Review

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Chimeric antigen receptor (CAR) T-cell therapy: Engineering immune cells to treat liver diseases

Elmar Jaeckel[1], Scott L. Friedman[2], Michael Hudecek[3,4,5], Ulrike Protzer[6,7,*]

Summary

Endogenous T cells recognise antigens through human leukocyte antigen (HLA)/peptide complexes. However, HLA polymorphism poses a major challenge to the development of broadly applicable adoptive T-cell therapies. Engineered T cells can circumvent this barrier by targeting surface antigens independently from HLA through a synthetic chimeric antigen receptor (CAR) with an antibody-derived recognition domain fused to intracellular signalling motifs. CAR T-cell therapies have transformed the treatment of B-cell malignancies in haematology, and recent studies demonstrate therapeutic potential against solid tumours. In this review, we provide an overview of the fundamental principles and key achievements of CAR technology, with a focus on its applications in hepatic viral infections, autoimmune liver diseases, and hepatobiliary tumours. We also highlight emerging senolytic therapies targeting senescent cells and hepatic fibrosis, as well as regulatory CAR T cells designed to induce liver-specific immune tolerance in transplantation. Finally, we discuss ongoing and future research aimed at improving the specificity, efficacy, and safety of CAR-based therapies as "living drugs" for targeted, durable, and personalised treatment of liver diseases.

Introduction

T cells are crucial players in the adaptive immune system and have long been known to play a critical role in controlling HBV and HCV infections.[1] They also play an important role in attacking hepatocellular carcinoma (HCC), as indicated by the successful implementation of checkpoint inhibitors in HCC therapy.[2] Therefore, it seems intuitive to exploit T cells as the key substrate in a new generation of cellular immunotherapies that are genetically engineered to redirect and control their antigen specificity and confer the attributes required for a reproducible and therapeutically beneficial immune response against liver diseases. Approved immune-based interventions for liver diseases or liver cancer include interferon alpha, immune sera containing neutralising antibodies, and monoclonal antibodies inhibiting immune checkpoints and growth factors. With the current pace of translational research and clinical development of cell-based therapies, we expect engineered immune cells to be approved for the treatment of liver diseases in this decade. In this review, we provide an overview of the principles of immune cell engineering, exemplified by T cells that are modified with a synthetic chimeric antigen receptor (CAR), with a particularly focus on applications in viral hepatitis, hepatobiliary tumours, hepatic fibrosis, autoimmune liver diseases, and liver transplantation.

T-cell therapy for liver diseases

Endogenous T cells recognise human leukocyte antigen (HLA)/peptide complexes through their T-cell receptor (TCR). Although the adaptive T-cell response allows for rapid and broad, as well as specific, immune reactivity against infections, the restriction of T cells by the highly polymorphic HLA system complicates the development of adoptive T-cell therapies. TCRs recognising hepatitis viruses with high specificity and avidity were cloned from donors with resolved infections and have become available for T-cell engineering.[3,4] TCR-engineered (TCR-) T cells follow the physiological path of antigen recognition and very sensitively recognise antigen-derived peptides in the context of an individual's HLA molecules. However, the fact that individual HLA subtypes vary significantly prevents application of the same TCR-T cells to all patients and requires careful pre-selection.

For cancer therapy, it has been difficult to reproducibly isolate T cells from the endogenous repertoire recognising peptide epitopes derived from tumour-associated antigens (TAAs) or mutated neoantigens expressed by a tumour with sufficient specificity and affinity to allow for the recognition and elimination of primary tumour cells. A key challenge that has limited the application of TCR-T cells for HCC is that most TAAs are also expressed by "normal" developing or regenerating liver tissues. T cells that recognise such antigens with

* Corresponding author. Address: Institute of Virology, Trogerstr. 30, 81675 Munich, Germany; Tel.: +49-89-4140-6886, fax: -6823.
E-mail address: protzer@tum.de (U. Protzer).
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Keypoints

- A chimeric antigen receptor (CAR) is a synthetic receptor that recognises an antigen on the surface of a target cell independent of the patient's HLA molecules.
- CAR T cells are T cells engineered to express a CAR. The CAR activates the T cell upon binding to the target antigen.
- CAR T-cell transfer after lymphodepletion marked a breakthrough in treating haematologic malignancies and is increasingly used, e.g., in autoimmune diseases and fibrosis.
- CAR T cells are an interesting, novel approach to treating liver diseases such as hepatobiliary cancers and autoimmune liver diseases, as well as hepatitis B and liver fibrosis.
- Regulatory T cells grafted with a CAR have the potential to support the acceptance of a liver graft after transplantation.
- Cytokine release syndrome, immune effector cell-associated neurotoxicity, cytopenia, and infectious complications are the most frequent but usually transient side effects of CAR T-cell therapies.

high affinity are eliminated during thymic selection in T-cell development.[5] An exception is virus-derived antigens, which vary significantly from the endogenous cellular antigens as discussed below.

CARs are synthetic immune receptors that target antigens on the surface of cells

A potential solution is the generation of CARs that recognise their antigen on their target cell's surface independently of HLA, first described in pioneering reports in the late 80s.[6,7] In 1991, Irving *et al.* showed that the intracellular CD3ζ domain of a TCR is sufficient to activate a T cell using a chimeric receptor.[8] However, developing clinically successful CAR T-cell products took over two decades of preclinical investigation.

In most cases, CARs carry an extracellular antibody-derived recognition domain consisting of a single-chain variable fragment (scFv). This scFv-based recognition domain is fused to an intracellular signalling moiety composed of CD3ζ and secondary costimulatory domains, such as CD28 or 4-1BB, which enhance and sustain CAR T-cell function.[9,10] This secondary, costimulatory domain varies in different CAR constructs, and over the years, several generations of CARs have been developed. The latest developments included receptors in which the extracellular binding domains of CARs were fused to the physiological, intracellular signalling domains of a TCR (Fig. 1).

Initially, the recognition domains that direct a CAR to its target were scFv from selected, murine or human monoclonal antibodies detecting the extracellular portion of a target antigen. Alternatively, scFv can be chosen from pre-existing libraries, or natural ligands or receptors can be used as binders. Nanobodies derived from camelidae provide another interesting alternative.[11]

Clinical application of CAR T cells

The use of gamma-retroviral or lentiviral vectors and the implementation of manufacturing schemes that enable the production of therapeutic CAR T cells in sufficient amounts and within an acceptable timeframe have supported clinical translation. Clinical observations informed subsequent iterations of CAR T-cell products to improve their efficacy and tolerability: for example, using humanised CAR binding domains to reduce immunogenicity,[12] or optimised CAR spacer and transmembrane domains,[13] and defined T-cell subpopulations to confer consistent pharmacokinetic and pharmacodynamic attributes.[14–17] This led to CAR T-cell therapies

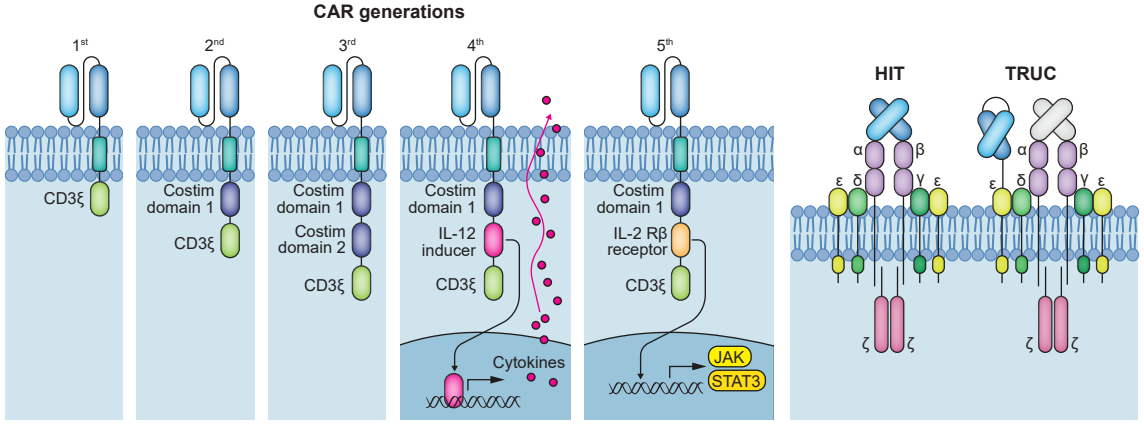

Fig. 1. The evolution of chimeric antigen receptors. The first generation of CARs consists of an antibody recognition domain fused to an intracellular CD3ζ activation domain, providing signal 1 only. The second generation combines signal 1 with a costimulatory signal 2. The third generation includes two costimulatory domains. The fourth generation combines the CAR with cytokine or antibody secretion to modify the tumour microenvironment. The fifth generation activates intracellular signalling independently of the TCR. Antibody recognition domains can also be directly fused to TCR components, forming HIT or TRUC constructs, which provide enhanced sensitivity by exploiting the physiological TCR signalling. CAR, chimeric antigen receptor; HIT, HLA-independent TCR receptors; TRUC, T-cell receptor fusion construct, e.g. with an antibody fragment added to the TCR ε-chain.

that have transformed treatments for haematologic malignancies and established the foundation for engineered T-cell applications. CAR-modified T cells directed against the B-lineage molecules and CD19 are now routinely used to treat acute lymphoblastic leukemia,[18,19] B-cell lymphoma,[20–22] and multiple myeloma.[23,24] As of spring 2025, seven autologous CAR T-cell products have been FDA approved.

