Review Article

Indian J Med Res 142, December 2015, pp 647-654
DOI:10.4103/0971-5916.174543

Multiple sclerosis - New treatment modalities

Rocco Totaro[*], Caterina Di Carmine[*], Carmine Marini[**] & Antonio Carolei[†]

[*]Multiple Sclerosis Center, Department of Neurology, San Salvatore Hospital, [**]Department of Medicine, Health & Environment Sciences, University of L'Aquila & [†]Department of Clinical & Applied Sciences & Biotechnology, University of L'Aquila, L'Aquila, Italy

Received September 28, 2015

Ever since the introduction of the first disease modifying therapies, the concept of multiple sclerosis treatment algorithms developed ceaselessly. The increasing number of available drugs is paralleled by impelling issue of ensuring the most appropriate treatment to the right patient at the right time. The purpose of this review is to describe novel agents recently approved for multiple sclerosis treatment, namely teriflunomide, alemtuzumab and dimethylfumarate, focusing on mechanism of action, efficacy data in experimental setting, safety and tolerability. The place in therapy of newer treatment implies careful balancing of risk-benefit profile as well as accurate patient selection. Hence the widening of therapeutic arsenal provides greater opportunity for personalized therapy but also entails a complex trade-off between efficacy, tolerability, safety and eventually patient preference.

Key words Multiple sclerosis - therapy - safety - tolerability - relapsing - remitting MS

Introduction

Just over 20 years ago there was no approved therapy for multiple sclerosis (MS) and patients could be offered only off-label and symptomatic treatments. Ever since their introduction, interferons and then later glatiramer acetate have been the mainstay of MS treatment for over a decade until the introduction of novel therapeutic agents has changed therapeutic scenario. Immunomodulatory drugs of the so-called 'platform therapy' have also been shown equal in efficacy in a post-marketing observational study[1]. First natalizumab and then fingolimod provided physicians with additional valuable options for patients with suboptimal disease control or breakthrough disease. Clinical trials and data derived from real life studies proved their efficacy[2-7]. The remarkable impact of second-line therapies on inflammatory measures of disease activity is offset by safety concern about treatment emergent serious adverse events limiting their use in the very early phase of disease when inflammatory damage seems to predominate. Nevertheless, early treatment with highly active drugs is warranted as a reflection of early 'window of opportunity', *i.e.* the chance to prevent the accumulation of long-term disability maximizing therapeutic impact during inflammatory phase. In such a steeply changing landscape, the complex trade-off between efficacy and safety remains a challenge for drug developers. Thus, newer agents have to face many challenges before definite approval as these must prove to be safer, more tolerated or more effective than the currently available drugs. This review will focus on drugs that have been recently licensed for treatment of relapsing-remitting multiple sclerosis (RRMS), outlining proposed mechanisms of action, efficacy, safety and tolerability profiles.

Teriflunomide

Teriflunomide is the active metabolite of leflunomide, a drug with immunosuppressant and anti-inflammatory properties licenced for the use in rheumatoid arthritis. Teriflunomide proved to be effective in mice experimental models of multiple sclerosis (experimental autoimmune encephalomyelitis) delaying the onset and hastening clinical recovery of disease in rats[8]. Teriflunomide confirmed its efficacy and safety profile in clinical trials finally receiving FDA (Food and Drug Administration) approval in 2012 for the treatment of relapsing-remitting multiple sclerosis[9].

Mechanism of action

Teriflunomide exerts its biological function through inhibition of dihydroorotate dehydrogenase (DHODH), a key mitochondrial enzyme in *de-novo* pyrimidine synthesis pathway required by rapidly dividing cells such as proliferating B and T cells[10]. Resting lymphocytes recycle pyrimidines from intracellular pool through an alternative DHODH independent "salvage pathway". Thus, teriflunomide leaves basic homeostatic lymphocyte functions unaffected and limits lymphocyte overactivation contributing to detrimental immune response in multiple sclerosis. Pyrimidines seem to be involved in various biological functions other than DNA and RNA synthesis like lipid and protein glycosylation, phospholipid synthesis and DNA repair which may account for additional immunomodulatory properties of teriflunomide. Teriflunomide also disrupts the JAK-STAT pathway causing downstream reduction of pro-inflammatory cytokines synthesis (TNF and IL-17)[11]. In addition, cyclooxygenase-2 function and intracellular calcium signalling pathway are also affected by teriflunomide, eventually contributing to its mechanism of action[12,13].

