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CAR-modified Cellular Therapies in Chronic Lymphocytic Leukemia: Is the Uphill Road Getting Less Steep?

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ABSTRACT

The clinical development of chimeric antigen receptor (CAR) T-cell therapy has been more challenging for chronic lymphocytic leukemia (CLL) compared to other settings. One of the main reasons is the CLL-associated state of immune dysfunction that specifically involves patient-derived T cells. Here, we provide an overview of the clinical results obtained with CAR T-cell therapy in CLL, describing the identified immunologic reasons for the inferior efficacy. Novel CAR T-cell formulations, such as lisocabtagene maraleucel, administered alone or in combination with the Bruton tyrosine kinase inhibitor ibrutinib, are currently under investigation. These approaches are based on the rationale that improving the quality of the T-cell source and of the CAR T-cell product may deliver a more functional therapeutic weapon. Further strategies to boost the efficacy of CAR T cells should rely not only on the production of CAR T cells with an improved cellular composition but also on additional changes. Such alterations could include (1) the coadministration of immunomodulatory agents capable of counteracting CLL-related immunological alterations, (2) the design of improved CAR constructs (such as third- and fourth-generation CARs), (3) the incorporation into the manufacturing process of immunomodulatory compounds overcoming the T-cell defects, and (4) the use of allogeneic CAR T cells or alternative CAR-modified cellular vectors. These strategies may allow to develop more effective CAR-modified cellular therapies capable of counteracting the more aggressive and still incurable forms of CLL.

INTRODUCTION

T cells engineered to express a chimeric antigen receptor (CAR) are undoubtedly among the most promising therapeutic modalities in oncology, and particularly in hematology. The first evidence of successful in vivo expansion, antitumor activity, and long-term persistence of a second-generation anti-CD19 CAR T-cell product came from a patient with a relapsed form of chronic lymphocytic leukemia (CLL) who during the course of the disease had acquired 17p deletion and had become refractory to third-line chemoimmunotherapy.[1] Since then, several advances have been achieved in the field of genetically modified T-cell immunotherapy for lymphoid malignancies. These have led to the approval of 4 second-generation anti-CD19 CAR T-cell products, with indications that are covering an expanding range of diseases, including acute lymphoblastic leukemia,[2,3] large B-cell lymphoma (BCL),[4–6] mantle cell lymphoma,[7,8] and follicular lymphoma.[9,10] Despite the promising preclinical and early clinical results and the meaningful progress in the field, the clinical development of CAR T cells has been more challenging in the context of CLL due to some disease-specific critical issues.

The first limitation to the clinical applicability of CAR T cells in CLL is the patients' population that mainly consists of elderly and comorbid subjects.[11] Still, although a high level of concern about a lower age–related tolerability is justified, evidences from the pivotal ZUMA-1 trial and from early real-world experiences indicate that CAR T-cell therapy can be delivered to older patients with comorbidities, at least in the more explored setting of large BCLs.[12–15]

The second critical issue for the development of CAR T-cell therapy for CLL is the significant expansion of the treatment options that has occurred in the last years, which may limit the clinical need for cellular immunotherapy. Since the early 2000, the addition of anti-CD20 monoclonal antibodies to chemotherapy has shown the potential to achieve prolonged remissions and significant improvement in overall survival (OS) compared to chemotherapy alone, especially in previously untreated patients without relevant comorbidities and presenting a disease with low-risk biological features.[16,17] More recently, the clinical testing of Bruton tyrosine kinase (BTK) inhibitors and of the BCL2 protein inhibitor venetoclax, variably administered in sequence and/or in combination and in presence or absence of anti-CD20 monoclonal antibodies, has provided remarkable clinical results and superiority compared to chemoimmunotherapy for the treatment of all risk groups.[18–23] That notwithstanding, targeted agents have some important limitations, which affect their curative potential, such as (1) the loss of efficacy and possible development of drug resistance mechanisms, frequently reported for

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continuous treatment regimens,[24–27] and (2) the inferior efficacy observed in high-risk subtypes (such as those carrying *TP53* aberrations or multiple cytogenetic abnormalities), in particular in the context of fixed-duration therapy. Furthermore, the use of targeted agents has not proven to be particularly beneficial for those cases of CLL that have transformed into an aggressive lymphoma (Richter syndrome), a clinical situation where treatment options are still very limited and prognosis remains definitely poor, with a median survival of less than 1 year.[28] Accordingly, for patients who have failed treatment with BTK inhibitors and venetoclax, or who develop Richter syndrome, the identification of immune-based therapeutic strategies is worth investigation.

The third and most likely the main issue that has limited the development of successful CAR T-cell therapies for CLL is the difficulty in producing effective CAR T-cell formulations. This is mainly due to the intrinsic immunologic deterioration that characterizes the disease.[29,30] Indeed differently from other hematological disorders, where the acquisition of antigen (Ag)-negative tumor variants represents a relevant cause of CAR T-cell therapy failure,[31–33] in CLL the main reasons for treatment resistance can be found more likely in the production of defective or shortly persisting CAR T cells that are not effective in eradicating the tumor cells and therefore favor the development of Ag-positive relapses. CLL is characterized by a wide range of deep immune alterations and complex intrinsic T-cell defects, which already occur in the very early phases of the disease—at some degree even in the preneoplastic phase of monoclonal B-cell lymphocytosis[34]—and then progressively accumulate, in parallel with the increase in tumor burden and under the pressure of multiple lines of treatment (Figure 1). From the clinical standpoint, the relevance of these immunologic alterations is demonstrated by some manifestations that frequently occur

