.P. Prens*, T. van Gelder*

* *Both authors contributed equally.*

Accepted for publication

ABSTRACT

Background

Biologics are usually licensed according to the "*one dose fits all*" principle. It is therefore suspected that a significant number of psoriasis patients are overtreated. However, evidence for successful dose reduction of biologics in psoriasis is scarce. The aim of this study was to investigate whether the dosing interval of three biologics, adalimumab, etanercept or ustekinumab could be prolonged successfully in patients with plaque psoriasis.

Methods

In a prospective exploratory cohort study, 59 psoriasis patients on maintenance treatment with adalimumab, etanercept or ustekinumab were included. After a run-in period of six weeks, the dosing interval of the biologics was prolonged according to a predefined schedule. Our primary objective was to determine the proportion of patients that could maintain a successful prolongation of the per label dosing interval. Secondary objectives were to evaluate the predictive value of baseline trough concentrations for successful dosing interval prolongation and to explore the feasibility of dosing interval prolongations in off label treated patients.

Results

In the per label group, 7 out of 16 (44%) adalimumab patients, 5 out of 16 (31%) etanercept patients and 2 out of 10 (20%) ustekinumab patients achieved a successful dosing interval prolongation. Baseline trough concentrations did not differ significantly between patients with successful dosing interval prolongation and failures. In the off label group, prolongation in patients with already extended intervals was unsuccessful. For patients with shortened intervals, minor prolongation was successful in 3 out of 17 (17.6%) patients.

Conclusion

Prolongation of the per label biologic dosing interval was feasible in approximately 30% of psoriasis patients with stable minimal disease activity and can reduce costs in clinical practice. Baseline trough concentrations were not predictive for successful dosing interval prolongation.

BACKGROUND

Biologics are effective drugs for the treatment of moderate to severe plaque psoriasis. As a result, their use is increasing and many new biologics and biosimilars are being developed. Biologics are usually licensed and marketed according to the "*one dose fits all*" principle, and dose regimens with fixed dosing intervals are recommended. However, a number of studies in patients with stable rheumatoid arthritis (RA) have shown that the per label biologic dose can be reduced without a subsequent increase in disease activity (1-3). Recently, biologic dose reduction and dosing interval prolongation have also been described in patients with psoriasis (4, 5).

Measurement of high trough concentrations supports these dose reductions in RA, since in RA patients adalimumab levels above 8 μg/mL were found to have no clear additional beneficial effect on disease activity (6). Similar findings were reported in psoriasis where one-third (44 out of 135) of psoriasis patients had adalimumab concentrations above 7 μg /ml without a further increase in clinical improvement (7). Chen et al. also showed that after 24 weeks of adalimumab dose reduction with 50%, the trough concentrations above the cut-off value of 6.4 μg/ml predicted persistent remission in patients with RA (3). The adalimumab trough concentrations were significantly higher in RA patients with persistent remission (median 10.5 μg/ml) or with low disease activity (4.5 μg/ml) than in those with disease flare (0.9 μg/ml). These data suggest that a substantial proportion of patients on biologics are indeed overtreated. In view of these studies in RA and psoriasis, we hypothesized that biologic trough concentrations could be a useful indicator for dose reduction of adalimumab and possibly other biologics that are currently being used for patients with psoriasis.

Adalimumab phase III studies have shown that complete discontinuation of biologic treatment in patients with psoriasis who are in remission is not recommendable. Papp et al. showed that after discontinuation of adalimumab treatment 69% (178/256) of psoriasis patients showed a relapse after a median of five months (8). Similarly, Ortonne et al. demonstrated a time to relapse of only two months in 240/359 psoriasis patients in whom etanercept was discontinued (9). Complete discontinuation in ustekinumab-treated patients was also unsuccessful, 60% had a recurrence of disease activity after a median time of 15 weeks (10). Therefore, tapering of biologics appears to be a better option than complete discontinuation of the biologic.

In Europe, off-label dosing of biologics is quite common in daily clinical practice (5, 11). This usually means dose escalation (e.g. doubling the dose), but also dose reductions, primarily by dosing interval prolongation. In daily practice intensified dosing regimens are often applied in the early treatment phase, and then continued as maintenance treatment, even when patients have been in remission for quite some time (12-14). Disadvantages of long term unnecessary or excessive doses are not only the potentially higher risk of treatment-related side effects, such as serious infections and development of malignancies, but also the high costs that are associated with the use of biologic drugs (15, 16). In Europe, pharmaceutical companies have negotiated

prices as high as 15,000 Euro per patient annually (17). As a result of the large numbers of patients now being treated with biologics, the pharmaceutical costs are a substantial proportion of the total health care budget. At present, evidence of successful prolongation of the per label dosing interval of biologics in psoriasis is scarce. Therefore our primary objective was to determine the proportion of patients that could maintain a successful prolongation of the per label dosing interval. Secondary objectives were to evaluate the predictive value of baseline trough concentrations for successful dosing interval prolongation. In addition we explored the feasibility of dosing interval prolongations in off label treated patients that were treated with either shorter of longer intervals according to the treatment label.

METHODS

Study design

This was a prospective exploratory cohort study conducted at the department of Dermatology in the Erasmus Medical Center, Rotterdam, the Netherlands between June 2013 and March 2016. The study was approved by the Institutional Review Board of the Erasmus University Medical Center Rotterdam (MEC-2013-050). The study was conducted according to the guidelines of Good Clinical Practice. The trial is registered in the European Clinical Trials Database (EudraCT) under EudraCT number 2012-005809-53.

Patients and treatments.

All included patients were 18 years or older, and received maintenance treatment with adalimumab, etanercept or ustekinumab for at least six months. In order to be eligible for the study patient were required to have an absolute PASI score below 8. Patients were excluded if they used other anti-psoriatic co-medication such as methotrexate, fumarates or acitretin. Pregnant or lactating women were not eligible. All patients provided written informed consent before any of the study procedures were executed. Patients were recruited from the dermatology outpatient clinic of our hospital.

"Per label use" was defined as the use of the biologic for the approved indication, in the approved dosage and route of administration. In our study "off label use" implies that the dose or dose interval of either of the three biologics used in this study was not according to the package insert approved by our national drug regulatory agency.

Study procedures

All patients were on maintenance treatment with adalimumab, etanercept, or ustekinumab with a dose and dosing interval prescribed by their dermatologist. Patient visits were scheduled at week 0, 6, 18, 30, 42, 54, 66 and 78. At each study visit, data were collected on biologic trough concentration and PASI scores, adverse events, and laboratory testing (full blood count,

aspartate aminotransferase, alanine aminotransferase, bilirubin, gamma-GT, serum creatinine, sedimentation rate, C-reactive protein, and urine analysis). The drug adherence was self-monitored, and empty syringes were collected during the outpatient visits. To determine whether a patient had stable disease activity, a run-in period of 6 weeks was used before start of the study where the PASI score of the patient was not allowed to fluctuate more than three points. In all patients, the dosing interval of the biologic was prolonged every 12 weeks during a 42 week period (see supplementary table 1 for predefined schedule). In case of an unacceptable increase in disease activity judged by the patient or a rise of the PASI score above 8, the last effective treatment regimen was resumed, and was maintained during the remainder of the study period. Patients were followed up to week 78.

Patients in whom the dosing interval could be prolonged according to protocol without loss of self-reported efficacy or deterioration of the PASI score above 8 were designated "successful". Patients in whom the dosing interval could not be prolonged according to our protocol were designated "failure". This includes patients who objected to continuing with the dosing interval prolongation because of self and investigator reported exacerbation of their psoriasis or for other (personal) reasons. In the majority of patients tapering consisted of dosing interval extensions (Table 1) and in only a minority as dose reductions (with unchanged interval). In this paper tapering will only be referred to as dosing interval prolongations.

Table 1. Predefined schedule for the biologic dosing interval prolongation

Adalimumab subcutaneous injections				
maintenance dose at baseline	**6 weeks**	**18 weeks**	**30 weeks**	**42 weeks**
40mg/2wk*	**40mg/2wk**	**40mg/3wk**	**40mg/4wk**	**40mg/4wk**
40mg/3wk	40mg/3wk	40mg/4wk	40mg/5wk	40mg/6wk
Etanercept subcutaneous injections				
maintenance dose at baseline	**6 weeks**	**18 weeks**	**30 weeks**	**42 weeks**
2x50mg/1wk	2x50mg/1wk	50mg/1wk	50mg/1wk	50mg/1wk
50mg/1wk*	**50mg/1wk**	**50mg/1wk**	**50mg/2wk**	**50mg/2wk**
Ustekinumab subcutaneous injections				
maintenance dose at baseline	**6 weeks**	**18 weeks**	**30 weeks**	**42 weeks**
90mg/8wk	90mg/8wk	90mg/10wk	90mg/12wk	45mg/8wk
90mg/12wk*	**90mg/12wk**	**90mg/12wk**	**45mg/12wk**	**45mg/12wk**
45mg/8wk	45mg/10wk	45mg/12wk	45mg/14wk	45mg/16wk
45mg/10wk	45mg/10wk	45mg/14wk	45mg/16wk	45mg/20wk
45mg/12wk*	**45mg/12wk***	**45mg/16wk**	**45mg/20wk**	**45mg/24wk**

*=per label group

Psoriasis Activity and Severity Index (PASI) and Physician Global Assessment (PGA)

The Psoriasis Activity and Severity Index (PASI) is a validated tool for monitoring the disease activity. The PASI is calculated based on the intensity of redness, thickness, scaling, and the affected surface area of the skin (18). The PASI score is the main outcome measure for success of treatment in clinical practice and clinical trials. The 5-point Physician Global Assessment (PGA) scale is a modified and more simplified tool for evaluating plaque psoriasis severity and improvement in clinical trials. It is divided in clear, almost clear, mild, moderate and severe score for the disease activity (19).

Dermatology Life Quality Index (DLQI)

The DLQI is a ten-item questionnaire used to measure the impact of a skin disease on the quality of life of an affected person. Each question refers to the impact of the skin disease on the patient's life over the previous week. The DLQI can provide more insight into the impairment of quality of life of psoriasis patients and supports more appropriate clinical decisions.

Biologic assay

Anti-TNFa biologic (adalimumab and etanercept) concentration was determined by an ELISA-based assay of Sanquin (Sanquin Reagents, Sanquin, Amsterdam, The Netherlands), according to manufacturer's instructions. Briefly, ELISA plates pre-coated with TNFa specific murine monoclonal antibody (CLB-TNF5, Sanquin) were incubated with recombinant TNFa, followed by biologic containing calibrators, controls, and samples, diluted in HPE (High Performance ELISA) buffer (Sanquin). Binding of anti-TNFa biologic was detected with a horse-radish peroxidase-labeled monoclonal antibody specific for the adalimumab or etanercept idiotype. Both assays were in-house validated by Sanquin (Diagnostic Services, Biologics Lab). The adalimumab assay is a CE-IVD kit of Sanquin Reagents B.V. (*http://www.sanquin.nl/repository/reagentia/ifu/M2910_2_level-adalimumab_en.pdf)*, whereas the etanercept assay is a research assay and has no CE-IVD label. The adalimumab assay had 99% accuracy at 2 ug/mL and and etanercept had 94% accuracy at 1 ug/mL of a spiked human serum sample. The lower limit of detection was 0.06 µg/mL for adalimuab and 0.15 µg/mL for etanercept. The lower limit of quantification for adalimumab was 0.06 ug/mL and 0.18 ug/mL for etanercept and the calibrator range was 0 – 25 ng/mL for adalimumab and 0 – 289 ng/mL for etanercept. The manual has been published at the website of sanquin: http://www.sanquin.nl/en/products-services/reagents/product-categories/biologicals/. Day-to-day imprecision of these assays was 10,0% and 5,6% and performed in 20 days for adalimumab concentration, and 11,3% and 8,1% and performed in 19 days for etanercept concentration, based on repetitive testing of low and high controls, respectively. For ustekinumab measurements an analogous ELISA based assay was used, however, this time executed by Sanquin (Diagnostic Services, Biologicals Lab). In this in-house validated assay plates are pre-coated with one of the ustekinumab targets, interleukin-12, to capture ustekinumab, and rabbit polycloncal antibody specific for

ustekinumab idiotype is used for detection (20). For ustekinumab PK measurements prior to Feb 2017: the accuracy was 97% at 0.4 ug/mL of spiked human serum sample. The lower limit of detection was 0.02 ng/mL. The lower limit of quantification was 0.02 ug/mL, the calibrator range was 0.0004 – 0.025 µg/mL and the reportable range was 0.02 – 16 µg/mL. Day-to-day imprecision of the ustekinumab assay was 9%, based on 3 days of repetitive testing of a single (medium) control.

Measurement of anti-drug antibody against a biologic (ADA) was only performed in patients with undetectable biologic trough levels, as it is more likely to find ADA in absence of detectable drug (21). ADA was also determined by Sanquin (Diagnostic Services, Biologicals Lab), using in house validated ELISA-based assays. Etanercept has no clinically relevant immunogenity, and therefore measurement of ADA was not indicated (22).

Statistical analysis

For numeric variables that were not normally distributed according to the Shapiro-Wilk test, we used the Mann Whitney U-test to compare groups. For the analysis of the associations between categorical data, we used Pearson's Chi Square test or Fisher's exact test, as appropriate. The difference between the biologic baseline trough concentration of the successes and the failures were analyzed with the Mann Whitney U-test. Statistical significance was defined as a two-tailed p-value of 0.05 or less. For statistical data analysis, SPSS statistics 23 software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) was used.

RESULTS

Patients

This study started in June 2013, and the last patient completed follow-up in March 2016. In total 64 patients were enrolled and after the run-in period of six weeks 59 patients were included (Fig.1). Patient demographics and baseline disease characteristics are presented in Table 2. The BMI, PASI, PGA and DLQI were significantly higher in the ustekinumab group compared with the adalimumab and etanercept group.

Seventeen out of 20 patients on adalimumab therapy (85%) completed the 78-week study, in the etanercept therapy group 18 of 21 patients (86%) completed the study and in the ustekinumab therapy 17 of 18 patients (94%) completed the study. Seven patients did not complete the study because of withdrawal of consent (n=3) or because they were lost to follow up (n=4) because of migration (moved to a different city or country). In each biologic group, patients had different maintenance dosing intervals, but the main cohort started with the per label dose and dosing interval (Fig. 1). The per label dosing interval for adalimumab was 40mg once every two weeks, for etanercept 50mg every week and for ustekinumab 45mg (<100 kg)

or 90mg (>100 kg) every 12 weeks. For etanercept and adalimumab the dose was unchanged during the study protocol, only the dose interval was prolonged step by step. In ustekinumab treated patients the dose was also unchanged, except in 3 patients, in whom the dose was reduced from 90mg to 45mg.

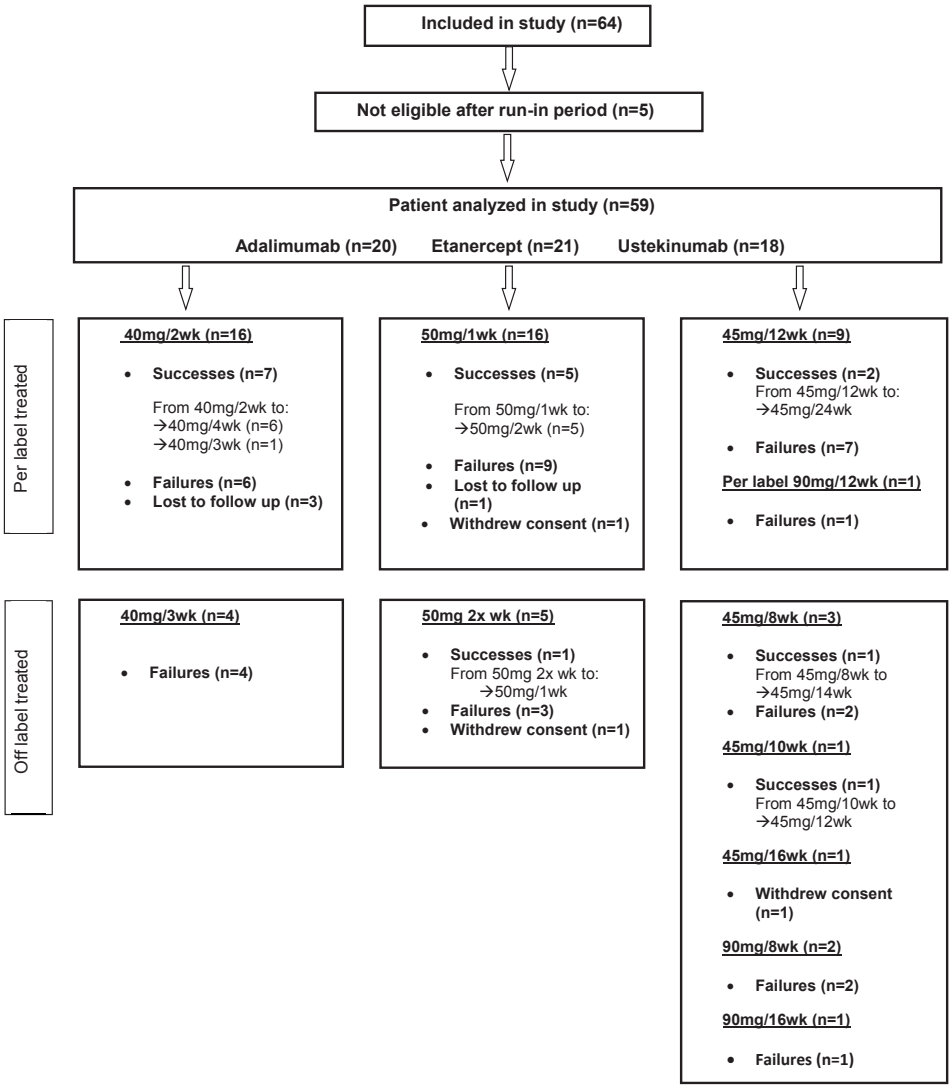

Figure 1: Study flow chart showing included patients per biologic and dosing interval groups. The number of patients with successful prolongation of the dosing interval and failures are depicted. Patients who did not complete the study are also shown.

Table 2. Baseline patient characteristics.

Patient characteristics	Adalimumab (n= 20) n (%)		Etanercept (n= 21) n (%)		Ustekinumab (n= 18) n (%)	
Gender, n (%)						
Males	16	(80)	12	(57)	11	(61)
Females	4	(20)	9	(43)	7	(39)
Race, n (%)						
Caucasian	13	(65)	15	(71)	14	(78)
Black	1	(5)	4	(19)	2	(11)
Asian	5	(25)	2	(10)	2	(11)
Other	1	(5)				
Age (years), mean (SD)	43.2	(13.2)	50.6	(14.4)	52.0	(15.3)
Body mass index (kg/m2), median (Q1, Q3)	24	(23, 29)	26	(23, 29)	28	(26, 31)
PASI score, Median (Q1, Q3)	1.7	(0.3, 3.2)	3.2	(1.3, 4.8)	2.9	(1.8, 3.9)
PGA score						
Clear	6	(30)	4	(19)	3	(17)
Almost clear	14	(70)	15	(71)	13	(72)
Mild			2		2	(11)
DLQI score, Median (Q1, Q3)	0.0	(0.0 , 2.5)	1.0	(0.0 , 5.0)	1.5	(0.0 , 5.3)
Duration of psoriasis, years, Median (Q1, Q3)	15	(8 , 28)	20	(14, 34)	22	(14, 39)
History of psoriatic arthritis, n (%)	5	(25)	2	(10)	2	(11)
Biologic-naïve before start current biologic , n (%)	12	(60)	15	(71)	4	(22)

Prolongation of the dosing interval

Per label dosing

At week 78, in the adalimumab group (40mg every two weeks), seven out of 16 (44%) patients had achieved a successful dosing interval prolongation. Six of these seven patients had a successful interval prolongation of 100% (40mg every four weeks) and one of 50% (40mg every 3 weeks). In the etanercept group (50mg every week), 5 out of 16 (31%) patients had a successful dosing interval prolongation of 100% (50mg every two weeks).

In the ustekinumab group (45mg every 12 weeks), 2 out of 9 (22%) patients had a successful dosing interval prolongation of 100% (45mg every 24 weeks).

Off-label dosing

The dosing interval could not be extended for the patients who were already being treated with adalimumab 40mg every three weeks at baseline (n=4). In all four patients the severity of psoriasis increased after dosing interval prolongation, and patients requested to be treated with their original regimen of adalimumab 40mg every three weeks.

In the escalated dose group etanercept 50mg twice a week, one out of five (20%) patients had a successful interval prolongation of 100% (50mg once a week; per label dosing interval).

In the escalated dose group of patients on ustekinumab treatment with 45mg every eight weeks (n=1) was successfully prolonged to 45mg every 14 weeks and the dose of 45mg every 10 weeks (n=1) was successfully prolonged to 45mg every 12 weeks. In the other off-label treated patients dosing interval prolongations were unsuccessful.

Trough concentrations

The median baseline trough concentration of adalimumab in patients in whom the dosing interval was successfully prolonged was higher compared to the concentrations in the failures, although the difference was not statistically different (8.8 μg/mL, 95% CI [5.2; 10.6] vs. 5.3 μg/mL, 95% CI [4.5; 10.9] P=0.37) (Fig. 2A).

The median baseline trough concentration of etanercept did not differ significantly between the successful prolongations vs. failures (4.1μg/mL, 95% CI [0.0; 4.7] vs. 3.6 μg/mL, 95% CI [1.3; 6.9], P= 1.0) (Fig. 2b).

Also the median baseline trough concentration of ustekinumab did not differ significantly between the successful prolongations vs. failures (0.6 μg/mL, 95%CI [0.3;0.9] vs. 0.2 μg/mL, 95% CI [0.2; 0.5] P=0.33) (Fig. 2c).

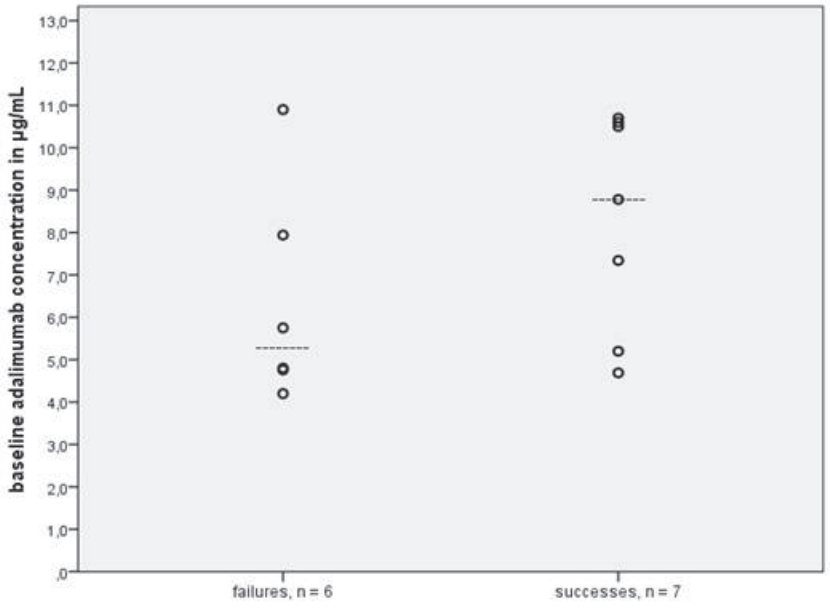

Figure 2a: Comparison of adalimumab trough concentrations at baseline after the run-in period of six weeks in the per label patients with a successful dosing interval prolongation and the failures. The concentrations at baseline of each patient are represented by an circle (○).Some circles (○) are on the same place in the graph because some patients have the same concentrations at baseline. The horizontal line in the graph represents the median. The three patients who failed to complete the study were not included in this graph.

