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T- and B-Lymphocyte Modulation Associated With Teriflunomide Treatment in Patients With Relapsing-Remitting MS: Analysis of the Phase 3b Teri-DYNAMIC Study

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OBJECTIVE

- **To study the effects of teriflunomide on T and B lymphocytes, and their functions, in patients with relapsing-remitting MS (RRMS)**

INTRODUCTION

- Teriflunomide, a once-daily oral immunomodulator approved for the treatment of patients with RRMS, selectively and reversibly inhibits dihydroorotate dehydrogenase, a key mitochondrial enzyme in de novo pyrimidine synthesis required by rapidly dividing lymphocytes[1]
  - Unlike other currently available oral disease-modifying therapies (DMTs), teriflunomide limits the expansion of activated T and B cells underlying the damaging inflammatory processes associated with MS[2]
  - Resting lymphocytes can still undergo homeostatic proliferation as their pyrimidine requirements are met through the salvage pyrimidine pathway, which is unaffected by teriflunomide[1]
  - In clinical trials of teriflunomide, lymphocyte and neutrophil counts were reduced, on average, by 10%–15% from baseline over a 6–12-week period and remained stable during treatment, with mean counts staying within the normal range ($3.8–10.7 \times 10^9/L$)[3,4]
- Long-term safety observations and results from vaccination studies indicate that the effects of teriflunomide on activated T and B cells do not significantly impact protective immunity[3,5,6]
  - There was no increased risk of malignancy or serious infection observed in patients receiving teriflunomide[3]
  - Individuals treated with teriflunomide mounted an effective immune response to seasonal influenza vaccination,[5] and had seroprotective responses to rabies neoantigen and memory responses to recall antigens[6]
- In a rat experimental autoimmune encephalomyelitis model of MS, teriflunomide treatment significantly reduced the percentages of T cells in the cervical spinal cord and circulating B cells in the peripheral blood mononuclear cell (PBMC) population during acute attack phases[7]
  - Prior to this trial, the effects of teriflunomide on the size and function of lymphocyte subpopulations have not been explored in humans; therefore, investigation of these parameters in patients with RRMS will provide greater insight into the mechanism of action of teriflunomide

METHODS

Study Design

- Teri-DYNAMIC (NCT01863888) is an exploratory, open-label, phase 3b clinical trial that included patients with RRMS, 18–56 years of age, meeting the McDonald 2010 criteria[8] and either:
  - Naïve to DMT/no DMT for ≥2 years; or
  - Received interferon (IFN) B or glatiramer acetate (GA) therapy (with interruption of ≤3 months) and a period of ≥2 weeks without IFNB or GA
- Patients with RRMS were treated with teriflunomide 14 mg for 24 weeks
- Age- and sex-matched healthy individuals (reference population) remained untreated

Sample Collection and Handling

- Blood was collected at baseline, Week 12, and Week 24
- PBMCs were isolated by Ficoll® (Sigma-Aldrich Inc., St Louis, MO, USA) density gradient centrifugation

Sample Analysis

Immune Phenotyping

- Adaptive immune cell subsets were analyzed by flow cytometry as follows:
  - $CD4^+$ T helper, $CD4^+$; Th1, $CD4^+IFN\gamma^+$; Th17, $CD4^+IL–17^+$; natural T regulatory [nTreg], $CD4^+Helios^+FoxP3^+$; induced T regulatory [iTreg], $CD4^+Helios^-FoxP3^+$; T cytotoxic, $CD8^+$; B, $CD19^+$

T-Cell Function

- The effects of teriflunomide treatment on T-cell proliferation and cytokine production were assessed following stimulation in culture as follows:
  - T-cell proliferation was measured by flow cytometry using eFluor670 dye dilution following 72-hour stimulation with anti-CD3/anti-CD28
  - Cytokine production was measured by Luminex assay® following 24-hour stimulation with anti-CD3/anti-CD28.
  - In vitro assay with exogenous teriflunomide: PBMCs collected at baseline, with or without the addition of exogenous teriflunomide (100 µM)
  - Ex vivo assay without exogenous teriflunomide: PBMCs collected at baseline and Week 24

Statistical Methods

- Data for patients with RRMS using comparisons of Week 12 and Week 24 with baseline are presented
- The change from baseline to Week 24 was analyzed using a mixed-effect model with repeated measures, adjusted for baseline
  - Adjusted least squares means (standard error) at Week 24 of changes from baseline are presented
  - Where data did not follow a Gaussian distribution, rank-transformed data were used and median changes from baseline (with interquartile ranges) are presented
  - Owing to the exploratory nature of the trial, no adjustment of *P* values for multiplicity was performed
- The per-protocol population comprised all patients with no major/critical pharmacodynamic-related protocol deviations and with evaluable samples at baseline and Week 24

