UNIVERSITÁ DEGLI STUDI DI PADOVA

Scuola di Medicina e Chirurgia

Corso di Laurea Magistrale in Medicina e Chirurgia

DIPARTIMENTO MEDICINA DEI SISTEMI

Direttore: Prof. Paolo Simioni

U.O.C. Reumatologia

Direttore: Prof. Andrea Doria

TESI DI LAUREA

Efficacy and safety of belimumab in lupus patients with hematological involvement in a multicenter prospective cohort study

Relatrice: Prof.ssa Margherita Zen

Laureanda: Kristi Brushtulli

Anno accademico: 2023/2024

Ai miei nonni…

INDEX

ABSTRACT

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by a disfunction of the immune system and widespread inflammation.

One common manifestation of SLE is hematological involvement. Lymphopenia, especially, is related to high disease activity and difficult prognosis.

This study aims to assess the efficacy and safety of Belimumab, a monoclonal antibody that binds the B Lymphocyte Stimulator (BlyS), in SLE patients manifesting hematological involvement across two cohorts.

In the first part of the study, 107 patients manifesting lymphopenia, enrolled in the BeRLiSS cohort, were considered. Lymphocyte count, complement levels, prednisone dosage, and disease activity indicators were taken into consideration to evaluate the general hematological response and disease activity over time. Belimumab efficacy in lymphopenic-at baseline patients was assessed by lymphocyte counts evaluated at 6, 12, 24, 36, and 48 months.

The second study involves thirteen patients from the Padua Lupus Clinic (Division of Rheumatology, University of Padua, Italy), with the objective of extending the evaluation of Belimumab's safety to a second cohort with a longer follow-up period to assess the long-term effects.

A total of 107 patients from the BeRLiSS cohort, of which 88.8% females, with a disease duration of 11.27 ±8.42 years pre belimumab were considered. Over a treatment period of 31.1±16.3 months, we observed a significant improvement in lymphocyte count (baseline 720±208.75, 6 months 778±325, 12 months 910.17±436.43, 24 months 1042±570, 36 months 1098.78±557.82, 48 months 1144±447, $p<0.001$). In addition, prednisone doses were significantly reduced by the final follow up (PDN at baseline 10.63±8.59, 6 months 6.27±4.7, 12 months 6.29±6.29, 24 months 4.25±4.19, 36 months 3.79±3.91, 48 months 3.13±3.16, $p<0.001$).

In the second cohort, including patients with longer treatment with belimumab, we confirmed the efficacy and the safety in the long-term, as we did not observe any increase in infections or other adverse events.

These outcomes accentuate the potential of Belimumab as a safe and efficient treatment strategy for SLE patients manifesting lymphopenia as hematological involvement.

Chapter 1- Systemic Lupus Erythematosus (SLE)

1.1 Definition

Systemic Lupus Erythematosus is a chronic autoimmune disease caused by immune system dysfunction that can affect different organs and systems. It is characterized by periods of remission and relapse. The variety of clinical manifestations of SLE is due to the abnormal activity of the immune system during the active phase. The severity of the disease may vary considerably between different individuals. **(1)(2)**

1.2 Epidemiology

For the overall population, it is estimated that the incidence of SLE globally is 5.14 per 100000 person-years. Meanwhile, the prevalence is estimated to be around 43.7 per 100000 persons and the affected population is 3.41 million people. **(3)**

Incidence and prevalence are significantly greater in some ethnicities. Among Asians, American Indians, blacks, Hispanics, the rates are higher, confirming that racial and ethnic minorities confront an elevated risk of developing SLE. **(3)(4)**

According to some studies, countries with the highest rates of incidence and prevalence in Europe are the ones located in Central Europe with a rate of 13.74 per 100000 person-years. **(3)**

Women are more likely to be affected by SLE than men. The F:M ratio is 9:1. The global incidence in women is 8.82 per 100000 person-years and there are 3.04 million women affected. In men, the incidence is estimated about 1.53 per 100000 person-years with 0.36 million men affected. **(3)**

In addition to regional and gender differences, age is another variable that influences values. The prevalence in young adults is estimated to be around 1.4-fold higher than the global prevalence. **(4)**

As for Italy, a nationwide study proved that the incidence rate in 2022 was 6.51 cases per 100000 person-years and the prevalence was 60.57 per 100000 people.

The prevalence resulted five times higher in women than men. In comparison to the rates from 2017, it has been noticed an upward trend. **(5)**

More specifically, the Veneto region has a prevalence of 70.6 per 100000 residents, with an annual increase of 1.14% in the last 10 years. The highest prevalence was noticed in females in their sixth decade of life. The incidence amounts to 2.8 per 100000 person-years, with an annual decline of 7.3%. **(6)**

1.3 Etiology

The etiology of SLE is still unknown, but there are different factors that play a role in its etiopathogenesis, such as genetics, hormones, immunological causes and environmental factors. They cause the loss of immunological tolerance against self-antigens. As a result, pathogenic autoantibodies are formed, causing tissue damage in different organs through various mechanisms. **(7)**

1.3.1 Genetic

There is indirect evidence that endorses genetic etiology in SLE. Monozygotic twins have a SLE concordance rate estimated at 24-35%, meanwhile dizygotic twins have a 2-5% rate. Technological advances have permitted genome-wide searches for genetic polymorphisms that could lead to SLE and its complications. Currently, approximately 30 genetic regions have been verified as predisposing to SLE. **(8)**

Various mutations in genetic regions associated with SLE have been reported to disseminate wrongful clearance of apoptotic cells and increased apoptosis. **(9)**

The genetic regions associated with SLE code for many proteins. Some of them are involved with lymphocyte differentiation, like ETS1 and IKZF1. More specifically, ETS1 is a negative regulator of B-cell differentiation and T helper 17 proliferation. According to research, ETS1 is reduced in patients affected by SLE. This may lead to abnormal differentiation of the B-cells. **(9)**

Also, in patients with SLE, low levels of IKZF1 were found in peripheral blood. This gene sequence contributes to SLE etiopathogenesis by interacting with

other genes. One example of these interactions is its role in trans-activating STAT4, which has been proved to be a risk locus in SLE. **(9)**

1.3.2 Endocrine involvement

Hormones such as estrogen, progesterone, androgens, GH, and PRL play an important role in regulating different immunologic events. Also, cytokines released by immune cells affect the endocrine system, regulating its function. The cytokines are able to pass the blood-brain barrier. The loss of immune tolerance is a characteristic that describes all the autoimmune diseases. When the micro-environment is composed of cytokines and pro-inflammatory hormones, the result will be an aggressive immune system activation and eventually a loss of tolerance, leading to autoimmune diseases. **(10)**

Estrogen is the main hormone related to SLE. The predominance of females in the epidemiology of SLE might be influenced by the female sex hormones, estrogen. According to some studies, the hydroxylation of estrone towards 16 hydroxy metabolites was the main factor contributing to SLE. These results made researchers try to treat patients with sex steroids, like DHEA and 19-nortestosterone, but the treatment was not successful because of collateral effects and overall lack of efficacy. **(11)**

During pregnancy, women affected by SLE might have relapses, especially those who have active disease at conception. These relapses can lead to increased risk of adverse pregnancy outcomes, like pre-eclampsia and fetal loss. **(12)**

1.3.3 Environmental factors

Environmental factors play an important role in the etiology of SLE. According to recent studies, cigarette smoking, alcohol and exposure to silica crystals have the strongest linkage to developing SLE. Other factors that have been hypothesized to cause SLE, like infections (Epstein-Barr virus), pollution and pesticides have yet to be proven. These elements may lead to epigenetic regulation and subsequently to modifications. **(13)**

Some experimental studies involving lead and cadmium have been conducted. During the studies, rodent models were exposed to lead and cadmium and the results imply that metals may play a contributory role in SLE. **(14)**

Another case-control study conducted near Alexandria University Hospital revealed that most SLE patients (89.7%) didn't prefer physical activity compared to 40.7% in the control group. **(14)**

As well as contributing to SLE etiology, smoking also plays a role in cutaneous manifestations during the disease. Current smoking is associated with active rashes, while past smoking is related to discoid rash and photosensitivity. **(15)**

As for infectious agents, EBV has been evidenced to contribute to the pathogenesis of SLE. Infective mononucleosis and active SLE have clinical symptoms in common. EBV infection may lead to antinuclear antibody (ANA) positivity and the production of other antibodies associated with SLE, such as anti-Sm. **(15**)

Another factor that has been found to contribute to SLE etiology is insufficiency of vitamin D. It is curious that the same ethnic differences noted in the prevalence of vitamin D deficiency are also seen in the prevalence of SLE. African Americans and Hispanics present a higher risk for developing SLE and having more severe symptoms. **(15)**

1.4 Pathogenesis

The loss of self-tolerance activates the adaptive immune system with autoreactive T cells and B cells. This abnormal activation leads to the production of pathogenic autoantibodies and tissue injury. **(16)**

1.4.1 B cells

B cells are the central factor in SLE given their ability to produce antibodies, which are present in blood years before there is other evidence of immune dysregulation. **(17)**

Other than producing pathogenic antibodies, B cells can secrete both pro- and anti-inflammatory cytokines. This pathogenic role of B cells was demonstrated in the MRL/lpr murine model. In this model, all B cells were eliminated, leading to the disease being repealed. **(17)**

Additionally, B cells activate T cells by expressing peptide-MHC complexes. Eventually, these complexes interact with T cell receptor (TCR). Cytokines can be secreted also by B cells, including IL-4, IL-6, IL-10, IFN-gamma, TGF-β. IL-4 and IL-6 are pro-inflammatory cytokines, while IL-10 has been shown to reduce inflammation. The production of IL-10 by B cells is used to regulate disease severity. Unfortunately, IL-10 role in humans with SLE is still controversial, as most studies suggest that it does not reduce the disease, but rather enhances it. This result is supported by the fact that blocking IL-10 with an antibody reduced the activity of the disease. **(17**)

B cells may become autoreactive after somatic hypermutations in the germinal center. **(17**)

B cells in patients with SLE may present various abnormalities. First of all, there is an increase in class-switched memory B cells, causing an imbalance of subtypes. A higher number of memory B cells leads to an excessive risk of disease, as they present a diminished activation threshold. As a result, even minimal antigen contact may bring the autoreactive B cells to activation. Another factor that increases this type of effect is the BCR response in SLE, which is exaggerated. **(18)**

1.4.2 T cells

T cells are also involved in the mechanisms of pathogenesis of SLE. They have a crucial role in affecting peripheral tolerance in case of abnormalities, leading to inappropriate B cells activation. Another effect of T cells, which is the elevated production of proinflammatory cytokines, brings to recruitment of immune cells. In this case, this recruitment leads to autoantibody production. **(19)**

CD8+ T cells in patients with SLE present a diminished cytolytic activity. This factor contributes to the increased risk of infection as well as the progressive development of autoimmunity. In addition, CD4+ T-cells are responsible for the autoantibodies' response. **(20)**

1.4.3 Innate immune cells

The innate immune system includes macrophages, neutrophils, dendritic cells (DCs), basophils, and innate lymphoid cells (ILCs).

Studies in patients with SLE demonstrate various abnormalities in the activation and secretory function of macrophages, associated with deregulation of T cell activity and autoantibody production in SLE. Also, SLE macrophages are defective in their capacity to remove apoptotic cell debris, leading to a higher risk of exposing auto antigens to the adaptive immune cells.

SLE neutrophils present an aberrant activity. Their phagocytosis is diminished, and they present an increased oxidative activity.

Mature DCs are able to activate T cells, while immature DCs can induce T cell hyporesponsiveness and lead to immune tolerance.

SLE basophils promote tissue damage when engaged into skin lesions of SLE patients. Some studies have shown that basophils from SLE patients can promote antibody production by B cells. **(21)**

1.4.4 Interferon (IFN) type 1

IFN type I can be produced in small amounts by all cells, but the plasmacytoid dendritic cells are the principal cell type that produces larger quantities of IFN-I, originally called the natural IFN-producing cell. IFN is usually released in conditions of infections by viruses, bacteria or microbial nucleic acids, when sensed by pattern recognition receptors (PRRs) **(22)**

Type I IFN is one of the main pathogenic factors in SLE. Approximately 50% of patients affected by SLE present increased levels of IFN in blood. **(23)**

Immune complexes formed by SLE-associated antibodies lead to type 1 IFN production. **(22)**

Observational studies have shown that using IFN-α to treat patients for malignancies could lead the very same patients to develop a lupus-like disease. They also developed autoantibodies to nuclear antigens. These results demonstrate that type I IFN can break the tolerance and lead to autoimmune diseases. **(22)**

Younger SLE patients have more elevated IFN activity compared to older patients. **(22)**

One mechanism of type I IFN induction in SLE patients proven in vitro relays in interferogenic ICs. The immune complexes are endocytosed in the dendritic cells and then are brought to the endosome where the nucleic part of the immune complexes binds TLR7 or TLR9. Eventually, transcription factors get activated and IFN-α is produced. **(22)**

In murine models of lupus, it was noticed that diminishing the number of pDCs leads to an improvement of the disease. **(22)**

IFN-1 is not only important for the beginning of the disease, but it also affects its progression. Elevated IFN-1 levels in blood are associated with higher disease activity, lupus nephritis, arthritis, and mucocutaneous manifestations. **(23)**

1.4.5 Autoantibodies

SLE is characterized by the production of various types of autoantibodies. These antibodies are not constant during the disease. Depending on the phase, remission or a relapse, there are modifications in quantities and type of autoantibodies in blood. Each type recognizes a different antigen and their binding leads to tissue damage. **(24)**

The main autoantibodies in SLE, important for their role in pathogenesis, are anti double stranded (ds)-DNA autoantibodies **(24)**

Their pathogenic effect is achieved by forming immune complexes that precipitate in tissues where these autoantibodies can activate the complement system, triggering an inflammatory response. **(24)**

Antiphospholipid antibodies are found in almost 50% of people affected by SLE. They are directed against phospholipids and their presence is associated with a higher risk of blood clots. Other severe complications of antiphospholipid antibodies in SLE include fetal loss/miscarriage, strokes, deep vein thrombosis, Libman-Sacks endocarditis. The most common antiphospholipid antibodies are anticardiolipin antibodies (aCL) and beta2 Glicoprotein 1 antibodies. Through a coagulation test called lupus anticoagulant, other antiphospholipid antibodies can

be indirectly detected, including anti-prothrombin and anti-annexin V antibodies. **(25)**

Other autoantibodies expressed in SLE are antibodies against extractable nuclear antigens, the so-called anti-ENA antibodies. The antigens represent 6 main proteins: Ro, La, Sm, RNP, Scl-70, Jo1. The anti-ENA antibodies are often used to screen for mixed connective tissue disease, Sjorgen's syndrome and SLE.

The detection of anti-Sm autoantibodies is very specific for SLE. It is part of the revised American Rheumatism Association criteria for the classification of SLE. **(26)**

1.4.6 BLyS/BRAFF

BLyS is a cytokine that is a member of the TNF family. Its role is inducing B cell proliferation. The BLyS expression is controlled by the levels of IFN-gamma. **(27)**

BLyS levels affect the survival and apoptosis of B cells that produce antibodies. An increase in BLyS levels may induce the production of autoantibodies from B cells, aggravating SLE. **(28)**

Also known as BAFF (B-cell activating factor), this cytokine binds its receptors BAFF-R, B-cells maturation antigen (BCMA), and TACI. BAFF is the only ligand that binds BAFF-R, while BCMA and TACI can bind either BAFF or another TNF family ligand called APRIL, a proliferation-inducing ligand.