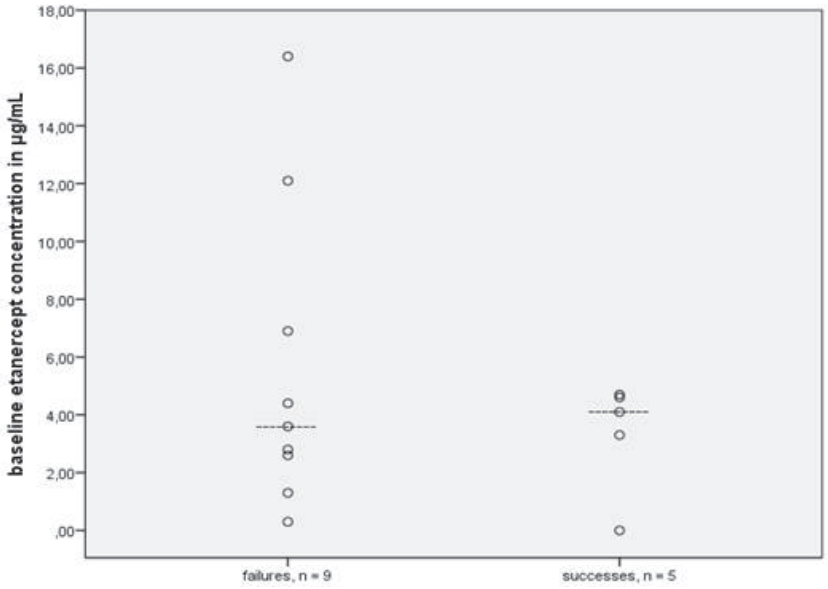

Figure 2b: Comparison of etanercept trough concentrations at baseline after the run-in period of six weeks in the per label patients with a successful dosing interval prolongation and the failures. The concentrations at baseline of each patient are represented by an circle (○). Some circles (○) are on the same place in the graph because some patients have the same concentrations at baseline. The horizontal line in the graph represents the median. The two patients who failed to complete the study were not included in this graph.

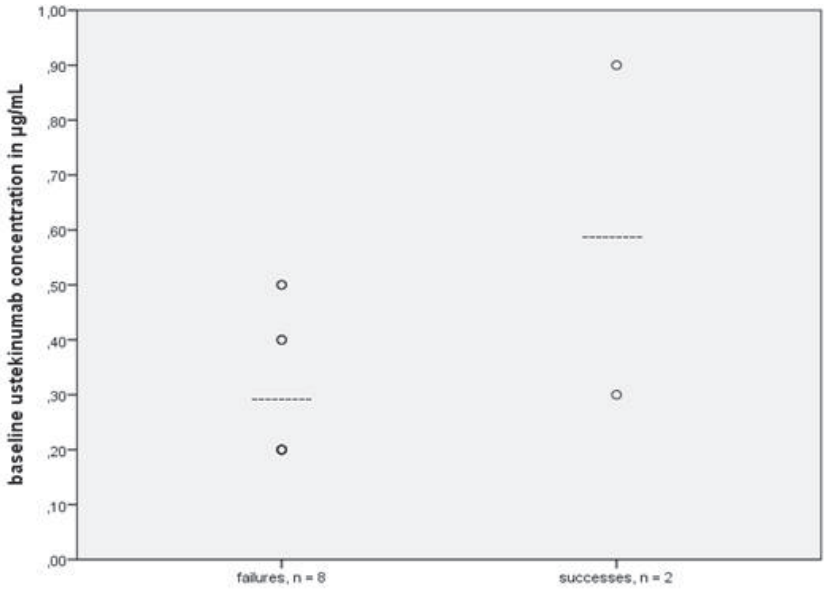

Figure 2c: Comparison of ustekinumab trough concentrations at baseline after the run-in period of six weeks in patients with a successful dosing interval prolongation and the failures. The concentrations at baseline of each patient are represented by an circle (○).Some circles (○) are on the same place in the graph because some patients have the same concentrations at baseline. The horizontal line in the graph represents the median.

Undetectable trough concentrations and presence of ADA

In the adalimumab group in three patients concentrations below the detection limit were found on two occasions. In one of these patients the presence of ADA was detected once. For this patient the psoriasis was in remission. In the etanercept group two patients had, each on two occasions, undetectable concentrations, and four patients showed an undetectable concentration at a single occasion. In total 9 patients had 14 occasions in which undetectable trough concentrations were found. In the ustekinumab group all patients had detectable trough concentrations and measurement of ADA was therefore not indicated.

Loss of efficacy

In the majority 93% (n=55) of patients, the PASI score remained below 8. In one patient in the per label adalimumab group and in two ustekinumab treated patients on off-label escalated dose, prolongation of the dosing interval caused an increase in disease activity that led to a PASI above 8. In one patient on off-label escalated etanercept dose the PASI increased above 8 before any interval prolongation, and in these cases, a dose prolongation was not performed. All of the four patients mentioned in this paragraph reached remission after they were retreated according to their original dosing regimen.

Occurrence of adverse events (AE)

The most frequent minor adverse events were respiratory and urinary tract infections. In the per label adalimumab group three severe adverse events (SAE) occurred. One patient developed meningitis and his psoriasis exacerbated. His PASI score reached 7.6 and at the end of study he was switched from adalimumab to ustekinumab. The other two patients suffered from rhabdomyolysis and had a coronary artery stent placement, respectively. In the per label ustekinumab group two SAEs occurred (cerebrovascular accident and miscarriage). In the off-label group, one patient on ustekinumab was admitted to the hospital with a myocardial infarction. All patients recovered without sequelae.

DISCUSSION

The main finding of this study is that in approximately 30% of psoriasis patients on maintenance treatment with adalimumab, etanercept or ustekinumab, the per label dosing interval could be prolonged without loss of clinical response.

The adalimumab group had the highest percentage of patients (44%) in whom the dosing interval could be prolonged successfully. Baniandres et al. also showed in a cross-sectional study among 112 psoriasis patients, that the adalimumab dose could be successfully reduced in 57.7% of their patients (5). Rodrigo-Nicolas et al. showed 75% successful adalimumab dosing interval prolongations in 12 patients (4). Their follow-up time of 46.5 weeks was shorter than

our follow-up time of 78 weeks. In our study 50% (3 out of 6) of the failures occurred after a follow-up of 52 weeks, potentially explaining the higher success rate of Rodrigo-Nicolas et al.

In the ustekinumab per label group a low rate (22%) of successful interval prolongation was achieved. The ustekinumab-treated patients had a significantly higher PASI and DLQI score at baseline and they had previously been treated with significantly more biologics before they started using ustekinumab. Possibly the lower success rate in the ustekinumab group was related to more treatment resistant disease.

Trough concentrations of etanercept and ustekinumab at baseline did not correlate with the success of subsequent dosing interval prolongations. Concentration-effect relationships have not been shown for these two biologics. Neither Mahil et al. nor Menting et al. found a correlation between etanercept or ustekinumab trough concentrations and clinical response in newly treated psoriasis patients (20, 23, 24). Van Herwaarden et al. also showed that adalimumab and etanercept trough concentrations were not predictive for successful dose reductions(24). In the adalimumab group of our study cohort there was a trend towards higher trough concentrations in the patients in whom dosing interval could be prolonged, although the difference was not statistically significant. Our results support the finding of Menting et al. who showed that adalimumab concentrations above 7 μg/mL do not provide additional clinical efficacy (7). The majority (71%) of adalimumab treated patients in whom the dosing interval could be prolonged had an adalimumab trough concentration above 7 μg/mL at baseline.

Another important secondary aim of our study was the safety of dosing interval prolongations. Reported risk of biologic dose reductions such as irreversible loss of efficacy and immunogenicity (25) appeared to be low/absent in this study. Dose reductions have a major influence on the costs of treatment, if patients can be treated with considerably less injections per year because the dosing interval is doubled. Potentially, this can result in treatment cost savings of 7,021 euros per adalimumab treated patient; 6,939 euros per etanercept-treated patient and 7,130 euros per ustekinumab-treated patient per year. For a formal and more accurate cost-effectiveness evaluation also the indirect costs should be taken into account, however this was beyond the scope of our study. Some studies in rheumatoid arthritis patients on treatment with biologics have promising data on major savings in the costs for biologics after dose reduction (26, 27).

In clinical practice patients are often treated off-label, therefore we also included these patients in our study. Successful prolongations were less often observed in the off-label group. In the off-label adalimumab and ustekinumab group who were already on a prolonged dosing interval, further dosing interval prolongation was unsuccessful.

A limitation of this exploratory study was the relatively small number of patients per biologic. A control group of patients who continued maintenance treatment without dose interval prolongation was not part of the current study design. For larger prospective trials inclusion of a control group will help to estimate the incidence of psoriasis flares, in patients treated with unchanged dose and dose interval. Moreover, we chose a relatively high PASI

of 8 as cut-off for inclusion in this study and to define the maximum allowed deterioration in disease activity upon dosing interval prolongation. In retrospect, a PASI of 5 would have been more appropriate since this has recently been suggested as a cut-off for intervention (28). However, the higher PASI cutoff does not appear to have influenced our results in this light, since all included patients had low disease activity with the highest PASI at baseline of 4.8 (etanercept group). Furthermore, most patients that experienced deterioration of their psoriasis upon dosing interval prolongation chose to return to their previous dosing interval before a PASI of 5 was reached. Also, none of the patients that were designated as 'successful' upon dosing interval prolongations had a PASI above 5. Despite these limitations, this is one of the first observational biologic tapering studies reflecting a real-life setting in a clinical dermatology practice. A definition of clinical criteria to select patients in whom dose interval prolongation can be applied would be helpful. Intuitively one would think that a longer duration of remission, no failed biologic treatments prior to the current treatment, a very low PASI score, and higher biologic concentrations would increase the chance of successful dose interval prolongation. However, larger cohorts of patients are needed to evaluate the predictive value of the mentioned clinical criteria.

In conclusion, safe and robust prolongation of biologic dosing intervals was achieved in almost 30% of patients. This can reduce the treatment costs without clinically relevant loss of efficacy. Larger (cost-effectiveness) studies are warranted to corroborate our findings and to investigate whether adalimumab baseline trough levels can indeed predict the successful interval prolongation in psoriasis patients with already stable mild disease. Moreover, these investigations could possibly be extended to the newly introduced anti-IL17 biologics for which the data on safe dosing interval prolongations in psoriasis are also lacking.

REFERENCES

1. Den Broeder AA, Creemers MC, van Gestel AM, van Riel PL. Dose titration using the Disease Activity Score (DAS28) in rheumatoid arthritis patients treated with anti-TNF-alpha. Rheumatology (Oxford). 2002;41(6):638-42.
2. van der Maas A, Kievit W, van den Bemt BJ, van den Hoogen FH, van Riel PL, den Broeder AA. Down-titration and discontinuation of infliximab in rheumatoid arthritis patients with stable low disease activity and stable treatment: an observational cohort study. Ann Rheum Dis. 2012;71(11):1849-54.
3. Chen DY, Chen YM, Hsieh TY, Hung WT, Hsieh CW, Chen HH, et al. Drug trough levels predict therapeutic responses to dose reduction of adalimumab for rheumatoid arthritis patients during 24 weeks of follow-up. Rheumatology (Oxford). 2016;55(1):143-8.
4. Vogelzang EH, Pouw MF, Nurmohamed M, Kneepkens EL, Rispens T, Wolbink GJ, et al. Adalimumab trough concentrations in patients with rheumatoid arthritis and psoriatic arthritis treated with concomitant disease-modifying antirheumatic drugs. Ann Rheum Dis. 2015;74(2):474-5.
5. Baniandres O, Rodriguez-Soria VJ, Romero-Jimenez RM, Suarez R. Dose Modification in Biologic Therapy for Moderate to Severe Psoriasis: A Descriptive Analysis in a Clinical Practice Setting. Actas Dermosifiliogr. 2015;106(7):569-77.
6. Pouw MF, Krieckaert CL, Nurmohamed MT, van der Kleij D, Aarden L, Rispens T, et al. Key findings towards optimising adalimumab treatment: the concentration-effect curve. Ann Rheum Dis. 2015;74(3):513-8.
7. Menting SP, Coussens E, Pouw MF, van den Reek JM, Temmerman L, Boonen H, et al. Developing a Therapeutic Range of Adalimumab Serum Concentrations in Management of Psoriasis: A Step Toward Personalized Treatment. JAMA Dermatol. 2015;151(6):616-22.
8. Papp K, Crowley J, Ortonne JP, Leu J, Okun M, Gupta SR, et al. Adalimumab for moderate to severe chronic plaque psoriasis: efficacy and safety of retreatment and disease recurrence following withdrawal from therapy. Br J Dermatol. 2011;164(2):434-41.
9. Ortonne JP, Taieb A, Ormerod AD, Robertson D, Foehl J, Pedersen R, et al. Patients with moderate-to-severe psoriasis recapture clinical response during re-treatment with etanercept. Br J Dermatol. 2009;161(5):1190-5.
10. Leonardi CL, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). Lancet. 2008;371(9625):1665-74.
11. Carrascosa JM, Garcia-Doval I, Perez-Zafrilla B, Carretero G, Vanaclocha F, Dauden E, et al. Use of off-label doses is frequent in biologic therapy for moderate to severe psoriasis: A cross-sectional study in clinical practice. J Dermatolog Treat. 2015;26(6):502-6.
12. Moots RJ, Haraoui B, Matucci-Cerinic M, van Riel PL, Kekow J, Schaeverbeke T, et al. Differences in biologic dose-escalation, non-biologic and steroid intensification among three anti-TNF agents: evidence from clinical practice. Clin Exp Rheumatol. 2011;29(1):26-34.
13. Mrowietz U, Kragballe K, Reich K, Spuls P, Griffiths CE, Nast A, et al. Definition of treatment goals for moderate to severe psoriasis: a European consensus. Arch Dermatol Res. 2011;303(1):1-10.
14. Bultman E, de Haar C, van Liere-Baron A, Verhoog H, West RL, Kuipers EJ, et al. Predictors of dose escalation of adalimumab in a prospective cohort of Crohn's disease patients. Aliment Pharmacol Ther. 2012;35(3):335-41.
15. Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. JAMA. 2006;295(19):2275-85.

16. Singh JA, Christensen R, Wells GA, Suarez-Almazor ME, Buchbinder R, Lopez-Olivo MA, et al. Biologics for rheumatoid arthritis: an overview of Cochrane reviews. Cochrane Database Syst Rev. 2009(4):CD007848.
17. Gelfand JM, Weinstein R, Porter SB, Neimann AL, Berlin JA, Margolis DJ. Prevalence and treatment of psoriasis in the United Kingdom: a population-based study. Arch Dermatol. 2005;141(12):1537-41.
18. Feldman SR, Krueger GG. Psoriasis assessment tools in clinical trials. Ann Rheum Dis. 2005;64 Suppl 2:ii65-8; discussion ii9-73.
19. Langley RG, Feldman SR, Nyirady J, van de Kerkhof P, Papavassilis C. The 5-point Investigator's Global Assessment (IGA) Scale: A modified tool for evaluating plaque psoriasis severity in clinical trials. J Dermatolog Treat. 2015;26(1):23-31.
20. Menting SP, van den Reek JM, Baerveldt EM, de Jong EM, Prens EP, Lecluse LL, et al. The correlation of clinical efficacy, serum trough levels and antidrug antibodies in ustekinumab-treated patients with psoriasis in a clinical-practice setting. Br J Dermatol. 2015;173(3):855-7.
21. Rispens T, Hart MH, Ooijevaar-de Heer P, van Leeuwen A, Vennegoor A, Killestein J, et al. Drug interference in immunogenicity assays depends on valency. J Pharm Biomed Anal. 2013;85:179-85.
22. de Vries MK, van der Horst-Bruinsma IE, Nurmohamed MT, Aarden LA, Stapel SO, Peters MJ, et al. Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis. Ann Rheum Dis. 2009;68(4):531-5.
23. Mahil SK, Arkir Z, Richards G, Lewis CM, Barker JN, Smith CH. Predicting treatment response in psoriasis using serum levels of adalimumab and etanercept: a single-centre, cohort study. Br J Dermatol. 2013;169(2):306-13.
24. van Herwaarden N, Bouman CA, van der Maas A, van Vollenhoven RF, Bijlsma JW, van den Hoogen FH, et al. Adalimumab and etanercept serum (anti)drug levels are not predictive for successful dose reduction or discontinuation in rheumatoid arthritis. Ann Rheum Dis. 2015;74(12):2260-1.
25. Chan CK, Holroyd CR, Mason A, Zarroug J, Edwards CJ. Are there dangers in biologic dose reduction strategies? Autoimmun Rev. 2016;15(7):742-6.
26. Murphy CL, Awan S, Sullivan MO, Chavrimootoo S, Bannon C, Martin L, et al. Major cost savings associated with biologic dose reduction in patients with inflammatory arthritis. Ir Med J. 2015;108(1):19-21.
27. Raffeiner B, Botsios C, Ometto F, Bernardi L, Stramare R, Todesco S, et al. Effects of half dose etanercept (25mg once a week) on clinical remission and radiographic progression in patients with rheumatoid arthritis in clinical remission achieved with standard dose. Clin Exp Rheumatol. 2015;33(1):63-8.
28. Dauden E, Puig L, Ferrandiz C, Sanchez-Carazo JL, Hernanz-Hermosa JM, Spanish Psoriasis Group of the Spanish Academy of D, et al. Consensus document on the evaluation and treatment of moderate-to-severe psoriasis: Psoriasis Group of the Spanish Academy of Dermatology and Venereology. J Eur Acad Dermatol Venereol. 2016;30 Suppl 2:1-18.

PART III

Conditions for TDM of biologics

Chapter 6

A comparison of three assays to quantify infliximab, adalimumab and etanercept serum concentrations

J.S. van Bezooijen, B.C.P. Koch M.B.A. van Doorn, E.P. Prens, T. van Gelder, M.W.J. Schreurs

Ther Drug Monit. 2016 Aug;38(4):432-8.

ABSTRACT

Background

To optimize treatment of inflammatory diseases, interest in the measurement of serum drug concentrations of anti-TNFα biologics is increasing. Preferably, assays for the detection of these biologics should be compared using the same reference material. In the current study two commercially available ELISAs and a commercially available bioassay for the determination of anti-TNFα biologics are compared.

Method

Serum samples from infliximab-, adalimumab- and etanercept-treated patients 40 each, control samples from ustekinumab-treated patients and healthy donors 10 each were obtained. ELISAs manufactured by Sanquin and Theradiag, and the iLite reporter gene-based bioassay from Biomonitor were used.

Results

Sanquin, Theradiag and iLite assays concordantly (100%) detected the compounds infliximab, adalimumab and etanercept in the relevant patient groups. The Sanquin ELISAs specifically detected the anti-TNFα biologic they were designed for, whereas the Theradiag and iLite showed cross-reactivity with other anti-TNFα biologics. Ustekinumab, the compound representing an anti-IL12/23 biologic, was not detected in any of the assays. Sanquin, Theradiag and iLite showed linear quantitative correlation in all respective biologic concentration assays. However, there were statistically significant quantitative differences in detected concentrations.

Conclusions

All three commercially available assays appear suitable for therapeutic drug monitoring of anti-TNFα biologics, allowing sensitive and comparable detection of infliximab, adalimumab and etanercept concentrations, however with differences in specificity and recovery.

BACKGROUND

The currently available anti- tumor necrosis factor alpha (TNFα) biologics infliximab (chimeric antibody), adalimumab (human antibody) and etanercept (human soluble receptor - Fc fusion protein) are used for long-term control of immune mediated inflammatory diseases (IMID) including psoriasis, rheumatoid arthritis and inflammatory bowel disease. Unfortunately, anti-TNFα biologics can lose efficacy over time. For the therapeutic antibodies this appears at least partly related to the development of anti-drug antibodies (ADA) (1). ADA can mediate neutralization of biologic's therapeutic activity and can initiate the formation of immune complexes that are rapidly cleared from the circulation. Consequently, there is a loss of response due to low or undetectable serum trough concentrations. However, low or undetectable serum trough concentrations could also be caused by poor compliance as well as poor quality of the drug, poorly stored and counterfeit drug products and differences in pharmacogenomics.

Therapeutic drug monitoring (TDM) could optimise treatment with biologics by measuring drug concentrations and ADA (2). At present, analysis of drug concentrations appears to be clinically most relevant. ADA could be measured as a second step to provide an explanation for low or undetectable drug concentrations. The ultimate goal of TDM is personalized medicine for treatment with drugs, thereby potentially increasing efficacy and reducing costs (3).

Custom-made immunoassays for monitoring drug concentrations have been developed successfully. To detect therapeutic drugs in plasma or serum, specific and sensitive enzyme-linked immunosorbent assay (ELISA)-based assays have been described. Several studies have been published measuring drug concentrations and ADA, especially in rheumatoid arthritis (4-6).

Unfortunately, testing drug concentrations and ADA is currently hampered by lack of assay standardization (7). Preferably, assays should be cross-validated and standardized using the same reference material or a standard to allow direct comparison of the results. Cross-validation and standardization are of utmost importance for reliable determination of the therapeutic windows of drugs. Several assays (primarily ELISA-based) for testing drug concentrations and ADA are currently commercially available for routine diagnostic use. In the literature discussion exists regarding the performance of particular commercially available assays (8-10). To further investigate such issues, we performed a comparative analysis of the standard performance of three commercially available assays designed for anti-TNFα drug TDM in routine practice. We compared the two most frequently used commercially available ELISA systems for the detection of anti-TNFα drugs (infliximab, adalimumab and etanercept) in the Netherlands. In addition, we included a bioassay as a third objective, non-ELISA based comparator.

MATERIALS AND METHODS

Samples

Blood samples for routine diagnostic use were collected from infliximab-, adalimumab- and etanercept-treated patients, n=40 each. The adalimumab and etanercept samples were obtained from patients with psoriasis, whereas the infliximab samples were obtained from patients with sarcoidosis, uveitis and Behcet's disease. As control samples we used blood samples derived from ustekinumab-treated psoriasis patients and healthy subjects ("not receiving a biologic"), n=10 each. Clotted blood samples were centrifuged for 10 minutes at 1800 g at room temperature. Serum was subsequently isolated in fresh dry tubes and stored at -80°C until further use.

The serum samples used in this study represented left over material of routine diagnostics requested by medical doctors. The samples were anonymized before inclusion in this study.

Furthermore, normal human serum derived from a single healthy subject was spiked with specific amounts of clinical grade infliximab, adalimumab (1, 2.5 and 5 μg/ml) or etanercept (1, 2 and 4 μg/ml), obtained from the Pharmacy Department. The experiments using spiked samples were performed once and at a random sequence, as is the case with routine diagnostic tests.

