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Review

The enchanting canvas of CAR technology: Unveiling its wonders in non-neoplastic diseases

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SUMMARY

Chimeric antigen receptor (CAR) T cells have made a groundbreaking advancement in personalized immunotherapy and achieved widespread success in hematological malignancies. As CAR technology continues to evolve, numerous studies have unveiled its potential far beyond the realm of oncology. This review focuses on the current applications of CAR-based cellular platforms in non-neoplastic indications, such as autoimmune, infectious, fibrotic, and cellular senescence-associated diseases. Furthermore, we delve into the utilization of CARs in non-T cell populations such as natural killer (NK) cells and macrophages, highlighting their therapeutic potential in non-neoplastic conditions and offering the potential for targeted, personalized therapies to improve patient outcomes and enhanced quality of life.

BACKGROUND

The chimeric antigen receptor (CAR) is a hybrid antigen receptor that redirects T cells to cells or tissues expressing specific markers and allows for antigen presentation independent of the major histocompatibility complex (MHC), enabling direct activation of T cells against tumor-associated antigens (TAAs).[1,2] In 2017, the United States Food and Drug Administration (FDA) approved the first CAR-T product, Kymriah, for the treatment of adult patients with relapsed or refractory (r/r) large B cell lymphoma and patients up to 25 years of age with (r/r) B cell precursor acute lymphoblastic leukemia (ALL).[3] Since then, countless clinical trials and preclinical projects have been launched globally, greatly advancing the development of CAR-T technology. As of 2023, 10 CAR-T products associated with hematological malignancies have been successfully approved for marketing,[4–13] while attempts are ongoing to apply CAR-T cell therapy in the treatment of solid tumors such as breast cancer, lung cancer, prostate cancer, colorectal cancer, gastric cancer, and ovarian cancer.[14] In recent years, with the continued advancement of CAR technology, several preclinical investigations and clinical trials have been initiated for various non-neoplastic diseases, including autoimmune disorders, chronic infectious conditions, fibrotic disorders, and a multitude of non-neoplastic ailments associated with cellular senescence. Several successful case reports have emerged in this respect, highlighting the potential of CAR technology in this respect. In our own clinical practice, we are actively exploring the applications of CAR technology in non-neoplastic diseases, as discussed in this review. We aim to translate the promising preclinical research findings and clinical trial advancements into practical therapies for a range of medical conditions, beyond just cancer, and are investigating the potential use of non-T cell carriers to enhance the effectiveness of CAR therapies.

CAR-T CELL THERAPY FOR AUTOIMMUNE DISEASES

Autoimmune diseases are a heterogeneous group of disorders characterized by the presence of autoantibodies and immune reactions initiated by disease-associated

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autoreactive lymphocytes. Autoantibodies participate in tissue damage through various mechanisms, including complement-dependent and antibody-dependent cell-mediated cytotoxicity, as well as immune complex deposition.[15] In contrast to the abundant autoantibodies, autoreactive lymphocytes primarily accumulate in target organs and circulate at low levels, providing unique targets for disease treatment.[16] Current pharmacological interventions for these disorders can be broadly classified into two categories: (1) immunosuppressive agents, such as corticosteroids, methotrexate, mycophenolate mofetil, and cyclosporine; and (2) biologic immunosuppressive drugs selectively targeting specific pathways or localized targets, such as belimumab (an anti-B cell-activating factor neutralizing antibody) and rituximab (an anti-CD20 antibody) for depleting B cells, and tocilizumab for blocking interleukin (IL)-6 signaling. Compared with the former, biologic immunosuppressive drugs offer more suitable options for long-term treatment owing to their reduced toxicity and side effects. Nevertheless, they fail to restore immune tolerance permanently.[17]

Recent advances have demonstrated that CAR-T therapy can achieve non-specific elimination of B cells by targeting pan-markers of B cells (CD19, CD20, BCMA) as well as specific elimination of autoreactive immune cells by targeting self-antigens (Table 1). Additionally, the use of CAR regulatory T cells (CAR-Tregs) to suppress autoimmune manifestations and self-inflammatory events, thereby restoring immune tolerance, has emerged as a promising approach for curing autoimmune diseases (Table 2).

Non-specific B cell depletion by CAR-T cells

Systemic lupus erythematosus

In systemic lupus erythematosus (SLE), immune tolerance to nuclear antigens is disrupted, leading to the sustained production of autoantibodies against double-stranded DNA and other nuclear antigens by aberrantly activated B cells. This, in turn, triggers immune-complex-induced inflammation in various organs such as the kidneys, heart, lungs, and skin.[50]

In 2019, Kansal et al.[18] conducted a groundbreaking study investigating the preventive and therapeutic potential of anti-CD19 CAR-T cells in two distinct mouse models of SLE. The study yielded compelling evidence that anti-CD19 CAR-T cells exhibited long-lasting efficacy in depleting B cells within the mice, thereby effectively preventing SLE recurrence in both disease models. Building upon these results, Jin and his team engineered two murine-derived anti-CD19 CARs, each incorporating either CD28 or 4-1BB as the intracellular co-stimulatory motif. These CARs were administered to MRL-lpr mice, a well-established spontaneous SLE model characterized by severe lupus nephritis revealing the robust efficacy of CD19 CAR-T cell therapy in preventing and treating SLE. Particularly noteworthy was the superior performance of CAR-T cells with the 4-1BB co-stimulatory motif, which exhibited prolonged therapeutic effects and lower levels of cellular exhaustion compared to those with CD28 co-stimulation.[19]

Based on preclinical research, Mougiakakos et al.[30] pioneered the clinical use of anti-CD19 CAR-T cells in severe SLE, achieving remarkable therapeutic efficacy in a 20-year-old female patient with refractory disease, including reduction in anti-dsDNA antibodies, proteinuria levels, and restoration of complement levels to normal, with a drop in SLE Disease Activity Index (SLEDAI) score to 0 after 44 days post infusion.

Table 1. Studies on the depletion ability of CAR-T cell therapy in autoimmune diseases

Preclinical studies

Disease	Target	CAR-T cell	Gene delivery system	Significant outcome	Reference
SLE	CD19	murine CD19-targeted CAR-T ($CD28^{-}CD3\zeta$)	lenti- and retroviral vectors/transfection	• long-lasting efficacy in depleting B cells and autoantibody • reduced manifestations of lupus pathogenesis	Kansal et al.[18]
SLE	CD19	murine CD19-targeted CAR-T ($CD28^{-}CD3\zeta$; 4-1BB- $CD3\zeta$)	lentiviral vectors/transfection	• 4-1BB CAR-T cells exhibited prolonged therapeutic effects and lower levels of cellular exhaustion	Jin et al.[19]
MS	CD19	murine CD19-targeted CAR-T ($CD28^{-}CD3\zeta$)	retroviral vectors/transfection	• enhanced clearance of meningeal B cell aggregates but exacerbated EAE symptoms	Mitsdoerffer et al.[20]
MS	CD19	murine CD19-targeted CAR-T ($CD28^{-}CD3\zeta$)	retroviral vectors/transfection	• achieved sustained elimination of CNS B cells compared to anti-CD20 mAb therapy • ameliorated EAE-associated symptoms	Gupta et al.[21]
T1D	IA^{g7}-B:10–23 R3	murine 287-CAR-T ($CD28^{-}CD3\zeta$ or $CD28^{-}$ 4-1BB-$CD3\zeta$)	retroviral vectors/transfection	• only retarded rather than entirely prevented T1D progression	Zhang et al.[22]
T1D	clonotypic TCRs of pMHC-II-specific $CD4^{+}$ T cells	murine ^{5M} CAR-CD8 + T	retroviral vectors/transfection	• attenuated diabetes incidence and restrained insulitis	Kobayashi et al.[23]
RA	citrullinated autoantigens	human universal anti-FITC CAR-T (4-1BB- $CD3\zeta$)	lentiviral vectors/transfection	• redirected anti-FITC CAR-T to corresponding hybridoma cells or autoreactive B cells	Zhang et al.[24]
RA	HLA-DRB1*01:01 (DR1)	murine DR1-CII CAR-CD8 + T ($CD28^{-}CD3\zeta$)	retroviral vectors/transfection	• efficiently recognized and killed CII-specific $CD4^{+}$ T cells • reduced self-reactive antibody responses	Whittington et al.[25]
MuSK MG	disease-relevant anti-MuSK B cell epitopes	human MuSK-CAAR-T (4-1BB- $CD3\zeta$)	lenti- and retroviral vectors/transfection	• Achieved MuSK-specific B cell depletion without decreasing B cells or total IgG levels	Oh et al.[26]
GvHD	CD83	human CD83 targeted CAR-T (4-1BB- $CD3\zeta$)	retroviral vectors/transfection	• significantly increased the Treg/conventional T cell (Tconv) ratio and provided sustained prophylaxis against xenogeneic GvHD	Shrestha et al.[27]
PV	anti-Dsg3 BCRs	human Dsg3 CAAR-T (4-1BB- $CD3\zeta$)	lentiviral vectors/transfection	• Exhibited specific eradication of Dsg3-specific B cells and achieved histological and serological remission in the murine PV model	Ellebrecht et al.[28]

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Table 1. *Continued*

Preclinical studies

Disease	Target	CAR-T cell	Gene delivery system	Significant outcome	Reference
PV	anti-Dsg3 BCRs	human Dsg3 CAAR-T (4-1BB- CD3ζ)	lentiviral vectors/transfection	• inhibited antibodies responses with clinical and histological regression of blisters in a rhDSG3 active immune model • exhibited specific lysis of autologous anti-Dsg3 B cells derived from PV patients	Lee et al.[29]

Case reports

Disease	Target	CAR-T cell	Gene delivery system	Significant outcome	References
SLE	CD19	human CD19-targeted CAR-T (4-1BB- CD3ζ)	lentiviral vectors/transfection	• reduced anti-dsDNA antibodies and proteinuria level • restoration of complement level • SLEDAI score drops to 0	Mougiakakos et al.[30]
SLE	CD19	human CD19-targeted CAR-T (4-1BB- CD3ζ)	lentiviral vectors/transfection	• eliminated autoantibody production • achieved profound resetting of the immune system • achieved complete drug-free remission over 17 months • 60% chance of developing cytokine release syndrome (CRS), grade 1, no ICANS	Mackensen et al.[31]
ASS	CD19	human CD19-targeted CAR-T	lentiviral vectors/transfection	• vanished anti-Jo-1 antibodies entirely • complete resolution of ASS	Müller et al.[32]

Clinical trials

Disease	Study start	CAR-T cell	Current status	Primary objectives/outcome	NCT
SLE	2017-03	CD19-targeted CAR-T	phase I unknown status	• assess anti-CD19-CAR-T cells safety and efficacy in treating patients with SLE	NCT03030976
SLE	2021-09	CD19/BCMA CAR-T	early phase I unknown status	• assess the safety and effectiveness of CD19/BCMA CAR-T cell in patients with relapsed/refractory SLE	NCT05030779
SLE	2022-04	BCMA-CD19 cCAR-T	phase I recruiting	• evaluate the safety and tolerability of BCMA-CD19 cCAR-T cells in patients with relapsed/refractory SLE	NCT05474885
SLE	2023-02	CD19-targeted CAR-T (Relma-cel)	phase I recruiting	• assess the safety tolerability pharmacokinetics and pharmacodynamics of Relma-cel in moderate or severe active SLE	NCT05765006
SLE	2023-04	universal CD19-targeted CAR-T (BRL-301)	recruiting	• assess the efficacy and safety of BRL-301 in the relapse or refractory autoimmune diseases	NCT05859997
SLE	2023-05	CD19-BCMA CAR-T (GC012F)	early phase 1 recruiting	• determine the maximum tolerated dose of GC012F injection in patients with refractory SLE	NCT05858684
SLE	2023-06	CD19-targeted CAR-T (CNCT19)	early phase 1 enrolling by invitation	• evaluate the safety and preliminary efficacy of CNCT19 in patients with refractory SLE	NCT05930314

