Poster No. 32

Effect of evobrutinib, a Bruton's tyrosine kinase inhibitor, on immune cell and immunoglobulin levels over 48 weeks in a phase 2 study in relapsing multiple sclerosis

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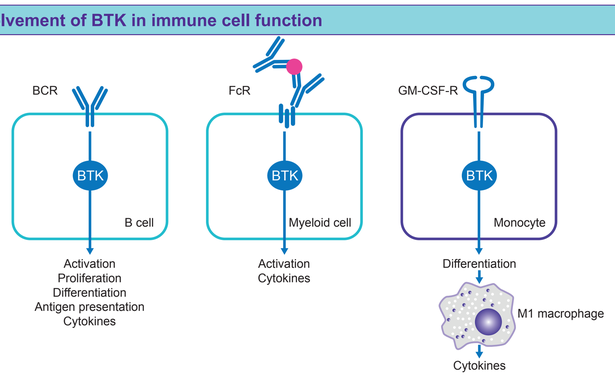
INTRODUCTION

- Bruton's tyrosine kinase (BTK) is expressed in B cells, macrophages, and myeloid cells, but not in T cells.[1]
- BTK deficiency in humans leads to X-linked agammaglobulinaemia, which is characterized by nearly complete loss of serum immunoglobulins (Ig) and circulating B cells,[2] while targeted deletion of *Btk* in knockout mice results in defects in B cell development and proliferation.[3]
- BTK is involved in both the adaptive and innate immune responses and mediates signaling through the B cell receptor (BCR), Fcγ receptor (FcγR), and GM-CSF receptor (**Figure 1**).[2]
- BTK plays an important role in pro-inflammatory pathways potentially involved with multiple sclerosis (MS).[4]
- Evobrutinib, a highly selective BTK inhibitor, has a dual mechanism of action, impacting both the adaptive and innate immune response through inhibition of BCR, FcγR and GM-CSF receptor signaling.[5,6]
- Evobrutinib inhibits primary B cell responses, such as proliferation and antibody and cytokine release, without directly affecting T cells. Indirect effects on pathological T cells may be mediated by BTK inhibition through the blocking of the B cell antigen presentation function.[7]
- Evobrutinib is the first BTK inhibitor to demonstrate clinical efficacy in MS in a Phase 2 study.[8]
- Here we report the effect of evobrutinib on B cells and immunoglobulins (Ig) and other immune cells in MS patients over 48 weeks.

Figure 1. Involvement of BTK in immune cell function

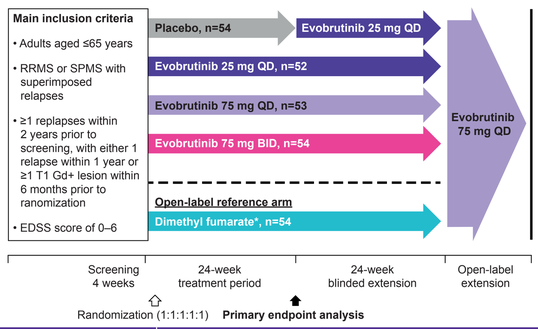


BCR, B cell receptor; **BTK,** Bruton's tyrosine kinase; **FcR,** Fc receptor; **GM-CSF-R,** Granulocyte-macrophage colony stimulating factor receptor.

METHODS

- Patients (18–65 years) with active relapsing-remitting MS or secondary progressive MS with superimposed relapses were randomized to receive double-blind evobrutinib 25 mg QD, 75 mg QD, 75 mg BID, placebo, or open-label dimethyl fumarate 240 mg (reference arm) (**Figure 2**).
- After 24 weeks, placebo-treated patients were switched to evobrutinib 25 mg QD; other treatment arms continued under original allocation.
  - After the 24-week blinded extension, there was an optional open-label extension (not a focus for the analysis presented here).
- Safety of evobrutinib, including assessment of B cell numbers and Ig levels, was a key secondary endpoint; investigations of the effects of evobrutinib on B cell subsets, T cell subsets, and natural killer (NK) cells in peripheral blood over 48 weeks were exploratory (**Table 1**).
- Assessments made at early treatment discontinuation were assigned to planned visits via windowing, prior to descriptive statistics and modeling.

