Perspective

CAR T therapy beyond cancer: the evolution of a living drug

https://doi.org/10.1038/s41586-023-06243-w

Received: 2 February 2023

Accepted: 22 May 2023

Published online: 26 July 2023

Check for updates

Daniel J. Baker[1,2,3,4]✉, Zoltan Arany[4,5], Joseph A. Baur[6], Jonathan A. Epstein[4,5,7,8] & Carl H. June[1,2,3]✉

Engineering a patient's own T cells to selectively target and eliminate tumour cells has cured patients with untreatable haematologic cancers. These results have energized the field to apply chimaeric antigen receptor (CAR) T therapy throughout oncology. However, evidence from clinical and preclinical studies underscores the potential of CAR T therapy beyond oncology in treating autoimmunity, chronic infections, cardiac fibrosis, senescence-associated disease and other conditions. Concurrently, the deployment of new technologies and platforms provides further opportunity for the application of CAR T therapy to noncancerous pathologies. Here we review the rationale behind CAR T therapy, current challenges faced in oncology, a synopsis of preliminary reports in noncancerous diseases, and a discussion of relevant emerging technologies. We examine potential applications for this therapy in a wide range of contexts. Last, we highlight concerns regarding specificity and safety and outline the path forward for CAR T therapy beyond cancer.

In the past decade, CAR T therapy has transformed the field of oncology, treating previously incurable haematologic cancers[1–3]. Although this therapy rose to prominence owing to its success in cancer, this therapeutic rationale stretches back to its initial development to redirect T cells to treat human immunodeficiency virus (HIV)[4–6]. These initial trials were unsuccessful in treating HIV but demonstrated the long-term persistence of engineered T cells in immunocompromised patients. The subsequent application of this approach to refractory B cell and plasma cell malignancies propelled the field of adoptive cell therapy forward[7–10]. Since, there has been substantial effort to apply this approach to other cancers[11]. Early results indicate that the next advances may be in fields beyond cancer, in which CAR T cells could find widespread application (Fig. 1).

A new pillar of therapy

The principle at the foundation of CAR T therapy is to couple the potency of a T cell with the specificity of an antibody to precisely kill diseased cells. The single-chain variable fragment confers specificity while the intracellular signalling domains activate T cell-mediated cytotoxicity (Fig. 2). Synthetic engineering allows for refinement of CARs in each specific context. For example, first-generation CAR constructs were composed of a CD4 extracellular domain in combination with the CD3ζ signalling domain[4]. These constructs lacked potency and spurred second-generation CARs that include a co-stimulatory domain, such as CD28 or 4-1BB. This improved the effector function and persistence, and these CAR T cells are approved by the US Food and Drug Administration (FDA) for the treatment of various cancers at present[12] (Fig. 2). Fine-tuning these receptors has led to new generations of CARs that eliminate inhibitory domains, introduce dominant-negative receptors or mutate co-stimulatory domains to improve efficacy[13].

The success of CAR T cells in contexts in which other therapies failed suggests that cell and gene therapy is a new pillar of treatment for cancer[14]. CAR T cells have many distinctive features in comparison to traditional therapies. The intrinsic specificity of a CAR is unrivalled by current small-molecule approaches. CAR T cells rely on the cytotoxicity of a T cell, taking advantage of this highly efficient endogenous pathway. The additional benefit of a living drug is that a single CAR T cell can exponentially expand and kill hundreds, if not thousands, of target cells. Conversely, the pool of CAR T cells can contract when antigen is no longer present and remain on patrol for years[15]. Last, CAR T cells have demonstrated clinical feasibility. With more than 15,000 patients dosed, we have yet to see any autologous products transform into malignant cells. This safety profile in a condition for which the disease burden is high bodes well for application to other diseases with less target burden. Thus, the possibility to leverage the specificity, potency and clinical safety of CAR T cells to treat other diseases is compelling.

Challenges facing CAR T therapy

Despite the clinical efficacy of CAR T therapy in blood cancers, serious clinical complications can occur. High levels of CAR T cell expansion and target cell killing over a short time can result in cytokine release syndrome (CRS)[16]. In severe cases of CRS, patients may experience high-grade fevers, hypotension and even multi-organ failure. Another major impediment is 'on-target, off tumour toxicity'[17]. This occurs when

[1]Center for Cellular Immunotherapies, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. [2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. [3]Parker Institute for Cancer Immunotherapy at University of Pennsylvania, Philadelphia, PA, USA. [4]Cardiovascular Institute, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. [5]Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. [6]Department of Physiology and Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. [7]Institute for Regenerative Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. [8]Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. ✉e-mail: bakerda@pennmedicine.upenn.edu; cjune@upenn.edu

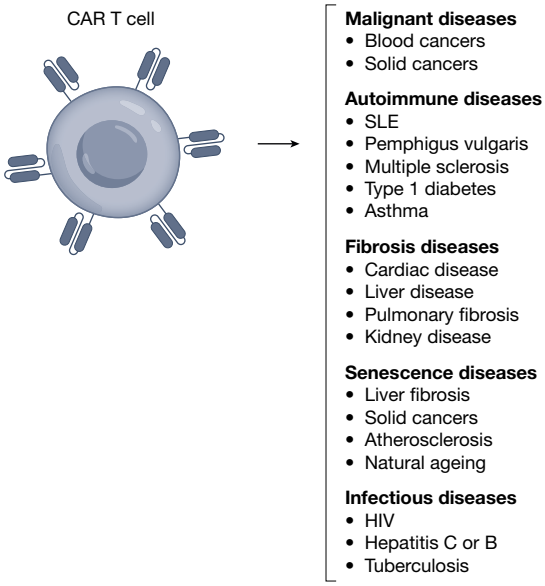

Fig. 1 | CAR T cells are poised to target a wide variety of diseases and pathological substrates. CAR T cells may find widespread use in a multiplicity of contexts beyond cancer.

the target antigen is present in vital tissues and can result in severe toxicity and even death[18]. Additionally, to facilitate the engraftment and expansion of the engineered T cells, lymphodepleting chemotherapy is administered before CAR T cell infusion. These chemotherapies are genotoxic, leading to increased risk for cancer and other diseases[19].