Assays

Two commercially available ELISA-based test systems were used for this study: the ELISA tests produced by Sanquin (Amsterdam, The Netherlands) and the Lisa Tracker tests produced by Theradiag (Marne La Vallee, France). The tests of Sanquin (referred to as SQ) and Theradiag (referred to as LT) were both used according to the manufacturer's instructions without modification.

In addition, the commercial reporter gene-based bioassay (iLite) produced by Biomonitor/Eurodiagnostica (Copenhagen, Denmark) was used. The iLite test was also used according to the manufacturer's instructions without modification. For calibration purposes, the following standards were included in the SQ and iLite assay: Remicade ® (Janssen Biologics, Leiden, The Netherlands) for the infliximab assay, Humira ® (AbbVie, Maidenhead, United Kingdom) for the adalimumab assay and Enbrel (Pfizer, Kent, United Kingdom) for the etanercept assay. The calibrators included in the LT assay were not disclosed in the manufacturer's instructions.

The control samples provided by the manufacturer (kit controls) were included in every assay run (three runs for the LT assay and two runs for the iLite assay). The precision (inter-test variation) of the assays used was calculated using the kit control results. In the SQ assay kit control samples were not included. With all assays, standards and kit control samples were handled in an identical manner as the patient derived serum samples. All assay measurements were performed in duplicate.

Statistical analysis

To determine the quantitative correlation between infliximab, adalimumab and etanercept concentrations detected by all three assays, the Pearson's correlation coefficients was calculated whereby a value of 1 represents a perfect correlation between two methods. In addition, Bland-Altman (BA) analysis was performed to evaluate agreement between the assays. The statistical software package GraphPad Prism version 5 for Windows (GraphPad Software, San Diego, CA, USA) was used for all analyses.

RESULTS

Assay characteristics

The differences in characteristics between the three assays used in this study are presented in Table 1. Each assay has its own detection system to quantify the TNFα concentration. The SQ assay uses an idiotype-specific secondary antibody whereas the LT assay uses an anti-human IgG Fc as secondary antibody. The iLite assay detects the drug concentration by measuring its neutralization of TNFα bioactivity as a result of interference with TNFα binding to its receptor. In addition, the assay ranges are clearly different.

Table 1. Assay characteristics of the Sanquin Biologic Level (SQ), Theradiag Lisa Tracker (LT) and Eurodiagnostica iLite assays for anti-TNFα concentration testing.

Assay	Test system	TNFα formulation	Bound biologic detection	Precision (inter-test variation)*	Assay range
SQ	ELISA	Plate bound via mAb	Anti-biologic idiotype	Not available	0.1- 30 *μg*/ml
LT	ELISA	Directly plate bound	Anti-human IgG Fc	infliximab: 9.6 adalimumab: 9.3 etanercept: 17.6	infliximab and adalimumab: 0.1-8 *μg*/ml etanercept: 0.2-5 *μg*/ml
iLite	Bioassay	Free in solution	Neutralization of TNF bioactivity	infliximab: 8.8 adalimumab: 16.0 etanercept: 15.4	0.65-19 *μg*/ml

* Inter-test variation is expressed as %CV based on kit control samples measured in 3 (LT) or 2 (iLite) experiments. The SQ kit did not contain a control sample

Sensitivity and specificity

Detection of infliximab, adalimumab and etanercept in patients treated with anti-TNFα biologics, and in ustekinumab treated and untreated controls is shown in Figure 1.

Sensitivity (qualitative agreement of positive results between the assays): in all samples tested, each drug is detected in all assays above the lower limit of detection as defined by the assay's manufacturer, except in four samples derived from infliximab treated patients. In these four

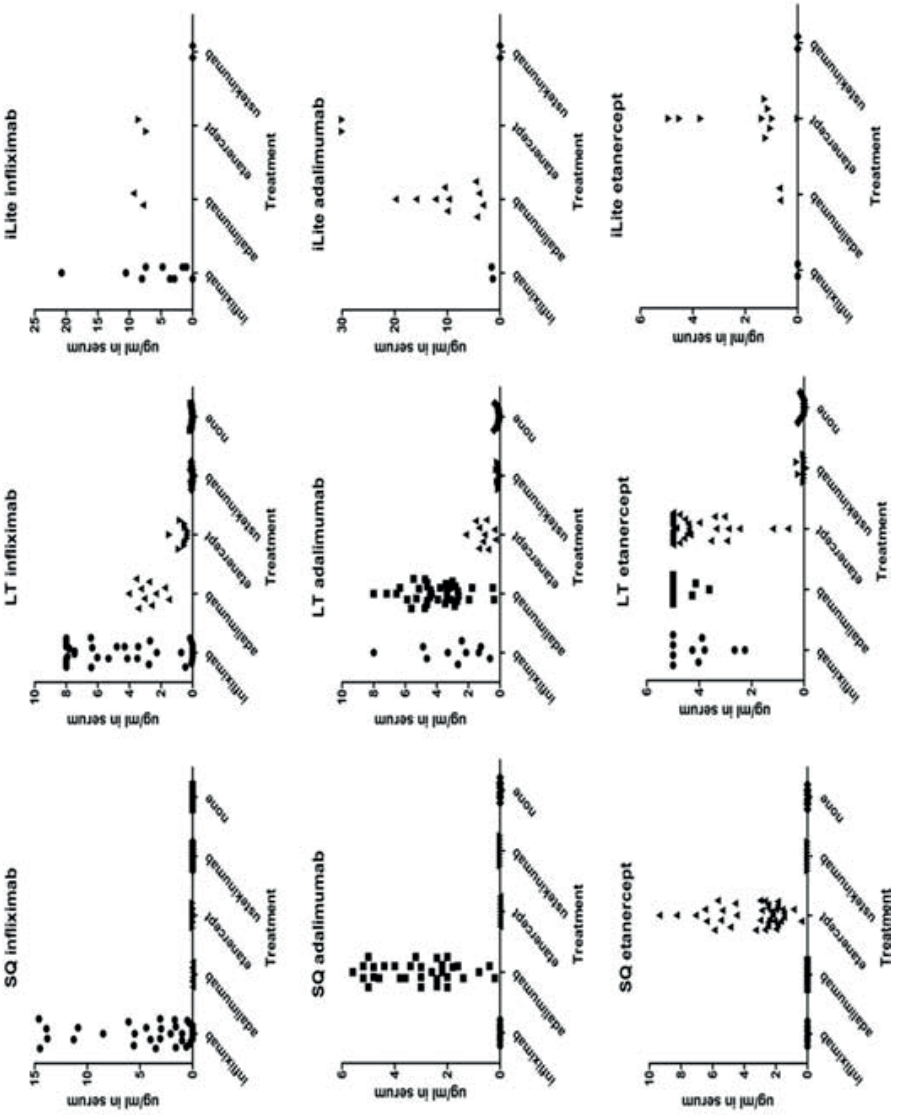

Figure 1. Sensitivity and specificity of infliximab, adalimumab and etanercept detection in serum is shown for all three assays, Sanquin Biologic concentration (SQ), Theradiag Lisa Tracker (LT) and Eurodiagnostica iLite. Serum samples obtained from infliximab, adalimumab and etanercept treated patients(n=40 for each) and controls (ustekinumab treated patients and healthy subjects ("none"), n=10) were used. LT results above the assay range, >8 µg/ml and >5 µg/ml for infliximab/adalimumab and etanercept, respectively, are shown as 8 µg/ml and 5 µg/ml. Due to the restricted availability of iLite reagents for this study, healthy controls were not tested in the iLite assay.

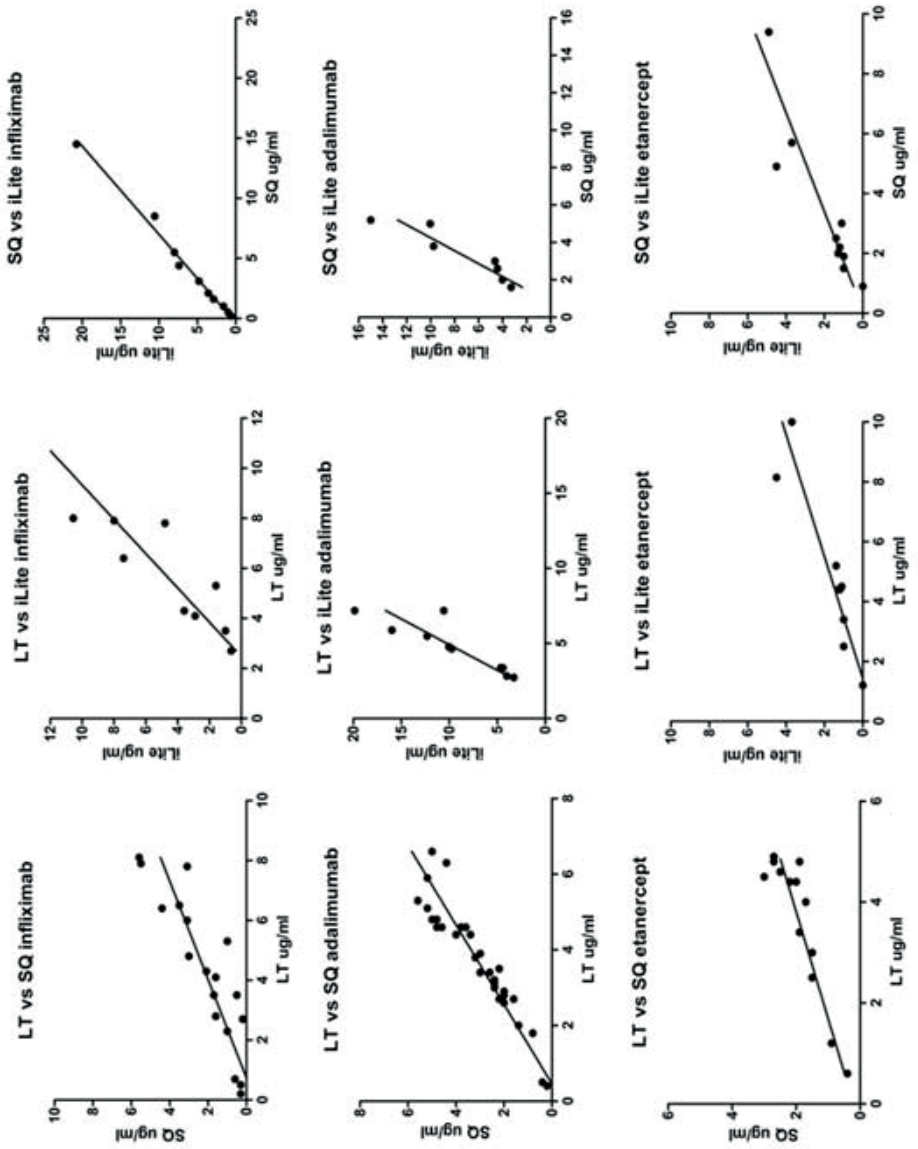

Figure 2A. Concordance of infliximab, adalimumab and etanercept detection in serum is shown for all three assays, Sanquin Biologic concentration (SQ), Theradiag Lisa Tracker (LT) and Eurodiagnostica iLite. LT vs SQ: all samples that yielded a result within the LT assay range (0.1-8 µg/ml and 0.2-5 µg/ml for infliximab/adalimumab and etanercept, respectively) were selected. LT vs iLite: 9 samples that yielded a result within the LT assay range were selected. SQ vs iLite: 10 samples across the SQ assay range were selected.

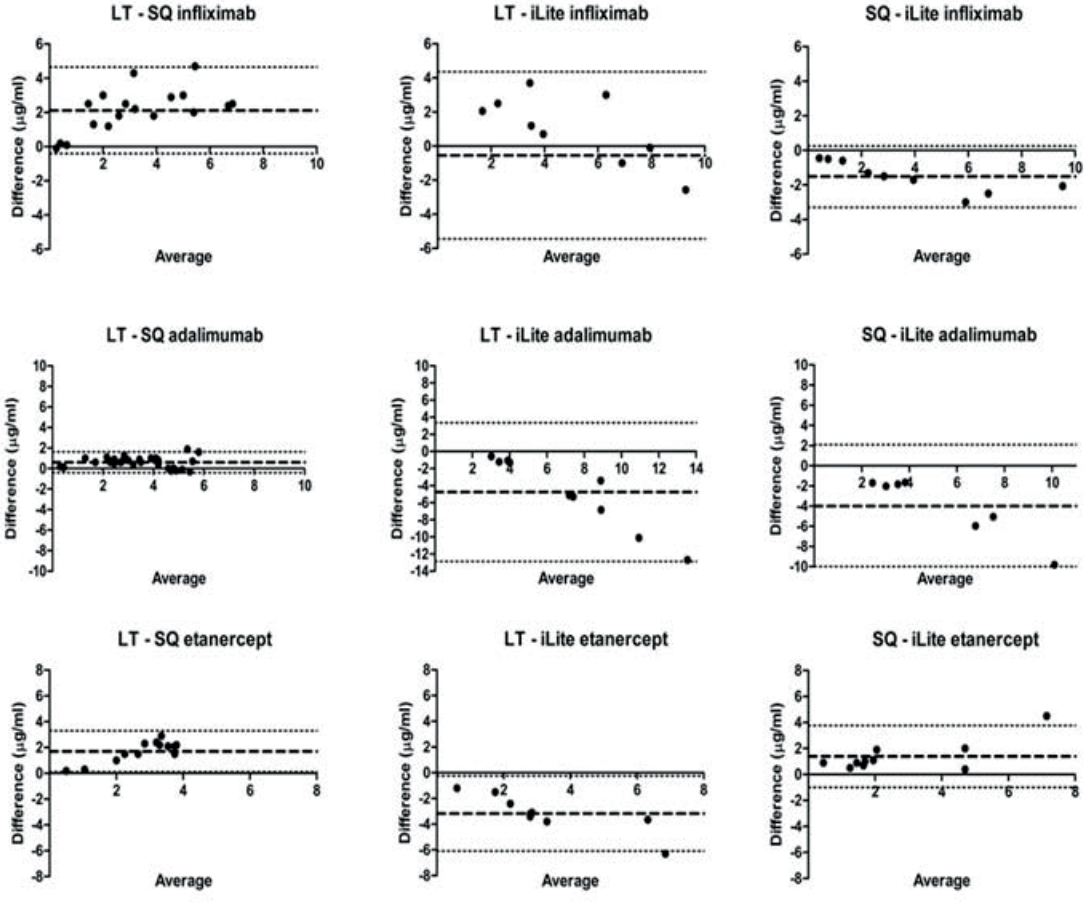

Figure 2B. Bland-Altman (BA) plots of infliximab, adalimumab and etanercept concentrations to evaluate agreement between the three assays. The difference between the Sanquin Biologic concentration (SQ), Theradiag Lisa Tracker (LT) and Eurodiagnostica iLite assays are plotted on the y-axis and their averages are plotted on the x-axis. The dotted lines represent the 95% limits of agreement and the bold dotted line represents the mean difference

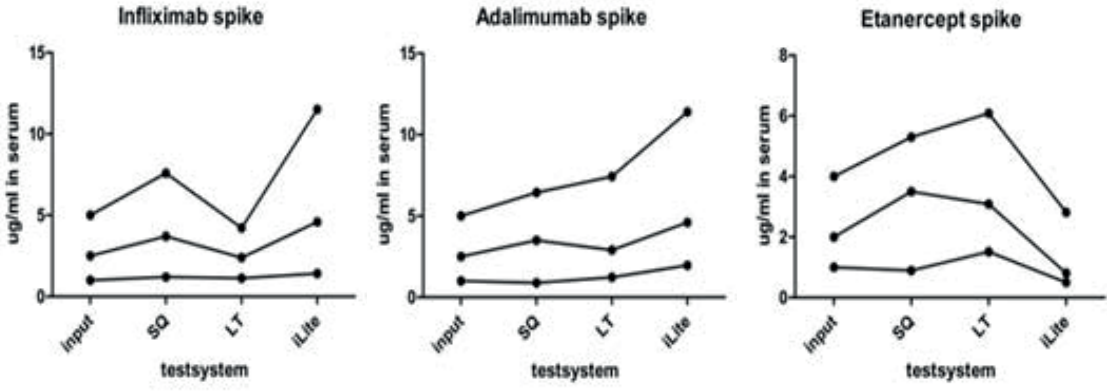

Figure 3. Recovery of infliximab, adalimumab and etanercept in spiked serum is shown for all three assays, Sanquin Biologic concentration (SQ), Theradiag Lisa Tracker (LT) and Eurodiagnostica iLite. The results are derived from a single experiment in accordance with regular patient-derived sample analysis.

samples infliximab was not detected in any of the assays (below the lower limit of detection). Overall, the assays showed no discrepancy in (qualitative) detection of drugs.

Specificity (cross reactivity of the of anti-TNFα biologics in the different assays): only the SQ assay showed a selective detection of anti-TNFα drugs, in contrast to the cross-reactivity observed in the LT and iLite assays. In this case, lack of specificity of the assays may also be interpreted as lack of selectivity. The control samples derived from ustekinumab treated patients and healthy blood donors were negative in all assays.

Concordance and agreement

All three assays showed a pairwise significant quantitative correlation in all cases for detected concentrations of infliximab, adalimumab and etanercept (Figure 2A).

This correlation was expressed using a Pearson r correlation coefficient, including concentration of significance (Table 2). The best correlation was obtained when comparing SQ vs. ILite, which gave a significant Pearson correlation of 0.99 in the infliximab concentration test. Clearly, quantitative differences in detected drug concentration are observed, depending on the assay used.

Table 2. Pearson correlation and equation of the regression, including level of significance, between biologic detection by the Sanquin Biologic Level (SQ), Theradiag Lisa Tracker (LT) and Eurodiagnostica iLite assays for anti-TNFα concentration testing

	LT vs SQ	P-value	LT vs iLite	P-value	SQ vs iLite	P-value
Infliximab	R^2= 0.77 y=0.61x-0.44	.0001	R^2= 0.92 y=1.46x-3.60	.0001	R^2= 0.99 y=1.36x+0.49	.0001
Adalimumab	R^2= 0.88 y=0.95x-0.43	.0001	R^2= 0.78 y=2.93x-4.41	.0007	R^2= 0.86 y=2.89x-2.25	.0024
Etanercept	R^2= 0.82 y=0.47x+0.21	.0001	R^2= 0.86 y=0.49x-0.69	.0004	R^2= 0.85 y=0.60x-0.05	.0002

The BA plots demonstrated restricted agreement in case the SQ and LT assays were directly compared. Only the BA plot LT –SQ adalimumab showed a good agreement with a small 95% confidence interval. In contrast, low agreement is observed when comparing the performance of the iLite assay with both ELISA systems. As shown in the BA plots, the iLite measures systematic higher concentrations compared to the LT and SQ assay, especially at higher drug concentrations (Figure 2B).

Recovery

To further investigate potential quantitative differences in recovery between different assays, we subsequently tested normal human serum, spiked with known amounts of anti-TNFα drugs (Figure 3). Again, differences in recovery depending on the assay used are observed when using spiked samples, in addition to the patient samples used in this study (Figure 2A). As shown in

Figure 3, the results of the spiking experiment merely suggests increased variability at higher drug concentrations, however this cannot be substantiated based on the current small data set.

DISCUSSION

In this study we compared the standard performance of three commercially available assays designed for anti-TNFα drug TDM; two ELISAs from Sanquin (SQ) and Theradiag (LT) and one reporter gene-based bioassay (iLite) from Eurodiagnostica. The potential clinical relevance of our study is suggested by the increasing use of drug concentration testing (11, 12).

Our results indicate that all three assays are sensitive and show concordant detection of infliximab, adalimumab and etanercept concentrations in treated patients. In four infliximab treated patients no drug concentration was detected in any of the assays, probably caused by the presence of neutralizing anti-drug antibodies (ADA) as opposed to insensitivity of the assays used in this study. The role of ADA presence and subsequent assay sensitivity for ADA interference is currently not completely clear for all assays used in this study but probably affects detection of drug concentrations. Analysis of ADA interference requires further investigation, but this is beyond the scope of this study.

The SQ assay exclusively shows selective detection of anti-TNFα drugs in this study. The use of a specific anti-idiotype secondary antibody in this assay, in contrast to the other assays, is responsible for this high specificity. In clinical practice, a patient who is switched from adalimumab to infliximab will be negative in the infliximab SQ assay before infliximab treatment is initiated, but will be (false) positive in both LT and iLite infliximab assays at that point. In such cases use of the LT and iLite assays hampers a realistic measure of the switched drug concentration, at least as long as the previous drug is still detectable.

Besides specificity, the assay ranges differ between manufacturers, with potential consequence for their application. The LT assay includes the most restricted upper range at recommended serum dilution and thus initially appears less suitable for the detection of high drug concentrations. In clinical practice, an additional serum dilution and subsequent concentration test is required for the LT assay to allow detection of concentrations above 8 μg/ml (Table 1). However, when the assay upper limit is within the defined therapeutic window of the particular drug treatment, such additional dilution and re-testing will not be required.

Although all three assays significantly correlated with regard to the amount of drug detected in the samples included in this study, we did observe differences in the absolute concentrations of infliximab, adalimumab and etanercept detected with the different assays, as evidences by our BA plots which showed a low agreement between the three assays. In spite of calibrated drug detection in μg/ml, the actual detection does not appear to be harmonized between assays. The analysis of spiked samples further supports this, indicating differences in drug recovery. In addition, when the amount of spiked drug concentration was increased, the observed vari-

ability in recovered concentration also increased. Apparently, the recovery is not similar in the three assays used in this study. The reasons for this discrepancy can be based on the assay itself and may include differences between the way TNFα is present in the assay. In the SQ and LT assays, TNFα is coated on the solid phase, either directly or monoclonal antibody-mediated, in contrast to the iLite assay whereby TNFα is in solution. Secondly, the use of ELISA versus bioassay most probably plays a role in the observed differences in recovery. Thirdly, as discussed above, the detection of TNFα bound drug differs substantially between the ELISA systems, using an anti-idiotype mAb as opposed polyclonal anti-human IgG Fc. Finally, the absolute ratio between all assay components, at the molecular concentration, could affect the final amount of detected drug concentration. Clearly, the different assays are not interchangeable during follow-up of an individual patient. Although interesting, detailed evaluation of the observed differences between assay performance is beyond the scope of this study.

Besides these differences in assay component constitution, potential effects of ADA interference on the observed differential recovery cannot be excluded. However, since ADA interference is not a relevant issue when using spiked samples, it cannot be fully responsible for the observed differential recovery. Taken together, the observed differences in recovery have important implications for the therapeutic windows of the respective anti-TNFα drugs in treated patients. In the Netherlands the therapeutic windows for infliximab, adalimumab and etanercept have (mainly) been defined using the Sanquin (SQ) assay (2). Based on our study, therapeutic windows are not interchangeable and need to be redefined individually for different assays.