In some patients, CD19 CAR T cells have been detected more than 10 years after therapy, supporting the notion of CAR T cells as a "living drug". Loss of CAR T-cell persistence has been attributed to T-cell intrinsic factors, such as terminal differentiation or compromised "fitness" after prior chemotherapy in patients with cancer, or suboptimal stimulation with antigens that are either expressed at low density and therefore fail to induce a productive CAR T-cell response or at too higher density resulting in explosive CAR signalling and activation-induced cell death or T-cell exhaustion.[25,26] Primary and secondary cancer resistance mechanisms include antigen downregulation or loss, acquisition of mutations in apoptosis pathways, and cellular dormancy that results in cancer relapse. The rapid clinical development and success of CAR T-cell therapy in haematology spurred its application to treat solid tumours and applications in non-malignant diseases. In the following review, we will discuss specific applications of CAR T cells in various liver diseases.

Clinical safety and specific adverse reactions after CAR T-cell therapy

Cytokine release syndrome (CRS) is the most prevalent and well-characterised toxicity. CRS occurs due to the massive release of pro-inflammatory cytokines, such as IL-6 and IL-1, mainly triggered by the activation of myeloid cells.[27] Symptoms range from mild fever and fatigue to severe manifestations, including hypotension, hypoxia, and multi-organ failure. Severe CRS necessitates interventions such as tocilizumab and corticosteroids, with early administration of these agents shown to significantly reduce mortality. Predictive biomarkers, including pre-infusion levels of C-reactive protein and ferritin, and scoring systems like "EASIX" have improved the stratification of patients at high risk for CRS.

Immune effector cell-associated neurotoxicity syndrome (ICANS) is another critical toxicity with a diverse presentation, including confusion, aphasia, tremors, seizures, and, in severe cases, cerebral oedema.[27] The exact mechanisms involve the disruption of the blood-brain barrier and local cytokine surges in the central nervous system. Notably, ICANS often correlates with the severity of CRS, although it can also occur independently. Corticosteroids remain the cornerstone of ICANS management, with dexamethasone being commonly employed. Anakinra, an IL-1 receptor antagonist, has demonstrated efficacy in preclinical models and early clinical use, representing a potential adjunct therapy.

Long-term toxicities, such as prolonged cytopenia, pose significant challenges. Prolonged grade 3-4 neutropenia and thrombocytopenia can persist for months, increasing susceptibility to severe infections. Recent findings suggest that persistent bone marrow inflammation, mediated by IFN-γ-expressing T cells, may suppress haematopoietic recovery. Strategies under evaluation include thrombopoietin receptor agonists and autologous stem cell boosts, with growing evidence supporting their efficacy.

Emerging toxicities, such as movement disorders associated with anti-BCMA (B-cell maturation antigen) CAR T cells, have added complexity to toxicity profiles. These delayed neurologic syndromes include Parkinson-like symptoms, such as rigidity and gait disturbances, which may result from the interaction of CAR T cells with basal ganglia neurons. Enhanced monitoring and early interventions, such as bridging therapies to reduce pre-infusion tumour burden, have decreased their incidence.

Secondary haemophagocytic lymphohistiocytosis represents a severe hyper-inflammatory state distinct from CRS. Characterised by hyperferritinemia, coagulopathy, and organ dysfunction, haemophagocytic lymphohistiocytosis often necessitates aggressive immunosuppressive therapy, including corticosteroids and IL-1 inhibitors. Its shared mechanisms with CRS highlight the need for differential diagnosis and tailored management strategies.

Infections and immunosuppression are pervasive concerns, driven by lymphodepletion regimens, B-cell aplasia, and hypogammaglobulinemia. Persistent infections, such as those caused by the reactivation of herpesviruses like cytomegalovirus (CMV), Epstein-Barr virus (EBV), or human-herpesvirus-6, are particularly concerning, necessitating vigilant monitoring and prophylactic therapies. Immunoglobulin replacement or antiviral prophylaxis is increasingly used to mitigate these risks, especially in patients with prolonged B-cell depletion.

Obstacles to CAR T-cell therapy in liver disease

CAR T cells are in clinical trials for HCC, hepatobiliary malignancies, and autoimmune liver disease, as well as to prevent rejection of liver grafts after transplantation. These approaches have also shown promise in treating viral hepatitis and liver fibrosis (Fig. 2), but significant obstacles to the use of CAR T-cell therapy for infectious and other non-malignant liver diseases remain. Lymphodepletion precedes T-cell therapy in almost all clinical trials because it creates a favourable immune environment for CAR T cells, avoiding rejection and improving expansion, persistence, and clinical activity.[28] However, lymphodepletion is associated with side effects and poses a particular problem for the endogenous control of viruses by T cells. Thus, clinical trials must develop strategies to avoid lymphodepletion in patients with infectious and non-malignant diseases.[29]

In addition, CAR T cells may face the exact immune modulatory mechanisms as endogenous T cells (Fig. 3). CAR T cells reach the liver via the hepatic sinusoids lined by liver sinusoidal endothelial cells (LSECs). T cells can reach and kill hepatocytes through fenestrae in LSECs.[30] However, with increasing tissue disruption, this access is impaired, as a continuous endothelium is formed[31] and extracellular matrix (ECM) accumulates in the space of Disse, generating a physical barrier and hindering the contact of CAR T cells with their target cells. The same hurdle applies to effector T and CAR T cells trying to reach their target. This is particularly relevant for targeting HCC, stellate cells, or fibroblasts in advanced liver fibrosis or cirrhosis (Fig. 3B).

CAR T cells are also confronted with the exact immune regulatory mechanisms in the liver as "natural" T cells

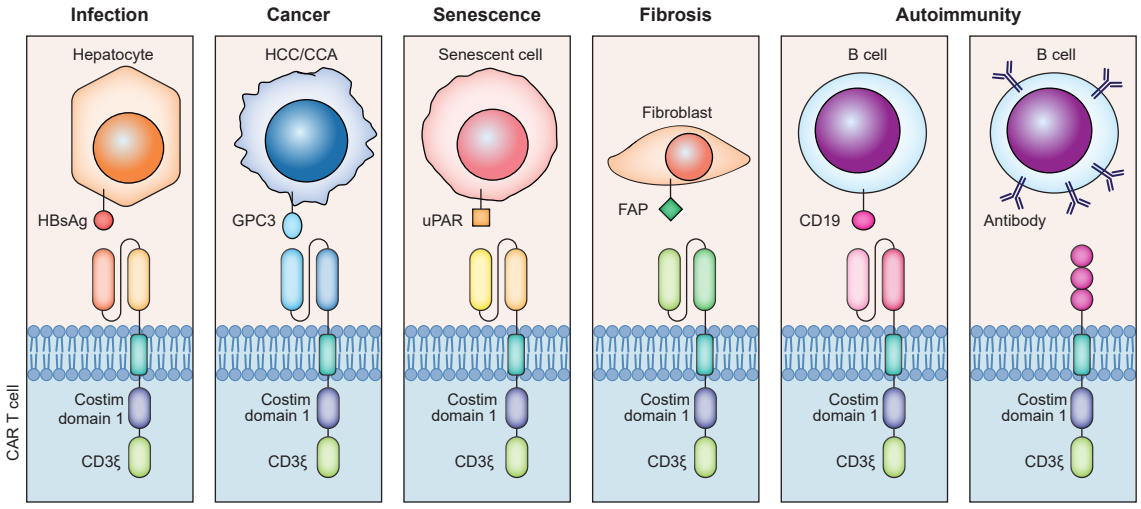

Fig. 2. Applications of CAR T cells in liver diseases. CAR T cells can be directed against virus-infected hepatocytes, tumour cells, senescent cells, and fibrogenic cells. They can also deplete all B lymphocytes or specifically target autoantigen-specific B cells in autoimmune liver diseases. CAR, chimeric antigen receptor; CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma.

