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Is there a place for CAR-T cells in the treatment of chronic autoimmune rheumatic diseases?

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Abstract :

Chimeric-Antigen-Receptor T cell therapy or CAR-T cell is based on a specific targeting of tumor antigen leading to lysis and destruction of tumor cells development. CAR-T cells have demonstrated high potency for the management of B cell malignancies. This successful story was followed by the development of new CAR-T cell-derived constructions that have the ability to eradicate pathogenic B cells or restore tolerance. The objective of the herein manuscript is to review and discuss how the knowledge and technology generated by the use of CAR-T cells may be translated and integrated in the ongoing therapeutic strategies of autoimmune rheumatic diseases.

To this end, we will introduce CAR-T cell technology, describe the meaningful achievements of CAR-T cells observed in onco-hematology and discuss preliminary data obtained with CAR-T cells and their derivative constructions in experimental models of autoimmune diseases. Then, we will focus on how CAR-T cell engineering is interfering with the pathogenesis of three chronic autoimmune rheumatic disorders - rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis - and discuss whether these constructs may permit to gain efficacy compared to current treatments and overcome their adverse events.

Keywords: CAR-T cells, CAAR-T cells, CAR-Tregs, autoimmune rheumatic diseases.

Autoimmune rheumatic diseases (ARD) have a high impact on patients' morbi-mortality and quality of life, given their chronicity, various, organ manifestations and association with co-morbidities. Over the last several decades, earlier diagnosis, new classification criteria and development of novel therapeutic approaches have allowed a better management of ARD (1,2). However, there are still great challenges that need to be addressed. Indeed, standard of care treatments are based on immunosuppression to control disease activity and dampen immune system overactivation. Conventional synthetic disease-modifying anti-rheumatic drugs (DMARDs) and biologic/synthetic targeted DMARDs have dramatically improved the prognosis of ARD (2), but, despite major therapeutic advances, several patients may not adequately respond to these therapies and long-term drug therapy is required to maintain long-term remission.

A recent breakthrough in oncology brought new hopes for cancer therapies. Genetic engineering of our immune cells, and especially T cells, has improved the prognosis of B cell cancers that were refractory to conventional therapies (3,4). This technology, called Chimeric-Antigen-Receptor T cell therapy or CAR-T cells, is based on a specific targeting of tumor antigen leading to lysis and destruction of tumor cells by modified autologous T cells. CAR-T cells are becoming of great therapeutic interest for autoimmune diseases, given their ability to delete pathologically activated immune cells or re-establish tolerance in an organ affected by dysregulated immunity.

This Review intends to introduce CAR-T cell technology, describe the achievements of CAR-T cells in onco-hematology and preliminary data obtained with CAR-T cells and their derivative constructions in experimental models of autoimmune diseases. Finally, we will describe how CAR-T cells may interfere with the pathogenesis of chronic autoimmune rheumatic disorders and discuss whether these constructs may permit to gain efficacy compared to current treatments and overcome their adverse events. To these ends, we searched on Pubmed following terms "CAR-T cells", "CAR-T cells AND autoimmunity", "CAAR-T cells" and "CAR-Treg" between 1989 and 2021. We found 3795 references and, we selected 42 articles for the purpose of this review after the reading of the abstract and/or the full text.

CAR-T CELL TECHNOLOGY

T cells are main effectors of our adaptive immune system. One of their key functions is to kill tumor or infected cells after recognition of a specific antigen via their T-cell receptor (TCR). This

observation led to the emergence of a new therapeutic strategy: redirecting T cells to antigens of interest to overcome natural barriers that they encounter. This concept has emerged in the 1990s and Eshhar's team showed that combination of TCR constant variant domains and antibody variable domains on T cells induced T cell activation and the lysis of target cells (5). These works further led to the development of genetic engineered autologous T cells, which expressed a chimeric antigen receptor (CAR), allowing redirection of these cells to the targeted antigen without major histocompatibility complex (MHC) restriction.

CAR-T construct contains four main components: an extracellular antigen-binding domain, a hinge, a transmembrane domain and an intracellular signaling domain leading to T cell activation, as shown in **Figure 1A** (6). All these components are crucial for CAR signaling and different combinations should be tested to analyze and determine the optimal CAR-T cell response. Recognition of CAR target is mediated by an antigen-binding domain composed of variable heavy and light chains of a monoclonal antibody connected by a flexible linker (scFv). scFv must have high affinity to the target antigen to induce CAR signaling and T cell activation. The hinge of CAR-T cells should be flexible to avoid steric hindrance and allow the recognition of the targeted antigen and T cell activity (7). The CAR hinge sequence is similar to amino acid sequences of CD8, CD28, IgG1 or IgG4 hinge. The transmembrane domain anchors the scFv in T cell membrane and is designed from amino acid sequences derived from CD3 ζ , CD28, CD4 and CD8 α transmembrane domains. The choice of the sequence is critical, since it influences CAR activity (8). Compared to CD28 sequences, CD8 α sequences chosen for the hinge and the transmembrane domain were associated with decreased IFN- γ and TNF- α production (8). Once the CAR has recognized its target, it induces phosphorylation of immunoreceptor tyrosine based activation motifs (ITAM) located on intracellular domains. Once phosphorylated, ITAM induce the recruitment of effector molecules conducting to T cell activation, cytokine production and target cell lysis, as achieved by unmodified T cells after antigen recognition by the TCR. This intracellular domain has been engineered to improve its function since several years. The first generation of CAR was composed of only one intracellular activation domain, which was the CD3 ζ domain. However, this domain was insufficient to induce activation of resting T cells *in vivo* (9). This has led to the development of second-generation CAR, characterized by an intracellular domain composed of both activation and co-stimulation domains. Indeed, human T

