

Clinical Case Report of CAR-T Therapy of Patient with Refractory Burkitt's Lymphoma

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Abstract

Approximately 20% of adult patients with Burkitt's lymphoma (BL) have relapsed/refractory disease, which is characterized by poor overall survival (OS) and virtually no therapeutic options after failure of two lines of chemotherapy, due in part to the peculiarities of tumor cell biology. Improved treatment strategies are an unmet need for these patients. There have been individual attempts to use chimeric antigen receptor (CAR)-T therapy in this patient population using anti-CD19, CD20, and CD22 cells. In our study, a 42-year-old patient with Burkitt's lymphoma received CAR-T cell therapy after failure of previous treatment, with a complete response at one month (as measured by positron emission tomography (PET)/computed tomography (CT) scan) and no significant adverse events. However, five months later, the patient experienced a rapid CD19+ relapse and died of disease progression while attempting to repeat CAR-T therapy. We believe that real therapeutic success for this group of patients is possible in the case of CAR-T therapy with a complete response followed by consolidation with hematopoietic stem cell transplantation (HSCT). Further studies are needed to obtain statistically significant data.

Keywords: Burkitt's lymphoma, Non-Hodgkin's lymphoma, CAR-T therapy, CD19, Relapsing/refractory, Lymphodepleting conditioning

Abbreviations: BL: Burkitt's Lymphoma; CAR-T: Chimeric Antigen Receptor T cells; r/r: Relapsed/Refractory; PD- Progressive Disease; PET/CT: Positron Emission Tomography and Computed Tomography; EGFR: Epidermal Growth Factor Receptor; CRS: Cytokine Release Syndrome; NHL-BFM-95- Non-Hodgkin Lymphoma Berlin–Frankfurt–Münster-95; FDG: Fluorodeoxyglucose; Da-EPOCH-R: Dose-adjusted Rituximab, Etoposide, Cyclophosphamide, Doxorubicin and Vincristine; HSCT: Hematopoietic Stem Cell Transplantation; HDCT: High-Dose Chemotherapy; OS: Overall Survival; CR: Complete Response

Introduction

Burkitt's lymphoma (BL) is an aggressive form of B-cell lymphoma. Compared to diffuse large B-cell lymphoma, BL rarely affects adults.

The molecular hallmark of BL is the t(8;14) translocation

of MYC proto-oncogene from chromosome 8 to the immunoglobulin heavy-chain region on chromosome 14, in 70 to 80% of cases; the t(2;8) translocation from chromosome 8 to the immunoglobulin kappa locus on chromosome 2, in 15% of cases; or the t(8;22) translocation from chromosome 8 to the immunoglobulin lambda locus on chromosome 22, in 5% of cases. MYC translocations are nearly universal across BL

variants. The deregulated MYC expression led to uncontrolled tumor proliferation [1].

Treatment strategies for adults with BL have been inspired by approaches with highly dose-intensive chemotherapy regimens of short duration that are designed to achieve high peak drug concentrations, which were originally adopted from pediatric protocols. This treatment strategy is the current state of the art, as it allows to achieve a 5-year overall survival (OS) in approximately 60-70% of patients [1-3]. Oncological outcomes (i.e survival rates) in relapsed/refractory (r/r) settings are usually poor [4]. It was reported that 37% of patients with chemosensitive recurrence survived for 3 years, while only 7% of patients with chemoresistant disease survived at this time point [5].

Over the last years, the use of chimeric antigen receptor T cells (CAR-T) has achieved unprecedented success in the treatment of B-cell malignancies, with complete remissions of up to 86% in acute lymphoblastic leukemia and 68% in B-cell non-Hodgkin's lymphoma [6]. However, there are a few cases reported of the potential benefit along with failure of CAR-T therapy with BL [7-16].

In this article we describe a case of academic designed and manufactured anti-CD19 CAR-T cell therapy in primary refractory BL.

