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Review Article

CAR-T Cells in Chronic Lymphocytic Leukemia

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Abstract. The treatment outcomes of patients with chronic lymphocytic leukemia (CLL) have considerably improved with the introduction of targeted therapies based on Bruton kinase inhibitors (BTKIs), venetoclax, and anti-CD20 monoclonal antibodies. However, despite these consistent improvements, patients who become resistant to these agents have poor outcomes and need new and more efficacious therapeutic strategies.
Among these new treatments, a potentially curative approach consists of the use of chimeric antigen receptor T (CAR-T) cell therapy, which achieved remarkable success in various B-cell malignancies, including B-cell Non-Hodgkin Lymphomas (NHLs) and B-acute lymphoblastic Leukemia (ALL). However, although CAR-T cells were initially used for the treatment of CLL, their efficacy in CLL patients was lower than in other B-cell malignancies. This review analyses possible mechanisms of these failures, highlighting some recent developments that could offer the perspective of the incorporation of CAR-T cells in treatment protocols for relapsed/refractory CLL patients.

Keywords: Chronic Lymphoid Leukemia; CLLia; CAR-T Cells; CLL relapsed/refractory.

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Introduction. Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in adult subjects in Western countries, with a mean age at diagnosis around 69 years.[1] At the cellular level, this leukemia is characterized by the progressive accumulation of mature CD5-positive B-lymphocytes in the peripheral blood, bone marrow, and secondary lymphoid organs.[2] The clinical stratification of CLL patients currently relies on the Rai and Binet classification: low-risk (Rai 0/Binet A), intermediate-risk (Rai I and II or Binet B), and high-risk (Rai III and IV or Binet C). The molecular stratification relies on molecular prognostic markers that include cytogenetic abnormalities and the assessment of the mutational status of immunoglobulin heavy-chain variable (IGVH) and TP53 genes. High-risk markers are represented by the presence of *TP53* mutations, del(17p), a complex karyotype, or unmutated IGVH.[2]

The treatment algorithm of CLL radically changed over the past few years, becoming almost completely chemo-free. Therefore, according to the current treatment recommendations, patients with the symptomatic disease receive a covalent Bruton tyrosine kinase inhibitor (i.e., ibrutinib, acalabrutinib, zanubrutinib), the BCL-2 inhibitor venetoclax +/- anti-

CD20 monoclonal antibody, or a combination of the classes.[2,3] In relapsed/refractory (R/R) patients, exposure to one class of drug does not prevent the patient from being treated with the other one, with effective results.[1] However, the outcomes of double-resistant patients are poor, with an overall survival of only a few months.[4-7]

Therefore, there is an absolute need to develop novel therapies for these double-resistant patients who have a dismal prognosis. Concerning cellular therapy, slightly better results were obtained from allogeneic stem cell transplantation ().[7,8] Studies carried out in the last years have suggested that chimeric antigen receptors (CAR) designed to target T cells to antigens expressed on CLL cells may offer a therapeutic opportunity for these patients. In this review, we explored and analyzed the current available evidence on the use of CAR-T cell therapy in CLL patients. The most significant results of trials with CAR-T in CLL and Richter's transformation are summarized in **Table 1**.

CD19-targeted CAR-T Cells in CLL. CD19-targeted CAR-T cell therapy was successfully experimented with for the treatment of several B-cell malignancies, including B-cell lymphomas and B-acute lymphoblastic leukemia.[9-19] Four CAR-T cell products are commercially available and approved by the FDA for clinical use: Axicabtagene ciloleucel (Axi-Cel), Brexucabtagene-autoleucel (Brexu-Cel), Tisagenlecleucel (Tisa-Cel) and Lisocabtagene maraleucel (Liso-Cel). These CAR-T products were also evaluated in CLL patients.

