

# Progressive multifocal leukoencephalopathy in patients treated with fumaric acid esters: a review of 19 cases

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**Abstract** Progressive multifocal leukoencephalopathy (PML) is a rare and potentially fatal condition caused by a brain infection with JC polyomavirus (JCV). PML develops almost exclusively in immunocompromised patients and has recently been associated with use of fumaric acid esters (FAEs), or fumarates. We reviewed the literature and the Dutch and European pharmacovigilance databases in order to identify all available FAE-associated PML cases and distinguish possible common features among these

patients. A total of 19 PML cases associated with FAE use were identified. Five cases were associated with FAE use for multiple sclerosis and 14 for psoriasis. Ten patients were male and nine were female. The median age at PML diagnosis was 59 years. The median duration of FAE therapy to PML symptom onset or appearance of first PML lesion on brain imaging was 31 months (range 6–110). In all cases a certain degree of lymphocytopenia was reported. The median duration of lymphocytopenia to PML symptom onset was 23 months (range 6–72). The median lymphocyte count at PML diagnosis was 414 cells/ $\mu$ L. CD4 and CD8 counts were reported in ten cases, with median cell count of 137 and 39 cells/ $\mu$ L, respectively. Three patients died (16% mortality). The association between occurrence of PML in patients with low CD4 and CD8 counts is reminiscent of PML cases in the HIV population and suggests that loss of T cells is the most important risk factor.

The opinions and conclusions of the authors do not necessarily reflect those of the various National Pharmacovigilance Centers or the European Medicines Agency (EMA).

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## Introduction

Progressive multifocal leukoencephalopathy (PML) is a rare and potentially fatal disease involving white matter and neuronal cells and is caused by an opportunistic infection with JC polyomavirus (JCV) [1, 2]. Infection with this virus is common in the general population worldwide with seroprevalence rates of about 50% in adulthood [3]. Infection with JCV leads to a lifelong infection that is regarded as harmless in immunocompetent hosts. In immunocompromised patients, such as patients with HIV/

AIDS, certain haematological malignancies, transplant recipients and patients using several immunomodulatory drugs, most notably natalizumab [1, 2, 4], PML can develop as a consequence of immune escape and viral evolution. The diagnosis of PML, if not biopsy proven, is based on the presence of three key features: symptoms suggestive of PML, lesions on brain magnetic resonance imaging (MRI) suspicious for PML and the detection of JCV DNA in the cerebrospinal fluid (CSF) by polymerase chain reaction (PCR) [5].

In 2013, the first PML cases associated with treatment with fumaric acid esters (FAEs) were published [6–10]. Three PML patients were reported in Germany and one in the Netherlands. In these countries, dimethyl fumarate (DMF) alone or FAE formulations that contain a mixture of monoethyl fumarate (MEF) salts and DMF are used to treat psoriasis. Since 2013 DMF has also been used as treatment for relapsing–remitting multiple sclerosis (MS) [11, 12]. In 2015 four PML cases were reported of patients with MS that were treated with DMF [13, 14].

After uptake in the body, DMF is rapidly metabolized to S-(1,2-dimethoxycarbonyl)ethyl glutathione and the bioactive metabolite monomethyl fumarate (MMF). MMF has a wide range of immunomodulatory effects (reviewed by Dubey et al.) [15]. This includes a reduction of about 50% of peripheral blood lymphocyte counts after one year of treatment [16], which might be the result of its anti-proliferative and pro-apoptotic effects, and a shift towards Th2-like cytokine secretion. FAEs also influence the CD4/CD8 ratio because the number of CD8 cells decreases more than the number of CD4 cells. In one study, after one year of therapy with FAEs the majority of recipients developed CD8 counts below the lower limit of normal in peripheral blood [16]. Finally, FAEs reduce the number of T memory cells and inhibit antigen presentation and expression of  $\alpha 4$  integrin in T cells [17, 18]. Studies with Tecfidera (DMF) indicate that grade 3 lymphocytopenia (lymphocytes between 200 and 500 cells/ $\mu$ L) develops in at least 5% of the patients; however, this number may increase up to 20% in patients above 55 years of age [19–21]. Despite these observations, little is understood about how DMF and MEF modify disease course and affect the immune system [22].

