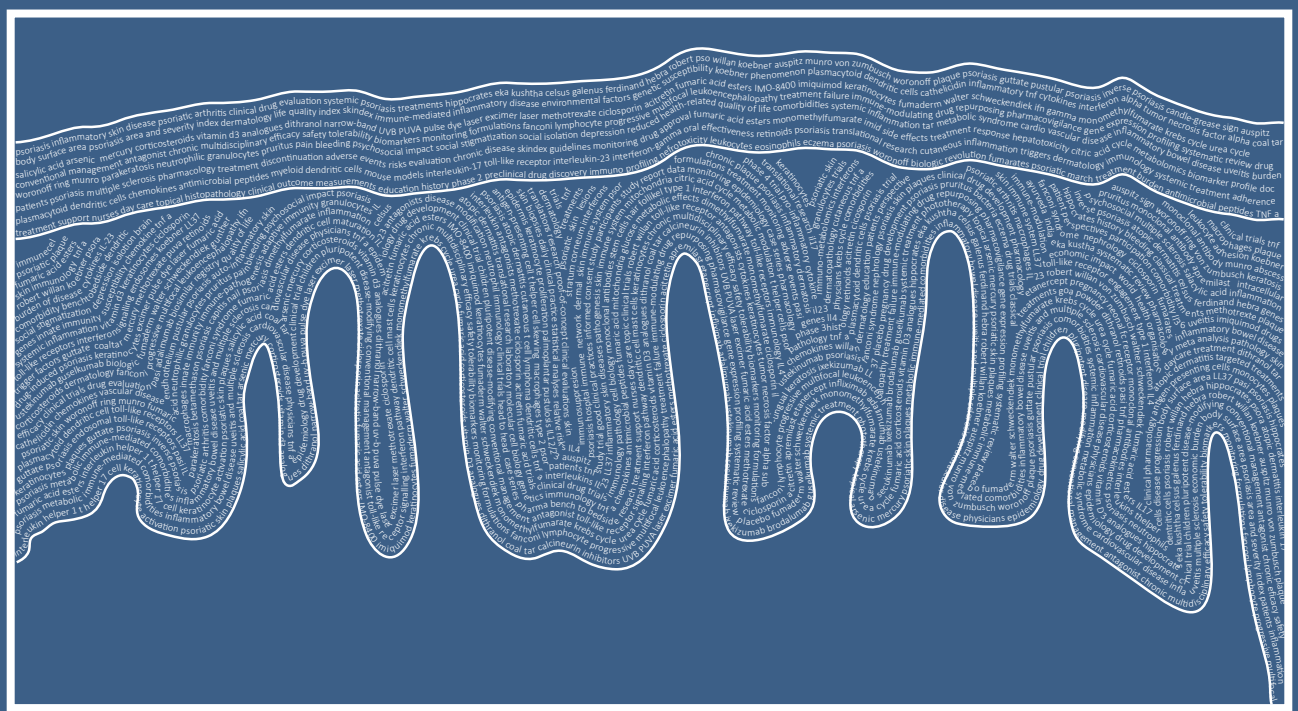
ADVANCING PSORIASIS TREATMENT

Clinical drug evaluation of fumaric acid esters and TLR-antagonists

D.M.W. Balak



ADVANCING PSORIASIS TREATMENT

Clinical drug evaluation of fumaric acid esters and TLR-antagonists

Deepak M.W. Balak

Design D.M.W. Balak
Printed by New Energy Drukwerk

ISBN 978-90-828709-0-9

Advancing Psoriasis Treatment

Clinical drug evaluation of fumaric acid esters and TLR-antagonists

Voortschrijdende inzichten in de behandeling van psoriasis

Geneesmiddelenontwikkeling van fumaraten en TLR-antagonisten

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

dinsdag 19 juni 2018 om 9.30 uur

Deepak Mukesh Wieshwaykumar Balak

geboren te Leiderdorp op 3 juli 1985

Erasmus University Rotterdam
Make it happen.

PROMOTIECOMMISSIE

Promotor:	Prof.dr. H.A.M. Neumann
Copromotor:	Dr. H.B. Thio
Overige leden:	Prof.dr. E.P. Prens Prof.dr. P.C. van de Kerkhof, Radboud Universiteit Nijmegen Prof.dr. T. van Gelder
Uitbreiding:	Prof.dr. M.A. de Rie, Universiteit van Amsterdam Prof.dr. A.F. Cohen, Universiteit Leiden Prof.dr. E.M.G.J. de Jong, Radboud Universiteit Nijmegen Dr. M.S. de Bruin-Weller, Universiteit Utrecht

TABLE OF CONTENTS

Prof.dr. Hermann W. Siemens

"Men zou daarom van meening kunnen zijn, dat wij van geen andere huidziekte zooveel afwisten als van psoriasis. De ironie der wetenschap heeft het echter anders gewild, want juist het omgekeerde is waar. Psoriasis behoort tot de raadselachtigste ziekten die er bestaan."

Vraagstukken der psoriasisbehandeling. *Ned Tijdschr Geneeskd*. 1938;82:1290-8.

| PART I |
GENERAL INTRODUCTION
&
AIMS OF THIS THESIS

Paul E. Bechet

"Psoriasis is an antidote for dermatologists' ego."

Psoriasis: A brief historical review. *Arch Derm Syphilol.* 1936;33(2):327–334

CHAPTER| 1

A short introduction into psoriasis, fumaric acid esters, and toll-like receptor antagonists

Deepak M.W. Balak[1]

H. Bing Thio[1]

H.A. Martino Neumann[1]

[1]Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

Based in part on:

Br J Dermatol. 2017 Oct;177(4):897-898.

Br J Dermatol. 2017 Mar;176(3):563-564.

Psoriasis (Auckl). 2015 Jan 5;5:9-23.

Bijblijven. Nr 1. 2012:33-40

GENERAL INTRODUCTION

Psoriasis is a common chronic, immune-mediated, inflammatory skin disease that is frequently encountered by dermatologists and general physicians in daily clinical practice.[1] The psychosocial impact of psoriasis can be significant and individuals with psoriasis often experience a decreased health-related quality of life.[2,3] In addition, psoriasis patients may suffer from extra-cutaneous comorbidities associated with systemic inflammation such as psoriatic arthritis, commonly face unmet treatment needs, and seem to have a decreased life expectancy compared to the general population.[4-6] Hence, psoriasis is a dermatological condition that carries a considerable disease-burden.[7] In line, the World Health Organization (WHO) has acknowledged psoriasis as a serious non-communicable disease in need of more awareness and further research.[8] The psoriasis treatment landscape has seen major breakthrough developments and consequently significant advances have been made in the last two decades. However, even today the treatment of psoriasis remains challanging.[9] Hence, there is a continuing medical need to develop novel treatments for psoriasis, but also for improvement of the use of established psoriasis therapies.[10]

In this thesis, the focus was set on advancing psoriasis treatment by undertaking clinical drug evaluation of two different systemic psoriasis treatments: i) fumaric acid esters (FAEs), which are an established classical systemic treatment for psoriasis, but important questions regarding their efficacy and mechanisms of action remain unanswered; and ii) a novel oligonucleotide-based antagonist of toll-like receptor (TLR) 7, 8 and 9, which is a potential targeted biologic treatment for psoriasis. In this introduction chapter, an overview of the treatment of psoriasis is given and the aims of the thesis are outlined.

History of psoriasis Arguably already early on in human history psoriasis was recognized as a skin disease.[11] Historical descriptions of psoriasis, however, have been surprisingly scarce. Hippocrates (460 – 370 BC) coined the term '*psora*' meaning itch to describe a group of skin diseases, but these cases probably did not involve psoriasis.[12] The ancient Greek physicians did distinguish '*lopoi*' (Greek for epidermis) as a separate group of scaly dermatoses that may have included psoriasis.[13] In ancient Indian Ayurveda medicine, skin diseases classified as '*eka kushtha*' bear close resemblance to psoriasis.[14] Aulus Cornelius Celsus (25 BC – 50 AD) distinguished in his '*De Medicina*' several forms of impetigo, and some of his descriptions match to psoriasis. The Roman physician Galenus (129 – 216

AD) is credited by many to be the first who used the term psoriasis, but he likely described seborrheic dermatitis - another chronic, scaly skin disease that is distinctive from psoriasis.[12] A second misclassification with more significant consequences occurred during the Middle Ages, when psoriasis was considered to be a form of leprosy, so that patients with psoriasis had to face severe stigmatization and isolation.[15] It was not until late in the 19th century that psoriasis was irrevocably separated from leprosy by Austrian dermatologist Ferdinand Hebra (1816 – 1880). The first clinical and morphological description of psoriasis as it is recognized today was provided earlier in 1808 by the Englishman Robert Willan in his textbook titled '*On Cutaneous Diseases*'.[16] Thereafter, physicians such as Koebner, Auspitz, Munro, von Zumbusch, and Woronoff described clinical or histopathological signs that are now considered to be pathognomic for a diagnosis of psoriasis.[17]

In the following early days of modern dermatology, the cutaneous morphology of psoriasis was clearly defined and psoriasis has remained ever since a major focus for clinical and scientific efforts.

Epidemiology of psoriasis Psoriasis is one of the most common inflammatory skin diseases encountered by physicians in clinical practice. Epidemiological data on psoriasis, however, have been quite limited.[18] The global prevalence of psoriasis is estimated to be 2 to 4%.[19] There is presumably a geographical gradient in the occurrence of psoriasis; the prevalence of psoriasis is largest in countries closest to the poles and tends to decrease in areas closer to the equator.[19] The reasons for this gradient in prevalence are incompletely understood, but geographical variations in average UV exposure may play a role. In the Netherlands the self-reported number of people with psoriasis in 2014 was about 400.000 people, equal to 2.4% of the total population.[20]

Psoriasis is a dermatologic disease that can be seen in virtually anyone. Men and women are affected equally and psoriasis occurs in almost all ethnicities.[21] Furthermore, psoriasis can present at any age. Nonetheless, two major peaks of age of onset are noted: one peak is around 20 years of age, which is defined as early-onset psoriasis, whereas late-onset psoriasis is typically seen around 50 years. Psoriasis is not uncommon in children. The prevalence of psoriasis among children is about 1%, ranging from 0.2% among 2-year old children to 1.7% among those aged 18 years.[22]

In most patients with psoriasis, the disease has a chronic, relapsing-remitting course. The severity of psoriatic skin lesions typically varies over time. Systematic investigations have been lacking, but overall most individuals with psoriasis seem to have a mild disease severity.[21] Approximately a third of all psoriasis patients has moderate-to-severe

disease activity.[23]

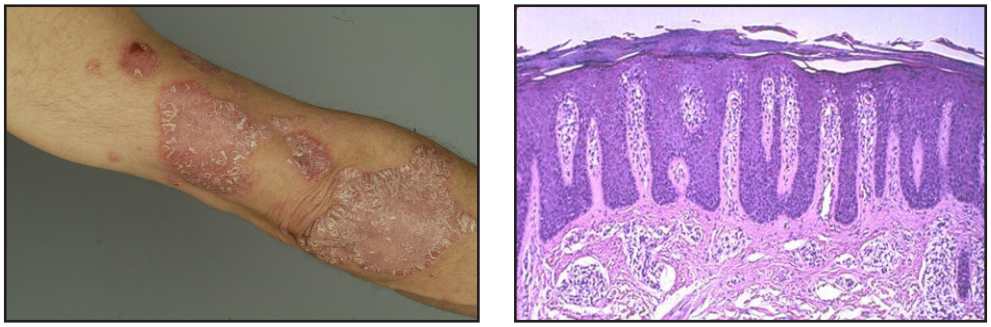
Clinical and histopathological features of psoriasis Psoriasis is characterized by typical skin lesions. Several subtypes of psoriasis are distinguished clinically based on the morphology and body site location of the skin lesions. The most common type is plaque psoriasis or psoriasis vulgaris, which entails approximately 85% of all psoriasis cases.(*See Figure 1*) Plaque psoriasis is defined by sharply demarcated skin plaques with erythema and silvery-white coarse scaling.[21] These plaque type psoriatic lesions preferentially occur symmetrically on arms, knees, lumbar region and scalp, but in principle any body site can be affected. Less frequently occurring psoriasis subtypes include: guttate psoriasis with drop-like erythematous papules located on trunk and extremities; inverse psoriasis with flat, erythematous skin lesions without much scaling that are located at the body fold areas; pustular psoriasis that characteristically involve the occurrence of sterile pustules on the palmar and plantar sides of hand and feet, respectively; and erythrodermic psoriasis which presents as generalized erythematous skin lesions.

In addition to the skin lesions, psoriasis can be associated with extra-cutaneous manifestations. Up to 30% of patients with psoriasis has inflammatory joint involvement, termed psoriatic arthritis.[24] Other potential manifestations of psoriatic arthritis include enthesitis and dactylitis. Furthermore, about 50% of patients with psoriasis has involvement of the nails, which can range from slight nail-discoloration to complete dystrophy of the nail.[21] The diagnosis of psoriasis is based primarily on the clinical assessment of skin lesions. Three clinical signs may aid to diagnose psoriasis. The candle-grease sign or 'signe de la tache de bougie' is defined by whitening of the stratum corneum when scales are scratched off superficially. The second characteristic finding of psoriasis is the Auspitz sign, which is characterized by the occurrence of small pinpoint bleeding upon deeper scraping of scales.[25] The third clinical sign consistent with a diagnosis of psoriasis is the Woronoff ring: a white-colored halo surrounding an erythematous plaque.[26]

Differential diagnoses of psoriasis include seborrheic dermatitis, nummular eczema, pityriasis rubra pilaris, cutaneous forms of lupus erythematosus (LE) - mainly subacute cutaneous LE - and cutaneous T-cell lymphoma. In rare cases, histopathologic analysis of a skin biopsy may be helpful to differentiate psoriasis from these differential diagnoses. Histopathologic features of psoriasis include: regular epidermal hyperplasia and parakeratosis, Munro microabscesses, elongation of the epidermal rete ridges with thinning of the

supra-papillary epidermis, dilated capillary vessels in the papillary dermis, and an infiltrate of immune cells that include neutrophilic granulocytes.[27](*See Figure 1*)

Figure 1: Clinical and histopathological features of chronic plaque psoriasis



Impact of psoriasis The impact of psoriasis is multi-fold. First, there are the physical symptoms exerted related to psoriatic skin lesions, such as pruritus, pain, and bleeding.[28] Second, patients with psoriasis often experience a considerable psychosocial impact due to the visibility of the skin lesions, which can result in social stigmatization.[29] Adding to this, psoriasis can be associated with feelings of social isolation and depression.[30] As a result, patients with psoriasis can have a significantly reduced health-related quality of life to an extent that is comparable to that of major chronic internal diseases like type 2 diabetes mellitus and asthma.[7] Third, psoriasis is more than a mere skin disease. There are multiple extra-cutaneous comorbidities, which may be owing to psoriasis-related systemic inflammation.[21] There is an association of psoriasis with the metabolic syndrome and cardiovascular disease, but whether this is due to a direct causal relationship remains unclear.[31] Other psoriasis-associated comorbidities include inflammatory bowel disease, uveitis, and multiple sclerosis.[21,32,33] These immune-mediated comorbidities may be linked to shared genetic pathways with psoriasis.[34,35] Fourth, there is evidence suggesting that psoriasis is associated with an increased mortality compared to the general population.[6,36] Both all-cause mortality and cardiovascular mortality risk specifically seem increased among patients with psoriasis compared to those without psoriasis.[6,37] Lastly, psoriasis poses a considerable substantial economic burden to society.[38,39] For instance, the total costs of psoriasis for the United States in 2013 was estimated at $35.2 billion, of which 35% was related to medical costs, 34% to a reduced health-related quality of live, and 32% stemming from losses in productivity.[40] Sparse data are available for the Dutch health-care setting. In a single-center retrospective cohort study from Nijmegen, mean direct costs related to psoriasis was estimated

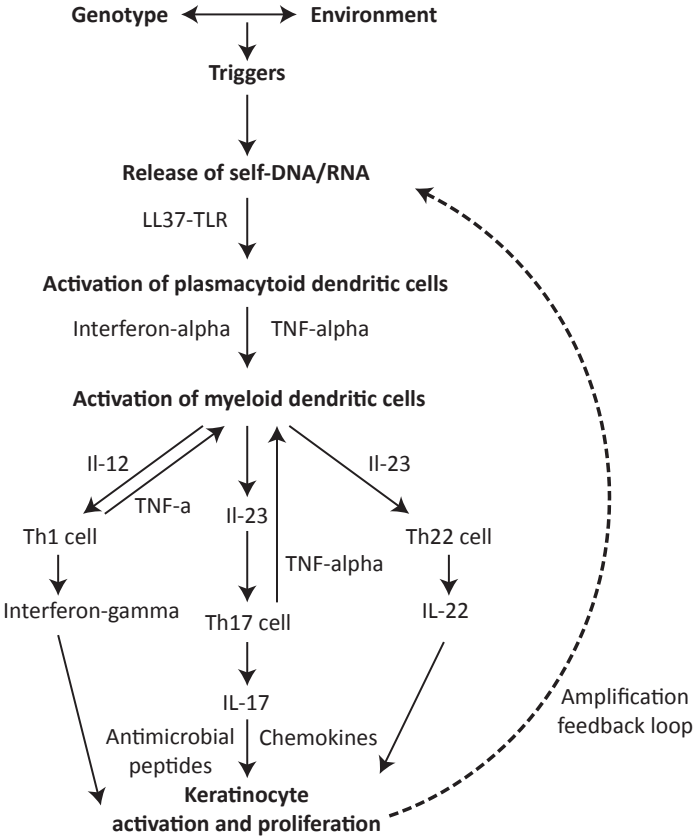
to almost € 18.000 per patient per year.[41]

Measuring disease severity of psoriasis There are several tools available and in use to measure the disease severity of psoriasis. Severity of psoriasis can be assessed based on the extent of the psoriasis plaques: severe psoriasis is defined as more than 10% of body surface area (BSA) affected, moderate psoriasis affects 5-10% of BSA, and mild psoriasis includes up to 5% of BSA.[10] A common outcome measure applied in clinical trials is the Psoriasis Area and Severity Index (PASI), which assesses BSA, but also takes into account the degree of erythema, induration and desquamation. The PASI scale ranges from zero to 72, with higher scoring indicating more severe disease activity. A PASI of 10 or higher is considered indicative of moderate-to-severe disease. Major limitations of BSA and PASI are that both grading systems do not always necessarily correlate with the patient's perceived impact of the disease. A limited number of psoriasis skin lesions in directly visible body sites (e.g. face, hands) or involving the genital area or scalp can still significantly affect an individual's quality of life.[42] Furthermore, a second disadvantage of PASI and BSA is that the patient's perspectives and impact of symptoms such as pruritus and pain are not taken into consideration in the scoring.[43] Therefore, patient-reported outcome measures (PROMs) on psoriasis severity and health-related quality of life are becoming more important parameters not only in clinical trials, but also in daily clinical practice.[44] Available patient-reported outcomes for psoriasis include general assessment tools intended for patients with a skin disease, such as the Dermatology Life Quality Index (DLQI) and the Skindex.[45,46] In addition, multiple specific tools for psoriasis have been developed, such as the Psoriasis Symptom Inventory.[47,48]

Pathogenesis of psoriasis The etiology of psoriasis has been a subject of debate for multiple decades.[49] Initial hypotheses focused on metabolic disturbances, parasitic infections, and a primary keratinocyte-dysfunction as the underlying cause of psoriasis.[50-52] These theories have been abandoned and psoriasis is now generally accepted to be a primary immune-mediated inflammatory disease.[53] Central in the current concept of psoriasis pathogenesis is an interplay between environmental factors and genetic susceptibility.[1] Genome-wide association studies (GWAS) have identified several psoriasis susceptibility loci, which mostly involve genes involved in immunity.[54,55] A clear mode of inheritance, however, is not observed. Environmental factors known to trigger psoriasis include streptococcal infection, exposure to certain drugs such as beta-blockers and lithium, physical trauma to skin (i.e., the Koebner phenomenon), and emotional stress.[56]

Experimental studies from the last two decades have established a well-characterized but complex immune-based pathogenesis of psoriasis.[53] Driving psoriasis pathogenesis are positive pro-inflammatory amplification feedback loops and interactions between multiple mediators, including the innate and adaptive immune system, the keratinocytes, and the vascular endothelium. One of the initiating events is cell damage leading to release of self-DNA and self-RNA, which form damage associated molecular patterns and bind to antimicrobial peptides like cathelicidin (also known as LL37). Via endosomal toll-like receptors 7, 8, and 9 engagement these complexes then activate plasmacytoid dendritic cells in the skin to induce production of multiple pro-inflammatory cytokines, such as interferon alpha, tumor necrosis factor (TNF) alpha, and interleukin (IL)-23. In turn, naïve T-lymphocytes are recruited and T helper 1 and T helper 17 cell responses are generated, leading to production of pro-inflammatory cytokines such as interleukin-17. The self-sustained pro-inflammatory loops subsequently cause keratinocyte activation and proliferation, which ultimately lead to formation of psoriatic skin plaques.(*See Figure 2*)

Figure 2: Schematic overview of the pathogenesis of plaque psoriasis



Landscape of psoriasis treatment Perhaps no other skin disease has had greater development of its therapeutic arsenal than has psoriasis. In particular the last two decades has seen significant improvements with the development of targeted treatments for psoriasis.[57] Still, no definitive cure is available for psoriasis. Early on, a multitude of different treatments were applied. Some of these treatments are now obsolete, such as arsenic and mercury, whereas other historical treatment modalities such as salicylic acid and coal tar are still applied today. Current treatment modalities for psoriasis can be broadly clustered into four broad categories: topical treatments, photo-(chemo)therapy, classical or conventional systemic treatments, and biologics.(*See Table 1*) The choice for treatment depends on factors such as the severity and impact of psoriasis, patient comorbidities and medical history, patient preference, expected treatment adherence, and treatment-characteristics.[10,58]

Topical treatments are the preferred treatments in mild and limited cases of psoriasis.[59] Available topical treatments include corticosteroids and vitamin D3 analogues. Dithranol and coal tar are also used as topical treatments, but mostly in context of an outpatient day-care clinic setting or in-hospital setting. Furthermore, topical calcineurin inhibitors - approved for atopic dermatitis - are used as an off-label drug for psoriasis.

A second-line therapy is photo-(chemo)therapy with ultraviolet (UV) radiation.[60] Narrow-band UV-B is the most commonly applied type of phototherapy for psoriasis. Less frequently used are photo-chemotherapy modalities, i.e. UV-A combined with either oral or topical psoralen (PUVA). Limitations of phototherapy include the relatively short-term disease control - with remission of psoriasis often only lasting several months - and the increased risk of cutaneous carcinogenesis due to cumulative UV exposure.[58] Laser-based treatments are also applied in psoriasis treatment, albeit quite limited in use. Still, the 308 nm excimer laser and the pulse dye laser (PDL) are both considered effective and safe treatment options for psoriasis.[61,62]

In case of moderate-to-severe psoriasis, systemic treatment is often indicated. Since the 1960s, several oral treatments became available for psoriasis: methotrexate, ciclosporin, acitretin, and fumaric acid esters (FAEs).[63] These four systemic agents are often classified as the classical or conventional psoriasis treatments.(*See Table 2*) Several adverse events potentially limit the use of these systemic treatments. For instance, important adverse events associated with methotrexate include hepatotoxicity and bone marrow depression. Ciclosporin is a fast-acting drug, but nephrotoxicity and development of hypertension limit the long-term use of ciclosporin in psoriasis. Acitretin, which is a vitamin A derivative, normalizes epidermal differentiation, but is typically thought to have a low efficacy as monotherapy for plaque type psoriasis.[58]

A new class of oral treatments became available in 2014 with the approval of apremilast, a small molecule drug that inhibits intracellular phosphodiesterase 4-activity.[64] Apremilast acts as an immunomodulator that decreases pro-inflammatory cytokine-production and at the same time stimulates cytokines having anti-inflammatory effects.

The most recently developed treatments are the biologics, i.e. monoclonal antibodies or fusion proteins that target specific pro-inflammatory cytokines. The introduction of biologics constituted a great breakthrough, as for the first time psoriasis could be treated with targeting drugs having a high efficacy and often a rapid onset of action. The first-generation biologics were introduced in the early 2000s and included three different tumor necrosis factor (TNF)-alpha inhibitors: infliximab, etanercept, and adalimumab. Of note, two other first-generation biologics targeting lymphocytes, i.e. efalizumab and alefacept, were withdrawn from the drug market in 2009 and 2011, respectively. The next-generation biologics target specific interleukin (IL) cytokines, i.e., in order of market approval date: anti-IL-12/23 (ustekinumab), anti-IL-17 (secukinumab, ixekizumab, brodalumab), and IL-23 (guselkumab). While biologics are effective in inducing rapid reduction of psoriasis severity in a majority of patients, the use of this class of drugs is associated with several disadvantages. First, the relatively high costs of biologics necessitate rational and restricted use of these treatments, especially in current times of restricted health care budgets.[65] Second, biologic treatment can be associated with loss of efficacy over time, which for some of these drugs may be related to the development of neutralizing antibodies.[66] Third, given the relatively recent introduction of biologics, data on their long-term effects of continuous treatment is lacking.

Need for improvement of psoriasis treatment While the number of different psoriasis treatments has vastly increased during the last decades, there is still a continued need for improvement of the treatment of psoriasis. First, the currently available treatment options are associated with disadvantages, such as primary non-response and secondary loss of efficacy over time.[66] Furthermore, adverse events and cumulative toxicity may lead to premature treatment discontinuation.[67] Such limitations also hamper long-term treatment, which is a significant issue in the management of psoriasis.[68] Given that psoriasis is a chronic disease, long-term treatment is often indicated to allow a continued disease control. A third limitation is the high costs of several psoriasis therapies, in particular the biologics. In context of present-day health care, in which cost-control is essential, high costs significantly decreases the availability of these treatments. In addition, access to biologic therapy for psoriasis does not seem equally distributed.[69] Moreover, in most low- and middle-income

countries, biologics are either not available at all or very limited in availability. In addition, from a patient's perspective there is a great extent of treatment dissatisfaction with current treatment options. Moreover, non-treatment and under-treatment are major issues in the care for psoriasis.[9] Taken together, there are clear unmet treatment needs for both psoriasis patients and physicians alike.

Table 1: Overview of treatment options for psoriasis anno 2018

Class of treatment		**Treatment**
Topical		
		Corticosteroids
		Vitamin D3 analogues
		Dithranol
		Coal tar
		Calcineurin inhibitors
Photo(chemo-)therapy		
		UVB
		PUVA
		Laser excimer / pulse dye laser
Classical systemic treatments		
		Fumaric acid esters
		Methotrexate
		Ciclosporin
		Acitretin
Small molecule		
		Apremilast
Biologic:		
	anti-TNF-alpha	Etanercept
		Infliximab
		Adalimumab
	anti-IL-12/23	Ustekinumab
	anti-IL-17	Secukinumab
		Ixekizumab
		Brodalumab
	anti-IL-23	Guselkumab

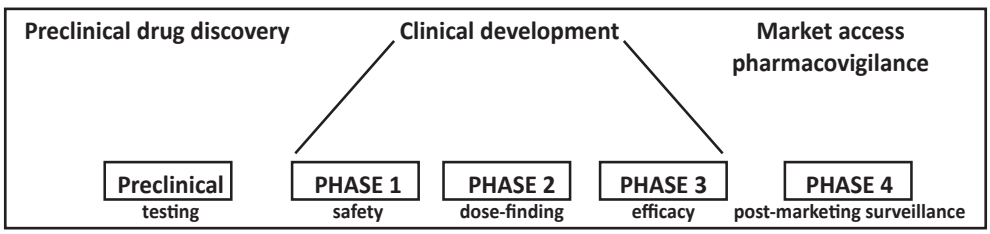
Table 2: Overview of the classical treatment options for psoriasis

Systemic treatment	Dosing	Important side effects	Comments
Fumaric acid esters, including dimethyl-fumarate	Up to 720 mg per day per os	Gastrointestinal complaints, lymphocytopenia, proteinuria	Unlicensed in most countries
Methotrexate	Up to 25 mg per day per week per os or subcutaneous injection	Hepatotoxicity, bone marrow depression	Not suitable for patients at risk for liver fibrosis, multiple drug-drug interactions
Ciclosporin	3.0-5.0 mg/kg per day per os	Nephrotoxicity, hypertension	Not suitable for long-term treatment
Acitretin	0.5-3.0 mg/kg per day per os	Xerosis cutis, dyslipidemia, teratogenic effects	Low efficacy as monotherapy

Clinical drug development and evaluation in psoriasis

Before a new drug can receive market approval and thus be prescribed in clinical practice, an extensive drug development and evaluation period is required. Contemporary drug development in general follows a linear approach with 4 specific phases.(*See Figure 3*) Following pre-clinical evaluations, a new compound enters clinical evaluation in healthy volunteers to assess drug tolerability and pharmacokinetics (Phase 1). Subsequently, phase 2 studies are performed to assess preliminary efficacy and dose-finding in a small number of patients, which are subsequently followed by large phase 3 trials to confirm efficacy and tolerability. After market approval, post-marketing surveillance studies or phase 4 trials are conducted to evaluate (long-term) efficacy and safety in daily clinical practice.

Figure 3: Overview of the linear phases of contemporary clinical drug development



Focus of this thesis The focus of this thesis was set on clinical drug development and evaluation in psoriasis treatment. The overall aim was to identify drawbacks and areas for improvements and optimization of current drug development practices in the context of psoriasis. Two different psoriasis drugs were investigated as examples for clinical drug development practices in psoriasis. First, fumaric acid esters (FAEs), an established treatment for psoriasis that are in use for over three decades but whose efficacy, safety, and mechanisms are still poorly understood.[70,71] The second drug investigated was IMO-8400, a novel, first-in-class oligonucleotide-based antagonist of TLRs 7, 8, and 9, which is a potential treatment modality for psoriasis.[53] Here, a short introduction of both FAEs and TLR-antagonists is given.

Fumaric acid esters: a classical psoriasis treatment The ester derivatives of fumaric acid, also known as fumarates, are small molecules derived from fumaric acid.[72] In terms of chemical structure, both fumaric acid and FAEs are dicarboxylic acids.(*See Figure 4*) Fumaric acid is a naturally occurring compound in the human body, in which it is involved in two basic cellular processes: the citric acid cycle and the urea cycle.(*See Figure 5*) The citric acid cycle, also known as the Krebs cycle, is an essential metabolic process that generates energy within the mitochondria.[73] Fumaric acid is one of the intermediate metabolites of the citric acid cycle. In the urea cycle, ammonia is converted into urea through fumaric acid.

Oral administration of fumaric acid has no clear anti-psoriasis effects. Fumaric acid is in use as a food additive (E-number E297); it is known for its acid, fruit-like taste. In contrast to fumaric acid, FAEs do have broad immunomodulating, anti-inflammatory, and anti-oxidative effects upon oral administration.[72] Hence, there is a clear potential of FAEs as therapeutic drug.

Figure 4: Chemical structure of fumaric acid (left) and dimethylfumarate, an fumaric acid ester derivative (right)

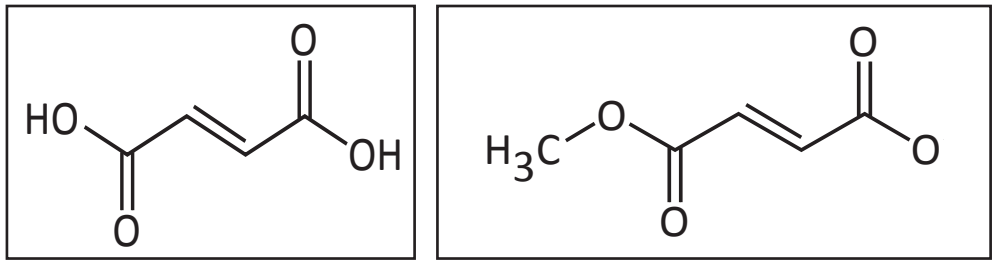
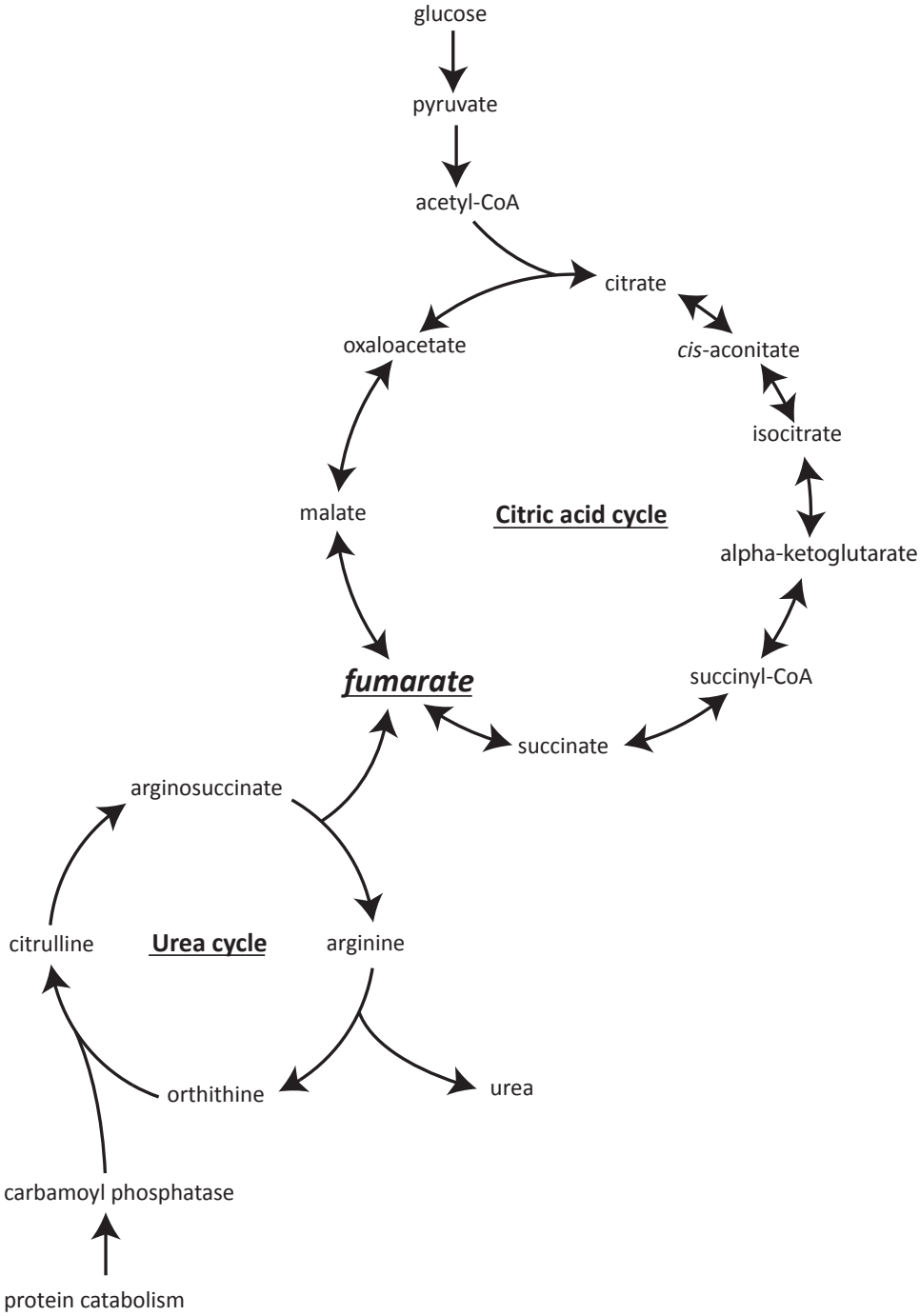


Figure 5: Fumaric acid is involved in two basic cellular processes: the citric acid cycle and the urea cycle



Development of fumaric acid esters for psoriasis There is a long-standing tradition to treat psoriasis with FAEs, but the development was somewhat peculiar done and not evidence-based.(*See Box 1*)[74,75]

The German chemist Walter Schweckendiek was the first to report the potential use of FAEs as psoriasis treatment in 1959.[76] Schweckendiek postulated that psoriasis occurred due a deficiency in serum levels of fumaric acid leading to a dysfunctional citric acid cycle and that oral supplementation of fumaric acid might neutralize these defects. In several self-experiments – Schweckendiek himself had psoriasis – he noted improvement of his psoriasis skin lesions following oral administration of fumaric acid. Given that fumaric acid caused too much gastrointestinal irritation, Schweckendiek instead tried to use the ester derivatives of fumaric acid.[77] In the 1960s and 1970s Schweckendiek together with the German general practitioner Schäfer continued to develop FAEs as a treatment for psoriasis.[78,79] A mixture of different FAEs was used in combination with topical application of FAEs and a specific elimination diet. The applied FAEs included dimethylfumarate (DMF) and monoethylfumarate (MEF). Using a standardized FAEs treatment regimen, Schäfer reported good effectiveness results in treating psoriasis patients with FAEs on a large scale in the psoriasis clinic Beau Réveil in Leysin, Switzerland. Initial clinical studies by German dermatologists, however, could not confirm the beneficial effects of FAEs in psoriasis treatment. Moreover, there were growing concerns on the safety of FAE as several cases were reported of acute renal toxicity in patients treated with FAEs. This led to a halt in the development of FAEs as psoriasis treatment for over a decade. Of note, there were no phase 1-3 trials conducted that evaluated FAE treatment in psoriasis.

In the mid-1980s there was a revival of interest in FAE treatment among academic dermatologists, partly under the influence of psoriasis patient associations. The first clinical observational studies were conducted by dermatology centers in Switzerland and in the Netherlands, which were published in 1987 and 1989, respectively.[80,81] Around the same time, the Dutch physician Leonard Kunst developed and treated psoriasis patients with a novel FAE formulation containing only DMF, thereby omitting the MEF-salts.[82]

In the early 1990s, the first randomized, placebo-controlled trials from the Netherlands and Germany that evaluated FAEs in psoriasis were published, in which efficacious responses and a good safety profile were observed in patients with chronic plaque psoriasis.[83,84] Based on these clinical trials, FAE treatment became approved in 1994 in Germany for the systemic treatment of severe psoriasis in adult patients. The licensed FAE formulation contained a mixture of DMF and MEF-salts, which was marketed as Fumaderm (Fumapharm AG, Switzerland). Fourteen years later, in 2008, the German registration for

FAE treatment with Fumaderm was expanded to include moderate psoriasis in adults.

Box 1: Overview of the development of fumaric acid esters as psoriasis treatment

Year	Event
1959	First published description of FAEs as potential psoriasis drug by Walter Schweckendiek, a German biochemist who performed several experiments on himself using fumaric acid and fumaric acid esters
1970	Gunther Schäfer, a German physician and psoriasis patient, further developed FAEs and treated patients with a standardized treatment protocol using DMF and MEF.
1985	Leonard Kunst, a Dutch physician, develops a FAE-formulation containing only DMF.
1987	Dermatologists from Bern, Switzerland, report the clinical effects of FAE treatment among patients with psoriasis.
1989	Several randomized studies involving different FAEs were conducted by Cornelis Nieboer and colleagues from Amsterdam, The Netherlands.
1990	The first randomized, placebo-controlled on FAEs in psoriasis is published by the dermatology from Leiden, The Netherlands.
1994	A large, German multicenter, randomized, placebo-controlled among 100 patients with psoriasis published by Altmeyer and colleagues.
1994	Registration of FAEs in Germany (Fumaderm) for the treatment of severe psoriasis.
2009	Inclusion of FAEs as systemic treatment option for psoriasis in the 2009 European S3-guidelines psoriasis treatment by Pathirana and colleagues.
2011	Expansion of Fumaderm registration for the treatment of moderate psoriasis in Germany.
2017	Publication of the BRIDGE trial, a large randomized controlled trial comparing Fumaderm with a DMF-formulation and placebo.

Pharmacological features of fumaric acid esters

The mechanisms underlying the efficacy of FAEs in improving psoriasis are not yet completely understood. DMF is considered the most active FAE and thought to improve psoriasis via various immune-modulating, antiproliferative, and anti-angiogenic effects.[72,85] Experimental studies have demonstrated a broad range of immune-modulating effects of FAEs. One on the mechanisms is FAEs' ability to deplete intracellular levels of glutathione in circulating immune cells, which induces the expression of the anti-inflammatory protein heme oxygenase 1 (HO-1). In turn, this inhibits pro-inflammatory cytokine production of TNF-α, interleukin (IL)-12, and IL-23, which could explain the beneficial response by FAEs in psoriasis treatment.[86]

Other lines of in vitro investigations have shown FAEs are capable of inhibiting the maturation of dendritic cells, inducing T-cell apoptosis, shifting T helper cell responses from a T helper 1 to a T helper 2 profile, and interfering with leukocyte extravasation by reduction of endothelial adhesion molecule expression.[87-94] Other potential mechanisms of action of FAE have been ascribed to inhibition of keratinocyte proliferation and downregulating angiogenesis by reducing vascular endothelial growth factor receptor-2 expression.[95-98] While numerous effects of FAEs have been observed in vitro, it remains unclear which of these are responsible of the clinical efficacy of FAEs in improving psoriasis severity.

There is relatively little data available on the pharmacokinetic properties of FAEs. DMF is considered a prodrug for monomethylfumarate (MMF).[99,100] Following oral administration, DMF is rapidly hydrolyzed in the small intestines into MMF. Serum levels of MMF rise as would be expected following oral intake of FAEs, whereas DMF levels are undetectable in serum.[101,102] However, DMF is likely not completely metabolized into MMF in the small intestines. Instead, DMF is able to reach the systemic circulation, and then DMF rapidly enters circulating cells to react and conjugate with intracellular glutathione.[103] In line, DMF-glutathione metabolites were detected in portal veins in rats after DMF administration into the small intestine and DMF-glutathione metabolites were detected in urine in psoriasis patients who were treated with FAEs.[104-105]

Position of fumaric acid esters in the treatment of psoriasis FAEs are considered one of the traditional systemic treatments for psoriasis, next to methotrexate, ciclosporin, and acitretin. As such, FAEs are included as one of the treatment options for psoriasis in the European S3-guidelines on psoriasis treatment.[106] Yet there are several issues involving FAEs in psoriasis treatment and uncertainties exist on the suitability of FAEs as psoriasis treatment, leading to a limited use of FAEs.

First, the quality of the available evidence to support the efficacy and safety of FAEs in psoriasis treatment is relatively low; the development of FAEs was mostly empirical without the rigorous evaluation standards that are adhered to today.[106] As a result, FAEs are not generally fully accepted by all dermatologists as suitable treatment option. In line, in the European guidelines no consensus could be reached for a unanimous recommendation for FAEs as a long-term treatment.[106] In addition, FAEs may be used, but rather as a second- or third-line option for systemic psoriasis treatment.[107-108]

Second, FAEs are not widely available in most countries.[71] At present, FAEs are approved only in Germany. There is a longstanding tradition in Germany to treat psoriasis patients with FAEs as a first-line systemic treatment and, as a result, FAEs are currently one

of the most frequently used systemic treatments in Germany.[109] In the Netherlands, where part of the development of FAEs as psoriasis therapy took place, FAEs are - albeit limited - in use as unlicensed treatment for psoriasis. FAEs are also available in Austria and Switzerland and there are reports of FAEs in other European countries, such as the U.K., Ireland, and Italy.[110-114] By contrast, FAEs are not in use in the U.S.A.[115]

Third, another limitation of FAEs is the relatively high incidence of inconvenient adverse events, such as gastrointestinal complaints and flushing symptoms. These complaints occur frequently during the first weeks of treatment; A significant proportion of patients on FAEs need to discontinue FAE-treatment premature due to intolerable adverse events.[116]

Finally, another current drawback of FAEs is that the mechanisms of action underlying its beneficial effects on psoriasis are not fully characterized. Accurate prediction of potential side effects by FAEs is therefore challenging.

Taken together, there is currently a limited understanding and use of FAEs as systemic treatment of psoriasis.

Toll-like receptor antagonism: a potential treatment for psoriasis The last two decades have seen significant advances in our understanding of the immune-based pathogenesis of psoriasis and the potential role of TLRs. Recent experimental studies have shown that one of the earliest and initiating events in the inflammatory cascade driving the pathogenesis of psoriasis is aberrant activation of TLR 7, 8, and 9 signaling. The TLRs are a family of pattern recognition receptors that initiate innate immune responses by recognizing pathogen-associated molecular patterns. TLR7, TLR8, and TLR9 are intracellular receptors located on endosomes within dendritic cells, B cells, and keratinocytes. These TLRs are responsible for generating antiviral and antibacterial responses through sensing of microbial-derived nucleic acids. TLR7 and TLR9 are expressed in plasmacytoid dendritic cells and B cells, whereas TLR8 is expressed in monocytes, dendritic cells, and neutrophils. Located within the endolysosomal compartments, TLR7 and TLR8 can be activated by ligands containing single-stranded RNA derived from viruses, whereas TLR9 recognizes unmethylated CpG dinucleotides that are present in bacterial DNA. Alternatively, in certain circumstances these endosomal TLRs can be engaged by self-nucleic acids released from cell death (so-called danger-associated molecular patterns), which can cause inappropriate immune activation and induction of pro-inflammatory cytokine-production.

In psoriasis, TLRs 7, 8, and 9 can be engaged by complexes of self-nucleic acids that are bound to antimicrobial peptides such as cathelicidin (also known as LL37), which

then enter and activate plasmacytoid dendritic cells in the skin to induce production of multiple pro-inflammatory cytokines including type 1 interferons, interleukin (IL)-12 and IL-23, maturation of conventional dendritic cells, and generation of T helper (Th) 1 and Th 17 cell responses. The self-sustained pro-inflammatory loop subsequently causes keratinocyte proliferation, which ultimately leads to the formation of psoriatic skin plaques.

Multiple lines of investigation underline the relevance of TLR-mediated inflammation in psoriasis. First, experimental studies have demonstrated increased expression of self-RNA-cathelicidin complexes in association with myeloid dendritic cells in lesional psoriatic skin compared to non-lesional skin. Second, keratinocytes in lesional psoriatic skin express increased levels of TLR9 that in combination with cathelicidin produce type 1 interferons. TLR7 and TLR9 are strongly expressed in keratinocytes from lesional psoriasis skin, and the number of activated plasmacytoid dendritic cells and the type 1 interferon signaling pathway are upregulated in psoriatic skin. Third, expression of TLRs 7, 8, and 9 is increased in peripheral blood mononuclear cells of psoriasis patients compared to healthy controls. Fourth, in a randomized phase 2 clinical trial treatment with an oligonucleotide antagonist of TLR7 and TLR9 resulted in disease improvement in patients with psoriasis. Fifth, in clinical practice topical use of the TLR7/8 agonist imiquimod can trigger or exacerbate psoriasis. Finally, imiquimod also induces a psoriasiform skin inflammation when applied topically to murine skin, which is now widely in use as an experimental mouse model for psoriasis.

Given the accumulating evidence on the involvement of TLR-activation in psoriasis pathogenesis, TLRs 7, 8, and 9 are potentially targets for the treatment of psoriasis. IMO-8400 is a first-in-class, second-generation, synthetic oligonucleotide-based antagonist of TLR7, TLR8 and TLR9 developed by Idera Pharmaceuticals, Inc. (Cambridge, MA). In in vitro studies using human cell-based assays and in in vivo studies involving mice and primates, IMO-8400 inhibited cytokine responses mediated by TLRs 7, 8 and 9. Furthermore, in an IL-23-induced psoriasis mouse model, treatment with IMO-8400 reduced epidermal hyperplasia and inhibited the induction of Th 1 and Th 17 cytokines. Gene expression profile analyses in the same IL-23 induced psoriasis model showed that IMO-8400 reduced IL-17A expression and normalized several IL-17-induced genes. In a phase 1 study among healthy adult volunteers, IMO-8400 administered by subcutaneous injection at single doses up to 0.6 mg/kg and multiple doses with 0.3 mg/kg for 4 weeks was well tolerated without any systemic reactions or laboratory changes.

AIMS OF THIS THESIS

The focus of this thesis was set on clinical drug development and evaluation in the field of psoriasis treatment. Two systemic immunomodulatory therapies were investigated.

First, fumaric acid esters, an established treatment for psoriasis in use for over three decades but whose efficacy, safety, and mechanisms of action in psoriasis are poorly understood.[70-71] In several studies, we assessed the efficacy, safety, tolerability, and mechanisms of action of FAEs in the treatment of psoriasis.

The second drug investigated was IMO-8400, a novel, first-in-class oligonucleotide-based antagonist of toll-like receptors 7, 8, and 9, which is a potential novel targeted treatment for psoriasis.[53] In a first-in-patient phase 2a clinical trial, we evaluated the clinical effects, short-term safety, and tolerability of IMO-8400 in the treatment of psoriasis.

Key research questions leading to this thesis were:

1. How does clinical drug development and evaluation of conventional systemic psoriasis treatments, in particular that of FAEs, compare to contemporary drug development?
2. What is the evidence for the efficacy and safety of FAEs in psoriasis?
3. What are clinically important adverse reactions associated with FAEs?
4. How is FAE treatment best monitored in daily clinical practice?
5. What are the mechanisms of action by which FAEs lead to improvement of psoriasis?
6. What are the clinical effects of TLRs 7, 8, and 9 antagonism in psoriasis?
7. What are the short-term safety and tolerability of TLRs 7, 8, and 9 antagonism in psoriasis?

In the first part of this thesis, we performed a systematic review to critically appraise the available evidence on the efficacy and safety of FAEs in the treatment of psoriasis (*Chapter 2*). One of the gaps in the current literature is a lack of evidence on combination treatments in psoriasis. We evaluated the efficacy and safety of adding FAEs to etanercept compared to etanercept monotherapy in a small, randomized pilot study (*Chapter 3*).

In the second part, we focused on the safety profile of FAEs. We undertook a randomized, double-blind, placebo-controlled trial to evaluate whether adding the oral histamine 1 receptor antagonist cetirizine to FAE treatment would improve the tolerability of FAEs (*Chapter 4*). Furthermore, we described two rare, but clinically important adverse re

actions associated with FAE treatment: progressive multifocal leukoencephalopathy (PML) and Fanconi syndrome (*Chapter 5*).

In part 3 of this thesis, we assessed the mechanisms of action of FAEs in psoriasis. Using an oligonucleotide-based microarray profiling assay, we evaluated changes in gene expression profiles in lesional skin of psoriasis patients after 12 weeks of treatment with FAEs (*Chapter 6)*.

Finally, we undertook a first clinical evaluation of a potential drug for psoriasis with a novel mechanism of action. In collaboration with the Centre for Human Drug Research (CHDR) based in Leiden, The Netherlands, we performed a first-in-patient phase 2a trial to evaluate the safety of pharmacodynamics of IMO-8400, a novel toll-like receptor 7, 8, and 9 antagonist for the treatment of plaque psoriasis (*Chapter 7*).

REFERENCES

1. Nestle FO et al. Psoriasis. N Engl J Med. 2009;361(5):496-509.
2. Kimball AB et al. The psychosocial burden of psoriasis. Am J Clin Dermatol. 2005;6(6):383-92.
3. Schmid-Ott G et al. Quality of life in patients with psoriasis and psoriasis arthritis with a special focus on stigmatization experience. Clin Dermatol. 2007;25(6):547-54.
4. Stern RS et al. 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.
5. Mrowietz U et al. Psoriasis: to treat or to manage? Exp Dermatol. 2014;23(10):705-9.
6. Springate DA et al. Incidence, prevalence and mortality of patients with psoriasis: a U.K. population-based cohort study. Br J Dermatol. 2017;176(3):650-8.
7. Rapp SR et al. Psoriasis causes as much disability as other major medical diseases. J Am Acad Dermatol. 1999;41(3 Pt 1):401-7.
8. World Health Organization WHO, Agenda item 13.5. [cited 2015 30 Oktober 2015]. Available from: http://apps.who.int/gb/ebwha/pdf_files/WHA67/A67_R9-en.pdf.
9. Armstrong AW et al. Undertreatment, treatment trends, and treatment dissatisfaction among patients with psoriasis and psoriatic arthritis in the United States: findings from the National Psoriasis Foundation surveys, 2003-2011. JAMA Dermatol. 2013;149(10):1180-5.
10. Menter A et al. Current and future management of psoriasis. Lancet. 2007;370(9583):272-84.
11. Holubar K. Psoriasis--100 years ago. Dermatologica. 1990;180(1):1-4.
12. Glickman FS. Lepra, psora, psoriasis. J Am Acad Dermatol. 1986;14(5 Pt 1):863-6.
13. Fry L. Psoriasis. Br J Dermatol. 1988;119(4):445-61.
14. Menon IA et al. Dermatological writings of ancient India. Med Hist. 1969;13(4):387-92.
15. Bechet PE. Psoriasis: A brief historical review. Archives of Dermatology and Syphilology. 1936;33(2):327-34.
16. Farber EM. The language of psoriasis. Int J Dermatol. 1991;30(4):295-302.
17. Menter A et al. Psoriasis. Manson Publishing, Ltd. 2010;London, UK.
18. Michalek IM et al. A systematic review of worldwide epidemiology of psoriasis. J Eur Acad Dermatol Venereol. 2016.
19. Parisi R et al. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. J Invest Dermatol. 2013;133(2):377-85.
20. CBS. https://www.cbs.nl/nl-nl/nieuws/2015/46/ruim-1-2-miljoen-nederlanders-hebben-eczeem-of-psoriasis. 2016.
21. Boehncke WH et al. Psoriasis. Lancet. 2015;386(9997):983-94.