Liso-Cel. Liso-Cel is a second-generation anti-CD19 CAR-T cell that utilizes the 4-1BB costimulatory domain and is produced from separated subsets of CD4- and CD8-positive cells to make a 1:1 CD4/CD8 ratio in CAR-T.[20]

The first study involving the investigation of Liso-Cel in CLL patients was performed on 24 R/R CLL patients, in large part with high-risk features (such as complex karyotype and/or 17p deletion).[21] The overall response rate (ORR) was 74%, with 21% complete response (CR) and 53% partial response (PR).[21] Recently, long-term follow-up of this study was reported, including the analysis of phase I and II of this study; the study included a total of 49 patients with R/R CLL and 10 patients with Richter's transformation.[22] Patients received CAR-T cell therapy without (30 patients) or with concurrent ibrutinib treatment (19 patients); patients were evaluated on day +28 for response by standard criteria, and their measurable residual disease (MRD) status was assessed by multiparameter flow cytometry (MFC) and IGH next-generation sequencing.[22] Of the enrolled patients, 96% had complex karyotype and/or 17p deletion, and 94% were ibrutinib-refractory. ORR and CRR were 70% and 17%, respectively, and the median duration of response (mDoR) was 21.3 months; 5-year overall survival (OS) and progression-free survival (PFS) were 35% and 21%, respectively. MRD by MFC was observed in 75% of patients, and 72% of these patients were negative for NGS; in uMRD patients by NGS, the mDoR was 55.9 months.[22]

In 2018, Siddiqi et al. reported the first results of the monotherapy section of the TRASCEND CLL 004 phase I/II clinical trial, which initially involved 10 R/R CLL patients.[23] The patients with standard risk received 3 and those with high risk 2 prior lines of therapy, including a Bruton's tyrosine kinase inhibitor (BTKi); after lymphodepletion, the patients received a single-infusion of Liso-Cel at either dosage 1 (DL1, $5x10^7$CAR-T cells) or dosage 2 (DL2, $1x10^8$CAR-T cells).[23] At 30 days post-CAR-T cell infusion, 75% of patients displayed an OR, with 50% of CR; all patients with CR remained with a negative MRD status at 3 months post-dose.[4] In 2022, the same authors reported the results of phase I of the TRASCEND CLL 004 study, involving a total of 23 R/R CLL patients, including 100% of patients who had prior ibrutinib and 65% venetoclax treatment; 83% of these patients had high-risk features, including *TP53* mutations and del (17p).[24] In this study, 74% of patients had cytokine release syndrome (CRS, 9% of grade 3) and 39% of neurological events (22% of grade 3). ORR was 42.9%, and CR was 11%; the patients who achieved MRD negativity were 75% and 60% in peripheral blood and bone marrow, respectively.[24] The results of this study have represented the basis for a phase II study at $1x10^8$CAR-T cell dosage. A more recent report of this phase I/II study reported the results of 117 CLL patients.[25] In the patients treated with dosage level 2 (DL2, $1x10^8$CAR-T cells), 88% (95/108) were BTKi refractory, 78% (89/108) were venetoclax refractory, 61% (66/108) were double-refractory. The efficacy evaluation at DL2 showed an ORR of 42.9% with 18.4% CR; the median DoR was 35.3 months, with a median follow-up of 19.7 months.[25] The same efficacy was reported in the evaluable double-refractory patients [ORR was 42% (21/49), with 18% (9/49) of CR, median DoR 35.25 months]. Undetectable MRD was obtained in 64% of double refractory patients in peripheral blood and 59% in bone marrow. The median duration of response among patients with CR was not reached. In exploratory analyses of undetectable MRD and progression-free survival, PFS was 26.2 months in patients with uMRD and 2.8 months in those with detectable MRD in blood. Grade 3 CRS occurred in 8.5% of cases, and grade 3 neurological events in 17.9% of cases.[25] With a longer follow-up (median follow-up of 23.5 months), Lico-Cel continues to demonstrate durable CR, high MRD rates, and a manageable safety profile in this population of heavily pretreated CLL patients.[26]

Tisa-Cel. Tisa-Cel is a second-generation anti-CD19

Table 1. CAR-T cell therapy in chronic lymphocytic leukemia. The most relevant clinical studies are reported.

