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A Structured Classification and Evaluation of Current and
Future Treatments for Systemic Lupus Erythematosus

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Declaration of Authenticity

I, Ahmed Mohamed Safwat Ibrahim Abdelazim, declare that the research work presented here is from the best of my knowledge and belief, original and the result of my own investigations. The cooperation I got for this research work is clearly acknowledged. To the best of my knowledge, it does not contain any materials those are written by others or published already except mentioned with due references in the text as well as with the quotation marks.

This work has not been published, submitted, either in part or whole intended for reward, degree at this or any other University.

Kleve, 14-02-2023

A handwritten signature in black ink, appearing to read 'Ahmed Safwat', written in a cursive style.

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List of Abbreviations

ABC	Age-Associated B cell
ACLE	Acute cutaneous lupus erythematosus
ACR	American College of Rheumatology
AE	Adverse Events
APRIL	A Proliferation-Inducing Ligand
BAFF	B Cell Activation Factor
BCMA	B Cell Maturation Antigen
BCMA	B-cell maturation antigen
BILAG	British Isles Lupus Assessment Group
BM	Bone Marrow
BW	Body Weight
CD	Complementary Receptor
CNS	Central Nervous System
CS	Corticosteroids
DC	Dendritic Cell
DLE	Discoid lupus erythematosus
DMARD	Disease-Modifying Antirheumatic Drugs
DNA	Deoxyribonucleic acid
EBV	Epstein–Barr Virus
EMA	European Medicines Agency
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
GI	Gastrointestinal
GWAS	Genome Wide Associated Studies
H-Chain	Heavy Chain
HCQ	hydroxychloroquine
HLA	Human Leukocyte Antigen
ICU	Intensive Care Unit
IgA	Immunoglobulin A
IgM	Immunoglobulin M
INF	Interferon
JAK	Janus kinase inhibitors

L-Chain	Light Chain
LLDAS	Lupus Low Disease Activity State
LLPC	Long Lived Plasma Cells
LN	Lupus Nephritis
mAb	Monoclonal Antibody
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetil
MYD88	Myeloid differentiation primary response 88
NPSLE	Neuropsychiatric SLE
PC	Plasma Cell
RTX	Rituximab
SLE	Systemic Lupus Erythematosus
SLICC	Systemic Lupus International Collaborating Clinics
SNP	Single Nucleotide Polymorphism
TACI	Transmembrane Activator and CAML Interactor
TIGER	The Institute for Genomics Research
TLR7	Toll-Like Receptor 7
TNF	Tumor Necrosis Factor

Abstract

Systemic Lupus Erythematosus (SLE) is a heterogeneous autoimmune disease that has a variety of manifestations and is therefore difficult to find a treatment for. Several advances have occurred in the field of SLE drug discovery, especially since the publishing of the first Genome Wide-Associated Studies in 2005. More than one hundred loci were found to be involved in the disease. Subsequently, the treatments were aimed towards tackling the pathways that were found after the uses of modern genetic techniques. Historically, the FDA approved only two drugs for treatment until the approval of Belimumab in 2011. Ten years later, two more drugs were approved in the same year, with few more drugs expected to receive approval in the near future. This study aims to evaluate the efficacy of these future drugs in addition to the ones considered to be milestones in the treatment of SLE, finding Anifrolumab to be the most promising drug currently on the market for treatment of moderate to severe SLE and Obinutuzumab to be the drug to watch.

1. Introduction:

1.1 Systemic Lupus Erythematosus Overview:

Systemic Lupus Erythematosus (SLE), also known as lupus, is a condition in which the immune system attacks healthy cells and tissues throughout the body (Ma et al., 2019). Exaggerated B cell and T cell responses and a loss of immune tolerance to self-antigens are characteristics of immune system activation in SLE. Clinical manifestations can range from mild tiredness and joint pain to severe, life-threatening organ damage. Cytokine activation, the production and improper elimination of antibodies, immune complex circulation and tissue deposition and complement activation all impact these clinical manifestations (Kiriakidou & Ching, 2020).

1.2 Systemic Lupus Epidemiology

Studies in the USA reported higher prevalence and incidence rates of SLE in women compared to men, and in African Americans compared to Caucasians. The data also showed that in comparison to Caucasians, Hispanics and Asians had a higher incidence and prevalence of SLE however, less so than Black Americans (Stojan & Petri, 2018).

Little was known about the epidemiology of SLE among the Arab population globally or in the Middle East. One study, however, described a 2.1-fold higher incidence of SLE among Arab-Americans compared to non-Arab Caucasians and Black Americans in the Michigan. Another study in the United Arab Emirates showed similar incidence in the country to the one in Michigan (Stojan & Petri, 2018).

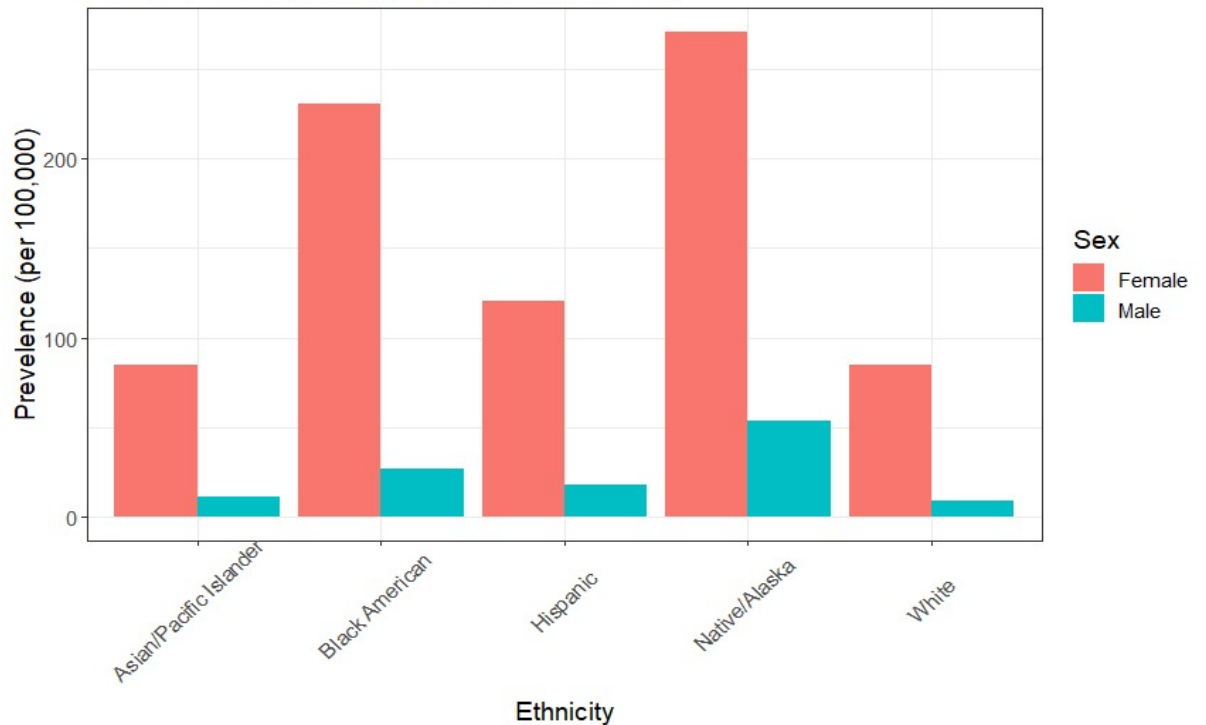


Figure 1 U.S. SLE Prevalence by Ethnicity and Gender

Another study (Tian, Zhang, Yao, Huang, & Lu, 2022) found that the global SLE prevalence and affected individuals were calculated to be 43.7 (15.87 to 108.92) per 100,000 people and 3.41 million, respectively, for the general population. SLE was more common in some regions than others, ranging from 15.9 (3.29 to 45.85 cases per 100,000 people) in southern Asia to 110.85 (26.74 to 314.1 cases per 100,000 people) in tropical Latin America.

The same study showed prevalence of SLE has varied greatly by nation. The four nations with the highest estimates of SLE prevalence for the general population were the United Arab Emirates (166.92, 139.01 to 198.54 per 100 000), Barbados (163.31, 35.41 to 391.57 per 100 000), Cuba (149.9, 26.05 to 424.12 per 100 000), and Brazil (147.37, 38.19 to 351.48 per 100 000). Argentina, on the other hand, has the lowest prevalence rate globally (5.05, 4.22 to 6.06 per 100 000 people).

In all the studies that have been reviewed, women showed significantly higher prevalence than men. This applies for all the different regions and ethnicities, with United Arab Emirates as the highest country, followed by Barbados, Cuba and Brazil.

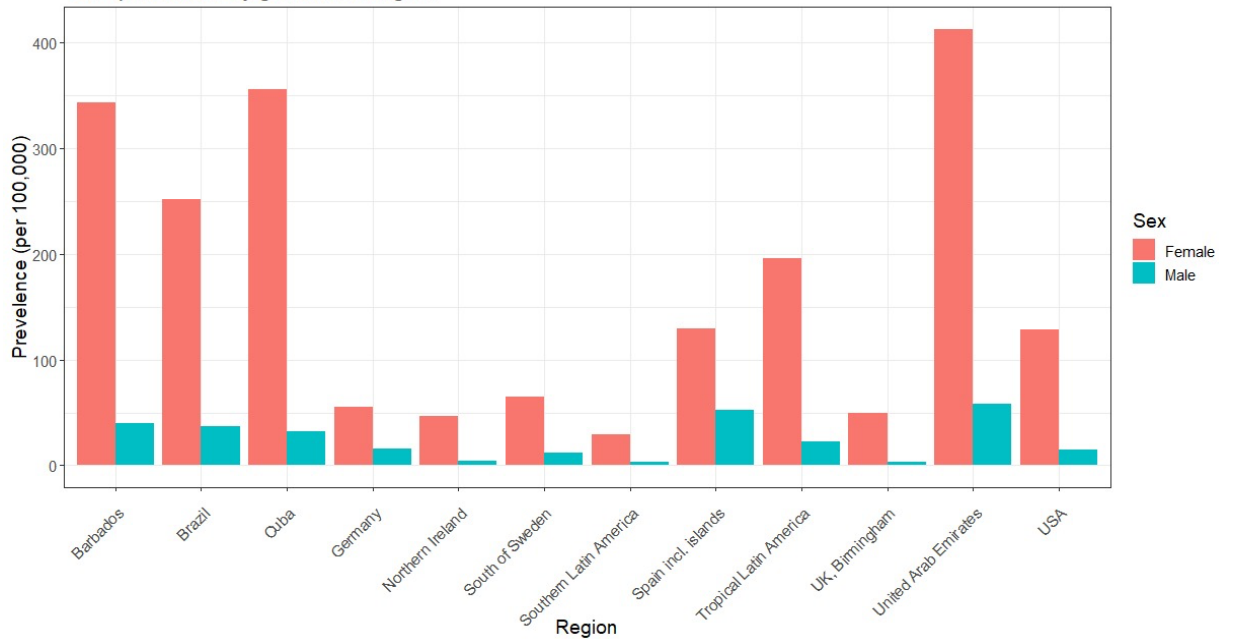


Figure 2 SLE Global Prevalence by Gender and Region

Table 1 The Prevalence of SLE across Regions

Study	Region	Prevalence per 100,000	Ethnicity	Sex
(Izmirly et al., 2021)	USA	72.8	N/A	N/A
(Izmirly et al., 2021)	USA	128.7	N/A	Female
(Izmirly et al., 2021)	USA	14.6	N/A	Male
(Izmirly et al., 2021)	USA	230.9	Black American	Female
(Izmirly et al., 2021)	USA	120.7	Hispanic	Female
(Izmirly et al., 2021)	USA	84.7	White	Female
(Izmirly et al., 2021)	USA	84.4	Asian/Pacific Islander	Female
(Izmirly et al., 2021)	USA	270.6	Native/Alaska	Female

