Efficacy of belimumab and targeting of rheumatoid factor-positive B-cell expansion in Sjögren's syndrome: follow-up after the end of the phase II open-label BELISS study

L. Quartuccio[1], S. Salvin[1], L. Corazza[1], S. Gandolfo[1], M. Fabris[2], S. De Vita[1]

[1]Rheumatology Clinic, Department of Medical and Biological Sciences, [2]Institute of Pathology, Department of Medical and Biological Sciences, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy.

Luca Quartuccio, MD, PhD
Sara Salvin, MD
Laura Corazza, MD
Saviana Gandolfo, MD
Martina Fabris, MD
Salvatore De Vita, MD, Prof.

Please address correspondence to:
Prof. Salvatore De Vita,
Clinica di Reumatologia,
Dipartmento di Scienze Mediche e Biologiche,
Azienda Ospedaliero-Universitaria S. Maria della Misericordia,
Piazzale Santa Maria Misericordia 1, 33100 Udine, Italy.
E-mail: devita.salvatore@aoud.sanita.fvg.it

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ABSTRACT

Objective. *Belimumab, a monoclonal anti-B lymphocyte stimulator (BLyS) antibody, appeared effective in Sjögren's syndrome (SS) in the phase II open-label 52-week BELISS study. Herein, the follow-up after the end of the BELISS study and suspension of the drug was reported in order to further verify the efficacy of belimumab in SS.*

Methods. *13 SS patients were followed after the end of the belimumab treatment. The patients were all female, aged 54±15 years; all the patients presented anti-SSA and/or anti-SSB positivity. Composite scores for SS disease activity were collected at month 6 and month 12 after the end of the trial. The changes of IgG, IgA, IgM immunoglobulin serum levels, and rheumatoid factor (RF) level were reported. BLyS serum levels were also analysed. Statistics for paired comparisons were used.*

Results. *ESSDAI score increased from 3.5±3.7 at week 52 (end of the trial) to 7.0±5.7 at month 12 after the end of the trial (p=0.003). RF level increased from 31.0 (8.0–224.6) IU/ml at week 52 to 69 (11–666) IU/ml at month 12 after the end of the trial (p=0.008). IgM level increased from 131.9±73.6 mg/dl at week 52 to 165±84.6 mg/dl at month 12 after the end of the trial (p=0.04). A significant increase of serum BLyS levels also increased from 1304 (667–3835) pg/ml at week 52 to 2882 (1353–6178) pg/ml twelve months after belimumab suspension (p=0,04).*

Conclusion. *Targeting BLyS by belimumab appears effective in SS, with the inhibition of RF-positive B cell proliferation.*

Introduction

Belimumab, a monoclonal anti-B lymphocyte Stimulator (BLyS) antibody is preliminary found to be effective in Sjögren's syndrome (SS) patients with moderate to high systemic activity in the phase II open-label BELISS study (1). Belimumab 10 mg/kg was administered monthly for 12 months in 30 SS patients coming from two Centres (Udine, Italy and Paris, France) (1).

Results at month +6 were recently published (1), and 52-week analyses in the responders at month +6 showed the persistence of response over time (2). Notably, the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) score (3), and the serum levels of rheumatoid factor (RF) and IgM immunoglobulins continued to decrease from month +6 to month +12 during treatment (1, 2).

The aim of this study is to report the follow-up after the end of the BELISS study, *i.e.* after the suspension of belimumab therapy, in the Italian cohort of patients.

Patients and methods

Clinical and laboratory data of 13 SS patients were collected at one year after the end of the belimumab treatment in the BELISS study. These patients were all the patients who completed the 52-week of the BELISS trial in the Italian cohort. No other immunosuppressors were employed in these patients after the end of the trial up to the evaluation at one year after suspension of the drug. Patients (all female, aged 54±15 years, disease duration 12.7±5.2 years) were all anti-SSA (13/13) and/or anti-SSB (12/13) positive, and RF positive in 7/13 (53.8%). BLyS serum levels were also analysed.

Serum levels of Ro/La autoantibodies, IgG, IgA, IgM immunoglobulins and IgM RF (nephelometry) were measured as part of standard diagnostic procedure. Anti-Ro/La autoantibody specificity was determined by ELISA using recombinant Ro60, Ro52 and La proteins, and sera from patients with anti-La were further tested by counterimmunoelectrophoresis, as previously described (4). The serum BLyS was measured by a quantitative sandwich enzyme immunoassay (Quantikine Human BAFF Immunoassay; R&D Systems, Minneapolis, MN, USA) using automated instruments and procedures (Dynex Technologies ELISA processor).