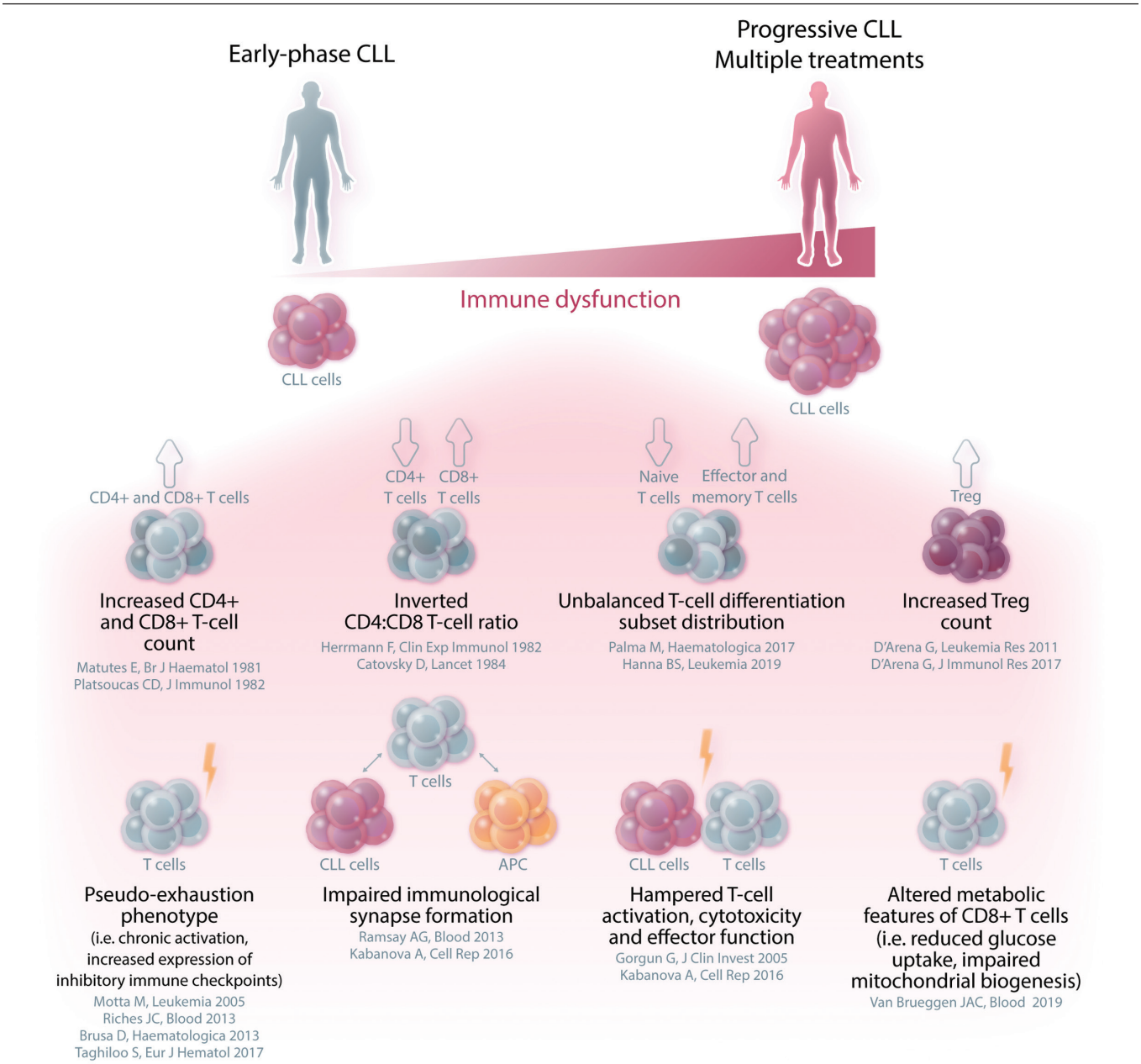

Figure 1. Immune system dysfunctions that characterize patients with CLL and can impact on CAR T-cell functionality. Phenotypic alterations and functional impairment characterize the T-cell compartment of CLL patients. Immune dysregulation is often present since the early stages of CLL, and exacerbates with disease progression and as a consequence of multiple lines of treatment, thus hampering immune competence. CAR = chimeric antigen receptor; CLL = chronic lymphocytic leukemia.

during the disease course and are considered a hallmark of CLL. These include the increased susceptibility to infections, the lower response to prophylactic vaccinations, the higher risk of second primary malignancies, and the high frequency of autoimmune phenomena.[35] Specifically, several T-cell alterations have been reported in CLL, such as the presence of an inverted CD4:CD8 T-cell ratio,[36–38] the unbalanced differentiation subset distribution,[39–42] the higher number of regulatory T cells (Tregs),[43–47] and the increased expression of pseudoexhaustion markers and transmembrane inhibitory receptors, including CTLA-4, PD-1, TIM-3, or LAG-3.[39–41,48–56] All of these T-cell alterations translate in functional impairments, including defects in immune synapse formation and in cytotoxicity.[57–61] This subversion of T-cell–related immunity results in altered features of CLL-derived CAR T cells, which often present expression of exhaustion markers that cause reduced cytokine secretion, limited expansion capacity, and consequently, poor antitumor function.[62] Of note, the manufacturing strategies developed up to now are not able to fully overcome the basic immunological alterations related to the disease and might even contribute to the acquisition of additional features of functional exhaustion and short in vivo persistence.

In this review, we provide a comprehensive overview of the clinical results achieved by CAR T-cell therapy in CLL and of the identified reasons, to date, of suboptimal efficacy. We specifically focus on immune-related mechanisms that contribute to the poor functionality and to the incomplete eradication or reappearance of Ag-positive leukemic cells. In addition, we describe strategies that aim to overcome the T-cell–related defects as well as strategies that are currently pursued to improve the clinical efficacy of CAR-modified cellular therapies. These efforts should lead to the development of novel cellular therapies capable of counteracting the more aggressive and still incurable forms of the disease.

SECOND-GENERATION ANTI-CD19 CAR T CELLS FOR THE TREATMENT OF CLL: MORE THAN 10 YEARS OF ATTEMPTS

Second-generation anti-CD19 CAR T-cell constructs—comprising an Ag-binding portion, a transmembrane domain, an intracellular signaling domain of CD3ζ chain, and a single costimulatory domain[2,6,63–66]—were the first to be broadly evaluated in the clinical settings and to be approved for use in the clinical practice. Patients with CLL were included in the heterogeneous patient populations enrolled in early studies exploring the safety and efficacy of second-generation anti-CD19 CAR T-cell products, but later clinical trials have mainly focused on aggressive BCLs. As of December 2023, no CAR T-cell product has been approved for the treatment of patients with CLL. A summary of the efficacy and safety results achieved in CLL patients with anti-CD19 CAR T-cell products is presented in Table 1.

In 2012, a group at the National Cancer Institute published the results obtained in 8 patients treated with their anti-CD19 CAR T cells (later called axicabtagene ciloleucel [axi-cel]) combined with a course of intravenous interleukin (IL)-2.[67] Four patients with extensively pretreated CLL (median number of prior therapies, 4.5) were included, among whom 3 had a response, including one who had a prolonged complete remission (CR, >15 months). A modified treatment plan that omitted IL-2 administration was later applied for the treatment of 4 additional patients with CLL, who all had a response with a CR rate of 75%.[68] At the last update presented in 2020, a persistent response of more than 3 years was reported in 4 of 8 patients with CLL, among whom 2 had a response lasting more than 6 years.[69]

A similar CAR T-cell product (brexucabtagene autoleucel [brexu-cel])—which differs from axi-cel only in the slightly different manufacturing process of removing circulating CD19-expressing malignant cells to avoid the possible activation and exhaustion of anti-CD19 CAR T cells during the production—is currently under evaluation for the treatment of relapsed/refractory CLL within the phase I ZUMA-8 study. Initial results from 15 patients, of whom 80% previously received >3 treatment lines, were recently presented, with an overall response rate (ORR) of 47% and a CR rate of 13%.[70] Cytokine release syndrome (CRS) developed in 12 of 15 patients but was only grade ≥3 in 1 patient, whereas all-grade and grade ≥3 neurotoxicity developed in 11 of 15 and 3 of 15 patients, respectively. Overall, the safety and preliminary efficacy results of this product appear limited, suggesting the need for further optimization.

Tisagenlecleucel (tisa-cel) was also evaluated for the treatment of patients with CLL: the tumor regression observed in the first patient with CLL was reported in 2011,[1] followed by preliminary results obtained in 3 patients, all of whom responded to the therapy.[71] Mature results from an expanded cohort of patients with relapsed/refractory CLL who received a median of 5 previous therapies were later presented (n = 14).[72] The ORR was 57%, and in 29% of the patients a CR with undetectable minimal residual disease (MRD) was reported. A persistent response was achieved in patients who obtained a CR (median duration of response [DOR], 40 months) with no relapses noted during the follow-up, whereas for patients who obtained a partial response (PR), median DOR was only 7 months. Nine of 14 patients developed CRS, which was grade 3-4 in 6 patients, and associated with tisa-cel peak expansion and clinical response.

Based on these encouraging results, and with the aim of determining an optimal cell dose for future application, the authors then performed a randomized, phase II study in patients with relapsed/refractory CLL.[73] Forty-two previously treated patients with CLL (median number of prior therapies, 3.5) were randomly assigned to receive 2 different doses of tisa-cel (5×10^8 or 5×10^7 CAR T cells). Thirty-eight patients received the treatment and were evaluable for safety, and 32 were evaluable for response. Despite the higher response rates achieved with high dose compared to low dose (ORR, 53% versus 31%; CR rate, 36% versus 15%), there were no differences in progression-free survival (PFS) and OS between the cohorts. Of note, PFS and OS were significantly longer in patients who achieved a CR versus those who did not (median PFS, 40 months versus 1 month, $P < 0.0001$; median OS not reached versus 64 months, $P = 0.035$), regardless of the dose received. Toxicity was similar in the 2 dose groups: 63% of patients overall developed all-grade CRS and 24% developed grade 3-4 CRS.