Study	Region	Prevalence per 100,000	Ethnicity	Sex
(Izmirly et al., 2021)	USA	26.7	Black American	Male
(Izmirly et al., 2021)	USA	18.0	Hispanic	Male
(Izmirly et al., 2021)	USA	11.2	Asian/Pacific Islande	Male
(Izmirly et al., 2021)	USA	8.9	White	Male
(Izmirly et al., 2021)	USA	53.8	Native/Alaska	Male
(Tian et al., 2022)	Southern Latin America	29.21	N/A	Female
(Tian et al., 2022)	Tropical Latin America	195.94	N/A	Female
(Tian et al., 2022)	United Arab Emirates	413.27	N/A	Female
(Tian et al., 2022)	Barbados	343.44	N/A	Female
(Tian et al., 2022)	Cuba	355.82	N/A	Female
(Tian et al., 2022)	Brazil	251.86	N/A	Female
(Danchenko, Satia, & Anthony, 2006)	North of Spain	57.9	White	Female
(Danchenko et al., 2006)	South of Sweden	64.8	N/A	Female
Danchenko et al., 2006)	UK, Birmingham	96.5	Asians	Female
Danchenko et al., 2006)	UK, Birmingham	36.3	White	Female
Danchenko et al., 2006)	UK, Birmingham	49.6	N/A	Female
Danchenko et al., 2006)	Northern Ireland	46.5	N/A	Female

Study	Region	Prevalence per 100,000	Ethnicity	Sex
Danchenko et al., 2006)	Spain incl. islands	130	N/A	Female
(Tian et al., 2022)	Southern Latin America	3.44	N/A	Male
(Tian et al., 2022)	Tropical Latin America	22.96	N/A	Male
(Tian et al., 2022)	United Arab Emirates	58.67	N/A	Male
(Tian et al., 2022)	Barbados	40.4	N/A	Male
(Tian et al., 2022)	Cuba	32.11	N/A	Male
(Tian et al., 2022)	Brazil	37	N/A	Male
(Danchenko et al., 2006)	North of Spain	8.3	White	Male
(Danchenko et al., 2006)	South of Sweden	11.7	N/A	Male
Danchenko et al., 2006)	UK, Birmingham	4.3	Asians	Male
Danchenko et al., 2006)	UK, Birmingham	3.4	White	Male
Danchenko et al., 2006)	UK, Birmingham	3.6	N/A	Male
Danchenko et al., 2006)	Northern Ireland	4.3	N/A	Male
Danchenko et al., 2006)	Spain incl. islands	52	N/A	Male
(Brinks et al., 2014)	Germany	55.4	N/A	Female
(Brinks et al., 2014)	Germany	15.4	N/A	Male

1.3 The Mortality and Morbidity Rates of SLE:

The 10-year survival rate for patients with SLE improved from 63.2% in the 1950s to 91.4% in the 2000s. However, mortality still exceeds that of the wider population. Patients are frequently hospitalized for either active disease, disease damage and comorbidities or side effects from medications (Marasco, Mucke, & Suurmond, 2022).

(Guo et al., 2021) conducted a study on 391 SLE patients who were admitted to the ICU from 2010 to 2019. 348 (89.0%) of the 391 patients were female. The median duration of SLE was six months, and the median age of the patients was 34. In-ICU mortality was 53.4% on average. 186 patients in total were admitted to the ICU as a result of infection. The most frequent clinical manifestation was pneumonia (81.8%), followed by renal disease (62.9%).

1.4 The Role of B Cells in Understanding the Pathogenicity and the Treatment:

Immune tolerance is maintained by the immune system's disregard for host-derived antigens and its recognition of pathogens from the outside environment. A breakdown in immune tolerance, however, causes the immune system to start attacking self-antigens and results in autoimmune diseases. Anti-nuclear antibodies and autoreactive B cells are imperative in the development of the severe autoimmune disease systemic lupus erythematosus (Tanaka, 2020), which results in damage to target organs (Ma et al., 2019).

The development of the B lymphocytes happens in the bone marrow from the hematopoietic precursor cells. Hematopoietic stem cells growing in the fetal liver seed the BM during embryonic life. Their precursor cells come from the aorta-gonad-mesonephros, which is made up of mesoderm descendants. Early stages of B-cell development that depend on the bone marrow are organized along the functional rearrangement of immunoglobulin gene segments (Pieper, Grimbacher, & Eibel, 2013).

The expression of a distinct immunoglobulin heavy and light chain, which allows for the recognition of antigen and is a component of the B cell's signaling system, in each cell is the main characteristic that distinguishes B cells from other lymphoid cells (Atisha-Fregoso, Toz, & Diamond, 2021). A B cell repertoire expressing antibodies capable of recognizing more than 53×10^{13} different antigens is produced by the rearrangements of

the heavy chain (H-chain) gene segments V_H , D_H , and J_H , as well as the light chain (L-chain) gene segments V_L - J_L . Three developmental stages are identified based on the rearrangement of the H-chain and L-chain gene segments. Pro-B cells rearrange the D and J segments of the H-chain in the first stage, then join an upstream V region to the rearranged DJ segment in the second stage. The rearrangement of μ -H-chain gene segments is opening the gate to the next stage, the pre-B cell phase. Pre-B cells in humans divide once or twice and rearrange the gene segments that code for the κ and λ chains. In combination with μ chain, an IgM molecule is created on the cell surface. We refer to these cells as immature B cells. After leaving the bone marrow and moving to the spleen, immature B cells complete their early development by differentiating into naive, follicular, or marginal zone B cells (Pieper et al., 2013).

In addition to allowing for the recognition of a wide variety of pathogens, the diversity of the B cell receptor (BCR) repertoire also leads to a significant number of autoreactive B cells, which are immature B cells that can recognize their own components (Atisha-Fregoso et al., 2021).

It is challenging for the immune system to balance between attacking pathogens and avoiding self-reactivation due to the diversity of antibody specificities. For that, B cells go through several checkpoints during their development to test their degree of self-reactivation (Pieper et al., 2013).

B cell response thresholds must be precisely calibrated to strike a balance between the risk of humoral autoimmunity and protective responses. From an evolutionary perspective, a wide antibody repertoire is necessary for efficient humoral responses against a variety of pathogens. As a result, eliminating all self-reactive B cells completely would leave "holes" in the naive repertoire that pathogens could take advantage of. Self-reactive anergic B cells can be recruited into an adaptive immune response through a procedure known as "clonal redemption," which is consistent with this hypothesis.

A significant portion of peripheral naive B cells in healthy individuals exhibit autoreactivity across a continuum of self-antigen affinities despite layered tolerance mechanisms such as clonal deletion, receptor editing, and induction of functional anergy. SLE is an example of how self-reactivation can manifest strongly within the naive repertoire.

1.4.1 B Cell Activation Factor BAFF and its Role in SLE Pathogenesis:

The impact of on the pathogenesis of the SLE is strongly related to B cell activating factor (BAFF). Cytokines B cell activating factor (BAFF) which is a novel member of the homologous tumor necrosis factor (TNF) ligand family together with A proliferation-inducing ligand (APRIL) have different effects on B cell differentiation and survival during development. It was found that that lupus-like disease is promoted in murine models by transgenic BAFF overexpression. It was observed that human patients with lupus show increase in the serum BAFF. Belimumab is an example of a drug that targets the BAFF. This is discussed in detail in the Belimumab chapter.

For B cells to survive and develop past the transitional stage of B cell development, BAFF signals are necessary. Contrarily, APRIL supports class-switch recombination to IgA and promotes the survival of long-lived plasma cells (LLPC) in the bone marrow but is redundant for normal B cell differentiation. These actions are carried out by different B cell surface receptors like BAFF and APRIL. For instance, soluble BAFF circulates as trimers and in 60-subunit multimers, which preferentially activate the BAFF receptor (BAFF-R), Transmembrane Activator and CAML Interactor (TACI), and, respectively, the BAFF receptor and TACI. APRIL, on the other hand, binds to both TACI and the plasma cell receptor BCMA, the latter of which promotes the persistence of LLPC in BM survival niches.

By encouraging T cell-independent activation of an autoreactive B cell clone, excess BAFF causes breaks in peripheral B cell tolerance. These events require activation of the downstream MyD88 signals and endosomal RNA-sensor TLR7. Both BAFF-R21, the canonical B cell survival receptor, and TACI22-24, the surface receptor driving humoral responses to T cell-independent antigens, transduce BAFF signals that result in the generation of pathogenic plasmablasts (Canny & Jackson, 2021).

In other words, on the surfaces of dendritic cells and macrophages, BAFF, a typical costimulatory molecule of B cells, is expressed. It is also produced as soluble BAFF. It binds to the BAFF receptor, the transmembrane activator and cyclophilin ligand interactor (TACI), and the B cell maturation antigen on B cells to cause class switching, differentiation into antibody-producing cells, and an inhibition of the apoptosis of autoreactive B cells. Increased serum levels of double-stranded DNA (dsDNA) antibodies and serum soluble BAFF correlate with disease activity in SLE patients and are strongly linked to pathogenesis (Tanaka, 2020).

1.4.2 The Impact of Age-Associated B Cells (ABCs):

The Age-Associated B cells (ABCs) subpopulation of B cells was recently described. Their number increases between the patients of autoimmune diseases. The activation of B cells by Toll-Like Receptor 7 (TLR7), Interleukin-21 (IL-21), and Interferon- γ (IFN- γ) is a factor in its genesis. ABCs are said to be enriched in autoreactivity. Some findings proposes that they are the precursors of Plasma Cells (PCs) in SLE patients. They exist with high numbers in the peripheral blood of patients with Lupus Nephritis (Atisha-Fregoso et al., 2021).

ABCs can be also described as the lack of expressing CD21 and CD23 while at the same time excessively expressing CD11c (Sachinidis et al., 2020), the immunoglobulins (IgM, Igk, IgD), an intracellular enzyme indoleamine 2,3-dioxygenase, and the B cell lineage transcription factor the paired box 5 (PAX5) (Ma et al., 2019).

2. Aim of The Study:

Almost a century and a half has passed since the discovery of the disease and yet the treatments are still novel. A wide range of treatments are being tested from combined monoclonal therapies to injection of gold. However, Belimumab is the only targeted treatment approved in the market as a treatment for Systemic Lupus. study aims to shed light on the heterogenicity of the disease, Systemic Lupus, by explaining the genetic findings regarding the disease that may lead to revolutionize the drug discovery research field. These genetic findings may help in reaching the ultimate goal of the study: evaluating the efficacy of the current and the future treatments. This is carried out, in addition to classifying these treatments into classes that can help define some specification for a disease that is extremely heterogenous and systematic in its pathogenicity.

3. Materials and Methods:

The first Genome-Wide Association Study was published in 2005 (Loos, 2020). Due to the massive impact this technique had on the study of complex autoimmune diseases like SLE, the current analysis focuses on studies published from 2004 onwards.

NCBI, Embase, Google Scholar, Clinicaltrials.gov and CINAHL were searched from January 2004 to January 30, 2023. The keywords used for the search are provided in the supplementary section. The GWAS Catalogue was also searched to conduct a

better understanding of the Genetic Polymorphisms. Another search was conducted based on the bibliography of the retrieved the articles. The including criteria of the studies are they must be in English, have used randomized controlled clinical trials, have passed phase I, and have used one of the drugs that are included in the study classification described in the subsequent paragraph. All primary research, opinions, and non-English studies were excluded due to lack of resources to translate.

The evaluation of the treatments will consider the efficacy using SLEDAI score and SELENA-SELDAI to test the disease activity and SRI-4 to test the response of treatments at the end point. SRI-4 is a clinical tool for evaluating how well patients with systemic lupus erythematosus respond to treatment (Queenan, Häsler, & Rushton). The British Isles Lupus Assessment Group (BILAG) index, the Physician's Global Assessment (PGA), the number of active systemic lupus erythematosus (Queenan et al.) disease items, and the number of new or worsening SLE disease items are all included in this modified version of the original SRI (Furie, Wang, Drappa, & Illei, 2016). The evaluation will also consider the quality-of-life assessment after the clinical trials. In addition to that, the steroid sparing will be assessed in the biologics.

The criterion for classification follows the EULAR guidelines (Antonis Fanouriakis et al., 2019) with classifying the treatments in relation to the manifestation. Those manifestation can be classified into Molecular and organ manifestations generally. Specifically, the Molecular manifestation will look into the biologics and classify them according to their mechanism of action while the organ manifestation will look into the Disease-Modifying Antirheumatic drugs (Classical Treatments) and connect them to each manifestation.

