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Review

Improving CAR T-cells: The next generation ☆

Andrew H. Marple [a], Challice L. Bonifant [b,*], Nirali N. Shah [c,*]

[a] Department of Medical Oncology, Johns Hopkins Hospital, Baltimore, MD
[b] Department of Pediatric Oncology, Johns Hopkins Hospital, Baltimore, MD
[c] Pediatric Oncology Branch, Center for Cancer Research (CCR), National Cancer Institute (NCI), NIH, Bethesda, MD

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ABSTRACT

The introduction of chimeric antigen receptor (CAR) T-cell therapy in acute lymphoblastic leukemia (ALL) has dramatically altered the landscape of treatment options available to children and adults with ALL. With complete remission induction rates exceeding 70% in most trials and FDA approval of one CD19 CAR T-cell construct in ALL, CAR T-cell therapy has become a mainstay in the ALL treatment algorithm for those with relapsed/refractory disease. Despite the high remission induction rate, with growing experience using CAR T-cell therapy in ALL, a host of barriers to maintaining long-term durable remissions have been identified. Specifically, relapse after, resistance to, or loss of long-term CAR T-cell persistence may all hinder CAR T-cell efficacy. In this review, we provide an overview of the current limitations which inform the design of the next generation of CAR T-cells and discuss advances in CAR T-cell engineering aimed to improve upon outcomes with CAR T-cell-based therapy in ALL.

Introduction

The introduction of chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of relapsed and refractory CD19 B-cell malignancies. With complete remission induction rates exceeding 70% in most trials [1-3], and FDA approval of one CD19 CAR T-cell construct in acute lymphoblastic leukemia (ALL) [4], CAR T-cell therapy has become a mainstay in the ALL treatment algorithm, particularly for children and young adults with relapsed/refractory disease.

With growing experience, however, a number of limitations to CAR T-cell therapy have emerged. Broadly divided into inherent leukemic factors vs intrinsic characteristics of the CAR T-cell product; relapse after, resistance to and failure of CAR T-cells collectively account for some of the greatest mechanisms limiting long-term durable remission. Equally important to efficacy outcomes, improving upon the toxicity profile of CAR T-cells remains a desired goal. Herein, we discuss recent advances in CAR T-cell engineering designed to improve upon the current outcomes with CAR T-cell-based therapy in ALL.

Overcoming intrinsic leukemia-associated factors limiting CAR T-cell efficacy

Limitation: Antigen negative escape

Approximately 35% to 45% of patients with ALL who achieve a response to treatment with CD19-CAR T-cell therapies eventually relapse [1,5]. Among those with relapse, 33% to 65% will relapse with CD19 negative disease [6,7]. Leukemic loss of CD19 expression has been shown to occur through a number of mechanisms including genetic disruption of CD19 membrane anchoring with acquired loss of heterozygosity at *CD19* [8], alternative splicing of *CD19* mRNA [9], lineage switch [10,11], antigen masking [12], and expansion of pre-existing minor CD19-negative subpopulations [13]. Given the frequency of CD19-negative relapses, targeting a single antigen in ALL is unlikely to provide curative therapy in a large proportion of treated patients. Indeed, in experiences with single antigen targeting of CD22, antigen escape with CD22 neg/dim disease was the primary reason precluding durable response [14].

Dual-targeting CAR T-cells

With the hypothesis that simultaneous targeting of more than one antigen on tumor cells will limit antigen-negative escape, a

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* Corresponding authors. Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. Tel.: (240) 760-6199

E-mail address: Nirali.Shah@nih.gov (N.N. Shah).

