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EDITED BY

Gaurav Prakash,
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REVIEWED BY

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University of Texas MD Anderson
Cancer Center, United States
Charanpreet Singh,
Post Graduate Institute of Medical
Education and Research (PGIMER),
India

*CORRESPONDENCE

R. Leo Sakemura
✉ Sakemura.Reona@mayo.edu

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Is CD19-directed chimeric antigen receptor T cell therapy a smart strategy to combat central nervous system lymphoma?

Kotaro Miyao¹, Hirofumi Yokota² and R. Leo Sakemura^{3,4*}

¹Department of Hematology and Oncology, Anjo Kosei Hospital, Anjo, Japan, ²Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan, ³T Cell Engineering, Mayo Clinic, Rochester, MN, United States, ⁴Division of Hematology, Mayo Clinic, Rochester, MN, United States

Primary central nervous system lymphoma (PCNSL) is a rare form and aggressive type of diffuse large B-cell lymphoma (DLBCL) that occurs in both immunocompetent and immunocompromised adults. While adding rituximab to chemotherapeutic regimens resulted in dramatic improvement in both progression-free survival and overall survival in patients with non-central nervous system (CNS) DLBCL, the outcomes of PCNSL are generally poor due to the immune-privileged tumor microenvironment or suboptimal delivery of systemic agents into tumor tissues. Therefore, more effective therapy for PCNSL generally requires systemic therapy with sufficient CNS penetration, including high-dose intravenous methotrexate with rituximab or high-dose chemotherapy followed by autologous stem cell transplantation. However, overall survival is usually inferior in comparison to non-CNS lymphomas, and treatment options are limited for elderly patients or patients with relapsed/refractory disease. Chimeric antigen receptor T (CAR-T) cell therapy has emerged as a cutting-edge cancer therapy, which led to recent FDA approvals for patients with B-cell malignancies and multiple myeloma. Although CAR-T cell therapy in patients with PCNSL demonstrated promising results without significant toxicities in some small cohorts, most cases of PCNSL are excluded from the pivotal CAR-T cell trials due to the concerns of neurotoxicity after CAR-T cell infusion. In this review, we will provide an overview of PCNSL and highlight current approaches, resistance mechanisms, and future perspectives of CAR-T cell therapy in patients with PCNSL.

KEYWORDS

CAR-T cell, PCNSL, CNS, CNS penetration, local administration

Introduction

Primary central nervous system lymphoma (PCNSL) is a rare malignancy with an annual incidence of 4-7 per one million people in the United States (1, 2). According to the World Health Organization classification, PCNSL is categorized as a sub-type of aggressive non-Hodgkin lymphoma (NHL) which develops in the central nervous system (CNS) (3–5). Unlike most other CNS malignancies, PCNSL is often responsive to chemotherapy and/or radiation therapy. However, outcomes are inferior compared to non-CNS lymphomas, with a median overall survival of 1.5 months when untreated (6). Furthermore, the prognosis for relapsed and/or refractory (R/R) PCNSL is significantly worse, especially when patients are not eligible for autologous stem cell transplantation (ASCT) or relapse after ASCT.

Chimeric antigen receptor T (CAR-T) cell therapy has emerged as a potent and potentially curative therapy in hematological malignancies (7–9). Pivotal clinical trials of CD19-directed CAR-T (CART19) cell therapy demonstrated unprecedented results in non-CNS B-cell lymphomas and acute lymphoblastic leukemia (ALL), leading to several FDA approvals of CART19 cell products (10–21). Although novel therapeutic approaches have improved overall response and survival in non-CNS lymphomas, the application of CAR-T cell therapy in PCNSL is not yet established. Nevertheless, recent clinical data have suggested efficacy and tolerable safety of CART19 cell therapy in secondary CNS lymphoma as well as PCNSL (22–25).

In this review article, we will focus on CAR-T therapy in patients with PCNSL, highlighting outcomes of reported cases and offering future perspectives to overcome resistance and improve CAR-T cell activity within the CNS.