Figure 2. Study design



Immune cell and Ig assessments	Visit					
	Day 1	Week 4	Week 16	Week 24	Week 48†	Week 52‡
Total B cell numbers	✓	✓		✓	✓	✓
Ig levels	✓	✓	✓	✓	✓	
B cell subtypes/NK cells/T cells and subtypes	✓	✓		✓	✓	✓

*120 mg BID for the first 7 days followed by 240 mg BID for the duration of treatment.
†Assessment either at Week 48 or premature end of treatment.
‡Assessment either at Week 52 or premature end of trial.

Table 1. Immune cell markers

Assay	Cell subset	Markers
TBNK Assay	T cells	CD3+
	T helper cells	CD3+CD4+
	Cytotoxic T cells	CD3+CD8+
	B cells	CD3–CD19+
	NK cells	CD3−CD56+/CD16+
B Cell & Plasma Subsets Assay	B cells	CD45+CD3–CD19+
	Mature-Naïve B cells	CD19+CD20+IgD+CD27−
	Memory B cells	CD19+CD20+IgD−CD27+CD38−

RESULTS

Patients

- Of 267 patients randomized, 227 patients completed 48 weeks of treatment.

B cells

- No clinically relevant changes were observed in the number of total B cells (**Figure 3A**), or subsets of memory B cells (**Figure 3B**) or mature-naïve B cells (**Figure 3C**) over 48 weeks.
  - For total B cells, the mean changes from baseline (CFB) at Week 48 for evobrutinib 75 mg QD (−61 cells/µL) and 75 mg BID (−47 cells/µL) were both numerically greater than that for placebo/evobrutinib 25 mg QD (−11 cells/µL) (**Table 2**).
  - There was no clear pattern to memory B cells mean CFB at Week 48 (**Table 2**).
  - Mature-naïve B cells showed a similar pattern to total B cells, with numerically greater decreases (mean CFB at Week 48) for evobrutinib 75 mg QD (−48 cells/µL) and 75 mg BID (−30 cells/µL) compared to placebo/evobrutinib 25 mg (−12 cells/µL) (**Table 2**).
  - Similar patterns were observed with the mixed effect model for repeated measures analysis (**Table 4**).

Table 2. B cells change from baseline at Week 48

Cells/µL, mean ± SD		Placebo/ Evobrutinib 25 mg (N=54)	Evobrutinib			DMF (N=54)
			25 mg QD (N=52)	75 mg QD (N=53)	75 mg BID (N=54)	
Total B cells	Baseline	209±134.4	178±82.9	215±157.9	206±123.1	191±82.8
	CFB at W48	−11±101.7	−22±76.7	−61±193.6	−47±130.6	−41±92.2
Memory B cells	Baseline	24±19.2	22±17.8	24±22.2	24±23.4	23±17.2
	CFB at W48	2±14.0	−3±18.0	3±39.8	−3±22.7	−12±11.4
Mature-naïve B cells	Baseline	138±108.7	111±66.3	141±114.2	127±89.0	121±60.0
	CFB at W48	−12±80.8	−10±54.6	−48±113.6	−30±97.5	−10±74.3

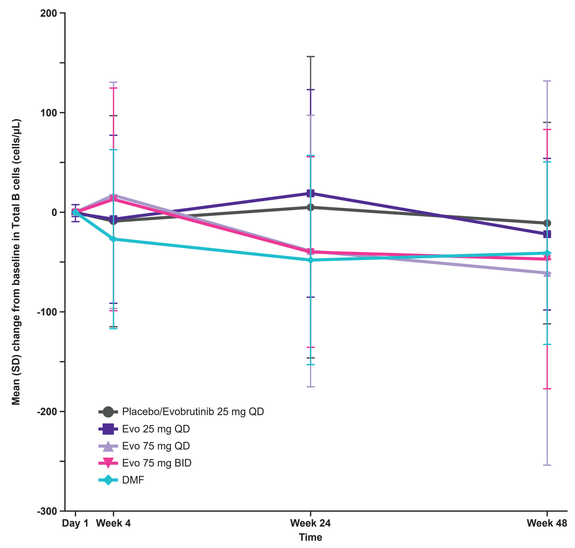
CFB, change from baseline; **DMF,** dimethyl fumarate.

