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REVIEW

Therapeutic peptides for the treatment of systemic lupus erythematosus: a place in therapy

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ABSTRACT

Introduction: Studies *in vitro* and *in vivo* have identified several peptides that are potentially useful in treating systemic lupus erythematosus (SLE). The rationale for their use lies in the cost-effective production, high potency, target selectivity, low toxicity, and a peculiar mechanism of action that is mainly based on the induction of immune tolerance. Three therapeutic peptides have entered clinical development, but they have yielded disappointing results. However, some subsets of patients, such as those with the positivity of anti-dsDNA antibodies, appear more likely to respond to these medications.
Areas covered: This review evaluates the potential use of therapeutic peptides for SLE and gives an opinion on how they may offer advantages for SLE treatment.
Expert opinion: Given their acceptable safety profile, therapeutic peptides could be added to agents traditionally used to treat SLE and this may offer a synergistic and drug-sparing effect, especially in selected patient populations. Moreover, they could temporarily be utilized to manage SLE flares, or be administered as a vaccine in subjects at risk. Efforts to ameliorate bioavailability, increase the half-life and prevent immunogenicity are ongoing. The formulation of hybrid compounds, like peptibodies or peptidomimetic small molecules, is expected to yield renewed treatments with a better pharmacologic profile and increased efficacy.

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1. Introduction

Systemic lupus erythematosus (SLE) is the prototypical autoimmune connective tissue disease, affecting 5 million individuals worldwide, mainly women during the fertile age [1]. Clinical presentation broadly varies from patient to patient, with kidney and central nervous system (CNS) involvement representing the most severe complications [2]. The disease has usually an unpredictable course with phases of activity followed by phases of remission, and patients with a low disease activity can experience during their life disease flares following triggering events, like infections.

SLE is characterised by a multifactorial pathogenesis, in which the combination of a favourable genetics and the intervention of external agents may induce the chronic activation of the innate (neutrophils, macrophages, complement system) and the adaptive (T and B lymphocytes, plasma cells, autoantibodies) immune system. The most salient events include an impaired apoptosis of dying cells, a type I interferon (IFN) signature, the uncontrolled activation of T and B lymphocytes and the production of autoantibodies mainly directed against nucleic acids or ribonucleoproteins (RNP), *Figure 1* [3–7].

Contrary to other autoimmune diseases, such as rheumatoid arthritis (RA) or spondyloarthritis, whose prognosis has noteworthy been improved by the advent of biologic agents and small molecules, the treatment of SLE still relies on the combination of traditional and symptomatic drugs and usually shows less successful results, *Figure 2* [8]. Several immunologic pathways are, in fact, concomitantly activated in SLE, and this justifies the use of medications like steroids, immunosuppressants and disease-modifying anti-rheumatic drugs (DMARDs), which unselectively counteract the immune response. Such a combo-therapy can indeed have many summing side effects that can be further exacerbated by SLE-related organ failure, coagulopathy, or cytopenia. In addition, some drugs, like anti-epileptic agents, can induce lupus-like skin manifestations or the production of antinuclear antibodies (ANA), and worsen the cutaneous or serologic manifestations of SLE [9]. To date, the use of biological agents, having higher specificity for a molecular target, is limited to belimumab, a human monoclonal antibody that neutralizes B-lymphocyte stimulator (BLyS) and inhibits B cell survival and function [10]. Despite the failure of previous randomized controlled trials (RCTs) [11–14], the results of more recent studies testing novel classes of biologic agents in SLE appear overall positive [15–17]. Similar encouraging data have been reported in clinical trials with the use of the small molecules Janus kinases (JAK)-inhibitors [18,19]. The advancement in the knowledge of SLE pathogenesis will indeed provide novel potential targets to be specifically addressed by medications. The latter may include drugs neutralizing or interfering with the proteasome of B cells, complement fragments,

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Article highlights

- Unlike other rheumatic diseases, the therapeutic armamentarium for SLE has been poorly impacted by the advent of biological agents and small molecules; hence, treatment mainly relies on the combination of traditional approaches which includes corticosteroids, antimalarials, disease-modifying anti-rheumatic drugs and immunosuppressive agents.
- The potential use of therapeutic peptides in SLE is justified by their cost-effective production, target selectivity, low rate of adverse events, and an overall immunomodulatory effect.
- Although no therapeutic peptide has been licensed for SLE treatment, the 21-mer peptide P140, the CDR1-based peptide and AMG623 have entered phase II or III clinical trials; they show a good safety profile but have mostly failed to achieve the primary endpoints despite positive results observed in some subsets of SLE patients.
- Preliminary findings regarding other peptides (pConsensus, laminin-derived peptide, nucleosomal peptides, DWEYS peptide, glatiramer acetate, and thymopentin-5) are encouraging; they show amelioration of glomerulonephritis, reduction in autoantibody titres, prevention of neuronal damage and an overall prolonged survival of SLE animal models.
- A better understanding of SLE pathogenesis and the improvement in biotechnologies should increase the number of peptides that can specifically neutralize a pathogenic pathway thus allowing a more personalized treatment.
- Because of their acceptable safety profile, therapeutic peptides could be added to standard of care of specific subsets of SLE patients potentially resulting in a sparing effect on immunosuppressants and glucocorticoids.

This box summarizes the key points contained in the article.

toll-like receptors (TLR), or metabolic pathways [20]. Target selectivity should result in a better efficacy profile and in a low risk of widespread side effects.

In this light, the formulation of human-derived or synthetic peptides, able to prevent specific steps of the immunologic cascade occurring in SLE, appears a fascinating alternative way to address this complicated disease. Therapeutic peptides consist of short amino acid chains [21] synthesized on the basis of a known human genetic sequence and designed in order to mimic the functional portion of native endogenous proteins or specific epitopes. This class of medications shows many favorable aspects, like the high target selectivity, the cost-effectiveness, the easily manufacturing, and an acceptable safety profile [22].

Thanks to genetic engineering and proteomics, it has been possible to build libraries containing a large collection of human peptides, all potentially screenable for the use in disease. In SLE, attention focuses on disease-specific T or B immunogenic amino acid fragments or epitopes. Epitopes, identified by epitope mapping, play a crucial role in the activation of the adaptive immune system by binding the T cell receptor (TCR) of T cells, the major histocompatibility complex (MHC) cleft of antigen-presenting cells (APC) or the combining site of autoantibodies [23]. Therapeutic peptides mimicking self-epitopes may modulate the immune response, counteracting the expansion of autoreactive cell clones.

The aim of this review is to report the evidence concerning the rationale, the efficacy, and the safety of therapeutic peptides developed or under development for SLE, and to discuss the future place in therapy of these innovative drugs.

2. Therapeutic peptides

2.1. Therapeutic peptides in SLE: what is good and what is bad?

Therapeutic peptides include a class of pharmaceutical compounds consisting of amino acid chains of various length (usually less than 40 amino acids) [21], isolated from natural sources, or artificially synthesized [24]. Though having its roots in the past, the panorama of therapeutic peptides appears highly dynamic and continuously evolving. In more recent times, therapeutic peptides are easily obtained by means of recombinant DNA technology (like microbial fermentation) and more than 100 therapeutic peptides, having various clinical indications, have already been approved worldwide [25]. The rationale for using therapeutic peptides in SLE lies in the cost-effective production, high potency, target selectivity, low toxicity, and a peculiar mechanism of action mainly based on the induction of immune tolerance [26]. These compounds share, in fact, some characteristics with both small molecules and biologic agents. Like small molecules, they can be chemically produced in the laboratory; however, contrary to them, are usually parenterally administered and show a negligible risk of drug–drug interaction and of off-target binding [21]. On the other hand, peptides share with biologic agents the high selectivity and the route of delivery, but have a more rapid clearance and a lower risk of tissue accumulation [22,27]. Globally, these features confer to pure peptides a unique pharmacologic profile, characterized by a high affinity for the target and a good safety profile, but, at the same time, a high metabolic instability, a poor membrane permeability, a scarce oral bioavailability and an accelerated biodegradation. These issues have partly been solved through drug engineering. Consequently, according to chemical properties, therapeutic peptides can be subdivided into native, analog, and heterologous peptides [24]. While unmodified native peptides, isolated from tissues or artificially synthesized, are used as replacing therapy in deficient patients affected by genetic or metabolic diseases, analogs, which consist of modified native peptides, and heterologous peptides, derived from synthetic library screening or phage display techniques, have also found a place in the experimental and clinical treatment of autoimmune diseases [24]. In many cases, peptides paved the way for the formulation of peptidomimetic oral small molecules, able to interact with peptide receptors [28]. In addition, peptides can be conjugated to the fragment crystallizable (Fc) of human immunoglobulins (Ig), cytotoxic payloads and polyethylene glycol (PEG), and acquire a better pharmacokinetic and pharmacodynamic profile [29].

Given the high specificity for their target and the low toxicity, therapeutic peptides would ideally represent the therapy of choice in SLE patients. Therapeutic peptides may, in fact, selectively counteract several steps of the immunological cascade aberrantly activated in SLE, meanwhile preserving the normal biologic functions of the immune cells. Most therapeutic peptides designed for SLE treatment are

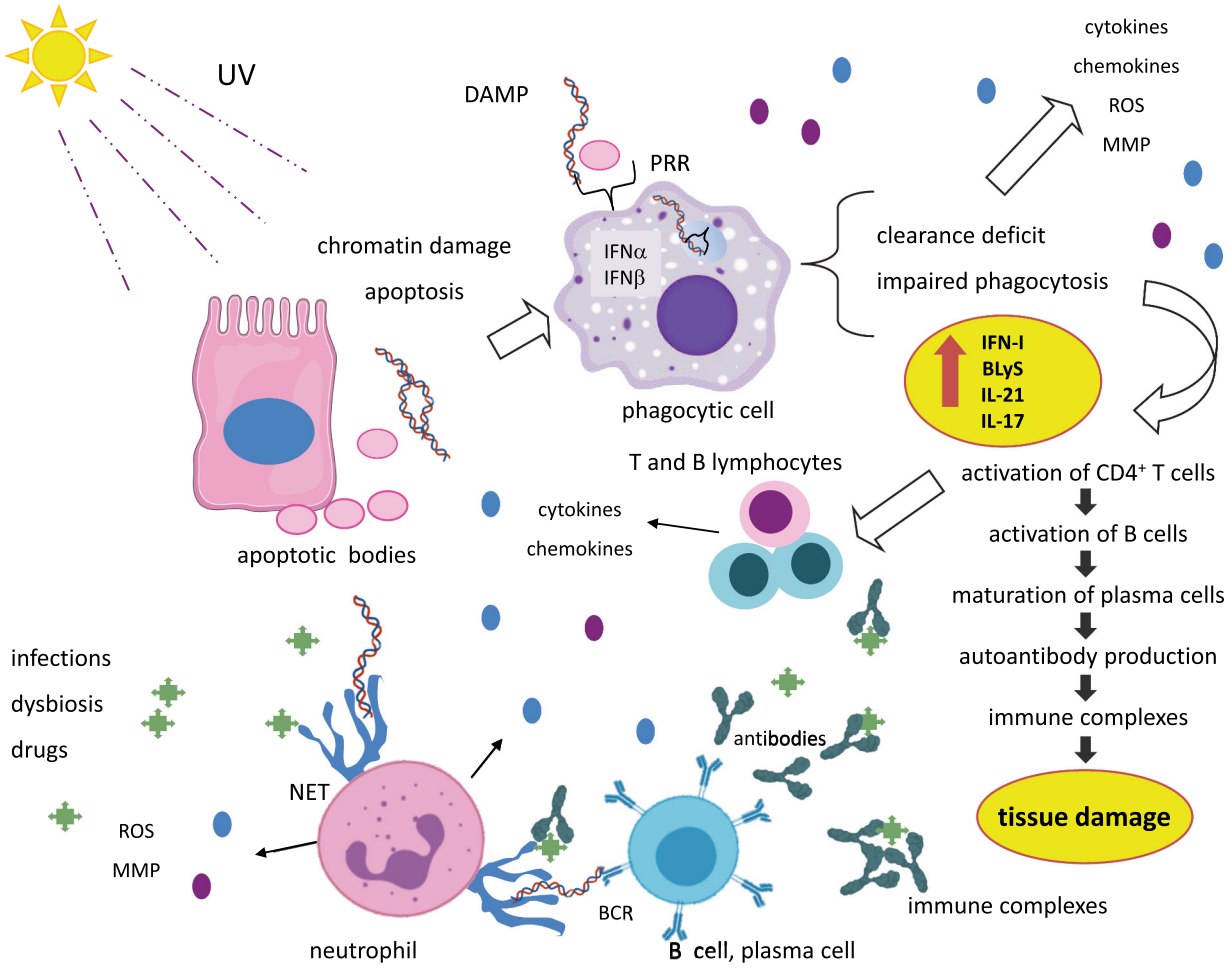

Figure 1. Overview of SLE pathogenesis.