Overview of PCNSL

PCNSL is a highly aggressive and rare NHL which includes lesions in the brain, spinal cord, cerebrospinal fluid (CSF), or eyes (3). Although rare, the incidence of PCNSL has been increasing in recent years, especially in patients older than 65 (1). Patients with immunosuppressive conditions such as human immunodeficiency virus 1 (HIV-1) infections can develop PCNSL, but the etiology and treatment are separate from immunocompetent patients with PCNSL (26). In this review article, we will mainly discuss PCNSL developed in immunocompetent patients and briefly review PCNSL in patients with HIV-1 infection.

Frontline treatment strategies of PCNSL have been improving. In the past, whole brain radiotherapy (WBRT) alone was the standard therapy for decades. WBRT showed a strong initial response, but the relapse rate was high and showed poor survival (27). Chemotherapy alone, particularly high-dose

methotrexate (MTX), showed better efficacy and less neurotoxicity than WBRT. For example, prospective analyses of treatment strategies in PCNSL revealed the safety and efficacy of high-dose MTX-based induction therapy and cytarabine (AraC)-based consolidation even in patients older than 60 (28, 29). These findings demonstrated the importance of high-dose MTX-based induction and other consolidation therapy. WBRT is also a selective consolidation approach especially for patients who are not eligible for high dose chemotherapy followed by ASCT. Some trials reported that the combination therapy of chemotherapy and WBRT achieved higher response rates and lower toxicity compared with chemotherapy alone. In a prospective multicenter study, patients underwent rituximab, MTX, vincristine, and procarbazine (R-MVP) and demonstrated 2-year overall survival (OS) of 67%, and no treatment-related neurotoxicity was observed (30). However, there are concerns about an increased risk of neurotoxicity after WBRT in long-term survivors (31). Therefore, dose reduction of WBRT should be considered when used as consolidation therapy. An alternate approach for consolidation therapy to improve efficacy without an increased risk of neurotoxicity is high dose chemotherapy followed by ASCT for eligible patients. ANOCEF-GOELAMS Randomized Phase 2 PRECIS Study reported that ASCT showed superior event free survival after consolidations to WBRT (32). Another prospective study showed WBRT and ASCT are both feasible and effective consolidation after high dose MTX based chemotherapy (33). The conditioning regimen is designed to allow the chemotherapeutic drugs to penetrate the CNS and thereby exert anti-tumor effects. One common conditioning regimen used for this particular scenario was primarily a combination of carmustine, etoposide (VP16), AraC, and melphalan (L-PAM) (BEAM regimen) or thiotepa-based treatments (34, 35). GOELAMS group reported an OS of 64% at 4 years in patients treated with BEAM regimen (34). Thiotepa-based and/or busulfan (BU)-based regimens have also demonstrated high efficacy (35–38). Busulfan and thiotepa (BuTT regimen) had an OS at 2 years of 48% (35). BuTT plus cyclophosphamide (TBC regimen) may improve the efficacy without neurotoxicity. Although TBC regimens demonstrated CR rates of >80%, they were associated with high treatment-related mortality, particularly in elderly patients (36, 38, 39). Among various other conditioning regimens, upfront L-PAM, cyclophosphamide, VP16, and dexamethasone (LEED) followed by ASCT was reported for patients with newly diagnosed PCNSL (40); no neurotoxicity was observed in the study. As a more intensive treatment strategy, combination of a high-dose regimen followed by ASCT and response-adapted WBRT was attempted, but WBRT increased the incidence of severe neurotoxicity (35, 41).

The prognosis of R/R PCNSL is poor. The median OS without treatment was reported to be only 2 months (42). Although there is no standard treatment strategy for R/R PCNSL, numerous studies have been reported. A retrospective

analysis revealed that the overall response rates of high dose MTX rechallenge were 85% or higher (43, 44). However, this study included patients only who achieved CR after first line MTX-based therapies, and seven of 16 patients relapsed after the salvage therapy. High dose chemotherapy followed by ASCT is one of the available treatment strategies for R/R PCNSL for patients who are eligible for intensive treatment, but the 2-year overall survival probability was 45% (45, 46). Although these conventional chemotherapy-based approaches commonly showed high response rates, the duration of efficacy was insufficient. Target kinase inhibitors may be a tolerable approach for patients with R/R PCNSL. Ibrutinib, which is a first-in-class Bruton's tyrosine kinase (BTK) inhibitor, showed 77% of clinical responses in patients with R/R PCNSL (47). Tirabrutinib, a second-generation BTK inhibitor, demonstrated an overall response rate of 64% in a phase 1/2 study (48). Although these therapies have shown varying degrees of efficacy with acceptable safety profiles, other treatment approaches, such as immunotherapy, are needed for R/R PCNSL to achieve a durable response. Rubenstein et al. conducted a phase I clinical trial of lenalidomide, a second-generation immunomodulatory agent, maintenance therapy in patients with PCNSL. They reported that the maintenance therapy with lenalidomide after rituximab plus lenalidomide therapy was feasible and prolonged the duration of response (49). This study served as a proof of concept for immunotherapy in PCNSL.