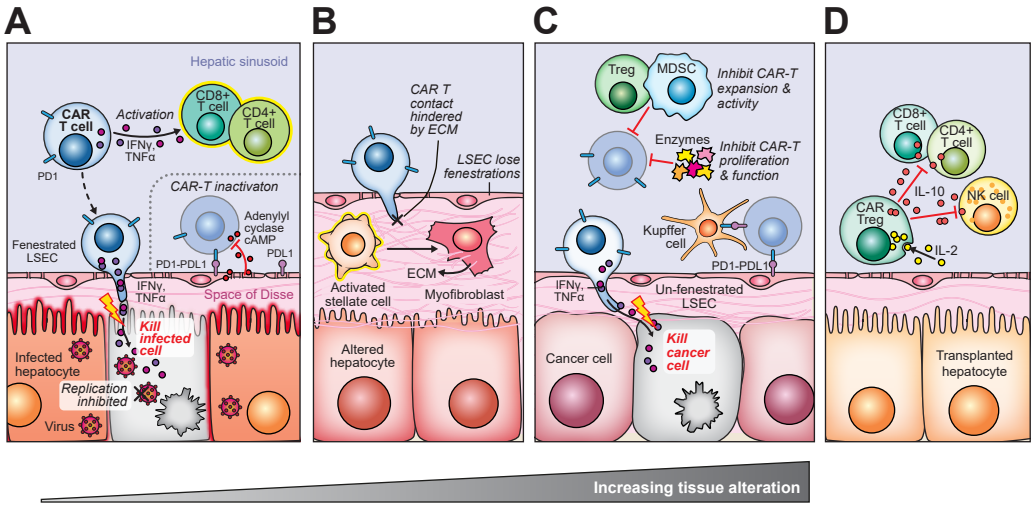

Fig. 3. The function of CAR T cells in the liver. CAR T cells can reach their target (hepatocytes, cancer or stellate cells, or fibroblasts) through the fenestrae in the sinusoidal endothelium. (A) Hepatocytes infected with HBV can be targeted by CAR T cells that kill infected hepatocytes and secrete cytokines (*e.g.* IFNγ, lymphotoxin, TNFα) that inhibit virus replication and activate endogenous CD4 and CD8 T-cell responses. However, the effector function of CAR T cells may be altered by the liver microenvironment. (B) Over time, increasing tissue alteration is observed, and the extracellular matrix accumulates and hinders the contact of CAR T cells with their target cells. CAR T cells can target myofibroblastic cells, potentially diminishing the extracellular matrix, reversing liver fibrosis, and reducing aging processes in the liver. (C) CAR T cells directed against cancer antigens kill cells and alter the tumour microenvironment by secreting cytokines. However, they are also confronted with immunoregulatory cell populations like Tregs or MDSCs. Enzymes such as IDO, TDO, and arginase are expressed at high levels and may hinder CAR T-cell proliferation and function. Co-inhibitory signalling by binding of PD-1 on T cells to PD-L1 on Kupffer cells, LSECs, stellate, and dendritic cells may restrict CAR T-cell effector functions, rendering them anergic or exhausted. (D) CAR Tregs blunt the immune response against transplanted livers by snatching IL-2 and secreting IL-10 to inhibit NK cells, CD4, and CD8 T cells. CAR, chimeric antigen receptor; LSECs, liver sinusoidal endothelial cells; MDSCs, myeloid-derived suppressor cells; NK, natural killer; Tregs, regulatory T cells.

(summarised in[32]). Immunoregulatory cell populations, such as regulatory T cells (Tregs) or myeloid-derived suppressor cells (MDSCs), prevent the local expansion of CAR T cells and restrict their cytotoxic activity (Fig. 3C); in the case of MDSCs, via the production and release of methylglyoxal. Enzymes such as IDO (indoleamine 2,3-dioxygenase), TDO (tryptophan 2,3-dioxygenase) and arginase are expressed at high levels in the liver and metabolise amino acids essential for local CAR T-cell proliferation and function. Co-inhibitory signalling via PD-1 [programmed cell death 1] on T cells binding to PD-L1 [programmed cell death ligand 1] on Kupffer cells, LSECs, stellate cells, and dendritic cells may restrict CAR T-cell effector functions, leading to anergy or exhaustion.

CAR T-cell therapy to treat viral hepatitis

Although the development of immunotherapies and proof-of-concept studies has been pursued mainly in haemato-oncology, infectious diseases and cancers related to chronic

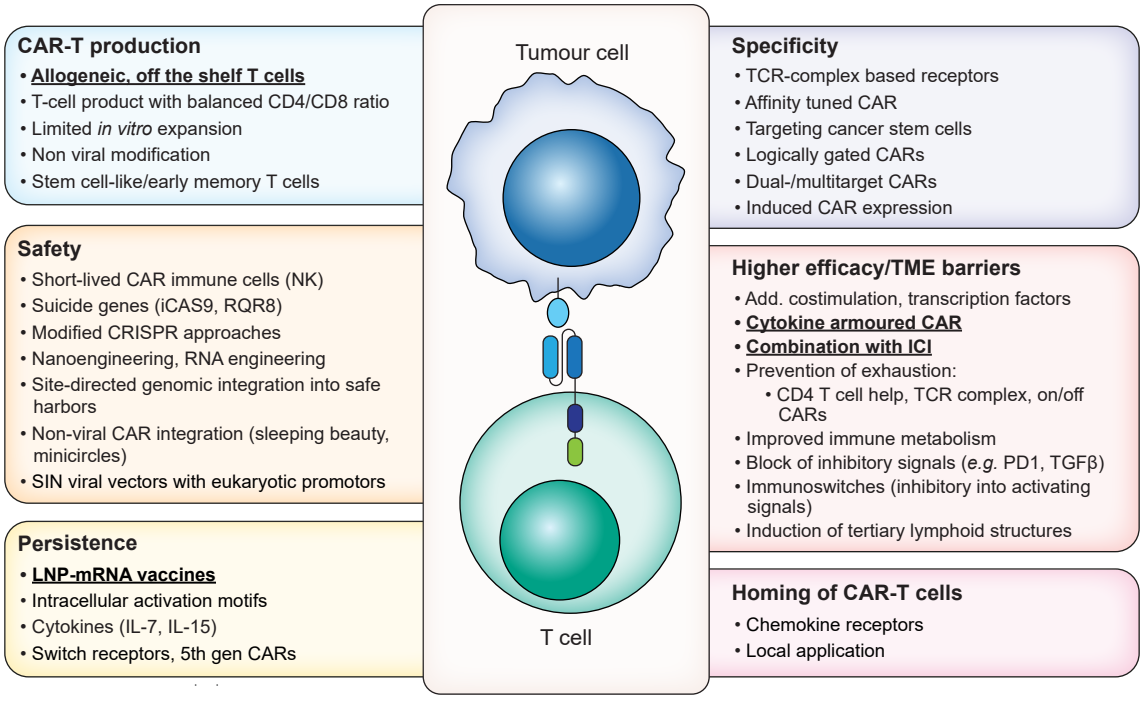

Fig. 4. Overview of improvement options for CAR T-cell therapies. This figure summarises advancements in improving antigen recognition, functionality, tumour and tissue accessibility, CAR T cell persistence, and safety. The underlined mechanisms highlight promising modifications for clinical application. CAR, chimeric antigen receptor; ICI, immune checkpoint inhibitor; LNP, lipid nanoparticle; NK, natural killer cells; SIN, self-inactivating; TCR, T-cell receptor; TME, tumour microenvironment.

infections are very appealing targets. Chronic viral infections may be an interesting application for T-cell therapy. Infected cells express pathogen-specific antigens that are unique, in contrast to TAAs that may also be expressed in healthy tissue and only vary in their antigen expression level.

