Supplementary material 2

Table 1. Patient characteristics

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Total (n=42) | RTX (n=16) | RTX+BLM (n=15) | BTZ (n=11) |
| **Organ involvement** | |  |  |  |  |
|  | Mucocutaneous (%) | 93% | 100% | 80% | 100% |
|  | Neuropsychiatric (%) | 31% | 38% | 20% | 36% |
|  | Musculoskeletal (%) | 88% | 100% | 67% | 100% |
|  | Cardiorespiratory (%) | 64% | 75% | 67% | 46% |
|  | Renal involvement (%) | 69% | 56% | 93% | 55% |
|  | Hematological (%) | 76% | 100% | 53% | 73% |
| **Concurrent Medication** | | |  |  |  |
|  | Prednison (%) | 90% | 81% | 100% | 82% |
|  | Hydroxychloroquine (%) | 76% | 53% | 100% | 64% |
|  | Mycophenolate mofetil (%) | 46% | 20% | 100% | 9% |
|  | Azathioprine (%) | 7% | 20% | 0% | 0% |
|  | Methotrexate (%) | 5% | 6% | 7% | 0% |
|  | Cyclophosphamide | 40% | 100% | 0% | 9% |
| **Previous immunosuppressants** | | | |  |  |
|  | Mycophenolate mofetil (%) | 57% | 31% | 100% | 36% |
|  | Cyclophosphamide (%) | 43% | 19% | 40% | 82% |
|  | Azathioprine (%) | 50% | 44% | 53% | 55% |
|  | Rituximab (%) | 24% | 0% | 27% | 55% |
|  | Methotrexate (%) | 21% | 31% | 7% | 27% |
| 1RTX vs RTX+BLM | |  |  |  |  |
| 2RTX vs BTZ | |  |  |  |  |
| 3RTX+BLM vs BTZ | |  |  |  |  |

Table 2. Treatment schedules

|  |  |  |  |
| --- | --- | --- | --- |
|  | RTX | RTX+BLM | BTZ |
| B cell targeted therapies | 2 infusions of 1000mg RTX 2 weeks apart with antihistamines and one infusion of 500-750mg CYC | 2 infusions of 1000mg RTX 2 weeks apart with antihistamines. 10 mg/kg BLM at weeks 4, 6, 8 and then every 4 weeks. MMF was initiated or continued at baseline.1 | One cycle of BTZ at doses of 1.3mg/m2 on day 0-4-8-11, 10-14 days treatment free interval. Total: 1-3 cycles. |
| Corticosteroids | 100-250mg methylprednisone every RTX infusion | 100mg methylprednisone every RTX infusion | 20mg dexamethason day 0,1 & every BTZ infusion |

BLM belimumab, BTZ – bortezomib, MMF – mycophenolate, RTX – rituximab

1MMF was rapidly tapered within 4-12 weeks and stopped at 24 weeks after baseline in 15 of 16 SLE patients treated with RTX+BLM7.

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Figure 1. Avidity of anti-dsDNA IgG and anti-TT-IgG determined by elution assays. A) Curve for anti-dsDNA IgG (AU/ml) and anti-TT-IgG (IU/ml) of a SLE patient with increasing amount of NaSCN (M). B) Example of the analysis of the serum titer of low, medium and high-avidity anti-dsDNA IgG autoantibodies (AU/ml). Low avidity antibodies are eluted from the antigen with < 0.25M NaSCN, medium avidity antibodies are eluted from the antigen with 0.25M to <1 M NaSCN and high avidity antibodies are eluted from the antigen with ≥1 M NaSCN. In the circle diagram the distribution of the low, medium and high avidity within the total of anti-dsDNA autoantibodies is displayed.

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Figure 2. Disease activity and C3 serum levels of SLE patients after B-cell targeted therapies. Absolute values of A) SLEDAI/BILAG and B) C3 serum levels for all individual patients per cohort before and after B-cell targeted therapies.. Dotted line indicates lower normal cut-off value for each local laboratory, which is 0.79g/L for RTX and 0.9g/L for RTX+BLM and BTZ. Wilcoxon matched-pairs signed rank test was used to test statistical differences between baseline and after different targeted therapies in paired patient serum samples. \*\*\*p<0.001. BLM – belimumab, BTZ – bortezomib, RTX – rituximab

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Figure 3. Absolute autoantibody serum levels before and after B cell targeted therapies.Each line represents serum levels of A) anti-dsDNA B) anti-histone, C) anti-nucleosome and D) anti-C1q autoantibodies in an individual patient at baseline and after therapy. Wilcoxon matched-pairs signed rank test was used to test statistical differences between baseline and after different targeted therapies in paired patient serum samples. \*p<0.05, \*\*p< 0.01, \*\*\*p<0.001. BLM – belimumab, BTZ – bortezomib, HIS – histones, NUC – nucleosomes, RTX – rituximab

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Figure 4. Autoantibody repertoire of SLE patients before and after B-cell targeted therapies. The presence of autoantibodies against dsDNA, histones, nucleosomes and C1q was assessed in the SLE patients at baseline and after B cell targeted therapies. A) The number of autoantibodies positive in the SLE patients at baseline in the RTX-treated patients (black bars), RTX+BLM-treated patients (white bars) and BTZ-treated patients (grey). B) The number of autoantibodies that turned negative per patient in each cohort after B-cell targeted therapy, each dot represents one SLE patient.

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Figure 5. Absolute serum levels of low-, medium- and high-avidity anti-dsDNA before and after B cell targeted therapies.Each line represents serum levels of A) low-avidity B) medium-avidity, C) high-avidity anti-dsDNA autoantibodies in each individual patient at baseline and after B-cell-targeted therapy, RTX (squares), RTX+BLM (triangles), BTZ (circles). Wilcoxon matched-pairs signed rank test was used to test statistical differences between baseline and after different targeted therapies in paired patient serum samples. \*p<0.05, \*\*p< 0.01, \*\*\*p<0.001. BLM – belimumab, BTZ – bortezomib, RTX – rituximab

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Figure 6. BTZ does not always have long lasting effects on autoantibodies, B cells, C3 levels and NET formation. Individual data are displayed for each BTZ-treated patient at baseline, 6 and 12 weeks after BTZ for A) Anti-dsDNA, B) Anti-histone, C) anti-nucleosome, and D) anti-C1q autoantibodies, E) CD19+ B cells F) C3 levels and G) NET formation. Wilcoxon matched-pairs signed rank test was used to test statistical differences between baseline and after BTZ in paired patient serum samples. \*p<0.05, \*\*p< 0.01, HIS – histones, NET – neutrophil extracellular trap, NUC – nucleosomes