CD19 CAR-T cell therapy in PCNSL

Pivotal clinical trials of CART19 demonstrated unprecedented efficacies in patients with R/R large B cell lymphoma or ALL, which led to the FDA approval of tisagenlecleucel (tisa-cel), axicabtagene ciloleucel (axi-cel), and lisocabtagene ciloleucel (liso-cel) (16, 50). Most recently, the FDA has approved axi-cel for the treatment of R/R follicular lymphoma and brexucabtagene autoleucel (brexu-cel) for R/R mantle cell lymphoma. Currently, various investigational drugs of CAR-T cell products are in the pipeline of clinical development. Clinical trials evaluating CAR-T cell therapy for large B cell lymphoma have mainly excluded patients with CNS lymphoma due to the risk of potential neurotoxicity with CAR-T cells, except for TRANSCEND NHL001 (22), which allowed secondary CNS involvement. According to Abramson et al., seven patients who enrolled in this study had secondary CNS lesions, and six of these patients were evaluable for CART19 efficacy. Three out of six patients (50%) achieved a CR, no patients had Grade 3-4 cytokine release syndrome (CRS), and two patients (33%) had Grade 3 neurological events. Some case studies have also evaluated the efficacy and safety of CART19 for secondary CNS lymphoma (15, 22-24, 51). Similar to the TRANSCEND study, these cases also demonstrated ORR of 30-50% with no cases of severe CRS and 0-

30% of patients experiencing Grade 3-4 neurotoxicity. Based on the favorable effect of CART19 in secondary CNS lymphoma, CART19 cell therapy is becoming a potential treatment option for PCNSL. Table 1 summarizes CART19 cell therapy approaches in PCNSL. Frigault et al. recently reported a prospective study of CART19 cell therapy mainly targeting PCNSL (52). This phase 1/2 study of tisa-cel in adults (median age 63 years, range 34-81 years) with R/R PCNSL enrolled a total of 13 patients and administered 12 patients with tisa-cel. One patient was excluded from the trial due to disease progression during CAR-T cell manufacturing. The majority of patients had the nongerminal center B-cell subtype of DLBCL. All patients had prior history of ibrutinib-based therapy, and patients were allowed to continue these therapies up to 3 months after CAR-T cell infusion. Patients received standard lymphodepletion chemotherapies of fludarabine (25 mg/m²/day) and cyclophosphamide (250 mg/m²/day) on days -5, -4, and -3 of CAR-T cell infusion. CAR-T cells were administered intravenously. Among 12 patients who received tisa-cel, seven patients responded to the treatment (ORR: 58.3%), which consisted of one partial response (PR) and six CRs (CR rate: 50%). Three patients have remained in remission within the follow-up period. CRS and immune cell-associated neurotoxicity syndrome (ICANS) were graded based on the American Society for Transplantation and Cellular Therapy (ASTCT) criteria (53) and occurred in seven (58%, Grade 1/2/3/4 = 7/0/0/0) and six patients (50%, Grade 1/2/3/4 = 3/2/1/0), respectively. No patient required tocilizumab to control CRS, and Grade 3 ICANS was reversible.

