ORIGINAL ARTICLE

Fumaric acid ester-induced T-cell lymphopenia in the real-life treatment of psoriasis

H. Dickel,[1,*] T. Bruckner,[2] S. Höxtermann,[1] B. Dickel,[3] E. Trinder,[1] P. Altmeyer[1,4]

[1]Department of Dermatology, Venereology and Allergology, Ruhr University Bochum, Bochum, Germany
[2]Institute of Medical Biometry and Informatics (IMBI), University Hospital Heidelberg, Heidelberg, Germany
[3]Dermatology Practice Peter Wenzel, MD, Hattingen, Germany
[4]Dermatology Practice at City Park, Bochum, Germany
*Correspondence: H. Dickel. E-mails: heinrich.dickel@rub.de and h.dickel@klinikum-bochum.de

Abstract

Background Fumaric acid esters (FAEs) are used to treat psoriasis and are known to cause lymphopenia in roughly 60% of the patients. Much remains to be elucidated about the biological effects of FAEs on lymphocytes.

Objective To evaluate the influence of long-term FAE (Fumaderm®) treatment on peripheral blood $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells and $CD56^+$ natural killer (NK) cells in psoriasis.

Methods In this single-centre retrospective observational subcohort study, we obtained leucocyte and lymphocyte subset counts before initiating FAE therapy in 371 psoriasis patients (mean age, 47.8 years; 63.3% males) and monitored them during treatment (mean treatment duration, 2.9 years). Multiparametric flow cytometry was used for immunophenotyping.

Results FAEs significantly reduced the numbers of $CD4^+$ T, $CD8^+$ T, $CD19^+$ B and $CD56^+$ NK cells. Among lymphocyte subsets, the mean percentage reduction from baseline was always highest for $CD8^+$ T cells, with a peak of 55.7% after 2 years of therapy. The risk of T-cell lymphopenia increased significantly with the age of the psoriasis patients at the time that FAE therapy was initiated. It was significantly decreased for the combination therapy with methotrexate and folic acid (vitamin B9) supplementation. Supporting evidence was found suggesting that T-cell lymphopenia enhances the effectiveness of FAE therapy.

Conclusions Monitoring distinct T-cell subsets rather than just absolute lymphocyte counts may provide more meaningful insights into both the FAE treatment safety and efficacy. We therefore suggest optimizing pharmacovigilance by additionally monitoring $CD4^+$ and $CD8^+$ T-cell counts at regular intervals, especially in patients of middle to older age. Thus, further prospective studies are needed to establish evidence-based recommendations to guide dermatologists in the management of psoriasis patients who are taking FAEs and who develop low absolute T-cell counts.

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Conflicts of interest

H. Dickel has received travel grants for lecturing activities from Biogen GmbH. P. Altmeyer has received grants and/or honoraria as a consultant and/or speaker from Biogen GmbH. T. Bruckner, S. Höxtermann, B. Dickel and E. Trinder declare no conflicts of interest.

Introduction

Fumaric acid esters (FAEs) have been used in systemic psoriasis treatment since 1959.[1] Some retrospective observational studies have shown that FAEs are safe and beneficial for long-term clinical use.[2–5] According to the European[6] and the recently updated German[7] evidence- and consensus-based guidelines, FAEs are recommended for the induction and long-term treatment of adult patients with moderate-to-severe psoriasis. The reference product Fumaderm®, which is a defined mixture of dimethyl fumarate (DMF) and three salts of monoethyl fumarate,[8] received marketing approval in Germany in 1994.[9] As a new DMF-only drug with a European registration for moderate-to-severe psoriasis, Skilarence® became available in 2017.[10,11]

Psoriasis is a multi-factorial autoimmune disease that involves the activation of many pathways driven by numerous cells.[12,13] Among these, T lymphocytes ($CD3^+$) control and orchestrate inflammation. Peripheral blood $CD3^+$ T cells are thought to be activated and subsequently recruited from circulation during the

development of psoriatic lesions.[14] Thus, in psoriatic plaques, T helper lymphocytes (CD4^{+}) are found predominantly in the upper dermis, and T cytotoxic lymphocytes (CD8^{+}) are predominantly observed in the epidermis. DMF and its metabolite monomethyl fumarate (MMF), which is rapidly formed by DMF hydrolysis, are currently thought to be the pharmacologically active FAEs that are used to treat psoriasis.[15–17] Numerous experimental studies, which have mostly concentrated on the effects of DMF *in vitro*, have described pleiotropic immunomodulating properties that particularly propagate development of an inflammatory T_h1/T_h17 immune response towards an anti-inflammatory T_h2 immune response in psoriasis patients.[17–20]

Under treatment with FAEs, leukopenia development that is mainly attributed to lymphopenia is commonly observed[2–5,21–23] and is of significant clinical importance.[15,16] Our group[24,25] and others[23,26,27] showed that the number of peripheral blood lymphocyte subsets is considerably reduced and inflammatory CD3^{+} T cells disappear from psoriatic lesions,[28] thus limiting the harmful effect mediated by these cells in the skin. However, the *in vivo* mechanism remains to be elucidated.[15,16]

Recently, several cases of progressive multifocal leukoencephalopathy (PML) in lymphopenic FAE-treated patients have raised concerns about drug safety.[29,30] This rare, but life-threatening opportunistic infection, which is caused by reactivation of the John Cunningham virus, seems to be related particularly to FAE-induced CD4^{+} and CD8^{+} T-cell lymphopenia. Therefore, in compliance with the newly adapted drug safety requirements of the European Medicines Agency (EMA),[31] the manufacturer of Fumaderm[®32] currently recommends monitoring of the blood count every 4 weeks and immediate discontinuation of the treatment if the absolute lymphocyte count (ALC) drops below 500/µL. If the ALC drops below 700/µL, the dose should be halved; if the ALC remains below this value during a follow-up check after 4 weeks, then treatment should be discontinued. However, regular monitoring of the lymphocyte subset count is not essential.[7,32]

The present substudy of our recently published single-centre retrospective observational study[2] aimed to evaluate the biological effects of FAEs on peripheral blood lymphocyte subsets (CD4^{+} T cells, CD8^{+} T cells, B lymphocytes (CD19^{+}) and natural killer (NK) cells (CD56^{+})) in a large subcohort of psoriasis patients during continuous long-term therapy of up to 11.7 years. Reliable immunological data from long-term clinical use of FAEs in psoriasis are scarce[27] and will provide a better understanding of FAE-based therapy management.