It is still uncertain why the association between FAE use and PML has been recognized only very recently. Perhaps cases have passed unrecognized, or maybe expanding treatment indications, increased long-term usage or confounding factors are responsible for this newly recognized side-effect. Since the number of patients with relapsing–remitting MS who are treated with FAEs is rapidly increasing (>170,000 patient treatment years), awareness of risk of PML is of utmost importance for treating physicians. To identify possible common features in the PML patients associated with FAE use and to increase

awareness among physicians that prescribe FAEs, we reviewed the characteristics of all cases we could find in the literature and Dutch and European pharmacovigilance databases.

## Methods

### Search strategy and inclusion criteria of PML cases

To identify FAE-associated PML cases, we searched the PubMed database and Google Scholar for: “Dimethyl Fumarate neurological disorders”, “Dimethyl Fumarate PML”, “PML, DMF”, “DMF Progressive Multifocal Leukoencephalopathy”, “Monomethyl Fumarate PML”, “Monomethyl Fumarate progressive multifocal leukoencephalopathy”, “BG-12 PML”, “Tecfidera PML”, “Tecfidera Progressive Multifocal Leukoencephalopathy”, “Multiple Sclerosis lymphocytopenia”, “PML case report”, “Monomethyl Fumarate”, “Fumaric Acid Esters PML”, and “Fumarates PML”. This search was performed in the last week of November 2016. From the case reports, we distilled age, sex, comorbidities, medication, FAE treatment duration, presence of lymphocytopenia, CD4/CD8 ratio, subset deficiency, onset of PML and the method of laboratory confirmation of PML. In addition, we reviewed the available imaging data on these cases. Only cases with laboratory confirmation of PML were included in the case series.

In addition to cases described in the literature, reports from the Dutch and European pharmacovigilance databases concerning FAEs and PML were analysed [23, 24]. This search was performed in December 2016. These pharmacovigilance databases (Eudravigilance and Lareb database) contain information concerning possible adverse drug reactions reported by patients, health care professionals, manufacturers or others. It is important to realize that in general the likelihood of a causal relationship can differ between cases.

## Results

### Demographic and epidemiological features

We identified 14 PML cases associated with FAE treatment in the published literature and five additional cases in the Eudravigilance or Lareb database (Table 1) [6–10, 13, 14, 23–31]. Fourteen patients were treated with FAEs for psoriasis and five for MS. The patients were treated with different FAE preparations: 11 patients used Fumaderm<sup>®</sup> (tablets containing a mixture of DMF and MEF salts), three patients used Psorinovo<sup>®</sup> (DMF,

