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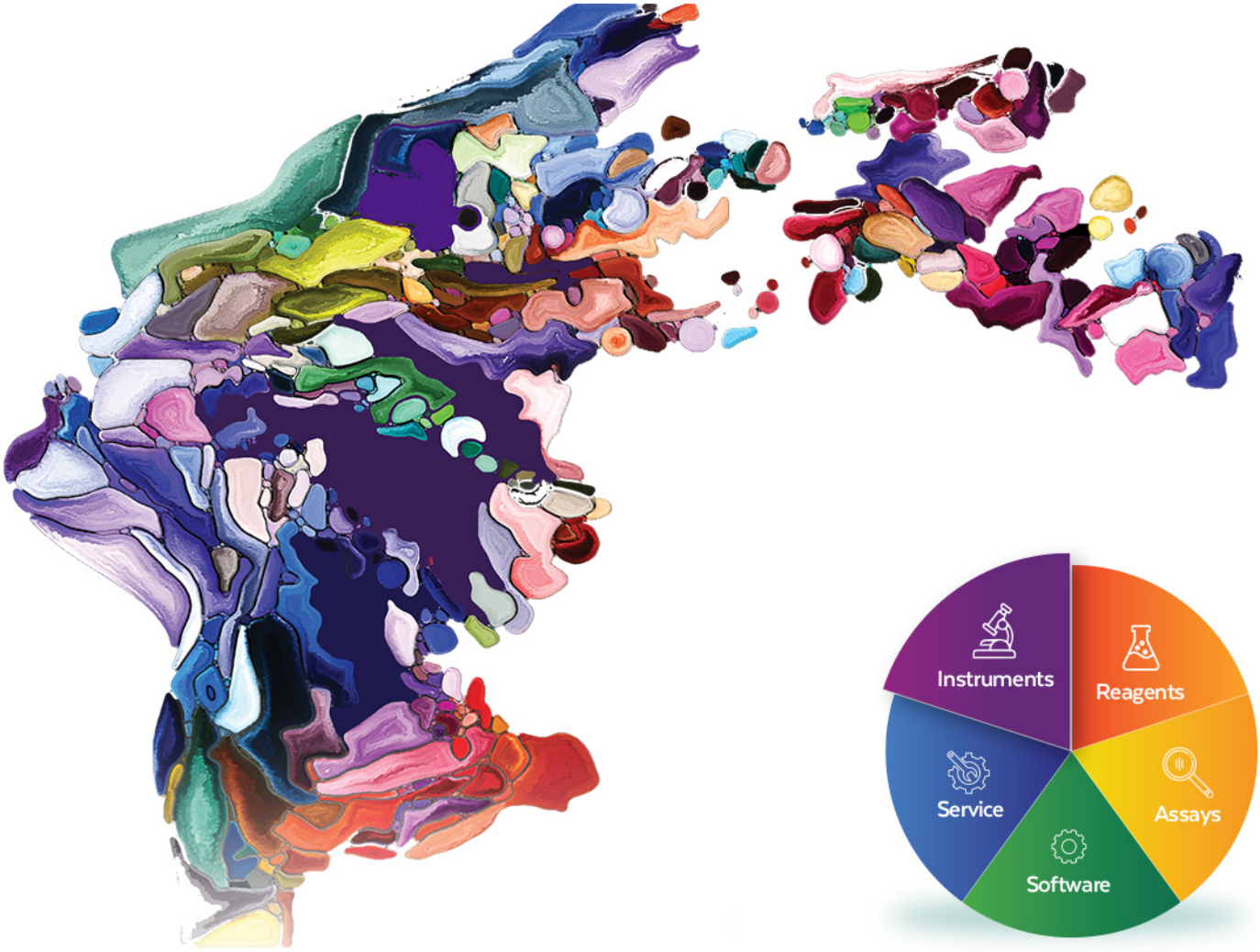

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HIGHLIGHTS

REVIEW

Chimeric antigen receptor–based therapies beyond cancer

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Adoptive cell transfer (ACT) therapies have gained renewed interest in the field of immunotherapy following the advent of chimeric antigen receptor (CAR) technology. This immunological breakthrough requires immune cell engineering with an artificial surface protein receptor for antigen-specific recognition coupled to an intracellular protein domain for cell activating functions. CAR-based ACT has successfully solved some hematological malignancies, and it is expected that other tumors may soon benefit from this approach. However, the potential of CAR technology is such that other immune-mediated disorders are beginning to profit from it. This review will focus on CAR-based ACT therapeutic areas other than oncology such as infection, allergy, autoimmunity, transplantation, and fibrotic repair. Herein, we discuss the results and limitations of preclinical and clinical studies in that regard.

Keywords: Adoptive cell transfer · Chimeric antigen receptors · Engineering immune cells · Immunotherapy

Introduction

Adoptive cell transfer (ACT) emerged in the 1960s as a potential cancer immunotherapy by demonstrating that lymphocyte transfer could inhibit carcinogen-induced rat sarcoma growth [1]. Since then, ACT immunotherapy has progressed steadily [2], and recently been fueled by the success of the chimeric antigen receptor (CAR-T) cell technology and the approval by the U.S. Food and Drug Administration of engineered CAR-T cells for diffuse large B-cell lymphoma and relapsed and refractory acute lymphoblastic leukemia [3].