To date, there have been only a few studies that have compared different assays designed for anti-TNFα drug concentration testing (7). In 2012 Casteele et al. compared three different assays including the SQ and LT assays (9). Their results are comparable to our data, however the authors suggested that the LT detected false positive infliximab concentrations in 18% of the samples. In a letter to the editor the manufacturer subsequently disputed these results (6). In our experience, the LT assays did not show any false positive detection of infliximab, adalimumab or etanercept. Another study by Steenholdt et al. in 2013 suggested that the same assay should be used in individual patients during follow-up because infliximab concentrations and ADA titers showed systematic differences between different assays (13). Our data, based on our quantitative analysis of patient- and spiked samples, fully support this suggestion.

Our study had some limitations. Firstly, we tested only a limited number of samples with the iLite assay. Secondly, all measurements were performed only once for each drug, although in duplicate within the assay and including appropriate kit controls. However, in clinical practice an assay will be validated to measure the drug concentration of a patient only once per sample. Thirdly, we did not perform an in depth analysis of the observed quantitative differences in results obtained with the different assays. Potentially, cross measurement of the calibrators used in the three assays could contribute to an explanation, however such detailed evaluation of difference in assay performance is beyond the scope of our study. Furthermore, not all

measured drug concentrations are trough concentrations, potentially resulting in increased interference by ADA. In conclusion, our study results indicate that all three commercially available assays are suitable for TDM of anti-TNFα drugs when the specific characteristics of the different assays are well recognized. Given the observed differences in drug recovery, therapeutic windows require an assay-specific definition and use of the same assay in individual patients for longitudinal TDM is warranted.

ACKNOWLEDGMENTS

The authors thank Diana Dufour-van den Goorbergh, Cindy Kneppers and Elham Torabi-Azhandeh for excellent technical assistance.

REFERENCES

1. Carrascosa JM, van Doorn MB, Lahfa M, et al. Clinical relevance of immunogenicity of biologics in psoriasis: implications for treatment strategies. *J Eur Acad Dermatol Venereol*. 2014;28(11):1424-30.
2. Zandvliet ML, van Bezooijen JS, Bos MA, et al. Monitoring antigen-specific biologics: current knowledge and future prospects. *Ther Drug Monit*. 2013;35(5):588-94.
3. Mosli MH, Sandborn WJ, Kim RB, et al. Toward a personalized medicine approach to the management of inflammatory bowel disease. *Am J Gastroenterol*. 2014;109(7):994-1004.
4. Jamnitski A, Bartelds GM, Nurmohamed MT, et al. The presence or absence of antibodies to infliximab or adalimumab determines the outcome of switching to etanercept. *Ann Rheum Dis.* 2011;70(2):284-8.
5. van Schouwenburg PA, van de Stadt LA, de Jong RN, et al. Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation. *Ann Rheum Dis.* 2013;72(1):104-9.
6. Bartelds GM, Krieckaert CL, Nurmohamed MT, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA.* 2011;305(14):1460-8.
7. Schmitz EM, van de Kerkhof D, Hamann D, et al. Therapeutic drug monitoring of infliximab: performance evaluation of three commercial ELISA kits. *Clin Chem Lab Med*. 2016 Jul 1; 54(7):1211-9.
8. Vande Casteele N, Buurman DJ, Sturkenboom MG, et al. Detection of infliximab levels and anti-infliximab antibodies: a comparison of three different assays. *Aliment Pharmacol Ther.* 2012;36(8):765-71.
9. Parussini E. Letter: detection of infliximab levels and anti-infliximab antibodies--comparison of three different assays. *Aliment Pharmacol Ther*. 2013;37(2):281.
10. Buurman DJ, Vande Casteele N, Sturkenboom MG, et al. Letter: detection of infliximab levels and anti-infliximab antibodies--comparison of three different assays; authors' reply. *Aliment Pharmacol Ther.* 2013;37(2):282.
11. Colombel JF, Feagan BG, Sandborn WJ, et al. Therapeutic drug monitoring of biologics for inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18(2):349-58.
12. Menting SP, Coussens E, Pouw MF, et al. Developing a therapeutic range of adalimumab serum concentrations in management of psoriasis: A step toward personalized treatment. *JAMA Dermatol*. 2015;151(6):616-22.
13. Steenholdt C, Ainsworth MA, Tovey M, et al. Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. *Ther Drug Monit*. 2013;35(4):530-8.

Chapter 7

Intra-patient variability in the pharmacokinetics of etanercept maintenance treatment

J.S. van Bezooijen, M.W.J. Schreurs, B.C.P. Koch, H. te Velthuis, M.B.A. van Doorn, E.P. Prens, T. van Gelder

Accepted for publication

ABSTRACT

Background

Etanercept has shown to mediate a favourable effect on Immune Mediated Inflammatory Diseases (IMID), including plaque psoriasis. Therapeutic drug monitoring (TDM) of etanercept could improve clinical outcome and cost-effectiveness. A high intra-patient variability (IPV) of etanercept trough concentrations at standard dosing would reduce the feasibility of TDM. Studies have focussed on the inter-patient differences associated with the exposure to biologics. The aim of this study was to determine the IPV of etanercept and to correlate etanercept trough concentrations and IPV with treatment response.

Methods

Repetitive serum samples of 29 psoriasis patients on standard etanercept maintenance treatment were collected. In these samples, etanercept trough concentrations were determined and IPV was assessed, in relation to response to treatment.

Results

The median IPV of etanercept trough concentrations was 33.7 % (Q1 21.3 % and Q3 51.7 %) ranging from 8% to 155%. All six non-responders showed an IPV at or above the median value of 33.7 %. The six non-responders showed a higher IPV as compared to the 23 responders (53.9% vs. 24.2 %; P= 0.031). The mean etanercept trough concentration for each patient ranged from 0.7 to 6.8 μg/ml, with a median of 2.7 μg/ml. Patients with an IPV above the median had lower mean etanercept trough concentrations compared to patients with an IPV below the median (1.96 μg/mL, 95% CI [1.7 ; 2.4] vs. 3.2 μg/mL, 95% CI [2.7 ; 4.0]; P = 0.001).

Conclusion

The median IPV of etanercept trough concentrations in this study population was 33.7 %. A higher IPV was correlated with lower etanercept trough concentrations and with non-responsiveness. Prospective trials are required to demonstrate the value of adjusting the etanercept dose based on drug trough concentrations. The relatively high IPV observed in this study may complicate TDM.

BACKGROUND

Several biologics have revolutionized the treatment of Immune Mediated Inflammatory Diseases (IMID), including psoriasis, rheumatoid arthritis (RA) and Crohn's disease. Following their introduction biologics have typically been used in standard dosing regimens in which all patients receive the same dose, at fixed intervals. More recently several studies have shown that the treatment response is related to the biologic trough concentration (1-3). Consequently, dose adjustments based on drug trough concentration may improve clinical outcome, and/or reduce the cost of treatment (4).

Etanercept treatment has been shown to have a favourable effect on treatment resistant plaque psoriasis (5). Despite more recently introduced treatment with IL12, IL-23 and IL-17 inhibitors, TNFα inhibition with etanercept is still widely used in clinical practice (6). According to current treatment guidelines the recommended maintenance etanercept dose is 50mg s.c. once a week. Whether or not therapeutic drug monitoring (TDM) of etanercept improves clinical outcome and/or cost-effectiveness in psoriasis remains to be demonstrated. However, related studies in other IMID suggest that TDM of biologics can indeed improve clinical outcome and/or cost-effectiveness (7). In general, required conditions for TDM to be useful in clinical practice include the presence of a concentration-effect relationship, availability of reliable assays to determine drug concentration and a low intra-patient variability (IPV) of concentrations at standard treatment dose. In case that the biologic concentrations within individual patients on standard dosing vary too much, TDM may not result in bringing patients into the therapeutic window, although TDM may still be able to identify grossly non-adherent individuals.

In the current literature, pharmacokinetic studies have mainly focussed on the differences of biologic trough concentration between patients as opposed to within individual patients in a certain time frame (8-10). In most studies a cross-sectional study design was chosen in which biologic trough concentration were assessed at a single time point and subsequently correlated to clinical outcome (11, 12). In our prospective, longitudinal study of psoriasis patients on standard etanerept maintenance treatment, repetitive samples of individual patients were evaluated at predefined time points. The aim of this study was to evaluate the IPV of etanercept trough concentration and to correlate this to treatment response.

METHODS

Study Design

Serum samples of etanercept treated psoriasis patients were collected as part of the FUMBREL study (13) , conducted at Erasmus MC, University Medical Centre Rotterdam, The Netherlands (EudraCT number 2011-005685-38). This study was approved by the Institutional

Review Board of the Erasmus University Medical Center Rotterdam (MEC-2011-500). All patients provided written informed consent. The study was conducted according to the guidelines of Good Clinical Practice. In this clinical trial patients with psoriasis were randomized at baseline in a 1:1 ratio to receive either etanercept with fumarates (combination group) or etanercept only (monotherapy group). All patients received 50mg etanercept subcutaneously twice weekly for 12 weeks followed by 50mg once weekly for an additional 36 weeks. Subjects in the combination group were treated with additional fumarate tablets of 215mg (120mg dimethylfumarate combined with 95mg monoethyllfumarate) up to four times a day. Subjects visited the outpatient clinic every month throughout the one-year study period. All patients administered etanercept themselves at home. Patients were asked to inject the etanercept every Thursday evening because the outpatient visits were scheduled on Thursdays during office hours. To improve adherence and to check for the actual times of administration, diaries were kept and empty syringes were collected. Date and time of last administration, next administration and date and time of sample collection at trough concentration moment, prior to the administration of the subsequent etanercept dose (trough concentration), were recorded in the electronic case report form (eCRF, Open Clinica). For the analysis of IPV only trough concentrations drawn between weeks 12 and 48 of the clinical study were included. At each study visit also Psoriasis Area Severity Index (PASI) scores were collected.

Study Objectives

The primary objective was to evaluate IPV in patients on standard etanercept dose maintenance treatment. Secondary objectives were to correlate etanercept trough concentrations and the IPV to treatment response, to assess the minimal trough concentration for etanercept to increase the likelihood of reaching a clinical response, to investigate if etanercept concentrations are correlated to body mass index (BMI) and to study the relationship between drug concentrations and clinical response expressed as PASI score. Furthermore, the influence of the concomitant use of fumarates on etanercept concentrations was assessed.

Psoriasis Activity and Severity Index (PASI)

In this study the validated PASI was used as a tool for monitoring patients disease activity. The PASI is calculated based on the intensity of redness, thickness, scaling, and the affected surface area of the skin (14). Responders were defined as patients who achieved PASI improvement of 75% and continued etanercept up to 48 weeks. Non-responders were defined as patients who did not achieve PASI improvement of 75 % at 48 weeks or patients in whom etanercept treatment was discontinued before 48 weeks.

Etanercept assay

To measure the etanercept concentration an ELISA based assay of Sanquin (Sanquin Reagents, Amsterdam, The Netherlands) was used. For the etanercept specific ELISA (15), ELISA plates

were coated with specific murine antibody (CLB-TNF5, Sanquin, Amsterdam). In the second step, recombinant TNFα was bound to the coated antibody. Etanercept containing calibrators, controls and samples were diluted in HPE (*High Performance ELISA)* buffer (Sanquin, Amsterdam) and loaded on the coated plates. The binding of etanercept was detected with a horse-radish peroxidase-labeled etanercept (idiotype) specific monoclonal antibody. The accuracy of the test was > 95% with a spiked sample at a concentration of 0.1 μg/mL. The lower limit of quantification was approximately 0.15 μg/mL.

Statistical analysis

For calculation of the IPV of etanercept concentrations the standard deviation of the observed etanercept trough concentrations was divided by the mean and multiplied by 100%. For each patient a minimum of at least 4 etanercept trough concentration measurements had to be available in order to calculate the IPV. As the variables were not normally distributed according to the Shapiro-Wilk test, we used the Mann Whitney U-test to compare groups. Bivariate correlations were expressed as Pearson's correlation coefficients. P-values < 0.05 were considered to be statistically significant. For exploring the minimal trough concentration for etanercept relating to a good clinical response, only descriptive statistics was used. For statistical data analysis, SPSS statistics 23 software (IBM Corp) was used.

RESULTS

Patients

In the clinical trial 33 patients were enrolled. For the current pharmacokinetic substudy four patients were excluded because less than four etanercept trough concentrations were available. Three patients had discontinued the study earlier (at week 24) because they withdrew consent for personal reasons. The fourth patient was not adherent to the study protocol and had repetitively failed to self-administer etanercept. For the analysis of IPV a total of 29 patients was available. Twenty patients were concomitantly treated with fumarates (maximum of 215mg four times a day). At week 48, 23 patients achieved PASI-75 and continued with etanercept treatment (responders). The remaining 6 patients did not reach PASI-75 or had already discontinued etanercept treatment (non-responders).

The baseline characteristics of the included patients are shown in Table 1. Of the 29 patients the majority (22 patients) was of Caucasian descent, and the other 7 were non-Caucasian (black, asian, other). Mean etanercept concentrations were not significantly different between the two groups (2.8 and 3.2 μg/mL respectively). Ethnicity also was not correlated with reaching a treatment response. In the patients of Caucasian descent 18/22 (82%) reached a positive response, compared to 5/7 (71%) of the non-Caucasians.

Intra-patient variability (IPV) of etanercept

A total of 194 samples were available (range 4-9 trough concentrations per patient). The median IPV was 33.7% (Q1 21.3% and Q3 51.7%) ranging from 8% to 155%. Figure 1 shows on the x-axis the IPV of the 29 individual patients. The six non-responders had an IPV at or above the median value of 33.7%. In these six non-responders the IPV was higher as compared to the 23 responders (53.9%, 95% CI [36.1 ; 110.9] vs 24.2 %, 95%CI [21.6 ; 43.8], and this difference was statistically significant (P= 0.031).

Differences between the responders vs. non-responders are shown in table 2.

Table 1. Baseline patient characteristics.

Patient characteristics	Patients (n=29)	
Males n (%)	20	(69)
Females n (%)	9	(31)
Age (years), mean (SD)	45.4	(3.0)
Weight (kg), median (Q1,Q3)	85	(75,97)
Height (m), mean (SD)	1.75	(0.2)
Body mass index (kg/m2), mean (SD)	28.5	(1.0)
Disease duration in months, mean (SD)	20.3	(1.9)
PASI score, median (Q1,Q3)	14.2	(10.7,20.8)
Biologic-naïve, n (%)	18	(62)
Concomitantly use of fumarates, n (%)	20	(69)
Etnicity : Caucasian vs other, n (%)	22	(76) vs 7 (24)

Table 2. Comparison of etanercept trough concentration, intra-patient variability (IPV), Psoriasis Activity and Severity Index (PASI) and body mass index (BMI) between responders and non-responders.

	Responders (N=23)	Non-Responders (N=6)	P-value
Mean etanercept trough concentration (µg/mL)	Median 2.77 95%CI[2.28 – 3.64]	Median 2.19 95%CI [0.93 – 3.03]	0.16
IPV	Median 24.2 95%CI[21.1 – 43.8]	Median 53.9 95%CI [36.1 – 110.9]	.031
PASI score	Median 0.8 95%CI[0.4 – 1.3]	Median 7.2 95%CI [5.3 – 13.5]	.000
BMI	Median 26.9 95%CI [25.2 – 28.1]	Median 31.6 95%CI [25.7 – 33.5]	.127

Mann-Whitney U-test

Etanercept trough concentrations and treatment response

Figure 1 also shows on the y-axis the mean etanercept concentrations measured in the 29 individual patients. The mean etanercept trough concentrations for each patient ranged from 0.7 to 6.8 µg/mL, with a median of 2.7 µg/mL. The concentration-effect relationship shown in Figure 1 suggests that a trough concentration above 1.6 µg /mL increases the likelihood of

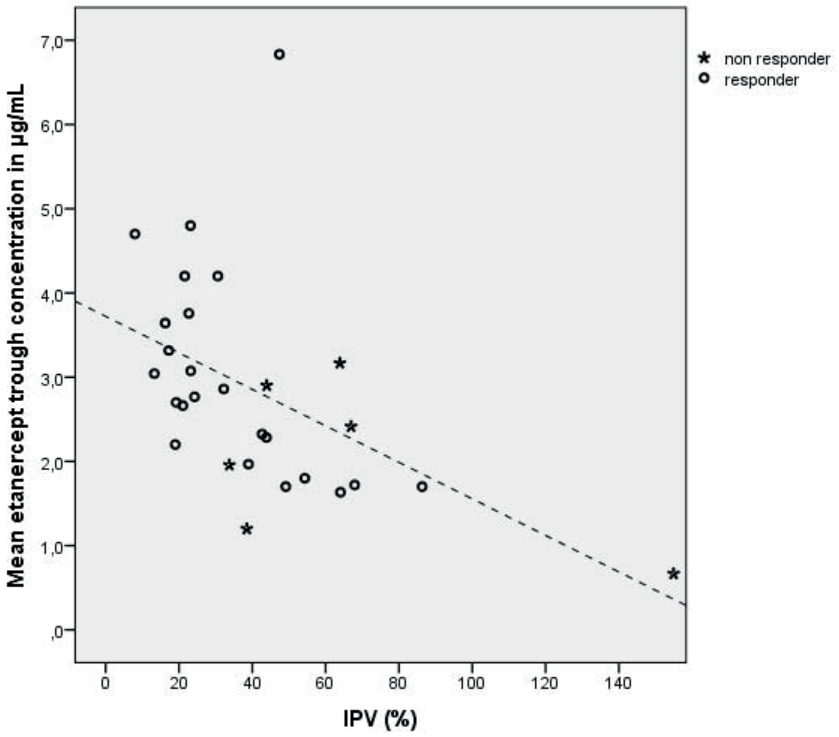

Figure 1: The correlation between the intra-patient variability of etanercept trough concentrations and the mean etanercept concentration in each of 29 individual patients. Responders are represented by an circle (○), and the non-responders by an asterix (*).

reaching a good clinical response (at least PASI improvement of 75%). However, there were only two patients in this study with a mean etanercept trough concentration below 1.6 µg/mL, and we do not want to make any recommendation regarding a lower threshold of the therapeutic window.

Figure 1 also shows a negative correlation of r=0.50, 95% CI [-0.76; -0.16] (P=0.006) between the mean etanercept trough concentration and IPV. Patients with an IPV above the median had lower mean etanercept trough concentrations compared to patients with an IPV below the median (1.96 µg/mL, 95% CI [1.7 ; 2.4] vs 3.2 µg/mL, 95% CI [2.7 ; 4.0]; P = 0.001). Patients with an IPV at or above the median of 33.7% had etanercept trough concentrations between ≥ 1 and ≤ 4 µg/mL in 69 % (68 out of the 99) samples, while patients with lower IPV had etanercept trough concentrations between ≥ 2 and ≤ 5 µg/mL in 92 % (87 out of the 95) samples.

A weak positive correlation (r= 0.27) is observed between the PASI % improvement and mean etanercept trough concentration, however this was not statistically significant (P= 0.15). There was a statistically significant negative correlation between etanercept trough concentra-

tion and BMI (r = -0.47), 95% CI [-0.63; -0.31] (P= 0.01) (Figure 2). The correlation between the IPV and BMI was r= 0.37, 95% CI [0.14 ; 0.68] (P=0.046)

The mean trough concentration in patients treated with concomitant fumarates (n=20) did not differ from the trough concentration in patients on etanercept monotherapy (median 2.78, 95% CI [2.14 ; 3.24] vs. 2.28, 95% CI [1.70 ; 2.90] µg/mL), P=0.76. The concomitant treatment with fumarates did not influence the clinical response at 48 weeks, as shown by the PASI score that did not differ between fumarate users and non-users (15).

Also the median IPV did not differ between the two groups (33.0 %, 95% CI [22.1 % ; 50.9 %] vs. 39 %, 95% CI [19.0 % ; 49.1 %] , P = 0,7). Therefore we do not think that the randomization into the two patients groups has contributed to the IPV observed in this study.

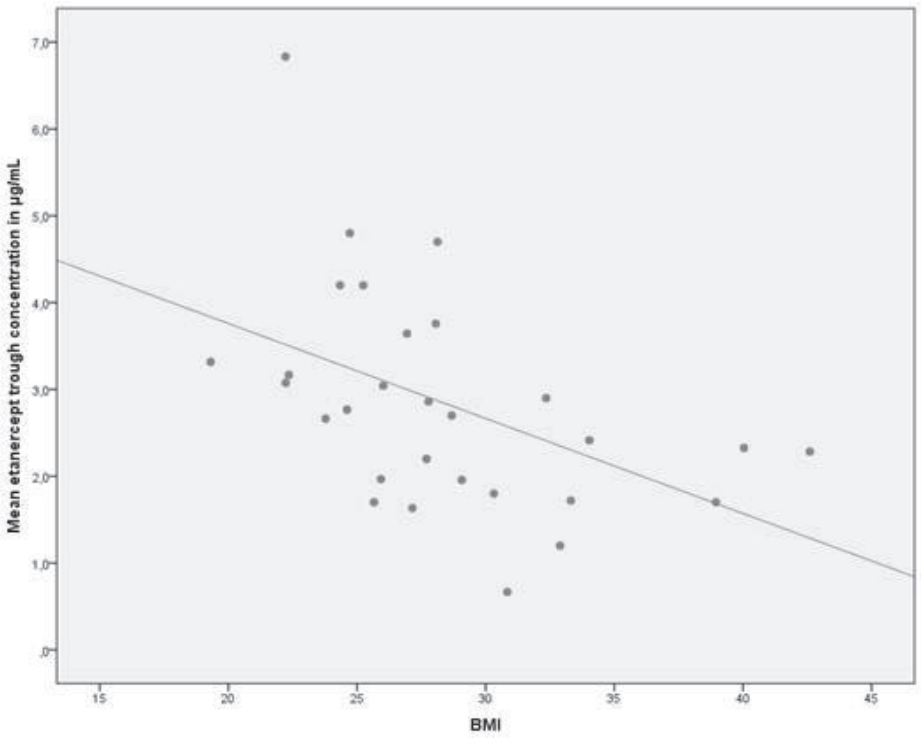

Figure 2: Correlation between etanercept trough concentrations and BMI.

DISCUSSION

In this longitudinal study of psoriasis patients on a stable dose of etanercept we show that the median intra-patient variability (IPV) of etanercept trough concentrations is 33.7%. IPV was not normally distributed, and included some patients having a IPV above 50%. Regulatory agencies tend to classify drugs with an intra-patient coefficient of variation maximum concentration and/or area under the curve of 30% or more as highly variable drugs (16).

In our study we have monitored trough concentrations only, and thus we cannot extrapolate these numbers to our study. However, we anticipated that IPV would be low, as etanercept is a subcutaneously administered drug, and is not metabolized by liver or kidney. Typical causes of variability for small molecule drugs are the absorption from the gut and differences in renal function and/or liver metabolism between patients. Don et al. also showed data on intra-patient variability, but only based the effect of hemodialysis sessions on etanercept pharmacokinetics was reported. They concluded that the pharmacokinetics of etanercept in patients with chronic renal failure on hemodialysis are similar to patients with normal renal function (17). Other than intravenous administration, the bioavailability of subcutaneous etanercept is not 100% and variable due to the complex absorption of etanercept and due to differences between injection sites.