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Table 1. *Continued*

Clinical trials

Disease	Study start	CAR-T cell	Current status	Primary objectives/outcome	NCT
SLE	2023-07	CD19-targeted Nex-T CAR-T (CC-97540)	phase I recruiting	• establish the tolerability, preliminary efficacy, and pharmacokinetics of CC-97540 in participants with severe, refractory SLE	NCT05869955
SLE	2023-09	universal CD19-targeted CAR-T (BRL-301)	not yet recruiting	• assess the efficacy and safety of BRL-301 in the refractory SLE	NCT05988216
SLE	2023-10	CD19-BCMA CAR-T (GC012F)	phase I recruiting	• determine the recommended phase II dose of GC012F injection in patients with refractory SLE	NCT05846347
Immune Nephritis	2021-11-05	CD19/BCMA CAR-T	early phase I recruiting	• assess the safety and effectiveness of CD19/BCMA CAR-T cell in patients with refractory immune nephritis	NCT05085418
Lupus Nephritis	2023-04	fully human Anti-CD19 CAR-T (KYV-101)	phase I recruiting	• assess the efficacy and safety of KYV-101 in patients with refractory lupus nephritis	NCT05938725
MG	2019-12	BCMA-targeted rCAR-T (Descartes-08)	phase II recruiting	• MG severity scales showed clinically meaningful declines over 9 months • no dose-limiting toxicities, CRS and ICANS	NCT04146051
MG	2023-04	CD19-targeted CAR-T	phase I recruiting	• evaluate the safety of CD19 CAR-T therapy for patients with refractory MG	NCT05828225
MuSK MG	2022-11	MuSK-CAAR-T	phase I recruiting	• evaluate the safety of various dosing regimens of MuSK-CAAR-T cell therapy	NCT05451212
NMOSD	2018-08	tandem CD19/20-targeted CAR-T	phase I withdrawn	• assess the safety of the tanCAR-T-19/20 cells in treating NMOSD patients • determine duration of *in vivo* survival of tanCART-19/20 cells	NCT03605238
NMOSD	2020-09	BCMA-targeted CAR-T (CT103A)	early phase 1 recruiting	• the mid-term results as of January 2023 indicate11 patients showed no relapse with 17% of patients maintaining a response beyond 6 months post-infusion	NCT04561557
NMOSD	2023-04	CD19-targeted CAR-T	phase I recruiting	• evaluate the safety and the pharmacokinetics of CD19 CAR-T therapy for patients with relapsed or refractory NMOSD	NCT05828212
Sjogren's Syndrome	2021-11	CD19/BCMA CAR-T	early phase I recruiting	• assess the safety and effectiveness of CD19/BCMA CAR-T cell in patients with refractory Sjogren's Syndrome	NCT05085431
PV	2020-09	DSG3-CAAR-T	phase I recruiting	• find the maximum tolerated dose and optimal fractionated infusion schedule of DSG3-CAAR-T in patients with mPV	NCT04422912

Table 2. Studies on the CAR-Treg therapy in autoimmune diseases

Preclinical studies

Disease	Target	CAR-Treg cell	Gene delivery system	Significant outcome	Reference
Vitiligo	ganglioside D3 (GD3)	murine GD3 CAR-Tregs (CD28$^-$CD3ζ)	retroviral vectors/transfection	• increased IL-10 secretion in response to antigen • significantly delayed depigmentation compared to untransduced Tregs	Mukhatayev et al.[33]
IBD	Flagellin	human FliC-CAR-Tregs (CD28$^-$CD3ζ)	lentiviral vectors/transfection	• drove preferential migration Tregs to the colon and expression of the activation marker PD-1 • promoted the reconstitution of colonic epithelial monolayers	Boardman et al.[34]
IBD	TNP	murine TNP-targeted CAR-Tregs (CD28$^-$CD3ζ)	unknown	• mitigated TNBS-triggered colitis through bystander immune suppression	Elinav et al.[35]
IBD	CEA	murine CEA CAR-Tregs (CD28$^-$CD3ζ)	retroviral vectors/transfection	• exhibited the capacity to effectively alleviate DSS-induced colitis • exerted suppressive effects on colorectal carcinogenesis	Blat et al.[36]
T1D	insulin	murine insulin-specific CAR-Tregs (derived from CD4$^+$ T cells converted with foxP3 gene) (CD28$^-$CD3ζ)	retroviral vectors/transfection	• exhibited sustained functionality, suppressive activity, and prolonged persistence • failed to prevent spontaneous diabetes due to the rapid degradation of insulin *in vivo*	Tenspolde et al.[37]
T1D	HPi2	human HPi2-CAR-Tregs (CD28$^-$CD3ζ)	retroviral vectors/transfection	• failed to maintain expansion due to a persistent tonic signaling from the CAR engagement to unexpectantly HPi2 antigen	Radichev et al.[38]
T1D	GAD65	human GAD65 CAR-M/N-Tregs	unknown	• infiltrated the islets, and yielded a substantial reduction in blood glucose levels	Imam et al.[39]
T1D	InsB $_{10–23;}$ IA^{g7}	murine InsB-g7 CAR-Tregs (CD28$^-$CD3ζ)	retroviral vectors/transfection	• sustained the Foxp3 phenotype and effectively averted spontaneous diabetes onset	Spanier et al.[40]
GvHD	HLA-A2	human HLA-A2 targeted CAR-Tregs (CD28$^-$CD3ζ)	lentiviral vectors/transfection	• exhibited superiority over polyclonal Tregs in inhibiting HLA-A2+ PBMC proliferation *in vitro* and preventing GvHD *in vivo*	MacDonald et al.[41]
GvHD	MHC class I proteins	murine anti-FITC CAR-Tregs (mAbCAR) (CD28$^-$CD3ζ)	transient transfection	• prolonged islet allograft survival and the survival of secondary skin grafts specifically matched to the original islet allograft	Pierini et al.[42]
GvHD	CD19	murine CD19 CAR-Tregs (4-1BB-CD3ζ)	retroviral vectors/transfection	• achieved GvHD inhibition without compromising GVT effects	Bolivar-Wagers et al.[43]
MS	MOG	murine MOG-FoxP3 CAR-Tregs (derived from CD4$^+$ T cells converted with foxP3 gene) (CD28$^-$CD3ζ)	lentiviral vectors/transfection	• curtailed immune aggression against MOG+ oligodendrocytes • mitigated sustained inflammation and ameliorated clinical manifestations of the EAE	Fransson et al.[44]
MS	MBP/MOG	human MBP/MOG targeted CAR-Tregs (CD28$^-$CD3ζ)	retroviral vectors/transfection	• constrained the progression of EAE in murine models	De Paula Pohl et al.[45]

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Table 2. *Continued*

Preclinical studies

Disease	Target	CAR-Treg cell	Gene delivery system	Significant outcome	Reference
Hemophilia A	factor VIII	human FVIII-targeted CAR-Tregs (ANS8 CAR) (CD28$^-$CD3ζ)	retroviral vectors/transfection	• restrained the proliferation of FVIII-specific effector cells and curbed the recall antibody response against FVIII in FVIII knockout mice	Yoon et al.[46]
Hemophilia A	factor VIII	murine FVIII-targeted CAR-Tregs (derived from CD4$^+$ T cells converted with murine foxP3 gene) (CD28-4-1BB-CD3ζ)	lenti- and retroviral vectors/ transfection	• effectively blocked the immune response against FVIII and developed tolerance to FVIII for up to 8 weeks	Fu et al.[47]
Hemophilia A	anti-FVIII BCRs	murine A2-BAR-Tregs (CD28$^-$CD3ζ)	retroviral vectors/transfection	• exhibited the capacity to selectively target FVIII-specific memory B cells and suppress the formation of antibody secreting cell (ASC) engendering FVIII antibodies	Pohl et al.[48]
Asthma	CEA	murine CEA-CAR-Tregs (CD28$^-$CD3ζ)	Cre/*loxP* rosa 26 vector/ transfection	• diminished AHR, reduced eosinophilic airway inflammation, mitigation of excessive pulmonary mucus production • curtailed levels of allergen-specific IgE and Th2 cytokines	Skuljec et al.[49]

Clinical trials

Disease	Study start	CAR-Treg cell	Current status	Primary objectives/outcome	NCT
Kidney transplant rejection	2021-03	HLA-A2 targeted CAR-Tregs (TX200-TR101)	phase I/phase II recruiting	• evaluate the safety and tolerability of TX200-TR101 and its effects on the donated kidney in living donor kidney transplant recipients	NCT04817774
Kidney transplant rejection	2023-08	HLA-A2 targeted CAR-Tregs (TX200-TR101)	enrolling by invitation	• collect long-term (up to 15 years post-infusion) safety and tolerability data from subjects enrolled in studies evaluating TX200-TR101	NCT05987527
Liver transplant rejection	2022-01	HLA-A2 targeted CAR-Tregs (QEL-001)	phase I/phase II recruiting	• evaluate the safety and tolerability of QEL-001 in the prevention of liver transplant rejection following immunosuppression withdrawal	NCT05234190
GvHD	2023-10	CD6-CAR-Tregs	phase I not yet recruiting	• determine if CD6-CAR-Tregs administration is safe and tolerable in patients who developed chronic graft-versus-host disease (cGvHD) • evaluate the feasibility to produce donor derived CD6-CAR-Tregs	NCT05993611

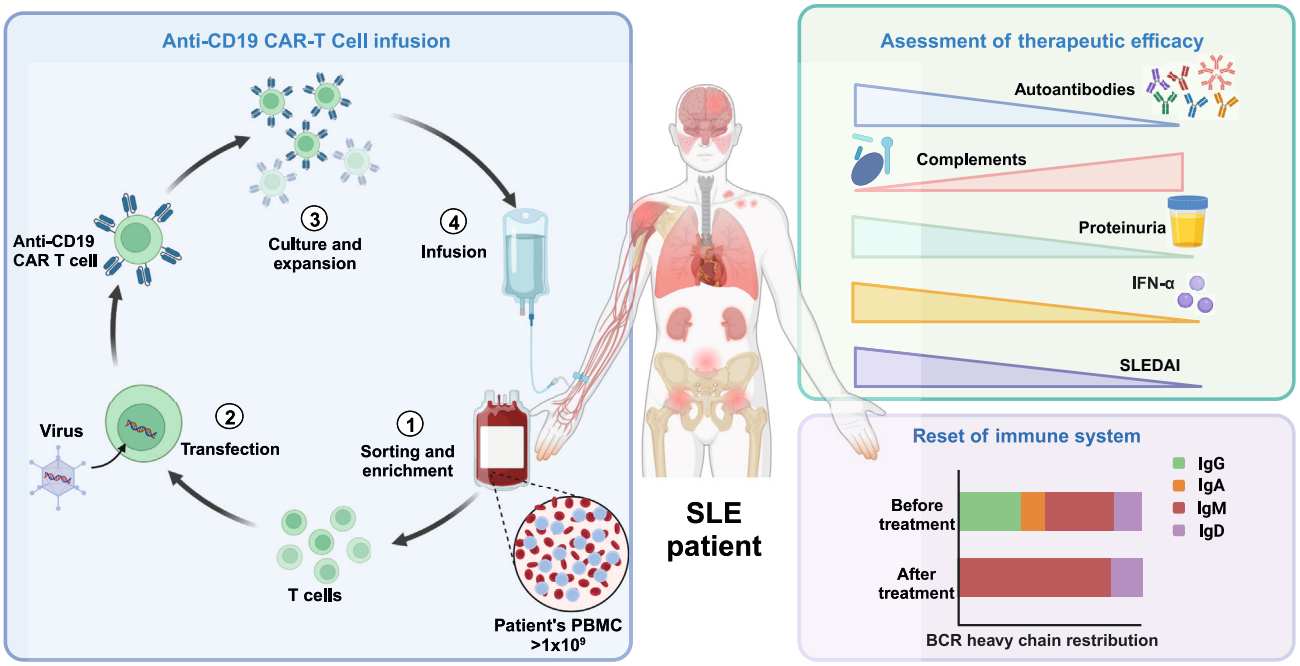

Figure 1. Anti-CD19 CAR-T cell therapy in patients with SLE and associated therapeutic efficacy assessment

T lymphocytes were isolated and enriched from the blood of SLE patients, followed by lentiviral transduction to obtain anti-CD19 CAR-T cells. The transduced CAR-T cells were expanded *in vitro* and subsequently infused into patients after lymphodepletion. The therapeutic efficacy of CAR-T cells *in vivo* was assessed based on the clinical severity of SLE, including levels of autoantibodies, complement, proteinuria, IFN-α, and the overall SLEDAI score. Additionally, the disappearance of BCR clonotypes and the emergence of B cells expressing only IgD and IgM indicated the capacity of CD19 CAR-T cells to reset the immune system (this figure was created with BioRender.com).

Subsequently, Mackensen et al.[31] extended CAR-T cell therapy to five patients with refractory SLE. During the treatment, additional parameters were monitored.[51] Figure 1 illustrates the comprehensive evaluation. The final results demonstrated CD19 CAR-T cell therapy rapidly resolved B cell-mediated autoimmune responses in SLE and facilitated profound immune system resetting, as shown by disappearance of enriched B cell receptor (BCR) clones and emergence of immunoglobulin (Ig) D/IgM-expressing B cells upon reconstitution. However, it is important to note that both trials administered fludarabine (a commonly used chemotherapy drug in cancer treatment) for lymphodepletion prior to CAR-T cell infusion. Thereby, in practical treatment, we need to assess the real-world cost-benefit ratio of CAR-T therapy and carefully consider the risks associated with lymphodepletion in comparison to potential benefits. Additionally, determining the necessity and ethical standards for the application of CAR-T cells in early-stage SLE treatment remains a significant challenge.

