DX28

Teriflunomide Mechanism of Action: Linking Preclinical Evidence to Clinical Activity

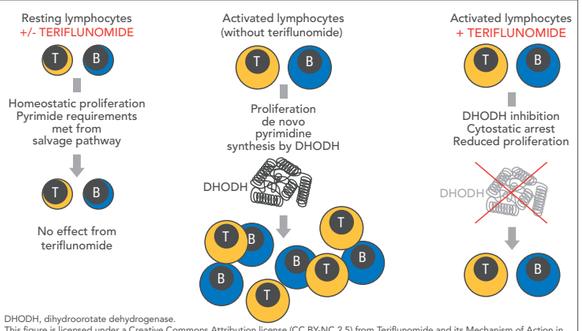
Heinz Wiendl,[1] Andrew R Pachner,[2] Johanne Kaplan,[3] Françoise Menguy-Vacheron,[4] Philippe Truffinet,[5] Amit Bar-Or[6]

[1]University of Münster, Münster, Germany; [2]Dartmouth-Hitchcock Medical Center and Geisel School of Medicine at Dartmouth, Lebanon, NH, USA; [3]Genzyme, a Sanofi company, Framingham, MA, USA; [4]Sanofi, Chilly-Mazarin, France; [5]Genzyme, a Sanofi company, Chilly-Mazarin, France; [6]Montreal Neurological Institute and Hospital, Montreal, QC, Canada

INTRODUCTION

- Teriflunomide is a once-daily oral immunomodulator approved for the treatment of relapsing–remitting MS (RRMS)
- Teriflunomide reversibly inhibits the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), required for de novo pyrimidine synthesis in rapidly dividing lymphocytes, thereby limiting proliferation of activated T and B cells.[1] Teriflunomide has no direct effects on DNA.[1] Through its effect on proliferation, teriflunomide has the potential to limit pathogenic immune responses that can contribute to MS disease activity
- Resting and slowly dividing cells, including lymphocytes and nonlymphoid cells, rely on the pyrimidine salvage pathway to meet their pyrimidine needs.[1] Because this pathway is not affected by teriflunomide, basic homeostatic functions of resting and slowly dividing cells appear to be preserved and immune cells remain available for surveillance (**Figure 1**)

Figure 1. Hypothesized Mechanism of Action of Teriflunomide



DHODH, dihydroorotate dehydrogenase.

OBJECTIVE

- To present the current understanding of teriflunomide's mechanism of action based on preclinical and clinical data

METHODS

Preclinical

- The impact of teriflunomide on the proliferation and viability of stimulated human peripheral blood mononuclear cells was measured in vitro[2]
- The Dark Agouti Experimental Autoimmune Encephalomyelitis (DA EAE) rat model of RRMS was used to assess the effect of teriflunomide on disease progression (neurological scores)[3]
- The DA EAE model was also used to investigate the impact of teriflunomide on central nervous system (CNS) lymphocyte infiltration (measured by flow cytometry of cell preparations and by image analysis of stained sections),[4,5] and on neuronal conduction (transcranial magnetic motor-evoked potentials[3] [tcMMEPs] and somatosensory-evoked potentials[6] [SSEPs])

Clinical

- In the double-blind, placebo-controlled, phase 3 clinical trials TEMSO (NCT00134563) and TOWER (NCT00751881), patients with relapsing MS were randomized (1:1:1) to receive teriflunomide 14 mg, teriflunomide 7 mg, or placebo once daily. Patients received treatment for 108 weeks in the TEMSO trial, and for a variable period of at least 48 weeks in the TOWER trial[7,8]
- The primary endpoint of both trials was annualized relapse rate (ARR). The key secondary endpoint was disability progression, and magnetic resonance imaging (MRI) measures of disease were included as an additional endpoint in TEMSO (MRI measures were not included in TOWER)

RESULTS

Preclinical

- In vitro, teriflunomide inhibited proliferation of stimulated human T and B cells, an effect reversed by the addition of uridine, thereby confirming DHODH dependency (**Table 1**).[4] Teriflunomide had no significant impact on lymphocyte viability[4]