The other two retrospective studies discussed in Table 1 also suggested favorable outcomes of CART19 cell therapy in PCNSL. Alcantara et al. reported nine cases of R/R PCNSL treated with CART19 (seven cases of tisa-cel and two cases of axi-cel). Overall response at one month was observed in six of nine patients (67%), including CR in three of nine patients (30%). Median progression-free survival (PFS) was 122 days, and PFS increased to 210 days for responders. Toxicities were mild and manageable. Seven patients experienced CRS (77.8%, Grade 1/2/3/4 = 2/4/1/0), and five patients developed ICANS (55.6%, Grade 1/2/3/4 = 2/1/1/1). Siddiqi et al. also discussed a similar case cohort study (54) with five patients with PCNSL. Overall response was seen in three of five patients (60%), and all three of these patients achieved CR. It should be noted that patients were monitored for CRS and ICANS with Lee criteria (55) and Common Terminology Criteria for Adverse Events v4.0, respectively. CRS and ICANS were seen in all patients; the highest grade CRS was 2 (Grade 1/2/3/4 = 3/2/0/0), and the highest grade ICANS was 3 (Grade 1/2/3/4 = 3/1/1/0). All toxicities were reversible and tolerable, and there were no treatment-related deaths.

Although these are small studies, CART19 cell therapies in PCNSL were well tolerated and showed promising efficacy similar to non-CNS lymphomas. Table 2 shows ongoing clinical trials of CAR-T cell therapy in patients with PCNSL.

TABLE 1 Summary of CAR-T cell therapy in PCNSL.

Author	CAR-T cell product(s)	Co-stimulatory domains	Study Design	Number of patients	Median age (range)	Number of prior regimen (range)	Prior transplantation	Median follow-up period from infusion (range)	Response ORR/CR	Median duration of response (range)	CRS	ICANS	Ref (PMID)
Frigault, M. J.	Tisa-cel	4-1BB-CD3 ζ	Phase 1/2	12	63 (34-81)	4 (2-6)	3	12.2 months (3.64-23.5)	58.3%/50%	N/A 3/7 patients have not progressed at data cut off	58.3% Grade 1/2/3/4=7/0/0/0 ASTCT criteria	50% Grade 1/2/3/4=3/2/1/0 ASTCT criteria	35167655
Alcantara M.	Tisa-cel Axi-cel	4-1BB-CD3 ζ or CD28-CD3 ζ	Retrospective	9 Tisa-cel: 7 Axi-cel: 2	67 (48-75)	3 (2-5)	7	8.5 months (1.2-15.3)	66.7%/55.6%	5.9 months (2.0-11.7) 4/6 patients have not progressed at data cut off	77.8% Grade 1/2/3/4=2/4/1/0 ASTCT criteria	55.6% Grade 1/2/3/4=2/1/1/1 ASTCT criteria	34871363
Siddiqi T.	CART19	CD28-CD3 ζ	Retrospective	5	49 (42-53)	5 (2-12)	0	N/A	60%/60%	9.1 months (1.4-17.3)	42% Grade 1/2/3/4=3/2/0/0 Lee criteria	100% Grade 1/2/3/4=3/1/1/0 CTCAE 4.0	34492703
Li T.	CART19 CART22	4-1BB-CD28-CD3 ζ	Prospective	1	49	3	0	N/A	PD	N/A	Grade 1 Lee Criteria	none CTCAE 5.0	32903866
Wu J.	CART19 CART22 followed by ASCT	4-1BB-CD3 ζ	Prospective	4	44.5 (39-55)	3 (3-4)	0	14.2 months (1.4-24.2)	100%/75%	9.35 months (0.23-19.3)	75% Grade 1/2/3/4=3/0/0/0 ASTCT criteria	25% Grade 1/2/3/4=0/0/1/0 ASTCT criteria	34267187
Tu S.	CART19 CART70	CD28-CD27-CD3 ζ	Case report	1	67	4	0	17 months	CR	17 months	none	none	31867275

CAR-T cell, chimeric antigen receptor-T cell; tisa-cel, tisagenlecleucel; axi-cel, axicabtagene ciloleuce; CART19, CD19 directed CAR-T cell; CART22, CD22 directed CAR-T cell; ASCT, autologous stem cell transplantation; CART70, CD70 directed CAR-T cell; ORR, overall response rate; CR, complete response; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; ASTCT, american society for transplantation and cellular therapy; CTCAE, common terminology criteria for adverse event.