Pioneering work with CAR T cells directed against viral antigens

Very early on, CAR T cells were exploited in clinical trials targeting HIV. First-generation CARs used the extracellular domain of human CD4, which targets the HIV envelope glycoprotein-120 expressed on the surface of infected cells, and later CARs used a scFv against glycoprotein-120 as a binder. However, only transient effects on viral load were observed in clinical trials, partially because the virus developed escape variants.[33] The next target were herpesviruses like CMV or EBV threatening immunosuppressed patients. EBV is associated with lymphoma, as well as nasopharyngeal and gastric cancers. Thus, EBV also provides an interesting target for CAR T-cell therapy. The first CAR T-cell therapies targeting EBV are currently in clinical trials for EBV-associated lymphoma and nasopharyngeal carcinoma, and results are eagerly awaited.

A key limitation in targeting HIV, EBV, and other herpesviruses and even coronaviruses is the early-late shift in their gene expression. Early genes express proteins that the viruses exploit to alter host cells and initiate viral genome replication. These early proteins are not displayed on the surface of infected cells and, therefore, are not suitable targets for CAR T cells. Viral envelope proteins would be good targets. However, the envelope proteins of HIV, CMV, and EBV are only expressed late in the infection cycle, limiting the window during which CAR T cells or antibodies can recognise and target infected cells before the release of newly formed virions, which is frequently accompanied by cell lysis.[34] The situation is different for the hepatitis viruses, HBV, HCV, and HDV. These hepatitis viruses, unlike most other viruses, do not undergo an early-late shift. HBV and HCV express their envelope proteins continuously after productive infection of a hepatocyte.

CAR T-cell therapy for the treatment of HBV infection

HBV expresses its small surface protein S constantly, not only in infected cells but also from most viral genomes randomly integrated during long-term infection, which is a hallmark of premalignant cells, rendering it an interesting therapeutic target. The expression of HBV envelope proteins is clinically detected by continuous secretion of the hepatitis B surface antigen (HBsAg). Because HBV envelope proteins are embedded in the endoplasmic reticulum and plasma membranes, they are displayed on the surface of infected cells where they can serve as targets for CAR T cells[35] (Fig. 2). This finding encouraged the development of CAR T cells and T-cell engager antibodies suitable to treat hepatitis B and hepatitis D using the same surface proteins.[36–39] CAR T cells targeting the E2 envelope protein of HCV have also been developed.[40] However, with curative treatments using direct-acting antivirals now available, CAR T-cell therapy for hepatitis C has not been further pursued.

Bohne *et al.* first attempted to re-target T cells against HBV-infected cells. They generated second-generation CAR T cells that recognise HBV S and L surface proteins on the surface of replicating cells, enabling them to selectively target HBV-

infected cells and effectively kill HBV-positive HCC cells.[36] An scFv that recognises a conformational epitope in the external loop of the HBV S protein of a broad range of HBV genotypes was selected as best suited.[41] Thus, these CAR T cells recognise all three HBV envelope proteins: S, M, and L. T cells engineered with the S-CAR were able to clear HBV from infected autologous primary hepatocyte cultures,[36] infiltrate the liver in immunocompetent HBV-transgenic animals, and control HBV replication in the liver.[42] In subsequent studies, new binders were cloned from human B cells allowing to optimise CAR T-cell function,[38,39] and a preS1-targeting CAR T cell was shown to control HBV in HBV-infected humanised mice.[38]

The CAR T cells described killed HBV-infected hepatocytes and secreted cytokines. These cytokines, including IFNγ, lymphotoxin, and TNFα inhibit viral replication in a non-cytopathic fashion[43] and activate endogenous CD4 and CD8 T-cell responses.[44] However, the effector function of virus-specific T cells, including the CAR T cells, may be shut off by immune checkpoints (*e.g.* PD-1 on T cells interacting with its ligand [PD-L1]). In addition, a liver rheostat may influence the effector function of T cells, which, upon prolonged contact with LSECs, shut off their intracellular TCR signaling[45] (Fig. 3A).

Because HBV causes about half of all HCC worldwide, T cells engineered with HBV-specific TCRs have been used to treat HBV-associated HCC. In these HCCs, HBV genomic sequences are integrated, expressing the viral antigens HBs and HBx. HBs can serve as targets for TCR- or CAR T-cell therapy.[46,47] HBsAg-targeting TCR-T cells applied as second- or third-line therapy were reported to reduce tumour load, slow tumour progression,[44,47] and eliminate HBsAg, curing HBV and the underlying chronic infection.[47]

CAR T-cell therapy to treat hepatobiliary cancers

HCC is susceptible to antibody- and cellular immune recognition

The application of CAR T-cell therapy in solid tumours, including HCC, remains challenging due to the absence of true tumour-specific antigens, the underlying liver fibrosis or cirrhosis, and the highly inhibitory tumour microenvironment (TME)[48] (Fig. 3B,C). The TME of HCC is notably hostile to effective immune responses, being characterised by a deficiency in the metabolic factors necessary for immune cell function (due to the activity of immune modulatory enzymes), the dominance of anti-inflammatory cytokines, the local enrichment of inhibitory immune cells (such as Tregs or MDSCs), the overexpression of immune checkpoint molecules that inhibit T-cell activity[48] and the lack of fenestrae in the endothelium (Fig. 3B).

Initially, HCC was considered a low-immunogenic tumour, supported by the minimal efficacy observed in early immune checkpoint inhibitor (ICI) trials. However, this view changed significantly with the IMbrave150 trial, which demonstrated that the combination of an anti-VEGF agent (bevacizumab) with a PD-L1 inhibitor (atezolizumab) led to substantial response rates, with long-term survival in a subset of patients.[49,50] This pivotal study highlighted the importance of modifying the TME and activating the adaptive immune system to overcome HCC's inherent resistance to immune-mediated destruction.

Several TAAs have been identified in HCC that present viable targets for T cells (Table 1). However, the endogenous T-cell repertoire is often tolerant to these antigens, or T cells become exhausted due to chronic antigen exposure.[51] In addition, it is hard to identify neoantigens because the mutation rate in HCC is relatively low compared to other cancers.[52,53] Thus, CAR T cells targeting TAAs could have potent therapeutic effects, provided these engineered T cells can overcome the barriers imposed by the TME and the liver tissue alteration observed in most livers developing HCC (Fig. 3). Ideally, T-cell therapy could be used to target minimal residual disease after liver transplantation or resection, and thereby prevent frequent HCC relapses.

Proof-of-concept with GCP3 CAR T cells in HCC

A key target for CAR T-cell therapy in HCC is glypican-3 (GPC3), a cell surface proteoglycan overexpressed in approximately 70% of HCCs (Table 1). GPC3 is minimally expressed in normal tissues, including normal and cirrhotic livers, making it an attractive target for immunotherapy. GPC3 plays a significant role in HCC pathophysiology by stimulating the Wnt signalling pathway, which is crucial for tumour growth and survival. Thus, the loss of GPC3 expression could reduce the malignant potential of HCC.

Gao et al. demonstrated the effectiveness of these CAR T cells *in vitro* and in humanised mouse models of HCC xenografts.[61] Given the challenges posed by the TME in HCC, GPC3-specific CAR T cells developed for clinical application were designed to resist the suppressive effects of the TME. For instance, GPC3-specific CAR T cells engineered to secrete IL-7 and CCL19 achieved complete tumour disappearance in a single patient within 30 days of administration, demonstrating the potential of these advanced CAR designs.[35]

Several larger phase I clinical trials have explored the efficacy of GPC3-specific CAR T cells. In one study, fourth-generation IL-15-armoured GPC3-specific CAR T cells achieved a disease control rate of 66%, with an antitumor response rate of 33%.[62] Infusing these IL-15-enhanced CAR T cells was associated with increased CRS, a common side effect of CAR T-cell therapy, which was rapidly controlled using an inducible caspase 9 safety switch. Another study utilised affinity-tuned GPC3-specific CAR T cells co-expressing a dominant-negative TGF-β receptor II to neutralise the abundant TGF-β in the tumour microenvironment. This approach resulted in a disease control rate of 91%, with 42% of patients experiencing a tumour size reduction of more than 30%, even after failing 2-3 lines of prior therapy (http://www.clinicaltrials.gov/ct2/show/NCT05155189). Both studies highlight the critical need for CAR T-cell therapies that target tumour-specific antigens and modulate the tumour microenvironment to enhance efficacy.