Interestingly, investigators from the University of Pennsylvania have recently reported on the long-lasting persistence of circulating CAR T cells, together with sustained disease remission, in 2 of the patients with CLL who were treated with tisa-cel and achieved a CR in 2010,[74] thus providing the proof of concept evidence that CAR T cells may potentially be considered curative for this disease.

An additional second-generation anti-CD19 CAR T-cell construct was developed by a group at Memorial Sloan Kettering Cancer Center. Initially reported data showed no response in 8 patients with purine analog refractory CLL.[75] The protocol was later modified, optimizing the conditioning regimen by adding fludarabine (Flu) and permitting the inclusion of ibrutinib pretreated patients. Sixteen patients with relapsed or refractory CLL were treated (median number of prior therapies, 4) and an objective response was observed in 6 of 16 (38%) CLL patients. Three of 12 (25%) evaluable patients with CLL who received conditioning chemotherapy achieved CR by International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria.[76] Alongside the unsatisfactory efficacy, toxicity of this CAR T-cell formulation was relevant. Indeed, all 16 patients experienced CRS, although only 2 had a grade ≥3 event.

As it emerges from data presented to date, the clinical efficacy of second-generation CAR T cells has been initially disappointing in CLL, despite the nonnegligible toxicities, thus prompting investigation of the immunological and biological mechanisms

Table 1

Summary of Results Obtained in Patients with CLL Using Anti-CD19 CAR T Cells

Product Administered	Product Specifics	Patients Evaluable for Response, Number	Lymphodepleting Chemotherapy	ORR	CR Rate	Median DOR	Median PFS	Median OS	Safety	Reference
Axi-cel (KTE-C19) + IV IL-2	Second generation	CLL = 4	Flu/Cy	75% (3/4)	25% (1/4)	7 months	NA	NA	Grade ≥3 AEs 100% (4/4)	67
Axi-cel (KTE-C19)	Second generation	CLL = 4 RS = 1	Flu/Cy	CLL: 100% (4/4) RS: 100% (1/1)	CLL: 75% (3/4) RS: 0% (0/1)	CLL: 14 months RS: 1 months	NA	NA	CLL: grade ≥3 AEs 50% (2/4) RS: grade ≥3 AEs 100% (1/1)	68
Brexu-cel (KTE-X19)	Second generation; depletion of circulating CD19-expressing malignant cells	CLL =15	Flu/Cy	47% (7/15)	13% (2/15)	NA	NA	NA	Grade ≥3 AEs 100% (15/15) CRS 80% (12/15), grade ≥3 CRS 7% (1/15) NE 73% (11/15), grade ≥3 NE 20% (3/15)	70
Tisa-cel (CTL019)	Second generation	CLL = 3	Different regimens (bendamustine ± rituximab, pentostatin/Cy)	100% (3/3)	33% (1/3)	10 months	NA	NA	NA	71
Tisa-cel (CTL019)	Second generation	CLL = 14[a]	Different regimens (Flu/Cy, bendamustine, pentostatin/Cy)	57% (8/14)	29% (4/14)	CR patients, 40 months PR patients, 7 months	7 months	29 months	CRS 63% (9/14), grade ≥3 CRS 43% (6/14) NE 36% (5/14), grade ≥3 NE 7% (1/14)	72
Tisa-cel (CTL019)	Second generation	CLL = 32 (5×10^8 CAR T cells, HD = 19; 5×10^7 CAR T cells, LD = 13)	Different regimens (bendamustine, Flu/Cy, pentostatin/Cy, oxaliplatin/Flu/cytarabine/ofatumumab, gemcitabine/oxaliplatin)	HD: 53% (10/19) LD: 31% (4/13)	HD: 37% (7/19) LD: 15% (2/13)	NA	1 months[b]	64 months[b]	CRS 63% (24/38), grade ≥3 CRS 24% (9/38)[b] Grade≥3 NE 8% (3/38)[b]	73
MSKCC CD19 CAR T cells	Second generation	CLL = 8	None or Cy	0%	0%	NA	NA	NA	Grade ≥3 AEs 50% (4/8)	75
MSKCC CD19 CAR T cells	Second generation	CLL = 16[a]	None or different regimens (Cy, bendamustine, Flu/Cy)	38% (6/16)	19% (3/16)	NA	3 months	17 months	CRS 100% (16/16), grade≥3 CRS 12% (2/16) NE 38% (6/16), grade ≥3 NE 6% (1/16)	76
JCAR014	Second generation; 1:1 ratio of CD4+:CD8+ CAR T cells; ex vivo stimulation with antigen-presenting cells	CLL = 18 RS = 5	Cy, Flu, or Flu/Cy	CLL: 72% (13/18) RS: 60% (3/5)	CLL: 11% (2/18) RS: 40% (2/5)	NA	8.5 months[b]	NR	CLL: CRS 95% (18/19), grade ≥3 CRS 10% (2/19). NE 37% (7/19), grade ≥3 NE 26% (5/19) RS: CRS 40% (2/5), grade ≥3 CRS 0. NE 20% (1/5), grade ≥3 NE 20% (1/5)	86
Liso-cel (JCAR017)	Second generation; 1:1 ratio of CD4+:CD8+ CAR T cells; removal of non-T-cell impurities before activation and transduction; ex vivo stimulation with cytokines	CLL = 96	Flu/Cy	48% (46/96)	18% (17/96) (CR/CRi)	35 months	18 months	NA	CRS 85% (99/117), grade ≥3 CRS 10% (10/117) NE 45% (53/117), grade ≥3 NE 19% (22/117)	89
CD19 CAR T cells (Baylor College of Medicine)	Third generation with 2 costimulatory domains (CD28 and 4-1BB)	CLL = 2	Flu/Cy	50% (1/2)	0%	NA	13 months	>40 months	CRS 2% (3/15), grade ≥3 CRS 7% (1/15)[b] NE grade ≥3 13% (2/15)[b]	91
CD19 CAR T cells (Heidelberg University Hospital)	Third generation with 2 costimulatory domains (CD28 and 4-1BB)	CLL = 4	Flu/Cy	43%[c]	29%[c]	NA	NA	NA	CRS grade ≥3 0.7% (2/27)[b] NE grade ≥3 0%[b]	97

(Continued)

Table 1 (Continued)

Product Administered	Product Specifics	Patients Evaluable for Response, Number	Lymphodepleting Chemotherapy	ORR	CR Rate	Median DOR	Median PFS	Median OS	Safety	Reference
MSKCC CD19 CAR T cells	Fourth generation with 2 signaling domains (CD28 and CD3ζ) and 4-1BB ligand expression	CLL = 9 RS = 3	Flu/Cy or Cy alone	CLL: 44% (4/9) RS: 67% (2/3)	CLL: 33% (3/9) RS: 67% (2/3)	NA	NA	NA	CRS 39% (11/28), grade ≥3 CRS 3% (1/28)[b] CRS 39% (11/28), grade ≥3 CRS 10% (3/28)[b]	98
JCAR014 and ibrutinib	Second generation	CLL = 18	Flu/Cy	83% (15/18)	22% (4/18, all CRi)	NA	NA (1-year PFS, 38%)	NA (1-year OS, 64%)	CRS 74% (14/19), grade ≥3 CRS 0% NE 26% (5/19), all grade 3	112
CTL119 and ibrutinib	Second generation	CLL = 16	Flu/Cy or bendamustine	69% (11/16)	44% (7/16)	NA	NR (48-months PFS, 70%)	NR (48-months OS, 84%)	CRS 95% (18/19), grade ≥3 CRS 11% (2/19) NE 26% (5/19), grade ≥3 NE 5% (1/19)	113
Liso-cel (JCAR017) and ibrutinib	Second generation	CLL = 19	NA	95% (19/19)	47% (9/19) CR/CRi	NA	NA	NA	CRS 74% (14/9), grade ≥3 CRS 5% (1/19) NE 32% (6/19), grade ≥3 NE 16% (3/19)	114

[a] Including patients from the previous series.
[b] Data available for the whole cohort of the patients enrolled in the study.
[c] Data available for the NHL/CLL cohort of the patients enrolled in the study.
AEs = adverse events; Axi-cel = axicabtagene ciloleucel; Brexu-cel = brexucabtagene autoleucel; CAR = chimeric antigen receptor; CLL = chronic lymphocytic leukemia; CR = complete response; CRS = cytokine release syndrome; Cy = cyclophosphamide; DOR = duration of response; Flu = fludarabine; HD = high dose; IV = intravenous; LD = low dose; Liso-cel = lisocabtagene maraleucel; MSKCC = Memorial Sloan Kettering Cancer Center; NA = not available; NE = neurologic events; NHL = non-Hodgkin lymphoma; NR = not reached; ORR = overall response rate; OS = overall survival; PFS = progression-free survival; RS = Richter syndrome; Tisa-cel = tisagenlecleucel.

responsible for the limited functionality and designing novel protocols of production and administration to achieve successful CAR T-cell therapies.