Anti-synthetase syndrome

Anti-synthetase syndrome (ASS) is a refractory autoimmune disease characterized by the presence of autoantibodies against one of several aminoacyl-tRNA synthetases (aaRSs), accompanied by interstitial lung disease, myositis, Raynaud's phenomenon, arthritis, mechanic's hands, and fever as clinical features.[52] Currently, there is no consensus regarding specific treatment guidelines for ASS. Clinical practice commonly involves the use of glucocorticoids, immunosuppressive agents, intravenous immunoglobulins, and other therapeutic approaches.[53] Recently, Müller et al.[32] achieved a successful treatment of ASS in a male patient who exhibited intolerance to current therapeutic interventions by employing CAR-T cells targeting $CD19^{+}$ B cells. After 6 months post CAR-T cell infusion, the patient

achieved complete restoration of muscle strength and elimination of autoantibodies associated with ASS, persisting even after discontinuing immunosuppressive agents and restoring B cell populations, but long-term monitoring is needed to assess therapy durability.

Multiple sclerosis

Multiple sclerosis (MS) is a central nervous system (CNS) inflammatory autoimmune demyelinating disease, often accompanied by severe neurological impairments, including weakness, vision loss, and cognitive decline.[54] The remarkable success of systemic anti-CD20 B cell depletion monoclonal antibodies in treating relapsing-remitting MS highlights the significant role of B cells in the immunopathology of this disease.[55] However, monoclonal antibodies, due to their large size, are unable to effectively penetrate the blood-brain barrier and eliminate CNS-resident B cells associated with meningeal and subpial inflammation in progressive MS, such as ectopic lymphoid follicles.[56] Recent research indicates that anti-CD19 CAR-T therapy achieves profound and sustained B cell depletion not only in the periphery but also within the CNS,[57] bolstering the concept of utilizing anti-CD19 CAR-T cells for MS treatment. In 2021, Mitsdoerffer et al. employed a murine model of spontaneous opticospinal encephalomyelitis (a commonly used model of MS) and found that, while anti-CD19 CAR-T cell therapy enhanced clearance of meningeal B cell aggregates, it exacerbated experimental autoimmune encephalomyelitis (EAE) in mice.[20] Moreover, some studies suggest that overexpression of CD19 on B cells can protect mice from EAE and is associated with elevated B10 cell levels, whereas CD19 deficiency exacerbates EAE.[58] Nevertheless, Gupta et al.[21] observed in a B cell-dependent EAE model (another MS model) that anti-CD19 CAR-T cells achieve more profound and sustained elimination of CNS B cells compared to anti-CD20 monoclonal antibody (mAb) therapy and effectively ameliorate EAE-associated symptoms in mice. Additionally, there is evidence supporting that treatment with humanized anti-CD19 monoclonal antibodies can also ameliorate MS.[59] These discrepancies among studies reflect the diverse autoimmune mechanisms driving tissue pathology in various EAE models and underscore the importance of employing multiple models when considering the translational potential of any therapeutic strategy for human disease.[60]

Neuromyelitis optica spectrum disorder

Neuromyelitis optica spectrum disorder (NMOSD), a variant of MS, is characterized by a relapsing course and severe sequelae. The pathogenicity of aquaporin-4-IgG antibodies (AQP4-IgG), demonstrated in NMOSD, has shed light on its role in disease development. Both the plasmablasts producing AQP4-IgG and the AQP4-IgG antibodies themselves can breach the blood-brain barrier, thus leading to complement-dependent cytotoxicity, immune cell chemotaxis, and various pathological outcomes.[61] Presently, a phase I single-arm clinical trial is underway, assessing the safety and efficacy of CT103A (a novel BCMA-targeting CAR construct) in AQP4-IgG serum-positive NMOSD patients. Mid-term results from BCMA-CAR-T cell infusion in 12 patients show promising outcomes, with 11 patients displaying no relapse and a decline in AQP-4 antibodies, correlating CAR-T cell expansion with response. This paves the way for further trials in neuroinflammatory disorders, indicating the potential of immunotherapy in treating neurological conditions.[62]

Myasthenia gravis

Myasthenia gravis (MG), an autoimmune disorder characterized by neuromuscular junction impairment, results from antibodies obstructing or dismantling nicotinic acetylcholine receptors (AChRs) at nerve-muscle synapses, culminating in

complement-mediated damage and muscular weakness.[63] While classical CAR-T cell therapy has shown efficacy in advanced cancer and refractory autoimmune conditions, its application to chronic autoimmune diseases such as MG appears less warranted due to potential severe side effects, such as cytokine release syndrome and neurotoxicity.[64,65] To circumvent these concerns, Cartesian Therapeutics has pioneered an innovative RNA-based therapy, employing the first-ever anti-BCMA RNA CAR-T (rCAR-T) cells, Descartes-08, for curing autoimmune disorders.[66] This rCAR-T approach employs mRNA to transiently reprogram T cells. As a result, the CAR+ cell burden is modulated and limited by dosing, gradually waning over time, enabling more precise control of therapeutic pharmacodynamics compared to DNA-modified CAR-T cells.

In assessing the safety and clinical activity of Descartes-08 for immune-suppressed adult patients with generalized MG, 14 subjects were administered varying doses of Descartes-08.[67] The outcomes demonstrated significant and sustained reductions in MG severity over a 9-month follow-up post infusion, with no observed toxicities, indicating the potential of rCAR-T cell therapy for treating MG and other autoimmune conditions.

CAR-T therapy for specific elimination of autoreactive immune cells

Muscle-specific tyrosine kinase MG

Muscle-specific kinase (MuSK) MG, a subtype of MG caused by autoantibodies against MuSK, remains a clinical challenge with unmet needs and limited treatment options. It is only present in a small subset of MG patients (6%–7.5%).[68] In efforts to circumvent the chronic immune suppression associated with current therapies, Oh et al.[26] engineered T cells to express MuSK autoantigen on their cell surface, the target of autoantibodies in MuSK MG, fused with 4-1BB and CD3ζ cytoplasmic domains, resulting in the creation of a novel CAR design, chimeric autoantibody receptor (CAAR) (Figure 2A). MuSK-CAAR-T cells effectively target and eliminate anti-MuSK autoantibody-producing B cells, demonstrating comparable efficacy to CD19 CAR-T cells, while specifically reducing anti-MuSK IgG levels without affecting overall B cell or IgG levels in an autoimmune MG mouse model. Presently, a phase I clinical trial of MuSK-CAAR-T therapy (ClinicalTrials.gov NCT05451212) is underway in California and Oregon. This innovative effort promises a hopeful approach for tailored treatment in this challenging subtype of MG.

Pemphigus vulgaris

Pemphigus vulgaris (PV) is a severe blistering disorder affecting the skin and mucous membranes, characterized by the presence of autoantibodies directed against desmoglein 3 (Dsg3), a component of desmosomes. Recurrent PV is typified by the persistence of antigen-specific B cell clones targeting Dsg3, as observed during active disease phases. Conversely, disease remission correlates with the elimination of anti-Dsg3 B cells from the circulating pool. Consequently, targeted eradication of memory B cells against Dsg3 presents a promising avenue for a definitive cure, avoiding the general risks associated with broad immunosuppression.[69,70]

A pioneering study by Ellebrecht et al. ingeniously devised CAAR-T cells engineered to express truncated Dsg3 peptides (EC1-3/EC1-4). These cells demonstrated remarkable specificity *in vitro*, exhibiting cytotoxicity selectively against cells expressing anti-Dsg3 BCRs. *In vivo*, these CAAR-T cells exhibited robust expansion, sustained presence, and specific eradication of Dsg3-specific B cells, leading to histological and serological remission in a physiologically relevant PV active immune model.[28] In addition, DSG3-CAAR-T cells also exhibited specific lysis of autologous

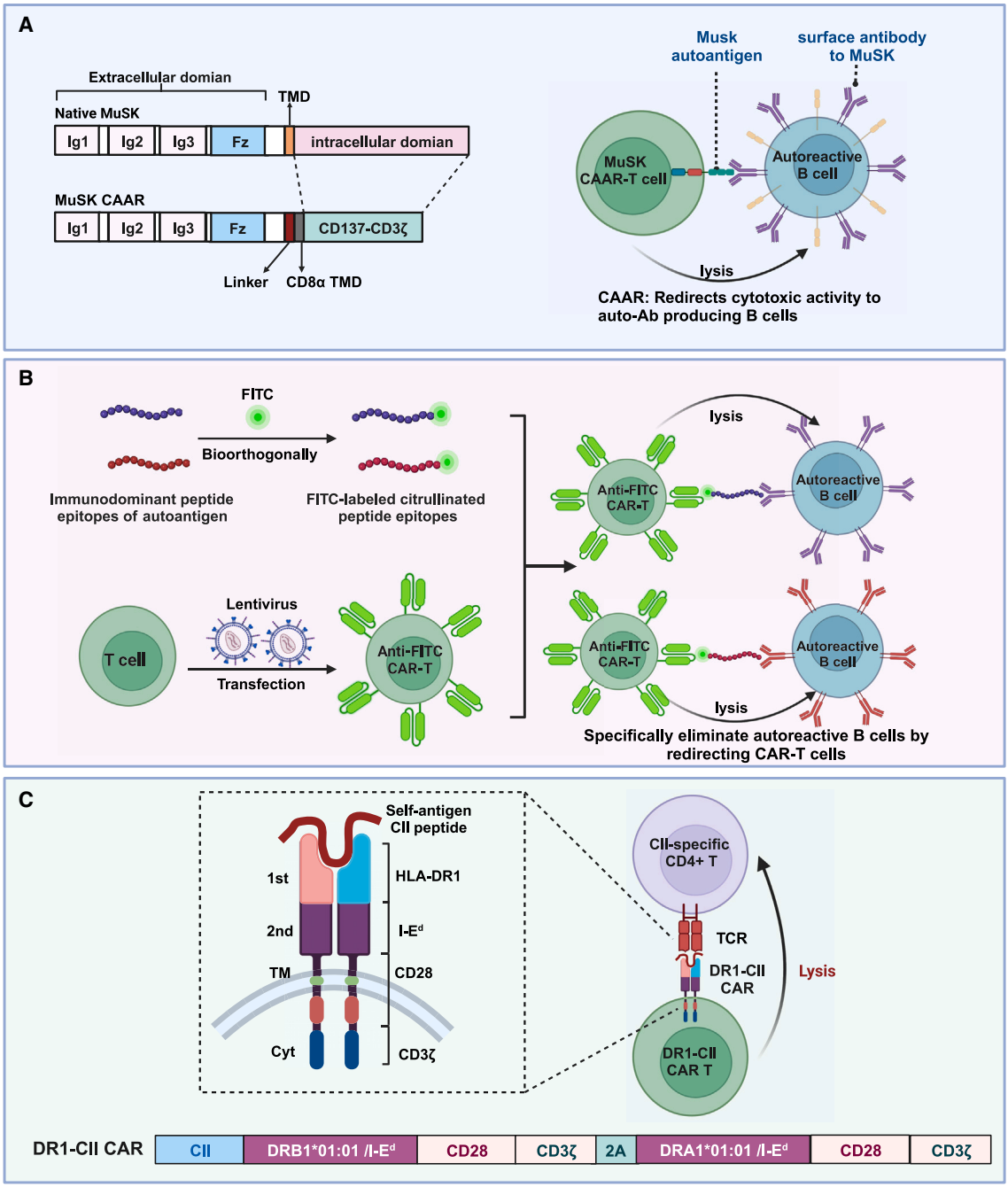

Figure 2. Multifaceted strategies for the targeted elimination of autoreactive immune cells

(A) The composition and functional mechanism of MuSK-CAAR-T cells. Native MuSK is a transmembrane tyrosine kinase with an ectodomain consisting of three immunoglobulin-like (Ig1–Ig3) and frizzled-like (Fz) domains. MuSK-CAAR includes the native MuSK ectodomain, followed by a glycine/serine-rich linker, the CD8α transmembrane domain (TMD), and 4-1BB-CD3ζ. MuSK-CAAR-T cells are capable of selectively eliminating self-reactive B cells bearing MuSK surface antibodies through CAR-mediated targeting.

(B) A schematic representation of the universal CAR-T-based approach for the eradication of autoreactive B cells. This approach involves the preparation of universal anti-FITC CAR-T cells and FITC-labeled autoantibody-positive peptides, which are subsequently utilized to eliminate autoreactive B cells by leveraging peptide-mediated CAR-T cytotoxicity.

(C) The structural and genetic composition of the DR1-CII CAR (this figure was created with BioRender.com).

anti-Dsg3 B cells derived from PV patients, reflecting its potential effectiveness at clinical dosages, making it a promising candidate for human trials in PV treatment.[29] However, the validation of the safety and efficacy of CAAR-T therapy through preclinical models remains inherently limited. Consequently, the current focus of this research team resides in the initiation of a phase I clinical trial (ClinicalTrials.gov NCT04422912) aimed at establishing the maximum tolerated dose of Dsg3-CAAR-T in patients with mucosal PV.