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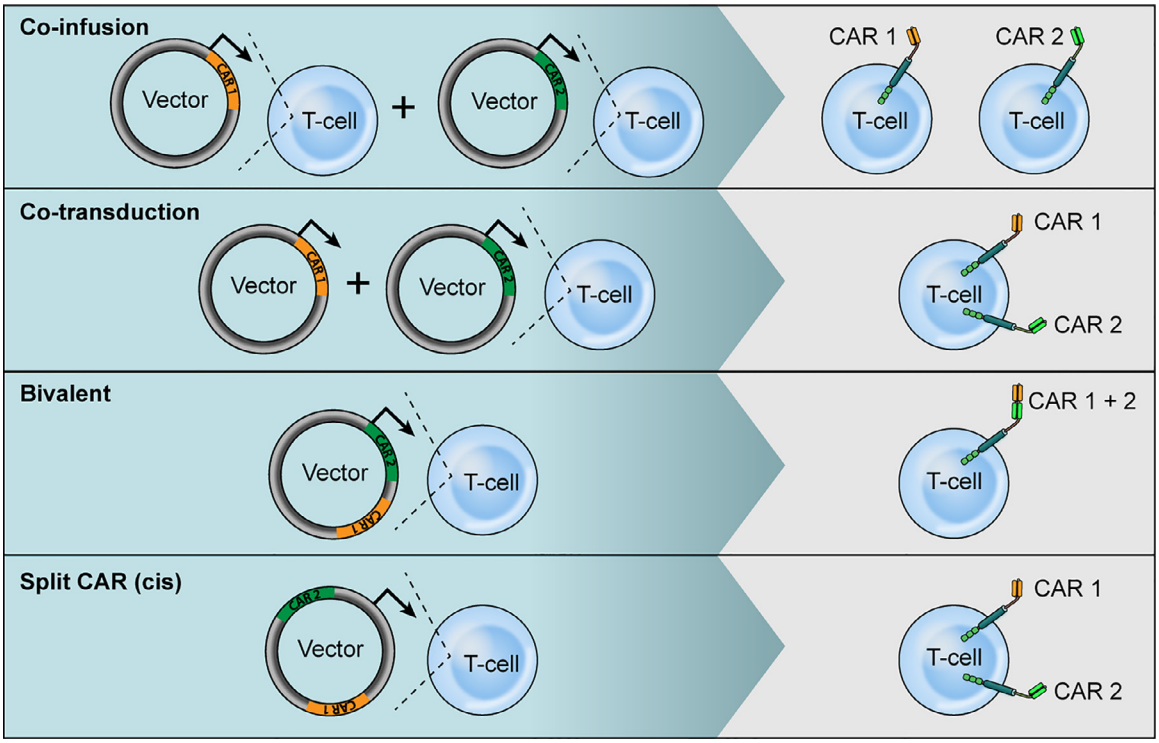

Fig 1. Model of dual-antigen targeting strategies.
Top panel: Co-infusion model.
Second panel: Co-transduction model.
Third panel: Tandem CAR T-cell model.
Fourth panel: Split (Cis) CAR T-cell model.

number of novel dual-targeting approaches have been developed. Beyond co-infusion strategies, CAR T-cell constructs incorporating co-transduction, tandem (bivalent) models or bicistronic (split CARs) [15] targeting CD19 and CD22 seek to improve upon remission durability (Fig. 1) Antigen targeting strategies beyond 2 antigens are also in active development [16]. The earliest clinical experiences with these various strategies have demonstrated efficacy rates comparable to each of these approaches, though long-term follow-up is needed to evaluate for both dual functionality and the ability to reduce antigen-negative relapses [17-19].

Dual-antigen targeting has also been achieved via engineered expression of synthetic notch (synNotch) receptors, which induce transcriptional activation following target binding [20]. SynNotch receptors are composed of the core regulatory domain of the cell-cell signaling receptor Notch, coupled to a synthetic extracellular recognition domain and synthetic intracellular transcriptional domains. Engagement of these receptors by their antigen target leads to cleavage-induced release of an intracellular transcription factor that can then be engineered to stimulate expression of a second CAR. Thus, synNotch receptors can be employed to target 2 antigens, one via the SynNotch and a second via a traditional CAR. T-cells expressing SynNotch receptors selectively target dual-antigen expressing cells in preclinical models [21]. This approach may ultimately prove to be a useful strategy to also limit on-target, off-tumor toxicity, given the expected increased specificity of SynNotch vs conventional CAR T-cells.