4. Results:

4.1 The Recent Findings in SLE Genetics:

SLE can be defined as when large amounts of antibodies are produced against common self-antigens, especially double-stranded DNA (dsDNA) and small nuclear RNA-binding proteins like Ro, La, Sm, and nRNP. SLE is distinguished by multisystem involvement, frequently affecting the hematopoietic, renal, skin, and musculoskeletal systems. UV rays, infections, and viruses are examples of environmental factors that can

interact with susceptibility genes to cause SLE. This causes an irreversible loss of immunologic self-tolerance (Goulielmos et al., 2018).

Although the precise pathogenic mechanism of SLE is still unknown, altered immune cell signaling, excessive activation of type I interferon (IFN-I) signaling via toll-like receptors (TLRs)/nucleic acid sensors, and impaired apoptosis/autophagy and clearance defects leading to increased exposure of nuclear autoantigens have been identified as crucial phases in the disease process (Deng & Tsao, 2017).

Early 2000s gene expression studies revealed that people with SLE overexpress more interferon pathway genes than healthy controls. The type I interferon (IFN) pathway's significance in the pathogenesis of SLE has also been highlighted in numerous recent studies. It was discovered that serum IFN-alpha (IFN- α) activity is a heritable risk factor for SLE. Additionally, lupus patients' and their healthy family members' serum IFN-activity exhibits age-related patterns that mirror the peak rates of SLE incidence, with female patients reaching their peak serum IFN- activity at a younger age than male patients (Goulielmos et al., 2018).

New loci that impact disease susceptibility have been discovered through candidate gene studies and genome-wide association scans (Ayukekbong, Ntemgwa, & Atabe). These findings highlight the significance of several pathways, such as those involved in lymphocyte activation and function, immune-complex clearance, innate immune response, and adaptive immune response (Harley et al., 2008).

The data shown from several epidemiological studies suggest that heredity plays an important role in SLE which underline the importance of the genetic studies. A study that included more 18,000 SLE patients and their parent-child relationships concluded that ratio of sibling's risk was significantly high (23.68%) (Kuo et al., 2015).

4.1.1 The Genetic Susceptibility to Systemic Lupus Erythematosus:

Genetic susceptibility is defined as the possibility of developing a pathogenic disorder due to the existence of one or more genetic variant or a heredity that might suggest increased risk of a particular disease. Genetically susceptible individuals are believed to have genomes that contain the right combination of susceptibility alleles that, when activated by the right environmental trigger, modulate the immune system and make them more likely to develop autoimmune diseases. By calculating a genetic risk ratio known as λ_s , the significance of a genetic predisposition to autoimmune disease has been roughly quantified. The λ_s ratio is calculated by comparing the disease

prevalence in a proband's first-degree relatives to the prevalence of the disease in the general population. Linkage analyses in pools of affected families, candidate gene association studies using collections of cases and controls, and genome wide association studies (GWAS), typically carried out by consortiums of researchers on collections of 1000s of patients and controls, have been the three main approaches used to identify autoimmune diseases susceptibility loci (Rai & Wakeland, 2011).

Nine genes were thought to be causal of SLE family-based approaches or candidate gene studies prior to the development of SNP-based genome-wide association technology. These genes, which include human leukocyte antigen (HLA), C2, C4, C1q, FCGR2A, FCGR3A, PDCD1, PTPN22, and IRF5, are all essential for immune-related processes (Ha, Bae, & Kim, 2022).

Recently, genome-wide association studies (GWAS) have significantly increased our understanding of the genetic causes of SLE. They can map associated variants with marginal effects and are more effective than traditional genetic linkage studies in this regard. They can also pinpoint the exact location of the disease-associated loci.

According to GWAS, the risk of developing SLE is linked to a large number of alleles, each of which carries a negligible risk and none of which is individually necessary to the risk of developing SLE. More than 100 loci (other studies identified 180 loci) (Ha et al., 2022) have been linked to SLE through GWAS, and as a result, the disease's heritability can now be explained for over half of the cases (Demkova, Morris, & Vyse, 2022).

The majority of identified risk variants are found in non-coding regions, making it challenging to interpret their functions and disease-relevant genes in SLE susceptibility loci and highlights the significance of the allele-specific regulatory effects of disease genes. Numerous genes that may potentially play pathogenic roles in aberrant immunity and cellular processes in SLE were suggested by genetic association analyses, which were followed by functional annotation and statistical analyses for gene prioritization. According to reports, genes like STAT4, IRF3, IRF5, IRAK1, and TNFA1P3 that positively regulate the type I (IFN-I) pathway are likely to be the causal genes for SLE risk, as mentioned in the previous chapter. Other genetic components involved in lymphocyte signaling, such as PTPN22, BLK, BANK1, and LRRK1, have also been proposed as SLE-causing genes. LRRK1, which codes for a multiple-domain leucine-rich repeat kinase, is one of them and plays a role in the pathogenesis of SLE by impairing B-cell function and altering the B-cell receptor-mediated NF- κ B signaling pathway (Ha et al., 2022).

The genetic relationships between SLE and genes that clear immune complexes and apoptotic cells (like FCGR2A, ITGAM, and NCF1) have been also well established (Harley et al., 2008).

4.1.2 The Genetic Polymorphisms in SLE patients:

The genetic susceptibility to SLE cannot be discussed without mentioning the genetic polymorphisms that might cause an individual to be vulnerable to certain diseases. When the human genome project started it was observed that 99.5% of the human gene does not go through any changes and is conserved. The remaining 0.1 to 0.4% can see variations between different individuals (Karki, Pandya, Elston, & Ferlini, 2015). These variations are called genetic polymorphisms if they occur in the population with a frequency more than 1% (Gummadi & Guddati, 2021). The difference between mutations and polymorphisms is in the rarity. A “mutation” is a rare change in the nucleotide that typically, but not always, has a disease-causing characteristic (Karki et al., 2015). A mutation exists in less than 1% of the population (Gummadi & Guddati, 2021). A variation in the nucleotide that exists in more than 1% of the population is called a polymorphism, on the other hand. Polymorphisms may involve one or more nucleotide changes. It is possible that a polymorphism is naturally occurring and has either a neutral or advantageous effect given the population's higher incidence of the condition. When a polymorphism happens in only one nucleotide it is called Single Nucleotide Polymorphism (SNP). The SNP is a prime example of the most prevalent polymorphism; it is estimated to occur every 1,000 base pairs in the human genome, is primarily found in regions flanking protein-coding genes and is now understood to be essential for microRNA binding and the control of gene and protein expression. SNPs can, however, also appear in intergenic regions, introns, and coding sequences (Karki et al., 2015).

Numerous factors can lead to genetic polymorphisms. These genetic polymorphisms can occasionally be the result of chance (as discussed in the last paragraph), and occasionally they can be the result of outside factors like radiation. No matter where they come from, genetic polymorphisms are important because they can act as disease markers (e.g. SLE) and, occasionally, as an explanation for differences in how patients react to various medications (Gummadi & Guddati, 2021).

By using several analytical methods, it was found that the non-coding variant rs2431697 is possibly a cause of SLE. This variant is located on the Chromosome number 5 (Hou et al., 2021).

Genome editing tests and transcription-activating/-inhibiting dCAS9 systems were used to validate the region's enhancing activity around the above mentioned SLE-risk variant. It is true that the variant-located enhancer formed cognate loops with the miR-146a promoter, and the SLE-risk rs2431697 allele downregulated the expression of miR-146a by differentially modulating the regional chromatin state and NF- κ B binding affinity to tune the activation of the IFN1 pathway in SLE patients (Hou et al., 2021). Another study identified 2 other polymorphisms in addition to rs2431697 to be modulating the miR-146a. These 2 other polymorphisms are rs2910164 and rs57095329 (El-Akhras et al., 2022).

(Qi et al., 2021) mentioned that a total of 28 single nucleotide polymorphisms (SNPs) were involved with SLE predisposition specifying the 5 polymorphisms withing the ZNF76 gene, rs10947540, rs9394289, rs2267663, rs1894650, and rs9366883.

The ZNF76 was observed at a lower lever in SLE patients. ZNF76, a novel transcriptional repressor that targets TATA-binding protein, significantly inhibits p53 in a variety of cell lines, including the HeLa, U2OS, MCF-7, and H1299 cell lines (Qi et al., 2021).

4.1.3 The Human Leukocyte Antigen Region:

All jawed vertebrates have a large gene complex called the major histocompatibility complex (MHC), which plays a crucial part in the immune system. Cell-surface glycoproteins that bind intracellular and extracellular peptides, respectively, are the antigen-presenting molecules that are encoded by the MHC class I and class II genes.

More than 200 genes make up the human MHC, which is located on chromosome 6. Human leukocyte antigen (HLA) is the name for the MHC-encoded glycoproteins that are specific for presenting short peptides to T cells and are essential for the body's immune defense (Mosaad, 2015).

The class III region of the human HLA contains many genes encoding immune-related proteins, while classes I and II of the HLA region encode HLA molecules involved in antigen presentation. HLA region genetic association with SLE shows complex and multi-locus effects (Deng & Tsao, 2017). The HLA-DRB1 gene, which codes for an HLA-DR β -chain of HLA-DR protein, has been associated with SLE most strongly in the MHC region. This protein would be crucial in determining the immune system's tolerance to self-antigens. The complex SLE association architecture of the MHC region is implied by the suggestion that, upon conditioning on the HLA-DRB1 associations, other HLA genes

(HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-A, and HLA-B) and non-HLA genes (C4, MICB, NOTCH4, TNXB, and SLC44A4) have secondary independent association signals contributing to susceptibility to SLE (Ha et al., 2022).

(Langefeld et al., 2017) is GWAS that consisted of the more than 27,000 cases divided into more than 11,500 SLE cases against around 16,000 as controls highlighted the general role of HLA region.

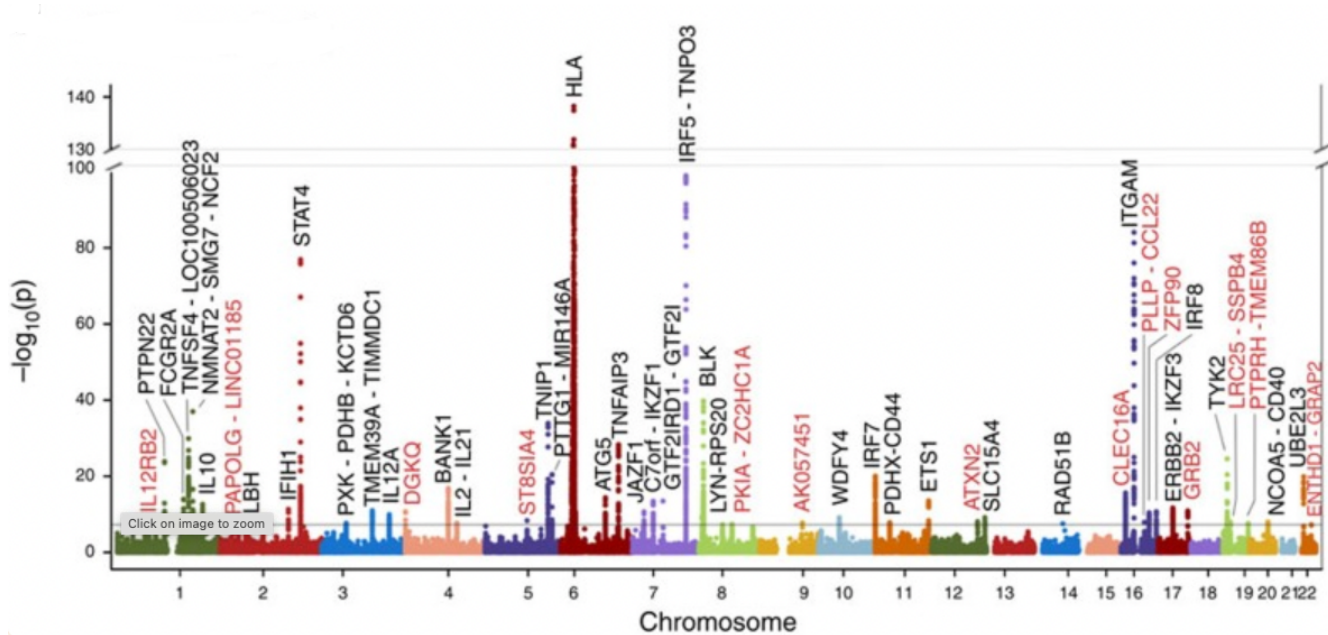


Figure 3 A Manhattan plot showing the significant loci related to SLE

Note. This Manhattan plot shows the result of a large study. The x axis represents the chromosomes of the human, and each set of genes belonging to the same chromosome is represented with one color. The y axis represents how these genes are associated with SLE. The lower line represents the threshold of the significance. From “Transancestral mapping and genetic load in systemic lupus erythematosus,” by Langefeld, C. D., Ainsworth, H. C., Cunninghame Graham, D. S., Kelly, J. A., Comeau, M. E., Marion, M. C., . . . Vyse, T. J., 2017, *Nat Commun*, 8, 16021, p. 3 (doi:10.1038/ncomms16021)

It worth noting that the HLA region in general is the most gene- dense region of the whole human genome with it being link to more than 120 functional genes (Y. Cui, Sheng, & Zhang, 2013).