In a genetically predisposed individual, environmental factors, including ultraviolet radiations, viral infections, or certain drugs, may induce cell degeneration and death, with the following release of ribonuclear debris. Impairment in cell apoptosis and debris clearance may activate phagocytic cells, like pDC and neutrophils. The subsequent steps include the generation of a type I IFN response in pDC, the release of cytokines, chemokines, ROS, and MMP and the recruitment of autoreactive T and B lymphocytes. This cascade finally leads to the maturation of plasma cells, the synthesis of autoantibodies, and the formation of immune complexes. By engaging with the Fc receptors of pDC, immune complexes may further stimulate type I IFN response. Alternatively, both membrane and secreted antibodies can recognize nucleic acids and ribonucleoproteins expressed in the neutrophil extracellular traps, fomenting NETosis, or prompting B lymphocytes to autoimmunity. Abbreviations: UV, ultraviolet rays; PRR, pattern recognition receptor; ROS, reactive oxygen species; MMP, matrix metalloproteinases; IFN-I, type I interferon; BLyS, B lymphocyte stimulator; IL, interleukin; CD, cluster of differentiation; NET, neutrophil extracellular trap; BCR, B cell receptor; DAMP, damage-associated molecular pattern.

synthesized on the basis of SLE-specific immunodominant epitope sequences. When given at high concentrations in already sick animals, these peptides exert an immunomodulatory effect, which is explicated through the direct interaction with MHC molecules, B cell receptors and TCR, leading to the polarization of plasmacytoid dendritic cells (pDC) or lymphocytes toward a tolerant phenotype, Figure 3. In addition, they can induce tolerance spreading, a phenomenon amplifying the tolerance of the immune cells to other structurally related peptides. Being designed on the basis of epitopes that are pathogenic in SLE alone, peptides should not affect the normal immune response against pathogens, but, on the other hand, they may display less efficacy in the quite common cases of overlapping clinical syndromes (like anti-phospholipid syndrome, APS). Furthermore, thanks to the recent advancements in proteomics and genomics, it is expected that this kind of therapy will be tailored on specific groups of patients who could have the highest beneficial effects. However, some uncertainties still exist on bioavailability, best route of administration, doses, and targets [30]. Modifications in their chemical structure may ameliorate the physicochemical profile and influence their biological activity, strengthening also the immunomodulatory function. For instance, the conjugation to albumin or the introduction of D-amino acids may increase half-life and stability, by reducing glomerular clearance and proteolysis, respectively. Nevertheless, some of these modifications may paradoxically result disadvantageous to therapeutic peptides designed for SLE patients. An active glomerulonephritis may accelerate the elimination of analog peptides conjugated to albumin, thus shortening their half-life. Similarly, the addition of D-amino acids in order to prevent proteolysis can stress immunogenicity, which is usually more pronounced in autoimmune diseases, due to the hyper-activation of the immune system. The conjugation of peptides to the Fc domain in the attempt to increase molecular stability may, instead, stimulate the immune cells bearing the Fc receptors (FcR) and accelerate the clearance of these drugs.

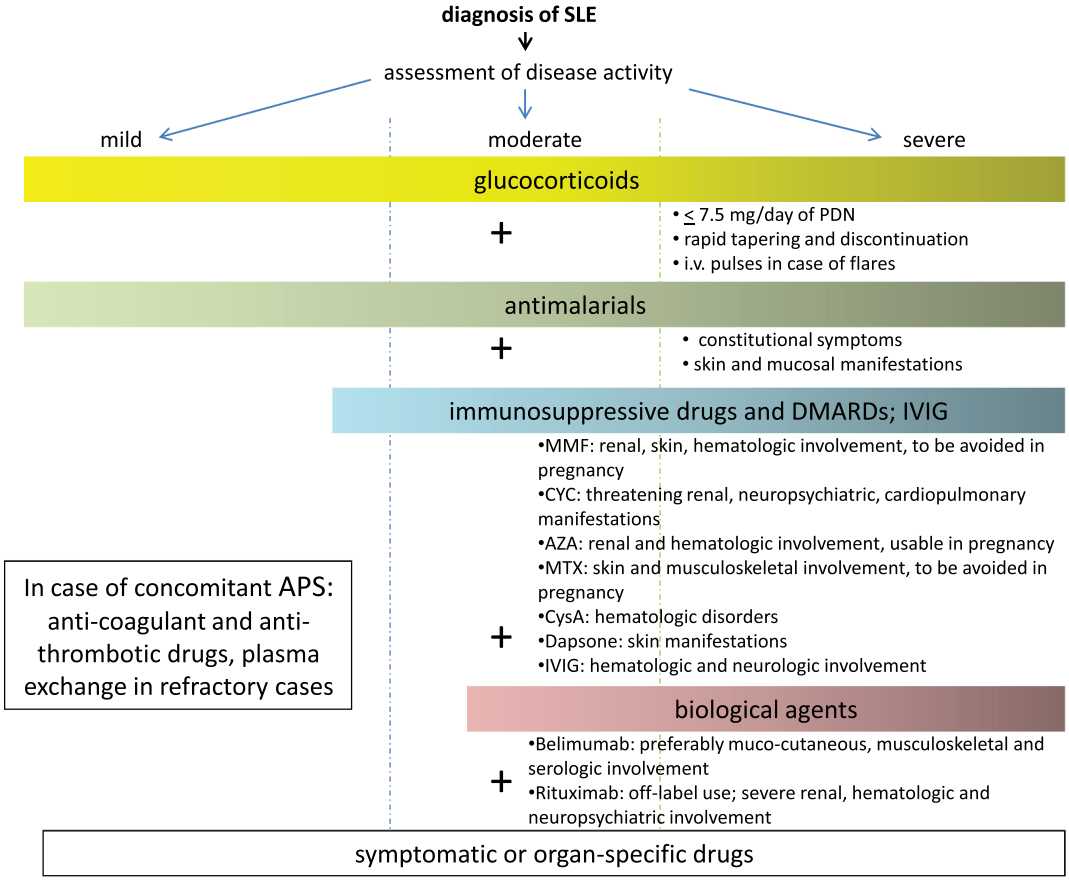

Figure 2. Current therapeutic algorithm for SLE patients according to the most recent European guidelines.

Following the diagnosis of SLE, patients are assessed for disease activity and organ involvement, both of which dictate the most appropriate therapy. SLE patients with a mild involvement can be easily managed with a low dose of oral steroids (to be discontinued as soon as possible), hydroxychloroquine, and symptomatic drugs. Moderate to severe manifestations usually require the addition of DMARDs and immunosuppressants, like methotrexate, mofetil mycophenolate, cyclophosphamide and azathioprine, or the administration of intravenous immunoglobulins. The use of biologic agents (belimumab or rituximab) is indicated in refractory forms of disease. SLE flares are treated with intravenous steroid pulses and prevented with long-term low doses of oral glucocorticoids. In addition, symptomatic or organ-specific drugs, like analgesics, antihypertensive or antiepileptic agents, are often co-prescribed. In case of a concomitant APS diagnosis, anti-thrombotic and anti-coagulant drugs, and, in severe forms, plasma exchange, may also be needed. Darker color in the bars indicates higher doses or a more common use of the referred medications. Abbreviations: SLE, systemic lupus erythematosus; DMARDs, disease-modifying anti-rheumatic drugs; PDN, prednisone; i.v., intravenous; APS, anti-phospholipid syndrome; MMF, mofetil mycophenolate; CYC, cyclophosphamide; AZA, azathioprine; MTX, methotrexate; CysA, cyclosporin A; IVIG, intravenous immunoglobulins.

To date, no therapeutic peptide has been licensed and marketed for the use in SLE patients, although some of them have entered the phase II or III of drug development.

The next paragraphs report and discuss the current evidence concerning unconjugated and conjugated therapeutic peptides under preclinical and clinical investigation, and potential novel candidates for SLE treatment. Results and targets are resumed and illustrated in Table1, Table2 and Figure 4.

2.2. Therapeutic peptides under development for SLE

2.2.1. 21-mer peptide P140

SLE patients and animal models are characterized by the production of autoantibodies reacting against epitopes of the spliceosome. The spliceosome is an intranuclear platform involved in RNA processing and is formed by several RNP [31]. Some of them, like U1RNP and Sm, contain epitopes that can be electively recognized by autoreactive T and B cells [32]. It has been estimated that about 5–30% of SLE patients have a seropositivity of anti-Sm antibodies that is associated to a more severe kidney involvement, while 25–47% of SLE patients are positive for serum anti-RNP antibodies, being associated with other overlapping autoimmune syndromes and a minor risk of glomerulonephritis [33]. Researchers argued that these spliceosomal epitopes may chronically stimulate B and T autoreactive cells, which would eventually recognize other structurally related or unrelated spliceosomal epitopes in a process known as intramolecular and intermolecular epitope spreading [32]. Based on this hypothesis, an amino acid sequence lying inside the RNA-binding site U1RNP was chosen from a collection of epitopes recognized by the TCR of cluster of differentiation (CD)4^+ T cells and IgG of murine SLE models. This peptide, known as the 21-mer peptide P140, includes the amino acid residues 131–151 of the spliceosomal U1-70K small nuclear RNP, and also interacts with T cells and autoantibodies of SLE patients [34]. By introducing a phosphorylation of the serine in position 140, the peptide acquires the property of selectively stimulating $CD4^+$ T regulatory (Treg) cells of SLE patients and restoring tolerance [34]. The mechanism of action of the 21-mer peptide P140 involves the prevention of T helper (Th)2 cell activation following antigen presentation by APC and the downstream expansion of B cells, while it up-regulates the Treg response [35]. These effects may be explicated through the antagonism or the partial agonism at the TCR of autoreactive cells or through the full agonism at that of Treg lymphocytes [36]. By

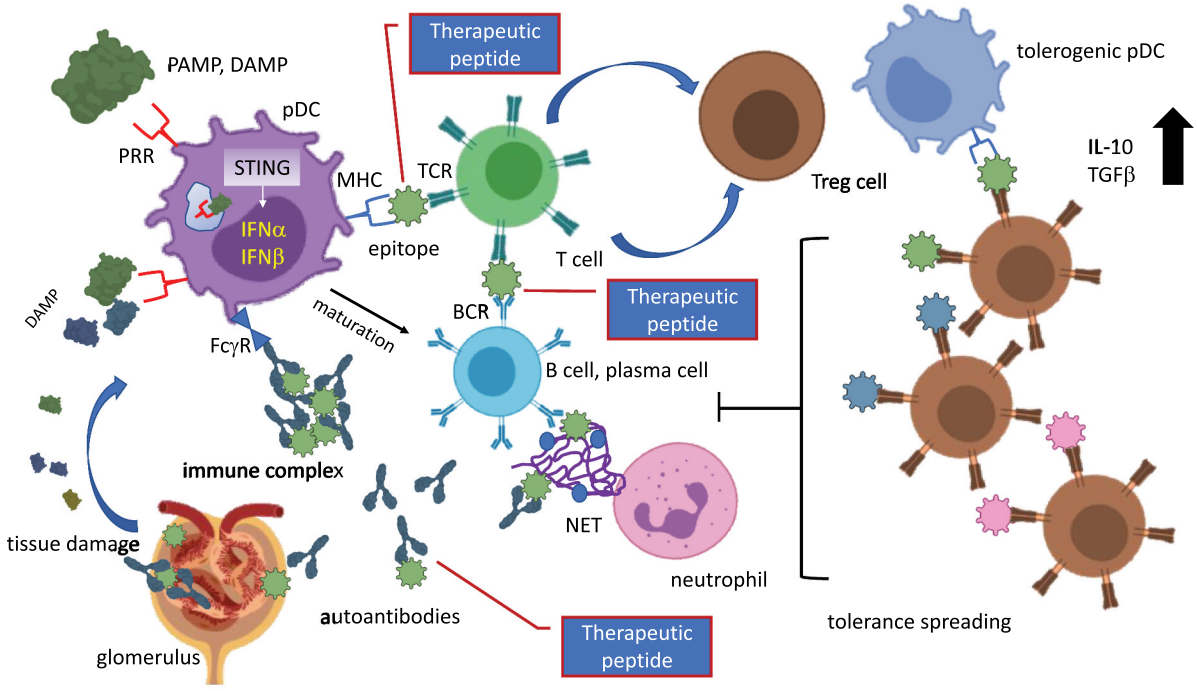

Figure 3. The immunomodulatory role of therapeutic peptides in SLE.