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CARs are synthetic protein receptors consisting of an extracellular recognition region directed against a desired target and an intracellular region endowed with one or several signaling domains to induce cell activation. The extracellular region is typically a single-chain variable fragment (scFv) from a monoclonal antibody (mAb). In first-generation intracellular regions, CARs incorporated the CD3ζ signaling domain. Second- and third-generation CARs subsequently added co-stimulatory domains from 4-1BB/CD137, CD28, DAP10, OX40/CD134, or ICOS/CD278 receptors to improve cytotoxicity and long-term surveillance out-puts [4].

As for other immunotherapeutic approaches, adverse effects associated with CAR-T cell infusions have been reported. Despite recent progress in safety during prophylaxis and therapy, cytokine

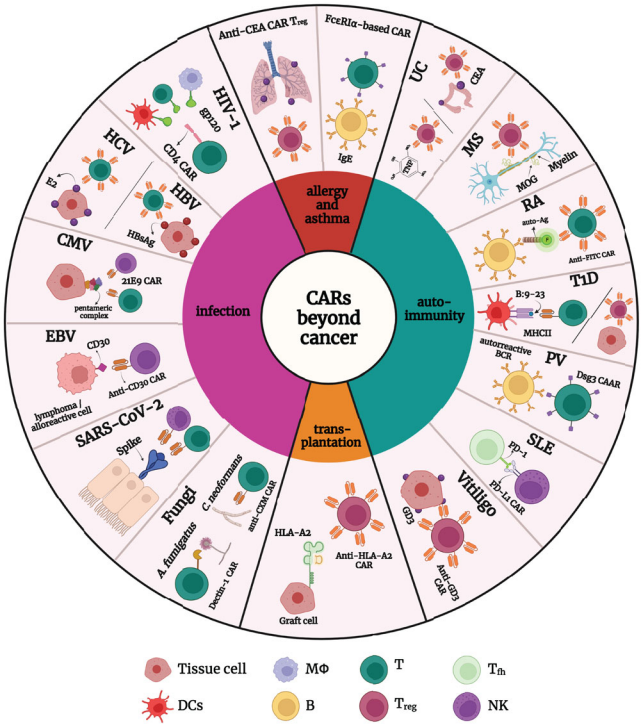

Figure 1. Graphic summary of CAR approaches to different nonneoplastic disorders. CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus; MS, multiple sclerosis; PV; pemphigus vulgaris; RA, rheumatoid arthritis; SARS-COV-2, severe acute respiratory syndrome coronavirus 2; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; UC, ulcerative colitis. Created with BioRender.com.

release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and cytopenia continue to be the most relevant adverse effects [5]. Some current drawbacks associated with autologous CAR-T cell therapies can be overcome by using natural killer (NK) cells [6–8]. In addition to their higher effector efficiency against tumors and pathogens, NK cells are suitable for off-the-shelf allogeneic ACT. NK cells do not induce graft-versus-host disease (GVHD) and have a limited lifespan. Thus, these cells induce lower long-term side effects and additional effector activity due to nonself or altered-self recognition by built-in NK cell receptors.

The versatility of CAR engineering expands its therapeutic use to other unmet medical needs beyond cancer in general and haematological malignancies (Figs. 1 and 2). Here, we present and discuss alternative applications of CAR-based ACT at the preclinical and clinical levels in medical areas as heterogeneous as infection, allergy, autoimmunity, or transplantation (Fig. 1).

Autoimmune Disorders

Autoimmune disorders (AD) and cancer are the two sides of the same coin, by way of a misbalance between effector and regulatory immune responses. AD arise from a combination of genetic and environmental factors (e.g., infections) that trigger a self-tolerance break leading to immune-mediated damage of self-cells and tissues [9–11]. Emerging therapeutic approaches to restore immune tolerance include CAR-based strategies (Figs. 1 and 2). The critical role of regulatory T cells (T_{reg}) in the maintenance of immune tolerance and our understanding of the tolerogenic mechanisms mediated by T_{reg} pave the way for CAR-T_{reg}-based ACT, whose therapeutic potential was hampered until efficient protocols for ex vivo expansion and genetic manipulation have become available [12].

Contrary to the tolerogenic effect of CAR-T_{reg} cells, chimeric auto-antibody receptors (CAAR)-T cells aim to exert a cytotoxic effect on autoimmunity-promoting cells (Fig. 2). This CAAR-T cell approach is based on engineering CARs with an intracellular signalling domain fused to an extracellular autoantigen that will be recognized by specific B-cell receptors (BCRs) on specific B cells [13]. This strategy can be adapted to redirect gene-modified T cells against pathogenic $CD8^+$ or $CD4^+$ T cells by fusing an intracellular signaling domain to extracellular self-peptide MHC class I or class II (pMHC-I or -II) complexes, respectively [14]. Alternatively, T cells can be engineered to express conventional CARs targeting antigen-presenting cells (APC) presenting self pMHC-II complexes [15].

As in other fields, the main challenges of CAR-based ACT in AD include the identification of *bona fide* tissue-specific autoantigens [16] and the plasticity between Th17 and T_{reg} in CAR-T_{reg} development. Inflammatory cytokines, especially IL-6, or Foxp3 instability can induce T_{reg} cells to trans-differentiate into Th17 cells, contributing to some AD. Combined therapies such as retinoic acid receptor-related orphan gamma t (RORγt) downregulation or disruption, immunosuppressive drugs, and Foxp3 overexpression and/or enhanced stability have been proposed to solve this issue [17].