Other immune cells

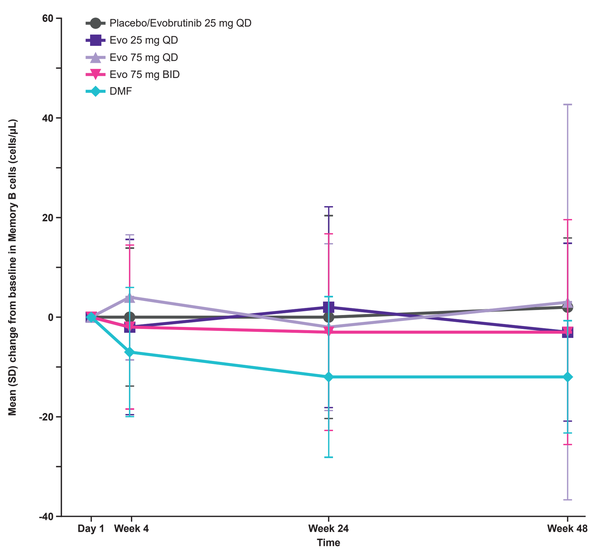
- The numbers of total T, helper T, cytotoxic T, and NK cells, showed no statistically significant changes over 48 weeks (data not shown).

Figure 3. Changes in total B cells, memory B cells and mature-naïve B cells over 48 weeks

A. Total B cells



B. Memory B cells



C. Mature-naïve B cells

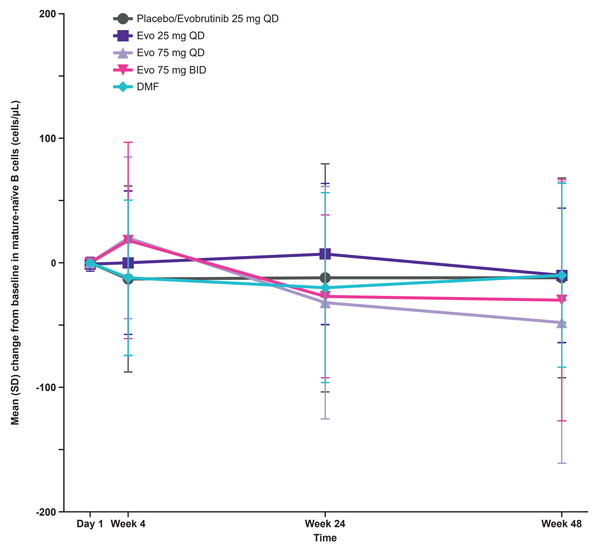
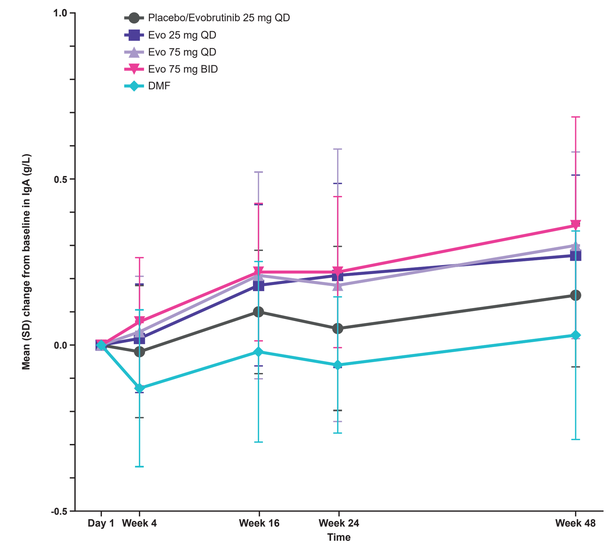
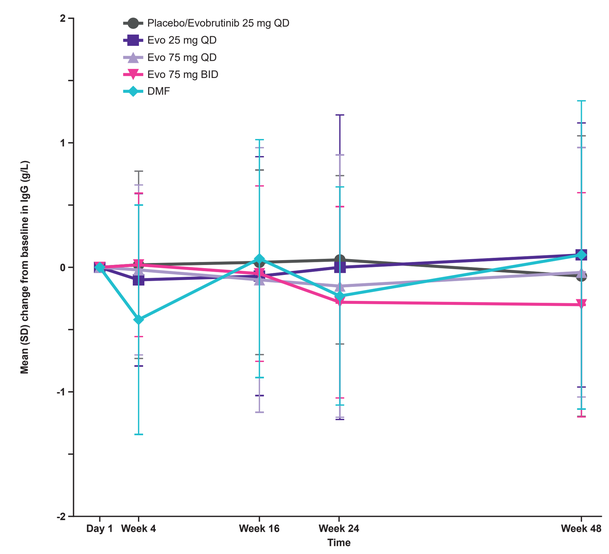