In studies investigating the importance of IPV in patients treated with small molecule drugs, monitoring the standard deviation of drug concentration measurements has been suggested as a method to detect non-adherence (18). A high IPV of the immunosuppressive drugs ciclosporin and tacrolimus has been linked to impaired survival of kidney transplants, probably because drug exposure below the recommended target concentrations leads to immune activation and hence development of allograft rejection (19, 20). In our renal transplant population we did find that patients with high IPV were more likely to be non-adherent (21). Also for biologics that are administered subcutaneously or intravenously, adherence may influence drug exposure. Patients who administer the drug themselves at home can miss or postpone doses, and when the drug is administered in the hospital they may not show up at scheduled visits. We suspect that some of the patients in our study were also not fully adherent to the treatment regimen. The higher etanercept trough concentrations in patients with lower IPV indeed suggest that better adherence leads to higher drug exposure.

In our study it is impossible to differentiate between the clinical relevance of IPV versus the absolute etanercept concentration. Others have found a significant association between clinical response and serum etanercept concentrations in patients with rheumatoid arthritis and in patients with ankylosing spondylitis (8,10). The primary objective of our study was to evaluate IPV in patients on standard etanercept dose maintenance treatment. The correlations with clinical outcome were secondary endpoints, and with the sample size of only 29 patients these correlations should be considered as exploratory.

Biologics are known to have immunogenic potential. Development of anti-drug antibodies has been shown to affect the concentration of several biologics. These anti-drug antibodies can lead to reduced drug concentrations, to neutralization of the biologic effect and to loss of therapeutic response. Reduced drug survival as a result of development of anti-drug antibodies has been reported to be more frequent in several monoclonal anti-TNFα antibody preparations, but almost not in patients treated with etanercept. For biologics prone to an anti-drug antibody response often immunosuppressive drugs are added as co-treatment, in order to prevent or attenuate the formation of these antibodies (22). Pouw et al. demonstrated that adalimumab concentrations are influenced by concomitant methotrexate use: patients on adalimumab monotherapy had a median adalimumab concentration of 4.1 μg/mL (IQR 1.3-7.7), whereas patients concomitantly taking MTX had a median concentration of 7.4 μg/mL (IQR 5.3-10.6, P<0.001) (12). Zhou et al. showed that the pharmacokinetics of etanercept were not altered by the concurrent administration of methotrexate in patients with rheumatoid arthritis, supporting the assumption that formation of anti-drug antibodies does not play a significant role in etancercept treatment (23).

Although in our study we did not measure the presence of anti-drug antibodies we do not think they have played an important role in contributing to IPV. There were no patients in whom a gradual decline of etanercept concentrations was observed, reflecting the potential gradual development of increasing concentrations of anti-drug antibodies. The already mentioned patient (number nine) did have etanercept trough concentrations below the lower limit of detection at several time points, but in this patient at later time points, etancercept concentrations increased again.

TDM for biologics is not routinely performed. These drugs are typically administered in a standard dose, at standard time intervals, and pharmacokinetic differences between patients are not recorded. In patients with rheumatoid arthritis there is clear evidence that trough concentrations are related to clinical outcome. Also for etanercept a clear association was shown between disease activity, inflammatory markers and etanercept concentrations in patients with ankylosing spondylitis (10). In studies with etanercept for rheumatoid arthritis a therapeutic window between 2 and 7 μg/mL was proposed (4). Based on our data we can not confirm that these target concentrations would also provide the best balance between efficacy and toxicity for psoriasis.

This study supports that patients with a high BMI have a lower trough concentration. This may be caused by a higher volume of distribution or by reduced bioavailability from the subcutaneous tissue. Physicians should take the influence of BMI into account and a dose adjustment could be considered when patients with a BMI in the upper range fail on their biologic. The data of this study do not allow to define a certain BMI above which dose should be increased.

This study has some limitations. First of all the number of patients for whom four or more etanercept trough concentrations were available was limited (n=29). Secondly, in this obser-

vational study we cannot define the margins of the therapeutic window of etanercept trough concentrations. In order to decide on the lower threshold we would need more patients, and for the upper limit an even larger sample size would be required. Thirdly the concomitant use of fumarates in a proportion of the patients may have affected the concentration-effect response, although a statistically significant difference in the clinical response after 48 weeks could not be shown in the clinical trial. In other studies investigating the concentration-effect response of biologics, patients also used concomitant immunosuppressives such as methotrexate (9, 11). Fourth, the influence of adherence on IPV not can be distinguished from the variability which is due to other factors. In clinical practice non-adherence will always play a role. A strict evaluation of IPV in a setting of 100% adherence (in a highly controlled pharmacokinetic-study) may provide interesting data for an investigator's brochure of a compound. For treatment in daily practice however, and for the correlation between variability and clinical outcome, our real life data are more relevant.

Prospective clinical trials are needed to demonstrate the added value of adjusting the etanercept dose based on drug trough concentrations. The intra-patient variability observed in this study will complicate TDM, and we recommend that decisions on dose adjustment or switching to another drug should be based on more than a single measurement and after exclusion of non-adherence as a cause for low or undetectable trough concentrations. We recommend to also evaluate the IPV for other biologics for which TDM is being considered, such as adalimumab, infliximab and secukinumab in the future. In such studies sequential samples should be taken over time, ideally while the patient is on a stable dose.

REFERENCES

1. Mok CC, van der Kleij D, Wolbink GJ. Drug levels, anti-drug antibodies, and clinical efficacy of the anti-TNFα lpha biologics in rheumatic diseases. Clin Rheumatol. 2013;32(10):1429-35.
2. Vogelzang EH, Kneepkens EL, Nurmohamed MT, et al. Anti-adalimumab antibodies and adalimumab concentrations in psoriatic arthritis; an association with disease activity at 28 and 52 weeks of follow-up. Ann Rheum Dis. 2014;73(12):2178-82.
3. Jani M, Chinoy H, Warren RB, et al. Clinical utility of random anti-tumor necrosis factor drug-level testing and measurement of antidrug antibodies on the long-term treatment response in rheumatoid arthritis. Arthritis Rheumatol. 2015;67(8):2011-9.
4. Zandvliet ML, van Bezooijen JS, Bos MA, et al. Monitoring antigen-specific biologics: current knowledge and future prospects. Ther Drug Monit. 2013;35(5):588-94.
5. Leonardi CL, Powers JL, Matheson RT, et al. Etanercept as monotherapy in patients with psoriasis. N Engl J Med. 2003;349(21):2014-22.
6. van den Reek JM, van Lumig PP, Driessen RJ, et al. Determinants of drug survival for etanercept in a long-term daily practice cohort of patients with psoriasis. Br J Dermatol. 2014;170(2):415-24.
7. Colombel JF, Feagan BG, Sandborn WJ, et al. Therapeutic drug monitoring of biologics for inflammatory bowel disease. Inflamm Bowel Dis. 2012;18(2):349-58.
8. Jamnitski A, Krieckaert CL, Nurmohamed MT, et al. Patients non-responding to etanercept obtain lower etanercept concentrations compared with responding patients. Ann Rheum Dis. 2012;71(1):88-91.
9. Mahil SK, Arkir Z, Richards G, et al. Predicting treatment response in psoriasis using serum levels of adalimumab and etanercept: a single-centre, cohort study. Br J Dermatol. 2013;169(2):306-13.
10. Kneepkens EL, Krieckaert CL, van der Kleij D, et al. Lower etanercept levels are associated with high disease activity in ankylosing spondylitis patients at 24 weeks of follow-up. Ann Rheum Dis. 2015;74:1825-9.
11. Menting SP, Coussens E, Pouw MF, et al. Developing a Therapeutic Range of Adalimumab Serum Concentrations in Management of Psoriasis: A Step Toward Personalized Treatment. JAMA Dermatol. 2015;151(6):616-22.
12. Pouw MF, Krieckaert CL, Nurmohamed MT, et al. Key findings towards optimising adalimumab treatment: the concentration-effect curve. Ann Rheum Dis. 2015;74(3):513-8.
13. van Bezooijen JS, Balak DM, van Doorn MB, et al. Combination Therapy of Etanercept and Fumarates versus Etanercept Monotherapy in Psoriasis: A Randomized Exploratory Study. Dermatology. 2016;1;232(4):407-14
14. Feldman SR, Krueger GG. Psoriasis assessment tools in clinical trials. Ann Rheum Dis. 2005;64 Suppl 2:ii65-8; discussion ii9-73.
15. van Bezooijen JS, van Doorn MB, Prens EP, et al. A comparison of three assays to quantify infliximab, adalimumab and etanercept serum concentrations. Ther Drug Monit 2016;Aug; 38(4):432-8
16. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm389370.pdf.
17. Don BR, Spin G, Nestorov I, et al. The pharmacokinetics of etanercept in patients with end-stage renal disease on haemodialysis. J Pharm Pharmacol. 2005;57(11):1407-13.
18. Stuber ML, Shemesh E, Seacord D, et al. Evaluating non-adherence to immunosuppressant medications in pediatric liver transplant recipients. Pediatr Transplant. 2008;12(3):284-8.

19. Borra LC, Roodnat JI, Kal JA, et al. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. Nephrol Dial Transplant. 2010;25(8):2757-63.
20. Waiser J, Dell K, Bohler T, et al. Ciclosporin A up-regulates the expression of TGF-beta1 and its receptors type I and type II in rat mesangial cells. Nephrol Dial Transplant. 2002;17(9):1568-77.
21. van Gelder T. Within-patient variability in immunosuppressive drug exposure as a predictor for poor outcome after transplantation. Kidney Int. 2014;85(6):1267-8.
22. Garces S, Demengeot J, Benito-Garcia E. The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis. Ann Rheum Dis. 2013;72(12): 1947-55.
23. Zhou H, Mayer PR, Wajdula J, et al. Unaltered etanercept pharmacokinetics with concurrent methotrexate in patients with rheumatoid arthritis. J Clin Pharmacol. 2004;44(11):1235-43.

Chapter 8

General discussion

The main objective of this thesis was to explore strategies that would allow more individualized treatment with biologics in psoriasis patients. Unfortunately, the current literature only provides limited evidence for such treatment and therefore most patients are still treated according to the 'one dose fits all' principle. In this chapter we discuss the outcomes of our studies and indicate their potential value for a more personalized biologic therapy in psoriasis in the future.

COMBINATION THERAPY (PART I)

A growing body of evidence indicates that in a variety of immune-mediated-inflammatory diseases (IMID), combination therapy of a biologic and methotrexate (MTX) is more effective than biologic monotherapy (1-10). This is partly due to the immunosuppressive effect of MTX on the development of anti-drug antibodies (ADA). Therefore, we have reviewed the literature with respect to this strategy used in psoriasis (**Chapter 2**). We refer to eight studies, showing favourable efficacy for the combination therapy of a biologic with MTX compared to biologic monotherapy. Combination therapy appeared to be well tolerated and was not associated with higher rates of clinically relevant adverse events. However, treatment with MTX is considered less suitable in patients with a history of liver disease, which applies to patients with psoriasis where a higher prevalence of non-alcoholic fatty liver disease has been reported (11, 12).

The main limitations of the reviewed studies were the relatively low number of patients and a short follow-up time or a retrospective design. We concluded that the available evidence on combination treatment of biologics with MTX in psoriasis is presently insufficient to propose an amendment of the current treatment guidelines. A similar conclusion was reached by Busard et al. (13). We argue that an adequately powered, long-term randomized clinical trial (RCT) to investigate the pharmacokinetics, efficacy, safety and drug survival of a combination therapy of biologic with MTX versus biologic monotherapy in psoriasis patients is required to support such an amendment. Therefore, we designed a multicenter RCT (the OPTIMAP study) to investigate this strategy for adalimumab combined with MTX. The OPTIMAP study is currently ongoing and in **Chapter 3** we describe the study protocol and discuss the rationale, pitfalls, and potential limitations of this RCT.

Since not all biologics appear to induce a clinically relevant ADA response, combination therapy with MTX may be less useful for such biologics for example etanercept (14). Therefore we also explored the combination treatment of etanercept with fumarates in the FUMBREL study (**Chapter 4**). At the time the study protocol was written, etanercept was among the most frequently used biologics for the treatment of psoriasis. In clinical practice today, although many other biologic treatment options have become available, a considerable number of patients are still being treated on etanercept (or its biosimilar) monotherapy. Unfortunately, most of these patients are eventually switched to another biologic therapy within the first few years after initiation, which reflects its limited drug survival as monotherapy (15).

We chose fumarate therapy as combination therapy because of its relatively favorable long-term safety profile and low costs. Monotherapy with fumarates is widely used in psoriasis patients, however it is not licensed for the treatment of psoriasis in the Netherlands. The combination therapy has therefore probably not been investigated previously, with the exception of a few smaller studies (16-18). The FUMBREL study is important because it provides useful data on the safety and tolerability of the combination therapy of etanercept with fumarates. Since a few cases of progressive multifocal leukoencephalopathy (PML) have occurred during treatment with fumarates, safety of fumarates in combination with etanercept is of particular interest (19-21).

The FUMBREL study showed that the difference in efficacy and drug survival between the combination therapy group and etanercept monotherapy group was not statistically significant. The latter was mainly due to an unexpected high PASI-75 percentage (almost 60%) in the etanercept monotherapy group. This could be explained by a high compliance in our patient group. We specifically instructed the patients to keep a treatment diary and collect empty etanercept syringes during their monthly visit to the out-patient clinic. Such intensified patient care may have increased drug adherence in this study. Drug adherence is very important given the high and still increasing cost of biologic treatment and needs careful monitoring (22).

In our FUMBREL study, the combination therapy group included significantly more patients who had previously used one or more biologics, namely 56% against 20% in the monotherapy group. This supports our hypothesis that combination therapy of a biologic with fumarates represents a good treatment strategy for patients who previously failed on one or more biologics. Current literature lacks evidence for this hypothesis. The timing of initiation of the concomitant use of fumarates may also influence efficacy. Based on our results it is difficult to assess if concomitant use of fumarates should be started directly or as add-on therapy when the efficacy of biologic monotherapy shows signs of deterioration. If patients are already treated with biologics and have not reached sufficient improvement, we recommend starting concomitant treatment with fumarates in some cases. Even though the sample size of the FUMBREL study was too low to draw definite conclusions although we experienced in clinical practice that fumarates did appear to improve the drug survival in patients who failed on etanercept monotherapy.

Since the recent arrival of biologics such as the anti p40 drugs (targeting IL-12 and IL-23) and the IL-17 inhibitors (23-25), we are cautious to recommend the above mentioned combination therapies due to the potential side effects and toxicity of MTX and fumarates. These new treatment options for psoriasis have emerged faster than the publication of the results of the FUMBREL study. If the more recently introduced anti-IL17 biologics will lose efficacy over time, combination therapy with MTX, fumarates or other systemic treatments may be investigated. Combination therapy with MTX or fumarates might also be considered as alternative treatment options in patients with a recalcitrant form of psoriasis who have failed several biologics.

BIOLOGIC DOSING INTERVAL PROLONGATION (PART II)

A second strategy to achieve a more personalized treatment with biologics is to prolong the biologic dosing interval in psoriasis patients on maintenance treatment with a biologic and who have a stable low disease activity. We investigated this approach in the POEMA study (**Chapter 5**). We showed that prolongation of the per label biologic dosing interval was feasible in approximately 30% of patients. Patients on adalimumab therapy (per label) showed the most successful prolongation when compared to etanercept and ustekinumab. This difference might be explained by the fact that etanercept has the lowest half-life (15) and is therefore less suitable for further prolongation of the dosing interval. The per label dosing interval of ustekinumab is already much longer than those of adalimumab and etanercept, potentially explaining the increased rate of successful prolongation of adalimumab (44%) and etanercept (31%) vs. ustekinumab (22%).

Patient characteristics may influence the treatment response, especially an increased BMI has been shown to affect the treatment response (26). BMI and disease activity may also have influenced successful prolongation, however our patient number was too small to specifically assess the impact of these factors on our results. We did however notice in our study, that in the majority of psoriasis patients in full remission or in an "almost clear" state at baseline, prolongation of the dosing interval was successful. Another important factor is the influence of individual pharmacokinetics on the treatment response since the efficacy of biologics shows a considerable heterogeneity among psoriasis patients. Recently, pharmacogenetic markers of treatment response to biologics have been investigated in psoriasis patients (27, 28). Several markers are associated with a good clinical response and thus might also predict successful dosing interval prolongation. However, these pharmacogenetics markers need to be more investigated and require further validation.

Recently, in rheumatoid arthritis (RA) successful biologic dose tapering studies have been published (29, 30). An important difference with psoriasis is the fact that RA patients are treated with combination therapy of a biologic and MTX. The biologic is started when patients fail on treatment with MTX, MTX treatment is continued during biologic therapy and finally the biologic dose is tapered. The combination therapy with MTX improves the pharmacokinetics of the biologic by reducing the systemic inflammation but also by reducing the formation of ADA and has an additive or synergistic effect on disease treatment (31). This strategy could also be applicable to psoriasis patients and could potentially allow biologic dose reduction.

The results from the POEMA study demonstrated that dosing interval prolongation is safe, did not lead to an increase in immunogenicity, or clinically relevant loss of response. Even "failure" patients in whom the dosing interval was prolonged only showed a temporarily increase in disease activity upon returning to the previous dosing interval. We therefore concluded that dosing interval prolongation should be considered by dermatologists for patients with low, stable psoriasis (a PGA clear/almost clear) disease activity.

Besides a significant cost reduction this approach can also be considered more patient-friendly because patients require less injections per year with a longer dosing interval. Dosing interval prolongations may potentially also decrease side effects such as respiratory and urinary tract infections. Theoretically, the costs can be decreased even more if patients with a successful interval prolongation are also switched to a biosimilar. At the moment several biosimilars for infliximab (Inflectra, Flixabi) and etanercept (Benepali) are already available and for adalimumab, several biosimilars are in their final stages of development.

A growing body of evidence demonstrates the benefits of therapeutic drug monitoring (TDM) of biologics. Chen et al. showed that trough concentrations were predictive for successful adalimumab dose reduction in RA patients (32). Furthermore, Mahil et al. showed that early adalimumab concentration monitoring at 4 weeks might be useful in predicting treatment response in psoriasis (33). Therefore we also investigated if the biologic baseline trough concentration has predictive value for successful dosing interval prolongation. In the POEMA study, higher adalimumab baseline trough concentrations were associated with successful prolongation, although the correlation was weak and not statistically significant. This is in contrast with other studies, that suggested a therapeutic range, a concentration-effect relationship of adalimumab treatment, and prognostic significance for trough concentrations in predicting the success of adalimumab dose reductions in psoriasis and RA (32, 34, 35). The result of the POEMA study for the adalimumab trough concentrations may differ because we included a relatively small number of patients.

TDM can also provide more information with regard to drug adherence, since poor adherence causes increased healthcare costs and reduced productivity (36). The results of the POEMA study demonstrate that a considerable number of patients had visits with undetectable biologic trough concentrations. In only one of these patients ADA were detected as probable cause of the undetectable trough concentration. The other patients might not have been fully drug adherent.

TDM should be considered in clinical practice during long-term use of biologics because it might be used to monitor drug adherence and can provide an explanation for the loss of clinical response. We believe this information can be a valuable tool in the communication with patients and can aid in the explanation as to why the current treatment is failing or in which case should be switched to a specific other drug.

CONDITIONS FOR THERAPEUTIC DRUG MONITORING (TDM) OF BIOLOGICS IN PSORIASIS (PART III)

TDM is used for several drugs, including tacrolimus, voriconazol and many others (37-39). The goal of TDM is to measure exposure to the drug (mostly by measurement of trough concentrations) at predefined intervals and to adjust the dose in order to reach target concen-

trations. With this strategy the drug concentrations are kept within the therapeutic window as much as possible, and the best clinical outcome can be achieved. TDM is especially used for drugs with a narrow therapeutic window and with a marked pharmacokinetic variability. For the interpretation of drug concentrations, several factors need to be considered including the time of sampling in relation to the dose, dosage history, half-life of the drug and desired target concentration (40).

A reliable and widely available assay is an important condition for widespread implementation of TDM. **Chapter 6** demonstrated that all three commercially available assays evaluated for TDM of the anti-TNFα biologics that are used for the treatment of psoriasis are suitable. The most important message of this study is that given the observed differences in drug recovery, therapeutic windows require assay-specific definition and use of the same assay in individual patients is warranted. Harmonization of assays is essential to allow comparison of data on biologic trough concentrations. Several studies have measured trough concentrations for adalimumab and infliximab and defined a therapeutic trough concentration range (34, 35, 41). However, these therapeutic ranges should be interpreted with caution and can only be compared with each other when the same assay was used.

Recently, Chen et al. used two different ELISA methods (Progenika and Sanquin) for detecting adalimumab and etanercept trough concentrations (42). They showed a significant correlation between both ELISA methods for adalimumab ($r=0.875$) and etanercept ($r=0.703$, $p<0.001$), but did not mention the degree of agreement. A correlation alone is not sufficient, but a Bland-Altman analysis as preformed in **Chapter 6** should have been included for estimation of the agreement. The difference in concentrations presented in the study of Chen et al. could be the result of inter-assay variation or inter-patient variability, however this remains unclear now.

At the moment measurement of trough concentrations is relatively expensive and the important question is whether TDM of biologics is cost effective. Since different assays are emerging on the market for measuring trough concentrations prices might drop substantially and cost effectiveness might be demonstrated more easily. Still, it will be difficult to motivate physicians in clinical practice to measure trough concentrations before switching to another biologic.

Measuring ADA appears to be less important than trough concentrations and therefore this aspect of treatment with biologics was not investigated in this thesis. We argue that detection of ADA is only indicated when trough concentrations are very low or undetectable in patients with a rebound of high disease activity (43). The technique to detect ADA is more complicated than measuring a trough concentration. A standard ELISA to measure ADA is less suitable due to immune complex formation of the circulating drug and ADA. Consequently bridging ELISA, radioimmunoassay and bioassay are necessary. We believe that in case of secondary loss of response (especially when treating with immunogenic drugs such as infliximab or adalimumab), measurement of trough concentrations and ADA (when indicated) can be helpful.

Currently most patients showing loss of response are switched from one biologic to another without investigating the cause of loss of efficacy.

Feldman et al. showed that one-third of psoriasis patients experienced a dose escalation of their biologic during the first 6 months. Half of these patients had a discontinuation or a dose reduction over the 12-month post-titration period (44). Patients who discontinued with their biologic had a useless dose escalation which results in an increase of the costs. These useless dose escalations can be prevented by measuring trough concentrations and ADA. If loss of response is caused by the development of ADA, the physician should not escalate the dose but start with another biologic that has less risk of ADA development such as etanercept or ustekinumab (14). If the loss of response is related to non-adherence this can more readily be discussed with the patient and may also prevent unnecessary dose or interval adjustments.

In order for TDM of a biologic to be feasible, the intra-patient variability (IPV) of the biologic concentration should be limited. When fluctuations of a biologic concentration within one patient on a stable dose are substantial, TDM will often show concentrations outside the therapeutic window. In this thesis we report the analysis of etanercept trough concentrations of the patients in our FUMBREL study to define the IPV of etanercept. **Chapter 7** showed a median IPV of 33.7% for etanercept in the patients participating in the FUMBREL study. The IPV did not depend on the co-administration of fumarates. Our study indicated that the IPV was relatively high, potentially complicating TDM. Decisions on dose adjustment or switching to another biologic should therefore be based on more than a single measurement after exclusion of non-adherence.

