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Poster No. P095

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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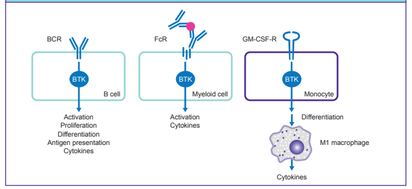
INTRODUCTION

- 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 agammaglobulinemia, which is characterized by nearly complete loss of serum immunoglobulins 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 granulocyte–macrophage colony-stimulating factor (GM-CSF) receptor (**Figure 1**).[2]

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.

- BTK plays an important role in proinflammatory 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 pathologic 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]

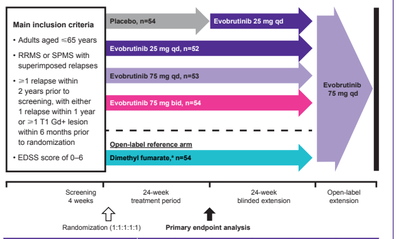
OBJECTIVE

- To report the effect of evobrutinib on B cells, immunoglobulins, and other immune cells in patients with MS over 48 weeks.

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 once daily (qd), 75 mg qd, 75 mg twice daily (bid), placebo, or open-label dimethyl fumarate (DMF) 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.
- Safety of evobrutinib, including assessment of B-cell numbers and immunoglobulin 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 immunoglobulin assessments	Visit					
	Day 1	Week 4	Week 16	Week 24	Week 48[b]	Week 52[c]
Total B-cell numbers	✓	✓		✓	✓	✓
Immunoglobulin levels	✓	✓	✓	✓	✓	✓
B cells and subtypes/NK cells/T cells and subtypes	✓	✓		✓	✓	✓

[a]120 mg bid for the first 7 days followed by 240 mg bid for the remainder of treatment.
[b]Assessment either at Week 48 or premature end of treatment.
[c]Assessment either at Week 52 or premature end of trial.
bid, twice daily; **EDSS**, Expanded Disability Status Scale; **Gd+**, gadolinium-enhancing; **NK**, natural killer; **qd**, once daily; **RRMS**, relapsing–remitting MS; **SPMS**, secondary progressive MS.

Table 1. Immune cell markers

Assay	Cell subset	Markers
T-cell, B-cell, and NK-cell panel	T cells	CD3+
	Helper T cells	CD3+CD4+
	Cytotoxic T cells	CD3+CD8+
	B cells	CD3–CD19+
	NK cells	CD3–CD56+/CD16+
B-cell and plasma subsets	B cells	CD45+CD3–CD19+
	Mature-naïve B cells	CD19+CD20+IgD+CD27–
	Memory B cells	CD19+CD20+IgD–CD27+CD38–

CD, cluster of differentiation; **NK**, natural killer.

RESULTS

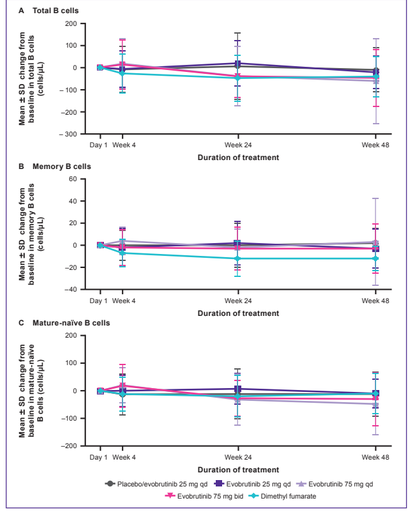
Patients

- 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 in the number of memory B-cell (**Figure 3B**) or mature-naïve B-cell (**Figure 3C**) subsets 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 the mean CFB for placebo/evobrutinib 25 mg qd (−11 cells/μL) (**Table 2**).
  - There was no clear pattern in the mean CFB for memory B cells 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 with placebo/evobrutinib 25 mg (−12 cells/μL) (**Table 2**).
  - Similar patterns were observed with the mixed-effects model for repeated measures analysis (**Table 4**).

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



bid, twice daily; **qd**, once daily; **SD**, standard deviation.

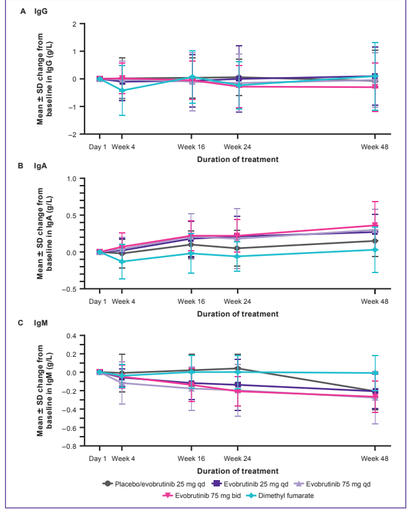
Other immune cells

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

Immunoglobulins

- No clinically relevant changes in immunoglobulin G (IgG) levels were observed over 48 weeks (**Figure 4A; 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 4B; Table 3**) and decreases in IgM (**Figure 4C; Table 3**) levels in all evobrutinib groups that were numerically greater than those in the placebo/evobrutinib 25 mg qd or DMF arms.

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



bid, twice daily; **Ig**, immunoglobulin; **qd**, once daily; **SD**, standard deviation.

Table 2. B cells: change from baseline at Week 48

Cell type	Cells/μL, mean ± SD	Placebo/ evobrutinib 25 mg qd (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

bid, twice daily; **CFB**, change from baseline; **DMF**, dimethyl fumarate; **qd**, once daily; **W**, Week.

Table 3. Immunoglobulins: change from baseline at Week 48

Ig type	g/L, mean ± SD	Placebo/ evobrutinib 25 mg qd (n=54)	Evobrutinib			DMF (n=54)
			25 mg qd (n=52)	75 mg qd (n=53)	75 mg bid (n=54)	
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
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

bid, twice daily; **CFB**, change from baseline; **DMF**, dimethyl fumarate; **Ig**, immunoglobulin; **qd**, once daily; **W**, Week.

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

LSM of Week 48 CFB (95% CI)*	Placebo/ evobrutinib 25 mg qd (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)**

Values in **bold** denote statistical significance.
*MMRM analysis 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 immunoglobulins), and treatment-by-visit interaction, a covariate for parameter value at baseline and unstructured covariance matrix for repeated measures.
bid, twice daily; **CFB**, change from baseline; **CI**, confidence interval; **Ig**, immunoglobulin; **LSM**, least squares mean; **MMRM**, mixed-effect model for repeated measures; **qd**, once daily.

CONCLUSIONS

- **Patients with MS who were treated with the BTK inhibitor evobrutinib showed no evidence of clinically relevant changes in memory or mature-naïve B-cell subsets over 48 weeks.**
- **IgG levels remained relatively stable over 48 weeks, although slight elevations in IgA levels and reductions in IgM levels were observed with evobrutinib over 48 weeks.**
- **These results demonstrate that, in contrast to genetic deficiency of BTK, continued pharmacologic 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 program.**

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DISCLOSURES

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

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

Status: February 2020

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