22. Augustin M et al. Epidemiology and comorbidity of psoriasis in children. Br J Dermatol. 2010;162(3):633

23. Yeung H et al. Psoriasis severity and the prevalence of major medical comorbidity: a population-based study. JAMA Dermatol. 2013;149(10):1173-9.

24. Ritchlin CT et al. Psoriatic Arthritis. N Engl J Med. 2017;376(10):957-70.

25. Bernhard JD. Clinical pearl: Auspitz sign in psoriasis scale. J Am Acad Dermatol. 1997;36(4):621.

26. Varma S et al. The Woronoff ring in psoriasis. Br J Dermatol. 2003;148(1):170.

27. Murphy M et al. The histopathologic spectrum of psoriasis. Clin Dermatol. 2007;25(6):524-8.

28. Martin ML et al. The experience of pain and redness in patients with moderate to severe plaque psoriasis. J Dermatolog Treat. 2015;26(5):401-5.

29. Donigan JM et al. Psoriasis and herpes simplex virus are highly stigmatizing compared with other common dermatologic conditions: A survey-based study. J Am Acad Dermatol. 2015;73(3):525-6.

30. Cohen BE et al. Psoriasis and the Risk of Depression in the US Population: National Health and Nutrition Examination Survey 2009-2012. JAMA Dermatol. 2016;152(1):73-9.

31. Mansouri B et al. Comparison of Coronary Artery Calcium Scores Between Patients With Psoriasis and Type 2 Diabetes. JAMA Dermatol. 2016.

32. Egeberg A et al. Risk of Multiple Sclerosis in Patients with Psoriasis: A Danish Nationwide Cohort Study. J Invest Dermatol. 2015.

33. Egeberg A et al. Association of Psoriatic Disease With Uveitis: A Danish Nationwide Cohort Study. JAMA Dermatol. 2015.

34. Takeshita J et al. Psoriasis and comorbid diseases: Epidemiology. J Am Acad Dermatol. 2017;76(3):377-90.

35. Sundarrajan S et al. Comorbidities of Psoriasis - Exploring the Links by Network Approach. PLoS One. 2016;11(3):e0149175.

36. Svedbom A et al. Increased cause-specific mortality in patients with mild and severe psoriasis: a population-based Swedish register study. Acta Derm Venereol. 2015;95(7):809-15.

37. Horreau C et al. Cardiovascular morbidity and mortality in psoriasis and psoriatic arthritis: a systematic literature review. J Eur Acad Dermatol Venereol. 2013;27 Suppl 3:12-29.

38. Brezinski EA et al. Economic Burden of Psoriasis in the United States: A Systematic Review. JAMA Dermatol. 2015;151(6):651-8.

39. Feldman SR et al. The economic burden of psoriasis: a systematic literature review. Expert Rev Pharmacoecon Outcomes Res. 2014;14(5):685-705.

40. Vanderpuye-Orgle J et al. Evaluating the economic burden of psoriasis in the United States. J Am Acad Dermatol. 2015;72(6):961-7 e5.

41. Driessen RJ et al. The economic impact of high-need psoriasis in daily clinical practice before and after the introduction of biologics. Br J Dermatol. 2010;162(6):1324-9.

42. Hawro M et al. Lesions on the back of hands and female gender predispose to stigmatization in patients with psoriasis. J Am Acad Dermatol. 2017;76(4):648

43. Heydendael VM et al. The burden of psoriasis is not determined by disease severity only. J Investig Dermatol Symp Proc. 2004;9(2):131-5.

44. Feldman SR et al. Psoriasis assessment tools in clinical trials. Ann Rheum Dis. 2005;64 Suppl 2:ii65-8.

45. Finlay AY et al. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994;19(3):210-6.

46. Chren MM et al. Skindex, a quality-of-life measure for patients with skin disease: reliability, validity, and responsiveness. J Invest Dermatol. 1996;107(5):707

47. Prinsen CA et al. Measurement of health-related quality of life in dermatological research and practice: outcome of the EADV Taskforce on Quality of Life. J Eur Acad Dermatol Venereol. 2013;27(10):1195-203.

48. Revicki DA et al. Reliability and validity of the psoriasis symptom inventory in patients with moderate-to-severe psoriasis. J Dermatolog Treat. 2014;25(1):8-14.

49. Cumming J. Remarks on Psoriasis. Br Med J. 1878;2(923):353-4.

50. Thin G. On the Pathology of Psoriasis. Br Med J. 1881;2(1074):146-9.

51. Heaney JH. The Etiology and Treatment of Psoriasis. Br Med J. 1927;2(3493):1136-7.

52. Ingram JT. Problems in Psoriasis. Br Med J. 1938;1(4033):881-4.

53. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol. 2014;32:227-55.

54. Tsoi LC et al. Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. Nat Commun. 2015;6:7001.

55. Mahil SK et al. Genetics of psoriasis. Dermatol Clin. 2015;33(1):1-11.

56. Camargo CM et al. Isomorphic phenomenon of Koebner: facts and controversies. Clin Dermatol. 2013;31(6):741-9.

57. Noda S et al. The translational revolution and use of biologics in patients with inflammatory skin diseases. J Allergy Clin Immunol. 2015;135(2):324-36.

58. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol. 2009;23 Suppl 2:1-70.

59. van de Kerkhof PC. An update on topical therapies for mild-moderate psoriasis. Dermatol Clin. 2015;33(1):73-7.

60. Nakamura M et al. Recent advances in phototherapy for psoriasis. F1000Res. 2016;5.

61. de Leeuw J et al. A comparative study on the efficacy of treatment with 585 nm pulsed dye laser and ultraviolet B-TL01 in plaque type psoriasis. Dermatol Surg. 2009;35(1):80-91.

62. Goldinger SM et al. Excimer laser versus narrow-band UVB (311 nm) in the treatment of psoriasis vulgaris. Dermatology. 2006;213(2):134-9.

63. Naldi L et al. Traditional therapies in the management of moderate to severe chronic plaque psoriasis: an assessment of the benefits and risks. Br J Dermatol. 2005;152(4):597-615.

64. Vangipuram R et al. Apremilast for the management of moderate to severe plaque psoriasis. Expert Rev Clin Pharmacol. 2017;10(4):349-60.

65. Welsing PM et al.[Cost effectiveness of biologicals: high costs are the other face of success. Ned Tijdschr Geneeskd. 2011;155(29):A3026.

66. Gniadecki R et al. Comparison of long-term drug survival and safety of biologic agents in patients with psoriasis vulgaris. Br J Dermatol. 2015;172(1):244-52.

67. Arnold T et al. Drug survival rates and reasons for drug discontinuation in psoriasis. J Dtsch Dermatol Ges. 2016;14(11):1089-99.

68. van de Kerkhof PC et al. Physician perspectives in the management of psoriasis and psoriatic arthritis: results from the population-based Multinational Assessment of Psoriasis and Psoriatic Arthritis survey. J Eur Acad Dermatol Venereol. 2015;29(10):2002-10.

69. Geale K et al. Evaluating equality in psoriasis healthcare: a cohort study of the impact of age on prescription of biologics. Br J Dermatol. 2016;174(3):579-87.

70. Mrowietz U et al. Treatment of severe psoriasis with fumaric acid esters: scientific background and guidelines for therapeutic use. The German Fumaric Acid Ester Consensus Conference. Br J Dermatol. 1999;141(3):424-9.

71. Barker JN. Methotrexate or fumarates: which is the best oral treatment for psoriasis? Br J Dermatol. 2011;164(4):695.

72. Mrowietz U et al. Dimethylfumarate for psoriasis: more than a dietary curiosity. Trends Mol Med. 2005;11(1):43-8.

73. Raimundo N et al. Revisiting the TCA cycle: signaling to tumor formation. Trends Mol Med. 2011;17(11):641-9.

74. Altmeyer P et al. Vorwort. Journal der Deutschen Dermatologischen Gesellschaft. 2009;7:s1-s.

75. Mrowietz U et al. Fumarsäureester für die systemische Behandlung der Psoriasis vulgaris. Journal der Deutschen Dermatologischen Gesellschaft. 2009;7:s3-s16.

76. Schweckendiek W. Heilung von Psoriasis vulgaris. Med Monatsschr. 1959;13(2):103-4.

77. Schweckendiek W. Behandlung von psoriasis mit lipoidloslichen fumarsaureverbindungen. Medizin Heute. 1966;15:219-20.

78. Schäfer G et al. Dem Problem Psoriasis auf der Spur. Arztl Prax. 1971;18:1034.

79. Schafer G. Fumarsaure lindert die schuppenflechte. Selecta. 1984;15:1260-1.

80. Bayard W et al. Perorale Langzeitbehandlung der Psoriasis mit Fumarsaurederivaten. Hautarzt. 1987;38(5):279-85.

81. Nieboer C et al. Systemic therapy with fumaric acid derivates: new possibilities in the treatment of psoriasis. J Am Acad Dermatol. 1989;20(4):601-8.

82. Kunst L. Psoriasis behandeling. Ned Tijdschr Int Geneesknd 1985;7(16):24-9.

83. Nugteren-Huying WM et al. Fumaric acid therapy for psoriasis: a randomized, double-blind, placebo-controlled study. J Am Acad Dermatol. 1990;22(2 Pt 1):311-2.

84. Altmeyer PJ et al. Antipsoriatic effect of fumaric acid derivatives. Results of a multicenter double-blind study in 100 patients. J Am Acad Dermatol. 1994;30(6):977-81.

85. Meissner M et al. Dimethyl fumarate - only an anti-psoriatic medication? J Dtsch Dermatol Ges. 2012;10(11):793-801.

86. Ghoreschi K et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. J Exp Med. 2011;208(11):2291-303.

87. Litjens NH et al. Effects of monomethylfumarate on dendritic cell differentiation. Br J Dermatol. 2006;154(2):211-7.

88. Zhu K et al. Inhibition of dendritic cell differentiation by fumaric acid esters. J Invest Dermatol. 2001;116(2):203-8.

89. Treumer F et al. Dimethylfumarate is a potent inducer of apoptosis in human T cells. J Invest Dermatol. 2003;121(6):1383-8.

90. Hoxtermann S et al. Fumaric acid esters suppress peripheral CD4- and CD8-positive lymphocytes in psoriasis. Dermatology. 1998;196(2):223-30.

91. Ockenfels HM et al. The antipsoriatic agent dimethylfumarate immunomodulates T-cell cytokine secretion and inhibits cytokines of the psoriatic cytokine network. Br J Dermatol. 1998;139(3):390-5.

92. de Jong R et al. Selective stimulation of T helper 2 cytokine responses by the anti-psoriasis agent monomethylfumarate. Eur J Immunol. 1996;26(9):2067-74.

93. Wallbrecht K et al. Downregulation of endothelial adhesion molecules by dimethylfumarate, but not monomethylfumarate, and impairment of dynamic lymphocyte-endothelial cell interactions. Exp Dermatol. 2011;20(12):980-5.

94. Rubant SA et al. Dimethylfumarate reduces leukocyte rolling in vivo through modulation of adhesion molecule expression. J Invest Dermatol. 2008;128(2):326-31.

95. Gesser B et al. Dimethylfumarate inhibits MIF-induced proliferation of keratinocytes by inhibiting MSK1 and RSK1 activation and by inducing nuclear p-c-Jun (S63) and p-p53 (S15) expression. Inflamm Res. 2011;60(7):643-53.

96. Thio HB et al. Fumaric acid derivatives evoke a transient increase in intracellular free calcium concentration and inhibit the proliferation of human keratinocytes. Br J Dermatol. 1994;131(6):856-61.

97. Garcia-Caballero M et al. Dimethylfumarate inhibits angiogenesis in vitro and in vivo: a possible role for its antipsoriatic effect? J Invest Dermatol. 2011;131(6):1347-55.

98. Meissner M et al. Suppression of VEGFR2 expression in human endothelial cells by dimethylfumarate treatment: evidence for anti-angiogenic action. J Invest Dermatol. 2011;131(6):1356-64.

99. Werdenberg D et al. Presystemic metabolism and intestinal absorption of antipsoriatic fumaric acid esters. Biopharm Drug Dispos. 2003;24(6):259-73.

100. Litjens NH et al. In vitro pharmacokinetics of anti-psoriatic fumaric acid esters. BMC Pharmacol. 2004;4:22.

101. Litjens NH et al. Pharmacokinetics of oral fumarates in healthy subjects. Br J Clin Pharmacol. 2004;58(4):429-32.

102. Rostami-Yazdi M et al. Pharmacokinetics of anti-psoriatic fumaric acid esters in psoriasis patients. Arch Dermatol Res. 2010;302(7):531-8.

103. Schmidt TJ et al. Reactivity of dimethyl fumarate and methylhydrogen fumarate towards glutathione and N-acetyl-L-cysteine--preparation of S-substituted thiosuccinic acid esters. Bioorg Med Chem. 2007;15(1):333-42.

104. Dibbert S et al. Detection of fumarate-glutathione adducts in the portal vein blood of rats: evidence for rapid dimethylfumarate metabolism. Arch Dermatol Res. 2013;305(5):447-51.

105. Rostami-Yazdi M et al. Detection of metabolites of fumaric acid esters in human urine: implications for their mode of action. J Invest Dermatol. 2009;129(1):231-4.

106. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol. 2009;23(SUPPL. 2):5-70.

107. Jabbar-Lopez ZK et al. Newer agents for psoriasis in adults. Bmj. 2014;349:g4026.

108. Anstey AV. Fumaric acid esters in the treatment of psoriasis. Br J Dermatol. 2010;162(2):237-8.

109. Reich K et al. Drug safety of systemic treatments for psoriasis: results from The German Psoriasis Registry PsoBest. Arch Dermatol Res. 2015;307(10):875-83.

110. Inzinger M et al. Methotrexate vs. fumaric acid esters in moderate-to-severe chronic plaque psoriasis: data registry report on the efficacy under daily life conditions. J Eur Acad Dermatol Venereol. 2013;27(7):861-6.

111. Kolios AG et al. Swiss S1 Guidelines on the Systemic Treatment of Psoriasis Vulgaris. Dermatology. 2016;232(4):385-406.

112. Wain EM et al. Treatment of severe, recalcitrant, chronic plaque psoriasis with fumaric acid esters: a prospective study. Br J Dermatol. 2010;162(2):427-34.

113. Ismail N et al. Drug survival of fumaric acid esters for psoriasis: a retrospective study. Br J Dermatol. 2014;171(2):397-402.

114. Carboni I et al. Fumaric acid esters in the treatment of psoriasis: an Italian experience. J Dermatolog Treat. 2004;15(1):23-6.

115. Menter A et al. Guidelines of care for the management of psoriasis and psoriatic arthritis: section 4. Guidelines of care for the management and treatment of psoriasis with traditional systemic agents. J Am Acad Dermatol. 2009;61(3):451-85.

116. Ormerod AD et al. Fumaric acid esters, their place in the treatment of psoriasis. Br J Dermatol. 2004;150(4):630-2.

George Thin, M.D.

"Of the unknown materies morbi in psoriasis, something may be learned from the effects of treatment."

On the Pathology of Psoriasis. *Br Med J.* 1881 Jul 30;2(1074):146-9.

| PART II |
EFFICACY OF FUMARIC ACID ESTERS IN PSORIASIS

Prof.dr. Th. van Joost

"...reeds voordat onderzoek naar het werkingsmechanisme en wetenschappelijke toetsing van het effect van deze stoffen plaats kon vinden, werd toepassing van fumaarzuurderivaten ook in ons land al enthousiast ontvangen bij een waarschijnlijk niet onbelangrijke groep van psoriasispatiënten. De behandeling met fumaarzuurderivaten als nieuw te onderzoeken mogelijkheid binnen de dermatologie heeft daardoor aanvankelijk een andere weg gevolgd dan de meeste behandelwijzen."

Toepassing van fumaarzuurderivaten bij psoriasis. *Ned Tijdschr Geneeskd*. 1990 Dec 8;134(49):2371-3.

CHAPTER| 2

Efficacy, effectiveness, and safety of fumaric acid esters in the treatment of psoriasis: a systematic review of randomized and observational studies

Deepak M.W. Balak[1]

Enes Hajdarbegovic[1]

Carlijn A.F. Hagemans[1]

Wichor M. Bramer[2]

H. Bing Thio[1]

H.A. Martino Neumann[1]

[1]Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

[2]Medical Library, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

Br J Dermatol. 2016 Aug;175(2):250-62.

ABSTRACT

Background: Fumaric acid esters (FAEs) are increasingly used as a systemic treatment for psoriasis, but there are still uncertainties regarding their suitability. The objective of this systematic review was to assess the evidence for the efficacy and safety of FAEs in psoriasis treatment.

Methods: A systematic literature search was performed in seven databases up to 17 August 2015. Inclusion criteria were studies that reported clinical effects of FAEs in patients with psoriasis without restrictions in study design, language or publication date. Methodological quality of randomized controlled trials (RCTs) and overall level of quality were assessed using the Cochrane risk of bias tool and the Grading of Recommendation, Assessment, Development and Evaluation (GRADE) approach, respectively.

Results: A total of 68 articles were included. There were seven RCTs (total 449 patients) that had an unclear risk of bias and were too clinically heterogeneous to allow a meta-analysis. Overall, mean Psoriasis Area and Severity Index (PASI) decreased by 42–65% following 12–16 weeks of treatment. There were 37 observational studies (a total of 3457 patients) that supported the RCT findings, but most were uncontrolled with a high risk of bias. Commonly reported adverse events included gastrointestinal complaints and flushing, leading to treatment withdrawal in 6–40% of patients. Several case reports described rare adverse events, such as renal Fanconi syndrome and progressive multifocal leukoencephalopathy. There was a lack of studies focusing on long-term use and comparisons with other treatments.

Conclusions: There is low-quality evidence to recommend the use of oral FAEs to treat plaque psoriasis in adult patients. Unclear and high-risk studies reported favourable efficacy and tolerability of FAEs. Studies focusing on long-term safety and comparison with systemic psoriasis treatments could lead to a better understanding of the role of FAEs as a treatment for psoriasis.

INTRODUCTION

Fumaric acid esters (FAEs) are small molecules that have immunomodulating properties.[1] Oral FAEs have been used to treat psoriasis for five decades. There is a long-standing tradition in Germany and the Netherlands to treat patients with psoriasis with FAEs as a first-line systemic treatment.[2,3] In other countries such as the U.K., FAEs are increasingly reported as treatment for psoriasis.[4,5] Globally, FAEs are limited in availability and unlicensed for the treatment of psoriasis, primarily owing to a lack of a high-quality evidence-based development with well-performed randomized controlled trials (RCTs). In contrast, the development of FAEs was mostly carried out on an empirical basis.

FAEs were introduced in 1959 as potential antipsoriatic drugs by the German chemist Schweckendiek, who reported improvement of psoriasis using different FAEs in several selfexperiments.[6] In the following two decades, FAEs were empirically developed further with favourable treatment effects.[7–10] However, initial dermatological observations regarding treatment using FAEs showed variable improvements and concerns about safety.[11–13] Hence, for a long time FAEs were regarded as a controversial psoriasis treatment.[14]

In the mid-1980s, there was a revival of interest in FAEs as a potential drug for the treatment of psoriasis, which was partly driven by requests from patients' associations.[15–17] The first randomized, double-blind, placebo-controlled trials were published in the early 1990s.[18,19] Subsequently, FAEs were approved by German regulatory agencies for the treatment of severe psoriasis in 1994 and for moderate psoriasis in 2011. The licensed FAE formulation (Fumaderm, Biogen Idec GmbH, Ismaning, Germany) is a mixture of dimethylfumarate (DMF) and lower concentrations of monoethylfumarate (MEF) salts.[20]

The mechanisms of action of FAEs are not completely understood. DMF is considered the most active FAE and thought to improve psoriasis via various immunomodulating, antiproliferative and antiangiogenic effects.[21–24] Importantly, DMF is a prodrug. The metabolites monomethylfumarate (MMF) and S-(1,2-dimethoxycarbonylethyl)glutathione are the in vivo moieties; MMF is the bioactive metabolite.[25,26]

Currently, FAEs are one of the most commonly prescribed treatments for psoriasis in Germany.[27] In other European countries, such as the Netherlands and the U.K., FAEs are increasingly used for psoriasis treatment albeit as an unlicensed drug. In the U.K., FAEs are considered to be a second-line systemic therapy for psoriasis.[28] The 2009 European S3-guidelines recommended FAEs as systemic treatment for plaque psoriasis, but no consensus was reached for a recommendation regarding the use of FAEs as a maintenance treatment.[29] In

the 2015 update of the European S3 guidelines, FAEs are recommended for the long-term treatment of psoriasis, but the recommendation is based on expert opinion only.[30] Hence, there are uncertainties about the suitability of FAEs as a psoriasis treatment.

In this systematic review, we aimed to summarize comprehensively and critically appraise the evidence for the efficacy, effectiveness and safety of the use of oral FAEs to treat patients with psoriasis.

MATERIALS AND METHODS

Literature search strategy The databases Embase, Medline (Ovid), Cochrane central registry of trials (CENTRAL), Web of Science, Scopus, PubMed (the subset as supplied by publisher, containing nonindexed citations) and Google Scholar were searched from inception to 17 August 2015. The searches, conducted by an experienced biomedical information specialist (W.B.), combined multiple thesaurus terms and words in titles/abstracts for FAEs with terms for psoriasis. Details of the search strategy are summarized in the Appendix.

Selection criteria Articles were screened for relevance according to the title and abstract. The remaining articles were assessed using the full text. Articles describing clinical effects (i.e. efficacy, effectiveness and/or safety outcomes) of oral FAEs in psoriasis treatment were eligible for inclusion. To obtain the most comprehensive overview possible, we did not apply restrictions for publication date, study design or publication language.

Data extraction Using a predefined data form, we extracted data regarding study design, study setting, sample size, study analyses, FAE formulation and dosage, efficacy or effectiveness outcomes and safety outcomes.

Quality assessment The methodological quality of RCTs and observational studies was assessed using the Cochrane risk of bias tool and the risk of bias assessment tool for nonrandomized studies, respectively.[31,32] Overall level of quality of evidence was assessed according to the Grading of Recommendation, Assessment, Development and Evaluation (GRADE) approach.[33]

Outcomes and data analysis We aimed to compare treatment effects of FAEs vs. placebo, FAEs vs. other systemic treatments, different FAEs formulations and different FAEs dosage levels. In addition, we looked at treatment effects of FAEs in combination with other

psoriasis treatments. The efficacy and effectiveness outcomes of interest included changes in psoriasis disease activity as measured by the Psoriasis Area and Severity Index (PASI), body surface area (BSA) affected or global psoriasis assessments. Additional outcomes included changes in arthritis, nail symptoms and health-related quality of life. The safety outcomes included proportions of patients reported with serious adverse events, subjective adverse events, laboratory abnormalities and adverse events requiring withdrawal of treatment. We classified observational studies that assessed treatment with FAEs for 12 months or longer as long-term studies.

Two researchers (D.B. and C.H.) independently assessed articles for eligibility for inclusion, extracted data and evaluated methodological quality. Any disagreements were resolved by consensus.

Descriptive statistics were used to analyse data. Reporting of findings was in line with the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines.[34] A predefined review protocol was used, but not registered beforehand.

RESULTS

Literature search The literature search yielded 2.515 results, of which 275 articles were assessed using the full article text. A total of 68 articles were included (Fig. 1).

Randomized controlled trials on the use of fumaric acid esters for the treatment of psoriasis

Characteristics of randomized controlled trials Our search results found seven RCTs published in the period 1990–2014.[18,19,35–38] Of these, three trials compared FAEs with placebo, one trial compared two different FAE formulations with placebo, one trial compared FAEs with methotrexate, one trial compared the combination of FAEs and topical calcipotriol with FAEs monotherapy, and the most recent trial compared FAEs plus an oral histamine antagonist with FAEs monotherapy. Two RCTs from the Netherlands were published additionally in an extended version in a Dutch journal.[39,40] The characteristics of study design and study population of each RCT are summarized in Table 1 and Table S1 (see Supporting Information).

The sample sizes of the RCTs were relatively small, ranging from 27 to 134 patients. Overall, 449 patients were included. The majority of the RCTs included patients with chronic

plaque psoriasis. One RCT enrolled patients with psoriatic arthritis.[41] All patients included were aged 18 years or older. There was considerable clinical heterogeneity among the RCTs in the efficacy outcomes, time of efficacy assessment and FAE formulations used. Frequently used efficacy outcomes were changes in PASI or in BSA. The treatment duration was relatively short, ranging from 28 months to 4 months.

There were differences in the evaluated FAE formulations. Most RCTs applied the standardized incremental dosage regimen up to 215 mg FAEs six times per day (720 mg DMF). Nieboer et al.[18] used a different dosage regimen up to 215 mg FAEs four times per day (480 mg DMF).

Methodological quality assessment of randomized controlled trials Assessment of the methodological quality of the RCTs using the Cochrane risk of bias tool yielded an unclear risk of bias, often owing to insufficient reporting (Table S2; see Supporting Information).

The overall level of quality of the RCTs included in the GRADE approach was therefore downgraded to moderate.

Efficacy outcomes reported in randomized controlled trials Owing to significant clinical heterogeneity and the small number of RCTs available (n = 7), we decided not to pool the efficacy data in a meta-analysis. All RCTs reported statistically significant efficacy results for FAEs. Overall, mean PASI decreased by 42–65% following 12–16 weeks of treatment. The efficacy results are summarized in Table 1 (Table S3; see Supporting Information).

All placebo-controlled RCTs reported a statistically significant improvement in psoriasis severity following the use of FAEs compared with placebo.[19,35] The placebo-controlled RCT for the treatment of psoriatic arthritis using FAEs found significant improvement in skin lesions but only modest improvement in arthritis.[41] Only one RCT reported improvements in health-related quality of life following treatment with FAEs.[38] The only head-to-head RCT compared FAEs with methotrexate and reported similar efficacy results following 16 weeks of treatment.[36] The RCT that directly compared an FAE formulation containing DMF and MEF with a DMF formulation reported equal short-term efficacy.[18]

The addition of a topical vitamin D analogue (i.e., calcipotriol) resulted in greater and faster improvement of psoriasis severity compared with treatment using FAEs alone.[37] In contrast, the addition of an oral histamine-1 receptor antagonist (cetirizine) did not increase the efficacy of FAEs.[38]

Safety outcomes in randomized controlled trials Treatment using FAEs was not associated with an increased risk of serious or severe adverse events. There was only one serious adverse event reported: adnexitis in a subject who received FAEs and calcipotriol, which was rated as unlikely to be related to the study medication.[37] The proportion of patients with adverse events was relatively high, ranging from 69% to 92% (Table 1). The most commonly reported adverse events were gastrointestinal complaints (up to 100%) and flushing (up to 92%) (Table S3; see Supporting Information). Commonly reported laboratory abnormalities included elevated liver enzymes (up to 62%), eosinophilia (up to 46%) and lymphocytopenia (up to 38%), but rarely resulted in treatment discontinuation. Definitions and grading of laboratory abnormalities were not reported in the individual studies. There was one case of reversible renal insufficiency reported.[39]

Overall, 8–39% of patients discontinued FAEs treatment owing to adverse events, mostly relating to intolerable gastrointestinal or flushing complaints.

Observational studies on fumaric acid esters treatment for psoriasis

Characteristics of observational studies There were 37 observational studies included from the period 1987–2015 with a total of 3457 patients. There was considerable clinical heterogeneity in formulations of FAEs and treatment duration. The characteristics of the observational studies included are summarized in Table 2 and Table S2 (see Supporting Information). The majority (73%) of these studies were open-label, single-center, cohort studies that were often uncontrolled. There were two cross-sectional studies.[42,43] The rest of the observational studies were case series (n = 8). The majority of studies included patients with moderate-to-severe plaque psoriasis. Two studies evaluated FAEs in mild cases of plaque psoriasis. In some studies a small number of patients with subtypes other than plaque psoriasis, such as guttate or palmoplantar pustular psoriasis, were included. Almost all studies involved adult patients, except for two studies that included paediatric patients with psoriasis.[44,45] Sample sizes ranged from six to 984 patients. The treatment duration ranged from 1 month to 14 years. There were 18 studies that described long-term treatment using FAEs for a period of 1–14 years. Most studies assessed Fumaderm with the recommended dosage schedule. There was variation in the effectiveness outcomes used. PASI, Physician's Global Assessment and global psoriasis severity assessments were used.

Quality assessment of observational studies Most of the observational studies were retrospective and uncontrolled single-center studies with a high or unclear risk of bias

(Table S5; see Supporting Information). Following the GRADE approach, there were insufficient grounds to upgrade the quality of evidence. Hence, the overall level of quality using GRADE was evaluated as having a very low quality of evidence.

Effectiveness in observational studies The effectiveness data are summarized in Table 2 and Table S6 (see Supporting Information). There was a wide range in the reported effectiveness outcomes. Overall, mean reductions in PASI ranged from 13% to 86% following 3–4 months of treatment. Reported PASI-75 responses ranged from 8% to 33%. One retrospective, single-center cohort study reported a drug survival rate of 60% after 4 years of treatment with FAEs.[46] Several studies reported improvements in patient-reported quality of life.[47–49] There were anecdotal data on combination treatment with other systemic treatments.[50,51] One registry study from Austria found similar effectiveness of FAEs and methotrexate.[52]

Two small retrospective case series from the Netherlands and Germany assessed the effects of FAEs in children with psoriasis.[44,45] The effectiveness results of FAEs were in line with results reported in adult patients.

Safety outcomes in observational studies No deaths or serious adverse events were reported in the observational studies. The adverse events profile was, in general, similar among the studies. The most commonly reported adverse events were gastrointestinal complaints and flushing (Table 3 and Table S6; see Supporting Information). Commonly reported laboratory abnormalities included lymphocytopenia, elevated liver enzymes and eosinophilia.

Overall, 45–87% of patients had experienced an adverse event. The proportion of patients discontinuing treatment with FAEs owing to adverse events ranged from 6% to 47%. The most common cause for early treatment discontinuation was intolerable gastrointestinal symptoms and, to a lesser extent, severe flushing symptoms. There were few reported treatment discontinuations owing to laboratory abnormalities.

There were few studies that specifically evaluated long-term treatment with FAEs. The available data indicated no increased risk for infections, malignancies or other serious adverse events associated with long-term FAE treatment. In a small, retrospective single-center study among patients treated with FAE continuously for up to 10–14 years, no serious adverse events or malignancies were observed.[53] Similar safety results were reported in a large German study among nearly 1000 patients treated with FAEs for a mean duration of 35 years.[42]

Case reports on adverse events of fumaric acid esters Twenty-four case reports described adverse events associated with treatment with FAEs (Table 4). Of these, several involved immunosuppressive events linked to FAE-induced lymphocytopenia, including Kaposi sarcoma,[54] organizing pneumonia,[55] tuberculous lymphadenitis,[56] squamous cell carcinoma,[57] melanoma[58] and progressive multifocal leukoencephalopathy (PML).[59–65] Seven cases of PML associated with treatment with FAEs have been reported. In most cases the development of PML was linked to exposure to severely low lymphocyte counts for prolonged periods of time. However, there was one case of PML linked to moderate lymphocytopenia.[65] Furthermore, there were several renal adverse events reported including six cases of drug-induced Fanconi syndrome linked to FAEs.[66–71] Fanconi syndrome is characterized by proximal renal tubular dysfunction and can lead to proteinuria, glycosuria and low serum levels of phosphate. Furthermore, there were nine cases of acute renal insufficiency linked to FAEs. These cases of acute renal insufficiency were all reported in the 1990s and involved uncontrolled use of oral and topical FAEs. Lastly, there was one case reported of collagenous colitis during treatment with FAEs, which may be associated with an FAE-induced T helper 2 immune response.[72]

DISCUSSION

FAEs have a long history as a systemic psoriasis treatment, but their development was not based on high-quality evidence. Here, we assessed studies on the efficacy and safety of FAEs in psoriasis treatment. The available evidence was limited and included seven RCTs with relatively small sample sizes and an unclear risk of bias. Overall, mean PASI decreased by 42–65% following 12–16 weeks of treatment. The number of observational studies (n = 37) was much larger, but the majority were uncontrolled and had a high risk of bias. The safety profile of FAEs was well characterized. Intolerable gastrointestinal complaints and flushing led to early treatment withdrawal in 6–40% of patients. Lymphocytopenia, eosinophilia, increased liver enzymes and proteinuria were commonly observed, but rarely resulted in the discontinuation of FAEs. Studies with long-term data were lacking.

To appreciate our results, several aspects of this systematic review need to be considered. Strengths of our study include the extensive literature search involving multiple bibliographic databases and the fact that we did not exclude specific study types or publication dates, making this the largest systematic review on FAEs in psoriasis to date. In addition, we included articles written in languages other than English, thereby decreasing language bias. Furthermore, the quality of the included studies was critically evaluated using the

GRADE approach. A limitation of our study was that most included studies were open-label and uncontrolled studies with a low level of evidence. Moreover, owing to considerable clinical heterogeneity among the studies, a meta-analysis was not possible. Furthermore, the majority of the RCTs from the 1990s did not adhere to reporting guidelines that are now considered standard (e.g. the Consolidated Standards of Reporting Trials guidelines).[73] Moreover, there was a lack of standardization of efficacy outcomes across the RCTs. A recent Cochrane review of the effects of FAEs in psoriasis could not carry out a meta-analysis because of incomplete and heterogeneous reporting of outcomes.[74] Several previous studies did apply a meta-analysis on a limited number of studies. These meta-analyses reported similar efficacy of FAEs to methotrexate,[75] superior efficacy of FAEs compared with the biologic efalizumab76 and significant differences of FAEs compared with placebo.[77]

Most studies assessed the FAE formulation Fumaderm that has had German marketing authorization since 1994. The choice of the components of Fumaderm (i.e. DMF and MEF salts) was not based on rational pharmacological studies. Recent preclinical studies suggest that DMF is the most active of the FAEs with antipsoriatic effects.[78,79] In particular, DMF is a prodrug for which MMF is the bioactive metabolite.[20] Two small studies from the 1990s compared an FAE formulation containing DMF plus MEF with a DMF formulation and found no statistically significant differences.[18,80] However, these studies applied different dosage schedules and did not use validated efficacy outcomes. Consequently, clear conclusions cannot be made based on these results. A novel DMF formulation BG-12 was assessed in several psoriasis RCTs,[81] but these studies have yet to be published. The BG-12 formulation later became approved for treatment of multiple sclerosis by the Food and Drug Administration in 2013.[82,83] Several novel FAE formulations are now in development, e.g. an MMF-linker formulation and a DMF formulation (clinicaltrials.gov numbers NCT02173301 and NCT01230138, respectively).

Since the mid-1990s, FAEs are increasingly being used and regarded as a systemic treatment with a favourable risk–benefit ratio. FAEs have several advantages. FAEs seem suitable for patients with psoriasis with comorbidity and there are no known drug interactions. Furthermore, FAEs appear to have no increased risk of significant immunosuppressive adverse events, in contrast to other systemic classical treatments.[84] Although lymphocytopenia is relatively frequently observed during treatment with FAEs in about 50% of patients, in most cases the lymphocyte reductions are mild.[30] A small proportion of patients (approximately 3%) had a severe lymphocytopenia during treatment with FAEs.[30] FAE-induced lymphocytopenia does not seem to cause significant immunosuppression as long as lymphocyte counts are closely monitored according to the guidelines.[85] FAE dosage reduc-

tion is recommended if lymphocyte counts fall below 700 per mm^3 and direct FAE discontinuation is recommended in cases where lymphocyte counts fall below 500 mm^3.[29,30] The occurrence of opportunistic infections during treatment with FAEs was linked to exposure to prolonged severe lymphocytopenia or to other known risk factors.

It is noted that the use of FAEs for the treatment of psoriasis is more prevalent than the published data suggests.[86] It is interesting to compare the level of evidence relating to the use of FAEs to that of methotrexate. Methotrexate is globally the most commonly used classical systemic treatment for psoriasis.[87] However, the available evidence is limited even though methotrexate has been in use since 1958.[29] Results from an RCT and a registry-based observational study indicated that methotrexate and FAEs produce similar clinical improvements in short-term treatment.[36,52] Excluding methotrexate, FAEs have not been compared head-to-head with other systemic psoriasis treatments. Such comparative studies are needed to gain a better understanding of the role of FAEs as a treatment for psoriasis.[4]

In conclusion, FAEs are considered to be suitable as a systemic treatment for moderate-to-severe plaque psoriasis, but the quality of the available evidence is low. Future studies could focus on optimizing FAE formulations and comparing FAEs with other systemic treatments in order to define the position of FAEs in the landscape of psoriasis treatment.

REFERENCES

1. Mrowietz U et al. Dimethylfumarate for psoriasis: more than a dietary curiosity. Trends Mol Med 2005; 11:43–8.
2. Mrowietz U et al. 15 years of fumaderm: fumaric acid esters for the systemic treatment of moderately severe and severe psoriasis vulgaris. J Dtsch Dermatol Ges 2009; 7(Suppl. 2):S3–16.
3. Fallah Arani S et al. Treatment of psoriasis with non-registered fumaric acid esters in The Netherlands: a nationwide survey among Dutch dermatologists. J Eur Acad Dermatol Venereol 2014; 28:972–5.
4. Anstey AV. Fumaric acid esters in the treatment of psoriasis. Br J Dermatol 2010; 162:237–8.
5. Iskandar IY et al. Demographics and disease characteristics of patients with psoriasis enrolled in the British Association of Dermatologists Biologic Interventions Register. Br J Dermatol 2015; 173:510–18.
6. Schweckendiek W. Treatment of psoriasis vulgaris. Med Monatsschr 1959; 13:103–4.
7. Schweckendiek W. Behandlung von Psoriasis mit lipoidloslichen fumarsaureverbindungen. Medizin Heute 1966; 15:219–20.
8. Schafer G et al. Dem Problem Psoriasis auf der Spur. Arztl Prax 1971; 18:1034.
9. Kunst L. Psoriasis behandeling. Ned Tijdschr Geneesknd 1985; 7:24–9.
10. Schafer G. Fumarsaure lindert die schuppenflechte. Selecta 1984; 15:1260–1.
11. Vonkennel J et al. Is a therapy of psoriasis through intermediate metabolism possible? Med Welt 1961; 34:1697–701.
12. Dubiel W et al. Experimental treatment with fumaric acid monoethylester in psoriasis vulgaris. Z Haut Geschlechtskr 1972; 47:545–50.
13. Roodnat JI et al. Acute renal failure in patients treated for psoriasis with fumaric acid esters. Ned Tijdschr Geneesknd 1989; 133:2623–6.
14. Raab W. Psoriasis therapy with fumaric acid and fumaric acid esters. Z Hautkr 1984; 59:671–9.
15. van Dijk E. Fumaric acid in the treatment of patients with psoriasis. Ned Tijdschr Geneeskd 1985; 129:485–6.
16. Bayard W et al. Peroral long-term treatment of psoriasis using fumaric acid derivatives. Hautarzt 1987; 38:279–85.
17. Nieboer C et al. Systemic therapy with fumaric acid derivates: new possibilities in the treatment of psoriasis. J Am Acad Dermatol 1989; 20:601–8.
18. Nieboer C et al. Fumaric acid therapy in psoriasis: a double-blind comparison between fumaric acid compound therapy and monotherapy with dimethylfumaric acid ester. Dermatologica 1990; 181:33–7.

19. Altmeyer PJ et al. Antipsoriatic effect of fumaric acid derivatives. Results of a multicenter double-blind study in 100 patients. J Am Acad Dermatol 1994; 30:977–81.

20. Rostami Yazdi M et al. Fumaric acid esters. Clin Dermatol 2008; 26:522–6.

21. Ghoreschi K et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. J Exp Med 2011; 208:2291–303.

22. Onderdijk AJ et al. Regulated genes in psoriatic skin during treatment with fumaric acid esters. Br J Dermatol 2014; 171:732–41.

23. Helwa I et al. The antipsoriatic agent monomethylfumarate has antiproliferative, prodifferentiative, and anti-inflammatory effects on keratinocytes. J Pharmacol Exp Ther 2015; 352:90–7.

24. Garcia-Caballero M et al. Dimethylfumarate inhibits angiogenesis in vitro and in vivo: a possible role for its antipsoriatic effect? J Invest Dermatol 2011; 131:1347–55.

25. Litjens NH et al. In vitro pharmacokinetics of anti-psoriatic fumaric acid esters. BMC Pharmacol 2004; 4:22.

26. Rostami-Yazdi M et al. Pharmacokinetics of anti-psoriatic fumaric acid esters in psoriasis patients. Arch Dermatol Res 2010; 302:531–8.

27. Augustin M et al. German psoriasis registry PsoBest: objectives, methodology and baseline data. J Dtsch Dermatol Ges 2014; 12:48–57.

28. Jabbar-Lopez ZK et al. Newer agents for psoriasis in adults. BMJ 2014; 349:g4026.

29. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol 2009; 23(Suppl. 2):1–70.

30. Nast A 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; 29:2277–94.

31. Higgins JPT et al. Cochrane handbook for systematic reviews of interventions version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available at http://http://handbook.cochrane.org/ (last accessed 15 April 2016).

32. Kim SY et al. Testing a tool for assessing the risk of bias for nonrandomized studies showed moderate reliability and promising validity. J Clin Epidemiol 2013; 66:408–14.

33. Guyatt GH et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008; 336:924–6.

34. Moher D et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009; 339:b2535.

35. Nugteren-Huying WM et al. Fumaric acid therapy for psoriasis: a randomized, double-blind, placebo-controlled study. J Am Acad Dermatol 1990; 22:311–12.

36. Fallah Arani S et al. Fumarates vs. methotrexate in moderate to severe chronic plaque psoriasis: a multicentre prospective randomized controlled clinical trial. Br J Dermatol 2011; 164:855–61.

37. Gollnick H et al. Topical calcipotriol plus oral fumaric acid is more effective and faster acting than oral fumaric acid monotherapy in the treatment of severe chronic plaque psoriasis vulgaris. Dermatology 2002; 205:46–53.

38. Balak DM et al. Addition of an oral histamine antagonist to reduce adverse events associated with fumaric acid esters in the treatment of psoriasis: a randomized double-blind placebo-controlled trial. Br J Dermatol 2014; 172:754–9.

39. Nugteren-Huying WM et al. Fumaric acid therapy in psoriasis; a double-blind, placebo-controlled study. Ned Tijdschr Geneeskd 1990; 134:2387–91.

40. Peeters AJ et al. Favourable effect of fumaric acid treatment in psoriatic arthritis: a double-blind placebo-controlled study. Ned Tijdschr Geneeskd 1992; 136:2428–31

41. Peeters AJ et al. Fumaric acid therapy for psoriatic arthritis. A randomized, double-blind, placebo-controlled study. Br J Rheumatol 1992; 31:502–4.

42. Reich K et al. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis–a retrospective study (FUTURE). J Dtsch Dermatol Ges 2009; 7:603–11.

43. Thaci D et al. Efficacy and safety of fumaric acid esters in patients with psoriasis on medication for comorbid conditions – a retrospective evaluation (FACTS). J Dtsch Dermatol Ges 2013; 11:429–35.

44. Balak DM et al. Effectiveness and safety of fumaric acid esters in children with psoriasis: a retrospective analysis of 14 patients from the Netherlands. Br J Dermatol 2013; 168:1343–7.

45. Steinz K et al. Systemic treatment with fumaric acid esters in six paediatric patients with psoriasis in a psoriasis centre. Dermatology 2014; 229:199–204.

46. Ismail N et al. Drug survival of fumaric acid esters for psoriasis: a retrospective study. Br J Dermatol 2014; 171:397–402.

47. Wain EM et al. Treatment of severe, recalcitrant, chronic plaque psoriasis with fumaric acid esters: a prospective study. Br J Dermatol 2010; 162:427–34.

48. Walker F et al. Fumaderm in daily practice for psoriasis: dosing, efficacy and quality of life. Br J Dermatol 2014; 171:1197–205.

49. Schmieder A et al. Impact of fumaric acid esters on cardiovascular risk factors and depression in psoriasis: a prospective pilot study. Arch Dermatol Res 2015; 307:413–24.

50. Balasubramaniam P et al. Fumaric acid esters in severe psoriasis, including experience of use in combination with other systemic modalities. Br J Dermatol 2004; 150:741–6.

51. Wilsmann-Theis D et al. Systemic antipsoriatic combination therapy with fumaric acid esters for plaquetype psoriasis: report on 17 cases. Dermatology 2015; 230:119–27.

52. Inzinger M et al. Methotrexate vs. fumaric acid esters in moderate-to-severe chronic plaque psoriasis: data registry report on the efficacy under daily life conditions. J Eur Acad Dermatol Venereol 2013; 27:861–6.

53. Hoefnagel JJ et al. Longterm safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol 2003; 149:363–9.

54. Philipp S et al. Immunological changes in psoriasis patients under long-term treatment with fumaric acid esters: risk of Kaposi sarcoma occurrence? Eur J Dermatol 2013; 23:339–43.

55. Deegan AP et al. Organising pneumonia associated with fumaric acid ester treatment for psoriasis. Clin Respir J 2010; 4:248–51.

56. Ahmad K et al. Does prior treatment with fumaric acid esters predispose to tuberculosis in patients on etanercept? Clin Exp Dermatol 2007; 32:329.

57. Jennings L et al. Squamous cell carcinoma as a complication of fumaric acid ester immunosuppression. J Eur Acad Dermatol Venereol 2009; 23:1451.

58. Barth D et al. Malignant melanoma during treatment with fumaric acid esters – coincidence or treatment-related? J Dtsch Dermatol Ges 2011; 9:223–5.

59. Ermis U et al. PML in a patient treated with fumaric acid. New Engl J Med 2013; 368:1657–8.

60. van Oosten B et al. PML in a patient treated with dimethyl fumarate from a compounding pharmacy. New Engl J Med 2013; 368:1658–9.

61. Stoppe M et al. Cerebellar manifestation of PML under fumarate and after efalizumab treatment of psoriasis. J Neurol 2014; 261:1021–4.

62. Bartsch T et al. Progressive neurologic dysfunction in a psoriasis patient treated with dimethyl fumarate. Ann Neurol 2015; 78:501–14.

63. Dammeier et al. Case report of a patient with progressive multifocal leukoencephalopathy under treatment with dimethyl fumarate. BMC Neurol 2015; 15:108.

64. Hoepner R et al. Progressive multifocal leukoencephalopathy during fumarate monotherapy of psoriasis. Neurol Neuroimmunol Neuroinflamm 2015; 2:e85.

65. Nieuwkamp DJ et al. PML in a patient without severe lymphocytopenia receiving dimethyl fumarate. New Engl J Med 2015; 372:1474–6.

66. Fliegner L et al. Osteomalacia as an apparently rare side effect of oral fumaric acid therapy. Secondary DeToni-Debre Fanconi syndrome in the adult. Hautarzt 1992; 43:554–60.

67. Haviv YS et al. Fumaric acid ester-induced diffuse renal tubular injury presenting as Fanconi syndrome and osteomalacia. Clin Drug Invest 1999; 17:333–5.

68. Raschka C et al. Longterm treatment of psoriasis using fumaric acid preparations can be associated with severe proximal tubular damage. Hum Exp Toxicol 1999; 18:738–9.

69. Schilling F, et al. Adult Debre-de Toni-Fanconi syndrome with osteomalacia, acquired through longterm psoriasis therapy with fumaric acid ester – and a contribution to malacic osteoarthropathy. Akt Rheumatol 1999; 24:174–9.

70. Warzecha J et al. Multiple pathological fractures in acquired Fanconi's syndrome after treatment of psoriasis with fumaric acid. Unfallchirurg 2001; 104:448–51.

71. Reid C et al. de Toni-Fanconi syndrome secondary to fumaric acid esters. Br J Dermatol 2013; 169(Suppl.1):P10.

72. Hoffmann K et al. Collagenous colitis during treatment with fumaric acid esters. J Dtsch Dermatol Ges 2014; 12:1138–40.

73. Schulz KF et al; CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010; 340:c332.

74. Atwan A et al. Oral fumaric acid esters for psoriasis. Cochrane Database Syst Rev 2015; 8:CD010497.

75. Schmitt J et al. Efficacy and tolerability of biologic and nonbiologic systemic treatments for moderate-to-severe psoriasis: meta-analysis of randomized controlled trials. Br J Dermatol 2008; 159:513–26.

76. Schmitt J et al. Efficacy and safety of systemic treatments for moderate-to-severe psoriasis: metaanalysis of randomized controlled trials. Br J Dermatol 2014; 170:274–303.

77. Griffiths CE et al. A systematic review of treatments for severe psoriasis. Health Technol Assess 2000; 4:1–125.

78. Treumer F et al. Dimethylfumarate is a potent inducer of apoptosis in human T cells. J Invest Dermatol 2003; 121:1383–8.

79. Lehmann JC et al. Dimethylfumarate induces immunosuppression via glutathione depletion and subsequent induction of heme oxygenase 1. J Invest Dermatol 2007; 127:835–45.

80. Kolbach DN et al. Fumaric acid therapy in psoriasis: results and side effects of 2 years of treatment. J Am Acad Dermatol 1992; 27:769–71.

81. Anonymous. BG 12: BG 00012, BG 12/Oral Fumarate, FAG-201, second-generation fumarate derivative–Fumapharm/Biogen Idec. Drugs R D 2005; 6:229–30.

82. Gold R et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med 2012; 367:1098–107.

83. Fox RJ et al. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med 2012; 367:1087–97.

84. Naldi L et al. Traditional therapies in the management of moderate to severe chronic plaque psoriasis: an assessment of the benefits and risks. Br J Dermatol 2005; 152:597–615.

85. Mrowietz U et al. Case reports of PML in patients treated for psoriasis. N Engl J Med 2013; 369:1080–2.

86. Nast A et al. German S3-guidelines on the treatment of psoriasis vulgaris (short version). Arch Dermatol Res 2012; 304:87–113.

87. Dogra S et al. Systemic methotrexate therapy for psoriasis: past, present and future. Clin Exp Dermatol 2013; 38:573–88.

88. Lijnen R et al. Long-term safety and effectiveness of high-dose dimethylfumarate in the treatment of moderate to severe psoriasis: a prospective single-blinded follow-up study. J Dermatolog Treat 2015; 27:31–6.

89. Brewer L et al. Fumaric acid esters in the management of severe psoriasis. Clin Exp Dermatol 2007; 32:246–9.

90. Carboni I et al. Fumaric acid esters in the treatment of psoriasis: an Italian experience. J Dermatol Treat 2004; 15:23–6.

91. Litjens NHR et al. Beneficial effects of fumarate therapy in psoriasis vulgaris patients coincide with downregulation of type 1 cytokines. Br J Dermatol 2003; 148:444–51.

92. Boesken WH et al. Nephrotoxic effects of fumaric acid therapy of psoriasis. Nieren- Hochdruckkr 1998; 27:145–50.

93. Thio HB et al. Long-term systemic therapy with dimethylfumarate and monoethylfumarate (Fumaderm) in psoriasis. J Eur Acad Dermatol Venereol 1995; 4:35–40.

94. Gambichler T et al. Glutathione-S-transferase T1 genotyping and phenotyping in psoriasis patients receiving treatment with oral fumaric acid esters. J Eur Acad Dermatol Venereol 2014; 28:574–80.

95. Heelan K et al. Fumaric acid esters as a suitable first-line treatment for severe psoriasis: an Irish experience. Clin Exp Dermatol 2012; 37:793–5.