Teriflunomide bioavailability after oral administration is nearly 100 per cent and food intake does not alter intestinal absorption. Time to peak plasma concentration ranges from 1 to 4 hours and mean plasma half-life is 10-12 days[14]. Teriflunomide is only moderately metabolized in the liver with limited CYP450 involvement, being largely secreted unchanged in bile and, to a lesser extent, in urine. Besides, teriflunomide undergoes extensive enterohepatic recirculation so that wash-out procedure (*i.e.* cholestyramine or activated charcoal) is necessary when accelerated elimination is needed[15].

Phase III clinical trials

The Teriflunomide Multiple Sclerosis Oral (TEMSO) trial was the first pivotal trial assessing teriflunomide efficacy in a cohort of patient diagnosed with relapsing remitting multiple sclerosis[9]. TEMSO trial was a phase III, randomized, double blind, placebo-controlled, parallel-group study enrolling 1088 patients worldwide. Patients were randomly assigned to placebo, teriflunomide 7 mg daily or teriflunomide 14 mg daily in a 1:1:1 ratio. Primary endpoint was the annualized relapse rate; secondary endpoint was 12-weeks confirmed disability progression. Teriflunomide reduced annualized relapse ratio (ARR) with relative risk reduction of 31.2 and 31.5 per cent for teriflunomide at 7 and 14 mg daily, respectively. Confirmed disability progression occurred in 27.3 per cent of patients with placebo, 21.7 per cent with teriflunomide at 7 mg (P=0.08), and 20.2 per cent with teriflunomide at 14 mg (P=0.03). Teriflunomide also yielded a favourable profile on radiological activity outcome measures such as the number of gadolinium enhancing lesions per scan, unique active lesions per scan and total lesion volume.

These favourable results were confirmed in the Teriflunomide Oral in People with Relapsing-Remitting Multiple Sclerosis (TOWER) study, a randomized, double-blind, placebo-controlled trial enrolling 1169 patients with relapsing-remitting multiple sclerosis in 26 countries[16]. Participants were assigned to placebo, teriflunomide 7 mg or teriflunomide 14 mg daily in a 1:1:1 ratio. Primary endpoint was ARR; secondary endpoint was time to sustained disability progression. Both doses of teriflunomide were superior to placebo in lowering ARR (0.50=placebo vs. 0.37 and 0.32= teriflunomide 7 mg and 14 mg, respectively); but teriflunomide 14 mg was associated with a 31.5 per cent risk reduction of sustained disability progression compared to placebo (log-rank=0.0442) while no effect on disability was noted in the 7 mg group.

The Teriflunomide Versus Placebo in Patients with First Clinical Symptom of Multiple Sclerosis (TOPIC) study assessed efficacy and safety of teriflunomide in delaying conversion to definite multiple sclerosis in a cohort of patients with a first clinical event suggestive of multiple sclerosis[17]. Patients were recruited and randomly assigned to placebo, teriflunomide 7 mg or teriflunomide 14 mg daily. The primary endpoint was time to relapse, while secondary endpoint was time to relapse or time to new gadolinium enhancing lesion

or time to new T2 lesion, whatever occurred first. Teriflunomide at both doses reduced the risk of relapse by 42.6 per cent (teriflunomide 14 mg) and 37.2 per cent (teriflunomide 7 mg) compared to placebo. Secondary endpoint was also met since teriflunomide proved effective in reducing the risk of new relapse or new MRI lesion (risk reduction: 34.9% teriflunomide 14 mg; 31.4% teriflunomide 7 mg).

Thus far, the only head-to-head trial is the Teriflunomide and IFNb-1α (interferon beta-1alpha) in Patients with Relapsing Multiple Sclerosis (TENERE) study comparing teriflunomide at both doses with IFNb-1α 44 mcg three times weekly[18]. Primary endpoint was time to failure as defined by treatment discontinuation for relapse or any other cause. Secondary endpoints were ARR, Fatigue Impact Scale (FIS) and Treatment Satisfaction Questionnaire for Medication (TSQM). Teriflunomide 14 mg failed to show superiority with regards to time to relapse or with ARR (0.259 vs. 0.216 in teriflunomide 14 mg and IFNb-1α, respectively). ARR was significantly higher in the 7 mg group.