In our study the adherence to the treatment regimen may have influenced the IPV substantially. A more exact determination of the IPV would only be possible when the drug is administered under supervised conditions. For example, most hospitals in the Netherlands combine subcutaneous ustekinumab injections with a regular outpatient control visit because of the length of the dose interval. In that setting IPV due to patient related factors might be substantially lower. We have learned from **Chapter 7** that IPV may be influenced by drug adherence issues. In non-responders the IPV was higher compared to the responders, and these high fluctuations are mainly caused by a number of undetectable trough concentrations in non-responders.

Realizing that the IPV of etanercept trough concentrations will complicate TDM, assessment of IPV's of other biologics may also be of interest. For example, the IPV of adalimumab is unknown and investigated However, Pouw et al. and Menting et al. did suggest a therapeutic range for adalimumab for the indications RA and psoriasis, based on cross-sectional measurement (34, 35). We believe that an evaluation of the Etanercept IPV and IPV of other biologic drugs is important to better evaluate the feasibility of TDM in psoriasis.

FUTURE PROSPECTS

In the future a more personalized approach to the treatment with biologics might be facilitated by a clear treatment algorithm that can be used in daily clinical practice. The flow chart presented in figure 1 shows a treatment algorithm that is based on the current literature with regard to the treatment with biologics in different IMID's as well as the results derived from the studies described in this thesis. This algorithm may aid the clinician in choosing and managing biologic therapies after conventional (systemic) treatment have failed.

The first step in the algorithm is choosing the first treatment based on predictive biomarkers for treatment response. This can be single nucleotide polymorphisms (SNP's) (27, 28), clinical characteristics or other measurements such as cytokine profiles. Unfortunately, the currently available biomarkers are not validated for use in clinical practice. After initiation of the biologic, depending on the clinical response TDM can be used to guide the decision for the consecutive treatment steps.

For example a patient that is selected based upon the predictive biomarkers to be a suitable candidate for anti-TNF treatment may be started on infliximab [1]. If the patient has a good initial response but fails the treatment after some months, the algorithm shown in Figure 1 advises to measure a drug trough concentration [2]. If this concentration is not detectable

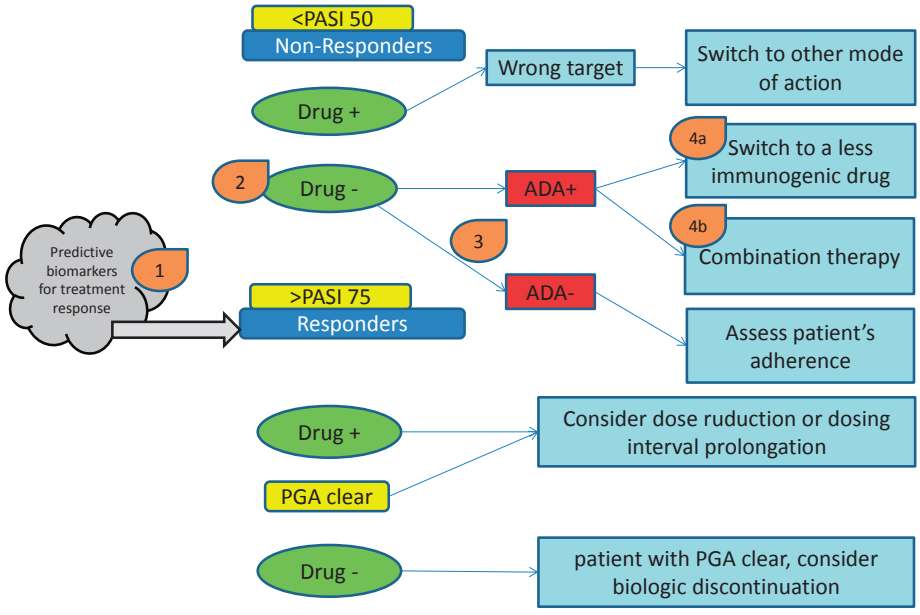

Figure 1. A proposed treatment algorithm for patients that are candidates for biologic treatment modified from Garces et al. Drug + means an adequate trough concentration and drug – means an undetectable trough concentration.

a second step will be to determine ADA [3]. If these are present, indicating failure of the drug due to immunogenicity, the patient should be switched to a less immunogenic drug [4a]. Alternatively, depending on the results of our ongoing RCT investigating the effects of adalimumab and co-treatment with MTX, the patient could be switched to this combination therapy, which would reduce the production of ADA and prolong the drug survival [4b].

Furthermore, for patients treated with the per label biologic dose with stable low disease activity and detectable trough concentrations tapering or prolongation of the dosing interval could be considered. However, in our POEMA study, the data appeared to indicate that the disease activity at baseline is a better predictor for successful dosing interval prolongation than the biologic trough concentration in psoriasis.

This proposed treatment algorithm could be further enhanced with an advice on how often measurement of trough concentrations (TDM) is needed in case of detectable (drug +) or undetectable (drug -) concentrations. Also, the data on IPV of more biologics should be evaluated before an advice on the frequency of TDM can be given.

Other authors have suggested to also incorporate a therapeutic range in clinical practice or to even guide decisions on dose reductions (32, 34, 35). However, we believe the data supporting this more sophisticated TDM assisted approach to drug tapering is currently insufficient for application in clinical practice. Importantly, in our study investigating the IPV of etanercept it is demonstrated that the IPV was higher than expected and may complicate TDM.

Ongoing research will be crucial to validate the recommendations depicted in the proposed treatment algorithm above, and to truly reach individualized treatment with biologics in psoriasis. Moreover, this treatment strategy should preferably be investigated in a prospective manner with cost-effectiveness as main outcome, as has been performed in RA (45).

We hope that the explorative studies in this thesis will be a good starting point for the development of more extensive studies on this topic.

REFERENCES

1. Emery P, Breedveld FC, Hall S, Durez P, Chang DJ, Robertson D, et al. Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis (COMET): a randomised, double-blind, parallel treatment trial. Lancet. 2008;372(9636):375-82.
2. Klareskog L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet. 2004;363(9410):675-81.
3. van der Heijde D, Klareskog L, Rodriguez-Valverde V, Codreanu C, Bolosiu H, Melo-Gomes J, et al. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. Arthritis Rheum. 2006;54(4):1063-74.
4. van Riel PL, Taggart AJ, Sany J, Gaubitz M, Nab HW, Pedersen R, et al. Efficacy and safety of combination etanercept and methotrexate versus etanercept alone in patients with rheumatoid arthritis with an inadequate response to methotrexate: the ADORE study. Ann Rheum Dis. 2006;65(11):1478-83.
5. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med. 1999;340(4):253-9.
6. Weisman MH, Moreland LW, Furst DE, Weinblatt ME, Keystone EC, Paulus HE, et al. Efficacy, pharmacokinetic, and safety assessment of adalimumab, a fully human anti-tumor necrosis factor-alpha monoclonal antibody, in adults with rheumatoid arthritis receiving concomitant methotrexate: a pilot study. Clin Ther. 2003;25(6):1700-21.
7. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum. 2003;48(1):35-45.
8. Bang LM, Keating GM. Adalimumab: a review of its use in rheumatoid arthritis. BioDrugs. 2004;18(2):121-39.
9. Keystone EC, van der Heijde D, Kavanaugh A, Kupper H, Liu S, Guerette B, et al. Clinical, Functional, and Radiographic Benefits of Longterm Adalimumab Plus Methotrexate: Final 10-year Data in Longstanding Rheumatoid Arthritis. J Rheumatol. 2013; 40(9)1487-1497.
10. St Clair EW, van der Heijde DM, Smolen JS, Maini RN, Bathon JM, Emery P, et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum. 2004;50(11): 3432-43.
11. Gisondi P, Targher G, Zoppini G, Girolomoni G. Non-alcoholic fatty liver disease in patients with chronic plaque psoriasis. J Hepatol. 2009;51(4):758-64.
12. van der Voort EA, Koehler EM, Dowlatshahi EA, Hofman A, Stricker BH, Janssen HL, et al. Psoriasis is independently associated with nonalcoholic fatty liver disease in patients 55 years old or older: Results from a population-based study. J Am Acad Dermatol. 2014;70(3):517-24.
13. Busard C, Zweegers J, Limpens J, Langendam M, Spuls PI. Combined use of systemic agents for psoriasis: a systematic review. JAMA Dermatol. 2014;150(11):1213-20.
14. Hsu L, Snodgrass BT, Armstrong AW. Antidrug antibodies in psoriasis: a systematic review. Br J Dermatol. 2014;170(2):261-73.
15. Gniadecki R, Bang B, Bryld LE, Iversen L, Lasthein S, Skov L. Comparison of long-term drug survival and safety of biologic agents in patients with psoriasis vulgaris. Br J Dermatol. 2015;172(1):244-52.

16. Wilsmann-Theis D, Frambach Y, Philipp S, Weyergraf AJ, Jacobi A, Mössner R, et al. Systemic Antipsoriatic Combination Therapy with Fumaric Acid Esters for Plaque-Type Psoriasis: Report on 17 Cases. Dermatology. 2015;230(2):119-27.
17. Balasubramaniam P, Stevenson O, Berth-Jones J. Fumaric acid esters in severe psoriasis, including experience of use in combination with other systemic modalities. British Journal of Dermatology. 2004;150(4):741-6.
18. Balak DM, Fallah Arani S, Hajdarbegovic E, Hagemans CA, Bramer WM, Thio HB, et al. Efficacy, effectiveness and safety of fumaric acid esters in the treatment of psoriasis: a systematic review of randomized and observational studies. Br J Dermatol. 2016;175(2):250-62.
19. van Oosten BW, Killestein J, Wattjes MP. Case reports of PML in patients treated for psoriasis. N Engl J Med. 2013;369(11):1081-2.
20. Balak D, Hajdarbegovic E. PML in Patients Treated with Dimethyl Fumarate. N Engl J Med. 2015;373(6):582-3.
21. Bartsch T, Rempe T, Wrede A, Leypoldt F, Bruck W, Adams O, et al. Progressive neurologic dysfunction in a psoriasis patient treated with dimethyl fumarate. Ann Neurol. 2015;78(4):501-14.
22. Cheng J, Feldman SR. The cost of biologics for psoriasis is increasing. Drugs in Context. 2014;3:212266.
23. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, et al. Secukinumab in plaque psoriasis--results of two phase 3 trials. N Engl J Med. 2014;371(4):326-38.
24. Gottlieb AB, Lacour JP, Korman N, Wilhelm S, Dutronc Y, Schacht A, et al. Treatment outcomes with ixekizumab in patients with moderate-to-severe psoriasis who have or have not received prior biological therapies: an integrated analysis of two Phase III randomized studies. J Eur Acad Dermatol Venereol. 2016(ISSN:1468-3083).
25. Menter A, Papp KA, Gooderham M, Pariser DM, Augustin M, Kerdel FA, et al. Drug survival of biologic therapy in a large, disease-based registry of patients with psoriasis: results from the Psoriasis Longitudinal Assessment and Registry (PSOLAR). Journal of the European Academy of Dermatology and Venereology. 2016;30(7):1148-58.
26. Edson-Heredia E, Sterling KL, Alatorre CI, Cuyun Carter G, Paczkowski R, Zarotsky V, et al. Heterogeneity of Response to Biologic Treatment: Perspective for Psoriasis. Journal of Investigative Dermatology.134(1):18-23.
27. Onderdijk AJ, Ijpma AS, Menting SP, Baerveldt EM, Prens EP. Potential serum biomarkers of treatment response to ustekinumab in patients with psoriasis: a pilot study. Br J Dermatol. 2015;173(6):1536-9.
28. van den Reek JM, Coenen MJ, van de L'Isle Arias M, Zweegers J, Rodijk-Olthuis D, Schalkwijk J, et al. Polymorphisms in CD84, IL12B and TNFAIP3 are associated with response to biologics in patients with psoriasis. Br J Dermatol. 2016.
29. Kuijper TM, Luime JJ, de Jong PH, Gerards AH, van Zeben D, Tchetverikov I, et al. Tapering conventional synthetic DMARDs in patients with early arthritis in sustained remission: 2-year follow-up of the tREACH trial. Ann Rheum Dis. 2016;75(12):2119-23.
30. Kuijper TM, Lamers-Karnebeek FB, Jacobs JW, Hazes JM, Luime JJ. Flare Rate in Patients with Rheumatoid Arthritis in Low Disease Activity or Remission When Tapering or Stopping Synthetic or Biologic DMARD: A Systematic Review. J Rheumatol. 2015;42(11):2012-22.
31. Krieckaert CL, Nurmohamed MT, Wolbink GJ. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. Ann Rheum Dis. 2012;71(11):1914-5.
32. Chen DY, Chen YM, Hsieh TY, Hung WT, Hsieh CW, Chen HH, et al. Drug trough levels predict therapeutic responses to dose reduction of adalimumab for rheumatoid arthritis patients during 24 weeks of follow-up. Rheumatology (Oxford). 2016;55(1):143-8.

33. Mahil SK, Arkir Z, Richards G, Lewis CM, Barker JN, Smith CH. Predicting treatment response in psoriasis using serum levels of adalimumab and etanercept: a single-centre, cohort study. Br J Dermatol. 2013;169(2):306-13.
34. Menting SP, Coussens E, Pouw MF, van den Reek JM, Temmerman L, Boonen H, et al. Developing a Therapeutic Range of Adalimumab Serum Concentrations in Management of Psoriasis: A Step Toward Personalized Treatment. JAMA Dermatol. 2015;151(6):616-22.
35. Pouw MF, Krieckaert CL, Nurmohamed MT, van der Kleij D, Aarden L, Rispens T, et al. Key findings towards optimising adalimumab treatment: the concentration-effect curve. Ann Rheum Dis. 2015;74(3):513-8.
36. Goldberg EL, Dekoven M, Schabert VF, Coyle A. Patient Medication Adherence: The Forgotten Aspect of Biologics. Biotechnology healthcare. 2009;6(2):39-44.
37. Borra LC, Roodnat JI, Kal JA, Mathot RA, Weimar W, van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. Nephrol Dial Transplant. 2010;25(8):2757-63.
38. van Gelder T. Within-patient variability in immunosuppressive drug exposure as a predictor for poor outcome after transplantation. Kidney Int. 2014;85(6):1267-8.
39. Ferrier KR, van Elk CE, Bunskoek PE, van den Broek MP. Dosing and therapeutic drug monitoring of voriconazole in bottlenose dolphins (Tursiops truncatus). Med Mycol. 2016.
40. Kang J-S, Lee M-H. Overview of Therapeutic Drug Monitoring. The Korean Journal of Internal Medicine. 2009;24(1):1-10.
41. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015;148(7):1320-9.
42. Chen DY, Chen YM, Tsai WC, Tseng JC, Chen YH, Hsieh CW, et al. Significant associations of antidrug antibody levels with serum drug trough levels and therapeutic response of adalimumab and etanercept treatment in rheumatoid arthritis. Ann Rheum Dis. 2015;74(3).
43. http://www.sanquin.nl/en/products-services/biologicals/available-assays/.
44. Feldman SR, Zhao Y, Navaratnam P, Friedman HS, Lu J, Tran MH. Patterns of medication utilization and costs associated with the use of etanercept, adalimumab, and ustekinumab in the management of moderate-to-severe psoriasis. J Manag Care Spec Pharm. 2015;21(3):201-9.
45. Krieckaert CL, Nair SC, Nurmohamed MT, Van Dongen CJ, Lems WF, Lafeber FP, Bijlsma JW, Koffijberg H, Wolbink GJ, Welsing PM. Personalised treatment using serum drug levels of adalimumab in patients with rheumatoid arthritis: an evaluation of costs and effects. Ann Rheum Dis. 2015;74(2):361-8.

Chapter 9

Summary / Samenvatting

SUMMARY

Chapter 1 is an introductory chapter in which the outline of the thesis and an introduction to the subjects described in this thesis is given. Psoriasis is a chronic immune-mediated skin disease with a prevalence of 2 to 3% in the Caucasian population. Since there is a better understanding of the pathogenesis of psoriasis, specific targeted therapies have been developed. Tumor necrosis factor alpha (TNFα) is an important cytokine in the inflammatory pathways involved in the pathogenesis of psoriasis. Therefore the first generation of biologics targeted this cytokine: the TNFα –inhibitors (adalimumab, infliximab and etanercept). Recently, other cytokines such as interleukine (IL)-17, IL-12 and IL-23 were found to also play an important role in the development of psoriasis and were chosen as targets for therapy. However, biologics have dramatically changed the management and outlook of patients with psoriasis. The biologics have the risk of being recognized as foreign by the host immune system. If this is the case, anti-drug antibodies (ADA) will be formed against a biologic. The presence of neutralizing ADA results in a reduced efficacy of a biologic because these ADA have an accelerated clearance. Development of ADA can be reduced by intermittent or continuous use of immunosuppressant drugs as co-medication, such as methotrexate (MTX), prednisone or azathioprine (AZA). However, published evidence showing that this combination therapy is of added value is scarce for psoriasis compared to rheumatoid arthritis.

There are some difficulties with the treatment of biologics in psoriasis. One problem is that biologic monotherapy appears to lose efficacy over time which results in reduced drug survival. Drug survival refers to the period patients use a drug. This is caused partly because of the formation of ADA which reduce the efficacy and some patients thus become refractory to a biologic treatment. Another problem is that a proportion of patients with stable low disease activity on long-term maintenance treatment with biologics may be overtreated. We believe that a more individualized treatment of biologics is necessary to overcome these problems. In addition, monitoring of biologics trough concentrations (therapeutic drug monitoring, TDM) could be an useful tool to optimize individualized treatment of biologics. A trough concentration is measured just prior to the next administration of the biologic. In this thesis, we have explored three strategies towards individualized treatment of biologics in psoriasis.

- **Combination therapy (part I)**
- **Biologic dosing interval prolongation (part II)**
- **Conditions for therapeutic drug monitoring (TDM) (part III)**

Part I: Combination therapy

In **Chapter 2** we have searched in the current literature for evidence on the efficacy and safety of combined therapy of biologics and MTX in psoriasis. Eight studies were found which demonstrated in general favourable results with regards to efficacy for the combination therapy

of a biologic and MTX compared to biologic monotherapy. The selected studies showed that combination therapy was well tolerated and did not appear to be associated with higher rates of clinically relevant adverse events. However, most of these studies had limitations such as short follow-up time and a retrospective design. There was also no consensus with respect to the most effective and safe dose of MTX for combination treatment. We concluded that the available evidence on combination treatment of biologics and MTX in psoriasis is not sufficient to propose an amendment of the current treatment guidelines. However, our findings do support the initiation of adequately powered randomized controlled trials (RCTs) to compare combination therapy of a biologic with MTX versus biologic monotherapy in psoriasis.

In **Chapter 3** we describe the design of a prospective RCT (OPTIMAP study), which is still ongoing at the moment and is a cooperation of three hospitals (Erasmus Medical Centre in Rotterdam, Academic Medical Centre in Amsterdam and Radboud University Medical Centre in Nijmegen) . The OPTIMAP study is the first investigator-initiated, multi-center RCT designed to compare combination treatment of adalimumab and MTX with adalimumab monotherapy in patients with psoriasis. The primary aim is to assess adalimumab drug survival at week 49 for both groups. Other aims are to collect long-term data on the efficacy and safety of adalimumab combined with MTX compared to adalimumab monotherapy; to assess the impact of concomitant MTX on adalimumab immunogenicity and serum concentrations; to test appropriate candidate genes and correlate genotypes with trial outcomes. With the OPTIMAP study we aim to improve the body of evidence for combination treatment in psoriasis and to optimize currently available biologic treatment strategies.

In **Chapter 4** we describe the results of the FUMBREL study. In this study we prospectively compared the clinical efficacy, safety and tolerability of etanercept and oral fumarates combination therapy with etanercept monotherapy per label up to 48 weeks of treatment. In total 33 patients were enrolled: 18 patients were randomized to combination therapy with etanercept and fumarates and 15 patients to etanercept monotherapy. Our results indicated that the combination treatment led to a numerically higher efficacy compared to etanercept monotherapy (78% vs 57% Psoriasis Activity Severity Index (PASI) 75) at week 24, P=0.27. The longitudinal analysis showed a PASI reduction of 6% per week for the combination therapy group and 4.8% for the monotherapy group (P=0.11). When using the Physician Global Assessment (PGA) score in this explorative study, the combination therapy showed a trend towards a faster rate of improvement in the first 24 weeks. However, none of these differences were statistically significant. We concluded that combination therapy of etanercept and fumarates appears to be relatively safe and has an acceptable tolerability up to 48 weeks of treatment. Larger studies are needed to assess the true added value of fumarates as additional treatment in patients receiving a biologic.

Part II: Biologic dosing interval prolongation

In **Chapter 5** we describe the results of the POEMA study in which we included 59 psoriasis patients on maintenance treatment with adalimumab (n=20), etanercept (n=21) and ustekinumab (n=18) therapy. We assessed the proportion of patients that could maintain a successful prolongation of the per label dosing interval and also explored dosing interval prolongations in off label treated patients as secondary aim. In addition, we evaluated the predictive value of baseline trough concentrations for successful dosing interval prolongation. In the per label group, seven out of 16 (44%) adalimumab patients, five out of 16 (31%) etanercept patients, and two out of ten (20%) ustekinumab patients achieved a successful dosing interval prolongation. In the off label group, prolongation in patients with already extended intervals was unsuccessful. For patients with shortened intervals, minor prolongation was successful in three out of 17 (17.6%) patients. Baseline trough concentrations were not predictive for a successful dosing interval prolongation. In four patients, the PASI score increased above 8. All of these four patients reached remission (clear/almost clear) after they were retreated according to their original dosing regimen. The most frequent minor adverse events were respiratory and urinary tract infections. Six severe adverse events (SAE) occurred during the study all patients recovered without sequelae.

In conclusion, prolongation of the per label biologic dosing interval was feasible in approximately 30% of psoriasis patients with stable minimal disease activity and can reduce costs in clinical practice. In this limited cohort, biologic baseline trough concentrations were not predictive for successful dosing interval prolongation.

Part III: Conditions for therapeutic drug monitoring (TDM)

In **Chapter 6** we compared the diagnostic performance of three commercially available assays designed for TDM of TNFα-inhibitors; two enzyme-linked immunosorbent assays (ELISAs) from Sanquin and Theradiag and one reporter gene-based bioassay (iLite) from Eurodiagnostica. Blood samples for routine diagnostic use were collected from infliximab-, adalimumab- and etanercept-treated patients, n=40 each. Control samples from ustekinumab-treated patients and healthy donors, n=10 each, were also obtained. Sanquin, Theradiag and iLite assays concordantly (100%) detected the compounds infliximab, adalimumab and etanercept in the relevant patient groups. Given the fundamental assay difference in anti-TNFα biologic detection of the different assays, only the Sanquin ELISAs showed specific detection of individual anti-TNFα biologics by employing anti-idiotype specific detection. In contrast, the Theradiag assay cross-reacted between different anti-TNFα biologics by detection of anti-human IgG Fc, present in all anti-TNFα biologics used. The iLite assay is based on interference with TNFα binding to its receptor, resulting in similar cross-reactivity between individual anti-TNFα biologics.