Inflammatory bowel disease

Ulcerative colitis and Crohn's disease are IBD forms believed to arise from several exogenous and endogenous factors, which include T_{reg} dysfunction [18, 19]. To prove the latter, a therapeutic CAR-T_{reg} against 2,4,6-trinitrophenol (TNP) was developed in a murine model of colitis induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) [20] (Fig. 1). These engineered CAR-T_{reg} mouse cells accumulated at the site of the target antigen promoting bystander suppression followed by colitis amelioration. Similarly, based on carcinoembryonic antigen (CEA) overexpression in both human colitis and colorectal cancer, CEA-specific CAR-T_{reg} cells showed potential therapeutic effect in two different models: T-cell-transfer colitis and an azoxymethane–dextran sodium sulphate (AOM-DSS) model for colitis-associated colorectal cancer [21] (Fig. 1). Blat and colleagues designed a second-generation CAR with CD28 and CD3ζ moieties in the intracellular region based on the mAb SCA431. CEA-specific CAR-T_{reg} cells specifically homed in and accumulated in the colon of sick mice, suppressing colitis severity and decreasing colorectal tumor burden.

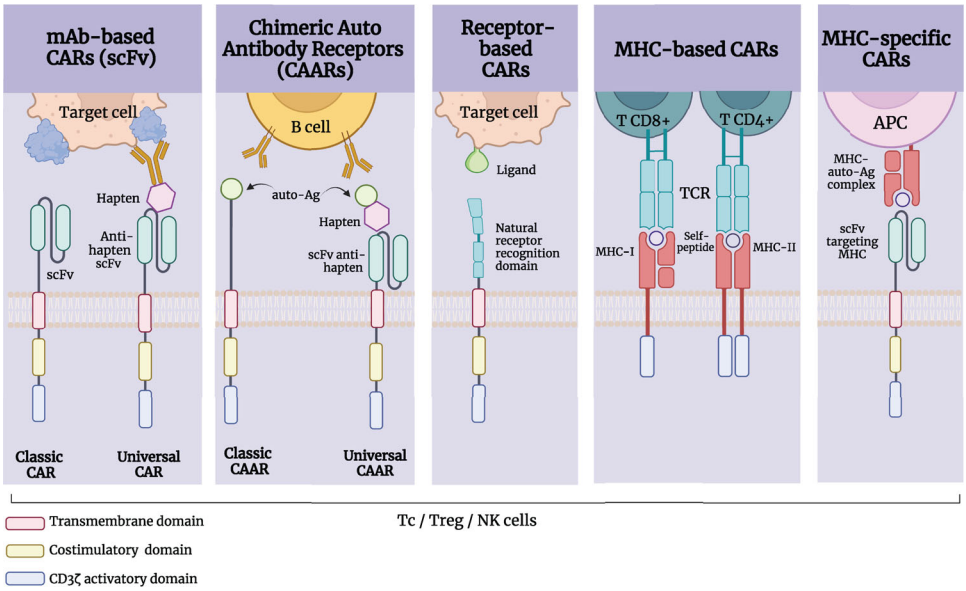

Figure 2. Schematic representation of different CAR-based strategies. CARs exhibiting extracellular regions containing scFv (mAb-based), autoantigens (CAARs), natural receptors (receptor-based) or self pMHC-I/II (MHC-based) are depicted. mAb- and autoantigen-based CARs can be made universal (meaning multiuse) by directing them against haptens or similar small molecules conjugated to specific mAbs or autoantigens. Created with BioRender.com.

Additionally, the relationship between abnormal T-cell function and the onset and progress of disease has prompted several studies on T-cell reduction. In this sense, one clinical trial (NCT05239702) is currently recruiting patients to study the administration of anti-CD7 CAR-T cells in refractory AD, including Crohn's disease, ulcerative colitis, and dermatomyositis.

Autoimmune demyelinating and neurodegenerative disorders

Multiple sclerosis (MS) is the most common immune-mediated demyelinating disease of the CNS with myelin-derived antigens as the main autoreactive targets [22]. Accordingly, myelin oligodendrocyte glycoprotein (MOG)-specific-Foxp3-CAR-T_{reg} cells have been tested in the experimental autoimmune encephalomyelitis (EAE) mouse MS model [23] (Fig. 1). MOG-CAR-T_{reg} cells efficiently reduced active inflammation, decreasing axon damage, and reducing effector cytokine levels (IL-12 and IFN-γ) in the brain. Moreover, in a second EAE challenge, MOG-CAR-T_{reg} cells protected against disease, indicating a sustained effect. Due to the affinity of MOG-specific $CD4^+$ T cells relevance in the EAE ongoing [24], a peptide-MHC-II CAR targeting lower affinity MOG-specific T cells has being designed improving CAR T cells sensitivity and clinical parameters in EAE mice models [25].

Rheumatoid arthritis

Rheumatoid arthritis (RA) is one of the most common chronic autoimmune diseases. It is characterized by the presence of autoantibodies against citrullinated antigens that promote aberrant inflammation at the synovial membrane, causing irreversible joint and bone damage [26]. To overcome the selective and persistent issues associated to rituximab therapy in RA, universal anti-fluorescein isothiocyanate (FITC) CAR-T cells combined with FITC-labeled citrullinated autoantigens (e.g., vimentin, type II collagen, fibrinogen or tenascin-C) were used to eliminate autoreactive B cells in vitro [27] (Figs. 1 and 2). Zhang and colleagues demonstrated that anti-FITC CAR-T cells targeted autoreactive hybridomas and B-cell subsets from RA patients via specific recognition of FITC-labeled citrullinated peptide epitopes, providing a customized approach to treat RA and possibly other systemic AD.