Methods

Study design

This investigator-initiated subcohort study is based on continuously recorded clinical and laboratory findings on psoriasis patients followed at the Department of Dermatology, Venereology and Allergology of the Ruhr University Bochum in Germany who were prescribed Fumaderm[®] between January 1996 and October 2012. The single-centre retrospective observational study's goals and methods have been previously described in detail.[2]

Study population

There were 371 psoriasis patients enrolled into the substudy, and we quantitatively analysed peripheral blood lymphocyte subsets data using flow cytometry before and during FAE treatment.

Briefly, FAEs were initiated using a gradually increasing dosing regimen.[8] Maintenance doses were individually adjusted based on therapeutic response and tolerability. FAEs were commonly administered as monotherapy (267 patients; mean average daily DMF dose, 345.8 mg ± 167.0 mg) or, less frequently, in combination with methotrexate (MTX; 53 patients; mean average daily DMF dose, 416.8 mg ± 196.2 mg) or phototherapy (51 patients; UVB, PUVA or UVA; mean average daily DMF dose, 362.4 mg ± 151.9 mg). MTX (dose range, 5–20 mg/week) was administered orally with folic acid (vitamin B9) supplementation 24 h afterwards at an equivalent dose.[33]

All patients typically attended our outpatient department monthly for clinical assessment and quarterly for complete blood count tests. As a non-routine part of our follow-up examinations, flow cytometric analyses were performed at baseline (371 patients) and, whenever possible, 3 ± 1 months (172 patients), 6 ± 1 months (148 patients), 12 ± 1 months (218 patients), 24 ± 1 months (203 patients) and 36 ± 1 months (119 patients) after initiating FAE treatment and at irregular intervals thereafter (77 patients).

Flow cytometry

Peripheral blood leucocyte counts were routinely determined using an automated particle counter. Flow cytometric methods were used to identify and determine the peripheral blood lymphocyte subset percentages and absolute counts. The following antibodies (BD Biosciences, San Jose, CA, USA), directed against mature human lymphocyte subsets, were used: T lymphocytes (CD3^{+}), helper T lymphocytes (CD3^{+}CD4^{+}), cytotoxic T lymphocytes (CD3^{+}CD8^{+}), B lymphocytes (CD19^{+}) and NK cells (CD3^{−}CD16^{+}CD56^{+}). The antibodies were conjugated to the following fluorescent dyes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein cyanine 5.5 (PerCP-Cy5.5), phycoerythrin-cyanine 7 (PE-Cy7), allophycocyanin (APC) and allophycocyanin-cyanine 7 (APC-Cy7). Additionally, Trucount™ tubes (BD Biosciences, San Jose, CA, USA) were used to determine the ALCs. A digital FACSCanto II™ flow cytometer (BD Biosciences, San Jose, CA, USA) equipped with three excitation lasers (635 nm (red), 488 nm (blue) and 405 nm (violet)), computer hardware, and FACSDiva™ and FACSCanto™ software was used to acquire and analyse the lymphocyte subsets in one tube only using six different fluorescent antibodies. The reference values for the lymphocyte subsets are presented in Table 5.

Flow cytometry was introduced in our laboratory in December 1995.[24] At that time, we used a FACScan™ flow cytometer equipped with a single blue laser. For standard immunophenotyping, six different tubes with different antibody combinations were required. Later, we worked with a FACSCalibur™ flow cytometer equipped with two lasers (red and blue). Standard immunophenotyping was performed with antibody combinations in two separate tubes. Generally, the basic immunophenotyping quality has not changed much over the last decades, but today the results of multicolour experiments are faster and provide higher grades of information density. Over the 17-year study period, flow cytometry was used in our laboratory in close compliance with the guidelines of the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards).[34–36] All laboratory personnel who performed immunophenotyping were blinded to the patients' clinical status.

Data sources and handling

Information about patient demographics, disease and treatment characteristics, reasons for treatment discontinuation, adverse events (AEs; including serious adverse events (SAEs)) and clinical efficacy (static Physician's Global Assessment (sPGA, distinguishing on a 5-point scale between 'light', 'moderate', 'moderate-to-severe', 'severe' and 'very severe' psoriasis[37]; data available for 371 patients, 100%) and Psoriasis Area and Severity Index (PASI, ranging from 0 to 72 with higher score indicating more severe psoriasis[38]; data available for 309 patients, 83.3%)) were extracted from the digital patient records.

In healthy individuals, the ALC consists of approximately 70% $CD3^+$ T cells, 20% $CD19^+$ and $CD20^+$ B cells, and 10% $CD56^+$ NK cells. $CD3^+$ T cells usually include approximately 70% $CD4^+$ and 30% $CD8^+$ T cells.[39] Grades of cytopenia are assigned according to the Common Terminology Criteria for Adverse Events (CTCAE v5.0; available at: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_5.0, last accessed 30 June 2018). In adults, lymphopenia is divided into grade 1 (<lower limit of normal (LLN)–800/μL), grade 2 (<800–500/μL), grade 3 (<500–200/μL) and grade 4 (<200/μL), with higher grades being associated with a higher risk of infections.[39] $CD4^+$ T-cell lymphopenia is divided into grade 1 (<LLN–500/μL), grade 2 (<500–200/μL), grade 3 (<200–50/μL) and grade 4 (<50/μL). However, the CTCAE do not provide a distinction between the $CD8^+$ T-cell lymphopenia. However, $CD8^+$ T cells <140/μL have been described with FAE-associated PML and are likely to be relevant.[29,30] We therefore stratified the patients for $CD4^+$ T cells <200/μL (comprising CTCAE grade 3 and 4) and/or $CD8^+$ T cells <140/μL developed during FAE therapy in these analyses.

Statistical methods

For descriptive purposes, we report categorical variables as absolute and relative frequencies and continuous variables as the mean, standard deviation (SD), median and range (minimum, maximum). Statistical significance was evaluated using a chi-square test, one-sample t-test or the Kruskal–Wallis test, depending on the underlying distribution of data. A P-value ≤0.05 was considered statistically significant. However, all P-values were purely descriptive and not confirmatory. Missing values were not included in the calculations.

Univariate and multivariate Cox proportional hazards models[40,41] were performed to identify possible determinants for T-cell lymphopenia. Patients were considered to be lymphopenic when, during FAE therapy, the $CD4^+$ T-cell count was first below 200/μL and/or the $CD8^+$ T-cell count first below 140/μL. Clinically significant baseline variables and therapy regimens were included in the analyses. The results were presented as hazard ratios with 95% confidence intervals (CI).