Autologous CAR T cell manufacturing is an emerging technology, and the costs to engineer and generate T cells and treat patients are high[15]. Current prices for patients are hundreds of thousands of US dollars, and even for a potentially curative treatment this is a substantial financial barrier[15]. Coupled closely with these high costs is the relative inaccessibility of CAR T therapy. A bespoke living drug requires specialized facilities and expertise restricting it to a select number of major centres. Together these challenges mean that many patients who may be optimal candidates for CAR T therapy are unable to access it at present.

CAR T therapy in solid tumours

Solid tumours comprise the most prevalent cancers and the largest unmet medical need. As such, CAR T cells targeting solid tumours have received an enormous amount of scientific and clinical attention[3,11,13,20,21]. The results of clinical trials in solid tumours have largely been disappointing so far. Solid cancers present unique challenges for CAR T therapy. In contrast to B cell malignancies, for which lineage-specific epitopes are relatively ubiquitous, cells within solid tumours are heterogenous, and with rare exceptions, tumour-specific epitopes have not been identified. To circumvent this, antigens that are overexpressed in tumours have been targeted; however, the low but non-negligible level of antigen expression in healthy tissues has resulted in toxicity[18]. The tumour microenvironment (TME) also presents challenges including physical barriers that resist CAR T cell trafficking and infiltration and an immunosuppressive environment that suppresses CAR T cell activity (Fig. 3a). To overcome obstacles to trafficking and infiltration, groups are exploring intratumoural injections of CAR T cells, peptide and nanoparticle vaccines to boost CAR T cell numbers, and engineered cytokine-driven CAR T cell expansion[17,22–27]. Approaches to target the TME include the use of oncolytic viruses to modulate the immunosuppressive nature of the TME and fortifying CAR T cells ex vivo using CRISPR–Cas9 and other modes of genome editing[11,28,29].

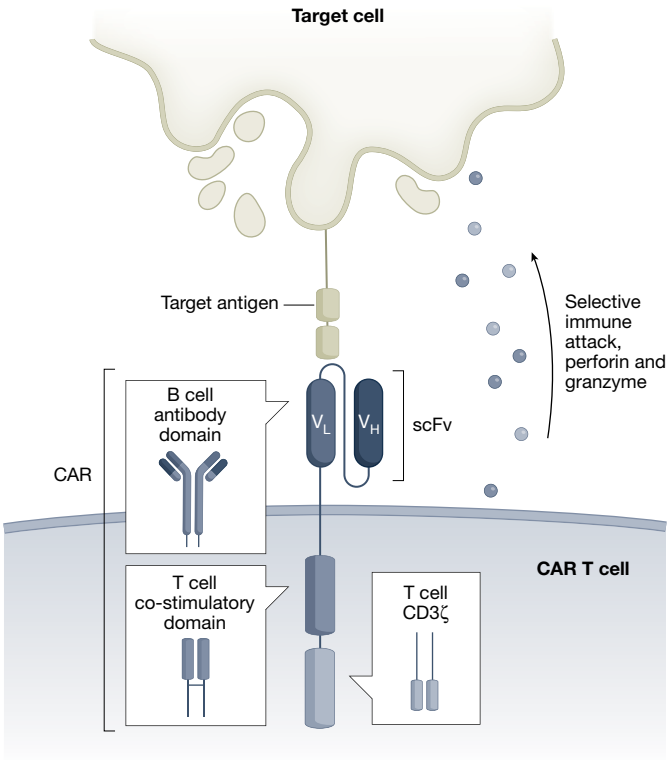

Fig. 2 | CAR T cells are engineered to be precise and powerful killers. The components of a CAR, including the variable light (V_L) and variable heavy (V_H) chains that comprise the single-chain variable fragment (scFv), which is fused to a co-stimulatory domain and CD3ζ. This synthetic receptor confers antigen-specific cytotoxicity.

A platform for innovation

An expanding universe

The clinical success of CAR T therapy has incited the imagination of those outside the field of cancer[30]. Theoretically, CAR T cells will find utility in contexts in which: eliminating a particular population of cells is therapeutic; and such a population of cells can be targeted specifically.

These two premises, although simple, are a limiting hurdle for many diseases. B cell and plasma cell malignancies reveal that such an approach is possible, but identifying a targetable antigen for other cancers has proved difficult. Emerging clinical and preclinical evidence suggests that CAR T cells may be used to treat autoimmune disease, chronic infections, heart failure and other chronic conditions.

Many of the challenges faced in solid tumours are less pronounced in noncancerous contexts (Fig. 3b). For example, tumour cell burden is often large, requiring large infusions of CAR T cells, associated with high levels of cytotoxicity, increased risk of CRS and on-target, off tumour toxicity due to the increased likelihood of CAR T cell trafficking. By contrast, noncancerous diseases have a markedly lower population of target cells. Additionally, in cancer nearly 100% of neoplastic cells must be eliminated, whereas in other diseases partial clearance of pathologic cells may prove therapeutic. Cancer cells also often carry a high mutational burden driving genomic heterogeneity. This can precipitate antigen escape (that is, the loss of the target antigen)[13,31]. By contrast, mutational load and genetic heterogeneity are relatively low in most chronic diseases. The TME is also a substantial physical barrier to CAR T cell efficacy (see above), whereas most noncancerous diseased tissues preserve physiologic architecture and perfusion, leading to the assumption that these tissues should be more accessible to a synthetic immune cell. The immunosuppressive environment of the TME is also