Type 1 diabetes

Type 1 diabetes (T1D) is a T-cell-mediated AD in which both $CD4^+$ and $CD8^+$ T cells are involved in the destruction of insulin-producing islet β cells. The feasibility of CAR-based strategies for T1D treatment has been experimentally proved recently. Based on the 287 mAb, CAR-T cells specific for a complex encompassing an insulin peptide (B:9-23) bound to a MHC class II molecule (I-A^{g7}) were developed to target APC bearing the I-A^{g7}-B:9-23 complex [15] (Fig. 1). A single infusion of 287-CAR $CD8^+$ T cells to young nonobese diabetic (NOD) mice delayed the onset of overt hyperglycemia, but over time, the overall incidence of diabetes was similar to that of control mice. Improved results have been recently reported using a new first-generation biomimetic five-module CAR (^{5M}CAR) [28] consisting of extracellular pMHCII complexes assembled to intracellular CD3 signaling modules (ζ, ε, γ, δ) and a surrogate co-receptor composed of CD80 fused to

Lck tyrosine kinase (Fig. 2). With this strategy, the specificity and function of adoptively transferred ^{5M}CAR-cytotoxic T lymphocyte (CTL) were redirected against clonotypic autoimmune CD4$^+$ T cells to prevent and mitigate T1D in NOD mice.

In another approach, insulin-specific CAR-T_{reg} cells were developed in order to improve the modest efficacy previously observed with polyclonal T_{reg} [29] (Fig. 1). Strong immune-suppressive capacity was achieved in vitro, though the authors did not observe curative effects in vivo despite effective migration and prolonged persistence in pancreas.

Autoimmune skin disorders

Pemphigus vulgaris

Pemphigus vulgaris is a life-threatening autoimmune blistering disease caused by autoantibodies against desmoglein 3 (Dsg3), a keratinocyte adhesion protein [30]. T cells expressing a CAAR consisting of Dsg3 fused to the CD137-CD3ζ signaling domains (Dsg3-CAAR-T cells) (Fig. 1) were administered to diseased mice and specifically killed anti-Dsg3 B cells without off-target toxicity even in the presence of circulating autoantibodies [13]. Currently, an open-label study (NCT04422912) to determine the maximum tolerated dose of Dsg3-CAAR-T in mucosal-dominant pemphigus vulgaris patients is in its recruiting phase.

Vitiligo

Vitiligo consists of the progressive destruction of epidermal melanocytes that leads to white patches in the skin. Oxidative stress in melanocytes triggers inflammatory response and innate immune cells activation which, in genetically predisposed individuals, results in melanocyte-specific cytotoxic responses [31]. Specifically, oxidative stress triggers inducible heat shock protein 70 (hsp70) release that can cause immune cell activation and act as a chaperone for melanocyte antigens. As a result, APC recruit auto-reactive T cells that mediate melanocyte destruction and leads to vitiligo clinical expression. Also, vitiligo patients display lower T_{reg} levels, which can lead to increased T CD8$^+$ cells with anti-melanocyte activity [32]. Thus, ACT using polyclonal T_{reg} in mouse models showed lasting remission of the disease [33]. More recently, a CAR-T_{reg} approach targeting ganglioside D3 (GD3), a molecule overexpressed by perilesional epidermal cells and stressed melanocytes, has been developed [34] (Fig. 1). Infusion of GD3-specific CAR-T_{reg} cells in a TCR transgenic mouse model of spontaneous vitiligo could infiltrate the skin and respond to GD3 expression, thereby suppression of cytotoxic T cells and yielding a localized immune tolerance in vitiligo skin. Moreover, GD3-specific CAR-T_{reg} cells also displayed increased IL-10, higher control of cytotoxic activity toward melanocytes and delayed skin depigmentation.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic, occasionally life-threatening, and multisystem immune-mediated disorder. Individuals with SLE have increased follicular T helper (T_{FH}) cell numbers, especially during disease flares, which correlate with autoantibody titers [35]. As T_{FH} cells express higher programmed death 1 (PD-1) receptor levels than other leukocyte subsets, a CAR construct encompassing the extracellular region of PD-1 ligand (PD-L1) was expressed on NK cells [36] (Fig. 1). PD-L1-CAR-NK cells selectively eliminated PD-1^{high} human T_{FH} cells and reduced memory B-cell proliferation, differentiation, and Ig secretion in vitro. Moreover, infusion of PD-L1-CAR-NK cells decreased PD-1^{high} CD4$^+$ T cells and splenomegaly in a humanized mouse model of lupus-like disease while sparing B cells and other PD-1^{low} T cell subsets, including T_{reg}.

An alternative strategy based on the elimination of the whole B-cell population has been proposed for different AD. In this sense, the administration of anti-CD19 CAR-T cells to a patient with a severe and refractory SLE showed serological and clinical remission with no adverse effects [37]. Moreover, Mackensen and colleagues recently reported SLE remission with no remarkable adverse effects in five patients resistant to conventional immunosuppressive drugs and treated as a compassionate use with anti-CD19 CAR-T cells [38]. Currently, clinical trials based on CAR-T cells targeting CD19 or B-cell maturation antigen are in place for the treatment of SLE (NCT05030779 and NCT03030976). The same approach is being evaluated in other AD such as refractory immune nephritis (NCT05085418), neuromyelitis optica (NMO, NCT03605238), Sjogren's syndrome (NCT05085431), refractory scleroderma (NCT05085444), and myasthenia gravis (MG, NCT04146051). Furthermore, another clinical trial (NCT04561557) is currently recruiting patients from NMO, MG, chronic inflammatory demyelinating polyradiculoneuropathy and immune-mediated necrotizing myopathy to evaluate the safety and efficacy of a novel anti-B-cell maturation antigen CAR-T cell in patients with relapsed/refractory antibody-mediated idiopathic inflammatory diseases.