Study	Product	Patients (n)	Median age (years)	Median previous lines of therapy	TP53 disrupted patients	IGHV unmutated patients	Ibrutinib-refractory	Venetoclax-refractory	Double-refractory	ORR	CR	uMRD	Survival variables (months)	CRS (any grade/ grade 3-5)	Neurotoxicity (any grade/ grade 3-5)
Turtle CJ, JCO 2017 (Phase I) [21]	Lisocabtagene maraleucel	49	61 (40-73)	5 (3-9)	96% (46/49)	N/A	96% (47/49)	25% (6/24)	N/A	70%	17%	75%	mPFS 8.5 mDOR 21.3	83%/8%	33%/25%
Liang EC, Blood Adv 2023 [22]	Lisocabtagene maraleucel	49	61 (55-67)	5 (4-7)	67% (33/49)	N/A	88% (43/49)	39% (19/49)	N/A				mPFS 8.9 mDOR 18.9		
TRANSCEND CLL 004 (Phase I/II) [25]	Lisocabtagene maraleucel	117	65 (59-70)	5 (3-7)	46% (54/108)	46% (54/108)	88% (95/108)	78% (89/108)	61% (66/108)	47%	18%	65%	mPFS 11.9 mDOR 35.3	85%/9%	45%/18%
Frey NV, JCO 2020 (phase I) [28]	Tisagenlecleucel	38	62 (49-76)	3.5 (2-7)	28% (9/38)	72% (23/38)	28% (9/38)	3% (1/38)	N/A	44%	28%	N/A	mPFS 1.8	63%/24%	16%/8%
ZUMA-8 (Phase I) [32]	Brexucabtagene-autoleucel	15	63 (52-79)	>2	27% (4/15)	N/A	N/A	N/A	N/A	47%	13%	N/A	N/A	80%/7%	73%/20%
Cappell KM, JCO 2020 (Phase I) [35]	Axicabtagene ciloleucel	7	61 (48-68)	4 (1-7)	N/A	N/A	N/A	N/A	N/A	88%	63%	N/A	mEFS 40.5	NA/50%	NA/25%
Euplagia-1/CP0101-CLL (Phase I/II) [36]	GLPG 5201	7	66 (58–71)	>1	N/A	N/A	N/A	N/A	N/A	100%	86%	N/A	N/A	57%/0%	0/0
Gauthier J, Blood 2020 (Phase I/II) [46]	antiCD19 CAR T-cell + ibrutinib	19	65 (56-69)	5 (4-7)	74% (14/19)	N/A	100% (19/19)	32% (6/19)	N/A	83%	22%	68%	1y PFS 38%	74%/0%	26%/26%
Gill S, Blood Adv 2022 (Phase II) [47]	anti-CD19 humanized binding domain CAR-T cells (hu-CART 19) + ibrutinib	19	62 (42-76)	2 (1-17)	68% (13/19)	N/A	Exposed 32% (6/19)	N/A	N/A	79%	44%	72%	4y PFS 70%	84%/10%	21%/5%

IGHV: immunoglobulin heavy chain variable region genes; ORR: overall response rate; CR: complete response; uMRD: undetectable measurable residual disease; CRS: cytokine release syndrome; N/A: not available; mPFS: mefian progression free survival; mDOR: median duration of response; mEFS: median event free survival.

CAR-T cell with 4-1BB costimulatory domain and CD8a hinge region.[27]

The initial study carried out using autologous T cell transduced with a CD19-directed CAR (CTL-109, Tisa-Cel) reported the results observed in 14 R/R CLL patients: the ORR was 57%, with 4 CR and 4PR.[28] A final evaluation of this study involved the treatment of 38 R/R CLL patients with CTL109, showing an ORR of 44%, with 28% of CR.[29] Patients achieving a CR showed an OS longer than those who did not, with a mOS not reached vs 64 months.[8] The median PFS was 40.2 months in patients with CR, compared to 1 month in those without CR.[29]