The majority of therapeutic peptides designed for SLE treatment are synthesized on the basis of immunodominant T and B epitopes, which are chemically modified in order to acquire tolerogenic properties (tolerogenic epitopes). Immune tolerance is restored after the binding of peptides to the combinatory site of MHC molecules, BCR and TCR of autoreactive cells, with the subsequent inhibition of effector T and B lymphocytes and the expansion of Treg cells. Therapeutic peptides can also interact with the combinatory site of autoantibodies and prevent the recognition of self-epitopes. In addition, they impart a reverse tolerogenic signal to pDC, and indirectly counteract the secretion of autoantibodies, the formation of immune complexes, NETosis, type I IFN production and final tissue damage. As the latter is responsible for the release of DAMPs that further stimulate pDC, therapeutic peptides may definitely interrupt this circuit. Abbreviations: PAMP, pathogen-associated molecular pattern; DAMP, damage-associated molecular pattern; PRR, pattern recognition receptor; pDC, plasmacytoid dendritic cell; STING, stimulator of interferon genes; IFN, interferon; FcγR, fragment crystallizable gamma receptor; MHC, major histocompatibility complex; TCR, T cell receptor; BCR, B cell receptor; NET, neutrophil extracellular trap; Treg, T regulatory; IL-10, interleukin-10; TGFβ, transforming growth factor-beta instead of factor beta.

selectively contrasting the recognition of ribonuclear autoantigens, this peptide does not prevent the normal immune response toward pathogens and its mechanism is solely active in SLE. In addition, it can bind heat shock 70 kDa protein 8 (HSPA8) and interfere with the process of autophagy, which represents a crucial pathway in receptor and other cellular component recycling, such as MHC class II, on the cell surface [37,38]. Another mechanism of action includes the killing of autoreactive T lymphocytes by means of γδT cells. Experiments in treated mice evidenced a prolonged life expectancy, the amelioration of glomerulonephritis, and a reduction in autoantibody titers [39].

Following these enthusiastic preliminary data, a number of phase II and phase III RCTs tested the efficacy and safety profile of the 21-mer peptide P140 in active SLE patients.

Among them, the 57-day open-label dose-escalation early phase II trial was conducted in a small cohort of 20 Bulgarian SLE patients and showed encouraging serologic and clinical findings [36]. The 21-mer peptide P140 was parentally administered at a dose of 200 mcg or 1,000 mcg every 2 weeks for a total of 3 subcutaneous (s.c.) injections. An anti-double stranded (ds) DNA antibody titer >50 IU/mL at screening was a prerequisite to entry the trial and patients on treatment with mycophenolate mofetil (MMF) or cyclophosphamide (CYC) were excluded. While the rate of adverse events was slightly increased in the group receiving the highest dose, the anti-dsDNA antibody titer significantly decreased from baseline in the group of subjects assigned to the 200 mcg dose, with a significant change detectable even since the first injection. The reduction in autoantibody titers was paralleled by significant improvements in clinical domains measured through the SLE Disease Activity Index (SLEDAI) and physician's global assessment (PGA). Remarkably, other immunologic parameters, including the titers of anti-U1RNP, anti-SmD1, anti-Ro-SSA, anti-La-SSB, and anti-cardiolipin antibodies, as well as the serum levels of the pro-inflammatory cytokines interleukin (IL)-2 and of tumor necrosis factor (TNF)α, remained unchanged. These results may be justified by the small cohort and the short period of exposure to active treatment.

Two-phase IIb studies with similar standard protocols were independently conducted in SLE patients randomized to receive 200 mcg of the 21-mer peptide P140 conditioned in 5.4% mannitol every 2 or 4 weeks or placebo, and sponsored by Immu-Pharma and Teva Pharmaceutical Industries (Cephalon) ([40] and ClinicalTrial.gov ID NCT01135459, respectively). Inclusion criteria were largely overlapping: they comprised an active disease (SLEDAI-2K ≥ 6); ANA or anti-dsDNA positivity; the allowance of the concomitant use of oral glucocorticoids (not exceeding the weekly cumulative dose of

Table 1. List of the therapeutic peptides investigated or currently under investigation for SLE and main results from studies.

			Results from studies		
Peptide	Molecular structure	Mechanism of action	Preclinical studies	Clinical trials	Reference
21-mer peptide P140	21-mer peptide (131e151) of the U1-70 K small nuclear ribonucleoprotein (snRNP) containing a phosphoserine residual at position 140	• Antagonism or partial agonism at the TCR of autoreactive cells or full agonism at the TCR of Treg lymphocytes • Interference with HSPA8-mediated autophagy and prevention of autoantigen processing and MHC-mediated presentation in APC • Killing of autoreactive T cells through γδT lymphocytes • Expansion of Treg lymphocytes at the detriment of autoreactive Th2 lymphocytes • Prevention of B cell activation • Intramolecular and intermolecular tolerance spreading	*Animal models* - Reduction in autoantibody titers, amelioration of glomerulonephritis, prolonged survival *In vitro and ex vivo assays* - Increased secretion of IL-10 by PBMCs of SLE patients - Polarization of $CD4^+$ T lymphocytes toward a tolerogenic phenotype	*Phase II RCTs* - Early phase IIb RCT: Improvement in SLEDAI and PGA scores at 43 and 57 days from baseline and reduction in anti-dsDNA antibody titers in patients receiving 200 mcg of the 21-mer peptide P140 every other week; no safety issues - Phase IIb RCTs: Achievement of SRI response at 12 weeks in patients receiving the 21-mer peptide P140 at a dose of 200 mcg every 4 weeks; endpoint not met with the dose of 200 mcg every 2 weeks (ITT analysis); lack of efficacy when 10% trehalose is used as excipient instead of mannitol (potentiation of autophagy) *Phase III RCTs* No significant difference in the SRI response rate at week 52 in patients assigned to the 21-mer peptide P140 at a dose of 200 mcg every 4 weeks compared to those taking PBO; better response in anti-dsDNA antibody positive patients; no safety issues	[32,34–41,43]
hCDR1 (*Edratide*)	Heavy chain CDR1 of a human monoclonal anti-DNA antibody bearing the idiotype 16/6	• Reduction of pro-inflammatory cytokines (IL-1β, IL-2, IFNα, IFNγ, TNFα) and up-regulation of TGFβ • Reduced expression of LFA-1 and CD44 • Inhibition of the NF-kB pathway • Prevention of apoptosis • Prevention of the expression of adhesion and costimulatory molecules on APC • Normalization of the BLyS and IFNγ pathways	*Animal models* - Amelioration of serological, renal and CNS manifestations -Independent and additional immunomodulatory effect played by the peptide compared to dexamethasone and CYC *In vitro and ex vivo assays* - Down-regulation of pro-inflammatory cytokines and increased polarization into Treg cells of SLE PBMCs - Reduced gene expression of IFNα in PBMCs of SLE patients but not in those of primitive APS patients or controls	*Phase II RCTs* Primary endpoints (significant difference in the SLEDAI-2K and AMS scores) not met at last office visit until week 26; significant amelioration of the BILAG scores with *Edratide* 0.5 mg/week; amelioration of BILAG scores in anti-dsDNA antibody positive patients receiving 0.5 mg/week of *Edratide* and low (< 20 mg/day) or no doses of prednisolone at baseline; lower occurrence of BILAG flares in the group assigned to *Edratide* 0.5 mg/week compared to PBO; reduced expression of IL-1β, TNFα, IL-10, IFNγ, BLyS, caspase 3 and caspase 8 transcripts and increased expression of TGFβ and FoxP3 transcripts in PBMCs of patients treated with *Edratide* compared to PBO; no safety issues	[49–57,60–62]
pConsensus	15-mer peptide derived from the VH region of murine anti-DNA immunoglobulins	• Cytokine modulation with augmented levels of TGFβ • Increase in $CD4^+CD25^+FoxP3^+$ and $CD8^+FoxP3^+$ Treg cells • Suppression of the synthesis of anti-dsDNA antibodies	*Animal models* - Attenuation and delay of the disease and reduction in autoantibody titers either with the i.v. or the oral route of administration *In vitro and ex vivo assays* - Increased differentiation toward a Treg phenotype of PBMCs of SLE patients exposed to the compound		[66,67]
Laminin-derived peptide	VRT101 (21-mer peptide within the globular part of the laminin-α1chain) and VRT102	• Prevention of the binding of autoantibodies to extracellular components in the glomerular mesangial matrix, subepithelium, and subendothelium	*Animal models* - Reduced renal damage and prolonged survival of MRL/lpr/lpr mice parenterally treated with VRT101-VRT102		[73]

(*Continued*)

Table 1. (Continued).

			Results from studies		
Peptide	Molecular structure	Mechanism of action	Preclinical studies	Clinical trials	Reference
Nucleosomal peptides	T helper and B lymphocyte dominant auto-epitopes in nucleosomal histones (H10 22e42, H2B10e33, H3 85e102, H416e39, and H471e94; IIIM1 nonapeptide)	• Suppression of type I IFN signaling and IL-6 secretion and increase in TGFβ expression • Down-regulation of IL-12 and IL-17 • Interaction with MHC class I and class II • Polarization toward a tolerogenic phenotype of pDC, B cells, $CD4^+$ and $CD8^+$ T lymphocytes • Disruption of T-B cross-talking • Expansion of $CD4^+CD25^+FoxP3^+$ Treg cells • Tolerance spreading	*Animal models* - Delay in nephritis development in pre-nephritic mice and prolonged survival in already glomerulonephritic mice - Reduction in anti-dsDNA antibody titers and lymphadenosis - Amelioration of the glomerular and tubulointerstitial histological patterns - Effects of IIIM1 mediated by the catabolic product UBE - Clinical amelioration, better kidney histological patterns, rebalance in cytokine *milieu* and T lymphocyte subsets in lupus-prone mice intra-peritoneally injected with 1 mcg/kg of UBE compared to untreated animals *In vitro and ex vivo assays* - Increased differentiation of PBMCs of SLE patients in $CD4^+CD25^{high}FoxP3^+$ T cells, $CD4^+CD45RA^+FoxP3^{low}$ T cells, and $CD8^+CD25^+FoxP3^+$ T cells - Reduction in IFNα gene expression		[26,78–80]
D-DWEYS peptide	Pentameric peptide sequence placed within the NR2A and NR2B subunits of the NMDAR	• Prevention of the binding of anti-dsDNA/NMDAR antibodies to NMDAR	*Animal models* -Neuroprotective effect overlapping that of memantine - Additional nephroprotective role - Protection of fetal neurons from the insult of maternal anti-NMDAR antibodies when given to gestating mice - No interference with the biological function of the NMDAR		[85,86]
Glatiramer acetate	Pool of heterologous peptides sharing the four natural amino acids L-glutamic acid, L-alanine, L-tyrosine and L-lysine, mimicking MBP	• Receptor antagonist of the MBP immunodominant epitope peptide 82–100, recognized by the combinatory site of TCR, antibodies and MHC • Rebalance of APC and $CD8^+$ cell function • Proliferation of Th2 and $FoxP3^+$ Treg lymphocytes • Potentiation of cytotoxicity and phagocytosis mediated by NK and macrophages	*Animal models* - No benefits in terms of autoantibody production, peripheral monocytosis, glomerulonephritis histological grading and survival in NZBxBXSB F1 mice treated with s.c. injections of glatiramer acetate plus mannitol compared to untreated animals *In vitro and ex vivo assays* - Polarization of drug-exposed SLE patients' $CD19^+$ B memory cells toward an immunomodulatory phenotype, characterized by the expression of the CD5 marker and IL-21 receptor and by the production of IL-10 - Prevention of both proliferation and differentiation of T lymphocytes into IFNγ-producer Th1 effectors		[89,90]

(*Continued*)

Table 1. (Continued).