Expanding the target tumour antigen portfolio for CAR T cells in hepatobiliary cancer

Beyond GPC3, other antigens have been identified as potential targets for CAR T cell therapy in HCC and cholangiocarcinoma, such as the epithelial cell adhesion molecule,

Table 1. CAR targets in hepatobiliary malignancies.

Target	% of hepatic cancers/tumour specificity	Expression in normal tissue	Involved in tumour pathogenesis	Effect *in vitro*/mouse xeno-grafts	Clinical trials	Comments	Clinical trial No.
GPC3	70-80% HCC <10% CCA +++	Minimal	Proliferation Invasion stimulates wnt pathway	+/+	+	Combination with ICI or anti-PD1 secretion	Multiple NCT05155189 NCT05103631
CD133	10-40% +++	Stem/pro-genitor cells	Expressed in cancer stem cells	+/+	+	Disease control in 14/21 patients reported[54]	NCT02541370
EpCAM	15-50% HCC 60-80% CCA +	Epithelial cells	Cancer stem cell Growth Invasion	+/+	+	Also tested in colo-rectal cancer[55]	NCT02729493 NCT03013712 NCT05028933
MUC1	25-65% HCC 40-80% CCA (+)	Epithelial cells	Immune evasion	+/+	+	Target for CCC[56]	NCT02587689
HBsAg	50-60% HCC HBV-assoc 50% CCA +	HBV-infected hepatocytes	Indicates virus integration driving clonal cell proliferation	+/+	No CAR-, but TCT-T cell trials	Human trial with TCRs against HLA-A2/HBsAg[44,47]	NCT06617000 NCT05339321 NCT06251115 NCT05195294 NCT02719782
CD147	80-90% HCC 50-70% CCA +++	Various tissues	Promotes tumour progression, invasion and metastasis	+/+	Preclinical[57]	Used in dual CARs (GPC3/CD147)	
AFP	60-70% +++	Regenerating hepatocytes	Immunosup-pression Apoptosis	+/+	Preclinical	CAR against peptide AFP158-166 pre-sented on HLA*A02:01	
c-Met	20-50% HCC 50-60% CCA +	Hepatocytes	Receptor tyrosine kinase in HGF dependent proliferation	+/+	Preclinical	Proto-oncogene[58]	
Claudin-4	80% CCA +++	Minimal	Tight junction formation	–/–	Preclinical	Clinical trials for solid cancers with mRNA vaccine against clau-din-6[59,60]	NCT04503278

AFP, alpha-fetoprotein; CAR, chimeric antigen receptor; CCA, cholangiocarcinoma; EpCAM, endothelial cell adhesion molecule; GPC3, glypican-3; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; MUC1, mucin 1.

mucin 1, the HBsAg, CD147, alpha-fetoprotein, the hepatocyte growth factor receptor c-Met, and claudin-4 or its analogue claudin-6. Their properties are summarised in Table 1. However, in most cases, solid clinical proof-of-concept is still missing (Table 2). In addition to these established targets, other potential antigens such as NKG2D (natural killer group 2D), DLK1 (delta-like 1 homolog), and carcinoembryonic antigen are being investigated. However, the scientific data supporting their efficacy in CAR T-cell therapy for solid cancers is limited.

Combination therapies to augment the efficacy of CAR T cells against HCC

T-cell therapies against HCC showed limited efficacy when used as monotherapies, necessitating the development of combination therapies to enhance their therapeutic potential. One of the most frequently used combinations is the co-application of ICIs, which help to counteract the immunosuppressive TME and prevent T-cell exhaustion. Combining CAR T-cell therapy with established HCC treatments has shown promise in improving tumour targeting.[66]

Another promising approach uses a fourth-generation CAR or TCR fusion constructs (Fig. 1). Here, T cells are engineered to secrete cytokines upon antigen binding, such as IL-15, which enhances T-cell survival, and IL-12 or IL-18, which boost anti-tumour immunity.[67] *In vivo* amplification of CAR T cells using mRNA vaccines is a promising novel strategy.[59,60] This method enables the expansion and activation of CAR T cells directly within the patient's body. It can potentially reduce the need for prolonged *ex vivo* cell culture and enhance the overall efficacy of the treatment.

HCC is one of the few cancers that can be cured through liver transplantation. CAR T-cell therapy offers a unique advantage in this context, as the therapeutic goal may be partial remission to enable downstaging to liver transplantation.[66] However, this combined approach depends on the availability of donor organs. CAR T-cell therapy for HCC after transplantation is challenged by the need to maintain T-cell function despite immunosuppression, while avoiding alloreactive T-cell responses that could lead to transplant rejection.

CAR T-cell therapy to treat degenerative liver diseases

Targeting hepatic stellate cells to treat liver fibrosis

Fibrosis, a wound healing response during chronic liver injury, is associated with progressive accumulation of ECM that ultimately impairs organ function and creates a stromal environment that confers a risk of cancer. The cellular source of ECM in the liver and other tissues is well established as resident pericytes that transdifferentiate or 'activate' into fibrogenic, contractile myofibroblasts.[68] In the liver, hepatic stellate cells (HSCs) are the origin of these fibrogenic cells. Thus, activated HSCs are an appealing target for clearance by CAR T cells. However, the targeting of HSCs and fibrotic cells by CAR T cells may be hindered by the loss of endothelial fenestration and the accumulation of ECM during the development of liver fibrosis (Fig. 3B).

Single-cell sequencing studies highlight the remarkable heterogeneity and plasticity of HSCs in the liver.[69,70] Among activated stellate cells, a subset with features of senescence is a particularly appealing target because this subset drives exuberant inflammation and tissue injury and promotes a carcinogenic milieu. Amor *et al.* sought to uncover cell surface markers of senescent HSCs using informatics, identifying urokinase plasminogen-activated receptor (uPAR) as an appealing candidate[71] (Fig. 2). Administration of CAR T cells targeting uPAR in two murine models of liver fibrosis significantly reduced ECM and improved liver function. In a subsequent study, the phenotype and ontology of senescence HSCs in mouse and human liver injury were more thoroughly characterised.[72] While uPAR is restricted to HSCs in early experimental injury, its expression expands to other cell types as the disease progresses. This finding indicates that collateral clearance of uPAR-expressing macrophages may amplify the efficacy of this CAR T-cell strategy, a point discussed further below.

Targeting fibrotic tissue with FAP CAR T cells

A related approach in cardiac fibrosis has utilised CAR T cells directed at the cell surface protein fibroblast activation protein (FAP)[73] (Fig. 2), which is restricted to fibrogenic cells in the heart as well as in other fibrotic tissues, including the liver.[74,75] CAR T cell-mediated clearance of FAP-expressing cells in the heart reduces fibrosis and improves cardiac function, reinforcing the appeal of this strategy in patients with fibrotic cardiac disease; in principle, this strategy should be effective in liver fibrosis, where HSCs express cell surface FAP.[74,75] More recently, CAR T-cell therapy has been explored for myelofibrosis by targeting a mutated surface protein, calreticulin, and additional fibrosis targets that are likely to emerge in other tissues.

Whereas the long-term persistence of conventional CAR T cells ensures ongoing surveillance of carcinogenesis, this durability may be less desirable in treating non-malignant diseases, including liver fibrosis. Unrestrained HSC clearance may be detrimental if the underlying disease is abrogated, *e.g.* after the cure of hepatitis C infection. It may also be detrimental if the clearance of HSCs is too complete. This concern is underscored by a recent study in which >99% depletion of HSCs was accomplished in mice by administering recombinant CD8 T cells directed towards green fluorescent protein,[76] which was transgenically expressed in HSCs.[77] Complete HSC depletion dramatically impaired liver regeneration, pointing to a homeostatic role of HSCs that must be preserved when subsets of this cell type are depleted.

The concern about the impact of unchecked HSC depletion on liver homeostasis has been circumvented by developing a CAR T-cell strategy that generates target-specific CAR T cells *in vivo,* whose long-term activity is constrained by the expression of a CAR through non-integrating mRNA instead of DNA. Specifically, lipid nanoparticles (LNPs) are administered to target T lymphocytes in the circulation, carrying instructions to reprogramme T cells into CAR T cells. Reprogrammed CAR T cells then target FAP-expressing cells for clearance, yielding the same beneficial effects as conventional *ex vivo* CAR T cells. This approach is exciting because: 1) The magnitude of CAR T-cell generation can be titrated based on the dose of

Table 2. Clinical trials using CAR T cells in liver diseases.