BAFF-R plays a role in the positive regulation during B cell development. The interaction of BAFF with BAFF-R promotes important signals for the formation and maintenance of B cells. Signaling through these receptors acts as a main factor for maintaining mature B cell homeostasis. The most important source of BLyS are innate immune cells, such as monocytes, macrophages, neutrophils, and DC. Also, fibroblast-like cells are capable of producing BLyS. **(29)**

In many autoimmune diseases, high levels of BAFF are found in blood. SLE, Sjorgen syndrome, rheumatoid arthritis, multiple myeloma are some of the most important diseases where BAFF is found in higher levels than normal. **(29)**

In SLE, the beginning of the disease is marked by the class switching of autoreactive B cells from IgM to pathogenic IgG. BAFF's role in this step is its

collaboration with cytokines and TLRs to promote Ig class switching. To support this affirmation, some studies of BAFF transgenic mice have concluded that the B-cells located in the marginal zone undergo T-independent class switching. In addition, they produce antinuclear antibodies, leading to the development of SLE. **(30)**

The reasons behind high levels of BAFF found in SLE patients may be due to BAFF production from inflammatory sites, induction by high type I interferon or B-cell lymphopenia. **(30)**

Clinical trials have evidenced that baseline levels of BAFF in blood do not correlate with clinical response to belimumab. **(30)**

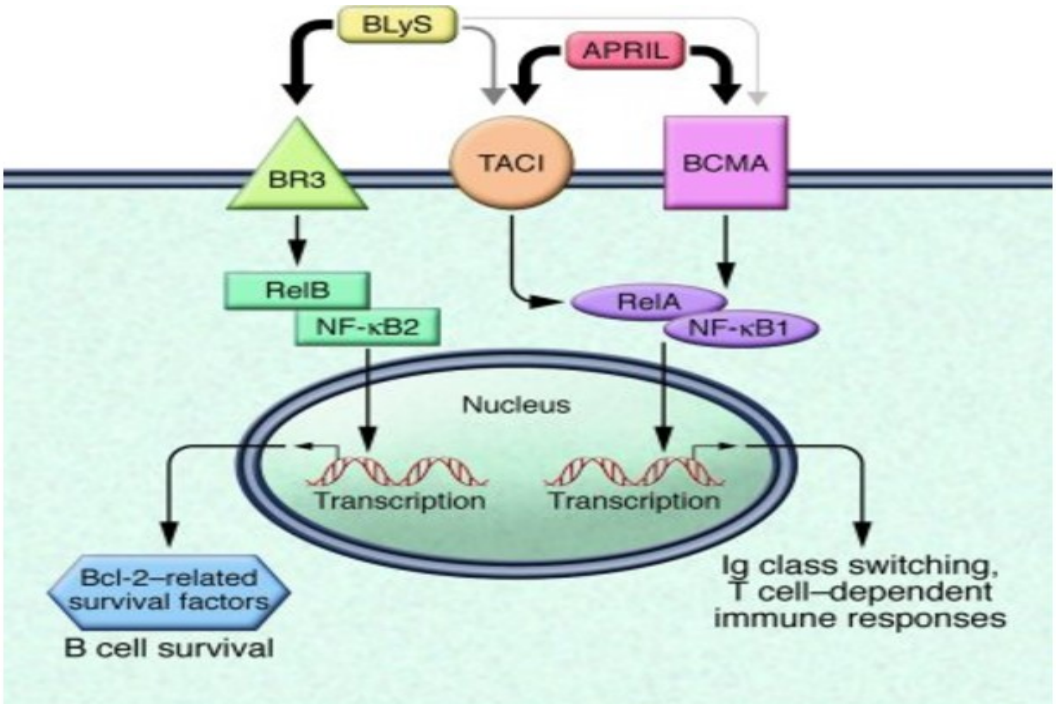


Figure 1: [BlyS and APRIL ligands and receptors]

Source: https://doi.org/10.1172/JCI38010

1.5 Manifestations

SLE can affect any organ of the body. Clinical manifestations vary from patient to patient and can be mild, moderate or severe. **(31)**

1.5.1 Musculoskeletal involvement

Musculoskeletal involvement is one of the most common clinical manifestations of SLE. Phenotypically, it can manifest in different forms, from minor arthralgia to severe arthritis. **(32)**

Arthritis in SLE is described as symmetric polyarthritis that affects the small joints. The majority of SLE patients with arthritis present non-deforming-non-erosive (NDNE) arthritis. **(32)**

Other musculoskeletal manifestations include inflammation of tendons and myositis. **(32)**

One specific musculoskeletal involvement in some SLE patients is Jaccoud's arthropathy. It presents joint deformities such as ulnar deviation, "swan neck" and "Z-thumb". The clinical manifestations of Jaccoud may resemble rheumatoid arthritis but the difference is noted with a simple radiography that demonstrates the lack of bone erosion. **(33)**

The only condition that includes bone erosion in patients with SLE is an overlap between SLE and Rheumatoid Arthritis called Rhupus. It is characterized by the presence of symmetrical erosive polyarthritis. The deformities in these patients remain permanent. Rhupus patients are less likely to have renal involvement than other SLE patients. They also have positive antibodies such as anti-dsDNA, anti-Smith, anti-citrullinated proteins and rheumatoid factor. **(34)**

Musculoskeletal involvement is the first presenting symptom in almost 50-70% of cases.

1.5.2 Hematological involvement

Hematological involvement is common in SLE. The most frequent hematological manifestations include cytopenia (anemia, leukopenia, thrombocytopenia), lymphadenopathy and hemostasis alterations. These manifestations may be a result of the disease itself or may be related to therapy with immunosuppressants.

1.5.2.1 Leucopenia

Leucopenia is usually related with disease activity in SLE patients. Leucopenia is defined as a low total white blood cell (WBC) count, <4000/ mmc on two or more occasions. It includes a scarcity of granulocytes and lymphocytes. As the majority of granulocytes are neutrophils, the paucity of granulocytes is also defined as neutropenia**. (35)**

Approximately 50% of patients affected by SLE present leucopenia, but only 17% have a WBC count <1000/ mmc.

Some causes that lead to leucopenia in SLE are destruction of neutrophils in peripheral blood due to antibodies that attack them, changes in marginal and splenic pool, and diminished bone marrow production. Leucopenia can also be a result of the use of immunosuppressive drugs. The most common drugs to cause leucopenia are azathioprine, methotrexate, and cyclophosphamide. **(36)**

1.5.2.2Neutropenia

Neutropenia is defined by a count of neutrophils below 1000/mmc. Most frequently, it is caused by viral infections, hypersplenism, and treatment with immunosuppressive drugs, such as azathioprine and cyclophosphamide.

Association with anti-Ro/SSA antibodies may lead to a higher predisposition to neutropenia. Mild or moderate neutropenia does not require therapy, while severe neutropenia can be treated with G-CSF. **(35)**

1.5.2.3 Lymphopenia

Lymphopenia is defined as a count of lymphocytes <1000/mmc on two or more occasions. Lymphopenia is more frequently observed in patients with an active disease. Notably, lymphopenia may be aggravated by the use of glucocorticoids or immunosuppressive drugs. **(35)**

Even though both subtypes of lymphocytes may be affected, T cells are more likely to be low, especially the CD4+ T cells.

The pathogenesis of lymphopenia is still uncertain. However, studies have evidenced the presence of IgM, cold reactive, cytotoxic anti-lymphocyte antibodies that may be the cause of destruction of lymphocytes in the blood. Anti-Ro, anti-

DNA, and anti-ribonucleoprotein titers are also seen to be higher in patients with lymphopenia. Lymphopenia is also a criteria in the SLEDAI-2K score and is a predictor of flare at one year of follow-up. **(36)**

As for the treatment, lymphopenia does not have a specific treatment other than disease control. **(35)**

1.5.2.4 Thrombocytopenia

A platelet count <100000/mmc without any other cause (blood aggregation, drugs) indicates a state of thrombocytopenia. The pathogenesis behind thrombocytopenia in SLE patients is due to compromised production in the bone marrow, sequestration in the spleen, and excessive platelet destruction. Under physiological conditions, platelets are formed from megakaryocytes in bone marrow through thrombocytopoiesis and then released into peripheral blood. A disfunction of this process contributes to SLE related thrombocytopenia. It may be due to the presence of anti-TPO and anti-c-Mpl antibodies, that block the activity of TPO and c-Mpl, leading to inhibition of platelet production. **(37)**

Platelet consumption could also be related to macroangiopathic disorders or the use of immunosuppressive drugs that cause abnormal platelet production.

Immune thrombocytopenia (ITP) is a disorder caused by autoimmunity characterized by isolated thrombocytopenia or related to other autoimmune diseases, such as SLE.

Antiplatelet antibodies are present up to 60% of SLE patients. They bind to platelets, leading to their phagocytosis in the spleen. The antigens for antiplatelet antibodies are glycoprotein IIb/IIIa and membrane glycoprotein.

Antiphospholipid antibodies are also associated with thrombocytopenia in SLE patients. They often result positive for lupus anticoagulant and have higher titer of IgM anticardiolipin. Studies have shown that the relative risk of thrombocytopenia is higher than four in patients presenting aPL antibodies, especially patients with positive lupus anticoagulant. **(38)**

Mild ITP (platelets 80-150000/mmc) in SLE does not require specific therapy other than disease control, while moderate ITP (PLT:30-80000/mmc) is usually treated with Prednisone 0.5mg/kg/day and immunosuppressive drugs can

be added, such as MMF 2g/day or AZA 2g/kg/day. Severe ITP is initially treated with IV Methylprednisolone 0,5g daily for 3 days, Cyclophosphamide, with or without IV immunoglobulin G (IVIG) and Rituximab. **(51)**

Evan's syndrome is a rare syndrome that includes both autoimmune thrombocytopenia and autoimmune hemolytic anemia. It can sometimes precede SLE diagnosis.

1.5.2.5 Anemia

Anemia is the most frequent hematological manifestation in SLE. More than 50% of patients become anemic during the course of the disease. Although the result may be the same, there are different causes behind this manifestation in SLE.

Anemia of chronic disease is the most common type of anemia in SLE patients. In this case, iron does not get incorporated into the red blood cell because of the upregulation of hepcidin. Inflammatory cytokines, such as IL-6, play a role in its synthesis and activation, leading to high levels of hepcidin. Anemia of chronic disease does not require specific treatment other than disease control.

Iron deficiency anemia is usually a result of hemorrhage, especially gastrointestinal bleeding. Laboratory exams accentuate low ferritin and/or transferrin. A risk factor that may lead to internal bleeding in SLE patients is treatment with glucocorticoids and non-steroidal anti-inflammatory drugs. Patients with iron deficiency in SLE may be treated with iron and erythropoietic agents simultaneously**. (36)**

Autoimmune hemolytic anemia (AIHA) manifests in about 10% of SLE patients. Laboratory exams show high levels of indirect bilirubinemia and lactate dehydrogenase, as well as low levels of haptoglobin. In some cases, AIHA can develop years before the diagnosis of SLE. As for treatment, high doses of GCs are administered to patients manifesting AIHA, and then the dosage gets gradually diminished when the hemoglobin levels reach 10g/dL. Other forms of treatment include immunosuppressive drugs, such as cyclosporine, mycophenolate, IV immunoglobulin, and splenectomy. Rituximab is a monoclonal antibody being used to treat AIHA in SLE patients. Its advantage is avoiding splenectomy.

Aplastic anemia is another type of anemia that can be manifested in SLE patients in which autoantibodies directed against erythrocyte precursors interfere with red blood cells formation. Autoimmunity dysfunctions are related to the genesis of aplastic anemia, among other factors, such as genetic defects, and viral infections. Aplastic anemia is treated with GCs. Cyclophosphamide of cyclosporine may be added to the treatment in cases of autoimmune pathogenesis.

Macroangiopathic hemolytic anemia is a rare but severe manifestation in SLE patients, caused by excessive turbulence in circulation, leading to fragmented red blood cells, called schistocytes.

Anemia may also be a consequence of drug treatment in SLE patients. Immunosuppressants can be a risk factor. Cyclophosphamide, mycophenolate and azathioprine may cause anemia because of bone marrow suppression, while hydroxychloroquine may also lead to hemolysis in rare cases. NSAIDs should be considered in cases of gastrointestinal blood loss, which leads to iron deficiency. **(36)**

1.5.3 Cutaneous manifestations

Skin is the second most frequent organ impacted by SLE. Over 80% of the patients have involvements of skin or mucous membranes.

Skin manifestations of SLE may be specific or non-specific. The specific ones are classified as acute, subacute, or chronic. The acute type can be manifested as a “butterfly” rash, which involves bridge of the nose, cheekbones and rarely, the eyelids. Notably, it does not involve the glabella and the nasolabial folds. As histology is concerned, the rash is an interface dermatitis. **(39)**

The subacute cutaneous lupus erythematosus in present in about 10% of the patients. These lesions do not scar or itch. SCLE often resists treatment with steroids and antimalarials. The lesions may appear as annular and polycyclic or similar to psoriasis. **(39)**

Chronic lesions in Lupus appear as discoid, bullous, or profundus, and are characterized by scarring. Usually, skin biopsy is necessary to diagnose the lesion. They are thick and scaly and usually appear in regions exposed to the sun.

Other non-specific cutaneous manifestations in SLE include alopecia, vasculitis, photosensitivity in about 50% of patients, livedo reticularis, oral and nasal ulcers. **(39)**

1.5.4 Renal involvement

The involvement of kidneys in SLE is also known as lupus nephritis. Usually, patients begin having renal involvement within 5 years from the onset of SLE. To monitor the condition, serial creatinine, urine album-to-creatine ratio, and urinalysis are necessary periodically. Lupus nephritis is often associated with proteinuria. **(40)**

The classification for LN indicates 6 classes of nephritis:

Class 1: Minimal mesangial lupus nephritis. This type of nephritis does not show lesions on the optical microscope, but there is evidence of immune complexes on the mesangial cells found with immunofluorescence and electron microscope.

Class 2: Mesangial proliferative lupus nephritis. It is characterized by the proliferation of mesangial cells.

Class 3: Focal proliferative lupus nephritis. There is a proliferation of mesangial cells in less than 50% of the glomerulus. It can be active or chronic and present proliferative or sclerosing lesions.

Class 4: Diffuse proliferative lupus nephritis. Distinguished by a proliferation of mesangial cells in more than 50% of the glomerulus. It can be further divided into two subtypes: segmental and global.

Class 5: membranous lupus nephritis. It is characterized by thickening of the basement membrane.

Class 6: Advanced sclerosis lupus nephritis. Over 90% of the glomerula present sclerosing lesions. **(41)**

It is crucial in patients with Lupus Nephritis to maintain a normal kidney function or at least try to prevent function loss. Kidney biopsy is necessary to classify the lesion and decide the best treatment for lupus nephritis. The first and

second class usually do not need treatment and only monitoring is enough. For class 3 and 4, treatment consists of immunosuppressive drugs and steroids. **(40)**

All patients with lupus nephritis should be prescribed hydroxychloroquine unless there are contraindications. **(40)**

1.5.5 CNS involvement

Patients affected by SLE often show symptoms of CNS involvement. These symptoms vary from patient to patient and may be aspecific, including signs such as headaches, and cognitive impairment. On the other hand, some of the symptoms may be more severe, like memory loss, seizures and stroke.

The pathogenesis of neuropsychiatric SLE is still uncertain. However, various intrinsic brain elements have been related as contributing to NSLE, such as resident microglia or the blood-brain barrier. **(42)**

NSLE prevalence in unknown because of difficulties in selecting the criteria to develop studies. Although, it is estimated that about 20% of SLE patients manifest CNS involvement. **(42)**

Focal NSLE involves certain CNS regions and is generally a consequence of either venous thrombosis or arterial ischemia**. (42)**

Another complexity of NSLE is the fact that often it is unclear whether the symptoms are due to the disease, the medications used for treatment, the psychological burden or other problems. **(43)**

CNS involvement is often present during relapses. If it affects peripheral nerves, symptoms may include foot weakness or numbness.