Coprimary effectiveness outcomes were defined as follows: (i) the proportion of patients achieving an sPGA score of 'light' (comprising 'clear', 'almost clear' and 'mild')[42] and at least a two-point reduction in baseline sPGA score; and (ii) the proportion of patients with PASI 75 (considered a less stringent reasonable therapeutic goal)[43] compared with baseline. We estimated the proportion of psoriasis patients who reached these defined 'events' of effectiveness in their respective varying observation periods using the Kaplan–Meier method.[44] The corresponding event dates were defined as the date of first occurrence during treatment. Censorship occurred at the last available follow-up date at which the effectiveness event had failed to occur. All Kaplan–Meier failure curves of event by group are depicted with the number of patients 'at risk'.[45,46] 'Patients at risk' were defined as patients receiving treatment at a certain time point who had not yet reached the effectiveness event. We summarized the Kaplan–Meier failure curve results by presenting the median event with a 95% CI.[47] This is the time point at which the cumulative response rate exceeds 50%. We used log-rank tests to descriptively compare the event between groups, taking the whole observation period into account.[48] Because of the retrospective study design, no imputation of missing values was performed.

Data were analysed using SAS® statistical analysis software, version 9.4 (SAS Institute, Cary, NC, USA).

Ethical approval

The Ethics Committee of the Medical Faculty of the Ruhr University Bochum approved the study protocol of the single-centre retrospective observational study (registration no. 4203-12; 6 February 2012).[2]

Results

Patient and treatment characteristics

Baseline demographic characteristics and comorbidities of the subset of 371 psoriasis patients are shown in Table 1. The mean

Table 1 Baseline demographic characteristics of patients with psoriasis, stratified by absolute CD4+ and CD8+ T-cell counts under FAE therapy; total number of patients = 371

	All	CD4+ T cells ≥200/µL and CD8+ T cells ≥140/µL	CD4+ T cells <200/µL or CD8+ T cells <140/µL	CD4+ T cells <200/µL and CD8+ T cells <140/µL	*P*-value
Patients initiating FAE therapy, *N* (%)	371 (100.0)	176 (47.4)	109 (29.4)	86 (23.2)	
Sex					
Male, *n* (%)	235 (63.3)	115 (65.3)	66 (60.6)	54 (62.8)	0.71*
Female, *n* (%)	136 (36.7)	61 (34.7)	43 (39.4)	32 (37.2)	
Age (years)					
Mean ± SD	47.8 ± 14.6	43.7 ± 14.1	49.8 ± 13.9	53.6 ± 14.0	**<0.0001****
Median (range)	47.0 (9.0−90.0)	43.5 (9.0−85.0)	50.0 (21.0−89.0)	53.0 (21.0−90.0)	
BMI (kg/m²), *n* (% of *N*)	99 (26.7)	47 (26.7)	37 (33.9)	15 (17.4)	
Mean ± SD	28.9 ± 7.1	28.8 ± 6.3	29.2 ± 8.9	28.3 ± 5.0	0.90**
Median (range)	27.2 (18.0−60.0)	27.2 (18.6−46.0)	26.6 (18.0−60.0)	28.0 (20.0−43.0)	
Smoking status, *n* (% of *N*)	118 (31.8)	59 (33.5)	39 (35.8)	20 (23.3)	
Positive/current, *n* (%)	69 (58.5)	36 (61.0)	23 (59.0)	10 (50.0)	0.69*
Negative, *n* (%)	49 (41.5)	23 (39.0)	16 (41.0)	10 (50.0)	
Comorbidities					
No comorbidity, *n* (%)	215 (58.0)	110 (62.5)	65 (59.6)	40 (46.5)	0.15*
1–2 Comorbidities, *n* (%)	25 (6.7)	9 (5.1)	8 (7.4)	8 (9.3)	
≥3 Comorbidities, *n* (%)	131 (35.3)	57 (32.4)	36 (33.0)	38 (44.2)	

*P-value derived from chi-square test; **P-value derived from Kruskal–Wallis test; bold, P ≤ 0.05.
BMI, body mass index; FAE, fumaric acid ester; *n*, number of patients with available data; SD, standard deviation.
Comorbidity includes any of the following: hypertension, type 2 diabetes mellitus, hyperlipoproteinemia, chronic liver disease, degenerative joint disease, alcohol/drug abuse, mental illness, coronary heart disease, other pulmonary disease, other chronic gastrointestinal disease, malignant neoplasm, chronic kidney disease, heart failure, chronic obstructive pulmonary disease, peptic ulcer disease, cerebrovascular disease, chronic viral infection, osteoporosis and other disease.

patient age was 47.8 years (range, 9–90 years), and 235 (63.3%) of the patients were male. In the subgroups with decreased CD4+ and/or CD8+ T-cell counts below 200 and 140/µL, respectively, patients were more likely to be older when initiating FAE therapy. Table 2 shows baseline disease and treatment characteristics of the total subcohort. In the subgroups with decreased CD4+ and/or CD8+ T-cell counts below 200 and 140/µL, respectively, patients spent a longer time under continuous FAE therapy. However, many values for body mass index, smoking status and PASI were missing.

A non-significantly greater proportion of patients prematurely discontinued FAE therapy in the 'CD4+ T cells <200/µL and CD8+ T cells <140/µL' subgroup compared with other groups (Table 3). Premature discontinuation occurred most often as a result of the T-lymphocyte alterations. However, the proportions of patients who discontinued FAE therapy because of clinical remission and lack of efficacy were lowest and highest, respectively, in the 'CD4+ T cells ≥200/µL and CD8+ T cells ≥140/µL' subgroup.

Table 4 provides the frequencies of abnormal leucocyte and lymphocyte subset counts of different thresholds during FAE

therapy. In the subcohort, 60.9% of the psoriasis patients developed lymphopenia. Severe lymphopenia (CTCAE grade 3 or 4) occurred in 14.3% of the patients. Changes in leucocyte and lymphocyte subset counts measured within a 3-year period to monitor the safety of the FAE therapy are displayed in Table 5. The mean lymphocyte count decreased significantly by 23.9% within the first 3 months of treatment. Over the course of treatment, mean lymphocyte counts further decreased significantly by about 40% of baseline values. The reduction in the mean leucocyte count was comparable but less pronounced, with a total reduction reaching 20% over the course of therapy. Among subsets, the mean CD4+ T-cell count declined significantly by 49.9%, which was similar to the nadir after 2 years of therapy, whereas the mean CD8+ T-cell count declined significantly by 55.7%. Over the course of therapy, the reduction in the mean CD8+ T-cell count was generally more pronounced compared with the reduction in the mean CD4+ T-cell count, which was also reflected by a significantly increased mean CD4/CD8 ratio, with a maximum mean percentage change of 49.5% from the baseline. Reaching the lowest mean values after 1 year of therapy, CD19+ B-cell and CD56+ NK cell counts were significantly

Table 2 Disease and treatment characteristics of patients with psoriasis, stratified by absolute CD4$^+$ and CD8$^+$ T-cell counts under FAE therapy