cells transduced with a CAR containing a fusion of intracellular CD3 ζ and CD28 domains display an increased proliferation rate, IL-2 production and cytotoxic activity (10).

Other studies have investigated the use of other co-stimulatory intracellular domains like 4-1BB on CAR-T cells function and fate. The 4-1BB domain induces CAR-T cell persistence and a central memory cell profile, whereas the CD28 domain is associated with less CAR-T cell persistence and an effector memory cell profile (11). It was also shown that a single amino acid residue in CD28 drove T cell exhaustion and hindered the persistence of CD28-based CAR-T cells; and changing asparagine to phenylalanine (CD28-YMFM) promoted increased CAR-T cell persistence *in vivo* and antitumor activity (12). To face with lack of persistence of second-generation CAR T cells, some groups have proposed to combine the activation CD3 ζ domain to two intracellular co-stimulations domains among CD27, CD28, ICOS, 4-1BB, MYD-88-CD40, KIR2DS2 or OX40 creating third-generation CAR-T cells (13–17). For example, CAR-T cells engineered with a combination of 4-1BB and CD28 displayed increased cytotoxicity and persistence compared to CAR-T cells composed of the 4-1BB intracellular domain only (18). Moreover, increased persistence of mesothelin CAR-T cells designed with ICOS and 4-1BB costimulation domains was observed compared to CAR-T cells composed of 4-1BB intracellular domain only (19). Further studies are still needed to further evaluate the efficacy and safety of third generation CAR-T cells. Actually, only the second-generation of CAR-T cells is approved by the Food and Drug Administration (FDA) as shown in **Table 1**.

Recent genetic modifications of CAR-T cells have been performed to enhance their cytokine production. This was particularly described for IL-12. Genetic modification of CD19-CAR T cells to express IL-12 was associated with enhanced anti-tumor activity *in vivo* without a preconditioning chemotherapy in mice infused with EL4 thymoma cell line expressing human CD19 (20). CAR-T cells expressing constitutively IL-12 and targeting vascular endothelial growth factor receptor-2 (IL-12/VEGFR2-CAR-T) were associated with a regression of established tumors together with long-term CAR-T cell persistence in the tumoral tissue (21). Moreover, CAR-T cells targeting carcinoembryonic antigen (CEA) and constitutively expressing IL-12 (IL-12/CEA-CAR-T cells) led to a more pronounced reduction of tumor volume compared to CEA-CAR-T cells (22). Other CAR-T cells have been armed to express IL-7, IL-15, IL-18 or IL-33 and results regarding their efficacy are reviewed elsewhere (6). These studies showed that CAR-T cell persistence and functions are modulated according to study objectives.

CAR-T CELLS IN ONCOLOGY

CAR-T cells were first employed in oncology to overcome the failure of natural T cells to eradicate tumor cells. Redirecting T cells against specific antigen expressed by tumor cells has shown great results in hematological cancers and specifically B cell malignancies. CD19 was chosen as the preferential B cell target since it is expressed during all major stages of B cell maturation (from pro-B cell state to late plasmablasts) conversely to CD20, which is mainly expressed on peripheral B cell maturation (naïve B cell to early plasmablasts) (23). Moreover, CD19 is highly expressed on tumoral B cells and preclinical studies showed a total eradication of systemic lymphoma cells after a single infusion of human CAR-CD19 cells in mice (24), providing the rationale to translate the use of these cells to the clinics. Meaningful results were obtained in chemotherapy-refractory patients with diffuse large B cell lymphoma and acute lymphoblastic leukemia (ALL) treated with CD19-CAR-T cells. Patients with diffuse large B cell or follicular lymphoma had a complete remission rate of 57% (4), and a complete remission rate ranging from 68% to 100% had been observed in patients with ALL (25). These results have conducted to the FDA approval of CD19-CAR-T cell therapy for patients with B cell lymphoma and ALL. The successful story of CAR-T cells in hematologic malignancies was not replicated in patients with solid cancers, which may be related to the lack of specific tumor antigens, leading to off-tumor toxicities, the loss of the targeted antigen or the complexity of immunosuppressive tumor microenvironment, preventing CAR-T cell penetration and activity. New engineering strategies to improve CAR-T cell activity in solid tumors are currently under investigation, such as development of CARs with multi-specificity, improvement of CAR trafficking and modification of their sensitivity to inhibitory signals of tumor microenvironment (6).