96. Gambichler T et al. Serum levels of antimicrobial peptides and proteins do not correlate with psoriasis severity and are increased after treatment with fumaric acid esters. Arch Dermatol Res 2012; 304:471–4.

97. Gambichler T et al. Monitoring peripheral blood CD4+ intracellular adenosine triphosphate concentration in patients with psoriasis treated with fumaric acid esters. Acta Derm Venereol 2012; 92:364–6.

98. Boehncke S et al. Systemic therapy of plaque- type psoriasis ameliorates endothelial cell function: results of a prospective longitudinal pilot trial. Arch Dermatol Res 2011; 303:381–8.

99. Haring N et al. Early detection of renal damage caused by fumaric acid ester therapy by determination of urinary b2-microglobulin. Br J Dermatol 2011; 164:648–51.

100. Kokelj F et al. Fumaric acid and its derivatives in the treatment of psoriasis vulgaris: our experience in forty-one patients. Acta Dermatovenerol Croat 2009; 17:170–5.

101. Sladden MJ et al. Fumaric acid esters for severe psoriasis: the Leicestershire experience. Br J Dermatol 2006; 155:843–4.

102. Fika Z et al. Fumaric acid esters in psoriasis. Br J Dermatol 2006; 154:567–8.

103. Harries MJ et al. Fumaric acid esters for severe psoriasis: a retrospective review of 58 cases. Br J Dermatol 2005; 153:549–51.

104. Stander H et al. Efficacy of fumaric acid ester monotherapy in psoriasis pustulosa palmoplantaris. Br J Dermatol 2003; 149:220–2.

105. Friedrich M et al. Addition of pentoxifylline could reduce the side effects of fumaric acid esters in the treatment of psoriasis. Acta Derm Venereol 2001; 81:429–30.

106. Mrowietz U et al. Treatment of psoriasis with fumaric acid esters: results of a prospective multicenter study. Br J Dermatol 1998; 138:456–60.

107. Hoxtermann S et al. Fumaric acid esters suppress peripheral CD4- and CD8-positive lymphocytes in psoriasis. Dermatology 1998; 196:223–30.

108. Altmeyer P et al. Efficacy and safety profile of fumaric acid esters in oral long-term therapy of severe psoriasis vulgaris. An investigation of 83 patients. Hautarzt 1996; 47:190–6.

109. Altmeyer P et al. Long term observations of lymphocyte subsets in psoriatic patients treated with fumaric acid esters. Aktuel Dermatol 1996; 22:272–7.

110. Dalhoff K et al. Acute renal failure during treatment of psoriasis with fumaric acid derivatives. Dtsch Med Wochenschr 1990; 115:1014–17.

111. Stuhlinger W et al. Nephrotoxic effects of fumaric acid ester during treatment of psoriasis. Dtsch Med Wochenschr 1990; 115:1712–15.

112. Ogilvie S et al. Proteinuria with fumaric acid ester treatment for psoriasis. Clin Exp Dermatol 2011; 36:632–4.

113. Philipp S et al. Kaposi-sarcoma during therapy with fumaric acid esters. Eur J Immunol 2009; 39 (Suppl. 1):PC08/32.

TABLES AND FIGURES

Table 1: Summary of characteristics and outcomes from randomized controlled trials of fumaric acid esters (FAEs) used in the treatment of psoriasis

No	Study	Sample size	Treatment duration, weeks	Risk of bias	Treatment arm	FAE dosage per day	PASI-75 (%)	Mean change in PASI (%)	Proportion with AEs (%)	Withdrawal rates due to AEs (%)
	FAEs in combination with other treatments compared with FAEs alone									
1.	Balak et al.[38]	50	12	Low	FAEs + placebo	720 mg DMF + 570 mg MEF	20	- 65	84	32
					FAEs + cetirizine	720 mg DMF + 570 mg MEF	20	- 66	84	24
2.	Gollnick et al.[37]	134	13	Low	FAEs + placebo ointment	720 mg DMF + 570 mg MEF	NR	- 52	79	30
					FAEs + calcipotriol ointment	720 mg DMF + 570 mg MEF	NR	- 76	82	21
	FAEs compared with other systemic psoriasis treatments									
3.	Fallah Arani et al.[36]	54	16	Unclear	FAEs	720 mg DMF + 570 mg	19	- 42	92	8
					MTX	NA	24	- 54	100	16
	FAEs compared with placebo									
4.	Altmeyer et al.[19]	100	16	Unclear	FAEs	720 mg DMF + 570 mg MEF	NR	- 50	76	39
					Placebo	NA	NR	NR	16	2
5.	Peeters et al.[41]	27	16	Unclear	FAEs	720 mg DMF + 570 mg MEF	NR	NR	69	15
					Placebo	NA	NR	NR	NR	0
6.	Nieboer et al.[18]	45	16	Unclear	FAEs (DMF + MEF)	480 mg DMF + 380 mg MEF	NR	NR	87	35
					FAEs (DMF)	480 mg DMF	NR	NR	86	18
7.	Nugteren-Huying et al.[35]	39	16	Unclear	FAEs (DMF + MEF)	720 mg DMF + 570 mg MEF	NR	NR	NR	8
					FAES (OF + MEF)	1704 mg OF + 48 mg MEF	NR	NR	NR	23
					Placebo	NA	NR	NR	NR	8

AEs, adverse events; DMF, dimethylfumarate; MEF, monoethylfumarate; MTX, methotrexate; NA, not applicable; NR, not reported; OF, octylfumarate; PASI, Psoriasis Area and Severity Index.

Table 2: Summary of characteristics and outcomes from observational studies of fumaric acid esters (FAEs) used in the treatment of psoriasis

No	Study	Study design	Sample size	Treatment duration, months	Treatment arm	Max. FAE dosage per day	PASI-75 (%)	Mean change in PASI (%)	Proportion with AEs (%)	Withdrawal rates due to AEs (%)
	Long-term studies (treatment duration > 12 months)									
1.	Wilmann-Theis et al. (2015)[47]	Retrospective multi-centre case series	17	Mean 21	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	82	12
2.	Lijnen et al. (2015)[83]	Prospective single-centre cohort study	176	Median 28	DMF + MEF (Fumaderm)	1680 mg DMF	NR	NR	86	25
3.	Steinz et al. (2014)[41]	Retrospective single-centre case series	6	Mean 18	FAEs	720 mg DMF + 570 mg MEF	33	NR	80	0
4.	Walker et al. (2014)[44]	Prospective multi-centre cohort study	249	12	FAEs	720 mg DMF + 570 mg MEF	NR	-50	76	39
5.	Ismail et al. (2014)[42]	Retrospective single-centre cohort study	249	Mean 28	FAEs	720 mg DMF + 570 mg MEF	NR	NR	NR	47
6.	Balak et al. (2013)[40]	Retrospective multi-centre case series	14	Median 10	DMF + MEF (Dutch formulations)	720 mg DMF + 570 mg MEF	NR	NR	64	14
7.	Thaci et al. (2013)[39]	Retrospective multi-centre cross-sectional study	69	Mean 27	DMF + MEF (Fumaderm)	NR	NR	NR	64	6
8.	Wain et al. (2010)[43]	Prospective single-centre cohort study	80	3 – 60	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	8	19	74	36

Table 2 (continued)

9.	Reich et al. (2009)[38]	Retrospective multi-centre, cross-sectional study	984	Mean 44	DMF + MEF (Fumaderm)	NR	NR	79	NR	2
10.	Brewer et al. (2007)[84]	Retrospective single-centre case series	31	Mean 8	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	87	26
11.	Balasu-bramani et al. (2004)[46]	Retrospective single-centre case series	12	Mean 10	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	83	8
12.	Carboni et al. (2004)[85]	Prospective single-centre cohort study	40	Mean 15	DMF + MEF (Fumaderm)	360 mg DMF + 285 MEF	NR	80	27	10
13.	Hoefnagel et al. (2003)[49]	Retrospective single-centre cohort study	66	0 - 168	FAEs	720 mg DMF + 570 mg MEF	NR	NR	73	40
14.	Litjens et al. (2003)[86]	Prospective single-centre cohort study	12	24	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	NR	42
15.	Boesken et al. (1998)[87]	Prospective single-centre cohort study	47	Mean 17	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	45	NR
16.	Thio et al. (1995)[88]	Retrospective single-centre cohort study	83	1-36	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	NR	11
17.	Kolbach et al. (1992)[76]	Prospective single-centre cohort study	129	1-24	DMF + MEF (Fumaderm)	480 mg DMF + 380 mg MEF	NR	NR	NR	18
			67		DMF	240 mg DMF	NR	NR	NR	26

Table 2 (continued)

18.	Bayard et al. (1987)[16]	Prospective single-centre cohort study	30	12-14	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	NR	17
			18	3	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	NR	11
	Short-term studies (treatment duration < 12 months)									
19.	Schmieder et al. (2015)[45]	Prospective multi-centre cohort study	39	4	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	27	59	77	13
20.	Gambichler et al. (2014)[89]	Prospective single-centre cohort study	106	6	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	28	71	NR	16
21.	Inzinger et al. (2013)4[8]	Retrospective single-centre cohort study	200	3-12	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	27	13	NR	31
22.	Heelan et al. (2012)[90]	Retrospective single-centre cohort study	45	Median 10	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	66	33
23.	Gambichler et al. (2012)[91]	Prospective single-centre cohort study	32	3	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	54	NR	13
24.	Gambichler et al. (2012)[92]	Prospective single-centre cohort study	21	4	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	67	NR	NR
25.	Boehncke et al. (2011)[93]	Prospective single-centre cohort study	13	6	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	31	71	NR	8
26.	Häring et al. (2011)[94]	Prospective single-centre case series	23	NR	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	NR	NR

Table 2 (continued)

27.	Kokelj et al. (2009)[95]	Prospective single-centre cohort study	41	4	DMF + MEF (Fumaderm)	216 mg DMF + 126 mg MEF	NR	32	NR	7
28.	Sladden et al. (2006)[96]	Retrospective single-centre cohort study	30	NR	DMF + MEF	720 mg DMF + 570 mg MEF	NR	NR	NR	30
29.	Fika et al. (2006)[97]	Retrospective single-centre case series	11	NR	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	NR	18
30.	Harries et al. (2005)[98]	Retrospective single-centre case series	58	NR	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	66	26
31.	Ständer et al.(2003)[99]	Prospective single-centre cohort study	13	6	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	75	NR	15
32.	Friedrich et al. (2001)[100]	Prospective single-centre randomized cohort study	21	2	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	76	19
			23	2	DMF + MEF (Fumaderm) + pentoxifylline	720 mg DMF + 570 mg MEF	NR	NR	52	17
33.	Mrowietz et al. (1998)[101]	Prospective multi-centre cohort study	101	4	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	80	69	7
34.	Höxter-mann et al. (1998)[102]	Prospective single-centre cohort study	10	12	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	86	NR	0
35.	Altmeyer et al. (1996)[103]	Prospective single-centre cohort study	83	12	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	76	62	13

Table 2 (continued)

36.	Altmeyer et al. (1996)[104]	Prospective single-centre cohort study	16	3	DMF + MEF (Fumaderm)	720 mg DMF + 570 mg MEF	NR	NR	NR	NR
37.	Nieboer et al. (1989)[17]	Prospective single centre cohort studies	36	Mean 10	DMF + MEF	720 mg DMF + 570 mg MEF	NR	NR	NR	8
			38	4	MEF	240 mg MEF	NR	NR	NR	5
			42	4	DMF	240 mg DMF	NR	NR	NR	27
			20	3	MEF	240 - 720 mg MEF	NR	NR	NR	0
			56	4-9	DMF	240 mg DMF	NR	NR	NR	27

Table 3: Adverse events associated with fumaric acid esters in psoriasis treatment reported in ≥ 5 patients in randomized controlled trials and observational studies

Adverse event	Combined total number of patients
Lymphocytopenia	1115
Gastrointestinal complaints	670
Flushing	626
Increase in liver enzymes	341
Eosinophilia	254
Proteinuria	242
Leukocytopenia	218
Increase in creatinine	79
Pruritus	55
Fatigue	55
Headache	34
Malaise	33
Increase in urea	19
Dizziness	15
Increase in cholesterol	12
Hypertension	10
Dermatitis/rash	9
Hyperkalaemia	8

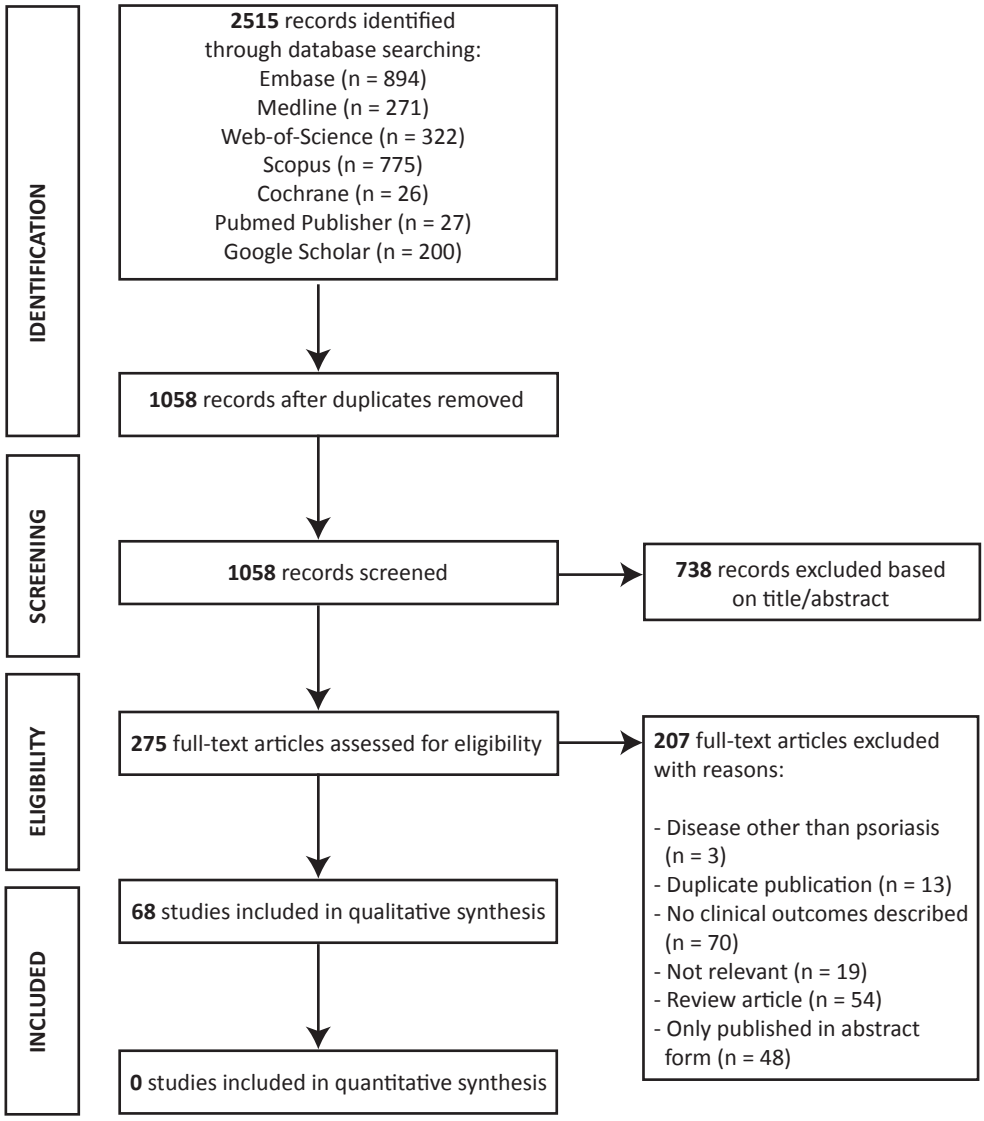
Table 4: Summary of safety outcomes of FAEs in psoriasis treatment reported in case reports

Adverse event	No. of cases	References
Related to renal events		
Acute renal insufficiency	7	Roodnat (1989)[13], Dalhoff (1990)[105], Stuhlinger (1990)[106]
Fanconi syndrome	6	Fliegner (1992)[62], Haviv (1999)[63], Raschka (1999)[64],Schilling (1999)[65], Warzecha (2001)[66], Reid (2013)[67]
Proteinuria	3	Ogilvie (2011)[107]
Potentially related to immunosuppression		
Progressive multifocal leuko-encephaopathy (PML)	7	Ermis (2013)[55], van Oosten (2013)[56],Stoppe (2014)[57], Bartsch (2015)[58], Dammeier (2015)[108], Hoep-ner (2015)[60], Nieuwkamp (2015)[109]
Malignant melanoma	2	Barth (2011)[54]
Tuberculous lymphadenitis	1	Ahmad (2007)[52]
Organizing pneumonia	1	Deegan (2010)[51]

Table 4 (continued)

Squamous cell carcinoma	1	Jennings (2009)[53]
Kaposi sarcoma	1	Phillips (2009, 2013)[50,110]
Other adverse events		
Collagenous colitis	1	Hoffmann (2014)[68]

Figure 1: Overview of literature search and selection



Menno A. de Rie & Jan D. Bos

"..it can be anticipated that new compounds will be encountered by serendipity, as in the case of cyclosporine A and sirolimus. In addition, immunology and biochemistry are showing us where points of interest in T-cell activation pathways are located."

Cyclosporine immunotherapy. *Clin Dermatol.* 1997 Sep-Oct;15(5):811-21

Jay Frank Schamberg

"If we can gain increased light on the modus operandi of remedies in a disease of unknown origin, it may lead later to a more accurate interpretation of the nature of the morbid process involved."

The known and the unknown about psoriasis. *JAMA*. 1924;83(16):1211-1214.

CHAPTER| 3

Combination therapy of fumaric acid esters and etanercept versus etanercept monotherapy in psoriasis: A randomized exploratory study

J. Sun van Bezooijen[1,2]

Deepak M.W. Balak[1]

Martijn B.A. van Doorn[1]

Casper W.N. Looman[3]

Marco W.J. Schreurs[4]

Birgit C.P. Koch[2]

Teun van Gelder[2,5]

Errol P. Prens[1]

[1]Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

[2]Department of Hospital Pharmacy, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

[3]Department of Public Health, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

[4]Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

[5]Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

Dermatology. 2016;232(4):407-14.

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. In this study, we aimed 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 fumaric acid esters 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 Physician Global Assessment (PGA) of clear/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 versus 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 of 4.76% for the monotherapy group ($p = 0.11$). In the combination therapy group, 94% (17 out of 18 patients) of patients had a PGA of clear/almost clear versus 64% (9 out of 14 patients) in the monotherapy group ($p = 0.064$). The incidence of mild gastrointestinal complaints was higher in the combination group than in the monotherapy group.

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

INTRODUCTION

Psoriasis is a chronic, immune-mediated inflammatory skin disease affecting approximately 1-3% 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-tumour necrosis factor (TNF)-α 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 TNF-α via a recombinant soluble p75 TNF-α receptor fused to an IgG1 constant chain and combines a satisfactory efficacy with a favourable safety profile.[9] Despite the increasing use of interleukin-12/23 and interleukin-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 × 50 mg/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 × 50 mg weekly, current guidelines and the label recommend reducing the dose of etanercept to 1 × 50 mg weekly from week 12 onwards.[13] However, at this once weekly dosage, the 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 (FAEs), or fumarates, have been in use for the treatment of psoriasis for over 4 decades and are considered safe and effective as long as the treatment guidelines are followed.[18-22] The fumaric acid ester derivative dimethylfumarate is metabolized in the body to monomethylfumarate, which is regarded as the most bio-active metabolite. In daily clinical practice, we noted in some patients that the addition of oral fumarates to etanercept 50 mg once weekly improved the clinical response and drug survival (pers. unpubl. observation). At present, evidence supporting the safety of the combination therapy of etanercept and FAEs 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 and tolerability of the combination therapy of etanercept with FAEs in psoriasis.

PATIENTS AND METHODS

Study design This was an investigator-initiated, single-centre, randomized, assessor-blinded study conducted in the Department of Dermatology in the Erasmus Medical Centre, Rotterdam, the Netherlands, between July 2013 and June 2015. This study was approved by the Institutional Review Board of the Erasmus University Medical Centre Rotterdam (MEC-2011-500). 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 No. 2011-005685-38. This investigator-initiated study was supported by a grant of Pfizer Pharmaceuticals. Pfizer was not involved in any study procedure, but 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 had stable, moderate-to-severe plaque psoriasis for more than 6 months, affecting more than 10% of the body surface area, had a PASI >10 at screening and at baseline, and were candidates for biologic treatment according to the approved product labelling and to Dutch guidelines.
Patients were recruited from the dermatology outpatient clinic from our hospital. Patients were excluded if they had any other subtype of psoriasis or previous treatment failure on etanercept or fumarates or had a clinically significant adverse event with prior use of both drugs. Pregnant or lactating women were not eligible.

Patients with severe recalcitrant psoriasis who had experienced a lack of efficacy during prior use of other biologics were also eligible, in order to represent real-life daily practice. The wash-out period for a TNF-blocking agent or any other biologic was 3 months, and for other systemic treatments (including fumarates) or UV therapy it was 4 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 weeks 12 and 48, the proportion of patients with a PGA clear or almost clear, the change in DLQI score, and treatment satisfaction (visual analogue scale) scores after 12, 24 and 48 weeks. Drug survival after 1 year was assessed by a post hoc analysis and was defined as the proportion of patients who were still under the treatment they had originally been randomized to and who also achieved at least a 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 (E.P.P.) was blinded to treatment throughout the course of the study.

All patients received etanercept 50 mg subcutaneously twice weekly for 12 weeks followed by 50 mg 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 within 4 weeks from 215 mg once daily up to a maximum of 215 mg 4 times a day. A large batch of enteric-coated tablets containing a total of 215 mg fumaric acid esters (120 mg dimethylfumarate and 95 mg calcium mono-ethylfumarate) was specifically manufactured for this trial by Fagron (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 (suppl. fig. 1, 2). Patient visits were scheduled at weeks 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, γ-glutamyltransferase, serum creatinine, sedimentation rate, C-reactive protein and urine analysis). Patients were asked to fill in the DLQI questionnaire and a visual analogue scale for 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 analysed using a $\chi 2$ or Fisher exact test, 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 in 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 the unpaired t test for comparing changes in DLQI and visual analogue scale 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

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

Patient demographics and baseline disease characteristics are shown in table 1. At baseline only the body mass index and previous use of biologics differed significantly between the combination and monotherapy groups ($p < 0.05$). The flow chart in figure 2 shows the number of patients who were enrolled and dropped out from 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%; $p = 0.71$).

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. The longitudinal analysis demonstrated a reduction of the PASI score for the combination therapy and monotherapy group from baseline up to week 48. All observations were made in a 4-week period separated into two groups; The medians per group per time are shown in figure 3. The reduction in the PASI score per week for the combination therapy was 5.97% (95% confidence interval, CI: 5.08-6.85) and in the monotherapy group 4.76% (95% CI: 3.57-5.93; $p = 0.11$). In the online supplementary data, figures 1 and 2 show the changes in the 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 1 out of 5 switchers achieved a PASI-75 improvement after 24 weeks of combination treatment. The proportion of patients with a Physician Global Assessment (PGA) score of 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 under etanercept therapy after 48 weeks. The drug survival in the combination group was 72%. Thirteen out of 18 patients (72%) remained under etanercept with FAE 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) versus 60% (95% CI: 35-85), with p = 0.71.

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

Quality of life and treatment satisfaction The results of the median change in the Dermatology Quality of Life Index (DLQI) score are shown in table 3.3. In both groups, DLQI scores decreased significantly over time. The difference between the two groups at 24 and 48 weeks was not statistically significant. The results of the visual analogue scale 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 5 switchers were excluded from the analysis.

Adverse events The most frequently reported adverse event in the monotherapy group was flu-like symptoms, present in 9 versus 14 in the combination therapy group. Gastrointestinal complaints (38), consisting of diarrhoea and abdominal cramps, were most frequently observed in the combination therapy group compared to the monotherapy group (1). One patient developed iron deficiency anaemia, 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) adverse events led to discontinuation of the study. No leucopenia and/or lymphopenia were observed in either treatment group. All adverse events are listed in table 5.

DISCUSSION

In this exploratory randomized study we prospectively compared the clinical efficacy, safety, and tolerability of etanercept with oral FAEs in combination therapy with etanercept monotherapy per label up to 48 weeks of treatment. The assumption was that addition of

FAEs 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 FAEs 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 the combination group were remarkably high, and the difference with the monotherapy group approached statistical significance. Furthermore, the DLQI and visual analogue scale scores did not differ between the two groups, suggesting that concomitant use of FAEs (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 the study of Gottlieb et al., in which 239 patients were randomized to etanercept monotherapy or etanercept with methotrexate (MTX).[9,12] 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%; $p < 0.0001$).

In daily practice 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 neutralizing antidrug 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 etanercept, inhibition of antidrug 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 FAEs could be safely combined with biologics in an off-label real-life setting.[26] Although a similar FAE dose of 4 times 215 mg a day as in our study was used, their study comprised only 4 patients with FAEs in combination with etanercept. In 2 cases, FAEs were added to etanercept treatment, while the other 2 patients had started on FAEs and were subsequently treated additionally with etanercept. Only 2 of the 4 cases showed a good clinical response to the combination treatment after 6 months and 2 years.[26] FAEs 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 FAEs appears to be relatively safe and to have an acceptable tolerability up to 48 weeks. Since higher doses of FAEs are known to be associated with an increased incidence of side effects, an adjusted dose of up to 215 mg 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 FAEs because of side effects. A common and potential serious side effect of FAEs, leucopenia and/or lymphopenia, was not observed in any of our patients during the 1-year course of the study. This is particularly of interest because of the recently reported cases of progressive multifocal leukoencephalopathy in long-term FAEs users. It has to be noted that most of the reported progressive multifocal leukoencephalopathy cases were lymphopenic for a prolonged period.

It is remarkable that the occurrence of gastrointestinal 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 FAE-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 (1 in the monotherapy and 2 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 group. 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 FAE 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 FAEs. Finally, in the etanercept monotherapy group, 5 patients were switched to the combination therapy because of inefficacy. However, only 1 of these 5 patients showed a clear improvement after the addition of FAEs to etanercept, the other 4 had persistent brittle psoriasis and did not achieve a PASI-75 response and had to be switched to other biologics after week 48. These 4 patients had a notorious therapy-resist-

ant psoriasis who had failed several other antipsoriatic therapies including biologics.

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

REFERENCES

1. Raychaudhuri SP et al. The prevalence of psoriasis in the world. J Eur Acad Dermatol Venereol 2001; 15: 16–17.
2. Rapp SR et al. Psoriasis causes as much disability as other major medical diseases. J Am Acad Dermatol 1999; 41: 401–407.
3. Stern RS et al. 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: 136–139.
4. Mrowietz U 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: 438–453.
5. Zandvliet ML et al. Monitoring antigen-specific biologics: current knowledge and future prospects. Ther Drug Monit 2013; 35: 588–594.
6. Gniadecki R et al. Comparison of drug survival rates for adalimumab, etanercept and infliximab in patients with psoriasis vulgaris. Br J Dermatol 2011; 164: 1091–1096.
7. Van den Reek JM et al. Determinants of drug survival for etanercept in a long-term daily practice cohort of patients with psoriasis. Br J Dermatol 2014; 170: 415–424.
8. Gniadecki R et al. Comparison of long-term drug survival and safety of biologic agents in patients with psoriasis vulgaris. Br J Dermatol 2015; 172: 244–252.
9. Leonardi CL et al. Etanercept Psoriasis Study Group: Etanercept as monotherapy in patients with psoriasis. N Engl J Med 2003; 349: 2014–2022.
10. Cassano N et al. 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: 225–229.
11. Papp KA et al. Etanercept Psoriasis Study Group: A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. Br J Dermatol 2005; 152: 1304–1312.
12. Tyring S et al. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. Lancet 2006; 367: 29–35.
13. Nast A et al. Deutsche Dermatologische Gesellschaft, Berufsverband Deutscher Dermatologen: S3 – guidelines on the treatment of psoriasis vulgaris (English version). Update. J Dtsch Dermatol Ges 2012; 10(suppl 2):S1–S95.
14. Foley PA 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: 1135–1143.
15. Heffernan MP. Combining traditional systemic and biologic therapies for psoriasis. Semin Cutan Med Surg 2010; 29: 67–69.
16. Wilsmann-Theis D et al. Systemic antipsoriatic combination therapy with fumaric acid esters for plaquetype psoriasis: report on 17 cases. Dermatology 2015; 230: 119–127.
17. Calzavara-Pinton PG et al. Synergism between narrowband ultraviolet B phototherapy and etanercept for the treatment of plaquetype psoriasis. Br J Dermatol 2013; 169: 130–136.
18. Warren RB et al. Biologic therapies for psoriasis: practical experience in a UK tertiary referral centre. Br J Dermatol 2009; 160: 162–169.
19. Reich K et al. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis – a retrospective study (future). J Dtsch Dermatol Ges 2009; 7: 603–611.
20. Hoefnagel JJ et al.Long-term safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol 2003; 149: 363–369.
21. Wain EM et al. Treatment of severe, recalcitrant, chronic plaque psoriasis with fumaric acid esters: a prospective study. Br J Dermatol 2010; 162: 427–434.
22. Fallah Arani S et al. Fumarates vs methotrexate in moderate to severe chronic plaque psoriasis: a multicenter prospective randomized controlled clinical trial. Br J Dermatol 2011; 164: 855–861.
23. Van Bezooijen JS et al. Combining biologics with methotrexate in psoriasis: a systematic review. Br J Dermatol 2015; 172: 1676–1680.
24. Armstrong AW et al. 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: 432–438.

25. Krieckaert CL et al. Comparison of long-term clinical outcome with etanercept treatment and adalimumab treatment of rheumatoid arthritis with respect to immunogenicity. Arthritis Rheum 2012; 64: 3850–3855.

26. Wilsmann-Theis D et al. Systemic antipsoriatic combination therapy with fumaric acid esters for plaque-type psoriasis: report on 17 cases. Dermatology 2015; 230: 119–127.

27. Balasubramaniam P et al. Fumaric acid esters in severe psoriasis, including experience of use in combination with other systemic modalities. Br J Dermatol 2004; 150: 741–746.

28. Busard C et al. Combined use of systemic agents for psoriasis: a systematic review. JAMA Dermatol 2014; 150: 1213–1220.

29. Lopez-Ferrer A et al. 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: 1141–1147.

30. Piaserico S et al.Psocare Study Group: Efficacy of switching between tumor necrosis factor-alfa inhibitors in psoriasis: results from the Italian Psocare registry. J Am Acad Dermatol 2014; 70: 257–262.

TABLES AND FIGURES

Table 1: Baseline demographic and clinical characteristics of the study population

Patient characteristic	Etanercept monotherapy (n = 15)	Etanercept combined with FAEs (n = 18)
Mean age ± SD	45 ± 16	43 ± 17
Sex		
Male	8 (53%)	14(78%)
Female	7 (47%)	4 (22%)
Mean BMI ± SD	30 ± 6	26 ± 6
Mean duration of psoriasis in years ± SD	19 ± 10	22 ± 10
History of psoriatic arthritis	1 (7%)	5 (28%)
Previous psoriasis treatments		
Phototherapy	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%)
Median PASI at baseline	14 (11-21)	12 (10-16)
Median DLQI at baseline	9 (5-20)	8 (3-13)

Data are numbers (percentages), means ± SD, or medians (interquartile ranges)

Table 2: Efficacy analyses of proportion of patients achieving a PASI-75 response and a PGA clear/almost clear

	Etanercept monotherapy (n = 15)	**Etanercept combined with FAEs (n = 18)**	**P value**
PASI-75 response			
Week 12	64 (39-89)	67 (41-91)	1.00
Week 24	57 (31-83)	78 (59-97)	0.27
Week 48	64 (42-86)	87 (70-100)	0.22
PGA clear/almost clear			
Week 12	57 (31-83)	89 (74-100)	0.01
Week 24	64 (39-89)	94 (84-105)	0.06
Week 48	64 (39-89)	87 (70-100)	0.22

Data are means with 95% confidence intervals. P values obtained with Fisher's exact test.

Table 3: Change in DLQI

	Etanercept monotherapy (n = 15)	**Etanercept combined with FAEs (n = 18)**	**P value**
Week 12	5.5 ± 6.8	7.1 ± 6.9	0.53
Week 24	5.4 ± 7.9	7.1 ± 6.4	0.56
Week 48	9.4 ± 8.6	7.3 ± 6.2	0.51

Data are means ± SD. P values obtained with unpaired t test.

Table 4: Treatment satisfaction measured by visual analogue scale (VAS)

	Etanercept monotherapy (n = 15)	**Etanercept combined with FAEs (n = 18)**	**P value**
Week 12	8.0 ± 1.5	8.1 ± 1.6	0.92
Week 24	8.1 ± 1.5	8.1 ± 1.4	0.95
Week 48	8.9 ± 1.2	8.2 ± 1.7	0.30

Data are means ± SD. P values obtained with unpaired t test.

Table 5: Overview of adverse events

	Etanercept mono- therapy (n = 15)	**Etanercept combined with FAEs (n = 18)**
Major adverse events		
Serious adverse event	1	2
Severe adverse event	-	-
Minor adverse events		
Gastrointestinal complaints	1	11
Flushing	1	4
Elevation of liver enzymes	-	1
Headache	-	1
Influenza-like symptoms	7	10
Fatigue	1	1
Pruritus	1	1
Injection-site reactions	1	1
Other	1	2

Figure 1: Study design overview

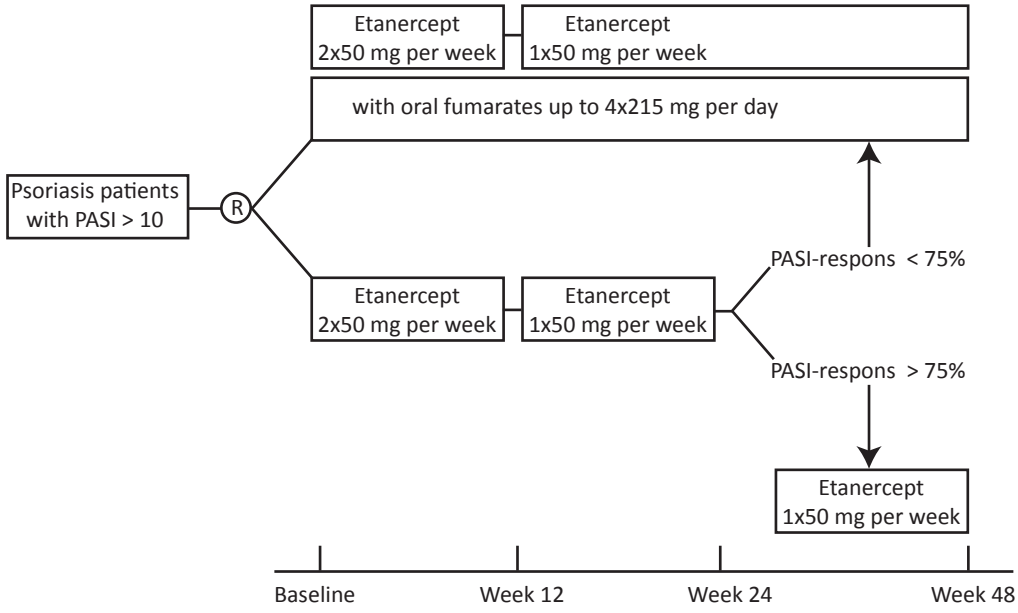


Figure 2: Study enrollment overview

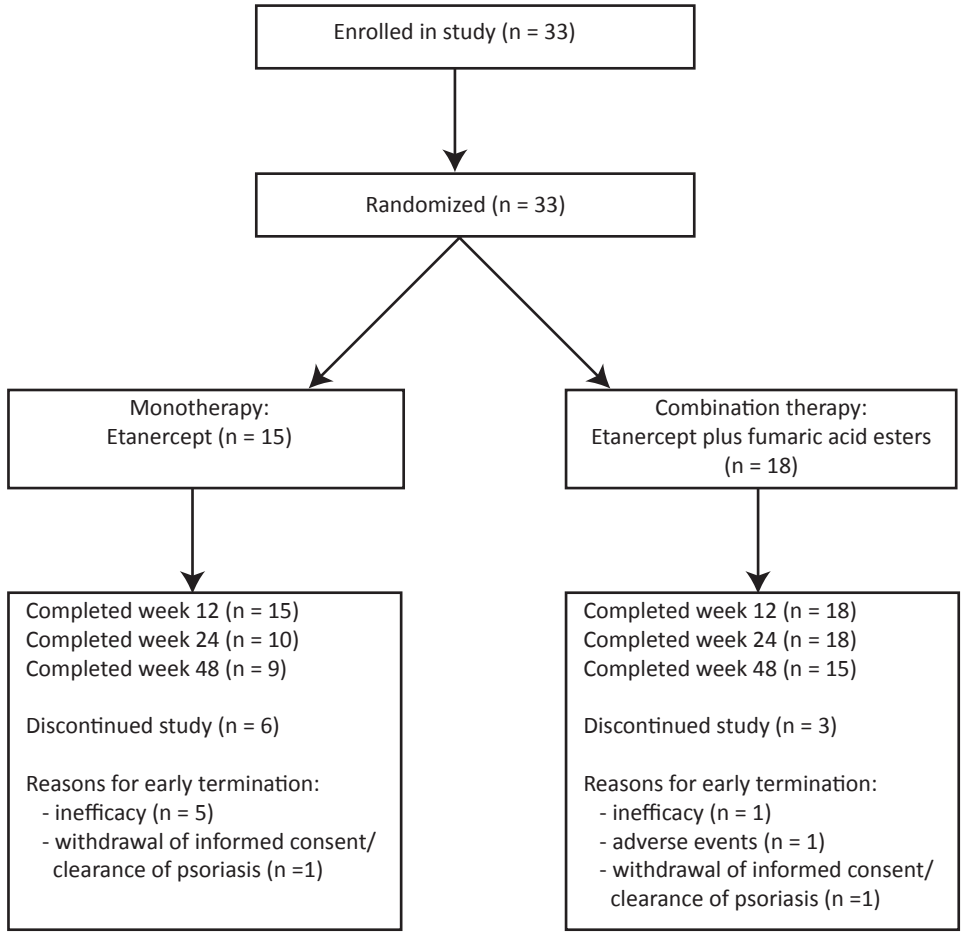
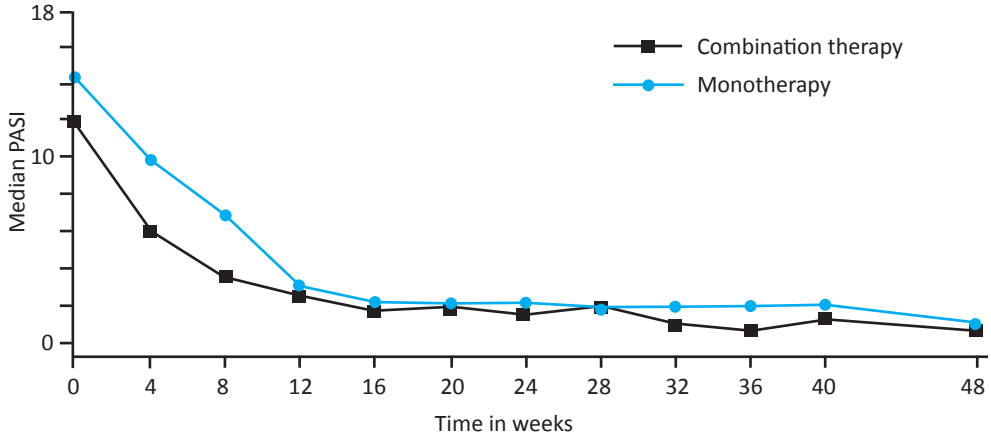


Figure 3: Efficacy over time of combination therapy of etanercept with fumaric acid esters versus etanercept monotherapy



| PART III |
SAFETY OF FUMARIC ACID ESTERS IN PSORIASIS

Arthur van Harlingen

"For the drug has not yet been discovered which will surely take away all tendency to the recurrence of psoriasis, and whoever promises a cure, in the wider sense of the word, to his patient, will in a very great number of cases find that he has been too sanguine."

The treatment of psoriasis. *JAMA*. 1883; I(18):530-532.

CHAPTER| 4

Addition of an oral histamine antagonist to reduce adverse events associated with fumaric acid esters in the treatment of psoriasis: A randomized double-blind placebo-controlled trial

Deepak M.W. Balak[1]

Shiva Fallah-Arani[1]

Clarieke M. Venema[1]

H.A. Martino Neumann[1]

H. Bing Thio[1]

[1]Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

Br J Dermatol. 2015 Mar;172(3):754-9.

ABSTRACT

Background: Fumaric acid esters (FAEs) are considered an effective and safe long- term treatment for psoriasis. However, 30–40% of patients need to discontinue FAE treatment due to intolerable adverse events. In this study, we aimed to assess whether the addition of cetirizine, an oral histamine-1 receptor antagonist, to FAEs would reduce the incidence of adverse events.

Methods: In a randomized, double-blind, placebo-controlled trial, patients with psoriasis with a Psoriasis Area and Severity Index ≥ 10 starting an FAE up to a dose of dimethylfumarate 720 mg per day were randomized 1 : 1 to receive either additional cetirizine 10 mg once daily (n = 25) or placebo (n = 25) for 12 weeks. Randomization and treatment allocation were done at our hospital trial pharmacy. Primary outcomes were the incidence of adverse events and the proportion of patients discontinuing treatment.

Results: Fifty patients (33 male, 17 female; median age 44 years) were enrolled. Addition of cetirizine did not reduce the incidence of adverse events compared with placebo (84% vs. 84%, P = 1·00). The types of adverse events were not different between the cetirizine and placebo groups, the most common being gastrointestinal complaints (68% vs. 64%) and flushes (60% vs. 48%). The proportion of patients discontinuing treatment was not statistically different between the cetirizine and placebo groups (24% vs. 32%, P = 0·53).

Conclusions: Addition of oral cetirizine 10 mg once daily to FAE treatment did not reduce adverse events in patients with psoriasis during the first 12 weeks of treatment. The mechanisms underlying FAE-induced gastrointestinal and flushing symptoms likely involve mediators other than histamine.

Trial registration: Dutch Trial Registry (http://www.trialregister.nl/) registration number NTR744.

INTRODUCTION

Fumaric acid esters (FAEs), or fumarates, are small molecules with immunomodulatory properties.[1] For over three decades, FAEs have been used as an oral treatment in patients with chronic plaque psoriasis, with a favourable long-term efficacy and safety profile.[2,3] The efficacy of FAEs is comparable with that of methotrexate.[4,5] To date, there are no indications that long-term FAE treatment is associated with an increased risk of malignancies or infections, making FAEs particularly suitable for maintenance therapy of psoriasis.[3,6]

An important limitation of FAE treatment is intolerable adverse events, which result in treatment discontinuation in 30–40% of patients.[7,8] The most commonly occurring adverse events of FAEs are gastrointestinal complaints and flushing. These relatively mild but inconvenient side-effects occur predominantly during the first 3 months of FAE treatment. In order to improve the tolerability of FAEs, the current guidelines recommend slowly increasing the dosage of the FAE using a standardized progressive dosing regimen.[2] In this dosing regimen, the maximum daily dosage of 720 mg dimethylfumarate is reached within 9 weeks. If patients have adverse events, it is advised that they increase the dosage more slowly, decrease the dosage, or temporarily stop the FAE.

Another strategy pursued in daily clinical practice when patients on FAEs experience adverse events is to prescribe an oral histamine antagonist in order to decrease these symptoms.[9,10] The adverse events frequently reported during FAE treatment are similar to several histamine-mediated symptoms, such as diarrhoea, abdominal complaints and flushing.[11] Blocking histamine might therefore be helpful in decreasing these adverse events during FAE treatment. Furthermore, treatment with a histamine antagonist could have an additional clinical benefit in patients with psoriasis, as histamine antagonists may have antipsoriatic effects.[12,13] However, to date there is no clear evidence that the addition of a histamine antagonist to FAE treatment improves the tolerability or the efficacy of FAEs in the treatment of psoriasis.

In this randomized placebo-controlled trial, we aimed to determine whether the addition of cetirizine, an oral histamine-1 receptor antagonist, to FAE treatment would reduce the incidence of adverse events in patients with moderate-to- severe plaque psoriasis. In addition, we aimed to assess whether the addition of cetirizine to FAE treatment would

increase the efficacy of FAEs.

PATIENT AND METHODS

Study design This was an investigator-initiated, single-centre, randomized, double-blind, placebo-controlled trial conducted at the dermatology outpatient clinic of Erasmus Medical Center, Rotterdam, The Netherlands in the period 2009–2012. This study was approved by the medical ethics committee of the Erasmus Medical Center (MEC 2005-500). The study was conducted according to the principles of the Declaration of Helsinki. All patients gave written informed consent. The trial protocol was registered at the Dutch Trial Registry (http://www.trialregister.nl/) under registration number NTR744. There were no funding sources.

Study population Eligible for inclusion were patients aged 18 years or older with moderate-to-severe chronic plaque psoriasis, who had a Psoriasis Area and Severity Index (PASI) ≥ 10 and who were candidates to start FAE treatment. The exclusion criteria were renal disease, liver disease, a medical history of malignancies, pregnancy and lactation. Patients had to discontinue all psoriasis treatments before enrolment, with a washout period of 4 weeks for systemic treatment and phototherapy, or 2 weeks for topical psoriasis treatment. During the study psoriasis treatments were not allowed expect for bland emollients.

Study procedures Enrolled subjects were randomized at baseline in a 1: 1 ratio to receive either FAEs plus cetirizine 10 mg once daily or FAEs plus a matching placebo for 12 weeks, followed by a follow- up period of 8 weeks. Randomization and treatment allocation were done at the hospital trial pharmacy. Patients and physician assessors were both blinded for the allocated treatment group.

All patients were treated with a Dutch FAE formulation with enteric-coated tablets containing 105 mg FAEs (30 mg dimethylfumarate and 75 mg calcium monoethylfumarate) for the first 3 weeks, followed by tablets of 215 mg FAEs (120 mg dimethylfumarate and 95 mg calcium monoethylfumarate) (Pharmacy ‘De Magistrale Bereider’, Oud-Beijerland, The Netherlands). In previous studies we analysed this FAE formulation for pharmacokinetic properties and for efficacy compared with methotrexate.[4,14] FAEs were given according to a standardized progressive dosing regimen, starting at 30 mg dimethylfumarate per day with an incremental increase up to a maximum daily dosage of 720 mg dimethylfumarate within 9 weeks.[2]

Patients were seen at weeks 0, 4, 8, 12, and 20. At each study visit adverse events were reviewed and routine laboratory testing was performed: full blood count with differential, liver function tests, serum creatinine levels, cholesterol levels and urine analysis. Furthermore, PASI was recorded at each study visit, and health-related quality of life was measured using the Skindex-29 questionnaire at week 0 and week 12.

Skindex-29 is a validated questionnaire that measures dermatology-specific health-related quality of life on a scale ranging from 0 to 100, with higher scores indicating lower quality of life.[15] There are currently several instruments available to measure dermatology-specific health-related quality of life, but there is no clear consensus on which instrument to use.[16] The Dermatology Life Quality Index is a widely used instrument, but there are some limitations to its use.[17] At our department the Skindex-29 questionnaire is routinely used in daily clinical practice to measure dermatology-specific health-related quality of life. Therefore, we chose to use Skindex-29 in this study.

Outcomes The primary outcomes were the incidence of adverse events and the proportion of patients discontinuing FAE treatment. Secondary outcomes were changes in PASI and changes in health-related quality of life.

Statistical analysis Data are presented as mean ± SD, or as median with interquartile range (IQR) when not normally distributed. Analysis was by intention to treat. Differences in categorical variables and continuous variables between the two groups were tested with the Kruskal–Wallis or $\chi 2$-test, respectively. A P-value $\leq 0{\cdot}05$ was considered statistically significant.

We calculated a sample size of 50 patients to give 90% power with a two-sided 5% significance level to detect a reduction of 55% in the incidence of adverse events.

RESULTS

Patient characteristics In total, 50 patients were enrolled and included in the trial (Fig. 1). The demographic and clinical characteristics of the study population are summarized in Table 1. The median age was 44 years (IQR 30–56) and the median duration of psoriasis was 11 years (IQR 5–21). Most patients had been treated previously with phototherapy (36%). Few patients had been previously treated with a classical systemic psoriasis treatment (28%) or with a biological (4%).

Incidences of adverse events during fumaric acid ester treatment Overall, 21 (84%) of 25 patients in the cetirizine group and 21 (84%) of 25 patients in the placebo group experienced an adverse event during the first 12 weeks of treatment (Table 2). The addition of cetirizine to the FAE did not reduce the incidence of adverse events in the cetirizine group com- pared with the placebo group (84% vs. 84%, P = 1·00).

The most common adverse events in the cetirizine group were gastrointestinal complaints (68%), flushes (60%) and pruritus (28%). Similar frequencies were observed in the placebo group, with the most commonly reported adverse events being gastrointestinal complaints (64%), flushes (48%) and headache (28%). The incidences of the different types of adverse events were not significantly different between the cetirizine and placebo groups. There were no differences between the two groups in the 8-week follow-up period following week 12.

Tolerability Six (24%) of 25 patients in the cetirizine group had discontinued FAE treatment. The reasons for discontinuing treatment were intolerable gastrointestinal complaints (n = 3), an increase of psoriasis (n = 1), arrhythmia (n = 1) and myocardial infarction (n = 1). In the placebo group the number of patients discontinuing FAE treatment was slightly higher, with eight (32%) of 25 patients discontinuing treatment. The reasons for discontinuing treatment were gastrointestinal complaints (n = 3), an increase in liver enzymes (n = 1), arrhythmia (n = 1), pregnancy (n = 1), tinnitus (n = 1) and chest pain (n = 1). The proportion of patients discontinuing FAE treatment was not statistically different between the cetirizine and placebo groups (P = 0·53). There were two (8%) patients in the placebo group and two (8%) patients in the cetirizine group who were lost to follow-up.

Safety Abnormal laboratory tests were observed in 24 patients (96%) in the cetirizine group vs. 21 (84%) in the placebo group (Table 3). The most common abnormal laboratory tests seen in both groups were an increase in liver enzymes, eosinophilia and proteinuria. A decrease in lymphocyte counts was seen in five patients (20%) treated with additional cetirizine vs. four (16%) in the placebo group. In all cases the laboratory abnormalities were mild, with changes of less than twofold the limit of normal value, and all laboratory abnormalities normalized without any intervention and while continuing FAE treatment. There was only one patient (4%) in the FAE plus cetirizine group who had a more than twofold increase in alanine transaminase and aspartate transaminase following 4 weeks of treatment with FAE, and who therefore had to discontinue FAE treatment. The increase in transaminases normalized within 2 weeks.

Efficacy The median improvement in PASI at week 12 compared with baseline was 65% (IQR 52–79%) among patients treated with FAE plus cetirizine (Fig. 2). In the group of patients receiving FAE plus placebo a similar improvement in PASI at week 12 was seen, with a median improvement of 66% (IQR 49–78%). The proportion of patients achieving at least 75% improvement in PASI at week 12 was 20% in both groups.

Health-related quality of life The health-related quality of life as measured by Skindex-29 improved during the 12-week FAE treatment (Fig. 3). In the group of patients treated with FAE plus cetirizine the median improvement at week 12 was 39% (IQR 20–62%). Among patients who received FAE plus placebo the median improvement at week 12 was 55% (IQR 21–77%). The improvements in Skindex-29 scores were not statistically different between the cetirizine and placebo groups (P = 0·87).

DISCUSSION

In this randomized double-blind placebo-controlled trial among 50 patients with moderate-to-severe psoriasis, treated with FAEs for 12 weeks, the addition of the oral histamine-1 receptor antagonist cetirizine 10 mg once daily to FAEs did not reduce the incidence of adverse events or improve the tolerability of FAE treatment. Furthermore, the addition of cetirizine did not increase the efficacy of FAE compared with treatment with FAEs alone.

To translate these findings into implications for clinical practice, several aspects of our study need to be considered. Firstly, we used a randomized, double-blind study design and a matching placebo for cetirizine so that both the patients and the physician assessors were blinded for the allocated treatment group. Secondly, we used a suitable histamine-1 receptor antagonist that has been shown to be well tolerated with favourable pharmacological properties in other patient populations.[18] In addition, the effects of cetirizine have been studied previously in patients with psoriasis.[12,19] A limitation of our study is the relatively small sample size, owing to our pre- study power calculation. However, if a greater sample size were required to detect a statistically significant difference, the clinical value of adding a histamine antagonist to FAEs would likely be small. Furthermore, we did not quantify the severity of flushing and gastrointestinal symptoms. However, we did look specifically for the incidence of adverse events requiring FAE treatment discontinuation, for which we found no statistically significant differences between the cetirizine and placebo groups. Lastly, we chose short-term study duration of 12 weeks, considering that adverse events occur predominantly at the beginning of FAE treatment.