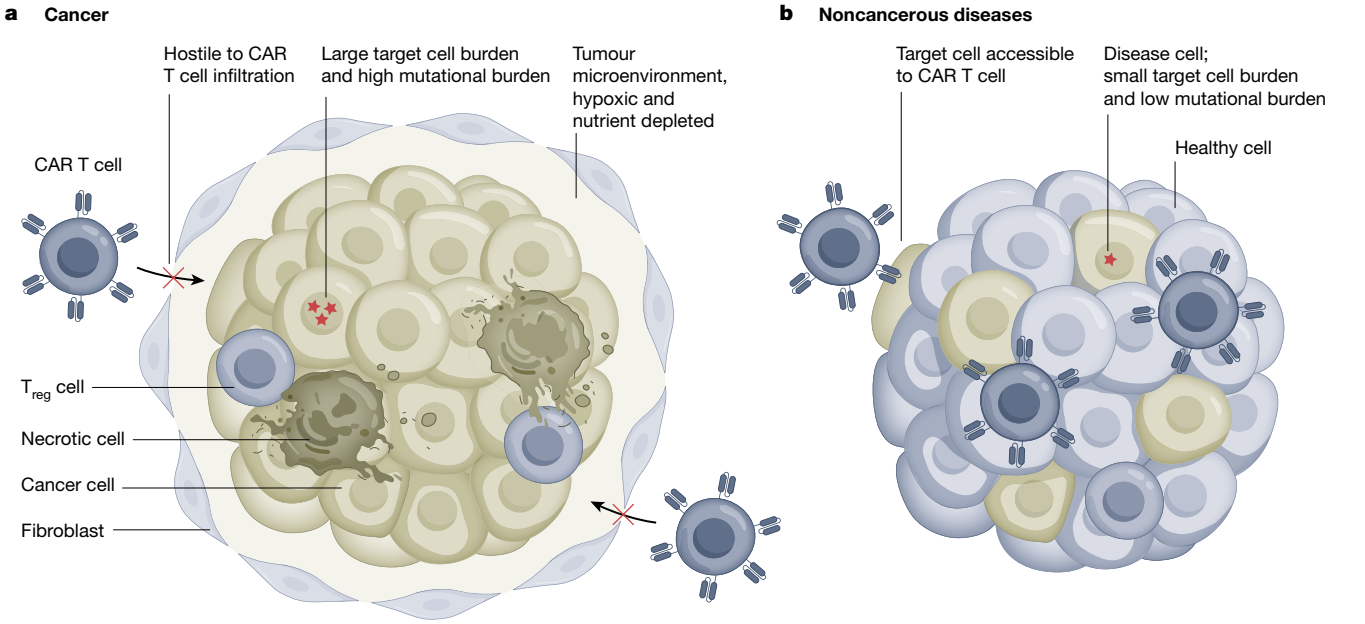

Fig. 3 | Advantages of CAR T therapy in other diseases in comparison to cancer. a, Cancer presents several substantial barriers to CAR T cell efficacy including a large target burden, nearly total clearance of malignant cells, a high mutational load and a hostile TME. **b**, By contrast, other diseases generally have a much smaller target burden, partial clearance of diseased cells can be therapeutic, a lower mutational load, and are generally not localized in such an inhospitable environment.

absent in most chronic diseases, and often the diseased tissue is targeted by the immune system physiologically. Thus, a synthetic immune approach to aid in the clearance of the pathologic cells is both feasible, and quite possibly, synergistic with endogenous immune function[32]. Hence, the notion of using CAR T cells to target diseases other than cancer is compelling.

Autoimmune and inflammatory diseases. CAR T therapy in autoimmune disease is already showing signs of clinical efficacy. A case report in 2021 repurposed approved CD19 CAR T cells to target B cells in a patient with systemic lupus erythematosus (SLE), a life-threatening autoimmune disease[33]. In 2022, results from five patients were published[34]. Remarkably, all patients showed expansion of the CAR T cells in vivo, rapid depletion of B cells, and a resolution of SLE symptoms and of markers of end-organ damage. All five patients discontinued their immunosuppressive drug regimen and were declared to be in drug-free remission. Surprisingly, short-term follow-up revealed that naive B cells re-emerge a few months following CAR T cell infusion without return of disease symptoms. The administration of CAR T cells was well tolerated, with only mild cases of CRS, consistent with the notion that a lower target cell burden may reduce the severity of CRS. Large trials and long-term follow-up will be necessary to elucidate whether the resolution of immunopathology is temporary or durable. These patients received lymphodepletion and it is difficult to disentangle whether this independently or coordinately with CAR T cells may have improved symptoms. With so few patients treated, it is still too early to tell whether chronic B cell aplasia may occur in some patients with SLE. The paradox raised by the rapid B cell recovery in SLE in contrast to the durable B cell aplasia in some patients with leukaemia after therapy with the same CAR T cells may be explained by understanding the kinetics and niches of CD19 CAR T cells in SLE as compared to those of patients with cancer[35].

CD19 CAR T cells have similarly been deployed in a patient with an inflammatory myopathy due to antisynthetase syndrome[36]. The patient presented with severe weakness and a computerized tomography (CT) scan revealed alveolitis and interstitial lung disease. The patient's T cells were collected, engineered and reinfused. A rapid expansion of CAR T cells was seen coinciding with a rapid depletion of B cells. Soon after CAR T cell infusion, the patient had increased myalgia and elevated serum creatinine kinase; however, this was followed by a sharp improvement in physical function and normalization of creatinine kinase and other clinical and laboratory measurements over the 180 days post CAR T cell infusion. Further imaging at 3 months post CAR T cell infusion revealed resolution of myositis in quadriceps and hamstrings and an amelioration of alveolitis. The patient did have mild CRS, which was managed and resolved in 3 days. It is possible that the acute increases in myalgia and serum creatinine kinase are a result of CRS; however, this needs to be explored in more detail. In this patient, once again B cells reappeared after a few months with no report of symptom recurrence. This is a single case and thus caution must be emphasized.

This clinical work was built on earlier preclinical reports using CD19 CAR T cell products to treat autoimmune diseases in which B cells are the source of pathology. CD19 CAR T cells in mouse models of SLE could both prevent and treat the disease[37,38]. Other engineered immune approaches include the use of a chimaeric autoantibody receptor. This strategy fuses an epitope, rather than a single-chain variable fragment, to intracellular signalling domains, to selectively eliminate pathologic B cells that recognize the given epitope. This has shown promise in mouse models of pemphigus vulgaris and myasthenia gravis[39,40]. A similar approach specifically targeted dysfunctional B cells in a mouse model of haemophilia[41]. These approaches allow for the selective depletion of only pathologic B cells rather than inducing B cell aplasia. Trials are underway to test the safety and efficacy of these approaches in humans (NCT04422912).