Safety and adverse events

Teriflunomide is a safe drug with only mild to moderate treatment adverse events (AE). Safety analysis derived from pooled data of four double blind, placebo-controlled clinical trials has been conducted. Pooled data were obtained from one phase II (NCT01487096) and three phase III trials (TEMSO, TOWER and TOPIC)[19]. Cumulative treatment exposure was >3070 patient-years in each group. Hair thinning, diarrhoea, alanine aminotransferase (ALT) increase, headache and nausea were AEs most commonly reported, being transient and mild to moderate in severity. Yet, discontinuation rate was higher in teriflunomide group at both doses compared to placebo (12.5%= teriflunomide 14 mg; 11.2%= teriflunomide 7 mg; 7.5%= placebo). Alanine aminotransferase increase was the most common reason for discontinuation, even so the proportion of patients with ALT elevation> X3 upper limit of normal (ULN) was similar between groups. Asymptomatic mild ALT elevation< X3 ULN was more common in the teriflunomide group. Decrease in neutrophil and lymphocyte counts was small in magnitude and unrelated to increased infection risk. Besides, mean lymphocyte and neutrophil counts remained within normal ranges in the TEMSO study[9]. Serious infections occurred with similar frequency in all groups (≤ 2.7% of patients). Malignancy occurred in ≤ 0.5 per cent of patients in all groups (14 mg, n=3; 7 mg, n=2; placebo, n=5). A recently published case of fatal toxic epidermal necrolysis in a patient on teriflunomide treatment suggests that strict pharmacovigilance is warranted[20].

Women with childbearing potential

The teratogenic potential of leflunomide and teriflunomide is well documented from animal model studies. Because of limited experience in humans, FDA assigned teriflunomide to pregnancy risk category X. Reliable contraception is needed for females with childbearing potential, women whishing to become pregnant should discontinue the drug and male patients are advised to plan family during treatment since small amounts of the drug are excreted into semen and the degree of transvaginal absorption is not known. Women who become pregnant while taking the drug should undergo accelerated elimination procedure through cholestyramine or activated charcoal washout until teriflunomide plasma level falls below 0.02 g/l which is thought to be relatively safe in pregnancy. Without the washout procedure, it can take up to two years to reach serum concentration as low as 0.02 g/l. In TEMSO trial, 11 pregnancies occurred, followed by four spontaneous and six induced abortions. One patient underwent washout procedure and delivered a healthy newborn[9]. In TOWER study, 14 females became pregnant, 10 underwent induced abortions and four pregnancies (one in the placebo group, two in the 7 mg group, one in the 14 mg group) resulted in live births of healthy babies[10]. A recently published retrospective analysis of pregnancy outcome derived from pharmacovigilance data identified 83 pregnancies in female patients enrolled in teriflunomide clinical trials. Out of these, 70 pregnancies (including one twin pregnancy) were actually exposed to teriflunomide, resulting in 26 live births, 13 spontaneous abortions, and 29 induced abortions. The majority of patients underwent accelerated elimination procedure[21].

ALEMTUZUMAB

Alemtuzumab is a humanized monoclonal antibody recently approved for the treatment of multiple sclerosis[22]. It is directed against the glycoprotein CD52 expressed on immune cells inducing a profound lymphocyte depletion early after administration which is followed by a slow recovery of a different subset of circulating lymphocytes, shifted towards a more tolerogenic profile[23]. Yet, its powerful impact on disease

activity measures is somehow offset by its safety and tolerability profile.

Mechanism of action

Alemtuzumab targets CD52, a 12 amino acid glycoprotein expressed on cell surface of T and B cells and, to a lesser extent, on monocytes, macrophages and eosinophils. Precursor stem cells, mature NK (natural killer) cells and plasma cells show little or no expression of CD52. Despite being largely expressed on lymphocytes, the exact biological function of CD52 is largely unknown; it has been shown to be involved in activation and migration of T lymphocytes and induction of regulatory T cells[24]. A few minutes after alemtuzumab administration a depletion of CD52 positive cells occurs, largely driven by antibody-dependent cell-mediated cytolysis and complement-dependent cytolysis. Cytolysis results in cytokine release and a marked drop of circulating lymphocyte levels. Lymphopenia ensues with nadir cell count at one month in phase III clinical trial but probably occurring as early as a few days after alemtuzumab administration. Then a slow and steady repopulation of a distinct subset of immune cells follows with distinctive time-course: B cells recover by three months followed by CD8 (31 months) and CD4 cells (60 months)[25]. Notably, the recovery of a distinct subset of T and B cells may underpin durable effect of alemtuzumab on disease activity measures as a reflection of sustained reprogramming of the immune system itself.