Immune cells	Phase	CAR target[Reference]	Amplification	Status	Start	Patients	Outcome	Location	NCT
Liver transplantation									
CAR Treg	1/2	MHC A2	FOXP3	Recruiting	2023	n = 20	Operational tolerance	UK, EU	NCT05234190
Hepatocellular carcinoma									
CAR T cell	1	GPC3[63]		Completed	2019	n = 13	2PR/2SD	Shanghai, China	NCT03884751, NCT02395250, NCT03146234
CAR T cell	case report	GPC3[50]				n = 1	1 patient CR	Renji, China	NCT03146234
CAR T cell	1	GPC3[62]	IL-15 armoured	Completed	2019	n = 121	PR 33%, 33%SD	Baylor, US	NCT02905188
CAR T cell	1	GPC3[64]	RUNX3	Completed	2019	n = 6	PR17%, SD33%	Zhejiang, China	NCT03980288
CAR T cell	1/2	GPC3[45]	dnTGFbRII	Interim report	2025	n = 24	ORR 75%	Zhengzou, China	NCT05155189
CAR T cell	1/2	GPC3[45]	dnTGFbRII	Recruiting	2024	n = 121	Tumour response rate	Shanghai, China	NCT06590246
CAR T cell	1/2	GPC3		Recruiting	2022	n = 105	Tumour response rate	Multicenter China	NCT05652920
CAR T cell	1	GPC3[65]		Recruiting	2021	n = 38	Tumour response rate	NIH, USA	NCT05003895
CAR T cell	1	GPC3		Recruiting	2024	n = 48	Tumour response rate	Multicenter, Korea/Australia	NCT06478693
CAR T cell	1/2	GPC3		Recruiting	2023	n = 94	Tumour response rate	US multicenter	NCT06084884
CAR T cell	1	GPC3		Recruiting	2023	n = 12	Tumour response rate	Korea	NCT05783570
CAR T cell	1	GPC3		Recruiting	2024	n = 15	Tumour response rate	Zhejiang, China	NCT06461624
CAR T cell	1	GPC3		Active, not recruiting	2021	n = 3	Tumour response rate	Tongji, China	NCT05070156
CAR T cell	1	GPC3	IL-15, IL_21	Not yet recruiting	2026	n = 21	Tumour response rate	Baylor, US	NCT06198296
CAR T cell	1-2	B7H3/CD276		Recruiting	2022	n = 15	Tumour response rate	Xuzhou, China	NCT03993743
CAR T cell	1	EpCAM		Recruiting	2021	n = 48	Tumour response rate	Zhejiang, China	NCT05028933
Macrophages	1	HER2		Active, not recruiting	2021	n = 48	Tumour response rate	Portland, US	NCT04660929
CAR T cell	1	IL1RAP		Recruiting	2025	n = 18	Tumour response rate	Shanghai, China	NCT06757881
Cholangiocarcinoma									
CAR T cell	1	CEA		Recruiting	2023	n = 36	Tumour response rate	Nanchang, China	NCT06010862
CAR T cell	1	CEA		Recruiting	2023	n = 60	Tumour response rate	Wanan, China	NCT06126406
CAR T cell	1	unknown		Not yet recruiting	2024	n = 60	Tumour response rate	Hangzhou, China	NCT06196658
CAR T cell	1	CEA		Recruiting	2023	n = 30	Tumour response rate	Hangzhou, China	NCT06043466
CAR T cell	1	Mesothelin		Recruiting	2023	n = 42	Tumour response rate	Multicenter, USA	NCT05568680
CAR T cell	1	Mesothelin		Recruiting	2024	n = 24	Tumour response rate	Bejing, China	NCT06256055

B7H3, B7 homolog 3 protein; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CR, complete response; EpCAM, endothelial cell adhesion molecule; GPC3, glypican-3; HER2, human epidermal growth factor receptor 2; IL1RAP, interleukin-1 receptor accessory protein; ORR, objective response rate; PR, partial response; SD, stable disease.

LNP; 2) CAR T cell-mediated clearance of target cells is self-limited because the effect of dosing with LNP-containing mRNAs is transient; 3) The approach can be scaled more readily than *ex vivo* CAR T cells, since frozen LNPs targeting FAP can be widely distributed, much like the LNP-mRNA vaccines that were used successfully for SARS-CoV-2.

CAR T cells may combat aging

A remarkable study has raised the prospect of using CAR T cells to prevent or treat aging-related metabolic dysregulation.[78] The same uPAR CAR T cells used to clear senescent HSCs[71] and reduce fibrosis, described above, were administered to systemically clear senescent cells marked by uPAR expression. This approach improved glucose intolerance in naturally aged mice or animals fed high-fat diets. Even more impressively, a single prophylactic administration of uPAR CAR T cells prevented features of age-dependent metabolic dysregulation.

The relevance of these findings to MASLD (metabolic dysfunction-associated steatotic liver disease) is particularly compelling. With one-third of the world's population affected by MASLD,[79] this potential senolytic therapy links senescent cells directly to the pathogenesis of liver disease, affecting not only HSCs but also other cell types, including epithelial, immune, and other mesenchymal cells. Moreover, in this instance, the long-term activity of senolytic CAR T cells could provide enduring benefits for aging and perhaps chronic diseases characterised by tissue injury and inflammation. While uPAR-directed CAR T cells appear to have broad benefits, no universal signature for senescence is preserved in all cells. Thus, responsiveness to this treatment may vary across different tissues and cell types.

CAR T cells in autoimmune liver diseases

The success of CAR T-cell therapy in oncology has spurred its application in autoimmune diseases. Pioneering work by Georg Schett and Andreas Mackensen demonstrated the efficacy of autologous CD19 CAR T cells in treating refractory systemic lupus erythematosus, with patients achieving long-term remission over 3 years.[80–82] CAR T-cell therapy was well tolerated in patients with lupus, with only mild CRS and no immune effector cell-associated neurotoxicity reported. Remarkably, B cells with a naïve, diverse repertoire re-emerged approximately 100 days post-therapy, as CAR T cells became undetectable, suggesting a transient yet profound "deep tissue depletion" of B cells. This contrasts with the persistent B-cell depletion observed in cancer treatments and highlights a unique therapeutic mechanism in autoimmunity.

Given these findings, similar CAR T-cell therapies are now being explored in refractory cases of myasthenia gravis, systemic sclerosis, idiopathic inflammatory myositis, and multiple sclerosis,[83] as well as in autoimmune hepatitis (AIH) (Fig. 2). Given the promising results of CAR T-cell therapy in other autoimmune diseases, there is a strong rationale to explore profound tissue B-cell depletion with CAR T cells in patients with advanced, refractory AIH, particularly those unable to achieve stable remission and facing progressive disease. Patients with AIH often exhibit strong humoral immune activation with elevated IgG levels, autoantibodies, and potentially misfolded polyreactive antibodies.[84] Rituximab has shown some success in treating AIH,[85,86] and therapies targeting BAFF (B-cell activating factor) are currently under clinical investigation.[87] For these patients, CD19 CAR T-cell therapy presents an interesting therapeutic option. This approach might also be extended to liver transplant recipients with severe antibody-mediated rejection, who typically have a poor prognosis.

An alternative CAR T-cell strategy in autoimmunity involves ligand CARs called "chimeric autoantibody receptors" (CAARs, Fig. 2), designed to specifically target and deplete autoantibody-producing B cells. In this approach, the extracellular domain of the CAR consists of a driver autoantigen, such as the desmoglein 3 in pemphigus vulgaris, rather than the traditional scFv. This allows the CAR T cells to target and deplete only those B cells that produce antibodies against the specific autoantigen.[88] This approach has shown promise in preclinical models. Still, several challenges remain, including whether these CAAR-T cells would also target cells with bound autoantibodies and the impact on plasma cells lacking surface immunoglobulins. In AIH, where liver-specific driver autoantibodies are generally absent, the clinical application of ligand CAARs may be limited. However, primary biliary cholangitis, which is characterised by a highly specific humoral immune response against PDC-E2, represents a potential target for this approach.

In summary, CAR T-cell therapy has rapidly transitioned from an innovative cancer treatment to a promising therapeutic option for refractory autoimmune diseases. Its success in systemic lupus erythematosus, with minimal toxicity and profound tissue B-cell depletion, highlights the potential of CAR T cells to transform the treatment landscape for autoimmune conditions and autoimmune liver disease.