Rheumatoid arthritis

Rheumatoid arthritis (RA), a prevalent systemic autoimmune disorder, is characterized by autoantibodies targeting citrullinated antigens, often culminating in chronic synovial joint inflammation and articular degradation. Protein citrullination has long been implicated in eliciting distinctive immune responses associated with RA.[71] The emergence of anti-citrullinated protein antibodies (ACPAs) in serum stands as one of the most specific serological markers for RA, intricately linked with disease progression and pathogenesis.[72] Therapies involving B cell depletion using agents such as rituximab have proved efficacious in RA treatment. However, transient B cell depletion presents considerable safety challenges tied to global immune suppression, thus heightening the risks of infections and oncogenesis.[73] Moreover, CAR-T cell therapies, including CAAR-T cells and other monospecific CAR-T constructs pertinent to various immune disorders, have proved insufficient in targeting the diverse array of self-reactive lymphocytes existing in RA patients.[71]

To tackle this quandary, Zhang et al.[24] ingeniously harnessed four citrullinated peptide epitopes derived from the self-antigen citrulline. These epitopes targeted self-reactive B cells, which were then bioorthogonally conjugated with fluorescein isothiocyanate (FITC). The resulting anti-FITC CAR-T cells were thereby empowered to selectively recognize and eradicate self-reactive B cell subsets in RA patients (Figure 2B). Concurrently, CAR-T cell cytotoxicity relied upon the presence of FITC-conjugated antigenic peptides, showing dose-dependent effects. However, the therapeutic efficacy of this method in living organisms remains uncertain and the impact of this approach on normal plasma cell differentiation, antibody secretion, and the stability of these peptide moieties *in vivo* requires further investigation.

Susceptibility to RA is linked to selected DR alleles, including DR1 (DRB101:01) and DR4 (DRB104:01, 04:04, and 04:05).[74] Consequently, targeting pathogenic $CD4^+$ T cells that recognize self-antigens presented by RA-associated HLA-DR alleles represents a remarkably effective strategy for treating RA while circumventing the widespread immune suppression often associated with current RA therapies. Whittington et al.[25] designed a CAR construct centered on HLA-DRB1*01:01 (DR1). This construct integrated a self-antigen (type II collagen [CII]) within its molecular framework, and adeptly tethered the HLA-DRB1 and DRA1 chains to CD28 and CD3ζ positioned within the transmembrane and intracellular segments of each DR1 chain. Post transduction, the resulting DR1-CII CAR-T cells effectively targeted and eliminated autoimmune CII-specific $CD4^+$ T cells (Figure 2C) and significantly reduced self-reactive antibody responses in B6.DR1 mice, thus reducing the severity and frequency of RA. In summation, these findings highlight the promise of MHC class II-based CAR-T cells for precise treatment of autoimmune diseases.

Type 1 diabetes

The etiology of type 1 diabetes (T1D) remains elusive, with prevailing evidence suggesting a central role of autoimmune T cells in the destruction of pancreatic β cells, consequently diminishing insulin production. Although lifelong insulin administration and

pancreatic islet transplantation stand as efficacious modalities for T1D management, the formidable barriers of exorbitant costs, limited donor availability, and immunosuppressive challenges underscore the imperative for innovative, antigen-specific therapeutic strategies.[75] In the T1D non-obese diabetic (NOD) mouse model, pathological cascade is triggered by $CD4^+$ T cells specific for the B:9-23 epitope (a peptide derived from the insulin β-chain) as presented by the MHC class II molecule IA^{g7}.[76]

Zhang et al.[77] have pioneered a monoclonal antibody, mAb287, that selectively targets the IA^{g7}-B:10-23 complex and effectively retards or prevents the onset of T1D. Emerging evidence suggests that mAb287-reconfigured cytotoxic T cells may outperform mAb287 itself in safeguarding recipients against diabetes.[78] Through the deployment of 287-CAR, Zhang et al.[22] redirected cytotoxic T cells and found that 287-CAR-T cells can respond to stimulation from IA^{g7}-B:10-23 complexes presented by artificial antigen-presenting cells (APCs). This response involves interferon (IFN)-γ secretion and APC elimination. Nevertheless, the limitation lies in the selective homing of transferred 287-CAR-T cells to pancreatic lymph nodes, causing the initial protective effect to wane over time, eventually only retarding rather than entirely preventing T1D progression.

Beyond the indirect eradication of APCs via pathogenic MHC class II (pMHC-II) peptide complexes, direct targeting and elimination of pathological T cells constitute another pivotal avenue of investigation. Kobayashi et al.[23] have ingeniously engineered a first-generation five-module CAR (^{5M}CAR) that assembles the extracellular domain of pMHC-II with CD3 signaling modules. This receptor efficiently alters the cytotoxic T lymphocyte (CTL) specificity and functionality in response to $CD4^+$ T cell receptors (TCRs) targeting pMHC-II. In the T1D NOD mouse model, ^{5M}CAR-T effectively eliminates pathogenic T cells, attenuating diabetes incidence and restraining insulitis. This innovative framework of the ^{5M}CAR offers a valuable tool to dissect the impact of antigen-specific T cells on immune responses and holds promise in ameliorating T cell-mediated pathologies.

CAR-Treg therapy for restoration of the immune microenvironment

Autoimmune skin conditions

Vitiligo is an autoimmune disorder characterized by the loss of melanocytes, orchestrated primarily by T cells. Within the skin of vitiligo patients, a substantial reduction in regulatory T cells is observed. Replenishing Tregs in the vicinity of affected skin has therefore been proposed as a protective measure against pigment loss.[79] Concurrently, there is an excessive expression of ganglioside D3 (GD3) in epidermal cells, including melanocytes, around the lesions. In a novel approach, Mukhatayev et al.[33] engineered GD3-specific CAR-Tregs for intervention in a murine model of vitiligo. This strategic augmentation of Tregs at the lesion site effectively suppressed $CD8^+$ T cell-mediated melanocyte destruction, resulting in a remarkable delay in depigmentation progression. GD3 is also implicated in promoting keratinocyte proliferation, with overexpression of O-acetylated GD3 in psoriatic skin.[80] Furthermore, transient depletion of Treg cells in psoriasis triggers new lesion formation and exacerbates the condition, underscoring the potential of GD3 CAR-Tregs as a prospective therapeutic avenue for psoriasis.[81] Recent evidence also implicates compromised Treg cells within hair follicles as pivotal contributors to the pathogenesis of alopecia areata (AA), fostering localized immune dysregulation and hindering hair follicle regeneration. Thus, targeting antigens associated with melanocytes and keratinocyte differentiation also holds promise for CAR-Treg cell therapy for AA, with preliminary studies exploring their impact on AA animal models.[82]

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic, relapsing-remitting gastrointestinal disorder, encompassing two primary subtypes: ulcerative colitis (UC) and Crohn's disease. Tregs have emerged as important orchestrators in the local intestinal milieu, fostering tissue repair, regeneration, and maintenance of immune equilibrium through immunosuppressive functions and the secretion of key mediators such as IL-22 and amphiregulin. Quantitative and qualitative perturbations in Tregs have been linked to the initiation and progression of IBD.[83,84]

Flagellin proteins, ubiquitously expressed by a spectrum of commensal and pathogenic bacteria, are recognized by the human innate immune system, predominantly via Toll-like receptor 5 (TLR5). IBD patients manifest an augmented frequency of flagellin-specific B cells and T cells compared to healthy subjects, rendering flagellin an immunogenic antigen of clinical interest and an ideal target for Treg-based interventions.[85] Boardman et al.[34] engineered FliC-CAR-Tregs targeting flagellin from *Escherichia coli* H18. In a humanized murine model, FliC-CAR showed strong ability to guide Tregs to inflamed intestines. Responsive to flagellin in the colonic lamina propria, FliC-CAR-Tregs suppressed immune responses while aiding colonic epithelial monolayer reconstitution. This discovery highlights FliC-CAR-Tregs' potential as a therapy for IBD and strengthens the promise of CARs using microbial antigens. Antecedently, explorations into CAR-Treg therapies for colitis had already been initiated. One study artfully redirected CAR-Tregs toward 2,4,6-trinitrophenol (TNP), a model antigen, and employed these Tregs to ameliorate 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, conclusively demonstrating the capacity of CAR-Tregs to mitigate TNBS-triggered colitis through bystander immune suppression.[35] Blat et al.[36] extended on this finding by means of CAR-Tregs specific for murine carcinoembryonic antigen (CEA). Remarkably, these cells exhibited the dual capacity to effectively alleviate dextran sodium sulfate (DSS)-induced colitis while concurrently exerting suppressive effects on colorectal carcinogenesis.

T1D

Given the prevailing impairment of Treg function within the majority of T1D patients, therapeutic approaches for this disease extend beyond eliminating autoimmune cells, as discussed in the section "Type 1 diabetes" above. Strategies involving the augmentation of Treg quantity and function are therefore being explored in preclinical models.[86] Tenspolde et al.[37] engineered an insulin-targeted CAR-Treg and evaluated its therapeutic potential in the NOD/LtJ murine model of diabetes. While the constructed CAR-Tregs exhibited sustained functionality, suppressive activity, and prolonged persistence within the host, this cellular therapy fell short of effectively preventing diabetes onset in the mice due to the rapid *in vivo* degradation of insulin. To address this challenge, Radichev et al.[38] developed CAR-Tregs addressing human pancreas endocrine islet cell marker 2 (HPi2). This targeting strategy is not restricted to β cells but encompasses all endocrine cell subtypes within the pancreas, aimed at enhancing the retention of HPi2-CAR-Tregs around the islets. However, this CAR construct resulted in functional impairment of the engineered Tregs caused by persistent tonic signaling due to the unanticipated expression of HPi2 on the Tregs themselves.

Exploring an alternative target, Imam et al.[39] devised two CAR-Treg variations targeting glutamic acid decarboxylase 65 (GAD65), autoantibodies against which are an important diagnostic T1D biomarker, and demonstrated that GAD65-specific CAR-Tregs could infiltrate the islets, yielding a substantial reduction in blood glucose levels. Furthermore, Spanier et al.[40] conceived a CAR derived from a

monoclonal antibody targeting an MHC/peptide complex (IA^{g7}-B:10-23). In the NOD murine model, InsB-g7 CAR-Tregs demonstrated the ability to sustain the Foxp3 phenotype and effectively prevent spontaneous diabetes onset.

MS

Emerging evidence underscores the pivotal role of Tregs in both the protective and recuperative mechanisms within the animal model of EAE. Depletion of Tregs has been shown to impede the intrinsic recovery process of EAE, whereas adoptive transfer of Tregs into recipient mice has demonstrated a propensity to mitigate the severity of the disease.[87] However, the development of stable CNS-targeted Tregs requires further refinement, with CAR-Tregs presenting as a promising option. Currently, conventional CAR-Tregs are mostly made from peripheral blood Tregs, which have limitations due to low Treg levels and potential phenotype changes.[88,89] However, studies show that introducing the FoxP3 gene into naive T cells can produce Tregs similar to natural ones,[90] overcoming the challenges of low Treg numbers and FoxP3 expression absence.[91]

In light of these findings, Fransson et al. [44] ingeniously co-expressed FoxP3 with CAR targeting myelin oligodendrocyte glycoprotein (MOG), a key antigen in CNS demyelination, and effectively transduced naive $CD4^+$ T cells utilizing a retroviral vector system. The resulting Tregs showed stable phenotype and effectively suppressed immunity against MOG+ oligodendrocytes, reducing inflammation and ameliorating the disease. A parallel investigation achieved analogous success by fabricating CAR-Tregs targeting myelin basic protein (MBP) or MOG, further constraining the progression of EAE in murine models.[45]

Asthma

Asthma is a chronic respiratory ailment characterized by airway inflammation, airway hyperresponsiveness (AHR), wheezing, coughing, breathlessness, and reversible airway obstruction. Among its subtypes, allergic asthma stands as a predominant classification.[92] Emerging investigations have revealed that, in murine asthma models, the adoptive transfer of Treg cells can suppress the initiation and effector phases of allergic airway inflammation.[93] CAR-Treg cells targeting CEA, a glycoprotein present on the epithelial surfaces of the lungs and gastrointestinal tract, were shown to accumulate and become activated within the inflamed lungs of transgenic asthmatic mice, resulting in diminished AHR, reduced eosinophilic airway inflammation, and lower levels of allergen-specific IgE and Th2 cytokines. CEA CAR-Tregs exhibited enhanced efficacy in allergic inflammation symptoms compared to unmodified Tregs.[49]

IgE plays a central role in the pathogenesis of allergic ailments. In susceptible individuals with allergic asthma, exposure to allergens prompts cytokine-activated B cells to synthesize membrane-bound IgE (mIgE), which subsequently binds vigorously to high-affinity receptors (FcεRIs) on immune cells such as mast cells and eosinophils (allergenic sensitization process), thereby precipitating allergic manifestations.[94] The approval of the IgE-neutralizing antibody omalizumab for treatment of asthma points toward the use of CARs for silencing IgE-producing B cells.[95] CARs utilizing the extracellular domain of FcεRI chain (FcεRIα), as transduced into $CD8^+$ T cells for eliminating B cells expressing membrane IgE, have demonstrated promising outcomes in *in vitro* experiments.[96] However, a known risk of CAR-mediated effector T cell therapy is strong inflammation-related adverse events accompanying the potent response by the engineered T cells, which should be avoided in patients already affected by immune-related disorders. In view of this, one could also

envision the use of FcεRIα-based CAR-Treg toward management of IgE-driven immune disorders.