Figure 4. Changes in levels of IgA, IgG, and IgM over 48 weeks

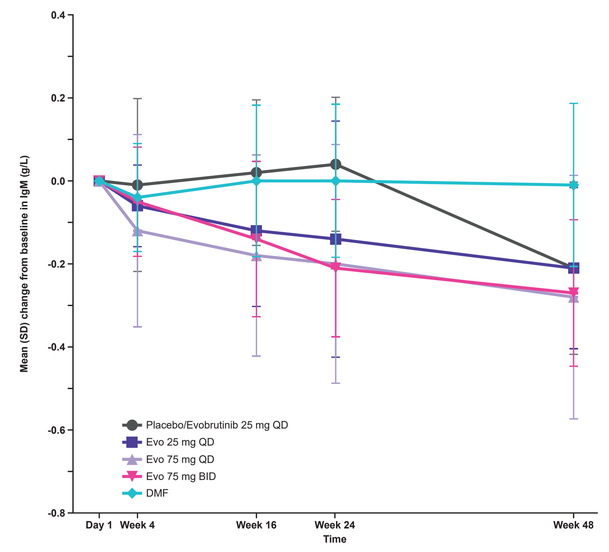
A. IgA



B. IgG



C. IgM



Immunoglobulins

- No clinically relevant changes in IgG levels were observed over 48 weeks (**Figure 4B; Table 3**).
  - The evobrutinib 75 mg BID arm showed larger numerical decreases in IgG levels at Week 48 than did the other arms
- No significant changes in the levels of IgG subtypes were observed over 48 weeks (**Table 3**).
- At Week 48, there were slight increases from baseline in IgA (**Figure 4A; Table 3**), and reductions in IgM (**Figure 4C; Table 3**) in all evobrutinib groups that were numerically greater than those in the placebo/evobrutinib 25 mg QD or DMF arms.

Table 3. Immunoglobulins change from baseline at Week 48

g/L, mean ± SD		Placebo/ Evobrutinib 25 mg (N=54)	Evobrutinib			DMF (N=54)
			25 mg QD (N=52)	75 mg QD (N=53)	75 mg BID (N=54)	
IgA	Baseline	1.99±0.777	1.89±0.771	1.90±0.722	1.87±0.675	2.03±0.763
	CFB at W48	0.15±0.218	0.27±0.244	0.30±0.283	0.36±0.329	0.03±0.316
IgM	Baseline	1.42±0.692	1.27±0.547	1.44±0.716	1.33±0.684	1.27±0.589
	CFB at W48	−0.21±0.209	−0.21±0.196	−0.28±0.295	−0.27±0.178	−0.01±0.198
IgG	Baseline	9.61±1.897	9.46±2.138	9.81±1.841	9.62±1.960	9.47±1.839
	CFB at W48	−0.07±1.132	0.10±1.066	−0.04±1.007	−0.30±0.905	0.10±1.244
IgG_1	Baseline	5.22±1.241	5.15±1.401	5.21±1.190	5.12±1.327	5.03±1.155
	CFB at W48	−0.18±0.661	0.01±0.750	−0.13±0.941	−0.31±0.568	0.08±0.882
IgG_2	Baseline	3.72±1.161	3.48±1.323	3.77±1.212	3.73±0.991	3.62±1.133
	CFB at W48	0.17±0.615	0.23±0.506	0.23±0.538	0.17±0.497	0.14±0.490
IgG_3	Baseline	0.59±0.253	0.48±0.228	0.58±0.228	0.59±0.305	0.58±0.256
	CFB at W48	0.04±0.173	0.03±0.103	0.02±0.131	0.01±0.108	0.05±0.156
IgG_4	Baseline	0.42±0.304	0.38±0.288	0.37±0.268	0.48±0.313	0.41±0.250
	CFB at W48	0.02±0.149	0.06±0.148	0.07±0.113	0.05±0.120	0.04±0.117

Table 4. B cells and immunoglobulins change from baseline at Week 48 (MMRM analysis)