Ustekinumab, the compound representing an anti-IL12 and IL-23 biologic, was not detected in any of the assays. Sanquin, Theradiag and iLite showed linear quantitative correlation in all

respective biologic concentration assays. However, there were statistically significant quantitative differences between the individual assays in detected concentrations. In conclusion, our study results indicate that all three commercially available assays are suitable for TDM of anti-TNFα biologics when the specific characteristics and performance of the different assays are well recognized. Given the observed differences in drug recovery, therapeutic windows require assay-specific definition and use of the same assay in individual patients for longitudinal TDM is warranted.

In **Chapter 7** we report on a study during which we have collected repetitive serum samples of 33 psoriasis patients in the FUMBREL study, while on stable dose etanercept maintenance treatment. In these samples, etanercept trough concentrations were determined and the intra-patient variability (IPV) was assessed in relation to response to treatment. In this longitudinal study we show that the median IPV of etanercept trough concentrations is 33.7% (Q1 21.3 % and Q3 51.7 %) ranging from 8% to 155%. The six non-responders showed a higher IPV when compared to the 23 responders (53.9% vs 24.2 %; P= 0.031). The mean etanercept trough concentration for each patient ranged from 0.7 to 6.8 μg/ml, with a median trough concentration of 2.7 μg/ml. Patients with an IPV above the median had lower mean etanercept trough concentrations compared to patients with an IPV below the median (1.96 μg/mL, 95% confidence interval (CI) [1.7 ; 2.4] vs 3.2 μg/mL, 95% CI [2.7 ; 4.0]; P = 0.001).

The IPV observed in this study will complicate TDM, and we recommend that decisions on dose adjustment or switching to another drug should be based on more than a single measurement. First non-adherence should be excluded as a cause for low or undetectable trough concentrations. We recommend to also evaluate the IPV for other biologics for which TDM is being considered, such as adalimumab, infliximab,ustekinumab and secukinumab in the future.

In **Chapter 8** the results of all studies in this thesis are discussed in light of the literature and recommendations for future research are given. We believe that individualized treatment of biologics in psoriasis will lead to more effective and more cost-effective treatment than the currently applied per label treatment following the “one dose fits all” principle. An individualized treatment hopefully will result in an extended drug survival and an increase of the quality of life and treatment satisfaction. TDM of biologics can be used as a tool for individualized treatment of biologics and increase drug adherence. However first, evaluation of conditions for TDM is warranted before implementation of TDM in psoriasis.

Current literature shows that the evidence is scarce for individualized treatment strategies of biologics in psoriasis, and we hope that this thesis will be a good start. Our final conclusions are as follows:

Combination therapies (part I):

- Combination therapy of biologics with MTX may increase the drug survival by reducing the formation of ADA. Therefore combination therapy of adalimumab with MTX is currently being investigated in a RCT.
- Combination therapy of etanercept with fumarates results in a higher efficacy in psoriasis patients, but may be inferior to the novel IL-12 and IL-23 and IL-17 inhibitors.

Biologic dosing interval prolongations (part II):

- In patients with sustained remission biologic dosing interval prolongations should be considered, as cost reductions are substantial.

Conditions for therapeutic drug monitoring (TDM) (part III)

- Harmonization of assays for measurement of biologics is warranted to compare data on biologic trough concentrations.
- The intra-patient variability of etanercept concentrations complicates TDM and should also be investigated for other biologics.

The explored strategies in this thesis are just the first steps towards individualized treatment. More studies are needed for implementation of individualized treatment of biologics in psoriasis.

NEDERLANDSE SAMENVATTING

In **Hoofdstuk 1** worden de onderwerpen die in dit proefschrift worden besproken ingeleid. Psoriasis is een chronische immunologische huidziekte met een prevalentie van 2-3% in de Westerse populatie. Doordat meer inzichten zijn in het ontstaan van psoriasis, zijn er specifieke doel gerichte therapieën ontwikkeld. Tumor necrose factor alfa (TNFα) is een belangrijk cytokine (chemische boodschapper binnen het immuunsysteem), dat betrokken is bij het ontstekingsproces dat zich afspeelt bij actieve psoriasis. Daarom was de eerste generatie biologische geneesmiddelen (hierna: biologics) gericht tegen dit cytokine: de TNFα –remmers (adalimumab, infliximab en etanercept). Onlangs zijn er nog andere cytokinen zoals interleukine (IL)-17, IL-12 en IL-23 ontdekt die een belangrijke rol spelen in het ontstaan van psoriasis. Als gevolg hiervan zijn er nu ook biologics die specifiek gericht zijn tegen deze cytokinen: de IL-12 en IL-23 remmers (ustekinumab) en IL-17 remmers (secukinumab en ixekizumab). De biologics hebben de prognose van psoriasis patiënten aanzienlijk verbeterd. Biologics hebben het risico als " lichaamsvreemd eiwit" te worden herkend door het immuunsysteem. Daardoor kunnen er anti-drug antilichamen (ADA) tegen een biologic ontstaan. Door de neutraliserende werking van de ADA resulteert dit in een verminderde werkzaamheid van een biologic vanwege een versnelde klaring. Ontwikkeling van ADA kan worden verminderd door af en toe of continue immunosuppressieve co-medicatie te geven, zoals methotrexaat (MTX), predinison of azathioprine. In de literatuur is het bewijs voor de werkzaamheid hiervan bij psoriasis echter schaars, in vergelijking met reumatoïde artritis.

Er zijn naast de gunstige effecten ook problemen met de behandeling van biologics van psoriasis. Een probleem is dat een biologic na verloop van tijd zijn werkzaamheid verliest, hetgeen resulteert in een verminderde 'drug survival' van een biologic. (drug survival is de tijdsperiode waarin patiënten een geneesmiddel gebruiken). Dit wordt mede veroorzaakt door de vorming van ADA, waardoor de werkzaamheid van de biologic wordt verminderd. Een andere oorzaak voor verlies van effectiviteit is dat sommige patiënten ongevoelig worden voor een behandeling met een biologic zonder aantoonbare oorzaak.

" Overbehandeling " van biologics is een ander probleem dat zich voordoet. Patiënten die al jarenlang een stabiele lage ziekteactiviteit hebben worden veelal nog steeds met dezelfde dosering behandeld als bij aanvang van de behandeling. Bij deze patiënten kan onderzocht worden of de dosering van een biologic kan worden verlaagd. Een individuele behandeling op maat voor de biologics bij psoriasis patiënten kan de veiligheid van de behandeling ten goede komen en de kosten reduceren. Bovendien zou het monitoren van biologic dalconcentraties in het bloed, (ook wel genoemd therapeutic drug monitoring, TDM) een nuttig instrument kunnen zijn voor het optimaliseren van geïndividualiseerde behandeling met biologics. Een dalconcentratie is het moment waarop de concentratie van de biologic in het bloed wordt gemeten net voor de volgende toediening van een biologic. In dit proefschrift hebben we drie

strategieën onderzocht die een geïndividualiseerde behandeling met biologics voor psoriasis mogelijk zouden kunnen maken:

- **Combinatie therapie (deel I)**
- **Doseerinterval verlenging van een biologic (deel II)**
- **Voorwaarden om concentraties van een biologic te monitoren (TDM) (deel III)**

Deel I: Combinatie therapie

In **Hoofdstuk 2** worden de uitkomsten van een literatuurstudie beschreven over werkzaamheid en veiligheid van de combinatie therapie van een biologic met MTX voor psoriasis. In totaal werden er acht geschikte artikelen geselecteerd, die laten zien dat combinatietherapie van een biologic met MTX in vergelijking met biologic monotherapie mogelijk een gunstiger werkzaamheid heeft. Tevens werd deze combinatie therapie goed verdragen en leek niet geassocieerd te zijn met meer klinisch relevante bijwerkingen. De meeste van deze studies hadden echter beperkingen zoals een korte follow-up tijd en/of een retrospectieve opzet. Er is bovendien geen consensus over de meest effectieve en veilige dosering van MTX voor de combinatie behandeling met een biologic. Op dit moment is er onvoldoende bewijs in de literatuur voor combinatie behandeling met een biologic en MTX in psoriasis. Huidige behandelingsrichtlijnen voor psoriasis kunnen nog niet worden aangepast, ook al laten onze bevindingen zien dat deze combinatie therapie wel een gunstig effect zal hebben. We pleiten daarom voor een adequate grote gerandomiseerde klinische studie die vergelijkt of een combinatie therapie met een biologic en MTX beter is dan biologic monotherapie.

In **Hoofdstuk 3** beschrijven we het protocol van een gerandomiseerde klinische studie (OPTIMAP studie), die wij in het vorige hoofdstuk nodig achtten. De OPTIMAP studie is momenteel nog steeds gaande en patiënten worden geïncludeerd. Het onderzoek is geïnitieerd door de samenwerking van verschillende medische centra (Erasmus MC in Rotterdam, Academisch Medisch Centrum in Amsterdam en Radboud Universitair Medisch Centrum in Nijmegen). De OPTIMAP studie vergelijkt of combinatie behandeling met adalimumab en MTX beter is dan adalimumab monotherapie bij patiënten met psoriasis. Het primaire doel is om de 'drug survival' van adalimumab te evalueren na 49 weken in beide groepen. Andere doelen zijn om data van meerdere jaren te verzamelen over de werkzaamheid en veiligheid van de combinatie behandeling met adalimumab en MTX in vergelijking met adalimumab monotherapie; om te evalueren of MTX de vorming van ADA tegen adalimumab vermindert (omdat dat mogelijk ook invloed heeft op de dalconcentraties van adalimumab in het bloed); en om te onderzoeken of een geselecteerde groep kandidaatgenen correleert met de uitkomsten van de studie. Het doel van de OPTIMAP studie is om voldoende bewijs te verzamelen voor combinatie therapie met adalimumab en MTX voor psoriasis. Deze combinatie therapie kan mogelijk als strategie in de toekomst gebruikt worden om de behandeling met biologics te optimaliseren.

In **Hoofdstuk 4** beschrijven we de resultaten van de FUMBREL studie. In deze studie is prospectief de klinische werkzaamheid, veiligheid en verdraagbaarheid van combinatie therapie met etanercept en orale fumaraten vergeleken met etanercept monotherapie over een behandelperiode van 48 weken. In totaal werden 33 patiënten geïncludeerd: 18 patiënten werden gerandomiseerd voor combinatietherapie met etanercept en fumaraten en 15 patiënten voor etanercept monotherapie. Het primaire eindpunt van deze studie was de werkzaamheid. De combinatie therapie leidde numeriek tot betere werkzaamheid in vergelijking met monotherapie etanercept (78% vs 57% Psoriasis Activity Severity Index (PASI) 75) na 24 weken, echter dit verschil was niet significant (P= 0.27). De longitudinale analyse toonde een PASI reductie van 5.97% per week voor de combinatietherapie groep en 4.76% voor de monotherapie groep (p = 0.11). In deze exploratieve studie, liet alleen de Physician Global Assessment (PGA) score een trend zien dat de patiënten in de combinatietherapie een snellere verbetering van de psoriasis hadden in de eerste 24 weken. Geen van al deze waargenomen verschillen waren echter statistisch significant. Dit was de eerste prospectieve gerandomiseerde studie die aantoonde dat combinatietherapie met fumaraten relatief veilig bleek te zijn met een aanvaardbare tolerantie na 48 weken. Echter, grotere studies zijn nodig om daadwerkelijk de toegevoegde waarde van fumaraten aan te tonen bij patiënten die behandeld worden met een biologic.

Deel II: Doseerinterval verlenging van een biologic

In **Hoofdstuk 5** hebben we voor de POEMA studie 59 psoriasis patiënten geïncludeerd, die onderhoudsbehandeling met adalimumab (n = 20), etanercept (n = 21) of ustsekinumab (n = 18) hadden. Het primaire doel was om te onderzoeken bij hoeveel patiënten het doseerinterval succesvol kon worden verlengd en vervolgens gehandhaafd kon worden in de 'per label' behandelde patiënten. Een secundair doel was om te onderzoeken of bij 'off-label' behandelde patiënten het doseerinterval ook verlengd kon worden. Daarnaast onderzochten we of dalconcentraties van een biologic bij inclusie in de POEMA studie een voorspellende waarde hadden voor het bereiken van een succesvolle doseerinterval verlenging.

In de 'per label' groep hadden 7 van de 16 (44%) adalimumab patiënten, 5 van de 16 (31%) etanercept patiënten en 2 van de 10 (20%) ustekinumab patiënten een succesvolle doseerinterval verlenging. In de off-label groep was verlenging van het doseerinterval niet mogelijk bij patiënten met reeds verlengde doseerintervallen. Patiënten met doseerintervallen die bij aanvang van de studie korter waren dan in het label vermeld, waren kleine doseerinterval verlengingen mogelijk in 3 van de 17 (17.6%) patiënten. De dalconcentraties van de biologics bij inclusie in de studie waren niet voorspellend voor een succesvolle doseerinterval verlenging. Bij vier patiënten steeg de PASI score boven de 8, maar bij alle vier nam de psoriasis weer af, nadat ze weer terug waren gezet op hun oorspronkelijke doseringsschema. De meest voorkomende milde bijwerkingen waren infecties van de lucht- en urinewegen. Zes ernstige bijwerkingen zijn opgetreden tijdens de POEMA studie, waarvan alle patiënten herstelden zonder restverschijnselen. Concluderend kan worden gesteld dat doseerinterval verlenging van een biologic 'per

label' haalbaar is bij ongeveer 30% van psoriasis patiënten met lage stabiele ziekteactiviteit. Als gevolg hiervan kunnen de geneesmiddelkosten in de klinische praktijk verminderd worden. In deze beperkte groep patiënten was de dalconcentratie van de biologics bij start van de studie helaas niet voorspellend voor een succesvolle doseerinterval verlenging.

Deel III: Voorwaarden om concentraties van een biologic te monitoren (TDM)

In **Hoofdstuk 6** vergeleken we drie commercieel verkrijgbare testen ontworpen voor TDM van biologic TNFα remmers. Twee enzyme-linked immunosorbent assays (ELISAs) van Sanquin en Theradiag en één bioassay (iLite) van Eurodiagnostica werden met elkaar vergeleken. Bloedmonsters werden verzameld van met infliximab, adalimumab- en etanercept behandelde patiënten (van elke biologic 40 bloedmonsters). Controle monsters waren afkomstig van patiënten die behandeld werden met ustekinumab en gezonde donoren, waarbij we van ieder 10 bloedmonsters hadden. De Sanquin, Theradiag en iLite testen waren 100% sensitief en detecteerden altijd de infliximab, adalimumab en etanercept concentratie in bloed van de bedoelde patiënten. De Sanquin ELISA detecteerde de specifieke concentraties van de TNFα remmers waarvoor deze ontworpen zijn, terwijl de Theradiag en iLite assays kruisreactiviteit vertoonden met andere TNFα remmers. De aanwezigheid van de IL-12 en IL-23 remmers (ustekinumab) werd niet gedetecteerd in het bloed door deze assays. Sanquin, Theradiag en iLite testen toonden een lineaire kwantitatieve correlatie voor de biologics concentraties. Er waren echter statistisch significante kwantitatieve verschillen in de gedetecteerde concentraties tussen de drie afzonderlijke testen. Concluderend, geven onze studieresultaten aan dat alle drie commercieel verkrijgbare testen geschikt zijn voor TDM van de TNFα remmers wanneer rekening wordt gehouden met de specifieke eigenschappen van de verschillende testen. Vanwege de aangetoonde verschillen in biologic sensitiviteit, dienen therapeutische streefconcentraties specifiek per testsysteem vastgesteld te worden. Voorts is het gebruik van dezelfde test om biologics concentraties in een patiënt in de tijd te vervolgen essentieel.

In **Hoofdstuk 7** hebben we meerdere bloedmonsters van de 33 psoriasis patiënten van de FUMBREL studie op etanercept onderhoudsbehandeling verzameld. In deze bloedmonsters werden de etanercept dalconcentraties bepaald en de intra-patiënt variabiliteit (IPV) werd beoordeeld met betrekking tot respons op de behandeling. In deze longitudinale studie tonen we aan dat de mediane IPV van de etanercept dalconcentraties 33.7% (Q1 21.3% en Q3 51.7%) is, en dat deze varieert van 8% tot 155%. De zes 'non-responders' hadden een hogere IPV in vergelijking met de 23 'responders' (53.9% vs. 24.2%; P= 0.031). De gemiddelde etanercept dalconcentratie voor elke patiënt varieerde van 0.7 tot 6.8 ug/ ml, met een mediane dalconcentratie van 2.7 ug/ml. Patiënten met een IPV boven de mediaan hadden een lagere gemiddelde etanercept dalconcentratie in vergelijking met patiënten met een IPV onder de mediaan (1,96 ug / ml, 95% (betrouwbaarheid interval) CI [1.7, 2.4] vs. 3.2 ug / ml, 95% CI [2.7; 4.0] ; P = 0.001).

De IPV, waargenomen in deze studie zal TDM bemoeilijken, en we adviseren daarom dat beslissingen over dosis aanpassingen of het over schakelen naar een ander biologic, moet worden

gebaseerd op meer dan één enkele meting. Eerst moet therapieontrouw worden uitgesloten als een oorzaak voor de lage of niet-detecteerbare dalconcentraties, of voor de grote fluctuaties in concentraties die wij bij sommige patiënten hebben waargenomen. We raden aan om de IPV ook te evalueren voor de andere biologics waarvoor TDM wordt overwogen, zoals adalimumab, infliximab, ustekinumad en secukinumab in de toekomst.

In **Hoofdstuk 8** worden de resultaten van alle studies in dit proefschrift besproken en aanbevelingen gedaan voor toekomstig onderzoek. Wij geloven dat geïndividualiseerde behandeling met biologics zal leiden tot een meer effectieve, kostenbesparende en doelmatige behandeling voor psoriasis. Als gevolg van een behandeling op maat zou dit moeten leiden tot verlengde "drug survival" , toename van kwaliteit van leven en tevredenheid over de behandeling. TDM van biologics kan als hulpmiddel worden gebruikt voor geïndividualiseerde behandeling met biologics en kan ook de therapietrouw verhogen. Eerst is een evaluatie nodig voor de voorwaarden waaraan moet worden voldaan om TDM zinvol in te kunnen zetten, voordat TDM geïmplementeerd kan worden in de behandeling voor psoriasis. In de huidige literatuur is er te weinig bewijs voor geïndividualiseerde behandeling met biologics voor psoriasis. We hopen dat dit proefschrift een goed begin zal zijn. Onze uiteindelijke conclusies zijn als volgt:

Combinatie therapie (deel I):

- Combinatie therapie met biologics en MTX verlengt mogelijk de drug survival, doorat MTX de ontwikkeling van ADA vermindert. Daarom wordt de toegevoegde waarde van combinatie therapie met adalimumab en MTX momenteel onderzocht in een grote gerandomiseerde klinische studie.
- Ondanks het feit dat combinatietherapie met etanercept en fumaraten resulteert in een betere werkzaamheid, is de werkzaamheid waarschijnlijk minder goed dan de zeer effectieve IL-12 en IL-23 remmer en IL-17-remmers.

Doseerinterval verlenging van een biologic (deel II):

- Bij psoriasis patiënten die een lage ziekteactiviteit hebben, moet verlenging van het doseerinterval van een biologic overwogen worden, omdat het kan leiden tot substantiële kostenbesparingen.

Voorwaarden voor therapeutische drug monitoring (TDM) (deel III):

- Harmonisatie van betrouwbare testen zijn nodig om data over biologic dalconcentraties met elkaar te kunnen vergelijken.
- Een hoge intra-patient variabiliteit (IPV) in de concentraties van etanercept compliceert het uitvoeren van TDM voor dit geneesmiddel. De IPV's van andere biologics moeten ook geëvalueerd worden.

Concluderend, zijn de onderzochte strategieën in dit proefschrift slechts de eerste stappen richting geïndividualiseerde behandeling van biologics. Meer studies zijn nodig voordat geïndividualiseerde behandeling van biologics voor psoriasis geïmplementeerd kan worden.

Chapter 10

Appendices

Abbrevations
List of co-authors
Publications in this thesis
Curriculum vitae
PhD portfolio
Dankwoord

ABBREVATIONS

ADA	anti-drug antibodies
AZA	azathioprine
AMP	antimicrobial peptide
BSA	body surface area
DC	dendritic cells
DLQI	Dermatology Life Quality Index
DMF	dimethylfumarate
ELISA	enzyme-linked immunosorbent assays
FcRn	Fc receptor
GI	gastro-intestinal
hBD-2	β-defensin
IFN	interferon
IGA	Investigator global assessment
IgG	immunoglobulin G
IL	interleukine
ILC	innate lymphoid cells
IMID	immune-mediated inflammatory disease
IPV	Intra-patient variability
LC-MS/MS	liquid chromatography coupled with tandem mass spectrometry
LT	Lisa Tracker
MAb	monoclonal antibody based
MTX	methotrexate
PASI	Psoriasis Activity and Severity Index
PGA	Physician Global Assessment
PML	progressive multifocal leukoencephalopathy
PSA	psoriatic arthritis
RA	rheumatoid arthritis
RIA	radioimmunoassay
(S)AE	(severe) adverse event
SNP	single nucleotide polymorphisms
SQ	Sanquin
TDM	therapeutic drug monitoring
TNFα	tumor necrosis factor alpha
TNFi	TNFα inhibitors
VEGF	vascular endothelial growth factor

LIST OF CO-AUTHORS

Affiliations at the time at which the research was conducted

R. Atiqi, Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands

D.M. Balak, Department of Dermatology, Erasmus University Medical Center, Rotterdam, the Netherlands

I. Bijen, Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands

M. A. Bos, Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands

C.I.M. Busard, Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

M.B.A. van Doorn, Department of Dermatology, Erasmus University Medical Center, Rotterdam, the Netherlands

T. van Gelder, Departments of Hospital Pharmacy and Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands

B.A. Hutten, Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, the Netherlands

E.M. de Jong, Department of Dermatology, Radboud University Medical Center, Nijmegen, the Netherlands

B.C.P. Koch, Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands

C.N. Looman, Department of Public Health, Erasmus University Medical Center, Rotterdam, the Netherlands

S.P. Menting, Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

M.S. Pradeepti, Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands

E.P. Prens, Departments of Dermatology and Immunology, Erasmus University Medical Center, Rotterdam, the Netherlands

J.M. van den Reek, Department of Dermatology, Radboud University Medical Center, Nijmegen, the Netherlands

M.W.J. Schreurs, Department of Immunology, Erasmus University Medical Center, Rotterdam, the Netherlands

Ph.I. Spuls, Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

H.J. van der Velden, Department of Immunology, Erasmus University Medical Center, Rotterdam, the Netherlands

H. te Velthuis, Department of R&D, Sanquin Reagents; Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, Amsterdam, the Netherlands

M. L. Zandvliet, Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands

PUBLICATIONS IN THIS THESIS

Intra-patient variability in the pharmacokinetics of etanercept maintenance treatment.
van Bezooijen J.S, Schreurs M.W, Koch B.C, te Velthuis H, van Doorn M.B, Prens E.P, van Gelder T.
Manuscript accepted for publication

Combination Therapy of Etanercept and Fumarates versus Etanercept Monotherapy in Psoriasis: A Randomized Exploratory Study.
van Bezooijen J.S, Balak D.M, van Doorn M.B, Looman C.W, Schreurs M.W, Koch B.C, van Gelder T, Prens E.P.
Dermatology. 2016;232(4):407-14.