CONCLUSIONS

- **The effects of teriflunomide on various immune cell types enhance our understanding of the teriflunomide unique mechanism of action, involving both activated T and B cells**
  - **Treatment with teriflunomide in patients with RRMS reduced the absolute numbers and frequencies of total lymphocytes, $CD8^+$ T cells, and $CD19^+$ B cells, consistent with observations from the phase 3 teriflunomide trials, TEMSO, TOWER, and TOPIC.[4] However, total lymphocyte counts remained within normal range throughout the study**
  - **Teriflunomide exerted a selective effect on different $CD4^+$ T-cell subsets, suggesting a shift in T-cell populations from proinflammatory to regulatory, potentially anti-inflammatory, T cells**
- **The contrast between effects on proliferation ex vivo vs in vitro supports a reversible effect of teriflunomide**
- **The results of the Teri-DYNAMIC trial further support the efficacy of teriflunomide as an immunomodulatory agent to treat patients with RRMS**

RESULTS

Participants

- The safety population (n=50) consisted of patients with RRMS with a median (min, max) Expanded Disability Status Scale score of 1.5 (0, 4.0) at baseline and a median time since first symptoms of MS of 6.67 (0.3, 37.0) years
- Most patients (68%) had a recent history of IFNB-1 or GA treatment prior to the protocol-mandated treatment discontinuation (≥2 weeks) and study enrollment
- The per-protocol pharmacodynamic population consisted of 39 patients

Immune Phenotyping

Overall Lymphocyte Populations

- Total lymphocyte counts remained within the clinically normal range after 24 weeks of teriflunomide treatment, although treatment significantly altered absolute cell counts and percentages of immune cell subsets, as follows:
  - Percentage and absolute numbers of lymphocytes were reduced compared with baseline (**Table 1**). However, decline in total lymphocytes was not clinically significant, as lymphocyte counts remained within normal range for adults (1000–4800 cells/µL)[9]
  - Percentage of $CD19^+$ B cells declined compared with baseline (**Table 1**)
  - Relative size of $CD4^+$ population increased, while $CD8^+$ population decreased (**Table 1**), resulting in an elevated $CD4^+/CD8^+$ ratio (median [interquartile range]: 0.6 [–0.2, 3.9], $P=0.008$)

Table 1. Total Lymphocytes and Lymphocyte Subsets in Patients With RRMS After 24 Weeks of Teriflunomide 14 mg (n=39)[10]

Absolute counts (cells/µL)	Total Lymphocytes	$CD4^+$ T Cells	$CD8^+$ T Cells	$CD19^+$ B Cells
Change from baseline[a]	–284.94 (47.3)[c]	–101.14 (30.91)[d]	–34.7 (–55.5, –5.3)[d]	N/A
P value[b]	<0.001	0.002[c]	0.005[c]	
Percentages	**Total Lymphocytes (% of PBMCs)**	**$CD4^+$ T Cells (% of $CD3^+$ Cells)**	**$CD8^+$ T Cells (% of $CD3^+$ Cells)**	**$CD19^+$ B Cells (% of Lymphocytes)**
Change from baseline[b]	–3.05 (1.43)[d]	1.6 (–0.5, 3.7)[d]	–1.0 (–3.5, 0.4)[d]	–2.0 (–4.1, 0)[d]
P value[c]	0.040[d]	0.006[d]	0.009[d]	0.001[d]

[a]Represented as LSM change (SE) or median change (IQR); [b]*P* value from MMRM on change from baseline to Week 24, adjusted for baseline; [c]n=38; [d]n=37. IQR, interquartile range; LSM, least squares mean; MMRM, mixed-effect model for repeated measures; PBMC, peripheral blood mononuclear cell; RRMS, relapsing-remitting MS; SE, standard error.

Regulatory and T-Helper Cells

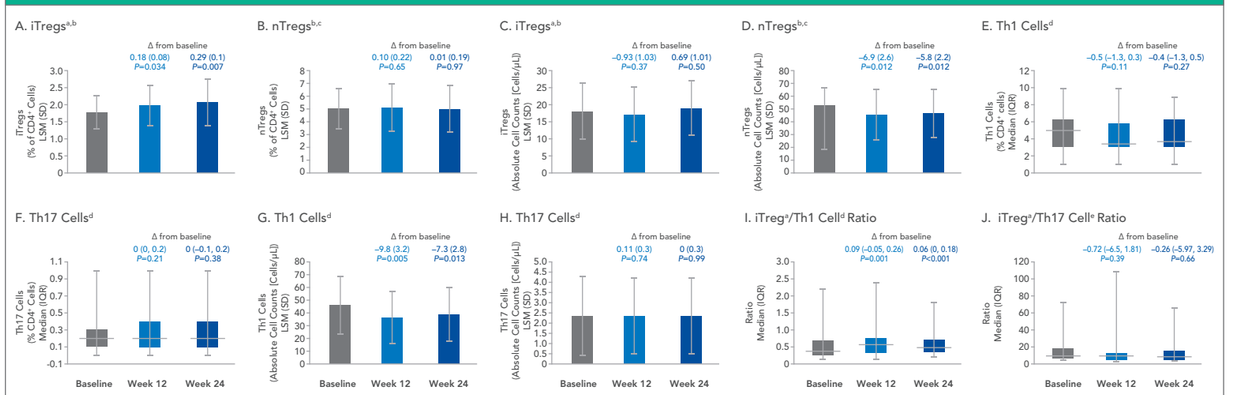
- Within the $CD4^+$ T-cell population, teriflunomide treatment resulted in significant changes from baseline through:
  - Increased percentage of iTregs ($Helios^-FoxP3^+$, **Figure 1A**)
  - Decreased absolute counts of nTregs ($Helios^+FoxP3^+$, **Figure 1D**)
  - Decreased absolute counts of Th1 cells (**Figure 1G**)
  - No significant change in percentage or absolute count of Th17 cells (**Figures 1F** and **1H**)
  - Elevated iTreg/Th1 ratio (**Figure 1I**)

