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Harnessing the potential of CAR-T cell in lupus treatment: From theory to practice

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ABSTRACT

Systemic Lupus Erythematosus (SLE) is a rare, heterogeneous, potentially life-threatening autoimmune disease. Presence of kidney or other major organ (brain, heart or lung) involvement are predictors of poor outcome and in a subset of patients resistant to 1st or 2nd line conventional treatment. The 10-year mortality remains around 10–15 %.

Chimeric Antigen Receptors (CAR) are molecules that allow to redirect the engineered immune cells towards specific target antigens and to simultaneously boost their activation. Following breakthrough results observed in the treatment of hematological malignancies, conventional CAR T-cell therapy has recently been applied to refractory SLE patients. Compared to the use of monoclonal antibodies, anti-CD19 CAR T-cells allow to achieve deeper depletion of autoreactive B cells, notably at site of inflamed tissues and lymphoid organs (i.e. lymph node), to suppress interferon signature and to restore the immune tolerance with the reemergence of naïve B-cells with a new repertoire.

All clinical data reported in SLE patients so far showed that autologous anti-CD19 CAR T-cell treatment allowed impressive short- and longer-term resolution of lupus nephritis and other severe disease-related manifestations, without major toxicities and only mild cytokine-release syndrome. These clinical effects persisted after B-cell reconstitution and were associated with normalization of double-stranded DNA antibodies and complement levels in drug-free patients until three years after the procedure. Overall, these pioneering experiences show unique clinical and immunological response to CAR T-cell therapy in SLE, and the need for extended follow-up to determine its long-term efficacy.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a rare and highly heterogeneous multi-systemic autoimmune disease [1,2] with a prevalence from 20 to 150 cases for 100,000 persons according to countries, ethnicity,

sex and age [1–5]. It affects a population of more than 3.4 million worldwide, predominantly young women before the 4th decade of life, all races, with a higher incidence and more severe forms among African, Hispanic, or Asian descendants [3–5]. SLE is characterized by successive periods of flares and remission, which “wax and wane” in duration and

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severity. Most of these variations are related to the patients' genetic background and exposure to local environments.

SLE is diagnosed on clinical grounds in the presence of characteristic serological abnormalities. Classification into SLE requires a score of 10 or higher (theoretical maximum of 91) to meet the new European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) classification criteria [6], that are also applicable to early SLE. Skin and articular symptoms are common, and at the other end of the spectrum patients present with severe lupus nephritis (LN) [7], autoimmune cytopenia, heart, lung, or central nervous system involvement [8,9], which can lead to life-threatening complications and even death. Renal involvement occurs in 30 to 60 % of adult patients. LN is based on glomerular immune complex deposition [7] with five histological types of glomerulonephritis according to the type and extent of active inflammatory and chronic lesions [10]. Despite important progress in earlier diagnosis and treatment of LN [11], diagnosis of a class III (focal proliferative), IV (diffuse proliferative) or V (membranous) glomerulonephritis can still lead to long-term renal damage.

The disease course varies highly among individual patients and can be relapsing remitting or persistently active. Consequent morbidity, altered quality of life and disability accumulate over time, while damage accrual contributes to progressive deterioration in renal, cardiac or pulmonary functions and eventually lead to organ failure and premature death [12]. SLE can become clinically quiescent, but serologically active, or going into prolonged remission [13]. Overall, SLE patients survival rates range from 83 % to 93 % after 10-years and decrease to 76–80 % at 15 years [12,14]. Evolution remains poor in a subset of patients with a 10-year mortality of 10–15 % [15]. In a recent 10 years retrospective (2012–2020) population-based study of 4283 prevalent SLE cases in Italy, accelerated cardiovascular disease, neoplasm, infections were the leading causes of death, followed by kidney disease and SLE per se [14]. These numbers stress the importance of long-term perspectives when initiating SLE therapy. Major organ involvement, and persistent disease activity are predictors of poor outcome, with increased risk of death in female patients aged <40 years and in African–American ethnicity. In the Euro-Lupus cohort, the respective 5- and 10-year survival rates were 95 % and 93 % and nephropathy was the only risk factor associated with a lower survival probability [16]. Active LN progresses to end stage renal disease (ESRD) in 10 to 30 % and LN and ESRD remain the most important predictors of death in this population [14,16].

Despite extensive efforts to develop novel therapies for SLE, refractory patients and those requiring life-long medical treatment suffer from cumulative acute and long-term treatment-related toxicities [9,17]. For SLE subjects resistant to 1st and 2nd line conventional treatment, more effective therapies with fewer long-term side effects are needed. Based on new immune-effector cell therapeutic strategies [18], the use of Chimeric Antigen Receptor T-cell therapy (CAR T-cell) has recently been applied to reset tolerance in such patients.

2. SLE pathogenesis and loss of tolerance

SLE is a multifactorial disease with individual genetic susceptibility, where various epigenetic, environmental exposure and hormonal factors all contribute to abnormal activation of both the innate and adaptive immunity responses [1,19,20]. Defective apoptotic cell clearance with accumulation of apoptotic remnants contribute to the loss of self-tolerance against nuclear antigens and the production of antinuclear antibodies (ANA), chronic activation of autoreactive lymphocytes, that are normally quiescent, with consequent tissue infiltration and damage [20,21].

Loss of tolerance after stimulation by environmental factors in genetically susceptible individuals may be the initiating event in SLE [19]. Recognition and internalization of apoptotic bodies and nucleic acids by monocytes, classical (cDCs) and plasmacytoid dendritic cells (pDCs) through their membrane Fc-Receptor are considered as early

event [22]. In parallel, activation of the innate immune system triggers cytokine secretion, resulting in enhanced antigen presentation to T-cells and release of proinflammatory cytokines (IFN-alpha, Tumor Necrosis Factor (TNF), Interleukin (IL)-7, IL-6, IL-1, IL-18). This first cycle can induce activation of the adaptive immune system and autoantibody production [19]. Complexes of autoantibodies and nucleic acids or cellular debris are formed, which provide the second level of innate immune system stimulation via Toll-like receptors. Subsequently, autoreactive B-cells, recognizing nucleic acid or apoptotic cells through their B cell receptor (BCR), can serve as antigen-presenting cells and contribute to recruit more autoreactive T-cells. These positive feedback loops of innate and adaptive immune systems amplify the proliferation of autoreactive lymphocyte clones during SLE preclinical stage. Initiation of the clinical phase requires systemic inflammation and injury of target organs, which further amplify the immune activation process. Overtime, SLE becomes increasingly resistant to immune-modulating therapies with non-reversible changes, related to organ damage [23].