Statistical comparisons by paired sample *t*-test or Wilcoxon test were performed, as appropriate, between baseline data and month +12 after the end of the trial, and between the last evaluation in the trial (*i.e.* week 52, *i.e.* four weeks after the last belimumab infusion) and after a follow-up of 12 months (+12F-UP) after the end of

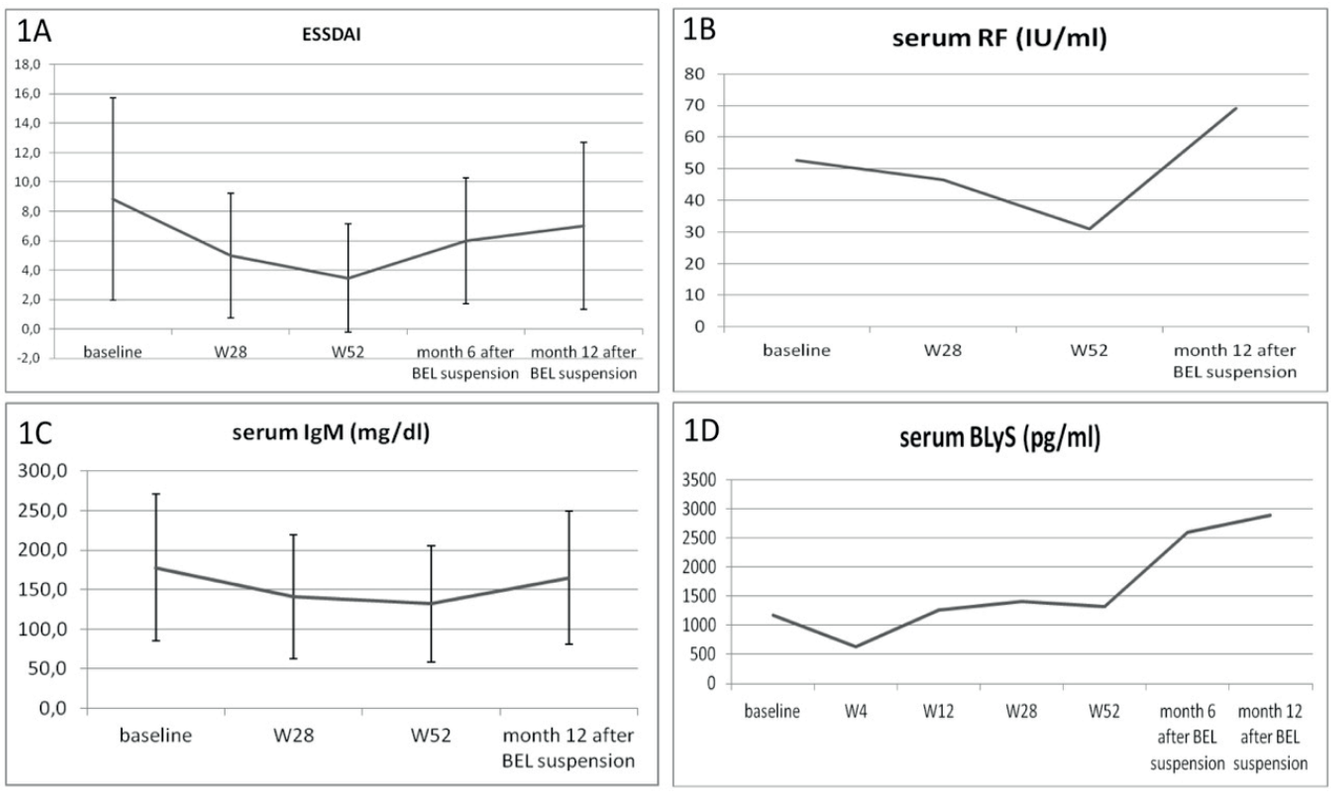

Fig. 1A. ESSDAI changes (mean±SD) during belimumab (BEL) treatment, 6 months and 12 months after drug suspension. **1B.** Serum rheumatoid factor (RF) changes (median) during belimumab (BEL) treatment, 6 months and 12 months after drug suspension. **1C.** Serum IgM changes (mean±SD) during belimumab (BEL) treatment, 6 months and 12 months after drug suspension. **1D.** Serum BLyS changes (median) during belimumab (BEL) treatment, 6 months and 12 months after drug suspension.