CAR-modified Tregs for the treatment of liver diseases

The concept of suppressor cells counteracting effector immune cells has been recognised for a long time. Yet, it was not until 1996 that Sakaguchi described Tregs as a distinct and stable regulatory immune cell population[89] characterised by the expression of Foxp3 as a master transcription factor. It has since become evident that Tregs play crucial roles in various liver conditions.

Bluestone *et al.* demonstrated the safety and tolerability of large-dose adoptive Treg transfer in patients with recent-onset type 1 diabetes as an exemplary autoimmune disease. They showed the phenotypic stability of the transferred Tregs over a year,[90] but the therapy had no clinical efficacy, underscoring the need for antigen-specific Tregs. Initial attempts to use Tregs as therapeutic agents in autoimmune liver disease[91,92] and liver transplantation[93] revealed that polyspecific Tregs are significantly less potent than antigen-specific Tregs.[94–96]

This poses a significant challenge, as only a few Tregs from the natural repertoire can recognise liver-specific target antigens. Even if 8-12% of Tregs are allospecific,[97] transferring these cells has not been sufficient to induce tolerance after liver transplantation.[93] For autoimmune liver diseases, it is estimated that only one in a million Tregs can recognise autoantigens.[98] Therefore, transferring 400 million cells ($5x10^6$/kg) would yield only around 400 antigen-specific Tregs, highlighting the urgent need to engineer more Tregs specific for hepatic or biliary antigens.

As for effector T cells, two primary strategies exist to engineer liver-specific Tregs. The first is a transfer of a liver-specific TCR. This method faces limitations due to HLA restriction with the highly diverse major histocompatibility complex class II repertoire and the risk of mispairing with endogenous TCR chains. The second is the engineering of Tregs with CARs, which can generate large amounts of liver-specific Tregs. While various intracellular signalling domains are currently used for CARs in effector T cells, "classical" second-generation signalling domains using CD28 and CD3ζ seem best suited for Tregs[99] (Fig. 3D).

CAR Tregs after liver transplantation

The application of CAR Tregs in liver diseases is being pioneered in liver transplantation. It was initially shown that CARs targeting the mismatched HLA-A2 molecule – expressed in the donor liver but not in the graft recipient – could induce allotolerance without immunosuppression in humanised mouse models.[100–102] This tolerance extended to highly immunogenic HLA-A2-positive skin grafts transplanted onto HLA-A2-negative recipients, leading to the development of clinical programmes using HLA-A2-directed CAR Tregs for tolerance induction post-liver transplantation.[100] In the LIBERATE clinical trial, HLA-A2-directed CAR Tregs are administered to patients with minimal hepatic inflammation more than 1 year after liver transplantation (NCT05234190). The goal is to wean these patients from tacrolimus to everolimus monotherapy, with the ultimate aim of achieving operational tolerance without immunosuppression.

CAR Tregs accumulate locally and persist long-term,[100,102] creating local immune tolerance without compromising the patient's overall immune competence. As living drugs, they can potentially survive for life. In mouse models, transferred Tregs have been observed for over 200 days post-transfer. Tregs can modulate multiple immune cells and suppress T cells recognising various targets (cross-suppression). Tregs can also induce other T cells to become Tregs, creating new regulatory immune networks. While long-term survival of transferred Tregs might be beneficial, it is not yet clear if it is necessary.

CAR Tregs to combat autoimmune and metabolic liver diseases

In AIH, Tregs increase during active disease but are insufficient to control autoimmunity. Current steroid-based therapies may preferentially deplete Tregs, explaining the high relapse rates post-therapy. Therefore, liver-specific CAR Treg therapies could re-establish local immune tolerance in AIH, potentially eliminating the need for chronic immunosuppressive therapy. Oo *et al.* were the first to demonstrate the safety of Tregs in patients with AIH, paving the way for liver-specific CAR Treg therapies.[92] In contrast, adoptive Treg transfer increased metabolic inflammation and steatosis.[103] This discouraged the use of Tregs for the treatment of metabolic liver disease but encouraged its use for AIH.

The asialoglycoprotein receptor is being explored as a potential target for a pan-liver-specific CAR, which could be used in autoimmunity and liver transplantation. Still, proof of its efficacy in relevant liver-inflammatory models is lacking. In primary biliary cholangitis, the target antigen PDC-E2 is highly disease-specific but not organ-specific, complicating CAR Treg generation. Similarly, generating CAR Tregs for primary sclerosing cholangitis is challenging due to the lack of biliary epithelial cell-specific surface proteins. However, conventional CD19-CAR T cells may provide an option to treat these diseases.[104]

Current developments with CAR Tregs

Future CAR Treg products for inflammatory liver disease will focus on directing specificity and stabilising the Treg phenotype. Overexpression of FOXP3 could stabilise the regulatory phenotype under inflammatory or low IL-2 conditions.[105] Making CAR Tregs more independent of external IL-2 could stabilise their phenotype and enhance their suppressive function. Membrane-attached IL-2 molecules[106] or chimeric cytokine receptors activated by rapamycin might provide a benefit.[107] Liver-specific CAR Tregs could be used as Trojan horses, delivering immune regulatory, regenerative, and anti-fibrotic molecules for AIH. Tregs with amplified effector functions ("TRAF" cells) might be an option to improve clinical efficacy in the future.

In summary, CAR Tregs represent a promising new therapeutic option for achieving long-lasting tissue-specific tolerance without compromising overall immune competence. They offer potential benefits for local tissue regeneration and homeostasis, marking a significant advance in the treatment of inflammatory liver diseases and transplantation tolerance.

Improvements of CAR T-cell therapies

CAR T-cell specificity and sensitivity

Improving CAR T cells' sensitivity without losing specificity is crucial to maximise their on-tumour effects while minimising off-tumour toxicities (Fig. 4). The affinity of the CAR for its target antigen plays a significant role in this regard. A high-affinity binder enables CAR T cells to recognise and bind to tumour cells even at low antigen densities. However, this may lead to trogocytosis, a process where the CAR T cell strips the target antigen from the tumour cell. In contrast, lower-affinity binders in CARs require higher antigen densities for activation but exhibit less trogocytosis and exhaustion, resulting in prolonged T-cell survival.[108]

Another major avenue of investigation is dedicated to improving the intrinsic attributes of CAR T cells. On the one hand, the desire is to prevent rapid, explosive CAR signalling and the ensuing clinical inflammatory side effects. On the other hand, there is an intention to endow T cells with the ability to persist long-term and unfold memory capable of protecting patients from relapse. Significant progress has been made in conferring these desired properties to T cells by modulating specific transcription factors such as c-Jun.[109,110] Combining several transcription factors is currently being explored. This illustrates the need to fine-tune the levels and timing of transcription factor expression to achieve the desired T cell attributes.[64]

CAR recognition domains have been fused to other TCR/CD3 signalling complex molecules, generating TCR fusion constructs (Fig. 1), which are currently undergoing clinical testing.[111,112] An exciting development in this field is the creation of HLA-independent TCRs (HIT receptors), which fuse the variable regions of the CAR recognition domains to the

CD3 complex, *i.e.* the constant regions of the TCR alpha and beta chains (Fig. 1). These HIT receptors reduced exhaustion, improved T-cell survival, and increased antigen sensitivity to <200 target molecules per cell.[108] However, conventional CARs may also recognise target cells with <100 target molecules,[113] demonstrating that the binder plays a crucial role. An alternative concept to the HIT receptors is STAR receptors, which have recently been described. A scFv is fused to each TCR alpha and beta chain in STAR receptors. Thus, using different scFv even allows for the generation of bispecific receptors targeting two different antigens.[114]

Another area of interest is targeting cancer stem cells, as these cells are thought to play a crucial role in tumour recurrence and resistance. Targeting antigens expressed on cancer stem cells may achieve more durable tumour remissions. Lastly, artificial intelligence is now being used to improve CAR design and optimise known CAR binders or even generate entirely new binders that can be used in CARs.

CAR T cells targeting multiple targets

Multitarget CAR T cells, which can recognise two or more antigens on their target cell, are being explored to prevent tumour escape due to antigen loss and to enhance the overall on-tumour effect (Fig. 4). These multitarget CARs represent a logical OR gate (targeting target A OR B), activating the T cell if either of the target antigens is present on the tumour cell.[115,116] Alternatively, they require both target antigens to be activated, representing a logical AND gate. These logical gating strategies are being developed to improve the specificity and safety of CAR T-cell therapies by ensuring that the engineered T cells target only tumour cells while sparing normal tissues.[117] They certainly represent the next frontier in the evolution of CAR T-cell therapy.