Toxicities of CAR-T cell therapy in PCNSL

According to the ASTCT, CRS is characterized by fever, hypotension, hypoxia, and end organ dysfunction (53). CRS is associated with massive expansion of infused cells *in vivo* as well as extreme elevation of multiple cytokines/chemokines. CRS develops in 50-100% of cases after CART19 cell therapy (15, 56, 57) and is significantly correlated with higher disease burden at baseline and *in vivo* CAR-T cell proliferation (58–61). ICANS is the second-most noted life-threatening adverse event associated with CAR-T cell therapy and is characterized by generalized cerebral edema, confusion, obtundation, aphasia, motor weakness, and occasionally, seizures (53, 62). Any grade of ICANS occurs in up to 70% of patients, and Grade 3-4 is reported in 20-30% of patients (15, 16, 56, 60). Overall, CRS and ICANS are common and can be fatal.

While the exact mechanisms of ICANS remain unknown, Parker et al. have recently shown that pericyte populations in the brain express CD19, pointing to one potential mechanism of ICANS (63). The incidences of ICANS also reported in patients treated with CD19-targeting bispecific antibodies (64). However, cases of ICANS have been reported with CD22 (65) and BCMA (66) directed CAR-T cell therapy, which makes difficult to conclude that ICANS occurrence is owing to the presence of the target antigen within the CNS.

Historically, patients with PCNSL or secondary CNS lymphoma were excluded from most pivotal CAR-T cell therapy trials due to concerns about the increased risk of ICANS. A recent CART19 clinical study in PCNSL reported by Frigault et al. (52) and two retrospective analyses from Alcantara et al. (25) and Siddiqi et al. (54) showed that patients with PCNSL treated with CART19 cell therapy developed reversible and tolerable ICANS.

Local administration of CAR-T Cells

Most clinical trials of CAR-T cell therapy in hematological malignancies as well as solid tumors have been conducted using intravenous administration (67). However, limited efficacies were reported in cases with bulky diseases, PCNSL, or solid tumors due to the poor trafficking of CAR-T cells to the tumor site (68). However, there is increasing evidence to suggest that local application of CAR-T cells may increase tumor penetration and efficacy in some cases.

Local administration of CAR T-cells was recently tested in an immunocompromised NOD-SCID- $\gamma^{-/-}$ mouse model of PCNSL (69). The researchers established an orthotopic PCNSL mouse model by intracranial injection of human CD19⁺ lymphoma cell lines (Daudi or JeKo-1). The CART19 cells (CD28 ζ co-stimulated) were administered through a single

infusion, either intracerebroventricular or intravenous. Interestingly, bioluminescence imaging revealed intracerebroventricularly delivered CAR-T cells were able to completely and durably eradicate both CNS and systemic lymphoma. On the other hand, CAR-T cells delivered through intravenous injection failed to show anti-tumor effects. They also showed that intracerebroventricularly infused CAR-T cells exhibited similar trafficking but significantly better proliferation and persistence compared to intravenously infused CAR-T cells. Interestingly, intracerebroventricularly delivered CAR-T cells exhibited a higher percentage of memory phenotype than intravenously administered CAR-T cells. The authors concluded that exposure of CAR-T cells to CSF leads to a metabolic reprogramming that favors the formation of memory T cells, as inhibition of glycolysis enhances memory T cell phenotypes (70, 71).

Locally administered CAR-T cells in PCNSL are now being tested in a phase I clinical trial. All patients will be treated first with CAR T-cells intravenously in this study. If patients do not show response (stable disease (SD) or progressive disease (PD) at day 28) to the first round of CAR-T cell infusion and in the absence of severe CART-related toxicity, patients will be potentially eligible for a second round of CAR-T cells administered intracerebroventricularly *via* an Ommaya reservoir (Table 2) (NCT04443829).

Similar to local CART19 injection in the PCNSL mouse model by Wang et al. (69), B7H3-targeted CAR-T cell therapy was tested in orthotopic atypical teratoid/rhabdoid tumor xenografts with either intracerebroventricular or intravenous injection. Theruvath et al. demonstrated that intracerebroventricular administration of B7H3 CAR-T cells resulted in significantly better overall survival and anti-tumor effects, with faster CAR-T expansion *in vivo* and reduced systemic inflammatory cytokines compared to intravenous injection (72).

These recently reported preclinical data provide rationale to further assess local administration of CAR-T cell to treat PCNSL.