Bacterial Infections

Bacterial infections are still a worldwide health problem due to their elevated morbidity and mortality rates. The emergence of antimicrobial-resistant strains together with the inability to eliminate infected cell reservoirs highlights the need for alternative therapies. There is little progress, however, regarding ACT in bacterial infections. The most feasible candidate are chronic infections, such as tuberculosis (Tbc). Feasible strategies in this regard would include engineered T cells displaying TCR$\alpha\beta$ recognizing Tbc-specific pMHC-I or -II complexes expressed by infected cells, as well as unmodified T$\gamma\delta$ cells targeting nonpeptidic Tbc antigens [39, 40].

Viral Infections

Human immunodeficiency virus type 1

Human immunodeficiency virus type 1 (HIV-1) infection still constitutes a major global health concern. Although current combined antiretroviral therapy (cART) can control HIV-1 replication, latent virus eradication in cellular reservoirs remains unresolved forcing life-long treatments. Additionally, cART reduces HIV-1 antigen expression on the surface of infected cells impairing CAR-based therapy efficacy.

Promising in vitro and in vivo results have been reported for engineered CAR-T cells targeting HIV-1. Two main strategies have been developed for anti-HIV CAR design: those based on CD4-gp120 interactions and those based on broadly neutralizing antibodies (BNAbs) targeting viral envelope-glycoproteins. First-generation CARs containing the CD4 extracellular region expressed on $CD8^+$ T cells inhibit viral replication and induce lysis of $gp120^+$ cells [41] (Fig. 1). Nonetheless, no clinical improvement on viral reservoir was observed despite the beneficial effects on viremia [42]. In vitro killing of Env^+ cells but also latently infected cells was achieved by using a second-generation CAR construct containing the CD28 co-stimulatory domain [43]. Further replacement of that co-stimulatory domain by 4-1BB substantially potentiated the in vitro activity CD4-CAR T cells [44]. Moreover, in a HIV-humanized mice model, CD4-CAR T cells infusion showed higher expansion numbers as well as HIV replication control.

CAR-T cell therapies based on BNAbs have also been promising, obtaining a high reduction in viral replication by targeting the gp120 and gp41 HIV-1 glycoproteins [45]. In line with this, a third-generation CAR based on the VRC01 BNAb induced T-cell-mediated cytolysis of Env^+ cells and inhibited HIV-1 rebound in a cell culture that mimics the termination of the cART in the clinic [46]. A further improvement was achieved with BNAb-based CARs after gene disruption of the primary HIV-1 co-receptor CCR5 [47]. This second-generation CAR construct containing 41-BB and CD3ζ as signaling domains allowed higher specific activation and cytotoxic effects avoiding CAR-T cell infection by HIV-1.

In order to promote an even more potent anti-HIV CAR-T cell response, bispecific CARs combining CD4- and BNAb-based constructions have been designed [48]. Similar improved anti-viral responses have been reported by replacing the scFV of the BNAb by a human C-type lectin-carbohydrate recognition domain [49].

Nowadays, CAR-T cell therapy for HIV is under evaluation in several phase I clinical trials (NCT03980691, NCT03240328, NCT04648046, and NCT04863066) but no results have yet been reported.

Some disadvantages of CAR-T cells can be overcome by the use of NK cells as effector cells [7, 8]. Two ongoing phase I clinical trials are awaiting results on the effect of haploidentical NK cell administration on HIV-infected patients (NCT03346499 and NCT03899480). In the 1990s, a first-generation CAR (CD4ζ CAR-NK) expressed on the human cell line NK3.3 [50] allowed specific and efficient killing of HIV-infected T cells and $gp120^+$ cell lines. However, this higher efficacy of CD4ζ CAR-NK expressing cells disappeared after in vivo administration [51].

One of the main concerns of HIV-1 is its high diversity and mutability. Recently, an alternative strategy has been proposed based on a universal CAR-NK cell, recognizing 2,4-dinitrophenyl (DNP), to target various epitopes of gp160 using DNP-conjugated antibodies as adaptor molecules [52].

Hepatitis C virus

Hepatitis C virus (HCV) is a leading cause of chronic liver disease [53]. Recently approved drugs show promise compared to conventional interferon α (IFN-α) and ribavirin treatment [54]. However, viral escape mechanisms and resistance as well as side effects of novel drugs remain as therapeutic concerns. Two CARs targeting HCV have been designed based on a human mAb specific for the HCV E2 glycoprotein (HCV/E2) (Fig. 1). Anti-HCV CAR-T cells showed good anti-viral in vitro activity and lysed HCV/E2-transfected as well as HCV-infected cells [55].

Hepatitis B virus

Chronic hepatitis B virus (HBV) infection, together with HCV infection, constitutes an important cause of liver disease leading to liver cirrhosis and hepatocellular carcinoma with high mortality rates. Current treatments with nucleoside analogues suppress viral replication but not reservoir eradication, a main concern in HBV-infected patients [56]. Unlike antiviral treatments in HIV, the use of nucleoside analogues does not affect antigen expression, allowing combined therapies. Thus, second-generation CAR-T cells recognizing hepatitis B surface antigens (HBsAg) eliminated infected hepatocytes, although HBV core protein and HBV rcDNA were still detectable [57] (Fig. 1). Similarly, infused HBsAg-CAR-T cells in a human liver chimeric mice resulted in decreased HBsAg and HBV-DNA plasma levels without complete virus elimination [58]. By using a S protein-specific CAR expressed on $CD8^+$ T cells, viral replication in vivo was efficiently controlled in a murine model of HBV infection [59].