Although CAR-T cells can produce durable remissions in hematologic malignancies that are not responsive to standard therapies, the use of CAR T cells is limited by potentially severe toxicities. Cytokine release syndrome (CRS) has been reported in 54-91% of patients treated with CD19 CAR-T cells, and the occurrence of severe CRS ranged from 8.3% to 43% of patients among series (26). Patients usually developed CRS one week after CAR-T cell infusion and could experience fever, hypotension and respiratory insufficiency leading in severe cases to multi-organ

failure. CRS is triggered by CAR-T cell activation after tumoral antigen recognition. Indeed, CAR-T cells secrete pro-inflammatory chemokines and cytokines leading to the recruitment of immune cells and the production of IL-1 and IL-6 by macrophages (27). Tumor volume, and CAR-T cell expansion are important determinants of the severity of CRS through the amount of released proinflammatory cytokines (28). To counteract CRS, CAR-T cell infusion is associated with the delivery of corticosteroids, anti-IL6, anti-IL1, or anti-GM-CSF (27).

CAR-T CELLS IN AUTOIMMUNITY

Most of standard of care treatments used in autoimmune diseases are not curative, may cause side effects, and may be associated with a primary or secondary lack of clinical response. Autoimmune diseases are characterized by the activation of the immune system against self-antigens leading to autoantibody production by B cells and tissue destruction by cytotoxic T cells. Successful development of CAR-T cells in cancers has led to the development of three strategies in autoimmunity (**Table 2**).

CAR-T cells

Given the successful use of CD19-CAR-T cells in B cell malignancies, a first strategy would be to reposition these cells in autoimmune diseases in which B cell depletion is currently used (26). Among existing therapies, Rituximab, an anti-CD20 antibody is the main B cell depletion strategy used in autoimmune diseases. Rituximab has demonstrated efficacy in patients with rheumatoid arthritis, multiple sclerosis and ANCA-associated Vasculitis (29–31). However, its efficacy was not conclusive in other autoimmune diseases like systemic lupus erythematosus (SLE) (32). Incomplete response to rituximab may be related to an incomplete B cell depletion due to resistant B cells or long-lived plasma cells sequestered in the bone marrow which might be less accessible to Rituximab (33). Some patients may develop antibodies directed against murine parts of anti-CD20 antibody which could limit its efficiency (34,35). According to these results, anti-CD20 humanized antibodies with increased complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC) have been designed, including Ocrelizumab or Ofatumumab, and are currently approved for the treatment of multiple sclerosis.

Another interesting B cell target is the CD19 antigen, which is expressed on more B cell subsets compared to CD20 antigen. We could speculate that use of CD19 antibody may lead to deeper B cell depletion by eradicating pro-B cell, antibody-secreting plasmablasts and some plasma cells (33). Monoclonal anti-CD19 antibodies are currently investigated in multiple sclerosis or anti-N-methyl D-aspartate (NMDA) receptor encephalitis (respectively NCT04372615 and NCT01585766). The expression of CD19 on numerous B cell subsets and successful B cell eradication by CD19-CAR T cells in B cell acute lymphoblastic leukemia drove physicians to investigate CD19-CAR T cells in murine models of autoimmune diseases. In experimental SLE, the infusion of CD19-CAR T cells in (NZBxNZW) F1 and MRL^{fas/fas} mouse models led to complete depletion of CD19⁺ B cells and autoantibody production, a reversion of disease manifestations in target organs and an extended mouse lifespan (36) (**Table 2**). Moreover, clinical trials using CD19-CAR T cells showed a persistence of B cell aplasia with a single dose compared to B cell depleting antibodies which need to be infused regularly to maintain B cell aplasia (37).

Besides B cell depletion, other types of CAR-T cells have been designed to target autoreactive immune cells in preclinical autoimmune diabetes. NOD mice develop autoimmune diabetes due to presentation of B:9-23 peptide from insulin B chain by the major histocompatibility complex II (I-A^{g7}) to pathogenic B:9-23 specific CD4⁺ cells. A single infusion of CAR-T cells directed against this MHC II complex in NOD mice delayed the onset of diabetes compared to control CAR-T cell-treated mice (38). However, this infusion did not provide long-term protection, likely due to a lack of CAR-T cell persistence.

An important point to consider before initiation of CAR-T cell therapy in patients affected by autoimmune diseases is their basal inflammatory state due to inflammatory cytokines produced by M1 macrophages infiltrating lesioned tissues (39). For this reason, it is tempting to speculate that CRS will be more harmful in these patients. Thus, the use of CAR-T cells in these patients will be conceivable after several lines of immunosuppressive drugs or targeted therapies.