Clinical trials

Alemtuzumab efficacy and safety was tested against an active comparator in two pivotal phase III clinical trials (CARE-MSI and CARE-MSII)[26,27]. Patients were randomized to receive intravenous (iv) alemtuzumab 12 mg daily for five consecutive days at baseline and 12 mg daily for three days at 12 months, or SC interferon β-1α 44 μg three times a week. A third arm enrolling patients in iv alemtuzumab 24 mg was early discontinued to accelerate recruitment in other treatment arms. Double-blinding was unattainable for definite side effect profile of the two treatment arms. Clinical and efficacy data were gathered through rater-blinded study design. CARE-MSI enrolled treatment naïve RRMS patients while CARE-MSII enrolled patients who had relapsed on prior interferon or glatiramer acetate therapy. Alemtuzumab reduced ARR by 55 per cent in CARE-MSI (P<0.0001) and by 49 per cent in CARE-MSII (P<0.0001) compared to IFNb-1α. Alemtuzumab has been shown superior over interferon on sustained accumulation of disability (SAD) achieving a risk reduction of SAD of 42 per cent (alemtuzumab 13% vs. SC IFNb-1α, 21%; P=0.0084) in CARE-MSII. In CARE-MSI alemtuzumab also yielded a 30 per cent reduction of SAD not reaching statistical significance (P=0.22).This result was most likely due to the far lower than expected rate of progression of disability in the interferon group (11% in CARE-MSI vs. 26% in phase II trial CAMMS223)[28]. In CARE-MSII mean EDSS (Expanded Disability Status Scale) improved by -0.17 from baseline in alemtuzumab group while interferon β-1α group experienced a 0.24 EDSS points deterioration from baseline with a net benefit of 0.41 EDSS points in alemtuzumab group. Alemtuzumab also proved superior to interferon in MRI outcome measures, *i.e.* patients with new or newly enlarging lesions, patients with gadolinium enhancing lesions and the rate of brain volume loss. The extension study of CARE-MSI and CARE-MSII showed sustained efficacy of alemtuzumab with 64 per cent (CARE-MSI) and 55 per cent (CARE-MSII) of patients having NEDA (No evidence of disease activity) at 3-years follow up[29].

Safety and adverse events

Long term safety data are derived from 4-year extension study of pivotal trials (CARE-MS extension) and 12-year follow up of the Cambridge cohort without new safety warnings other than those emerged from phase III trials[30]. Adverse events were reported by 96-98 per cent of patients and serious adverse events occurred in 18-20 per cent of cases in alemtuzumab 12 mg arm of CARE-MSI and CARE-MSII. Most of patients experienced mild to moderate infusion-associated reactions (IARs) including headache, nausea, rash, pyrexia, urticaria, flush and chills. Serious IARs occurred in 3 per cent of cases with tachycardia, bradycardia and palpitations. Some patients may experience transient exacerbation or re-awakening of pre-existing symptoms bolstered by the effect of cytokine release on partially demyelinated pathways[31]. Premedication with iv methylprednisolone 1 g daily for the first three days of any treatment course, antihistamines and antipyretics lower the incidence of IARs. Infections were more common in alemtuzumab group compared to interferon and were mild to moderate in severity, being mostly represented by oral herpes, herpes zoster, urinary tract and upper respiratory tract infections, influenza, bronchitis, and localized superficial mycotic infections. Prophylaxis with oral acyclovir 200 mg twice daily should be

started at the time of alemtuzumab first administration and continued for one month. Two cases of active tuberculosis in patients coming from endemic regions were reported in clinical trials, and one case of *Listeria* meningitis a few days after alemtuzumab infusion was recently reported[32]. Alemtuzumab has also been shown to yield an increase risk of autoimmune diseases (AID) such as thyroid disorders, immune thrombocytopenia (ITP), and Goodpasture disease. Family history of autoimmune diseases and smoking habit seem to be predictive of AID susceptibility[33]. Autoimmune thyroid disorders occurred in 35-38 per cent of patients during the 4-year extension study of CARE-MS trials, with peak incidence at year 3, in line with previous findings from literature[34]. Immune thrombocytopenia cumulative incidence ranged between 1 to 2.5 per cent of cases. Prompt recognition of ITP ensures immediate referral for urgent care, decreasing the risk of poor outcome. In clinical trial 0.3 per cent of patients also suffered from glomerulonephritis (including Goodpasture syndrome) with good recovery of renal function after treatment. Though an increased risk of opportunistic infections and malignancy has not emerged so far, real life long-term safety data are warranted to address this issue.

Pregnancy

Pregnancy outcomes of alemtuzumab exposed women during clinical studies have been recently reported[35]. Despite contraception was required to enter the studies, 139 pregnancies occurred most of which (133/139) >4 months after alemtuzumab exposure. They resulted in 67 live births, 14 elective abortions, 24 miscarriages, one stillbirth, four unknown outcomes, and 29 are ongoing. To date alemtuzumab is assigned to pregnancy risk category C and women are advised to undergo strict contraception up to four months after each treatment cycle[36].

DIMETHYLFUMARATE

Dimethylfumarate (DMF) is a fumaric acid derived chemical compounds whose introduction for moderate to severe forms of psoriasis dates back to 1950s. The understanding of immunomodulatory properties as the basis of its mechanism of action paved the way to its utilization in other immune diseases. After the first pilot study conducted in a small cohort of RRMS patients, DMF has been shown to deeply impact MRI disease activity measures proving a promising therapeutic agent for MS treatment[37].