Treatment in this case consists in treating the relapse of the disease, specifically with immunosuppressants and steroids. Also, in case of seizures due to lupus, the patient receives anti-seizure medications. Finally, psychosis due to lupus is treated with mood stabilizers. **(43)**

1.5.6 Cardiovascular manifestations

The most common cardiac manifestation in SLE is pericarditis. Other cardiac involvements are rare, but still relevant, such as myocarditis, coronary lesions, or heart valve pathologies. Echocardiography is the gold standard to detect these lesions due to its high sensitivity and specificity. **(44)**

Libman-Sacks endocarditis is a rare form of endocarditis associated with positive anti-phospholipid autoantibodies in SLE. It affects the heart valves, especially the aortic and mitral ones, which present sterile lesions. The lesions may lead to the formation of embolus. **(45)**

As for the treatment of cardiovascular manifestations, oral or parental steroids are given for first-time myocarditis or pericarditis. Recurring pericarditis is usually treated with DMARDS like aziathioprine, methotrexate, and mycophenolate. **(46)**

1.5.7 Pulmonary manifestations

SLE can cause different pulmonary diseases, including acute pneumonitis, diffuse alveolar hemorrhage, pleural effusion/pleuritis, shrinking lung syndrome, and pulmonary hypertension.

Acute Lupus Pneumonitis (ALP) is characterized by acute onset of fever, cough, and dyspnea. ALP patients also present hemoptysis, sometimes accompanied by tachypnea, tachycardia. Histopathological examination accentuates diffuse alveolar damage, edema, and mononuclear cell infiltration.

Pleuritis is the main manifestation of acute pulmonary involvement in SLE. Patients present chest pain, dyspnea, cough, and fever. Pleural effusion is often bilateral in SLE patients. The pleural fluid is an exudate with increased lactate dehydrogenase and a WBC count of 3000-5000 cells/mL.

Diffuse alveolar hemorrhage (DAH) is another manifestation of SLE. Patients present dyspnea, cough, fever, and hemoptysis. Chest X-rays or CT is used to make diagnosis of DAH as it demonstrates diffuse lung infiltrates.

Shrinking lung syndrome (SLS) is a rare SLE manifestation. This condition is characterized by a progressive decrease in lung volume without interstitial or pleural disease visible on chest CT. **(47)**

1.5.8 Other manifestations

Other less common SLE manifestations include other organs, like pancreas, intestine, eyes, etc.

Acute pancreatitis is a rare but severe complication of SLE, often associated with other visceral involvement. Its pathogenesis is multifactorial, including autoimmunity and drug injury induced by corticosteroids and azathioprine. **(48)**

The most common ocular manifestation of SLE is keratoconjuctivitis sicca. A high count of proinflammatory cytokines, such as IL-17 can be found in the tear film of SLE patients. Other ocular manifestations include retinal vasculitis and optic neuritis, being the most severe conditions as they involve the optic nerve. **(49)**

The gastrointestinal tract may also be affected by SLE and present conditions in up to 40-50% of patients. Lupus mesenteric vasculitis is a rare presentation of SLE. In some cases, this condition can precede the common manifestations of SLE. It presents with acute, diffuse abdominal pain, and nausea with vomiting or diarrhea. **(50)**

1.6 Therapy

The purpose behind therapy for SLE is to achieve a remission of the disease and keep its symptoms under control. In 2023, the EULAR recommendations for the management of patients with SLE were updated. HCQ is suggested for all patients, unless contraindicated, at a target dose of 5mg/kg/day, but personalized based on retinal toxicity.

The dosage of GCs, if needed, is decided based on the type and severity of organ involvement. In any case, the dosage should be reduced to maintenance dose of ≤5mg/day, and, when possible, withdrawn.

Patients who do not respond to HCQ, with or without GCs, or patients who cannot reduce GCs below maintenance dose for chronic use, are eligible for addition of immunomodulating/immunosuppressive agents.

Intravenous cyclophosphamide should be considered in patients with life-threatening diseases. In refractory cases, rituximab may be evaluated. **(51)**

1.6.1 Antimalarials

The mechanism of action of antimalarials is the inhibition of lysosomal activity and autophagy, blocking T-cell proliferation and Toll-like receptors signaling. Chronic HCQ treatment reduces organ damage, but its blood concentration must not go over 800-1000 ng/ml. HCQ is efficient in reducing renal flare in lupus nephritis. **(52)**

HCQ is prescribed as a background treatment for all patients with SLE if they do not have contraindications, which include retinopathy or cardiomyopathy. **(52)**

Hydroxychloroquine has been seen to diminish disease activity, especially mild and moderate levels of disease. It also helps prevent SLE flares and decreases the long-term need for glucocorticoids.

HCQ is a safe drug to be used even during pregnancy or breastfeeding.

Also, according to studies, an early treatment in patients who present positive antinuclear antibodies with hydroxychloroquine, might delay the progression to active SLE. **(53)**

The suggested daily dose of HCQ is ≤5mg/kg/day, taking into consideration the high risk of developing retinopathy due to HCQ administration. This dosage has been studied to be the best compromise between efficacy and safety. Also, patients with renal involvement and filtration rate lower than 30ml/min request a reduction of 50% of the dosage. Another change in dosages is made for patients weighing more than 80 kg. However, a maximum daily dose of 400 mg is recommended. **(53)**

A recent study showed that HCQ might have a regulating action by blocking B cell activating factor (BAFF) and interferon. **(53)**

1.6.2 Glucocorticoids

Glucocorticoids remain one of the most important treatments for SLE, as they reduce the expression of the cytokines, interfering with the organism's capability to induce a state of inflammation. Glucocorticoids bind the cytosolic GC receptor. Afterwards, the complex is translocated into the nucleus where it blocks genes that promote cytokine synthesis, leading to an anti-inflammatory role. With the increase of GCs levels in the nucleus, there appears another phenomenon called transactivation, which is primarily associated with the activation of gluconeogenesis, insulin resistance, and inhibition of bone formation. All these effects are well known adverse events (AEs) of glucocorticoids. They also inhibit leucocyte traffic and endothelial cell function. In addition, they cause lymphopenia in all lymphocyte subpopulations and block the proliferation of T cells. **(54)**

Low doses of prednisone (≤7.5 mg/day) are related to a saturation of GC receptors up to 50%. The complete saturation of the receptors is noted at approximately 30-40 mg/day. With higher doses reaching up to 100% saturation, the main effect is the transactivation, therefore leading to AEs of glucocorticoids. The use of glucocorticoids should be minimized as they lead to several side effects, such as osteoporosis, fractures, and higher risk for heart disease. **(52)(54)**

Often, SLE patients suffer organ damage. GC usage is a fundamental factor that contributes to this state. A cohort study revealed that the damage caused by glucocorticoid treatment was associated with the cumulative dose of prednisone. **(54)**

During pregnancy, given the scarcity of treatment possibilities, glucocorticoids are one the most important treatment resources in case of flares. The AEs of GC during pregnancy are similar to those observed out of pregnancy, but other severe side effects include hypertension, preeclampsia, and insulin resistance. **(54)**

1.6.2.1 Leukopenia from GC

Glucocorticoids are used as immunosuppressants and anti-inflammatory agents as a treatment for various autoimmune and inflammatory diseases. This anti-inflammatory effect of GCs influences white blood cells by regulating their

proliferation, differentiation and apoptosis. GCs can also control activation and secretion of white blood cells. Glucocorticoids inhibit the production of various pro-inflammatory cytokines and encourage the production of anti-inflammatory cytokines. **(55)**

Specifically, glucocorticoids increase the number of neutrophils in peripheral blood, while the number of lymphocytes, eosinophils, basophils and monocytes is reduced. **(55)**

Neutrophils increase in the peripheral blood because GCs promote their attachment to the blood vessel walls and eventually, they manage to pass in the blood circulation. Also, GCs block neutrophils apoptosis and delay their clearance.

On the other hand, lymphocytes diminish after short or long term use of GC as they promote the apoptosis of lymphocytes. They promote T-cells apoptosis by affecting the activity of transcription factors of T cell receptor activation. GCs influence T cells, by regulating the functions of DCs, macrophages and mast cells. Some studies have proved that glucocorticoids selectively inhibit the responses of Th1 cells and Th-17 cells. As for Th2 cells and regulatory T cells, glucocorticoids may even promote their functions. **(55)**

Glucocorticoids present a dual effect on macrophages. Based on the concentration of GCs, there may be an immunosuppressive or immunostimulatory effect. The immunosuppressive effect is reached by high doses of GCs. On the contrary, low doses of GCs lead to an immunostimulatory effect. **(55)**

Glucocorticoids may promote the apoptosis of eosinophils. A research has evidenced that the reduction of eosinophils count mediated by glucocorticoids is caused by CXCR4-dipendent movement of eosinophils toward the bone marrow. **(55)**

GCs can block the release of histamine from basophils and decrease their count in peripheral blood.

As for dendritic cells, the use of GCs can block their maturation and influence their activity by making it weaker.

The mechanism of action of GCs includes the drug's effect on intracellular transcription and protein expression. **(55)**

1.6.3 Cyclophosphamide

CYC is an immunosuppressive drug that targets naive and pre-switching memory B-cells. It acts by interfering with DNA and inhibiting replication in proliferating cells. An association of CYC and GCs is used as first-line treatment to induce remission in Lupus nephritis. **(52)**

Combination therapy can be more effective in preventing an increase of serum creatinine level rather than glucocorticoids therapy alone.

The most frequent adverse effects of cyclophosphamide are nausea, vomiting, and leukopenia. **(56)**

As for the pharmacokinetics of the drug, cyclophosphamide is an alkylating agent. Cytochrome P450 oxidizes cyclophosphamide in the liver and eventually produces its metabolite, 4-hydroxycyclophosphamide, which can be further oxidized. Cyclophosphamide mainly acts during the S phase. It inhibits cell division before G2 phase. **(56)**

1.6.4 Mycophenolate mofetil (MMF)

MMF is a prodrug that is metabolized in the liver. Then it becomes an active molecule, the mycophenolic acid. It inhibits DNA replication, especially T cells and B cells. MMF blocks an enzyme called inosine-5'-monophosphate dehydrogenase, whose purpose is the synthesis of guanosine-5'-monophosphate. **(52)**

Mycophenolate was first approved to prevent transplant graft failure.

It is recommended for patients with lupus nephritis. Several randomized studies have evidenced MMF efficacy in preventing flares in patients affected by lupus nephritis. The main adverse effects include gastrointestinal disturbances, such as nausea, vomiting and diarrhea. **(52)(57)**

1.6.5 Azathioprine

Azathioprine is authorized by the Food and Drug Administration for the treatment of rheumatoid arthritis. It is used as an off-label drug to treat various

autoimmune conditions, including lupus nephritis, multiple sclerosis, severe myastenia gravis, etc. **(58)**

Azathioprine is a prodrug. Once converted to 6-mercaptopurine, it modifies DNA replication and blocks purine synthesis in lymphocytes. AZA is usually prescribed as a maintenance therapy. **(52)**

The drug is absorbed quickly by the gastrointestinal system and cannot cross the blood-brain barrier. The starting dosage of azathioprine is 2 to 2.5 mg/kg/day. Frequent side effects include nausea, fever, arthralgia, hepatotoxicity, and infections. AZA can be used in pregnancy, plan for pregnancy, breastfeeding. **(58)**

1.6.6 Methotrexate

Methotrexate is an immunosuppressive drug that blocks the growth of cells that are rapidly reproducing.

According to a study in 2014, methotrexate was efficient in reducing SLEDAI score and the average dose of steroids in patients with SLE. **(59)**

Methotrexate is used as a treatment for moderate disease activity. Its main prescription is in patients who manifest joint and cutaneous involvement. As a result, it allows for diminished steroid doses. **(60)**

1.6.7 Anifrolumab

Anifrolumab is a humanized monoclonal antibody. Its mechanism of action consists in its binding to the type-1 interferon receptor. In this way, Anifrolumab interferes with the formation of IFN/IFNAR complex. As a difference from other monoclonal antibodies that only attack IFN-α, anifrolumab attacks IFN receptor, blocking its binding even to other molecules like INF-β, INF-ε, INF-κ. **(61)**

Real life studies for the efficacy of Anifrolumab are still limited, meanwhile trials have suggested a higher remission rate in patients administered with this monoclonal antibody. **(62)**

Chapter 2: Clinical measurement evaluation

Disease activity indicators are essential in the management and studying of SLE by providing standardized measures to evaluate the dynamic nature of the disease. These indices, such as the SLE Disease Activity Index (SLEDAI), British Isles Lupus Assessment Group (BILAG) index, and others, are validated tools created to differentiate active disease manifestations from chronic damage, infections, and co-existing conditions. They are important not only in everyday clinical settings for managing therapeutic decisions but also in observational studies and randomized controlled trials. By objectively measuring disease activity, these indices allow physicians and researchers to monitor changes over time, compare patient cohorts, and examine the efficacy of treatments, ultimately contributing to improved patient care and outcomes in SLE. (**63)**

2.1 SELENA-SLEDAI

It is a score that measures disease activity and consists of 24 clinical and laboratory variables. The maximum score is 105. The score takes into consideration the presence of specific manifestations or conditions at the time of the visit or within 10 days from the visit. The 24 variables included in the score are: seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, vasculitis, myositis, arthritis, cerebrovascular accident, urinary casts, hematuria, proteinuria, pyuria, rash, alopecia, mucosal ulcers, pleurisy, pericarditis, low complement, increased DNA binding, fever, thrombocytopenia and leukopenia. **(64)**

According to some studies, a decrease of seven points from the baseline SELENA-SLEDAI score is associated with a significant clinical improvement. On the other hand, an increase of 8 points in the score was related to a clinically worsening condition of the patient. **(64)**

Table I: SLEDAI score

Score	Description
8	Psychosis
8	Organic brain syndrome
8	Visual disturbance
8	Cranial nerve disorder
8	Lupus headache
8	Cerebro vascular accident (excluding atherosclerosis)
8	Vasculitis
4	Arthritis
4	Myositis
4	Heme-granular or red blood cells casts in urine
4	Hematuria (excluding stone, infection or other cause)
4	Proteinuria
4	Pyuria (excluding infection)
2	New onset or recurrence of inflammatory rash
2	Alopecia
2	Mucosal ulcers
2	Preurisy
2	Pericarditis
2	Low Complement
2	Increased DNA binding
1	Fever (excluding infection cause)
1	Thrombocytopenia
1	Leukopenia (excluding drug causes)

2.2 BILAG index

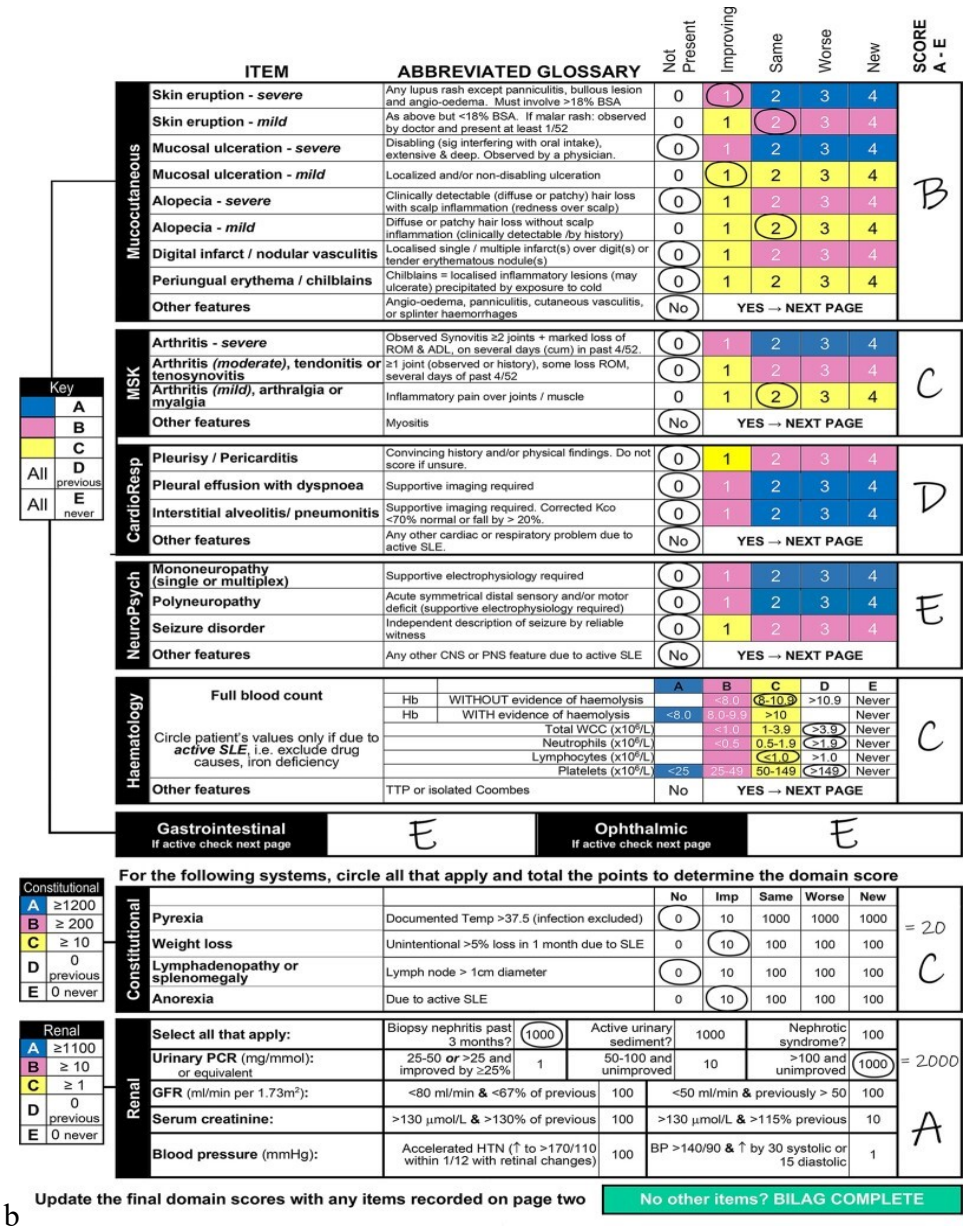
BILAG Index is a scoring system that takes into consideration both current lupus disease activity and changes in the disease activity since the last visit. It was

created according to the principle of the 'physician's intention to treat'. The BILAG score has a sensitivity of 87% and specificity of 99%. **(65)**