REASONS FOR SUBOPTIMAL EFFICACY AND DETERMINANTS OF CAR T-CELL FUNCTIONALITY IN CLL

Over time, several groups have investigated the possible reasons underlying the suboptimal clinical efficacy obtained with second-generation anti-CD19 CAR T-cell products in CLL, focusing on the identification of T-cell–related determinants capable to predict CAR T-cell functionality.

One of the main factors affecting the clinical efficacy of CAR T cells is the cellular composition of the infused genetically modified T lymphocytes. Reported data show that the ability of CAR T cells to expand in vivo positively correlates with the frequency, within the infused product, of a CAR-expressing CD8+CD45RA+CCR7+ T-cell population: a cell subset phenotypically close to T memory stem cells (T_{SCM}), which have self-renewal capacity and the ability to rapidly differentiate into effector T cells upon Ag exposure.[77] However, in this study, no patient achieved a sustained clinical response, thus precluding the possibility to correlate the composition and phenotype of CAR T cells with the clinical outcome.

A further demonstration of the importance of the subset composition of the administered cellular products was provided by Sommermeyer et al, who were able to recognize the enhanced potency of anti-CD19 CAR T cells composed of defined T-cell subsets compared with those produced from unselected peripheral blood mononuclear cells (PBMCs) obtained from patients with BCL.[78] Specifically, individual CD8+ and CD4+ T-cell subsets from both patients with B-cell malignancies and from normal donors were purified and used to produce CAR T cells, whose functional activity was then assessed in vitro and in vivo. Overall, CAR T cells derived from less-differentiated naive (T_N) and central memory (T_{CM}) CD4+ or CD8+ T cells were functionally more effective in terms of cytokine production than those derived from more differentiated effector memory (T_{EM}) CD4+ or CD8+ T cells. In addition, in vivo experiments show that a third-generation CAR T-cell product containing the CD28 and 4-1BB costimulatory domains administered to immunodeficient mice (nonobese diabetic/severe combined immunodeficiency/$\gamma c^{-/-}$) engrafted with a CD19+ Burkitt lymphoma–derived cell line was more potent in eradicating the tumor when administered as a formulation containing a 1:1 ratio of CD8+ CAR T cells derived from T_{CM} with CD4+ CAR T cells, particularly if derived from the T_N subset. Based on these observations, one possible reason for the reported heterogeneous efficacy of CAR T-cell therapy in patients with lymphoproliferative disorders could be found in the patient-to-patient variability in terms of subset distribution within the CAR T-cell population produced from unselected T-cell sources. Since the quality of the CAR T-cell product strictly depends on the characteristics of the initial T-cell population, it is important to take into account that CLL patients have an underrepresented compartment of circulating T_N cells, which are also characterized by a limited expansion potential—2 hallmarks that may relevantly challenge the generation of an effective CAR T-cell population.[79]

Precisely with the purpose of recognizing parameters predictive of CAR T-cell functionality, Fraietta et al performed an extensive analysis evaluating several T-cell–related features in a large cohort of 41 patients with CLL treated with 4-1BB-based anti-CD19 CAR T cells (CTL019).[62] Clinical efficacy was not predicted by patient and disease characteristics (ie, tumor burden, presence of *TP53* gene aberration, and number of previous therapies), whereas parameters predictive of successful treatment could be identified both at the level of the premanufacturing T cells and of the infused CAR T cells. Results from this analysis showed that premanufacturing

unmanipulated T cells from nonresponding patients display upregulation in genes involved in T-cell exhaustion (ie, *DUSP4, CXCL13*), activation (ie, *IL1A, STAT3*), glycolysis (ie, *B4GALNT1, DNAJC12*), and apoptosis (ie, *EMP1, DRAM1*).[62] Additionally, leukapheresis products from responding patients were characterized by an elevated frequency of CD8+ T_{SCM} cells, thus confirming that the cellular composition of the initial T-cell source also has an impact on the functional properties of the administered cellular product. In line with these observations also in other hematologic and solid tumors,[80,81] both the presence of specific T-cell subsets and the phenotypical and functional characteristics of the premanufacturing T cells were identified as informative parameters of the functionality of the infused CAR T cells. In this context, a point that is still a matter of debate is the impact of previous chemoimmunotherapy, which can certainly affect the initial composition and fitness of the T-cell compartment, and the possibility to effectively generate fully functional CAR T cells.[82]

In their comprehensive analyses, Fraietta et al have also strengthened the concept that the characteristics of the final CAR T-cell formulation have a relevant impact on patients' clinical outcome. They observed that in CAR T-cell–treated patients, the therapeutic efficacy was primarily associated with parameters of preserved functionality at the level of the infused CAR T cells, as defined by their in vivo expansion and persistence. In addition, CAR T cells from responding versus nonresponding patients were characterized by a different transcriptomic profile (early memory differentiation versus late memory/effector T-cell differentiation, respectively) and by a decreased dependency from aerobic glycolysis. The impact of the metabolic asset on the functionality of CAR T cells was also confirmed by van Bruggen et al, who demonstrated that patients with CLL achieving a CR after treatment with CTL019 have a higher CAR T-cell mitochondrial mass compared with nonresponder patients.[83]

Another factor that has shown to exert a relevant impact on the clinical outcome is the expression profile of inhibitory immune checkpoints on the infused CAR T cells. Kong et al reported significantly higher levels of CTLA-4 and TIM-3 or LAG-3 expression on CAR T cells of nonresponding patients compared with responding patients.[84] Moreover, in nonresponding patients, at the peak of the in vivo expansion phase, a higher proportion of CAR T cells coexpressing PD-1 and TIM-3 was observed, and the upregulation of these markers of exhaustion was paralleled by the acquisition of defects in expansion capacity and in perforin-mediated cytotoxicity.

Overall, these observations highlight the meaningful impact of the composition and quality of both the premanufacturing T-cell source and the final CAR-modified T-cell products in determining the postinfusion expansion, the in vivo persistence, and, ultimately, the antitumor functions and clinical efficacy of CAR T cells.

STRATEGIES TO IMPROVE CAR T-CELL EFFICACY IN CLL: OVERCOMING T-CELL DYSFUNCTIONS

Given the disappointing clinical efficacy achieved in CLL by the same anti-CD19 CAR T-cell formulations that are instead successful in other lymphoid malignancies,[10,13,85] different strategies have been tested and are currently under investigation with the aim of reversing CLL-related immunological dysfunctions and obtaining a cellular product with more powerful antitumor functions (Figure 2).