**Table 1** Clinical and demographic information of all identified FAE-associated PML cases

Case	Age	Sex m = male f = female	Underlying illness and comorbidities <sup>a</sup>	FAE treatment (maximum daily dose)	Other immunosuppressive drugs in previous 5 years (no. of months stopped before PML diagnosis)	Duration of FAE use to lab diagnosis PML, in months	Duration of FAE start to onset symptoms, in months	Duration of lymphopenia to PML symptom onset, in months
<b>1</b> [7]	42	f	<b>Psoriasis</b>	DMF (420 mg reported-likely 480 mg) <i>Psorinovo</i>	None	67	61	58
<b>2</b> [6]	74	m	<b>Psoriasis</b>	DMF (240 mg) + MEF (190 mg)	Methotrexate (36)	36	35	26
<b>3</b> [8, 9]	60	f	<b>Psoriasis; pulmonary sarcoidosis</b>	DMF (480 mg) + MEF (380 mg) <i>Fumadem</i>	Prednisolone (41), methotrexate (42)	39	37	20
<b>4</b> [8, 10]	NA	m	<b>Psoriasis superficial spreading melanoma (surgically removed)</b>	<i>Fumadem</i>	Efalizumab (±34), steroids topical/tablets (NA)	34	32	NA
<b>5</b> [26]	69	m	<b>Psoriasis; arterial hypertension, aortic valve replacement</b>	DMF (720 mg) + MEF (570 mg) <i>Fumadem</i>	None	58	53	16
<b>6</b> [23]	64	f	<b>Psoriasis</b>	DMF (720 mg) <i>Psorinovo</i>	Topical steroids (NA), triamcinolone acetonide (>36)	26	24	2
<b>7</b> [24]	68	m	<b>Psoriasis; rectal carcinoma (8 years before PML)</b>	DMF (600 mg) + MEF (475 mg) <i>Fumadem</i>	Topical steroids (NA), 5-fluorouracil (±95)	50	30	27
<b>8</b> [25]	53	f	<b>Psoriasis; arterial hypertension, hypothyroidism</b>	DMF (360 mg) + MEF (285 mg) <i>Fumadem</i>	Topical therapy; not specified	19	16	13
<b>9</b> [13]	54	f	<b>Multiple sclerosis</b>	DMF (720 mg) <i>Tecfidera</i>	Glatiramer acetate (≥54)	54	53	42
<b>10</b> [27]	46	m	<b>Psoriasis; SLE</b>	DMF (NA—stopped 5 months before onset PML) <i>Fumadem</i>	Cyclophosphamide (up to PML diagnosis)	6	NA	NA
<b>11</b> [27]	54	f	<b>Psoriasis</b>	<i>Fumadem</i>	None	6	NA	NA
<b>12</b> [14]	61	f	<b>Multiple sclerosis</b>	DMF <i>Tecfidera</i>	Natalizumab (24)	22	NA	6

Table 1 continued

Case	Age	Sex	Underlying illness and comorbidities <sup>a</sup>	FAE treatment (maximum daily dose)	Other immunosuppressive drugs in previous 5 years (no. of months stopped before PML diagnosis)	Duration of FAE use to lab diagnosis PML, in months	Duration of FAE FAE start to onset symptoms, in months	Duration of lymphopenia to PML symptom onset, in months
<b>13</b> [22]	58	m	<b>Multiple sclerosis</b> ; hypertension, hypercholesterolaemia, Paroxysmal atrial fibrillation	DMF <i>Tecfidera</i>	None	24	16	9.5
<b>14</b> [22]	57	m	<b>Psoriasis</b> ; hypertension, chronic atrial fibrillation, actinic keratosis	DMF + MEF <i>Fumadem</i>	NA	50	48	47
<b>15</b> [22]	50	f	<b>Psoriasis</b> ; depression, Nicotine abuse, gonarthrosis left knee	DMF (120 mg) + MEF (95 mg) <i>Fumadem</i>	NA	112	110	72
<b>16</b> [21]	71	m	<b>Psoriasis</b>	DMF (120 mg)	NA	25	19	NA
<b>17</b> [28]	59	m	<b>Multiple sclerosis</b>	DMF <i>Tecfidera</i>	Interferon-β (12)	17	12	8
<b>18</b> [29]	64	m	<b>Multiple sclerosis</b>	DMF <i>Tecfidera</i>	None	26	23	14
<b>19</b> [22]	64	f	<b>Psoriasis</b> ; breast carcinoma (10 years before PML), hypertension, nicotine abuse, hyperthyroidism, partial thyroidectomy, myocardial infarction	DMF (120 mg) + MEF (95 mg) <i>Fumadem</i>	Topical steroids (NA), cyclophosphamide (NA)	7	6	NA
Lymphopenia grade <sup>b</sup>	Range of lymphocyte counts, per mm <sup>3</sup>	Lymphocyte subsets at PML diagnosis, per mm <sup>3</sup>	CD4/CD8 ratio	Initially misdiagnosed as stroke?	Lab diagnosis of PML (detection of JCV)	PML-IRIS	Duration FAE stop to PML-IRIS, in weeks	Outcome
Grade 3	200–500	Lymphocytes: 200; CD4: 40; CD8: 20; B cells: 98	2	NA	CSF	Yes	4	Survived
Grade 3	410–450	Leukocytes: 4600; lymphocytes: 410; CD4: 143; CD8: 23	>6	Yes	CSF + brain biopsy	Yes	5	Survived
Grade 2–3	>445	NA	NA	NA	NA	NA	NA	Survived
Grade 1–2	450–800	CD4: 131; CD8: ±130	1	No	CSF	No	NA	Survived
Grade 2	724–738	Leukocytes: 4800; lymphocytes: 288	NA	No	CSF	Yes	4	Survived
Grade 1	792–1000	Leukocytes: 4000; lymphocytes: 792; CD4: 270; CD8: 40	>2	Yes	Brain biopsy (CSF JCV negative)	Yes	4	Death
Grade 2	500–1000	Lymphocytes: 670; CD4: 154; CD8: 117	1	Yes	Brain biopsy (CSF JCV negative)	Yes	±12	Survived
Grade 2	450–700	Leukocytes: >3000; lymphocytes: 450–700	NA	No	CSF + brain biopsy	No	NA	Survived
Grade 2–3	290–580	NA	NA	NA	CSF	No	NA	Death
Grade 3	NA	Leukocytes: 5580; lymphocytes: 246; CD4: 13; CD8: 14	1	NA	CSF	No	NA	Death
Grade 3	NA	Lymphocytes: 481; CD4: 106; CD8: 38	>2	NA	Brain biopsy	No	NA	Survived