In this study, treatment with an oral histamine antagonist had no effect on the occurrence of the adverse events during FAE treatment. Therefore, the mechanisms underlying FAE-induced gastrointestinal and flushing symptoms likely involve mediators other than histamine. Alternatively, histamine may play only a minor role in the generation of adverse events associated with FAEs. In recent experimental studies it has been shown that FAE-induced flushing is mediated through activation of the G-protein-coupled receptor GPR109A, located on Langerhans cells and on keratinocytes, which via cyclooxygenase-1 and -2 enzymes leads to prostaglandin release.[20] Further evidence for this mechanism comes from a clinical trial involving 56 healthy volunteers, in which aspirin, a cyclooxygenase inhibitor, decreased the incidence and severity of flushing symptoms from dimethyl-fumarate.[21]

The mechanisms leading to gastrointestinal complaints in FAE treatment are not yet understood. One hypothesis involves the FAE-triggered release of tumour necrosis factor (TNF)-a as a mechanism leading to gastrointestinal complaints.[22] In a previous clinical study, cotreatment with FAE and pentoxifylline, a methylxanthine derivative with some anti-TNF-a properties, reduced the frequency of gastrointestinal complaints.[23] However, this study was open label and uncontrolled, so bias of these results cannot be excluded. Another potential mechanism may involve dimethylfumarate-induced allergic contact mucositis of the gastrointestinal tract.[24] Hessam et al. described three patients who had to discontinue FAE treatment due to gastrointestinal complaints and who had a positive patch test reaction to dimethylfumarate.[24] Considering that dimethylfumarate is a potent contact sensitizer, systemic treatment with it could induce an allergic contact mucositis, thus causing gastrointestinal symptoms. Future studies could focus on these mechanisms to find new strategies to improve the tolerability of FAEs.

In this study, cetirizine at a daily dosage of 10 mg did not reduce FAE-induced adverse events. One can speculate whether higher dosages of cetirizine may be more effective. In the current guidelines on the treatment of chronic urticaria, the daily dose of cetirizine may be increased up to fourfold when a single dose of cetirizine 10 mg is ineffective.[25] Similarly, cetirizine in higher doses might be effective to decrease FAE-induced adverse events. We did not test this hypothesis in this current study.

It has been suggested that the histamine antagonist cetirizine might be beneficial in psoriasis treatment.[12,26] In this study, we could not detect an additional improvement by cetirizine. The improvement in PASI in the FAE plus cetirizine group was comparable with that in the placebo group. Other histamine antagonists such as ranitidine, a histamine-2 receptor antagonist, did show improvements of psoriasis in several observational studies, but failed to demonstrate efficacy in randomized controlled trials.[27,28]

FAEs have a favourable long-term efficacy and safety profile, but the induction phase of FAE treatment can be challenging for patients because of problems with tolerability. Therefore, a gradual increase in the dose of FAEs is considered standard. A large disadvantage of this approach is that it takes longer to reach the dosage that gives a satisfactory clinical response. In daily clinical practice, the first clinical response is usually observed following 6–8 weeks of treatment.[2] If FAE- induced adverse events can be decreased or even prevented, the optimal dosage of FAEs could be reached faster and would thereby increase patient treatment satisfaction. There is evidence that aspirin and nonsteroidal anti-inflammatory drugs decrease flushing symptoms, but these drugs to do not affect gastrointestinal complaint. It is our experience that patients on FAEs find the gastrointestinal symptoms to be more bothersome than the flushing symptoms. In this study, six of 50 patients discontinued FAEs due to gastrointestinal complaints, and none discontinued FAE treatment due to flushing. In daily clinical practice, gastrointestinal complaints can be managed by symptomatic treatment with proton pump inhibitors, antiemetics or antidiarrhoeal drugs.[10] Given the results of this study, adding a histamine antagonist to FAEs is not helpful in reducing the adverse events.

In conclusion, the addition of the histamine antagonist cetirizine 10 mg once daily to FAE treatment did not reduce adverse events or improve the tolerability of FAE in patients with psoriasis. Our results do not support a beneficial effect of adding a systemic histamine antagonist to FAE treatment in patients with psoriasis.

REFERENCES

1. Mrowietz U et al. Dimethylfumarate for psoriasis: more than a dietary curiosity. Trends Mol Med 2005; 11:43–8.
2. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol 2009; 23 (Suppl. 2):1–70.
3. Reich K et al. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis – a retrospective study (FUTURE). J Dtsch Dermatol Ges 2009; 7:603–11.
4. Fallah Arani S et al. Fumarates vs. methotrexate in moderate to severe chronic plaque psoriasis: a multicentre prospective randomized controlled clinical trial. Br J Dermatol 2011; 164:855–61.
5. Inzinger M et al. Methotrexate vs. fumaric acid esters in moderate-to-severe chronic plaque psoriasis: data registry report on the efficacy under daily life conditions. J Eur Acad Dermatol Venereol 2013; 27:861–6.
6. Hoefnagel JJ et al. Longterm safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol 2003; 149:363–9.
7. Wain EM et al. Treatment of severe, recalcitrant, chronic plaque psoriasis with fumaric acid esters: a prospective study. Br J Dermatol 2010; 162:427–34.
8. Heelan K et al. Fumaric acid esters as a suitable first-line treatment for severe psoriasis: an Irish experience. Clin Exp Dermatol 2012; 37:793–5.
9. Zweegers J et al. Summary of the Dutch S3-guidelines on the treatment of psoriasis 2011. Dermatol Online J 2014; 20:21769.
10. Phillips JT et al. Management strategies for flushing and gastrointestinal events associated with BG-12 (dimethyl fumarate): expert panel recommendations. Multiple Sclerosis 2013; 19 (11 Suppl.):228.
11. Maintz L et al. Histamine and histamine intolerance. Am J Clin Nutr 2007; 85:1185–96.
12. Pestelli E et al. Cetirizine modulates adhesion molecule expression in a double-blind controlled study conducted in psoriatic patients. Int J Tissue React 2003; 25:1–8.
13. Namazi MR. Cetirizine and allopurinol as novel weapons against cellular autoimmune disorders. Int Immunopharmacol 2004; 4: 349–53.

14. Litjens NH et al. Pharmacokinetics of oral fumarates in healthy subjects. Br J Clin Pharmacol 2004; 58:429–32.

15. Chren MM et al. Improved discriminative and evaluative capability of a refined version of Skindex, a quality-of-life instrument for patients with skin diseases. Arch Dermatol 1997; 133:1433–40.

16. Prinsen CA et al. Measurement of healthrelated quality of life in dermatological research and practice: outcome of the EADV Taskforce on Quality of Life. J Eur Acad Dermatol Venereol 2013; 27:1195–203.

17. Nijsten T. Dermatology Life Quality Index: time to move forward. J Invest Dermatol 2012; 132:11–13.

18. Zhang L et al. The clinical use of cetirizine in the treatment of allergic rhinitis. Pharmacology 2013; 92:14–25.

19. Pestelli E et al. Cetirizine reduces the number of tryptase-positive mast cells in psoriatic patients: a double-blind controlled study. Int J Tissue React 2001; 23:97–103.

20. Hanson J et al. Nicotinic acid- and monomethyl fumarate-induced flushing involves GPR109A expressed by keratinocytes and COX-2-dependent prostanoid formation in mice. J Clin Invest 2010; 120:2910–19.

21. Sheikh SI et al. Tolerability and pharmacokinetics of delayed-release dimethyl fumarate administered with and without aspirin in healthy volunteers. Clin Ther 2013; 35:1582–94.e9.

22. Asadullah K et al. Influence of monomethylfumarate on monocytic cytokine formation – explanation for adverse and therapeutic effects in psoriasis? Arch Dermatol Res 1997; 289:623–30.

23. Friedrich M et al. Addition of pentoxifylline could reduce the side effects of fumaric acid esters in the treatment of psoriasis. Acta Derm Venereol 2001; 81:429–30.

24. Hessam S et al. Dimethylfumarate in the treatment of psoriasis – a hitherto unnoticed source of sensitization? A case series and review of the literature. Allergologie 2013; 36:19–27.

25. Zuberbier T et al. The EAACI/GA2LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. Allergy 2014; 69:868–87.

26. Caproni M et al. Pharmacologic modulation by cetirizine of some adhesion molecules expression in psoriatic skin lesions. Int J Dermatol 1995; 34:510–13.

27. Kristensen JK et al. Systemic high-dose ranitidine in the treatment of psoriasis: an open prospective clinical trial. Br J Dermatol 1995; 133:905–8.

28. Zonneveld IM et al. Ranitidine does not affect psoriasis: a multicenter, double-blind, placebo-controlled study. J Am Acad Dermatol 1997; 36:932–4.

TABLES AND FIGURES

Table 1: Baseline demographic and clinical characteristics of the study population

Patient characteristic	Fumaric acid esters with cetirizine (n = 25)	Fumaric acid esters with placebo (n = 25)
Age	46 (31-62)	38 (30-54)
Sex		
Male	15 (60%)	18 (72%)
Female	10 (40%)	7 (28%)
Duration of psoriasis in years	11 (5-19)	12 (5-24)
History of psoriatic arthritis	8 (32%)	5 (20%)
Previous psoriasis treatments		
Phototherapy	10 (40%)	8 (32%)
Classical treatment (methotrexate, ciclosporin)	8 (32%)	6 (24%)
Biologic	2 (8%)	0 (0%)
PASI at baseline	12.7 (10.8-16.0)	14.5 (12.0-16.7)

Data are numbers (percentages) or medians (interquartile ranges)

Table 2: Subjective adverse events

	Fumaric acid esters with cetirizine (n = 25)	Fumaric acid esters with placebo (n = 25)
Gastrointestinal complaints	17 (68%)	16 (64%)
Flushing	15 (60%)	12 (48%)
Pruritus	7 (28%)	6 (24%)
Headache	2 (8%)	7 (28%)
Fatigue	5 (20%)	4 (16%)
Lower extremity edema	2 (8%)	2 (8%)
Dermatitis	0 (0%)	1 (4%)
Chest pain	0 (0%)	1 (4%)
Tinnitus	0 (0%)	1 (4%)
Flu-like symptoms	1 (4%)	0 (0%)
Dizziness	1 (4%)	0 (0%)

Data are numbers (percentages)

Table 3: Laboratory adverse events

	Fumaric acid esters with cetirizine (n = 25)	Fumaric acid esters with placebo (n = 25)
Increase in liver enzymes	13 (52%)	15 (60%)
Eosinophilia	10 (40%)	8 (32%)
Proteinuria	8 (32%)	5 (20%)
Lymphocytopenia	5 (20%)	4 (16%)
Decrease in creatinine	4 (16%)	0 (0%)
Increase in urea	2 (8%)	1 (4%)
Increase in cholesterol	2 (8%)	1 (4%)
Decrease in thrombocytes	0 (0%)	1 (4%)
Increase in haemoglobin	1 (4%)	0 (0%

Data are numbers (percentages)

Figure 1: Flow diagram

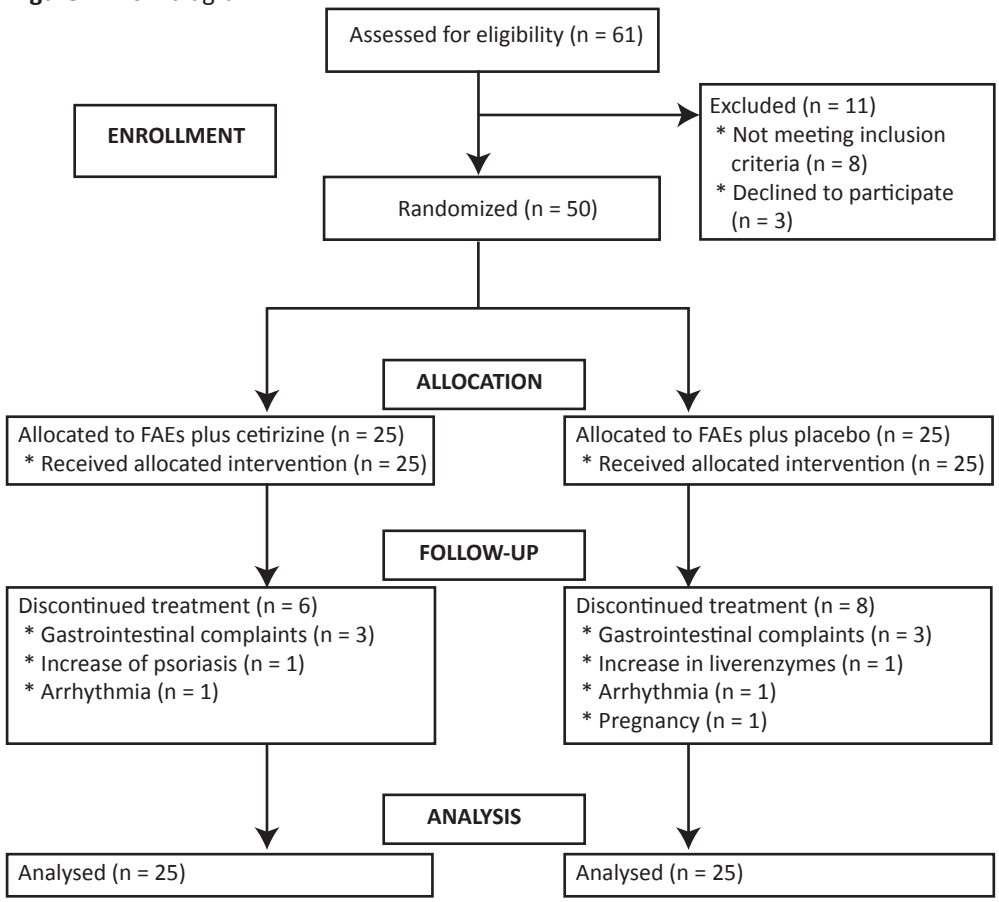


Figure 2: Changes in PASI during 12 weeks of FAE treatment in patients treated with FAEs plus cetirizine (n=25) and in patients treated with FAEs plus placebo (n=25). Error bars show median and interquartile range

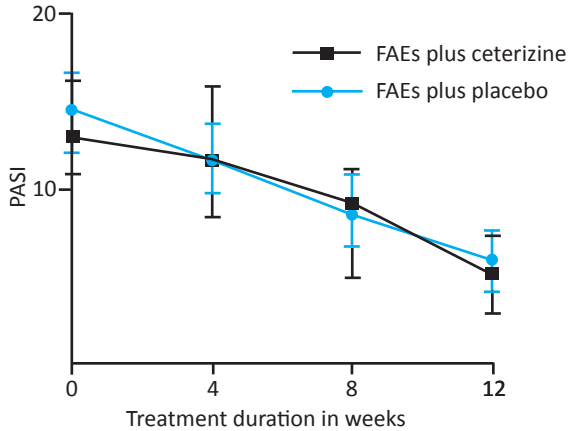
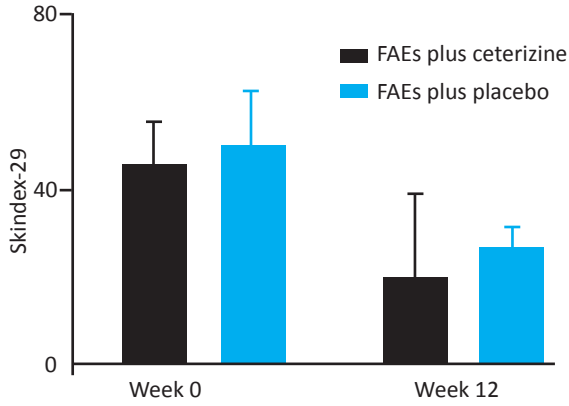


Figure 3: Changes in Skindex-29 scores during 12 weeks of FAE treatment in patients treated with FAEs plus cetirizine (n=25) and in patients treated with FAEs plus placebo (n=25). Error bars show median and interquartile range



J.D Bos & R.H. Cormane

"Virtually no cell observed in the skin lesions of psoriasis has found to be completely normal."

Immunocompetent cells in psoriasis. *Arch Dermatol Res*. 1983;275(3):181-9.

CHAPTER| 5.1

Progressive multifocal leukoencephalopathy associated with fumaric acid esters treatment in psoriasis patients

Deepak M.W. Balak[1]

Enes Hajdarbegovic[1]

Wichor M. Bramer[2]

H.A. Martino Neumann[1]

H. Bing Thio[1]

[1]Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

[2]Medical library, Erasmus Medical Center, Rotterdam, the Netherlands

J Eur Acad Dermatol Venereol. 2017 Sep;31(9):1475-1482.

N Engl J Med. 2016 Jan 21;374(3):295.

N Engl J Med. 2015 Aug 6;373(6):582-3.

ABSTRACT

Background: Fumaric acid esters (FAEs) are a systemic treatment for psoriasis considered to have a favourable long-term safety profile without an increased risk for immunosuppression. However, progressive multifocal leukoencephalopathy (PML), a rare, opportunistic viral infection of the central nervous system, has been linked anecdotally to FAE treatment. Here, we aimed to assess clinical features and outcomes of FAE-associated PML cases.

Methods: Systematic literature search in multiple databases up to 25th February 2016 for reports of PML in psoriasis patients treated with FAEs.

Results: Eight cases (four male, four female) of FAE-associated PML were identified. Median age was 64 years (range 42–74 years); median FAE treatment duration was 3 years (range 1.5–5 years). Six patients were treated with a formulation containing dimethyl fumarate (DMF) and monoethyl fumarates, and two patients with a DMF formulation. Patients exhibited neurological symptoms, such as aphasia, hemiparesis and dysarthria. PML diagnosis was based on MRI findings and presence of JC virus in cerebrospinal fluid and/or brain tissue.

All cases were linked to moderate-to-severe reductions in absolute lymphocyte counts, with nadirs ranging from 200 to 792 cells per mm^3. Median exposure to lymphocytopenia was 2 years (range 1–5 years).

In all cases, FAE treatment was discontinued; PML was treated with mefloquine plus mirtazapine. Three patients improved, two had stable disease, two had residual symptoms, and one patient died to an immune reconstitution inflammatory syndrome (IRIS).

Conclusions: Progressive multifocal leukoencephalopathy is infrequently linked to FAE treatment, but underreporting cannot be excluded. Physicians treating patients with FAEs should be vigilant for the occurrence of PML, and both clinicians and patients should be alert for onset of new neurological symptoms. Periodic monitoring of lymphocyte counts and FAE discontinuation in case of moderate-to-severe lymphocytopenia is recommended to minimize the risk for PML.

INTRODUCTION

Fumaric acid esters (FAEs), also termed fumarates, are small molecules with pleiotropic immune-modulating properties that have been developed for the treatment of psoriasis.[1,2] FAE-formulations containing dimethyl fumarate (DMF) have been in use for the systemic treatment of psoriasis since 1959, mainly in Europe.[3] Fumaderm, an FAE-formulation containing a mixture of DMF and three monoethyl fumarate (MEF) salts, has held German market authorization since 1994 and is currently the most prescribed systemic treatment for psoriasis.[4] In other European countries such as the UK, Ireland, the Netherlands, Italy, Austria and Switzerland, FAEs are unlicensed in use for psoriasis treatment.[5-10]

The 2015 European psoriasis S3-guidelines recommend FAEs as a long-term maintenance treatment for psoriasis on the basis of a favourable long-term safety profile of FAEs.[11] Observational studies have not reported clinically significant safety issues or an increased risk for malignancy or immunosuppressive adverse events.[10,12,13] However, in 2013 two cases were published of progressive multifocal leukoencephalopathy (PML) in patients with psoriasis treated with FAEs.[14,15] PML is a rare, opportunistic viral infection of the central nervous system.[16] PML occurs due to reactivation of the John Cunningham polyomavirus (JC virus), a human polyomavirus which is latently present in up to 60% of the general population.[17] Initial JC virus-infection typically occurs during childhood without any symptoms. However, in the context of an immunocompromised status, JC virus-reactivation can lead to a demyelinating infection of the central nervous system that can be potentially fetal.[18] Established risk factors for the development of PML include infection with human immunodeficiency virus, haematological malignancies, and certain immune-mediated diseases, such as sarcoidosis and systemic lupus erythematosus.[16] In addition, the occurrence of PML can be drug-related with immunosuppressive treatments, such as natalizumab, rituximab, efalizumab and ciclosporine.[19-20]

FAEs seem to be a new association of drug-induced PML. Of note, the development of FAE-associated PML is thought to be related to lymphocytopenia and not directly to the compound itself. Only a subpopulation of DMF-treated patients show lymphocytopenia and are thus potential at increased risk for PML. This is different to other compounds such as natalizumab, where the risk of PML is presumably linked directly to the drug's mechanism of action, that is, inhibition of leukocyte migration into tissues leading to a decreased immunosurveillance. As a result, all patients treated with natalizumab and similar drugs would have an increased risk to develop PML, in contrast to only the subpopulation of DMF-treated

patients having a lymphocytopenia.

Following the first case report of FAE-associated PML, several other cases were published.[21-23] Assessment of these cases is of clinical importance in order to define the risk factors for the development of PML, to support better-informed management and monitoring of FAE treatment, and to improve treatment outcomes. Here, we systematically reviewed the literature to assess clinical features and outcomes of cases of FAE-associated PML, and to generate suggestions for recommendations for treatment monitoring to minimize the risk of PML.

MATERIALS AND METHODS

Literature search strategy The databases Embase.com, Medline (Ovid), Cochrane central registry of trials (CENTRAL), Web-of-Science, PubMed (the subset as supplied by publisher, containing non-indexed citations) and Google Scholar were searched from inception up to 25th February 2016. The searches, conducted by an experienced biomedical information specialist (WB), combined multiple thesaurus terms and words in title/abstract for FAEs with terms for PML and JC virus. Details of the search strategy terms are summarized in Appendix 1.

Literature search strategy Articles were first screened by title and/or abstract, which were followed by a full-text evaluation by one assessor (DB). We included articles describing the occurrence of PML in patients with psoriasis treated with a FAE formulation. There were no limitations applied for publication language or date.

Data extraction and analysis Data were extracted on demographics, FAE formulation, dosage, treatment duration, laboratory assessments, including leukocyte counts, clinical features and diagnostic assessments of PML, and treatment outcomes.

Descriptive statics were used. Median range and interquartile range (IQR) were reported when appropriate.

RESULTS

Literature search The literature search yielded 524 hits, of which eight articles were included after evaluation of abstract and full text (Fig. 1). All articles consisted of single case reports that were published in the period 2013–2015 in English, peer-reviewed jour-

nals (Table 1).[14,15,21-26]

Case descriptions A total of eight cases (four male, four female) of FAE-associated PML in patients with psoriasis were reported (Table 1). Median age at time of diagnosis of PML was 64 years [interquartile range (IQR) 55–69 years].

All patients received FAE treatment for a minimum period of 1.5 years. Median FAE treatment duration was 3 years (IQR 2.4–3.5 years). Six patients were treated with Fumaderm, a German licensed FAE-formulation containing DMF and three MEF-salts. The other two patients were treated with Psorinovo, a Dutch unlicensed FAE-formulation containing slow-release DMF. Median daily dosage of FAEs was 645 mg (IQR 423–1022 mg).

Presenting signs of PML included neurological symptoms, mainly aphasia (38% of cases), hemiparesis (38%) and dysarthria (25%) (Table 2). In all cases, the neurological symptoms were progressive over time.

Diagnosis of PML was based on abnormal MRI findings, the presence of JC virus in cerebrospinal fluid and/or brain tissue and histopathological analysis of brain biopsy specimens. A brain biopsy was performed in five cases. In several cases, initial testing of cerebrospinal fluid for JC virus DNA with a polymerase chain reaction (PCR) assay was negative.[22,23,26] This led to an initial misdiagnosis of ischemic stroke or multiple sclerosis in four cases.[14,23,25,26]

Risk factors for PML Four cases were associated with established risk factors for PML, such as a medical history of malignancies, auto-immune diseases or exposure to previous immunosuppressive treatments.

All cases of FAEs-associated PML were linked to exposure to moderate-to-severe lymphocytopenia of varying periods (Table 3). Six patients had a grade 3 (severe) lymphocytopenia. Two patients were exposed to only moderate grade 2 lymphocytopenia. Median exposure to FAEs-induced lymphocytopenia was 2 years (IQR 1.5–2.8 years). The nadir of the absolute lymphocyte count ranged from 200 to 792 per mm^3. Several reports involved data on lymphocyte subsets. A low $CD4^+$-cell count was observed in four cases, while a low $CD8^+$-cell count was seen in three cases.

The lag time between start of FAE treatment, onset of lymphocytopenia and onset of PML varied in the reported cases (Fig. 2).

Treatment outcomes In all eight cases, FAE treatment was discontinued, and treatment for PML with mefloquine and mirtazapine was initiated. This led to improvement of

symptoms in three cases and a stable condition in two cases. There were two patients with residual symptoms of PML.

An immune reconstitution inflammatory syndrome (IRIS) following FAE treatment discontinuation was reported in five cases. One patient died due to complications following IRIS.[26]

DISCUSSION

Drug-related PML has been reported anecdotally in patients with psoriasis treated with FAEs.[27] The association between PML and FAE treatment is of clinical importance, considering the increasing use of FAEs as psoriasis treatment and the progressive and potential fatal course of PML. Here, we assessed eight confirmed cases of FAEs-associated PML.

Several important points can be made from this case series. First, several cases were not linked to known risk factors for PML, thus strengthening the potential associated between FAEs and PML. However, in four of the cases, there were established risk factors for PML other than FAE treatment present. Second, all confirmed cases were exposed to periods of varying lymphocytopenia. Importantly, in all reported cases, the monitoring rules for lymphocytopenia advised in current guidelines were not adhered to, which resulted in long periods of unknown lymphocyte counts. Third, severe lymphocytopenia does not seem critical for the development of FAEs-associated PML, as there were two cases with exposure to only moderate grade 2 lymphocytopenia. Fourth, diagnosing FAE-associated PML can be challenging due to non-specific neurological symptoms, which in some cases initially led to a misdiagnosis. In addition, a negative PCR assay on JC virus does not rule out PML. Further testing, for example brain biopsy, should therefore be considering when the clinical suspicion of a diagnosis of PML remains high despite a negative PCR result for JC virus.

This case series is limited due to insufficient data on causal and pathophysiological relationships between PML and FAEs. In addition, risk estimates for FAE-related PML were not available in this data set. The reported cases involved different FAE-formulations, that is, formulations containing DMF with MEF and formulations containing DMF. The number of cases (n = 8), however, was too low to allow assessment of differences in risks or outcomes of the various FAE-formulations.

The risk of PML related to FAE treatment has yet to be determined, considering that studies assessing the incidence of PML are not available. Observational studies so far have not reported any opportunistic adverse events associated with FAEs.[3,13] Despite the relatively common occurrence of lymphocytopenia during FAE treatment, the incidence of

associated immunosuppressive adverse events has been surprisingly low with eight reported PML cases to date set against 180.000 patient-years of FAE treatment.[28] However, underreporting of the incidence of PML in FAE treatment cannot be excluded.

The exact mechanisms by which exposure to FAEs may lead to PML are unknown. Given that PML usually occurs in the context of cellular immune-incompetence, FAE-induced lymphocytopenia is implicated.[17] In line, all reported cases of FAE-associated PML were linked to lymphocytopenia. Experimental studies have shown that FAEs induce lymphocytopenia, including reduction of $CD4^+$, $CD8^+$ and B lymphocytes.[29] CD4+ lymphocytopenia, occurring as in idiopathic $CD4^+$-lymphocytopenia, has been linked to PML.[30] FAEs are known to reduce $CD4^+$ and $CD8^+$ lymphocytes, and in all 8 cases of PML reported in patients on FAE treatment exposure to moderate-to-severe lymphocytopenia was present.[31,32] FAE-induced moderate lymphocytopenia with more pronounced selective reductions in lymphocyte-subsets could predispose to an increased risk for PML.[31] Unfortunately, data on lymphocyte-subsets were not available for all cases. Although the immunosuppressive effects of FAEs have been noted in experimental studies, in daily clinical practice no significant increased immunosuppressive risk is observed.[33] The safety profile of FAE treatment is considered favourable.[11–13] In context of potential immunosuppressive effects of FAEs, 7 cases have been published of uncommon adverse events potential related to immune-incompetence.[3] These include the occurrence of Kaposi sarcoma, tuberculous lymphadenitis, and organizing pneumonia, and disseminated varicella zoster.[34,35] Whether and how exactly FAE-induced lymphocytopenia plays a pivotal role in the development of PML is subject for future studies. Furthermore, rare genetic variants may predispose to an increased risk for PML. Several cases of PML have also been described in patients with no apparent immune-deficiency. In one of such cases, analyses showed a genetic deficit in interferon-gamma production as a possible underlying cause for PML.[36]

The occurrence of PML linked to FAE treatment has been reported in patients with multiple sclerosis.[37] As of 2013, a FAE-formulation containing DMF is approved by the U.S. Food and Drug and the European Medicines Agency for the treatment of relapsing-remitting multiple sclerosis. Four cases have been reported of PML in patients with multiple sclerosis treated with DMF.[38] In response to the reported cases of PML associated with various FAE formulations, the European Medicines Agency has formulated different recommendations to minimize risks for PML during treatment with the DMF formulation in multiple sclerosis and the FAE formulation in psoriasis.[39] It is of importance to note that there are no clear differences in the DMF formulation used for MS and the DMF formulations in psoriasis as these compounds are identical in terms of chemimal structure. While there may be intrinsic

differences between patients with psoriasis compared to those having multiple sclerosis, there is no clear scientific rationale to have differential monitoring guidelines for lymphocytopenia for the various DMF formulations. For psoriasis, monitoring blood cell counts, including lymphocyte counts, every 4 weeks is now recommended. In case of an absolute lymphocyte count below 0.5 * 10^9/L, treatment with FAEs is to be discontinued. In case of an absolute lymphocyte count below 0.7 * 10^9/L, a 50% dosage reduction is advised and treatment discontinuation when following 4 weeks the lymphocyte counts persist below 0.7 * 10^9/L.39 Although these drug-discontinuation rules are in line with the current European guidelines, such strategies remain to be validated.[11,40] Moreover, there is currently no sufficient evidence to support monthly monitoring of lymphocyte counts during FAE treatment in psoriasis. In previous psoriasis guidelines, monitoring a full blood count once every 3 months was advised.[41] We suggest that an interval of 3 months for blood count monitoring is acceptable when no leukocyte abnormalities are found during initial monthly testing (Table 4). Importantly, more frequent testing may be indicated when leukocyte and/or lymphocyte count values show a decreasing trend. Other suggested recommendations to minimize risks for the development of PML during treatment with FAEs are summarized in Table 4.

In case of FAE-induced leukocytopenia and/or lymphocytopenia, care should be given to follow-up the leukocyte and/or lymphocyte count until normalization. This is of clinical importance as starting a systemic treatment with a known association with leukocytopenia should preferably by avoided until the low counts are resolved. For instance, methotrexate would be a less suitable treatment option in view of its potential for bone marrow suppression. Logical next options for patients who had to stop FAE due to the development of lymphocytopenia would include optimal topical treatment and/or UVB phototherapy.

In conclusion, PML is an infrequently reported adverse event linked to FAE treatment in patients with psoriasis. The neurological symptoms of PML are non-specific and often lead to initial misdiagnosis. Physicians treating patients with FAEs should be vigilant for the occurrence of PML. Patients on FAEs presenting with neurological symptoms or signs should undergo evaluation for PML. Periodic monitoring of lymphocyte counts, treatment discontinuation in case of moderate-to-severe lymphocytopenia and clinical alertness for new onset of neurological symptoms is recommended to minimize risks for PML.

REFERENCES

1. Mrowietz U et al. Dimethylfumarate for psoriasis: more than a dietary curiosity. Trends Mol Med 2005; 11:43–8.
2. Onderdijk AJ et al. Regulated genes in psoriatic skin during treatment with fumaric acid esters. Br J Dermatol 2014; 171: 732–741.
3. Balak DM 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: 250–262.
4. Reich K et al. Drug safety of systemic treatments for psoriasis: results from The German Psoriasis Registry PsoBest. Arch Dermatol Res 2015; 307: 875–883.
5. Fallah Arani S et al. Treatment of psoriasis with non-registered fumaric acid esters in The Netherlands: a nationwide survey among Dutch dermatologists. J Eur Acad Dermatol Venereol 2014; 28: 972–975.
6. Jabbar-Lopez ZK et al. Newer agents for psoriasis in adults. BMJ 2014; 349: g4026.
7. Kolios AG et al. Swiss S1 guidelines on the systemic treatment of psoriasis vulgaris. Dermatology 2016; 232: 385–406.
8. Kokelj F et al. Fumaric acid and its derivatives in the treatment of psoriasis vulgaris: our experience in forty-one patients. Acta Dermatovenerol Croat 2009; 17: 170–175.
9. Inzinger M et al. Methotrexate vs. fumaric acid esters in moderate-to-severe chronic plaque psoriasis: data registry report on the efficacy under daily life conditions. J Eur Acad Dermatol Venereol 2013; 27: 861–866.
10. Ismail N et al. Drug survival of fumaric acid esters for psoriasis: a retrospective study. Br J Dermatol 2014; 171: 397–402.
11. Nast A 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: 2277–2294.
12. Hoefnagel JJ et al. Long-term safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol 2003; 149: 363–369.
13. Reich K et al. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis–a retrospective study (FUTURE). J Dtsch Dermatol Ges 2009; 7: 603–611.
14. van Oosten BW et al. PML in a patient treated with dimethyl fumarate from a compounding pharmacy. N Engl J Med 2013; 368: 1658–1659.
15. Ermis U et al. PML in a patient treated with fumaric acid. N Engl J Med 2013; 368: 1657–1658.
16. Tan CS et al. Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis. Lancet Neurol 2010; 9: 425–437.
17. Jelcic et al. Immunology of progressive multifocal leukoencephalopathy. J Neurovirol 2015; 21: 614–622.
18. Berger JR. Progressive multifocal leukoencephalopathy. Handb Clin Neurol 2014; 123: 357–376.
19. Chahin S et al. A risk classification for immunosuppressive treatment-associated progressive multifocal leukoencephalopathy. J Neurovirol 2015; 21: 623–631.
20. Ladizinski B et al. Progressive multifocal leukoencephalopathy and reversible progressive leukoencephalopathy syndrome in dermatologic therapy. J Drugs Dermatol 2013; 12: e20–e24.
21. Stoppe M et al. Cerebellar manifestation of PML under fumarate and after efalizumab treatment of psoriasis. J Neurol 2014; 261: 1021–1024.
22. Dammeier N et al. Case report of a patient with progressive multifocal leukoencephalopathy under treatment with dimethyl fumarate. BMC Neurol 2015; 15: 108.
23. Metz I et al. Increased nuclear Nrf2 expression after fumarate treatment for multiple sclerosis and psoriasis. Mult Scler 2015; 23: 259–260.
24. Buttmann M et al. Case reports of PML in patients treated for psoriasis. N Engl J Med 2013; 369: 1081.
25. Hoepner R et al. Progressive multifocal leukoencephalopathy during fumarate monotherapy of psoriasis. Neurol Neuroimmunol Neuroinflamm 2015; 2: e85.
26. Nieuwkamp DJ et al. PML in a patient without severe lymphocytopenia receiving dimethyl fumarate. N Engl J Med 2015; 372: 1474–1476.
27. Balak DM 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: 250–262.
28. Mrowietz U et al. Case reports of PML in patients treated for psoriasis. N Engl J Med 2013; 369: 1080–1081.
29. Hoxtermann S et al. Fumaric acid esters suppress peripheral CD4- and CD8-positive lymphocytes in psoriasis. Dermatology 1998; 196: 223–230.
30. Grewal J et al. Progressive multifocal leukoencephalopathy in a patient without apparent immunosuppression. J Neurovirol 2016; 22: 683–687.
31. Balak D et al. PML in patients treated with dimethyl fumarate. N Engl J Med 2015; 373: 582–583.

32. Khatri BO et al. The effect of dimethyl fumarate (Tecfidera) on lymphocyte counts: a potential contributor to progressive multifocal leukoencephalopathy risk. Mult Scler Relat Disord 2015; 4: 377–379.
33. Lehmann M et al. Fumaric acid esters are potent immunosuppressants: inhibition of acute and chronic rejection in rat kidney transplantation models by methyl hydrogen fumarate. Arch Dermatol Res 2002; 294: 399–404.
34. Philipp S et al. Immunological changes in psoriasis patients under long-term treatment with fumaric acid esters: risk of Kaposi sarcoma occurrence? Eur J Dermatol 2013; 23: 339–343.
35. van Kester MS et al. PML in patients treated with dimethyl fumarate. N Engl J Med 2015; 373: 583–584.
36. van der Kolk NM et al. Progressive multifocal leukoencephalopathy in an immunocompetent patient. Ann Clin Transl Neurol 2016; 3: 226–232.
37. Rosenkranz T et al. PML in a patient with lymphocytopenia treated with dimethyl fumarate. N Engl J Med 2015; 372: 1476–1478.
38. Baharnoori M et al. Nonfatal PML in a patient with multiple sclerosis treated with dimethyl fumarate. Neurol Neuroimmunol Neuroinflamm 2016; 3: e274.
39. European Medicines Agency (EMA). Updated recommendations to minimize the risk of the rare brain infection PML with Tecfidera. 2015. URL http://www-wemaeuropaeu/docs/en_GB/document_library/Press_release/2015/10/WC500196017pdf (last accessed: 31 October 2016).
40. Balak D et al. More on PML in patients treated with dimethyl fumarate. Author reply. N Engl J Med 2016; 374: 295.
41. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol 2009; 23(Suppl 2): 1–70.

TABLES AND FIGURES

Figure 1: Flow diagram

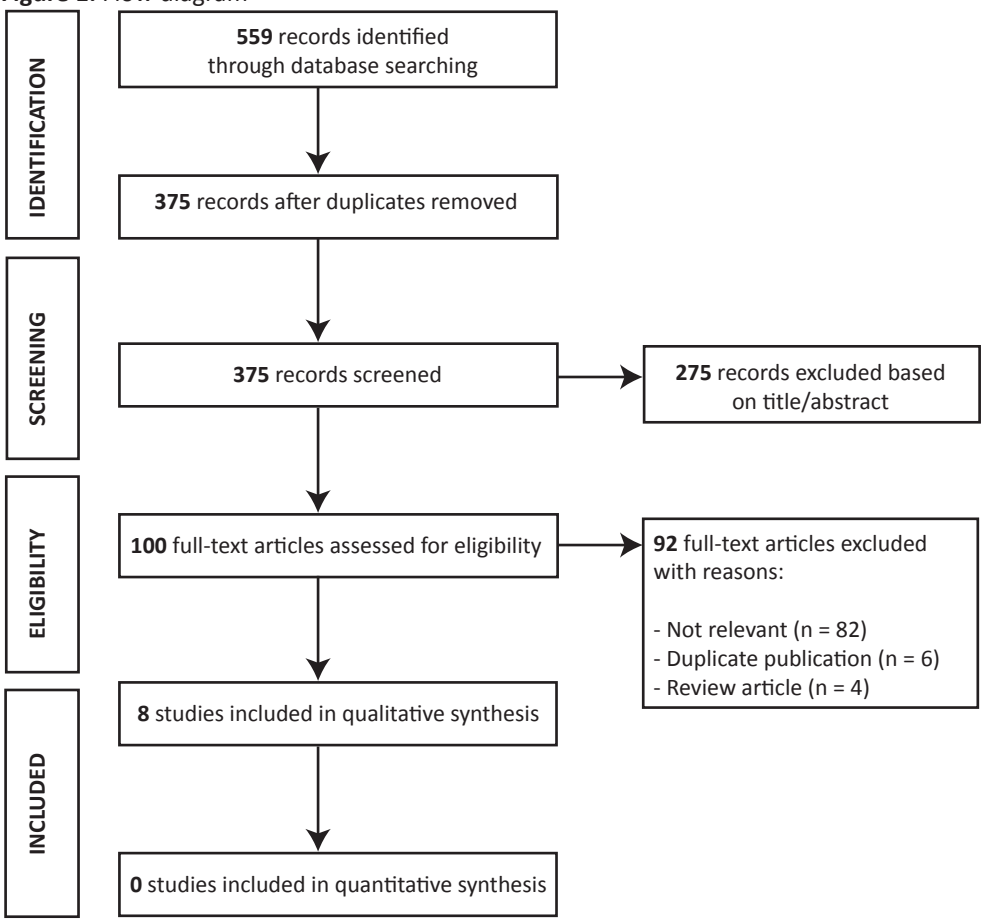
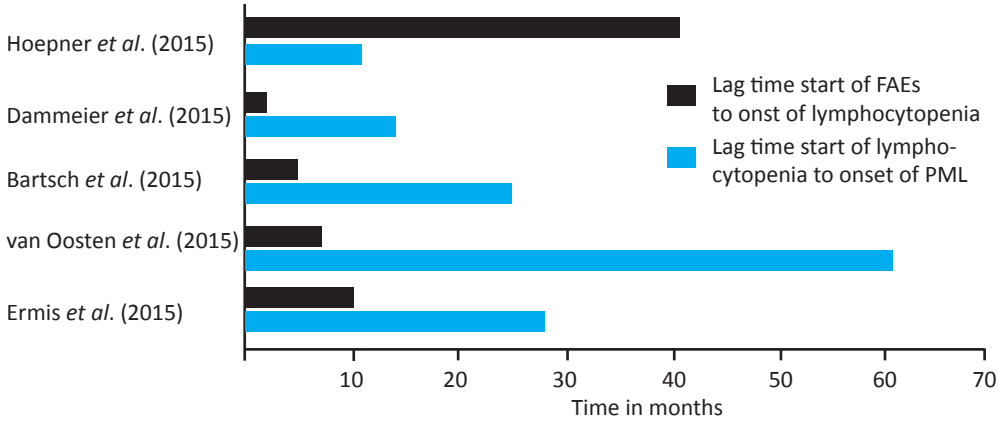


Figure 1: Overview of estimated lag times in months between start of FAE treatment and onset of lymphocytopenia and lag times between start of lymphocytopenia and onset of PML



Abbreviations: FAE, fumaric acid esters; PML, progressive multifocal leukoencephalopathy. All reported cases were associated with exposure to grade 2–3 lymphocytopenia, with absolute lymphocyte counts ranging between 200 and 800/mm^3 [Common Terminology Criteria for Adverse Events version 4.0, National Cancer Institute (NCI) of the National Institutes of Health (NIH)]; In three cases (i.e. Buttmann[24], Stoppe[21], and Nieuwkamp[26]) the time of onset of lymphocytopenia was not specified.

Table 2: Presenting neurological symptoms in fumaric acid ester (FAE)-associated PML cases

Symptom	Frequency
(Sensory) aphasia	3
Hemiparesis	2
Dysarthria	1
Hemiataxia	1
Dysphagia	1
Dysesthesia	1
Apraxia	1
Hemihypesthesia	1
Confusion	1
Left-sided weakness	1
Focal motor seizures	1
Headache	1
Somnolence	1

Table 1: Overview of published cases of progressive multifocal leukoencephalopathy (PML) associated with fumaric acid ester (FAE) treatment in patients with psoriasis

No Ref	Sex Age	Treatment duration, years	Dose per day, mg	Formulation	Symptoms	Diagnosis	Treatment	Outcome	Known risk factors for PML
1. Ermis (2013)[15]	M 74	3	430	DMF + MEF #	Sensory aphasia	MRI findings, brain biopsy, PCR JCV in CSF	Mefloquine, mirtazapine	Improvement, persistence of sensory aphasia, IRIS	-
2. van Oosten (2013)[14]	F 42	5	420	DMF slow release ‡	Right-sided hemiparesis	MRI findings, PCR JCV in CSF	Mefloquine, mirtazapine, methylprednisone	Iprovement	-
3. Buttmann (2013)[24]	F 57	3	860	DMF + MEF #	NR	NR	NR	Residual symptoms	Pulmonary sarcoidosis
4. Stoppe (2014)[21]	M NR	3	NR	DMF + MEF #	Left-sided hemiataxia, dysarthria	MRI findings, PCR JCV in CSF	Immune-globulin, mirtazapine, mefloquine, mirtazapine, mefloquine	Improve-ment	Superficial spreading melanoma
5. Bartsch (2015)[23]	M 68	2.5	1075	DMF + MEF #	Left-sided hemihypesthesia, dysesthesia, left-sided weakness, dysphagia, dysarthria, focal motor seizures	MRI findings, brain biopsy, PCR JCV in CSF	Mirtazapine, mefloquine	Stable	Adenocarcinoma of the rectum
6. Dammeier (2015)[22]	F 53	1.5	360	DMF + MEF #	Transient confusion, sensory aphasia, headache	MRI findings, brain biopsy, PCR JCV in brain tissue	Mirtazapine, mefloquine	Stable	-
7. Hoepner (2015)[25]	M 69	5	1290	DMF + MEF #	Right-sided hemiparesis, aphasia	MRI findings, brain biopsy, PCR JCV in CSF	Mirtazapine mefloquine, levetiracetam, glucocorticoids	Improve-ment	Monoclonal gammopathy
8. Nieuwkamp (2015)[26]	F 64	2	NR	DMF slow release ‡	Apraxia, hemiparesis, somnolence	MRI findings, brain biopsy, PCR JCV in CSF/brain tissue	Mefloquine, mirtazapine, glucocorticoids	Died due to PML-IRIS	-

Fumaderm is a licensed FAE formulation in Germany with tablets of 105 mg (30 mg DMF plus 75 mg MEF-salts) and 215 mg (120 mg DMF and 95 mg MEF-salts). ‡ Psorinovo is a Dutch unlicensed, pharmacy-made slow-release FAE formulation with tablets of 30 and 120 mg DMF. CSF, cerebrospinal fluid; DMF, dimethyl fumarate; F, female; FAEs, fumaric acid esters; IRIS, immune reconstitution inflammatory syndrome; JCV, JC virus; M, male; MEF, monoethyl fumarate; NR, not reported; PML, progressive multifocal leukoencephalopathy; PCR, polymerase chain reaction.

Table 3: Summary of lymphocyte counts in published cases of progressive multifocal leukoencephalopathy (PML) associated with fumaric acid ester (FAE) treatment in patients with psoriasis

Case Ref	Duration of lyphocytopenia in years	Grade of lyphocytopenia†	Absolute lymphocyte count at time of diagnosis (per mm^3)	Absolute nadir of lymphocyte counts (per mm^3)	Absolute Leukocyte count (*10^9/L)	$CD4^+$ cell count (*1000/mL)	$CD8^+$ cell count (*1000/mL)	Ratio $CD4^+$/$CD8^+$
1. Ermis (2013)[15]	2	Grade 3	410	410	4.6	NR	NR	6.1
2. van Oosten (2013)[14]	5	Grade 3	200	200	NR	40	20	2.0
3. Buttmann (2013)[24]	Ca. 3	Grade 2-3	NR	445	NR	NR	NR	NR
4. Stoppe (2014)[21]	NR	Grade 3	Ca. 500	NR	NR	131	NR	NR
5. Bartsch (2015)[23]	2	Grade 2	500	500	3.0	154	117	1.3
6. Dammeier (2015)[22]	Ca. 1	Grade 2-3	450	450	> 3.0	NR	NR	NR
7. Hoepner (2015)[25]	Ca. 1.3	Grade 2-3	288	288	NR	NR	NR	NR
8. Nieuwkamp (2015)[26]	NR	Grade 2	792	792	4.0	270	40	6.8

†According to the Common Terminology Criteria for Adverse Events version 4.0, National Cancer Institute (NCI) of the National Institutes of Health (NIH): Grade 1 (mild) < lower limit of normal (LLN) – 800/mm^3 (<LLN – 0.8 * 10^9/L); Grade 2 (moderate) < 800–500/mm^3 (<0.8–0.5 * 10^9/L); Grade 3 (severe) < 500–200/mm^3 (<0.5–0.2 * 10^9/L); Grade 4 (life-threatening) < 200/mm^3 (<0.2 * 10^9/L).

Ca., circa; NR, not reported.

Table 4: Suggested recommendations to minimize risks for the development of progressive multifocal leukoencephalopathy during treatment with fumaric acid esters (FAEs)

Suggested recommendation	Comment
Before starting FAE treatment	
* Check for a medical history of recurring or opportunistic infections and neurological diseases, such as multiple sclerosis	Consider starting FAE treatment with more caution for blood cell counts and possible PML
* Obtain baseline measurements of absolute leukocyte counts and differential, including lymphocyte counts	In case of unknown leukocytopenia or lymphocytopenia, considering postponing FAE treatment until further hematological or immunological evaluation
During FAE treatment	
* In case of new onset of neurological symptoms, stop FAE treatment and consider referral to a neurologist for an evaluation for PML	See Table 2 for potential neurological symptoms
* Monitor leukocyte count and differential, including lymphocyte counts	Initially, monitoring once every months is advised, and after 6 months once every 3 months if no leukocyte abnormalities are present
* Adjust or stop FAE treatment in case of moderate-to-severe leukocytopenia or lymphocytopenia	Immediate stop FAE treatment in case of an absolute leukocyte count of 3.0 * 10^9/L or an absolute lymphocyte count of 0.5 * 10^9/L Reduce FAE dosage by 50% in case of an absolute lymphocyte count of 0.7 * 10^9/L and follow-up in 4 weeks
After FAE treatment discontinuation	
* In case of leukocytopenia or lymphocytopenia follow-up leukocyte- and lymphocyte counts until reaching normal values	Until leukocyte and/or lymphocyte count normalization, consider postponing start of systemic treatments with a known association with leukocytopenia, e.g. methotrexate; Consider starting optimal topical treatment and/or UVB phototherapy

Prof.dr. Hermann W. Siemens

"Psoriasis wordt met alles behandeld, wat langs velden en wegen en in den schoot der bergen groeit."

Vraagstukken der psoriasisbehandeling. *Ned Tijdschr Geneeskd*. 1938;82:1290-8.

Jay Frank Schamberg

"Psoriasis, the great dermatologic mystery, is an affliction that has been known since the days of the early Greeks."

The known and the unknown about psoriasis. *JAMA*. 1924;83(16):1211-1214.

CHAPTER| 5.2

Drug-induced Fanconi syndrome associated with fumaric acid esters treatment for psoriasis: a case series

Deepak M.W. Balak[1]

Jan Nico Bouwes Bavinck[2]

Aiko P.J. de Vries[3]

Jenny Hartman[4]

H.A. Martino Neumann[1]

Robert Zietse[5]

H. Bing Thio[1]

[1]Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands

[2]Department of Dermatology, Leiden University Medical Center, Leiden, the Netherlands

[3]Division of Nephrology, Department of Medicine, Leiden University Medical Center, Leiden, the Netherlands

[4]Netherlands Pharmacovigilance Centre Lareb, 's-Hertogenbosch, the Netherlands

[5]Division of Nephrology and Transplantation, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

Clin Kidney J. 2016 Feb;9(1):82-9.

ABSTRACT

Background: Fumaric acid esters (FAEs), an oral immunomodulating treatment for psoriasis and multiple sclerosis, have been anecdotally associated with proximal renal tubular dysfunction due to a drug-induced Fanconi syndrome. Few data are available on clinical outcomes of FAE-induced Fanconi syndrome.

Methods: Descriptive case series with two cases of Fanconi syndrome associated with FAE treatment diagnosed at two Dutch university nephrology departments, three cases reported at the Dutch and German national pharmacovigilance databases and six previously reported cases.

Results: All 11 cases involved female patients with psoriasis. The median age at the time of onset was 38 years [interquartile range (IQR) 37–46]. Patients received long-term FAEs treatment with a median treatment duration of 60 months (IQR 28–111). Laboratory tests were typically significant for low serum levels of phosphate and uric acid, while urinalysis showed glycosuria and proteinuria. Eight (73%) patients had developed a hypophosphataemic osteomalacia and three (27%) had pathological bone fractures. All patients discontinued FAEs, while four (36%) patients were treated with supplementation of phosphate and/or vitamin D. Five (45%) patients had persisting symptoms despite FAEs discontinuation.