Targeting multiple antigens with CAR-T cells

To prevent tumor relapse after CAR-T cell therapy due to loss of the target antigen, there have been efforts to establish CAR-T cell strategies to recognize multiple tumor antigens in preclinical models (73–78), and some dual-targeted CAR-T products have already been tested in clinical trials in patients with systemic lymphoma or multiple myeloma (79–84). A similar concept is also being applied to PCNSL. Wu et al. have conducted a clinical trial of sequential therapy with ASCT followed by anti-CD19 and CD22 cocktail CAR-T cell therapy. Thirteen patients with CNS lymphoma were enrolled in this

TABLE 2 Clinical trials of CAR-T cell therapy in PCNSL.

Clinical Trial Identifier (ClinicalTrials.gov)	Title	Disease(s)	Population	Location	Study Design	Study Design	Interventions	Conditioning Regimen
NCT04443829	Immunotherapy Using CAR T-cells to Target CD19 for Relapsed/Refractory CD19+ PCNSL Lymphoma (CAROUSEL)	R/R PCNSL	>16 years	University College, London	Phase 1	Single-center, non-randomised, open label	Dose 1: CART19 250 x 10 ⁶ cells iv At Day 28 response SD or PD →Dose 2: 25 x 10 ⁶ cells icv	Cy 60mg/kg on Day -6 Flu 30mg/m ² on Day -5 to Day -3 Pembrolizumab 200mg on Day -1
NCT04134117	Pilot Study of Tisagenlecleucel, CD19-targeted CAR T Cells, in Patients With PCNSL	R/R PCNSL	>18 years	Massachusetts General Hospital	Phase 1	Single-center, non-randomised, open label	One time single predetermined dose level of tisa-cel will be infused intravenously	Flu/Cy
NCT04608487	A Phase I Study of Anti-CD19 CAR T-cell Therapy With Axi-cel in Patients With Relapsed/Refractory Primary and Secondary CNS Lymphoma	Cohort 1: R/R PCNSL, secondary CNSL without DLBCL, HGBL, PMBL, tFL Cohort 2: R/R systemic DLBCL, HGBL, PMBL, tFL with either active CNSL or previously treated CNSL	>18 years	Dana Farber Cancer Institute	Phase 1	Single-center, non-randomised, open label	One time single predetermined dose level axi-cel will be infused intravenously	Flu/Cy
NCT04464200	A Phase I Study of CD19-Targeted 19(T2)28z1xx CAR Modified T Cells in Adult Patients With Relapsed or Refractory B-cell Malignancies	DLBCL, PMBL, tFL, CLL, indolent NHL, MZL, Waldenstrom Macroglobulinemia, Burkitt lymphoma, PCNSL	>18 years	Memorial Sloan Kettering Cancer Center	Phase 1 Dose escalation study	Single-center, non-randomised, open label	CD19-Targeted 19 (T2)28z1xx CAR T cells will be infused intravenously	Flu/Cy

CAR-T cell, chimeric antigen receptor-T cell; tisa-cel, tisagenlecleucel; axi-cel, axicabtagene ciloleuce; CART19, CD19 directed CAR-T cell; R/R PCNSL, relapsed refractory primary central nervous system lymphoma; DLBCL, diffuse large B cell lymphoma; HGBL, high-grade B cell lymphoma; PMBL, primary mediastinal B cell lymphoma; tFL, transformed follicular lymphoma; NHL, non-Hodgkin lymphoma; MZL, marginal zone lymphoma; SD, stable disease; PD, progressive disease; iv, intravenous injection, icv, Intracerebroventricular injection; Cy, cytarabine; Flu, fludarabine.

study (four PCNSL and nine secondary CNS lymphoma). Two patients (one PCNSL and one secondary CNS lymphoma) achieved CR at the time of CAR-T cell infusion and maintained durable remission. Overall response was observed in nine of 11 patients (82%, three PCNSL and six secondary CNS lymphoma), including CR in six of 11 patients (55%, two PCNSL and four secondary CNS lymphoma). CRS and ICANS occurred in 11 patients (85%, Grade 1/2/3/4 = 9/2/0/0) and three patients (23%, Grade 1/2/3/4 = 2/0/1/0), respectively. All adverse events were reversible and tolerable, and there were no deaths related to the treatment (85).