It is important to consider the deleterious effect of CAR-T cell administration in vital organs such as the liver. To avoid this side effect, a second-generation CAR against the S protein together with an inducible caspase 9 gene reduced toxic effects both in vitro and in vivo by limiting the CAR-T cell life span [60].

Human cytomegalovirus

Cytomegalovirus (CMV) infection has no clinical impact in most of the adult population. However, immunosuppressed transplant recipients can develop overt CMV disease and subsequent complications such as graft rejection and graft versus host disease (GvHD) enhancement. The antiviral therapy is limited by the emergence of resistant strains, nephrotoxicity or bone marrow

suppression [61], highlighting the need for alternative therapies. Accordingly, a second-generation CAR-T directed against human CMV glycoprotein B (gB) containing the CD28 co-stimulatory domain was developed [62]. Despite significant TNF-α and INF-γ cytokine production and degranulation activity of gB-CAR-T cells, a low cytolytic activity was observed [63]. Later on, a new gB-CAR-T version including the 4-1BB co-stimulatory domain showed increased cytokine secretion, CD107a expression and proliferation, as well as higher activation and cytotoxicity in vitro compared to the CD28 only construct [64]. Moreover, humanized mice infected by HCMV responded to a single-dose gB-4-1BB-CAR-T cell administration.

Recently, anti-CMV CAR-T constructs targeting the pentameric complex, which is composed of gH, gL, UL128, UL130, and UL131A viral glycoproteins, essential for CMV entry to host cells, have been developed [65] (Fig. 1). One of them based on the 21E9 mAb excelled in activity against CMV-infected cells as assessed by cytokine release (IFN-γ and TNF–α), up-regulation of surface CD107a, proliferation, cytolysis of infected cells, and suppression of viral replication. However, the efficacy of these CAR-T cells has not been tested in vivo.

Epstein–Barr virus

EBV is a herpesvirus that, after a primary infection, produces a latent infection with oncogenic properties [66]. EBV^+ lymphoproliferative disease (EBV-LPD) arises in immunocompromised hosts after hematopoietic stem cell transplantation (HSCT) or solid organ (SOT) transplantation and provides an excellent model for evaluating the clinical potential of T-cell therapies targeting viral antigens (e.g. Epstein–Barr nuclear antigen 1 [EBNA-1], latent membrane protein 1 [LMP-1], or LMP-2). Accordingly, a clinical trial (NCT00058812) has reported safe and effective prophylaxis or treatment for LPD by ACT of polyclonal EBV-CTLs generated from the transplant donor in HSCT patients [67, 68]. Promising results have been also reported from a phase I clinical trial testing safety and efficacy of autologous LMP2A-specific 4-1BB-based CAR-T cells in patients with relapsed/refractory EBV-LPD [67, 68]. Allogeneic EBV-specific T cells engineered to express a CD30-specific CAR eliminate $CD30^+$ lymphomas and avoid GvHD [69] (Fig. 1), and two different clinical trials are in place for relapsed or refractory $CD30^+$ lymphomas (NCT04288726 and NCT04952584).

Furthermore, CAR therapy has emerged as an alternative treatment for EBV-related malignancies. For instance, second-generation CAR against LMP1 [70] or against gp350 EBV envelope glycoprotein [71] showed tumor growth reduction.

Severe acute respiratory syndrome coronavirus 2

Since its appearance in 2019, the infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has concentrated the interest of the scientific community. In addition to vaccine development, approaches involving CARs redirected against this virus have been developed [72]. Second-generation CAR-T against the spike protein of SARS-CoV-2 promotes cell activation and killing activity both in vitro and in vivo in a mouse infection model [72] (Fig. 1). However, the cytokine storm syndrome (CSS) is a clinical complication in SARS-CoV-2 infection similar to the main side effects in CAR-T cell therapy (CRS), so an alternative approach is needed.

The role of NK cells as effector cells against tumor and infected cells positions them as an attractive tool for alternative ACT therapies. Several clinical trials are currently evaluating NK cells in SARS-CoV-2 infection (NCT04365101, NCT04280224, and NCT04344548). The use of NK cells allows for allogenic and off-the-shelf therapy. CAR-NK cells targeting the spike protein by the scFv domain of the neutralizing antibody (NAb) CR3022 recognize SARS-CoV-2 viral particles and kill SARS-CoV-2 infected cells in vitro [73] (Fig. 1). A similar CAR-NK carrying the scFv fragment of the NAb S309 also binds to pseudotyped SARS-CoV-2 virus and its mutants and exerts higher killing activity and cytokine production [74]. To our knowledge, there is only one phase I/II clinical trial evaluating CAR-NK cells in the context of SARS-CoV-2 infection (NCT04324996). In this case, the biological effect of a bispecific CAR construct targeting NKG2D ligands (e.g., MIC-A/B and ULBP1–ULBP6 in human) and the spike protein on the surface of infected cells (NKG2D-ACE2 CAR-NK cells) will be compared with that of NKG2D CAR-NK and ACE2 CAR-NK cells.