Specific considerations deserve attention to understand the longevity and persistence of the aforementioned cycles:

- 1 Cell damage, favored by environmental factors, exposes the immune system to self-antigens, thereby leading to T- and B-cell activation, which become self-sustained by a chronic self-aimed immune response. Cytokine release, complement activation, and autoantibody production all contribute to SLE organ damage.
- 2 The innate immune system activation is either Toll-like receptor (TLR) dependent or independent. Both self-DNA/RNA and foreign DNA/RNA, such as from viruses, can lead to TLR-independent pathway activation.
- 3 NETosis has gained attention in SLE pathogenesis [24]. Once activated by various factors, namely cytokines, activated platelets and vascular endothelial cells, the neutrophils, in particular low-density granulocytes (LDGs), systematically release their nuclear aggregates in the extracellular environment. These nuclear aggregates or neutrophil extracellular traps (NETs) contain immunostimulatory proteins and auto-antigens, including double-stranded deoxyribonucleic acid (DNA), and contribute to promote interferon (INF)-alpha production by the dendritic cells. They also mediate vascular thrombosis and damage [25] with increased levels of proinflammatory cytokines, and serve as self-antigens for T-lymphocytes [22].
- 4 T and B-lymphocytes play a central role in SLE. T-cells in SLE display distorted gene expression and lower IL-2 production, that contribute to altered regulatory T-cell production [26] and to a pro-inflammatory immune response [20]. Increased Interferon- γ leads to activation of autoreactive B-cells by CD40L and cytokine production. Upon interaction with DNA and RNA, TLRs directly activate B-cells, and the nucleic acid and protein-containing intranuclear complexes are the most prominent autoantigens leading to B-cell activation [27]. The pathogenic role of autoantibodies is supported by the correlation of autoantibody titers and SLE flare. B-cells can also serve as antigen-presenting cells and activate T-cells by presenting internalized soluble antigens to T-cells. This process creates a loop where both B- and T-cells activate each other, with enhanced autoimmunity [19,20,26]. Another important aspect of pathogenic B-cell activation in SLE involves germinal center (GC) and extra-follicular pathways [28–30].

3. SLE treatment at a glance

SLE treatment classically relies on the use of corticosteroids and conventional or biological immunosuppressive drugs to control disease activity and to prevent relapses [9]. Despite treatment, disease activity flares and relapses may recur over time and require treatment escalation with more potent immunosuppressive regimen. The definition of Refractory SLE (RL) may be contrived, as it depends on a treatment

response, while adequate SLE management depends on choosing the most appropriate therapeutic agent, but also on its availability and prompt administration [9,17]. Risk factors for poor prognosis include RL, delayed or unjustified aggressive treatments [31,32], and also therapeutic non-compliance. Additionally, drug-drug interference influence the bioavailability of the ongoing treatments. Also, toxicities observed with some treatments has dampened their expected benefits [33].

3.1. Conventional immunosuppressants

Anti-malarial drugs, such as hydroxychloroquine, are recommended for all patients, unless contraindicated, to maintain low disease activity and prevent recurrent flares. Glucocorticoids, when needed, are dose-based on the type and severity of organ involvement and should be reduced to maintenance dose of ≤5 mg/day (prednisone equivalent) and ultimately withdrawn. In patients with (i) severe organ involvement, (ii) no response or relapse despite hydroxychloroquine alone or in combination with glucocorticoids or (iii) in those unable to reduce glucocorticoids below acceptable doses, the addition of immunomodulating/ immunosuppressive agents (eg, methotrexate, azathioprine, or mycophenolate mofetil (MMF)) and/or biological agents (eg, belimumab as

anti-BAFF, anifrolumab) or voclosporin should be considered [8,9]. For the last 20 years, only the anti-BAFF monoclonal antibody has gained indication in moderately active SLE [34], and more recently anifrolumab (a monoclonal antibody against type I IFN-receptor subunit 1) for severe cutaneous forms [35,36] and voclosporin (a novel calcineurin inhibitor) for active LN [37] in addition to MMF and steroids. Of note, these three drugs were approved as add-on to standard of care (SOC). In patients with more severe organ-threatening or life-threatening disease, intravenous cyclophosphamide (CYC) should be considered according to the Euro lupus protocol [8,9,16].

3.2. B cells and plasma cells as therapeutic targets to restore tolerance x

During their differentiation, B cells express different surface molecules, which can be used as therapeutic targets, namely: a) CD20, which is expressed from the pre-B cell state to memory B cells, but is lost early during plasma cell (PC) differentiation; b) CD19, which is expressed on a larger spectrum of B-cell subsets, from pro-B cells to plasmablasts, and on short-lived PC unlike on terminally differentiated bone marrow long-lived PC; c) BCMA, and CD138 (syndecan-1), that are conversely induced during PC differentiation and are highly expressed on long-lived PC [38]. SLE pathogenic B cells include in particular atypical

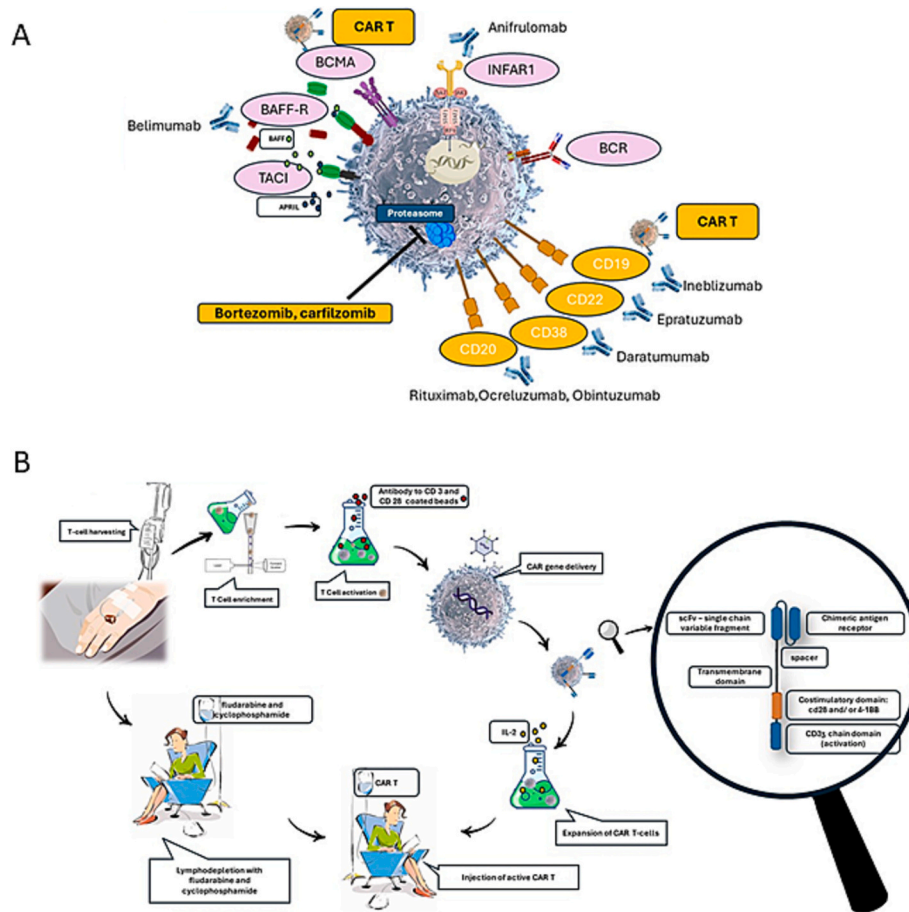


Fig. 1. Chimeric Antigen Receptor (CAR)T-cells therapy for Systemic Lupus.