4.2 The Environmental and Sex Influences of SLE:

Strong epidemiological data support the link between SLE and a number of environmental factors, such as exposure to crystalline silica, alcohol use, current cigarette smoking, and exogenous estrogens (oral contraceptives and postmenopausal hormones). Other exogenous factors may be associated with one another, according to the evidence. (For instance, UV rays, solvents, and pesticides;). Birthweight, reproductive history (such as parity, age at menarche and menopause), endometriosis, and latent infections like EBV are all associated with SLE and may indicate a secondary role for environmental exposure (Parks, de Souza Espindola Santos, Barbhaiya, & Costenbader, 2017).

4.2.1 Silica (Silicon Dioxide) Exposure:

The crystalline form of quartz-derived respirable silica dust is a known risk factor for SLE and other systemic autoimmune diseases (e.g., scleroderma and rheumatoid arthritis) (Miller et al., 2012).

The so-called "dusty trades," which include sandblasting, rock drilling, sand factory work, granite cutting, construction work, brick laying, tilling, and cement work, also release freshly fractured particulate silica (crystalline silica or quartz). Agricultural exposure levels may also vary depending on the amount of silica in the soil. Studies on animals and in the basic sciences suggest that silica may act as a T-cell adjuvant and a potential autoimmunity trigger (Miller et al., 2012).

Large population studies confirmed the relationship between occupations and SLE. For example, (Gold, Ward, Dosemeci, & Roos, 2007) showed that mining machine workers were at risk of death from all systemic autoimmune diseases in general and SLE in particular, which could point to the relationship between silica and the disease.

Crystalline silica may act as an immune adjuvant by inducing apoptosis and the release of intracellular antigens, raising levels of pro-inflammatory cytokines, oxidative stress, and T cell responses, and lowering levels of regulatory T cells, according to experimental studies (Pollard, 2016).

In murine models, exposure to silica worsens lupus by raising serum levels of immune complexes, glomerulonephritis, and proteinuria.

There is strong evidence referring to the involvement of the nanoparticles of silica with the increase in the immunostimulatory effects (Parks et al., 2017).

4.2.2 Cigarettes Smoking:

There is mechanistic evidence linking smoking to the pathogenesis of SLE. Exposure to the harmful substances found in cigarette smoke, such as tars, nicotine, carbon monoxide, polycyclic aromatic hydrocarbons, and free radicals, can cause oxidative stress, directly harm endogenous proteins and DNA, and result in genetic mutations and gene activation that may contribute to the onset of SLE. Smoking increases the production of pro-inflammatory cytokines and stimulates the expression of CD95 on B and CD4 T cell surfaces, which may lead to autoimmunity (Barbhaiya & Costenbader, 2016).

One of the largest GWAS studies that investigated the interactions between smoking status and genetic risk factors for SLE concluded that, in comparison to non- and more distant past smokers, those who smoke now or recently quit had a roughly 50% higher risk of developing SLE.

The study found strong and distinct associations between current smoking and the subtype of dsDNA autoantibody+ SLE in particular, indicating that smoking is involved in the pathogenesis of SLE and that its effects are reduced after quitting. Smoking may affect DNA and proteins, cause oxidative stress, stimulate the release of pro-inflammatory cytokines like TNF- and IL-6, increase CD95 expression on B and CD4 T cells, cause apoptosis, and trigger autoimmunity as potential biologic mechanisms that may contribute to or hasten the pathogenesis of SLE (J. Cui et al., 2020).

4.2.3 UV Radiation:

Several studies of human populations and experimental studies have shown that ultraviolet radiation (UVR) is a significant environmental factor causing SLE. By causing a proinflammatory environment and abnormal long-lasting photoreactivity via inflammatory mediators, such as proinflammatory cytokines, chemokines, and adhesion molecules, it plays a crucial role in the pathogenesis of lupus. UVR exposure increases the expression of proinflammatory cytokines like IFN- α , IL-1, IL-6, and TNF- α (Chen et al., 2022).

(Abdulahad et al., 2013) revealed that UVR exposure caused the release of the protein high-mobility group protein B1 (HMGB1), which is associated with the number of apoptotic cells in SLE patients. When exposed to UVR, HMGB1 released from apoptotic keratinocytes causes inflammation by binding to its receptors, leading to the development of inflammatory lesions in the skin of SLE patients.

4.2.4 Effect of Infections on Inducing SLE:

Numerous mechanisms by which viral infections can interact with the host immune system result in the loss of tolerance, the generation of autoantibodies, the deposition of immune complexes in the tissues, and ultimately tissue damage. These include altered apoptosis and clearance deficit, epigenetic factors, structural or functional molecular mimicry, superantigen production, bystander activation, persistent or recurrent viral infection, and innate immunity activation with type I IFN production (Quaglia, Merlotti, De Andrea, Borgogna, & Cantaluppi, 2021).

Epigenetic mechanisms have gained attention in the last ten years as a crucial mode of how viruses and the immune system interact in SLE because they enable viruses to produce gene expression profiles that predispose the host to autoimmunity. They consist of histone modifications, microRNA (miRNA) modulation, and DNA methylation changes in SLE susceptibility genes (for instance, IFN-related genes) (Long, Yin, Wang, Gershwin, & Lu, 2016).

Herpesviruses and the hepatitis C virus are examples of viruses that can evade immune clearance for an extended period of time and cause persistent viral infection (HCV). This determines chronic inflammation and polyclonal T- and B-cell proliferation, ultimately resulting in the production of autoantibodies and the emergence of self-reactive clones. Numerous non-retrovirus RNA viruses cause persistent infections "within the host," which can sporadically lead to autoimmune diseases (Quaglia et al., 2021).

Ninety five percent of people on the planet have latent Epstein–Barr Virus (EBV) in their memory B cells. It first infects pharyngeal epithelial cells after being spread by saliva, then resting B lymphocytes, T lymphocytes, NK cells, and neutrophils. The virus is linked to a number of neoplasms and AIDs, even though a primary infection is typically asymptomatic (during childhood) or results in a self-limiting illness called infectious mononucleosis (IM) (during adolescence) (Quaglia et al., 2021).

Potential mechanisms include molecular mimicry between Epstein–Barr Virus (EBV) and SLE antigens and EBV RNA/SSB protein complexes that activate type 1

interferon through Toll-like receptor 3. Furthermore, plasmacytoid dendritic cells, CD69+CD4+ T cells, impaired CD8+ cytotoxic T cells, and irregular cytokine production in response to EBV are all observed in SLE patients (Parks et al., 2017).

To determine a lifelong infection in memory B cells, EBV can significantly alter physiological B cell differentiation which could explain its impact on SLE patients (Münz, 2019).

4.2.5 Sex Hormones and the X Chromosome:

Female sex is a major risk factor for developing SLE. The overwhelming majority of SLE cases in women occur during the years when they are capable of having children. The startling sex disparity in SLE is thought to be caused by both sex hormones and X chromosome factors. A potential explanation for the higher prevalence of SLE in women is epigenetic modification of genes on the "inactive" X chromosome. Sex steroid hormones, such as 17- β -estradiol, testosterone, prolactin, progesterone, and dehydroepiandrosterone, affect how the immune system is regulated and how severe a patient's condition is in SLE (Barbhaiya & Costenbader, 2016).

Sex hormones regulate the immune system's development and function in addition to the reproductive system. Hormones affect the innate, adaptive, humoral, and cell-mediated immune responses, and dysregulation of these mechanisms underlies immune-mediated diseases like autoimmune disease (Klein & Flanagan, 2016).

Studies have shown that female hormones control T and B lymphocyte development, homeostasis, gene expression, and signaling processes to affect how well or poorly they function in health and disease. However, the precise molecular mechanisms by which female hormones regulate the immune system are still not fully understood (Moulton, 2018).

Numerous immunomodulatory cytokines are activated or suppressed by estrogen and ER signaling, which aids in the development of the lupus disease and the pathology of its organs (Kassi & Moutsatsou, 2010).

The production of cytokines and other cytokine regulatory molecules (such as calcineurin and CD40L) in humans and animals with SLE disease is shown to be mediated by the estrogen receptor in existing data. Additionally, it has been suggested that the production of cytokines and the pathogenesis of disease may be correlated with ER gene polymorphisms, as well as quantitative and qualitative alterations in the receptor proteins (Kassi & Moutsatsou, 2010).

Many genes with significant immunoregulatory functions can be found on the X chromosome. Multiple autoimmune diseases are more likely to strike females as they have two X chromosomes. A second X chromosome in women may be the cause of the increased expression of X-linked genes seen in autoimmunity, which is an intriguing finding considering that elevated expression of some X-linked immune-related genes is also linked to autoimmune pathogenesis. In light of this, it may be crucial to understand how X-linked genes are regulated in order to comprehend the gender disparity in autoimmune disease risk (Syrett & Anguera, 2019).

Not only men show significantly low prevalence in comparison with women but also, female SLE cases of Turner syndrome with one X Chromosome (45,X) are underrepresented in women, supporting the idea that having multiple X chromosomes increases autoimmune susceptibility (Cooney et al., 2009) (Syrett & Anguera, 2019).

Similar to 46, XX females, men with extra X chromosomes, such as those with Klinefelter syndrome (47, XXY), are predisposed to autoimmunity. In comparison to 46, XY males with a single X chromosome, the risk of SLE in 47, XXY males is 14 times higher. Men with Klinefelter syndrome are more likely to develop SS, which is also heavily biased toward women. Additionally, female trisomy (47, XXX) is overrepresented in SLE, with a 2.5:1 prevalence rate in comparison to karyotypically normal 46, XX women. Patients with polysomy X have been reported as well to have SLE (48, XXXX) (Syrett & Anguera, 2019).

4.3 Clinical Manifestation of SLE:

“SLE is a chronic multi system inflammatory autoimmune disease” that varies in its clinical presentation and may fluctuate in intensity (Tsokos, 2011). From mild symptoms to serious, life-threatening conditions, SLE displays a wide range of presentations. Adults who are diagnosed with the disease before the age of 50 typically have cutaneous symptoms (malar rash) and renal abnormalities (lupus nephritis), have a higher 10-year survival rate, and use more immunosuppressive therapies. The timing of presentation and the start of therapy are also influenced by a number of variables, including age, race, gender, genetic predisposition, and socioeconomic status (Ameer et al., 2022).

Some patients can be at a higher risk but still asymptomatic. They might still be at the stage of developing Lupus (10 to 20% of them develop into Lupus), and

autoantibodies will likely be detected in their serum. These patients are in the Preclinical Phase (Gatto, Saccon, Zen, Iaccarino, & Doria, 2019).

In the subsequent section the most common SLE manifestation is discussed, as they play a very important role in developing a proper treatment protocol.

4.3.1 Cutaneous Manifestation:

About 75% of SLE patients experience cutaneous symptoms over the course of the illness, and in 25% of cases, they are the initial symptom (Kuhn et al., 2015). James N. Gilliam (1936–1984) proposed a classification scheme for the various skin manifestations of LE, “distinguishing between LE-specific and LE-nonspecific cutaneous manifestations based on histological criteria” (Kuhn, Sticherling, & Bonsmann, 2007).