LSM of Week 48 CFB (95% CI)[a]	Placebo/ Evobrutinib 25 mg (N=54)	Evobrutinib		
		25 mg QD (N=52)	75 mg QD (N=53)	75 mg BID (N=54)
Total B cells (cells/µL)	0.66 (−19.83, 21.16)	−17.93 (−38.96, 3.11)	−19.07 (−39.57, 1.42)	**−25.00 (−45.33, −4.66)**
Memory B cells (cells/µL)	0.90 (−3.34, 5.15)	−1.11 (−5.44, 3.21)	1.06 (−3.21, 5.33)	−2.43 (−6.68, 1.81)
Mature-naïve B cells (cells/µL)	−6.47 (−19.75, 6.81)	−11.16 (−24.78, 2.47)	−13.00 (−26.28, 0.29)	**−14.89 (−28.03, −1.75)**
IgA (g/L)	**0.06 (0.01, 0.11)**	**0.17 (0.11, 0.22)**	**0.17 (0.12, 0.23)**	**0.22 (0.17, 0.28)**
IgG (g/L)	−0.00 (−0.19, 0.18)	−0.01 (−0.20, 0.17)	−0.10 (−0.29, 0.08)	−0.15 (−0.33, 0.03)
IgM (g/L)	−0.03 (−0.08, 0.01)	**−0.14 (−0.18, −0.09)**	**−0.19 (−0.23, −0.14)**	**−0.16 (−0.20, −0.12)**

[a]MMRM model for CFB in score includes fixed effects for treatment, visit (Weeks 4, 24, and 48 for B cells and Weeks 4, 16, 24, and 48 for Ig) and treatment-by-visit interaction, a covariate for parameter value at baseline and unstructured covariance matrix for repeated measures.
LSM, least squares mean; **MMRM,** Mixed effect Model for Repeated Measures.

CONCLUSIONS

- **MS patients treated with the BTK inhibitor, evobrutinib, showed no evidence of clinically relevant changes in memory or mature-naive B cell subsets over 48 weeks**
- **IgG levels remained relatively stable over 48 weeks, though slight elevations in IgA levels and reductions in IgM levels were observed with evobrutinib over 48 weeks**
- **These new results demonstrate that, in contrast to genetic deficiency of BTK, continued pharmacological BTK inhibition does not lead to B cell depletion or significant reductions in circulating immunoglobulins over 48 weeks of treatment**
- **Overall, these findings may have favorable implications for the relative safety of evobrutinib in MS compared with other B cell targeting therapies; however, this will be further investigated in larger numbers of patients with longer follow up in the Phase 3 trial programme**

REFERENCES

1. Brunner C, et al. *Histol Histopathol* 2005;20:945–55
2. Tsukada S, et al. *Cell* 1993;72:279–90
3. Khan WN, et al. *Immunol Res* 2001;23:147–56
4. Gregson A, et al. *F1000Research* 2019;8(F1000 Faculty Rev):245
5. Satterthwaite AB, et al. *Semin Immunol* 1998;10:309–16
6. Haselmeyer P, et al. *J Immunol* 2019;202:2888–906
7. Jelcic I, et al. *Cell* 2018;175:85–100
8. Montalban X, et al. *N Engl J Med* 2019;380:2406–19

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DISCLOSURES

XM has received speaking honoraria and travel expenses for participation in scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past 3 years with Biogen, Merck Serono, Sanofi-Genzyme, Novartis, Sanofi-Aventis, Teva Pharmaceuticals, Roche, Celgene, Actelion, NMSS, MSIF and Excemed. **MSW** receives research support from the Deutsche Forschungsgemeinschaft (DFG; WE 3547/5-1), from Novartis, TEVA, Biogen-Idec, F. Hoffmann-La Roche, Merck and the ProFutura Programm of the Universitätsmedizin Göttingen. MS Weber is serving as an editor for PLoS One. He received travel funding and/or speaker honoraria from Biogen-Idec, Merck Serono, Novartis, Roche, TEVA, Bayer and Genzyme. **JS, SS, FD, ECM,** and **RG** are employed by EMD Serono Research & Development Institute, Inc. (an affiliate of Merck KGaA, Darmstadt, Germany), Billerica, MA, USA

Evobrutinib is currently under clinical investigation and has not been approved by any regulatory authority.

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