A. B-cell targeting therapies in Systemic Lupus Erythematosus (SLE):

TACI, Transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor; BAFF, B cell activating factor; BCMA, B cell maturation antigen receptor; CAR T cell, Chimeric antigen receptor T cell; INFAR1, Interferon-alpha/beta receptor 1; BCR, B cell receptor;

B. CAR T cell therapy step by step.

Lymphocytes are isolated from the peripheral blood (PB) through leukapheresis. Cell enrichment is done by density gradient separation, counterflow elutriation, and antibody-based selection. Subsequently, T-cells are activated using CD3 and CD28 coated beads. Clinically applied CD19-specific CARs are generated by the fusion of a scFv, derived from an anti-CD19 monoclonal antibody, to an intracellular signaling. CAR gene is then delivered to T-cells using lentiviral or gamma-retroviral vectors. Expansion of CAR T cells is induced by interleukin 2 (IL-2). Finally, the patient is treated with cyclophosphamide and fludarabine to achieve lymphodepletion before the activated CAR T cells are injected back to the patient.

IgD^{neg}CD27^{neg} double negative (DN) memory B cells, which are activated i) in part through the germinal center reaction, producing most of autoreactive long-lived PCs, ii) preferentially through the extrafollicular response in an IFN- and TLR7-dependent manner which differentiate mainly into short-lived PCs producing autoantibodies. The exact role of follicular versus extrafollicular reactions and the subset of antibody-producing cells involved the generation of the various pathogenic autoantibodies in SLE remains unclear [39], but is a key determinant of the variable impact of the targeting of CD20, CD19, or BCMA [22,28–30]. (Fig. 1A). The pathogenic role of B cells in SLE has focused many therapeutic targets.

3.2.1. B cell-directed therapies

Despite its wide use in SLE, rituximab, which target the surface molecule CD20, failed to achieve consistent and long-term outcome results in two randomized controlled trials [9,40,41]. Rituximab, which depletes B lymphocytes *in vivo*, from the pre-B-cell stage in the bone marrow, when CD20 is first expressed, until the mature-B-cell stage, has good depleting-activity on circulating B-cells but results in only partial depletion within tissues [42]. Deep peripheral blood B-cell depletion with anti-CD20 monoclonal antibodies [43] (rituximab, ocrelizumab or obinutuzimab) or anti BAFF-therapy that allows partial naïve B-cell depletion and inhibition of B-cell activation can reduce the disease activity towards either a Lupus Low Disease Activity State (LLDAS) [44] or a clinical status corresponding to the Definition Of Remission In SLE (DORIS) [45].

Antibody-dependent cell death requires complement activation (CDC) or effector cells for phagocytosis (macrophages) or cytotoxicity (NK cells), that may be altered in SLE patients. Therefore, persistence of autoreactive resident B cells, within secondary lymphoid organs, inflamed tissues, and bone marrow, may limit the efficacy of rituximab [43]. Furthermore, CD20 is not expressed by plasmablasts nor by PCs, which are involved in autoantibody production. After B-cell depletion with rituximab, the reconstituting B-cell compartment may be functionally immature with a preponderance of naïve and transitional B-cells and a paucity of memory B-cells [46]. The magnitude, duration and clinical effects of these factors are highly variable among patients and hard to predict in clinical practice [43,46,47].

3.2.2. Plasma cells directed therapies

The use of bortezomib (a proteasome inhibitor) and daratumumab (humanized anti-CD38 monoclonal antibody) aim to target the long-lived autoreactive PCs. Their relevance for using bortezomib [48] and daratumumab [49] in SLE was established in preclinical studies. Few SLE treated patients have yet been reported with substantial clinical benefit in several cases, but also with important adverse effects (AE) [35,37,48,50–53].

Proteasome inhibition, with bortezomib and also with carfilzomib, was shown to deplete short-lived and long-lived PCs and to reduce nephritis and prolong lupus-prone mice survival [48]. In twelve severe SLE patients with sustained disease activity and autoantibody production despite immunosuppressors [51], bortezomib administered with steroids, according to the approved protocol for multiple myeloma, reduced or stabilized disease activity on maintenance therapy at 6 months, with reduced anti-double strand DNA (dsDNA) and vaccine-induced protective antibody titers and reduced numbers of peripheral blood and bone marrow PCs (~50 %). AE in 92 % of patients, including infections and polyneuropathies, resulted in premature drug discontinuation in almost half of patients.

Daratumumab has been used in eight severe refractory SLE patients so far [50,53]. In two cases, clinical and serologic responses occurred until one year under daratumumab monotherapy with anti-dsDNA antibodies seroconversion, decreases in levels of IFN-gamma and soluble B-cell maturation antigen (BCMA) and CD163 and increases in C4 and interleukin-10 (IL10) levels [50]. In the six other patients with refractory LN, treatment had no effect and was discontinued after 6

months [53]. These findings illustrate the interest of targeting PCs, but results must be confirmed in controlled trials and tolerance improved.

3.3. Hematopoietic stem cell transplantation and reset of immune tolerance

The rationale for Hematopoietic Stem Cell Transplantation (HSCT) in SLE derived from Ikehara pioneering work in 1985, who used experimental bone marrow (BM) transfer from SLE-prone mice into normal recipients to demonstrate that autoimmune diseases originate from the BM, and not from the thymus [54]. In genetically determined New Zealand Black/White (NZB/B) lupus-prone mice, non-myeloablative conditioning and allogeneic HSCT from a normal mouse prevent SLE disease and autoantibody production, while complete lymphodepletion and allogeneic HSCT allowed regression of proteinuria and LN [55]. Similar results were obtained in the Murthy Roth large-lymphoproliferative (MRL-lpr) mouse model, where myeloablative conditioning and also syngeneic HSCT allowed prolonged remissions, although anti-DNA antibodies decreased, but did not disappear, and mice eventually died after one year. Considering the risk of Transplant Related mortality (TRM) associated with allogeneic HSCT, autologous HSCT (aHSCT) was preferred for autoimmune diseases, unless a hematological malignancy coexisting with the autoimmune disease would indicate an allogeneic HSCT. Since the first successful aHSCT for SLE [56], around 350 aHSCT for SLE patients were performed worldwide in three decades with the two largest experiences from the EBMT multi-center data registry ($n = 53$) [57] and Chicago single center trial ($n = 50$) [58]. The 5-year disease free survival varied between 29 % and 50 %, while the overall and TRM ranged from 0 % to 25 % [58,59]. Durable treatment-free responses were noticeable in patients refractory to all immunosuppressors and in those not achieving remission, increased responsiveness to treatment which had previously failed was noted. TRM gradually improved. A recent Chinese study in 22 refractory LN patients reported 5 % TRM, with 82 % progression free survival at 6 years and 45 % relapse rate at 10 years [60]. While patient selection, conditioning regimen and center effect all contribute to TRM after aHSCT for AD [61], these severe refractory SLE patients are highly fragile and at high risk of mortality.