Interestingly, Melenhorst et al. reported the cellular and molecular analysis of two CLL patients exhibiting long-term remission after a CTL 109 infusion performed in 2010.[9] The anti-leukemia activity of anti-CD19 CTL 109 cells displayed two distinct phases: an initial phase characterized by $CD8^+$or $CD4^-CD8^-Helios^{hi}$ γδ CAR-T cells, followed by the predominance of proliferative $CD4^+$CAR-T cells in the ensuing years.[30] Surprisingly, $CD4^+$T cells seem responsible for mediating anti-CD19 cytotoxic activity; functional characterization of these cells, as well as upregulation of antigen-mediated signaling pathways and upregulation of G 2MK, G2MA, and PRF1, support this view.[30]

Brexu-Cel. Brexu-Cel is a second-generation anti-CD19 CAR-T cell that utilizes the CD28 costimulatory domain and CD28 hinge region; an additional step is introduced in the manufacturing of these CAR-T cells to remove malignant cells from the leukapheresis product.[31]

The ZUMA-8 phase I/II clinical trial explored the safety and efficacy of Brexu-Cel (KTE-19), anti-CD19 CAR-T cells. In phase I, the enrolment of 12-18 patients was planned to assess dose-limiting toxicities, while in phase II, 60 patients would be enrolled to assess safety and efficacy.[32] Phase I results of the ZUMA-8 trial involving 15 R/R CLL patients have involved four cohorts of patients: cohorts 1 and 2 were treated with $1x10^6$ and $2x10^6$ KTE-19 cells/Kg, cohort 3 involved the enrolment of low-tumor burden patients and cohort 4 any patients.[33] The enrolled patients were heavily pretreated. Seven out of fifteen patients exhibited an objective response, with 3 CR; two of these patients with a low tumor burden had a CR.[33] Significant CAR-T cell expansion occurred in 4 patients, mostly in those with low tumor burden.[33]

Axi-Cel. Axi-Cel is a second-generation anti-CD19 CAR-T cell utilizing CD28 costimulatory domain and CD28 hinge region.[34] Axi-Cel, autologous anti-CD19 CAR-T cells have been explored in a few R/R CLL patients. An initial study by Kochenderfer et al. also included 4 R/R CLL patients.[35] A later study included 8 R/R CLL patients and explored the long-term effects of Axi-Cel CAR-T cell therapy. The ORR was 88%, with 63% of CR and 25% of PR; 3/5 of the patients who achieved a CR maintained a response ≥3 years.[36] The median duration of ongoing responses was 82 months; median EFS was 40.5 months.[36]

CAR-T GLPG 5201. A recent study reported the first results obtained using GLPC 5201, a second-generation anti-CD19/4-1BB CAR-T product, administered as a fresh product in a single dose, without the need for cryopreservation within 7 days of apheresis.[36] Seven patients were enrolled in the ongoing phase I study; all these 7 patients were diagnosed as R/R CLL, and 4/7 had Richter's transformation (RT); no grade 3-4 CRS or neurologic events were observed; 7/7 patients responded to the treatment, with 6/7 CRs; responding patients remained in CR at a 7-month follow-up.[37] These results, although still preliminary, are considered very promising.

A more extensive report of this study was presented at the ASH Meeting 2023 and involved a total of 12 patients: 5 with R/R CLL and 7 with RT.[38] ORR and CRR were 86% and 7!% for RT patients.[38] Robust expansion of CAR-T cells was observed, with detectable CAR-T cells up to 9 months after CAR-T cell infusion.[38] Infused CAR-T cells exhibited a preserved early memory phenotype for $CD4^+$ and $CD8^+$ CAR-T cells.[38]

Varnicambtagene autoleucel. Varnicambtagene autoleucel (Var-Cel), an academic anti-CD19 CAR product manufactured by the Hospital Clinic of Barcelona, Spain, was used primarily for the treatment of non-Hodgkin lymphoma in the CART19-BE-01 trial. Recently, the safety profile and the efficacy of Var-Cel were explored in 20 R/R CLL patients, including 63% of patients with RT.[39] Var-Cel manufacturing was performed in a median of 8 days; with a median follow-up of 8.4 months, the ORR was 83%, with 83% of patients achieving an MRD negativity; CR in BM and PB was 67%, achieving CR at the level of extramedullary sites.[39] PFS was shorter in patients with RT and with higher BM infiltration.[39] These preliminary observations support further development of Var-Cel in these high-risk populations of CLL patients.