A recent report also implicated the potential of CAR T cells in the context of severe asthma[42]. In two separate animal models, CAR T cells targeted eosinophils and protected from asthma attack. This protection was durable, providing a potential advantage over antibody-based therapies for chronic allergic disorders with pathogenic eosinophils. Emerging evidence also suggests potential efficacy in type 1 diabetes, using CAR T cells that target the antigen-presenting cells that activate autoimmune T cells[43]. However, the resolution of diabetes was not durable.

CD19 CAR T cells used thus far target both harmful and healthy B cells. Using chimaeric autoantibody receptor T cells to specifically eliminate pathologic B cells is promising but requires knowledge of the

pathologic autoantibody. Other approaches that could be explored include the use of other B cell- and plasma-lineage antigens (that is, BCMA, GPRC5D, CD20 and CD22). These targets may be leveraged in the event of antigen negative relapse or in the case that both B cells and plasma cells need to be targeted. It is possible that pathologic B cells and plasma cells may have a specific targetable surface protein, although none has been identified so far. An alternative approach to eliminating inflammatory cells is to redirect regulatory T (T_{reg}) cells to a target tissue of interest. These cells are not cytotoxic but provide paracrine signals to dampen inflammation and autoimmune response[44,45]. Adoptive transfer of T_{reg} cells shows potential in mouse models of SLE, graft versus host disease (GvHD), allograft transplantation, type 1 diabetes and multiple sclerosis[3]. Engineering a CAR into T_{reg} cells allows for increased specificity and reduces the risk of system-wide immunosuppression. The feasibility and technical challenges of CAR T_{reg} cell engineering have been reviewed[45]. This approach has shown preclinical promise in mouse models of transplantation, rheumatoid arthritis, multiple sclerosis and metabolic disease[44,46].

With robust evidence in various preclinical models, and now early but strong clinical evidence in SLE, there is compelling rationale for CAR T therapy to be tested in other autoimmune diseases. Conditions that use a B cell-depleting approach at present could be strong candidates for a CAR T therapy, including antineutrophil cytoplasmic antibody-associated vasculitis, rheumatoid arthritis, myositis, multiple sclerosis, pemphigus vulgaris, immune thrombocytopenia, myasthenia gravis and neuromyelitis optica[47]. Owing to the deeper clearance of B cells, CD19 CAR T cells have proved effective in cancer contexts refractory to monoclonal antibodies; this raises the possibility that CD19 CAR T cells may prove effective in treating B cell-mediated autoimmunity, even if monoclonal antibodies have shown minimal efficacy. Autoimmune diseases seem to be one of the most promising areas for investigating the potential for CAR T therapy outside oncology. The financial cost associated with autoimmune diseases is another rationale to develop a one-time therapy, as 6 of the top 20 drugs by worldwide sales are for autoimmunity[48].

Cardiac disease. Cardiac fibrosis occurs with acute injury, chronic disease or ageing[49]. The stiffening of cardiac muscle leads to near-term morbidity and long-term mortality. Despite this serious pathology, few therapies approved at present directly target cardiac fibrosis. Recent preclinical work demonstrated that CAR T cells could target fibrosis and rescue cardiac function in mice following hypertensive injury[50]. Hearts from patients with cardiomyopathy were profiled for fibroblast-specific antigens, and fibroblast activation protein (FAP) was identified as the most upregulated surface-expressed target. Infusion of FAPCAR T cells, which had already been generated to target the tumour stroma in solid cancers, led to a resolution of fibrosis after heart injury, with improved measures of cardiac function[51–53]. No toxicities were observed. This work has been replicated with a transient CAR T cell approach (see discussion below) with similar therapeutic results[54].

FAP is a promising target antigen for cardiac fibrosis but may not be as highly expressed in other fibrotic diseases. Potential targets for other fibrotic diseases include LRRC15, a cell membrane protein that has been noted as a cancer-associated fibroblast marker[55]. Antibody–drug conjugates against LRRC15 have been shown to have anti-tumour effects in multiple contexts[55]. Recently a human atlas of perturbed fibroblasts revealed that $LRRC15^{+}$ fibroblasts are present in multiple pathologies[56]. Emerging single-cell datasets from a variety of diseased tissues identify numerous fibroblast cell states and these types of study may provide additional candidate antigens for targeting in fibrotic diseases.

Targeting pathologic fibrosis using CAR T cells has implications beyond cardiac disease. Deposition of extracellular matrix and fibrosis is pathologic in a wide range of maladies. Liver disease, chronic kidney disease, lung diseases, skeletal muscle diseases and many other contexts may be suitable for anti-fibrotic CAR T cells[49,57]. First-in-human clinical trials will require additional safety and toxicity preclinical studies and selection of specific targets for initial clinical investigation, but an RNA approach to make a transient CAR T cell (discussed below) may have a favourable safety profile. With a dearth of therapies targeting fibrosis directly, CAR T cells may provide a potent and selective way of treating such diseases, perhaps in combination with therapies or interventions that address underlying pro-fibrotic conditions associated with the specific disorder.

Senescence-associated diseases. A diversity of stressors can cause cells to undergo an irreversible proliferative arrest, termed cellular senescence. In this state, cells remain metabolically active, secreting a cadre of pro-inflammatory and proteolytic molecules termed the senescence-associated secretory phenotype (SASP)[58]. Genetic and pharmacological studies have demonstrated that removal of these cells, termed senolysis, is beneficial in various models of chronic disease. Small molecules that preferentially target intracellular pathways in senescent cells seem promising, but important questions regarding their potency, specificity and side-effects have been raised[58–60]. CAR T cells are an attractive alternative owing to their intrinsic specificity and potency. Senescent cells are cleared physiologically by the immune system early in life, and an approach to augment and extend this process with synthetic immune cells could have substantial benefit[61].