Unique immunological changes with effective reset of *de novo* immune tolerance were demonstrated after aHSCT in these SLE patients, with: a decrease or disappearance of autoantibodies, normalization of the restricted T-cell repertoire, a sustained shift in B-cell subpopulations from memory to a naïve B-cell dominance, disappearance of circulating plasmablasts, True immunological remission was demonstrated for the first time in this disease, and both CD4^{pos} CD25^{hi}FoxP3^{pos} and an unusual CD8^{pos} FoxP3^{pos} Treg subsets returned to normal levels after aHSCT with almost complete inhibition of pathogenic T-cell response to peptide autoepitopes from histones. In addition, the posttransplant CD8^{pos} Tregs showed autoantigen-specific and non-specific suppressive activity, which was contact independent and predominantly TGF-dependent [62]. Such findings suggest that HSCT treatment in SLE allows to 'reset the immunological clock' with prolonged clinical remission, that were life-long in a few cases.

4. Chimeric antigen receptor (CAR) cells: a new adoptive cell and gene therapy (CGT)

CARs are engineered synthetic receptors, that redirect lymphocytes, generally T-cells, to recognize and eradicate the targeted cells that express a specific antigen [63–65]. The effectiveness of anti-CD19 CAR T-cell therapy against some B cell malignancies, such as B cell acute lymphocytic leukemia (B-ALL) and aggressive lymphomas, led to their first therapeutic approval by the US Food and Drug Administration (FDA) in 2017 [66].

4.1. CAR T-cell structure

CARs consist essentially of four main components: (a) *the CAR extracellular target antigen-binding domain*, is the portion of the CAR that confers its target antigen specificity towards CD19, BCMA or another antigens; (b) *the CAR extracellular hinge or structural region* extends the binding units to the transmembrane domain, and also provides increased flexibility to overcome steric hindrance and with extended length to allow the antigen-binding domain to access the targeted epitope; (c) *the CAR transmembrane domain*, which major function is to anchor the CAR to the T-cell membrane; (d) *the CAR intracytoplasmic signaling domain* is the functional end of the receptor, composed of i) the CD3 zeta-chain of the T-cell receptor (CD3 ζ), that includes three immunoreceptor tyrosine-based activation motifs (ITAMs) and ii) the intracellular portion of one or more co-stimulatory domains: CD28, 4-1BB, and OX-40, which are critical to initiate signal transduction and T-cell activation when the CAR binds to its target antigen [67].

4.2. CAR T-cell manufacturing

Manufacturing clinical-grade CAR T-cells is a multi-step process (Fig. 1B), including:

1. CAR vector design and production: lentiviral or gamma-retroviral vectors are used to transfer CAR genes to T-cells. Protocols are safe and optimized, but require time and expense needed to ensure that all viral vectors are replication-deficient. Therefore production of CAR T-cells is mostly centralized [68]. Non-viral transposon-based gene delivery systems, used in some pre-clinical studies, appear simpler with no infectious potential, and allow CAR T-cells to be produced by hospitals [69]. Genome editing tools such as CRISPR/Cas systems allow to overcome many limitations associated with virus-based gene-delivery techniques and to facilitate off-the-shelf production with increased efficacy, and minimize CAR T-associated toxicities [70].
2. T-cell harvesting: Autologous or allogeneic lymphocytes are isolated from the peripheral blood (PB) through leukapheresis or directly from the whole blood. Autologous lymphocytes are currently the only source used on clinical level [71].
3. Cell enrichment: by density gradient separation, counterflow elutriation, or antibody-based selection.
4. T-cell activation: T-cell traditional activation, proliferation, and differentiation through agonizing and triggering both CD3 and CD28 pathways in vitro can expand cells in a unique manner based on how both signals are presented to human T-cells and which cytokines are provided [72].
5. CAR gene delivery: The CAR is responsible for cell recognition and lysis, cytokine secretion [68,73,74]. T-cell isolation may be divided into cell collection from leukapheresis or from whole blood and subsequent cell enrichment. T-cells are then activated using CD3/CD28 antibodies. Similar process is applied for manufacturing other types of CAR T-cell, such as anti-BCMA CAR T-cells.
6. Expansion of CAR T-cells during cell culture processing: nutrients, growth factors and constituents used to maintain the osmolarity and pH buffering are essential for optimal growth of the expanded cells. Cytokines, as IL-2, can also be added to enhance the differentiation of naïve T-cells towards terminally differentiated T-effector cell with higher cytotoxic function [75].

4.3. CAR T-cell generations and subtypes

Several generations of CAR T-cells have been designed for clinical applications. *First generation CAR T-cells*, that contained a CD3 ζ or FcR γ signaling domain for signal transduction, had low in-vivo persistence [76] and are no longer used [76–78]. *Second-generation CAR T-cells* are equipped with an extracellular antigen-recognizing domain combined

with two intracellular domains: CD3 ζ plus a costimulatory domain, e.g., CD28-CD3 ζ or 4-1BB-CD3 ζ , which represent the two most frequent costimulatory molecules in use. *The third-generation CAR T-cells* are combined with three intracellular domains: CD3 ζ along with two (or more) costimulatory domains, such as CD28, 4-1BB (CD137) and OX40 (CD134) [73,79]. Fourth and fifth generations are yet to be used in pre-clinical in-vivo studies. *The fourth generation CAR T-cells* include a diversified group of CAR T constructs embracing armored CAR T-cells in order to disrupt the programmed cell death protein 1 (PD-1) pathway, cytokine-expressing CAR T-cells, switchable CAR T-cells, and universal CAR T-cells. *Fifth-generation CARs* consist of a novel co-stimulatory domain to activate some specific signaling pathways along cytokine signaling, that can support their own growth and persistence [76–80].

In SLE patients so far, only second generation autologous CAR T-cells [81,82] have been infused for compassionate care or are currently being tested in a number of clinical trials (Table 1). The first eight SLE patients treated in Germany received the Myltenyi Biotec (MB)-19.1 CAR T-cell, using a second generation lentiviral vector, that includes a single-chain variable fragment sequence derived from the murine anti-human CD19 FMC63 (MB) for the transduction [81,82]. Second-generation fully human antiCD19 CAR vector construct, as proposed by Kyverna Hu19-CD828Z, aims to maintain target cytotoxicity with the CD28+ co-stimulatory domain and a CD3zeta activation domain. This product has been successfully used in oncology with a lower risk of neurotoxicity and similar efficacy [83,84], and is also being tested in LN patients in the US and in Europe. Interestingly, Hu19-CD828ZCART-cells generated from SLE patient lymphocytes demonstrate CAR-mediated and CD19-dependent activity against autologous primary B cells with reduced inflammatory cytokine production [85].