Graft-versus-host disease

Graft-versus-host disease (GvHD) arises from a multifaceted response of allogeneic immune effector cells in early-surviving recipients of allogeneic hematopoietic stem cell transplantation (alloHSCT), affecting various tissues. The therapeutic potential of Tregs in GvHD management has been substantiated in human studies.[97] However, owing to the risk of off-target toxicity, the focus of adoptive Treg therapy is progressively shifting toward engineered antigen-specific Tregs. In 2016, MacDonald et al.[41] pioneered the feasibility of CAR-Tregs in GvHD treatment. They developed HLA-A2-specific CAR-Tregs targeting the common mismatched alloantigen in transplants. These CAR-Tregs exhibit superiority over polyclonal Tregs in effectively inhibiting HLA-A2+ peripheral blood mononuclear cell (PBMC) proliferation *in vitro* and preventing HLA-A2+ PBMC-induced xenogeneic GvHD *in vivo*. Furthermore, a systematic comparison of 10 CAR designs, each comprising various signaling domains, using an HLA-A2-specific CAR platform revealed that the CD28 signaling domain was superior to 4-1BB-based configurations in achieving a stable, helios-positive Treg phenotype.[98]

In another study, Pierini et al.[42] engineered a modular system consisting of an FITC-specific CAR capable of mediating tissue-specific Treg activation through the binding of FITC-conjugated monoclonal antibodies (mAbCAR). Infusion of mice carrying allogeneic pancreatic islet transplants with mAbCAR-Tregs loaded with anti-allo-MHC-I antibodies significantly extended the survival of islet allografts as well as of matched secondary skin grafts. More recently, Bolivar-Wagers et al.[43] employed anti-human CD19 CAR-Tregs to suppress antibody production in immunodeficient mice reconstituted with human PBMCs, achieving GvHD inhibition without compromising graft-versus-tumor (GVT) effects.

Hemophilia A

Hemophilia A is an X-linked coagulation disorder caused by a deficiency of clotting factor VIII (FVIII), commonly treated with FVIII replacement therapy, the efficacy of which can be countered by the development of inhibitory antibodies.[99] In efforts to enhance patients' tolerance to exogenous FVIII, Yoon et al.[46] designed a CAR using FVIII-specific single-chain variable fragments (scFv) obtained from a synthetic phage display library. Through *in vitro* and *in vivo* experiments, they demonstrated that FVIII CAR-Tregs could exert bystander suppression, restraining the proliferation of FVIII-specific effector cells and curbing the recall antibody response against FVIII in FVIII knockout mice, culminating in a remarkable 8-week period of FVIII tolerance.[47]

Furthermore, the research team engineered a chimeric B cell antibody receptor (BAR) encompassing the FVIII immunodominant A2 domain. The resultant A2-BAR-Tregs effectively target and suppress FVIII-specific memory B cells, inhibiting the production of FVIII antibodies. This suppressive effect manifests in a contact-dependent manner, presumably involving direct interaction between A2-BAR expressed by Tregs and BCR on FVIII-specific B cells. Notably, terminally differentiated plasma cells cease to express the BCR, an aspect that may limit the applicability of BAR-Tregs.[48]

CAR-T CELL THERAPY IN INFECTIOUS DISEASES

Viral and opportunistic fungal infections pose significant threats to immune-compromised individuals. In current antiviral therapies, the treatment of chronic viral

infections remains a challenge, primarily due to the existence of a viral reservoir within infected cells that can maintain a latent phase for several years and reemerge as infectious viruses with the potential to develop drug resistance at any given time.[100] Pathogen-specific effector T cells play a pivotal role in controlling acute viral and fungal infections in individuals with robust immune competence. This has rendered adoptive T cell therapy an attractive alternative approach to the currently employed antiviral treatments.[101,102] However, the frequency of pathogen-specific T cells in the blood of patients is typically quite low, making their isolation and expansion a challenging endeavor. Consequently, CAR-T cells represent an appealing choice in this context. In the realm of combating human immunodeficiency virus (HIV), CAR-T cell therapy has made remarkable strides. These advancements encompass diverse antigen-targeting strategies, including portions of the HIV envelope protein, broadly neutralizing antibodies, bi-specific targeting approaches, and innovative methods to counteract viral escape and host antigen downregulation.[103] Concurrently, for other pathogens, such as hepatitis viruses, fungi, cytomegalovirus, and Epstein-Barr virus (EBV), which give rise to chronic infectious diseases, CAR-T cell therapy has exhibited promising therapeutic efficacy (Figure 3). In this discourse, we present a compilation of clinical trials pertaining to CAR-T therapy for HIV as well as relevant *in vitro* experiments and preclinical investigations for other infectious diseases (Table 3).

HIV

Combination antiretroviral therapy (cART) has significantly reduced the morbidity and mortality of HIV-1 infection, transforming AIDS into a manageable chronic condition.[135] However, despite its success in controlling viral replication, cART falls short in eradicating the viral reservoir, as various cell types, including $CD4^+$, CCR5+, and CXCR4+ cells, can serve as latent reservoirs in different tissues.[136] Recent attention has turned to CCR5Δ32 mutation-based allogeneic hematopoietic stem cell transplantation (HSCT), aimed at reconstitution of the hematopoietic system with cells lacking a functional CCR5 receptor for HIV entry, which has shown promise in a select group of patients but remains limited to those requiring allogeneic HSCT and suffering from HIV.[137] Furthermore, HIV itself employs mechanisms to evade immune responses, such as downregulating MHC-I expression on infected cells.[138] Herein, CAR-T cell therapy emerges as a novel beacon of hope for achieving HIV cure, boasting MHC-independent recognition,[139] sustained persistence,[140] precise targeting, and extensive infiltration capabilities.[141]

Two predominant structures have emerged for anti-HIV CAR design: the CD4 co-receptor[142] and broadly neutralizing antibodies (bNAbs).[109] CD4-based CARs, exhibiting high affinity for gp120 on HIV-infected cells, enable broad neutralization capacity, although their expression does render engineered T cells susceptible to HIV infection. CARs based on bNAbs mediate specific elimination of HIV-infected cells, while their interaction with cell-free HIV does not lead to infection.

In 2000 and 2002, two phase II clinical trials were conducted employing CAR-T therapy against HIV using a CD4-based CAR,[133,134] which substantiated the feasibility of CAR-T therapy against HIV and underscored the role of HIV-specific helper T cells in prolonging engineered T cell survival. Overall, both trials affirmed the safety and potential of CD4 CAR-T cell therapy, and multiple clinical trials are currently underway for HIV-positive patients after cART therapy. While CAR therapy represents a promising avenue for HIV treatment, precision targeting comes hand in hand with unique challenges, such as toxicity, off-target effects, viral escape mechanisms, and host-related factors. Meanwhile,

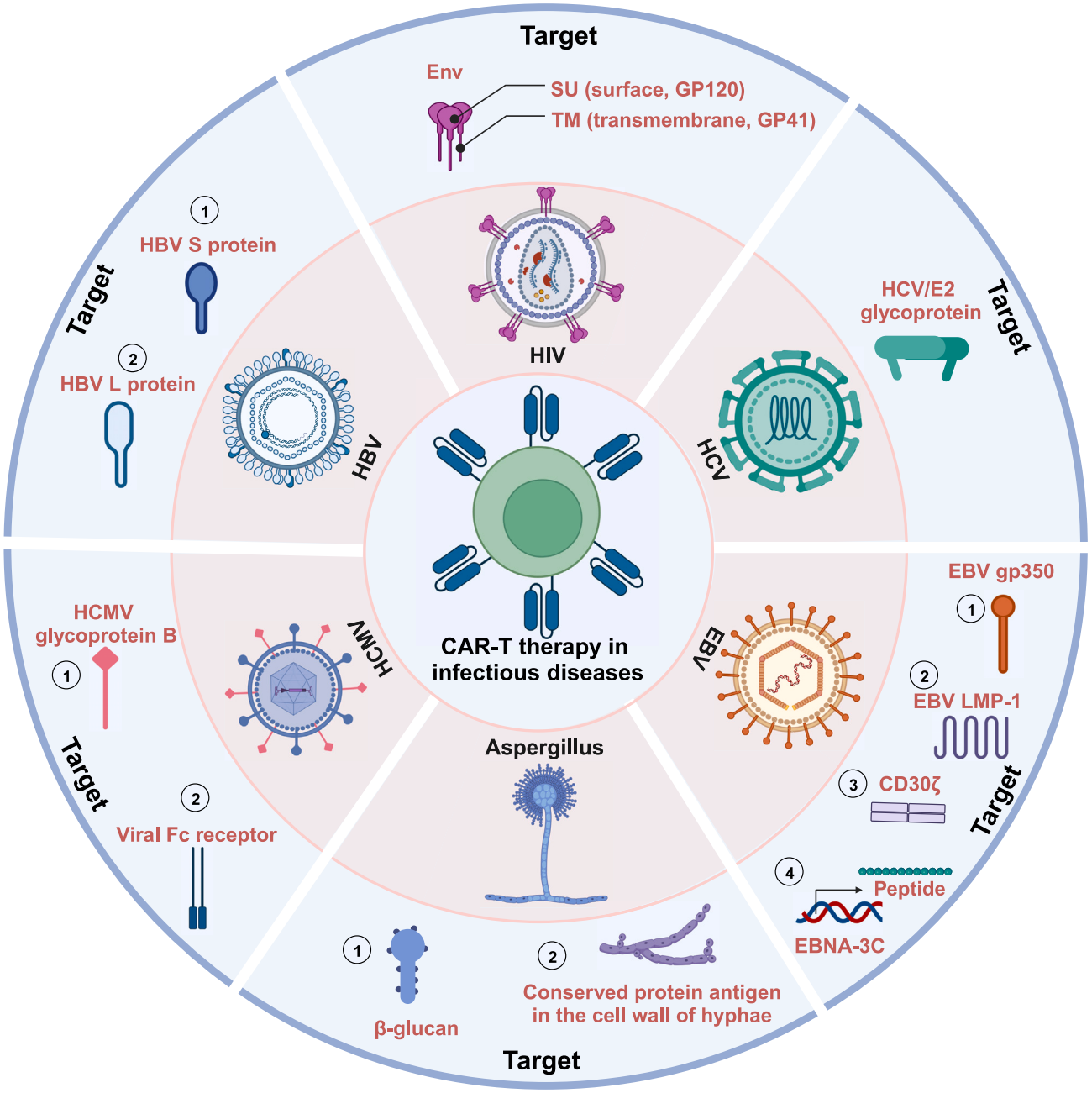

Figure 3. Application of CAR-T cell therapy in infectious diseases and target selection

CAR-T cell therapy holds promise for the treatment of infections caused by various pathogens, including HIV, HBV, HCV, HCMV, EBV, and *Aspergillus*. In the context of HIV treatment, the primary target is its envelope glycoprotein (Env). For HBV therapy, the key target is its surface protein (S or L protein). In the case of HCV treatment, the primary focus is on the surface glycoprotein E2. HCMV therapy primarily targets glycoprotein B and viral Fc receptors. EBV therapy revolves around key targets such as CD30ζ, EBNA-3C-derived peptides, LMP-1, and gp350. As for *Aspergillus*, the primary treatment targets include β-glucan and conserved protein antigens present in the cell wall of *A. fumigatus* hyphae (this figure was created with BioRender.com).

significant optimization efforts are still required to ensure the safety and efficacy of CAR structures.