Conclusions: FAE treatment can cause drug-induced Fanconi syndrome, but the association has been reported infrequently. Female patients with psoriasis treated long term with FAEs seem to be particularly at risk. Physicians treating patients with FAEs should be vigilant and monitor for the potential occurrence of Fanconi syndrome. Measurement of the urinary albumin:total protein ratio is a suggested screening tool for tubular proteinuria in Fanconi syndrome.

INTRODUCTION

Fumaric acid esters (FAEs) are small molecules with immunomodulating effects that have been used as an oral treatment for psoriasis for four decades.[1] Since 1994, FAEs are an approved treatment for psoriasis in Germany. The licensed FAE formulation Fumaderm® (Biogen Idec, Ismaning, Germany) is a mixture of dimethylfumarate and monoethylfumarate salts.[2] Currently, Fumaderm is one of the most commonly used first-line systemic treatments in Germany.[3] In other countries, FAEs are increasingly used as an unlicensed treatment for psoriasis. The European S3 guidelines recommend FAEs as a treatment for moderate-to-severe psoriasis on the basis of their positive efficacy and safety profile.[4] The U.S. Food and Drug Administration and the European Medicines Agency approved an FAE formulation containing dimethylfumarate (Tecfidera®; Biogen Idec, Cambridge, MA, USA) as a treatment for multiple sclerosis in 2013 and 2014, respectively.[5]

FAEs have been anecdotally linked to renal adverse events, such as acute kidney injury and Fanconi syndrome (FS).[6-9] FS is defined by a generalized dysfunction of the proximal renal tubules, which can lead to an impaired resorption of glucose, amino acids and phosphate. A complication of FS is osteomalacia, which can lead to bone fractures and bone pain.[10] Several drugs can induce FS, such as tenofovir, ifosfamide, aminoglycoside antibiotics and FAEs (see Table 1).[7,10] There have been only a limited number of cases described of FS associated with FAEs. Consequently, few data are available on the clinical presentation and treatment of FAE-induced FS.

Here, we assessed clinical and treatment outcomes of drug-induced FS associated with FAEs treatment.

MATERIALS AND METHODS

This is a descriptive case series of FAE-associated FS in patients with psoriasis. We described two new cases that were diagnosed at our department. We reviewed clinical features, laboratory findings and treatment outcomes. Measurements of serum levels of substances such as phosphate, calcium and 1,25-dihydroxyvitamin D were performed according to standard operating procedures at the Department of Clinical Chemistry. Additional testing to exclude the differential diagnoses of FS involved genetic testing or radiologic evaluation.

We searched for additional cases in pharmacovigilance databases of the Netherlands Pharmacovigilance Centre Lareb and the German Federal Institute for Drugs and Med-

ical Devices [Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)] up to August 2015. We chose these two national pharmacovigilance databases given that FAEs are extensively used in these countries.[3,11] In addition, Medline and Embase databases were searched up to August 2015 for published cases.

We used descriptive statistical methods to analyse data.

RESULTS

Case characteristics We included a total of 11 patients who developed FS during treatment with FAEs. Two cases were diagnosed at the nephrology department of two Dutch university medical centres, three cases were identified in German and Dutch pharmacovigilance databases and six case reports were found through a literature search. The clinical characteristics of the cases are presented in Table 2.

All cases involved female patients with psoriasis. The median age at the time of onset of FS was 38 years [interquartile range (IQR) 37–46]. The median treatment duration with FAEs was 60 months (IQR 28–111) and the median daily FAEs dosage was 840 mg (IQR 720–1290). Data on body mass were available for four patients [median 20.9 kg/m^2 (IQR 20.3–23.6)]

In most cases, patients did not exhibit any symptoms. The most frequently reported presenting symptoms of FS were generalized weakness (27%), myalgia (27%) and arthralgia (27%) (see Table 3). Laboratory tests often displayed low serum levels of phosphate (73%), high alkaline phosphatase (45%) and low uric acid (45%). Urinalysis typically showed glycosuria (73%), proteinuria (64%), aminoaciduria (27%) and phosphaturia (27%). Diagnosis of FS was based on laboratory test results. Five (45%) patients were diagnosed with a hypophosphataemic osteomalacia, of whom three (27%) patients suffered from multiple pathological bone fractures. FAEs treatment was discontinued in almost all cases; two reports did not describe whether FAEs were discontinued. Four (36%) patients were treated with supplementation of phosphate and three (27%) with vitamin D supplementation.

Complete improvement was reported in four (36%) patients, partial improvement in one (9%) patient and there were five (45%) patients who had no improvement. In one (9%) case, the symptoms of FS recurred twice upon rechallenges with FAEs.[12]

Below, we describe detailed information on clinical features and outcomes of the novel FS cases.

New cases of FAE-associated FS A 37-year-old female patient with plaque psoriasis developed FS during FAE treatment (patient no. 7; see Tables 2 and 4). This patient had a history of psoriasis from the age of 20 years and had been previously treated with phototherapy and topical treatments. She had been treated with oral FAEs for 15 years and had only temporarily stopped FAEs during pregnancy. Her medical history was remarkable for two miscarriages, migraine and recurrent urinary tract infections. She used FAEs (Fumaderm) 215 mg dimethylfumarate once per day, rizatriptan 5 mg as needed, an ethinylestradiol/gestodene contraceptive and calcipotriol/betamethasone dipropionate ointment as needed. She had no history of drug use known to cause drug-induced FS. The psoriasis was well controlled with FAEs and there were no adverse events. Routine urinalyses throughout FAE treatment indicated persisting proteinuria and glycosuria, therefore she was referred to the nephrology department for further analysis.

Additional laboratory testing showed a hypophosphataemia (serum phosphate 0.86 mmol/L, RR 0.90–1.50) with a normal serum creatinine level (74 µmol/L, RR 49–90) and a normal estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m^2 (see Table 3). The fractional phosphate excretion was 22%, suggesting renal phosphate wasting. There was also a low serum level of uric acid of 0.09 mmol/L (RR 0.14–0.34); the fractional uric acid excretion was >73%. Further urinalysis revealed a tubular proteinuria (0.86 g protein/24 h). There was also glycosuria (1+ glucose on dipstick) in the face of a normal glycated haemoglobin level of 32 mmol/mol Hb (RR 20–42). She also had hypercalciuria and nephrolithiasis. Her serum calcium level was normal. The parathyroid hormone (PTH) level was decreased (0.7 pmol/L, RR 1.4–7.3) and PTH-like peptide was not present. Differential diagnoses as Dent's disease and sarcoidosis were excluded by genetic mutation testing and by chest radiograph evaluation, respectively. The findings in this case were consistent with a drug-induced FS. However, although unlikely, a new genetic entity cannot be excluded. FAE treatment was subsequently discontinued. However, the laboratory and urine abnormalities persisted.

A second case of FAE-associated FS involved a 40-year-old female patient who had plaque psoriasis since the age of 8 years (patient no. 8; see Tables 2 and 4). Her medical history was remarkable for hypercholesterolaemia and recurrent vaginal yeast infections, and she was recently diagnosed with a vitamin B12 deficiency. Her medication use included atorvastatin 10 mg once daily, fluconazole 150 mg as needed and vitamin B12 injections. There was no prior use of drugs linked to drug-induced FS. She had previously been treated with topical psoriasis treatments and phototherapy. In May 2008, she started FAE treatment 215 mg three to six times per day. Her psoriasis was well controlled and she reported no

adverse events. She had used FAEs continuously for 7 years. Routine urinalysis showed persisting proteinuria, and therefore she was referred to the nephrology department.

Additional testing was significant for hypophosphataemia (serum phosphate 0.58 mmol/L, RR 0.80–1.40), hypocalcaemia (serum calcium 2.16 mmol/L, RR 2.20–2.65), an increased PTH level of 9.1 pmol/L (RR 1.4–7.3) and a slightly decreased serum bicarbonate level of 19.9 mmol/L (RR 21.0–27.0) (see Table 3). The fractional phosphate excretion was 30.6%, which is in line with renal phosphate wasting. The serum creatinine level was within the normal range (87 µmol/L, RR 55–90), as was the eGFR of 63 mL/min/1.73 m^2 (RR >60). Urinalysis was significant for glycosuria (3+ glucose on dipstick) and tubular proteinuria (0.38 g protein/L). Based on these findings, a diagnosis of FAE-induced FS was made. It is likely that the patient's vitamin B12 deficiency is part of FS, considering that vitamin B12 is taken up at the proximal renal tubule.[17] Pernicious anaemia as an alternative reason for the vitamin B12 deficiency was excluded, as antibodies to intrinsic factor were not present. Vitamin D is also taken up in the proximal tubule[17], but in this case serum 1,25-dihydroxyvitamin D was within normal limits (69 pmol/L, RR 38–183). Following the diagnosis of FS, FAEs were stopped. There was partial improvement of the symptoms with normalization of serum phosphate levels and proteinuria.

Cases reported in Dutch and German national pharmacovigilance databases One case of FS linked to FAEs was reported to Lareb. This case involved a 31-year-old female psoriasis patient. Her medical history was remarkable for endometriosis, and she used celecoxib and a lynestrenol contraceptive. She had been treated with FAEs for 5 years (dosage not reported). She developed FS with severe osteomalacia. She had no symptoms except for a spontaneous bone fracture. FAE treatment was discontinued and the patient was treated with supplementation of phosphate and vita- min D. The patient did not recover and became wheelchair dependent.

Two cases of FS were reported by BfArM. A female patient between 45 and 49 years of age was reported with FS. She had been treated since 1989 with Fumaderm (dosage not reported) and topical fumaric acid. In 1999, she developed FS, from which she did not recover. She also developed osteomalacia, which led to a pathologic fracture. The second case involved a female psoriasis patient between 29 and 35 years of age who had been treated with Fumaderm (dosage not reported) since 1995. She was reported with FS (year of onset not reported) with a complete recovery. She was diagnosed in 1998 with osteomalacia.

DISCUSSION

FS is a renal disorder that is characterized by proximal tubular dysfunction, which can lead to inappropriate urinary losses of phosphate, glucose, bicarbonate and amino acids. Here, we report 11 psoriasis patients who developed an FAE-induced FS. Presenting symptoms of FS included generalized weakness and myalgia. Laboratory tests typically showed low serum levels of phosphate and uric acid and a proximal tubular acidosis, while urinalysis showed glycosuria and proteinuria. Eight (73%) patients had hypophosphataemic osteomalacia and five (45%) patients had persisting symptoms despite FAE discontinuation.

The reported cases of FAE-associated FS share several phenotypical similarities. All cases involved females. Sex differences in renal proximal tubular function and in the expression of organic anion receptors have been described and such differences could underlie differential risks for male and female patients to develop FAE-associated FS.[18,19] However, we cannot exclude selective reporting due to the relatively small sample size described in our case series. Whether there is a true predominance for females to develop FS remains to be determined. Furthermore, all patients reported with FS were treated long term with FAEs. The median duration of FAEs treatment was 60 months (IQR 28–111). The five cases that were published in the 1990s were linked to the use of unlicensed FAEs formulations with dimethylfumarate and monoethylfumarate salts. Moreover, the doses of FAEs applied in these cases exceeded the maximum daily dose of 1290 mg that is recommended in current psoriasis guidelines.[4] However, several of the patients were treated with lower dosages of FAEs and still developed FS (see Table 2). Therefore, a clear correlation between FAE dosage and proximal tubular dysfunction cannot be made. Higher doses of FAEs per body weight has been proposed as a potential factor in the nephrotoxicity of FAEs.[9] The body mass index (BMI) was reported for only four cases, with a median BMI for these cases of 20.9 kg/m^2 (IQR 20.3–23.6). More data are needed to identify risk factors for the development of FS during FAEs treatment.

Limitations of our study are the relatively small sample size and the fact that causality between FAEs and drug-induced FS cannot be proven in this data set. In addition, the data available for some cases were incomplete for findings of proximal tubular dysfunction. Also, some cases had limited data on alternative causes of FS or use of prior and concomitant drugs that are known to result in drug-induced FS. Two recent epidemiological studies have shown an independent association between moderate-to-severe psoriasis and an increased risk for glomerulonephritis or chronic kidney disease.[20,21] Psoriasis itself does not

seem to be associated with FS.

The incidence of FAE-associated FS has not been studied. In randomized controlled trials that evaluated FAEs treatment in psoriasis patients, no renal toxicity was reported.[4] This could be due to the relatively short follow-up period in these trials. Long-term observational studies have not indicated an increased risk for nephrotoxicity during continuous FAE treatment.[22,23] FS could be underdiagnosed, especially mild forms of proximal tubular dysfunction, given that proteinuria is a recognized and common adverse event of FAEs.[24] Up to 30% of patients display proteinuria.[25] On the other hand, FAE-induced proteinuria is typically transient, even with continued FAE treatment.[4]

The current psoriasis guidelines recommend regular urinalysis during FAEs, but there is no consensus on the optimal frequency. The 2009 European S3 guidelines on psoriasis treatment recommend monitoring serum creatinine and urine sediment every 4 weeks.[4] In the 2011 German psoriasis guidelines, it is advised to monitor serum creatinine and urine status every 4 weeks for the first 4 months of treatment, followed by once every 8 weeks.[26] Urinalysis is typically performed with a urine dipstick, but this method is sensitive for albumin and probably not reliable for detection of tubular proteinuria.[27] Tubular dysfunction leads to increased urinary levels of proteins other than albumin, such as β2-microglobulin. Haring et al. assessed the use of β2-microglobulin in urine as a marker for proximal tubular damage during FAEs treatment in psoriasis patients.[9] Urinary β2-microglobulin was increased in 3 of 23 patients receiving FAEs, which normalized upon discontinuation of the FAEs. β2-microglobulin does not seem to be a sensitive marker, which may be due to the collection method. β2- microglobulin is not stable in acid urine and is only measurable after alkalinization of the urine. A new method is measurement of the urinary albumin:total protein ratio, which is an inexpensive and simple screening test. If albumin constitutes <40% of the total protein in urine, this is a good indication for tubular proteinuria.[28] In our data set, we had no data available on the urinary albumin:total protein ratio. Use of the urinary albumin:total protein ratio as a screening test for tubular proteinuria needs to be tested and validated for FAEs-induced FS.

FAEs are increasingly being used, also for conditions other than psoriasis.[29] An FAE formulation containing dimethylfumarate (Tecfidera®, Biogen Idec) was approved for the treatment of multiple sclerosis in 2013. Although the FAEs formulation and the dosage schedule are different than those used in the treatment of psoriasis, the safety profile of this FAE formulation seems similar to that of FAE treatment in psoriasis. There have been no reports of proximal tubular dysfunction with dimethylfumarate in multiple sclerosis patients.[30,31]

The mechanisms leading to FAE-induced proximal tubular dysfunction in FS are not understood.7 One potential mechanism involves FAE-induced glutathione depletion. FAEs are able to enter proximal tubular cells via the organic anion transporter (OAT1)[32] and deplete intracellular levels of glutathione levels[33], which can cause FS.[9,34] Maleic acid, a cis isomer of fumaric acid, induces FS in rats through similar mechanisms of intracellular depletion of glutathione and adenosine triphosphate.[35]

In conclusion, FS seems to be an infrequent adverse event of FAE treatment. Female patients treated long term with FAEs seem to be particularly at risk. Physicians treating patients with FAEs should be aware of and vigilant for proximal tubular dysfunction during FAEs treatment. Quantitative total protein measurement to calculate the albumin:total protein ratio is suggested as a screening test for tubular proteinuria.

REFERENCES

1. Mrowietz U et al. Dimethylfumarate for psoriasis: more than a dietary curiosity. Trends Mol Med 2005; 11: 43–48.
2. Rostami Yazdi M et al. Fumaric acid esters. Clin Dermatol 2008; 26: 522–526.
3. Augustin M et al. German psoriasis registry PsoBest: objectives, methodology and baseline data. J Dtsch Dermatol Ges 2014; 12: 48–57.
4. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol 2009; 23(Suppl 2): 1–70.
5. Sheridan C. Second oral MS drug wins FDA nod. Nat Biotechnol 2013; 31: 373.
6. Raschka C et al. Longterm treatment of psoriasis using fumaric acid preparations can be associated with severe proximal tubular damage. Hum Exp Toxicol 1999; 18: 738–739.
7. Izzedine H et al. Drug-induced Fanconi's syndrome. Am J Kidney Dis 2003; 41: 292–309.
8. Roodnat JI et al. Acute kidney insufficiency in patients treated with fumaric acid esters for psoriasis. Ned Tijdschr Geneeskd 1989; 133: 2623–2626.
9. Haring N et al. Early detection of renal damage caused by fumaric acid ester therapy by determination of urinary beta2-microglobulin. Br J Dermatol 2011; 164: 648–651
10. Hall AM et al. Drug-induced renal Fanconi syndrome. QJM 2014; 107: 261–269
11. Fallah Arani S et al. Treatment of psoriasis with non-registered fumaric acid esters in the Netherlands: a nationwide survey among Dutch dermatologists. J Eur Acad Dermatol Venereol 2014; 28: 972–975.
12. Fliegner L et al. Osteomalacia as an obviously rare secondary effect of oral fumaric acid therapy. Hautarzt 1992; 43: 554–560.
13. Haviv YS et al. Fumaric acid ester-induced diffuse renal tubular injury presenting as Fanconi syndrome and osteomalacia. Clin Drug Investig 1999; 17: 333-5
14. Schilling F et al. Adult Debre-de Toni-Fanconi syndrome with osteomalacia, acquired through long-termpsoriasis therapy with fumaric acid ester. Aktuelle Rheumatol 1999; 24: 174–179.
15. Warzecha J et al. Multiple pathological fractures within the scope of DeToni-Debre-Fanconi syndrome after fumarate therapy in psoriasis. Unfallchirurg 2001; 104: 448–451.
16. Reid C et al. De Toni-Fanconi syndrome secondary to fumaric acid esters. Br J Dermatol 2013; 169: 24.
17. Birn H. The kidney in vitamin B12 and folate homeostasis: Am J Physiol Renal Physiol 2006; 291: F22–36
18. Sharma N et al. Sex differences in renal and metabolic responses to a high-fructose diet in mice. Am J Physiol Renal Physiol 2015; 308: F400–F410.
19. Sekine T et al. Molecular physiology of renal organic anion transporters. Am J Physiol Renal Physiol 2006; 290: F251–F261.
20. Wan J et al. Risk of moderate to advanced kidney disease in patients with psoriasis: population based cohort study. BMJ 2013; 347: f5961.
21. Chiu HY et al. Increased risk of glomerulonephritis and chronic kidney disease in relation to the severity of psoriasis, concomitant medication, and comorbidity: a nationwide population-based cohort study. Br J Dermatol 2015; 173: 146–154.
22. Reich K et al. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis—a retrospective study (FUTURE). J Dtsch Dermatol Ges 2009; 7: 603–611.
23. Hoefnagel JJ et al. Long-term safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol 2003; 149: 363–369

24. Ogilvie S et al. Proteinuria with fumaric acid ester treatment for psoriasis. Clin Exp Dermatol 2011; 36: 632–634.

25. Balak DM et al. Addition of an oral histamine antagonist to reduce adverse events associated with fumaric acid esters in the treatment of psoriasis: a randomized double-blind placebo-controlled trial. Br J Dermatol 2015; 172: 754–759.

26. Nast A et al. German S3-guidelines on the treatment of psoriasis vulgaris (short version). Arch Dermatol Res 2012; 304: 87–113.

27. Sise ME et al. Nonalbumin proteinuria predominates in biopsy-proven tenofovir nephrotoxicity. AIDS 2015; 29: 941–946.

28. Smith ER et al. The value of simultaneous measurements of urinary albumin and total protein in proteinuric patients. Nephrol Dial Transplant 2012; 27: 1534–1541.

29. Meissner M et al. Dimethyl fumarate—only an anti-psoriatic medication? J Dtsch Dermatol Ges 2012; 10: 793–801.

30. Fox RJ et al. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med 2012; 367: 1087–1097.

31. Gold R et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med 2012; 367: 1098–1107.

32. Kaler G et al. Structural variation governs substrate specificity for organic anion transporter (OAT) homologs. Potential remote sensing by OAT family members. J Biol Chem 2007; 282: 23841–23853.

33. Ghoreschi K et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. J Exp Med 2011; 208: 2291–2303.

34. Abraham et al. Depletion of the cellular antioxidant system contributes to tenofovir disoproxil fumarate-induced mitochondrial damage and increased oxido-nitrosative stress in the kidney. J Biomed Sci 2013; 20: 61.

35. Zager RA et al. Maleate nephrotoxicity: mechanisms of injury and correlates with ischemic/hypoxic tubular cell death. Am J Physiol Renal Physiol 2008; 294: F187–F197.

TABLES AND FIGURES

Table 1: Overview of drugs that can cause a drug-induced Fanconi syndrome[7,10]

Drug	Class of drug	Indications
Adefovir	Antiviral	Hepatitis B
Aminoglycosides, e.g. gentamicin, tobramycin, amikacin	Antibiotic	Bacterial infections
Aspirin	Cyclooxygenase inhibitor	Pain, fever
Azacitidine	Cytostatic	Types of cancer
Carboplatin	Cytostatic	Types of cancer
Cidofovir	Antiviral	Cytomegalovirus retinitis
Cisplatin	Cytostatic	Types of cancer
Deferasirox	Iron chelator	Chronic iron overload
Didanosine	Antiviral	HIV
Fumaric acid esters, e.g. dimethyl fumarate	Immunomodulator	Psoriasis, multiple sclerosis
Ifosfamide	Cytostatic	Types of cancer
Imatinib mesylate	Tyrosine-kinase inhibitor	Leukemia
Mercaptopurine	Cytostatic	Leukemia
Ranitidine	Histamine antagonist	Gastroesophageal reflux, peptic ulcer disease
Streptozocin	Cytostatic	Types of cancer
Suramin	Antiparasitic	Trypanosomiasis, onchocerciasis
Tenofovir	Antiviral	HIV, hepatitis B
Tetracyclines	Antibiotic	Bacterial infections, acne
Valproic acid	Anticonvulsant	Epilepsy, bipolar disorder, migraine

Table 2: Overview of reported cases of drug-induced Fanconi syndrome associated with FAEs treatment in patients with psoriasis

No Ref	Sex Age	BMI	FAE treatment duration, months	FAE dosage per day	Complaints	Lab deviations	Therapy	Outcome
1. Fliegner[12] (1992)	F 46	NR	16	1736 mg (960 mg DMF+ 776 mg MEF)	Arthralgia, myalgia, difficulties with walking, immobility	Serum: low phosphate, low uric acid, increased alkaline phosphatase Urine: glycosuria, proteinuria	FAE treatment discontinuation. Oral phosphate and vitamin D3 supplementation	Complete improvement. Recurrence after re-treatment with FAEs. Osteomalacia +
2. Haviv[13] (1999)	F 48	20.3	12	NR	Generalized weakness, dyspnoea	Serum: increased alkaline phosphatase, low phosphate, increased PTH, vitamin B12 deficiency, low total calcium Urine: glycosuria, aminoaciduria, phosphaturia, uric aciduria	FAE treatment discontinuation. Oral phosphate supplementation	Improvement of the respiratory capacity Osteomalacia +
3. Raschka[6] (1999)	F 38	20.2	60	840 mg	Fatigue, weakness, polydipsia	Serum: low uric acid, low phosphate Urine: proteinuria, glycosuria	FAE treatment discontinuation. Oral phosphate supplementation	No improvement within 6 months No osteomalacia
4. Schilling[14] (1999)	F 35	NR	Ca 36	Max. 1290 mgb (720 DMF + 570 MEF)	Pain in feet, myalgia	Serum: increased alkaline phosphatase, low phosphate, low uric acid Urine: glycosuria, proteinuria, hypercalciuria	FAE treatment discontinuation	Complete improvement within 8 months Osteomalacia +
5. Warzcha[15] (2001)	F 48	NR	Ca 120	NR	Multiple pathological bone fractures, myalgia	Serum: increased alkaline phosphatase, low phosphate, low vitamin D3 Urine: proteinuria, aminoaciduria, glycosuria	FAE treatment discontinuation. Oral vitamin D3 supplementation	Complete improvement within 3 months Osteomalacia +
6. Reid[16] (2013)	F 37	NR	25	720 mg	Generalized weakness and pain in her feet, pathologic bone fractures	Serum: increased serum alkaline phosphatase, low phosphate, low uric acid, low potassium Urine: proteinuria, glycosuria, aminoaciduria, hypercalciuria	FAE treatment discontinuation	Complete improvement within 4 weeks Osteomalacia +

Table 2 (continued)

No Ref	Sex Age	BMI	FAE treatment duration, months	FAE dosage per day	Complaints	Lab deviations	Therapy	Outcome
7 This report	F 37	21.5	180	120–480 mg DMFb	None	Serum: low phosphate, low uric acid, low PTH Urine: Proteinuria, glycosuria, hypercalciuria, phosphaturia	FAE treatment discontinuation	No improvement No osteomalacia
8 This report	F 40	NR	84	720 mg DMF	None	Serum: low phosphate, low calcium, increased PTH, low bicarbonate, low vitamin B12 Urine: proteinuria, glycosuria, phosphaturia	FAE treatment discontinuation	Partial Improvement No osteomalacia
9 This report (Lareb)	F 31	29.8	60	NR	Spontaneous bone fracture	NR	FAE treatment discontinuation. Phosphate and vitamin D supplementation	No improvement Osteomalacia +
10 This report BfArM	F 45-49	NR	120	NR	NR	NR	NR	No improvement Osteomalacia +
11 This report BfArM	F 29-35	NR	NR	NR	NR	NR	NR	NR Osteomalacia +

BMI, body mass index; DMF, dimethylfumate; F, female; FAEs, fumaric acid esters; M, male; MEF, monoethylfumarate; NR, not reported. a Unlicensed FAE formulation containing dimethylfumarate plus monoethylfumarate salts. b Licensed German FAE formulation containing dimethylfumarate plus monoethylfumarate salts (Fumaderm).

Table 3: Overview of characteristics of drug-induced FS observed in cases associated with FAE treatment for psoriasis (n = 11)

Characteristic	Frequency in cases linked to FAEs
Subjective symptoms	
Myalgia	3
Generalized weakness	3
Arthralgia/pain in feet	3
Fatigue	1
Polydipsia	1
Immobility	1
Dyspnoea	1
Laboratory abnormalities	
Low phosphate	8
High alkaline phosphatase	5
Low uric acid	2
High PTH	2
Low vitamin B12	2
Low calcium	2
Low potassium	1
Low vitamin D3	1
Low bicarbonate	1
Low PTH	1
Urine analysis abnormalities	
Glycosuria	8
Proteinuria	7
Aminoaciduria	3
Hypercalciuria	3
Phosphaturia	3
Uric aciduria	1
Complications	
Osteomalacia	8
Pathological bone fractures	3

Table 4: Laboratory data characteristics from the two new cases of drug-induced FS associated with FAE treatment

Characteristics (reference values)	**Patient no. 7**	**Patient no. 8**
Serum		
eGFR (>60 mL/min/1.73m^2)	>60	63
Creatinine (49–90 µmol/L)	74	87
Uric acid (0.14–0.34 mmol/L)	0.09	NA
Phosphate (0.90–1.50 mmol/L)	0.86	0.58
1,25-Dihydroxyvitamin D3 (38–183 pmol/L)	259	69
25-Hydroxyvitamin D3 (50–250 nmol/L)	161	71
PTH (1.4–7.3 pmol/L)	0.7	9.1
PTH-like peptide	Negative	NA
Alkaline phosphatase (0–97 U/L)	43	NA
Calcium (2.20–2.65 mmol/L)	2.50	2.16
Bicarbonate (21.0–27.0 mmol/L)	26	19.9
HbA1c (26–42 mmol/mol)	32	NA
Glucose (4.0–6.1 mmol/L)	NA	4.8
Urine		
Proteinuria (dipstick)	Positive (2+)	Positive (1+)
Proteinuria (g protein/24 h)	0.86	0.38
Glycosuria	Positive (1+)	Positive (3+)
Hypercalciuria (mmol/L)	4.5	NA
Creatinine (mmol/L)	5.1	4.7
Phosphate (mmol/L)	16	9.6
Fractional phosphate excretion	22%	31%
Fractional uric acid excretion	>73%	NA

eGFR, estimated glomerular filtration rate; NA, not available.

| PART IV |
MECHANISMS OF ACTION OF FUMARIC ACID ESTERS IN PSORIASIS

Christopher E.M. Griffiths

"The impressive ability of infliximab, a monoclonal antibody against TNF-alpha, to clear severe psoriasis might perhaps have been predicted but serendipity took the lead."

Immunotherapy for psoriasis: from serendipity to selectivity. *Lancet.* 2002 Jan 26;359(9303):279-80.

CHAPTER| 6

Regulated genes in psoriatic skin during treatment with fumaric acid esters

Armanda J. Onderdijk[1,2*]

Deepak M.W. Balak[1*]

Ewout M. Baerveldt[1]

Edwin F. Florencia[1]

Marinus Kant[1]

Jon D. Laman[2]

Wilfred F.J. van IJcken[3]

Emoké Racz[1]

Dick de Ridder[4]

H. Bing Thio[1]

Errol P. Prens[1,2]

[1]Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands

[2]Department of Immunology, Erasmus Medical Center, Rotterdam, the Netherlands

[3]Center for Biomics, Erasmus Medical Center, Rotterdam, the Netherlands

[4]The Delft Bioinformatics Lab, Faculty of Electrical Engineering, Mathematics and Computer Science, Delft University of Technology, Delft, the Netherlands

[*]A.J.O. and D.M.W.B. share first authorship

Br J Dermatol. 2014 Oct;171(4):732-41.

ABSTRACT

Background: Fumaric acid esters (FAEs) are widely used in Europe for the treatment of psoriasis because of their clinical efficacy and favourable safety profile. However, the mechanisms of action by which FAEs improve psoriasis remain largely unknown. In this study, we aimed to identify pathways and mechanisms affected by FAE treatment and to compare these with pathways affected by treatment with the antitumour necrosis factor (anti-TNF)-a biologic etanercept.

Methods: In a prospective cohort study, 50 patients with plaque psoriasis were treated with FAEs for 20 weeks. Nine patients were randomly selected for gene expression profiling of plaque biopsies from week 0 and week 12. The groups consisted of FAE responders [> Psoriasis Area and Severity Index (PASI) 75% improvement] and nonresponders (< PASI-50 improvement). Changes in gene expression profiles were analysed using Ingenuity Pathway Analysis and the outcome was compared with gene expression affected by etanercept.

Results: Response to FAE treatment was associated with a ≥ 2-fold change ($P < 0{\cdot}05$) in the expression of 458 genes. In FAE responders the role of interleukin-17A in the psoriasis pathway was most significantly activated. Glutathione and Nrf2 pathway molecules were specifically induced by FAE treatment and not by etanercept treatment, representing an FAE-specific effect in psoriatic skin. In addition, FAE treatment specifically induced the transcription factors PTTG1, NR3C1, GATA3 and NFκBIZ in responding patients.

Conclusions: FAE treatment induces glutathione and Nrf2 pathway genes in lesional skin of patients with psoriasis. In responders, FAEs specifically regulate the transcription factors PTTG1, NR3C1, GATA3 and NFκBIZ, which are important in normal cutaneous development, and the T-helper (Th) 2 and Th 17 pathways, respectively.

INTRODUCTION

Psoriasis is a common chronic inflammatory skin disease, characterized by hyperproliferation of keratinocytes and an increased dermal infiltration by immune cells, notably neutrophils and T helper (Th) 1 and Th 17 cells. Most patients with moderate-to-severe disease require long-term systemic treatment to control their psoriasis. Fumaric acid esters (FAEs) are small molecules that have been used as oral treatment in psoriasis for more than 25 years, mainly in Western Europe.[1] Of patients with psoriasis treated with FAEs, 50–70% show a clinical improvement of at least 75% following 16 weeks of treatment.[2] This treatment response is comparable to the efficacy of first-generation anti- tumour necrosis factor (TNF)-alpha biologics, but at a fraction of the costs. Data from long-term observational studies on treatment of patients with psoriasis with FAEs indicate a favourable safety profile without evidence for an increased risk of infections or malignancies.[3-5] In vitro, FAEs inhibit dendritic cell maturation and keratinocyte proliferation.[6,7]

Anti-TNF-alpha biologics, including etanercept, are commonly used effective systemic treatments for moderate-to-severe psoriasis.[8] In recent years, gene expression profiling studies have provided insights into the mechanisms of action and the signalling pathways by which anti-TNF-a biologics improve psoriasis.[9,10] However, the molecular pathways by which FAEs improve psoriasis remain largely unknown. FAEs have not been studied by gene expression profiling nor have they been compared with anti-TNF-alpha biologics.

In this study we investigated pathways and mechanisms targeted by FAE treatment, and assessed whether successful FAE treatment invoked different molecules and pathways from etanercept treatment. Gene expression profiling was performed on RNA derived from biopsies of psoriatic plaques taken before and after 12 weeks of FAE treatment. We then compared the molecules and pathways that were differentially affected by FAE treatment with those affected by etanercept treatment.

MATERIALS AND METHODS

Study design and skin biopsies In a prospective, single-centre clinical study, 50 patients with a Psoriasis Area and Severity Index (PASI) ≥ 10 were treated with oral FAE for 20 weeks. In the Netherlands, the import of Fumaderm tablets from Germany is often not reimbursed by insurance companies. We therefore used a Dutch FAE formulation with enteric-coated tablets containing 105 mg FAEs (30 mg dimethylfumarate, 75 mg calcium mo-

noethylfumarate) and 215 mg FAEs (120 mg dimethylfumarate, 95 mg calcium monoethylfumarate.[11] The short-term efficacy of this FAE formulation is comparable with that of oral methotrexate 15 mg weekly.[12]

Eligible patients were at least 18 years of age, had a diagnosis of plaque psoriasis for at least 6 months, and were candidates for phototherapy or systemic therapy. Patients were excluded when they had received systemic psoriasis therapy or phototherapy within the previous 4 weeks, or had received topical psoriasis treatment within 2 weeks. All patients were dosed according to the German S-3 guideline on systemic treatment of psoriasis (see Supporting Information TableS1).[13] Lesional skin biopsies (3 mm) were taken at baseline from the edge of a well-defined psoriasis plaque on the legs and after 12 weeks, from the same plaque, near to the previous biopsy site at baseline. The interval of 12 weeks was chosen because subjects had by then reached the maximum daily dosage of FAEs and after 12 weeks of treatment a clinically meaningful improvement is expected in daily clinical practice. Responders were defined as having a PASI improvement ≥ 75% at week 12 compared with baseline, while nonresponders were defined as having a PASI improvement < 50%, as defined in the European treatment goal consensus.[14]

Patients were randomly selected for gene expression profiling based on clinical improvement. The intermediate responders (PASI 50–75; n = 19) and dropouts (n = 13) were not included in the analysis.

The clinical study protocol was approved by the local medical ethical committee (MEC 2005-105), and all patients gave written informed consent prior to study enrolment. The study was conducted according to the principles of the Declaration of Helsinki.

cDNA preparation hybridization cDNA was made using 1 lg of total RNA template, with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, U.S.A.) and oligo(dT). qRT-PCR was performed using the ABI PRISM 7900 sequence-detection system (Applied Biosystems, Foster City, CA, U.S.A.; Supporting Information Table S2).

Immunofluorescent staining Cryosections from normal healthy skin and lesional skin biopsies were thaw-mounted on slides for immunofluorescent staining. Slides were fixed for 15 min in 4% paraformaldehyde in phosphate-buffered saline. A rabbit affinity-purified antiphospho-Stat-3 antibody (Tyr705) (1:100; Cell Signaling Technology, Danvers, MA, U.S.A.) was used as the primary antibody. Goat antirabbit antibody A488 IgG (1:1600, Invitrogen) was used as secondary antibody. Nuclei were stained with DAPI (1:2000). Photomicrographs were taken with an Axio Imager fluorescence microscope (Carl Zeiss Microimag-

ing GmbH, Jena, Germany).

Statistical analysis A quality check on the microarray data was performed using the R package 'affyQCReport' and analysis was done as previously described.[15] A gene was considered differentially expressed when its adjusted P-value was < 0·05 and its fold change was > 2. We utilized IPA (Ingenuity Pathway Analysis, Ingenuity Systems 2012, Redwood City, CA, U.S.A.) to identify biological functions and pathways affected by FAEa in psoriasis lesions and to understand more thoroughly the roles of the genes uniquely identified in this study as possible targets of FAE treatment. Clinical data were analysed with SPSS Statistics (IBM, Armonk, NY, U.S.A.) and mRNA data was analysed with GRAPHPAD PRISM version 5 (Graphpad Soft- ware, La Jolla, CA, U.S.A.).

Etanercept comparison data The gene expression pathways affected by FAE treatment were compared with previously published data10 that identified pathways affected by etanercept treatment (NCBI Gene Expression Omnibus GSE11903). In this study GeneChip HG-U133A v2 gene arrays were used to analyse gene expression in lesional skin biopsies at baseline and at week 12 from 11 responding patients who were treated with etanercept 50 mg twice a week for 12 weeks. In that study responders were defined as having histological disease resolution at week 12 marked by decreased epidermal thickening and normalization of Ki67 and K16 expression. For comparison purposes, we selected the 22.277 probe sets present on both these microarrays and our HG-U133 Plus 2.0 microarrays and analysed the data using the same methods as used on our full FAE data.

RNA processing and microarray hybridization From the 10 patients showing a clinical PASI improvement of more than 75% at week 12 (PASI > 75 response), four patients were randomly selected as clinical responders and from the eight patients with a PASI < 50 response, five patients were randomly selected as clinical nonresponders for microarray analysis. RNA was extracted from whole-skin biopsies (epidermis and dermis) of these nine patients and from 11 additional patients for validation of the microarray results by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). RNA was processed as previously described.15 In short, 1 µg of total RNA of four responding and five nonresponding patients was hybridized to GeneChip HG-U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA, U.S.A.). Array hybridization and scanning was done as previously described.[16]

RESULTS

Clinical response to oral fumaric acid ester treatment The baseline characteristics of the selected patients with psoriasis (six male and three female patients) are summarized in Table S3 (see Supporting Information). The median PASI reduction after 12 weeks of FAE treatment in the total group of patients (n = 50) was 65·2% [interquartile range (IQR) 50·6–77·8%]. The median PASI at baseline was 13·6 (IQR 11·3–16·4), which decreased to 5·5 (IQR 3·3–7·6) following 12 weeks of FAE treatment (P < 0·001). The clinical responders, randomly included for array analysis, had a median decrease in PASI of 84·3% (IQR 77·5–89·4%) whereas the clinical nonresponders had a median PASI decrease of 40·4% (IQR 34·8–44·7%). The difference in PASI reduction was statistically significant (P = 0·02; see Supporting Information Figure S1).

Differentially expressed genes in the skin of psoriasis patients treated with fumaric acid esters After 12 weeks of FAE treatment, 24 genes were differentially expressed in the lesional skin of patients with psoriasis (responders and nonresponders). Seven of these were downregulated [including psoriasin (S100A7), calgranulin-B (S100A9), pentraxin 3 (PTX3), matrix metallopeptidase 1, lipocallin 2 (LCN2), desmocollin 2 (DSC2) and 5-hydroxytryptamine (serotonin) receptor 3A]. Seventeen molecules were upregulated including dermcidin, secretoglobulin, prolactin-induced protein (PIP), keratins and NADPH dehydrogenase (NQO1; Table 1).

Most of the genes that were differentially expressed in the skin of patients with psoriasis are known markers of psoriatic inflammation, such as psoriasin, calgranulin-B, LCN2, DSC2 and PTX3. PIP is an immunosuppressive molecule.[17,18]

Differentially expressed genes in responders to fumaric acid esters We also analysed gene expression changes separately in the group of responders and in the group of nonresponders, in order to identify molecules and pathways that might be responsible for the clinical improvement of the psoriasis lesions. In responders, 458 genes were differentially expressed in lesional skin before treatment vs. 12 weeks after the start of the treatment (166 upregulated, 292 downregulated, ≥ 2-fold, P < 0·05; Supporting Information Table S4). In the FAE-treated responders several upregulated keratin genes were detected, including keratin (K)15, which is downregulated in activated keratinocytes and in psoriatic skin.19,20 Thus the upregulation of K15 in FAE-treated responding patients was illustrative for the induced transition to normal skin. Similarly, the expression of K16 and K17, markers

of keratinocyte hyperproliferation that are upregulated in psoriatic skin, were significantly downregulated in FAE responders after 12 weeks (Supporting Information Table S4). Several molecules of the epidermal differentiation complex were significantly downregulated after 12 weeks in responders to FAE treatment including LCE3D (late cornified envelope 3D), involucrin and several members of the small proline-rich (SPRR) family. The expression of inducible nitric oxide synthase and interleukin (IL)-20 was significantly reduced after 12 weeks of FAE treatment (Supporting Information Table S4).

The list of differentially expressed genes was analysed by IPA. In responders, the IL-17A pathway was the most significantly affected, with a downregulated expression of the chemokines CCL20, CXCL1 and CXCL6, the antimicrobial peptides b-defensin 2 (DEFB4), psoriasin (S100A7), calgranulin-A (S100A8) and calgranulin-B (S100A9) and the cytokines IL-8 and IL-17A (Table 2). When validating these findings with qRT-PCR, we confirmed a significant decrease in the expression of these genes after 12 weeks of FAE treatment (Supporting Information Figure S2). Expression of the signal transducer and activator of transcription (STAT)3 gene was significantly (–2·3-fold, $P < 0{\cdot}001$) downregulated in biopsies of lesional psoriatic skin of responders after 12 weeks of FAE treatment (Supporting Information Table S4). To verify that STAT3 protein was also downregulated, immunofluorescent staining of phosphorylated STAT3 on these skin biopsies was performed. This showed a clear reduction of phosphorylated STAT3 at week 12 in responders, which is indicative of repression of the Th17 pathway (Fig. 1).

Furthermore, a downregulation of the expression of the proinflammatory cytokines IL-1b, IL-22, IL-36a (IL-1F6) and IL-36c (IL-1F9) and an upregulation of the anti-inflammatory IL-37 (IL-1F7) was associated with successful FAE treatment (Supporting Information Table S4).

Differentially expressed genes in nonresponders to fumaric acid esters In the nonresponding patients, a differential expression of 35 genes was found: two of these were downregulated and 33 were upregulated. IPA showed activation of the glutathione signalling pathway [microsomal glutathione S-transferase (MGST)1, glutathione peroxidase 2 (GPX2)], the Nrf2 pathway (MGST1, NQO1 and GPX2), superoxide radical degeneration (NQO1) and that of xenobiotic mechanism signalling [MGST1, NQO1, peroxisome proliferator-activated receptor gamma]. These pathways were enriched in both nonresponders and responding patients (Table 2).

Comparison of gene expression profiles between fumaric acid esters and etanercept The differentially expressed genes in the skin of FAE responders before and after treatment were compared with the differentially expressed genes before and after successful treatment with etanercept.10 When using the same cut-off values (> 2- fold change, P < 0·05), we found 112 upregulated and 208 downregulated genes (Fig. 2a). We compared the change in gene expression of psoriasis-related molecules during FAE and etanercept treatment and found an overlap of 122 significantly downregulated genes and of 35 significantly upregulated genes. Overlapping downregulated genes included CCL20, CXCL1, DEFB4A and several S100 family genes (Fig. 2a). All overlapping genes are known markers of the psoriatic transcriptome and are likely important for lesion improvement as they were downregulated during successful treatment with both FAEs and etanercept.[21–23]

Several molecules and pathways that were differentially expressed in FAE-treated patients did not alter during etanercept treatment. FAE downregulated 170 genes and upregulated 131 genes that were not differentially affected by etanercept treatment (Tables 3 and 4). FAE-specific pathways included the Nrf2, superoxide radicals degradation, eicosanoid signalling and IL-17 signalling in fibroblast pathways (Fig. 2). NQO1 is part of the Nrf2 pathway.24 The aryl hydrocarbon receptor (AHR) can regulate the Nrf2 pathway and AHR knockdown in keratinocytes partly inhibits NQO1 induction by coal tar.[25] During FAE treatment the AHR was not differentially expressed; however, the AHR signalling pathway was differentially expressed in FAE responders, but not in etanercept responders.

Transcription factors The differential expression of transcription factors due to FAE treatment was compared with that for etanercept treatment. In etanercept responders five transcription factors were differentially expressed, whereas in the FAE responders nine transcription factors were differentially expressed (Table 5). FAE treatment specifically reduced the transcription regulator NFκBIZ (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta) and PTTG1 (pituitary tumour transforming gene 1) in lesional psoriatic skin (Table 5). In addition, FAE specifically upregulated the transcription factors NR3C1 (nuclear receptor subfamily 3, group c, member 1/glucocorticoid receptor) and GATA3, which is an important regulator in T-cell differentiation.[26] These transcription factors were not differentially expressed during etanercept treatment.

DISCUSSION

This study assessed the mode of action of FAEs by analysing their effects on the gene ex-

pression profile in lesional psoriatic skin before and after 12 weeks of FAE treatment. We compared our findings of FAE treatment with a previously published study that investigated gene expression during etanercept treatment.[10] The comparison shows that FAE and etanercept have a considerable overlap in the affected pathways leading to psoriasis improvement, including the IL-17 pathway. Responders to FAE treatment showed a differential expression after 12 weeks of many antimicrobial peptide genes. Antimicrobial peptides are significantly upregulated in psoriatic skin representing a disturbance in innate immunity, an important aspect of the pathogenesis of the disease.[27] Human β-defensin 2 even serves as a psoriasis disease severity biomarker.[28] The differential expression during successful FAE treatment is similar to the molecular effects of other systemic treatments for psoriasis and may represent a fingerprint of a successful psoriasis treatment response.

In vitro, dimethylfumarate and its primary metabolite monomethylfumarate induce the expression of the Nrf2/ NQO1 pathway in endothelial cells.[29] The Nrf2 pathway can be regulated by FAE in neurons, and the neuroprotective effects of fumarates are dependent on Nrf2-mediated antioxidative pathways.[24] An FAE formulation containing dimethyl-fumarate (BG-12, Biogen Idec, Cambridge, MA, U.S.A.) was recently approved by the U.S. Food and Drug Administration for the treatment of multiple sclerosis.[30,31] The nervous system and neuronal factors promote inflammation in psoriasis lesions, which are characterized by a high density of nerves and an increased expression of neurotrophins.[32] The Nrf2 pathway has an important antioxidative function and is involved in epidermal barrier function.[33] Our results show that in FAE-treated responding as well as nonresponding patients the Nrf2 pathway is activated and the expression of its major effector molecule NQO1 is induced. The AHR can regulate the Nrf2 pathway, and AHR knockdown in keratinocytes partly inhibits the induction of NQO1 by coal tar.[25] However, during FAE treatment the AHR was not differentially expressed; therefore additional mechanisms might play a role in FAE-induced activation of the Nrf2 pathway. The Nrf2 pathway was differentially expressed in FAE responders as well as nonresponders, but not in etanercept-treated patients, which suggests an FAE-specific effect.

In addition, the glutathione pathway was activated only in the FAE-treated patients and not in the etanercept group. This was evidenced by an upregulation of several glutathione transferases and depletion enzymes in both responding and nonresponding patients, including GPX2, MGST1 and glutathione S-transferase (GST) mu 3. The activation suggests a specific effect of FAE; however, it is not critical for substantial psoriasis plaque clearance. Therefore, FAE-dependent glutathione depletion might not be required for improvement of clinical disease. In vitro, dimethylfumarate has been shown to interfere with

the glutathione pathway in human monocytes and T cells as well as in mouse dendritic cells.[34,35] Dimethylfumarate binds to glutathione, and the consequent functional depletion of intracellular glutathione leads to the induction of haem oxygenase-1 (HO-1).35 HO-1 induction leads to an inactivation of STAT1, which prevents IL-12p35 transcription. Transcription of IL-23p19 is also impaired due to binding of a part of HO-1 to the IL-23p19 promoter.[34] In previous in vitro studies, HO-1 was identified as a key molecule induced by FAE. However, a study that determined GST genotyping and phenotyping found no differences in allelic variants and enzymatic activity of GSTT1 and responder status to FAE treatment.[36]

FAEs have shown in vitro effects on T cells and Th-cell differentiation.[37-39] GATA3 is an important transcription factor and regulator in T-cell development, and regulation by GATA3 leads to Th2 cell differentiation;[26] in addition, GATA3 is involved in normal epidermal development.[15] GATA3 was significantly upregulated only in the FAE-treated responders, but not in the etanercept-treated responders. This suggests that Th2 cell development/skewing is an important target of FAE, which is also illustrated by the known induction of IgE by FAE. In addition, NR3C1 was significantly up-regulated. This glucocorticoid receptor is important for normal cutaneous development, and in keratinocyte-specific glucocorticoid receptor knockout mice the epidermis shows altered levels of many innate immunity genes including S100A8 (calgranulin-A) and S100A9 (calgranulin-B).[40] The FAE-specific upregulation represents a shift from an abnormal to normal phenotype of the epidermis in responders to FAE treatment. The transcription factor PTTG1 is involved in keratinocyte proliferation and differentiation and is overexpressed in psoriatic skin. Overexpression of PTTG1 results in an overproduction of TNF-alpha.[41] FAE specifically reduced the expression of PTTG1, which was not reduced in etanercept-treated patients.

The NFκBIZ (IκBζ) gene encodes the transcription factor IκBζ, which is required for Th17 development in mice. NFκBIZ-deficient mice are not able to produce Th17 cells.[42,43] The observed downregulation of IκBζ expression in our human psoriatic skin samples is likely important in the inhibition of the Th17 pathway, although this should be confirmed in further experiments. Interestingly, this transcription factor was not differentially expressed in etanercept-treated patients, but only in FAE-treatment responders.

In conclusion, FAE-specific induced pathways in the skin include activation of the Nrf2 and glutathione pathways. FAE- specific molecules that are related to response to treatment are the transcription factors PTTG1 and NR3C1, which are important in keratinocyte regulation and normal cutaneous development, , respectively, and GATA3 and NFκBIZ, which are important in Th2 and Th17 cell development, respectively.

REFERENCES

1. Mrowietz U et al. Dimethylfumarate for psoriasis: more than a dietary curiosity. Trends Mol Med 2005; 11: 43–48.
2. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol 2009; 23(Suppl. 2):1–70.
3. Hoefnagel JJ et al. Long-term safety aspects of systemic therapy with fumaric acid esters in severe psoriasis. Br J Dermatol 2003; 149:363–9.
4. Reich K et al. Efficacy and safety of fumaric acid esters in the long-term treatment of psoriasis – a retrospective study (FUTURE). J Dtsch Dermatol Ges 2009; 7:603–11.
5. Wain EM et al. Treatment of severe, recalcitrant, chronic plaque psoriasis with fumaric acid esters: a prospective study. Br J Dermatol 2010; 162:427–34.
6. Litjens NH et al. Effects of monomethylfumarate on dendritic cell differentiation. Br J Dermatol 2006; 154:211–17.
7. Thio HB et al. Fumaric acid derivatives evoke a transient increase in intracellular free calcium concentration and inhibit the proliferation of human keratinocytes. Br J Dermatol 1994; 131:856–61.
8. Griffiths CE et al. Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. N Engl J Med 2010; 362:118–28.
9. Suarez-Farinas M et al. Resolved psoriasis lesions retain expression of a subset of disease-related genes. J Invest Dermatol 2011; 131:391–400.
10. Zaba LC et al. Effective treatment of psoriasis with etanercept is linked to suppression of IL-17 signaling, not immediate response TNF genes. J Allergy Clin Immunol 2009; 124:1022-10.e1–395.
11. Litjens NH et al. Pharmacokinetics of oral fumarates in healthy subjects. Br J Clin Pharmacol 2004; 58:429–32.
12. Fallah Arani S et al. Fumarates vs. methotrexate in moderate to severe chronic plaque psoriasis: a multicenter prospective randomized controlled clinical trial. Br J Dermatol 2011; 164:855–61.
13. Nast A et al. German S3-guidelines on the treatment of psoriasis vulgaris (short version). Arch Dermatol Res 2012; 304:87–113.
14. Mrowietz U et al. Definition of treatment goals for moderate to severe psoriasis: a European consensus. Arch Dermatol Res 2011; 303:1–10.
15. Racz E et al. Effective treatment of psoriasis with narrow-band UVB phototherapy is linked to suppression of the IFN and Th17 pathways. J Invest Dermatol 2011; 131:1547–58.
16. Staal FJ. Gene expression profiling in acute lymphoblastic leukemia (ALL). Lab Hematol 2004; 10:178–81.
17. Sugiura S et al. Immunosuppressive effect of prolactin-induced protein. Chem Immunol Allergy 2012; 96:100–7.
18. Sugiura et al. Immunosuppressive effect of prolactin-induced protein: a new insight into its local and systemic role in chronic allergic contact dermatitis. Br J Dermatol 2010; 162:1286–93.
19. Korver JE et al. Assessment of epidermal subpopulations and proliferation in healthy skin, symptomless and lesional skin of spreading psoriasis. Br J Dermatol 2006; 155:688–94.
20. Waseem A et al. Keratin 15 expression in stratified epithelia: downregulation in activated keratinocytes. J Invest Dermatol 1999; 112:362–9.
21. Capon F et al. Psoriasis and other complex trait dermatoses: from loci to functional pathways. J Invest Dermatol 2012; 132:915–22.
22. Gudjonsson JE et al. Assessment of the psoriatic transcriptome in a large sample: additional regulated genes and comparisons with in vitro models. J Invest Dermatol 2010; 130:1829–40.
23. Suarez-Farinas M et al. Expanding the psoriasis disease profile: interrogation of the skin and serum of patients with moderate-to-severe psoriasis. J Invest Dermatol 2012; 132:2552–64.
24. Linker RA et al. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain 2011; 134:678–92.
25. van den Bogaard EH et al. Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. J Clin Invest 2013; 123:917–27.
26. Zeng WP. 'All things considered': transcriptional regulation of T helper type 2 cell differentiation from precursor to effector activation. Immunology 2013; 140:31–8.
27. Nestle FO et al. Psoriasis. N Engl J Med 2009; 361:496–509.
28. Jansen PA et al. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLOS ONE 2009; 4:e4725.
29. Benardais K et al. Effects of fumaric acid esters on blood–brain barrier tight junction proteins. Neurosci Lett 2013; 555:165–70.
30. Fox RJ et al. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med 2012; 367:1087–97.
31. Gold R et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med 2012; 367:1098–107.
32. Raychaudhuri SK et al. NGF and its receptor system: a new dimension in the pathogenesis of psoriasis. Ann N Y Acad Sci 2009; 1173:470–7.
33. Schafer M et al. Nrf2 links epidermal barrier function with antioxidant defense. EMBO Mol Med 2012; 4:364–79.
34. Ghoreschi K et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. J

Exp Med 2011; 208:2291–303.