	All	CD4$^+$ T cells ≥200/μL and CD8$^+$ T cells ≥140/μL	CD4$^+$ T cells <200/μL or CD8$^+$ T cells <140/μL	CD4$^+$ T cells <200/μL and CD8$^+$ T cells <140/μL	P-value
Patients initiating FAE therapy, N	371	176	109	86	
sPGA score at baseline					
Mean ± SD	3.7 ± 0.8	3.7 ± 0.8	3.7 ± 0.9	3.7 ± 0.8	0.72**
Median (range)	4 (1−5)	4 (2−5)	4 (1−5)	4 (2−5)	
PASI score at baseline, n (% of N)	309 (83.3)	146 (83.0)	88 (80.7)	75 (87.2)	
Mean ± SD	22.3 ± 8.1	22.9 ± 7.9	22.1 ± 9.3	21.3 ± 6.8	0.35**
Median (range)	22.4 (2.4−43.2)	22.8 (2.4−43.2)	23.4 (2.4−43.2)	21.6 (3.0−35.2)	
Prior topical and/or phototherapy					
Topical therapy, n (%)	177 (47.7)	88 (50.0)	46 (42.2)	43 (50.0)	0.54*
Topical therapy + phototherapy, n (%)	193 (52.0)	87 (49.4)	63 (57.8)	43 (50.0)	
Phototherapy, n (%)	1 (0.3)	1 (0.6)	0	0	
Prior systemic therapy					
No systemic therapy, n (%)	327 (88.1)	152 (86.4)	101 (92.7)	74 (86.0)	0.22*
Systemic therapy, n (%)	44 (11.9)	24 (13.6)	8 (7.3)	12 (14.0)	
Time between initial diagnosis and FAE therapy initiation [years]					
Mean ± SD	13.8 ± 12.9	12.2 ± 11.5	14.7 ± 13.8	15.6 ± 14.2	0.18**
Median (range)	10.1 (0−69.9)	8.3 (0−63.0)	10.1 (0−60.0)	10.2 (0−69.9)	
Years on continuous therapy with FAEs					
Mean ± SD	2.9 ± 2.7	2.5 ± 2.7	3.5 ± 3.0	2.9 ± 2.4	**0.0006****
Median (range)	1.8 (0.1−11.7)	1.4 (0.1−11.4)	2.5 (0.2−11.7)	2.1 (0.4−11.0)	
Therapy regimen					
FAE monotherapy, n (%)	267 (72.0)	127 (72.2)	76 (69.7)	64 (74.4)	0.81*
FAEs + phototherapy, n (%)	51 (13.7)	23 (13.0)	15 (13.8)	13 (15.1)	
FAEs + MTX, n (%)	53 (14.3)	26 (14.8)	18 (16.5)	9 (10.5)	

*P-value derived from chi-square test; **P-value derived from Kruskal–Wallis test; bold, P ≤ 0.05.
FAE, fumaric acid ester; MTX, methotrexate; n, number of patients with available data; PASI, Psoriasis Area and Severity Index; phototherapy, ultraviolet A, ultraviolet B or psoralen + ultraviolet A; SD, standard deviation; sPGA, static Physician's Global Assessment.
Systemic therapy includes any of MTX, acitretin, biological (alefacept, efalizumab, etanercept and infliximab), cyclosporine and glucocorticoid.

reduced by 27.7% and 21.9%, respectively, compared with mean baseline values.

Determinants for FAE-induced T-cell lymphopenia

Table 6 shows the results from the Cox regression analyses. Based on univariate analysis, the statistically significant factors possibly associated with T-cell lymphopenia, as defined, were older age ($P < 0.0001$; age group 40–55 years, $P = 0.012$; age group >55 years, $P < 0.0001$) and a lower absolute leucocyte ($P = 0.05$) or lymphocyte ($P = 0.002$) count when initiating FAE therapy.

Based on the multivariate analysis, the age factor remained statistically significant for the development of T-cell lymphopenia ($P < 0.0001$). The therapy regimen 'FAEs + MTX' showed an inverse effect on the development of T-cell lymphopenia ($P = 0.03$). A similar statistically non-significant trend could already be observed in the univariate analysis ($P = 0.07$). The robustness of these results was confirmed using a sensitivity analysis (data not shown).

Impact of T-cell lymphopenia on FAE effectiveness

Although not statistically significant, sPGA and PASI assessments showed a tendency towards a faster improvement in the severity of psoriatic skin lesions in the two groups with decreased CD4$^+$ and/or CD8$^+$ T-cell counts below 200 and 140/μL, respectively (Figs 1,2). In both groups, the time from FAE therapy initiation to a cumulative sPGA response rate of 50% for achieving 'light' status and at least a 2-point reduction in baseline PGA was 0.49 years (95% CI: 0.49–0.99 years) compared with 0.99 years (95% CI: 0.49–0.99 years) in the group with CD4$^+$ and CD8$^+$ T-cell counts greater than or equal to 200 and 140/μL (Fig. 1). The sPGA results were not significantly different among the three groups ($P = 0.07$).

In the group with decreased CD4$^+$ and CD8$^+$ T-cell counts below 200 and 140/μL, the time from FAE therapy initiation to a cumulative PASI 75 response rate of 50% was 3 years (95% CI: 2–4 years) compared with 3.8 years (95% CI: 3–6.3 years) in the group with CD4$^+$ and CD8$^+$ T-cell counts greater than or equal

Table 3 Reasons for FAE treatment discontinuation, stratified by absolute CD4$^+$ and CD8$^+$ T-cell counts under FAE therapy

	All	CD4$^+$ T cells ≥200/μL and CD8$^+$ T cells ≥140/μL	CD4$^+$ T cells <200/μL or CD8$^+$ T cells <140/μL	CD4$^+$ T cells <200/μL and CD8$^+$ T cells <140/μL	*P*-value*
Patients initiating FAE therapy, *N*	371	176	109	86	
Total patients terminating therapy, *n* (% of *N*)	117 (31.5)	53 (30.1)	29 (26.6)	35 (40.7)	0.09
Reasons for discontinuation, *n* (% of *N*)					
Clinical remission	24 (6.5)	5 (2.8)	10 (9.2)	9 (10.5)	**0.002**
Lack of efficacy (or lower than expected improvement)	31 (8.4)	21 (11.9)	6 (5.5)	4 (4.7)	
AE (including SAE)	43 (11.6)	15 (8.5)	10 (9.2)	18 (20.9)	
Blood and lymphatic system disorders	18 (4.9)	2 (1.1)	2 (1.8)	14 (16.3)	
Gastrointestinal disorders	13 (3.5)	7 (4.0)	3 (2.8)	3 (3.5)	
Hepatobiliary disorders	5 (1.3)	3 (1.7)	2 (1.8)	0	
Flush/hot flushes	4 (1.1)	2 (1.1)	1 (0.9)	1 (1.2)	
Renal and urinary disorders	1 (0.3)	1 (0.6)	0	0	
Skin and subcutaneous tissue disorders	1 (0.3)	0	1 (0.9)	0	
Neoplasms benign, malignant and unspecified (including cysts and polyps)	1 (0.3)	0	1 (0.9)	0	
Other reasons	19 (5.1)	12 (6.8)	3 (2.8)	4 (4.7)	