Hepatitis viruses: Hepatitis B virus and hepatitis C virus

Chronic hepatitis B virus (HBV) infection remains a global health concern, necessitating novel therapeutic approaches. The ultimate goal of treatment is the

Table 3. Studies on the CAR-T cell therapy in infectious diseases

Preclinical studies

Pathogen	Target	CAR-T cell	Gene delivery system	Significant outcome	Reference
HIV	Env	human Env-targeted CD4-ζ CAR-T ($CD28^{-}CD3ζ$)	retroviral vectors/transfection	• targeted and killed HIV Env-expressing cells • secreted effector cytokines, lead to reactivate latent HIV in a cell line model	Sahu et al.[104]
HIV	Env	rhesus Env-targeted CD4-ζ CAR-T ($CD28^{-}CD3ζ$)	retroviral vectors/transfection	• CD4-ζ-CAR-transduced T cells specifically killed Env (+) 293 T cells with maC46 expressing vectors	MacLean et al.[105]
HIV	Env	human Env-targeted CD4-ζ and 447D- ζ CAR-T ($CD28^{-}CD3ζ$)	retroviral vectors/transfection	• CD4-ζ CAR-T cells exhibited the better lytic activity and cytokine production • modifications to the extracellular protein domains of the anti-HIV CARs had a notable effect on both receptor stability and substrate binding affinity	Patel et al.[106]
HIV	Env	human bNAb (VRC01) CAR-T (CD28-4-1BB-CD3ζ)	lentiviral vectors/transfection	• induced T cell-mediated cytolysis of cells expressing HIV-1 Env proteins • suppressed the resurgence of HIV-1 upon discontinuation of antiviral inhibitors in a cell culture viral infectivity model • induced the cytolysis of LRA-reactivated HIV-1-infected $CD4^{+}$ T	Liu et al.[107]
HIV	Env	human bNAb (10E8, 3BNC117, PG9, PGT126, PGT128, VRC01, and X5) CAR-T (4-1BB-CD3ζ)	lentiviral vectors/transfection	• bNAbs represent promising candidates for the creation of innovative CARs aimed at combating HIV-1	Ali et al.[108]
HIV	Env	human bNAb (PGT128, PGT145, VRC07-523, 10E8) CAR-T (4-1BB-CD3ζ)	lentiviral vectors/transfection	• specific activation and killing of HIV-infected cells • enhanced suppression of replicating virus was achieved through homology-directed recombination of the HIV CAR gene expression cassette into the CCR5 locus	Hale et al.[109]
HIV	Env	human Env-targeted CD4-ζ CAR-T and bNAb CAR-T ($CD28^{-}CD3ζ$; 4-1BB-CD3ζ)	lentiviral vectors/transfection	• CAR-T cells containing 4-1BB outperform CAR-T cells containing CD28 in an HIV-treatment model • CD4-based CARs control HIV more effectively than bNAb-based CARs	Leibman et al.[110]
HIV	Env	human $CD4^{-}$ ζ CAR-T and Bi-specific CD4-scFv(17*b*)-ζ CAR-T (CD4-35-17b & CD4-10-17b) ($CD28^{-}CD3ζ$)	retroviral vectors/transfection	• the CD4-10-17b CAR exhibited greater effectiveness in comparison to the CD4 CAR, whereas the CD4-35-17b CAR showed reduced effectiveness	Liu et al.[111]
HIV	Env	human Bi-specific CD4-CRD (DCSIGN) CAR-T ($CD28^{-}CD3ζ$)	retroviral vectors/transfection	• minimized concerns of mutational escape and anti-idiotypic immune responses • exhibited strong stimulation when encountering Env+ cells	Ghanem et al.[112]
HIV	Env	human multi-specific anti-HIV CAR-T, using a two-molecule CAR architecture, termed duoCAR (mD1.22, m36.4, and C46) (4-1BB-CD3ζ)	lentiviral vectors/transfection	• reduced *in vitro* HIV infection by over 99% and *in vivo* by over 97%. • sustained management of HIV infection *in vivo* • selective elimination of PBMCs hosting HIV strains resistant to bNAb	Anthony-Gonda et al.[113]
HIV	Env	human anti-HIV duoCAR-T (4-1BB-CD3ζ)	lentiviral vectors/transfection	• efficiently detected and eliminated $CD4^{+}$ T cells and monocytes/macrophages infected with HIV	Anthony-Gonda et al.[114]

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Table 3. *Continued*

Preclinical studies

Pathogen	Target	CAR-T cell	Gene delivery system	Significant outcome	Reference
HIV	Env and PD-1	human 3BNC117-E27(3BE) CAR-T (4-1BB-CD3ζ)	pseudoviruses/transfection	• enabled the expression of PD-1-blocking scFv E27 and scFv of bNAb • showed greater cytotoxic activity, stronger proliferation capability, higher killing efficiency, and enhanced cytokine secretion	Pan et al.[115]
HBV	HBV S or L protein	human S/L-protein targeted CAR-T ($CD28^{-}$CD3ζ)	retroviral vectors/lipofection	• eliminated primary HBV-infected liver cells, liberating IFN-γ and IL-2 • S-CAR-T cells exhibited superior activation kinetics and cytokine secretion compared to L-protein-targeting counterparts	Bohne et al.[116]
HBV	HBV S protein	human S protein-targeted CAR-T ($CD28^{-}$CD3ζ)	retroviral vectors/lipofection	• significant reduction in cccDNA-positive cells within the liver, accompanied by minimal hepatic side effects	Krebs et al.[117]
HBV	HBV S protein	fully human S protein-targeted CAR-T ($CD28^{-}$CD3ζ)	retroviral vectors/transfection	• persisted in substantial numbers and elicited sustained antiviral effects	Festag et al.[118]
HBV	PreS1 region	human preS1-targeted CAR-T (A14 CAR-T) (CD28-4-1BB-CD3ζ)	retroviral vectors/transfection	• exhibited potent cytotoxicity against HBV-infected liver cells and reduced viral biomarkers in humanized HBV-infected mice	Guo et al.[119]
HCV	HCV/E2 glycoprotein	human anti-HCV/E2 CAR-T ($CD28^{-}$CD3ζ)	retroviral vectors/transfection	• exhibited the capability to lyse E2-expressing cells and HCV-infected hepatocytes	Sautto et al.[120]
HCMV	HCMV glycoprotein B	human gB-targeted CAR-T ($CD28^{-}$CD3ζ)	lentiviral transfection or RNA mediated gene transfer	• robust lysis of gB-transfected 293T cells and provoked cytokine release in response to HCMV infection in HFFs	Full et al.[121]
HCMV	HCMV glycoprotein B	human gB-targeted CAR-T ($CD28^{-}$CD3ζ)	electroporation	• cells infected with HCMV evaded CAR-T cell cytotoxicity through viral effector proteins (UL37x1 and UL36)	Proff et al.[122]
HCMV	Viral Fc receptors and gB	human gB-targeted CAR-T with mutated CH2-CH3 IgG spacer domains ($CD28^{-}$CD3ζ)	electroporation	• mutated gB CAR-T selectively engaged viral FcRs while evaded interactions with endogenous cellular FcRs • exhibited generally diminished cytokine-producing capacity	Proff et al.[123]
HCMV	HCMV glycoprotein B	human gB-targeted CAR-T (SM5-1 CAR-T) (CD28/4-1BB-CD3ζ)	retroviral vectors/transfection	• eliminate HCMV-infected cells and confer herapeutic benefits in a humanized mouse model of HCMV infection	Olbrich et al.[124]
HCMV	HCMV glycoprotein B	human anti-CMV neutralizing antibody CAR-T (4-1BB-CD3ζ)	lentiviral vectors/transfection	• escalated cytokine release, exerted cytotoxicity against CMV-infected cells, and curtailed viral replication	Ali et al.[125]

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Table 3. *Continued*

Preclinical studies

Pathogen	Target	CAR-T cell	Gene delivery system	Significant outcome	Reference
EBV	CD30ζ	human CD30-targeted CAR-CTL	retroviral vectors/transfection	• demonstrated cross-stimulation with EBV-infected cells and exerted anti-tumor effects against EBV(−)/CD30(+) tumors	Savoldo et al.[126]
EBV	LMP-1	human LMP-1-targeted CAR-T ($CD28^{-}CD3\zeta$)	lentiviral vectors/transfection	• elicited anti-tumor responses against LMP1-positive NPC cells, both in *in vitro* and *in vivo* settings	Tang et al.[127]
EBV	EBNA-3C-derived peptide	human TÜ165 CAR-T and TÜ165 TRUCKs (with iIL-12 expression cassette) ($CD28^{-}CD3\zeta$)	lentiviral vectors/transfection	• TÜ165 CAR-T recognized EBNA-3C-derived peptides in a TCR-like manner, facilitating targeted cell elimination • TRUCKs induced IL-12 release upon target engagement and enhanced immune cell proximity to post-transplant lymphoproliferative disease (PTLD) cells	Dragon et al.[128]
EBV	EBV gp350	human gp350-targeted CAR-T ($CD28^{-}CD3\zeta$)	retroviral vectors/transfection	• reduced EBV-associated B cell lymphoproliferation, curtailed tumor growth, and mitigated inflammation	Slabik et al.[129]
EBV	EBV gp350	human gp350-targeted CAR-T ($CD28^{-}CD3\zeta$)	lentiviral vectors/transfection	• the results highlighted the capacity of gp350 CAR-T as innovative tools for managing patients suffering from EBV-related malignancies	Zhang et al.[130]
A. fumigatus	β-glucan	human Dectin 1-CAR-T ($CD28^{-}CD3\zeta$)	electroporation	• demonstrated a selective affinity for β-glucan, resulting in the impairment and inhibition of Aspergillus hyphal growth both *in vitro* and *in vivo*	Kumaresan et al.[131]
A. fumigatus	conserved protein antigen in the cell wall of *A. fumigatus* hyphae	human anti-Af (AB90-E8) CAR-T ($CD28^{-}CD3\zeta$)	sleeping beauty transposon/transfection	• recognized both *A. fumigatus* strains and clinical isolates and exerted direct antifungal effects against *A. fumigatus* hyphae • CD8+ Af-CAR-T cells demonstrated enhanced antifungal efficacy compared to $CD4^{+}$ Af-CAR-T cell	Seif et al.[132]

Clinical trials

Pathogen	Study start	CAR-T cell	Current status	Primary objectives/outcome	Reference/NCT
HIV	1997–05	CD4ζ -CAR-T (CD4+T/CD8+T)	phase II completed	• CD4ζ -CAR-T cells exhibited sustained and high-level persistence • demonstrated the feasibility of *ex vivo* T cell gene therapy in HIV-infected adults	Mitsuyasu et al.[133]
HIV	Unknown	CD4ζ -CAR-T (CD4+T/CD8+T)	phase II completed	• reduced HIV viral load but had no impact on the size of the viral reservoir	Deeks et al.[134]
HIV	2017–10	HIV- targeted CAR-T	phase I recruiting	• evaluate the safety and effectiveness of CAR-T Cell therapy on HIV patients whose plasma HIV has been successfully suppressed after cART	NCT03240328
HIV	2017–12	HIV- targeted CAR-T	phase I completed	• evaluate the safety and efficacy of the combination of Chidamide with CAR-T on HIV-1 latent reservoir	NCT03980691
HIV	2019–07	CD4 CAR+CCR5 ZFN T	phase I active, not recruiting	• evaluate the impact of CCR5-disrupted CD4 CAR-T cells on HIV drug resistance	NCT03617198

(*Continued on next page*)

Table 3. *Continued*

Clinical trials

Pathogen	Study start	CAR-T cell	Current status	Primary objectives/outcome	Reference/NCT
HIV	2021–03	LVgp120 duoCAR-T	phase I/phase II recruiting	• evaluate the safety and anti-HIV activity of LVgp120 duoCAR-T in anti-retroviral drug-treated HIV-1 infection	NCT04648046
HIV	2021–05	third-generation HIV-targeted CAR-T	phase I Unknown status	• assess the safety of CAR-T cell treatment, potential therapeutic efficacy, and kinetics of the third-generation HIV-targeted CAR-T cell	NCT04863066
HIV	2024–01	CD19-targeted CAR-T (axicabtagene ciloleucel)	phase I not yet recruiting	• demonstrate safety and feasibility of axicabtagene ciloleucel for relapsed/refractory HIV-associated aggressive B-NHL in participants with well-controlled HIV	NCT05077527
EBV	2024–03	CD30-CAR-EBVST	phase I not yet recruiting	• find out the highest safe dose of allogeneic CD30-CAR-EBVST cells given following chemotherapy and used to treat lymphoma	NCT04952584
EBV	2023–05	EBV-specific CAR-T(BRG01)	phase I not yet recruiting	• evaluate the safety and efficacy of BRG01 in subjects with relapsed/metastatic EBV-positive NPC	NCT05864924
EBV	2022–01	EBV-specific CAR-T	early phase I recruiting	• investigate the safety and preliminary efficacy of EBV CAR-T cells in the treatment of relapsed/refractory NPC	NCT05654077
EBV	2022–12	EBV-specific CAR-T	early phase I recruiting	• investigate highest safe dose of EBV CAR-T/TCR-T cells in the treatment of relapsed/refractory NPC	NCT05587543
EBV	2016–11	LMP1-CAR-T	phase I/phase II unknown status	• evaluate the safety of the designed LMP1-CAR-T cells and determine whether the CAR-T cells are effective in the treatment of EBV-associated malignant tumors	NCT02980315

eradication of the HBV replication template, known as covalently closed circular DNA (cccDNA). In chronic HBV patients, sustained exposure to HBV antigens results in dysfunction or exhaustion of HBV-specific T cells, characterized by elevated expression of immune checkpoint molecules such as programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4), coupled with defects in proliferation and cytokine production.[143] Therefore, restoration of HBV-specific immunity through adoptive T cell transfer holds promise for treating chronic HBV infection. While clinical trials for HBV infection using CAR-T cells are lacking, preclinical studies have made significant strides. Researchers such as Bohne et al.[116] have designed CAR constructs targeting the large (L) and small (S) HBV envelope proteins on infected cells. The resulting S-CAR-T and L-CAR-T cells could eliminate primary HBV-infected liver cells, releasing IFN-γ and IL-2 to eliminate cccDNA-positive cells. Intriguingly, S-CAR-T cells exhibited superior activation and cytokine secretion, likely attributable to the higher expression of the S protein on HBV-infected cells. However, it is noteworthy that, despite both CAR-T variants effectively clearing HBV-infected liver cells, residual HBV core protein and relaxed circular DNA (rcDNA) persisted, possibly due to the localization of HBV rcDNA within the viral capsid, rendering it impervious to caspase-activated DNases.