Peptide	Molecular structure	Mechanism of action	Results from studies: Preclinical studies	Results from studies: Clinical trials	Reference
Thymopentin (TP-5)	Analog pentapeptide including the five amino acid residues Arg^1-Lys^2-Asp^3-Val^4-Tyr^5 of thymopoietin 2	• Immunopotentiation • Immunomodulatory effect in autoimmunity • Interaction with HLA-DR molecules of APC	*Animal models* - Increased life-span and reduced proteinuria when subcutaneously given at a dose of 10–100 mg/kg 5 days a week to MRL/lpr mice - No significant changes in the titers of anti-DNA antibodies nor in the histological grading of glomerulonephritis *In vitro and ex vivo assays* - Induction of the lectin-dependent cell-mediated cytotoxicity and release of IL-6 and IFNγ following the stimulation with mitogens in PBMCs of patients with SLE		[93–96]
AMG623 (*Blisibimod*)	Peptibody carrying four high affinity BLyS-binding peptides grafted onto the Fc portion of a IgG_1	• Neutralization of soluble and membrane-bound BLyS	*Animal models* - Constrained number of peripheral and spleen B lymphocytes in healthy mice - Reduced proteinuria and increased survival of NZBxNZW F1 mice intraperitoneally treated with AMG623 for 5 months - Relapse after drug discontinuation	*Phase I RCTs* - Early phase I trials: Acceptable safety profile; flares of disease reported; ADA detected in both treated and untreated patients at any time, but mostly non-neutralizing; decrease in peripheral B lymphocyte count and unbalance in the memory/naive B cell ratio at detriment of the latter *Phase II RCTs* Primary endpoint, consisting of the achievement of the SRI-5 response at 24 weeks, not met; significant amelioration of fatigue and positive trend in the time to flare, glucocorticoid needed dose, proteinuria, anti-dsDNA antibody titers and hypo-complementemia, especially in patients with severe disease activity, with the dose of 200 mg administered once weekly; no safety issues *Phase III RCTs* Primary outcome (SRI-6 response at 52 weeks) not achieved; significant tapering in steroid dose since week 24 onward, increase in C3 and C4 serum levels, reduction in total Ig and in anti-cardiolipin IgM and IgG titers in patients assigned to blisibimod *vs*. PBO; positive trend concerning the decrease in proteinuria and anti-dsDNA antibody titers in blisibimod-treated patients *vs*. PBO; good safety profile, mild local reactions reported	[99–104]
Romiplostim	Peptibody containing a 14 amino acid domain binding the TPO receptor attached to the Fc of a IgG_1	• TPO receptor agonist • Stimulation of megakaryopoiesis		*Case report – case series* - Effectiveness in refractory SLE-related autoimmune thrombocytopenia - Effectiveness in pregnancy without safety concerns - Cases of arterial, venous and microvessel thrombosis, including the occurrence of catastrophic APS, especially in anti-phospholipid antibody positive individuals	[108–112]

Abbreviations: SLE, systemic lupus erythematosus; MHC, major histocompatibility complex; PBMCs, peripheral blood mononuclear cells; SLEDAI, SLE disease activity index; BILAG, British Isles lupus assessment group; CDR, complementarity determining region; IL, interleukin; IFN, interferon; TGFβ, transforming growth factor-beta; LFA, lymphocyte function-associated antigen; CD, cluster of differentiation; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; BLyS, B lymphocyte stimulator; VH, heavy chain variable domain; instead of VH, variable heavy chain; FoxP3, fork-head box protein 3; H, histone; HSPA8, heat shock 70 kDa protein 8; APC, antigen presenting cell; Treg, T regulatory; Th, T helper; snRNP, small nuclear ribonucleoprotein; RCT, randomized controlled trial; PGA, physician global assessment; anti-dsDNA, anti-double stranded DNA; PBO, placebo; ITT, intention to treat; SRI, SLE responder index; CNS, central nervous system; CYC, cyclophosphamide; AMS, adjusted mean SLEDAI; MBP, myelin basic protein; NMDAR, N-methyl-D-aspartate receptor; TCR, T cell receptor; NK, natural killer; Fc, fragment crystallizable; ADA, anti-drug antibodies; Ig, immunoglobulin; APS, anti-phospholipid syndrome.

Table 2. List of the untested but potentially useful peptide candidates for SLE treatment.

Peptide	Target	Rationale	Evidence in favor of a therapeutic role in autoimmunity	References
T140 analog	CXCR4-CXCL12 pathway	• Hyper-activation of the CXCR4-CXCL12 pathway in SLE patients and significant association with disease activity, severity of glomerulonephritis and neuropsychiatric symptoms • Hyper-expression of CXCL12 in kidney specimens of patients with lupus nephritis • Efficacy of the s.c. CXCR4 antagonist small molecule plerixafor, injected at a dose of 5 mg/kg 3 times a week in female NZB/W F1 mice, in counteracting the activity of plasma cells, reducing the production of autoantibodies and proteinuria and increasing animal survival	The bio-stable T140 analog acts as inverse agonist of CXCR4, and prevents the development of collagen-induced arthritis in mice and the CXCL12-mediated chemotaxis of human Jurkat cells and mouse splenocytes *in vitro*	[114–117]
Astin C cyclopeptide	STING signalosome	• Hyper-activation of STING signaling pathway in SLE and association with type I IFN signature	Astin C binds the C-terminal domain of STING and prevents the IRF3 recruitment in the STING signalosome. The exposure to the peptide of Trex1-/- BMDM cells and Trex1-/- mice constrains cGAS-STING-mediated inflammation triggered by cytosolic DNA and reduces the antiviral response	[118,119]
TTV ORF2	Molecular mimicry between viral and self-antigens	• Increased serum levels of TTV DNA in SLE patients • Cross-reactivity between the HRES-1-derived B cell epitope p28 and the TTV ORF2a peptide	The TTV ORF2 protein suppresses *in vitro* the NF-kB canonical pathway in a dose dependent manner	[125,126]
4B4 idiotype peptide	Molecular mimicry between retroviral and self-antigens	• Detection of antibodies reacting against the epitopes of the HIVp24core in the sera of one-third of SLE patients • Identification of peptide sequences of the HIVp24 core (E, H, P) electively recognized by antibodies of SLE individuals, but not by those of patients affected by Sjögren's syndrome • Isolation of the 4B4 idiotype common to anti-p24 and anti-Sm antibodies of SLE subjects	To be investigated	[127,128]
Env59-GP3	HERV reactivation	• High rates of antibodies cross-reacting against the U1-70K snRNP autoantigen and a p30 gag protein of HRES-1 in SLE patients • Integration site of HERV-K10 within the complement C2 gene, which could lead to the misactivation of the complement system • Increased expression of the viral *gag* protein of HERV-E clone 4–1 in SLE patients eliciting a humoral response, in turn related to the synthesis of anti-RNP antibodies • Hyper-expression of the *env59* gene in PBMCs of SLE patients and inverse association with the expression of the IL-6 and TLR7 genes	Env59-GP3, synthesized on the basis of the immunosuppressive domain of the *env* protein of HERV-H, is effective in preventing the development of EAE in animals and in polarizing *in vitro* human macrophages toward a M2 tolerant phenotype	[134,135]

Abbreviations: CXCR4-CXCL12, C-X-C motif chemokine receptor 4-C-X-C motif chemokine ligand 12; SLE, systemic lupus erythematosus; s.c., subcutaneous; STING, stimulator of interferon genes; IFN, interferon; IRF3, interferon regulatory transcription factor 3; cGAS, cyclic guanosine monophosphate–adenosine monophosphate (GMP-AMP) synthase; TTV, Torque teno virus; ORF, open reading frame; HRES-1, human T lymphotropic virus type 1 (HTLV-1)-related endogenous sequence; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; HIV, human immunodeficiency virus; RNP, ribonucleoprotein; HERV, human endogenous retrovirus; EAE, experimental autoimmune encephalomyelitis; PBMCs, peripheral blood mononuclear cells; TLR, toll-like receptor; IL, interleukin.

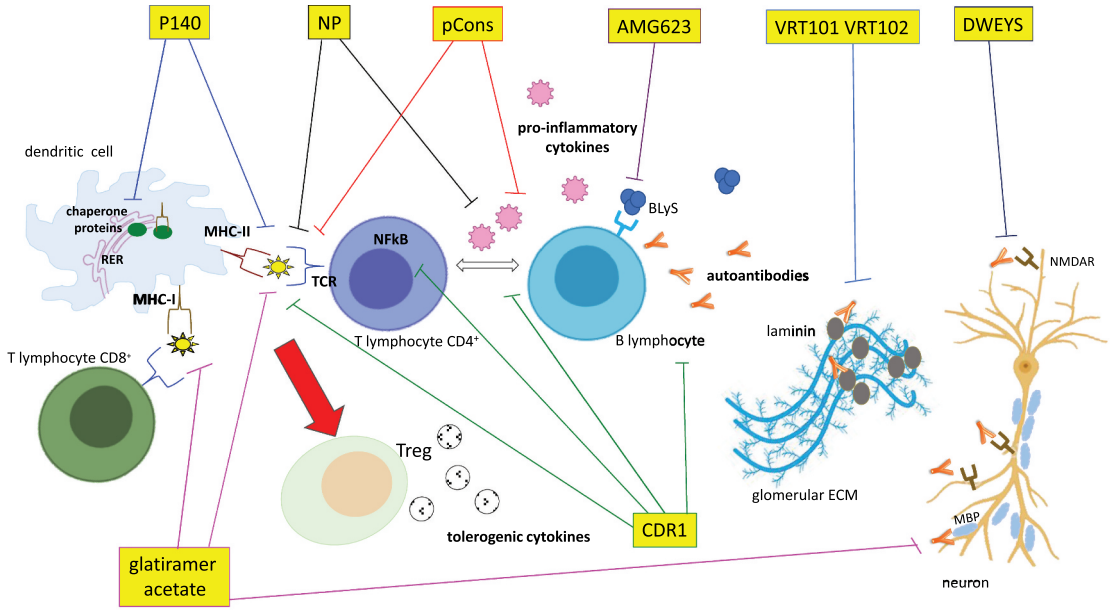

Figure 4. Targets and mechanism of action of therapeutic peptides under investigation in SLE.

The figure summarizes the mechanism of action of eight peptides targeting different SLE-specific pathways. NP, P140, pCons, CDR1, and glatiramer acetate inhibit the activation of effector T cells by interfering with antigen presentation. Consequently, these peptides lead to a lower production of pro-inflammatory cytokines and to the switch of T cells toward a tolerogenic phenotype. P140 prevents the recycling of MHC molecules in RER acting on chaperone proteins. NP and pCons interfere with T-B cell interaction, and impede the maturation of B cells into plasma cells and, therefore, the production of autoantibodies. CDR1 inhibits the nuclear translocation of NF-kB and the subsequent expression of genes involved in inflammation. VRT101 and VRT102 antagonize the binding of anti-laminin pathogenic antibodies to glomerular extracellular matrix. DWEYS prevents the interaction of anti-dsDNA/NMDAR antibodies with the NMDAR of neurons and counteracts neuronal depolarization and apoptosis. Glatiramer acetate, instead, inhibits the binding of autoantibodies to MBP, thus also exerting a neuroprotective effect. Finally, the peptibody AMG623 neutralizes both soluble and membrane-bound BLyS and constrains the further activation of B cells. Abbreviations: P140, 21-mer peptide P140; NP, nucleosomal peptides; CDR1, complementarity determining region-1-based peptide; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TCR, T cell receptor; MHC, major histocompatibility complex; RER, rough endoplasmic reticulum; Treg, T regulatory cell; ECM, extra-cellular matrix; MBP, myelin basic protein; NMDAR, N-methyl-D-aspartate receptor; BLyS, B lymphocyte stimulator.

80 mg of prednisone equivalent), antimalarials, methotrexate (MTX), leflunomide, MMF, or azathioprine (AZA). Patients affected by severe nephritis or cerebritis were excluded, as well as those previously treated with cyclosporin A (CysA), CYC, biologic agents, and intravenous immunoglobulins (IVIG) unless an appropriate wash-out period was guaranteed. The NCT01135459 trial primarily aimed to assess changes in SLE responder index (SRI) at week 24, and, secondly, in other items including the SLEDAI-2K, the British Isles Lupus Assessment Group (BILAG)-2004 and the Systemic Lupus International Collaborating Clinics (SLICC) scores, after a 20 week treatment period. Though completed in 2012, to our knowledge, no results were posted or even published to date. Instead, the overall intention-to-treat (ITT) analysis of the phase IIb RCT sponsored by ImmuPharma, randomizing 149 patients to placebo or s.c. injections of the 21-mer peptide P140 at a dose of 200 mcg every 2 or 4 weeks in addition to standard of care, showed the achievement of the primary endpoint (SRI response at 12 weeks) in 53.1% of patients treated every 4 weeks *vs*. 36.2% of those assigned to placebo [40]. Interestingly, the highest contribution to the amelioration of composite indexes was attributed to the improvement of articular and cutaneous symptoms. One death, due to pneumonia, was reported in a patient receiving the active treatment every 4 weeks, but, in the investigator's opinion, it was related to concomitant immunosuppressive medications (AZA and steroids) rather than to the experimental drug.