**Table 1** continued

Lymphopenia grade <sup>b</sup>	Range of lymphocyte counts, per mm <sup>3</sup>	Lymphocyte subsets at PML diagnosis, per mm <sup>3</sup>	CD4/CD8 ratio	Initially misdiagnosed as stroke?	Lab diagnosis of PML (detection of JCV)	PML-IRIS	Duration FAE, stop to PML-IRIS, in weeks	Outcome
Grade 2	600	NA	NA	No	NA	NA	NA	Survived
Grade 3	476–486	NA	NA	No	Brain biopsy	No	NA	Survived
Grade 1–3	>391	Lymphocytes: 1148; CD4: 391; CD8: 72; B cells: 583; NK cells: 161	>5	Yes	CSF	NA	NA	Survived
Grade 2–3	398–631	Lymphocytes: 584; CD4: 202; CD8: 54; NK cells: 177	>3	NA	CSF	Yes	±4	Survived
Grade 3	420	NA	NA	No	Brain biopsy	No	NA	Survived
Grade 2–3	414–728	Lymphocytes: 414	NA	No	Brain biopsy + CSF	Yes	NA	Survived
Grade 3	NA	CD4: 96; CD8: 14	6.77	No	CSF	No	NA	Survived
Grade 3	414	Leukocytes: 6670; lymphocytes: 414	NA	No	CSF	No	NA	Survived

FAE fumaric acid ester, PML progressive multifocal leukoencephalopathy, IRIS immune reconstitution inflammatory syndrome, DMF dimethyl fumarate, MMF monomethyl fumarate, CSF cerebrospinal fluid, JCV JC virus, NA not available

<sup>a</sup> In bold is the illness for which FAE treatment was given

<sup>b</sup> Grade 1 lymphocytopenia = 800–1000 cells per mm<sup>3</sup>; grade 2 lymphocytopenia = 500–800 cells per mm<sup>3</sup>; grade 3 lymphocytopenia ≤500 cells per mm<sup>3</sup>

compounded pharmacy) and five patients used Tecfidera<sup>®</sup> (DMF).

Ten patients were male and nine were female. The median age (years) at PML diagnosis was 59 for all patients with known age, 54 for women and 64 for men. The median age at PML diagnosis was 60 years in psoriasis patients and 59 years in MS patients.