Li et al. also reported five patients with CNS lymphoma (one patient with PCNSL and four patients with secondary CNS lymphoma) who underwent anti-CD19 and CD22 cocktail CAR-T cell therapy with a follow-up of 6-16 months. Two patients achieved CR, and three other patients achieved PR. However, four patients developed relapse within 3 to 8 months after CAR-T cell therapy. Unlike the study from Wu et al., this trial did not perform ASCT prior to CAR-T cell infusion. Therefore, the baseline tumor burden at the time of CAR-T cell administration was higher. Furthermore, the authors mentioned that the early relapse was seen due to the immunosuppressive tumor microenvironment (TME) of the CNS, which was unrelated to antigen escape (86). Tu et al. reported a case report of dual-targeting CD19/CD70 CAR-T cells in a patient with R/R PCNSL. Durable remission at 17 months was observed after the treatment (Table 1) (87).

Given these results, dual targeting of different antigens on tumor cells may not contribute to favorable outcomes. To achieve long-term durable response, it may be crucial to decrease the tumor burden before CAR-T cell treatment. Moreover, targeting not only the tumor cells but also the TME may enhance anti-tumor activity of CAR-T cells and prevent an early relapse after CAR-T cell treatment.

Combination with other immunotherapies

The contribution of the TME to tumor growth and therapy resistance has been recognized in most malignancies and also applies to PCNSL. In the last decade, the field of oncology has been transformed by immunotherapies, including antibodies directed against immune checkpoints or ligands, such as PD-1/PD-L1 or CTLA-4 (88, 89). Many studies have demonstrated that the presence of PD-1⁺ tumor-infiltrating lymphocytes and PD-L1⁺ microglial cells, tumor-associated macrophages, and tumor cells within the TME correlate with patient outcomes. Specifically, Chapuy et al. reported PCNSL and primary testicular lymphoma (PTL) showed a higher 9p24 amplification compared to systemic DLBCL. A 9p24

amplification in malignant lymphoma correlates with PD-L1/PD-L2 deregulation and results in increased PD-L1 expression on tumor cells (90–92). Takashima et al. performed next generation sequencing on PCNSL samples and discovered that high expression of LAG3, PD-1, and PD-L2 were associated with poor prognosis (93). PD-1 blocking therapy in preclinical PCNSL models as well as early clinical data indicate its efficacy in PCNSL. Qiu et al. created a mouse PCNSL model by injecting the murine B-cell lymphoma cell line, A20, to the periventricular area. PD-1 antibody treatment resulted in prolonged overall survival, increased CD8⁺ tumor-infiltrating lymphocytes, and complete eradication of tumor cells (94). Nayak et al. treated four patients with PCNSL and one patient with CNS relapse of PTL with the PD-1 blocking antibody, nivolumab. Overall response was 100%, including CR rates of 80% (three patients with PCNSL and one patient with CNS relapse of PTL). One patient developed Grade 2 pruritus, and another patient experienced Grade 2 fatigue. The authors concluded that these are nivolumab-related toxicities. However, one patient developed worsening of baseline renal functions (Grade 4) and required hemodialysis. Renal biopsy showed no evidence of interstitial nephritis, so this event was considered to be unrelated to nivolumab treatment (95). Another study reported a case with R/R PCNSL who was successfully treated with nivolumab and dendritic cell vaccination (96).

These checkpoint inhibitors demonstrate anti-tumor effects *via* activated T lymphocytes; therefore, combination therapy of PD-1 blocking antibody and CAR-T cell therapy may increase CAR-T cell anti-tumor efficacy in the treatment of PCNSL. Based on these preclinical and clinical data, the combination of CAR-T cell therapy with the PD-1 inhibitor, pembrolizumab, to overcome the negative effects of immunosuppressive cells in the TME is being evaluated in patients with R/R PCNSL (Table 2) (CAROUSEL Trial, phase I clinical trial, NCT04443829).

Can CAR-T cell therapy be applied to human immunodeficiency virus (HIV)-1-related PCNSL?