Mechanism of action

Even though the exact mechanism of action of DMF has not been fully understood and probably affects multiple cellular pathways, DMF enables activation of nuclear factor E2 (erythroid derived 2)-related factor-2 (NRF2) which in turn promotes transcription of many genes involved in the antioxidative stress cell machinery[38]. In addition, DMF induces a shift towards a Th2 profile in lymphocytes and dendritic cells and enhances Th2-related cytokine (IL-4 and IL5) release. Dimethylfumarate is licensed in a delayed release oral formulation known as BG12 to allow it to bypass the stomach and be absorbed in the intestine. When tables are given with a fat meal, mean lag time to peak plasma concentration is about a few hours which is useful to temper gastrointestinal side effects[39].

Clinical trials

Dimethylfumarate was first tested against placebo in a phase III clinical trial, the Determination of the Efficacy and Safety of Oral Fumarate in Relapsing-Remitting Multiple Sclerosis (DEFINE) study[40]. Patients diagnosed with RRMS were randomized to receive BG-12 240 mg twice daily (BID), BG-12 240 mg thrice daily (TID) or placebo for two years. Dimethylfumarate met primary endpoint reducing the proportion of patients who relapsed on DMF treatment compared to placebo (27% BG-12 240 mg BID, 26% BG-12 240 mg TID and 46% placebo, $P<0.001$); ARR at two years dropped to 0.17 in BG-12 240 mg BID and 0.19 in BG-12 240 mg TID compared to 0.36 in the placebo group, with relative risk reduction of 53 and 48 per cent, respectively. Dimethylfumarate also reduced the risk of 12 wk confirmed disability progression compared to placebo by 38 per cent in BG-12 240 mg BID and 34 per cent in BG-12 240 mg TID group, respectively ($P=0.01$). In the second pivotal trial, the Comparator and an Oral Fumarate in Relapsing–Remitting Multiple Sclerosis (CONFIRM) study, BG-12 at both dosages was tested against placebo or open-label active comparator glatiramer acetate[41]. The ARR at two years was lower in BG-12 group (0.22 BG-12 240 mg BID, 0.20 BG-12 240 mg TID) and glatiramer acetate group (0.29) compared to placebo (0.40) ($P=0.01$). Dimetylfumarate and glatiramer acetate also decreased the number of T2W new or newly enlarging lesions, gadolinium enhancing lesions and T1W hypointense lesions compared to placebo. Yet, either BG-12 and glatiramer acetate failed to prove superior to placebo in relative risk

reduction of disability progression (21% BG-12 240 mg BID, 24% BG-12 240 mg TID, 7% glatiramer acetate vs. placebo). Efficacy and safety analysis of pooled data from newly diagnosed RRMS patients enrolled in DEFINE and CONFIRM studies disclosed a more powerful impact on treatment naïve population having received RRMS diagnosis 1 year prior to study entry[42]. In such a cohort DMF seemed to reduce the risk of 12 wk disability progression by 71 per cent in BG-12 240 mg BID and 47 per cent in BG-12 240 mg TID and to lower the ARR by 56 per cent in BG-12 240 mg BID and 60 per cent in TID. In the subgroup undergoing MRI scan BG-12 also yielded a reduction of the number of new/newly enlarging lesions and gadolinium enhancing lesions by 80-92 per cent at both dosages. The ongoing phase III ENDORSE extension study aims at evaluating long-term efficacy and safety of BG-12. Integrated interim analysis of efficacy data from DEFINE, CONFIRM and ENDORSE confirmed sustained low ARR in BID/BID cohort compared to placebo/BID group suggesting greater beneficial effect in early treatment group[43].

Safety and adverse events

Dimethylfumarate exhibited a good tolerability profile in clinical trials suggesting a favourable trade-off between safety and efficacy. Gastrointestinal complaints including diarrhoea, nausea, vomiting and upper abdominal pain were more common in the BG-12 group compared to placebo (27 vs. 17%). Patients in BG-12 group were also more likely to suffer from flushing, compared to placebo (32 vs. 4%)[44]. The incidence of gastrointestinal complaints and flushing peaked in the first three months, declining afterwards. An ongoing trial is set to test usefulness of premedication with aspirin to mitigate gastrointestinal side effects. The incidence of infection was similar between BG-12 and placebo. Infections with cumulative incidence >2 per cent compared to placebo were nasopharyngitis, urinary tract infection, upper respiratory tract infection, bronchitis, sinusitis and gastroenteritis in CONFIRM study. Though no opportunistic infections occurred in clinical trial, five case reports of progressive multifocal leukoencephalopathy (PML) have been reported in four patients taking oral dimethylfumarate for psoriasis and in one patient taking BG-12 for many years after entering DEFINE study[45-49]. Severe lymphopenia is associated with increased risk of PML, though some cases developed with normal total lymphocyte cell count. Thus monitoring for leukocytopenia, lymphocytopenia and fall in specific lymphocyte subpopulations is mandatory for the potential for opportunistic infections in BG-12.