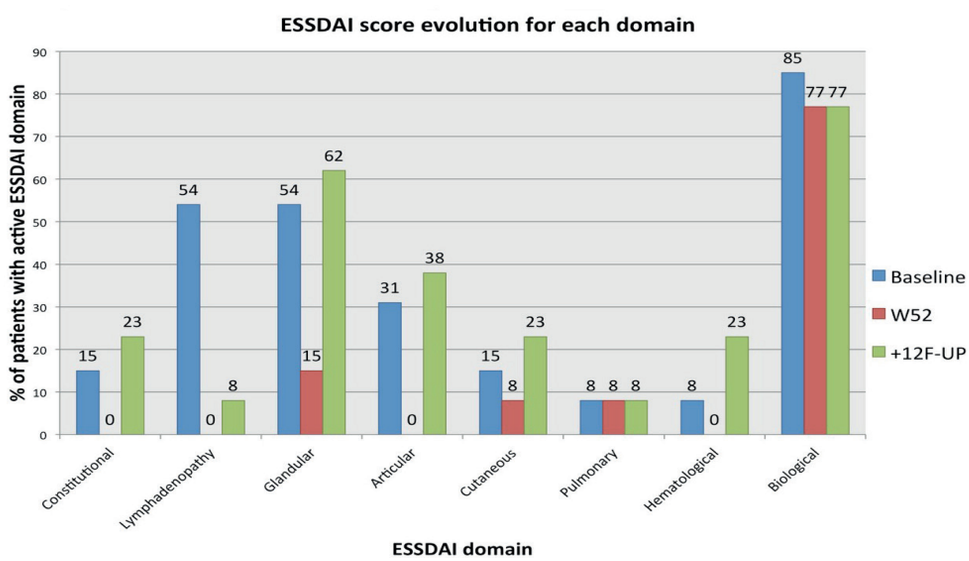

Fig. 2. The ESSDAI score evolution for each domain from baseline to 12 months after the end of the trial.

the trial. Results are reported as mean ± standard deviation (SD), or median (range).

Results

Clinical follow-up

The ESSDAI score was 8.8±6.9 at baseline, 3.5±3.7 at week 52 (end of the trial) and 7.0±5.7 at +12F-UP (baseline *vs*. +12F-UP, p=0.2; week 52 *vs*. +12F-UP, p=0.003) (Fig. 1A).

Thus, a significant increase in the ESSDAI score was observed between week 52 (*i.e*. end of the trial, 4 weeks after the last infusion of belimumab) and month +12 after the end of the trial, with the mean score coming back in the range of moderate disease activity from the low level.

As shown in Figure 2, at +12F-UP, a worsening was recorded in all the ESSDAI domains, which were mainly affected by belimumab during the trial, *i.e*. constitutional, lymphadenopathy,

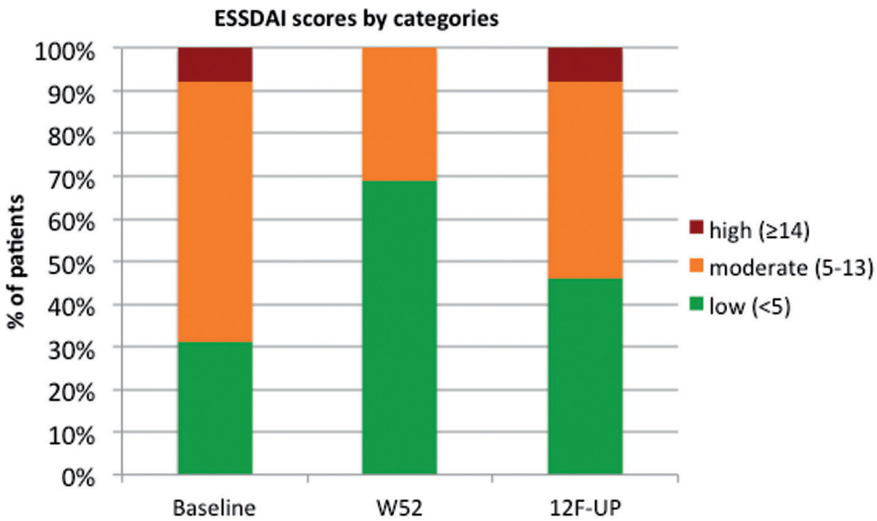

Fig. 3. The ESSDAI score evolution from baseline to baseline to 12 months after the end of the trial displayed by categories (low-moderate-high).