35. Lehmann JC et al. Dimethylfumarate induces immunosuppression via glutathione depletion and subsequent induction of heme oxygenase 1. J Invest Dermatol 2007; 127:835–45.

36. Gambichler T et al. Glutathione-S-transferase T1 genotyping and phenotyping in psoriasis patients receiving treatment with oral fumaric acid esters. J Eur Acad Dermatol Venereol 2014; 28:574–80.

37. Treumer F et al. Dimethylfumarate is a potent inducer of apoptosis in human T cells. J Invest Dermatol 2003; 121:1383–8.

38. Litjens NH et al. Monomethylfumarate affects polarization of monocyte-derived dendritic cells resulting in down-regulated Th1 lymphocyte responses. Eur J Immunol 2004; 34:565–75.

39. Moed H et al. Identification of anti-inflammatory drugs according to their capacity to suppress type-1 and type-2 T cell profiles. Clin Exp Allergy 2004; 34:1868–75.

40. Sevilla LM et al. Epidermal inactivation of the glucocorticoid receptor triggers skin barrier defects and cutaneous inflammation. J Invest Dermatol 2013; 133:361–70.

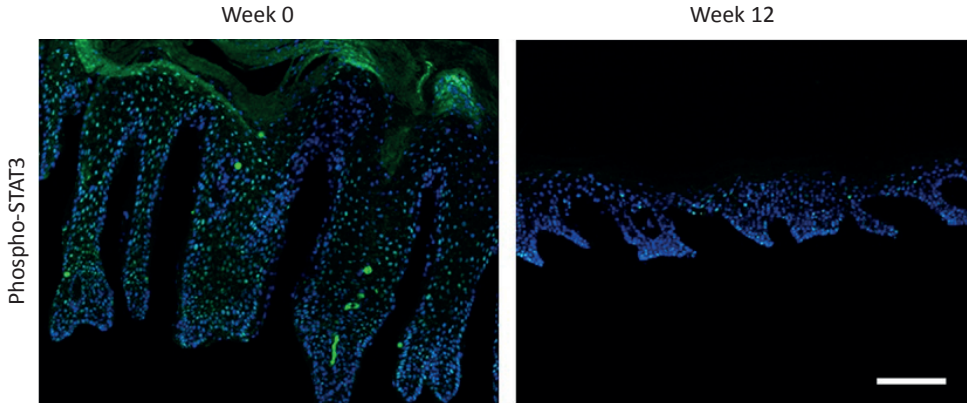
41. Ishitsuka Y et al al. Pituitary tumor transforming gene 1 induces tumor necrosis factor-alpha production from keratinocytes: implication for involvement in the pathophysiology of psoriasis. J Invest Dermatol 2013; 133:2566–75.

42. Okamoto et al. IkappaBzeta regulates T(H) 17 development by cooperating with ROR nuclear receptors. Nature 2010; 464:1381–5.

43. Yamamoto M et al. Regulation of Toll/IL- 1-receptor-mediated gene expression by the inducible nuclear protein IkappaBzeta. Nature 2004; 430:218–22.

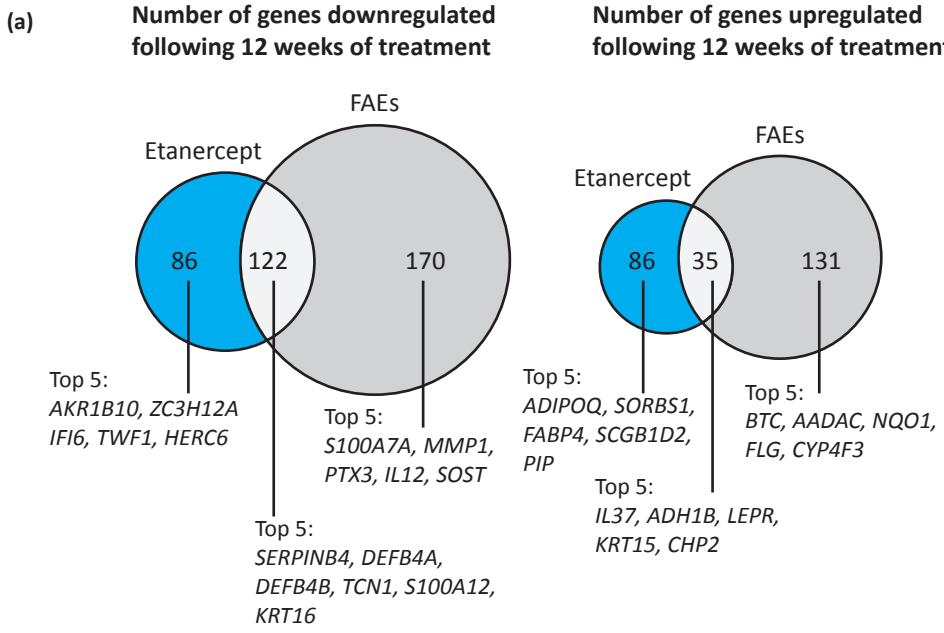
TABLES AND FIGURES

Figure 1: Reduction of phosphorylated signal transducer and activator of transcription (STAT)3 protein in the psoriatic epidermis during fumaric acid ester (FAE) treatment indicative of inhibition of the T-helper 17 pathway.



Immunofluorescent staining in lesional psoriatic skin of a representative responder patient to FAE treatment at week 0 and at week 12. Nuclei were stained with DAPI. Scale bar: 10 µm.

Figure 2: Overlap in gene expression profiles of responders to treatment with fumaric acid ester (FAE) or etanercept. (a) Venn diagram comparing the overlap in genes significantly (> 2-fold change and P < 005) downregulated or upregulated following 12 weeks of treatment. (b) Complete list of canonical pathways affected by FAE in responders following 12 weeks of treatment.



(b) **Complete list of FAE-specific canonical pathways following 12 weeks of treatment**

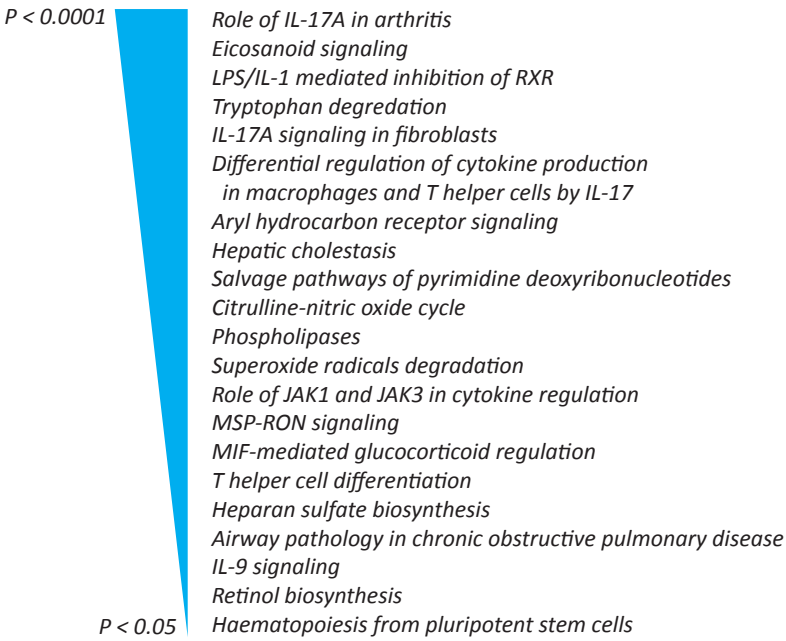


Table 1: Complete list of differentially expressed genes in patients treated with fumaric acid esters (responders and nonresponders) week 0 vs. week 12

Gene name	Description	Fold change	P-value
DCD	Dermcidin	7.521	< 0.001
SCGB2A2	Secretoglobin, family 2A, member 2	6.227	< 0.001
SCGB1D2	Secretoglobin, family 1D, member 2	4.055	< 0.001
PIP	Prolactin-induced protein	3.797	0.034
KRT19	Keratin 19	3.153	< 0.001
CA6	Carbonic anhydrase VI	2.783	< 0.001
THRSP	Thyroid hormone responsive	2.695	0.043
ATP6V0A4	ATPase, H+ transporting, lysosomal V0 subunit a4	2.464	< 0.001
LGR5	Leucine-rich repeat containing G protein-coupled receptor 5	2.399	0.011
CLDN10	Claudin 10	2.342	0.008
DNER	Delta/notch-like EGF repeat containing	2.331	0.005
NQO1	NAD(P)H dehydrogenase, quinone 1	2.244	< 0.001
PPARGC1A	Peroxisome proliferator-activated receptor gamma	2.157	< 0.001
RHPN2	Rhophilin, Rho GTPase binding protein 2	2.116	< 0.001
KRT7	Keratin 7	2.083	< 0.001
BTC	Betacellulin 2	2.04	< 0.001
SLC12A2	Solute carrier family 12, member 2	2.03	< 0.001
S100A9	S100 calcium binding protein A9 (calgranulin-A)	- 2.065	< 0.001
HTR3A	5-Hydroxytryptamine (serotonin) receptor 3A, ionotropic	- 2.138	< 0.001
DSC2	Desmocollin 2	- 2.227	< 0.001
LCN2	Lipocalin 2	- 2.414	0.039
PTX3	Pentraxin 3, long	- 2.48	< 0.001
MMP1	Matrix metallopeptidase 1 (interstitial collagenase)	- 2.589	< 0.001
S100A7A	S100 calcium binding protein A7A (psoriasin)	- 4.443	< 0.001

Table 2: Pathways affected by fumaric acid esters (FAEs) in responders (top 10) and nonresponders (all) following 12 weeks of FAE treatment

Canonical pathways	P-value	Upregulated	Downregulated
FAE responders			
Role of IL-17A in psoriasis	< 0.001		CCL20, CXCL1, CXCL6, DEFB4A/DEFB4B, IL8, IL17A, S100A8, S100A9
Role of cytokines in mediating communication between cells	< 0.001	IL37	IL8, IL20, IL24, IL12B, IL17A, IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN
Atherosclerosis signaling	< 0.001	IL37, PLA2R1	ALOX12B, ALOX15B, IL8, IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN, MMP1, PLA2G3, PLA2G2A, PLA2G4D, S100A8, SERPINA1
Dendritic cell maturation	< 0.001	IL37, LEPR, PIK-3C2G, PLCB4	CCR7, FCGR1A, FCGR1B, FCGR3B, IL12B, IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN, LTBR, STAT1
LXR/RXR activation	< 0.001	IL37	ARG2, CCL7, IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN, LDLR, NOS2, S100A8, SAA1, SERPINA1
IL-10 signalling	< 0.001	IL37	ARG2, IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN, IL4R, STAT3
p38 MAPK signaling	< 0.001	EEF2K, HSPB3, IL37	IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN, PLA2G3, PLA2G2A, PLA2G4D, STAT1
Eicosanoid signaling	< 0.001	AKR1C3, PLA2R1	ALOX12B, ALOX15B, FPR2, LTB4R, PLA2G3, PLA2G2A, PLA2G4D
Communication between innate and adaptive immune cells	< 0.001	IL37	CCR7, IL8, IL12B, IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN
LPS/IL-1 mediated inhibition of RXR function	< 0.001	ABCC3, ALDH3A2, ALDH6A1, GSTM3, HS3ST6, IL37, SULT1E1, UST	ALAS1, ALDH1A3, HMGCS1, HS3ST3A1, IL1A, IL1B, IL1RN, IL36A, IL36G, IL36RN
FAE nonresponders			
Glutathione redox reactions	< 0.001	GPX2, MGST1	
Putrescine biosynthesis III	0.006	ODC1	
Nrf2-mediated oxidative stress response	0.013	GPX2, MGST1, NQO1	
Superoxide radicals degradation	0.017	NQO1	
Xenobiotic metabolism signalling	< 0.001	MGST1, NQO1, PPARGC1A	

IL, interleukin; LXR, liver X receptor; RXR, retinoid X receptor; PLS, lipopolysaccharide.

Table 3: Fumaric acid ester-specific induced downregulated molecules (top 20), not regulated by etanercept therapy in responders at 12 weeks of treatment

Gene name	Description	Fold change	Adjusted P-value
S100A7A	S100 calcium binding protein A7A	- 47.656	< 0.001
MMP1	Matrix metallopeptidase 1	- 10.493	< 0.001
PTX3	Pentraxin 3	- 5.56	< 0.001
IL20	Interleukin 20	- 5.481	< 0.001
SOST	Sclerostin	- 4.78	< 0.001
LRG1	Leucine-rich alpha-2-glycoprotein 1	- 4.748	< 0.001
GJB2	Gap junction protein, beta 2, 26 kDa	- 4.608	< 0.001
TNIP3	TNFAIP3 interacting protein 3	- 4.578	< 0.001
SPRR3	Small proline-rich protein 3	- 4.558	< 0.001
FPR1	Formyl peptide receptor 1	- 4.541	< 0.001
XDH	Xanthine dehydrogenase	- 4.246	< 0.001
HAS3	Hyaluronan synthase 3	- 3.845	< 0.001
IL36A	Interleukin 36, alpha	- 3.845	< 0.001
C15orf48	Chromosome 15 open reading frame 48	- 3.525	< 0.001
ACTA1	Actin, alpha 1, skeletal muscle	3.483	0.004
IL17A	Interleukin 17A	- 3.379	< 0.001
LCE3D	Late cornified envelope 3D	- 3.333	0.014
SLPI	Secretory leucocyte peptidase inhibitor	- 3.175	< 0.001
TDO2	Tryptophan 2,3-dioxygenase	- 3.084	< 0.001
CDH26	Cadherin	- 3.067	< 0.001

Table 4: Fumaric acid ester-specific induced upregulated molecules (top 20), not regulated by etanercept therapy in responders at 12 weeks of treatment

Gene name	Description	Fold change	Adjusted P-value
BTC	Betacellulin	4.705	< 0.001
AADAC	Arylacetamide deacetylase	4.154	< 0.001
NQO1	NAD(P)H dehydrogenase, quinone 1 3_	3.995	< 0.001
FLG	Filaggrin	3.765	< 0.001
CYP4F3	Cytochrome P450, family 4, subfamily F3	3.588	< 0.001
LOR	Loricrin	3.557	0.004
SLC1A6	Solute carrier family 1, member 6	3.552	< 0.001
AKR1C1/C2	Aldo-keto reductase family 1, member C1/C2	3.551	< 0.001

Table 4 (continued)

SPINK7	Serine peptidase inhibitor, Kazal type 7	3.151	<0.034
SGCG	Sarcoglycan, gamma	3.03	< 0.001
CLDN11	Claudin 11	3.008	< 0.001
CYP39A1	Cytochrome P450, family 39, subfamily A1	2.979	< 0.001
SOSTDC1	Sclerostin domain containing 1	2.957	< 0.001
ANGPTL1	Angiopoietin-like 1	2.95	< 0.001
SCEL	Sciellin	2.836	0.004
SCARA5	Scavenger receptor class A, member 5	2.834	< 0.001
GSTM3	Glutathione S-transferase mu 3	2.832	0.014
IL17D	Interleukin 17D	2.781	< 0.001
DCT	Dopachrome tautomerase	2.7	< 0.001

Table 5: Differentially expressed transcription factors in patients treated with etanercept and fumaric acid esters (FAEs)

FAEs			**Etanercept**		
Upstream regulator	**Fold change**	**P-value**	**Upstream regulator**	**Fold change**	**P-value**
NFKBIZ	- 2.96	0.003	EHF	- 5.33	0.005
EHF	- 2.53	0.014	STAT1	- 3.11	< 0.001
STAT3	- 2.28	< 0.001	TP63	- 2.49	< 0.001
PTTG1	- 2.22	0.046	STAT3	- 2.18	< 0.001
ELF3	- 2.06	0.001	ZEB1	2.03	0.001
STAT1	- 2.00	< 0.001			
NR3C1	2.12	0.046			
GATA3	2.12	0.046			
ZEB1	2.30	0.046			

A.S. Myrtle

"In common with others, I have been struck with the number of cases where the disease is confined entirely to parts of the body which are always under cover, and often its existence is only known to the individual, who keeps his secret even from his ordinary medical attendant as well as his relatives."

Remarks on psoriasis. *Br Med J. 1*873 Mar 1;1(635):225.

| PART V |
TOLL-LIKE RECEPTOR ANTAGONISM IN PSORIASIS

Lotus Mallbris & Brian J. Nickeloff

"In conclusion, there is little doubt that psoriasis epitomizes the essence of translational medicine that is impacting not only dermatology, but other medical specialties as well."

Psoriasis: the poster child for bench-to-bedside translational medicine. *J Clin Aesthet Dermatol.* 2015 Jul;8(7):14-6.

CHAPTER| 7

IMO-8400, a toll-like receptor 7, 8, and 9 antagonist, demonstrates clinical activity in a phase 2a, randomized, placebo-controlled trial in patients with moderate-to-severe plaque psoriasis

Deepak M.W. Balak[1]

Martijn B.A. van Doorn[1]

Robert D. Arbeit[2]

Rianne Rijneveld[3]

Erica Klaassen[3]

Tim Sullivan[2]

Julie Brevard[2]

H. Bing Thio[1]

Errol P. Prens[1]

Jacobus Burggraaf[3]

Robert Rissmann[3]

[1]Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

[2]Idera Pharmaceuticals, Inc., Cambridge, MA, USA

[3]Centre for Human Drug Research, Leiden, the Netherlands

Clin Immunol. 2017 Jan;174:63-72.

ABSTRACT

Background: Aberrant toll-like receptors (TLRs) 7, 8, and 9 activation by self-nucleic acids is implicated in immune-mediated inflammatory diseases (IMIDs) such as psoriasis. In preclinical IMID models, blocking TLR-activation reduced disease severity. IMO-8400 is a first-in-class, oligonucleotide-based antagonist of TLRs 7, 8, and 9. We evaluated the short-term safety and proof-of-concept for efficacy of IMO-8400 in a first-in-patient phase 2 trial.

Methods: Forty-six psoriasis patients were randomly assigned to IMO-8400 in four dose levels or placebo for 12 weeks. Post-treatment follow-up was seven weeks. Primary outcome was incidence of adverse events. Secondary, exploratory outcomes included changes in psoriasis area and severity index (PASI).

Results: IMO-8400 across all dose levels did not cause any serious or severe adverse events. The most common treatment-related adverse events were dose-dependent injection-site reactions. All IMO-8400 groups showed clinical improvement, but a clear dose-response relationship and statistically significant differences with placebo were not observed (P = 0.26). Eleven (38%) of 29 subjects on IMO-8400 achieved ≥50% PASI-reduction, compared to 1 (11%) of 9 subjects on placebo. Five (17%) and 2 (7%) IMO-8400-treated subjects achieved PASI-75 and PASI-90, respectively, compared to none on placebo.

Conclusions: Short-term IMO-8400-treatment was well tolerated and reduced psoriasis severity. These findings warrant further investigation of endosomal TLR-antagonism as a therapeutic approach in psoriasis and other TLR-mediated IMIDs.

Trial registration: EudraCT 2013-000164-28 and Clinicaltrials.gov NCT01899729.

INTRODUCTION

The toll-like receptors (TLRs) belong to a family of pattern recognition receptors that initiate innate immune responses by recognizing pathogen-associated molecular patterns.[1] TLR7, TLR8, and TLR9 are intracellular receptors located on endosomes in dendritic cells, B cells, and keratinocytes that specifically generate antiviral and antibacterial responses through sensing of microbial-derived nucleic acids.[2-4] TLR7 and TLR9 are expressed in plasmacytoid dendritic cells and B cells; TLR8 is expressed in monocytes, dendritic cells, and neutrophils.[5] Located within the endolysosomal compartments, TLR7 and TLR8 can be activated by ligands containing single-stranded RNA derived from viruses, whereas TLR9 recognizes unmethylated CpG dinucleotides that are present in bacterial DNA.[6,7] Alternatively, these endosomal TLRs can be engaged by self-nucleic acids released from cell death (so-called danger-associated molecular patterns), which causes inappropriate immune activation and induction of proinflammatory cytokines.[8,9] Aberrant endosomal TLR-mediated immune activation by self-nucleic acids is implicated in the pathogenesis of immune-mediated inflammatory diseases (IMIDs), such as psoriasis.[10,11]

In psoriasis, TLRs 7, 8, and 9 can be engaged by complexes of self-nucleic acids that are bound to antimicrobial peptides such as cathelicidin (also known as LL37), which then enter and activate plasmacytoid dendritic cells in the skin to induce production of multiple pro-inflammatory cytokines including type 1 interferons, interleukin (IL)-12 and IL-23, maturation of conventional dendritic cells, and generation of T helper (Th) 1 and Th 17 cell responses.[12] The self-sustained pro-inflammatory loop subsequently causes keratinocyte proliferation, which ultimately leads to formation of psoriatic skin plaques. Aberrant activation of TLRs 7, 8, and 9 is now recognized as an initiating event in the inflammatory cascade driving the pathogenesis of psoriasis.[13]

Several observations underline the relevance of TLR-mediated inflammation in psoriasis. First, experimental studies have demonstrated increased expression of self-RNA-cathelicidin complexes in association with myeloid dendritic cells in lesional psoriatic skin compared to non-lesional skin.[14] Second, keratinocytes in lesional psoriatic skin express increased levels of TLR9 that in combination with cathelicidin produce type 1 interferons.12 TLR7 and TLR9 are strongly expressed in keratinocytes from lesional psoriasis skin and the number of activated plasmacytoid dendritic cells and the type 1 interferon signaling pathway are upregulated in psoriatic skin.[15-17] Third, expression of TLRs 7, 8, and 9 is increased in peripheral blood mononuclear cells of psoriasis patients compared to healthy

controls.[18] Fourth, in a randomized phase 2 clinical trial treatment with an oligonucleotide antagonist of TLR7 and TLR9 resulted in disease improvement in patients with psoriasis.[19] Fifth, in clinical practice topical use of the TLR7/8 agonist imiquimod triggers or exacerbates psoriasis.[20,21] Finally, imiquimod also induces a psoriasiform skin inflammation when applied topically to murine skin, which is now used as an experimental mouse model for psoriasis.[22,23]

Given the accumulating evidence on the involvement of TLR-activation in psoriasis pathogenesis, TLRs 7, 8, and 9 are potentially targets for the treatment of psoriasis.[24] Theoretically, this novel therapeutic approach has several advantages over currently available treatments including the biologics.[5] Targeting TLR-mediated inflammation would have broader activity than monoclonal antibodies such as TNF-α-inhibitors, which block only one of the multiple cytokines induced by TLR activation. Furthermore, TLR antagonists act on specific upstream activation mechanisms of immune cell activation and cytokine induction, and thus may represent a more leveraged treatment compared to cytokine-based antagonists that globally suppress cytokine-levels.[25] Theoretically, blockage of TLRs upstream of cytokine-induction likely does not compromise cytokine-levels as much as anti-cytokine agents (given that cytokine-induction by other non-TLR pathways are left undisturbed), and thus may be a safer alternative.[26,27]

IMO-8400 is a first-in-class, second-generation, synthetic oligonucleotide-based antagonist of TLR7, TLR8 and TLR9 developed by Idera Pharmaceuticals, Inc. (Cambridge, MA).[28] In in vitro studies using human cell-based assays and in in vivo studies with mice and primates, IMO-8400 inhibited cytokine responses mediated by TLRs 7, 8 and 9.[28] Furthermore, in an IL-23-induced psoriasis mouse model, treatment with IMO-8400 reduced epidermal hyperplasia and inhibited the induction of Th 1 and Th 17 cytokines.[24] In addition, gene expression profile analyses in the same IL-23 induced psoriasis model showed that IMO-8400 reduced IL-17A expression and normalized several IL-17-induced genes.[29] In a phase 1 study among healthy adult volunteers, IMO-8400 administered by subcutaneous injection at single doses up to 0.6 mg/kg and multiple doses at 0.3 mg/kg for 4 weeks was well tolerated without any systemic reactions or laboratory changes.[30]

In this first-in-patient, phase 2a, randomized, placebo-controlled, clinical trial, we evaluated the tolerability, pharmacodynamics, and clinical effects of IMO-8400 at dosages up to 0.6 mg/kg by weekly subcutaneous injections for 12 weeks in patients with moderate-to-severe plaque psoriasis.

MATERIAL AND METHODS

Study design This was a phase 2a, randomized, single-center, double-blind, placebo-controlled, parallel group, dose-ranging study of IMO-8400. The study was conducted at the Centre for Human Drug Research (CHDR), Leiden, the Netherlands. Subjects were recruited throughout the Netherlands via the Dutch Trial Network Dermatology and advertisements in newspapers. Date of first patient enrolled was 23 April 2013; date of last patient last visit was 2 April 2014. All subjects gave written and informed consent. The study protocol was approved by the Medical Ethics Committee "Medisch Ethische Toetsingscommissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek" (Assen, the Netherlands). The study was performed in compliance with Good Clinical Practice (ICH-GCP) and the principles of the Declaration of Helsinki. The study protocol was registered at EudraCT under number 2013-000164-28 and at Clinicaltrials.gov under number NCT01899729. There were no major protocol violations. Most protocol violations involved the use of prohibited medications such as topical corticosteroids. In such instances, observations of efficacy data gathered subsequent to initiation of prohibited medications were excluded from the analyses. Funding of the trial was provided by Idera Pharmaceuticals, Inc. (Cambridge, MA). A pre-study sample size calculation yielded 8 patients to be enrolled in each treatment arm to have a power of 0.80 to detect differences between IMO-8400 and placebo across the entire data set.

Study population Patients were eligible for inclusion if they had a clinical diagnosis of moderate-to-severe chronic plaque psoriasis and a PASI of 12.0 or higher and a BSA of at least 10% at screening. Patients with significant medical conditions or who had previously failed treatment due to lack or loss of efficacy to two different biologics were excluded. Ongoing treatments for psoriasis were discontinued before enrollment in the study. The minimal discontinuation intervals were 2 weeks for topical treatments, 4 weeks for phototherapy and systemic treatments including methotrexate and fumaric acid esters, and 4 to 26 weeks for biologics. Concomitant psoriasis treatments were not allowed for the duration of the study, except for bland emollients. Subjects were instructed to avoid excessive sun-exposure.

Treatment assignment and study procedures Patients were randomly assigned to treatment regimen using a computer-generated randomization schedule prepared by a study-independent statistician. The study was executed in 2 parts. The main cohort was de-

signed to randomize 32 patients with psoriasis 1:1:1:1 to receive IMO-8400 in 0.075 mg/kg, 0.15 mg/kg, or 0.30 mg/kg, or placebo. The protocol was subsequently amended to evaluate the 0.60 mg/kg dose of IMO-8400, randomizing 9 patients to 0.60 mg/kg IMO-8400 and 3 to placebo.

Following enrollment, subjects underwent a medical screening consisting of a physical examination, medical history, and laboratory testing, including hepatitis and HIV serology. Study treatment was administered on Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78 by study site personnel as subcutaneous injections rotated among the 4 quadrants of the abdomen and upper thigh. There were 4 posttreatment follow-up visits on Days 85, 99, 113, and 127. Subjects, study personnel, and investigators were blinded for allocated treatment throughout the study.

Study objectives The primary objective of this study was to evaluate the short-term safety and tolerability of four dose levels of IMO-8400 compared with placebo. Safety and tolerability outcomes were collected throughout the study period by adverse event monitoring, physical examination, measurement of vital signs, 12-lead electrocardiograms, and laboratory tests (i.e. hematology, chemistry, coagulation, and urinalysis). Laboratory outcomes were graded according to the Common Terminology Criteria for Adverse Events, version 4.02. Furthermore, each injection site was systematically assessed for ISRs, which were defined as erythema, induration, pruritus, tenderness, pain, blistering, ulceration, or necrosis. Severity of ISRs was graded from grade 1 (mild) to grade 3 (severe).

The secondary objective of this study was to evaluate the clinical response of IMO-8400 compared with placebo. Efficacy outcomes included changes in PASI, which is a validated composite score ranging from 0 (no disease activity) to 72 (extensive disease activity). All PASI measurements were performed at one site by two experienced assessors. Additional efficacy parameters included: body surface area (BSA), ranging from0% (no disease) to 100% (extensive disease activity); physician global assessment (PGA), graded in categories from 0 (clear skin) to 4 (very severe psoriatic skin lesions); and dermatology life quality index (DLQI), ranging from 0 to 30, with higher scores representing greater impairment.31 Exploratory outcome measures included plasma concentrations of IL-12, IL-23, human beta-defensin-2 (hBD2), and interferon-inducible protein 10 (IP-10), which were measured as potential biomarkers at specific time points.

Data analysis All efficacy endpoints were analyzed using a mixed-effect model repeated measures analysis for percentage reduction in PASI score relative to placebo by treatment

group for the intent-to-treat population and the clinically evaluable (CE) population. The CE population included all subjects considered to have completed at least 10 of the 12 doses of planned study treatment without major protocol deviation. The mixed-effect model includes percent reduction in PASI from baseline as the response variable, treatment, visit, and treatment-by-visit as fixed factors, patient as a random factor, and baseline measurement as a covariate. Comparisons were made between individual dose cohorts and placebo using the mixed model without the treatment by visit interaction term, as this was not statistically significant at the 0.05 level. The general treatment effect was reported with p-values from the Type 3 tests of fixed effects. Specific contrasts were reported with the least square mean estimates, 95% confidence interval, and p-value. A post-hoc responder analysis was done by Fisher's exact test to examine the proportion of patients in the CE population who achieved a 50%, 75%, and 90% reduction in PASI (PASI-50, PASI-75, and PASI-90 response, respectively) from baseline to end of treatment and to end of study by treatment group.

All statistical hypothesis tests were two-sided, conducted at the alpha=0.05 level. There was no adjustment for multiple comparisons. All analyses were performed using SAS for windows V9.1.3 (SAS Institute, Inc., Cary, NC).

RESULTS

Participant flow and characteristics One hundred-eighteen subjects were screened, of whom 71 (60%) were excluded based on the in- and exclusion criteria (Fig. 1). Most subjects were excluded because of an insufficient psoriasis severity. Forty-six subjects were enrolled within 6 months in this mono-center study. Eight (17%) subjects discontinued study treatment early due to: lack of efficacy (n=3), withdrawal of consent (n=3), and an adverse event unrelated to the study treatment (n = 2). Two of the 8 subjects who withdrew before week 6 were replaced per protocol. The demographic characteristics of the study population are summarized in Table 1. The majority (72%) was male, and most patients (80%) were of Caucasian origin.

Short-term safety and tolerability IMO-8400 treatment was not associated with any clinically significant changes in laboratory tests, ECG, or vital signs. Throughout the study, there were no deaths or serious adverse events. The incidence of treatment-emergent adverse events was similar among patients who received IMO-8400 and patients who received placebo (Table 2). Common adverse events included headache (37% of IMO-8400-treated patients), diarrhea (20%), and influenza-like illness (20%). The majority of the treat-

ment-emergent adverse events were of mild (grade 1) or moderate (grade 2) severity and self-limiting without the need for therapeutic intervention. Three patients experienced a severe adverse event and discontinued the study prematurely. In all 3 cases, the events (severe abdominal pain, transient ischemic attack, and hypothyroidism, respectively) were assessed as unlikely related to the study drug and resolved without sequelae.

Injection site reactions (ISRs) were common adverse events, reported in 23 (66%) of 35 of the IMO-8400-treated patients compared to 1 (9%) of 11 subjects on placebo (Table 3). The most common ISRs included erythema and induration of mild to moderate severity that resolved without treatment. No blistering, ulceration, or necrosis at the injection site was observed in any subject. There was a clear dose-related increase in the incidence of ISRs in IMO-8400-treated subjects (Table 3). The greatest incidences of ISRs were observed in the two highest dose groups. Furthermore, at the two highest dose levels of IMO-8400, the development of 2- to 4-cm psoriasis plaques (Fig. 2B) was observed at the site of injection in 2 (25%) subjects in the 0.30 mg/kg group and 4 (44%) subjects in the 0.60 mg/kg group.

Efficacy The mean PASI improved in all four IMO-8400 dose groups as well as the placebo group over the 12-week study treatment period (Fig. 3). However, there were no statistically significant treatment effects found for any IMO-8400 dose level compared to placebo in the repeated measures analysis (P = 0.26). Response to IMO-8400 treatment was heterogeneous (Fig. 3b). Eleven (38%) of 29 patients treated with IMO-8400 achieved at least a 50% reduction in PASI (i.e. PASI-50 response) compared to 1 of 9 (11%) patients treated with placebo (Table 4). Five (17%) and 2 (7%) IMO-8400-treated subjects achieved clinically significant clinical responses with PASI-75 and PASI-90 responses, respectively, compared to none treated with placebo.

In a responder analysis, no statistically significant differences compared to placebo were found (Table 4). The PASI-75 response through end of treatment was 29% in the 0.075 mg/kg group (P = 0.18 vs. placebo), 14% in the 0.15 mg/kg group (P = 0.44 vs. placebo), and 11% in the 0.60 mg/kg group (P = 1.00 vs. placebo). None of the subjects in the 0.30 mg/kg group achieved a PASI-75 response. A PASI-90 response was observed in 2 (29%) patients who had received the lowest IMO-8400 dose level of 0.075 mg/kg (P = 0.18 vs. placebo).
Secondary efficacy outcomes showed no statistical significant differences. Changes in BSA were comparable between any IMO-8400 treated group and placebo (P=0.53). Likewise, the proportion of patients with a PGA of 0 (clear disease) or 1 (minimal disease) was more or less similar across all treatment arms, ranging from 22 to 38%. Patient-reported DLQI-scores improved during treatment with IMO-8400, but no statistically differences were observed.

Exploratory analyses of serum biomarkers Across all IMO-8400 treatment groups, there were no statistically significant changes in the plasma concentrations of the cytokines IL-12, IL-23, human beta-defensin-2 (hBD2) and IP-10. However, hBD2 concentrations in the intent-to-treat population showed a significant correlation with changes in PASI ($r=0.57$, $p < 0.0001$), suggesting that hBD2 has the potential to be employed as a biomarker of psoriasis disease activity (See Fig. 4).

DISCUSSION

Antagonism of aberrant TLR-mediated inflammation is a potential novel therapeutic approach for psoriasis Accumulating experimental studies indicate that blocking TLR-mediated inflammation may be a relevant approach in the treatment of psoriasis. For example, a recent study found that thiostrepton, an antibiotic with specific inhibitory effects on TLR7 and TLR9, led to disease improvement in the imiquimod-induced psoriasis mouse model.[32] Another study reported that disruption of the ubiquitin-binding protein ABIN-1 in dendritic cells led to an increased psoriasis phenotype in a mouse model due to increased TLR-induced MyD88 signaling.[33] ABIN-1 has been linked to psoriasis susceptibility in genome-wide association studies.[34]

Limitations and strengths Several limitations of this trial need to be considered. The study had a relatively small sample size (n=8 per treatment group), with the primary goal of evaluating tolerability and short-term safety. The protocol was amended mid-study to add evaluation of the dose of 0.60 mg/kg, which involved enrollment and randomization of a separate population. A clinical trial with a larger sample size would be needed to characterize fully dose-related clinical activity. In addition, we did not have data available to demonstrate successful blockage of TLR-mediated responses at target sites, e.g. gene expression analysis of skin biopsies from lesional psoriasis skin. Strengths of our study include the randomized, double-blind, placebo-controlled study design and the use of multiple validated physician- and patient-reported psoriasis outcomes. Furthermore, all PASI assessments were scored by the same two assessors at a single center, in an effort to minimize variability due to assessor differences.

Interpretation These results of this phase 2 trial are in line with previous studies on IMO-8400 in healthy volunteers and a phase 2 study with a somewhat similar TLR-antagonist.[19,30] Our findings indicated a treatment effect of IMO-8400 in only some of the study

subjects. A clear linear dose-response relationship and statistically significant differences to placebo were not observed; secondary efficacy endpoints analyses showed essentially a flat dose-response relationship. Unexpectedly, with the lowest dose levels of 0.075 mg/kg IMO-8400 the most pronounced clinical effects were observed and some patients achieved PASI-reductions up to 90%. However, a clear trend towards a possible inverse dose response was not seen and interpretation of these observations remains difficult.

Overall, in this phase 2 trial the efficacy of IMO-8400 was modest. There are several reasons possible to explain the relatively low efficacy of IMO-8400 in this trial. First, the primary aim was to investigate safety, while the sample size was too low to assess efficacy adequately. Second, we believe the dosing regimen and duration were not optimized to establish and sustain immune modulation required to control psoriasis disease activity in all patients. Furthermore, it is possible that TLRs 7, 8, and 9 activation is only relevant in a subtype of psoriasis. Recent studies have identified several molecular subtypes of psoriasis based upon differences in gene expressions profiles of psoriasis skin lesions.[35,36] Such differential gene expression profiles could implicate differential responses to treatment.[37] Future studies may benefit from considering gene expression profile. Third, the type of psoriasis (i.e. chronic plaque psoriasis) that was studied in our trial might not be completely responsive to TLR antagonism. It is unknown whether blocking TLR signaling is clinically relevant in all patients with an established chronic psoriasis plaque. There is a possible distinction to be made between early and late immune activation in psoriatic plaques.[38] Aberrant activation of TLRs by self-nucleic acids is implicated as one the first events in the cascade leading to initiation of psoriasis.13,39 Theoretically, antagonism of TLRs 7, 8 and 9 activation may more readily prevent the onset of new psoriatic skin lesions relative to healing established lesions. This may be similar to treatment with fumaric acid esters FAEs treatment, which in daily clinical practice has a slow onset of efficacy.[38] Illustratively, blocking interferon-alpha, which is triggered by TLR-activated plasmacytoid dendritic cells, had no clinical efficacy in established psoriasis plaques in a randomized phase 1 trial with a monoclonal antibody targeting interferon-alpha.[40] This is in contrast with the clinical efficacy of inhibitors targeting more downstream cytokines like IL-17 and IL-23, for which high PASI response rates were demonstrated.[41,42] Thus, TLR-antagonists may impact the disease by preventing rather than improving psoriasis skin plaques. Based on the current knowledge on the transient activation of plasmacytoid dendritic cells by endosomal TLRs in psoriasis, TLR-antagonism might be more clinically relevant in acute forms of the disease (such as guttate psoriasis) or the prevention of new exacerbations of chronic plaque psoriasis.[43] In addition, other type 1 interferon driven diseases such as dermatomyositis may be promising targets.

The occurrence of ISRs, which were the most common treatment-emergent adverse event associated with IMO-8400, was dose-dependent. ISRs are a well-known, frequently occurring adverse event associated with oligonucleotide-based treatments and likely represent a class adverse effect of oligonucleotide-based drugs.[44,45] Oligonucleotide-associated ISRs are likely due to an immune-mediated reaction, but the underlying mechanisms are not clear.[46] The erythematous ISRs occurring at higher dosages may reflect an increased immune activation locally in the skin, which theoretically could impact the overall response. However, there was no correlation found between the occurrence of ISRs and PASI-response (data not shown). In addition, in this trial no skin biopsies were available to analyze the erythematous skin reactions. Of note, several subjects developed psoriasis-like skin lesions at the site of some of the ISRs. These reactions occurred infrequently and were observed only in the two highest dose levels of IMO-8400. The occurrence of a psoriatic skin lesion could be due to the Koebner phenomenon, i.e. a trauma to healthy skin evoking a psoriasis plaque.[47] The development of Koebner-like reactions specifically at the site of a drug-injection has been described only for subcutaneous injection of interferon-gamma and adalimumab.[46-50]

In this trial we assessed plasma concentrations of 4 potential biomarkers. Of these, only hBD2 showed a significant correlation with change in PASI. Hence, hBD2 could therefore be a suitable biomarker for psoriasis disease activity, which is in line with previous studies.[51]

Generalizability Findings from this trial have implications for future drug development. TLR antagonism by IMO-8400 has relevance as a potential treatment for other IMIDs. TLR-mediated inflammation are implicated in the pathogenesis of multiple IMIDs including dermatomyositis, polymyositis and lupus erythematosus.[52,53] In particular, dermatomyositis may be a promising indication for TLR antagonism as changes in type 1 interferon and chemokine expression have been correlated with disease activity.[54] Other potential future indications for IMO-8400 and similar TLR antagonist compounds include inflammatory, immune-mediated diseases, such as lupus erythematosus, rheumatoid arthritis, systemic sclerosis, multiple sclerosis, Sjögren's syndrome, and lichen planus.[5,8,26,55-57] Preclinical studies of TLR antagonists indicated ameliorating effects in mouse models of lupus and rheumatoid arthritis.[58] In addition, the drug hydroxychloroquine is used to treat dermatomyositis as well as cutaneous and systemic forms of lupus erythematosus, and may work via blockage of endosomal TLR signaling.[59,60] Based on these findings a phase 2 trial of IMO-8400 in patients with dermatomyositis has been initiated (Clinicaltrials.gov number NCT02612857).

Conclusions In conclusion, in this phase 2a trial blocking TLRs 7, 8 and 9 with the oligonucleotide-based antagonist IMO-8400 up to 12 weeks was well-tolerated and did show some clinical improvement of plaque psoriasis in several subjects. Our findings provide proof-of-concept that antagonism of TLRs 7, 8 and 9 has beneficial effects in at least a subgroup of patients with plaque psoriasis. Further investigations are warranted to assess TLR antagonism as a potential treatment for psoriasis and other IMIDs in which TLR-mediated inflammation is implicated.

REFERENCES

1. O'Neill LA et al. The history of Toll-like receptors. Nat Rev Immunol. 2013 Jun;13(6):453-60.
2. Gay NJ et al. Assembly and localization of Toll-like receptor signalling complexes. Nat Rev Immunol. 2014 Aug;14(8):546-58.
3. Lebre MC et al. Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. J Invest Dermatol. 2007 Feb;127(2):331-41.
4. Li ZJ et al. Roles of TLR7 in activation of NF-κB signaling of keratinocytes by imiquimod. PLoS One. 2013 Oct 11;8(10):e77159.
5. Junt T et al. Translating nucleic acid-sensing pathways into therapies. Nat Rev Immunol. 2015 Sep 15;15(9):529-44.
6. Hemmi H et al. A Toll-like receptor recognizes bacterial DNA. Nature. 2000 Dec 7;408(6813):740-5.
7. Heil F et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 2004 Mar 5;303(5663):1526-9.
8. Conrad C et al. Plasmacytoid dendritic cells in the skin: to sense or not to sense nucleic acids. Semin Immunol. 2009 Jun;21(3):101-9.
9. Pisetsky DS. The origin and properties of extracellular DNA: from PAMP to DAMP. Clin Immunol. 2012 Jul;144(1):32-40.
10. Ganguly D et al. The role of dendritic cells in autoimmunity. Nat Rev Immunol. 2013 Aug;13(8):566-77.
11. Chen JQ et al. Toll-Like Receptor Pathways in Autoimmune Diseases. Clin Rev Allergy Immunol. 2016 Feb;50(1):1-17.
12. Morizane S et al. Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. J Invest Dermatol. 2012 Jan;132(1):135-43.
13. Lowes MA et al. Immunology of psoriasis. Annu Rev Immunol. 2014;32:227-55.
14. Ganguly D et al. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J Exp Med. 2009 Aug 31;206(9):1983-94.
15. Suarez-Farinas M et al. Treatment of psoriasis patients with IMO-3100 shows improvement in gene expression patterns of meta-analysis derived-3 transcriptome. Arthritis Rheum. 2013;65:S495.
16. van der Fits L et al. In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. J Invest Dermatol. 2004 Jan;122(1):51-60.
17. Nestle FO et al. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. J Exp Med. 2005 Jul 4;202(1):135-43.
18. Kim HJ et al. Increased expression of Toll-like receptors 3, 7, 8 and 9 in peripheral blood mononuclear cells in patients with psoriasis. Exp Dermatol. 2016 Jun;25(6):485-7.
19. Kimball AB et al. IMO-3100, an antagonist of Toll-like receptor (TLR) 7 and TLR9, demonstrates clinical activity in psoriasis patients with 4 weeks of treatment in a phase 2a trial. J. Invest Dermatol. 2013;133:S26.
20. Patel U et al. Imiquimod 5% cream induced psoriasis: a case report, summary of the literature and mechanism. Br J Dermatol. 2011 Mar;164(3):670-2.
21. Gilliet M et al. Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch Dermatol. 2004 Dec;140(12):1490-5.
22. van der Fits L et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol. 2009 May 1;182(9):5836-45.
23. Swindell WR et al. Genome-wide expression profiling of five mouse models identifies similarities and differences with human psoriasis. PLoS One. 2011 Apr 4;6(4):e18266.
24. Jiang W et al. A Toll-like receptor 7, 8, and 9 antagonist inhibits Th1 and Th17 responses and inflammasome activation in a model of IL-23-induced psoriasis. J Invest Dermatol. 2013 Jul;133(7):1777-84.
25. Thwaites R et al. Emerging role of endosomal toll-like receptors in rheumatoid arthritis. Front Immunol. 2014 Jan 16;5:1.
26. Kirou KA et al. Anti-interferon alpha treatment in SLE. Clin Immunol. 2013 Sep;148(3):303-12.
27. Reizis B et al. Plasmacytoid dendritic cells: one-trick ponies or workhorses of the immune system? Nat Rev Immunol. 2011 Jul 22;11(8):558-65.
28. Kandimalla ER et al. Design, synthesis and biological evaluation of novel antagonist compounds of TLR 7, 8 and 9. Nucleic Acids Res. 2013 Apr 1;41(6):3947-61

29. Suárez-Fariñas M et al. Suppression of molecular inflammatory pathways by Toll-like receptor 7, 8, and 9 antagonists in a model of IL-23-induced skin inflammation. PLoS One. 2013 Dec 27;8(12):e84634.

30. Brenner L et al. IMO-8400, an antagonist of toll-like receptors 7, 8, and 9, in development for genetically defined B-cell lymphomas: Safety and activity in phase 1 and phase 2 clinical trials. Blood. 2014;124:21

31. Finlay AY et al. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994 May;19(3):210-6.

32. Lai CY et al. Identification of Thiostrepton as a Novel Inhibitor for Psoriasis-like Inflammation Induced by TLR7-9. J Immunol. 2015 Oct 15;195(8):3912-21.

33. Callahan JA et al. Cutting edge: ABIN-1 protects against psoriasis by restricting MyD88 signals in dendritic cells. J Immunol. 2013 Jul 15;191(2):535-9.

34. Nair RP et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. Nat Genet. 2009 Feb;41(2):199-204.

35. Ainali C et al. Transcriptome classification reveals molecular subtypes in psoriasis. BMC Genomics. 2012 Sep 12;13:472.

36 Swindell WR et al. Dissecting the psoriasis transcriptome: inflammatory- and cytokine-driven gene expression in lesions from 163 patients. BMC Genomics. 2013 Aug 1;14:527.

37. Onderdijk AJ et al. Potential serum biomarkers of treatment response to ustekinumab in patients with psoriasis: a pilot study. Br J Dermatol. 2015 Dec;173(6):1536-9.

38. Christophers E et al. Bimodal immune activation in psoriasis. Br J Dermatol. 2014 Jan;170(1):59-65.

39. Kim J et al. The immunopathogenesis of psoriasis. Dermatol Clin. 2015 Jan;33(1):13-23.

40. Bissonnette R et al. A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti-interferon-alfa monoclonal antibody, in subjects with chronic psoriasis. J Am Acad Dermatol. 2010 Mar;62(3):427-36.

41. Langley RG et al. Secukinumab in plaque psoriasis--results of two phase 3 trials. N Engl J Med. 2014 Jul 24;371(4):326-38.

42. Kopp T et al. Clinical improvement in psoriasis with specific targeting of interleukin-23. Nature. 2015 May 14;521(7551):222-6.

43. Conrad C et al. Type I IFNs at the interface between cutaneous immunity and epidermal remodeling. J Invest Dermatol. 2012 Jul;132(7):1759-62.

44. Voit T et al. Safety and efficacy of drisapersen for the treatment of Duchenne muscular dystrophy (DEMAND II): an exploratory, randomised, placebo-controlled phase 2 study. Lancet Neurol. 2014 Oct;13(10):987-96.

45. Thomas GS et al. Mipomersen, an apolipoprotein B synthesis inhibitor, reduces atherogenic lipoproteins in patients with severe hypercholesterolemia at high cardiovascular risk: a randomized, double-blind, placebo-controlled trial. J Am Coll Cardiol. 2013 Dec 10;62(23):2178-84.

46. Sjouke B et al. Is mipomersen ready for clinical implementation? A transatlantic dilemma. Curr Opin Lipidol. 2013 Aug;24(4):301-6.

47. Camargo CM et al. Isomorphic phenomenon of Koebner: facts and controversies. Clin Dermatol. 2013 Nov-Dec;31(6):741-9.

48. Fierlbeck G et al. Psoriasis induced at the injection site of recombinant interferon gamma. Results of immunohistologic investigations. Arch Dermatol. 1990 Mar;126(3):351-5.

49. Kowalzick L et al. Psoriasis induced at the injection site of recombinant interferons. Arch Dermatol. 1990 Nov;126(11):1515-6.

50. Wang TS et al. Occurrence of psoriatic plaques on previous adalimumab injection sites during disease relapse in a patient with pustular psoriasis. J Am Acad Dermatol. 2014 Jul;71(1):e24-6.

51. Jansen PA et al. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLoS One. 2009;4(3):e4725.

52. Celhar T et al. Toll-like receptors in systemic lupus erythematosus: potential for personalized treatment. Front Pharmacol. 2014 Dec 8;5:265.

53. Lorenz G et al. Toll-like receptor activation in the pathogenesis of lupus nephritis. Clin Immunol. 2017 Dec;185:86-94.

54. Greenberg SA et al. Relationship between disease activity and type 1 interferon-inducible gene expression in blood in dermatomyositis and polymyositis. Genes Immun. 2012 Apr;13(3):207-13.

55. Duffy L et al. Toll-like receptors in the pathogenesis of autoimmune diseases: recent and emerging translational developments. Immunotargets Ther. 2016 Aug 22;5:69-80.

56. Kalunian KC. Interferon-targeted therapy in systemic lupus erythematosus: Is this an alternative to targeting B and T cells? Lupus. 2016 Sep;25(10):1097-101.

57. Prinz M et al. Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis. J Clin Invest. 2006 Feb;116(2):456-64.

58. Zhu FG et al. A novel antagonist of Toll-like receptors 7, 8 and 9 suppresses lupus disease-associated parameters in NZBW/F1 mice. Autoimmunity. 2013 Nov;46(7):419-28.