Recently it was shown that CAR T cells could target senescence in mouse models of cancer and liver disease[62]. An initial search was conducted for a senescence-specific antigen (that is, senoantigen), using RNA-sequencing analyses of three separate senescence contexts. *PLAUR*, which encodes for the urokinase-type plasminogen activator receptor (uPAR), was identified as a possible senoantigen with a relatively low level of expression in vital tissues. uPAR CAR T cells targeted senescent cells in vitro and eliminated senescent cells in mouse models of oncogene-induced senescence in the liver, oncotherapy-induced senescence in the lung, drug-induced liver fibrosis and a diet-induced model of non-alcoholic steatohepatitis. uPAR CAR T cells cleared senescent cells in the cancer models and decreased fibrosis and markers of liver damage in the liver disease models. Despite the initial analysis indicating limited expression of uPAR on vital tissues, high doses of uPAR CAR T cells induced CRS-like symptoms in mice, including a rise of serum cytokines, hypothermia, weight loss and death. Strategies to mitigate these toxicities need to be developed as uPAR CAR T cells are evaluated in other senescence-associated contexts.

Identifying senoantigens will be both difficult and important as senescent cells are notoriously heterogeneous[58,59]. A universal surface senoantigen is unlikely to exist. This may be an advantage, as the identification of antigens specific to subpopulations of senescent cells could allow for fine-tuning and enhanced specificity. The specificity of uPAR in other senescence-associated diseases should be evaluated. Other targets that could be leveraged to generate senolytic CAR T cells include the NKG2D ligands, MICA, MICB and ULBP1–5, which are expressed in a variety of senescent cells, although these ligands are also expressed elsewhere, and this approach presents challenges in both manufacturing and toxicity[63–65]. Another potential target, glycoprotein non-metastatic melanoma protein B (GPNMB), has been described as a senoantigen in endothelial cells[66]. An immunization strategy against GPNMB was used in mouse models. This cleared senescent cells, improved metabolic parameters, reduced atherosclerotic plaques and extended lifespan in male progeroid mice. GPNMB may thus be a suitable senescence target for CAR T therapy.

Proof-of-concept work demonstrates that senolytic CAR T cells may prove useful in many chronic diseases if toxicities can be avoided, minimized or managed. Senolytic drugs have shown preclinical potential in progeria, renal dysfunction, musculoskeletal degeneration, respiratory diseases, neurodegenerative diseases, cardiovascular disease and many other chronic diseases[59]. In addition, the accumulation of senescent cells is implicated in the pathophysiology of ageing[67]. Senolysis

has been shown to increase muscular strength, health and lifespan of mice, and it is possible that a senolytic CAR T cell could have similar effects[60,68]. The contribution of senescence to a wide range of pathologies suggests that senolytic CAR T cells may have wide applicability.

Other diseases. The potential to target a non-human antigen greatly reduces the risks of on-target toxicity in vital tissues. As discussed, the first clinical trials with CAR T cells were conducted in patients with HIV/AIDS[4]. The recent successes of this approach in cancer reignited interest in exploring CAR T therapy for this disease[69]. Allogeneic haemopoietic stem cell transplants have provided the first cures for HIV/AIDS, and it is conceivable that engineered autologous T cells could have the same effects without the risk of GvHD[70,71]. Several strategies have been undertaken to improve the CAR T cells that were originally given to patients. This includes exploring second-generation CAR T cells and improving persistence through genetic engineering[72,73]. Such strategies for other infectious diseases are also being explored. For example, a CAR was also developed using the fungal pattern recognition receptor to recognize a carbohydrate antigen in the cell wall of fungi[74]. This approach had antifungal properties in vitro and in mouse models. Similarly, a recent study tested the efficacy of CAR T cells against invasive pulmonary aspergillosis, a condition that frequently occurs in severely immunocompromised patients[75]. A CAR was designed against an antigen present in the cell wall of *Aspergillus fumigatus*, and AfCAR T cells showed antifungal properties in vitro and in a mouse model of invasive pulmonary aspergillosis. A concern relating to CAR T cells in infectious disease is bystander cytolysis of vital infected tissue. Another limitation in these contexts is that an acute infection demands an urgent response and the current ex vivo manufacturing of autologous CAR T cells may not be feasible. Similar preclinical strategies have been undertaken for hepatitis B virus, hepatitis C virus, cytomegalovirus and tuberculosis[30].

Acute GvHD can be a fatal side-effect of haemopoietic stem cell transplants. Engineered CAR T cells against OX40, a protein upregulated on pathogenic T cells in acute GvHD, protected mice from acute GvHD without sacrificing antiviral efficacy[76]. Simultaneous expression of this CAR with an anti-leukaemic CAR protected against acute GvHD while preventing leukaemia relapse. Adoptive cell transfer has been proposed for several other conditions including obesity, diabetes, atherosclerosis and osteoporosis[62,77]. These numerous early examples of CAR T therapy in disparate fields engender optimism that this therapy will reach beyond oncology.

Emerging technologies

CAR T therapy is rapidly evolving, incorporating emerging technologies. CRISPR editing was described shortly after the initial reports of CAR T therapy. The ability to precisely edit the genome improves effector function and persistence of synthetic T cells[14]. CRISPR-edited CAR T cells have already entered the clinic and are being evaluated for safety and feasibility at present[78,79]. More recent improvement in gene editing, such as base or prime editing, has also allowed for the generation of gene-edited CAR T cells without introducing double-strand breaks[80].

Synthetic cellular circuits offer tremendous potential for CAR T therapy. These engineering strategies can improve potency, reduce toxicity or regulate CAR expression[13]. In Boolean terms, synthetic AND circuits have been developed in which two separate receptors must bind their respective antigens to activate the CAR T cell. NOT circuits express a canonical second-generation CAR with a receptor bound to an inhibitory domain. If the NOT receptor binds to its antigen, the T cell will not activate. Additionally, the synthetic Notch system (IF–THEN) is a strategy to express a protein or secrete a cytokine following binding to an antigen of interest[81]. These strategies are still being fine-tuned but hold tremendous therapeutic potential for CAR T therapy at large.