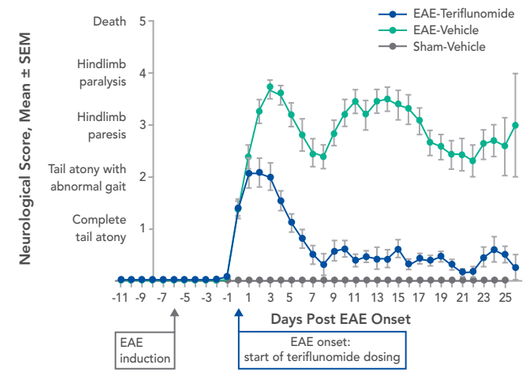
Table 1. Teriflunomide Inhibition of Stimulated Human T- and B-Cell Proliferation In Vitro

	CD4⁺ T Cells	CD8⁺ T Cells	B Cells
Teriflunomide 0 µM	0	0	0
Teriflunomide 0 µM + uridine	0.02 (0.07)	0.83 (0.92)	4.4 (10.05)
Teriflunomide 25 µM	51.39 (5.23)	52.59 (3.31)	60.63 (2.24)
Teriflunomide 25 µM + uridine	1.91 (1.43)	0.87 (1.04)	4.01 (4.33)
Teriflunomide 100 µM	99.64 (0.18)	98.93 (0.54)	70.42 (5.03)
Teriflunomide 100 µM + uridine	7.64 (2.82)	4.13 (0.77)	12.57 (5.05)

Data are presented as mean ± standard error for % inhibition of proliferation in response to anti-CD3 antibody (T cells) or CpG oligonucleotide (B cells), measured by carboxyfluorescein succinimidyl ester dye dilution and flow cytometry.

- DA EAE rats had reduced disease severity at attack, remission, and relapse when teriflunomide was administered prophylactically (not shown) or therapeutically (**Figure 2**)[3]

Figure 2. Neurologic Scores in DA EAE Rats



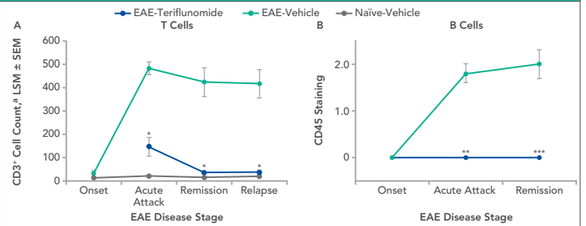
DA EAE, Dark Agouti Experimental Autoimmune Encephalomyelitis; SEM, standard error of the mean.
Mean neurological score (± SEM) of EAE and sham-treated DA rats treated with vehicle or teriflunomide (10 mg/kg/day) therapeutically from onset of EAE disease. n=11–13 animals per group per time point.
Iglesias-Bregna D, Hanak S, Ji Z, Petty M, Liu L, Zhang D, McMonagle-Strucko K. Effects of prophylactic and therapeutic teriflunomide in transcranial magnetic stimulation-induced motor-evoked potentials in the dark agouti rat model of experimental autoimmune encephalomyelitis. *J Pharmacol Exp Ther.* 2013;347(1):203-11. doi: 10.1124/jpet.113.205146.

- In teriflunomide-treated EAE animals, there was a significant reduction in T and B cells (**Figure 3**),[4,5] natural killer cells,[4] and microglial cell numbers in the CNS[4,5] at all stages of disease[4,5]
- Teriflunomide improved sensory and motor functional outcomes in the DA EAE rat model. In this system, both tcMMEP and SSEP, and measures of neuronal conduction, showed that neuronal conduction was preserved with therapeutic teriflunomide treatment (**Figure 4**)[3,6]

Clinical

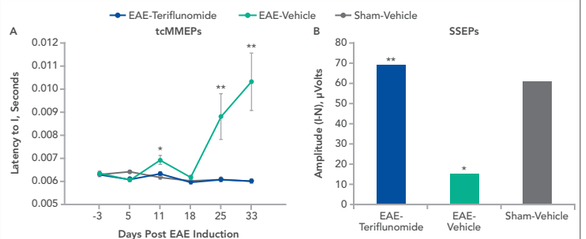
- In the TEMSO and TOWER pivotal clinical studies in patients with relapsing MS, teriflunomide reduced signs of inflammation, as shown by a significant decrease in ARR (**Figure 5**)[7,8]