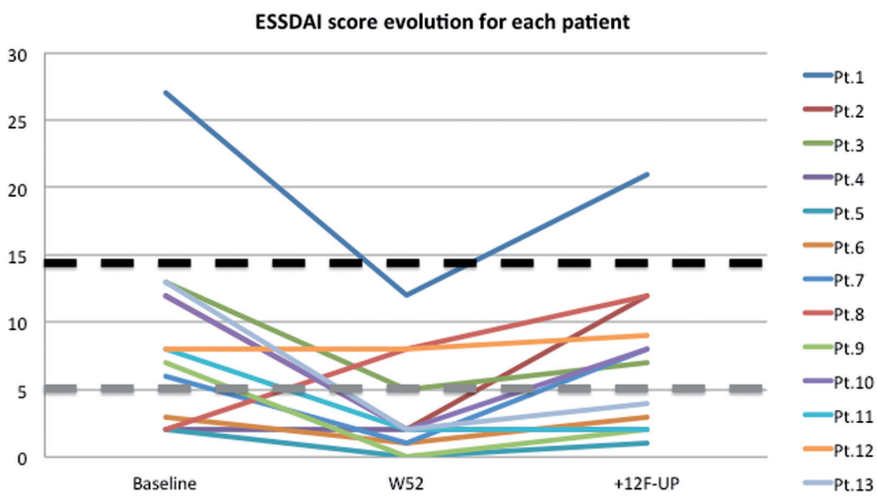

Fig. 4. The ESSDAI score evolution for each patient from baseline to 12 months after the end of the trial. The grey dashed line indicates the boundary between the level of low and moderate disease activity, while the black dashed line indicates the boundary between the level of moderate and high disease activity.

glandular, articular, cutaneous and haematological domains. Also, the loss of the effect of the drug was suggested by the higher number of patients showing a high or a moderate disease activity at +12F-UP if compared to W52 (Fig. 3 and Fig. 4).

Importantly, the development of B-cell lymphoma from non-neoplastic parotid sialadenitis (all biopsy-proven as non-neoplastic lymphoepithelial sialadenitis before belimumab treatment) was observed in two patients two years after the end of the trial. Another patient with low-grade lymphoma, who had received belimumab for 12 months due to the improvement of other SS-related manifestations (2), presented a progression of lymphoma after belimumab suspension, with new-onset loco-regional nodal involvement. Finally, in another patient the onset hypergammaglobulinaemic purpura was recorded three years after the end of the BELISS trial. No significant changes were recorded in the EULAR Sjögren's Syndrome Patients Reported Index (ESSPRI) score (data not shown).

Laboratory follow-up

RF level was 52,7 (11.1-327.2) IU/ml at baseline, 31 (8–224.6) IU/ml at week 52 (end of the trial), 69 (11–666) IU/ml at month 12 after the end of the trial (baseline *vs*. +12F-UP, p=0.5; week 52 *vs*. +12F-UP, p=0,008). Thus, a significant increase in the RF serum level was observed between week 52 (end of the trial) and month +12 after the end of the trial, serum RF almost doubling the baseline level (Fig. 1B).

IgM level was 177.6±92.8 mg/dl at baseline, 131.9±73.6 mg/dl at week 52 (end of the trial), 165±84.6 mg/dl at month 12 after the end of the trial (baseline *vs*. +12F-UP, p=0.3; week 52 *vs*. +12F-UP, p=0.04). Thus, a significant increase in the IgM serum level was observed between week 52 (end of the trial) and month +12 after the end of the trial, serum IgM coming back to the baseline level (Fig. 1C).

BLyS level was 1190 (875–2439) pg/ml at baseline, 1304 (667–3835) pg/ml at week 52 and 2882 (1353-6178) pg/ml twelve months after belimumab suspension. Interestingly, BLyS levels was higher even four weeks after the last belimumab infusion if compared to baseline levels (p=0.04), and increased 12 months later (baseline *vs*. +12F-UP, p=0.005; week 52 *vs*. +12F-UP, p=0.01) (Fig. 1D).