*P-value derived from chi-square test; bold, *P* ≤ 0.05.
AE, adverse event; FAE, fumaric acid ester; SAE, serious adverse event.
AE terminology based on MedDRA (Medical Dictionary for Regulatory Activities) system organ classification version 20.1 September 2017. Other reasons include any of patient choice, not specified, non-compliance, no reimbursement and switch to an alternative systemic drug, and pregnant or planning to become pregnant.

Table 4 Psoriasis patients with abnormal absolute counts of peripheral blood leucocyte and lymphocyte subsets of different thresholds at some point during treatment duration, stratified by absolute CD4$^+$ and CD8$^+$ T-cell counts under FAE therapy

	All	CD4$^+$ T cells ≥200/μL and CD8$^+$ T cells ≥140/μL	CD4$^+$ T cells <200/μL or CD8$^+$ T cells <140/μL	CD4$^+$ T cells <200/μL and CD8$^+$ T cells <140/μL	*P*-value*
Patients initiating FAE therapy, *N*	371	176	109	86	
Leucocytes, *n* (% of *N*)					
<4600 cells/μL (LLN)	158 (42.6)	44 (25.0)	56 (51.4)	58 (67.4)	**<0.0001**
<3000 cells/μL (reference 8,11)	16 (4.3)	4 (2.3)	5 (4.6)	7 (8.1)	0.09
Lymphocytes, *n* (% of *N*)					
<1000 cells/μL (LLN)	226 (60.9)	53 (30.1)	89 (81.7)	84 (97.7)	**<0.0001**
<700 cells/μL (reference 11,32)	133 (35.9)	17 (9.7)	46 (42.2)	70 (81.4)	**<0.0001**
<500 cells/μL (CTCAE grade 3 and 4)	53 (14.3)	3 (1.7)	7 (6.4)	43 (50.0)	**<0.0001**
T helper cells (CD3$^+$CD4$^+$), *n* (% of *N*)					
<410 cells/μL (LLN)	225 (60.7)	50 (28.4)	89 (81.7)	86 (100.0; by definition)	**<0.0001**
<200 cells/μL (CTCAE grade 3 and 4)	98 (26.4)	0 (by definition)	12 (11.0)	86 (100.0; by definition)	**<0.0001**
T cytotoxic cells (CD3$^+$CD8$^+$), *n* (% of *N*)					
<190 cells/μL (LLN)	232 (62.5)	44 (25.0)	102 (93.6)	86 (100.0; by definition)	**<0.0001**
<140 cells/μL (reference 29)	183 (49.3)	0 (by definition)	97 (89.0)	86 (100.0; by definition)	**<0.0001**
B lymphocytes (CD19$^+$), *n* (% of *N*)					
<90 cells/μL (LLN)	120 (32.4)	37 (21.0)	39 (35.8)	44 (51.2)	**<0.0001**
NK cells (CD3$^-$CD16$^+$CD56$^+$), *n* (% of *N*)					
<90 cells/μL (LLN)	84 (22.6)	25 (14.2)	26 (23.9)	33 (38.4)	**<0.0001**

*P-value derived from chi-square test; bold, *P* ≤ 0.05.
CTCAE, Common Terminology Criteria for Adverse Events; LLN, lower limit of normal; NK cells, natural killer cells.
Normal ranges for our laboratory: leucocytes, 4600−9500/μL; lymphocytes, 1000−4050/μL; T helper cells, 410−1590/μL; T cytotoxic cells, 190−1140/μL; B lymphocytes, 90−660/μL; natural killer cells, 90−590/μL.

Table 5 Changes in absolute counts of peripheral blood leucocytes, lymphocytes and their subsets in patients with psoriasis before and during FAE therapy for a period of up to 3 years

	Baseline Mean ± SD [cells/µL]	Month 3 Change† [%] (95% CI)	P-value‡	Month 6 Change† [%] (95% CI)	P-value‡	Month 12 Change† [%] (95% CI)	P-value‡	Month 24 Change† [%] (95% CI)	P-value‡	Month 36 Change† [%] (95% CI)	P-value‡
Patients, N (%)	371 (100.0)	172 (46.4)		148 (39.9)		218 (58.8)		203 (54.7)		119 (32.1)	
Leucocytes	7433.5 ± 2339.8	−9.3 (−13.1, −5.5)	<0.0001	−16.4 (−20.7, −12.1)	<0.0001	−20.7 (−24.0, −17.5)	<0.0001	−18.8 (−22.9, −14.7)	<0.0001	−17.6 (−24.2, −10.9)	<0.0001
Lymphocytes	1962.0 ± 679.9	−23.9 (−28.9, −19.0)	<0.0001	−39.3 (−45.1, −33.4)	<0.0001	−44.1 (−48.2, −40.0)	<0.0001	−39.4 (−46.8, −31.9)	<0.0001	−45.1 (−51.5, −38.7)	<0.0001
T lymphocytes (CD3$^+$)	1272.8 ± 574.6	−12.1 (−25.4, 1.1)	0.073	−43.0 (−48.4, −37.6)	<0.0001	−47.8 (−53.2, −42.3)	<0.0001	−54.5 (−58.5, −50.5)	<0.0001	−48.3 (−54.2, −42.3)	<0.0001
T helper cells (CD3$^+$CD4$^+$)	817.1 ± 364.9	−16.9 (−25.9, −7.9)	0.0003	−41.8 (−46.8, −36.8)	<0.0001	−47.3 (−51.4, −43.3)	<0.0001	−49.9 (−54.1, −45.6)	<0.0001	−46.7 (−52.4, −40.9)	<0.0001
T cytotoxic cells (CD3$^+$CD8$^+$)	444.9 ± 272.9	−19.7 (−29.1, −10.2)	<0.0001	−48.7 (−54.1, −43.4)	<0.0001	−55.2 (−59.7, −50.8)	<0.0001	−55.7 (−61.3, −50.1)	<0.0001	−51.6 (−60.1, −43.1)	<0.0001
CD4/CD8 ratio	2.2 ± 1.7	20.7 (7.4, 33.9)	0.003	31.7 (23.1, 40.4)	<0.0001	49.5 (35.7, 63.3)	<0.0001	45.2 (31.6, 58.8)	<0.0001	34.1 (24.3, 44.0)	<0.0001
B lymphocytes (CD19$^+$)	270.3 ± 202.8	−18.8 (−30.8, −6.8)	0.002	−23.5 (−38.1, −9.0)	0.002	−27.7 (−36.5, −18.8)	<0.0001	−24.5 (−30.1, −18.9)	<0.0001	−11.5 (−21.4, −1.5)	0.024
NK cells (CD3$^-$CD16$^+$CD56$^+$)	255.4 ± 154.8	−3.9 (−11.4, 3.6)	0.31	−15.3 (−23.2, −7.5)	0.0002	−21.9 (−27.9, −15.9)	<0.0001	−11.3 (−19.0, −3.5)	0.004	4.5 (−5.8, 14.8)	0.39