In two parallel phase IIb RCTs, the efficacy and safety profile of a 21-mer peptide P140 in 10% trehalose (*Forigerimod*, ImmuPharma) was also investigated [41]. The peptide was given at a dose of 200 mcg/month in addition to standard of care in active SLE (SLEDAI-2K score > 6) patients and the efficacy evaluated through the SRI score at 3 and 6 months. Noteworthy, the trial showed that the excipient trehalose reduced the efficacy of the 21-mer peptide P140, presumably *via* the potentiation of the process of autophagy [42].

Controversial results instead characterize phase III investigations. The ImmuPharma-sponsored phase III NCT02504645 trial was recently completed and results posted at www.clinicaltrials.gov. This was a 52-week randomized double-blind parallel group placebo-controlled study, in which 202 SLE patients were recruited. Patients were required to have an active disease (SLEDAI-2K ≥ 6) and may receive oral corticosteroids, antimalarials, MTX, leflunomide, MMF or AZA at stable doses before entering the study. Patients with renal damage were included, providing that the estimated glomerular filtration rate (eGFR) was above 30 mL/min/1.73 m^2 at screening. Surprisingly, the primary endpoint, consisting of the SRI response at week 52, was not met due to a high response rate in the placebo group (52.5% *vs*. 44.6% of responders assigned to the active treatment given at a dose of 200 mcg every 4 weeks and placebo, respectively). Also, in spite of the apparent superior response rate of the 21-mer peptide P140 over placebo (68.8% *vs*. 59.2%) in patients who completed the study, no statistical analysis was provided [43]. However, in line with the results of the early phase II trial, this study showed that the subgroup of patients with anti-dsDNA antibodies were more likely to respond to the experimental treatment and even achieve a complete remission when compared to placebo. The inclusion of patients with low

titers of anti-dsDNA antibodies at screening (30 IU/mL) or even seronegative might have biased the final results, suggesting the presumably coexistence of two different SLE patient populations (seropositive and seronegative) who may differently respond to the peptide. Unfortunately, to the best of our knowledge, no extended report of the results of the NCT02504645 trial is available in peer-reviewed literature, making data interpretation extremely risky. Neither it is possible to speculate on the plausible benefits of the 21-mer peptide P140 on anti-dsDNA antibody-mediated SLE manifestations, like glomerulonephritis or neuropsychiatric symptoms. The list of adverse events recorded during the trial was transparently reported at www.clinicaltrials.gov and globally shows no safety issues. On the contrary, the results of the completed phase III open-label NCT03427151 study, specifically aiming to assess the safety and tolerability of repeated injections of the peptide plus standard of care over a time of 7 months, were unpublished. Also, the single-arm open-label phase III NCT01240694 trial, aiming to evaluate the long-term (72 weeks) safety and tolerability of the 21-mer peptide P140 in SLE patients completing the previous phase IIb study (Teva Pharmaceutical Industries, Cephalon), was prematurely terminated in 2012 due to a business decision (no safety issues). Despite the winding developmental path, ImmuPharma and Avion Pharmaceuticals recently agreed to further investigate the efficacy and safety of the 21-mer peptide P140 in SLE in a new phase III RCT to be started in 2020. The trial will specifically address anti-dsDNA antibody positive cohorts and pave the way for the commercialization of the drug in the United States [44].

2.2.2. CDR1-based peptide (Edratide)

Complementarity determining regions (CDRs) encompass the variable portions of antibodies and TCR that recognize and interact with antigens. Antibodies can bear CDRs binding epitopes within the CDR amino acid structures of other antibodies, establishing the so-called idiotype-anti-idiotype network [45]. It has been shown that the CDRs of autoantibodies in SLE can interact with antibodies having the common idiotype 16/6 Id [46]. These autoantibodies can cross-react with nuclear antigens, and have been found increased in the sera of SLE patients [47]. Furthermore, when transferred to non-SLE prone mice they were capable of inducing a SLE-like phenotype (leukopenia, proteinuria, and immune complex deposits in kidneys) [48]. The idiotype 16/6 is an immunogenic epitope for T cells in BALB/c mice, and mediates, therefore, the activation of effector T lymphocytes that play a crucial role in SLE pathogenesis and in the onset of clinical manifestations. Researchers synthesized a peptide on the basis of the CDR1 sequence bearing the idiotype 16/6 of a pathogenic murine monoclonal anti-DNA antibody (5G12). This peptide showed an immunomodulatory effect in experiments on animal models. Immunization of BALB/c mice with this peptide rebalanced the cytokine secretion: on the one hand, it reduced the amount of the pro-inflammatory cytokines IL-2, IFNγ and TNFα and, on the other hand, it increased that of the tolerogenic cytokine transforming growth factor (TGF)β [49]. Similarly, the treatment of NZBxNZW F1 female mice with weekly s.c. injections of 50 mcg of human (h) CDR1 for 10 consecutive weeks resulted in the down-regulation of the p21Ras pathway and of c-Jun NH2-terminal kinase (JNK) activity, which are associated to T cell proliferation and apoptosis, respectively, and in the amelioration of clinical manifestations [50]. Other experiments on mice models further unraveled the immunomodulatory role played by the peptide, which encompasses the down-regulation of adhesion and costimulatory molecules on APC and the normalization of the IFNγ- and of BLyS-related pathways [51–53]. *Ex vivo* experiments conducted on CDR1-exposed peripheral blood mononuclear cells (PBMCs) of SLE patients showed a reduced expression of pro-inflammatory cytokines and a constrained T cell apoptosis whereas the up-regulation of Treg cells was reported [54,55]. An interesting *ex vivo* study confirmed the high selectivity of the hCDR peptide for the aberrant immunologic pathways specifically hyper-activated in SLE. By exposing PBMCs of 10 SLE subjects, 5 primitive APS patients and 5 healthy controls to 25 mcg/mL of the peptide for 48 hours, a significantly diminished expression of the IFNα gene was observed in PBMCs of SLE patients but not in those of APS individuals and controls [56].

The successful findings that emerged from preclinical research paved the way for further clinical studies in human disease. *Edratide* (Teva Pharmaceutical Industries), a hCDR1 peptide, subcutaneously injected once a week at a dose of 0.5 mg, 1.0 mg, and 2.5 mg, was tested in a phase II RCT (ClinicalTrial.gov ID NCT00203151) in a cohort of 340 SLE patients. Despite a favorable safety profile, the primary endpoints (SLEDAI-2K and Adjusted Mean SLEDAI or AMS response) were not met [57], and the reason may be traced back to the short period of observation (26 weeks) and to the design of the study, based on the last observed value (LOV) approach [58,59]. Nevertheless, the trial offered some interesting hints: 1) in the ITT, the dose of 0.5 mg significantly reduced the BILAG score, with a trend observed for the doses of 1.0 and 2.5 mg; 2) the post-hoc analysis revealed that efficacy, assessed through the BILAG questionnaire, was significantly higher in anti-dsDNA antibody-positive patients (baseline titer > 30 IU/mL) receiving 0.5 mg of *Edratide* and low (<20 mg/day) or no doses of prednisolone at baseline; 3) the occurrence of BILAG flares was significantly lower in the group assigned to *Edratide* 0.5 mg/week compared to placebo (ITT and post-hoc analysis); 4) the transcriptomic analysis of various genes coding for cytokines, conducted on 9 patients completing the study, showed that *Edratide*, but not placebo, reduced the expression of IL-1β, TNFα, IL-10, IFNγ, BLyS, caspase 3 and caspase 8, while augmenting that of TGFβ and forkhead box P3 (FoxP3) [60].

The apparently conflicting results concerning the efficacy of *Edratide* may be explained on the ground of the different scoring method (SLEDAI-2K/AMS and BILAG) used for the assessment of clinical response (with SLEDAI-2K only capturing complete response and BILAG capturing both partial and complete response). Moreover, despite a SLEDAI-2K score ≥ 6 at baseline was an entry criterion, enrolled patients were required to concomitantly receive only antimalarials, non-steroidal anti-inflammatory drugs (NSAIDs), and glucocorticoids, and not to have an active glomerulonephritis or an active involvement of the CNS. Enrolled patients were mainly affected by long-lasting (mean 7 years) musculoskeletal, mucocutaneous, and hematologic

manifestations. Therefore, the role of *Edratide* in more severe forms of SLE, as well as in other disease domains, remains to be elucidated. The *as per needed* use of glucocorticoids, permitting an increase in the daily dose of prednisone up to 60 mg, might further have masked the effect of *Edratide*, thus explaining the higher response rates to the peptide compared to placebo in subjects treated at baseline with low steroid doses or steroid-untreated. Experiments conducted in animal models showed, in fact, that *Edratide* may have some independent and additional tolerogenic effects to those exerted by glucocorticoids and CYC [61,62], which may privilege in the long-term the use of the peptide over that of traditional drugs burdened by a higher morbidity. Furthermore, the efficacy of *Edratide* may be corroborated by chemical modifications. For instance, it has been shown that single amino acid substitutions at the 7–14 positions seem to influence the capability of interacting with both TCR and MHC and thus affect the T cell response [48]: these data could be exploited for designing more efficacious isoforms of the CDR1 peptide. Nevertheless, the homology of the peptide with the immunogenic idiotopes and paratopes recognized by autoantibodies may be influenced by the CDR spatial conformation rather than by the primary amino acid sequence, and by the non-protein nature of self-antigens (like nucleic acids within RNP) placed upstream the idiotype-anti-idiotype network [63].

According to these considerations, the efficacy and safety of *Edratide* should be reassessed in long-term studies conducted in wider SLE cohorts, as well as in specific subsets of patients. Also, its drug-sparing effect on steroids, immunosuppressants, and DMARDs would deserve further clinical investigation.

2.2.3. pConsensus

On the basis of experiments on both animal and human cells, it has been shown that heavy chains (HC) of anti-dsDNA antibodies can behave as immunogenic epitopes and elicit a T cell response [64]. Using a computer algorithm, Hahn *et al*. elaborated the sequence of a 15 amino acid peptide, namely pConsensus, derived from the HC of anti-dsDNA antibodies extracted from 4 different NZBxNZW F1 (BWF1) murine groups [65]. Preliminary experiments included the testing of 439 12-mer and 15-mer peptides in the variable domain of HC of 4 different IgG_2a or IgG_2b anti-DNA monoclonal antibodies of nephritic BWF1 mice. These peptides were used to evaluate the response *in vitro* of spleen-derived T cells of not immunized BWF1 female mice, focusing on the induction of autoantibody production and antibody-mediated response to pathogenic agents. Among them, the stimulatory peptide pConsensus showed an immune tolerogenic effect when intravenously injected in BWF1 mice, reducing both the progression of renal damage and the autoantibody titers, and prolonging the survival without affecting defense against pathogens. These effects may be the result of a cytokine rebalance that is strictly interconnected with the differentiation of Treg lymphocytes. In fact, mice intravenously treated with pConsensus had an increased production of TGFβ that might mirror the up-regulation of $CD4^+CD25^+FoxP3^+$ Treg cells [66].

Noteworthy, using NZBxNZW F1 mice, Skaggs *et al*. evaluated the effect of the oral administration of pConsensus on clinical manifestations and serum autoantibody titers [67]. Indeed, the enteral route of delivery increases the compliance of patients to a drug and the long-term adherence to a therapeutic regimen. However, peptides are usually degraded by gastrointestinal enzymes before their intestinal absorption [68]. In this case, pConsensus was appositely modified in order to be more resistant to the attack of gut peptidases, while meantime maintaining tolerogenic properties. Modifications included the choice of chains of D-amino acids, peptide cyclization, and multimerization of tolerogenic and immunogenic sequences on an inert backbone of polylysine (MAP). Overall, six peptide isoforms were tested at different concentrations (10, 100, and 500 mcg) in animal models. Among them, L-MAP or D-MAP pConsensus, diluted in dimethyl sulfoxide (DMSO) at the proportion of 1/100 and given at a dose of 100 mcg for 30 consecutive weeks, decreased proteinuria and serum anti-dsDNA antibody titers in a similar way to the parenterally administered isoform.