Pregnancy

Dimethylfumarate is labelled as pregnancy risk category C. Pregnancy outcomes from clinical development programme and postmarketing experience showed no association between DMF exposure and increased risk of foetal abnormalities or miscarriages[50]. A total of 45 pregnancy occurred in clinical trials, of which 40 outcomes are known resulting in 27 live births, three miscarriages and 10 elective aborptions. The incidence of pregnancy loss did not differ significantly from that expected in general population.

Conclusive remarks

The tremendous widening of therapeutic arsenal provided neurologists with more valid options to favourably impact natural disease course of multiple sclerosis. This is paralleled by the urgency to address unmet needs: patient selection, risk prediction for serious adverse events, early recognition of treatment failure and need for therapeutic shift. The need to fill this informational gap will pave the way towards the concept of personalized medicine in multiple sclerosis. Every effort should be made to translate recent breakthroughs in multiple sclerosis treatment into a more personalized approach to MS disease.

References

1. Trojano M, Paolicelli D, Zimatore GB, De Robertis F, Fuiani A, Di Monte E, *et al*. The IFNβ treatment of multiple sclerosis (MS) in clinical practice: the experience at the MS Center of Bari, Italy. *Neurol Sci* 2005; *26* (Suppl 4) : S179-82.
2. Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, *et al*. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006; *354* : 889-910.
3. Rudick RA, Stuart WH, Calabresi PA, Confavreux C, Galetta SL, Radue EW, *et al*; SENTINEL Investigators. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med* 2006; *354* : 911-23.
4. Kappos L, Radue EW, O'Connor P, Polman C, Hohlfeld R, Calabresi P, *et al*; FREEDOMS Study Group. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med* 2010; *362* : 387-401.
5. Cohen JA, Barkhof F, Comi G, Hartung HP, Khatri BO, Montalban X, *et al*; TRANSFORMS Study Group. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* 2010; *362* : 402-15.
6. Totaro R, Lugaresi A, Bellantonio P, Danni M, Costantino G, Gasperini C, *et al*. Natalizumab treatment in multiple sclerosis patients: a multicenter experience in clinical practice in Italy. *Int J Immunopathol Pharmacol* 2014; *27* : 147-54.