CD19-targeted CAR-T Cell Therapy in Richter's Transformation. Richter's transformation is a relatively rare event involving the development of aggressive B-cell lymphoma in patients with CLL or SLL; the prognosis is dismal, with a short OS.[40] **Table 2** reports the results of the trials involving CAR-T use in Richter's transformation.

Benjamini and coworkers reported the analysis of the safety and efficacy of CD19 CAR-T cells in 8 patients with Richter's transformation generated using a retroviral vector encoding a CAR comprising FMC63 anti-CD19 ScFv, linked to a CD28 costimulatory domain

Table 2. CAR-T cell therapy in Richter's transformation. The most relevant clinical studies are reported.

Study	Product	Patients (n)	Median age (years)	Median previous lines of therapy	TP53 disrupted patients	IGHV unmutated patients	Ibrutinib-refractory	Venetoclax-refractory	Double-refractory	ORR	CR	uMRD	Survival variables (months)	CRS (any grade/ grade 3-5)	Neurotoxicity (any grade/ grade 3-5)
Benjamini O, Blood 2020 (Phase I/II) [38]	antiCD19 CAR T-cell	8	64 (54-73)	2 (1-3)	83% (5/6)	N/A	87% (7/8)	75% (6/8)	62% (5/8)	71%	71%	N/A	6m PFS 62%	87%/37%	37%/25%
Kittai AS, Blood Adv 2020 [39]	Axicabtagene ciloleucel	9	64 (40-77)	4 (2-6)	33% (3/9)	71% (5/7)	N/A	N/A	N/A	100%	62%		6m PFS 87%	100%/11%	44%/33%
Bensaber H, Blood 2022 [40]	Axicabtagene ciloleucel or Tisagenlecleucel	12	60 (42-76)	3 (0-3)	57% (4/7)	67% (4/6)	7/12 (58%)	5/12 (42%)	5/12 (42%)	50%	42%	N/A	N/A	83%/25%	42%/25%

IGHV: immunoglobulin heavy chain variable region genes; ORR: overall response rate; CR: complete response; uMRD: undetectable measurable residual disease; CRS: cytokine release syndrome; N/A: not available; PFS: progression free survival.

and CD3-zeta intracellular signaling domain.[38] All these patients had a prior history of CLL, and 83% of them had del 17p/*TP53* mutation.[41] Patients had at least 3 previous lines of therapy, including ibrutinib and venetoclax treatment.[41] Of these patients, 71% had a CR following treatment with CAR-t cells, and 2 patients proceeded to allo-HSCT.[38] Three patients had grade 3-4 CRS.[41]

Kittai et al. reported a retrospective analysis of 9 patients with Richter's transformation treated with Axi-Cel in the context of an institutional experience of off-label use of these CAR-T cells.[39] Eight of these 9 patients were evaluated for response, and most of them continued treatment with BTK inhibitor.[42] All of these patients displayed a response, with 5 CR and 3 PR.[42] The median follow-up was 6 months: one patient relapsed during these 6 months, while the remaining 7 patients remained in remission.[42]

Bensader et al. have retrospectively analyzed 12 patients with Richter's transformation treated with either Axi-Cel (5 patients) or Tisa-Cel (7 patients), with 50% ORR and 42 CRs.[43] Of the treated patients, 25% had grade 3 CRS. All the patients included in this analysis were heavily pretreated.