59. Chang AY et al. Response to antimalarial agents in cutaneous lupus erythematosus: a prospective analysis. Arch Dermatol. 2011 Nov;147(11):1261-7.

60. Kuznik A et al. Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. Immunol. 2011 Apr 15;186(8):4794-804.

TABLES AND FIGURES

Table 1: Baseline characteristics of the study population

	IMO-8400				
	0.075 mg/kg (n=9)	**0.15 mg/kg (n=9)**	**0.30 mg/kg (n=8)**	**0.60 mg/kg (n=9)**	**Placebo (n=11)**
Age (years)	48.8 (15.1)	35.0 (16.0)	42.3 (17.2)	47.8 (13.2)	47.2 (13.4)
Sex					
Female	4 (44%)	3 (33%)	2 (25%)	1 (11%)	2 (18%)
Male	5 (56%)	6 (67%)	6 (75%)	7 (78%)	9 (82%)
Race					
White	5 (56%)	8 (89%)	6 (75%)	9 (100%)	8 (73%)
Asian	0 (0%)	0 (0%)	2 (25%)	0 (0%)	2 (18%)
Other or un known	2 (22%)	1 (11%)	0 (0%)	0 (0%)	0 (0%)
Mixed	2 (22%)	0 (0%)	0 (0%)	0 (0%)	1 (9%)
BMI (kg/m^2)	25.8 (4.5)	24.1 (4.9)	27.1 (1.3)	28.1 (5.8)	29.8 (3.9)
PASI	14.09 (2.6)	14.09 (2.0)	14.78 (2.7)	14.23 (2.0)	14.12 (2.5)
Disease duration (years)	19.2 (14.1)	15.3 (10.6)	13.5 (9.7)	31.7 (14.9)	20.5 (11.7)
Previous treatments:					
Phototherapy	7 (78%)	6 (67%)	8 (88%)	5 (56%)	7 (64%)
Methotrexate	4 (%)	4 (44%)	0 (0%)	2 (22%)	2 (18%)
Fumaric acid esters	0 (0%)	4 (44%)	1 (13%)	2 (22%)	3 (27%)
Acitretin	0 (0%)	1 (11%)	0 (0%)	1 (11%)	2 (18%)
Ciclosporin	1 (11%)	1 (11%)	0 (0%)	1 (11%)	0 (0%)
Biologic	0 (0%)	1 (11%)	0 (0%)	1 (11%)	0 (0%)

Values are absolute numbers (percentages) or means (standard deviation).

Table 2: Summary of treatment-emergent adverse events reported in > 1 patient

	IMO-8400				
	0.075 mg/kg (n=9)	**0.15 mg/kg (n=9)**	**0.30 mg/kg (n=8)**	**0.60 mg/kg (n=9)**	**Placebo (n=11)**
Any adverse event	8 (89%)	8 (89%)	7 (88%)	7 (78%)	9 (82%)
Headache	4 (44%)	3 (33%)	2 (25%)	4 (44%)	5 (46%)
Fatigue	1 (11%)	3 (33%)	1 (13%)	1 (11%)	1 (9%)
Diarrhea	3 (33%)	1 (11%)	1 (13%)	2 (22%)	-
Influenza-like illness	1 (11%)	3 (33%)	2 (25%)	1 (11%)	1 (9%)
Upper respiratory tract infection	1 (11%)	2 (22%)	1 (13%)	1 (11%)	1 (9%)
Back pain	1 (11%)	1 (11%)	1 (11%)	1 (11%)	1 (9%)
Insomnia	-	2 (22%)	1 (13%)	-	1 (9%)
Nausea	2 (22%)	-	1 (13%)	-	-
Arthralgia	-	-	-	1 (11%)	2 (18%)
Somnolence	1 (11%)	1 (11%)	-	1 (11%)	-
Polyuria	-	1 (11%)	-	1 (11%)	1 (9%)
Abdominal discomfort	1 (11%)	1 (11%)	-	-	1 (9%)
Joint swelling	-	-	-	-	2 (18%)
Muscle spasms	-	-	-	-	2 (18%)
Upper respiratory tract infection	-	-	-	1 (11%)	1 (9%)
Oropharyngeal pain	1 (11%)	-	-	1 (11%)	-
Vomiting	1 (11%)	-	1 (13%)	-	-
Abdominal pain					
Dizziness	-	1 (11%)	-	1 (11%)	-
Myalgia	1 (11%)	-	-	-	1 (9%)
Pain in extremity	-	1 (11%)	-	-	1 (9%)

Table 3: Summary of incidence of injection-site reactions

	IMO-8400				
	0.075 mg/kg (n=9)	**0.15 mg/kg (n=9)**	**0.30 mg/kg (n=8)**	**0.60 mg/kg (n=9)**	**Placebo (n=11)**
Any ISR	4 (44%)	3 (33%)	7 (88%)	9 (100%)	1 (9%)
Erythema	4 (44%)	2 (22%)	6 (75%)	9 (100%)	1 (9%)
Induration	0 (0%)	1 (11%)	4 (50%)	8 (89%)	1 (9%)
Pruritus	2 (22%)	0 (0%)	5 (63%)	5 (56%)	0 (0%)
Pain	0 (0%)	0 (0%)	2 (25%)	1 (11%)	0 (0%)
Tenderness	0 (0%)	1 (11%)	4 (50%)	4 (44%)	0 (0%)

Table 4: Summary of efficacy analyses (clinically evaluable population)

	IMO-8400				
	0.075 mg/kg (n=7)	**0.15 mg/kg (n=7)**	**0.30 mg/kg (n=6)**	**0.60 mg/kg (n=9)**	**Placebo (n=9)**
Mixed effect model repeated measures analysis*					
PASI	P = 0.1535	P = 0.8978	P = 0.3775	P = 0.4556	NA
Responder analysis#					
End of treatment					
PASI-50 response	3 (43%) P = 0.26	2 (29%) P = 0.55	3 (50%) P = 0.24	2 (22%) P = 1.00	1 (11%) NA
PASI-75 response	2 (29%) P = 0.18	1 (14%) P = 0.44	0 (0%) NA	1 (11%) P = 1.00	0 (0%) NA
PASI-90 response	2 (29%) P = 0.1	0 (0%) NA	0 (0%) NA	0 (0%) NA	0 (0%) NA
End of study					
PASI-50 response	3 (43%) P = 0.26	3 (43%) P = 0.26	3 (50%) P = 0.24	2 (22%) P = 1.00	1 (11%) NA
PASI-75 response	3 (43%) P = 0.06	1 (14%) P = 0.44	0 (0%) NA	1 (11%) P = 1.00	0 (0%) NA
PASI-90 response	2 (29%) P = 0.18	0 (0%) NA	0 (0%) NA	0 (0%) NA	0 (0%) NA

* Model based on a repeated-measures mixed-model with response variable change from Baseline, fixed factors for treatment and visit, and Baseline as covariate. P-values for pair-wise comparison of IMO-8400 dose cohort vs. placebo obtained from the repeated measures model.

Compared to placebo; P-value is based on Fisher's exact test.

Figure 1: Flow chart of enrollment

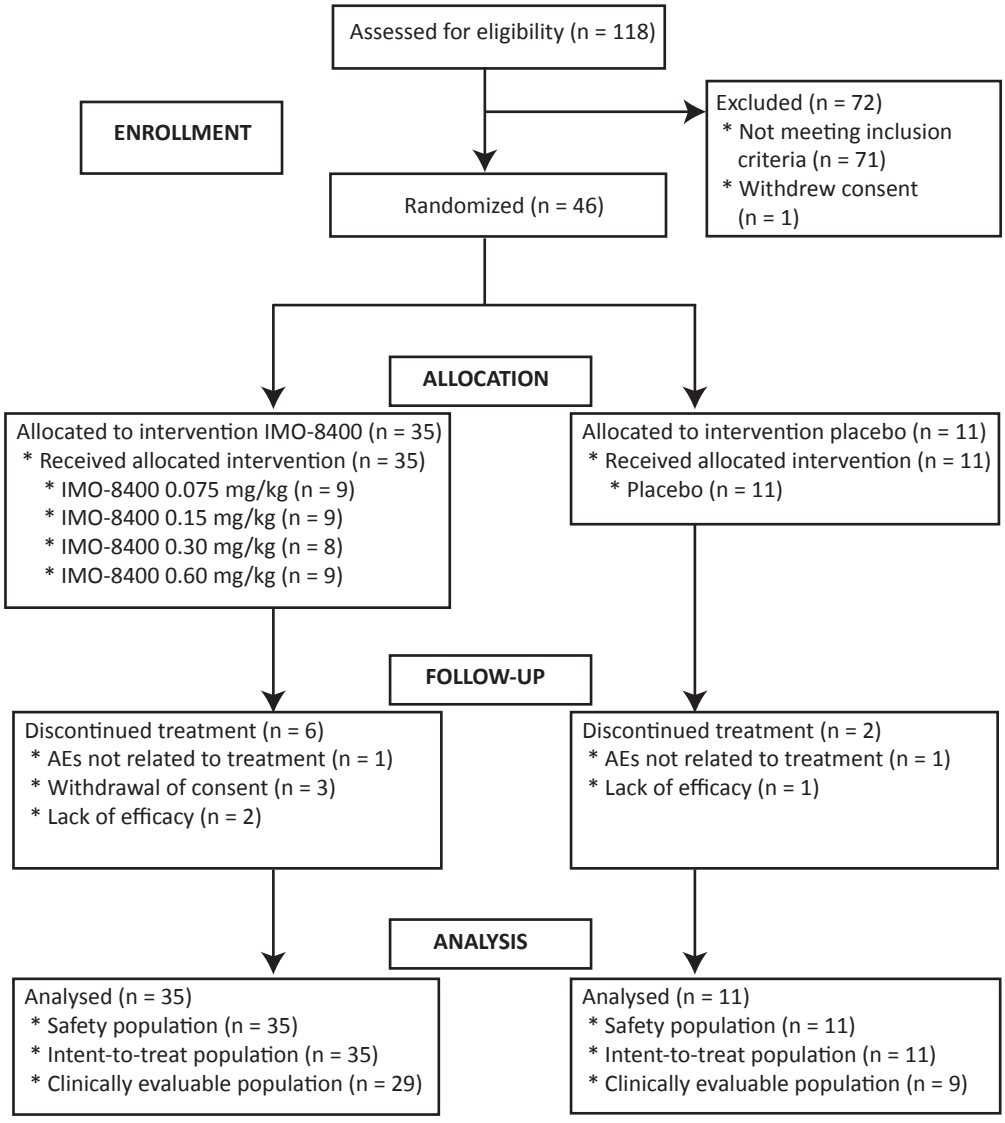


Figure 2: Example of a typical injection-site reaction with mild erythema at the lower abdomen (upper panel), and psoriasis-like plaques that occasionally developed at an injection-site (lower pannel)

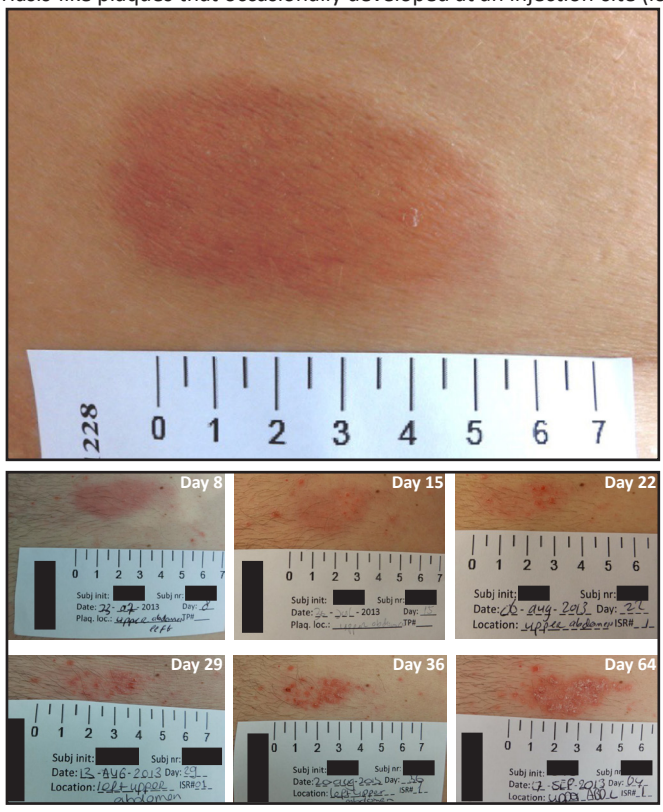
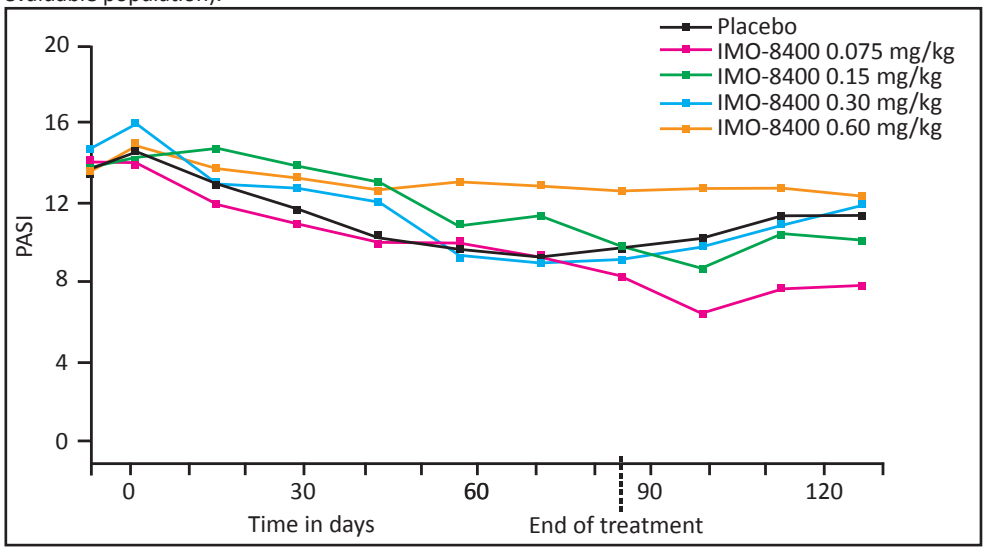


Figure 3a: Efficacy outcomes of IMO-8400. Mean PASI during the study by treatment arm (clinically evaluable population).



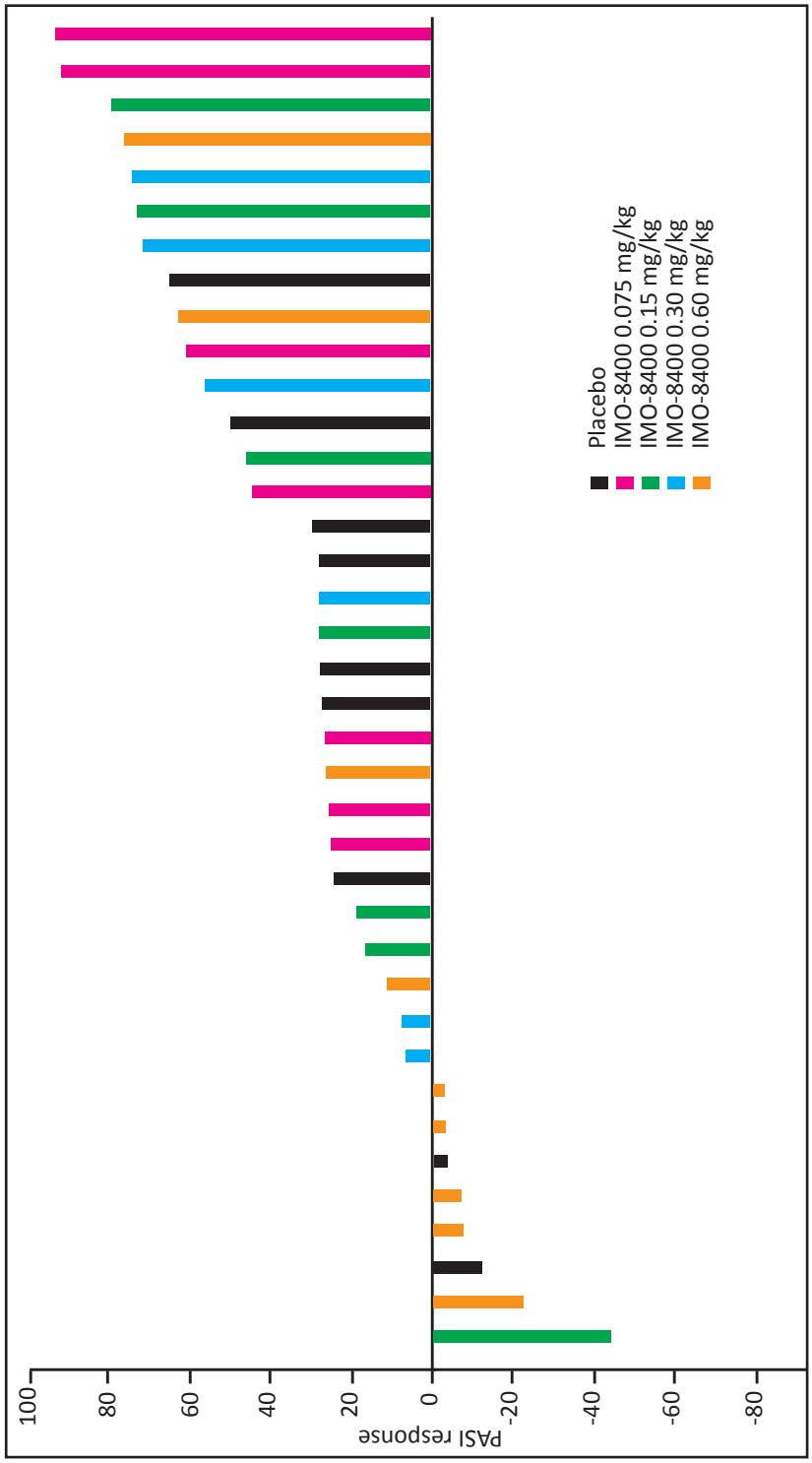


Figure 3b: Efficacy outcomes of IMO-8400. Individual PASI-responses at end of treatment (clinically evaluable population).

Walter Schweckendiek

“Dieser Erfolg machte nachdenklich. [...] Damit wäre für die Psoriasis eine sehr einfache Erklärung als Stoffwechselkrankheit gegeben, denn gerade die Schuppenflechte und die rheumatisch-arthritischen Krankheiten sollen ihre Ursache in einem fehlgesteuerten Stoffwechsel haben.”

Heilung von Psoriasis vulgaris. *Med Monatsschr.* 1959 Feb;13(2):103-4.

| PART VI |
SUMMARY, GENERAL DISCUSSION & MAIN CONCLUSIONS

SUMMARY

Psoriasis is a common chronic, relapsing inflammatory disease that from a clinical and socio-economical perspective can elicit a considerable high disease-burden on patients, their families, and society.[1] Improving established treatments and developing new treatments for psoriasis is therefore a relevant research subject. For the last 100 years or so the treatment of psoriasis has remained a major research topic.[2,3] These scientific efforts have accumulated in groundbreaking innovations making their entry in psoriasis treatment, perhaps unparalleled to any other skin disease. Significant advances made in the last two decades have led to the establishment of a wide array of treatment modalities for psoriasis.[4] In particular, novel insights in our understanding of the immune-based pathogenesis of psoriasis have resulted in the development of targeted therapies (i.e. the biologics). While such biologic monoclonal antibodies targeting specific cytokines have revolutionized the treatment of psoriasis - inducing quick and often near complete clearance of psoriasis in a majority of treated patients - their still expensive costs, primary treatment failure in a subset of patients, and potential secondary treatment failure over time necessitate the continued development of alternative treatment targets.[5-7] In comparison to the biologics, the 'older' classical or conventional systemic psoriasis treatments have been longer in use, but their supporting evidence is more limited.[8,9] Furthermore, a lack of understanding of their exact mechanisms of action, efficacy, and safety profile limits more widespread use of the conventional systemic treatments. Taken together, there is a clear clinical need for a continuous development and evaluation of emerging and established treatments of psoriasis. In this thesis, the focus was set on clinical drug development and evaluation of two different systemic psoriasis treatments: fumaric acid esters (FAEs) and toll-like receptor (TLR) antagonists.

FAEs, which are small molecules with broad, pluripotent immune-modulating properties, have been in use as a systemic psoriasis treatment for over three decades, but important questions on their efficacy, safety, and mechanisms of action remain unanswered. In this thesis, several clinical and experimental studies were conducted to assess the potential of FAEs in psoriasis. In contrast to the broad immune-modulating effects of FAEs, oligonucleotide-based antagonists of TLRs 7, 8, and 9 are a potential targeted psoriasis treatment. Preclinical evaluations and studies in healthy volunteers have yielded promising effects, but assessments in patients with psoriasis have not been performed yet. Here, we performed a first-in-patient phase 2a randomized clinical assessment of a novel, first-in-class TLR7, 8 and

9 antagonist in patients with psoriasis.

In this concluding chapter, the results of each of the studies are put into context, potential implications of the findings are discussed, and some perspectives on the future care for individuals with psoriasis are explored.

Main findings of this thesis

Efficacy of fumaric acid esters in psoriasis To assess the efficacy data on FAEs in psoriasis we performed a systematic review of the available literature up to August 2015 by searching multiple electronic databases. In order to include as much articles as possible we did not apply restrictions in study type, publication date, or article language. We could include 7 randomized controlled trials and 37 observational studies that evaluated the clinical effects of FAEs in patients with psoriasis.[10] All RCTs had relatively small sample sizes. Moreover, there were no formal phase 2 or 3 trials published. In the included studies, FAEs reduced psoriasis severity significantly compared to placebo; roughly 50-70% of patients had a favorable response to FAE treatment. Regrettably, high-quality studies evaluating FAE treatment in psoriasis were lacking, thus resulting in a low-to-moderate quality of evidence to support the use of FAEs in the treatment of psoriasis. Also, significant heterogeneity of the studies hampered to conduct a meta-analysis. Notwithstanding the low level of evidence, the clinical experience with conventional psoriasis treatments including FAEs is thought to be larger than the published data suggests.[8] A recent German multicenter RCT published in 2017 confirmed the efficacy of FAE treatment in psoriasis.[64]

A gap in the currently available literature is the paucity on efficacy and safety data of combination treatments in psoriasis.[11] In daily clinical practice, there is sometimes a clinical necessity to combine different systemic treatments to allow a sufficient disease control of psoriasis. Biologic treatments for instance may be combined with methotrexate or FAEs to enhance their efficacy and drug survival. High-quality evidence, however, to support the efficacy and safety of such combinations remains lacking.[11] Given this gap, we conducted a small randomized pilot study to assess the benefits of adding FAEs to etanercept, a first-generation anti-TNF alpha biologic.[12] The combination of etanercept with FAEs was safe and well-tolerated and did not lead to more adverse events with the exeption of gastrointestinal complaints. However, the combination treatment of FAEs and etanercept did not result in a robust increase in efficacy. These findings are in line with previously reported observational studies.[13-14] In a second placebo-controlled RCT, we evaluated the combination treatment of FAEs and the histamine-antagonist cetirizine.[15] There are some preclinical and clinical data

that suggest a potential beneficial role of cetirizine in psoriasis treatment. Our trial, however, demonstrated that adding cetirizine did not increase the efficacy of FAEs compared to treatment with FAEs alone. Taken together, combining FAEs with cetirizine does not seem clinically meaningful for daily clinical practice.

Safety of fumaric acid esters in psoriasis Ever since the introduction of FAEs as potential psoriasis treatment, concerns existed regarding the safety of FAEs. Back in the 1980s, several cases of severe acute renal toxicity were reported in psoriasis patients treated with FAEs.[16,17] For a long time FAEs were therefore considered by most dermatologists to be unsuitable for use as a systemic psoriasis treatment. However, these concerns were likely unjustified as we found no recent reports of acute nephrotoxic effects of FAEs.[10] The reported cases of renal failure from the 1980s were probably related to uncontrolled use of concomitant oral and topical FAEs leading to an overdose of FAEs. Rather, we did find several cases of another renal adverse event, namely Fanconi syndrome, that is likely underappreciated by dermatologists. Fanconi syndrome is characterized by renal proximal tubular damage, which can lead to urinary losses of amino acid, glucose and phosphate.[18] When left untreated, Fanconi syndrome can lead to a hypophosphataemic osteomalacia and associated pathological bone fractures.

We described two novel Dutch cases of a FAE-associated Fanconi syndrome from our department and the Leiden University Medical Center. In addition, we reviewed the clinical outcomes of all previously reported cases (n = 9). The median age at the time of onset was 38 years [interquartile range (IQR) 37–46]. Patients received long-term FAE treatment with a median treatment duration of 60 months (IQR 28–111). Laboratory tests were typically significant for low serum levels of phosphate and uric acid, while urinalysis showed glycosuria and proteinuria. Eight (73%) patients had developed a hypophosphataemic osteomalacia and three (27%) had pathological bone fractures. All patients discontinued FAEs, while four (36%) patients were treated with supplementation of phosphate and/or vitamin D. Five (45%) patients had persisting symptoms despite FAEs discontinuation. Of note, all reported cases of Fanconi syndrome were female psoriasis patients treated long-term with FAEs. A recent, retrospective study from Ireland also reported the occurrence of FAE-associated proximal renal tubular damage predominantly in female patients.19 The reasons for this sex-specific difference in risk for proximal tubular damage are still unknown.

A new, but potentially serious adverse event is the possible association of FAE treatment with progressive multifocal leukoencephalopathy (PML). PML is a rare, opportunistic viral infection of the central nervous system that is linked to reactivation of the John

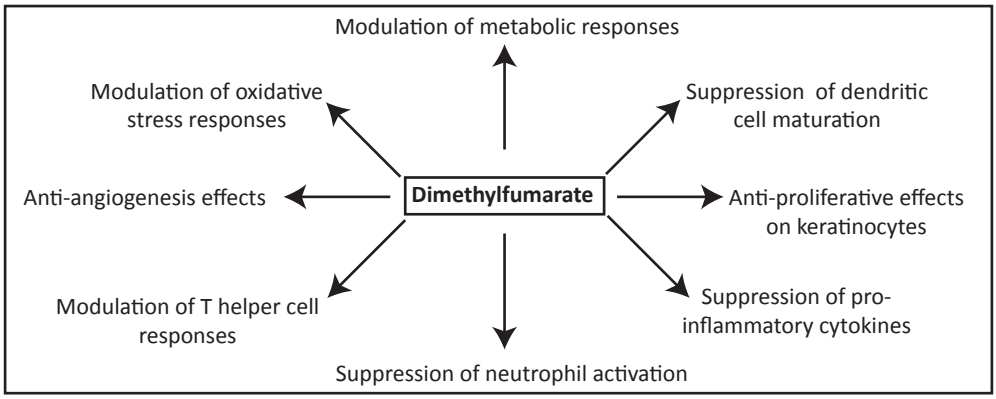
Cunningham (JC) virus, a human polyomavirus, due to a state of immunosuppression. The occurrence of PML can be linked to several drugs, which now includes FAEs. The incidence of FAE-associated PML remains unknown as systematic studies assessing the frequency of PML have not been performed yet. Searching multiple electronic databases, we identified 8 reports in the literature. We reviewed the clinical features and of all 8 reported cases of PML to define risk factors.[20] The median age was 64 years (range 42–74 years); the median FAE treatment duration was 3 years (range 1.5–5 years). Six patients were treated with a formulation containing dimethyl fumarate (DMF) and monoethyl fumarates, and two patients with a DMF mono-formulation. Patients exhibited neurological symptoms, such as aphasia, hemiparesis, and dysarthria. PML diagnosis was based on MRI findings and presence of JC virus in cerebrospinal fluid and/or brain tissue. All cases were linked to moderate-to-severe reductions in absolute lymphocyte counts, with nadirs ranging from 200 to 792 cells per mm^3. Of importance, all reported cases were exposed to varying lengths of lymphocytopenia. Median exposure to lymphocytopenia was 2 years (range 1–5 years). In all cases, FAE treatment was discontinued; PML was treated with mefloquine plus mirtazapine. Three patients improved, two had stable disease, two had residual symptoms, and one patient died to complications follwing an immune reconstitution inflammatory syndrome (IRIS).

Mechanisms of action of fumaric acid esters in psoriasis One major drawback associated with FAEs is that the exact mode of action underlying their effects in psoriasis is still incompletely understood. Insights into the drug-actions of FAEs are needed to better define their efficacy potential and to be able to predict potential adverse events. Experimental in vitro studies have reported numerous effects of FAEs, but the relevance of these for FAE's effects in vivo are disputable. For instance, DMF is considered a prodrug for MMF, but most studies have been performed with only DMF.[21] Notwithstanding those limitations, the in vitro effects of FAEs are broad. Next to various immune-modulating effects, FAEs have been linked to anti-angiogenic and anti-proliferating effects. Which of these are responsible for the clinical effects of FAEs in psoriasis treatment is unknown.

To evaluate potential pathways influenced by FAEs, we undertook a micro-array profiling study of lesional skin biopsies before and after 12 weeks of FAE treatment.[22] The study population included 9 patients with moderate-to-severe plaque psoriasis, of which 4 patients had a PASI-50 response and 5 who did not have a PASI-50 response. Our findings showed that FAE treatment reduces Th-17 responses, which is a clear mechanism of action given the pivotal pathogenetic role of IL-17 in psoriasis. In addition, our microarray analyses also showed FAE's capability to induce glutathione and Nrf2 pathway genes in psoriatic

lesional skin. Whether these effects are essential for FAEs to reduce psoriasis inflammation is unclear. The comparison with the effects of a non-related targeted treatment (i.e., the anti-TNF alpha inhibitor etanercept) was helpful to delineate precisely the potential effects of FAEs in psoriasis. In the current view, FAEs act via broad mechanisms of action in psoriasis. (See Figure 1)

Figure 1: Broad immune-related effects of fumaric acid esters relevant for psoriasis treatment



Efficacy and safety of a novel TLR-antagonist in psoriasis In a phase 2a clinical trial we assessed the effects of IMO-8400, a novel first-in-class oligonucleotide-based antagonist of TLRs 7, 8, and 9 among patients with moderate-to-severe plaque psoriasis.23 Four dose-levels ranging from 0.075 mg/kg to 0.60 mg/kg were included with weekly subcutaneous administration for a total treatment duration of 12 weeks. After the final study drug administration, there was a 6-week follow-up period. Treatment with all doses of IMO-8400 was well-tolerated and not associated with an increased risk of adverse events compared to placebo. Injection-site reactions were the most reported treatment-related adverse events. The overall efficacy was moderate, with 11 (38%) of 29 subjects on IMO-8400 who achieved ≥50% PASI-reduction, compared to 1 (11%) of 9 subjects on placebo. Five (17%) and 2 (7%) IMO-8400-treated subjects achieved PASI-75 and PASI-90, respectively, compared to none on placebo. There were no statistically significant differences compared to placebo, nor was there a clear dose-response relationship present.

GENERAL DISCUSSION

Clinical drug development and evaluation in psoriasis: past versus present

Example of drug development in the past: evaluation of FAEs as psoriasis treatment In this thesis we conducted a drug-evaluation of FAEs, which were introduced as one of the earliest developed systemic psoriasis treatments in the late 1950s.[24] The first published description of FAEs as potential psoriasis drug was provided in 1959 by Walter Schweckendiek.[25] Following his somewhat serendipitous discovery in a series of self-experiments that oral administration of FAEs reduces psoriasis severity, FAEs were empirically developed further by Schweckendiek and later by the German general practitioner Gunther Schäfer in the period 1960-1980. Regrettably, but not uncommon in that time period, the clinical development of FAEs was undertaken without published pre-clinical or pharmacological evaluations.[26-27] Combined with concerns on nephrotoxic potential of FAEs, this led to FAEs having a status of an alternative treatment in the eyes of dermatologists at that time. As a result, FAEs were prescribed only on a very limited scale in Germany and Switzerland. This began to change when - partly under pressure of psoriasis patient associations - the first dermatology-driven placebo-controlled randomized controlled studies with FAEs were conducted in the early 1990s.[28-31] While these early trials reported favourable results, all studies had small sample sizes and incomplete reporting of findings and statistical analyses.[10] Still, FAEs received German market approval (as Fumaderm) for the treatment of severe psoriasis in 1994 and for moderately severe psoriasis in 2008.[32] Outside Germany, the use of FAEs in psoriasis remains off-label treatment. To date, FAE-use is still limited, prescribed mainly in Western European countries, such as the Netherlands, the U.K., Ireland, and Austria.[33-36]

While the development of FAEs was quite unconventional in view of current drug development standards, it is a typical example of how the conventional psoriasis treatments were developed and introduced during that time period. Following a rather serendipitous drug discovery, therapies were evaluated without large, placebo-controlled randomized controlled trials prior to approval and market introduction. In general, the clinical experience would outweigh the limited number of trials published. While generally considered the golden era of the RCT, the sparse clinical trials in psoriasis from the 1960s to 1980s often did not adhere to methodological and reporting standards now considered standard, such as the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[8,37] In addition, at

that time the understanding of the immunological basis of psoriasis was limited, making pre-clinical evaluations to assess the mechanisms of action of a new compound understandably rather inadequate in modern-day view. To put FAE's drug development into context, the same holds true for the other conventional psoriasis agents developed in the same time period. For instance, methotrexate - introduced in the 1950s - is also associated with a low quality of evidence to support its clinical effects in psoriasis.[4,8] In addition, the drug actions of methotrexate leading to psoriasis improvement are only relatively recently elucidated. Yet, in general methotrexate is regarded as gold standard for the systemic treatment of psoriasis and is used commonly as a first-line treatment.[38] In contrast to the status of methotrexate, FAEs are not yet widely approved and available.[4] One of the underlying reasons for the differential status of FAEs compared to methotrexate is that FAEs were regarded as an alternative treatment due to their rather peculiar and non-dermatology-based development.

Taken together, the current limited evidence of the conventional psoriasis treatments like FAEs and methotrexate is a legacy from the past, given that at the time of their introduction and development, treatments were prior to approval not evaluated to the stringent standards of today. Without proper evaluations, the level of evidence of the conventional treatments will remain low, especially when compared to recently developed treatments, such as the biologics; Now-a-days, newly developed psoriasis treatments will always need to be evaluated with extensive preclinical evaluations and phase 1-3 assessments before consideration for approval by regulatory agencies.

Example of contemporary drug development: evaluation of a novel TLR-antagonist The development of TLR-antagonists as a potential new treatment for psoriasis is exemplarily for contemporary drug development. A multitude of experimental studies point towards a pivotal role of endosomal TLR engagement in the inflammatory cascade leading to psoriasis and possible other immune-mediated inflammatory diseases.[39-43] Aberrant over-activation of the TLRs 7,8, and 9 by self-nucleic acids is now recognized as one of the initial steps in the pathogenesis of psoriasis.[44-45] Hence, blocking TLRs 7, 8, and 9 would represent a viable therapeutic target in psoriasis. Pre-clinical interventional studies with TLR-blocking compounds in psoriasis mouse models have shown potential for targeting TLRs 7, 8, and 9 to reduce psoriatic skin inflammation.[46-47] Based on these findings, a clinical development path was initiated for evaluation of a novel TLR-antagonist in psoriasis. A synthetic oligonucleotide-based compound was developed named IMO-8400 that has antagonistic properties for

TLRs 7, 8, and 9 activation.[48] The first evaluations of IMO-8400 in several preclinical psoriasis mice models demonstrated ameliorating effects.[49-50] Subsequently, IMO-8400 administration was well-tolerated in a phase 1 clinical trial among healthy volunteers.[51] On the basis of these promising results, we conducted a first-in-patient, phase 2a clinical trial, evaluation of IMO-8400.[23] Unfortunately, the efficacy of the novel TLR-antagonist was overall modest in our phase 2 trial. Tolerability and short-term safety were favourable.

The development of the TLR-antagonist IMO-8400 is an example of modern drug development of a targeted therapy. Based on hypothesis-testing in pre-clinical disease models, the potential compound is then assessed in phase 1 and 2 evaluations. If well-evaluated, the development can continue to phase 3 trials to allow consideration by regulatory agencies for market authorization. Despite extensive preclinical evaluations suggesting 'the target is right', a compound still can fail to deliver upon entering the clinical phase development. An example is our phase 2 trial on TLR-antagonist in psoriasis. Despite the initial successful results, one of the most important disadvantages of contemporary drug development is the relatively low probability of success. Overall, less than 10% of developed compounds ultimately receive market approval. The long development period of novel treatments - which in general takes between 10 to 17 years - is another clear disadvantage of current drug development. Evidently, changes are needed in the way novel drugs with a novel mode of action are being developed.

Implications for clinical drug development and evaluation in psoriasis

Early drug development Adjustments in the way early clinical trials are designed are required to increase the success rates of drug development. One of the proposed alterations is to engage in more informative trials early on in drug development.[52](*See Figure 2*) The use of biomarkers in early clinical evaluations could also be of additive value. This could result in demonstrating proof-of-concept and establishing dose selection earlier on in the development phase and on the other hand earlier discontinuation of unsuccesful development of unsuitable compounds. In psoriasis trials, one could apply ex vivo analyses on serum samples to allow early establishment of target engagement and correlations to clinical effects. Also, gene expression profiling in lesional skin biopsies can be applied to predict earlier drug effects in psoriasis. Another potential improvement in clinical drug development in psoriasis is to stratify for the underlying type of psoriasis. It is increasingly appreciated that there seems to be genetic heterogeneity in the pathogenic pathways leading to psoriasis that is not directly mirrored by different clinical phenotypes. Stratifying for

such underlying pathways could enrich the study design. In our IMO-8400 trial, for instance, included patients could be stratified beforehand for type 1 interferon signaling or TLR engagement. Conducting such analyses would prove target engagement in target tissue (i.e., lesional skin) and could allow subgroup analyses. In the IMO-8400 trial we did perform an ex vivo challenge with synthetic TLR-agonists, but the assays were not robust enough for reliable analyses. Taken together, there are multiple possibilities to increase the potential success of early drug development in psoriasis.

Ongoing need for pharmacovigilance A lesson learned from the studies on FAEs in psoriasis is the continuous need for pharmacovigilance. RCTs are often insufficiently powered to assess infrequently occurring side adverse events. To detect signals for rare side effects, analyses of spontaneous reports to pharmacovigilance centers are pivotal. In this context, a local - and preferably nationwide - register that includes patients starting with FAE treatment is also of added value. Registration of clinical practice data may provide useful insights into the incidence of uncommon adverse events. Furthermore, such registers generate useful data on tolerability, effectiveness, and drug survival in daily clinical practice. These data are a useful addition to data generated from randomized clinical trials, which often have stringent in- and exclusion criteria. As a result, patients with for instance renal of hepatic insufficiency often are excluded in such clinical trials. Effectiveness and safety outcomes from daily clinical practice registries are therefore suitable complementary data to RCTs. Moreover, given the relatively paucity in data of FAEs, there is much to gain with optimal use of data generated from daily clinical practice. To maximize the usefulness and success, such a register should be a joint venture of the prescribing physician and pharmaceutical companies.

Psoriasis as a proof-of-concept disease model Psoriasis can be applied as a useful proof-of-concept disease model for evaluations of novel targets intended for immune-mediated inflammatory diseases (IMIDs).[53] There are several reasons for this. First, the immune-based pathogenesis of psoriasis is well-characterized, and there is a significant overlap of the immunologic background of psoriasis and other IMIDs, such as lupus erythematosus and dermatomyositis. Second, clinical effects can be easily evaluated as psoriatic skin lesions are directly visible. As a result, there is no absolute need for invasive biopsy procedures or use of proxy biomarkers. Taken together, psoriasis can serve as an useful model of other immune-mediated diseases, such as systemic lupus erythematosus (SLE). Our phase 2 trial on IMO-8400 in psoriasis is a good demonstration of the usefulness of pso-

riasis as suitable go-to model for pharmacological evaluations for IMIDs. On the basis of the findings of our phase 2 trial involving IMO-8400, a clinical development path was developed to assess TLR antagonism as potential treatment for dermatomyositis. A phase 2 clinical trial to evaluate IMO-8400 in dermatomyositis (ClinicalTrials.gov NCT02612857) is now ongoing in several sites in the US.

Reverse drug discovery and drug-repurposing of fumaric acid esters The current status of FAEs as psoriasis treatment is somewhat special, considering their relatively frequent use in spite of a lack of a clear understanding of the mechanisms of action. In this thesis, we attempted a reverse drug discovery approach by studying the effects of FAEs underlying their clinical effects in psoriasis. By a better understanding the drug-actions of FAEs, we might be able to better predict the effects of FAEs. Moreover, such understanding is also needed to better define the safety profile and predict adverse events. Our gene expression profiling study demonstrated multiple pathways being affected by FAE treatment. These results are in line with other experimental studies that showed effects of FAEs on multiple cell-types, including lymphocytes, neutrophils, endothelial cells, and mast cells.

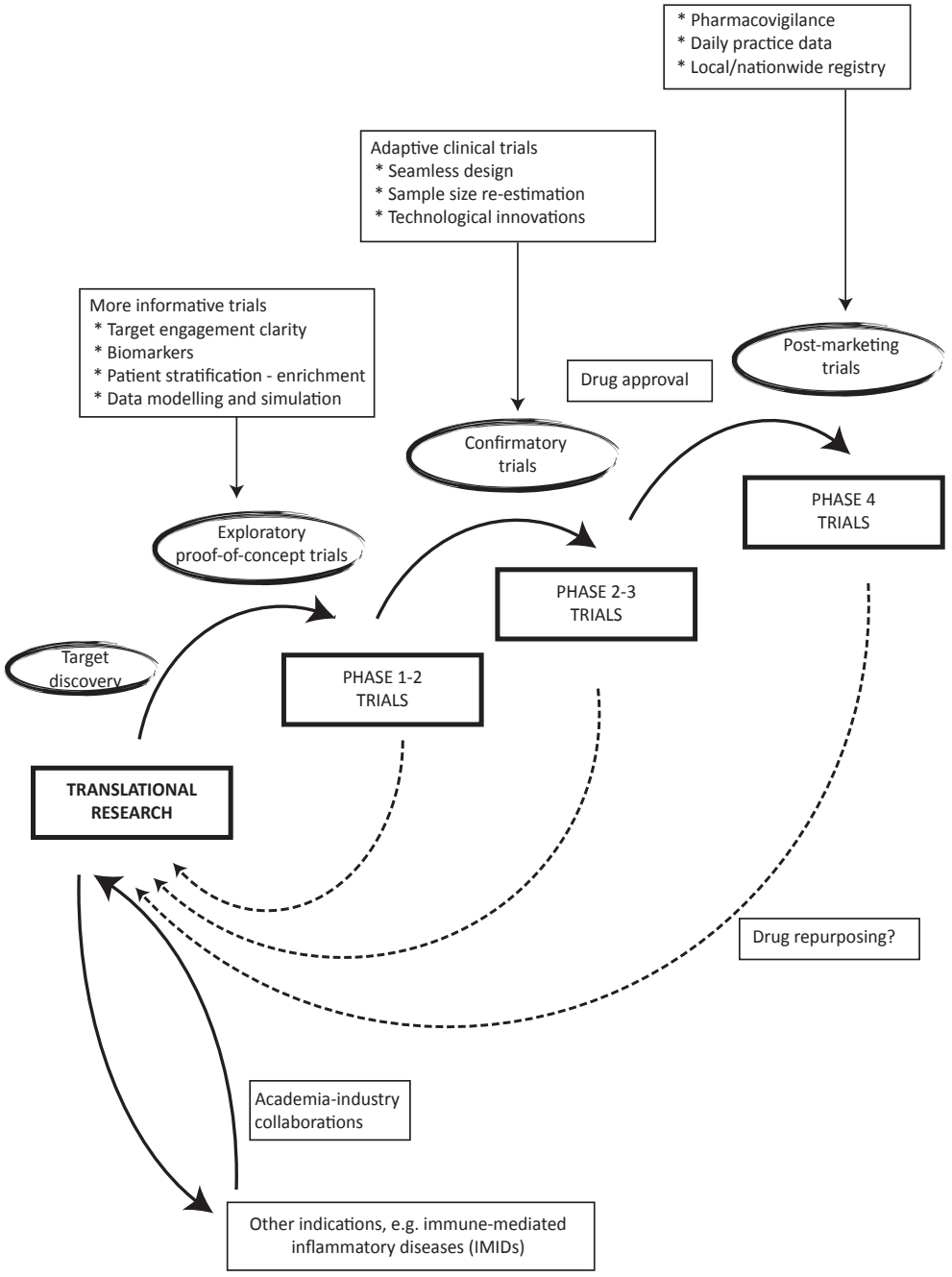
Given the broad immune-modulating properties of FAEs, they are good candidate drugs for drug repurposing. FAEs could have potential as treatment options for other indications. One successful example of FAE drug repurposing is represented by the treatment of multiple sclerosis. A DMF-formulation has been an FDA- and EMA-approved treatment for multiple sclerosis since 2013.

Drug repurposing of approved drugs hold several advantages. As the safety profile of approved drugs are well-characterized, the developmental time and the associated costs can be reduced considerable with drug repurposing.

Implications for the treatment of psoriasis

FAEs as primary option of first-line treatment of psoriasis We propose a more prominent role for FAEs as a first option for first-line systemic treatment for psoriasis. In spite of the limited evidence, the clinical experience with FAEs as psoriasis treatment is favourable. With proper patient-selection and adequate treatment monitoring FAEs treatment has the potential for long-term use and persistent disease control of psoriasis. In line, the drug survival of FAEs is also favourable; one Irish single-center study reported a drug survival of 60% following 5 years of treatment.[54] Next to a favourable safety profile without severe immunosuppressive effects, there are no known drug-drug interactions of FAEs.[55]

Figure 2: Drug-development 2.0 in psoriasis



This is a clear advantage in psoriasis treatment, considering that most patients with psoriasis require concomitant medications for comorbidity. We propose FAEs as the first choice of first-line systemic treatment in moderate-to-severe psoriasis, preferred over methotrexate and UV-B phototherapy.

FAEs vs. methotrexate Until now, methotrexate (MTX) is widely regarded as the primary drug for first-line therapy in psoriasis.[56] There are several arguments to favour the use of FAEs over MTX as first-line systemic agent. First, the short-term efficacy of FAEs seems similar to that of MTX.[36,57] Second, the risk of hepatotoxicity is a major drawback for long-term use of MTX. Moreover, the hepatotoxic effects of MTX are quite relevant, given that current monitoring options for MTX-associated hepatotoxicity seem limited, psoriasis itself is associated with liver fibrosis, and that concomitant use of alcohol with MTX is prohibited.[58,59] The latter is a somewhat serious issue for modern-day society, in which alcohol consumption has a rather prominent social position. Moreover, psoriasis in itself may be additionally associated with an increased alcohol consumption.[60] Furthermore, in rare cases MTX use has been associated with bone marrow suppression; even at low dosages, MTX can cause significant adverse events – especially in elderly patients.[61] Another drawback of MTX is the potential drug-drug interactions with for instance antibiotics. Finally, in daily clinical practice is it noted that many patients are reluctant to start MTX in fear of adverse events and toxicity. Taken together, MTX had a limited potential for long-term use.[4]

FAEs vs. phototherapy A next issue is the changing position of UV phototherapy. Phototherapy has been a hallmark therapy for psoriasis since its introduction in the 1980s and often applied as next therapy in case psoriasis is unresponsive or inadequately controlled with topical treatments.[62] Yet the position of phototherapy may become increasingly narrower due to several reasons. First, phototherapy often induces only short clinical remission as psoriasis often reoccurs within months after stopping photo-therapy. Continuous or frequent courses of phototherapy are not a viable option considering the risk of cutaneous malignancies associated with cumulative UV-exposure. Moreover, the risk of UV-associated skin cancer is now-a-days more relevant, given the increasing life expectancy and increasing UV exposure in current-day society. Finally, phototherapy poses a considerable logistic burden to patients - especially those with a day-time occupation - given the requirement of 2 to 3 weekly visits to the outpatient clinic for up to 3 months. Therefore, the use of phototherapy may become more restricted to a subgroup of patients with psoriasis, e.g. patients with uncontrolled severe psoriasis and who have contra-indications, intolerance, or treatment

failure to systemic treatments.

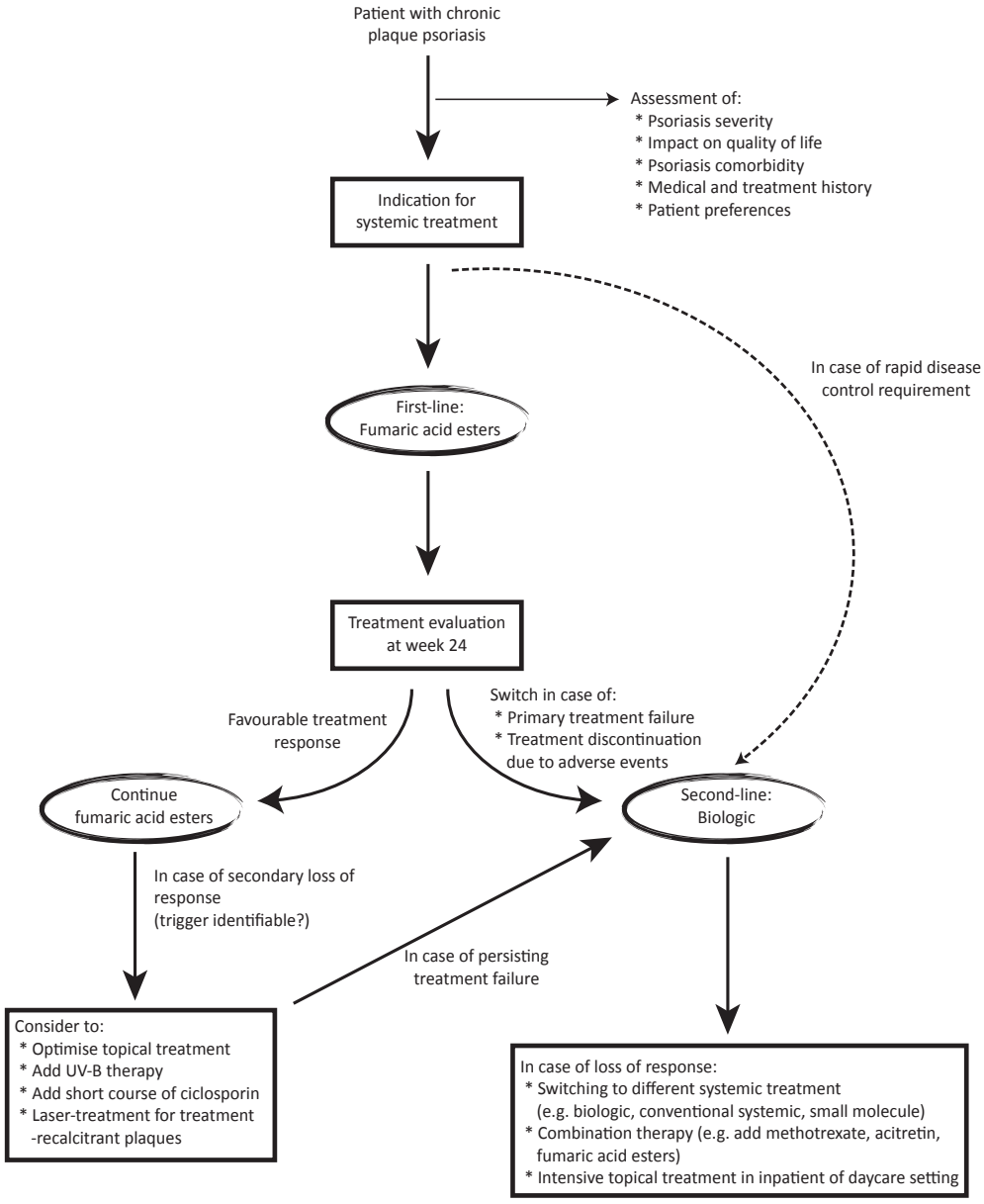
Position of FAEs in the psoriasis treatment landscape Based on the current evidence and our clinical experience, we propose a position of FAEs earlier in the treatment ladder as first-line systemic treatment before other classical systemic treatments – most notable MTX - and also before phototherapy.(*See Figure 3*)

In case of primary or secondary treatment failure of FAEs, a logical next step would be switching to a biologic. Antagonists of interleukin-12/23 and IL-17 are highly efficacious treatments, but their high costs are potential drawbacks. In current health care systems, budget control is essential for sustainability. As the costs of FAEs are considerable lower than that of biologic agents, it would by cost-reductive to start with FAEs before a biologic. A substantial proportion will respond favourable to FAEs, bypassing the need for a biologic, and this approach would therefore contribute significantly to costs reduction in health care. Our preferred position of FAEs relative to that of the biologics is not only financially driven. One issue is that the safety aspects of long-term continuous treatment with a biologic are still relatively unknown. In addition, biologics have a decreased drug survival in daily clinical practice due to progressive secondary loss of efficacy. In contrast, FAEs seem to have a favourable drug survival in daily clinical practice. Furthermore, there are several medical contra-indications for biologics, such as congestive heart failure for anti-TNF alpha antagonists. Also, patient-related factors may prohibit biologic use, such as belonephobia (i.e., fear for needles). Taken together, there are several lines of argumentation to support a preferred status of FAEs as first-line systemic treatment for psoriasis.