Studies by Krebs et al.[117] demonstrated S-CAR-T efficacy in a transgenic mouse model, demonstrating significant reduction in cccDNA-positive cells within the liver, accompanied by minimal hepatic side effects. Nevertheless, challenges pertaining to immune responses triggered by humanized CARs in murine hosts hindered CAR-T cell survival. Addressing this concern, Festag et al.[118] accomplished full humanization of the S-CAR construct in 2019. They employed immunocompetent mice engineered to tolerate alloreactive immune responses directed toward the homologous allo-CAR domain, thus overcoming cellular rejection of S-CAR-T cells. In this setting, S-CAR-T cells persisted in substantial numbers, eliciting sustained antiviral effects. As the field advances, investigations have diversified, yielding innovative strategies such as the utilization of antibodies targeting the HBV L-protein preS1 region. Guo et al.[119] showcased that A14 CAR-T cells, designed for this purpose, effectively killed HBV-infected liver cells and significantly reduced viral biomarkers in humanized HBV-infected mice. However, it is crucial to create a mouse model that better mimics real infections to thoroughly evaluate CAR-T cell effectiveness *in vivo*. Moreover, exploring potential synergies between CAR-T cells and frontline or secondary therapeutic interventions for HBV infections is a promising avenue for enhanced disease management.

For hepatitis C virus (HCV), another prominent hepatic infectious agent, parallel research endeavors have revolved around targeting the highly mutable viral protein, HCV E2, using CAR-T cells. These anti-HCV/E2 CAR-T cells have exhibited the capability to lyse E2-expressing cells and HCV-infected hepatocytes. Nevertheless, the effectiveness of these constructs necessitates rigorous preclinical validation.[120]

Human cytomegalovirus

Human cytomegalovirus (HCMV) infection and reactivation remain prominent culprits of morbidity and mortality following HSCT and solid organ transplantation.[144] Despite the advent of effective antiviral agents over the years, the quest for more efficacious and less toxic approaches to HCMV treatment persists.[145] Full et al.[121] successfully engineered CAR-T cells targeting the HCMV glycoprotein B (gB), mediating lysis of gB-transfected 293T cells and triggering cytokine release in response to HCMV-infected human foreskin fibroblasts (HFFs). However, it was found that cells infected with HCMV may evade CAR-T cell cytotoxicity through viral effector

proteins,[122] in particular UL37x1 and UL36, through interference with apoptotic pathways and the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STINGs) immune pathway. Furthermore, CAR-T immunoevasion in HCMV was delineated as a flaw in the gB CAR design, in that the CH2-CH3 fragment crystallizable (Fc) hinge exhibited intrinsic Fc receptor (FcR)-binding capacity, causing tonic signaling attenuating CAR-T cell function.[123] Introduction of specific mutations in the hinge domain, while alleviating this problem, diminished cytokine-producing capacity of gB CAR-T cells, possibly due to less stable expression of the CAR.[146]

In parallel, CAR-T cells based on a high-affinity gB-specific neutralizing antibody (SM5-1), generated from adult and cord blood T cells, were shown to eliminate HCMV-infected cells and confer therapeutic benefits in a humanized mouse model of HCMV infection.[124] Similarly, primary $CD8^+$ T cells transduced with CMV-specific CAR based on an antibody against HCMV glycoprotein H (gH; 21E9) mediated cytokine release and cytotoxicity against CMV-infected cells and curtailed viral replication.[125]

EBV

EBV, a widely prevalent pathogen, is associated with lymphoproliferative disorders, B/T/NK cell lymphomas, nasopharyngeal carcinoma (NPC), and gastric cancer (GC).[147] For some of these conditions, adoptive transfer of EBV-specific T cells has been explored as a therapeutic strategy, achieving promising clinical outcomes. Loss of EBV expression by a subset of malignant cells, however, imposes a high risk for treatment failure. In view of this, Savoldo et al.[126] endowed endogenous EBV-specific CTLs (EBV-CTLs) with a CAR targeting CD30, a cell-encoded molecule highly expressed on EBV-transformed cells. The resulting CD30CAR (+) EBV-CTLs were shown to exert anti-tumor effects against both EBV(+)/CD30(+) and EBV(−)/CD30(+) tumors in xenograft models, thereby holding potential for immune therapy in Hodgkin's disease.

Further studies involved CAR-T cells targeting EBV latent membrane protein 1 (LMP1).[127] Co-culture of these CAR-T cells with LMP1-overexpressing NPC cells revealed cytolytic activity along with IFN-γ and IL-2 production. Intratumoral injection of anti-LMP1 CAR-T cells in a murine xenograft model led to diminished tumor growth. Dragon et al.[128] engineered EBV-reactive CAR-T based on monoclonal antibody TÜ165, which specifically targets a peptide epitope derived from EBV nuclear antigen (EBNA). To enhance immune effector potency, they subsequently developed T cells redirected for universal cytokine killing (TRUCKs) based on this CAR, designed to release IL-12 upon target engagement. Recently, a CAR-T targeting EBV envelope glycoprotein 350 (gp350) was successfully employed in a fully humanized non-obese diabetic (NOD)-$Rag1^{null}$-γ $chain^{null}$ (NRG) mouse model infected with EBV to reduce EBV-associated B cell lymphoproliferation, curtail tumor growth, and mitigate inflammation.[129] Subsequent efforts encompassed guanosine monophosphate (GMP) development and preclinical validation of gp350 CAR-T cells, underscoring their potential as innovative agents for treating patients afflicted by EBV-related malignancies.[130]

Aspergillus fumigatus

Invasive pulmonary aspergillosis (IPA) is a severe infection commonly caused by *Aspergillus fumigatus*, particularly in immunocompromised patients. While adaptive and innate immune cells responsive to *A. fumigatus* exist within the endogenous immune repertoire of IPA patients, they are infrequent and therefore challenging to isolate and expand for adoptive immunotherapy.[148] In light of this, Iliev et al.[149]

explored CAR-T for direct targeting of this extracellular pathogen. The basis for this was the extracellular domain of the pattern-recognition receptor Dectin-1, which mediates recognition of *A. fumigatus* through binding of the fungal cell wall component β-glucan, as a targeting element to construct D-CAR. While D-CAR-T cells reduced fungal burden in an immunocompromised invasive aspergillosis mouse model, Dectin-1 may not be the optimal target for specifically directing T cells against *A. fumigatus*. This is because β-glucan is not *Aspergillus* specific and is also expressed on other commensal and pathogenic microorganisms, thus raising concerns about off-target CAR-T cell activity.[131]

To address this limitation, a novel CAR-targeting domain was engineered based on an antibody that selectively recognizes a conserved surface antigen on *A. fumigatus*.[132] T cells expressing the *A. fumigatus*-specific chimeric antigen receptor (Af-CAR) recognized *A. fumigatus* laboratory strains and clinical isolates, exerting direct antifungal effects against *A. fumigatus* hyphae and reducing lung fungal burden in an immunodeficient mouse model of IPA. This discovery highlights the potential of CAR-T cell therapy for invasive infectious diseases that are challenging to control with traditional antimicrobial treatments.

THE APPLICATION OF CAR-T IN OTHER NON-NEOPLASTIC DISEASES

Fibrotic diseases

Excessive cardiac fibrosis is a crucial factor in the progression of various heart diseases and heart failure. Following myocardial injury, cardiac fibroblasts initiate myocardial remodeling by depositing an excessive extracellular matrix, resulting in increased tissue stiffness and decreased compliance.[150] However, clinical interventions and treatments for fibrosis remain limited. Two studies in 2016 found that inducing gene ablation in activated cardiac fibroblasts in injured mice significantly alleviated cardiac fibrosis and improved heart function.[151,152] Aghajanian et al.[153] discovered that fibroblast activation protein (FAP) was strongly expressed in left-ventricular tissues of failing hearts in dilated cardiomyopathy and hypertrophic cardiomyopathy patients. Consequently, they targeted this protein to develop corresponding CAR-T cells and confirmed that FAP-CAR-T cells could target and eliminate activated cardiac fibroblasts, thereby restoring contractile and diastolic function following cardiac injury. However, fibroblast activation is a normal part of wound healing processes in many tissues, and the sustained anti-fibrotic effect of the CAR-T cells used in the above-mentioned study could pose risks for future injuries. To address this, they utilized LNP-mRNA technology to deliver therapeutic mRNA directly to T cells in the body via CD5-targeted lipid nanoparticles, producing transient and effective CAR-T cells *in vivo*. This approach significantly improved cardiac function in a murine heart failure model while avoiding long-term impacts on the mice's survival due to CAR-T cell presence.[154]

Given this groundbreaking achievement, it is conceivable that this approach may also be applicable to autoimmune fibrotic diseases characterized by FAP overexpression, such as RA,[155] systemic sclerosis,[156] idiopathic pulmonary fibrosis,[157] and Crohn's disease.[158] Additionally, extracellular matrix deposition and fibrosis are pathological processes in many diseases, including liver diseases, chronic kidney diseases, lung conditions, skeletal muscle disorders, and several solid cancers, which might also be suitable for anti-fibrotic CAR-T cells.[159]

Cellular senescence-associated diseases

Cellular senescence is a permanent cell-cycle arrest state in which cells maintain metabolic activity and secrete a range of pro-inflammatory and protein-degrading molecules,

referred to as the senescence-associated secretory phenotype (SASP). The accumulation of senescent cells in organs and tissues can lead to various age-related pathologies, including inflammatory diseases, tissue degeneration, and cancer.[160] Recent research has indicated that the removal of senescent cells can effectively ameliorate these age-related pathologies.[161] Amor et al.[162] conducted RNA sequencing analyses on three different senescence backgrounds and ultimately discovered a cell surface molecule, urokinase-type plasminogen activator receptor (uPAR), which is widely expressed in senescent cells.

However, due to the significant heterogeneity of senescent cells, a universally shared surface senoantigen is unlikely to exist. Therefore, it is crucial to assess the specificity of uPAR in other age-related diseases and explore alternative targets for generating senolytic CAR-T cells.[163] For instance, a vaccine targeting glycoprotein non-metastatic melanoma protein B (GPNMB) has been shown to eliminate senescent vascular endothelial cells and leukocytes in atherosclerotic mice, reducing atherosclerotic plaques and extending the lifespan of male premature aging mice.[164] Similarly, a vaccine targeting tumor necrosis factor (TNF) superfamily protein CD153 has been found to deplete senescent T cells in obese mice induced by a high-fat diet.[165] Additionally, research indicates that the natural killer (NK) group 2 member D ligand (NKG2DL) is highly expressed in senescent cells, allowing senescent cells to evade innate immune clearance mediated by endogenous NK cells.[166] To explore the latter finding, Yang et al.[167] developed CAR-T cells targeting human NKG2DL and demonstrated selective and effective reduction of cellular senescence induced by oncogenic stress, replicative stress, DNA damage, or P16INK4a overexpression in human cells. In irradiated or aged mice, NKG2D-CAR-T cells alleviated various age-related pathologies and improved their physical function. Furthermore, they effectively cleared naturally occurring senescent cells in non-human primates without any adverse reactions.

Currently, numerous anti-aging drugs have shown significant clinical potential in various chronic diseases such as premature aging syndromes, renal dysfunction, respiratory diseases, neurodegenerative disorders, and cardiovascular diseases.[168] The aforementioned studies collectively suggest that, in the future, senescence-specific CAR-T cells will have broad applicability.

THE APPLICATION OF NON-T CELL CAR TECHNOLOGY IN NON-NEOPLASTIC DISEASES

CAR-NK

Although CAR technology has achieved significant milestones in T cell therapy, challenges such as high manufacturing costs, low efficacy, treatment-related toxicities, and antigen escape remain crucial factors limiting its widespread application.[169] Consequently, recent research has started to apply CAR technology to non-T cell immune cells, such as CAR-NK cells and macrophages (MΦs). CAR-NK cells have gained attention due to their activation independent of the MHC pathway, thereby reducing the risk of TCR-mediated alloreactivity. NK cells can be generated from pre-existing cell lines or allogeneic NK cells.[170] Furthermore, CAR-NK cells, compared to CAR-T cells, can kill target cells through multiple pathways, including CAR-dependent and CAR-independent mechanisms.[171] Moreover, their shorter half-life (<10 days) results in reduced toxicity during therapy, particularly in terms of cytokine release syndrome and neurotoxicity.[172] While this characteristic may provide an advantage concerning the latter, it also presents challenges of potentially requiring repeated dosing to achieve a sustained response.[173] Currently, CAR-NK

cells have demonstrated efficacy in various solid tumors and hematologic malignancies, with numerous clinical trials underway.[174] Beyond cancer, research is also exploring the application of CAR-NK cells in autoimmune disorders. Meng et al.[175] developed a CAAR targeting the self-antigen La/SSB, associated with multiple autoimmune diseases. This CAAR was then introduced into NK92MI cells, enabling them to selectively target and destroy La/SSB-reactive autoreactive B cell clones.