Blackmon reported 7 patients who have been treated with Liso-Cel and relapsed after CAR-T cell therapy with Richter's syndrome.[41] All these patients had CLL with high-risk factors prior to CAR-T cell therapy (including *TP53* mutations, del17p, *NOTCH1* mutations, and S*F3B1* mutations.[44]

CD19-targeted CAR-T Cell Therapy in Combination with Ibrutinib. Several clinical observations have strongly supported the rationale of associating CAR-T cells with ibrutinib administration: (i) ibrutinib treatment of CLL patients increases *in vivo* persistence of activated T cells, decreases Treg/CD4$^+$T cell ratio, and decreases the immunosuppressive properties of CLL cells;[45] (ii) previous cycles of ibrutinib therapy improve the *in vivo* expansion of CD19-directed CAR-T cells, in association with decreased expression of the immunosuppressive molecules PD-1 and CD200 on the membrane of T-lymphocytes;[46] (iii) Ibrutinib administration after CAR-T cell infusion decreases cytokine release syndrome;[47] assays demonstrated enhanced anti-CLL T-cell killing function during ibrutinib-rituximab treatment, including a switch from predominantly CD4+ T-cell: CLL immune synapses at baseline to increased CD8+ lytic synapses on-therapy.[48]

Gauthier et al. have conducted a pilot study to evaluate the safety and feasibility of administering ibrutinib concurrently with CD19 CAR-T cell therapy.[49] Thus, 19 R/R CLL patients, with a median number of 5 prior therapies, have been treated with Liso-Cel and ibrutinib; 13 of these patients received the scheduled ibrutinib treatment and were evaluated for safety and response to the treatment. The 4-week ORR was 83%, with 61% of patients achieving an MRD-negativity in bone marrow, as assessed by NGS.[46] In these patients, the 1-yr OS and PFS were 86% and 59% respectively. Patients treated with ibrutinib and CAR-T cells displayed lower CRS severity and lower serum concentrations of CRS-associated cytokines.[49]

Gill et al. reported the results of a phase II clinical study in which anti-CD19 humanized binding domain CAR-T cells (hu-CART 19) were used in combination with ibrutinib in 19R/R CLL patients not in CR despite ≥6 months of ibrutinib.[50] CRR evaluated at 3 months was 44%; at 12 months, 72% of patients had no measurable MRD.[47] Of 15 patients with undetectable MRD at 6 months, 13 remained in CR at the last follow-up.[50]

Mechanisms of Resistance to CD19 CAR-T Cell Therapy in CLL. As mentioned above, clinical trials of CD19-targeted CAR-T cell therapy have shown a durable antitumor response in a limited proportion of R/R CLL patients, around 20-25%, while most patients are resistant to this treatment.

Several studies have explored the characteristics of CAR-T cells, as well as the genomic abnormalities and other molecular features of CLL that could correlate with response or resistance to CAR-T cells.[51]

Frazetta and coworkers have investigated the transcriptomic profile of CAR-T cells of R/R CLL patients undergoing CAR-T cell therapy: responding patients were enriched in memory-related genes, whereas T cells from non-responders upregulated the expression of genes involved in effector differentiation, glycolysis, exhaustion, and apoptosis.[52] Furthermore, patients with sustained mission showed an elevated frequency of CD27$^+$CD45RO$^-$CD8$^+$T cells with memory-like characteristics before CAR-T cell generation.[52] An additional feature of functionally active CAR-T cells consisted of the production of STAT3-related cytokines and serum IL6 levels correlated with CAR-T cell expansion.[52]

Resting CD8$^+$lymphocytes in CLL have an altered mitochondrial profile (increased mitochondrial respiration, membrane potential, and level of reactive oxygen species), which impairs the development of efficacious CAR-T cells in these patients.[53] In line with these observations, CD8$^+$CAR-T cells of CLL patients responding to CAR-T cell therapy have increased mitochondrial mass with respect to non-responders, and this property is correlated with CAR-T cell expansion *in vivo*.[53]

CAR-T cells harbor an engineered receptor that is delivered through lentiviral vector integration and may modify the cellular genome by insertional mutagenesis. In some patients, retroviral integration within the host genes, such as *the TET2* gene[50] or genes involved in cell

signaling and chromatin modification pathways,[54] may promote therapeutic T cell proliferation.