Fungal Infections

The emergence of anti-fungal resistant isolates together with the side effects of current antifungal drugs highlights the need for novel strategies. ACT therapies based on the infusion of in vitro-expanded *Aspergillus fumigatus*-specific T cells have shown promising results in mice and humans [75, 76]. However, this approach implies technical challenges related to cell handling, manipulation, and timing, a situation that also applies to autologous CAR-T cell-based strategies. Nevertheless, CAR-T cells using the extracellular domain of human Dectin-1 (Fig. 1), a fungal β-glucan receptor, exhibited specificity to laminarin and led to damage and inhibition of hyphal growth in *A. fumigatus* in vitro and in vivo [77]. Recently, a specific second-generation CAR based on a mAb against *A. fumigatus* hyphae has showed pro-inflammatory cytokines production after fungus-recognition in vitro as well as increased antifungal response, fungal burden reduction, and survival improvement in vivo. Moreover, the authors demonstrate safety in terms of no-off target effects [78]. Similarly, CAR-T cells targeting the *Cryptococcus neoformans* capsule component glucuronoxylomannan (GXM) based on the mouse 18B7 mAb [79] (Fig. 1) showed granzyme and IFN-γ production as well as reduced number of titan and giant cells upon to *C. neoformans* co-culture [80]. This is an important effect as cell enlargement is a main virulence factor of this pathogen [81]. Moreover, GXMR-CAR-T cells also recognized the resistant strain *C. gatti* [82].

Transplantation

Adaptive immune responses to human leukocyte antigens (HLA) are a major concern in the field of transplantation. HLA mismatching is associated with poor outcomes after HSCT and SOT. But HSCT can in turn give rise to GvHD, in which graft T-cells recognize host antigens. In order to overcome organ transplant rejection and GvHD, CAR-based treatments have emerged based on CAR-T_{reg} cells [83] (Fig. 1).

HLA-A*02 is present in 50% of the population, and tolerance induction for this allele is meaningful. Several ACT approaches have been developed using HLA-A*02-specific CAR-T_{reg} cells (A2-CAR-T_{reg}) that prevent xenogeneic GvHD in immunodeficient NOD SCID $\gamma_c^{-/-}$ (NSG) mice receiving HLA-A*02$^+$ human T cells (Figs. 1 and 2) [84]. In this line, another report showed that A2-CAR-T_{reg} cells completely prevent rejection of human skin allografts in immune-reconstituted humanized mice in the absence of immunosuppression [85]. The A2-CAR-T_{reg} cells led to stronger proliferation and upregulation of the CD39 effector molecule, inhibition of allo-specific T_{eff} cell proliferation in vitro, and suppressed delayed-type hypersensitivity allogeneic responses more effectively than unmodified natural T_{reg} (nT_{reg}) or control CAR-T_{reg} cells. Moreover, transferred A2-CAR-T_{reg} cells homed into skin grafts persisting long-term and preventing the rejection of HLA-A*02$^+$ skin grafts by HLA-A*02$^-$ T cells. In a more recent study, ACT of T_{reg} cells expressing a fully humanized HLA-A2-specific CAR (hA2-CAR-T_{reg}) suppressed HLA-A*02$^+$ cell–mediated xenogeneic GvHD and diminished rejection of human HLA-A2$^+$ skin allografts [86].

To our knowledge, there is only one clinical trial (NCT04817774) on CAR-based therapy in transplantation, evaluating the safety and tolerability of TX200-TR101, an autologous HLA-A*02-specific CAR-T_{reg} product, aimed at preventing the rejection of transplanted kidney.

HSCT patients develop an immunodeficient state after chemoradiotherapy and GvHD prophylaxis. This condition predisposes to bacterial, viral, and fungal infections, increasing the risk of nonrelapse mortality. Antimicrobial therapies are short-term, costly, show long-term related toxicity, and are heavily burdened by drug resistance. ACT of CTLs against microbial antigens (e.g., EBV, CMV or adenovirus; see Section 3) offers pathogen-specific cytotoxic responses, reduced organ toxicities, and long-term T-cell memory.

Allergy and asthma

Asthma is a chronic inflammatory lung disease characterized by airway obstruction and breathing difficulties. Allergen inhalation can promote an immune system overreaction characterized by dysregulated T_{reg} function and T helper 2 (Th2)-immune response dominance. Th2 cells promote immunoglobulin class-switch in B cells to produce immunoglobulin E (IgE) antibodies whose binding to high-affinity FcεRI receptors orchestrate the allergic reaction [87]. Useful bronchodilators and anti-inflammatory drugs control symptoms in most cases, but do not offer curative solutions.

Efficacy of T_{reg} ACT in allergic pathologies is observed, with reduced eosinophilia and Th2 responses as well as decreased asthma symptomatology [88, 89]. In keep with these results, redirected CAR-T_{reg} cells have been shown to suppress experimental allergic airway inflammation, a model of asthma [90] (Fig. 1). CAR-T_{reg} cells targeting CEA, expressed in the stromal site of lung epithelium in airway inflammation conditions, reduce a number of important clinical manifestations, such as, airway hyper-reactivity (AHR), eosinophilia inflammation, mucus secretion, Th2 cytokine release, and allergen-specific IgE titers.

The success of omalizumab, an IgE-specific mAb, in treating severe allergic asthma is hampered by the frequent need administration of an expensive drug. To overcome this limitation, CAR-T cells were engineered to express the extracellular domain of FcεRI for recognition of the transmembrane form of IgE (mIgE) present on all IgE-producing B cells [91] (Fig. 1). First-generation FcεRIα-based CAR-T cells specifically recognized cells expressing mIgE in vitro, but did not capture IgE secreting cells, thus holding promise for novel, specific, and effective ACT against severe allergic diseases.