CAR-MΦs

CAR-MΦs have also emerged as a potential alternative therapeutic approach. Similar to CAR-T cells, CAR-MΦs require specific antigens and can encounter challenges such as antigen escape, downregulation, and systemic cytokine toxicity during treatment. Importantly, CAR-MΦs exhibit superior capabilities in penetrating and infiltrating solid tumors as well as modulating the tumor microenvironment (TME).[176] Currently, CAR-MΦs are still in their nascent stage, with only one clinical trial (ClinicalTrials.gov NCT04660929) officially initiated, primarily targeting HER-2-positive solid tumors with no results yet reported.[177]

In the field of non-neoplastic diseases, CAR-MΦs are predominantly being explored for infectious diseases. Effective clearance of *Staphylococcus aureus* in the periprosthetic joint environment is crucial for preventing postoperative prosthetic joint infection (PJI). However, currently no effective methods are available.[178,179] Although MΦs play a pivotal role in clearing *S. aureus*,[180] the implant itself triggers a local tissue reaction, leading to an immunosuppressive niche,[181] while the evolution of immune escape mechanisms by *S. aureus* hinders the phagocytic and bactericidal activities of MΦs.[182] To address these hurdles, Li et al.[183] designed an implant that carries plasmid-loaded nanoparticles capable of delivering CAR genes targeting *S. aureus* surface protein A (SasA) and CASP11 short hairpin RNA (shRNA) into the nuclei of MΦs, thereby generating engineered CAR-MΦs *in situ*. CASP11 shRNA reduces CASP11 expression in CAR-MΦs and significantly enhances the phagolysosomal killing effect of SasA-specific CAR-MΦs (Figure 4). These super CAR-MΦs target and eradicate *S. aureus*, promoting rapid bone integration at the bone-implant interface, offering a novel therapeutic strategy for multidrug-resistant bacterial infections and providing innovative insights into the production and application of CAR-MΦs.

CONCLUSIONS AND FUTURE PERSPECTIVE

The application of CAR-engineered T cells for the treatment of hematopoietic malignancies has marked a breakthrough not only in cancer treatment but also in the management of a wide variety of non-neoplastic clinical indications, including autoimmune diseases, allergic asthma, chronic infections, hemophilia, fibrosis, and cellular senescence. With these new applications came the development of novel CAR-based concepts as summarized in Figure 5, such as CAAR-T cells that express a chimeric receptor the targeting domain of which is an autoantigen, allowing the selective elimination of autoantibody-producing B cells. Alternatively, the use of a targeting domain consisting of an MHC/self-peptide-epitope complex was shown to enable the $CD8^+$ T cell-mediated elimination of autoreactive $CD4^+$ T cells. The spectrum of creativity in this respect includes various further CAR designs and also extends to the application of CARs on other immune cell subtypes to generate alternative outputs downstream of CAR engagement, such as suppression rather than destruction of immune cells through targeted deployment of CAR-Tregs. CAR-equipped innate immune cells, such as NK cells and MΦs, offer further opportunities

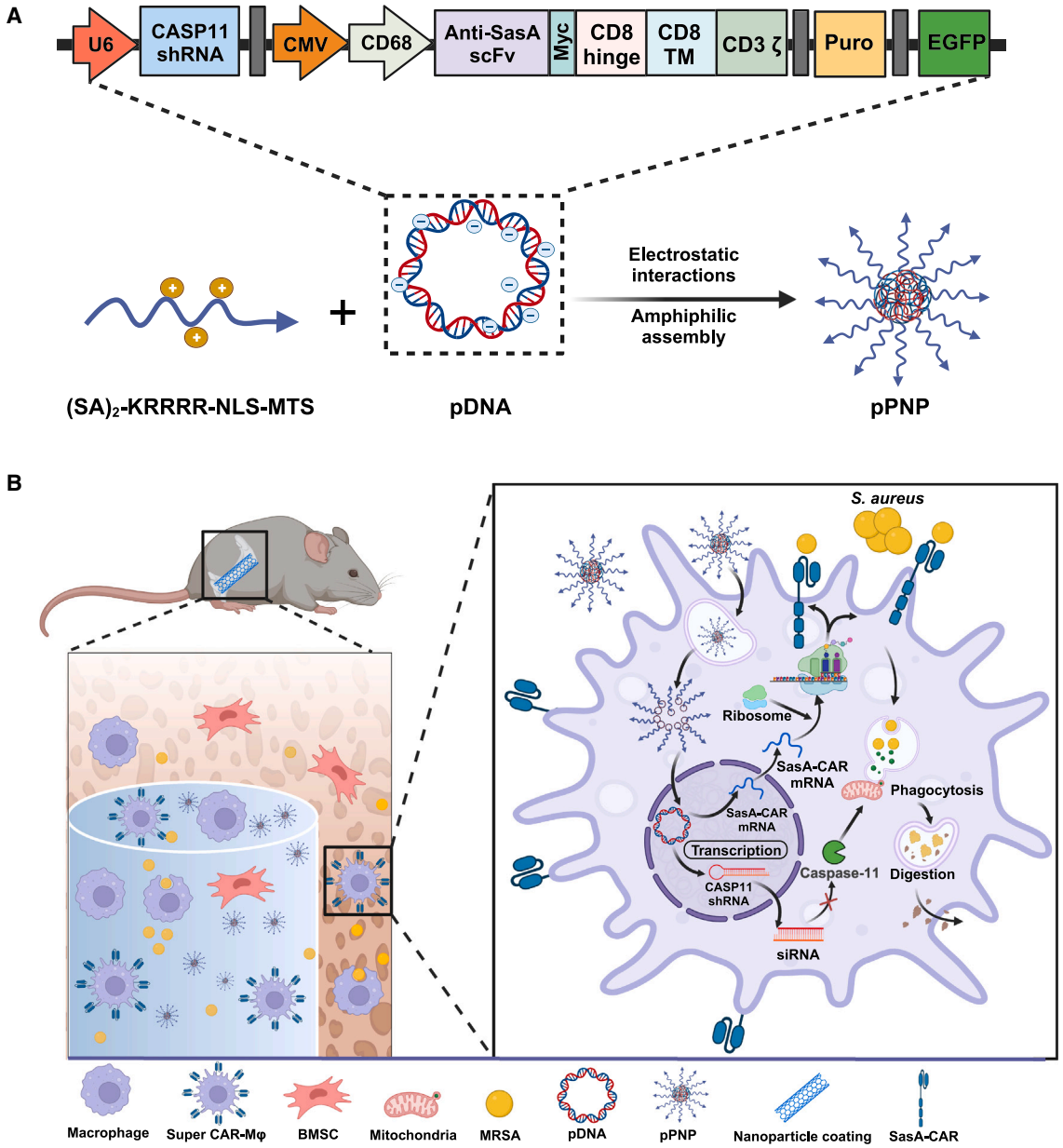

Figure 4. A schematic diagram of locally generated *S. aureus*-specific CAR-MΦs on a nanoparticle coating

(A) A schematic representation of the preparation of plasmid DNA (pDNA)-laden peptide nanoparticle (pPNP). An amphiphilic peptide, (SA)2-KRRRR-NLS-MTS, was synthesized with the hydrophilic sequences corresponding to the MΦ targeting sequence (MTS) and nuclear localization signal (NLS), along with a hydrophobic stearic acid (SA) domain. This peptide unit was used to create pPNP complexes with pDNA. Within the plasmid gene, CASP11 shRNA was expressed under the control of the U6 promoter, while the *S. aureus*-specific CAR gene was regulated by the MΦ-specific CD68 promoter. (B) A schematic illustration of *in situ* generation of *S. aureus*-specific CAR-MΦs. Super CAR-MΦs were generated in a murine model by introducing SasA-CAR genes and CASP11 shRNA into the macrophage nucleus. CASP11 shRNA enabled mitochondria to be recruited around the phagosome containing engulfed bacteria, facilitating the delivery of mitochondria-generated bactericidal reactive oxygen species (this figure was created with BioRender.com).

to broaden the range of applications. Subsequent to promising studies in preclinical disease models, several of these modalities are currently in phase I/II clinical testing with the intent to fulfill unmet medical needs.

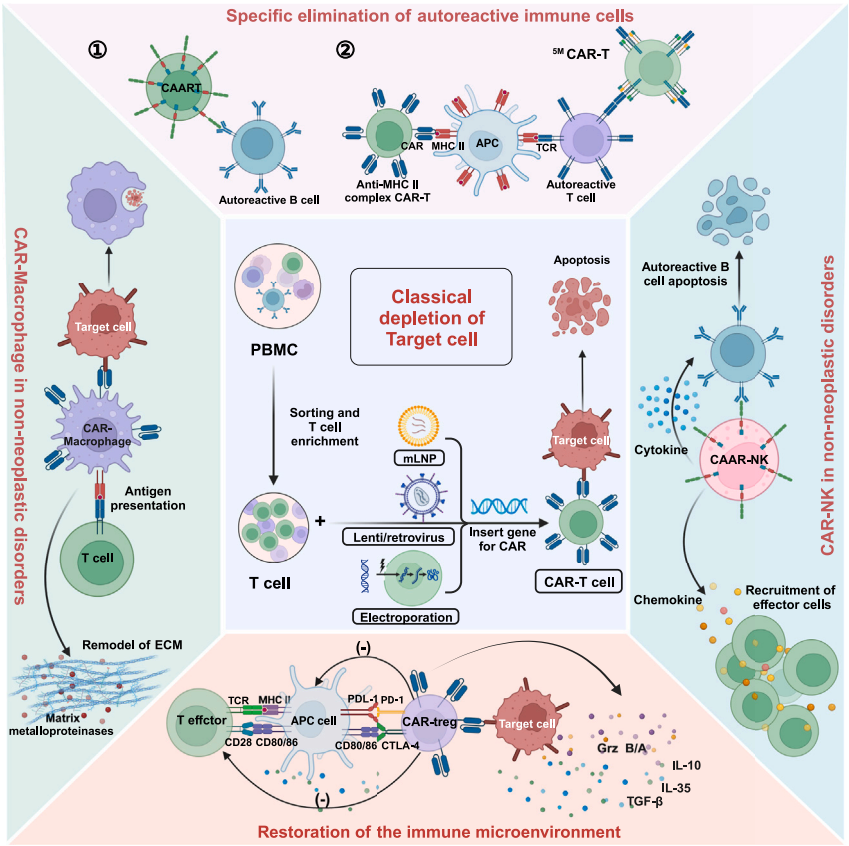

Figure 5. Mechanisms of CAR technology in non-neoplastic disorders

Five distinct CAR-based therapeutic strategies for non-neoplastic diseases. The central diagram depicts the most classical mechanisms of CAR-T cell production and targeted cell clearance, commonly employed for the broad elimination of B cells in autoimmune diseases and the specific clearance of pathogens and infected cells in infectious diseases. The upper panel demonstrates the utilization of CAR technology for the targeted depletion of autoreactive immune cells in autoimmune diseases. CAAR-T cells recognize autoreactive B cells bearing self-antigen-specific receptors and autoreactive APCs, exerting their cytotoxic effects. CAR-T cells expressing antigen-specific MHC-II can directly eliminate pathological $CD4^+$ T cells in a TCR-dependent manner. The lower panel illustrates the role of CAR-Tregs activated by antigens on target cells in restoring the immune microenvironment. The left panel displays CAR-MΦs with the ability to specifically kill target cells and promote antigen presentation and extracellular matrix remodeling. The right panel describes how CAAR-NK cells can achieve the specific clearance of autoreactive B cells and recruit corresponding effector cells through chemokines (this figure was created with BioRender.com).

As we gaze toward the future, several crucial trajectories merit attention. Refinement of target specificity and mitigation of adverse events stand as pivotal imperatives. Innovations in synthetic biology and bioinformatics hold promise for enhancing CAR design, minimizing collateral damage, and promoting patient safety. Additionally, expanding the current repertoire of validated CAR targets through systematic exploration will broaden the spectrum of treatable disorders. Furthermore, the convergence of CAR technology with emerging modalities such as gene editing and nanoparticle-based drug delivery opens vistas for synergistic therapeutic interventions. Nevertheless, a major challenge that stands in the way of widespread implementation of CAR-T therapy, even of the approved CAR-T products for treatment of hematological malignancies, are the complex logistics, lengthy manufacturing times (3–4 weeks), and high per-patient costs involved.[184,185]

In conclusion, the progress of CAR technology within the realm of non-neoplastic diseases is marked by substantial strides and tantalizing potential. The journey, though intricate, lays the groundwork for a future where precise immunomodulation revolutionizes therapeutic landscapes. Addressing current limitations through innovative strategies, CAR technology is poised to redefine treatment approaches, offering renewed hope for patients grappling with diverse medical conditions.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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