Improving the composition of infused CAR T-cell products

Based on previously cited preclinical data proving the key role of the T-cell subset composition on CAR T-cell efficacy,[78] a group at Fred Hutchinson Cancer Research Center focused their work on the development of an optimized second-generation anti-CD19 CAR T-cell formulation consisting of a precise dose of CD4+ and CD8+ CAR T cells. JCAR014 is manufactured from separate subsets of $CD4^+$ T cells and either bulk $CD8^+$ T cells or $CD8^+$ T_{CM} cells, and formulated for infusion in a 1:1 ratio of CD4+:CD8+ CAR T cells. This product was studied in a phase I/II clinical trial that enrolled 24 heavily pretreated and high-risk patients with aggressive CLL or Richter transformation (n = 19 and n = 5, respectively).[86] Of note, all patients had received previous ibrutinib therapy (median duration of ibrutinib administration, 13 months) and most of them had experienced ibrutinib failure. CAR T cells were administered after lymphodepleting chemotherapy consisting of cyclophosphamide (Cy), Flu, or Cy plus Flu. In the whole cohort, 16 patients responded (ORR, 70%) as per IWCLL imaging criteria assessed by the investigators, with a CR rate of 17% at 4 weeks after CAR T-cell infusion. Among patients who received Cy plus Flu lymphodepletion and $<2\times10^6$ CAR T cells/kg, 88% reached undetectable MRD by flow cytometry in the bone marrow 4 weeks after CAR T-cell administration, and 58% of them also had molecularly undetectable MRD (assessed by immunoglobulin heavy chain [IGH] sequence). At a median follow-up of 6.6 months, estimated median PFS was 8.5 months and median OS was not reached. The response to treatment correlated with outcome since patients who achieved either a CR or PR had longer PFS and OS compared with those who experienced treatment failure (CR: median PFS 9.8 months, median OS not reached; PR: median PFS not reached, median OS not reached; treatment failure: median PFS 1.1 months, median OS 11.2 months). Of note, in this cohort, MRD clearance by IGH sequencing was predictive of longer PFS, independently of the response achieved by IWCLL criteria. Regarding treatment-related toxicities, despite the high frequency of occurrence (83%), CRS presented as grade 1-2 in most patients (75%), with only 1 patient experiencing grade 4 and 1 experiencing grade 5 CRS. Eight of 24 patients (33%) had neurotoxicity (grade 1-2: 2 patients; grade 3: 5 patients; grade 5: 1 patient). Although the incidence of serious CRS was low, neurotoxicity could represent a limit for this CAR T-cell product, especially considering that patients with CLL are frequently old and with meaningful comorbidities.

Lisocabtagene maraleucel (liso-cel, JCAR017) is a more recently introduced CAR T-cell product that uses the same construct as JCAR014 and maintains a fixed 1:1 ratio of CD4+:CD8+ CAR T cells, but differs in the manufacturing process, because (1) cytokines instead of Ag-presenting cells are used during the ex vivo T-cell stimulation phase[87] and (2) the early selection phase of CD4+ and CD8+ T cells involves discharging from the leukapheresis the non-T-cell elements to limit the risk of transducing leukemic cells with the anti-CD19 CAR and to favor a more efficient expansion of CAR T cells.[87,88] JCAR017 is currently under evaluation for the treatment of relapsed/refractory CLL in the phase I/II TRANSCEND CLL 004 trial, whose primary efficacy analysis results were recently published.[89] In this study, 137 patients were enrolled, 117 of whom received liso-cel. Most of the patients (83%) were considered high-risk, carrying a *TP53* alteration and/or unmutated *IGHV* genes and/or complex karyotype. Notably, all treated patients had received previous ibrutinib treatment, with most of the patients developing resistance to BTK inhibitor (88%) and a consistent subset showing failure or disease recurrence upon venetoclax-based therapy (60%). Patients received liso-cel at 2 different dose levels (ie, 50×10^6 CAR T cells [n = 9] or 100×10^6 CAR T cells [n = 10^8]) and 96 of them were evaluable for efficacy: 46 achieved a response (ORR, 48%) and 17 achieved a CR (18%). These response rates were comparable to those obtained in the subcohorts of patients who progressed after BTK inhibitors and failed venetoclax therapy (ORR, 43%; CR, 18%). At a median follow-up of 21 months, the median PFS was

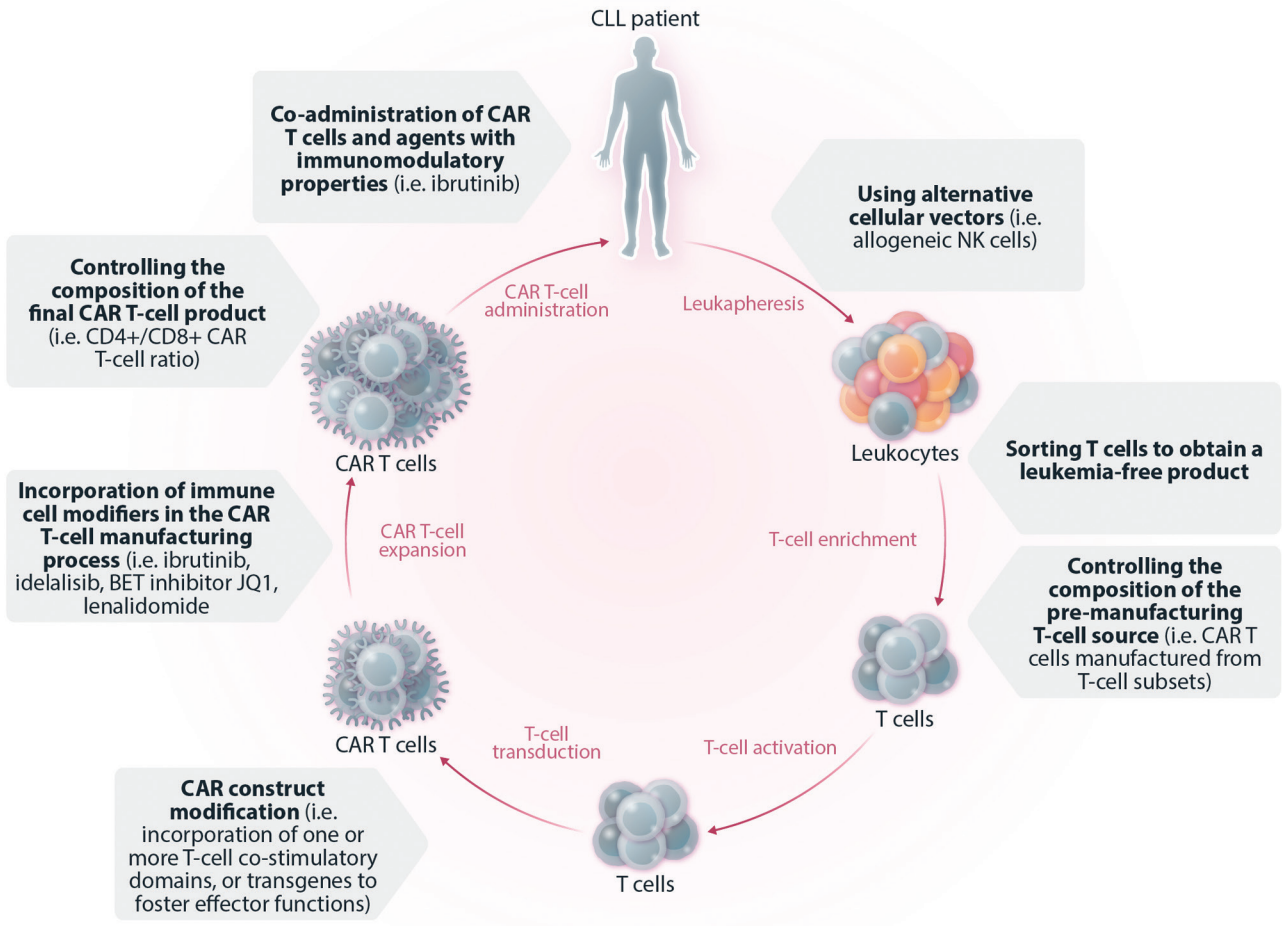

Figure 2. Overview of strategies to improve CAR T-cell efficacy in CLL through the targeting of disease-related immune dysfunctions. The steps for the generation of autologous CAR T cells from a CLL patient are depicted. Several strategies are currently under investigation with the aim to overcome T-cell alterations and to obtain CAR T-cell products with improved antitumor efficacy in CLL. CAR = chimeric antigen receptor; CLL = chronic lymphocytic leukemia.