No significant changes were observed in IgG or in IgA serum levels (data not shown).

Discussion

SS is both an autoimmune and a B-cell lymphoproliferative disorder, where interaction among epithelial cells, B and T-cells plays a critical role (5). Directly targeting the B-cells by rituximab has been proved effective in some SS systemic manifestations, especially in those related to cryoglobulinaemia (6). However, B-cells and local clonal expansion may persist after rituximab (7, 8), suggesting local mechanisms of resistance to rituximab in SS (9). By blocking T-cell activation, B-cells may be also indirectly affected (10). BLyS is a known anti-apoptotic cytokine that promotes survival of autoreactive B-cells in SS, as well as the proliferation of neoplastic B-cell in malignant B-cell lymphoma (11). Importantly, BLyS is expressed by monocytes, dendritic cells, T-cells, neutrophils and epithelial cells in SS, and BLyS transgenic mice can develop a lupus-like syndrome,

followed by sialadenitis, B-cell salivary gland infiltration and finally B-cell lymphoma (12). Thus, BLyS is a crucial cytokine to link the activated epithelial cells of salivary gland tissue to the innate and the acquired immunity in SS. Also, it could represent a very important cytokine in the process leading from inflammation to autoimmunity and to lymphoproliferation. Importantly, BLyS mediates resistance to B-cell depletion by rituximab in the MALT microenvironment (9).

In the BELISS trial, belimumab, a monoclonal anti-BLyS antibody, was particularly effective for some clinical manifestations of SS, such as salivary gland swelling and lymphadenopathy, with significant decrease of serum biomarkers of B-cell activation (1, 2). Targeting BLyS with belimumab has been proved also effective on B-cell homeostasis, by normalising B cell frequency, phenotype and functions in SS (13).

Indirectly, the present study further supports the benefit of belimumab on SS.

Overall, the systemic disease activity of the disease, measured by ESSDAI score, significantly increased after the end of belimumab therapy. Furthermore, the significant increase in serum RF and IgM supported the biologic effect of belimumab on RF-positive B-cells in SS. This may in turn be linked to a positive effect of the drug on the risk of B-cell lymphoproliferation in SS, which appears to be RF-positive (14).

This observation is particularly interesting in the light of the development (two cases) or the worsening (one case) of three MALT B-cell lymphomas after the end of the trial, and by the onset of hypergammaglobulinaemic purpura in another patient. Since salivary MALT lymphoma in SS appears to derive from RF-positive B-cell clones (14), belimumab could be useful in SS by slowing down the expansion of these RF-positive B-cells. However, while non-neoplastic B-cell proliferation may be sensitive, neoplastic lymphoproliferation appears resistant to belimumab alone, as shown by the lack of response to belimumab of two low-grade parotid lymphomas in SS in the BELISS study (1). In addition, the suspension of belimumab is likely followed by the reinstitution of BLyS stimulation on B-cells. We indeed observed a significant rebound of serum BLyS after such suspension in this study. While the increase of serum BLyS levels during belimumab treatment may be considered an artifact caused by the measurement of the immune complexes between serum BLyS and belimumab itself, the increase of BLyS after the end of the trial can be definitely considered a real change of the serum BLyS levels, since the terminal half-life of belimumab is estimated at 18.0 (11.6–28.0) days.

As we observed that two of our SS patients developed B-cell lymphoma and one patient with low-grade B-cell lymphoma worsened after the suspension of belimumab, we consider that a very prolonged therapy with belimumab may be, however, advisable in SS, in particular in patients at high risk for lymphoma (11), in whom a rebound of BLyS might represent a hazardous stimulation for B-cells. Alternatively, belimumab might be soon followed by rituximab in SS (15, 16). This sequential treatment led to overcome B-cell resistance to rituximab, with parotid lymphoma disappearance and persistent recovery from cryoglobulinemic vasculitis in a patient treated by our Group (15).

In conclusion, targeting BLyS by belimumab appears effective in SS also based on the worsening of disease manifestations after drug suspension. A possible control of RF-positive B cell proliferation is highlighted. Finally, a prolonged suppression of BLyS may be advisable in this disease.

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