**Clinical characteristics**

The most notable comorbidities were sarcoidosis in one patient, SLE in one patient and a history of malignancy in three patients. One of the patients with a history of malignancy had a superficial spreading melanoma (Clark-level II, excision in sano), which was removed surgically. The second patient had a rectal carcinoma (pT2 pN1 cM0 L0 V0) 8 years before PML diagnosis; it was treated with surgery followed by radiotherapy and chemotherapy. The third patient was diagnosed with breast cancer (cT2 cN0 cM0) 10 years before, for which she received chemotherapy (including cyclophosphamide), surgery and radiotherapy.

In three patients, no medication history was available. In seven cases, the medication history included additional systemic immunosuppressive drugs: steroids (3 patients), 5-fluorouracil (1 patient), cyclophosphamide (2 patients), glatiramer acetate (1 patient), efalizumab (1 patient) and natalizumab (1 patient). Efalizumab, natalizumab and glatiramer acetate were, respectively, stopped 34, 24 and 54 months before PML diagnosis. For two patients who had used systemic steroids the stop date was known. In both cases, steroid treatment had been stopped at least 36 months before PML diagnosis. The use of topical steroids was reported in four psoriasis patients (see Table 1). The patient with psoriasis and SLE (case 10) had switched from DMF to cyclophosphamide about 5 months before PML diagnosis.

The median duration of FAE therapy to PML diagnosis or appearance of first PML-related lesion on brain imaging was 31 months (range 6–110). In all cases, a certain degree of lymphocytopenia was reported. Total leukocyte counts were normal or almost normal in at least 6 of 17 patients (29%). The average lymphocyte count at PML diagnosis was 538 cells/μL (normal value 1000–4000 cells/μL), the median count was 414 cells/μL. The average and median duration of lymphocytopenia to PML symptom onset were, respectively, 29 and 23 months. Only moderate lymphocytopenia (grade 1 or 2) was noted in at least five PML cases (26%) in the months before PML development [14, 25–28]. CD4 and CD8 counts were reported in ten cases. The median CD4 and CD8 counts at PML diagnosis were, respectively, 137 (range 13–391) and 39 (range 14–130) cells/μL. The average CD4 and CD8 counts at