17.8 months and the median DOR was 35.2 months, with median DOR for CR patients not reached. Among 49 patients included in the primary efficacy analysis set and treated with the higher dose level, at any time during follow-up, 63% and 59% achieved an undetectable MRD in the blood and in the bone marrow, respectively, as assessed by next-generation sequencing. The concordance of detectable and undetectable MRD between blood and bone marrow was 96%, and all patients who responded to the therapy reached undetectable MRD in both compartments. Most of the adverse events were low grade. Among 117 patients evaluated for safety, 99 (85%) had CRS (grade 1-2: 76%; grade 3: 9%) and 53 patients (45%) had neurological events (grade 1-2: 26%; grade 3: 18%; grade 4: 1%). Forty-three patients died after CAR T-cell infusion: 27 due to progressive disease, 5 due to adverse events occurring within 90 days of CAR T-cell infusion (1 macrophage activation syndrome-hemophagocytic lymphohistiocytosis and 4 infectious complications considered unrelated to liso-cel treatment), and 11 due to other reasons (>90 days after CAR T-cell infusion).

Overall, a longer follow-up is certainly needed to fully ascertain the clinical benefit of liso-cel for patients with CLL, but these most updated results of the TRANSCEND CLL 004 trial did not fully meet the expectations set by the preliminary efficacy results reported for the phase I dose-escalation portion of the study, where ORR and CR rate were 82% and 45%, respectively.[90] Certainly liso-cel proved to be an effective therapy in a proportion of patients showing a difficult-to-treat disease and for whom there are no currently approved therapeutic options (ie, patients who failed both ibrutinib and venetoclax). However, current data show that the applied manufacturing strategy is not sufficient for fully overcoming the obstacles related to the dysfunctional T-cell compartment of patients with CLL, which leaves space for further improving CAR T-cell formulations in this disease.

Construct modifications and improvements: third- and fourth-generation CAR T cells

The possibility of bolstering T-cell functions and fine-tuning the antitumor efficacy of the final CAR T-cell product through modifications of the transduced CAR construct has been evaluated in different settings.[91,92] This approach is presumed to be particularly effective in CLL and other diseases where the suboptimal efficacy of second-generation CAR T cells can be attributed to an immunocompromised state.

CAR T-cell expansion, cytokine production, persistence and antitumor activity can be increased by incorporating in the CAR construct intracellular signaling domains from one or more T-cell costimulatory molecules, such as CD28 or 4-1BB.[64,93] It has been shown that specific costimulatory domains influence the effector functions of CAR T cells differently. For instance, CD28 has proven to induce a more rapid and intense signal but a shorter period of activity and in vivo persistence compared to 4-1BB.[94–96] With the aim of combining different properties,

third-generation CAR constructs containing 2 or more costimulatory domains (eg, CD28 and 4-1BB) have been designed and are being clinically assessed, but efficacy data in the context of CLL are only preliminary.

In a phase I/IIa study, a third-generation product containing both CD28 and 4-1BB costimulatory domains was administered to 11 patients with either relapsed/refractory non-Hodgkin lymphoma (NHL) or CLL (n = 2). Results showed a good tolerability profile but CR rates were in the lower range and comparable with those achieved in studies using second-generation CARs.[91]

A similar third-generation in-house manufactured anti-CD19 CAR T-cell product has been developed and clinically tested at Heidelberg University Hospital, Germany. Preliminary results of the phase I study have been presented, showing that academic CAR T-cell generation was feasible for all enrolled patients and that the administered cellular product had a favorable safety profile with a potential clinical efficacy. Overall, in patients with NHL/CLL (n = 15; CLL, n = 4) this third-generation CAR T-cell formulation produced an ORR of 43% and a CR rate of 29%, but efficacy results achieved in the small subgroup of patients with CLL were not reported.[97]

Fourth-generation CAR T cells are a further advancement, in which additional transgenes are transduced into T cells, leading to the expression of molecules that can foster supplementary effector functions or help the infused CAR T cells to be released from microenvironment-induced immunosuppression. A group at Memorial Sloan Kettering Cancer Center is currently evaluating the clinical use of CD28-costimulated CAR T cells that also express 4-1BB-L, with the aim of exploiting a concomitant enhancement of both CD28 and 4-1BB signals and potentiate the tumoricidal activity of effector cells. Preliminary data reported encouraging overall CR rates for patients with both CLL (3/9 patients, 33%) and Richter syndrome (2/3 patients, 67%).[98]

In line with this approach, CAR T cells can also be armored to counteract microenvironment-derived immune-suppressive signals, such as those involving the PD-1/PD-L1 axis,[99] or can be implemented to secrete specific cytokines (ie, IL-12, IL-15) with immunomodulatory properties (TRUCKs, T cells redirected for universal cytokine-mediated killing).[100–104] The potential advantage of TRUCKs is that the secreted cytokines, in addition to enhancing the efficacy of CAR T cells, exert a broad and beneficial immunomodulatory effect on the tumor microenvironment.[105] However, preclinical or clinical data on these fourth-generation CAR T cells in CLL are not yet available.

Combined treatment with CAR T cells and agents with immunomodulatory properties

An intuitive approach to improve CAR T-cell functionality and in vivo persistence implies the coadministration of CAR T cells with a drug with immunomodulatory functions. To date, the most studied compound in the context of CLL is the BTK inhibitor ibrutinib. Ibrutinib represents one of the fundamental drugs in the treatment armamentarium for B-cell lymphoproliferative diseases, and especially CLL.[106] Interestingly, besides its antitumor activity, ibrutinib also exerts modulatory effects on multiple immune compartments, particularly T cells.[107–109] Therefore, the possibility of combining a direct cytotoxic effect on leukemic cells with an indirect effect aimed at boosting the effector arms of cellular immunotherapy is certainly very appealing.

It has been demonstrated that previous treatment of CLL patients with ibrutinib—right before the T-cell apheresis or as conditioning regimen before CAR T-cell infusion—improves CAR T-cell expansion and positively modulates the phenotype and functional characteristics of the cellular product.[76,110,111] At a preclinical level, it has been shown in a murine xenograft model that the concomitant administration of ibrutinib can increase the antitumor effect of anti-CD19 CAR T cells.[110]

Based on these evidences, in a phase I/II trial from the Fred Hutchinson Cancer Research Center, 19 patients with relapsed/refractory CLL were treated with JCAR014 and ibrutinib (administered at least for 2 weeks before leukapheresis and continued for at least 3 months after CAR T-cell infusion).[112] All patients were considered bearing a high-risk disease, with a median of 5 prior therapies (range, 1–10), and all patients had failed ibrutinib before study entry. Eighteen patients were evaluable for response, with a 4-week ORR of 83% (15/18, of whom 4 had a CR with incomplete hematologic recovery [CRi]). At a median follow-up of 12 months, 1-year PFS and OS were 38% and 64%, respectively, with responding patients achieving superior long-term outcomes compared with nonresponding patients (1-year PFS: CRi = 67%, PR = 38%, nonresponding = 0; 1-year OS: CRi = 100%, PR = 58%, nonresponding = 33%). One patient died from a presumed ibrutinib-related cardiac arrhythmia during grade 2 CRS, 4 days after infusion. Overall, 14 of 19 patients (74%) had CRS (all grade 1-2), and 5 of 19 (26%) developed neurotoxicity (all grade 3). The authors also compared these results with those obtained in a cohort of 19 patients treated with the same CAR T-cell product without ibrutinib. Results from this comparison did not show an improvement in CAR T-cell expansion and in clinical outcome, but detected significantly lower serum concentrations of CRS-associated inflammatory cytokines (ie, monocyte chemoattractant protein-1 and IL-2Rα) and less severe CRS in the cohort of patients who received concomitant ibrutinib. In this study, the lack of a clear gain of efficacy achieved by CAR T cells administered with concurrent ibrutinib compared with CAR T cells alone might be partially attributable to the retrospective nature of the analysis and to the small size of the analyzed cohorts. In addition, it is relevant to consider that in this cohort all patients had experienced ibrutinib failure before CAR T-cell treatment and therefore the immune deterioration induced by an actively progressive disease could prevail on the potential ibrutinib-induced immunomodulation.