Figure 3. Staining of DA EAE Rat CNS for T- and B-Cell Markers



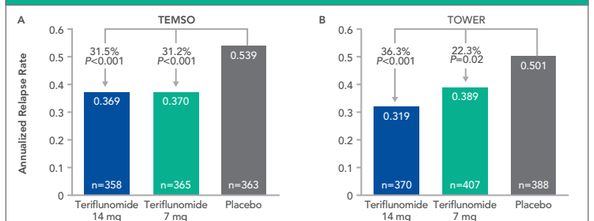
(A) CD3 staining of cervical spinal cord cell preparations, least squares mean (LSM) cell count, error bars show standard error of the mean (SEM). (B) CD45 staining of brain/spinal cord, staining scores for immunopositive cells: 0, none; 1, minimal number; 2, mild number; 3, moderate number. Teriflunomide dose 10 mg/kg/day starting at disease onset (A) or 1 day post EAE induction (B).
CNS, central nervous system; DA EAE, Dark Agouti Experimental Autoimmune Encephalomyelitis.
n=10–15 per treatment group per disease stage. ªTotal/10^5 events. *P<0.0005 EAE-teriflunomide vs EAE-vehicle; **P<0.05 vs EAE-vehicle, ***P<0.01 vs EAE-vehicle.

Figure 4. Measures of Neuronal Conduction in DA EAE Rats



(A) Latency to I in transcranial magnetic motor-evoked potentials (tcMMEPs) recorded at 60% magnetic output, n=11–13 animals per group per time point, except for sham-vehicle rats Day 33; n=7. (B) Amplitude N to I of somatosensory-evoked potentials (SSEPs) collected over a 10 minute period (approximately 21 responses), using slope of the diagonal line between the value for initiation (I) and the negative peak (N); single-pulse paradigm, n=9–12 animals per treatment group. EAE and sham-treated DA rats treated with vehicle or teriflunomide 10 mg/kg/day orally therapeutically, beginning at disease onset.
DA EAE, Dark Agouti Experimental Autoimmune Encephalomyelitis.
*P<0.05 sham-vehicle vs EAE-vehicle, **P<0.05 EAE-teriflunomide vs EAE-vehicle.
(A) Iglesias-Bregna D, Hanak S, Ji Z, Petty M, Liu L, Zhang D, McMonagle-Strucko K. Effects of prophylactic and therapeutic teriflunomide in transcranial magnetic stimulation-induced motor-evoked potentials in the dark agouti rat model of experimental autoimmune encephalomyelitis. *J Pharmacol Exp Ther.* 2013;347(1):203-11. doi: 10.1124/jpet.113.205146.

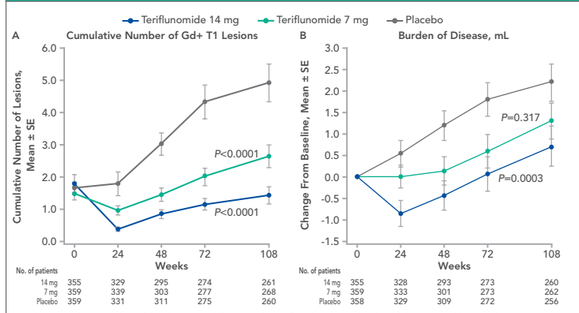
Figure 5. Annualized Relapse Rate in TEMSO and TOWER



Modified intent-to-treat population.
(A) *From: N Engl J Med.* O'Connor P, Wolinsky JS, Confavreux C, Comi G, Kappos L, Olsson TP, Benzerdjeb H, Truffinet P, Wang L, Miller A, Freedman MS; TEMSO Trial Group. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. 365(14):1293-303. Copyright ©2011 Massachusetts Medical Society. Reprinted with permission.

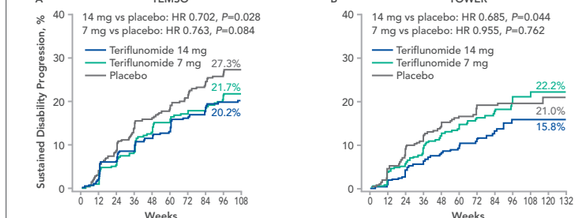
- In TEMSO, where MRI measures of disease were collected, the cumulative number of gadolinium-enhancing lesions and the increase in total lesion volume were significantly lower in patients receiving teriflunomide compared with those receiving placebo (**Figure 6**)[9]
- Consistent benefits of teriflunomide (at the 14-mg dose) were observed on disability progression in both studies. Teriflunomide 7 mg also showed a reduction in the risk of disability progression; this reduction was significant in the TEMSO study (**Figure 7**)[7,8]

Figure 6. MRI Measures of Disease in TEMSO



(A) Mean cumulative number of Gd+ lesions and (B) Mean change from baseline in burden of disease (total lesion volume, mL) in TEMSO.
Gd+, gadolinium-enhancing; MRI, magnetic resonance imaging; SE, standard error.
P values comparing teriflunomide with placebo, calculated from overall reduction in the number of Gd+ lesions per scan (Poisson model)
(A) and mixed-effects model for repeated measures analysis of cubic root transformed data at Week 108 (B).