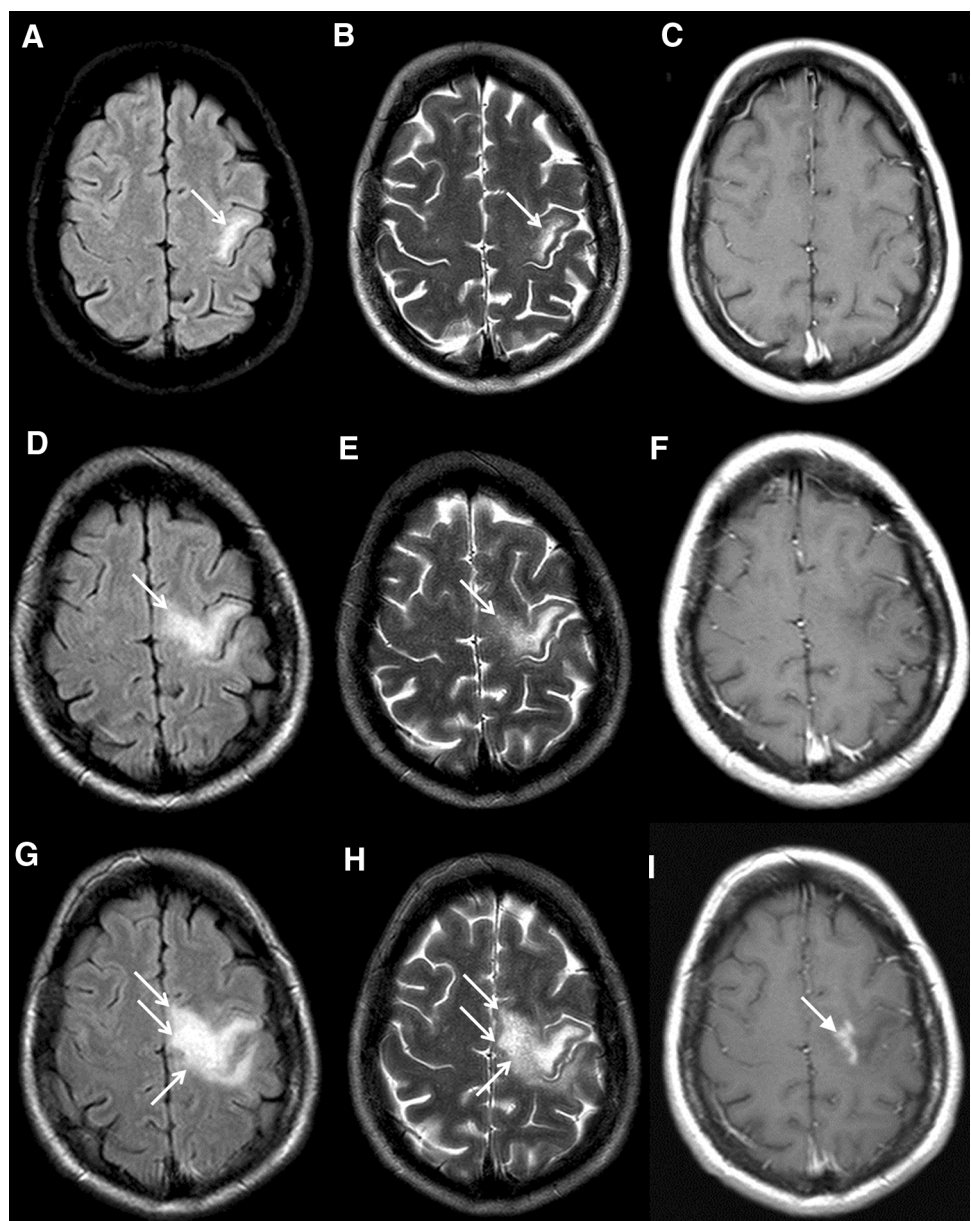
PML diagnosis were, respectively, 155 and 52 cells/ $\mu\text{L}$ . Normal values lie between 460 and 1600 cells/ $\mu\text{L}$  for CD4 cells and between 150 and 1000 cells/ $\mu\text{L}$  for CD8.

PML was initially misdiagnosed as ischemic stroke in four cases (21%). Laboratory confirmation of PML by detection of JCV in the central nervous system was obtained by detection of JCV DNA in CSF in nine patients (47%), in eight patients a brain biopsy was performed (42%) and in two cases the method of laboratory confirmation was not specified. In seven cases, PML was complicated by immune reconstitution inflammatory syndrome (IRIS), which occurred 4–12 weeks after discontinuation of FAEs. Eventually, three patients (16%) died due to PML or PML–IRIS related complications.

## Imaging findings

The appearance and evolution of PML lesions were heterogeneous; ranging from multiple smaller PML lesions slowly increasing in number over the course of 2 years (case 7), up to a very rapid and fatal confluent spread throughout a whole cerebral hemisphere in just a few months (case 6). Most of the presented PML lesions showed a classical sub-cortical white matter involvement in the cerebral hemispheres, some of which showed cortical grey matter involvement as well (Fig. 1). However, at least three patients also had cerebellar involvement (cases 4, 7, 9) and at least one patient primarily showed a deep grey matter PML lesion (case 7). At least five cases displayed