While all the aforementioned CAR T-cell therapies are autologous, manufacturing “universal” CAR T-cells derived from healthy allogeneic donor T-cells and isolated from peripheral blood or umbilical cord blood (UC) is being developed. The use of such allogeneic CAR T-cell appears attractive, especially for heavily pre-treated patients with treatment-or disease-related lymphopenia and insufficient lymphocytes count for CAR T production [71].

5. CAR T-cell therapy in SLE

CAR T-cells therapy has the following theoretical advantages over the other B-cell depleting therapies in SLE:

- *Wider range/ spectrum of B cell depletion*, as they target CD19, which has a functional role in B-cell activation and is a marker present on most B-cells throughout their development, including pro-B cells, pre-B cells, immature B cells, mature B cells, activated B cells, memory B cells, and plasmablasts.
- **Mechanism of Action:** Unlike traditional therapies that may rely on passive mechanisms, CAR T-cells direct cell-to-cell contact cytotoxic interaction can lead to more robust and sustained responses.
- **Deeper B cell Depletion** - CAR T-cells can achieve more comprehensive depletion of B-cells in various tissues due to their ability to invade tissues like skin, kidneys, and even bone marrow and lymph nodes.

5.1. CAR T-cell treatment in murine lupus models

5.1.1. Second generation standard CAR T-cells in experimental SLE

Two SLE mouse models (NZBxNZW and MRLfas/fas) were initially used to test CD19-targeted CAR T-cell, using an experimental design similar to a standard CAR-approach, except for the selected use of CD8+ T-cells, to avoid potential disease-enhancing effect of autoreactive CD4+ T-cells in this setting [86]. The 1D3 CD19-targeted CAR, consisted of the 1D3 extracellular single-chain Fv domains, linked to the CD28 transmembrane and cytoplasmic signaling domains, and of a variant

Table 1
Chimeric Antigen Receptor (CAR) T-cells in Systemic Lupus Erythematosus (SLE): main characteristics of the published cases.

	IUPUS and simultaneous diseases	CAR T-cell	Lymphodepletion	CRS/ ICANS	Other toxicities	disease response	follow up
Zhang et al. (2021) [99]	SLE + stage IV diffuse large B-cell lymphoma; (n = 1)	CD19-BCMA compound CAR	Flu (not specified) and Cy(not specified)	none	none	- SLE remains stable (lymphoma in remission) - Nuclear, cytoplasmic, and granular ANA reached undetectable levels by 9, 12, and 37 weeks post-CAR T	23 months
Mougiakakos et al. (2021) [81]	SLE (active LN); (n = 1)	CD19 CAR, 4-1BB co-stimulatory domain	Flu 25 mg/m ² /d i.v. on D -5, -4, -3 and Cy 1000 mg/m ² i.v. on D -3	none	none	Clinical remission (proteinuria, SLEDAI), serologic remission (dsDNA Antibody; C3/C4)	1.5 months
Mackensen et al. (2022) [92]	SLE (multiorgan involvement, LN in all); (n = 5)	CD19 CAR, 4-1BB co-stimulatory domain	Flu 25 mg/m ² /d i.v. on D -5, -4, -3 and Cy 1000 mg/m ² i.v. on D -3	CRS grade1 (3/5); no ICANS	no infections	Resolution of nephritis and disease-related symptoms, serologic remission (5/5)	8 months
Müller et al. (2024) [82]	SLE (n = 8),	CD19 CAR–transduced CD4- and CD8-enriched T-cells	Flu (25 mg/m ² D -5 -4 -3) and Cy (1000 mg/m ² D -3);	CRS grade1 (8/15) ICANS (1/15)	none	DORIS remission (100 %)	15 months
Krickau et al. (2024) [102]	SLE (n = 1), 15 years old, LN	CD19 CAR-T lentiviral vector, 2nd generation	Flu (12.5 mg/m ² D -5 -4 -3) and Cy (500 mg/m ² D -3);	CRS grade 1	transient grade 4 granulocytopenia	- Symptoms of arthritis resolved, - factors C3 and C4 normalized within 6 weeks, - anti-nuclear-antibody anti-dsDNA and all other autoantibodies disappeared - hemodialysis-free after day 17	6 months
Wang et al. (2024) [101]	SLE (n = 13), LN classes III–V	BCMA-CD19 CAR-T	- Flu (0.03 g/m ²) + Cy (0.3 g/m) (n = 2) - Cy alone (0.3 g/m) (n = 11)	CRS grade 1 or less (n = 13), no ICANS	Grade 1 urinary tract infection (n = 1)	- symptom and medication-free remission: (n = 12) ↓ SLEDAI	46 months

ANA: Antinuclear antibodies; BCMA: B-cell maturation antigen; CRS: Cytokine Release Syndrome; Cy: Cyclophosphamide; DORIS: consensus on a definition of remission in SLE; dsDNA: double strand DNA; Flu: Fludarabine; ICAN: immune effector cell-associated neurotoxicity syndrome; LN: Lupus nephritis; SLE: Systemic Lupus Erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

CD3ζ C terminus. Recipient mice were conditioned by irradiation. The CD19-targeted CAR was transduced into splenic CD8 + T-cells using either the amphotropic murine leukemia virus (A-MLV) retrovirus (n = 10), the vesicular stomatitis virus G glycoprotein (VSV-G)–pseudotyped MLV (n = 9) or the VSV-G–pseudotyped lentivirus (G-LV) (n = 10). The CD19-targeted CAR T-cells successfully expanded, with decreased lupus symptoms and splenomegaly and prolonged survival in both models. The various viral vectors showed different efficiencies of B cell depletion. Marked reduction of immunoglobulin light-chain message in kidneys and depletion of circulating anti-DNA antibodies in treated mice support the potential of CD19-targeted CAR T-cell treatment to mitigate one particular type of disease-related plasma cells, while sparing other long-lived plasma cells in the bone marrow. However, RNA analysis showed that CD19-targeted CAR T-cells do not deplete all splenic B cell populations or long-lived PC in the bone marrow. Over time, the CAR T-cell–treated mice accumulated a CD62L^{hi}CD44^{hi} double-positive CD8+ T-cell population, which expressed the CD19-targeted CAR, thereby arguing for its contribution to sustained B cell depletion. These markers serve to identify human T central memory cell population, which has the capacity for self-renewal and differentiation into effector T-cells. Using these approaches, it was shown that CD8+ T-cells from NZB/W and MRL-lpr mice correctly carry out the cytotoxic function of the CD19-directed CAR [86]. Overall results showed that CD19 CAR T-cells effectively deplete B cells in this mouse model and prevent disease progression when transferred before disease onset.

Another study using a syngeneic anti-mouse-CD19 CAR, with either CD28 or 4-1BB costimulatory domains injected in MRL-lpr mice, before and after SLE disease onset, showed effective in vivo, B cells depletion with prolonged mice survival, improved lupus symptoms and delayed SLE progression [87].