7. Totaro R, Di Carmine C, Costantino G, Fantozzi R, Bellantonio P, Fuiani A, *et al*. Fingolimod treatment in relapsing-remitting multiple sclerosis patients: a prospective observational multicenter postmarketing study. *Mult Scler Int* 2015; *2015* : 763418, 1-7.
8. Merrill JE, Hanak S, Pu SF, Liang J, Dang C, Iglesias-Bregna D, *et al*. Teriflunomide reduces behavioral, electrophysiological, and histopathological deficits in the Dark Agouti rat model of experimental autoimmune encephalomyelitis. *J Neurol* 2009; *256* : 89-103.
9. O'Connor P, Wolinsky JS, Confavreux C, Comi G, Kappos L, Olsson TP, *et al*; TEMSO Trial Group. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. *N Engl J Med* 2011; *365* : 1293-303.
10. Bruneau JM, Yea CM, Spinella-Jaegle S, Fudali C, Woodward K, Robson PA, *et al*. Purification of human dihydro-orotate dehydrogenase and its inhibition by A77 1726, the active metabolite of leflunomide. *Biochem J* 1998; *336* : 299-303.
11. Haghikia A, Gold R. Multiple sclerosis: TOWER confirms the efficacy of oral teriflunomide in MS. *Nat Rev Neurol* 2014; *10* : 183-4.
12. Hamilton LC, Vojnovic I, Warner TD. A771726, the active metabolite of leflunomide, directly inhibits the activity of cyclo-oxygenase-2 *in vitro* and *in vivo* in a substrate-sensitive manner. *Br J Pharmacol* 1999; *127* : 1589-96.
13. Zeyda M, Poglitsch M, Geyeregger R, Smolen JS, Zlabinger GJ, Hörl WH, *et al*. Disruption of the interaction of T cells with antigen-presenting cells by the active leflunomide metabolite teriflunomide: involvement of impaired integrin activation and immunologic synapse formation. *Arthritis Rheum* 2005; *52* : 2730-9.
14. Limsakun T, Menguy-Vacheron F, Mazarin C. Pharmacokinetics of oral teriflunomide, a novel oral disease-modifying agent under investigation for the treatment of multiple sclerosis. *Programs and abstracts of the 62nd American Academy of Neurology Annual Meeting*; Toronto, ON, Canada; 2010.
15. Limsakun T, Menguy-Vacheron F. Effects of cholestyramine on the elimination of teriflunomide in healthy male volunteers. *Mult Scler* 2010; *16* : 1004 (Abstract).
16. Confavreux, C, O'Connor P, Comi G, Freedman MS, Miller AE, Olsson TP, *et al*; TOWER Trial Group. Oral teriflunomide for patients with relapsing multiple sclerosis (TOWER): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol* 2014; *13* : 247-56.
17. Miller AE, Wolinsky JS, Kappos L, Comi G, Freedman MS, Olsson TP, *et al*; TOPIC Study Group. Oral teriflunomide for patients with a first clinical episode suggestive of multiple sclerosis (TOPIC): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol* 2014; *13* : 977-86.
18. Vermersch P, Czlonkowska A, Grimaldi LM, Confavreux C, Comi G, Kappos L, *et al*; TENERE Trial Group. Teriflunomide versus subcutaneous interferon beta-1a in patients with relapsing multiple sclerosis: a randomised, controlled phase 3 trial. *Mult Scler* 2014; *20* : 705-16.
19. Leist T, Freedman M, Kappos L, Olsson T, Miller A,Wolinsky J, *et al*. Pooled safety analyses from teriflunomide clinical studies. *Neurology* 2015; *84* (Suppl) : P7.268.
20. Gerschenfeld G, Servy A, Valeyrie-Allanore L, de Prost N, Cecchini J. Fatal toxic epidermal necrolysis in a patient on teriflunomide treatment for relapsing multiple sclerosis. *Mult Scler* 2015; *21* : 1476-7.
21. Kieseier BC, Benamor M. Pregnancy outcomes following maternal and paternal exposure to teriflunomide during treatment for relapsing-remitting multiple sclerosis. *Neurol Ther* 2014; *3* : 133-8.
22. Willis MD, Robertson NP. Alemtuzumab for the treatment of multiple sclerosis. *Ther Clin Risk Manag* 2015; *11* : 525-34.
23. Zhang X, Tao Y, Chopra M, Ahn M, Marcus KL, Choudhary N, *et al*. Differential reconstitution of T cell subsets following immunodepleting treatment with Alemtuzumab (anti-CD52 monoclonal antibody) in patients with relapsing-remitting multiple sclerosis. *J Immunol* 2013; *191* : 5867-74.
24. Watanabe T, Masuyama J, Sohma Y, Inazawa H, Horie K, Kojima K, *et al*. CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. *Clin Immunol* 2006; *120* : 247-59.
25. Coles AJ, Cox A, Le Page E, Jones J, Trip SA, Deans J, *et al*. The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol* 2006; *253* : 98-108.
26. Cohen JA, Coles AJ, Arnold DL, Confavreux C, Fox EJ, Hartung HP, *et al*; CARA-MSI Investigators. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* 2012; *380* : 1819-28.
27. Coles AJ, Twyman CL, Arnold DL, Cohen JA, Confavreux C, Fox EJ, *et al*; CARE-MS II Investigators. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* 2012; *380* : 1829-39.
28. CAMMS223 Trial Investigators, Coles AJ, Compston DA, Selmaj KW, Lake SL, Moran S, Margolin DH, *et al*. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med* 2008; *359* : 1786-801.
29. Traboulsee A, Coles A, Cohen J, Compston DA, Fox E, Hartung HP, *et al*. Durable effect of alemtuzumab on MRI outcomes in patients with relapsing-remitting multiple sclerosis who relapsed on prior therapy: 4-year follow-up of CARE-MS II. *Neurology* 2015; *84* (Suppl): P7.249.
30. Tuohy O, Costelloe L, Hill-Cawthorne G, Bjornson I, Harding K, Robertson N, *et al*. Alemtuzumab treatment of multiple sclerosis: long-term safety and efficacy. *J Neurol Neurosurg Psychiatry* 2015; *86* : 208-15.
31. Moreau T, Coles A, Wing M, Isaacs J, Hale G, Waldmann H, *et al*. Transient increase in symptoms associated with cytokine release in patients with multiple sclerosis. *Brain* 1996; *119* : 225-37.
32. Bayas A, Rank A, Naumann M. Listeria meningitis complicating alemtuzumab treatment for multiple sclerosis. *Neurology* 2015, *84* (Suppl) : P3.265.
33. Cossburn M, Pace AA, Jones J, Ali R, Ingram G, Baker K, *et al*. Autoimmune disease after alemtuzumab treatment for multiple sclerosis in a multicenter cohort. *Neurology* 2011; *77* : 573-9.