Recently, a group at the University of Pennsylvania presented data from their phase II study showing that the addition of the humanized anti-CD19 CAR T-cell product CTL119 to ≥6-month treatment with ibrutinib was effective in achieving frequent CR rates and even deep remissions in patients with CLL.[113] In this patient cohort, the median number of prior therapies was 2 (range, 1–17, including 6 patients for whom ibrutinib was the frontline treatment and 3 patients who previously received CAR T cells), and 15 of 19 patients (79%) were characterized by poor-risk cytogenetic or molecular aberrations. Among evaluable patients, the ORR at 3 months was 69% (11/16 patients), with a CR rate of 44% (7/16 patients). With a median follow-up of 41 months, the estimated PFS and OS at 48 months were 70% and 84%, respectively. CRS was experienced by 18 of 19 patients (95%) but was grade 3-4 in only 2 patients (11%). Five patients experienced neurotoxicity (grade 1-2: 4 patients; grade 4: 1 patient).

Notably, the already mentioned TRANSCEND CLL 004 trial includes—in the phase I dose-escalation portion—a specific cohort aimed at assessing the combination of JCAR017 and concurrent ibrutinib in patients who either progressed during ibrutinib or have a high-risk disease and are not in CR after ≥6 months of ibrutinib treatment, or carry a mutation-conferring resistance to ibrutinib. Preliminary results showed an ORR of 95% (18/19 patients) and a CR/CRi rate of 47% (9/19).[114] Fourteen patients (74%) experienced CRS (grade 1-2: 13 patients; grade 3: 1 patient) and 6 patients had neurotoxicity (grade 1-2: 3 patients; grade 3: 3 patients).

Overall, available data to date suggest the potential of combination strategies in the CAR T-cell therapy scenario for CLL patients, establishing that the simultaneous administration of ibrutinib and CAR T cells displays promising efficacy and safety

benefits. A number of evidences demonstrate that ibrutinib can restore CLL intrinsic T-cell dysfunctions thus leading to a more efficient CAR T-cell product.[109,115,116] In the future, new studies should further demonstrate the advantage of generating CAR T cells from patients who are still benefiting from ibrutinib treatment so as to avoid the risk that the immune deterioration induced by a progressive disease could overcome the immunomodulatory effects exerted by the drug.

Besides ibrutinib, alternative drugs could be coadministered with CAR T cells to boost their antitumor functions. For instance, anti-PD-1 or anti-PD-L1 monoclonal antibodies are being investigated in association with anti-CD19 CAR T cells for the treatment of relapsed/refractory high-grade NHL, reporting preliminary results of objective responses, together with a manageable safety profile.[117–121] Although single-agent PD-1 inhibitors have so far provided disappointing therapeutic results in CLL,[122] data derived from experiences gained in other BCLs could support the design of treatment strategies exploiting the combination of PD-1 or PD-L1 inhibitors with CAR T cells also in the setting of CLL.

Another potential partner drug that could be administered in combination with CAR T cells is lenalidomide, based on its pleiotropic activity on the immune system and its ability to restore immunologic synapse formation.[59,123,124] In the preclinical setting, it was shown that lenalidomide directly acts on anti-CD19 CAR T cells enhancing their in vivo antitumor functions in a murine NHL model.[125] More recently, lenalidomide has shown to potentiate the therapeutic efficacy of anti-CD23 CAR T cells in a MEC1-based xenograft model of CLL.[126] A note of caution is due, since it is conceivable that the improved antitumor effect, together with the well-known tumor flare reaction observed in lenalidomide-based clinical studies, may increase the CRS rates in CLL patients.[127,128] Despite the strong rationale and potential results, to the best of our knowledge, clinical data on the combination of CAR T cells with lenalidomide are not yet available in the setting of CLL.

Modifying the manufacturing process to optimize CAR T-cell properties

One possible limitation restricting the clinical applicability of a therapeutic regimen combining CAR T cells with immunomodulatory agents is the potential need to administer the selected compound outside of its approved indication, thus requiring to previously ascertain potential toxicities. Interestingly, some evidence already exists that an alternative approach relying on the use of compounds with immunomodulatory effects during the manufacturing process of CAR T cells is feasible and clinically applicable.[129] Although in an early exploratory phase, this approach may grant the possibility to exploit ex vivo the immunomodulatory effect of a selected compound, regardless of the disease for which CAR T cells are investigated.

It has been shown that ibrutinib can be added to the production process of anti-CD19 CAR T cells derived from patients with CLL, thus enhancing the transduction efficiency and the overall production yield of CAR T cells.[130] Moreover, ibrutinib-supplemented CAR T-cell manufacturing resulted in a final product characterized by improved functional features such as a less-differentiated naïve-like phenotype and a lower expression of exhaustion markers (ie, PD-1, TIM-3 and LAG-3).

The PI3K/AKT/mTOR axis is fundamental for T-cell differentiation and function, and the clinically approved PI3Kδ inhibitor idelalisib has demonstrated to have immunomodulatory functions on T cells, mostly consisting in an inhibitory effect on immunosuppressive Tregs.[131,132] Idelalisib was incorporated in the culture medium during the manufacturing process of third-generation anti-CD19 CAR T cells, producing an enrichment of less-differentiated naïve-like T cells, a decrease in exhaustion markers expression and a CD4+:CD8+ ratio normalization.[133] However, a lower production of tumor necrosis factor-α and interferon-γ upon Ag stimulation was noted, and preclinical results of in vivo murine experiments were not conclusive due to premature clearance of CAR T cells within the first days after administration. More recently, it was shown that the dual inhibition of PI3Kγ/δ with duvelisib during the generation process of CAR T cells normalizes the CD4+:CD8+ ratio, increases the frequency and absolute number of naive CD8+CD27+CD45RO– T cells and central memory CD4+ T cells, and also enhances the in vitro cytotoxic potential and the in vivo performance of the final product in a murine CLL model.[134]

The use of other small molecules not endowed with a proven antileukemia effect has also been explored in this preclinical setting. For instance, it has been shown that bromodomain and extraterminal (BET) proteins can modify gene transcription through the recruitment of transcriptional coactivators or repressors.[84] Based on data indicating that the blockade of BET proteins in healthy donor T cells endows properties of stem cell–like and central memory T cells with potent effector function in the setting of adoptive immunotherapy,[135] the BET inhibitor JQ1 was evaluated for its effect on CLL-derived second-generation anti-CD19 CAR T cells.[84] In vitro culture of CAR T cells in presence of the BET inhibitor led to the production of reinvigorated CAR T cells, characterized by improved phenotypical characteristics (eg, decreased expression of T-cell exhaustion markers), increased proliferative capacity upon the encounter of a CD19+ target and enhanced metabolic fitness, as determined by in vitro functional assays.

This type of approach may be of special interest in the context of different tumors where, similarly to CLL, patient-derived T cells exhibit features of exhaustion, especially if we consider that the standard manufacturing procedures of CAR T cells tend to further accelerate the senescence and drive the terminal differentiation of T lymphocytes. In multiple myeloma, for instance, the addition of lenalidomide during the production process improves the antitumor properties of anti-CS1 CAR T cells, also enhancing memory maintenance, Th1 cytokine production and immune synapse formation.[136]

Using alternative cellular vectors for CAR constructs: allogeneic CAR T cells and CAR NK

A further approach to improve CAR-based strategies in CLL is the use of off-the-shelf products, which involves using T cells from healthy donors instead of dysfunctional patient-derived T cells. Of note, additional advantages of this approach include the potential consistent reduction of manufacturing labor and costs, and the possibility to obtain a final product that can be used to treat multiple patients.