Fine-tuning CAR T-cell efficacy

The intracellular activation domain of CAR T cells is a critical determinant of their sensitivity, activation strength, *in vivo* survival, and susceptibility to exhaustion. Lower target molecule densities can activate CARs with CD28 costimulatory domains. This initially results in stronger T-cell activation and tumour killing, but fosters T-cell exhaustion.[118] In contrast, CARs with 4-1BB costimulatory domains require higher antigen densities but demonstrate slower exhaustion and more prolonged survival.[117] Therefore, further modifications to the signalling domains balancing initial activation with long-term survival are being investigated.

Strategies to prevent CAR T-cell exhaustion include modulation of CAR affinity, using early memory T cells for CAR T-cell production, and shortening *in vitro* manipulation times. Additionally, ongoing studies are investigating the provision of CD4+ T-cell help, the use of on/off CARs to prevent tonic signalling, the modulation of T-cell metabolism, and fourth and fifth generation CARs (Fig. 1). An emerging topic is the role of the gastric microbiome in influencing CAR-T cell efficacy.

Homing of CAR T cells

The effective homing of CAR T cells to the liver or into tumour sites is essential for their success. This can be enhanced by various strategies (Fig. 4), such as administering ICIs, expressing chemokines like CCL19 to attract other immune cells, and engineering CAR T cells to express chemokine receptors. Alteration of liver tissue including the accumulation of ECM in the Space of Disse and the formation of a continuous endothelium poses an obstacle to CAR T-cell therapy in liver fibrosis and HCC. An interesting approach is using a heparanase-secreting CAR to digest ECM and enable easier access for the CAR T cells.[119] Additionally, local delivery of CAR T cells via intratumoral injection or arterial supply can improve their accumulation at the tumour site.

Safety

Although CAR T-cell therapies are generally safe, concerns remain regarding acute side effects such as CRS and immune effector cell-associated neurotoxicity and on-target, off-tumour effects. Additionally, there is ongoing debate about the potential risk of CAR T cells inducing T cell lymphomas due to mutations caused by non-specific insertion of strong non-human promoters.[120] Genetic safety is being improved by using self-inactivating retroviral and lentiviral vectors with human promoters to introduce the CAR, modified CRISPR approaches for homologous recombination without double-strand breaks, and site-directed integration into safe harbours using integrases.[121,122] CAR insertion into the natural TCR locus also improves CAR T-cell functionality.[123]

Synthetic genetic switches are being evaluated to allow for the depletion of CAR T cells if needed. These include suicide genes, such as inducible caspase 9, depletion strategies via antibodies targeting a truncated receptor co-expressed on the cell surface, or using tyrosine kinase inhibitors to mitigate CAR T-cell function (Fig. 4).[34]

Alternative cells grafted with CARs

In addition to T cells, shorter-lived CAR natural killer (NK) cells are currently being investigated in clinical trials.[124] However, without further genetic modification to enhance potency, CAR-NK cells remain less effective and durable than CAR T cells.[125] Rare T-cell subsets, such as γ/δ T cells and invariant NKT cells, are under investigation to overcome tumour cells' primary and secondary resistance to conventional CAR T cells. Blended immune cell products combining CAR T cells with other synergistically engineered immune cells are of emerging interest.

To provide ready-to-use and affordable CAR T-cell products, allogeneic CAR T cells derived from a healthy donor gene-edited to reduce immunogenicity have been intensively studied.[126] However, their therapeutic potential is significantly lower than that of autologous CAR T-cell products because of the toxicity associated with multiplexed gene-editing and the limited clinical efficacy resulting from rejection by the host patient's immune system. Using stem cell-derived CAR T cells from a perpetual source, such as induced pluripotent stem cells, represents an interesting alternative.[127]

Scaled manufacturing and *in vivo* gene transfer

An important ambition is to increase overall patient access and to ascertain the sustainability of engineered immune cell therapy for our healthcare systems (Fig. 4). Accordingly, strategies for scalable, rapid, and affordable CAR T-cell

manufacturing are in focus. Since 2020, the field has shifted towards reduced-expansion protocols, low-activation protocols, and point-of-care manufacturing, allowing for a rapid turnaround while providing less differentiated and exhausted T cells.[63]

In vivo gene transfer into T cells may alleviate many of the logistical and operational constraints associated with current CAR T-cell therapies. To avoid handling blood and cells outside the patient's body, targeted lentiviral vectors[128] or LNPs[129,130] are administered that target T lymphocytes in the circulation, carrying instructions to reprogramme them into CAR T cells. These *in vivo* manipulations, however, face regulatory challenges as quality control is difficult. Most recent developments include imminently available methods of T-cell engineering that one can envision as modified haemodialysis machines. These would combine simplified T-cell engineering without requiring a specialised "good manufacturing process" laboratory, still allowing for the quality control needed to fully realise the clinical potential of CAR T-cell therapies.

Conclusions

CAR T-cell therapies have transformed the treatment of B-cell malignancies, and recent advances in CAR technology hold promise for solid tumours, including those in the liver. These developments open new avenues for targeted immune interventions in liver disease. CAR T cells may allow for the local elimination of HBV-infected hepatocytes and hepatobiliary tumours, potentially avoiding the need for systemic chemotherapy and its associated side effects; initial clinical trials in these areas are underway. Meanwhile, preclinical research on the use of CAR T cells to remove senescent and fibrogenic liver cells is advancing. Additionally, CAR Tregs can induce liver-specific immune tolerance in transplantation and autoimmune liver diseases, eliminating the need for lifelong immunosuppression. Current research aims to further refine the specificity, efficacy, and safety of CAR-based immune therapies. Ultimately, CAR-redirected immune cells represent "living drugs" that offer targeted, local, and sustained therapeutic solutions for the personalised treatment of hepatic diseases.

Affiliations

[1]*University of Toronto, Center for Liver Transplantation, Center for Islet Transplantation, Multi-Organ Transplant Center, Toronto, Canada;* [2]*Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, United States;* [3]*Chair in Cellular Immunotherapy, Department of Medicine II, University Hospital Würzburg, Würzburg, Germany;* [4]*Fraunhofer Institute for Cell Therapy and Immunology (IZI), Cellular Immunotherapy Branch Site, Würzburg, Germany;* [5]*National Center for Tumor Diseases (NCT), Site Würzburg-Erlangen-Regensburg-Augsburg (WERA), and Bayerisches Zentrum für Krebsforschung (BKFZ), Site Würzburg, Würzburg, Germany;* [6]*Institute of Virology, School of Medicine and Health, Technical University of Munich / Helmholtz Munich, Germany;* [7]*German Center for Infection Research (DZIF), Munich partner site, Munich, Germany*

Abbreviations

AIH, autoimmune hepatitis; CAR, chimeric antigen receptor; CAAR, chimeric autoantibody receptor; CMV, cytomegalovirus; CRS, cytokine release syndrome; EBV, Epstein-Barr virus; ECM, extracellular matrix; FAP, fibroblast activation protein; GPC3, glypican 3; HLA, human leukocyte antigen; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; ICI, immune checkpoint inhibitor; ICANS, immune effector cell-associated neurotoxicity syndrome; LNP, lipid nanoparticles; LSECs, liver sinusoidal endothelial cells; scFv, single-chain variable fragment; TAA, tumour-associated antigen; TCR, T-cell receptor; TME, tumour microenvironment; Treg, regulatory T cell; uPAR, urokinase plasminogen-activated receptor.

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Conflict of interest

EJ is a co-founder, shareholder, and board member of Quell Therapeutics, London, UK. UP is a co-founder of SCG Cell Therapy Inc., Singapore, and a shareholder and board member. UP was a consultant for AATech, Aligos, Arbutus, GSK, Gilead, Leukocare, Roche, Sanofi, Vaccitech, and VirBio. MH is listed as an inventor on patent applications and granted patents related to CAR technologies owned by the Fred Hutchinson Cancer Center, Seattle, WA, and the University of Würzburg, Würzburg, Germany, which have been partly licensed to industry. MH is a co-founder and equity owner of T-CURX GmbH, Würzburg, Germany. MH declares speaker honoraria from BMS, Janssen, Kite/Gilead, and research support from BMS.

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Authors' contributions

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