CAR T therapies approved at present are limited to target surface-bound proteins and glycans. Recently it was shown that CAR T cells could target peptides presented by the major histocompatibility complex[82]. For example, a CAR T cell was designed to target a peptide of p16, a canonical marker of senescence, that is presented by HLA-B*35:01 (ref. 83). This CAR T cell was activated following binding of this peptide in a genetic system in vitro. Several considerations limit the broad application of this approach: most intracellular peptides are not presented by HLA, their low level of surface expression may not reach the targeting threshold of CAR T cells and HLA alleles are highly polymorphic. Nevertheless, this work provides a proof of concept that CAR T cells could target known disease-associated intracellular peptides. This greatly expands the pool of potential targets.

Engineered T cells have received the bulk of attention so far, but efforts to engineer other immune cells have shown promise. CARs expressed in natural killer cells, macrophages, dendritic cells, B cells and γδ T cells have shown preclinical potential[84,85]. Engineering these cells could potentially reduce the risk for GvHD and CRS compared to that of αβ T cells. However, these approaches are still in their infancy, and it is unclear what the tradeoffs may be including potential risk for CAR cell rejection, transformation and toxicity.

Targeted mRNA delivery. mRNA therapeutics recently demonstrated widespread efficacy with the FDA-approved vaccines against coronavirus disease 2019. Although the current FDA-approved therapies are untargeted, there are multiple clinical trials underway using targeted lipid nanoparticles (tLNPs) to specifically deliver mRNA to selected cells; this is achieved by conjugating a targeting antibody to the surface of LNPs. Preclinical reports show that LNPs can be directed to endothelial cells, the inflamed brain and, importantly, T cells[86].

Delivering modified CAR mRNA to T cells has been demonstrated by multiple groups. CAR mRNA was delivered to T cells through nanocarriers in mouse models of leukaemia and prostate cancer[87]. The mRNA-engineered CAR T cells performed similarly to retrovirally engineered CAR T cells by controlling the tumour and improving survival of affected individuals. Separately, it was shown that tLNPs could deliver FAPCAR mRNA to T cells in mice, resulting in CAR expression, reduced fibrosis and improved cardiac function in a model of heart failure, similar to the results of previous work with retrovirally transduced FAPCAR T cells[54]. Thus, a single injection of encapsulated RNA can produce functional and therapeutic levels of CAR T cells in vivo.

tLNP-mediated delivery of CAR mRNA offers distinct advantages over methods used at present in the clinic. At present, CAR T therapy requires a costly ex vivo manufacturing process (Fig. 4). Generating CAR T cells in a patient would reduce manufacturing costs and allow for rapid scalability as is evidenced by the global deployment of the vaccine against coronavirus disease 2019. In vivo-generated CAR T cells may also improve safety. Current therapy requires lymphodepletion before CAR T cell infusion to optimize engraftment. This process increases the risks of CRS, due to acute CAR T cell expansion, and secondary malignancies, due to genotoxicity. These risks may be acceptable in contexts in which the short-term mortality rate is high such as cancer, but are unlikely to be acceptable in non-malignant diseases with long periods of morbidity but not imminent death. In vivo generation may obviate the need for conditioning protocols, improving long-term safety.

Another potential advantage of an mRNA approach is the transient nature of CAR expression by T cells, which is lost following dissipation of the delivered mRNA (Fig. 4). Current CAR T cells express CARs permanently and can persist for decades in some patients[15]. Persistence is an attractive feature in the context of cancer but may be a liability in diseases for which persistent clearance is undesired or unnecessary. For example, one-time removal of a fraction of cardiac fibrosis may suffice to improve cardiac contractility, whereas long-term inhibition of fibrosis may inhibit other important processes, such as wound repair, for which fibrosis is required. Further profiling of targeted mRNA CAR T cells will be necessary to evaluate kinetics and safety in vivo.