CAAR-T cells

CAR-T cells development highlighted that pathological cells, like tumor cells, could be targeted and then eradicated. B cells in autoimmune diseases produce autoantibodies that could cause organ dysfunction. To target these autoimmune B cells, chimeric AutoAntibody Receptor-T cells (CAAR-T) have been engineered. Conversely to the specific expression on CAR-T cells of

receptors for molecules expressed on pathologic cells, CAAR-T cells express an extracellular autoantigen recognized by the BCR as shown in Figure 1B. This extracellular domain is linked to a transmembrane domain, a co-stimulation and an activation domain, similar to the intracellular part of CAR receptors. Thus, autoantigen recognition by autoreactive B cell leads to CAAR activation and specific lysis of pathogenic B cells (40). The proof of concept of this approach was conducted in a preclinical mouse model of pemphigus vulgaris (PV). The aim of the study was to test the efficacy of CAAR-T cells expressing the PV autoantigen, desmoglein (Dsg)-3 fused to CD137-CD3 ζ signaling domains to deplete pathogenic B cells. After infusion of Dsg3 CAAR-T cells, specific elimination of B cells carrying a B cell receptor directed against Dsg3 antigen was observed, in presence or not of soluble Dsg3-IgG. Moreover, Dsg3 CAAR-T cells engrafted and persisted in mice even in the absence of their antigen (40) (**Table 2**). Recently, DSG-3 CAAR-T cells have been shown to lyse primary human anti-DSG3 IgG B cells isolated from PV patients. Moreover, injection of DSG-3 CAAR-T cells in a murine PV active model improved mucocutaneous erosions and induced a decrease in serum anti-DSG3 antibodies (41). This new CAR strategy may be promising to treat autoimmune diseases characterized by pathological B cells and autoantibodies. However, some disease aspects should be studied before initiating a CAAR-T cell therapeutic approach. Indeed, pathological role of autoantibodies should be demonstrated in the disease pathogenesis. Then, the sequence and molecular structure of autoantigen targeted by pathogenic autoantibodies must be characterized in order to design suitable CAAR-T cells (42). Future works need to focus on the pathogen role of autoantibodies and the characterization of target autoantigens in autoimmune diseases for which B cell contribute to disease development.

CAR-Treg

Regulatory T cell (Treg) number and function is altered in autoimmune diseases leading to a defect of tolerance, dysregulation of immunity, inflammation and emergence of autoimmune cells (43). Several therapeutic strategies based on Treg function have been proposed to restore tolerance in affected tissues. The infusion of low-dose IL-2 to stimulate Treg expansion has shown to increase the Treg/Teff ratio and improved the Clinical Global Impression (CGI) in a phase I-IIa study including patients affected by different autoimmune diseases (44). Other phase I/II studies showed increased Treg expansion after low-dose IL-2 infusion in SLE or type-1 diabetes (45).

Nevertheless, IL-2 therapy is limited by its short half-life, requiring repetitive injections, and anti-drug immunization.

Polyclonal Treg infusion has a good safety profile and has shown benefits in type-1 diabetes or GvHD, and other clinical trials in Crohn disease or pemphigus are ongoing (46). However, the use of these cells is limited by their lack of specificity, leading to reduced anti-infectious and anti-tumoral immune responses. Thus, Treg including a transgenic TCR or a CAR were further designed to target specific antigens, and they display a better ability to suppress effector responses compared to polyclonal Tregs infusion (46). However, transgenic TCR can recognize the antigen only in a MHC context, requiring a specific TCR design for each patient and thus limiting the large-scale development of this approach. This limitation has led to design CAR directed against a specific antigen of an affected tissues that could be recognized without MHC restriction. . Elinav *et al* identified that mouse CAR Tregs accumulate at the site of the target antigen and induce bystander suppression (47). This phenomenon offers to target antigens that are not necessarily expressed on cell surface, conducting to immune suppression in the affected organ. Moreover, Treg cells were able to inhibit DC differentiation and maturation (48,49) and limit migration of inflammatory DC in draining lymph nodes (50). We could hypothesize that CAR-Treg will also modulate DC function in affected tissues and secondary lymphoid organs. Restoring immune tolerance with CAR-Tregs may be a relevant strategy (**Table 2**), but CAR-Treg plasticity and stability should be maintained to avoid their conversion into inflammatory cells (51) and additional studies should be conducted regarding their persistence in targeted tissues.

CAR-Tregs in Experimental Autoimmune Encephalitis

CAR-expressing Tregs directed against the antigen myelin oligodendrocyte glycoprotein (MOG) showed suppressive capacity *in vitro* and could efficiently access various regions in the brain via intranasal cell delivery in the Experimental Autoimmune Encephalitis (EAE) mouse model. Mice displayed decreased disease symptoms and brain inflammation, and remained healthy after a second challenge with the MOG peptide, pointing the sustained effects of engineered Tregs (52).