Patients with HIV-1 are at increased risk for PCNSL compared to uninfected populations. From a retrospective study, among all patients with PCNSL, 19% had HIV-1 (97). As we discussed earlier, CAR-T cell therapy may be a feasible treatment option for patients with PCNSL. Since pivotal clinical trials of CAR-T cell therapy excluded patients who were positive for HIV-1, it is difficult to assess whether CAR-T cell therapy is safe and efficacious in patients with HIV-1-positive PCNSL. However, the use of CAR-T cell therapy in HIV-1-positive PCNSL is increasing. Exclusion criteria for CAR-T cell

products approved by the FDA or other regulatory agencies do not include HIV-1 positivity. In fact, two HIV-1-infected patients with high-grade B-cell lymphoma were successfully treated with CART19 cell therapy (98). In this report, CAR-T cells were successfully manufactured in HIV-infected patients who were receiving antiretroviral therapy. CAR-T cell products were administered safely and led patients into remission. One of the original concepts of CAR-T cell therapy was to target HIV-1-infected T cells with anti-HIV CAR-T cells (99). In 2002, Deeks et al. reported CD4 ζ -modified first-generation anti-HIV CAR-T cells in a phase II randomized study (100). Although no therapeutic efficacy was demonstrated, anti-HIV-1 CAR-T cells were successfully generated from HIV-1-infected patients, and long-term engraftment was reported. More recently, anti-HIV-1 CAR-T cell technology was reported by Liu et al. They developed HIV-1 broadly neutralizing antibody (bNAb)-derived CAR-T cells, which contain both CD28 and 4-1BB intracellular costimulatory domains, and administered them to 14 patients with HIV-1. These CAR-T cells significantly reduced cell-associated viral RNA and intact proviruses (101). Additionally, an *in vivo* study reported that bNAb-derived CAR-T cells could enhance the efficacy of PD-1 blockade (102). These results encourage the application of CART19 cell therapy in HIV-1-positive PCNSL. Novel and promising strategies can further improve efficacy and favorable outcomes. For example, dual targeting CD19 along with the membrane-proximal external region, which is a highly conserved region of the envelope glycoprotein gp41 subunit near the viral envelope (103–105), with CAR-T cells or combination of CAR-T cell cocktails targeting both CD19⁺ and HIV-infected cells might synergistically enhance the efficacy.

Discussion

As we discussed in this review article, the standard therapy for PCNSL has not been established, and numerous problems and obstacles must be overcome to induce durable remission. CAR-T cell therapy may be a promising solution to advance the treatment approach for PCNSL. Historically, radiation therapy, high-dose MTX or AraC, and consolidation therapy with autologous transplantation have been applied for the treatment of PCNSL due to the ability of these therapies to penetrate the blood-brain barrier. However, serious adverse events including leukoencephalopathy or neurotoxicity are common. Unlike these treatments, CAR-T cell therapies in patients with PCNSL has rarely resulted in cases of leukoencephalopathy. In terms of neurotoxicity, most cases of PCNSL treated with CAR-T cell therapy demonstrated that treatment-related toxicities were reversible and tolerable. Cook et al. have recently reported a

meta-analysis of CAR-T cell therapy in patients with PCNSL or secondary CNSL. Similar to the studies that we described in this review article that toxicities were comparable to that of systemic large B cell lymphoma with no increased incidence of neurotoxicity. They also described that encouraging efficacy of CAR-T cell therapy were demonstrated with PCNSL and secondary CNSL (106).

The major drawback of CAR-T cell therapy in PCNSL is low durable response, similar to systemic lymphoma or leukemia. The lack of durable responses has been widely attributed to the immunosuppressive TME and resultant T cell dysfunction. Lack of trafficking of CAR-T cells to the tumor site also correlates with the low durability of CAR-T cells in PCNSL. Novel combination therapies with other agents, including kinase inhibitors or checkpoint inhibitors, or local administration of CAR-T cell therapy are currently being investigated in clinical trials. CAR-T cell therapy in PCNSL has potential to change overall treatment strategy. For example, the combination of CAR-T cell therapy with conventional induction therapy with high-dose MTX may improve the response rate and durable response as a first line therapy for PCNSL. This would reduce patients' exposure to high-dose chemotherapies and thereby reduce the risk of associated leukoencephalopathy or neurotoxicity. Overall, emerging data discussed in this manuscript encourage further investigation of the use of CART19 cell therapy for the treatment of PCNSL.

Author contributions

KM, HY, and RS wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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