The scoring system consists of 86 questions. The questions are divided into 8 subgroups: general, mucocutaneous, neurological, musculoskeletal, cardiovascular and respiratory, vasculitis, renal, hematological. **(65)**

Table 2: BILAG score Oxford University

b



2.3 Physician Global Assessment (PGA)

The PGA is a scoring system that rates the overall disease activity. It considers the severity of active manifestations and clinical laboratory results. The score ranges from 0- “no disease activity” to 3- “most severe disease activity”. The values 1 and 2 are used to categorize the disease in mild (≥0.5 to 1), moderate (>1 and ≤2) and severe (>2 to 3). **(66)**

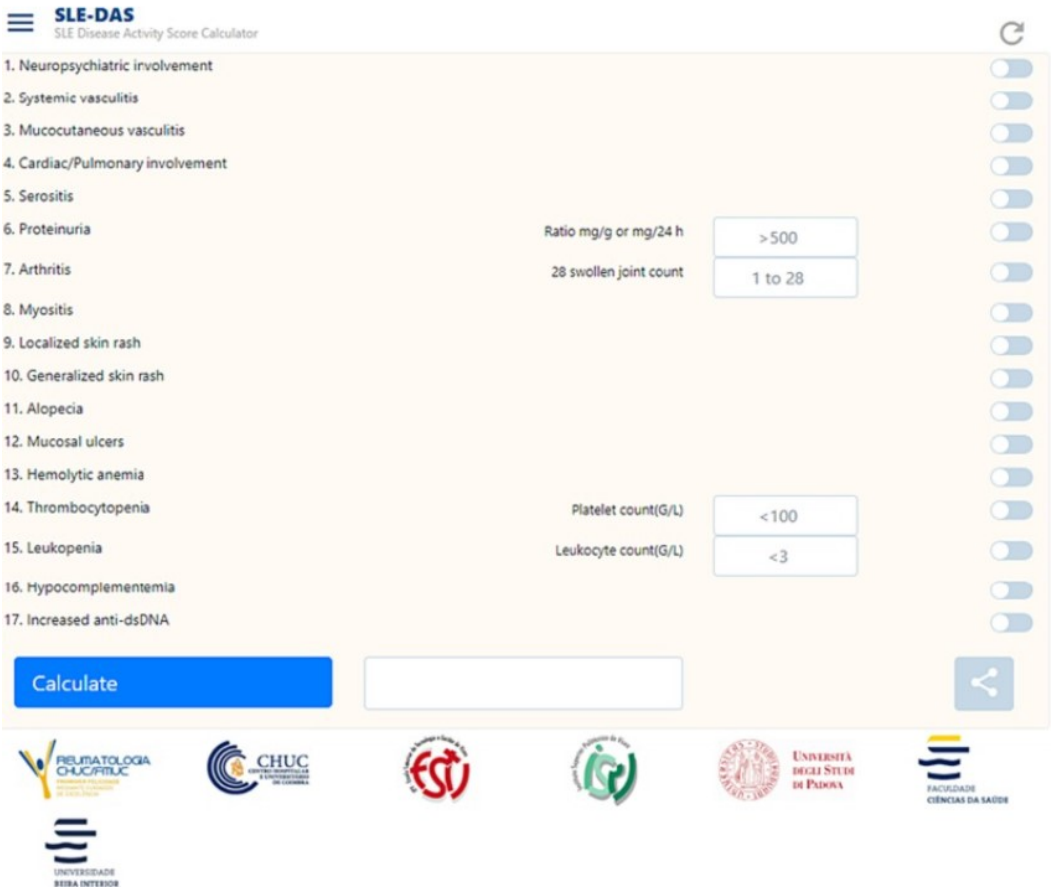
2.4 Systemic Lupus Erythematosus Disease Activity Score (SLE-DAS)

The SLE-DAS is an easy tool used to calculate clinical remission state in SLE patients. It includes 17 parameters with an online calculator. 4 of these 17 parameters are continuous variables (arthritis, proteinuria, leukopenia, and thrombocytopenia)

Studies have shown a higher sensitivity to change in comparison to SLEDAI-2K score.

SLE-DAS considered some rare SLE manifestations, such as hemolytic anemia, cardiopulmonary, gastrointestinal and ophthalmological involvement. Also, it changed the scoring of some variables in the SLEDAI score, giving higher scores to systemic vasculitis than mucocutaneous manifestations, and lower scores to localized skin rashes instead of generalized skin rashes. **(68)**

Table III: SLE-DAS score



2.5 Systemic Lupus Erythematosus Responder Index (SRI)

Systemic Lupus Erythematosus Responder Index is used to measure response to treatment. The score is dichotomous, it rates whether the patient has reached or not the response. **(64)**

The SRI score was created from an exploratory analysis of a phase II Belimumab trial.

It is a composite result related to SELENA-SLEDAI score, BILAG, PGA score. **(64)**

An SRI-4 response means a decrease in the SLEDAI score ≥4 points, without worsening of BILAG index (British Isles Lupus Assessment Group) or decrease from the baseline in PGA (physician global assessment) ≥0.3 points. As some researchers suggest, the SRI responder classification is almost always decided

by meeting the SLEDAI score. Whereas BILAG index and PGA criteria are meant to capture worsening conditions that are not distinguished by SLEDAI score. **(67)**

Chapter 3: BELIMUMAB

3.1 Mechanism of action

Belimumab is the first biological drug approved for the treatment of SLE.

Belimumab is a humanized monoclonal antibody. Its main target is the B-Lymphocyte Stimulator (BLyS), in charge of the development and proliferation of the B-cells. **(69)**

BLyS is a cytokine that induces survival of B cells. It is expressed and quickly cleaved by myeloid cells and other immune cells. BlyS binds its receptors that are expressed on the surface of normal and autoreactive B cells. As a result, B cells survive, mature and differentiate into antibody producing cells. **(70)**

The binding of belimumab with soluble BlyS blocks the interaction of BlyS with its three receptors. As a result, B cell survival and its production of autoantibodies is diminished. Belimumab adjusts the signaling downstream of BAFF-R, expressed on mature B cells, memory B cells and CD138+ plasmablasts. **(71)**

Consequently, inhibiting BLyS, Belimumab is effective in treating the disease. SLE patients are often characterized by a high level of circulating BLyS.

Currently, Belimumab is being tested for other autoimmune disorders, like Sjorgen syndrome, systemic sclerosis, myasthenia gravis, RA. **(72)**

The indication for receiving Belimumab is in patients with active SLE, with positive autoantibodies. It is used as a concomitant therapy in patients being treated with standard therapy, which includes antimalarials and glucocorticoids, with or without immunosuppressants. It is administered with a dosage of 10 mg/kg. The interval of therapy with Belimumab is day 0, day 14, day 28 and then 28 days after the last administration. **(72)**

Belimumab was approved by the US Food and Drug Administration (FDA) in 2011. Later that year, it was also approved by the European Medicines Agency. **(72)**

Belimumab is available as a lyophilized powder that is put in single use vials. These vials hold 120 or 400 mg of Belimumab. Next, the vials are

reconstituted with 1.5 and 4.8 ml of sterile water, respectively. Consequently, the solutions will contain 80 ml/mg of the drug. As a last step, the solution is dissolved in 250 ml of saline for IV infusion. The infusion, then, is administered over 1 h. **(72)**

Belimumab can also be injected subcutaneously with a dosage of 200mg. Currently, SC Belimumab can be injected via either auto-injector or prefilled syringes**. (73)**

The assumed metabolic pathway includes degradation to small peptides and amino acids by proteolytic enzymes. The distribution half life of IV belimumab is 1.75 days, while SC belimumab has a half-life of 1.1 days. The systemic clearance of IV belimumab is 215 ml/day, against 204 ml/day in case of SC belimumab. **(74)**

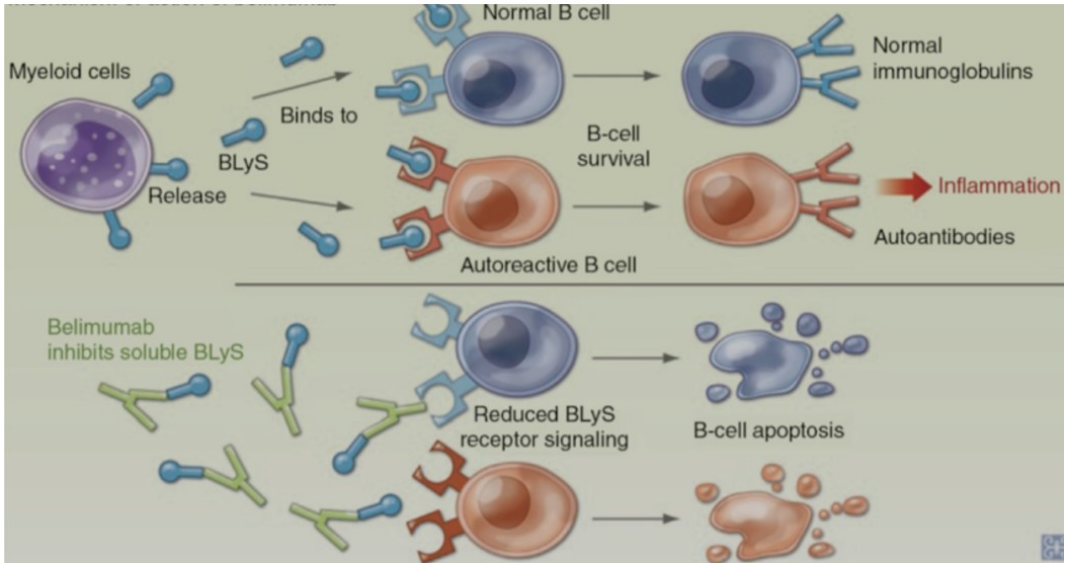


Figure 2. [Mechanism of action of Belimumab linking to Blys to inhibit BLyS receptor signaling and therefore promoting cell apoptosis]. Available via Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0). Source (https://www.researchgate.net/figure/Mechanism-of-action-of-Belimumab-linking-to-Blys-to-inhibit-BLyS-receptor-signaling-and_fig3_379947283)

3.2 Contraindications

At the moment, the only contraindication to the use of Belimumab is if the patient has experienced an anaphylactic reaction to the treatment before.

As for pregnancy, Belimumab is a Pregnancy Category C medication, which means that the drug may be administered to a pregnant woman only if the potential benefits surpass the risks. **(75)**

Another indication is that live vaccines should not be administered to the patient within 30 days before Belimumab is administered. The cause behind this indication is that the drug may interfere with the patient's reaction to immunizations. **(75)**

3.3 Adverse drug reactions

As for side effects of Belimumab, during the studies it was noticed that the patients who received the drug experienced more psychiatric AEs (depression, insomnia) than those who received the placebo.

Other AEs found during these trials are nausea, diarrhea, pyrexia, pharyngitis, bronchitis. These adverse reactions were not due to increased doses of Belimumab. **(75)**

3.4 BLiSS 52

It is a phase III, randomized study of Belimumab. This study was a placebo-controlled, RTC study. 819 patients were randomized to receive either the drug (1/10 mg/kg) or the placebo. Primary outcome in this study was the SLE responder Index (SRI) at week 52 (≥4 point decrease in SELENA-SLEDAI score). **(76)**

As a result, treatment with 10 mg/kg Belimumab and standard SLE therapy led to a significantly better SRI response than placebo. It also led to reduced corticosteroid use and prevented SLE flares. **(76)**

53% of the patients treated with belimumab 1mg/kg and 58% of those treated with belimumab 10mg/kg had their SELENA-SLEDAI score reduced by at

least 4 points during 52 weeks, against 46% of the patients who only received the placebo. **(77)**

The BLiSS 52 study was carried out primarily in Asia, South America, and Eastern Europe. It included patients who met the conditions: diagnosis of SLE according to ACR criteria, active disease, and seropositivity of autoantibodies in at least two counts. **(76)**

3.5 BLiSS 76

BLiSS 76 is a randomized study that followed the BLiSS 52 study. The patients in this study were randomized to receive either Belimumab at a dosage of 10 mg/kg or the placebo. The primary endpoint was to demonstrate the effectiveness and safety of the drug. **(76)**

Patients that received the treatment showed improvement of serological and clinical variables after 76 weeks.

SRI response rates at week 76 were 32.4% with placebo, 39.1% with 1mg/kg belimumab and 38.5% with 10mg/kg belimumab. **(76)**

3.6 BLiSS-NEA

BLiSS-NEA is a randomized phase III study that included more than 600 patients from Japan, China and Korea. The primary endpoint of this study was SLE Responder Index 4 (SRI-4) response at week 52. **(78)**

Patients were treated with belimumab at a dosage of 10 mg/kg IV or were given the placebo.

The response was analyzed according to gender, country of origin, concomitant medications, SELENA-SLEDAI score, antibodies positivity, complement levels, ACR score.

Patients with a baseline SELENA-SLEDAI score ≥10 had a better response with Belimumab than patients with a score ≤9. Also, patients with low C3/C4 and positive anti-dsDNA at baseline had a better response with Belimumab than those who were treated with standard of care alone.

53.8% of the patients that received belimumab were SRI4 week 52 responders, against 40.1% of those only treated with standard of care. **(78)**

3.7 BLiSS-SC

BLiSS-SC is a randomized phase III study. The objective of this study was the effectiveness of subcutaneous administration of Belimumab in SLE patients.

Out of 836 patients, 556 of them were treated with 200 mg of Belimumab plus standard therapy, while the others were given the placebo plus standard therapy. The trial enrolled adults with a diagnosis of active SLE, and positive autoantibodies. **(79)**

The primary endpoint was the SLE Responder Index (SRI4) after 54 weeks. Secondary endpoints included reduction of GC dosage and time to severe flare. As a result, 61.4% of the patients who received Belimumab were SRI4 responders versus 48.4% of the patients that received placebo. **(80)**

3.8 OBServe studies

OBSErve (evaluation of use of Belimumab in clinical practice settings) is an observational study program that has included a multinational cohort. Its results have been accounted from Canada, USA, Spain and Germany. **(81)**

The OBSErve studies had common results regarding Belimumab's efficacy, confirming the phase III trial.