Of note, no standard pre-clinical BCMA-CAR T-cell-based studies

using specifically SLE models was reported.

5.1.2. CAR Regulatory T-cells (Tregs) in experimental SLE

Other CARs involving antigen-specific regulatory T-cells (Tregs), a highly heterogeneous population with suppressive functions, are explored in autoimmune diseases [18]. Tregs are CD4+ T lymphocytes, which inhibit autoimmunity and support long term tolerance, notably through their homing ability towards the cognate antigen in target tissue and lymphoid organs. Tregs, which constitutionally express the IL-2 Receptor alpha (CD25) and are highly enriched in the fraction of CD4^{pos}CD25^{hi} cells, are identified as CD127^{lo} and FoxP3^{pos} cells.

Antigen specificity can be conferred by transducing T-reg cells with recombinant TCR. TCR-engineered T-reg cells recognize peptides from both the intracellular and the surface derived proteins and also show a high affinity to cognate and liaise with the major histocompatibility complex (MHC)-peptides. The transduced cells for creating the CAR Tregs may be isolated from polyclonal T-cells, CD4+ T-cells or Tregs. Additionally, Tregs have been engineered by co-transducing CD4+ or CD3+ T-cells with CAR constructs and FoxP3 cDNA. This strategy may resolve the low population of primary Tregs in peripheral blood and loss of FoxP3 expression in endogenous Tregs [88]. Recently, CAR-Tregs were successfully engineered to overexpress FoxP3 with harboring an anti-CD19 CAR after using a bidirectional lentiviral (LV) vector encoding for an anti-CD19 CAR constituted by a spacer region derived from the extracellular domain of the human low-affinity nerve growth factor receptor (LNGFR), the CD3zeta chain, and the CD28 intracellular portion as most active costimulatory domain. When using an *ad hoc* humanized mouse model of SLE, a single infusion of FoxP3CAR-Tregs reduced anti-dsDNA development, delayed lymphopenia, restored the immune system composition in the BM and the spleen, and reduced inflammation in SLE target organs showed with good safety profile.

Overall, CARs appear effective in redirecting the Tregs antigen specificity to boost their suppressive capacities and restore immune homeostasis in experimental SLE [89].

5.1.3. CAAR (chimeric autoantibody receptor) T-cell

Another targeted approach for antibody-mediated ADs can use chimeric autoantigen receptor (CAAR) engineered T-cells to deliver selective immunotherapy and specifically kill the autoimmune B-cells directed towards this antigen by the CAAR extracellular domain, as first envisioned for Pemphigus Vulgaris [90]. This approach was proposed for LN to specifically deplete the anti-DNA producing B-cells. Six human T-cells models were tested to express a CAAR, consisting of LN autoantigen Heparanase1/2, Histone1 and alpha actinin 1/2 CAAR T-cells alongside with C1q protein fused to CD19-CD3 signaling domains. In vivo imaging assay reveals that C1q or Heparanase2 CAAR-T-cells completely depleted B cells at 48 h (ratio: 1B cells / 10 CAAR T-cells) [91]. In conclusion, the use of an anti-dsDNA and anti-C1q CAAR T-cell could be considered as a promising option. Translational research must be accomplished to explore its feasibility in real-world scenario.

5.2. Clinical experience so far with CAR T-cells in SLE adult patients

5.2.1. CD19 single-targeted chimeric antigen receptor (CAR) T-cell therapies

The first successful use of CAR T-cells in SLE was reported by Mougiakakos et al. [81] in a 20-year-old woman who had been refractory to all therapies (hydroxychloroquine, high-dose glucocorticoids, cyclophosphamide, mycophenolate mofetil, tacrolimus, belimumab and rituximab) over nearly two years with severe active disease, including LN and pleuro-pericarditis. Results showed: a) the feasibility of manufacturing a second-generation anti-CD19 CAR T using this patient's apheresis product that contained 51 % CD3+ T-cells, which were enriched to 94 % CD3+ T-cells before transduction; b) the need to stop all medications but low-dose prednisolone during a critical time period until lymphodepletion with fludarabine and cyclophosphamide before the planned CAR T-cell infusion; c) the CAR T-cells rapid in vivo expansion up to ten days after injection followed by their decrease from peripheral blood thereafter; d) a rapid clinical and immunological efficacy with B-cells depletion, dsDNA autoantibodies disappearance, normalization complement levels [81], regression of proteinuria and remarkable disease remission without immunosuppression anymore. Subsequently, five other active SLE patients, aged 18 to 24 years after at least 1 to 9 years SLE duration, all with histologically-proven LN plus various multiorgan involvement at baseline (SLEDAI-2 K scores: 8 to 16) and refractory to previous immunosuppressors, were treated with the same CD19 CAR T-cells manufacturing process and protocol procedures [92]. The same unique results showed significant regression of SLEDAI-2 K scores, proteinuria, C3 complement and anti-dsDNA antibody levels within three months. All patients remained drug-free with LN cessation fulfilling DORIS remission criteria [45], with no flare during follow-up. The observed AE were limited to Grade-1 Cytokine Release Syndrome (CRS) in three patients and effectively managed. The in vivo dynamics of CD19 CAR T-cells expansion and B-cell elimination, similar to first report [81] showed that: a) the CAR T-cells constituted only a small proportion of circulating T-cells shortly after infusion (at day 1) with CAR T-cell peak expansion on average at day 9 and thereafter rapid decline in circulating CAR T-cell count, which phenotype had shifted to central memory T-cells, indicating their circulation to lymphoid organs and other tissue sites; b) the circulating B-cell numbers fully disappeared from day 2 after CAR T infusion for a median 110 ± 32 days duration, while other circulating cell lineages (CD4+/CD8+ T-cells, monocytes and neutrophils) only transiently decreased after lymphodepleting chemotherapy and white blood cell counts rapidly recovered within 10 days after treatment; c) B cell reconstitution, mostly consisted of naive B cells with low or no memory B cells and plasmablasts, without any CD11c + pathogenic extrafollicular B cells. Conventional and

immunohistochemistry staining analysis on lymph node biopsies after CD19 CAR T-cells in 3 SLE patients showed complete CD19+ and CD20+ B-cells depletion and disruption of the follicular structures, with disappearance of CD23+ Follicular Dendritic Cells and PD-1+ Follicular Helper Cells, while plasma cells and macrophages remain unchanged [42]. Vaccination-induced antibodies were not depleted by CAR-T cell treatment, in agreement with a lack of destruction of long-lived BM PCs generated by the classical germinal center reaction. In two patients with long disease-free status and no need to reinstall immunosuppression, BCR repertoire analysis showed loss of BCR clonotype enrichment after CART injection and exclusive expression of IgD and IgM in the emerging B cells. This was first evidence that immune system rebooting may occur after CAR T-cell treatment [92] in SLE patients, as previously obtained by aHSCT treatment only. It was also shown that selective CAR T-cell mediated B cell depletion suppresses interferon signature in SLE [93]. A newly published case series of AD patients, that included 8 SLE patients who received the MB-CART19.1 (autologous anti-CD19 CAR-transduced CD4- and CD8-enriched cells), with the same clinical protocol for leukapheresis and lymphodepletion, also showed DORIS clinical remission with seroconversion of antibodies against dsDNA, single-stranded DNA, nucleosomes, and Smith protein sustained until at least one year of follow-up in seven of the eight patients [82]. No moderate- or high-grade cytokine release syndrome or ICANS occurred and 5/8 SLE patients had grade 1 cytokine release syndrome, effectively treated with tocilizumab. Whether new vaccinations are necessary and how they will affect the recovery of the memory B-cell compartment is still not clear.