CAR-Tregs in colitis

A CAR-Treg directed against the 2,4,6-trinitrophenyl (TNP) antigen was assessed in the TNBS colitis mice model. Mice infused with TNP-CAR Tregs had a better survival (75% vs. 40%) and a decrease colitis score compared to mice infused with WT Tregs (47). The same group has induced

experimental colitis in carcinoembryonic antigen (CEA)-transgenic mice by irradiating mice and injected CEA-CD4⁺ T effectors cells. Then, mice were treated with CEA specific CAR-Tregs or CAR-Tregs not specific for target antigen (irrelevant CAR-Tregs). An accumulation of CEA-CAR-Tregs in the colon of diseased mice associated with an inhibition of abdominal accumulation of CEA-CAR CD4⁺ Teffs was observed at day 6 post-injection. They also noticed an increased lifespan of treated mice after lethal colitis induction compared to mice that received irrelevant CAR Tregs. The development of colorectal cancer induced by injected Dextran Sulfate Sodium (DSS) was also analyzed in mice treated with CEA-CAR Tregs or irrelevant CAR-Tregs. The tumor score, defined by the percentage of the tumor-occupied colon circumference, was significantly decreased in mice receiving CEA-CAR Tregs compared to irrelevant Tregs suggesting a protective effect of these cells against colorectal cancer development. This result is of importance given the increased risk of colorectal cancers in patients affected by ulcerative colitis (53).

CAR-Treg and type I diabetes

The first observations following polyclonal Treg infusion in patients with type I diabetes supported a persistence of transferred Tregs until one year after infusion and a good safety profile of the procedure (54). The infusion of CAR-Tregs directed against insulin in NOD mice was also associated with CAR-Treg persistence in mice, but showed no benefit to prevent diabetes occurrence, supporting the development of further studies (55).

CAR-Treg and graft versus host disease

Interestingly, a recent study performed by Imura Y *et al* has shown *in vitro* the suppression of IgG antibody production and differentiation of B cells by CD19 CAR-Treg. In the same study, infusion of CD19-CAR Treg in immunodeficient mice reconstituted with human PBMC, suppressed their antibody production and reduced the risk of graft-versus-host disease (56).

CAR-T CELLS APPLICATION IN AUTOIMMUNE RHEUMATIC DISORDER

Contribution of CAR-T cells to ARD current treatments

Several issues may be encountered by current treatments of ARD, including increased rate of infections due to a strong immunosuppression and the need of regular injections to maintain

remission. Moreover, the development of anti-drug antibodies (ADA), leading to treatment failure or injection-site reactions, has also been observed with the use of monoclonal antibodies. The development of CAR T cells may represent an innovative way to counteract several biologic therapy limitations, e.g., several issues encountered with short or long-term use of rituximab, including repeated Rituximab administration, incomplete B cell depletion (57) or immunogenicity (34,58). CD19-CAR T cell therapy requires only one infusion and induces total B cell aplasia during several years (37). Moreover, pre-existing humoral immunity before CD19-CAR-T therapy is conserved after treatment (59). CAAR-T cells could be used to deplete specifically pathogenic B cells and CAR-Tregs may be a promising therapy to promote localized tolerance in organs affected by ARD.

Several CAR T-cell preclinical approaches have been studied or are ongoing in RA, SLE and SSc.

CAR-T cells in rheumatoid arthritis

Anti-citrullinated protein antibodies (ACPA) are well described in RA and could have a pathological role and ACPA against citrullinated vimentin have been suggested to enhance osteoclastogenesis and high bone resorption in mice (60) suggesting a pathological role of B cells. The efficacy of Rituximab has been demonstrated in active RA, especially in patients with high ACPA titres (61). Thus, we may hypothesize that the development of CAAR-T cells expressing citrullinated antigens would allow a specific deletion of anti-citrulline B cells while saving protective B cells (**Figure 2**). Another original strategy to target pathogenic B cells has been proposed by the group of Zhang. Immunodominant antigens targeted by autoantibodies were identified and coupled to fluorescein isothiocyanate (FITC). Then, the authors engineered FITC-CAR T cells, which will recognize FITC-immunodominant antigens fixed on autoreactive B cells leading to lysis of pathogenic B cells. In this study, they analysed lysis of autoreactive B cells isolated from RA patients by FITC-CAR T cells. They observed a specific killing of B cells which recognized FITC-citrullinated autoantigens by FITC-CAR T cells *in vitro* supporting the efficacy of this approach (62). Induction of tolerance in the synovium of affected joints by developing specific CAR-Tregs also seems a promising strategy (**Figure 2**). Citrullinated vimentin (CV) has been identified as a specific antigen found exclusively in the extracellular matrix of the inflamed synovial tissue of RA patients (63). Preliminary unpublished data have suggested that engineered CAR-Tregs directed against citrullinated vimentin (CV-CAR-Tregs) may react with CV expressed

in RA synovial fluid. Future studies are now needed to investigate CV-CAR-Tregs effect in preclinical RA.