One of the main obstacles in this context is the need to minimize the risk of CAR T-cell rejection and/or graft-versus-host disease, and different genetic engineering strategies are currently under evaluation to improve the histocompatibility of the infused products. However, to date, the use of allogenic CAR T cells in CLL has only been reported in the post-allogenic stem cell transplant context (n = 5), where PBMCs from each original donor were used to produce second-generation anti-CD19 CAR T cells.[137]

Natural killer (NK) cells are also under investigation as alternative allogeneic CAR carriers, with the potential advantage of exploiting their major histocompatibility complex–independent cytotoxic functions. Compared to T cells, NK cells combine the innate immune effector functions with the possibility to avoid a full human leukocyte Ag matching, thus preventing the need of obtaining a specific CAR product for each individual patient.[138,139] In addition, they can be generated starting from different sources, including peripheral or umbilical cord blood, hematopoietic stem cells, induced pluripotent stem cells, and cell lines.[140,141]

A group at MD Anderson Cancer Center developed a cord blood-derived anti-CD19 CAR NK-cell product.[142] In this formulation the IL-15 gene was transduced together with the CAR construct, to support the in vivo expansion and persistence of the infused CAR NK cells. After the initial proof of efficacy was achieved with in vitro experiments and in a murine in vivo model,[142] a phase I/II study was initiated for the treatment of patients with relapsed or refractory B-cell malignancies. Preliminary results from this study describe the outcome of the first 11 treated patients,[143] among whom there were 5 patients with CLL including 2 patients with Richter syndrome or accelerated CLL, and who were all ibrutinib-refractory, heavily pretreated (ie, ≥4 prior lines of therapy), and with a high genetic risk disease. At a median follow-up of 13.8 months, the ORR was 73% (8/11 patients) and the CR rate was 64% (7/11 patients) in the entire cohort. Three of 5 patients with CLL (60%) achieved a CR. Unfortunately, DOR could not be evaluated due to the administration of postremission therapy in some of the patients. Since the main limitation of CAR NK-cell therapy is the unsatisfactory expansion and persistence of the administered genetically modified product, an additional interesting result of this study was the demonstration of low-number circulating CAR NK cells in the patients' peripheral blood for at least 12 months after the infusion. In terms of safety, promisingly, none of the patients developed CRS, neurotoxicity, or graft-versus-host disease, and treatment-related adverse events were mainly limited to hematological toxicities, with grade 3 anemia in 2 of 11 patients (18%) and grade 3 and 4 neutropenia in 2 of 11 (18%) and 8 of 11 (73%) patients, respectively.

CONCLUSIONS AND PERSPECTIVES

The treatment scenario of CLL has dramatically changed in the last 20 years with the almost full replacement of chemoimmunotherapy-based treatments with the more recent targeted therapies, including BTK and BCL2 inhibitors.[144] Despite these advances, patients may either show or develop resistance to these drugs, or may present intolerance preventing their use.[25,27] For the high-risk CLL patients, especially those who are refractory or relapse after treatment with targeted drugs, therapeutic options are very limited, and there is a recommendation to consider them eligible for allogeneic transplantation or more investigational cellular therapies.

CLL is one of the diseases where the possibility to successfully produce, even at a large scale, autologous CAR T cells has been first demonstrated, also for patients who had been previously exposed to chemotherapeutic agents potentially affecting the T-cell fitness.[145] However, initial results from clinical trials with CAR T-cell therapy have been somewhat disappointing. This makes it clear that a better definition of patient- and disease-related parameters that potentially compromise the clinical success of CAR T-cell therapy is of utmost importance. One factor affecting CAR T-cell efficacy within clinical trials is the inclusion of subjects who had been previously exposed to multiple lines of treatment. In these patients who are certainly the main candidates to receive a cellular therapy strategy in the clinical practice, the long disease history may have further increased the CLL-related immune system subversion, leading to the production of CAR T cells with intrinsic features of terminal differentiation and functional exhaustion. In the TRANSCEND CLL 004 clinical trial, extensively pretreated patients—including a meaningful proportion of patients who had failed both ibrutinib and venetoclax—were treated with liso-cel, obtaining considerable response rates. However, this cellular immunotherapy was not able to induce a long-term control of the disease in the majority of patients, thus providing evidence that more effective CAR T-cell formulation should be produced and administered for a successful treatment. Several strategies in this direction are currently under investigation, including not only the manufacturing of cellular products with an improved cellular composition and quality, but also the coadministration of immunomodulatory agents capable of reversing T-cell abnormalities and counteracting the immunosuppressive tumor microenvironment.

In addition, other potential approaches aimed at overcoming T-cell impairment and boost CAR T-cell antitumor functions consist in the design of improved CAR constructs (such as third- and fourth-generation CARs) or the incorporation of compounds with immunomodulatory effects within the production workflow leading to CAR T-cell generation. A summary of additional ongoing clinical trials investigating novel anti-CD19 CAR T-cell products in patients with CLL is reported in Table 2. All of these approaches aim to improve the intrinsic functionality of CAR T cells by acting on the patients' immune system or, more specifically, on a wide range of T-cell properties, and are paralleled by extensive studies—going beyond the scope of this article—aimed at identifying strategies to prevent or treat the onset of Ag-negative relapses, such as the production of CAR T cells targeting more than 1 Ag (eg, CD19 and CD20, CD19 and CD22) through the use of dual or tandem CARs (reviewed in ref. [146]).

From a clinical standpoint, in selected patients with adverse disease features, a step forward toward the achievement of better outcomes might be the positioning of CAR T-cell therapy in an earlier phase of the treatment algorithm, possibly in combination with targeted agents. This would allow production and

Table 2

Additional Ongoing Clinical Trials Evaluating New Anti-CD19 CAR T-cell Products in Patients With CLL

Product Administered	Modification of the CAR Product	NCT Number
SYNCAR-001 + STK-009	Administration of anti-CD19 CAR T cells expressing a mutated IL-2-Rβ plus a half-life extended pegylated IL-2 (STK-009)	NCT05665062
HuCART19-IL18	Fourth-generation 4-1BB CAR T product secreting IL-18	NCT04684563
CD19-CD34t metabolically programmed CAR T cells	Ameliorated purification of the CAR T-cell product via CD34 selection by adding a CD34 tag to the CAR construct; T cells exposed to priming conditions leading to a metabolically enhanced CAR T-cell product akin to a Th1/17 hybrid cell	NCT05702853
CAR19T2	Anti-CD19 CAR T cells incorporating CD28 and TLR2 costimulatory domains	NCT05613348
SAGAN	Anti-CD19 CAR T cells with CD28 and 4-1BB as costimulatory domains	NCT01853631
TBI-2001	Anti-CD19 CAR T cells including costimulatory sequences that lead to the activation of cytokine-related JAK/STAT signaling pathways.	NCT05963217
MB-CART19.1	Anti-CD19 CAR with CD4/CD8 enriched T cells	NCT03853616
CD19 CAR T cells	Anti-CD19 CAR T cells with CD28 and 4-1BB as costimulatory domains	NCT03676504

CAR = chimeric antigen receptor; CLL = chronic lymphocytic leukemia; NCT = National Clinical Trial number.

administration of CAR T cells with a more preserved or restored fitness and reliance on potentially synergistic antitumor activities. In line with the goal of administering more functional CAR T cells, a further future perspective may be represented by the possibility of generating allogeneic off-the-shelf cellular products. This approach has the potential advantage of obtaining a more streamlined production of CAR T cells, also avoiding the interpatient variability originating from an autologous source of T cells, and the reliance on effector arms that were unaffected by long-lasting and detrimental interactions with the tumor.

AUTHOR CONTRIBUTIONS

CV and MC designed the review, reviewed the literature, and wrote the article. VG and FP contributed to literature review and to figures preparation. All authors revised the article, read, and approved the submitted version.

DISCLOSURES

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