Prescription of fumaric acid esters for psoriasis in clinical practice

FAE-formulations in psoriasis At present, multiple different FAE-formulations are in use in daily clinical practice. The most studied formulation is the combination-formulation of DMF and MEF, which is also the licensed drug in Germany marketed as Fumaderm.[32] Historically, the combination formulations with DMF and MEF were the most applied. Newer formulations, however, contain only DMF, given that in experimental studies DMF appeared to be the most active FAE. In the Netherlands multiple FAE-formulations are in use, including combination-formulations with DMF and MEF and DMF-mono-preparations.[15,63] At present, multiple DMF-formulations are in clinical development. A recent phase III trial reported non-inferiority of a DMF-formulation compared to Fumaderm.[64] Based on the current available studies, no clear preference for a specific FAE formulation can be made. Until more data

Figure 3: Proposed treatment ladder for chronic plaque psoriasis



become available, the choice for a FAE-formulation is likely dependent predominantly on local availability. From a pharmacologic and regulatory view, it seems logical that ultimately the preference will be set for the DMF-formulations.

Monitoring FAEs in psoriasis treatment As with any systemic drug, monitoring for side effects is of importance during FAE treatment.(*See Table 1*) Two aspects of FAEs that require specific monitoring includes Fanconi syndrome and progressive multifocal leukoencephalopathy. Both seem infrequently occurring adverse events, but their potential severity and risks for serious complications justify a rigorous clinical vigilance.

To monitor for Fanconi syndrome, measurement of proteinuria during FAE treatment is recommended. Measurement of albumin:total protein ratio in urine is an additional tool to look specifically for tubular proteinuria. Referral to a nephrologist for further renal evaluating should be considered in case of persisting (tubular) proteinuria.

To monitor for PML, periodic monitoring of lymphocyte-counts and FAE discontinuation in case of moderate-to-severe lymphocytopenia are recommended. The 2015 European S3-guidelines on psoriasis treatment has recommended two drug-discontinuation rules. Direct FAE-treatment discontinuation is recommended upon an absolute lymphocyte count of 0.5 x 10^9/L and/or an absolute leukocyte count of 3.0 x 10^9/L. In case of an absolute lymphocyte count of 0.7 x 10^9/L, reduction of the FAE-dosage by 50% and re-evaluation within 4 weeks is indicated. Of equal importance to laboratory monitoring, clinical vigilance for onset of new neurological symptoms should be continued throughout the FAE-treatment. Upon new neurological symptoms, immediate FAE discontinuation and prompt evaluation by a neurologist are recommended.

Table 1: Recommendations for treatment monitoring of fumaric acid esters in psoriasis

	Start	Month 1	Month 2	Month 3, thereafter every 3 months
Full blood count including leukocytes and lymphocytes	x	x	x	x
Liver enzymes	x	x	x	x
Serum creatinine	x	x	x	x
Urine analysis	x	x	x	x

Recommendations for the use of FAEs in clinical practice It is essential that treatment with FAEs is combined with proper patient education. The initial treatment period with FAEs can be challenging for patients, as FAEs are associated with a relatively slow onset of efficacy. Moreover, the first weeks of FAE-treatment are commonly associated with a relatively high incidence of gastrointestinal complaints and cutaneous flushing symptoms that can be quite bothersome to tolerate. Therefore, the initial treatment phase of FAEs can be difficult to get through for patients. It is therefore essential to guide patients with proper education and counseling In this context, co-guidance of patients starting FAEs by a specialized physician assistant of a nurse practitioner can be helpful for clinical practice.

Towards approval of FAEs as psoriasis treatment One important current limitation of FAEs is the fact that it remains an unlicensed therapy in a majority of countries. At present, Germany is the only country in which FAEs have an approved status for the treatment of psoriasis.[32] For medicolegal reasons, the unlicensed status of FAEs can be a major drawback for their use in clinical practice. Having received German market authorization in 1994, FAEs are currently the most common prescribed systemic treatment in Germany.65 In contrast, in other countries such as the U.K. the use of FAEs is much more narrow. Results from the U.K. national psoriasis registry BADDIR showed that only 3% is prescribed FAEs.[34] There are unfortunately no data available for prevalence of FAE-use in the Netherlands. Based upon a nationwide survey in 2010, two-third of all dermatologists indicated to prescribe FAEs.[33] The other third stated the unlicensed status as a barrier for FAE-prescription. Taken together, there may be much to gain following approval of FAEs as psoriasis treatment. However, one disadvantage to consider in this context would be the pricing of the drug. Following approval, the costs of the licensed drug typically increases compared to the compounded formulation. Nonetheless, it would be advantageous when FAEs are granted market authorization for psoriasis treatment, as this will presumably lead to a wider availability of FAEs. A DMF-formulation has now received market approval by the European Medicines Agency (EMA) following publication of a successful phase 3 trial in 2017.[64]

Where to go: precise or pluripotent?

Anno 2018, the physician treating psoriasis can apply a multitude of treatment modalities with different treatment targets. As direct head-to-head studies comparing are largely lacking, the choice which treatment to start depends on factors such as physician experience and patient-bound factors. Conceptually, control of the systemic inflammation associated

with psoriasis would require long-term systemic treatment. In terms of treatment target, a distinction can be made between targeted biologic treatments and broad, pluripotent treatments. Examples of targeted treatments include antagonists of TNF-alpha, IL-23, and IL-17. These antagonists of downstream cytokines that play pivotal roles in psoriasis pathogenesis are effective therapies for psoriasis. In contrast to the targeted drugs, disease-modifying drugs act on multiple targets to decrease cutaneous inflammation. Examples of these include the classical systemic psoriasis treatments FAEs and MTX. At present, there is insufficient evidence for superiority of a particular class of treatment. Historically, conventional systemic psoriasis drugs (mostly MTX) are chosen as first-line systemic treatment for psoriasis. This preference was based on clinical experience - the (long-term) safety profile of biologics was yet to be established - and financial grounds. However, the first-line status of MTX and other classical systemic treatments may be undergoing a change, given that now-a-days the long-term effects of biologics are increasingly known. Furthermore, with the approval of biosimilars the costs of biologics will likely diminish.

Table 2: Targeted therapies versus broad, non-specific therapies in psoriasis.

	Precise, targeted therapies	**Broad, non-specific therapies**
Examples	Anti-TNF alpha biologics	Fumaric acid esters
	Anti-IL12/23 biologic	Methotrexate
	Anti-IL17 biologics	Apremilast
		JAK-STAT inhibitors
Advantages	Rapid onset of response	Multiple indications, suitable for co-morbidity
	High efficacy	Oral administration
		Relatively low costs
Disadvantages	Relatively high costs	Slow onset of response
	Primary non-response	Moderate efficacy
	Secondary loss of efficacy due to anti-drug antibody formation	Cumulative toxicity

Studies focusing on long-term safety and direct comparisons with other psoriasis treatments could lead to a better understanding of the role of conventional treatments such as FAEs relative to that of biologics in the treatment for psoriasis. For the time being, it is likely that the classical systemic psoriasis treatments will retain their first-line status. New insights into their mechanisms in action could lead to better prediction of efficacy and safety

outcomes. Future studies could be directed at identifying biomarkers to predict treatment response or side effects.

Some perspectives on the future care for individuals with psoriasis

As long as a definitive cure for psoriasis remains lacking, the treatment of psoriasis is a joint venture of the physician and the patient. In addition to factors such as efficacy, safety, and tolerability of a treatment, it is essential to include the patient's perspective in the treatment. Furthermore, a multidisciplinary approach is crucial for an optimal treatment of an individual with psoriasis.[66] A clear example includes rheumatology with regards to psoriatic arthritis. But other medical disciplines could also have a part in a 'psoriasis management team'. For instance, endocrinologists specialized in obesity and bariatric surgeons could co-manage patients having a severely elevated high body mass index (BMI). Furthermore, psychosocial support should also be central in the management of psoriasis.

CONCLUSIONS

Despite significant advances in the treatment of psoriasis seen in the last two decades, psoriasis remains a challenging and difficult-to-treat disease. There is a continuous clinical need for development and evaluation of emerging and established treatments of psoriasis. Contemporary drug development for psoriasis could be enhanced in order to increase the success rates.

In this thesis we studied the clinical drug development of fumaric acid esters and a toll-like receptor antagonist. In a drug rediscovery approach we attempted to elucidate further the effects and mechanisms of action of fumaric acid esters in psoriasis treatment; these insights will contribute to a more accurate prediction of their treatment responses and potential side effects. The toll-like receptor 7, 8, and 9 antagonist IMO-8400 failed to deliver in a phase 2 trial involving psoriasis, but the study results did provide useful insights into the potential mechanisms underlying psoriasis. Additionally, the phase 2 trial gave sufficient grounds for a continuous drug development of IMO-8400 for related immune-mediated inflammatory diseases (e.g. dermatomyositis).

Given that psoriasis is a chronic disease, long-term systemic treatment is pivotal for sustained disease control in most patients with moderate-to-severe plaque psoriasis. With proper monitoring, fumaric acid esters have the potential for effective and safe disease control of psoriasis in long-term treatment. Out of all currently available systemic treatment

options, fumaric acid esters may arguably have the most favourable risk-benefit ratio. Future direct head-to-head comparison with other systemic treatments and evaluations to establish biomarkers for early assessment of treatment response could all enhance the position of fumaric acid esters in the psoriasis treatment landscape.

Notwithstanding al the recent developments and innovations, the 'magic bullet' for psoriasis is yet to be discovered. The need remains to continue the development of new, better and safer treatment options for psoriasis, so that in the end the lives of those having psoriasis may be improved.

REFERENCES

1. Karimkhani C et al. Global Skin Disease Morbidity and Mortality: An Update From the Global Burden of Disease Study 2013. JAMA Dermatol. 2017.
2. Abraham PS. An Address ON PSORIASIS AND ITS TREATMENT: Delivered before the Ealing Division of the Metropolitan Counties Branch of the British Medical Association. Br Med J. 1906;1(2363):842-4.
3. Siemens HW. Vraagstukken der psoriasisbehandeling. Ned Tijdschr Geneeskd. 1938;82(12):1290.
4. Nast A 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.
5. Noda S et al. The translational revolution and use of biologics in patients with inflammatory skin diseases. J Allergy Clin Immunol. 2015;135(2):324-36.
6. Gniadecki R et al. Comparison of long-term drug survival and safety of biologic agents in patients with psoriasis vulgaris. Br J Dermatol. 2015;172(1):244-52.
7. Warren RB 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.
8. Pathirana D et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. J Eur Acad Dermatol Venereol. 2009;23 Suppl 2:1-70.
9. Naldi L et al. Traditional therapies in the management of moderate to severe chronic plaque psoriasis: an assessment of the benefits and risks. Br J Dermatol. 2005;152(4):597-615.
10. Balak DM 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.
11. Busard C et al. Combined use of systemic agents for psoriasis: a systematic review. JAMA Dermatol. 2014;150(11):1213-20.
12. van Bezooijen JS et al. Combination Therapy of Etanercept and Fumarates versus Etanercept Monotherapy in Psoriasis: A Randomized Exploratory Study. Dermatology. 2016;232(4):407-14.
13. Balasubramaniam P et al. Fumaric acid esters in severe psoriasis, including experience of use in combination with other systemic modalities. Br J Dermatol. 2004;150(4):741-6.
14. Wilsmann-Theis D et al. Systemic antipsoriatic combination therapy with fumaric acid esters for plaque-type psoriasis: report on 17 cases. Dermatology. 2015;230(2):119-27.
15. Balak DM et al. Addition of an oral histamine antagonist to reduce adverse events associated with fumaric acid esters in the treatment of psoriasis: a randomized double-blind placebo-controlled trial. Br J Dermatol. 2015;172(3):754-9.
16. Dalhoff K et al. Akutes Nierenversagen unter Psoriasistherapie mit Fumarsaurederivaten. Dtsch Med Wochenschr. 1990;115(26):1014-7.
17. Roodnat JI et al. Akute Niereninsuffizienz bei der Behandlung der Psoriasis mit Fumarsaure-Estern. Schweiz Med Wochenschr. 1989;119(23):826-30.
18. Balak DM et al. Drug-induced Fanconi syndrome associated with fumaric acid esters treatment for psoriasis: a case series. Clin Kidney J. 2016;9(1):82-9.
19. Menzies S et al. Renal dysfunction in patients taking fumaric acid esters - a retrospective cross-sectional study. J Eur Acad Dermatol Venereol. 2016.
20. Balak DM et al. Progressive multifocal leukoencephalopathy associated with fumaric acid esters treatment in psoriasis patients. J Eur Acad Dermatol Venereol. 2017.
21. Dibbert S et al. Detection of fumarate-glutathione adducts in the portal vein blood of rats. Arch Dermatol Res. 2013;305(5):447-51.
22. Onderdijk AJ et al. Regulated genes in psoriatic skin during treatment with fumaric acid esters. Br J Dermatol. 2014;171(4):732-41.
23. Balak DM et al. IMO-8400, a toll-like receptor 7, 8, and 9 antagonist, demonstrates clinical activity in a phase 2a, randomized, placebo-controlled trial in patients with moderate-to-severe plaque psoriasis. Clin Immunol. 2017;174:63-72.
24. Balak DMW. Fumaric acid esters in the management of psoriasis. Psoriasis: Targets and Therapy. 2015;5:9-23.
25. Schweckendiek W. Heilung von Psoriasis vulgaris. Med Monatsschr. 1959;13(2):103-4.
26. Schweckendiek W. Behandlung von psoriasis mit lipoidloslichen fumarsaureverbindungen. Medizin Heute 1966(15):219-20.
27. Schafer G. Fumarsaure lindert die schuppenflechte. Selecta. 1984(15):1260-1.
28. van Dijk E. Fumaarzuur voor de behandeling van patienten met psoriasis. Ned Tijdschr Geneeskd. 1985;129(11):485-6.
29. Nugteren-Huying WM et al. Fumaric acid therapy for psoriasis: a randomized, double-blind, placebo-controlled study. J Am Acad Dermatol. 1990;22(2 Pt 1):311-2.
30. Altmeyer PJ et al. Antipsoriatic effect of fumaric acid derivatives. Results of a multicenter double-blind study in 100 patients. J Am Acad Dermatol. 1994;30(6):977-81.

31. Nieboer C et al. Systemic therapy with fumaric acid derivates: new possibilities in the treatment of psoriasis. J Am Acad Dermatol. 1989;20(4):601-8.

32. Mrowietz U et al. 15 Jahre Fumaderm: Fumarsaureester fur die systemische Behandlung der mittelschweren und schweren Psoriasis vulgaris. J Dtsch Dermatol Ges. 2009;7 Suppl 2:S3-16.

33. Fallah Arani S et al. Treatment of psoriasis with non-registered fumaric acid esters in The Netherlands: a nationwide survey among Dutch dermatologists. J Eur Acad Dermatol Venereol. 2014;28(7):972-5.

34. Iskandar IY et al. Demographics and disease characteristics of patients with psoriasis enrolled in the British Association of Dermatologists Biologic Interventions Register. Br J Dermatol. 2015;173(2):510-8.

35. Heelan K et al. Fumaric acid esters as a suitable first-line treatment for severe psoriasis: an Irish experience. Clin Exp Dermatol. 2012;37(7):793-5.

36. Inzinger M et al. Methotrexate vs. fumaric acid esters in moderate-to-severe chronic plaque psoriasis: data registry report on the efficacy under daily life conditions. J Eur Acad Dermatol Venereol. 2013;27(7):861-6.

37. Moher D et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. Bmj. 2010;340:c869.

38. Gladman DD. Should methotrexate remain the first-line drug for psoriasis? Lancet. 2017;389:482-3.

39. Ganguly D et al. The role of dendritic cells in autoimmunity. Nat Rev Immunol. 2013;13(8):566-77.

40. Ganguly D et al. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J Exp Med. 2009;206(9):1983-94.

41. Gilliet M et al. Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch Dermatol. 2004;140(12):1490-5.

42. Wohn C et al. Langerin(neg) conventional dendritic cells produce IL-23 to drive psoriatic plaque formation in mice. Proc Natl Acad Sci U S A. 2013;110(26):10723-8.

43. van der Fits L et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol. 2009;182(9):5836-45.

44. Lowes MA et al. Immunology of psoriasis. Annu Rev Immunol. 2014;32:227-55.

45. Conrad C et al. Plasmacytoid dendritic cells in the skin. Semin Immunol. 2009;21(3):101-9.

46. Lai CY et al. Identification of Thiostrepton as a Novel Inhibitor for Psoriasis-like Inflammation Induced by TLR7-9. J Immunol. 2015;195(8):3912-21.

47. Huang SW et al. Azithromycin impairs TLR7 signaling in dendritic cells and improves the severity of imiquimod-induced psoriasis-like skin inflammation in mice. J Dermatol Sci. 2016;84(1):59-70.

48. Kandimalla ER et al. Design, synthesis and biological evaluation of novel antagonist compounds of Toll-like receptors 7, 8 and 9. Nucleic Acids Res. 2013;41(6):3947-61.

49. Jiang W et al. A Toll-like receptor 7, 8, and 9 antagonist inhibits Th1 and Th17 responses and inflammasome activation in a model of IL-23-induced psoriasis. J Invest Dermatol. 2013;133(7):1777-84.

50. Suarez-Farinas M et al. Suppression of molecular inflammatory pathways by Toll-like receptor 7, 8, and 9 antagonists in a model of IL-23-induced skin inflammation. PLoS One. 2013;8(12):e84634.

51. Brenner L et al. IMO-8400, an antagonist of toll-like receptors 7, 8, and 9, in development for genetically defined B-Cell lymphomas: Safety and activity in phase 1 and phase 2 clinical trials. Blood. 2014;124 (21)(0006-4971).

52. Cohen AF. Developing drug prototypes: pharmacology replaces safety and tolerability? Nat Rev Drug Discov. 2010;9(11):856-65.

53. Nestle FO et al. Psoriasis. N Engl J Med. 2009;361(5):496-509.

54. Ismail N et al. Drug survival of fumaric acid esters for psoriasis: a retrospective study. Br J Dermatol. 2014;171(2):397-402.

55. Thaci D et al. Efficacy and safety of fumaric acid esters in patients with psoriasis on medication for comorbid conditions - a retrospective evaluation (FACTS). J Dtsch Dermatol Ges. 2013;11(5):429-35.

56. Gyulai R et al. Current practice of methotrexate use for psoriasis: results of a worldwide survey among dermatologists. J Eur Acad Dermatol Venereol. 2015;29(2):224-31.

57. Fallah Arani S et al. 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.

58. Barker J et al. Assessment and management of methotrexate hepatotoxicity in psoriasis patients: report from a consensus conference to evaluate current practice and identify key questions toward optimizing methotrexate use in the clinic. J Eur Acad Dermatol Venereol. 2011;25(7):758-64.

59. Xu X et al. The Prevalence of Nonalcoholic Fatty Liver Disease and Related Metabolic Comorbidities Was Associated with Age at Onset of Moderate to Severe Plaque Psoriasis: A Cross-Sectional Study. PLoS One. 2017;12(1):e0169952.

60. Brenaut E et al. Alcohol consumption and psoriasis: a systematic literature review. J Eur Acad Dermatol Venereol. 2013;27 Suppl 3:30-5.

61. Rodenburg RJ et al. Ernstige complicaties tijdens methotrexaatbehandeling. Ned Tijdschr Geneeskd. 2017;161(0):D575.

62. Racz E et al. Phototherapy and photochemotherapy for psoriasis. Dermatol Clin. 2015;33(1):79-89.

63. Lijnen R et al. Long-term safety and effectiveness of high-dose dimethylfumarate in the treatment of moderate to severe psoriasis: a prospective single-blinded follow-up study. J Dermatolog Treat. 2016;27(1):31-6.

64. Mrowietz U et al. Efficacy and safety of LAS41008 (dimethyl fumarate) in adults with moderate-to-severe chronic plaque psoriasis: a randomized, double-blind, Fumaderm(R) - and placebo-controlled trial (BRIDGE). Br J Dermatol. 2017;176(3):615-23.

65. Augustin M et al. German psoriasis registry PsoBest: objectives, methodology and baseline data. J Dtsch Dermatol Ges. 2014;12(1):48-57.

66. Mrowietz U et al. Psoriasis: to treat or to manage? Exp Dermatol. 2014;23(10):705-9.

Prof.dr. E. van Dijk

"In Zwitserland, Duitsland en Nederland begint men zowel in universitaire als niet-universitaire medische kringen voor dit merkwaardige therapeutische verschijnsel te interesseren. [...] Verdere verbetering van deze behandeling lijkt niet onmogelijk."

Fumaarzuur voor de behandeling van patiënten met psoriasis. *Ned Tijdschr Geneeskd*. 1985 Mar 16;129(11):485-6.

| PART VII |
SUMMARY IN DUTCH (NEDERLANDSE SAMENVATTING)

SAMENVATTING

Hoofdstuk 1 van deze thesis start met een inwijding in de achtergronden en behandelopties van psoriasis, een veelvoorkomende chronische immuun-gemedieerde inflammatoire aandoening die een hoge ziektelast met zich meebrengt. De meeste patiënten met uitgebreide en ernstige vormen van psoriasis zijn veelal afhankelijk van systemische behandeling om hun aandoening afdoende te kunnen onderdrukken. In de praktijk blijkt de behandeling van psoriasis vaak lastig, maar de afgelopen twee decennia hebben zich grote ontwikkelingen in de behandelopties voorgedaan. Voortschrijdende inzichten in de onderliggende pathogenese van psoriasis hebben geleid tot een revolutionaire ontwikkeling van nieuwe, gerichte behandelopties. De huidig beschikbare psoriasis behandelingen zijn onder te verdelen in vier grote categorieën: i) lokale therapieën, waaronder corticosteroïdbevattende zalven en crèmes; ii) lichttherapie met UV-straling; iii) systemische immunosuppressieve en immunomodulerende medicatie, waaronder methotrexaat, fumaraten, ciclosporine, acitretine en apremilast; iv) biologics, bestaande uit monoklonale antilichamen die specifiek bepaalde componenten van het afweersysteem onderdrukken, waronder TNF-alpha, interleukine-17 en interleukine-23. Ondanks de diverse scala aan beschikbare behandelopties, blijft de behandeling van psoriasis in de praktijk een uitdaging. Derhalve is er nog steeds ruimte in het optimaliseren van psoriasis behandeling. In deze thesis werd de geneesmiddelen-ontwikkeling en -evaluatie van twee verschillende systemische psoriasis-behandelingen onderzocht: fumaraten en toll-like receptor (TLR)-antagonisten.

Fumaraten zijn ester derivaten van fumaarzuur, een lichaamseigen stof dat betrokken is bij de energiehuishouding in de cellen. Fumaarzuur zelf heeft geen anti-psoriasis effecten en wordt o.a. gebruikt als voedingsadditief (E-nummer 297). Daarentegen hebben de ester derivaten van fumaarzuur diverse anti-inflammatoire, immunomodulerende en antiproliferatieve effecten. De eerste beschrijving van fumaraten als mogelijke behandeling van psoriasis dateert uit 1959 toen de Duitse chemicus Schweckendiek in enkele zelf-experimenten - hij leed zelf aan psoriasis - aantoonde dat psoriasis in ernst afneemt met orale toediening van fumaarzuur-derivaten. Omdat verdere wetenschappelijk evaluaties helaas uitbleven, ontbrak de nodige bewijskracht en bleven belangrijke vragen omtrent de effectiviteit, veiligheid en werkingsmechanisme van fumaraten als systemische psoriasisbehandeling onbeantwoord. Mede daardoor werden fumaraten niet volledig geaccepteerd als valide behandeloptie voor psoriasis en bleef de toepassing van fumaraten voor lange tijd zeer

gelimiteerd.

In hoofdstuk 2 worden de resultaten beschreven van een systematisch uitgevoerd literatuuronderzoek die beoogde om de effectiviteit en veiligheid van fumaraten in de behandeling van psoriasis in kaart te brengen. Om zoveel mogelijk studies te kunnen includeren werden geen limitaties in studie-design, publicatie-periode of publicatie-taal gehanteerd. In totaal konden 68 artikelen worden geïncludeerd, gepubliceerd in de periode 1987–2015. Zeven van de geïncludeerde artikelen betroffen gerandomiseerde klinische onderzoeken (totaal patiëntenaantal 449). Opvallend was dat de ontwikkeling van fumaraten als psoriasis-behandeling niet verliep volgens de gewoonlijke fases van geneesmiddelenonderzoek. Er waren bijvoorbeeld geen fase 2 of 3 studies verricht die fumaraten evalueerden als psoriasis-behandeling. De 7 beschikbare studies waren relatief klein en vaak incompleet beschreven. De studies bleken daarnaast te heterogeen qua studie-opzet om een resultaten met een meta-analyse te kunnen onderzoeken. Gemiddeld genomen nam de ernst van psoriasis gemeten met de PASI af met 42-65% na 12 tot 16 weken behandeling. Naast de gerandomiseerde studies konden 37 observationele studies worden (totaal patiëntenaantal 3457) opgenomen in het systematisch literatuuronderzoek. Deze studies hadden een hoog risico op bias, maar ondersteunden over het algemeen de resultaten van de gerandomiseerde studies. Frequent gerapporteerde bijwerkingen betroffen gastro-intestinale klachten en flushing. In 6-40% van de patiënten leidden het optreden van bijwerkingen tot voortijdig staken van fumaraten-behandeling. Daarnaast werden enkele gevallen gerapporteerd omtrent het optreden van zeldzame bijwerkingen, waaronder progressieve multifocale leukencefalopathie (PML). Hiaten in de huidige literatuur bleken lange-termijn evaluaties en directe vergelijkende studies met andere systemische psoriasis-behandelingen.

In de praktijk worden fumaraten soms toegevoegd aan etanercept, een 1e generatie anti-TNF alpha biologic, om een verlies van effectiviteit op te vangen, maar bewijskracht voor deze combinatie-behandeling ontbreekt echter. Hoofdstuk 3 beschrijft de bevindingen van een gerandomiseerde exploratieve studie naar de toegevoegde waarde van combinatie-behandeling van fumaraten met etanercept versus etanercept monotherapie. In totaal werden 33 patiënten met psoriasis gerandomiseerd naar of behandeling met etanercept of behandeling met etanercept en fumaraten. Na 24 weken behandeling bleek de proportie die een PASI-75 respons behaalde numeriek groter te zijn in de combinatie-groep vergeleken met de etanercept monotherapie-arm (respectievelijk 78% en 57%), maar het verschil was niet statistisch significant. Qua bijwerkingen was er een hoger frequentie van

milde gastro-intestinale bijwerkingen in de groep behandeld met fumaraten. Op basis van een deze kleine studie is een combinatie-behandeling van etanercept met fumaraten niet direct aan te bevelen voor de dagelijkse praktijk.

Een belangrijk limitatie van fumaraten is het relatief frequent optreden van bijwerkingen, met name in de beginfase van behandeling met fumaraten. Dit kan leiden tot voortijdig staken tot 40% van de patiënten. Strategieën om fumaraat-gerelateerde bijwerkingen te kunnen minimaliseren zijn daarom van klinisch belang. Een deel van de bijwerkingen, met name de gastrointestinale klachten, zou mogelijk histaminerg bepaald zijn, waardoor het zinvol kan zijn om een antihistaminicum toe te voegen aan de fumaraten-behandeling. In hoofdstuk 4 worden de resultaten beschreven van een gerandomiseerde studie naar de potentie van de orale histamine-1 receptor antagonist cetirizine om bijwerkingen van fumaraten te reduceren. In een dubbelblinde, placebo-gecontroleerde studie onder 50 patiënten werd de helft van de studiepopulatie behandeld met fumaraten + cetirizine 10 mg/dag, de andere helft ontving fumaraten + placebo cetirizine. Na 12 weken behandelingen bleek de incidentie van bijwerkingen niet verschillend tussen beide groepen. Ook het percentage dat de fumaraten-behandeling voortijdig diende te staken was niet significant verschillend tussen beide groepen. Toevoeging van cetirizine ter voorkoming of vermindering van bijwerkingen tijdens fumaraten-behandeling lijkt derhalve niet klinisch zinvol.

Hoofdstuk 5 behandelt twee zeldzame, maar klinisch gezien belangrijke bijwerkingen die kunnen optreden tijdens fumaratenbehandeling: progressieve multifocale leuco-encephalopathie (PML) en het Fanconi syndroom. PML is een een zeldzame opportunistische infectie van het centrale zenuwstelsel t.g.v. reactivatie van het John Cunningham (JC) virus. Meerdere immunosuppresieve geneesmiddelen kunnen aanleiding geven tot het optreden van PML, waaronder ciclosporine en methotrexaat. In 2013 verschenen enkele case reports die de ontwikkeling van PML tijdens fumaraten-behandeling beschcreven. Hoofdstuk 5.1 beschrijft een systematische analyse van 8 gevallen van PML die gelinked zijn aan fumaraten-behandeling. Opvallend was dat alle gerapporteerde gevallen gekenmerkt werden door het optreden van een verlaging van het aantal lymfocyten. De diepte van de lymfocytopenie was wel wisselend. Het nadir van het absolute lymfocyten-aantal varieerde van 200 tot 792 per mm3. Gemiddeld bedroeg de blootstelling aan lymfocytopenie 2 jaar (range 1 tot 5 jaar). Tot nu toe zijn geen casus beschreven waarbij er geen sprake was van een lymfocytopenie. Derhalve lijkt nauwlettend controleren van het lymfocyten-aantal van belang

om het risico op PML te minimaliseren. In de huidige richtlijnen wordt aanbevolen om bij een absolute lymfocyten-aantal ≤ 0.5 x 109/L en/of een absolute leukocyten-aantal ≤ 3.0 x 109/L fumaraten-behandeling direct te staken. In geval van een absolute lymfocyten-aantal ≤ 0.7 x 109/L wordt aanbevolen de fumaraten-dosering te halveren en - indien na 4 weken de lymfocytopenie peristeert - de behandeling met fumaraten alsnog te stoppen. Naast monitoring van het lymfocyten-aantal is waakzaamheid op het optreden van neurologische symptomen raadzaam.

In hoofdstuk 5.2 wordt het Fanconi syndroom als bijwerking van fumaraten beschreven. Het Fanconi syndroom wordt gekenmerkt door schade aan de proximale niertubuli, waardoor een toegenomen verlies van aminozuren, glucose en fosfaat via de urine kan optreden. Indien persisterend kan het verlies van fostaat leiden tot een hypofosfatemische osteomalacie. Verschillende geneesmiddelen kunnen een Fanconi syndroom veroorzaken, waaronder fumaraten. Het Fanconi syndroom lijkt echter een zeldzame bijwerking en er heerst mede daardoor onbekendheid van dit ziektebeeld onder dermatologen. Dit gaf aanleiding om twee nieuwe casus te beschreven van een fumaraten-gerelateerde Fanconi syndroom. Daarnaast werd de literatuur systematisch onderzocht voor eerder gerapporteerde Fanconi syndroom gevallen. In totaal werden 9 casus geïdentificeerd. De klinische gegevens van alle 11 casus werden geanalyseerd om risicofacturen voor het optreden van Fanconi syndroom in kaart te brengen. Uit de analyse bleek dat alle 11 patiënten vrouwen waren van middelbare leeftijd, die relatief lang behandeld waren geweest met fumaraten. Met name in deze patiëntenpopulatie lijkt klinische alertheid op het optreden van Fanconi syndroom en monitoring van proteinurie aanbevolen. Daarnaast kan het meten van de albumine - totale eiwit ratio in de urine van additionele screenende waarde zijn.

Een belangrijke limitatie van fumaraten is dat de exacte werkingsmechanisme waarmee fumaraten tot psoriasis-verbetering leiden nog onopgehelderd zijn. In hoofdstuk 6 beschrijven wij de eerste studie die onderzocht in hoe verre fumaraten veranderingen op genexpressie-niveau in de huid induceren. In een groep van 9 psoriasis-patiënten die behandeld werden met fumaraat-behandeling werden lesionale huidbiopten afgenomen voor start en na 12 weken behandeling. Met genexpressieprofiling werd vervolgens onderzocht welke genen en pathways mogelijk worden beïnvloed door fumaraten. De resultaten toonden dat fumaraten onder andere de interleukine-17 pathway beïnvloeden, welke bekend staat als een belangrijk therapeutisch aangrijpingspunt voor psoriasis. De resultaten lieten daarnaast zien dat fumaraten twee andere pathways kunnen beïnvloeden: de glutathione en de Nrf2 pathway. Of deze betrokken pathways essentieel zijn voor de werking

van fumaraten bij psoriasis moet verder onderzoek uitwijzen.

Abnormale endosomale TLR-activatie speelt een belangrijk initiërende rol in de pathogenese van psoriasis. Preklinische data suggereren dat blokkade van deze TLR-receptoren een potentieel nieuw geneesmiddel-target voor psoriasis is. Een nieuwe oligonucleotide-antagonist van TLRs 7, 8 en 9 (IMO-8400) werd positief geevalueerd in enkele experimentele studies en een fase 1 trial onder gezonde vrijwilligers. Op basis van deze positieve resultaten werd een eerste klinische evaluatie in psoriasis patiënten uitgevoerd. In hoofdstuk 7 worden de resultaten beschreven van een fase 2a trial met IMO-8400 in patiënten met chronische plaque psoriasis. In deze studie werden 46 patiënten gerandomiseerd naar een van 4 doseringen IMO-8400 of placebo. Studiemedicatie werd 1 keer per week toegediend met subcutane injecties. De behandelduur was in totaal 12 weken, gevolgd door een follow-up periode van 7 weken. Tijdens de gehele studie-periode traden geen ernstige bijwerkingen op en alle doseringen van IMO-8400 bleken goed verdraagzaam. Overal gezien bracht IMO-8400 behandeling verbetering, maar een duidelijk dosis-respons relatie kon niet worden aangetoond. Bovendien waren er geen statistisch significante verschillen met placebo ($P = 0.26$). Van de 29 patiënten behandeld met IM0-8400 behaalde 11 (38%) een PASI-50 respons, vergeleken met 1 (11%) van 9 patiënten met placebo. Vijf (17%) behaalde een PASI-75 respons t.o.v. geen van de deelnemers in de placebo-groep. Concluderend toonde deze fase 2 studie aan dat TLR-antagonisme met IMO-8400 klinisch leidt tot psoriasis-verbetering in ten minste een subgroep van patiënten met plaque psoriasis. Verdere studies naar onder andere doseringen e.d. zijn noodzakelijk om de waarde van TLR-antagonisten in de behandeling van psoriasis nader te onderzoeken.

P.S. Abraham

"Most of us, it must be admitted, still treat many diseases absolutely empirically, and we often cannot well do anything else. [...]

Our empiricism may be in one sense unscientific, but it is obvious that a rational treatment founded upon pathology and etiology can only be possible when the facts connected with these latter are quite certain."

An address on psoriasis and its treatment. *Br Med J.* 1906 Apr 14;1(2363):842-4.

Aulus Cornelius Celsus

"Alterum genus peius est, simile papulae fere, sed asperius rubicundiusque; figuras varias habet; squamulae ex summa cute discedunt, rosio maior est; celerius et latius procedit certioribusque etiamnum quam prior temporibus et fit et desinit: rubrica cognominatur."

De Medicina, Liber V. From Loeb Classical Library (1935), Harvard University Press

| PART VIII |
APPENDICES

Eugene M. Farber & Richard P. McClintock Jr.

"The search for a 'magic bullet' that will resolve lesions of psoriasis without disturbing the normal metabolic functions is far from over."

A current review on psoriasis. *Calif Med.* 1968 Jun;108(6):440-57.

CHAPTER| 10

Bibliography
PhD Portfolio
Acknowledgements
Curriculum vitae

BIBLIOGRAPHY

1. **Balak DMW**. First-line systemic treatment of psoriasis: staying conventional or going biologic? Br J Dermatol. 2017 Oct;177(4):897-898.
2. **Balak DMW**, Hajdarbegovic E. Drug-induced psoriasis: clinical perspectives. Psoriasis (Auckl). 2017 Dec 7;7:87-94.
3. **Balak DMW**. De huid in auto-inflammatie. Update Immunologie, editie auto-inflammatie. 2017;1(1):1-7
4. **Balak DMW**, Hajdarbegovic E, Bramer WM, Neumann HAM, Thio HB. Progressive multifocal leukoencephalopathy associated with fumaric acid esters treatment in psoriasis patients. J Eur Acad Dermatol Venereol. 2017 Sep;31(9):1475-1482.
5. Hajdarbegovic E, **Balak DMW**. Anti-Interleukin-31 Receptor A Antibody for Atopic Dermatitis. N Engl J Med. 2017 May 25;376(21):2092.
6. **Balak DMW**. Dimethyl fumarate finally coming of age. Br J Dermatol. 2017 Mar;176(3):563-564.
7. **Balak DMW**, Zonnenberg BA, Spitzer-Naaijkens JMJ, Hulshof MM. A 28-year old male patient with nail tumors, skin lesions, and epilepsy. Case Rep Dermatol. 2017 Jan 26;9(1):12-19.
8. **Balak DMW**, van Doorn MB, Arbeit RD, Rijneveld R, Klaassen E, Sullivan T, Brevard J, Thio HB, Prens EP, Burggraaf J, Rissmann R. IMO-8400, a toll-like receptor 7, 8, and 9 antagonist, demonstrates clinical activity in a phase 2a, randomized, placebo-controlled trial in patients with moderate-to-severe plaque psoriasis. Clin Immunol. 2017 Jan;174:63-72.
9. Hajdarbegovic E, **Balak DMW**. Percutaneous ligature of the superficial temporal artery: A simple technique to reduce bleeding. J Am Acad Dermatol. 2017 Jan;76(1):e15.
10. **Balak DMW**, Fallah Arani S, Hajdarbegovic E, Hagemans CA, Bramer WM, Thio HB, Neumann HA. 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 Aug;175(2):250-62.
11. **Balak DMW**, Bouwes Bavinck JN, de Vries AP, Hartman J, Neumann HA, Zietse R, Thio HB. Drug-induced Fanconi syndrome associated with fumaric acid esters treatment for psoriasis: a case series. Clin Kidney J. 2016 Feb;9(1):82-9.

12. **Balak DMW**, Hajdarbegovic E. More on PML in Patients Treated with Dimethyl Fumarate. Author reply. N Engl J Med. 2016 Jan 21;374(3):295.
13. van Bezooijen JS, **Balak DMW**, van Doorn MB, Looman CW, Schreurs MW, Koch BC, van Gelder T, Prens EP. Combination Therapy of Etanercept and Fumarates versus Etanercept Monotherapy in Psoriasis: A Randomized Exploratory Study. Dermatology. 2016;232(4):407-14.
14. Lijnen R, Otters E, **Balak DMW**, Thio B. Long-term safety and effectiveness of high-dose dimethylfumarate in the treatment of moderate to severe psoriasis: a prospective single-blinded follow-up study. J Dermatolog Treat. 2016;27(1):31-6.
15. **Balak DMW**, Hajdarbegovic E. Science, spam, and scam. BMJ Blogs, 27 jan 2016.
16. Hajdarbegovic E, Bloem A, **Balak DMW**, Thio B, Nijsten T. The Association between Atopic Disorders and Keloids: A Case-control Study. Indian J Dermatol. 2015 Nov-Dec;60(6):635
17. **Balak DMW**, Hajdarbegovic E. PML in Patients Treated with Dimethyl Fumarate. N Engl J Med. 2015 Aug 6;373(6):582-3.
18. Deckers IE, van der Zee HH, **Balak DMW**, Prens EP. Fumarates, a new treatment option for therapy-resistant hidradenitis suppurativa: a prospective open-label pilot study. Br J Dermatol. 2015 Mar;172(3):828-9
19. **Balak DMW**, Fallah-Arani S, Venema CM, Neumann HA, Thio HB. Addition of an oral histamine antagonist to reduce adverse events associated with fumaric acid esters in the treatment of psoriasis: a randomized double-blind placebo-controlled trial. Br J Dermatol. 2015 Mar;172(3):754-9.
20. **Balak DMW**. Fumaric acid esters in the management of psoriasis. Psoriasis (Auckl). 2015 Jan 5;5:9-23
21. **Balak DMW**, Hajdarbegovic E. Towards harmonisation of referencing styles. BMJ Blogs, 29 July 2015.
22. Onderdijk AJ, **Balak DMW**, Baerveldt EM, Florencia EF, Kant M, Laman JD, van IJcken WF, Racz E, de Ridder D, Thio HB, Prens EP. Regulated genes in psoriatic skin during treatment with fumaric acid esters. Br J Dermatol. 2014 Oct;171(4):732-41.
23. Fallah Arani S, **Balak DMW**, Neumann HA, Kuipers MV, Thio HB. Treatment of psoriasis with non-registered fumaric acid esters in The Netherlands: a nationwide survey among Dutch dermatologists. J Eur Acad Dermatol Venereol. 2014 Jul;28(7):972-5.

24. Maška M, Ulman V, Svoboda D, Matula P, Matula P, Ederra C, Urbiola A, España T, Venkatesan S, **Balak DMW**, et al. A benchmark for comparison of cell tracking algorithms. Bioinformatics. 2014 Jun 1;30(11):1609-17.
25. **Balak DMW**, Oostveen AM, Bousema MT, Venema AW, Arnold WP, Seyger MM, Thio HB. Effectiveness and safety of fumaric acid esters in children with psoriasis: a retrospective analysis of 14 patients from The Netherlands. Br J Dermatol. 2013 Jun;168(6):1343-7
26. **Balak DMW**, Hengstman GJ, Hajdarbegovic E, van den Brule RJ, Hupperts RM, Thio HB. Prevalence of cutaneous adverse events associated with long-term disease-modifying therapy and their impact on health-related quality of life in patients with multiple sclerosis: a cross-sectional study. BMC Neurol. 2013 Oct 16;13:146
27. Sjouke B, **Balak DMW**, Beuers U, Ratziu V, Stroes ES. Is mipomersen ready for clinical implementation? A transatlantic dilemma. Curr Opin Lipidol. 2013 Aug;24(4):301-6
28. Hajdarbegovic E, **Balak DMW**. Images in clinical medicine. Metastatic inguinal lymphadenopathy. N Engl J Med. 2012 Apr 19;366(16):1526
29. Thio HB, **Balak DMW**, Meilof JF, Stegeman CA, Voskuyl AE; 'Consensus diagnostic small-vessel vasculitis' Workgroup. [Guideline 'Diagnostics of small-vessel vasculitis']. Ned Tijdschr Geneeskd. 2012;156(21):A4317
30. Hajdarbegovic E, Verkouteren J, **Balak DMW**. Non-melanoma skin cancer: the hygiene hypothesis. Med Hypotheses. 2012 Dec;79(6):872-4
31. **Balak DMW**, Thio HB. Jeuk bij psoriasis en wat daaraan te doen valt. Bijblijven. Nr 1. 2012:33-40
32. **Balak DMW**, Hengstman GJ, Çakmak A, Thio HB. Cutaneous adverse events associated with disease-modifying treatment in multiple sclerosis: a systematic review. Mult Scler. 2012 Dec;18(12):1705-17
33. Steeman ASM, **Balak DMW**, Seyger MMB, Thio HB, Bousema MT. Fumaraten bij een meisje van 15 jaar met psoriasis. Nederlands Tijdschrift voor Dermatologie en Venereologie. 2012. 22 (5): 308-310.
34. **Balak DMW**, Gouw SC, Plug I, Mauser-Bunschoten EP, Vriends AH, Van Diemen-Homan JE, Rosendaal FR, van der Bom JG. Prenatal diagnosis for haemophilia: a nationwide survey among female carriers in the Netherlands. Haemophilia. 2012 Jul;18(4):584-92.

35. **Balak DMW**, Thio HB. Treatment of lupus erythematosus with fumaric acid ester derivatives: two case-reports. J Transl Med. 2011 Nov 23;9 Suppl 2:P15.
36. Bezerra MA, Araujo AS, Phylipsen M, **Balak DMW**, Kimura EM, Oliveira DM, Costa FF, Sonati MF, Harteveld CL. The deletion of SOX8 is not associated with ATR-16 in an HbH family from Brazil. Br J Haematol. 2008 Jun;142(2):324-6.
37. Harteveld CL, Kriek M, Bijlsma EK, Erjavec Z, **Balak DMW**, Phylipsen M, Voskamp A, di Capua E, White SJ, Giordano PC. Refinement of the genetic cause of ATR-16. Hum Genet. 2007 Nov;122(3-4):283-92.

PhD PORTFOLIO

PhD student:	D.M.W. Balak
Erasmus MC Department:	Dermatology
Research school:	MolMed
PhD period:	December 2011 - December 2017
Promotor:	Prof.dr. H.A.M. Neumann
Co-promotor:	Dr. H.B. Thio

Activity	Year	Work-load (ECTS)
1. PhD training		
General and specific courses		
BROK cursus, Erasmus MC	2011	1.0
Boerhaave cursus safely working in the laboratory, LUMC	2011	10.0
Erasmus summer programme Introduction to epidemiology, EUR	2011	10.0
Microscopic Image Analysis: From Theory to Practice	2012	10.0
MolMed course on Indesign for PhD students	2013	10.0
MolMed course on Photoshop and Illustra tor for PhD students	2013	10.0
Boerhaave cursus klinisch onderwijs, LUMC	2015	10.0
Teach the Teacher II, Erasmus MC	2015	10.0
Update BROK, Erasmus MC	2016	1.0
Euroderm excellence training programme for residents, Nice, France	2017	10.0
ESDR Future Leaders in Dermatology	2017	10.0
Seminars and workshops		
EWIMID 2011, Lyon, France	2011	1.0
ESDR 2012, Venicy, Italy	2012	1.0
IID 2013, Edinburgh, Scotland	2013	1.0
EADV 2014, Amsterdam	2014	1.0
AAD 2016, Washington, USA	2016	1.0
Congress of the Psoriasis international Network, Paris, France	2016	1.0
EADV 2017, Geneva, Switzerland	2017	1.0

Presentations

DerMiS study results, Zwolle	2011	
Promoveren op fumaraten, PhD weekend, Noorbeek	2012	1.0
Experiences in treating psoriasis with bio logics, YoungJnJ event, Leiden	2013	1.0
Tolerability of injectable therapies, 6th EU Round table on FH, Berlin,	2013	1.0
Klaar met de trial, en dan? PhD weekend dermatology, Maastricht	2014	1.0
Resultaten IMO-8400 trial, Skintermezzo op reis, Leiden	2014	1.0
Emerging TLR-antagonists: proof-of-con cept in psoriasis patients, Leiden Pharma Science Symposium, Leiden	2014	1.0
Een nieuw therapeutisch target voor psoriasis? Skintermezzo, Rotterdam	2015	1.0
Treatment of psoriasis with biologics, Customer connection event, Leiden	2015	1.0
AAD 2015, IMO-8400 phase 2 trial in psoriasis, San Francisco, USA	2015	1.0
Domburg dagen 2016, Fumaraten in de praktijk, Domburg	2016	1.0
Clinical case presentation, Psoriasis International Forum, Kiel, Germany	2016	1.0
Oude en nieuwe orale behandelingen voor psoriasis, Brugge dagen 2017, Utrecht	2017	1.0
2. Teaching		1.0
Supervising Master's Thesis	2011-2013	1.0
Teaching to medical interns (eczema, acne, STD) Erasmus MC	2011-2017	1.0
Clinical drug development, biopharmaceutical sciences, Leiden University	2017-2018	1.0
3. Other		1.0
Organizing committee first Dermatology PhD weekend, Noorbeek	2011-2012	1.0
Member of committee Dutch guideline psoriasis update 2017, NVDV, Utrecht	2015-2017	10.0
Regular peer reviewer for medical journals, including British Journal of Dermatology and Journal of the European Academy of Dermatology and Venereology	2013-2017	1.0

ACKNOWLEDGEMENTS

I would like to express my gratitude to all of those who – directly or indirectly – have contributed and helped to the studies leading to this thesis. My deepest thanks to all my mentors who have guided me on the road becoming a scientist and a dermatologist. It is also with the support of many that I am able to stand here today. A very special word of gratitude to my promotor professor Martino Neumann, who from the very first beginning believed in me and gave me his trust to become a dermatologist, and to Bing Thio, for his help in introducing and supporting me in the field of dermatology.

CURRICULUM VITAE

Deepak Balak was born on July 3rd 1985 in Leiderdorp, The Netherlands. After completing pre-university secondary education (Gymnasium) at the Rijnlands Lyceum in Oegstgeest, Deepak started his medical training in September 2003 at Leiden University. Early on, Deepak did an elective research project at The Hemoglobinopathies Laboratory (Department of Human and Clinical Genetics, LUMC, Leiden) to characterize genomic deletions causing thalassemia (Supervisors Dr. Piero Giordano & Dr. Cornelis Harteveld). Captured by these early steps in science, Deepak deciced to start a second study in Biomedical Sciences. Additionally, he worked as a student-assistant in histology classes and participated in two Honours Classess organized by Leiden University: 'Health, science & technology' (2007) and 'For God's sake? Biomedicine, law and religion' (2010). Having obtained his Medical Degree in January 2010 and a Master of Science Degree in Biomedical Sciences in Oktober 2010, Deepak left Leiden for Rotterdam to start as a research physician at the Department of Dermatology of the Erasmus MC (Supervisor Dr. Bing Thio). At Erasmus MC, Deepak conducted clinical trials on psoriasis and other inflammatory skin diseases, and he commensed with the studies leading to this PhD thesis under supervision of Prof.dr. Martino Neumann and Dr. Bing Thio. In January 2013, Deepak began his residency in Dermatology at Erasmus MC (residency program directors Dr. Bing Thio and Mr.dr. Ellen de Haas) while continuing his research activities. At the Centre for Human Drug Research (CHDR) in Leiden, Deepak was involved in a novel phase 2 psoriasis trial (supervisors Dr. Martijn van Doorn & Dr. Robert Rissmann). In 2015-2016 Deepak spent a year of dermatology training at Reinier de Graaf Gasthuis in Delft/Voorburg (residency program directors Dr. Mieke Hulshof and Dr. Mirjam Brakman). At that time, he joined the Dutch guideline committee on psoriasis treatment. In his last year of residency, Deepak was selected to partipate in the Euroderm Excellence training program for European dermatology residents and the European Society for Dermatological Research Academy for Future Leaders in Dermatology. Furthermore, he received a ZonMw scholarship to attent the clinical development course of the Paul Janssen Futurelab in Leiden. Since november 2017 Deepak works as a dermatologist at the Department of Dermatology and Allergology of University Medical Center Utrecht (Department head Dr. Vigfús Sigurdsson), where his main clinical and research interests include translational research and clinical drug development in the field of inflammatory skin diseases.

ॐ

भूर्भुवःस्वः

तत्सवितुर्वरेण्यं

भर्गो देवस्य धीमहि

धियो यो नः प्रचोदयात्

All
Earth Intermedium Space
That light I utilize
On that extremely pure energy I concentrate
To lead my attentions towards bright clear rightnesses

Rigveda 3.62.10

ADVANCING PSORIASIS TREATMENT

Psoriasis is a common immune-mediated inflammatory skin disease that can elicit a significant high burden of disease on patients, theirs families, and society. In the last two decades significant advances have been made in the treatment of psoriasis, but psoriasis remains a challenging and difficult-to-treat disease. In this thesis, the focus was set on advancing psoriasis treatment by undertaking a clinical drug evaluation approach of two different systemic psoriasis treatments. The first studied therapy is fumaric acid esters, which are an established classical systemic treatment for psoriasis, but important questions regarding their efficacy and mechanisms of action remain unanswered. The second drug assessed is a novel oligonucleotide-based antagonist of toll-like receptor (TLR) 7, 8 and 9, which is a potential targeted biologic treatment for psoriasis.