**Fig. 1** Brain MRI scan of FAE PML case 1. Axial fluid attenuation inversion recovery (FLAIR; column *a*), T2-weighted (column *b*) and T1-weighted images with contrast administration (column *c*) of FAE-associated PML case 1.<sup>7</sup> The *top* row shows the initial MRI which displays a T2 and FLAIR hyperintense lesion in the sub-cortical white matter of the left frontal lobe (*open arrow heads*) suspicious for PML. The lesion appears hypointense on T1-weighted images and shows some microcysts on T2-weighted images. On follow-up MRI 2 months later (*middle row*), the PML lesion had progressed to the adjacent white matter. At that time the diagnosis of PML was confirmed by positive JC virus PCR in CSF and treatment with Psorinovo was discontinued. 1 month later, the lesion clearly shows contrast enhancement on the T1-weighted sequence (*bottom row*), consistent with the development of PML immune reconstitution inflammatory syndrome (PML–IRIS)



small punctate lesions, an imaging sign which has recently been shown to be very specific for PML (cases 2, 4, 5, 7, 8) [32, 33]. In addition, at least four cases already showed contrast enhancement prior to the cessation of FAE treatment, which is deemed a sign of inflammation in these patients (cases 4, 7, 8, 18) [34].

## Discussion

Here we summarized the characteristics of 19 cases of PML associated with FAE use, of which 14 were previously reported in the medical literature and five were collected from the Dutch and European pharmacovigilance database.

Several observations are noteworthy. First, all FAE-associated PML patients of which data were available, had very low CD4 and CD8 counts at the time of diagnosis. In HIV patients, the risk for PML is strongly increased when the CD4 count is <200 cells/ $\mu$ L, although in one cases series 13% of PML cases had a CD4 count >200 cells/ $\mu$ L [35]. It is striking that the average and median CD4 count in the FAE-associated PML cases are also below such a 'threshold' value. The reduction of CD8 counts was generally more pronounced than reduction of CD4 counts, which is reflected by an increased CD4/CD8 ratio. This effect of FAE use on the CD4/CD8 ratio has been described before [16]. In our case series, CD8 counts were all below 140 cells/ $\mu$ L. Low CD8 counts may also increase the risk for PML, because PML containment and survival appears to be more strongly correlated with CD8 cells than CD4 cells [36]. The mortality rate of 16% in the patients in our study is relatively low compared to HIV-associated PML, where survival in a group of patients with PML from 1996 to 2011 ranged from 50 to 80%, and depended on CD4 counts [35]. It is important to note that the low CD4 and CD8 counts were not always accompanied by low total leukocyte counts and can be more profound than the decrease in lymphocytes would suggest. In our case series, the observed median time to lymphocytopenia after start with FAEs was 9 months. This rate of development of lymphocytopenia appears to be in line with observations in non-PML patients that develop lymphocytopenia after FAE use [16, 17]. Additional research is necessary to validate this conclusion.

A second noteworthy observation is that the average age of FAE-associated PML diagnosis at 59 years is much higher than the average age of PML diagnosis in natalizumab-treated patients and AIDS patients, which is about 40–45 years [35, 37]. In a recent study, Longbrake et al. identified older age as a risk factor for development of FAE-associated lymphocytopenia. The authors found that >40% of patients above 55 years of age develop grade

2 or 3 lymphocytopenia [38]. In the DEFINE and CONFIRM phase three studies with Tecfidera only about 5% developed this degree of lymphocytopenia, but in these studies the average age was 37 and 38 years, respectively [19–21]. The reason for the apparently age-related increased vulnerability for FAE-elicited lymphocytopenia is unknown, but may be related to immunosenescence. If lymphocytopenia is a major risk factor for development of PML in FAE-treated patients, it is not surprising that FAE-associated PML cases are older than natalizumab-associated PML cases.

A third observation is that PML symptoms or first PML lesions on imaging appeared at a median of 31 months after start with FAEs. In PML cases associated with natalizumab, the median treatment duration until PML symptoms is 25 months [37]. Thus, long duration of FAE exposure appears to be necessary for PML to develop or become manifest.

An important difference between natalizumab and FAEs is that, in contrast to FAE-treated patients, natalizumab-treated patients are subject to strict pharmacovigilance via MRI screening programmes for high-risk patients [39, 40]. Therefore, natalizumab-associated PML is frequently detected early in the disease course, when PML lesion volume is still limited and patients can even be asymptomatic for PML. Hence, clinical and radiological findings of natalizumab and FAE-associated PML patients are difficult to compare. Although pending formal investigation, we hypothesize that asymptomatic FAE-associated PML cases in the context of strict pharmacovigilance would follow a similar pattern and outcome as observed in natalizumab-associated PML [41, 42]. In addition, as with natalizumab-treated and HIV-infected patients, other JCV-associated diseases such as granule cell neuronopathy might develop in FAE-treated patients [43, 44]. However, this has not yet been reported.