†Mean percentage change from baseline; ‡P-value derived from one-sample t-test.
CI, confidence interval; FAE, fumaric acid ester; NK cells, natural killer cells; SD, standard deviation.
Normal ranges for our laboratory: leucocytes, 4600–9500/µL; lymphocytes, 1000–4050/µL; T lymphocytes, 690–2540/µL; T helper cells, 410–1590/µL; T cytotoxic cells, 190–1140/µL; CD4/CD8 ratio, 0.8–2.0; B lymphocytes, 90–660/µL; natural killer cells, 90–590/µL.

Table 6 Univariate and multivariate Cox proportional hazard analysis for the event 'CD4+ T cells <200/µL and/or CD8+ T cells <140/µL under FAE therapy'; data presented as hazard ratio (95% confidence interval)

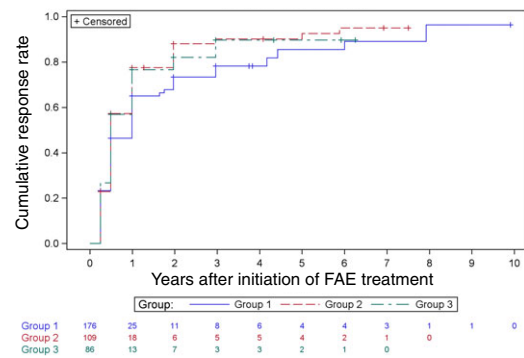| Variable	Cox proportional hazard analysis	
	Univariate	**Multivariate**
Demographics		
Age at the time of initiating FAE therapy	**1.03* (1.02, 1.04)**	**1.03* (1.02, 1.04)**
Age group 40–55 years† (reference 29,30)	**1.62* (1.12, 2.36)**	
Age group >55 years† (reference 49)	**2.42* (1.65, 3.55)**	
Female	1.11 (0.80, 1.53)	0.86 (0.56, 1.31)
Comorbidities‡		
1–2 Comorbidities	1.31 (0.94, 1.81)	1.22 (0.79, 1.90)
≥3 Comorbidities	1.05 (0.58, 1.88)	0.95 (0.48, 1.88)
Disease characteristics at baseline		
sPGA score	0.85 (0.68, 1.05)	1.00 (0.73, 1.38)
PASI score	0.98 (0.96, 1.00)	0.98 (0.95, 1.01)
Absolute leucocyte count	**1.00* (1.00, 1.00)**	1.00 (1.00, 1.00)
ALC	**1.00* (1.00, 1.00)**	1.00 (1.00, 1.00)
Therapy regimen§		
FAEs + phototherapy	1.17 (0.76, 1.78)	0.97 (0.57, 1.63)
FAEs + MTX	0.63 (0.38, 1.04)	**0.50* (0.27, 0.92)**

*$P \leq 0.05$, shown in bold.
†Reference category: age group <40 years. ‡Reference category: no comorbidities. §Reference category: FAE monotherapy.
ALC, absolute lymphocyte count; FAE, fumaric acid ester; MTX, methotrexate; PASI, Psoriasis Area and Severity Index; phototherapy, ultraviolet A, ultraviolet B or psoralen + ultraviolet A; sPGA, static Physician's Global Assessment.
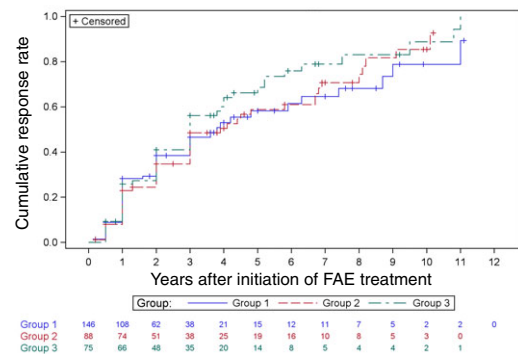

Figure 1 Cumulative percentage of sPGA responders (event: sPGA = 'light' and at least a 2-point reduction in baseline sPGA score) after initiation of FAE treatment by groups stratified for absolute CD4+ and CD8+ T-cell counts under FAE therapy: 1, 'CD4+ T cells ≥200/µL and CD8+ T cells ≥140/µL'; 2, 'CD4+ T cells <200/µL or CD8+ T cells <140/µL'; and 3, 'CD4+ T cells < 200/µL and CD8+ T cells <140/µL'. Kaplan–Meier failure curves with the number of patients 'at risk' (on therapy) in each group. Log-rank test: $P = 0.07$. FAE, fumaric acid ester; sPGA, static Physician's Global Assessment.

Figure 2 Cumulative percentage of PASI 75 responders after initiation of FAE treatment by groups stratified for absolute CD4+ and CD8+ T-cell counts under FAE therapy: 1, 'CD4+ T cells ≥200/µL and CD8+ T cells ≥140/µL'; 2, 'CD4+ T cells <200/µL or CD8+ T cells <140/µL'; and 3, 'CD4+ T cells <200/µL and CD8+ T cells <140/µL'. Kaplan–Meier failure curves with the number of patients 'at risk' (on therapy) in each group. Log-rank test: $P = 0.43$. FAE, fumaric acid ester; PASI, Psoriasis Area and Severity Index.

to 200 and 140/µL and 3.9 years (95% CI: 3–6.7 years) in the group with CD4+ or CD8+ T-cell counts below 200 and 140/µL (Fig. 2). The PASI 75 results were not significantly different among the three groups ($P = 0.43$).