Comparison of Three Assays to Quantify Infliximab, Adalimumab, and Etanercept Serum Concentrations.
van Bezooijen J.S, Koch B.C, van Doorn M.B, Prens E.P, van Gelder T, Schreurs M.W.
Ther Drug Monit. 2016 Aug;38(4):432-8.

Combining biologics with methotrexate in psoriasis: a systematic review.
van Bezooijen J.S, Prens E.P, Pradeepti M.S, Atiqi R, Schreurs M.W, Koch B.C, van Gelder T, van Doorn M.B.
Br J Dermatol. 2015 Jun;172(6):1676-80.

Monitoring antigen-specific biologics: current knowledge and future prospects.
Zandvliet M.L, **van Bezooijen JS**, Bos M.A, Prens E.P, van Doorn M.B, Bijen I, Schreurs M.W, van der Velden V.H, Koch B.C, van Gelder T.
Ther Drug Monit. 2013 Oct;35(5):588-94.

Prolongation of biologic dosing intervals in patients with stable psoriasis; a feasibility study.
van Bezooijen J.S, van Doorn M.B, Schreurs M.W, Koch B.C, te Velthuis H, Prens E.P, van Gelder T.
Manuscript accepted for publication

Optimising adalimumab treatment in psoriasis with concomitant methotrexate (OPTIMAP): study protocol of a pragmatic, single-blinded, investigator-initiated randomized controlled trial.
Busard C, Menting S.P, **van Bezooijen J.S**, van den Reek J.M, Prens E.P, Hutten B.A, de Jong E.M, van Doorn M.B, Spuls P.I.
Trials. 2017 Feb 2; 18(1):52.

CURRICULUM VITAE

Ji Sun van Bezooijen werd geboren op 10 april 1986 in Seoul (Zuid-Korea). Ze groeide op in Bergen op Zoom en in 2004 behaalde ze haar middelbare school examen aan het Gymnasium Juvenaat H. Hart. Datzelfde jaar begon ze met haar studie Geneeskunde aan de Erasmus Universiteit Rotterdam, waar ze in 2012 haar artsexamen behaalde. Tijdens haar Geneeskunde studie was ze in het studiejaar 2008-2009 'fulltime' Assessor externe van de Medische Faculteits Vereniging Rotterdam. Haar eerste interesse in wetenschappelijk onderzoek werd gewekt in 2009, tijdens een keuze-onderzoek naar het orbita volume in syndromale craniosynostose patiënten, op de afdeling Mondziekten, Kaak- en Aangezichtschirugie. In september 2012 werd gestart met het promotie onderzoek naar het individualiseren van de behandeling met biologics in psoriasis op de afdeling Apotheek en Klinische Farmacologie in samenwerking met de afdeling Dermatologie en Immunologie. Het promotie onderzoek deed zij onder begeleiding van haar promotor Prof. dr. T. van Gelder (afdeling Apotheek en Klinische Farmacologie), promotor Prof. dr. E.P. Prens (Dermatologie) en haar co-promotoren dr. M.B.A. van Doorn (Dermatologie) en dr. M.W.J. Schreurs (Immunologie). Per 1 januari 2017 is ze gestart met de opleiding Dermatologie in het Erasmus Medisch Centrum te Rotterdam.

PHD PORTOFOLIO

Name PhD student:	Ji Sun van Bezooijen
Departments:	Pharmacy and Dermatology Erasmus MC
PhD Period:	2012- 2016
Promotors:	prof. T. van Gelder, MD, PhD, prof. E.P. Prens, MD, PhD
Supervisors:	prof. T. van Gelder, MD, PhD, prof. E.P. Prens, MD, PhD

PhD training	Year	Workload
General academic skills		
- Introduction course SPSS (NIHES)	2015	1 ECTS
- Biostatistical Methods (NIHES)	2015	2 ECTS
- Biomedical English Writing and Communication	2014	3 ECTS
- BROK cursus (Good Clinical Practice)	2012	1 ECTS
In-depth courses		
- Clinical Pharmacology Meetings, Erasmus MC	2012-2014	50 hours
- Lab course ELISA techniques, Erasmus MC	2012	25 hours
- Open Clinica (building an e-CRF) course	2013	1 ECTS
- Biologic Masterclass Sanquin, Amsterdam	2015	1 ECTS
- Assay Masterclass Phadia, Rotterdam	2015	1 ECTS
- Research Meetings and Journal Clubs Dermatology, Erasmus MC	2013-2016	120 hours
(Inter)national conferences		
- Coral Gables Immunogenicity Conference 2013, Florida, USA	2013	1 ECTS
- 14th Annual meeting of the Nederlandse Vereniging voor Experimentele Dermatologie (NVED), Lunteren, The Netherlands	2013	1 ECTS
- 44th Annual European Society for Dermatological Research (ESDR), Copenhagen, Denmark	2014	1 ECTS
- 23rd Annual congress of the European Academy of Dermatology and Venereology (EADV), Amsterdam, The Netherlands	2014	1 ECTS
- 14th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), Rotterdam, The Netherlands	2015	1 ECTS

PhD training	Year	Workload
- Wetenschappelijke vergadering Nederlandse Vereniging voor Dermatologie en Venereologie (NVDV), Rotterdam, The Netherlands	2015	1 ECTS
- 17th Annual meeting of the Nederlandse Vereniging voor Experimentele Dermatologie (NVED), Lunteren, The Netherlands	2016	1 ECTS
(Inter)national presentations		
- Biologics in psoriasis, at Education day Pharmacist, Utrecht, the Netherlands. *Presentation*	2013	1 ECTS
- Open Clinica (building an e-CRF) tips and tricks, Dermatology Department Meeting, Erasmus MC, Rotterdam, the Netherlands. *Presentation*	2014	1 ECTS
- Pre-trail, 2nd PhD weekend Dermatology, Erasmus MC, Maastricht, the Netherlands. *Presentation*	2014	1 ECTS
- Therapeutic drug monitoring for biologics, Clinical pharmacology Department, Erasmus MC, Rotterdam, the Netherlands. *presentation*	2015	1 ECTS
- Assays: ELISA and bioassay for biologics, Immunology Department Research Meeting, Erasmus MC, Rotterdam, the Netherlands. *Presentation*	2015	1 ECTS
- 24th EADV : Combination therapy of etanercept with fumarates versus etanercept monotherapy in psoriasis: a randomized prospective pilot study. *Poster*	2015	1 ECTS
- 14th IATDMCT, "Determination of adalimumab, infliximab and etanercept trough concentrations: an assay comparison". *Poster*	2015	1 ECTS
Student coaching and teaching Year Workload		
- Lab course at the laboratory of the pharmacy, Erasmus MC	2013	30 hours
- Presentation "Therapeutic drug monitoring of biologics" for master students Infection & Immunity	2013	1 ECTS
- Supervising internship master student pharmacology from India	2014-2015	30 hours
- ICK education Auto-Immune diseases 4th year medical students	2015-2016	25 hours
- Supervising HLO student at the laboratory of the Immunology	2016	10 hours

PhD training	Year	Workload
Other activities		
- Reviewer for the Journal of the European Academy of Dermatology and Venereology	2013-2016	1 ECTS
- 1st PhD weekend Dermatology, Maastricht	2013	1 ECTS
- Geneticist of the month, Pharmacogenetics, Erasmus MC	2013	5 hours
- 2nd PhD weekend Dermatology, Maastricht	2014	1 ECTS
- 3rd PhD weekend Dermatology, Wassenaar	2015	1 ECTS
- 4th PhD weekend Dermatology, Antwerpen	2016	1 ECTS

DANKWOORD

Mijn proefschrift is klaar. Iets dat altijd zo ver weg leek is nu echt een feit. Ik kijk terug op een heel leerzame periode waarbij ik me niet alleen op wetenschappelijk maar ook op persoonlijk vlak heb kunnen ontwikkelen. Graag zou ik nog een paar personen willen bedanken die belangrijk voor me zijn en/of mij tijdens het promotietraject gesteund en geholpen hebben.

Beste Teun, onze eerst gesprek herinner ik me nog goed. Meteen voelde ik een klik en wilde ik graag je promovenda zijn. Ten eerste bedankt dat je me in dienst hebt genomen en mij deze kans hebt gegeven. Je hebt me heel erg goed begeleid als promotor en ook vond ik onze samenwerking erg prettig. Je hebt me altijd veel respect en zelfvertrouwen gegeven, door jouw waardering werd ik enorm gemotiveerd en voelde me altijd op mijn gemak. Ik bewonder je integriteit en zie je leiderschap als voorbeeld. Niet alleen ben je erg professioneel en heb je alle zaakjes goed op orde, ook je persoonlijke betrokkenheid bewonder ik zeer. Ik zal onze samenwerking echt missen.

Beste Errol, wat was ik blij dat je me opbelde en dat je me uitnodigde voor een sollicitatie gesprek. Van begin af aan behandelde je me als een gelijkwaardige en stimuleerde me door altijd veel complimentjes te geven als ik weer eens een patiënt had geïncludeerd. Daarnaast mocht ik bij jou ook mijn onzekerheid uiten zonder dat je me daarop beoordeelde. Ik kan bij jou mezelf zijn en onze fijne samenwerking heeft voor mij altijd heel erg motiverend gewerkt. Als er soms een artikel niet werd geaccepteerd had jij altijd positieve hoop dat het wel goed zou komen en daar had je gelijk in. Net zoals bij Teun voelde ik me ook echt een bofkont dat ik jouw promovenda mocht zijn.

Beste Martijn, ik bewonder je brede kennis in zowel de dermatologie als de klinische farmacologie. Ik heb daardoor veel van je geleerd, vooral ook omdat je mijn stukken altijd met een kritische blik las. Ik weet dat je er soms 'kromme tenen' van kreeg maar gelukkig heb je geduld gehad om mij veel te leren met schrijven. Soms was je wel wat streng voor mij maar je was altijd wel oprecht en eerlijk. Hierdoor kwam ik uit mijn 'comfort zone' en haalde ik net wat meer uit mezelf.

Beste Marco, wat was ik blij dat je erbij kwam na het vertrek van Maarten. Je hebt me ontzettend veel geleerd over een vakgebied waar ik weinig vanaf wist. Je hebt me ook in het lab goed wegwijs gemaakt en je analisten stonden voor me klaar als ik die nodig had voor mijn studie. Ik vond ik jouw analytische en technische blik altijd erg verfrissend. Dit hielp mij met nieuwe inzichten en ook met het schrijven van de artikelen. Ik vond het fijn dat ik altijd bij je je langs mocht komen en ik zal onze samenwerking missen. Op persoonlijk vlak vind ik het leuk dat je niet zo standaard bent en anders durft te zijn.

Beste Birgit, ondanks dat je niet mijn co-promotor bent voelde het wel een beetje zo. Je was altijd erg betrokken bij mijn promotieonderzoek en daarnaast ook persoonlijk geïnteresseerd. Bedankt hiervoor en ook dan dat je in de grote commissie plaats wilt nemen.

Beste Ivonne, heel veel dank voor al je hulp bij de statistiek en het meedenken. Jouw objectieve en frisse kijk op de resultaten waren altijd erg bruikbaar. Ook was je erg geduldig en flexibel als we weer eens bedacht hadden dat een figuur of tabel toch anders moest. Ik vond onze samenwerking erg prettig en vond het fijn dat ik je altijd mocht bellen of mailen.

Leden van mijn leescommissie; prof. dr. P.M. van Hagen, prof. dr. T.E.C. Nijsten en prof. dr. Ph.I. Spuls, ik zou u graag willen bedanken voor het doornemen en beoordelen van mijn proefschrift. Daarnaast wil dr. B.J.F. van den Bemt en dr. B.C.P. Koch bedanken voor het plaatsnemen in mijn grote commissie.

Beste Peter Roos, ook al heb ik niet zo veel met je samen gewerkt, ik vond je altijd geïnteresseerd en gastvrij. Ik vond het erg fijn om op jouw afdeling te mogen werken, destijds.

Graag wil ik alle patiënten bedanken die deel hebben genomen aan de FUMBREL, POEMA en OPTIMAP studie. Zonder hun inzet en medewerking was dit proefschrift niet mogelijk geweest.

Beste analisten van het apotheek lab en het immunologie lab, bedankt voor al jullie werk en het opslaan van de honderden buisjes bloed. Ruud en Najib, jullie hebben mij in het begin erg veel geleerd. Dit is al weer enige tijd geleden maar ik ben jullie zeker niet vergeten. Cindy, Diana en Elham bedankt voor het uitvoeren van de ELISA's voor al mijn studies.

Stef Menting, Juul van den Reek, Celine Busard, Gayle van der Kraaij, Phyllis Spuls en Elke de Jong, bedankt voor de samenwerking. De OPTIMAP studie vind ik een erg mooi initiatief en ik ben blij dat ik daar deel van uit mocht maken.

Lieve Nelleke, ik vond de samenwerking met jou altijd erg fijn en gezellig. We hebben veel PASI scores afgenomen en ik snap wel waarom prof. Neumann een spreekuur naar jou had vernoemd. De FUMBREL en POEMA studie had ik zonder jouw hulp niet kunnen uitvoeren.

Beste Henk en Annick, ik wil jullie bedanken voor de prettige samenwerking tussen Sanquin en het Erasmus MC. Via de mail kon ik altijd terecht bij jullie voor mijn vragen, dit stelde ik zeer op prijs.

Lieve onderzoekers, ik heb een leuke tijd gehad met jullie en kon altijd terecht met mijn vragen. Ik was er op het einde wel wat minder bij maar vond het altijd gezellig om samen te borrelen. Allard, Christine, Jill, Joan, Kelsey, Kirtie, Martijn, Merel en Sterre, bedankt dat jullie allemaal een hoofdstuk van mijn proefschrift hebben doorgelezen en de laatste foutjes eruit hebben gehaald.

A(n)IOS en staf van de afdeling en medewerkers Dermatologie van het Erasmus MC, dank voor de interesse in mijn promotie en jullie hulp en samenwerking voor de studies die zijn uitgevoerd op de dermatologie afdeling. Daarnaast wil ik jullie ook bedanken voor jullie collegialiteit en gezelligheid de afgelopen jaren.

Beste Elodie, bedankt voor je steun en vertrouwen in mij, de afgelopen maanden waren best pittig maar jouw persoonlijke begeleiding is ontzettend fijn geweest.

Party people: lieve Celine, Karin, Joan, hoewel er laatste tijd niet meer zoveel word gefeest kijk ik terug op een paar hele feestelijke momenten zoals bijvoorbeeld een geweldige skireis in

st. Anton. Daarnaast waren jullie echt mijn buddies tijdens mijn promotie en kon ik lekker sparren met jullie als ik tegen iets aanliep en kon ik bij jullie terecht met vragen. Jullie gaan nu ieder je eigen weg. Ik wens jullie daar heel veel geluk bij en ik heb genoten van onze promotietijd.

Lieve Nienke en Renilt, onze vriendschap terug gaat vanaf de middelbare school. Ik vind het bijzonder en fijn dat we belangrijke momenten in ons leven samen hebben kunnen delen en hoop dat we dat in de toekomst blijven doen. Ondanks dat wel elkaar wat minder zien, weet ik dat het altijd goed zit tussen ons.

JC Pikant, in 2004 is het allemaal begonnen.We zijn wat uitgedund maar dit clubje is nu wel 'friends for life'. We hebben een heerlijke studententijd gehad maar nu we al een paar jaar werken merk ik dat we er ook voor elkaar zijn in serieuzere tijden. Het is bijzonder om ons allemaal zo te zien opgroeien en ons eigen pad te kiezen. Ik hoop dat we samen nog veel mooie momenten zullen beleven.

Lieve Denise, wat ben ik trost op jou. Je doet het allemaal zo goed, je bent heel zorgzaam voor je familie en je doet het heel goed op werk. Ik bewonder je rust en wens je heel veel geluk in je nieuwe huisje.

Lieve Hester, soms verlang ik terug naar onze zorgeloze barcie tijd of dat we lekker bij Culpepper op het strand zaten te chillen. Onze vriendschap is erg gegroeid, ik kan met jou altijd goed praten en je laat altijd weten dat je aan me denkt door mij een appje te sturen. We hebben veel gesprekken gehad over onze carrières, Ik hoop dat we gelukkig worden van de keuzes die we durven te maken.

Lieve Jo, ook al zie ik je niet zo vaak, is het altijd fijn op de momenten als we elkaar wel zien. Je bent altijd zo vrolijk en een zelfstandig persoon, die altijd gezelligheid in de club brengt.

Lieve Lenny, met jou kan ik heerlijk genieten van het leven en even al mijn zorgen vergeten. We delen allebei de passie voor lekker eten, koken en drinken, hierdoor hebben we al vele gezellige avondjes samen gehad. Ik bewonder je positivisme en je zelfvertrouwen, daardoor laat ik me graag inspireren. Ik vind het lief dat je zo gek op me bent en altijd heel veel waardering geeft. Daarnaast heb je, niet geheel onbelangrijk, mij gekoppeld aan mijn eerste en grote liefde Dirk, waar ik nog steeds erg van geniet. Ik hoop dat we nog heel veel leuke dingen samen gaan doen en lekker genieten, lieve poes.

Lieve Farah, Inge en Jennifer, naast huisgenootjes zijn jullie hele dierbare vriendinnen gebleven. We waren een heerlijk geneeskunde huisje in de Kareldoormanstraat. Na onze studie hebben we alle een eigen weg binnen de geneeskunde gekozen. Ik bewonder jullie ambitie en doorzettingsvermogen. Ik vind het fijn dat ik altijd bij jullie terecht kan als ik vroeger met de studie of nu met werk tegen iets aanloop. Lieve Faar, ik vond heel bijzonder dat we samen naar Korea en Japan zijn geweest en ik ben blij dat ik mijn teckelliefde met jou kan delen. Lieve Inge, ik ben heel blij dat ik je aan je man Thijs heb gekoppeld en ik vond het een eer dat ik je getuige mocht zijn. Lieve Jen, met jou is het altijd een feestje en je bent super gastvrij, bedankt voor de gezellige jaarlijkse weekendjes in Friesland.

Lieve Paranimfen, ten eerste bedankt dat jullie mijn paranimf wilden zijn en zij aan zij willen staan tijdens de verdediging. Als ik terug denk aan afgelopen oud en nieuw hoe blij we toen waren toen ik jullie vroeg, dan kijk ik echt uit om de grote dag met jullie straks samen te vieren.

Lieve Bianca, eigenlijk kennen we elkaar al vanuit Bergen, maar de "vonk" is pas echt overgevlogen tijdens de decentrale selectie voor geneeskunde. Wat hebben we gelachen in de trein, collegebanken, in de Kareldoormanstraat, in de Beurs etc. De vonk is nog steeds niet over, want nog steeds kunnen we urenlang praten en alles analyseren. Ook in minder leuke tijden steun je me en geef je me advies in het maken van lastige keuzes. Je durft eerlijk en kritisch tegen me te zijn, dat stel ik erg op prijs. Ik weet dat je echt om me geeft en dat je me nooit veroordeelt, wat ik ook doe. Ik waardeer onze intense vriendschap die wij door de jaren hebben hebben opgebouwd enorm.

Lieve Maries, onze "liefde" begon tijdens het punniken in de ontgroening. We zagen er niet uit, maar dat maakte ons niet uit en sindsdien is een hele mooie en hechte vriendschap ontstaan. Samen hebben we ons ontwikkeld van best wel naïeve meisjes tot volwassen vrouwen. We hebben zoveel samen gedaan, soms zou ik onze een reis in Thailand nog wel eens een keer met je beleven, dan gaan we weer op pad met onze afritsbroekken en onze stokken. We hebben allebei ook moeilijkere periodes in ons leven gehad en toch sta je altijd voor mij klaar en kan jij me als beste troosten. Je bent echt een van de allerliefste personen die ik ken in mijn leven. Ik ben erg dankbaar voor je vriendschap.

Lieve Tjerk, Joana, Arnout, Marloes, Laurens, Judy, Els en Wim, het is al bijna 10 jaar geleden dat ik in de familie werd opgenomen en het voelt erg fijn zo'n grote familie erbij te hebben.

Lieve Youk, ik ben super trots op jou als persoon en wat je hebt bereikt! Ik hoop dat je dat nooit vergeet. Ik vond het heel bijzonder dat we samen naar ons geboorteland Korea zijn gegaan. We hebben prachtige bergen beklommen, heel veel vlees gegeten en ook hebben we je favoriete land Japan bezocht.

Lieve Pappa en Mamma, jullie geven me zoveel liefde en waardering. Als klein meisje en nu nog steeds. Tijdens mijn schooltijd, geneeskunde studie en promotie traject hebben jullie me altijd gesteund, ook als ik het even niet zag zitten. Altijd kan ik jullie bellen voor een luisterend oor en dan kan ik met goede moed weer doorgaan. Nog steeds hoop ik dat jullie om de hoek komen wonen, dan hoef ik niet meer te bellen maar kan ik even langs gaan. Ik waardeer dat ik zowel mooie als moeilijke momenten met jullie kan delen en altijd bij jullie terecht kan. Jullie liefde is onvoorwaardelijk en het maakt jullie niet uit wat ik doe, als ik maar gelukkig ben. Ik houd heel veel van jullie en ben heel blij dat jullie mijn ouders zijn.

Lieve Dirk, wat ben ik gek op jou. Jij verdient zo veel dank voor al je steun de afgelopen jaren tijdens mijn promotie traject. Soms kan ik me heel erg zorgen maken en dan moet jij me altijd maar gerust stellen en zeggen dat het goed komt, ik weet dat dit niet altijd makkelijk voor je is. Hoe sterk jij bent zie ik als voorbeeld, ik weet dat je niet van clichés houdt maar je bent toch wel echt mijn rots in de branding. Jouw stabiliteit heb ik af en toe nodig, als ik alle kanten

uitschiet. Je bent echt geweldig, soms zie je dat zelf niet eens .Ik ben zo blij dat we elkaar ooit ontmoet hebben. Sindsdien hebben we al veel meegemaakt en hebben onszelf ontwikkeld. Het is nooit saai met jouw en we durven elkaar uit te dagen. Samen maken we soms niet voor de hand liggende keuzes, zoals een teckel nemen en in Rhoon gaan wonen. Met jou zou ik graag de rest van de wereld ontdekken. Samen met onze lieve teckel Saartje vormen we al een leuk gezinnetje, hopelijk zal dit zich uitbreiden. Ik hoop dat we samen heel gelukkig oud worden, liefje.

Financial support for the publication of this thesis was generously provided by:

AbbVie
Artisan The Label
Aurobindo Pharma B.V.
BMD
Celgene
Chipsoft
Fagron
La Roche-Posay
LEO Pharma
Oldekamp Medisch
Pfizer
Pierre Fabre dermatologie
Psoriasis Vereniging Nederland
Sanquin
Tobrix
UCB Pharma
Van der Bend