CAR-T cells in systemic lupus erythematosus

SLE is characterized by pathological autoantibody production leading to immune complex formation which deposit on tissues leading to complement activation and then tissue destruction (64). Treatments include low-dose glucocorticoids, anti-malarial, immunosuppressive drugs and specific biologics targeting B cells (1). Belimumab which targets the cytokine BAFF, is the single licensed biologic drug for SLE management (65,66). To overcome side effects of current ARD treatments, repositioning CD19-CAR-T cells has been proposed by Kansal R *et al*. They reported a complete and sustained depletion of CD19+ B cells in two murine models of lupus associated with increased mouse survival. This is likely related to the significant decrease of anti-DNA IgG and IgM in the serum of treated mice. Moreover, they observed that CD8+ T cells isolated from mice who received a single injection of CD19-CAR-T cells seven months ago, and then re-injected in different lupus mice, still had the capacity to deplete CD19+ B cells, indicating the persistence and action of CD19-CAR-T cells (36). Another recent study showed that infusion of CD19-CAR-T cells in MRL-lpr mice before the onset of disease or after disease initiation, improved lupus symptoms, prolonged the life span of mice and delayed the progression of lupus (67). These results were promising and the translation to clinical trials now needs to be addressed. CD19-CAR-T cells are expected to deplete total B cells leading to decrease production of antibodies by healthy B cells of SLE patients. Based on recent studies performed in PV, the refinement of B cell targeting by engineering CAAR-T cells may also be proposed. However, SLE displays a huge diversity of autoantibodies (around 180) suggesting numerous autoantigens that could be targeted (68). Double-stranded DNA and nuclear antigens have been well described to take part in tissue injury. Design of CAAR-T cells directed against these antigens could be interesting and should be evaluated in lupus mouse models as they may bring potential effectiveness to deplete specific pathogenic B cells (**Figure 3**). Inducing tolerance in affected tissues by using Tregs or CAR-Tregs may also be a relevant strategy. Indeed, Dall'Era *et al* showed in a SLE patient with skin involvement that autologous Treg infusion increased Treg activation in inflamed skin and reduced its infiltration by IFN- γ CD4+ effectors cells (69). The study performed by He J *et al* showed that treatment with low-dose of interleukin-2 led to marked

reductions of disease activity and increased numbers of Tregs in the blood of in patients with SLE (70). This result was confirmed another group (44). Based on these studies, we hypothesize that targeting CAR-Treg to skin or kidney will induce tolerance and reduced inflammation in affected tissues (**Figure 3**).

CAR-T cells in systemic sclerosis

Significant progress has been achieved to better control the vascular component of the disease. In addition, nintedanib (tyrosine kinase inhibitor) (FDA and EMA) and tocilizumab (FDA) have been recently approved for the treatment of interstitial lung disease (ILD) associated with SSc (71). However, therapies of larger effects on SSc-ILD and others with broader disease modifying properties are eagerly awaited. CD19 CAR-T cells could be an interesting strategy given the dysregulation of B cell homeostasis in SSc (72–75) and the positive signal observed with rituximab in skin fibrosis (76–78). However, the deletion of total B cells including cells that produce protective antibodies could lead to increase the risk of infections. To overcome protective B cells deletion, we could consider targeting B cells responsible for the generation of autoantibodies involved in pathogenesis of systemic sclerosis like anti-endothelial or anti-fibroblast antibodies which contribute to increase activation of these cells (79). Identification of antigens targeted by these pathogenic B cells could lead to design CAAR-T cells allowing their deletion (**Figure 4**). Moreover, numbers and functional changes of Treg population have been described in SSc patients. Decreased Treg number has been assessed in SSc patients, except for those in early phase or active disease (80). It would be exiting to design CAR-Tregs directed to specific skin and lung antigens to investigate the induction of tolerance in early and late phases of SSc. We could hypothesize that early treatment with CAR-Tregs may alleviate inflammation and promote physiologic tissue repair in affected tissues (**Figure 4**). However, we could not speculate on the implication of late infusion of CAR-Tregs on the resolution of fibrosis. Further investigations are needed to assess the effect of tolerance induction at different fibrotic stages. Interestingly, a recent study has shown that targeting fibroblast activation protein (FAP) by CAR T cells induces a significant reduction of cardiac fibrosis and restoration of function after injury in mice due to FAP+ fibroblast deletion (81). It would be exciting to find a specific myofibroblast protein that could be targeted by CAR-T cells.

CONCLUSION

CAR-T cell therapies have demonstrated high potency during last years in B cell malignancies. This has caught the attention of researchers of other fields and especially autoimmunity. Dsg-3 CAAR-T cells in pemphigus vulgaris, CD19-CAR T cells in SLE and MOG-CAR Treg in EAE have shown promising results leading to new hopes especially for ARD treatment. Indeed, CD19-CAR T cells and BCMA-CAR T cells are respectively evaluated in myasthenia gravis (NCT04146051) and in relapsed/refractory antibody-associated idiopathic inflammatory diseases of the nervous system (NCT04561557). Evaluation of the maximal tolerated dose of DSG3-CAART cells in pemphigus vulgaris patients is ongoing (NCT04422912). Design of CAAR-T cells directed against antigens targeted by autoantibodies is a promising approach to delete pathogenic B cells in RA, SLE and SSc. Infusion of polyclonal Tregs or low dose IL-2 in patients affected by autoimmune diseases has shown that re-induction of tolerance was possible. Design of CAR-Tregs will allow their targeting in appropriated tissues affected by ARD. For this reason, it is necessary to identify antigens expressed by affected tissues in ARD. Attention must also be paid to improve the stability of CAR-Tregs to avoid any reversal plasticity, which could exacerbate autoimmune responses. Potential limitations of using CAR-T cell therapy could be some potential severe side effects, including the cytokine release syndrome and their cost. For example, CAR-CD19 and rituximab, used in diffuse large B-cell lymphoma have respectively a cost effectiveness of \$150000/Quality-Adjusted Life Years threshold and \$61984/Life-Year Gained (82,83). Even if a lot of research remains to be done, derived CAR T cells constructions could bring real therapeutic arsenal to treat ARD that have no curative treatment to date.