In our series of 19 patients, several FAE-treated patients had other risk factors that may have contributed to development of PML. Patient 4 had been treated with efalizumab for about 18 months, before FAE therapy was started. Although PML became manifest after about 32 months of treatment with FAE, it cannot be excluded that efalizumab contributed to PML development. The same applies to patient 12, who had been pre-treated with natalizumab. Pre-treatment with natalizumab appears to be a risk factor for development of FAE-induced lymphocytopenia [38]. In patient 10, FAE therapy appears to have been stopped 5 months before PML became manifest. In the following months the patient was treated with cyclophosphamide, a drug that is also known to increase the risk for PML [45]. Patient 19 received chemotherapy with cyclophosphamide for breast cancer 10 years before. 2 years later, her psoriasis was treated with Fumaderm for

one year and continued with topical steroids. Fumaderm was resumed 6 months before PML symptoms became manifest. An infection with HIV was excluded in this patient. Even if these cases are excluded from analysis, the general picture remains unaltered.

Why have FAE-associated PML cases only recently surfaced, even though FAEs have been used for such a long time and in so many patients? We see three possible explanations. First, only long-term use appears to be associated with PML development and the number of patients that have been treated with FAEs for long periods appears to be limited. There are only two studies that evaluate the long-term effects of FAE use in which patients are included with >2 years FAE therapy. Hoeffnagel et al. published a study that included 34 patients and Reich et al. published a study that included 984 patients [46, 47]. Numbers of MS patients with more than 2 years of treatment with DMF are also small at this moment. Second, the age of the majority of patients on long-term treatment may have been below 55 years and thus have had a comparatively low risk to develop lymphocytopenia. In Reich's study [47], the mean age of patients on long-term treatment was 50 years. As previously mentioned, the average age in the DEFINE and CONFIRM studies was below 40 years. Finally, the diagnosis of PML may have been missed because PML lesions on brain imaging can be misinterpreted (for instance as an ischemic stroke or multiple sclerosis lesions) [48], and JC virus PCR in CSF can be false negative in PML patients, as was the case in eight patients reported here, where a brain biopsy was performed following an initial negative CSF JCV PCR.

In conclusion, in our case series the occurrence of PML appears to be related to FAE-induced CD4 and CD8 cell cytopenia. The comparatively high age of FAE-associated PML patients may be related to the increased risk to develop FAE-induced lymphocytopenia at older age. The clear association between occurrence of PML in patients with low CD4 and CD8 counts is reminiscent of PML cases in the HIV population and suggests that low T-cell counts are the most important risk factor. Prospective studies are needed to confirm this association. Physicians should be aware that normal or mildly decreased total lymphocyte counts do not imply that CD4 and CD8 counts are in the normal range. Yearly monitoring of CD4 and CD8 counts in patients above 40 years of age may be considered until further monitoring guidelines, based on systematic studies, become available. Review of our case series suggests the risk for PML is increased when CD8 counts are below 140 cells/ $\mu$ L.

In 2015, the European Medicines Agency (EMA) issued advices to minimize the risk of PML associated with Tecfidera [49]. These recommendations included performance of a complete blood count prior to treatment with

Tecfidera and every 3 months during treatment, and availability of a baseline MRI as reference. Related recommendations were also issued for Fumaderm and Psorinovo. For a complete overview of current recommendations from EMA, we refer to the EMA website (<http://www.ema.europa.eu/ema/>).

#### Compliance with ethical standards

**Conflicts of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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**Ethical standards** All procedures performed were in accordance with the ethical standards stated in the Declaration of Helsinki.

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