2.2.4. Laminin-derived peptide

For a long time, it was believed that SLE glomerulonephritis could be the result of immune complex deposition, however, recent evidence suggests that it may also derive from the direct interaction of autoantibodies with autoantigens present in the glomerular extracellular matrix [69]. Laminin is normally found in mesangial matrix, but in SLE it may also be expressed in subepithelium, subendothelium, and fibrotic areas of glomeruli. Antibodies against laminin epitopes have been reported in several autoimmune diseases [70]. In SLE patients, these antibodies can be detected in urine, strengthening the pathogenic role exerted in kidney [71]. SLE patients have high titers of pathogenic autoantibodies directed against VRT101, a 21-mer peptide located at the globular part of the laminin-α1 chain, and their titers typically correlate with the SLEDAI-2K score [72]. Using a panel of peptides derived from the laminin-α1 chain (VRT101, R28, R30, R37, R18, R27, R26, and R35) and 13 mouse anti-laminin antibodies in two SLE mouse models, it was observed that the pathogenic anti-DNA antibodies, isolated in mice sera, highly recognized the peptide VRT101 [73]. However, most of the antibodies did not have the same avidity for DNA, underlining the coexistence of different kinds of autoantibodies mediating the pathogenic damage in SLE glomerulonephritis. Immunization of MRL/lpr/lpr mice by injecting the VRT101 peptide exacerbated glomerulonephritis, leading to the increase of proliferative lesions. On the contrary, the co-administration of 80 mcg of both VRT101 and VRT102 peptides 5 times weekly for 260 consecutive days to MRL/lpr/lpr mice with established glomerulonephritis brought to the amelioration of renal histological findings and clinical manifestations and prolonged mice survival.

Also, another isolated study identified a 12 amino acid peptide (ALW), recognized by a panel of murine PL9-11 IgG anti-DNA isotypes, which was able to prevent *in vitro* the binding of anti-DNA antibodies to DNA, as well as to laminin, mesangial cells, and isolated glomeruli in both SLE mice and patients [74].

Though still preliminary, research on peptides structurally related to laminin may carve out a niche for the treatment of

SLE glomerulonephritis, and studies evaluating the biological effect of these medications on the different glomerular injury patterns should be encouraged.

2.2.5. Nucleosomal peptides

Nucleosomes consist of chromatin packages around an octameric histone core. In SLE, antibodies reacting against components of nucleosomes represent a hallmark of the disease and their titers are associated with disease activity [75]. The five nucleosomal peptides *H10 22e42, H2B10e33, H3 85e102, H416e39,* and *H471e94* have been reported to be immunodominant epitopes for $CD4^+$ T lymphocytes [76]. When presented within the MHC class II, they can induce the activation of effector T lymphocytes. Particularly, the H1′(22–42) epitope can concomitantly activate T and B cells, and antibodies reacting against this epitope are associated with the severity of glomerulonephritis in lupus-prone SWRxNZB F1 mice [77]. Some experiments conducted on animal models evidenced that the parenteral administration of nucleosomal peptides led to a delay in the development of the disease in pre-nephritic SWRxNZB F1 mice and prolonged the survival of already glomerulonephritic mice [78]. Interestingly, the nucleosomal peptide H416-39, containing both B and T autoepitopes, showed a double tolerogenic effect on both Th and B lymphocytes. Nucleosomal epitopes can lead to tolerance spreading, perhaps by competing with the presentation of natural antigens by APC and by inducing an APC tolerogenic phenotype characterized by the production of TGFβ [26]. Other explanations may include the interaction with multiple TCR or the interference with T-B cell cognate interaction at several steps. Results obtained from animal models were confirmed in *ex vivo* experiments using PBMCs of SLE patients [79]. Stimulation of these cells with nucleosomal peptides resulted in a lower production of autoantibodies and in a modest increase in $CD4^+CD25^+FoxP3^+$ Treg cells.

An interesting experiment on MRL/LPR mouse models showed that the oral administration of the IIIM1 nonapeptide, derived from a human and murine T immunodominant epitope placed within H2A, was effective in treating both the early and late stages of the disease [80]. The peptide significantly prevented the deterioration of the renal function, suppressed the production of anti-dsDNA antibodies, reduced lymphadenosis, and prolonged animal survival. It was shown that these pharmacological effects were ultimately mediated by the catabolic 36 mer peptide product UBE, whose parenteral administration to lupus-prone mice induced similar clinical, immunologic, and histological benefits to those obtained with IIIM1.

To resume, nucleosomal peptides offer several attractive opportunities: 1) they don't elicit allergic reactions and are relatively atoxic, as already present in the T and B repertoire of recognized epitopes; 2) are effective at very low concentrations (1 mcg/kg in mice corresponding to 0.2–2 mg/kg in humans); 3) are highly soluble and easily absorbed; 4) though rapidly degraded, they impart long-lasting tolerogenic signals in pDC; 5) are recognized by both MHC class I and II molecules irrespective of the human leukocyte antigen (HLA) haplotype, thus polarizing both $CD8^+$ and $CD4^+$ T cells toward a regulatory phenotype; and 5) are effective at early and late stages of the disease, showing an additional synergistic effect with conventional drugs [81].

2.2.6. DWEYS peptide

It has been demonstrated that anti-dsDNA antibodies passing across the blood-brain barrier (BBB) are able to cross-react with the N-methyl-D-aspartate receptor (NMDAR) of neuronal cells, inducing neuron depolarization and apoptosis in both SLE individuals and mouse models [82,83]. Anti-dsDNA /NMDAR antibodies, detected in the sera of about 40% of SLE patients, are typically correlated to neuropsychiatric manifestations when also found in the cerebrospinal fluid [84]. Researchers showed that the nephritogenic murine anti-dsDNA antibodies R4A were able to bind a pentameric peptide sequence (DWEYS) placed within the NR2A and NR2B subunits of the NMDAR [83]. Animals immunized with DWEYS produced anti-NMDAR antibodies, which mediated hippocampal and amygdala damage following a breach in the BBB, with final repercussions on the cognitive and emotional behavior. Interestingly, the i.v. administration of the D isoform of the DWEYS peptide in SLE mouse models spared neuronal damage in a similar way to memantine [85]. Beside playing an additional nephroprotective role, the D-DWEYS peptide, given to gestating mice, also protected fetal neurons from the insult of maternal anti-NMDAR antibodies, without interfering with the biologic function of the NMDAR [86]. Although the D-DWEYS peptide appeared a promising candidate, the research in this field rather moved toward the generation of peptidomimetic small molecules, mimicking *in vitro* and *in vivo* the pharmacologic effect of the DWEYS peptide, but displaying better enteral bioavailability and molecular stability [28].

2.2.7. Glatiramer acetate

Glatiramer acetate (Teva Pharmaceutical Industries) is a heterologous peptide therapy approved for the treatment of multiple sclerosis. It consists of a pool of synthetic peptides sharing the four natural amino acids L-glutamic acid, L-alanine, L-tyrosine, and L-lysine [87]. These key amino acids, combined in a specific molar ratio, mimic the myelin basic protein (MBP), which represents one of the most pathogenic autoantigens in demyelinating diseases. Thanks to this homology, glatiramer acetate acts as receptor antagonist of the MBP immunodominant epitope peptide 82–100, recognized by the combinatory site of TCR, antibodies, and MHC. In line with other therapeutic peptides, the drug shows an overall immunomodulatory effect, explicated through the rebalance of APC and $CD8^+$ cell function, the shift of the immune response toward a higher proliferation of Th2 and $FoxP3^+$ Treg lymphocytes, and the potentiation of cytotoxicity and phagocytosis mediated by natural killer (NK) and macrophages. Anti-MBP antibodies with additional proteinase activity (abzymes) are commonly found in SLE patients' sera, and, once crossed the BBB, they seem to cause synaptic dysfunction of neurons and oligodendrocytes [88]. In order to evaluate the potential effectiveness in SLE, glatiramer acetate was tested in two preliminary studies. The first experiment, conducted on NZBxBXSB F1 mice, showed no benefits in terms of autoantibody production, monocytosis, glomerulonephritis histological grading, and survival in treated *vs*. untreated animals [89]. The second study, using an *in vitro* co-culture system, showed that drug-

exposed $CD19^+$ B memory cells of SLE patients acquired an immunomodulatory phenotype and prevented the polarization of T lymphocytes toward IFNγ-producer Th1 effectors [90]. It is however tricky to hypothesize a fair advantage derived from the use of glatiramer acetate *in vivo*, since the unbalance in the Th1/Th2 ratio in favor of the latter may be detrimental in a Th2-piloted disease, like SLE. Furthermore, the potential efficacy in the neurological manifestations of the disease remains uninvestigated.

2.2.8. Thymopentin

Thymopentin (TP-5) is an analog pentapeptide synthesized on the basis of hormones extracted from thymus gland, and includes the five amino acid residues 32–36 (Arg^1-Lys^2-Asp^3-Val^4-Tyr^5) of thymopoietin 2, influencing the biological activity [91,92]. Given their immune-potentiating function, thymic hormones and derived peptides have been licensed for the treatment of primitive and acquired forms of immunodeficiency. Of note, researchers demonstrated that TP-5 directly interacts with the HLA-DR molecules of APC [93] and exerts an immunomodulatory effect in autoimmunity [94]. A couple of studies investigated, in the past, the effect of the administration of TP-5 in lupus animal models and human cells of SLE individuals [95,96]. These experiments showed that TP-5 induces *in vitro* the lectin-dependent cell-mediated cytotoxicity and the release of IL-6 and IFNγ following the stimulation with mitogens in PBMCs of patients with SLE. Additionally, when subcutaneously given at a dose of 10–100 mg/kg 5 days a week to MRL/lpr mice, it increased life-span and reduced proteinuria, though the titers of anti-DNA antibodies and the histological grading of glomerulonephritis remained unchanged. Although promising, research in this field was discontinued.

2.3. Peptibodies

The theme of Fc-conjugated peptides, or peptibodies, is worth a special mention, since they represent an evolution of therapeutic peptides. Peptibodies, in fact, are characterized by a better physicochemical profile than that of simple peptides, and, therefore, may potentially replace the latter in future pharmaceutical research. In SLE, two peptibodies have so far been investigated: 1) blisibimod, specifically targeting a crucial step of SLE pathogenesis, and 2) romiplostim, licensed for other clinical indications and tested in real-life SLE case series.

2.3.1. Blisibimod

AMG623 is a peptibody carrying four high-affinity BLyS-binding peptides grafted onto the Fc portion of a IgG_1 [97]. The compound was electively designed for SLE on the basis of the results of a phage display library screening, yielding a group of peptides binding to both soluble and membrane-bound BLyS with high affinity [98]. BLyS plays a crucial role in SLE pathogenesis, as it is able to foment B cell auto-reactivity, being also strictly interconnected with the type I IFN pathway. In preclinical experiments [99], the compound constrained the number of peripheral and spleen B lymphocytes in healthy BALB/c mice, and these results were confirmed in a BLyS-mediated B lymphocyte proliferation assay. Using NZBxNZW F1 mouse models, researchers showed that the intraperitoneal administration of AMG623 for 5 months reduced proteinuria and increased animal survival. Noteworthy, these effects were not long-lasting and disappeared after the discontinuation of the treatment. The reason may be traced back to the mechanism of action of this molecule, which works by targeting and neutralizing BLyS but does not restore immune tolerance.

AMG623 (blisibimod, Anthera Pharmaceuticals) entered the clinical phase of drug development and was tested in a number of phase II and III RCTs with conflicting results. Early phase I trials (ClinicalTrial.gov ID NCT02443506 and NCT02411136) showed an acceptable safety profile when the drug was parentally (s.c. or i.v. route) administered in adult patients with mild or inactive disease in addition to standard of care [100]. However, lupus flares were reported, as well as fever, depression, and arrhythmia that are also described among SLE symptoms and might reflect a recrudescence of the disease. This may be due to the immunogenicity of the Fc tail of blisibimod, exacerbating inflammation through the FcR-mediated activation of immune cells and the subsequent generation of anti-drug antibodies (ADA). ADA were actually reported in many patients at any time, but they were mostly non-neutralizing and, surprisingly, detected also in drug-unexposed subjects. In addition, blisibimod appeared to decrease peripheral B lymphocyte count and unbalance the memory/naive B cell ratio at the detriment of the latter, though without any significant association with immunogenicity.