Discussion

This retrospective investigation of the largest-ever subcohort from our single-centre observational study[2] has provided more precise data on the dynamics of CD4+ T, CD8+ T, CD19+ B and CD56+ NK cell counts in psoriasis patients under long-term treatment with FAEs (Fumaderm®). This confirms and expands upon previous work[24–27] demonstrating that FAEs significantly reduced the numbers of both CD4+ and CD8+ T cells. Additionally, this substudy expands our understanding of FAE effects on CD19+ B and CD56+ NK cells by demonstrating that FAEs also significantly reduced their numbers, while all mean values remained within the normal ranges.[27] The risk of T-cell lymphopenia increased significantly with the psoriasis patient's age

at the time of initiation of FAE therapy, a correlation that has thus far only been described in multiple sclerosis (MS) patients receiving DMF therapy with Tecfidera®.[49,50] Unexpectedly, the risk of T-cell lymphopenia decreased significantly for the combination therapy with MTX and folic acid supplementation. An increased infection rate was not detected in our subcohort.[26,51]

No deaths occurred. Conversely, our data strongly suggest that T-cell lymphopenia is a condition necessary for an enhanced FAE therapy effectiveness,[24,26,52,53] a finding that was first reported by Nieboer et al.[23] in a smaller psoriasis patient cohort.

Biological effects of FAEs on peripheral blood lymphocytes

Leukopenia and lymphopenia occur frequently during FAE treatment,[7,54] but, in our experience, are usually reversible after FAE dose reduction, and in severe and persistent cases, treatment discontinuation is required to decrease the risk of infection.[55,56] Severe lymphopenia has been reported in about 3% of FAE-treated psoriasis patients, while in up to 63% of patients, the lymphocyte reductions were mild.[3,57] The changes in the absolute leucocyte and lymphocyte subset counts observed in the present substudy are consistent with those recently reported in smaller cohorts by Sondermann et al.[26] (B and NK cells were not monitored) and Philipp et al.[27] for psoriasis patients treated with FAEs (Fumaderm®), and by Spencer et al.[58] for MS patients treated with DMF (Tecfidera®). Similar to the 11.4% reported by Sondermann et al.,[26] we detected a relatively high rate of severe lymphopenia below 500 cells/µL in our subcohort. Equally comparable is the severe $CD4^+$ T-cell reduction below 200/µL that occurred in 25% of the investigated patients.[26] Flow cytometric analyses by our group had already demonstrated that FAEs have profound effects on the T-cell system.[24,59] Within the T-cell fraction, stronger suppression of $CD8^+$ compared with $CD4^+$ lymphocytes was associated with an increased CD4/CD8 ratio.[24] This effect on $CD8^+$ T cells was then confirmed by several authors in psoriasis patients treated with FAEs[26,27] and MS patients treated with DMF.[58,60–65] FAEs affect $CD8^+$ more than $CD4^+$ T cells, which is important because $CD8^+$, more than $CD4^+$ T cells, are crucial in the disease containment and clinical outcome of PML and other viral infections.[29,30,39,58,66,67] $CD8^+$ T cells participate in antiviral immunity by recognizing and destroying virus-infected cells through production of toxic chemokines, thereby preventing further spread of the infection.[39,68] However, $CD4^+$ T cells induce B-cell differentiation in antibody-secreting plasma cells, monocyte and macrophage phagocytic capabilities, and $CD8^+$ T-cell cytotoxic function.[39,69,70]

Comparable with these and our earlier results,[24,59] Spencer et al.[58] found a significant $CD19^+$ B-cell count reduction by 37.5% after 12 months of DMF treatment. Longbrake et al.[68] recently reported that DMF induces a phenotypic shift among circulating B cells, reducing the proportion of memory B cells and expanding the proportion of naïve B cells. Markers of B-cell function were also modulated by DMF. In contrast to our results, Spencer et al.[58] did not observe a significant altered mean $CD56^+$ NK cell count during DMF treatment. While altered circulating B- and NK cell frequencies may not be of direct relevance in the pathogenesis of psoriasis,[12,13,71] both

lymphocyte subsets contribute to the immune response against infections, including those of a viral nature.[72–74]

Drugs containing FAEs are used to treat psoriasis (Fumaderm®, FAEs; Skilarence®, DMF; and Psorinovo®, DMF, compounded pharmacy) and MS (Tecfidera®, DMF). All licensed FAE drugs contain DMF as the main active ingredient, which acts as an orally administered prodrug of MMF in vivo.[15,16] However, the exact mechanism by which MMF, as the main systemically active metabolite, produces the effects seen in the immune system, particularly lymphopenia, has not yet been fully elucidated. It may be possible that MMF directly induces apoptosis in peripheral blood T cells, although $CD4^+$ or $CD8^+$ T-cell culture experiments showed that MMF does not increase the frequency of apoptotic cells.[62,75] Instead, in vitro DMF leads to the inhibition of T-cell cytokine secretion, T-cell proliferation and, in higher concentrations, the induction of apoptosis in a subpopulation of activated, but not resting, human T cells by impairing the nuclear factor-kappa B signalling pathway in particular.[19,60,62,75–78] The clinical observation of the preferential reduction of peripheral $CD8^+$ compared with $CD4^+$ T cells was also confirmed in vitro. Ghadiri et al.[62] found that DMF-induced apoptotic cell death was approximately twice as marked for $CD8^+$ T cells compared with $CD4^+$ T cells. However, the exact mechanism of this effect in treated psoriasis[24,26,27] and MS patients[58,60–65] remains unknown.[15,39] Additional work showed that DMF-induced apoptosis also disproportionately affects circulating memory $CD4^+$ and $CD8^+$ T-cell subsets compared with naïve $CD4^+$ and $CD8^+$ T-cell subsets, independent of the ALC.[60,62–64] New insights into B- and T-cell metabolism suggest that aerobic glycolysis is a hallmark of activated cells, while naïve and memory cells favour oxidative phosphorylation and consume fatty acids.[79,80] Both DMF and MMF target and inactivate the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH).[81] GAPDH inhibition downregulates aerobic glycolysis in activated B- and T cells, which may explain why FAEs differentially affect distinct lymphocyte subsets, producing lymphopenia that selectively depletes highly glycolytic effector cells while sparing oxidative naïve and memory cells. In summary, all these presumed modes of action may partially contribute to lymphopenia in the FAE treatment of psoriasis and MS.

FAE-induced lymphopenia linked to infection and influencing factors

Gieselbach et al.[29,30] recently summarized the characteristics of 19 PML cases (52.6% males) associated with the use of different FAE-containing drugs (11 Fumaderm®, 3 Psorinovo® and 5 Tecfidera®) as follows: median age at PML diagnosis was 59 years (range, 42–74 years); median FAE therapy duration until PML symptom onset was 2.6 years (range, 0.5–9.2 years); median lymphopenia duration (reported in all cases) until PML symptom onset was 1.9 years (range, 0.5–6 years); median lymphocyte count at PML diagnosis was 414/µL (mean, 538/µL);

median CD4$^+$ T-cell count was 137/µL (range, 13–391/µL; mean, 155/µL); median CD8$^+$ T-cell count was 39/µL (range, 14–130/µL; mean, 52/µL); and mortality rate was 15.8%. Thus, older age when initiating FAE therapy and longer time under continuous FAE therapy appeared to be decisive for lymphopenia to develop and PML to become manifest. Finally, a CD8$^+$ T-cell count below 140/µL may be a risk for FAE-associated PML.