Figure 7. Disability Progression in TEMSO and TOWER



HR, hazard ratio. Modified intent-to-treat population.
(A) From: *N Engl J Med.* O'Connor P, Wolinsky JS, Confavreux C, Comi G, Kappos L, Olsson TP, Benzerdjeb H, Truffinet P, Wang L, Miller A, Freedman MS; TEMSO Trial Group. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. 365(14):1293-303. Copyright ©2011 Massachusetts Medical Society. Reprinted with permission.
(B) Reproduced from Confavreux C, et al. *Lancet Neurol.* 2014;13(3):247-56. Copyright © 2014, with permission from Elsevier.

CONCLUSIONS

- In vitro data show that teriflunomide inhibits proliferation of stimulated T and B cells
- Inhibition of lymphocyte proliferation, associated with a reduction in the number of lymphocytes in the CNS, may account for the reduction of disease severity and preserved neuronal function observed in teriflunomide-treated EAE rats
- These observations provide insight into the mechanisms underlying the consistent benefits associated with teriflunomide in patients with MS, notably reductions in CNS inflammatory lesions, relapses, and disability progression

REFERENCES

1. Bar-Or A, et al. *Drugs.* 2014: ePub ahead of print. 2. Li L, et al. *J Neuroimmunol.* 2013;265:82-90. 3. Iglesias-Bregna D, et al. *J Pharmacol Exp Ther.* 2013;347:203-211. 4. Ringheim GE, et al. *Front Neurol.* 2013;4:169. 5. Petty M, et al. *Neurology.* 2010;74:9. P05.033. 6. Merrill JE, et al. *J Neurol.* 2009;256:89-103. 7. O'Connor PW, et al. *N Engl J Med.* 2011;365:293-303. 8. Confavreux C, et al. *Lancet Neurol.* 2014;13:247-56. 9. Wolinsky JS, et al. *Mult Scler J.* 2013;19:1310-1319.

Acknowledgments

This poster was reviewed by Pascal Rufi, MD, of Sanofi, and Thierry Aupérin, PhD, of Genzyme, a Sanofi company. Editorial support for this poster was provided by Victoria Lawson, of Fishawack Communications, and was funded by Genzyme.

Disclosures

HW has received consulting fees from Bayer Healthcare, Biogen Idec, EMD Serono, Genzyme, a Sanofi company, Merck Serono, Novartis, sanofi-aventis, Teva; grant/research support from the German Research Foundation (DFG), Interdisciplinary Center for Clinical Research (IZKF) Münster, German Ministry of Education and Research (BMBF); intellectual property rights/patents: patent pending on CD62-L risk prediction. **AP** has received consulting fees from Biogen Idec, Novartis, Genzyme; fees for non-CME services from commercial interest or their agents from Biogen Idec, Novartis; and grant/research support from Sanofi. **JK** and **PT** are employees of Genzyme. **FM-V** is an employee of Sanofi. **AB-O** has received consulting fees from Amplimmune, Aventis, Bayhill Therapeutics, Berlex/Bayer, Biogen Idec, BioMS, Diogenix, Eli-Lilly, EMD Serono, Genentech, Genzyme, GlaxoSmithKline, Guthy-Jackson Greater Good Foundation, Medimmune, Mitsubishi Pharma, Novartis, Ono Pharmacia, Receptos, Roche, Teva Neuroscience, and Wyeth; grant/research support from Amplimmune, Aventis, Bayhill Therapeutics, Berlex/Bayer, Biogen Idec, BioMS, Diogenix, Eli-Lilly, EMD Sereno, Genentech, Genzyme, GlaxoSmithKline, Guthy-Jackson Greater Good Foundation, Medimmune, Mitsubishi Pharma, Novartis, Ono Pharmacia, Receptos, Roche, Teva Neuroscience, and Wyeth.

Presented at the 2014 Annual Meeting of the Consortium of Multiple Sclerosis Centers (CMSC) and the Sixth Cooperative Meeting with Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS), May 28–31, 2014, Dallas, TX, USA
Funding provided by Genzyme, a Sanofi company