Limitation: Primary resistance

There remains a sizable proportion of patients who do not achieve remission after CD19 CAR T-cell treatment. In long-term follow up, approximately 50% of patients with diffuse large B-cell lymphoma, and at least 1 in 5 patients with CD19+ ALL remain refractory to treatment with CD19-targeted CAR T-cell therapies [1,5,22]. In clinical trials, approximately 1 out of every 5 patients with CD19+ ALL does not achieve remission with CD19-directed CAR T-cell therapy [1,5,7]. Although lack of clinical response is primarily attributed to intrinsic T-cell intrinsic defects which CAR T-cell expansion and response [23,24], ALL intrinsic mechanisms also exist.

Targeting low antigen disease

Low levels of baseline leukemic surface target antigen expression (eg, low site density) have emerged as an important factor for CAR T-cell efficacy [25]. Specifically, low antigen density may promote limited CAR T-cell expansion leading to suboptimal responses [26], as seen in a phase I trial of CD22 CAR T-cells [14]. Attributes of the CAR T-cell construct, including higher affinity binding [26], selection of the co-stimulatory domain [27], and/or incorporation or deletion of immunoreceptor tyrosine-based activation motifs (ITAMs) may modulate the ability for a particular construct in its ability to target lower antigen density disease [27], and remain under active investigation.

ALL-mediated CAR T-cell dysfunction

An additional ALL-intrinsic mechanism of resistance to CAR T-cell therapy was recently identified using a CRISPR-based genome-wide loss-of-function screen in NALM6 ALL cells [28]. Under selective CAR T-cell pressure, impaired death receptor signaling was found in CAR T-cell-resistant leukemic cells. Their screen identified depletion of genes involved in activating the cell death pathway (eg, FADD, BID, and CASP8) and enrichment of genes important for resisting death (eg, CFLAR, TRAF2, BIRC2). Persistence of ALL led to progressive impairment of CAR T-cell function in vitro, as T-cells were unable to kill resistant leukemia cells. Together, this suggests that death receptor signaling may be a key regulator of primary

CAR T-cell resistance, a hypothesis that was further validated by clinical correlation. Reduced expression of death receptor genes in primary leukemia samples prior to CAR T-cell therapy was associated with worse overall survival and reduced T-cell fitness [28]. These data support a novel model of tumor-intrinsic biological resistance that complements previously observed CAR T-cell exhaustion. Engineering of CAR constructs that utilize killing mechanisms independent of the death receptor pathway could prevent this type of resistance and the subsequent leukemia-induced T-cell exhaustion, though alternate resistance mechanisms may then evolve.

Optimizing CAR T-cell design to promote functional CAR T-cell persistence

In addition to CD19 negative relapse, CD19+ relapses following CAR T-cell infusion are thought to occur primarily due to poor T-cell function or loss of CAR T-cell persistence which can no longer effectively provide ongoing immunosurveillance. Thus, designing CAR T-cell constructs which can maintain long-term functional persistence without undergoing rapid exhaustion has the potential to improve upon remission durability.

Limitation: CAR T-cell exhaustion

Study of dysfunctional CAR T-cells in the tumor microenvironment has provided evidence that T-cells exposed to continuous antigen-specific stimulation display an exhausted phenotype [23]. The unique structural design of individual CARs can also precipitate functional exhaustion. Antigen-independent signaling, or clustering of CAR soluble chain variable fragments (scFVs) in the absence of antigen, can result in tonic CAR-CD3ζ phosphorylation [29]. This constitutive signaling can lead to early CAR T-cell exhaustion, which ultimately limits antitumor efficacy. Exhausted CAR T-cells are likely to expand less efficiently ex vivo show higher rates of apoptosis and have higher expression of PD-1, TIM-3, and LAG-3 [30].