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Table 1: CAR-T cell therapies approved by the Food and Drug Administration

Drug	Provider	Target antigen	Costimulation Activation domains	Indication	Approval date
Tisagenlecleucel (Kymriah)	Novartis Pharmaceuticals	CD19	4-1BB CD3 ζ	Adults with acute lymphoblastic leukaemia	30th August 2017
Axicabtagene ciloleucel (Yescarta)	Gilead	CD19	CD28 CD3 ζ	Adults with relapsed or refractory large B-cell lymphoma	18th October 2017
Brexucabtagene autoleucel (Tecartus)	Gilead	CD19	CD28 CD3 ζ	Adult patients with relapsed or refractory mantle cell lymphoma	24th June 2020
Lisocabtagene maraleucel (Breyanzi)	Bristol Myers Squibb	CD19	4-1BB CD3 ζ	Adults with relapsed or refractory large B-cell lymphoma	05th February 2021

Table 2: CAR-T cells strategies developed in autoimmunity

Disease	Targeted Antigen	Mouse model	Costimulation/activation domains	Outcome	Reference
CAR-T cells					
Type I Diabete	MHC I-A ^{g7} -B:9-23(R3)	NOD	CD28/CD3 ζ or CD28/CD137/CD3 ζ	Delays occurrence of type I diabetes	Zhang <i>et al</i> (39)
Lupus	CD19	(NZBxNZW) F1	CD28/CD3 ζ	Sustained B cell depletion, decrease of autoantibodies	Kansal <i>et al</i> (37)

			or MRL ^{fas/fas}		production and increased survival	
CAAR-T cells						
Pemphigus Vulgaris (PV)	Dsg3	PV hybridoma (NOD-scid-gamma)		CD137/CD3 ζ	Specific deletion of Dsg3 B cells and decrease of Dsg3 antibodies	Ellebrecht <i>et al</i> (41)
CAR-Tregs						
Multiple Sclerosis (MS)	MOG	Experimental Autoimmune Encephalitis (EAE)		CD28/CD3 ζ	Migration of CAR-Tregs in brain. Reduced disease symptoms and decrease of pro-inflammatory cytokines in brain	Fransson <i>et al</i> (53)
Colitis	CEA	TNBS-induced colitis		CD28/FcR γ	Migration of CEA-CAR Tregs in colon mucosa. Amelioration of colitis and improved survival	Elinav <i>et al</i> (48)
Colitis	CEA	CEABAC or transfer of		CD28/CD3 ζ	Accumulation of CAR-Tregs in colon.	Blat <i>et al</i> (54)

			CEA-specific CD4+ cells		Suppression of colitis	
Colorectal cancer	CEA	DSS-induced colitis	CD28/CD3 ζ		Decreased of colorectal tumor burden	Blat <i>et al</i> (54)
Type I diabetes	Insulin	NOD	CD28/CD3 ζ		No prevention of diabetes occurrence	Tenspolde M <i>et al</i> (56)

MHC: Major Histocompatibility Complex ; NOD mice: Non-Obese Diabetic mice ; PV: Pemphigus Vulgaris ; RA: Rheumatoid Arthritis ; Dsg3: Desmoglein 3 ; MS: Multiple Sclerosis ; MOG: Myelin Oligodendrocyte Glycoprotein ; EAE: Experimental AutoImmune Encephalitis ; CEA: Carcino Embryonic Antigen ; TNBS: TriNitroBenzene Sulphonic acid ; DSS : Dextran Sulfate Sodium

FIGURE LEGENDS

Figure 1: Structure of Chimeric Antigen Receptor (CAR) and Chimeric AutoAntibody receptors (CAAR). Both receptors are composed of activation, co-stimulation and transmembrane domains linked to a flexible linker (ScFv) for CAR or an autoantigen for CAAR. White boxes listed protein sequences that have been used for design of CAR or CAAR. *ITAM: immunoreceptor tyrosine based activation motif.*

Figure 2: Potential future research direction strategies for targeting pathologic cells in rheumatoid arthritis

Presentation of citrullinated neoepitopes to autoimmune B cells lead to autoantibodies production that could be break with a CAAR-T bearing a citrullinated antigen. Use of CAR-Tregs directed against Citrullinated Vimentin (CV) could re-establish tolerance in joints. Dotted rectangle represented how CAAR-T cells delete pathogenic B cells.