Other clinical observational studies were made by different groups. One of them is the study leaded by Hui-Yuen et al. This study included data from 10 medical centers in USA and Sweden. The only contraindication for patients to be included in this study was history of severe renal involvement or neuropsychiatric symptoms. 3 months after the first administration of Belimumab, there were serological and clinical markers that showed improvement of the patients' general state. The serological markers included improvement of C3 and C4 levels, as well as a decrease in levels of dsDNA autoantibodies. Another important outcome of this

study was the ability to decrease the dosage of corticosteroids after approximately 6 months from the initiation of Belimumab administration.

In the OBServe studies, 2-9.6% of patients discontinued GCs and 72-88.4% reached ≥20% improvement at 6 months. **(81)**

3.9 Real life studies

In Italy, a real-life study including 11 prospective cohorts was performed, with the aim of evaluating the effectiveness and safety of Belimumab. The conditions of inclusion in the study were the satisfaction of ACR criteria for SLE, active and refractory SLE involvement, positive anti-dsDNA antibodies and/or low C3/C4 levels. On the other side, patients with severe renal involvement, neuropsychiatric manifestations, other life-threatening SLE symptoms and pregnant or planning to get pregnant patients were excluded from the study. After administrating Belimumab, serological and clinical variables were collected at baseline and then every 6 months, including C3/C4 count, blood test, dsDNA autoantibodies, dosage of glucocorticoids. The major part of adverse events in these patients was manifested with infections, while other AEs included hypersensitivity reactions, infusion reaction, depression. **(82)**

The results suggested that among SLE patients with positive ant-dsDNA antibodies and low C3/C4, the ones manifesting polyarthritis and/or skin involvement, are the best responders to Belimumab. Another outcome of the study was the decrease of flare rates in patients in treatment with the monoclonal antibody. **(82)**

BeRLiSS-LN is a real life study that analyzed the efficacy and safety of Belimumab in patients with Lupus Nephritis in a clinical practice setting. Patients included in the study presented active SLE with a clinical SLEDAI (cSLEDAI) score >0. They also had positive ds-DNA and/or low C3/C4. Another inclusion criteria was renal manifestations at the beginning of belimumab therapy, including eGFR≤60ml/min/1.73m^2, and fulfillment of SLEDAI-2K renal items, such as hematuria, proteinuria, and pyuria. These patients were treated with intravenous belimumab (10mg/kg at day 1, 14, 28 and then every 28 days) for at least 6 months.

Primary outcome was the accomplishment of primary efficacy renal response with proteinuria ≤0.7g/24h, eGFR≥60ml/min/1.73m^2 evaluated at 6, 12 and 24 months.

70.3% of the patients achieved primary efficacy renal response. Among these, 38.4% also achieved complete renal response as secondary outcome, which included proteinuria ≤0.5g/24h, eGFR≥90ml/min/1.73m^2 and no rescue therapy. **(83)**

BeRLiSS-JS is a real life study that included patients with skin and joint manifestations in therapy with belimumab. Patients with active joint involvement according to SLEDAI-2K score and skin involvement according to CLASI≥1 were included. The CLASI consists of 2 scores, activity of the disease and measure of the damage done by the disease. The activity is scored depending on erythema, hyperkeratosis, mucous membrane involvement, acute hair loss and alopecia. Damage is evaluated based on dyspigmentation and scarring.

51.6% of patients with joint manifestations were SRI-4 responders at 6 months, while 58.5%, 62.3% and 64.8% were SRI-4 responders at 12, 24 and 36 months, respectively.

As for patients with skin involvement, 16.5%, 26.1%, 33.7% and 36.7% achieved remission at 6, 12, 25 and 36 months, respectively. **(84)**

3.10 Belimumab predictors of response

Multivariate analyses were performed on the patients included in BLISS 56 and 76 trials to determine baseline factors that lead to a higher benefit of belimumab versus standard of care. These factors included SELENA-SLEDAI score ≥10, positive anti-dsDNA antibodies, treatment with GCs, and low C3/C4. Comparing SRI4 rates in the low C3/C4 and anti-dsDNA-positive subgroups, the results indicated a higher response rate in patients treated with belimumab 10mg/kg (51.5%) and belimumab 1mg/kg (41.5%), against placebo (31.7%). **(85)**

INTRODUCTION TO THE STUDY

Belimumab is the first monoclonal antibody approved for the treatment of SLE. Clinical trials and real-life studies have shown belimumab's efficiency in treating many organ manifestations, like BeRLiSS-JS for joints and skin, or BeRLiSS-LN for renal involvement**.(83)(84)**

Up to present, the efficacy and safety of belimumab in hematological manifestations has not been specifically studied yet. This gap accentuates the need for targeted studies to evaluate its role in regulating this specific manifestation of SLE.

The purpose of our study is to evaluate this aspect, trying to broaden the clinical benefits of the treatment and optimize its use in the future.

Chapter 4: Objective of the study

The objective of this study is to evaluate the efficacy and safety of Belimumab in patients with SLE who manifest hematological involvement, particularly lymphopenia at baseline. By analyzing changes in lymphocyte counts over time within a multicenter, prospective cohort, this research aims to assess Belimumab's role in attaining remission and improving lymphocyte counts, while also documenting its safety during the treatment period (*Study A*)

The second part of this study aims to evaluate the efficacy and safety of Belimumab in a separate cohort of SLE patients with baseline lymphopenia, monitored over an extended follow-up period. This longer term evaluation aims to track lymphocyte count trends to provide additional information into Belimumab's long-term impact and safety in patients with persistent hematological involvement. (*Study B*)

Chapter 5: Methods and materials

5.1 BeRLiSS

BeRLiSS (Belimumab in Real Life Setting Study) is a national multicenter cohort study. Inclusion criteria in the BeRLiSS study were:

1- accomplishment of the American College of Rheumatology (ACR) 1982 revised criteria for SLE or the Systemic Lupus International Collaborating Clinics (SLICC)/ACR classification criteria for SLE of 2012
2- active disease, defined by a clinical SLE Disease Activity Index (SLEDAI) score of >0, that is refractory to a standard of care regimen.
3- Therapy with belimumab IV or s.c. at standard dose for at least 6 months.

5.1.1 Data collection

Patients were followed up from 1st of January 2013 to the 31st of March 2019 prospectively based on EULAR 2019 recommendations for monitoring of SLE patients in clinical practice and observational studies. Patient data were registered anonymously in an ad hoc database since first administration of belimumab and were regularly updated.

Clinical and laboratory variables collected at baseline and every 6 months included: SLEDAI2K score, daily prednisone intake, complete blood cell count, proteinuria, levels of anti-dsDNA, levels of C3 and C4, SLEDAI2K and SRI4 score, and concomitant medications.

The study was approved by the University of Padua Ethics Committee and was achieved in accordance with the Declaration of Helsinki. Each patient signed an informed consent about personal data treatment.

5.2 Study A

5.2.1 Participants

The study was a post hoc analysis that involved patients included in the BeRLiSS study who manifested baseline lymphopenia. Additional inclusion criteria were:

1) Diagnosis of SLE based on the American College of Rheumatology criteria
2) Presence of hematological involvement, specifically lymphopenia (lymphocyte count < 1000 cells/mm^3) at baseline
3) Ongoing treatment with Belimumab for at least six months prior to inclusion
4) No concurrent participation in other SLE treatment trials

Patients included in the study were followed up for at least 6 months with data regarding hematological manifestations at baseline and at month six, twelve, twenty-four, thirty-six and forty-eight. Patients were included in the study until the last available infusion.

5.2.2 Methods

Lymphocyte counts were recorded at baseline, 6, 12, 24, 36, and 48 months. In addition to lymphocyte counts, other clinical parameters, such as disease activity scores, C3 and C4 complement levels, and prednisone dosage, were assessed at each time point to provide a better understanding of hematological response and disease progression.

The study's primary outcome was the change in lymphocyte count over time, aiming to assess remission, defined as reaching a lymphocyte count > 1000 cells/mm^3. Secondary outcomes included prednisone dose reduction and overall patient stability, as indicated by complement levels and disease activity indices.

5.3 Study B

5.3.1 Participants

A complementary analysis including patients treated with long-term Belimumab therapy and followed at the Padua Lupus Clinic (Division of Rheumatology, University of Padua, Italy) was carried out to evaluate response to treatment. Inclusion criteria were as follows: 1) Fulfillment of the American College of Rheumatology (ACR) 1982 revised criteria for SLE or the Systemic Lupus International Collaborating Clinics (SLICC)/ACR classification criteria for SLE of 2012; 2) Belimumab treatment plus standard of care; 3) lymphopenia at baseline; 4) more than 48 months of follow up.

5.3.2 Methods

Lymphocyte counts were recorded at baseline and then at the last follow up. To measure safety, number of infections during belimumab therapy was recorded. Incidence rate of infections in patients treated with belimumab was compared with incidence rate of infections in patients included in Padua Lupus Cohort not treated with belimumab. Other parameters, such as disease activity scores, number of infections, C3 and C4 complement levels, and prednisone dosage, were documented at baseline and at the last follow up.

5.4 Statistical analysis

Univariate analysis was used to describe baseline characteristics. Multivariate tests, including Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root, evaluated the effect of time on lymphocyte count across different time points. Mauchly's Test of Sphericity was carried out to assess the assumption of sphericity; violations were corrected using Greenhouse-Geisser and Huynh-Feldt adjustments.

Linear contrasts examined trends in lymphocyte count changes over time. The statistical significance of within-subjects and between-subjects effects was analyzed to determine whether prior immunosuppressive therapy and other baseline variables influenced the outcomes. All analyses were conducted using statistical software, with significance set at $p < .05$.

Chapter 6: RESULTS

6.1 Study A

This study involved 107 patients displaying lymphopenia (L<1000/mmc) at baseline from the BeRLiSS cohort.

Demographic, clinical and serological features at baseline are reported in Table IV.

Table IV: Demographic, clinical and serological features of the patients.

	Baseline
Number of patients	107
Females	95 (88.8%)
Males	12 (11.2%)
Chronic active disease	36 (33.6%)
Relapsing remitting disease	73 (68.2%)
Early_use_Belimumab	1.00 23 (21.5%)
	2.00 84 (78.5%)
Hematological manifestation	56 (52.3%)
Renal manifestation	21 (19.6%)
Serositis	8 (7.5%)
Smoke	19 (17.9%)
Previous arthritis	93 (86.9%)
Previous mucocutaneous involvement	78 (73.6%)
Previous hematological involvement	69 (64.5%)
Previous neurological involvement	6 (5.7%)
Previous renal involvement	39 (36.4%)
Previous serositis	30 (28.0%)

Previous immunosuppressor	61 (57.0%)
Motive_discontinuation	AEs: 12 (48.0%)
	Inefficacy: 4 (16%)
	Other: 9 (36%)
Previous/concomitant IS	95 (88.8%)
CQ (mg)	4 (3.7%)
ANA	106 (99.1%)
AntiDNA	102 (95.3%)
aPL	41 (38.3%)
Anti-P RIB	14 (13.2%)
Anti-Sm	36 (33.6%)
Anti-SSA	61 (57.0%)
Anti-SSB	17 (15.9%)
Anti-URNP	42 (39.3%)
APS	16 (15.0%)
SRI4_12	68 (69.4%)
SRI4_24	54 (79.4%)
Previous AZA	52 (48.6%)
Previous CsA	27 (25.2%)
Previous CYF	18 (16.8%)
Previous HCQ	50 (46.7%)
Previous MMF	51 (47.7%)
Previous MTX	53 (49.5%)
Previous RTX	12 (11.2%)

Median and percentiles of C3/C4 levels, lymphocytes, prednisone daily take, SLEDAI2K AND SLICC-DI at different time-points are reported in Table V, VI, and VII.

Table V: Mean, standard deviation, median, p-value,25th and 75th percentiles of C3, C4, nDNA at different time-points.

Variables	**Mean**	**Standard deviation**	**Median**	**25th percentile**	**75th percentile**	**p-value**
C3 (mg/dl)						
Baseline	69.56	16.80	70.00	60.00	78.00	
6 months	80.6	18.2	81.5	68.0	95.0	<0.05
12 months	83.71	21.02	84.50	72.00	95.00	
24 months	85.37	19.44	93.00	71.00	95.00	
36 months	87.60	19.20	91.00	74.00	95.00	
48 months	90.7	21.2	95.0	84.0	98.0	
C4 (mg/dl)						
Baseline	10.00	5.26	9.20	6.50	13.50	
6 months	13.65	6.67	15.00	10.00	16.00	
12 months	15.08	10.96	15.00	10.00	17.00	<0.05
24 months	14.76	5.87	15.00	13.00	17.00	
36 months	15.8	6.2	15.0	13.0	20.0	
48 months	16.82	5.62	15.00	15.00	20.00	
nDNA						
Baseline	.80	.39	1.00	1.00	1.00	
12 months	.72	.44	1.00	.00	1.00	<0.05
24 months	.70	.45	1.00	.00	1.00	
36 months	.55	.50	1.00	.00	1.00	
48 months	.61	.49	1.00	.00	1.00	

Table VI: Mean, standard deviation, median, p-value,25th and 75th percentiles of lymphocytes, PDN, WBC, Hb at different time-points.

Variables	**Mean**	**Standard deviation**	**Median**	**25th percentile**	**75th percentile**	**p-value**
Lymphocytes (mmc)						
Baseline	720.02	208.75	770.00	532.00	900.00	<0.001
6 months	778	325	775	575	935	
12 months	910.17	436.43	810.00	600.00	1200.00	
24 months	1042	570	950	680	1200	
36 months	1098.78	557.82	950.00	660.00	1210.00	
48 months	1144	447	1200	880	1200	
PDN (mg/die)						
Baseline	10.63	8.59	10.00	5.00	12.50	<0.001
6 months	6.27	4.70	5.00	3.75	7.50	
12 months	6.29	6.29	5.00	2.50	7.50	
24 months	4.25	4.19	5.00	1.25	5.00	
36 months	3.79	3.91	3.66	.00	5.00	
48 months	3.13	3.16	2.50	.00	5.00	
WBC (mmc)						
Baseline	4567.11	2591.44	4070.00	2970.00	5300.00	
6 months	4439	1809	4155	3200	5015	
12 months	4214.51	1430.22	4170.00	3120.00	4670.00	0.06
24 months	4300.88	1144.29	4500.00	3640.00	4800.00	
36 months	4580	1401	4500	3800	5160	
48 months	4770	1729	4500	3900	5330	
Hb (g/dl)						
Baseline	11.67	1.39	11.90	10.80	12.80	
6 months	11.70	1.68	11.90	11.00	12.70	
12 months	12.02	1.31	12.10	11.10	13.00	0.09
24 months	12.12	1.26	12.20	11.50	13.00	
36 months	12.44	1.27	12.80	11.80	13.30	
48 months	12.38	1.25	12.80	12.00	13.00	

Table VII: Mean, standard deviation, median, p-value,25th and 75th percentiles of SLEDAI-2K, PGA, SLICC-DI, fatigue at different time-points.

Variables	**Mean**	**Standard deviation**	**Median**	**25th percentile**	**75th percentile**	**p-value**
SLEDAI-2K						
Baseline	9.74	3.93	10.00	8.00	12.00	<0.05
6 months	5.35	3.90	4.00	2.00	6.50	
12 months	4.31	3.28	4.00	2.00	6.00	
24 months	3.39	2.94	3.50	2.00	4.00	
36 months	3.00	2.35	2.00	2.00	4.00	
48 months	2.62	2.44	2.00	1.00	4.00	
PGA						
Baseline	2.25	1.47	2.00	1.50	2.50	<0.05
6 months	1.44	1.47	1.00	.60	2.00	
12 months	1.20	1.38	1.00	.50	1.50	
24 months	.69	.82	.50	.00	1.00	
36 months	.61	.73	.50	.00	1.00	
48 months	.6	.6	.5	.0	1.0	
SLICC-DI						
Baseline	1	2	0	0	1	<0.05
12 months	1	2	0	0	1	
24 months	1	2	1	0	1	
36 months	1	2	1	0	2	
48 months	1	2	1	0	1	
Fatigue (VAS 0-10)						
Baseline	5.0	2.6	5.0	3.0	7.0	0.018
6 months	3.2	2.0	3.0	2.0	4.5	
12 months	2.9	2.2	2.0	1.0	5.0	
24 months	2	2	2	0	4	
36 months	2	2	2	1	3	

Activity indicators, such as SLEDAI-2K scores, PGA scores, and fatigue scores, all decrease significantly.