Acute cutaneous lupus erythematosus (ACLE), which can manifest as a butterfly rash or a generalized maculopapular exanthema, is the most common LE-specific manifestation. Discoid lupus erythematosus (Petri et al.) has a chronic course and is frequently characterized by inflammatory erythematous plaques, follicular hyperkeratosis, and scarring, which results in permanent alopecia in hair-bearing areas. There are two types of classic DLE: localized (above the neck) and generalized (above and below the neck, with typical involvement of the extensor forearms and hands). Five percent of DLE patients who initially exhibit no systemic organ involvement will go on to develop SLE. On sun-exposed areas of the back, chest, and extensor surfaces of the arms, subacute cutaneous lupus erythematosus (SCLE) is characterized by symmetric, annular, polycyclic, and/or papulosquamous/psoriasiform skin lesions without scarring (Walling & Sontheimer, 2009) (Kuhn et al., 2015).

“The LE-non-specific manifestations include, among others, vascular skin lesions (e.g., periungual telangiectasia, livedo racemosa, Raynaud syndrome)” (Kuhn et al., 2015).

Bullae, rheumatoid nodules, calcinosis cutis, anetoderma, thrombophlebitis, erythromelalgia, erythema multiforme, acanthosis nigricans, lichen planus, and leg ulcers are some additional lesions linked to LE that have been noted. Less than 5% of patients are reported to have cheilitis, episcleritis, or facial edema (Walling & Sontheimer, 2009).

4.3.2 Musculoskeletal Manifestation:

Ninety to one hundred percent of SLE patients experience musculoskeletal manifestations. At some point during the course of the disease, 83% of SLE patients experience joint pain, making it the most prevalent presenting symptom. The most frequently affected joints are the proximal interphalangeal joints, followed by the knees, wrists, and metacarpophalangeal joints. Late in the course of the disease, due to ligament laxity, joint deformity may develop (Jaccoud arthropathy). Typically, involvement is symmetrical. Up to 50% of patients experience morning stiffness, and 30% will feel pain in their muscles. In 10% of patients, tendonitis and tenosynovitis may develop. According to one series, fibromyalgia affects 10 out of 60 patients (17%) and is active independently of SLE disease activity (Kuhn et al., 2015; Walling & Sontheimer, 2009)

4.3.3 Lupus Nephritis:

Systemic lupus erythematosus patients are more than 50% likely to develop lupus nephritis (LN) (Queenan et al.), which raises the risk of renal failure, cardiovascular disease, and death (Bertsias et al., 2012).

Lupus nephritis happens at the glomerular of the kidney and it is characterized by proteinuria, erythrocyturia (especially with dysmorphic erythrocytes) in addition to the “erythrocyte cylinders in the urinary sediment” (Kuhn et al., 2015).

The prognosis for LE nephritis is not good. Up to 20% of patients with LE nephritis may develop end-stage renal failure (Walling & Sontheimer, 2009). However, 25% of SLE patients may have lupus nephritis (class III, IV, and V) without any outward signs of renal disease (Wakasugi et al., 2012).

A renal biopsy should be performed if the urine protein level is 500 mg/24 hours or higher, which is linked to histopathological lupus nephritis. A reliable indicator of proteinuria in lupus nephritis is the urine protein-to-creatinine ratio. It is also simpler to measure on a regular basis and has a strong correlation with 24-hour proteinuria. Additionally, higher risk histological features are linked to clinical and serological (hypocomplementemia and elevation of anti-dsDNA antibodies) activity that may further suggest lupus nephritis (Fava & Petri, 2019).

There are six classes of lupus nephritis and categorized by the Renal Pathology Society/International Society of Nephrology (RPS/ISN): minimal mesangial lupus

nephritis, mesangial proliferative lupus nephritis, focal lupus nephritis, diffuse lupus nephritis, membranous nephropathy (Weening et al., 2004).

4.3.4 Neuropsychiatric Systemic Lupus Erythematosus:

The central nervous system is impacted in 15 to 50% of SLE patients, but diagnosis of the condition is frequently challenging due to the symptoms' low specificity (such as headache) and high variability (Kuhn et al., 2015).

There is no formal system in place for establishing a diagnosis and directing treatment choices in neuropsychiatric SLE (NPSLE), nor are there any laboratory or radiological biomarkers (Magro-Checa, Zirkzee, Huizinga, & Steup-Beekman, 2016).

12 CNS and 7 PNS manifestations were included in a set of NPSLE case definitions published by the American College of Rheumatology (ACR) in 1999. The eight neurological syndromes and four psychiatric syndromes that make up the Central Nervous System (CNS) manifestations can be further broken down into focal (events that present as focal neurologic deficits) and diffuse categories “(including cognitive disorder, mood disorder, psychosis, acute confusional state, and anxiety disorder)” (Magro-Checa et al., 2016).

More and more research has shown a connection between SLE and other CNS manifestations not covered by the ACR definitions, like posterior reversible encephalopathy syndrome or Neuromyelitis Optica (Barber, Leclerc, Gladman, Urowitz, & Fortin, 2011).

There are two different main pathogenic mechanisms that could cause NPSLE. The first one is inflammatory or autoimmune which include key features like brain dysfunction that happens because of autoantibodies or inflammatory mediators with “either a disrupted blood–brain barrier (BBB) or intrathecal formation of immune complexes and the presence of inflammatory mediators”. These mediators can cause neuronal dysfunction directly or it can happen indirectly by activating other neural cells.

The second mechanism is Vascular injury and occlusion caused by immune complexes, complement deposition, leukoagglutination, and accelerated atherosclerosis, characterized by a thrombotic process of the small and large intracranial vessels (microangiopathy) (Magro-Checa et al., 2016).

4.3.5 Gastrointestinal Manifestation:

Numerous gastrointestinal (GI) symptoms, such as flatulence, diarrhea, abdominal pain, hematemesis, gastric atony, duodenal and jejunal ileus, oral ulcers, problems with esophageal motility, protein-losing enteropathy, and lupus enteritis, are also seen in SLE. Additionally, secondary to SLE and antiphospholipid antibody syndrome, mesenteric vessel thrombosis, Budd-Chiari syndrome, and hepatic veno-occlusive disease all manifest (Ameer et al., 2022).

4.3.6 Cardiovascular Manifestation:

Mortality because of cardiovascular disease between SLE patients is significantly high (Yazdany et al., 2020). SLE may affect all three layers of the heart—the pericardium, myocardium, and endocardium—as well as frequently the coronary circulation. Heart failure, cardiomyopathy, valvular diseases, and arrhythmia are among the commonly observed symptoms. Pericarditis caused by exudative pericardial effusions is the most common cardiac manifestation (Ameer et al., 2022).

4.4 Diagnosis and Classification of SLE:

Due to the complexity and heterogeneity of SLE and its manifestation, a classification criteria became a must to support the “diagnostical, epidemiological and research purposes” (Fava & Petri, 2019). The American College of Rheumatology's (ACR) standards, which were initially published in 1982 and updated in 1997 was the first to be developed. It listed 11 criteria. To diagnose SLE, four of the eleven criteria must be met. However, application of the ACR criteria without autoantibody analysis may lead to an overestimation of SLE because four of the criteria include mucocutaneous lesions (Kuhn et al., 2015).

The (SLICC) criteria from 2012 were evidence-based, included lupus nephritis as a "stand alone" criterion, and required at least 1 clinical (acute cutaneous lupus, chronic cutaneous lupus, oral or nasal ulcers, synovitis, serositis, proteinuria or red blood cell casts, neurologic manifestations, hemolytic anemia, leukopenia and thrombocytopenia) and another immunological criteria (ANA, anti-dsDNA, anti-Smith, anti-phospholipid antibodies, hypocomplementemia, and direct Coombs test) (Kuhn et al., 2015) (Fava & Petri, 2019).

To maintain sensitivity and improve specificity, the EULAR/ACR 2019 criteria were subsequently developed. ANA positivity with an immunofluorescence titer of 1:80 or higher is a requirement for entry into the EULAR/ACR 2019 criteria, which uses a scoring system. If the patient receives a weighted sum of 10 or more across the clinical and laboratory domains, SLE is considered to be the diagnosis (Aringer et al., 2019).

These new criteria performed better than older criteria in a validation cohort for adult-onset SLE in terms of sensitivity and specificity. The sensitivity of EULAR/ACR 2019 is similar to or lower than that of SLICC 2012, whereas the specificity of EULAR/ACR 2019 is comparable to or lower than that of ACR 1997 in adult-onset SLE, according to recent studies (Lerkvaleekul, Chobchai, Rattanasiri, & Vilaiyuk, 2022).

Previous studies on SLE with childhood onset have also produced contradictory findings. According to some authors, EULAR/ACR 2019 has a lower sensitivity than SLICC 2012 and a lower specificity than ACR 1997, making it a less effective tool for diagnosing childhood-onset SLE. However, some studies have discovered that, in contrast to SLICC 2012 and ACR 1997, EULAR/ACR 2019 performs better and advances over time (Lerkvaleekul et al., 2022).

4.5 Classification and Evaluation of Current and Future treatments for SLE:

Aspirin was the first medication to be authorized for use in treating lupus by the US Food and Drug Administration (FDA) in 1948. In 1950, cortisone was administered to one patient, marking the beginning of the use of glucocorticoids in the treatment of lupus. Following the FDA's approval of hydroxychloroquine and corticosteroids for lupus in 1955, chloroquine became commercially available in 1953 (Levy et al., 2021).

A task force of the European League Against Rheumatism (EULAR) published a report in 2014 outlining the treat-to-target (T2T) strategy for therapeutic objectives. Remission without any systemic symptoms or organ disorders was the therapeutic target and avoiding relapse or organ disorders was the realistic therapeutic goal. Despite the absence of remission criteria, evaluation utilizing indicators of organ disorders and systemic lupus activity was advised (Tanaka, 2020).

SLE has a very variable clinical course, so it's critical to determine the prognosis along with the creation of accurate markers for disease activity, severity, and damage accumulation. Numerous clinical signs (such as discoid lesions, arthritis, serositis, renal involvement, psychosis, or seizures), laboratory tests (such as anemia, thrombocytopenia, leucopenia, serum creatinine), immunological tests (such as anti-dsDNA, anti-C1q, antiphospholipid, anti-RNP, anti-Ro/SSA, anti-La/SSB antibodies, and serum complement concentrations), brain MRI7, and renal biopsy all decides on the outcome and the prognosis of the patient (Antonis Fanouriakis et al., 2019).

Exacerbations and flares of varying severity make SLE more challenging. In routine clinical practice and clinical trials, SLE patients are assessed using a variety of global and organ-specific activity indices. The Systemic Lupus Erythematosus Disease Activity Index, the European Consensus Lupus Activity Measure, and the British Isles Lupus Assessment Group Scale are more widely used. These indices, which were created in the context of extensive observational studies, accurately reflect changes in disease activity and are good indicators of damage and mortality. For the purpose of tracking disease activity, the EULAR encourages the use of at least one of these indices (Antonis Fanouriakis et al., 2019).

4.5.1 Drugs Targeting Molecular Manifestations:

It is not surprising that there is no one medication that can treat all manifestations of SLE due to the wide genetic and clinical heterogeneity of the condition. Biologics, including IFN-inhibitors and other drugs that can modulate the immune system are well mirrored by a better understanding of the pathogenic mechanisms causing SLE. Research is being conducted on biological therapies that concentrate on important SLE molecular mediators in an effort to better understand the pathogenesis. (Sciascia, Radin, Roccatello, Sanna, & Bertolaccini, 2018).

4.5.1.1 Drugs Targeting the B-Cell:

As mentioned before in the study, B-cell plays a critical role in the pathogenesis of SLE. The ability of B cells to produce autoantibodies, as well as their capacity to present autoantigens to T cells through the BCR (B cell receptor), secrete cytokines, and regulate dendritic cell (DC) activity, all play a role in the physiopathology of SLE. Thus, a B cell-targeted drug ought to inhibit pathogenic B cells and/or cause B cell expansion while enhancing the activity of protective cells (Samotij & Reich, 2019).