As discussed above, non-responding or partially responding CLL patients exhibited marginal or no expansion of their transformed CAR-T cells; in contrast, patients with full response possessed CAR-T cells with pronounced proliferative capacity and sustained persistence.[55] In addition to these observations, the level and the duration of CAR expression on the surface of T cells are a key determinant of clinical efficacy since patients exhibiting complete remission have a permanent CAR expression. In contrast, non-responding patients lost their cell-surface CAR detection at the time of relapse, thus suggesting that CAR extinction at the cell surface is an important mechanism of resistance to therapy.[56] Epigenetic mechanisms seem to be responsible for the extinction of CAR expression; particularly, the bromodomain and extra-terminal (BET) family of chromatin adaptors seem to be involved in this epigenetic silencing of CAR expression.[56] BET protein inhibition decreased TET2 levels and improved the proliferative capacity of exhausted CAR-T cells and their antitumor activity.[56]

The reduced CAR-T cell expansion and persistence may also be attributed to the activation of naturally occurring negative immune checkpoint molecules (such as PD-1, TM-3, LAG-3, and CTLA-4). Agarwal et al. have explored whether the disruption of the co-inhibitory receptors CTLA-4 or PD-1 could restore CAR-T function.[57] CRISPR-Cas9-mediated deletion of CTLA-4 in preclinical models of leukemia and in T-cells from patients with CLL who previously failed CAR-T cell treatment reinvigorates dysfunctional T cells, thus suggesting a strategy for increasing patient responses to CAR-T cell therapy.[57]

CD20-targeted CAR-T Cells in CLL. Only an ongoing phase I clinical trial is evaluating the safety and efficacy of CD20-targeted CAR-T cells in CLL patients.[54] MB-106 is a fully human third-generation CD20-targeted CAR-T product with both CD28 and 4-1BB costimulatory domains; an ongoing phase I/II clinical trial is evaluating the safety and the efficacy of MB-106 in R/R $CD20^+$ B-cell malignancies.[58] The results on the first 16 patients included 1 patient with R/R CLL achieving CR, with MRD-negative status following treatment with MB-106.[58]

Other clinical trials have started the evaluation of bispecific CAR-T cells targeting both CD19 and CD20 antigens. In phase I clinical trial enrolling patients with B-cell lymphomas and CC, it was evaluated the safety and the efficacy of LV20.19, a CAR construct targeting both CD20 and CD19: 3 patients with R/R CLL were treated, with two patients achieving CR and one patient achieving PR.[59]

Another study of tandem CAR-T targeting CD19 and CD20 also included one R/R CLL patient, who achieved a CR to this therapy.[60]

Park et al. reported the initial evaluation of a third-generation CAR-T targeting CD19 and containing two costimulatory domains, CD28 and 4-1BB [61]. In the context of a phase I evaluation of CAR-T cells developed using this CAR construct, 9 patients with R/R CLLL and 2 with Richter's transformation were evaluated: 2/9 R/R CLL and 1 / 2 patients with Richter's transformation achieved a CR.[61] In the same phase I study, 7/8 and 2/2 patients with large B cell lymphoma and follicular lymphoma, respectively, achieved a CR.[61]