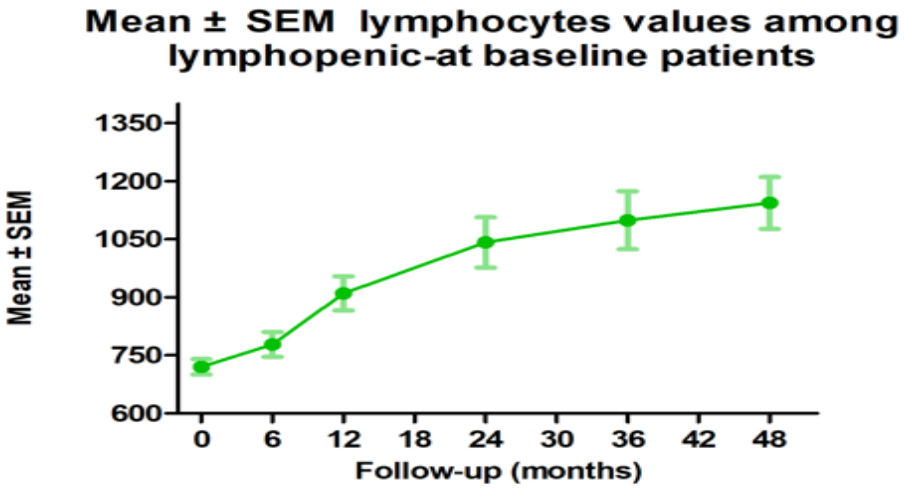
C3 and C4 gradually increase, and lymphocyte counts rise. Additionally, the prednisone daily dosage required for patients decreases.

- **Lymphopenia at baseline**

In this statistical analysis, the 107 patients displaying lymphopenia at baseline from the BeRLiSS cohort were taken in consideration.

The effect of the time factor on lymphocyte counts was statistically significant ($p < .001$). This confirms that there are significant differences in lymphocyte counts at different time points, with an increase in lymphocyte count during belimumab treatment.

Graphic 1: Mean of lymphocytes count at different time-points.



N°	107	104	99	77	55	44
Mean	720	778	910	1042	1099	1144
±SEM	20	32	44	65	75	67

Graphic 1 represents the linear progression of the mean of lymphocyte counts at each time point. Lymphocyte count rises from 720/mmc at baseline to 1143/mmc at the last follow up at 48 months.

- **Lymphocyte counts over time in patients with and without prior immunosuppressive therapy**

We also evaluated the contribution of concomitant conventional immunosuppressants on lymphocyte count

However, the interaction of time and immunosuppressive therapy history was not significant, suggesting similar trends in lymphocyte changes over time not depending on prior therapy.

At baseline, there was no significant difference between patients with and without prior immunosuppressive therapy regarding their overall lymphocyte levels ($p = .584$), suggesting similar baseline levels in both groups.

We found that the interaction of time and immunosuppressive therapy was not significant, suggesting similar trends in lymphocyte changes over time irrespective of the concomitant use of conventional immunosuppressive agents with a consistent trend, regardless of prior immunosuppressive therapy.

This suggests that Belimumab treatment impacts lymphocyte levels similarly in patients with and without immunosuppressive use.

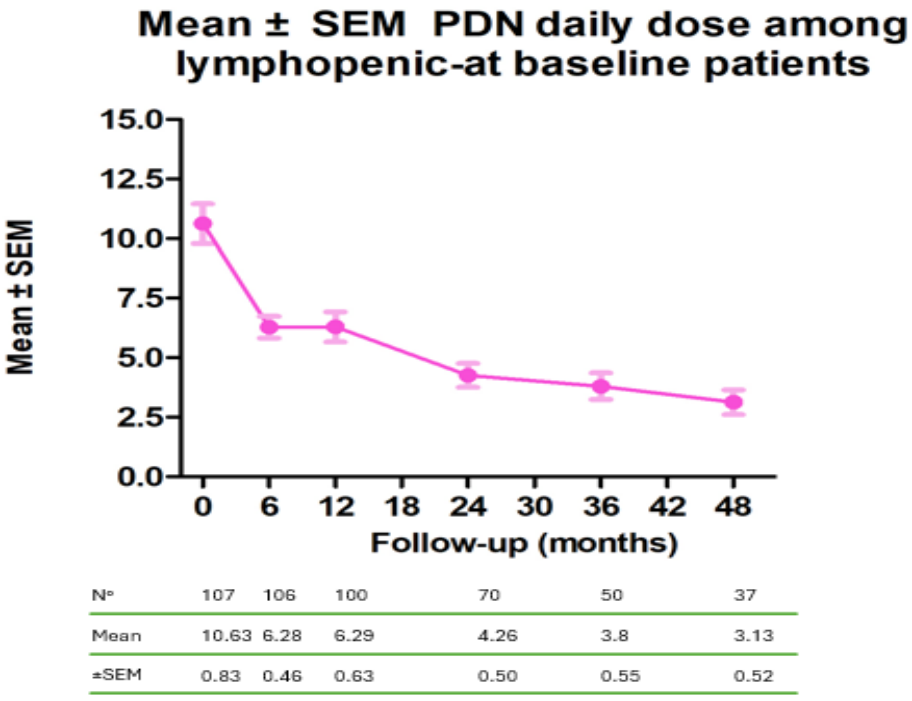
- **Prednisone dosage in patients with lymphopenia at baseline**

Table VIII: Prednisone dosage of patients with lymphopenia at different time-points.

PDN (mg/die)	N	Min	Max	Mean	Standard deviation	p-value
Baseline	107	.00	50.00	10.63	8.59	
6 months	106	.00	25.00	6.27	4.70	0.14
12 months	100	.00	50.00	6.29	6.29	0.44
18 months	81	.00	50.00	5.36	6.23	0.029
24 months	70	.00	25.00	4.25	4.19	<0.001
36 months	50	.00	20.00	3.79	3.91	<0.001
48 months	37	.00	15.00	3.12	3.16	<0.001

We also evaluated the dose of glucocorticoids that patients had taken at baseline and throughout the follow-up.

This analysis demonstrates a significant decrease in prednisone dosage over the study duration, which indicates a possible successful tapering in response to treatment.



Graphic 2: prednisone dosage at different time-points in patients with lymphopenia at baseline.

Graphic 2 summarizes the prednisone dosages trend in patients with lymphopenia treated with belimumab. Notably, we observed an initial (6 months) fast reduction in GC dose, as highlighted by the slope of the curve. In the subsequent follow-up, mean daily dose continued to decrease over time.

- **Prednisone dosage with/without immunosuppressants**

In addition, patients were categorized in two groups in table IX to study the prednisone dosages at different time point in patients with or without immunosuppressants. The results of this subanalysis are reported in Table IX.

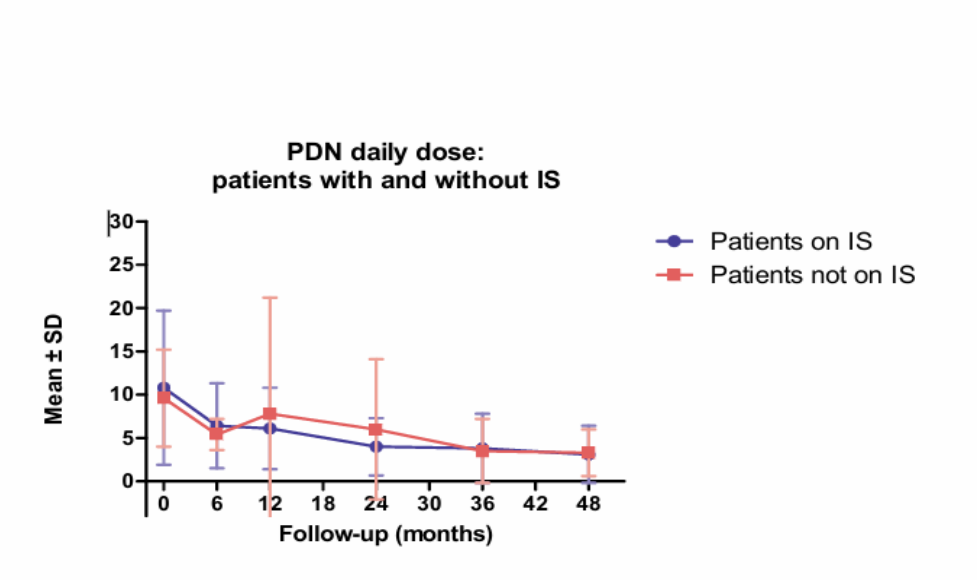
Table IX: mean and standard deviation of prednisone doses in patients with and without immunosuppressants at different time-points.

	Without immunosuppression (12 patients)			With immunosuppression (95 patients)		
PDN (mg/die)	**Mean**	**Standard deviation**	**p-value**	**Mean**	**Standard deviation**	**p-value**
Baseline	9.6	5.6	0.016	10.8	8.9	0.148
6 months	5.4	1.8		6.4	4.9	
12 months	7.8	13.4		6.1	4.7	
24 months	6.0	8.1		4.0	3.3	
36 months	3.5	3.7		3.8	4	
48 months	3.3	2.7		3.1	3.3	

Both groups show a progressive reduction in GC dosage over the follow-up period.

Notably, the group with prior or concomitant treatment with immunosuppressants generally maintains slightly lower prednisone doses after 6 months.

The differences between groups diminish by 36 and 48 months, indicating that both groups achieve similar control with lower prednisone use over time.



Graphic 3: Mean±SD of prednisone dosages in patients with and without immunosuppressants at different time-points.

Graphic 3 shows the reduction of prednisone dosage over the 48-month follow-up for both groups.

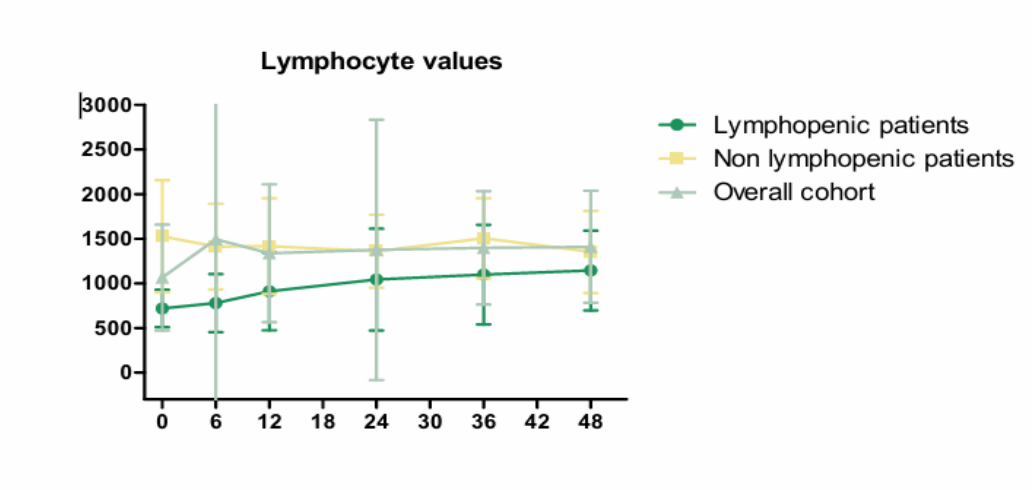
From 24 months onwards, the prednisone dosages in both groups converge, with minimal differences by the 48-month timepoint.

- **Lymphocyte count over time in non-lymphopenic patients at baseline**

Table X: lymphocyte counts in non-lymphopenic patients at different time-points

Lymphocytes (mmc)	**N**	**Min**	**Max**	**Mean**	**Standard deviation**	**p-value**
Baseline	80	1010.00	4600.00	1527.5	629.9	
6 months	74	700	3230	1411.9	480.1	
12 months	65	690.00	3870.00	1417.5	535.7	0.06
24 months	49	520	2550	1358.8	409.2	
36 months	27	800.00	2600.00	1505.2	448.9	
48 months	23	620	2500	1353.0	460.5	

Since some previous papers reported that belimumab can induce a reduction in lymphocyte count, we measured lymphocyte count in patients with baseline normal values. Lymphocyte counts did not show a meaningful change over time in this population (p=0.06), although a trend toward reduction was observed (Graphic 4).



Graphic 4: Mean of lymphocyte counts at different time-points in lymphopenic, non-lymphopenic, and overall cohort.

6.2 Study B

An additional study was carried out at the Padua Lupus Clinic (Division of Rheumatology, University of Padua, Italy) to assess the long-term efficacy and safety of belimumab. Thirteen patients treated with Belimumab for more than 4 consecutive years were included in the study.

Values of lymphocytes at baseline and at the last follow up for 93±24 months were integrated in the analysis, as well as other variables, including c3/c4 levels, prednisone dosages, SLEDAI-2K and SLICC-DI scores.

Demographic, clinical and serological features, and concomitant treatment at baseline are reported in Table XI

Table XI: demographic, clinical, and serological features of patients in study B.

TOTAL PATIENTS	**13 (100%)**
Females	10 (76.9%)
Males	3 (23.1%)
Rash	11 (84.6%)
Alopecia	4 (30.7%)
Arthritis	11 (84.6%)
Serositis	3 (23.1%)
Proteinuria	8 (61.5%)
Hematuria	6 (46.2%)
Thrombocytopenia	7 (53.8%)
Leukopenia	13 (100%)
Neurologic involvement	2 (15.4%)
Vasculitis	3 (23.1%)
C3C4 low	13 (100%)
AntiDNA	11 (84.6%)
SSASSB	8 (61.5%)
U1RNP	9 (69.2%)
sdrantiPL	1 (23.1%)

Prior IS use		
MMF	7 (53.8%)	
AZA	7 (53.8%)	
Cyclosporine	5 (38.5%)	
Cyclophosphamide	2 (15.4%)	
MTX	6 (46.2%)	
Leflunomide	0 (0%)	
Belimumab	13 (100%)	
Rituximab	6 (46.2%)	
Tacrolimus	3 (23.1%)	
Steroid bolus	5 (38.5%)	
Concomitant IS		
MMF	7 (53.8%)	
MTX	2 (15.4%)	
Tacrolimus	1 (7.7%)	
Leflunomide	1 (7.7%)	
CsA	1 (7.7%)	
On steroid at last follow up	6 (46.2%)	
GC ever suspended	8 (61.5%)	
Hospitalizations for infections ever	4 (30.7%)	
Overall severity (Mild=0, Moderate=2, Severe=3)	2	6 (46.2%)
	3	7 (53.8%)
Disease course (RR=1, Chronic Active=2, longstanding quiescent=3)	1	10 (76.9%)
	2	2 (15.4%)
	3	1 (7.7%)

In table XII, the mean±SD of various variables are reported, including activity index SLEDAI-2K, damage index SLICC-DI, lymphocyte count, C3/C4 levels and PDN dosage. The data were collected at baseline and at the last follow up.

Table XII: Mean and standard deviation of SLEDAI-2K and SLICC-DI, lymphocyte count, C3/C4 levels and PDN dosage at baseline and last follow up.

Variables	**Mean**	**Standard deviation**	**p-value**
SLEDAI-2K at baseline	8.1	3.6	0.004
SLEDAI-2K at last follow-up	2.8	3.1	
SLICC-DI at baseline	0.4	0.6	0.035
SLICC-DI at last follow-up	0.9	1.1	
Lymphocytes at baseline	745	194.3	0.001
Lymphocytes at last follow-up	1289	534	
C3 at baseline	.66	.15	0.005
C3 at last follow-up	.95	.15	
C4 at baseline	.198	.320	0.045
C4 at last follow-up	.08	.04	
GC dosage at baseline	10.9	7.2	0.002
GC dosage at last follow-up	2.3	0.5	
Year of diagnosis	1999	7.1	

The data shows reduction in disease activity (SLEDAI-2K), increased lymphocyte counts, and reduced use of steroids, as shown in Table XII.

- **Prednisone use**

Baseline

Table XIII indicates the number of patients administered with different dosages of GCs at baseline.