Miscellanea

Hemophilia A

A common side effect of factor VIII (FVIII) replacement therapy in hemophilia A patients is the development of NAbs by FVIII-specific B cells. The only clinically proven strategy to eradicate NAbs is repeated high-dose FVIII infusions until NAbs become undetectable, known as immune tolerance induction therapy, but it is not always successful. In light of this, a CAR harboring the immunodominant A2 or C2 domains of FVIII as B-cell antibody receptor (BAR) fused to intracellular CD28 and CD3ζ domains has been expressed on human and mouse CD8$^+$ T cells [92]. BAR-expressing CAR CD8$^+$ T cells killed FVIII-specific B cells in vitro and in vivo and lowered anti-FVIII NAbs levels in LPS-stimulated splenocytes. Similar suppression of anti-FVIII NAbs production was also experimentally achieved by using CAR-T_{reg} cells expressing the above-mentioned BAR, targeting FVIII-specific memory B cells [93]. In another report, anti-FVIII CAR-T_{regs} based on a mAb against the A2 subunit of FVIII also suppressed FVIII-specific T- and B-cell responses leading to lower anti-FVIII NAbs production [94]. These data suggest that engineered CAR-T_{reg} cells hold promise in future tolerogenic treatment of hemophilia A patients.

Cardiac fibrosis

Cardiac fibrosis results from the progressive accumulation of ECM proteins produced by cardiac fibroblast upon injury or disease. This leads to excessive stiffness and consequently to reduced

function of cardiac tissue. There is general interest in developing new treatments for cardiac fibrosis, but there are very limited therapeutic options to target excessive fibrosis directly [95].

The first study exploring the use of ACT therapy in cardiac fibrosis targeted activated cardiac fibroblasts by specifically expressing a xenogeneic antigen (OVA) in myofibroblasts [96]. Cardiac fibrosis was induced with angiotensin II (AngII) and phenylephrine. When $CD8^+$ T cells specifically recognizing OVA peptide (OT-I T cells) were injected, diminished cardiac hypertrophy and cardiac fibrosis progression were detected. The authors identified the fibroblast activation protein (FAP) as a suitable target for ACT therapy, as it is upregulated in hypertrophic cardiomyopathy, dilated cardiomyopathy, and in the AngII/phenylephrine cardiac fibrosis model. Anti-FAP CAR-T cells infiltrated the myocardium reducing cardiac fibrosis and partially rescuing systolic and diastolic functions. In the same line, anti-FAP CAR-T cells were recently generated in vivo by delivering mRNA in T-cell-targeted lipid nanoparticles (LNPs) [97], as continued CAR-T-driven antifibrotic activity may impair wound healing. mRNA sequences encoding an anti-FAP CAR structure were delivered to T cells by LNPs functionalized with an anti-CD5 mAb. The use of LNPs directed to specific cell types also represents an advantage in terms of specificity and controlled action. It should be noted here, however, that anti-FAP CAR-T cells targeting stromal fibroblasts in the microenvironment of solid tumors may also be myelotoxic [96].

Conclusions/future perspectives

There is growing evidence that CAR-based ACT offers versatile therapeutic strategies for many unmet clinical needs beyond cancer. Such versatility is achievable through diverse antigen binding domains and cell type combinations (Figs. 1 and 2). This implies that any immune-based disorder may potentially be targeted with CARs should appropriate antigens be identified. Thus, enabling strategies against chronic infections, especially those that are hard to eradicate and those resistant to antimicrobial therapies, which require long-term medication (e.g., HIV-1 infection or Tbc). With regard to AD, promising results are being challenged by issues of plasticity, stability, and source of T_{reg}. The identification of self-antigens through genome-editing technology for self-antigen-specific antibody-CAR construction will no doubt prompt new clinical trials in AD.

The immune system also plays an important role in the pathogenesis of asthma, allergy, and graft rejection. In this sense, redirecting T_{reg} enables regulation of the immune reaction at the base of the disease, generates long-term immune memory, and can help avoid immunosuppressive therapy risks.

Nowadays, CAR-based ACT runs on costly specialized personnel and infrastructure requirements, making it largely inaccessible and restricted to exceptional patients with no other therapeutic option and only in developed countries. However, cost-effectiveness depends heavily on cure rate and long-term outcomes. In this scenario, the cost of one curative dose of CAR therapy should be calculated against the economic burdens associated to chronic treatments, current drug toxicity, and multi-resistance to ascertain its overall financial viability and global availability.

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Abbreviations: **ACT**: adoptive cell transfer · **BAR**: B-cell antibody receptor · **BNAb**: broadly neutralizing antibodies · **CAAR**: chimeric auto-antibody receptors · **CAR**: chimeric antigen receptor · **cART**: combined antiretroviral therapy · **CEA**: carinoembryonic antigen · **CMV**: cytomegalovirus · **FAP**: fibroblast activation protein · **FVIII**: factor VIII · **gB**: glycoprotein B · **GD3**: ganglioside D3 · **GvHD**: graft versus host disease · **HBsAg**: hepatitis B surface antigen · **HBV**: hepatitis B virus · **HCV**: hepatitis C virus · **HLA**: human leukocyte antigens · **HSCT**: hematopoietic stem cell transplantation · **LMP**: latent membrane protein · **LNP**: lipid nanoparticles · **LPD**: lymphoproliferative disease · **MOG**: myelin oligodendrocyte glycoprotein · **NOD**: nonobese diabetic · **OVA**: ovoalbumin · **RA**: rheumatoid arthritis · **SARS-CoV-2**: severe acute respiratory syndrome coronavirus 2 · **scFv**: single-chain variable fragment · **SLE**: systemic lupus erythematosus · **T1D**: type 1 diabetes

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