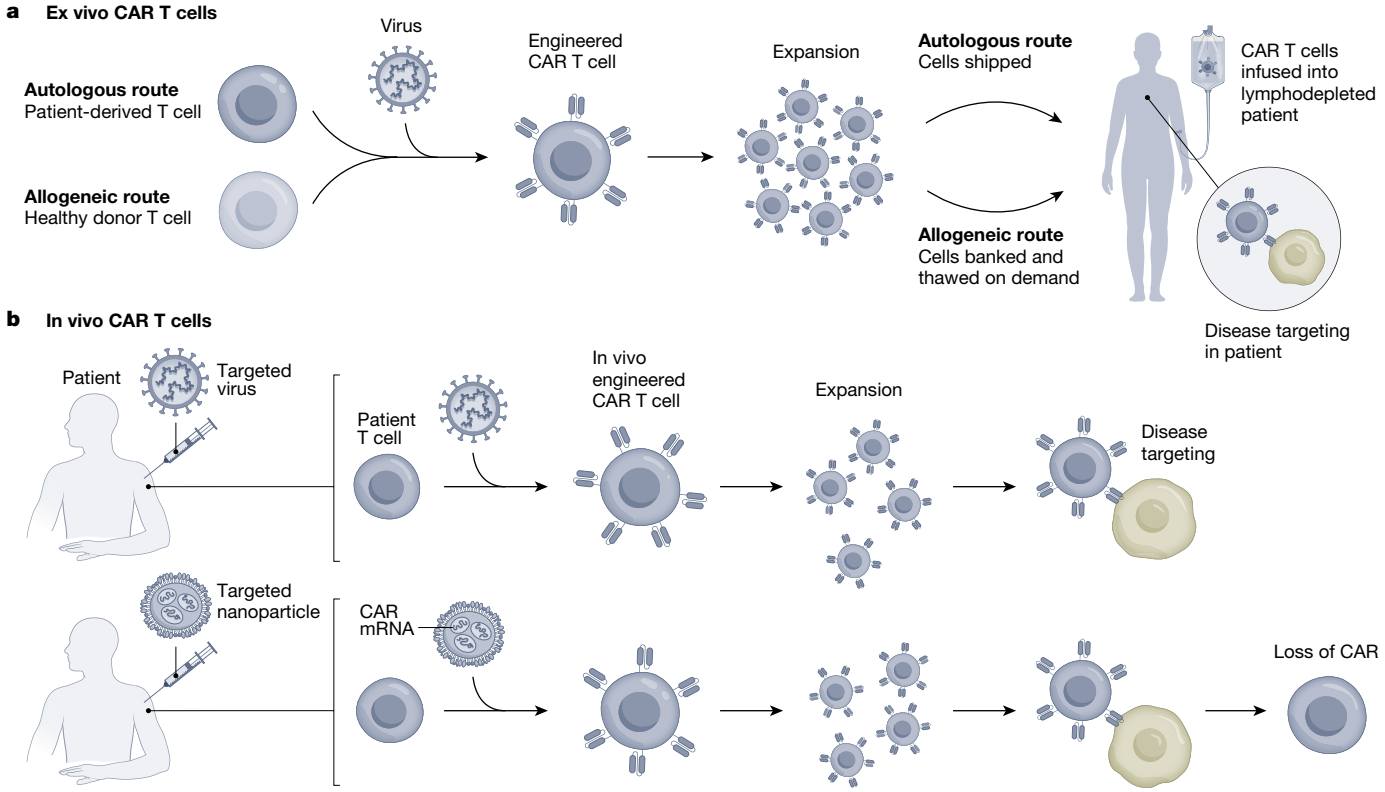

Fig. 4 | A comparison of ex vivo and in vivo platforms being explored for CAR T therapy. a, At present, autologous CAR T cell manufacturing requires extensive infrastructure, time and lymphodepletion of the patient. Allogeneic CAR T cell manufacturing can potentially reduce the associated costs and time required, but still requires lymphodepletion of the patient. **b**, In vivo platforms, such as targeted viral delivery and tLNP delivery, are much less mature, but have tremendous potential to disrupt this industry by potentially obviating the need for manufacturing facilities and lymphodepletion. tLNP delivery has the additional feature of only transiently expressing the CAR.

The long-term effects of T cells that were once CAR T cells should be profiled. Whether T cells have a memory of once being CAR T cells and whether there are functional consequences of this prior activity should be determined. Ways to gain temporal control of CAR T cells have been reviewed[13]. tLNPs delivering mRNA in vivo is an orthogonal way to regulate CAR T cells. The use of transient CAR T cells may also prove to be an effective way to evaluate safety of new CAR products, allowing for titration of both dose and duration of CAR T activity. Although certain cancer contexts may necessitate a virally transduced CAR T cell product, transient CAR T cells could enable widespread adoption of this modality for non-malignant indications.

Alternative platforms. Targeted in vivo viral transduction strategies are a different way to bypass the extensive and expensive CAR T cell manufacturing process (Fig. 4). Rather than transducing T cells ex vivo, a targeted virus delivers the CAR gene specifically to T cells in vivo. This approach produced functional human CAR T cells in humanized mice, using engineered adeno-associated virus or targeted lentivirus[88–91]. This approach may, like the tLNP approach, obviate the need for the current lymphodepletion protocols. Viral delivery in vivo has the potential to improve accessibility and cost in comparison with ex vivo autologous CAR T therapy; however, viral vector-mediated gene therapy approaches will probably remain more expensive than mRNA delivery. Concerns regarding this modality include the risk of off-target transduction, with germline transmission being the most serious concern. A potential immunogenic response to the viral vector may negate the possibility of redosing patients. In contrast to mRNA delivery, in vivo viral transduction probably will generate CAR T cells that persist. Engineered viral-like particles have the potential to deliver gene-editing proteins and overcome many limitations of in vivo viral transduction strategies[92]. This may prove attractive for conditions that require persistent CAR T cells but increases the likelihood of potential unwanted targeting.

Other approaches being explored include banking donor or stem cell-derived T cells for off-the-shelf CAR T therapy[2,3,93,94] (Fig. 4). This platform still relies on ex vivo transduction, but rather than collecting patient cells, cells can be obtained from healthy donors or from induced pluripotent stem cells. Using allogeneic CAR T cells in cancer has gained substantial traction owing to its potential to lower costs, simplify manufacturing and expand treatment to patients with low-quality T cells. Allogeneic approaches have already reached the clinic[95]. Despite the potential benefits of allogeneic CAR T cells, the process is more complex, requiring genetic alterations of allogeneic T cells to prevent GvHD and rejection of allogeneic CAR T cells[93]. It is likely that patients may still need to be lymphodepleted to see robust engraftment of allogeneic CAR T cells[95]. In urgent contexts, having banked CAR T cells may be a substantial advantage. Although clinical deployment of allogeneic CAR T cells is still in the early stages, at present these cells have a reduced duration of persistence in comparison with that of autologous CAR T cells. This is probably due to rejection by the host; however, these T cells may also have cell-intrinsic defects due to the required genetic modifications. This reduced persistence may be attractive for conditions for which persistent targeting is undesired. The transformation of allogeneic CAR T cells has been observed and is a major safety concern[96].

The promise of translation

A major reason for the interest surrounding CAR T therapy is its clinical success in leukaemia, lymphoma and myeloma. Most of the

work outside the field of cancer is in the early stages of development. As foundational work is carried out, it is paramount to incorporate clinical considerations up front.

Finding an antigen

A key bottleneck to designing CAR T therapy is identifying a targetable antigen. Current approaches include examining transcriptional and other omic datasets and histological analysis of target tissue. A crucial consideration as targets are chosen is the level of antigen expression on normal tissues. This may become less restrictive in the context of transient CAR T cells and with the incorporation of synthetic circuits. Methods to identify antigens in a disease of interest in an unbiased and high-throughput manner would have a major impact on the clinical pipeline.