Table XIII: Count of patients with different GC dosage at baseline.

GC dosage 5mg	4 (30.8%)
5mg<GC dosage≤7,5mg	4 (30.8%)
GC dosage > 7,5 mg	5 (38.4%)
Mean	10.96
SD	±2.8

Last follow-up

Table XIV summarizes the GC dosage at last follow up.

Table XIV: Count of patients with different GC dosage at last follow-up.

GC dosage 5 mg	6 (46.2%)
GCs suspended	7 (53.8%)
GC dosage > 5mg	0 (0%)
Mean	2.3
SD	±2.5

At the last follow-up, only 6 patients were still receiving a maintenance dosage of GCs, all below 5 mg. the other patients completely discontinued prednisone (53.8% of cases).

- **Infections during follow-up**

Table XV: count of patients with infections during follow-up

Infections	yes	9	69.2%
	no	4	30.8%

To further investigate the safety of belimumab in these patients, we studied the number of infections they experienced during the period of follow up. The follow up varies from 60 to 128 months, with a mean of 93 months.

During the period of follow up, 4 patients out of 13 registered an episode of infection by Herpes Zoster (HZ), which is caused by a reactivation of latent Varicella-zoster virus.

In our population, the incidence rate of infections was 0.038 infections/person-years.

Table XVI: Incidence rate of infections in our population during the period of follow-up

95% CI of number counted	1.0899 to 10.2416
Incidence rate	0.03883
95% Confidence Interval	0.01058 to 0.09943

CHAPTER 7: DISCUSSION

There are no recommendations for specific treatment for lymphopenia in SLE patients. Lymphopenia is often related to higher steroid doses and cyclophosphamide use. **(90)**

In this study, we evaluated the efficacy of Belimumab in patients with hematological manifestations, focusing on lymphopenia.

Belimumab is a humanized monoclonal antibody that binds the soluble BlyS cytokine, leading to BlyS not being able to bind its three receptors on B cells. As a result, B cell survival and their production of autoantibodies is reduced. **(71)**

BlyS is found in high levels in SLE patients, contributing to its pathogenesis by collaborating with cytokines and TLRs to promote Ig class switching. **(30)**

Besides the extensive research about Belimumab and its effects in reducing disease activity and preventing flares in SLE, its specific effects on hematological manifestations have not been properly studied.

BeRLiSS is a multicenter nationwide cohort, where patients' data is updated at each follow up.

The results of our study that included 107 patients with lymphopenia at baseline from the BeRLiSS cohort showed a trend of improvement in disease response over time, with lymphocyte counts rising and SLEDAI-2K and PGA scores decreasing. Overall, the time-based changes suggest a favorable response to treatment, with progressive reduction in disease activity and the need for steroids.

The analysis on lymphocyte counts through 48 months of follow up showed a significant response.

We also assessed prednisone dosage trend in patients treated with belimumab. The results showed a significant reduction in PDN doses among patients during the follow up, indicating a possible success in tapering due to treatment.

There is a significant reduction in the mean GC dosage from baseline to the last follow-up, with a mean decreasing from 10.9 mg to 2.3 mg. It suggests an improvement in disease management, as patients require lower doses of glucocorticoids over time. Lower GC dosages are generally preferable due to the

reduction in potential side effects associated with long-term steroid use. GCs are commonly prescribed in SLE patients because of their fast action in the immune system. Given the long-term side effects, reduction of GC doses is one of the most important goals in SLE management. **(87)**

To further investigate PDN tapering in our patients, we studied possible interference of concomitant immunosuppressive therapy. The group with prior or concomitant treatment with immunosuppressants generally maintains slightly lower prednisone doses after 6 months, suggesting a possible effect of these drugs in diminishing the dependence on corticosteroids.

The differences between groups diminish by 36 and 48 months, indicating that both groups achieve similar control with lower prednisone use over time.

We also studied the lymphocyte count trend in non-lymphopenic patients at baseline, in order to evaluate the effect of blocking BlyS in patients without hematological involvement, since a reduction in lymphocyte count has been reported in previous studies.**(76)** We did not find a significant reduction in lymphocyte count, suggesting relatively stable lymphocyte counts across the time points.

Since the patients considered for our study were followed up for 48 months, an additional study was conducted with patients followed up in Padua Lupus Clinic and treated with belimumab for at least 5 years, in order to further investigate the efficacy and safety of the drug in a longer term.

Thirteen patients with lymphopenia at baseline with at least 5 years of follow up were studied. The analysis showed a significant increase in lymphocyte count, with a mean of 745/mmc at baseline and then 1289 lymphocytes/mmc at the last follow up. In addition, the SLEDAI-2K showed substantial improvement at the last follow up and SLICC-DI did not worsen. Prednisone dosages were reduced at the last follow up, showing better management of the disease.

We also analyzed the incidence rate of infections in our patients treated with belimumab for at least 5 years. The incidence of infection was within the range observed in the general SLE population. This confirms previous data on the safety of belimumab, reinforces the importance of reducing glucocorticoid dose and disease activity, which contributes to reduction in infection risk.

Autoimmune diseases, such as SLE, present a higher risk for recurrent HZ infections, alongside with malignancies, and immune deficiency syndrome. As a matter of fact, the incidence of HZ in the general population varies from 1.2 to 4.9 cases per 1000 person-years. In SLE patients, the incidence is much higher, it ranges from 6.4 to 37.7 cases per 1000 person-years. **(86)**

Overall, HZ incidence rate in our study is comparable or slightly higher than the upper range of the general SLE population.

Our study provides important insights into Belimumab's role in reducing disease activity, and lowering GC dependence, and ensuring an acceptable safety profile.

It also underlines the importance of biological therapies such as Belimumab in SLE management to improve disease outcomes.

Our results confirm Belimumab's ability in reducing disease activity and achieving remission, with a good safety profile even in patients with lymphopenia.

REFERENCES

1. Fortuna, Giulio, and Michael T. Brennan. 'Systemic Lupus Erythematosus: Epidemiology, Pathophysiology, Manifestations, and Management'. *Dental Clinics of North America*, vol. 57, no. 4, Oct. 2013, pp. 631–55. *PubMed*, https://doi.org/10.1016/j.cden.2013.06.003.
2. Kiriakidou, Marianthi, and Cathy Lee Ching. 'Systemic Lupus Erythematosus'. *Annals of Internal Medicine*, vol. 172, no. 11, June 2020, pp. ITC81–96. *PubMed*, https://doi.org/10.7326/AITC202006020
3. Tian, Jingru, et al. 'Global Epidemiology of Systemic Lupus Erythematosus: A Comprehensive Systematic Analysis and Modelling Study'. *Annals of the Rheumatic Diseases*, vol. 82, no. 3, Mar. 2023, pp. 351–56. *ard.bmj.com*, https://doi.org/10.1136/ard-2022-223035
4. Lim, S. Sama; Drenkard, Cristinab. Epidemiology of lupus: an update. Current Opinion in Rheumatology 27(5):p 427-432, September 2015. | DOI: 10.1097/BOR.0000000000000198
5. Ferrara, Pietro, et al. 'Epidemiology of SLE in Italy: An Observational Study Using a Primary Care Database'. *Lupus Science & Medicine*, vol. 11, no. 1, May 2024, p. e001162. *PubMed*, https://doi.org/10.1136/lupus-2024-001162
6. Margherita Zen, Laura Salmaso, Claudio Barbiellini Amidei, Ugo Fedeli, Stefania Bellio, Luca Iaccarino, Alessandro Giollo, Andrea Doria, Mario Saia, Systemic lupus erythematosus incidence and prevalence in a large population-based study in northeastern Italy, *Rheumatology*, Volume 62, Issue 8, August 2023, Pages 2773–2779, https://doi.org/10.1093/rheumatology/keac685
7. Justiz Vaillant, Angel A., et al. 'Systemic Lupus Erythematosus'. *StatPearls*, StatPearls Publishing, 2024. *PubMed*, http://www.ncbi.nlm.nih.gov/books/NBK535405/
8. Ramos, Paula S., et al. 'Genetic Factors Predisposing to Systemic Lupus Erythematosus and Lupus Nephritis'. *Seminars in Nephrology*, vol. 30, no.

2, Mar. 2010, p. 164. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1016/j.semnephrol.2010.01.007

9. Guerra, Sandra G., et al. 'The Genetics of Lupus: A Functional Perspective'. *Arthritis Research & Therapy*, vol. 14, no. 3, May 2012, p. 211. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1186/ar3844
10. Jara, Luis J., et al. 'The Endocrine System and Autoimmunity'. *Autoimmunity: From Bench to Bedside [Internet]*, El Rosario University Press, 2013. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK459473/
11. 'The Affect of Hormones on SLE | Treating Lupus'. *Endocrine News*, 1 Dec. 2012, https://endocrinenews.endocrine.org/managing-lupus/
12. Dao, Kathryn H., and Bonnie L. Bermas. 'Systemic Lupus Erythematosus Management in Pregnancy'. *International Journal of Women's Health*, vol. 14, Feb. 2022, p. 199. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.2147/IJWH.S282604
13. Barbhaiya, Medha, and Karen H. Costenbader. 'Environmental Exposures and the Development of Systemic Lupus Erythematosus'. *Current Opinion in Rheumatology*, vol. 28, no. 5, Sept. 2016, p. 497. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1097/BOR.0000000000000318
14. Refai, Rania H., et al. 'Environmental Risk Factors of Systemic Lupus Erythematosus: A Case–Control Study'. *Scientific Reports*, vol. 13, no. 1, June 2023, p. 10219. *www.nature.com*, https://doi.org/10.1038/s41598-023-36901-y
15. Kamen, Diane L. 'Environmental Influences on Systemic Lupus Erythematosus Expression'. *Rheumatic Diseases Clinics of North America*, vol. 40, no. 3, Aug. 2014, p. 401. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1016/j.rdc.2014.05.003
16. Choi, Jinyoung, et al. 'The Pathogenesis of Systemic Lupus Erythematosus – An Update'. *Current Opinion in Immunology*, vol. 24, no. 6, Nov. 2012, p. 651. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1016/j.coi.2012.10.004

17. Nashi, Emil, et al. ‘The Role Of B Cells in Lupus Pathogenesis’. *The International Journal of Biochemistry & Cell Biology*, vol. 42, no. 4, Oct. 2009, p. 543. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1016/j.biocel.2009.10.011
18. Karrar, Sarah, and Deborah S. Cunninghame Graham. ‘Abnormal B Cell Development in Systemic Lupus Erythematosus: What the Genetics Tell Us’. *Arthritis & Rheumatology (Hoboken, N.j.)*, vol. 70, no. 4, Feb. 2018, p. 496. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1002/art.40396
19. Paredes, Jacqueline L., et al. ‘T Cells in Systemic Lupus Erythematosus’. *Rheumatic Diseases Clinics of North America*, vol. 47, no. 3, June 2021, p. 379. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1016/j.rdc.2021.04.005
20. Li, Hao, et al. ‘Abnormalities of T Cells in Systemic Lupus Erythematosus: New Insights in Pathogenesis and Therapeutic Strategies’. *Journal of Autoimmunity*, vol. 132, Oct. 2022, p. 102870. *ScienceDirect*, https://doi.org/10.1016/j.jaut.2022.102870
21. Herrada, Andrés A., et al. ‘Innate Immune Cells’ Contribution to Systemic Lupus Erythematosus’. *Frontiers in Immunology*, vol. 10, Apr. 2019, p. 772. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.3389/fimmu.2019.00772
22. Rönnblom LI8 The role of the interferon system in SLELupus Science & Medicine 2020;7:doi: 10.1136/lupus-2020-eurolupus.8
23. Postal, Mariana, et al. ‘Type I Interferon in the Pathogenesis of Systemic Lupus Erythematosus’. *Current Opinion in Immunology*, vol. 67, Nov. 2020, p. 87. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1016/j.coi.2020.10.014
24. Dema, Barbara, and Nicolas Charles. ‘Autoantibodies in SLE: Specificities, Isotypes and Receptors’. *Antibodies*, vol. 5, no. 1, Jan. 2016, p. 2. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.3390/antib5010002
25. ‘Antiphospholipid Antibodies’. *Johns Hopkins Lupus Center*, https://www.hopkinslupus.org/lupus-tests/antiphospholipid-antibodies/. Accessed 15 Nov. 2024

26. Banhuk, Fernanda Weyand, et al. ‘Relationships among Antibodies against Extractable Nuclear Antigens, Antinuclear Antibodies, and Autoimmune Diseases in a Brazilian Public Hospital’. *Autoimmune Diseases*, vol. 2018, Sept. 2018, p. 9856910. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1155/2018/9856910
27. Moore, P. A., et al. ‘BLyS: Member of the Tumor Necrosis Factor Family and B Lymphocyte Stimulator’. *Science (New York, N.Y.)*, vol. 285, no. 5425, July 1999, pp. 260–63. *PubMed*, https://doi.org/10.1126/science.285.5425.260
28. Cancro, Michael P., et al. ‘The Role of B Lymphocyte Stimulator (BLyS) in Systemic Lupus Erythematosus’. *The Journal of Clinical Investigation*, vol. 119, no. 5, May 2009, pp. 1066–73. *PubMed*, https://doi.org/10.1172/JCI38010
29. Carrillo-Ballesteros, Francisco Josué, et al. ‘B-Cell Activating Factor Receptor Expression Is Associated with Germinal Center B-Cell Maintenance’. *Experimental and Therapeutic Medicine*, vol. 17, no. 3, Jan. 2019, p. 2053. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.3892/etm.2019.7172
30. Davidson, Anne. ‘The Rationale for BAFF Inhibition in Systemic Lupus Erythematosus’. *Current Rheumatology Reports*, vol. 14, no. 4, Aug. 2012, p. 295. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1007/s11926-012-0258-2
31. Cojocaru, Manole, et al. ‘Manifestations of Systemic Lupus Erythematosus’. *Mædica*, vol. 6, no. 4, Oct. 2011, p. 330. *pmc.ncbi.nlm.nih.gov*, https://pmc.ncbi.nlm.nih.gov/articles/PMC3391953/
32. Shumilova, Anastasiia, and Edward M. Vital. ‘Musculoskeletal Manifestations of Systemic Lupus Erythematosus’. *Best Practice & Research Clinical Rheumatology*, vol. 37, no. 4, Dec. 2023, p. 101859. *ScienceDirect*, https://doi.org/10.1016/j.berh.2023.101859
33. Santiago, Mittermayer B., et al. ‘Severe Jaccoud’s Arthropathy in Systemic Lupus Erythematosus’. *Rheumatology International*, vol. 35, no.

10, Oct. 2015, pp. 1773–77. *PubMed*, https://doi.org/10.1007/s00296-015-3351-9

34. Upadhyaya, Susmita, et al. ‘Rhupus Syndrome: A Diagnostic Dilemma’. *Cureus*, vol. 14, no. 9, Sept. 2022, p. e29018. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.7759/cureus.29018
35. Fayyaz, Anum, et al. ‘Haematological Manifestations of Lupus’. *Lupus Science & Medicine*, vol. 2, no. 1, Mar. 2015, p. e000078. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1136/lupus-2014-000078
36. Santacruz, Juan Camilo, et al. ‘A Practical Perspective of the Hematologic Manifestations of Systemic Lupus Erythematosus’. *Cureus*, vol. 14, no. 3, Mar. 2022, p. e22938. *PubMed*, https://doi.org/10.7759/cureus.22938
37. Jiang, Ying, et al. ‘Systemic Lupus Erythematosus-Complicating Immune Thrombocytopenia: From Pathogenesis to Treatment’. *Journal of Autoimmunity*, vol. 132, Oct. 2022, p. 102887. *ScienceDirect*, https://doi.org/10.1016/j.jaut.2022.102887
38. Janoudi, Nahid, and Ekhlas Samir Bardisi. ‘Haematological Manifestations in Systemic Lupus Erythematosus’. *Systemic Lupus Erythematosus*, IntechOpen, 2012. *www.intechopen.com*, https://doi.org/10.5772/30612
39. ‘Lupus-Specific Skin Disease and Skin Problems’. *Johns Hopkins Lupus Center*, https://www.hopkinslupus.org/lupus-info/lupus-affects-body/skin-lupus/. Accessed 15 Nov. 2024
40. Musa, Rina, et al. ‘Lupus Nephritis’. *StatPearls*, StatPearls Publishing, 2024. *PubMed*, http://www.ncbi.nlm.nih.gov/books/NBK499817/
41. *Lupus Nephritis: Practice Essentials, Pathophysiology, Etiology*. Oct. 2024. *eMedicine*, https://emedicine.medscape.com/article/330369-overview?form=fpf
42. Schwartz, Noa, et al. ‘Neuropsychiatric Lupus: New Mechanistic Insights and Future Treatment Directions’. *Nature Reviews. Rheumatology*, vol. 15, no. 3, Mar. 2019, p. 137. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1038/s41584-018-0156-8.