The phase II PEARL-SC study (ClinicalTrial.gov ID NCT01162681) aimed to assess the efficacy and safety of s.c. blisibimod at three different dose regimens (100 mg every week, 200 mg every week or 200 mg every 4 weeks) in 547 recruited SLE patients [101]. An active disease at baseline corresponding to a safety of estrogens in lupus erythematosus national assessment (SELENA)-SLEDAI score ≥6 and the absence of severe renal and CNS complications represented inclusion criteria for the study. The primary endpoint, consisting of the achievement of the SRI-5 response at week 24, was not met. However, the dose of 200 mg administered once weekly significantly reduced fatigue [102], and tended to space SLE flares, spare the use of glucocorticoids and ameliorate some clinical and immunologic domains, especially in patients with severe disease activity. The safety profile was acceptable, and ADA not detected. Following the completion of this study, 382 participants entered the open-label extension phase (PEARL-OLE; ClinicalTrial.gov ID NCT01305746) [103]. This trial further confirmed the beneficial effect of blisibimod on proteinuria, anti-dsDNA antibody titers, and hypocomplementemia and reassured on the long-term safety profile.

The efficacy and safety profile of blisibimod was then tested in three phase III RCTs. The RCT CHABLIS-SC1 (ClinicalTrial.gov ID NCT01395745) was designed in order to selectively target those SLE subsets of patients who appeared more likely to respond to the drug in the previous phase II study. A total of 442 SLE patients were enrolled and randomized to receive blisibimod 200 mg subcutaneously injected once weekly or placebo plus standard of care. The primary

outcome consisted of the SRI-6 response at 52 weeks; while secondary endpoints focused on glucocorticoid tapering, bioumoral parameters, and safety [104]. Enrolled patients could be ANA or anti-dsDNA antibody positive and were required to have an active disease at SELENA-SLEDAI despite a background therapy with steroids, DMARDs, and immunosuppressants (antimalarials, MTX, MMF, AZA, leflunomide). Nephritic patients (proteinuria < 6 g/day) were included in the study and represented 29.9% of the cohort. The study failed to meet the primary endpoint at the modified ITT; however, a significant tapering in the steroid dose since week 24 onward, the increase in C3 and C4 serum levels and the reduction in total Ig and in anti-cardiolipin IgM and IgG titers were reported in patients assigned to blisibimod. Also, a positive trend was observed concerning the decrease in proteinuria and anti-dsDNA antibody titers in blisibimod- treated vs. placebo-treated patients. The drug was overall well-tolerated, except for mild skin reaction at the injection site, which compromised the compliance to the treatment in some cases. The phase III RCT CHABLIS-SC2 (ClinicalTrial.gov ID NCT02074020) aiming to assess the proportion of SRI-8 responders at week 52 was withdrawn in 2015. Also, the phase III RCT CHABLIS 7.5 (ClinicalTrial.gov ID NCT02514967), whose primary outcome was the rate of SRI-6 response at week 52 in seropositive and hypo-complementemic patients, was prematurely terminated in 2017.

2.3.2. Romiplostim

Contrary to other peptides, whose pharmacologic effects in SLE have been characterized in preclinical studies and RCTs, the evidence concerning the efficacy of AMG531 or romiplostim (Amgen) in SLE derives from real-life clinical experience. Romiplostim is a peptibody containing a 14 amino acid domain binding the thrombopoietin (TPO) receptor that is attached to the Fc of a IgG_1 [105,106]. By interacting with the TPO receptor, the drug works as a receptor agonist, thus stimulating megakaryopoiesis. Therefore, romiplostim has been approved for the treatment of refractory chronic immune (idiopathic) thrombocytopenic purpura (ITP). As an autoimmune thrombocytopenia is detectable in up to 30% of SLE patients [107], romiplostim has been used in real-life to treat thrombocytopenic SLE patients resistant to conventional treatments. In spite of an interesting efficacy profile [108] also reported during pregnancy [109], cases of arterial, venous, and microvessel thrombosis were described [110,111], including the occurrence of catastrophic APS [112]. These events especially developed in anti-phospholipid antibody positive individuals, who are already at risk of thromboembolism. Thus, the efficacy and safety profile of this peptibody in SLE remains poorly characterized and should be specifically investigated in SLE cohorts.

2.4. Therapeutic peptides on the way

2.4.1. CXCR4 antagonists

The molecule C-X-C motif chemokine receptor 4 (CXCR4) is a seven-transmembrane G-protein-coupled receptor expressed on blood cells, endothelial cells, epithelial cells, fibroblasts, and cancer cells [113]. This receptor binds C-X-C motif chemokine ligand 12 (CXCL12) and regulates cell proliferation and migration, hematopoiesis, neoangiogenesis, and embryogenesis. The CXCR4-CXCL12 pathway seems to be hyper-activated in SLE patients, and significantly associated with disease activity, severity of glomerulonephritis, and neuropsychiatric symptoms. Furthermore, CXCL12 is over-expressed in kidney specimens of patients with lupus nephritis [114]. In female NZB/W F1 mice, the s.c. use of the CXCR4 antagonist small molecule plerixafor significantly counteracted the activity of plasma cells, reduced the production of autoantibodies and proteinuria and increased animal survival [115]. Tamamura et al. showed that a bio-stable T140 analog, acting as an inverse agonist of CXCR4, prevented the development of collagen-induced arthritis in mice and the CXCL12-mediated chemotaxis of human Jurkat cells and mouse splenocytes *in vitro* [116]. T140 is an unstable 14-mer peptide extracted from horseshoe crabs, whose biological activity depends on the four amino acid residuals Arg^2, L-3-(2-naphthyl)alanine $(Nal)^3$, $Tyr,^5$ and Arg^{14} [117]. Chemical modifications, including the N-conjugation of a 4-fluorobenzoyl moiety and the cyclization or downsizing of the molecular structure, showed to enhance its stability in serum and tissues while conferring a stronger effectiveness. Given the interesting findings with the use of plerixafor in SLE mouse models, it is conceivable that the T140 analog may work in this disease, but to date no evidence is available.

2.4.2. STING antagonists

Due to the impaired clearance of nucleic acids, the endoplasmic reticulum (ER)-resident protein stimulator of interferon genes (STING)-cyclic GMP-AMP synthase (cGAS) pathway is typically hyper-activated in SLE and associated to the type I IFN response [118]. Li *et al*. reported interesting results from an *in vitro* experiment using the cyclopeptide astin C derived from the medicinal plant *Aster tataricus* [119]. Cyclopeptides, commonly found in nature, are characterized by a conformational rigidity, which augments receptor selectivity, resistance to proteolysis, and membrane permeability [120,121]. Astin C binds the C-terminal domain of STING and prevents the interferon regulatory transcription factor (IRF)3 recruitment in the STING signalosome. The authors observed that the exposure to the peptide of Trex1-/- BMDM cells and Trex1-/- mice constrained cGAS-STING-mediated inflammation triggered by cytosolic DNA, but reduced, at the same time, the antiviral response. Therefore, although appearing a promising strategy in autoimmune diseases, STING antagonism might put patients at risk of serious viral infections, especially in the quite common case of a coexistent iatrogenic immunosuppression.

2.4.3. Virus-derived peptides

The administration of peptides designed on the basis of specific viral sequences hyper-expressed in SLE may induce immune tolerance *via* a booster effect. In fact, SLE may be triggered by external viral infections or by the aberrant reactivation of human endogenous retroviruses (HERVs) through a molecular mimicry mechanism or other immunologic pathways [122–124].

The Torque teno virus (TTV) is a human circular DNA virus transmitted by blood transfusions. SLE patients have increased serum levels of TTV DNA, and Gergely *et al*. evidenced a cross-reactivity between the B cell epitope peptide p28, derived from human T lymphotropic virus type 1 (HTLV-1)-related endogenous sequence (HRES-1) and the open reading frame (ORF)2a peptide of TTV [125]. An *in vitro* study evidenced that the TTV ORF2 protein may suppress the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) canonical pathway in a dose-dependent manner [126]. Thus, despite no evidence is available in autoimmunity, TTV ORF2-derived peptides may be considered worth candidates for the treatment of immune-mediated diseases, including SLE.

Additionally, about one third of SLE patients even without any prior exposure to the human immunodeficiency virus (HIV), produce antibodies reacting against the epitopes of the HIVp24core. Of note, an enzyme-linked immunosorbent assay (ELISA) experiment identified some peptide sequences of the HIVp24 core (E, H, P) electively recognized by antibodies of SLE individuals, but not by those of Sjögren's syndrome patients [127]. The 4B4 idiotype is common to anti-HIVp24 and anti-Sm antibodies of SLE subjects and could be exploited for further research as a therapeutic peptide[128].

HERVs are ancient retroviral genetic insertions accounting for 8% of human genome, whose reactivation has been associated with autoimmunity [129]. Several studies link SLE pathogenesis to HRES-1 [130], HERV-K10 [131], HERV-E clone 4–1 [132,133] and HERV-H [134], which may elicit an aberrant immune response through the release of immunogenic proteins, the activation of cytosolic platforms or the misactivation of the complement system.

Bahrami *et al*. tested with success a peptide, called Env59-GP3 and synthesized on the basis of the immunosuppressive (ISU) domain of the *env* protein of HERV-H, in a mouse model of experimental autoimmune encephalomyelitis (EAE) [135]. Interestingly, the Env59-GP3 peptide was effective in preventing the development of EAE in animals and in polarizing *in vitro* human macrophages toward a M2 tolerant phenotype. The *env59* gene is typically hyper-expressed in PBMCs of SLE patients and inversely associated with the expression of the IL-6 and TLR7 genes [134]: these observations suggest that the Env59-GP3 ISU peptide could potentially play a beneficial role in counteracting SLE inflammation and in ameliorating SLE clinical manifestations.

2.5. Discussion

Human derived or artificially synthesized therapeutic peptides might appear a safe and efficacious alternative to conventional agents currently considered the gold-standard treatment for SLE. Their main strength lies in the capability of specifically targeting unbalanced pathways in disease [26] and in an overall immunomodulatory rather than immunosuppressive effect. Additionally, some of them (DWEYS peptide, glatiramer acetate, blisibimod, and romiplostim) have a peculiar mechanism of action and are consequently able to prevent specific organ involvements or pathogenic pathways either in SLE or other immune-mediated diseases.

For most of these compounds research is still ongoing at a preclinical stage, while only two peptides (the 21-mer peptide P140 and the CDR1-based peptide) and a peptibody (blisibimod) entered the clinical phase of the investigation. Of note, the successful results obtained in preclinical studies were not paralleled by similarly enthusiastic findings in RCTs. This may be due to several reasons.

Firstly, any comparison between preclinical and clinical data is indeed very risky and challenging, since the two contexts noteworthy differ in drug doses and routes of delivery, disease phenotype, serology, co-medications, assessment methods, study design, time of observation and statistical analysis. For instance, in preclinical studies on MRL/LPR mouse models, the 21-mer peptide P140 in saline solution was intravenously given at a high dose (5 mg/kg), whilst the s.c. route was inefficacious [38]. On the contrary, in humans, the i.v. administration of the 21-mer peptide P140 was not tested and the s.c. dose used was considerably lower (3 mcg/kg). Moreover, additives and excipients may also account for different pharmacologic properties of peptides *in vivo* and indirectly influence the biologic effects in organisms [41].

Secondly, the endpoints of preclinical and clinical studies were often unmatchable, as in the first case they focused on peripheral blood hyper-cellularity, glomerulonephritis, autoantibody titers, neuronal damage, perivascular inflammatory infiltrates, and survival of SLE animal models, whereas, in the second case, they were based on the overall clinical improvement, measured through validated scoring systems at prefixed time-points. A prolonged survival of experimentally treated SLE individuals is obviously unpredictable with phase II and phase III studies; moreover, patients with severe renal and CNS involvement were excluded from clinical trials and renal biopsies not performed.

Thirdly, SLE patients represent a non-homogeneous category with polyhedral manifestations reflecting the numerous pathogenic pathways being simultaneously activated. The achievement of a complete remission of the disease, which should be the ultimate goal of treatments, is still a matter of debate in clinical trials and in real-life [8,136], while the achievement of a low disease activity seems to be more realistic [137]. As a consequence, the design of RCTs for SLE cohorts of patients is often challenging and prefixed outcomes are less likely to be satisfied.