Biologic underpinnings of CAR T-cell exhaustion

Several seminal reports have further elucidated the underlying transcriptional mechanism of induced CAR T-cell exhaustion. Chen et al. employed cancer cell-specific CAR T-cells in 2 mouse models of solid tumors, noting that exhausted CD8+CAR+ tumor-infiltrating lymphocytes expressed higher levels of the NR4A family members (NR4A1, NR4A2, and NR4A3). N4RA expression correlated with increased PD-1 and TIM3 expression, and generation of NR4A triple knockout cells produced higher levels of effector cytokines and expressed fewer inhibitory receptors [31]. At the same time, Liu et al. conducted a genome-wide transcriptomic and epigenetic screen of in vitro-generated tolerant CD4 T-cells, identifying NR4A1 as a mediator of T-cell exhaustion. Overexpression of NR4A1 suppressed effector cytokine production (IL-2, IFNγ), whereas ablation enhanced T-cell production of effector cytokines and increased their proliferative capacity.

Mechanistically, NR4A1 was preferentially recruited to binding sites of the transcription factor AP-1, thereby repressing effector gene expression through inhibition of the AP-1 mediated transcriptional program [32]. Accordingly, CAR T-cells engineered to overexpress the canonical AP-1 factor c-Jun, exhibited the opposite phenotype. Overexpression of c-Jun rendered CAR T-cells with enhanced expansion potential, functional capacity, diminished terminal differentiation phenotype, and improved antitumor responses [33]. Together, these data warrant further clinical investigations making use of either NR4A depleted, or c-Jun overexpressed CAR T-cells to overcome T-cell exhaustion.

Incorporating checkpoint inhibition

Early clinical evidence supports checkpoint inhibition as a therapeutic strategy to enhance the efficacy and persistence of infused CAR T-cells. A small cohort of children with relapsed B-ALL, in whom treatment with CD19 CAR T-cells demonstrated only partial/no response to or poor CAR T-cell persistence, were treated with the PD-1 inhibitor pembrolizumab [34]. Treatment resulted in prolonged detection of circulating CAR T-cells in all 4 patients, in addition to objective responses in 2 of the 4 patients. Interestingly, 1 patient with widespread extramedullary disease despite marrow remission developed a second peak of CAR T-cell expansion following pembrolizumab treatment, resulting in a reported dramatic reduction in PET-avid disease [34]. These data support the notion that checkpoint pathway targeting may have clinical efficacy in patients without an initial response to CAR T-cell infusion.

Limitation: CAR T-cell persistence

Clinical experience with CAR T-cell therapies have further informed our understanding that persistence of the infused CAR T-cell product correlates with durable antitumor efficacy [35-37]. Indeed, monitoring for persistence of B-cell aplasia as a proxy for monitoring of ongoing functional CAR T-cell activity has been adopted as one approach for surveillance of emerging relapse [38].

CAR T-cell vector integration

Case reports of clonal expansion of CAR T-cells mediating antitumor effect have illustrated how the progeny of a *single* CAR T-cell can be sufficient to mediate a potent antitumor response [39,40]. As an example, a recent case report of a patient with delayed CAR T-cell expansion emerging from a single clone has provided mechanistic insight validating the importance of the CAR T-cell differentiation phenotype to clinical outcomes [39]. Further analysis of this clone revealed vector-mediated insertion of the CAR transgene into the *TET2* gene locus. A germline hypomorphic mutation affected his second *TET2* allele, and experimental knockdown of *TET2* in healthy donor CTL019 cells, conferred the ability for these cells to undergo repetitive expansion in response to serial re-stimulation [39]. Another example of disruptive vector integration accounting for an enhanced CAR T-cell response resulted from lentivector integration in the CBL gene [40]. Recent investigations into clonal kinetics of CD19-targeted CAR T-cells have similarly revealed novel insights into the role of vector integration in CAR toxicity and proliferation [41,42]. Future efforts in CAR T-cell design may be able to capitalize on these discoveries to improve upon the persistence of CAR T-cell therapy.