Allogeneic CAR-T Cells. Most of the studies performed with CAR-T cells in CLL, as well as in other tumors, involve the use of autologous, patient-derived CAR-T cells. However, allogeneic CAR-T (alloCAR-T) cells may represent a treatment alternative to autoCAR-T cells. Two types of allogeneic CAR-T cells can be utilized: donor-compatible donors and off-the-shelf CAR-T cells. Donor-derived CAR-T cells are CAR-T cells obtained from HLA-compatible donors or donors in patients with a history of allo-HSCT. HLA compatibility is required for donor-relative CAR-T, while "off-the-shelf" CAR-T cells require techniques to reduce the rejection risk of non-HLA-matched products. An example of the technology used for off-the-shelf CAR-T cells is given by UCART 19 cells, recently used for the treatment of B-ALL patients.[62] UCART 19 is a first-in-class "off-the-shelf" alloCAR-T cell based on genetic engineering of T cells from a normal donor to express an anti-CD19 (murine 4G7 scFv)/4-1BB/CD3ζ CAR together with RQR8 safety switch (a suicide gene); T cells were further genome-edited through disruption of T cell receptor alpha chain (to prevent GVHD) and CD52 gene knockout (to protect donor cells from early rejection).[63]

Studies with "off-the-shelf" CAR-T cells have not yet been reported in CLL. Only one study with donor-derived alloCAR-T cells reported the treatment of a few R/R CLL patients.[64] In fact, Brudno et al. reported the results of a phase I study involving the treatment of 20 patients with B-cell malignancies, including 5 R/R CLL patients.[60] In this study, CAR-T cells were generated from the patient's prior allo-HSCT donor. They were based on a CAR composed of a murine scFv anti-CD19, a CD28 costimulatory domain, and a CD3ζ T-cell activation domain.[64] None of the treated patients developed new onset GVHD. Among the 5 CLL patients included in the study, 1 achieved a CR, 1 a PR, and 3 did not respond to the treatment.[64] Interestingly, the CLL patient achieving a CR showed rapid elimination of leukemic cells after alloCAR-T at the level of bone marrow, peripheral blood, and lymph nodes.[64]

Conclusions. CLL was the first hematopoietic malignancy for which CAR-T cells were administered

for therapeutic purposes. However, subsequent development was challenged by the finding of inferior responses observed in CLL patients compared to those observed in other B-cell malignancies. These more limited responses are related to the presence of patient comorbidities, immunodeficiency, and immunosubversion of the CAR-T cell product.

Thus, none of the four approved CAR-T cell products, Axi-Cel, Brexu-Cel, Lisi-Cell, and Tisa-Cel for NHL and B-ALL, are currently approved for CLL. The recent data reported for Liso-Cel could provide sufficient support for its approval for the treatment of R/R CLL patients.

Although preliminary and based on only 12 R/R CLL patients, the results observed using fresh, unfrozen CAR-T cells GLPG 5201 were particularly promising, manufactured, and reinfused to the patients within 7 days of blood draw. Frozen peripheral blood mononucleated cells as starting material and frozen CAR-T infusion products maintain high antitumor activity, but fresh CAR-T infusion product exhibits higher antitumor reactivity.[65] Future studies will assess whether freshly manufactured CAR-T cells have a more potent antitumor activity compared to CAR-T cells manufactured using frozen cell preparations for the treatment of R/R CLL patients.

The development of allogeneic CAR-T cells and natural killer cells from healthy donors may represent a promising solution to address the reduced fitness of T cells observed in CLL patients.

Combined treatment with ibrutinib and CAR-T cells appears to be a therapeutic strategy associated with increased efficacy due to synergistic effects between BTKi and cell therapy and provides some safety benefits. Future studies will clarify whether next-generation BTK inhibitors in association with CAR-T cells could further improve the therapeutic benefit observed with ibrutinib.

At the moment, the role of CAR-T cell therapy is limited to CLL patients with relapsed/refractory disease. Additional improvements in the safety and efficacy of CAR-T cells are required to integrate CAR-T cell therapy in earlier lines of treatment and patients with Richter's transformation. It is of interest to note that some RT patients treated with CAR-T cell therapy achieved long-term remission with prolonged survival.[66]

Interestingly, a very recent study supports the targeting of CLL cells expressing a tumor-specific antigen, a B-cell receptor light chain neoepitope defined by a characteristic point mutation (IGLV3-21^{R110}), for selective targeting of a poor-risk subset of CLL with CAR-T cells.[67] CAR-T cells targeting this tumor neoantigen exert a significant anti-leukemia effect, sparing normal B cells.

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