Belimumab:

In order to use human DNA sequences in drug discovery, Human Genome Sciences (HGS; Rockville, MD, USA) and The Institute for Genomics Research (TIGR) were founded in 1992. HGS extensively screened countless human tissue-specific cDNA libraries. A single cDNA clone (HNEDU15) that encoded a new member of the tumor necrosis factor (TNF) ligand superfamily was found after a single search of a library made from primary human neutrophils. The HNEDU15 transcript produced the type II transmembrane protein BLyS (also known as BAFF) (Schneider et al., 1999), which can be found in both membrane-bound and soluble forms (Levy et al., 2021).

Belimumab is the first drug to be approved by the FDA and EMA since the approval of hydroxychloroquine in 1955 (Samotij & Reich, 2019). It was approved as an IV treatment for SLE by the FDA and EMA in 2011 (Levy et al., 2021). It functions as a human monoclonal IgG1 antibody that is specific for soluble BAFF. It prevents BAFF from tying up with B Cell Maturation Antigen (BCMA), TACI, and BAFF-R (Atisha-Fregoso et al., 2021).

Efficacy: In 2007 HGS and GlaxoSmithKline started the two pivotal multicenter, randomized, placebo-controlled BLISS-52 and BLISS-76 studies (Furie et al., 2011). Belimumab was linked to a significantly higher SRI-4 (Systemic Lupus Erythematosus Responder Index 4, a score used to evaluate the response of SLE in clinical trials mainly at end point) (Furie et al., 2016) response rate at 52 weeks in both studies compared to placebo, indicating a clinically significant reduction in SLE disease activity. According to the SFI (The SELENA-SLEDAI Flare Index) and BILAG (British Isles Lupus Assessment Group) domain scores, belimumab treatment also decreased the number of severe SLE flares and the risk of developing a new severe flare within a year when compared to placebo (Levy et al., 2021).

Belimumab at a dose of 10 mg/kg Body Weight (BW) significantly increased the proportion of patients achieving SRI-4 compared to placebo (58% vs. 44% in BLISS-52 and 43% vs. 34% in BLISS-76). Belimumab patients also were significantly more likely to experience relief from musculoskeletal and mucocutaneous symptoms (Samotij & Reich, 2019).

The medication also improved the quality of life and fatigue levels while having a positive impact on immunological markers of disease activity (reduction of Ig concentration and increase in complement components, reduction of autoantibody production) (Samotij & Reich, 2019).

In a randomized study conducted in Asia it was found that, Belimumab-treated patients significantly outperformed the placebo group in terms of achieving the primary endpoint of SRI-4 response at Week 52, and their risk of a severe SLE flare was cut in half. Belimumab showed a significant steroid-sparing effect in patients with baseline prednisone doses by 50% (Levy et al., 2021).

The effect of Belimumab on the steroid-sparing effect was also demonstrated in a study in Greece. It revealed a gradual reduction in the daily prednisone dosage, with more than 20% of patients able to stop corticosteroid use as early as three months after starting it and more than 40% achieving a lupus low disease activity state (LLDAS) by nine to twelve months after starting it (A. Fanourakis et al., 2018).

The subgroup of patients with Black African ancestry did not appear to benefit from IV belimumab treatment, according to the combined data from the BLISS-52 and -76 studies, whereas in the Phase 2 study, post hoc subgroup analyses of patients with Black African ancestry did show a benefit for IV belimumab (Levy et al., 2021).

Belimumab treatment was linked to an early reduction of both naive and autoimmunity-associated B cells (CD11c + CD21-) at 3 months but had little effect on

class-switched memory B cells and plasma cells over a 3-year follow-up period. Beyond 76 weeks of belimumab treatment, significant and ongoing reduction of B lymphocyte subsets was seen, with 80-90% reduction for naive plasmacytoid B cells, 70-75% decrease for CD19+/CD20+ B cells, and 50-60% drop for plasma cells, respectively (Yap & Chan, 2019).

Safety: A double-blind, randomised, placebo-controlled study that enrolled 4003 adults with active SLE and randomly assigned them to receive IV belimumab 10 mg/kg or placebo, plus ST, for 48 weeks found that, the incidences of all-cause mortality and most AESIs, including malignancies, serious infections, and opportunistic infections, were similar between belimumab and placebo groups. Belimumab was linked to a higher incidence of serious infusion and hypersensitivity reactions (n = 8 [0.40%], placebo n = 2 [0.10%]), serious depression (n = 7 [0.35%], placebo n = 1 [0.05%]), treatment-emergent suicidality (n = 28 [1.42%], placebo n = 23 [1.16%]), and sponsor-adjudicated serious suicide or self-injury (n = 15 [0.75%]), compared with placebo (Levy et al., 2021).

A study under the name PLUTO assessed the safety of Belimumab on children (5-17 years) confirmed the safety of Belimumab for children (Brunner et al., 2021).

Even when used for up to 13 years, long-term data indicate that it is well-tolerated and has a low risk of side effects (Wallace et al., 2019).

Tabalumab:

Tabalumab is an IgG4 monoclonal antibody recognizes and neutralizes BAFF in the soluble form and the membrane-bound one (D. A. Isenberg et al., 2016).

Efficacy: Two phase III trials were set to determine the effectiveness and safety of giving tabalumab subcutaneously. these 2 studies are ILLUMINATE-1 and ILLUMINATE-2 (Merrill et al., 2016).

A total of 1,164 patients with moderate to severe SLE and a SELENA-SLEDAI score of at least 6 were enrolled in the 52-week ILLUMINATE-1 which is a multi-center, randomized, double-blind, placebo-controlled study. Although the primary endpoint—an SRI of 5 at week 52—was not met, the treatment group's response rates were higher than those of the placebo group. The time to first severe SLE flare, GC-sparing effects, and changes in fatigue levels were the secondary endpoints that weren't met either. But as early as week 4 and continuing through week 52, tabalumab groups in ILLUMINATE-

1 results demonstrated a significant decline in anti-dsDNA levels compared to placebo groups (Sciascia et al., 2018). a decrease in the total B cell count, Ig concentration, and BAFF levels, as well as an increase in the serum levels of the C3/C4 complement components, were also noted (Samotij & Reich, 2019).

in the second study (ILLUMINATE-2) (n = 1124). At week 52, the 120 mg group every two weeks achieved the primary end point (the percentage of patients who achieved SRI-5) (38% vs. 28.7% in the treatment group and placebo, respectively; p = 0.002). However, no secondary end points have been reached. A statistically significant 11% difference in the response rate in favor of the group of patients taking tabalumab compared to placebo recipients was seen in post hoc analyses of the efficacy of tabalumab using the same clinical response rate that was used in the belimumab phase III trials (Considering SRI-4 instead of SRI-5) (Samotij & Reich, 2019).

Safety: Patients receiving treatment and those receiving a placebo experienced the same frequency of adverse events and mortality in ILLUMINATE-1 (Samotij & Reich, 2019).

Atacicept:

Atacicept is an Fc fragment of human IgG1(Tanaka, 2020). It blocks both BAFF and APRIL and by that inhibits the activation of B-cell. APRIL is a cytokine that is secreted by a variety of immune response-related cells, including monocytes, dendritic cells, macrophages, and T cells (All involved in the pathogenesis of SLE) (Sciascia et al., 2018).

Efficacy: In patients with active LN who also received a high dose of CS and mycophenolate mofetil (MMF) (3g/day), a 52-week phase II/III study with atacicept (150 mg SC twice weekly for 4 weeks, then once weekly) was set up. Unfortunately, a sudden, severe drop in serum IgG levels and serious infections forced the early termination of this trial. A further 461 patients were subjected to a double-blind phase II/III trial with randomization and placebo control. Participants in this study were randomized in a 1:1:1 ratio to placebo and two doses of atacicept if they had active SLE according to the BILAG index (≥ 1 item in the BILAG A and/or B domain), had previously been treated with CS with gradual dose reduction for 10 weeks, and reached the BILAG C or D domain (75 mg or 150 mg 2x per week for 4 weeks, then 1x per week for 48 weeks). The 75 mg patients

did not achieve the primary end point, which was a significantly lower proportion of patients who experienced a new flare based on BILAG A or BILAG B domain scores. Serious adverse effects caused the 150mg study to halt (Samotij & Reich, 2019). Regarding the disease's immunological parameters, the drug's administration in both doses produced an improvement (D. Isenberg et al., 2015).

Toxicity: The study APRIL-SLE was halted prematurely due to 2 infection related deaths. Ginzler et al. was terminated also because of safety considerations. hypogammaglobulinemia and severe infections were developed during the trial in half of the patients (N=6) (Sciascia et al., 2018).

Blisibimod:

The peptibody (fusion polypeptide protein) blisibimod (AMG623, also known as A-623) is made in Escherichia coli and targets both soluble and membrane BAFF. It's a new BAFF binding domain attach to the N-terminus of the human Fc domain of the IgG (Vincent, Saulep-Easton, Figgett, Fairfax, & Mackay, 2013).

Efficacy: 547 patients with serologically active SLE and a SELENA-SLEDAI score of at least 6 points were randomly assigned to 3 different doses of blisibimod or a placebo in a double-blind, randomized, placebo-controlled clinical trial (PEARL-SC). At week 24, the highest dose group (200 mg once weekly) outperformed the placebo group in terms of SRI-5 response rate. Treatment with blisibimod was linked to a significant improvement in biomarkers, including a decrease in anti-dsDNA antibodies, an increase in serum C3/C4 concentrations, and a decrease in B cell count (Sciascia et al., 2018). The primary endpoint of the study (SRI-6) was not significantly different between the blisibimod and placebo groups (47% vs. 42%, respectively) in a multicenter, placebo-controlled, phase III randomized, double-blind study (CHABLIS-SC1) that involved 442 seropositive SLE patients with persistent high disease activity (SELENA-SLEDAI 10 points) despite use of CS and other conventional treatments. SRI-4 and SRI-8, the secondary end points, were also not reached. Despite mixed results for the study's endpoint measurements, significant improvements in B cell count, Ig concentration, and complement component levels were seen that were comparable to those seen in the phase IIb clinical trial (Samotij & Reich, 2019).

Safety: Blisibimod was tolerated in all the different doses in the study (PEARL-SC) (Samotij & Reich, 2019).

Rituximab:

Rituximab is a glycosylated immunoglobulin (Ig) G1- κ monoclonal antibody (mAb) with human kappa and human IgG1 constant region sequences and murine light- and heavy-chain variable region sequences (MabThera®/Rituxan®/Rituxan HYCELATM, F. Hoffmann-La Roche Ltd.). Rituximab has a particular affinity for CD20, a B-lymphocyte transmembrane protein that is expressed on normal B cells (aside from stem cells, pro-B cells, and plasma B cells) and the majority of malignant B cells (Salles et al., 2017).

Rituximab was given regulatory approval for use in relapsed/refractory indolent non-Hodgkin lymphoma in 1997 by the US Food and Drug Administration and in 1998 by the European Medicines Agency. It then received approvals for use in Chronic lymphocytic leukaemia in 2009 and 2010, respectively. The first therapeutic mAb used in oncology was Rituximab, which was administered intravenously. This discovery led to the development of a new class of anticancer medications (Salles et al., 2017).

RTX specifically binds to CD20-positive cells, causing cell-cycle arrest and ultimately apoptosis. These cells are further eliminated by phagocytosis, complement-dependent lysis, and antibody-dependent cellular cytotoxicity; these processes all rely on the antibody's Fc portion binding to Fc γ Rs on immune cells (Pescovitz, 2006).

Efficacy: The earliest studies on RTX's efficacy in SLE patients date back to 2002. In later studies, patients with SLE and LN who also had joint and mucocutaneous symptoms, serositis, cytopenia, and neurological involvement benefited from this medication. The efficacy of RTX in this indication was confirmed by analysis of 188 cases of its use in SLE in small, uncontrolled studies. However, the primary endpoints of two sizable, double-blind, randomized, placebo-controlled studies in patients with nonrenal SLE (EXPLORER, phases II and III) and LN (LUNAR, phases III) were not met (Samotij & Reich, 2019).