Implications of current CAR T-cell constructs and costimulatory domains

There is evidence that the composition of a CAR can affect the differentiation state of the product. The binding affinity of the scFv, choice of costimulatory signal, and degree of constitutive signaling through the CD3ζ domain have a multifactorial impact on the differentiation state of the infused product. The first generation of CAR T-cells consisted of a scFv coupled to the ζ-chain of the CD3 complex, providing only signal 1 of T-cell priming. Not unexpectedly, this resulted in poor production of IFN-γ and anergic T-cells [43]. Addition of a co-stimulatory domain (signal 2), prevented CAR T-cell anergy and exhaustion, promoting clonal expansion and differentiation. The most commonly incorporated co-stimulatory domains in CAR T-cell products are CD28 [44] and 4-1BB [45]. The use of both of these has resulted in remarkable clinical responses. However, these domains exhibit different tumor

elimination kinetics with CD28 CARs conferring a brisk proliferative response and faster tumor reduction, and 4-1BB CARs greater persistence [46]. Phenotypically, T-cells from 4-1BB expressing constructs retain a central memory-like phenotype and increased oxidative metabolism. 4-1BB containing CAR T-cells demonstrate increased effector memory formation and glycolytic metabolism [47]. A comprehensive comparison of combinations of intracellular domains revealed that the addition of a 4-1BB signal in *trans* to the 1928z second-generation CAR potentiated in vivo tumor control. Combined CD28 and 4-1BB signaling in *trans* reduced expression of PD-1, LAG-3, and TIM-3 and exhibited *IRF7*-dependent activation of the T-cell IFN-I pathway [48].

Finally, the strength of the stimulus delivered by the CD3ζ domain has the potential to affect the differentiation state of the CAR T-cell product. T-cell signal strength can induce a state of terminal differentiation [49]. With this knowledge of endogenous TCR behavior, Feucht et al. hypothesized that CAR signal strength was similarly impactful. To test this hypothesis, they modified the CD3ζ ITAMS in a series of CARs and assessed the function, differentiation, and therapeutic potency of these constructs. Deletion of the 2 C-terminal ITAM motifs augmented T-cell potency and induced a long-lived memory T-cell phenotype with reduced expression of exhaustion markers [50]. Similarly, a novel CD19 CAR expressing a scFv with >40-fold lower affinity to CD19 with a much faster off-rate, showed enhanced proliferative and in vivo antitumor activity. A clinical trial utilizing this construct achieved molecular remission in 12/14 patients with ALL, demonstrating enhanced CAR T-cell expansion and persistence in 11/14 patients at 2-year follow-up [51]. These data foster the recurring notion that the strength of the signal imparted on the CAR T-cell affects the differentiation state of the infused product, which in turn may improve its antitumor efficacy. This has been suggested previously in preclinical models, where T-cell subsets with self-renewing capacity demonstrate improved antitumor control [52,53]. In summary, a number of structural components of a CAR T-cell appear to impact the differentiation state of the final product. The summation of these signals may play a more important role than initially realized, and are an important area of future investigation.

Impact of manufacturing strategies on CAR T-cell functionality

CAR T-cell therapy differs from anticancer cytotoxic chemotherapy, targeted drugs, antibodies, and antibody-based conjugates in that CAR T-cells are "living drugs," engineered from a patient's own T-cells. The infused product is not uniform, but rather is comprised of a heterogeneous conglomeration of T-cells, often of varying CD4/8 ratios, with variability in patient-derived T-cell subsets as well as in those induced by unique CAR constructs. A host of manipulations in the CAR T-cell manufacturing process also impacts the CAR T-cell performance.

Additionally, one of the major limitations to manufacture CAR T-cells from patients with leukemia is the need for collection of T-cells that will expand and maintain functionality. This need is challenged by patient receipt of prior therapy and/or progressive disease impacting T-cell fitness. This personalized approach is also highly expensive and may be cost-prohibitive [54,55]. In this section, we review strategies which are being employed to further optimize CAR T-cell manufacturing and will also discuss the impact of CAR T-cell construct modification on product characteristics.