T-Cell Function

Proliferation

- Inclusion of teriflunomide in the in vitro proliferation assays led to significant reductions in the proliferation of both $CD4^+$ and $CD8^+$ T cells (**Table 2**)
- However, ex vivo assays demonstrated that there were no significant changes in proliferation in isolated T cells following teriflunomide treatment when comparing baseline with Week 24 (**Table 2**)

Figure 1. Evolution and Change From Baseline in $CD4^+$ T-Cell Subpopulations in Patients With RRMS After 12 and 24 Weeks of Teriflunomide 14 mg (n=39)[10]



[a]$Helios^-FoxP3^+$; [b]n=38; [c]$Helios^+FoxP3^+$; [d]n=32; [e]n=30. Bar graphs represent mean and error bars show SD (Figures A–D, G, H). Box plots represent IQR, with horizontal line indicating median, and error bars showing maximum and minimum values (Figures E, F, I, J). Per-protocol population, patients with RRMS treated with teriflunomide 14 mg. *P* value calculated from MMRM on change from baseline, performed on raw or rank data. Δ, change; IQR, interquartile range; iTregs, inducible T-regulatory cells; LSM, least squares means; MMRM, mixed-effect model for repeated measures; nTregs, natural T-regulatory cells; RRMS, relapsing-remitting MS; SD, standard deviation; SE, standard error.

Table 2. Proliferation of T Cells From Patients With RRMS In Vitro at Baseline and Ex Vivo After 24 Weeks of Teriflunomide 14 mg (n=39)[10]

Experimental Conditions	$CD4^+$ T Cells[a]	$CD8^+$ T Cells[a]
In vitro assays (n=25)		
Change with addition of exogenous teriflunomide 100 µM		
LSM (SE)	–32.84 (1.84)	–32.15 (2.15)
P value[b]	<0.0001	<0.0001
Ex vivo assays (n=33)		
Change from baseline to Week 24		
LSM (SE)	–2.1 (1.36)	–1.53 (1.01)
P value[b]	0.132	0.138

Cells were stimulated with anti-CD3/CD28 for 72 hours. [a]As % of total proliferating cells; [b]*P* value calculated from MMRM on change from baseline, performed on raw data. LSM, least squares mean; MMRM, mixed-effect model with repeated measures; RRMS, relapsing-remitting MS; SE, standard error.

Cytokine Production

- In vitro exposure to teriflunomide significantly reduced T-cell production of pro-inflammatory cytokines IL-2, TNFα, GM-CSF, IFN-γ, and IL-1β (**Table 3**)
- Compared with baseline, after 24 weeks of treatment with teriflunomide 14 mg, T-cell production of IL-2 and TNFα were suppressed, whereas production of GM-CSF, IFN-γ, and IL-1β did not differ (**Table 3**)

Table 3. Cytokine Production (pg/mL) by T Cells From Patients With RRMS In Vitro at Baseline and Ex Vivo After 24 Weeks of Teriflunomide 14 mg (n=39)[10]

Experimental Conditions	IL-2	TNFα	GM-CSF	IFN-γ	IL-1β
In vitro assays (n=19)					
Change with addition of exogenous teriflunomide 100 µM					
Median (IQR)	–310.0 (–493, –151)	–1252.9 (17.9)[a]	–235.0 (–313, –94)	–624.0 (–1531, –73)	–3.0 (–34, 0)
P value[b]	<0.0001	<0.0001	<0.0001	<0.001	<0.001
Ex vivo assays (n=26)					
Change from baseline to Week 24					
Median (IQR)	–147.0 (–505, 106)	–459.6 (106.8)[a]	–60.0 (–224, 76)	–200.0 (–2172, 92)	0 (–10, 15)
P value[b]	0.002	<0.001	0.305	0.099	0.950

Cells (100,000 per well) were stimulated with anti-CD3/CD28 for 24 hours. [a]Data represent LSM (SE); [b]*P* value calculated from MMRM on change from baseline, performed on raw or rank data. GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IQR, interquartile range; LSM, least squares means; MMRM, mixed-effect model with repeated measures; RRMS, relapsing-remitting MS; SE, standard error; TNF, tumor necrosis factor.

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Teriflunomide is approved in many countries, including the US and the European Union, for the treatment of relapsing multiple sclerosis or relapsing-remitting multiple sclerosis.
This material may contain information that is outside of the approved labeling in some countries.

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