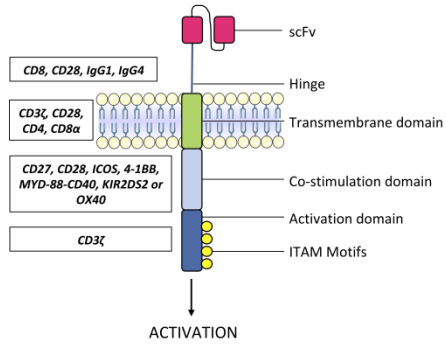
Figure 3: Potential future research direction strategies for targeting pathologic cells in systemic lupus erythematosus

Defect in clearance of apoptotic cells lead to dsDNA liberation, production of autoantibodies by B cells and immune complex formation. CAR-CD19 may drive B cell depletion and stop immune complex formation. CAR-Tregs specific of a renal antigen could secrete anti-inflammatory molecules limiting B cell activation and differentiation. Dotted rectangle represented how dsDNA-CAAR-T and CD19-CAR-T delete respectively pathogenic B cells and CD19-expressing B cells.

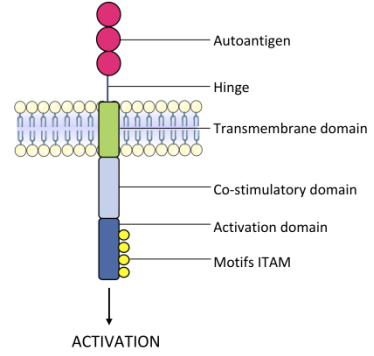
Figure 4: Potential future research direction strategies for targeting pathologic cells in systemic sclerosis

Macrophages phagocytosis of endothelial cell apoptotic bodies lead to pro-inflammatory molecules production, immune cells recruitment, autoantigen presentation and thus anti-fibroblast or anti-endothelial antibody production. Deleting pathogenic cells by CD19 CAR T cells and CAAR-T specific of fibroblast or endothelial antigen could limit fibroblast activation and extracellular matrix protein production. Design of CAR-Tregs against lung antigen could re-establish tolerance in the lung Dotted rectangle represented how endothelial/Fibroblast-CAAR-T and CD19-CAR-T delete respectively pathogenic B cells and CD19-expressing B cells.

A Chimeric Antigen Receptor (CAR)



B Chimeric AutoAntibody Receptor (CAAR)



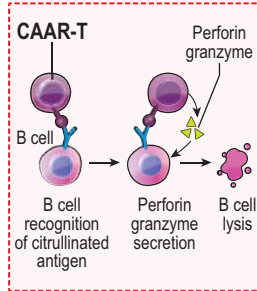
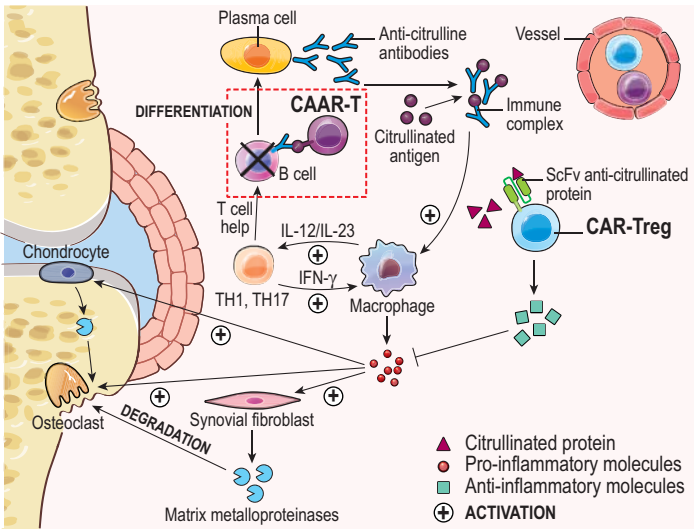
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JOINT

Healthy

Rheumatoid arthritis

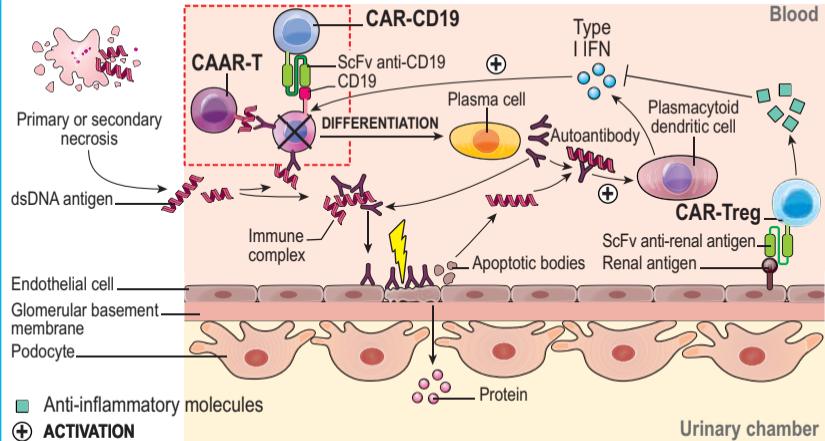
Capsule
Synovium
Joint space
Cartilage
Synoviocyte
Bone



- ▲ Citrullinated protein
- Pro-inflammatory molecules
- Anti-inflammatory molecules
- ⊕ ACTIVATION

RHEUMATOID ARTHRITIS

KIDNEY



SYSTEMIC LUPUS ERYTHEMATOSUS