CAR T-cell differentiation

The quality and differentiation profile of T-cells collected for manufacture impact outcomes, as T-cell populations enriched for naïve and early memory T-cells have demonstrated greater ability to expand during manufacturing [56]. Recent data suggest that adoptive transfer of less differentiated naïve and central memory T-cells confer enhanced T-cell expansion, persistence, and antitumor efficacy as compared to more differentiated effector memory and effector T-cell populations [57]. Notable to the case with *TET2*-disrupted CAR T-cells, these cells displayed an epigenetic profile consistent with a central memory phenotype which may have further contributed to the efficacy of this delayed expansion [39]. Based upon this knowledge, generation of CD19+ CAR-modified CD8+ memory stem cells represent yet another approach in CAR T-cell design. Whether this modification improves upon CAR T-cell persistence is being tested in the clinic [58]. Manufacturing techniques, including choice of exogenous cytokine, affect the final product composition. For instance, as opposed to expansion with interleukin-2 (IL-2), culture of activated T-cells with IL-7 and IL-15 enriches for naïve and central memory-like T-cells in the pre-infusion product [59]. This becomes of importance, as cumulative cycles of chemotherapy have been shown to deplete naïve T-cells, reducing the expansion potential associated with successful cellular therapies [60].

Modifications to the manufacturing platform

Although not strictly related to CAR T-cell design, considering the apheresis product and standardizing the CD4/CD8 ratio may also impact outcomes [61]. Specifically combining pre-established CD4/CD8 ratios for CAR T-cells allowed for synergistic antitumor effects in vivo and provided a more uniform potency than in products where the CD4/CD8 ratios were purely based on intrinsic patient factors [61]. Similarly, manipulation of the apheresis product has been another strategy to improve feasibility of CAR T-cell manufacturing [62]. One recent example emerged from a minor manufacturing change which incorporated CD4/CD8 selection of the apheresis product without any other downstream changes to the manufacturing on the CD22 CAR T-cell trial. While this optimized the ability to manufacture CAR T-cells in subjects whose apheresis product was heavily involved with leukemic blasts and/or immunosuppressive NK or myeloid derived cells, it also augmented the potency and toxicity, leading to a dose de-escalation. This provided a clear illustration that minor manufacturing changes can have significant clinical impact [63].

Recent advances in closed and distributive manufacturing models may decrease per-patient costs, ultimately improving feasibility and access to CAR T-cell manufacturing [64]. Product characteristics will need to be closely studied as these new platforms evolve, particularly in the context of new constructs.

Universal CAR and alternative CAR T-cell constructs

In addition to the inherent limitations of manufacturing autologous CAR T-cells from subjects with leukemia, a major obstacle to expanded use of CAR T-cell therapy is the expense associated with personalized manufacture. Creation of an "off-the-shelf" or "universal" CAR product may not only reduce the cost of treatment, but also could expand the number of patients eligible for CAR T-cell therapy. One hurdle limiting CAR T-cell universality is the risk of graft-vs-host disease (GVHD) following allogeneic T-cell transfer. One potential strategy to overcome this obstacle involves targeted integration of the CAR transgene to the TRAC locus using CRISPR/Cas9 mediated homologous recombination [65]. Targeted insertion prevented endogenous TCR expression and therefore prevented GVHD. This technique also resulted in enhanced CAR T-cell potency, potentially by averting tonic CAR signaling and subsequently also delaying effector T-cell differentiation and exhaustion. CRISPR/Cas9 edited CAR T-cells have recently been proven safe in a first-in-human phase I clinical trial. Multiplex CRISPR/Cas9 was utilized to target *TRAC*, *TRBC* and *PDCD1* on T-cells, followed by