34. Twyman C, Oyuela P, Palmer J, Margolin D, Dayan C. Thyroid autoimmune adverse events in patients treated with alemtuzumab for relapsing-remitting multiple sclerosis: four-year follow-up of the CARE-MS studies. *Neurology* 2014; *82* (Suppl): P2.199.
35. McCombe P, Achiron A, Brinar V, Margolin DH, Palmer J, Oyuela P, *et al*. Pregnancy outcomes in the alemtuzumab MS clinical development program. Pactrims invited lecture. *Mult Scler* 2015; *21* : 821-2.
36. Lemtrada (alemtuzumab) Summary of product characteristics. Oxford, UK: Gen- zyme Therapeutics; 2013.
37. Schimrigk S, Brune N, Hellwig K, Lukas C, Bellenberg B. Oral fumaric acid esters for the treatment of active multiple sclerosis: an open-label, baseline-controlled pilot study. *Eur J Neurol* 2006; *13* : 604-10.
38. Chen H, Assmann JC, Krenz A, Rahman M, Grimm M, Karsten CM, *et al*. Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE. *J Clin Invest* 2014, *124* : 2188-92.
39. Biogen Idec Inc. Tecfidera (dimethyl fumarate): US prescribing information; 2013. Available from: *http://www.tecfidera.com/pdfs/full-prescribing- information.pdf*, accessed on February 14, 2014.
40. Gold R, Kappos L, Arnold DL, Bar-Or A, Giovannoni G, Selmaj K, *et al*; DEFINE Study Investigators. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. *N Engl J Med* 2012; *367* : 1098-107.
41. Fox RJ, Miller DH, Phillips JT, Hutchinson M, Havrdova E, Kita M, *et al*; CONFIRM Study Investigators. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. *N Engl J Med* 2012; *367* : 1087-97.
42. Gold R, Giovannoni G, Phillips JT, Fox RJ, Zhang A, Meltzer L, *et al*. Efficacy and safety of delayed-release dimethyl fumarate in patients newly diagnosed with relapsing-remitting multiple sclerosis (RRMS). *Mult Scler* 2015; *21* : 57-66.
43. Gold R, Giovannoni G, Phillips T, Fox R, Zhang A, Kurukulasuriya N. Long-term efficacy of delayed-release dimethyl fumarate in newly diagnosed patients with RRMS: an integrated analysis of DEFINE, CONFIRM, and ENDORSE. *Neurology* 2015; *84* (Suppl) : P7.227.
44. Selmaj K, Gold R, Fox RJ, Havrdova E, Giovannoni G, Pace A, *et al*. Flushing and gastrointestinal tolerability events in relapsing remitting multiple sclerosis (RRMS) patients treated with oral BG-12 dimethyl fumarate in the phase 3 DEFINE and CONFIRM trials. *Mult Scler* 2013; *19* : 221.
45. Dammeier N, Schubert V, Hauser TK, Borneman A, Bischof F. Case report of a patient with progressive multifocal leukoencephalopathy under treatment with dimethyl fumarate. *BMC Neurol* 2015; *15* : 108.
46. Ermis U, Weis J, Schulz JB. PML in a patient treated with fumaric acid. *N Engl J Med* 2013; *368* : 1657-8.
47. Nieuwkamp DJ, Murk JL, van Oosten BW, Cremers CH, Killestein J, Viveen MC, *et al*; PML in Dutch MS Patients Consortium. PML in a patient without severe lymphocytopenia receiving dimethyl fumarate. *N Engl J Med* 2015, *372* : 1474-6.
48. Rosenkranz T, Novas M, Terborg C. PML in a patient with lymphocytopenia treated with dimethyl fumarate. *N Engl J Med* 2015; *372* : 1476-8.
49. van Oosten BW, Killestein J, Barkhof F, Polman CH, Wattjes MP. PML in a patient treated with dimethyl fumarate from a compounding pharmacy. *N Engl J Med* 2013; *368* : 1658-9.
50. Li J, Gold R, Fox R, Phillips JT, Havrdova E, Bar-Or A, *et al*. Delayed-release dimethyl fumarate and pregnancy: preclinical studies and pregnancy outcomes from clinical trials and postmarketing experience. *Neurology* 2015; *84* (Suppl) : P7.238

Reprint requests: Dr Rocco Totaro, Multiple Sclerosis Center, Department of Neurology
San Salvatore Hospital, Via Vetoio 1, 67100 L'Aquila, Italy
e-mail: rocco.totaro@univaq.it