Safety considerations

Cytokine release syndrome. CRS is one of the most problematic clinical toxicities of CAR T therapy. Careful efforts should be made preclinically to examine whether CRS-like symptoms manifest in new disease applications. Early clinical indications from CAR T therapy in SLE suggest that CRS may be milder in noncancer settings, in which the target cell burden is relatively low, but it will be important to evaluate whether this is true in larger SLE trials and for other autoimmune diseases. The preclinical CAR T investigations in cardiac failure reported no increase in serum cytokines, changes in body weight or delays in wound healing in treated mice[50,54], but higher levels of fibrotic clearance in humans may produce CRS-like symptoms. The toxicities seen with high doses of the uPAR CAR T cells should also be studied closely. These symptoms may not be CRS but due to on-target toxicity of tissues that express uPAR. The SASP-associated inflammatory state of senescent cells may exacerbate CRS-like symptoms, although the prevalence of senescent cells in diseased tissue is low.

Clearing physiologic cells. Eliminating subsets of healthy cells, such as B cells in SLE, is therapeutic. However, the long-term effects of clearing these cells are unknown. The preliminary results in SLE suggest that this clearance may be only temporary. As these trials expand, we will learn whether there are long-term complications. Although chronic fibrosis is pathologic, it remains to be seen whether contexts exist for which elimination of activated fibroblasts yields negative effects. Senescent cells play an important physiologic role in development, wound healing and regeneration[97]. The impact of senolytic CAR T cells on physiologic processes should be evaluated, and antigens should be preferentially selected to target pathologic senescent cells. Transient CAR T cells are particularly attractive as this would allow for a greater ability to control these undesired effects.

Key questions

Many questions remain unexplored in the noncancer CAR T cell space, and it will be critical to answer these in the coming years. The therapeutic index of CAR T cells remains an important area of study, as infusion dose is an important approach to manage safety concerns, particularly with mRNA CAR T cells. The potential tradeoffs of a transient approach in the context of acute and chronic disease should also be carefully inspected. The optimal time to dose patients for these diseases is unknown and is likely to be variable depending on the pathogenesis of each disease. Whether there is a point of no return at which treatment may no longer be helpful or may even be harmful should be established. For tissues that have regenerative capacity, it should be determined whether elimination of pathologic cells reinvigorates healthy tissue. Where CAR T cells traffic in noncancerous tissues should also be explored. It is possible that CAR T cells delivered locally may result in superior outcomes and reduce potential toxicities. Elucidating CAR intrinsic and extrinsic variables will be vital as the field develops.

A path forward

Concurrently with the ongoing innovation to develop new platforms for CAR T cells, there is substantial effort already underway to optimize the process of ex vivo CAR T cell production. Important considerations include shortening the time for expansion, reducing ex vivo exhaustion, regulating CAR T cell metabolism ex vivo and ensuring that expanded CAR T cells retain stem-like properties[98,99]. As this is a living drug, the manufacturing process is critical to its efficacy in patients.

For conditions described above, and many more not considered, it is possible that CAR T therapy may have applications. As identifying an antigen and constructing a CAR is complex and resource intensive, it may be useful to first use a genetic strategy in a model organism, such as the OVA/OT-1 system in mice, to provide proof of concept for functional benefit in new settings[50]. Following therapeutic validation, a search can be conducted for a target-specific antigen. A CAR T cell should be engineered with substantial consideration for both the extracellular and intracellular signalling domains as both play an important role in efficacy[100]. These cell products should be tested in small- and large-animal models for safety and efficacy as required by the FDA[101]. Once sufficient evidence is accumulated for a clinical trial, phase 0 or phase I trials can establish safety, a path forged by CAR T cells in cancer, and similarly deployed in the recent reports on autoimmune diseases[33,34,36]. Phase 0 trials testing small doses of CAR T cells injected into diseased tissues of interest may be a powerful approach to determine whether the CAR T cells have the desired pharmacologic effects[102].

Much will be learned in this decade as more CAR T cell products are used to treat solid cancers and as long-term follow-up data become abundant. In the same way that development of CAR T cells for blood cancers paved the way for application in solid tumours, advances in oncology will provide critical insight to guide translation beyond cancer. The intense research in the field to enhance CAR T cells for cancer has obvious interdisciplinary applications. Additionally, much of the innovation in development, engineering and clinical deployment for CAR T cells in cancer will have direct relevance to a wide diversity of non-malignant conditions.

Conclusion

Although CAR T therapy was initially developed for HIV, it has gained traction owing to its application to haematologic malignancies. CAR T therapy is now poised to treat a much broader spectrum of conditions. Emerging technologies in CAR design and delivery amplify this potential. The theoretical applications are vast, and the platform is powerful. CAR T therapy shows promise to treat autoimmunity, chronic infections, fibrosis and senescence, and may promote healthy ageing. We are only beginning to realize the full potential of this living drug.

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Acknowledgements This work was supported by 1P01CA214278 and R01CA226983 (C.H.J.), the Parker Institute for Cancer Immunotherapy and the Centurion Foundation Innovation Fund.

Author contributions Conceptualization: D.J.B., Z.A., J.A.B., J.A.E., C.H.J. Visualization: D.J.B., Z.A., J.A.B., J.A.E., C.H.J. Writing: D.J.B., Z.A., J.A.B., J.A.E., C.H.J.

Competing interests D.J.B. and Z.A. declare no competing interests. J.A.B. is a consultant to Pfizer and Cytokinetics. J.A.E. is a scientific founder and holds equity in Capstan Therapeutics, which develops therapeutics to reprogram immune cells in vivo. C.H.J. is an inventor on patents and/or patent applications licensed to Novartis Institutes of Biomedical Research and receives licence revenue from such licences. C.H.J. is a scientific founder of Tmunity Therapeutics and Capstan Therapeutics. C.H.J. is a member of the scientific advisory boards of AC Immune, Alaunos, BluesphereBio, Cabaletta, Carisma, Cartography, Cellares, Celldex, Danaher, Decheng, Kite Gilead, Poseida, Verismo, Viracta and WIRB-Copernicus.

Additional information

Correspondence and requests for materials should be addressed to Daniel J. Baker or Carl H. June.

Peer review information *Nature* thanks Kristen Hege and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.