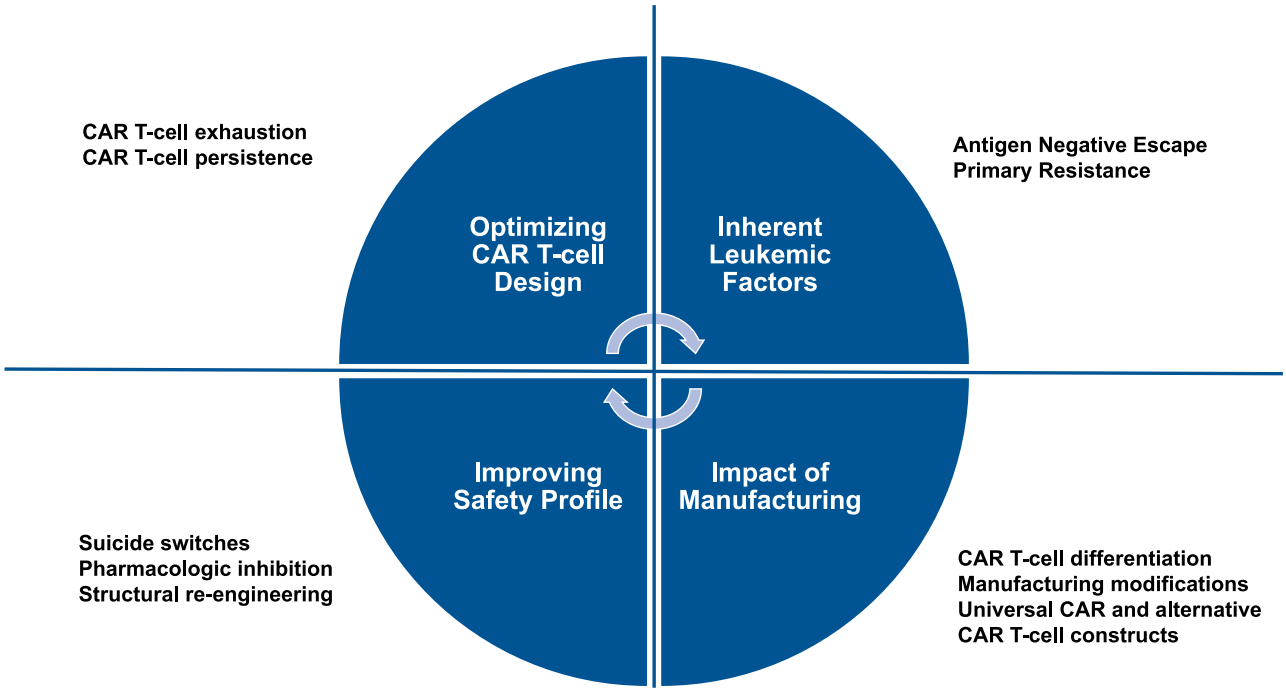

Fig 2. Summary of Barriers to Durable CAR T-cell Remission and Considerations for the Next-Generation CAR T-cell constructs.

lentiviral transduction of their CAR transgene, which resulted in durable engraftment and edits at all 3 genomic loci [66].

CAR natural killer (CAR-NK) cells are another promising "off-the-shelf" product with diminished risk of GVHD. Allogeneic NK cells can be adoptively transferred safely without the need for full HLA matching [67-69]. Earlier this year, results of a phase 1/2 clinical trial demonstrated the effectiveness of CD19 CAR NK cells derived from cord blood used to treat 11 patients with relapsed or refractory CD19-positive cancers. Of the 11 treated patients, 8 had a response, 7 with complete remission. Importantly, there was no increase in the levels of inflammatory cytokines, cytokine release syndrome (CRS), or neurotoxicity observed [70].

CAR T-cell-based modifications to improve the safety profile

The most severe and potentially use-limiting toxicities associated with CAR T-cell therapy are CRS and immune-effector cell associated neurotoxicity syndrome (ICANS) [71]. CRS correlates with T-cell expansion, and typically develops within the within the first few days after infusion, whereas ICANS presents with a more variable course, and can occur as early as the day following infusion or as late as several weeks after CAR T-cell infusion [71]. Both CRS and ICANS, when severe, can lead to death. Understanding the biological underpinning of CRS and ICANS is critical to further CAR T-cell clinical development. Preclinical models provide some clarity in the underlying mechanism of these toxicities. A rheuses macaque model of neurotoxicity demonstrated disproportionally high levels of IL-6, IL-2, GM-CSF, and VEGF in the cerebrospinal fluid. This was accompanied by CAR and non-CAR T-cell infiltration in the CSF and brain, resulting in pan-encephalitis [72]. In a humanized mouse model of CRS, it was revealed that monocytes were the major source of IL-1 and IL-6 during CRS, and that this syndrome could be prevented by monocyte depletion of by blocking of the IL-6 receptor with tocilizumab. However, tocilizumab treatment could not protect mice from delayed lethal neurotoxicity, which instead could be abrogated by the IL-1 receptor antagonist anakinra [73].