In conclusion, these pioneering publications established new evidence for the short- and long-term safety and efficacy of CD19 CAR T-cell therapy in refractory SLE patients and opened the gate to conducting controlled clinical studies [18,82], with still several questions. First the duration of response, as CAR T-cells must anchor to targeted cells to effectively kill them, and then expand in vivo and persist in patients to eliminate the targeted cells and prevent relapse. Second, disease related T-cell defects may prevent good manufacturing of autologous CAR T-cells in SLE patients and/or contribute to target antigen negative resistance (CD19 loss or downregulation) as widely studied in hematology [94,95]. with consequent lower efficacy of the antiCD19 CAR T-cells.

5.2.2. Anti-CD19 / BCMA CAR T-cell

As CD19 single-targeted CAR T-cell therapies are unable to attack CD19-negative long-lived PCs [96], other CAR T-cell approaches [97] are being explored in refractory SLE. CD19-BCMA compound CAR (cCAR) is a 2-unit CAR consisting of a complete BCMA-CAR fused to a complete CD19-CAR by a self-cleaving P2A peptide, which allow the independent expression of both CAR receptors separately on the T-cell surface [98]. This approach hypothesizes that cCAR can significantly reduce donor-specific antibody levels by dual targeting CD19 on B cells and BCMA (CD269) on PCs [99]. The efficacy of anti-BCMA (CAR) T-cells in relapsed/refractory multiple myeloma, such as idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel) [100] has led to their regulatory approval in some countries. The first reported patient, enrolled in the first phase I open clinical trial using cCAR (NCT04162353), had a 20-year-history of SLE and recently diagnosed stage IV diffuse large B-cell lymphoma with severe AE to R-CHOP therapy that had to be discontinued [99]. After prednisone discontinuation, fludarabine and cyclophosphamide were administered before patient was infused 5.3×10^6 /kg cCAR T cells, which expanded over two months. Thereafter, the patient's complement levels remained within normal, the anti-nuclear antibody (ANA) titers that were highly elevated prior to cCAR therapy remained undetectable until 37 weeks of follow-up without prednisone. BM aspiration and subsequent flow cytometry confirmed PC depletion [99].

In a Chinese single arm open phase-1 trial (NCT 04162353 and NCT 05474885), thirteen refractory SLE patients (P) (10 Female, median aged 31 years (range 16 to 58)) with either coexisting relapsed/refractory diffuse large B cell lymphoma (DLBCL) comorbid or biopsy

proven LN (P3–P13), while P11 had severe BM depression [101]. All (except P11) received 3×10^6 cCAR cells/kg after stopping all SLE medications and conditioning using cyclophosphamide and fludarabine (P1, P2) or Cy alone (P3–P13) to test if both conditioning may have similar efficacy. P1 and P2 achieved SLE symptoms and medication-free remission (MFR) plus complete lymphoma remission. Three months post-cCAR, P3–P13 were negative for all autoantibodies, with normal complement levels and fall in SLEDAI compared to baseline, while renal function had significantly improved in 10 patients. All patients showed reduction IgA, IgG and IgM levels within 30 days. B cell populations recovered in all patients but P11 within 2–6 months post-cCAR, with normal B lymphocyte as well as IgM counts within 6 months of cCAR treatment and no sign of SLE relapse. B cell and humoral immune reset were shown by flow cytometry for all patients (and BCR deep sequencing for P5) demonstrating the absence of IgG and IgA clonotypes with non-class-switched BCR repertoires >95% IgM heavy chain at the time of B-cell populations recovery and Interferon-alpha (IFN- α) back to physiologically normal ranges in eight patients. IgA levels within patient saliva samples returned to normal after 8 months. cCAR was well tolerated with only mild cytokine release syndrome (CRS) and no immune effector cell-associated neurotoxicity syndrome (ICAN) occurred. Neutropenia was observed in relation to pretreatment conditioning and resolved within 45 days of cCAR therapy.

5.3. CAR T-cell therapy in SLE pediatric patients

There is limited clinical experience regarding the use of CAR T-cell therapy in SLE pediatric patients, but there are emerging reports in childhood-onset SLE, which is a more aggressive disease [102,103].

A second-generation 4-1BB-based CD19 CAR T-cell therapy was successfully used in a 15-year-old adolescent girl with severe and rapidly progressive LN requiring hemodialysis and who had been refractory to therapy with high-dose methylprednisolone, azathioprine, mycophenolate mofetil, cyclophosphamide, and belimumab [102]. After administration of CD19-targeted CAR T-cell therapy [102], her disease activity rapidly decreased with improved renal function, cessation of hemodialysis 17 days after CAR T cell administration, and sustained clinical and laboratory remission. Compared with adult patients with SLE, the patient had a relatively low CAR T-cell peak in maximum cell expansion, but the cells persisted substantially longer and remained present at 6 months after infusion. Complete peripheral B-cell depletion was observed from day 7 after CAR T-cell therapy and persisted at 6 months after infusion. The patient developed mild cytokine release syndrome which was treated with antipyretics and a single infusion of tocilizumab on day 6 after CAR T-cell administration and experienced no other adverse events [102]. Presently, one ongoing clinical trial is assessing the safety and efficacy of CD19-targeted CAR T-cells in childhood-onset refractory SLE (NCT06222853), and one trial is evaluating the CD19/BCMA CAR T-cell therapy in both pediatric and adult SLE patients (NCT05030779).

5.4. Good clinical practices for the use of CAR-T cells in SLE patients

5.4.1. Referral for CART therapy

It is highly advised to respect numerous principles when referring SLE patients to be treated by CAR T-cell. Good clinical practices for innovative therapies in AD were recently proposed by an international group of experts under the auspices of EBMT and JACIE accreditation committee [18] to promote patient safety and harmonized procedures for patient selection, care and follow-up, clinical and immune monitoring before and after treatment delivery, and data collection, in accordance with Good Clinical (GCP) and Good Manufacturing (GMP) Practices and appropriate accreditation and regulatory requirements. The recommended criteria for SLE patients candidates specifically, include: EULAR-ACR classification criteria 2019 fulfillment [6], Anti-DsDNA or anti-histone or anti-SM or anti-nucleosome positivity, not

being in remission according to DORIS criteria [45] or in low disease activity state [LLDAS] [44], having at least one active organ system involvement, with one BILAG A score or more than 2 BILAG B scores (moderate disease activity), and no or insufficient response to glucocorticoids, despite at least 2 of the following treatments for at least three months each: cyclophosphamide, mycophenolate mofetil or its derivatives, belimumab, azathioprine, anifrolumab, methotrexate, rituximab, obinutuzumab, cyclosporin, tacrolimus or voclosporin [18]. Patients with life-threatening end-organ damage must not be referred for CART therapy. Given the fragility of SLE patients, related to both the underlying disease and past immunosuppressive treatments, a case-by-case evaluation is recommended by Multidisciplinary Team (MDT) evaluation. Fertility preservation must be considered prior to CAR T-cell, if not previously conducted [18,104,105]. Patients with pre-existing irreversible kidney damage must be treated with caution and all procedures should be adapted to this specific condition [18].