257 people with moderate to severe SLE (16–75 years old) were enrolled in the EXPLORER trial. Four of the American College of Rheumatology's criteria for SLE had to be met by participants, including the presence of antinuclear antibodies (Lerkvaleekul et al.), active disease at screening (defined as at least one domain with a British Isles Lupus Assessment Group [BILAG] disease activity index A score or at least two domains

with a BILAG B score), and stable use of one immunosuppressive medication that was maintained throughout the study. The primary endpoint compared how rituximab and placebo affected reaching and maintaining a clinical response at week 52 (Sciascia et al., 2018).

In patients with active LN, the effectiveness of RTX was assessed in the second clinical trial. The patients also received Corticosteroids CS and MMF (2 g/day), in addition to RTX. Between the RTX and placebo arms, there were no statistically significant differences in the proportion of patients achieving the primary and secondary end points at week 52. The proportion of responders in the RTX arm, however, was higher than in the placebo arm (57% vs. 46%). Additionally, the group of patients who received RTX showed greater improvement in terms of SLE-specific immunological markers, such as the concentration of anti-dsDNA antibodies and levels of C3/C4 complement component (Samotij & Reich, 2019). Early use of rituximab in children delayed the onset of the disease for a very long time. T cell activation was decreased as part of the mechanism of action (Atisha-Fregoso et al., 2021).

Toxicity: Rituximab (RTX) treatment for SLE resulted in the death of 2 patients from progressive multifocal leukoencephalopathy in the study (EXPLORER)(Tanaka, 2020). The RTX group experienced quantitatively higher rates of neutropenia, leukopenia, herpes zoster, opportunistic infections, hypotension, fever, skin rash, and other adverse events (AEs) connected to the IV infusion (Rovin et al., 2012).

Obinutuzumab:

It will not be wrong to describe Obinutuzumab as the new generation RTX with an optimism in the horizon that it will be successful. The phase III Obinutuzumab trial has a good chance of being successful. Obinutuzumab was successful in a phase II lupus nephritis trial when added once more to standard of care treatment. It's sought that one of the aspects of the failure of RTX was inefficiency to completely remove the B cells. Contrary, Obinutuzumab is designed to do this job (Aringer et al., 2022).

4.5.1.2 Drugs Targeting the Interferon Type I Pathway:

As mentioned before, Blood interferon (IFN) concentrations rise in SLE patients who have active disease, and elevated IFN signature gene expression is linked to critical

organ diseases like nephritis and lesions of the central nervous system (Tanaka, 2020). The so-called IFNAR (type I IFN- α/β receptor) mediates the cell signaling of all type I IFNs, including for example IFN α , IFN β , and IFN ω , leading to IFN-stimulated gene transcription, also known as IFN gene signature (Samotij & Reich, 2019). IFN encourages the development of DC, T cell activation, and B cell production of autoantibodies. Anti-dsDNA antibodies, complement components, and IL-10 levels all correlated with increased levels of IFN α and IFN-dependent cytokines, as well as IFN-regulated gene expression (Samotij & Reich, 2019).

With all the above mentioned, Multiple IFN-dependent signaling pathway polymorphisms have been also linked to increased susceptibility to SLE (Farivar & Shaabanpour Aghamaleki, 2018).

Sifalimumab:

Sifalimumab is a monoclonal IgG1 κ fully humanized antibody that can bind to and incapacitate the majority of the 13 known IFN α subtypes (Petri et al., 2013).

Efficacy: 431 patients with active SLE participated in multicenter phase II trials of Sifalimumab, with the percentage of patients achieving an SRI (4) response at the end of the 52-week period serving as the trial's primary end point. SRI (4) scores at week 52 revealed improvements compared to the placebo group in the Sifalimumab dosage groups of 200 mg, 600 mg, and 1200 mg. Additionally, it was discovered that Sifalimumab improved skin scores and significantly decreased the number of swollen and painful joints. Sifalimumab's clinical effectiveness was shown by improvements in the organ-specific outcomes, such as mucocutaneous, musculoskeletal, renal, haematological, and vascular manifestations of SLE (Sim, Ong, Mak, & Tay, 2022).

Safety: Adverse event rates were similar among groups and were mostly mild. No relationship was apparent between Sifalimumab dose and severity or frequency of adverse events (Sim et al., 2022).

Anifrolumab:

Anifrolumab is also a fully human monoclonal antibody (IgG1 κ) that has the capacity to bind to IFNAR, which enables it to prevent the assembly of the IFN-IFNAR

complex and downstream gene transcription. Anifrolumab antagonizes the receptor in charge of cellular signaling induced by all types of type I IFNs, in contrast to rontalizumab and sifalimumab, which were created to bind and neutralize IFN α (Khamashta et al., 2016) (Sim et al., 2022).

Efficacy: The effectiveness of Anifrolumab in the treatment of SLE was assessed in phase II trials. A cohort of 305 SLE patients with moderate to severe disease were randomized to receive IV Anifrolumab (300 mg or 1000 mg) or a placebo every four weeks for a total of 48 weeks in the Phase lib, double-blind MUSE trial. The subjects were divided into groups based on the SLEDAI-2K of disease activity, their oral corticosteroid dosage, and either a high or low IFN signature based on gene expression. The percentage of patients who had an SRI (4) response at week 24 and a sustained reduction in oral corticosteroids was the trial's primary endpoint. In comparison to the placebo group, a greater percentage of subjects in the treatment group (34.3% of 99 subjects in the 300 mg group and 28.8% of 104 subjects in the 1000 mg group) met the primary end point. Approximately 75% of trial participants had a high baseline IFN signature, and the IFN-high subgroup showed a larger response. At both 300 mg and 1000 mg, Anifrolumab was found to be more effective in this subgroup when compared to the placebo. Subjects with low baseline IFN signature response rates were comparable to those in the placebo group. Nevertheless, the interpretation of efficacy in this subset analysis might have been limited due to the small sample size of the IFN-low subgroup. By week 52 of the trial, several primary and secondary end points, including SRI (4), BILAG-Based Composite Lupus Assessment (BICLA), modified SRI (6), and BILAG-2004 clinical responses, had been met in the Anifrolumab group. Additionally, Anifrolumab-treated patients were shown to have experienced greater improvements in organ-specific disease measures and outcomes as compared to the placebo group at the end of the 52-week study, with a higher percentage of subjects demonstrating improvements in skin manifestations of SLE and the number of swollen and tender joints. (Sim et al., 2022). This study was followed by several other studies with TULIP-LN being the most recent in 2022. All of these studies showed general promising results with TULIP-2 in particular achieving the primary endpoint in addition to several other secondary endpoints (Jayne et al., 2022).

The TULIP SLE LTE (NCT02794285), a phase III, international, double-blind RCT in moderate to severe SLE subjects who completed TULIP-1 or TULIP-2, is still ongoing and is designed to compare the long-term safety and tolerability of intravenous Anifrolumab to the placebo (Felten, Scher, Sagez, Chasset, & Arnaud, 2019).

Safety: Anifrolumab's safety profile in the TULIP-2 trial was similar to that in the MUSE and TULIP-1 trials. Similar to the MUSE and TULIP-1 trials, Anifrolumab subjects had a 7.2% herpes zoster incidence rate. Pneumonia was the most common serious adverse effect, appearing in three subjects out of 362 in the Anifrolumab group of the TULIP-2 trial (Sim et al., 2022).

4.5.1.3 Drugs Targeting the Other Cytokines and their receptors:

Cytokines are essential for controlling the kind and intensity of immune response. There is mounting evidence that cytokines play a pathogenic role in the SLE-related processes that result in organ symptoms like organ lesions, inflammation, and skin lesions. It appears that the autoimmune processes may not only be influenced by one cytokine but also by the overall altered pattern of these markers. Due to their widespread accessibility, numerous practical and precise methods of measurement, and most importantly, their crucial capacity to mediate local inflammatory processes and tissue injury, many of them have been investigated as potential SLE biomarkers (Stypińska & Paradowska-Gorycka, 2015). T helper (Th)1, Th2, and Th17 cytokines are the typical categories into which cytokines are grouped for their functional effects. B-cell hyperactivity and humoral responses are typically encouraged by excess Th2 cytokines, whereas T cell hyperactivity and inflammation are frequently associated with excess Th1 and Th17 cytokines. IL-6, TNF and IFN are examples of Th-1 Cytokine (Lourenço & La Cava, 2009).

Tocilizumab:

It is a humanized mAb that blocks the binding of Interleukin 6 (IL-6) to membrane-bound and soluble IL-6 receptors by targeting the α -chain of the receptor (Illei et al., 2010). B cells' ability to differentiate into Ig-producing cells and the proliferation and differentiation of T cells can both be triggered by IL-6 alone or in combination with other cytokines. Studies have linked IL-6 to a number of autoimmune diseases, including SLE, RA, juvenile idiopathic arthritis, and Crohn's disease. IL-6 is essential for the host immune responses against pathogens (Lourenço, 2009 #86).

Efficacy: In order to examine the effectiveness and safety of tocilizumab, 16 SLE patients with mild to moderate disease activity were enrolled in an 8-week phase I dosage-escalation study. Disease activity significantly improved, with eight out of the 15 enrolled patients experiencing a decrease in SELENA score and a concurrent, significant drop in anti-dsDNA titers (Sciascia et al., 2018).

Toxicity: One of the patients had to be removed from the study because of dosage-related transient decreases in absolute neutrophil count brought on by tocilizumab treatment (Sciascia et al., 2018). The incidence of adverse events (Aes), mostly dose-dependent neutropenia and mild-to-moderate infections, was comparable to that reported in the rheumatoid arthritis trial (Samotij & Reich, 2019).

4.5.1.4 The JAK inhibitors treatments:

Tyrosine kinases Jaks (Jak1, Jak2, Jak3, and Tyk2) bind to the subunits of cell receptors and mediate the intracellular signaling that is triggered by IFN, numerous interleukins, colony-stimulating factors, and hormones like prolactin, erythropoietin, and growth hormone. The latent transcription factors known as signal transducers and activators of transcription are phosphorylated by activated Jak after receptor ligation (STATs). Then STATs translocate into the nucleus where they control gene transcription in homodimers or heterodimers. Human Jak or STAT mutations cause severe immune dysfunction, highlighting the crucial function of this pathway in the induction and control of immune responses. The Jak-STAT signaling involvement in SLE is established (Apostolidis, Lieberman, Kis-Toth, Crispín, & Tsokos, 2011). Two examples of Jak inhibitors are Tofacitinib and Ruxolitinib. The first targets mainly Jak3 and Jak1. It also targets Jak2 but to a lesser extent. It was found to be effective in treating RA in clinical phase III trials. The second targets only Jak2 and was approved by the FDA but to treat myelofibrosis (Sciascia et al., 2018).

In several experiments on the MRL/lpr mic, treatment with Jak 2 inhibitor led to the improvement of lupus. In mice treated with tofacitinib at 10 weeks of age, Furumoto et al. noted less pronounced LN histopathologic features as well as decreased immune complex deposition, accompanied by a lower level of proteinuria. In the mouse model of established disease, blood urea nitrogen, serum creatinine, and proteinuria all significantly decreased in fourteen-week-old mice (Furumoto et al., 2017). Currently a

study in phase III is evaluating the JAK inhibitor Baricitinib. The study has the number NCT03616964 and the name Brave II (Hannon, McCourt, Lima, Chen, & Bennett, 2021).

4.5.1.5 The T Cell costimulation inhibitors (Abatacept):

Abatacept is a fusion protein consist of the extracellular domain of CTLA-4 and the Fc region of immunoglobulin IgG1. It blocks the co-stimulatory interaction between T and B lymphocytes, preventing co-stimulatory T-cell activation and preventing B-cell response. It binds CD80 and CD86 with higher affinity than CD28 (Sciascia et al., 2018).

Efficacy: Patients with non-life-threatening SLE who had active polyarthritis, discoid lupus erythematosus skin lesions, and serositis (pleuritis and/or pericarditis) were given abatacept in combination with 30 mg/day of prednisone in a randomized, double-blind, placebo-controlled phase lib trial. The prednisone dose was then tapered 1 month into the treatment. The percentage of patients in the abatacept and placebo groups who met a primary or secondary end point after 12 months of treatment did not differ significantly. However, it was found that patients who had polyarthritis as their primary disease manifestation benefited the most from the treatment (Samotij & Reich, 2019).