In an effort to prevent and manage CRS, ICANS, and unidentified toxicities that would not be evident prior to human trial, some CAR T-cell constructs have been engineered with "suicide switches" (inducible Caspase 9) or depletion markers (tEGFR or CD20) [74,75]. Unfortunately, these treatments potentially abrogate the antitumor effect of the intended therapy. An alternate strategy employs the tyrosine kinase inhibitor dasatinib as a reversible CAR T-cell inhibitor. Dasatinib has been shown to effectively interfere with lymphocyte-specific protein tyrosine kinase (Lck) function, leading to suppression of CD3ζ and ZAP70 phosphorylation. As all current CAR designs incorporate CD3ζ as part of their signaling domain, dasatinib induces a function-off state of CD8+ and CD4+ CAR T-cells. This effect was immediate and could be sustained for several days without affecting T-cell viability. Upon withdrawal, the inhibitory effect was rapidly and completely reversed [76].

Intrinsic changes to the CAR itself are also important. Modifications to the design of the "classic" second-generation CD19 CAR have provided insight into the functional relevance of CAR structure. Addition of several amino acids to the CD8α hinge and transmembrane domains in the prototype CD19-BBz CAR can produce a CAR T-cell product that produces less cytokines while retaining potent cytolytic activity [77]. This construct was used to generate CD19-CAR T-cells for a phase I trial in which 35% of patients with B-cell lymphoma achieved a complete remission. No neurologic toxicity or CRS (greater than grade 1) occurred in any of the 25 treated patients [77]. While the mechanism underlying the observed functional difference remains unknown, these results underscore the potential great impact of seemingly minor structural changes. It is possible that the strength of the CAR signal influences the differentiation phenotype of CAR T-cells, as a substantial proportion of T-cells in this study demonstrated a central memory phenotype after in vivo expansion. Similar results were found in a first-in-human trial of T-cells expressing a fully human anti-CD19 CAR. In this trial, severe neurologic toxicity occurred in only 5% of patients [78]. This is stark comparison to the prior reported 50% incidence of neurologic toxicity seen in patients treated with a FMC63-28Z CAR T-cell product [79]. T-cells expressing the human-

ized scFv secrete less cytokine than those expressing a FMC63-28Z CAR, potentially explaining the lower level of neurologic toxicity [78].

Conclusion

Given the successes of CAR T-cell-based immunotherapy in ALL, it is imperative to continue to optimize this treatment strategy. In recognition of current limitations, a host of approaches (Fig. 2) are being actively pursued to improve upon the more well-established paradigm. By virtue of CAR T-cell construct design and/or modifications in the manufacturing process, the future of CAR T-cell therapy will focus on: improving CAR T-cell persistence, extending durable remissions, limiting antigen escape, enhancing the safety profile, increasing the feasibility of CAR T-cell manufacturing, and reducing cost. These active efforts will contribute to a host of novel CAR T-cell constructs as we enter the next decade and will further improve upon the therapeutic index of CAR T-cell therapy—and most importantly, continue to revolutionize the field.

Authorship contributions

A.H.M, C.B., and N.N.S., all contributed to the first version of the manuscript. No nonauthor wrote the first draft or any part of the paper. All authors contributed to reviewing the final manuscript and have agreed to be co-authors.

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Conflicts of interest

A.H.M. and N.N.S. have no conflicts of interest. C.B. has patents pending in the field of engineered cellular therapy.

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