5.4.2. Manufacturing CAR T-cells for SLE challenges

The source material for CAR T-cell production is autologous lymphocytes from leukapheresis, the quality of these cells has a crucial impact on successive manufacturing steps [106,107]. When aiming to produce CAR T-cells from SLE patients the challenge is the concomitant/previous treatment with immunosuppressive and T-cell-toxic drugs, such as glucocorticoids, mycophenolate mofetil, or tacrolimus, which are widely used to control the disease and cannot be easily stopped in these patients [106]. A, drug-specific washout periods before leukapheresis is important to ensure optimize T cell collection and fitness [18]. Despite chronic immune suppression for years, the retrieval of sufficient functional T-cells is feasible in SLE patients [106]. Analysis in six severe SLE patients using the same manufacturing process and clinical protocol, confirmed that peripheral blood cells derived from the leukapheresis product yielded sufficient number and fitness of autologous T-cell for clinical scale MBC19 CAR T-cell production [106]. In addition, in severe active SLE patients, with lymphopenia (<500/mm³) or organ involvement, that necessitate prompt treatment and high dose steroids, using cryopreserved leukapheresis product may become feasible.

After collecting, the cells are sent to a pharmaceutical laboratory either in a fresh state or cryopreserved. Points of care manufacturing in a closed system allow to use fresh leukapheresis product. A recent in vitro study showed that cryopreserved leukapheresis products from five SLE patients allowed to achieve a similar expansion and transduction efficacy as those from healthy donors [106]. In the laboratory, T-cells undergo modification through activation, transduction, expansion of CAR T-cells, and then are shipped back. The duration from the patient's blood samples delivery "on-site for manufacturing" until the CAR T-cell product return at bedside for infusion may vary between 8 days (decentralized) to 3–6 weeks (centralized) depending on the production platform, techniques and logistics.

5.4.3. Lymphodepletion chemotherapy before CART cell administration

Lymphodepletion (LD) is to be administered, usually on a 3-to-5 days schedule before the infusion of the CAR T cells. LD can only be administered in accredited centers to ensure attentive monitoring and appropriate hydration [18]. Fludarabine-cyclophosphamide based regimen is the most widely used LD method. The main purpose is to allow homeostatic CART cell expansion and survival in vivo by modulating cytokine and immune pathways through a) the reduction of endogenous lymphocytes and inflammatory burden to prevent rapid exhaustion of CAR-T; b) preparation of a "niche" for CAR-T optimal engraftment, homing and long-term survival of CAR-T. Forty-eight hours later, CAR T-cells are thawed and immediately intravenously infused into the patient [106,107].

5.4.4. CAR T cell toxicities

As defined per both national and international GCP guidelines [18],

it is of utmost importance to ensure access to specialized point of care for adequate early and long term clinical and immune monitoring of the patients. These guidelines for use of CAR T in AD recommend up to 14 days of hospitalization to monitor any early severe reactions, and that patients should be located within 60 min of the center with the continuous presence of a caregiver educated to identify the potential complications maintained for a year. As compared to the use of CART in patients with malignant diseases, the few SLE patients treated and reported so far [81,82,85,97,99] seems to be present a lower frequency and lower severity of cytokine release syndromes (CRS) and of ICANs. The most prominent medium- and long-term toxicities after CAR T cell therapy as in oncology include cytopenias and infections (de novo and reactivation). Adequate monitoring and prophylaxis in profoundly immunosuppressed patients should be maintained.

As for hypogammaglobulinemia, the incidence rates may vary across studies in SLE patients prior to CAR-T therapy due to differences in hypogammaglobulinemia definitions, timing, and study protocols. In general, pre-treatment with anti-CD 20 antibodies-based regimens and anti-CD 38 antibodies alone or in combination with steroids, may result in undetectable circulating B-cells accounts and decreased immunoglobulin levels, even prior to CAR T-cell infusion. This notion is of high importance in SLE patients because of the high probability to their exposure to one or more aforementioned agents. Interestingly, in four adult trials among patients treated with CAR T for malignancy, intravenous immunoglobulins supplementation varied from 11 % to 32 % according to clinician discretion or to institutional protocol [108]. Infection risk associated with leukopenia may be amplified by the persistent hypogammaglobulinemia occurring after CD19- and BCMA-targeting CAR-T, impacting the incidences of severe viral, bacterial, and fungal infections [109].

5.4.5. The limited access to CAR T cell therapy in some settings

CAR T cell is a relatively new method of treatment, dating less than 10 years, with maximum number of technically limited possible procedure per year. The imbalance between possible supplies and demand is magnified in countries with no or limited number of accredited/certified centers. This issue is also of concern regarding relatively rare cases of new indications as SLE. Limited access is multifactorial and mostly related to the cellular product such as cost, complex logistics and manufacturing platforms limitations. On the other hand, the multidisciplinary experience needed to validate this procedures, in a case-by-case basis, and to accomplish the multi-phase treatment are crucial but difficult to organize in some countries [110]. Due to the complexity of the treatment in combination with the underlying SLE disease, a joint follow-up period in a multidisciplinary team composed of disease specialist and a CAR T expert (hematologist) for at least 6 months is recommended after which, individual decisions can be made. General guidance for the management of short/medium term complications and long-term follow-up after CAR T and other CTs are well codified. Hematologists should be continued to be involved in monitoring of side effects according to EBMT handbook recommendations with a quarterly MDT assessment during the first year, and yearly thereafter with data collection and reporting in the adequate registries.

6. Conclusion and perspectives

Although immunomodulatory and lympho-depletive approaches are widely used to treat SLE, there is still an unmet need for more effective and sustained treatments. Severe patients are at risk for damage and disease related mortality. CD19-targeted CAR T cells may constitute an attractive treatment through its unique features including deep tissue depletion of B-lineage cells, effectiveness on plasmablast depletion and the capability to reset autoimmunity. CAR T-cells may be considered as a flexible platform which permits the development of new out-of-the-box solutions, like FOXP3-based CAR Treg. Beyond efficacy, this approach needs to be specifically developed for SLE, as most of advances on this

direction are originally based on tumor cell-directed CAR T products.

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Declaration of competing interest

We have no conflict of interest.

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