Toxicity: There were serious AEs noticed in the control group in comparison with the placebo in the above mentioned study (Samotij & Reich, 2019).

4.5.2 Disease-Modifying Antirheumatic drugs (DMARDs):

The EULAR recommends for patients with no, mild and or moderate organ manifestation to be treated with Antimalarial drugs as a first line. If the aim of the goal not achieved, glucocorticoids with a dose less than or equal to 7.5 mg dose is recommended. In case of autoantibody positivity or high disease activity, the EULAR recommends using adjacent treatment (Aringer et al., 2019).

4.5.2.1 Antimalarial Treatments:

The antimalarial drug hydroxychloroquine (HCQ), from which the name of the drug class derives, was first used to treat Plasmodium parasitic infections. Beyond its original use as an antimalarial, HCQ has also been used to treat autoimmune, infectious,

metabolic, and neoplastic disorders. However, a recent review found that systemic lupus erythematosus patients saw the most obvious benefits (SLE). It is one of the most effective treatments for SLE today, HCQ has been shown to have a number of positive effects on both the disease and its associated comorbidities. An affordable, widely accessible, and well-tolerated immunomodulator is HCQ (Dima, Jurcut, Chasset, Felten, & Arnaud, 2022).

Efficacy: The meta-analysis of multiple studies conducted on the use of HCQ showed that, patients with drug level greater of equal to 750 ng/ml are at 58% lower risk of active lupus compared to those who had lower levels of the drug. The analysis collected the mean SELDAI score between 6 studies and it was found that SLEDAI scores in patients with levels under 500 ng/mL are significantly lower (Garg, Unnithan, Hansen, Costedoat-Chalumeau, & Bartels, 2021). Studies have also shown that arthralgia can improve even without having a significant effect on arthritis, that corticosteroid dosage can be reduced while still preventing SLE flares, and that HDL-cholesterol can improve lipid metabolism by lowering total cholesterol and triglycerides while increasing HDL cholesterol (Dima et al., 2022).

4.5.2.2 calcineurin inhibitor (Voclosporin)

Voclosporin is an oral calcineurin inhibitor and powerful immunosuppressive drug. It was created by. Voclosporin received its first approval in the USA on January 22, 2021, for use in conjunction with a background immunosuppressive therapy regimen for adults with active lupus nephritis (Heo, 2021).

Efficacy: In comparison to MMF alone, voclosporin was tested in patients with LN who were also receiving mycophenolate mofetil (MMF). In comparison to the placebo group, patients in the VCS group experienced complete renal responses rates that were both higher and earlier (41% at 52 weeks vs. 23%) (Pappa et al., 2022).

Toxicity: When used in conjunction with standard-of-care therapy, Voclosporin was generally well tolerated. Voclosporin 23.7 mg twice daily (n = 267) was associated with a number of adverse events, the most frequent of which were decreased GFR (26% vs 9%), hypertension (19% vs 9%), diarrhea (19% vs 13%), headache (15% vs 8%), anemia (12% vs 6%), cough (11% vs 2%), and urinary tract infection (10% vs 6%) (Heo, 2021).

Table 2 Recent Treatments of SLE and their Clinical Trials

Drug	Target	Primary Endpoint	Outcome	Name of The Study
Belimumab	Soluble BAFF	SRI-4	Primary endpoint met in Both Studies	BLISS – 52 BLISS – 76
Tabalumab	Soluble and Membrane-bound BAFF	SRI-5	Primary endpoint was not met in the 1 st study but met in the 2 nd	ILUMINATE-1 ILUMINATE-2
Atacicept	BAFF and APRIL	BILAG	Primary endpoint was not met, and study stopped due to serious AE	APRIL-SLE

Drug	Target	Primary Endpoint	Outcome	Name of The Study
Blisibimod	Soluble and Membrane-bound BAFF	SRI-6	Primary endpoint was not met	PEARL-SC
Rituximab	CD20	BILAG	Primary endpoint was not met	EXPLORER
Sifalimumab	IFN α	SRI-4	Primary endpoint was met only in the 2 nd study (studies were halted in favor of Anifrolumab)	TULIP -1 TULIP -2
Anifrolumab	All types of IFN - I	SRI-4	Primary Endpoint was met at week 24 and the drug is approved by FDA in 2021	MUSE

5. Discussion:

5.1 GWAS Limitations and Benefits:

Since the first time it was used, GWAS have been pivotal in the drug discovery field in addition to research to understand SLE. Its cost decreased dramatically and that led to dramatic increase in the number of the studies. Langefeld et al was a turning point as it studied the genome of more than 27,000 SLE patients shedding the light on around 100 loci that are involvement in the pathogenesis of the disease. It became clearer how the HLA region is involved. However, Langefeld et al had a bias towards the European population.

That's a general issue related to GWAS as a study study showed that only 10% or less of the total number of GWAS are including non-European participants (Mills & Rahal, 2020).

Even though geneticists have worked extremely hard to increase the sample sizes in GWASs, it is doubtful that these studies will ultimately be able to account for all of the heritability in SLE due to the incomplete coverage of the GWAS arrays, the possibility of non-additive effects, and the insufficient statistical power.

The requirement to use a high level of significance to account for the multiple tests is a significant drawback of genome-wide approaches. The Bonferroni correction is frequently used in GWAS to keep the genome-wide false-positive rate at 5%, based on the assumption that 1 million independent tests for common genetic variation will be conducted thus (Tam et al., 2019), current guidelines have established extremely stringent thresholds for defining a significant association with disease susceptibility (i.e., $p < 5 \times 10^{-8}$) and, as a result, many genome scans provide a plethora of suggestive associations (SNPs having p value $< 5 \times 10^{-8}$) while detecting few or no associations that reach genome-wide "significant" threshold level (Rai & Wakeland, 2011).

Last, GWAS will always be a very important tool that can be used at least for a single assessment to examine the predisposition of a single individual. That can help in designing T2T strategy by using a preventative treatments like hydroxychloroquine which is believed to delay the onset of the disease as shown in the results section.

5.2 Intersecting in The Definitions between Polymorphisms and Mutations:

An arbitrary number is chosen to represent the polymorphism's 1% or higher frequency. suggested by scientists before the advent of next-generation sequencing. Redefining the population may change the classification because the threshold is arbitrary; depending on the population studied, polymorphisms may become rare variants and vice versa. For many years, developing population models using this frequency was preferred to using the labor- and error-intensive sequencing tools that were available at the time. A very different picture of population dynamics has started to emerge with the introduction of new sequencing technologies and the subsequent sequencing of individuals. It has been discovered that mutations that were previously believed to be uncommon in a population exceed the 1% frequency threshold. Even more unexpectedly, not all of these rare mutations have been linked to human diseases. A disease-causing mutation in one population is discovered to be harmless in another when populations separated by physical and geographic barriers are compared (Karki et al., 2015).

5.3 The Hardship in Designing a Clinical Trial for SLE Treatments:

As shown in the results section, most of the SLE treatments showed modest to disappointing results. This might be due to hardship in designing a clinical trial to evaluate a treatment efficacy. It is unethical to stop a patient from taking a treatment to a deadly disease. This will always lead to the imperfect control group which will often lead to inability to find statistical significance between the placebo and the treatment groups as both are already receiving treatment. For instance, Glucocorticoid use can boost the placebo group's response rate and thus affect trial outcomes. As a result, GC dosage and exposure should be restricted. To reduce introduced bias, the use of Glucocorticoids should be modified in relation to disease activity, and the dosage of Glucocorticoid should be balanced between arms.

The endpoint is crucial for the success of clinical trials. The discussion of a thorough disease activity index follows. Due to the positive results of belimumab clinical trials, SRI has been utilized most frequently recently. However, because SRI is primarily based on SLEDAI, it readily exposes SLEDAI's flaws. The biggest flaw in SLEDAI is that it can only evaluate each symptom or item in binary terms and cannot assess improvement. As stated above, the SELENA-SLEDAI mean % change from baseline was

significant at most points, but the rate of SRI4 responders between the belimumab group and placebo group was not significantly different at week 76 of the BLISS76 trial. As a result, continuous evaluation might be preferable to binary evaluation (Ohmura, 2021). Designing multiple studies using both SELADAI and BILAG could also be a solution.

5.4 The failure of Rituximab

The main reason behind the failure of RTX could be due to the clinical design of the study. The score system was used in study EXPLORE to assess the response of the disease was BILAG (Sciascia et al., 2018). Since BILAG is not a numeric system, some difficulties could stand in the way of constructing a statistical significance. Also, the efficacy of the immunosuppressants and the steroids taken by both the placebo and the treatment group could be underestimated. The sub analysis of EXPLORE showed that the difference between the placebo and treatment groups was significant for the Hispanic and the African sub-groups (Wise & Stohl, 2020) a group known to show resistance to steroids (Zhang, Levin, & Williams, 2019), which could indicate that RTX could be effective if the study was redesigned. A recommendation to repeat the studies on Rituximab using SRI-4 scoring system. Since RTX is licensed and sold in the market, it could be recommended for physicians to prescribe it after an individual assessment to the patients with severe renal manifestations as the drug shown promising result with renal cases.

5.5 The Future of the SLE Treatments

Despite the disappointing results of many of the treatments, there are indications for a slightly better near future. In July 2021 Anifrolumab was given the approval by the FDA to treat adults with severe or moderate SLE (Deeks, 2021). In January 2021, Voclosporin was also approved by the FDA as an immunosuppressant treatment for SLE (Heo, 2021). This, in one year, amounts to how many treatments were approved in the preceding fifty years.

Obinutuzumab is the next lupus nephritis medication that is most likely to be given the green light by the FDA. Obinutuzumab was successful in a phase II lupus nephritis trial when it was once more added to standard of care therapy. Rituximab, the organ-threatening, refractory SLE treatment standard for more than 20 years, has never been able to demonstrate such benefit in randomized controlled trials (Might be due to issues with the clinical trial designs). The effectiveness of rituximab may also be constrained by

relatively ineffective B cell depletion in this condition, and Obinutuzumab is created for more thorough tissue B cell removal (Aringer et al., 2022).

6. Conclusion and outlooks:

Despite many disappointing results from the studies of most of the biologic treatments, further sub-analysis needs to be done to detect the weakness points. Researchers should redesign their studies and direct it more towards specific manifestations rather than fighting the disease systematically. For example, Rituximab failed to meet the primary and the secondary endpoint of EXPLORER but showed a very promising results in fighting the immunological markers such as the concentration of anti-dsDNA antibodies and levels of C3/C4 complement component. The medicine is already in the market and should be used as an extra weapon at the hands of the physician in the process of developing a T2T strategy to fight such a complicated and heterogenous disease. In addition to that, bolder strategies should be considered, such as resetting the whole immunity by completely removing the memory B cells in order to stop producing the autoantibodies and considering repeating all the vaccination. Traditional drugs like hydroxychloroquine should be continued to be given especially to the genetically susceptible people in order to delay the disease prognosis. It is true that big advances happened in the field of fighting SLE, however, a lot of work needs to be done in the field of early diagnosis and monitoring the disease activity. In conclusion, the outlook for SLE is promising and a handful novel weapons and techniques are to be expected soon.

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Appendix:**Key words**

- Systemic lupus treatment classification
- SLE guidelines
- Systemic lupus guidelines
- BAFF role in SLE.
- B cell pathogenesis in SLE.
- B cell activation factor in SLE.
- Prevalence of SLE in the USA
- Prevalence of SLE in the Europe.
- Age-associating B cells SLE.
- SLE mortality rate
- SLE morbidity rate
- Systemic Lupus mortality rate.
- systemic lupus cd20
- Lupus siblings ratio
- SLE gene polymorphism
- rs2431697.
- environmental causes sle.
- Silica and SLE.
- RTX
- Belimumab
- Seldai
- SRI
- GWAS SLE
- Hydroxychloroquine SLE
- Baricitinib
- Abatacept
- Jak Inhibitors
- IL-6 targeting drugs
- Anifrolumab
- Sifalimumab
- INF alpha pathway
- Blisibimod

- EULAR regulations
- EULAR Classification
- Anti dsDNA.