Our results also showed that the reason for the age-related increased susceptibility for FAE-induced lymphopenia is not well understood,[49] but it may be related to 'immunosenescence'.[29,30] An additional lymphopenia predictor is a lower ALC when FAE therapy is initiated, which was described by Longbrake et al.[49]

While MTX induces apoptosis of *in vitro* activated T cells,[82] it only slightly increased the risk of leukopenia in randomized controlled trials (RCTs).[83] Dividing cytokine production into CD4$^+$IFN-γ$^+$ (T$_h$1), CD4$^+$IL4$^+$ (T$_h$2) and CD4$^+$IL17$^+$ (T$_h$17) cells, and CD8$^+$IFN-γ$^+$ (T$_c$1), CD8$^+$IL4$^+$ (T$_c$2) and CD8$^+$IL17$^+$ (T$_c$17) cells, MTX (up to 25 mg/week) significantly decreased the T$_c$1 subset and increased the T$_c$17 subset after 24 weeks of treatment in rheumatoid arthritis.[84] Frequencies were not altered in other subsets. Folic acid status also affects cell-mediated immunity by reducing circulating T cells.[85] In folic acid-sufficient conditions, both CD4$^+$ and CD8$^+$ T cells proliferated, whereas in lower folic acid concentrations, CD8$^+$ T-cell proliferation decreased more than CD4$^+$ T-cell proliferation; thus, the CD4/CD8 ratio increased. Folic acid supplementation was shown to rapidly restore the rate of T-cell proliferation and lower the CD4/CD8 ratio.[85] These results may advance our understanding on how the MTX-related folic acid supplementation might elicit a protective effect against lymphopenia. Thus, an RCT is warranted to substantiate our findings. Gold et al.[86] recently reported that the percentage of DMF-treated MS patients with low vitamin B9 (folic acid) and vitamin B12 baseline levels was slightly higher in those experiencing gastrointestinal-related events. The authors hypothesized that patients with higher serum vitamin B levels may experience fewer gastrointestinal symptoms.

FAE-induced lymphopenia linked to responder status

Although (T cell) lymphopenia may increase the risk of PML[29,30] and other severe or opportunistic infections,[27,49,65,87] it may also be a biomarker for the clinical efficacy of FAEs and DMF, respectively, in the treatment of psoriasis[24,52,53] and MS.[49,61] As early as 1989, a significant correlation between an improvement of at least 50% under antipsoriatic DMF therapy and lymphopenia was reported by Nieboer et al.[23] Sondermann et al.[26] recently found that psoriasis patients who developed lymphopenia under FAE treatment had a significantly higher chance of reaching a PASI 75 response. One may speculate that treatment responders are characterized not only by a broad and stronger reduction of all lymphocyte subsets but also by a preferential loss of CD8$^+$ T cells, indicating that they play a key role in

the FAE therapeutic mechanism of action.[24,61,88] However, little available data lead to contradictory results published by others and our own group.[49,89] While assessing only circulating lymphocytes, it might also be possible, for example, that DMF alters immune cell compartmentalization and that lymphocytes are being redirected to another location rather than being permanently lost.[63,70]

Limitations

The present subcohort study has some potential limitations. First, our data are limited in its retrospective nature, leading to missing values. Second, data reported here reflect the clinical experience of a single tertiary care centre and may not be representative of the entire psoriasis patient population. However, our real-life results can be extrapolated to psoriasis patients who have been exposed to FAEs for several years. Third, this study was limited by the risk of selection bias due to non-routine performance of flow cytometric analyses and the lack of a parallel control group, both of which complicate the interpretation of the causality between FAE treatment and T-cell lymphopenia. Fourth, despite the continuous strict compliance to high-quality standards in our laboratory throughout the entire study period[34–36] and the continuous use of the same laboratory team (S.H. and colleague) for the performance of flow cytometry analyses, we cannot strictly rule out an influence of the techniques developed in flow cytometry in the interim on lymphocyte phenotyping. Finally, examination of the lymphocyte subset count recovery over time after FAE discontinuation would have also been desirable, but these data were not interpretable because patients who discontinued FAE use as a result of lymphopenia typically began other disease-modifying therapies immediately thereafter.[63]

Conclusions

Fumaric acid ester-induced (T cell) lymphopenia does not seem to cause a significantly attenuated immunosurveillance[2,4,5] as long as ALCs are closely monitored in accordance with the guidelines.[7,32] The occurrence of severe or opportunistic infections such as PML during treatment with FAEs was mostly linked to exposure to prolonged uncontrolled lymphopenia.[57] However, severe or opportunistic infections can also occur in patients with unremarkable total lymphocyte counts;[27,66,87,90,91] thus, monitoring the ALC, which is not a good indicator of CD4$^+$ and CD8$^+$ T-cell counts, seems to be a crude way to measure the cellular immune response.[29,30,39] However, the CD8$^+$ T-cell count may be a better surrogate marker for risk of infection than the ALC.[66,68]

The current Summary of Product Characteristics for Fumaderm® requires a monthly complete blood count and lymphocyte count. In the current German guidelines,[7] 2-month intervals are considered to be sufficient enough for patient safety. Based on our long-term experiences, we mostly share

this view but suggest also monitoring $CD4^+$ and $CD8^+$ T-cell counts in intervals of 4 months or less. Periodic monitoring of $CD4^+$ and $CD8^+$ T cells seems to be clinically relevant for making therapeutic decisions regarding continual and long-term use of FAEs[26] or DMF,[58,60,62,90] thus reducing the risk of severe or opportunistic infections. For all T-cell subsets examined, most *in vivo* changes occurred within the first 6 months of FAE[27] or DMF treatment.[60–62,65] Primarily based on the recently published case series of 19 patients with FAE-related PML,[29,30] we recommend a safety threshold of 200/μL for a $CD4^+$ T-cell count[26] and 140/μL for a $CD8^+$ T-cell count. This increased vigilance in T-cell monitoring appears to be particularly warranted in older patients,[49] especially those who are older than 40 years of age.[29,30] However, further prospective investigation is required to establish evidence-based recommendations and thus overcome the discrepancies in leucocyte and lymphocyte count monitoring between licensed FAE-containing drugs[11,32,92] that are currently available.[93]

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