43. ‘Neuropsychiatric Lupus: SLE and the Brain | HSS Rheumatology’. *Hospital for Special Surgery*, https://www.hss.edu/conditions_neuropsychiatric-sle-lupus-and-brain.asp. Accessed 15 Nov. 2024
44. Doria, A., et al. ‘Cardiac Involvement in Systemic Lupus Erythematosus’. *Lupus*, vol. 14, no. 9, 2005, pp. 683–86. *PubMed*, https://doi.org/10.1191/0961203305lu2200oa
45. Kebar, Yousef Mohammadi, et al. ‘Libman-Sacks Endocarditis in Patients with Systemic Lupus Erythematosus with Secondary Antiphospholipid Syndrome’. *Caspian Journal of Internal Medicine*, vol. 10, no. 3, Summer 2019, p. 339. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.22088/cjim.10.3.339
46. Piranavan, Paramarjan, and Andras Perl. ‘Management of Cardiovascular Disease in Patients with Systemic Lupus Erythematosus’. *Expert Opinion on Pharmacotherapy*, vol. 21, no. 13, June 2020, p. 1617. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1080/14656566.2020.1770227
47. Shin, Jae Il, et al. ‘Systemic Lupus Erythematosus and Lung Involvement: A Comprehensive Review’. *Journal of Clinical Medicine*, vol. 11, no. 22, Nov. 2022, p. 6714. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.3390/jcm11226714
48. Ferjani, Maryem, et al. ‘Systemic Lupus Erythematosus-related Acute Pancreatitis: An Exceptional Form with Severe Exocrine and Endocrine Pancreatitic Failure in a Tunisian Child’. *Clinical Case Reports*, vol. 10, no. 2, Feb. 2022, p. e05423. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1002/ccr3.5423
49. Palejwala, Neal V., et al. ‘Ocular Manifestations of Systemic Lupus Erythematosus: A Review of the Literature’. *Autoimmune Diseases*, vol. 2012, July 2012, p. 290898. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1155/2012/290898
50. Alshehri, Abdullah Mohammed, et al. ‘Mesenteric Vasculitis as a Rare Initial Presentation of Systemic Lupus Erythematosus: A Case Report’. *Saudi Journal of Medicine & Medical Sciences*, vol. 8, no. 3,

Aug. 2020, p. 223. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.4103/sjmms.sjmms_206_19

51. Fanouriakis, Antonis, et al. 'EULAR Recommendations for the Management of Systemic Lupus Erythematosus: 2023 Update'. *Annals of the Rheumatic Diseases*, vol. 83, no. 1, Jan. 2024, pp. 15–29. *ard.bmj.com*, https://doi.org/10.1136/ard-2023-224762.
52. Katarzyna, Pawlak-Buś, et al. 'Current Treatment of Systemic Lupus Erythematosus: A Clinician's Perspective'. *Rheumatology International*, vol. 43, no. 8, May 2023, p. 1395. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1007/s00296-023-05306-5
53. Dima, Alina, et al. 'Hydroxychloroquine in Systemic Lupus Erythematosus: Overview of Current Knowledge'. *Therapeutic Advances in Musculoskeletal Disease*, vol. 14, Feb. 2022, p. 1759720X211073001. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1177/1759720X211073001.
54. Porta, Sabrina, et al. 'Glucocorticoids in Systemic Lupus Erythematosus. Ten Questions and Some Issues'. *Journal of Clinical Medicine*, vol. 9, no. 9, Aug. 2020, p. 2709. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.3390/jcm9092709
55. Jia, Wan-Yu, and Jian-Jiang Zhang. 'Effects of Glucocorticoids on Leukocytes: Genomic and Non-Genomic Mechanisms'. *World Journal of Clinical Cases*, vol. 10, no. 21, July 2022, p. 7187. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.12998/wjcc.v10.i21.7187
56. Quan, Xiao-ying, et al. 'Revisited Cyclophosphamide in the Treatment of Lupus Nephritis'. *BioMed Research International*, vol. 2022, May 2022, p. 8345737. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1155/2022/8345737
57. Trevisonno, Michael, et al. 'Mycophenolate Mofetil for Systemic Lupus Erythematosus: Our 20-Year Experience'. *Cureus*, vol. 15, no. 1, Jan. 2023, p. e34413. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.7759/cureus.34413

58. Mohammadi, Oranus, and Thamer A. Kassim. ‘Azathioprine’. *StatPearls*, StatPearls Publishing, 2024. *PubMed*, http://www.ncbi.nlm.nih.gov/books/NBK542190/
59. Sakthiswary, R., and E. Suresh. ‘Methotrexate in Systemic Lupus Erythematosus: A Systematic Review of Its Efficacy’. *Lupus*, vol. 23, no. 3, Mar. 2014, pp. 225–35. *PubMed*, https://doi.org/10.1177/0961203313519159
60. Sánchez, Yunchoy, and Aurelio Carvallo. ‘[Methotrexate use in patients with systemic lupus erythematosus]’. *Revista Medica De Chile*, vol. 132, no. 2, Feb. 2004, pp. 195–201. *PubMed*, https://doi.org/10.4067/s0034-98872004000200009
61. Bui, Albert, et al. ‘Anifrolumab’. *StatPearls*, StatPearls Publishing, 2024. *PubMed*, http://www.ncbi.nlm.nih.gov/books/NBK555979/
62. Tanaka, Yoshiya. ‘Viewpoint on Anifrolumab in Patients with Systemic Lupus Erythematosus and a High Unmet Need in Clinical Practice’. *RMD Open*, vol. 9, no. 3, Aug. 2023, p. e003270. *PubMed*, https://doi.org/10.1136/rmdopen-2023-003270
63. Griffiths, Bridget, et al. ‘Assessment of Patients with Systemic Lupus Erythematosus and the Use of Lupus Disease Activity Indices’. *Best Practice & Research. Clinical Rheumatology*, vol. 19, no. 5, Oct. 2005, pp. 685–708. *PubMed*, https://doi.org/10.1016/j.berh.2005.03.010
64. ‘Description and Appraisal of Outcome Measures’. *Clinical Review Report: Belimumab (Benlysta): (GlaxoSmithKline Inc.): Indication: Indicated in Addition to Standard Therapy for Reducing Disease Activity in Adult Patients with Active, Autoantibody-Positive Systemic Lupus Erythematosus [Internet]*, Canadian Agency for Drugs and Technologies in Health, 2020. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK565224/
65. Yee, C. S., et al. ‘BILAG-2004 Index Captures Systemic Lupus Erythematosus Disease Activity Better than SLEDAI-2000’. *Annals of the Rheumatic Diseases*, vol. 67, no. 6, June 2008, pp. 873–76. *ard.bmj.com*, https://doi.org/10.1136/ard.2007.070847

66. Physician Global Assessment International Standardisation COnsensus in Systemic Lupus Erythematosus: the PISCOS study; Piga, Matteo Appenzeller, Simone et al. The Lancet Rheumatology, Volume 4, Issue 6, e441 - e449
67. Connelly, Kathryn, et al. 'Associations of Improvement in Laboratory Tests with Clinical Outcomes in Patients with Active Systemic Lupus Erythematosus: A Multinational Longitudinal Cohort Study'. *The Lancet. Rheumatology*, vol. 4, no. 12, Dec. 2022, pp. e831–41. *PubMed*, https://doi.org/10.1016/S2665-9913(22)00307-1
68. Jesus, Diogo, et al. 'Systemic Lupus Erythematosus Disease Activity Score (SLE-DAS) Enables Accurate and User-Friendly Definitions of Clinical Remission and Categories of Disease Activity'. *Annals of the Rheumatic Diseases*, vol. 80, no. 12, Dec. 2021, pp. 1568–74. *PubMed*, https://doi.org/10.1136/annrheumdis-2021-220363
69. *Italian Journal of Public Health World*. https://www.ijph.it/hta-belimumab-lupus-eritematoso-sistemico. Accessed 15 Nov. 2024
70. Dennis, G. J. 'Belimumab: A BLyS-Specific Inhibitor for the Treatment of Systemic Lupus Erythematosus'. *Clinical Pharmacology & Therapeutics*, vol. 91, no. 1, Jan. 2012, pp. 143–49. *DOI.org (Crossref)*, https://doi.org/10.1038/clpt.2011.290
71. La Cava, Antonio. 'Targeting the BLyS-APRIL Signaling Pathway in SLE'. *Clinical Immunology*, vol. 148, no. 3, Sept. 2013, pp. 322–27. *ScienceDirect*, https://doi.org/10.1016/j.clim.2012.11.010
72. Srivastava, Ankita. 'Belimumab in Systemic Lupus Erythematosus'. *Indian Journal of Dermatology*, vol. 61, no. 5, Oct. 2016, p. 550. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.4103/0019-5154.190107
73. Ahmed, Hamdy MA, et al. 'Subcutaneous Formulation of Belimumab in Treatment of Systemic Lupus Erythematosus: A Critical Review with Focus on Safety and Satisfaction'. *Patient Preference and Adherence*, vol. 12, Nov. 2018, p. 2475. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.2147/PPA.S147163

74. Blair, Hannah A., and Sean T. Duggan. ‘Belimumab: A Review in Systemic Lupus Erythematosus’. *Drugs*, vol. 78, no. 3, Mar. 2018, pp. 355–66. *Springer Link*, https://doi.org/10.1007/s40265-018-0872-z
75. Raymond Lamore, I. I. I., et al. ‘Belimumab (Benlysta): A Breakthrough Therapy for Systemic Lupus Erythematosus’. *Pharmacy and Therapeutics*, vol. 37, no. 4, Apr. 2012, p. 212. *pmc.ncbi.nlm.nih.gov*, https://pmc.ncbi.nlm.nih.gov/articles/PMC3351861/
76. Furie, Richard, et al. ‘A Phase 3, Randomized, Placebo-Controlled Study of Belimumab, a Monoclonal Antibody That Inhibits BLyS, in Patients With Systemic Lupus Erythematosus’. *Arthritis and Rheumatism*, vol. 63, no. 12, Dec. 2011, p. 3918. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1002/art.30613
77. Navarra, Sandra V., et al. ‘Efficacy and Safety of Belimumab in Patients with Active Systemic Lupus Erythematosus: A Randomised, Placebo-Controlled, Phase 3 Trial’. *Lancet (London, England)*, vol. 377, no. 9767, Feb. 2011, pp. 721–31. *PubMed*, https://doi.org/10.1016/S0140-6736(10)61354-2
78. Zheng, Jie, et al. ‘Efficacy of Belimumab in Patients with Systemic Lupus Erythematosus from North East Asia: Results of Exploratory Subgroup Analyses’. *Modern Rheumatology*, vol. 33, no. 4, July 2023, pp. 751–57. *PubMed*, https://doi.org/10.1093/mr/roac076
79. Lokhandwala, Tasneem, et al. ‘Within-Trial Economic Analysis of Flare Data from the BLISS-SC Trial of Subcutaneous Belimumab in Systemic Lupus Erythematosus’. *Lupus Science & Medicine*, vol. 8, no. 1, Feb. 2021, p. e000438. *lupus.bmj.com*, https://doi.org/10.1136/lupus-2020-000438
80. Stohl, William, et al. ‘Efficacy and Safety of Subcutaneous Belimumab in Systemic Lupus Erythematosus: A Fifty-Two–Week Randomized, Double-Blind, Placebo-Controlled Study’. *Arthritis & Rheumatology (Hoboken, N.j.)*, vol. 69, no. 5, Apr. 2017, p. 1016. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1002/art.40049

81. Trentin, Francesca, et al. ‘Effectiveness, Tolerability, and Safety of Belimumab in Patients with Refractory SLE: A Review of Observational Clinical-Practice-Based Studies’. *Clinical Reviews in Allergy & Immunology*, vol. 54, no. 2, Mar. 2018, p. 331. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.1007/s12016-018-8675-2
82. Iaccarino, Luca, et al. ‘Clinical Predictors of Response and Discontinuation of Belimumab in Patients with Systemic Lupus Erythematosus in Real Life Setting. Results of a Large, Multicentric, Nationwide Study’. *Journal of Autoimmunity*, vol. 86, Jan. 2018, pp. 1–8. *PubMed*, https://doi.org/10.1016/j.jaut.2017.09.004
83. Gatto, Mariele, et al. ‘Durable Renal Response and Safety with Add-on Belimumab in Patients with Lupus Nephritis in Real-Life Setting (BeRLiSS-LN). Results from a Large, Nationwide, Multicentric Cohort’. *Journal of Autoimmunity*, vol. 124, Nov. 2021, p. 102729. *PubMed*, https://doi.org/10.1016/j.jaut.2021.102729
84. Zen, Margherita, et al. ‘Early and Late Response and Glucocorticoid-Sparing Effect of Belimumab in Patients with Systemic Lupus Erythematosus with Joint and Skin Manifestations: Results from the Belimumab in Real Life Setting Study—Joint and Skin (BeRLiSS-JS)’. *Journal of Personalized Medicine*, vol. 13, no. 4, Apr. 2023, p. 691. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.3390/jpm13040691
85. van Vollenhoven, Ronald F., et al. ‘Belimumab in the Treatment of Systemic Lupus Erythematosus: High Disease Activity Predictors of Response’. *Annals of the Rheumatic Diseases*, vol. 71, no. 8, Aug. 2012, pp. 1343–49. *PubMed*, https://doi.org/10.1136/annrheumdis-2011-200937
86. Kwan, Andrew, et al. ‘Herpes Zoster in SLE: Prevalence, Incidence and Risk Factors’. *Lupus Science & Medicine*, vol. 9, no. 1, Jan. 2022, p. e000574. *lupus.bmj.com*, https://doi.org/10.1136/lupus-2021-000574
87. Diane Apostolopoulos, Rangi Kandane-Rathnayake, Sudha Raghunath, Alberta Hoi, Mandana Nikpour, Eric F Morand - Independent association of glucocorticoids with damage accrual in SLE: Lupus Science & Medicine 2016;3:e000157.

88. Sun YS, Huang DF, Chen WS, Liao HT, Chen MH, Tsai MT, Yang CY, Lai CC, Tsai CY. Risk Factors and Incidence of Serious Infections in Patients With Systemic Lupus Erythematosus Undergoing Rituximab Therapy. J Rheumatol. 2024 Feb 1;51(2):160-167. doi: 10.3899/jrheum.2023-0623. PMID: 37839817.
89. He J, Li Z. Dilemma of immunosuppression and infection risk in systemic lupus erythematosus. Rheumatology (Oxford). 2023 Mar 29;62(Suppl 1):i22-i29. doi: 10.1093/rheumatology/keac678. PMID: 36987605; PMCID: PMC10050939.
90. Al- Rawi, S. S., Ibrahim, A. H., & Ahmed, G. B. (2023). Unraveling The Link Between Lymphopenia And Systemic Lupus Erythematosus: Implications For Disease Severity And Potential Treatment Strategies. *EURASIAN JOURNAL OF SCIENCE AND ENGINEERING*, *9*(2), 231-240. https://doi.org/10.23918/eajse.v9i2p18
91. Uitdehaag BM, Nillesen WM, Hommes OR. Long-lasting effects of cyclophosphamide on lymphocytes in peripheral blood and spinal fluid. Acta Neurol Scand. 1989 Jan;79(1):12-7. doi: 10.1111/j.1600-0404.1989.tb03702.x. PMID: 2784607.