Post-hoc analyses reported a better efficacy of clinically tested peptides in selected SLE cohorts, namely those patients having anti-dsDNA antibody positivity. Anti-dsDNA antibodies represent a marker of disease activity, being associated with some manifestations like glomerulonephritis and CNS symptoms [69]. Anti-dsDNA may, in fact, cross-react with several autoantigens, including components of the extracellular matrix of glomeruli or neuronal receptors and be responsible for organ-specific clinical manifestations [69,138]. However, it must be underlined that anti-dsDNA antibody titers broadly vary during the course of the disease, and that methodology for their measurement may further differ according to the laboratory (e.g. qualitative *vs*. quantitative methods) [139]. In addition, anti-dsDNA antibodies include Ig with variable isotypes and targets [140]. For instance, it has been shown that anti-dsDNA IgM are often associated with

skin manifestations, presumably due to the deposition of immune complexes, whereas the isotype IgG seems to be directly involved in the renal damage [141]. Also, it is debated whether antibodies binding DNA in its native conformation might have a true pathogenic role in SLE nephritis, or rather be a heterogeneous group of antibodies cross-reacting with native DNA and other components of glomeruli [140].

A separate story, instead, lies below the disappointing results observed during the clinical experimentation of the peptibody blisibimod [101,104]. This peptibody has an extremely different pharmacokinetic and pharmacodynamic profile compared to simple peptides. Differences include the mechanism of action, molecular structure, half-life, stability, and immunogenicity risk. In this regard, it may be argued that blisibimod, by counteracting the expansion and the activation of B cells, would constrain the synthesis of antibodies [101], including ADA. Nevertheless, immunogenicity is a complex matter, often involving immunologic mechanisms other than the solely humoral response [142]. In SLE, the engagement of the Fc of therapeutics with the FcR of phagocytic cells may unleash, through an impaired phagocytosis, the cascade of events leading to type I IFN production, and amplify, through this pathway, inflammation and tissue damage.

3. Conclusion

Better knowledge of the pathogenesis of SLE is expected to enrich the therapeutic armamentarium and facilitate the management of the disease. The use of peptides, specifically designed to target SLE-related epitopes or crucial pathways, may represent a novel fascinating opportunity. Given their good safety profile and immunomodulatory properties, therapeutic peptides could be added to standard of care, and, perhaps, allow the sparing of conventional drugs. In addition, their prescription might be tailored to specific subsets of patients having the highest likelihood of response. Nevertheless, despite the successful results observed in preclinical studies, RCTs showed a controversial efficacy profile concerning the use of these compounds in SLE. It is expected that future research, aiming at the amelioration of their physicochemical properties and at the improvement in the design of clinical trials, will bring more encouraging data on this innovative therapeutic panorama.

4. Expert opinion

The treatment of SLE still relies on a conservative approach, combining multiple unselective immunosuppressive agents [8] and, consecutively, increasing the risk of unwanted side effects. Unlike other rheumatic diseases, the licensed use of biologic agents, which electively inhibit a specific target, has been solely limited to belimumab. Rituximab failed to achieve the primary endpoints in RCTs conducted in SLE patients [12,13,143] but, due to encouraging real-life data [11], its off-label use is advised in resistant severe manifestations [8]. The potential use of novel biological agents and small molecules in SLE is still an object of clinical investigation [15–18]. Furthermore, several preliminary data on other small molecules acting as IFN receptor, NF-kB, or CXCR4 antagonists [115,144,145] spur research in this direction. Despite promising preliminary data, the immunogenicity of big molecules, like monoclonal antibodies, and the unselective effect of small molecules might represent a disadvantage in SLE. In the meantime, other innovative pharmaceutical compounds, including IL-2, artificially synthesized oligodeoxynucleotide (ASO) and retinoids, have been developed and tested in preclinical and clinical studies with encouraging results [146–149].

In this effervescent panorama, therapeutic peptides, able to interfere with the most crucial steps of SLE immunopathogenesis, may represent an additional intriguing pharmacologic strategy. Therapeutic peptides consist of short chains of amino acids displaying high target selectivity and potency, low toxicity, and a negligible risk of organ accumulation. On the other hand, due to their protein nature, these compounds are metabolically unstable, poorly bioavailable *per os*, rapidly biodegraded, and unable to cross plasma membranes [22,24]. Peptides can be easily produced from a known nucleotide sequence by means of recombinant DNA technology and then modified in order to enhance their pharmacologic properties. Most of therapeutic peptides designed for SLE treatment are synthesized on the basis of immunodominant epitope sequences that are pathogenic in SLE alone. Therefore, they exert an immunomodulatory effect on autoreactive pDC and lymphocytes, without affecting, instead, the immune response against pathogens [26].

To date, no therapeutic peptide has been licensed and marketed for the use in SLE patients. The 21-mer peptide P140 is the only one entering phase III RCTs, and, despite controversial results [36,40,41,43], its development is still ongoing. *Edratide*, synthesized on the basis of the hCDR1 expressing the major idiotype 16/6 Id, showed promising results in preclinical studies [48,50,54,55], but failed to meet the primary endpoint in a phase II RCT [57], with the following interruption of further clinical development. Both the two peptides appeared more effective in anti-dsDNA seropositive patients, and, although data are lacking, a beneficial role may be supposed in those SLE manifestations related to anti-dsDNA antibodies, such as glomerulonephritis.

Other peptides (pConsensus, laminin-derived peptide, nucleosomal peptides, DWEYS peptide, glatiramer acetate, TP-5) have been tested in SLE preclinical models, with promising findings [65,73,78,85,89,94]. The preliminary evidence gathered from these studies showed the amelioration of glomerulonephritis, the reduction in serum autoantibody titers, the prevention of neuronal damage, and a prolonged survival rate of SLE animal models. Whether these successful results may be translated in humans is an uncertain issue that needs to be addressed in future research.

The discovery of novel molecular targets is expected to enrich, in the next years, the panorama of therapeutic peptides for SLE, among which CXCR4 and STING antagonists and virus-derived peptides seem promising candidates [116,119,135].

Meanwhile, research is focusing on the optimization of the physicochemical structure of preexisting peptides with the intention to improve their pharmacologic properties, including bioavailability and half-life. One of these efforts consists of the

Table 3. Hypothetical future therapeutic indications of the peptides designed or designable for SLE.

Peptide	Plausible indications	Evidence
21-mer peptide P140	• Positivity and high titers of anti-dsDNA antibodies • Cutaneous and articular manifestations • Glomerulonephritis?	Preclinical and clinical studies (tested in RCTs on SLE patients; tested in SLE animal models and i*n vitro/ex vivo* experiments)
CDR1-based peptide	• Positivity and high titers of anti-dsDNA antibodies • Constitutional, cutaneous, and musculoskeletal symptoms • Hematologic involvement • Disease flares • Steroid sparing effect • Immunosuppressant sparing effect? • Glomerulonephritis? • Neurologic involvement? • Not effective in APS	Preclinical and clinical studies (tested in RCTs on SLE patients; tested in SLE animal models and i*n vitro/ex vivo* experiments)
pConsensus	• Positivity and high titers of anti-dsDNA antibodies • Poor compliance to parenteral therapy?	Preclinical studies (tested in SLE animal models and i*n vitro/ex vivo* experiments)
Laminin-derived peptide	• Positivity and high titers of anti-dsDNA antibodies • Glomerulonephritis	Preclinical studies (tested in SLE animal models and i*n vitro/ex vivo* experiments)
Nucleosomal peptides	• As vaccination in subjects at risk of SLE development (e.g. first-degree relatives) • Early and late stage of disease • Positivity and high titers of anti-dsDNA antibodies • Glomerulonephritis • Sparing effect on conventional agents	Preclinical studies (tested in SLE animal models and i*n vitro/ex vivo* experiments)
DWEYS peptide	• Neuropsychiatric lupus • Positivity and high titers of anti-dsDNA antibodies • Glomerulonephritis	Preclinical studies (tested in SLE animal models and i*n vitro/ex vivo* experiments)
Glatiramer acetate	• Neurologic involvement with demyelination	Preclinical and clinical studies (tested in RCTs and marketed for non-SLE patients; tested in SLE animal models and i*n vitro/ex vivo* experiments)
Thymopentin	• Highly immunosuppressed patients • Autoimmune lymphopenia • Glomerulonephritis?	Preclinical and clinical studies (tested in RCTs and marketed for non-SLE patients; tested in SLE animal models and i*n vitro/ex vivo* experiments)
Blisibimod	• Glomerulonephritis with moderate proteinuria • Hyper-gammaglobulinemia with high titers of anti-dsDNA and anti-cardiolipin antibodies • Hypo-complementemia • Fatigue • Steroid sparing effect	Preclinical and clinical studies (tested in RCTs in SLE patients; tested in SLE animal models and i*n vitro/ex vivo* experiments)
Romiplostim	• Autoimmune thrombocytopenia • To be avoided in APS	Preclinical and clinical studies, case series (tested in RCTs and marketed for non-SLE patients; tested in SLE patients in real-life)
CXCR4 antagonists	• HIV coinfection (anti-HIV activity) • Concomitant cancer • Neuropsychiatric lupus? • Glomerulonephritis? • To be avoided in pregnancy (risk of malformation)	Preclinical studies (tested in non-SLE animal models and i*n vitro/ex vivo* experiments); indirect evidence
STING antagonists	• Early phase of SLE? • To be avoided in severely immunosuppressed patients • Consider prophylactic anti-viral vaccinations?	Preclinical studies (tested in non-SLE animal models and i*n vitro/ex vivo* experiments); indirect evidence
Virus-derived peptides	• Concomitant viral infections (e.g. AIDS) • Anti-Sm and anti-RNP antibody positivity • Glomerulonephritis? • Neurologic involvement with demyelination?	Preclinical studies (tested in non-SLE animal models and i*n vitro/ex vivo* experiments); indirect evidence

Abbreviations: SLE, systemic lupus erythematosus; RCTs, randomized controlled trials; CDR, complementarity determining region; anti-dsDNA antibodies, anti-double stranded DNA antibodies; HIV, human immunodeficiency virus; APS, anti-phospholipid syndrome; AIDS, acquired immunodeficiency syndrome.

generation of peptidase-resistant compounds that could be administered *per os*, with a considerable impact on treatment adherence [67]. Additionally, the high target selectivity of peptides can be combined with the molecular stability and the optimal oral bioavailability of small molecules, thus giving rise to the formulation of hybrid compounds, known as peptidomimetic small molecules.

Peptibodies, consisting of fusion proteins retaining a peptide sequence grafted onto the Fc portion of a Ig, represent another fair example of peptide molecular engineering [29]. However, in spite of a reduced clearance and a better chemical stability, the interaction of the Fc of peptibodies with the FcR of immune cells may increase inflammation, trigger immunogenicity, and favor the removal of the drug by the reticuloendothelial system. This may explain the controversial efficacy profile in SLE of the peptibody blisibimod observed in RCTs [101,104]. However, despite failing to achieve the primary endpoints in clinical investigation, blisibimod showed

some interesting aspects, including the steroid sparing effect, the effectiveness in more severe forms of disease, and the capability of ameliorating some laboratory parameters, including proteinuria, anti-dsDNA and anti-cardiolipin antibody titers and hypo-complementemia. Although immunogenicity of peptibodies may represent a warning, RCTs on blisibimod showed no safety issues compared to placebo.

Unconjugated peptides might appear even safer than conjugated peptides, as they undergo a rapid proteolysis, generating harmless simple amino acids [40,57].

Based on these considerations, therapeutic peptides would eventually offer several advantages in SLE treatment that may be resumed in the following scenarios: 1) according to mechanism of action, they could be used for selected populations of SLE patients, like those characterized by active glomerulonephritis, CNS involvement [85] or high autoantibody titers, thus allowing a personalized medicine; 2) they could synergistically strengthen the efficacy of steroids, conventional DMARDs, and immunosuppressants [61,62], allowing dose-reduction or even discontinuation of these drugs once a low disease activity is achieved; 3) given their rapid catabolism, they could temporary be used to manage SLE flares, avoiding the risk of drug accumulation and undesired toxicity during the remission phases of the disease [57,119]; 4) they could be combined in a cocktail to be administered as a vaccine in subjects at risk of SLE, in order to restore the immune tolerance and prevent disease development [80]; 5) thanks to the acceptable safety profile, they could be associated with other conventional medications in those patients being concomitantly diagnosed with SLE and another autoimmune disorder, who may less benefit from highly SLE-specific compounds [56]. Finally, the introduction in future pipelines of more sophisticated hybrid compounds like peptibodies or peptidomimetic small molecules is expected to rewrite the chapter of SLE peptide therapy, providing renewed drugs with a better pharmacologic profile and increased efficacy.

A hypothetical list of specific therapeutic indications of the peptides designed or designable for SLE, whose preclinical and clinical evidence has been discussed in this review, is provided in *Table3*.

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