Ethical statement

All procedures performed in this study complied with ethical standards and were ratified by the institutional committee. Written informed consent was signed by the patient for publication of this clinical case.

Case Presentation

Patient M., 42 years old, was diagnosed with Burkitt's lymphoma in May 2022. The disease manifested with involvement of bilateral cervical, supraclavicular and mediastinal lymph nodes. The patient was admitted to the hospital in a serious condition due to a large tumor mass, intoxication, and high fever. Ann Arbor stage II was documented. The lactate dehydrogenase level was within normal limits. Neither cytopenic features in peripheral blood, nor bone marrow involvement was observed. Immunophenotype of lymphoma cell was: PanCK(-), CD56(-), bcl2(-), bcl6(+), CD3(-), CD5(-), CD10(+), CD20(+), CD23(-), CyclinD1(-), MUM(-), PAX5(+), IgM(+), IgG4(-), ALK(-), CD30(-), CD56(-), Ki67(~100%). A typical MYC gene rearrangement was detected by fluorescence in situ hybridization while the absence of rearrangements of the BCL2 and BCL6 genes was confirmed.

The patient was referred to the intermediate risk group and received 2 cycles of induction therapy according to the

Da-EPOCH-R regimen (dose-adjusted Rituximab, Etoposide, Cyclophosphamide, Doxorubicin and Vincristine).

Interim positron emission tomography and computed tomography (PET-CT) revealed disease progression in the form of increased size and metabolic activity of previously involved lymph nodes. The line of therapy was changed, and the patient received two cycles of chemoimmunotherapy according to the modified Non-Hodgkin lymphoma Berlin-Frankfurt-Münster-95 (NHL-BFM-95) protocol (including high-dose methotrexate and cytarabine). PET/CT at the end of treatment showed no remission (Deauville score 5).

In an attempt to control the disease, radiotherapy was administered to the affected area (4 Gy per fraction, total dose 28 Gy), followed by 2 cycles of immunochemotherapy (ifosfamide, carboplatin, etoposide, rituximab). However, the results of the next PET/CT scan showed a progression pattern with liver, nodal and bone marrow involvement (Deauville score 5), (**Figure 1A**).

The patient was considered eligible for CAR-T cell therapy and lymphocyte apheresis was performed. An additional immunohistochemical study was performed to evaluate the expression of CD19 on tumor cells. The detected level of CD19 expression was 90% (**Figure 2A**).

Steroid-based (dexamethasone 30 mg/m²/day) "bridging" therapy was required due to rapid deterioration and disease progression.

Following lymphodepleting conditioning with fludarabine and cyclophosphamide, the CAR-T cellular product was infused on November 09, 2022 (ratio CD4/CD8:1/1). The second generation anti-CD19 chimeric antigen receptor contained an anti-CD19 antibody scFv fragment, CD28 transmembrane domain, 4-1BB and CD3z signaling domains. The coding sequence was cloned into the lentiviral vector S4 (**Supplementary Figure 1**). The cell product was obtained by expansion of CD4⁺ and CD8⁺ positive lymphocyte populations in an Iscove's Modified Dulbecco's Medium, supplemented with 10% fetal bovine serum, 5% AB serum, 10 ng/ml IL-7 and IL-15 after initial activation and lentiviral transduction with vector S4. Analysis of the persistence and expansion of anti-CD19 CAR-T cells was performed against truncated epidermal growth factor receptor (EGFR) protein transduced within the expression cassette. The peak expansion of CAR T cells in peripheral blood was observed by flow cytometry on day +16 after infusion (absolute cell count = 47.05 cells/ μ l).

Despite the nature of the disease and the high tumor burden, no cytokine release syndrome (CRS) (blood interleukine-6 concentration in a peak - 212 pg/ml, starting from 68 pg/ml on day 0) or neurotoxicity was observed.

The PET/CT scan performed on day +30 showed remarkable

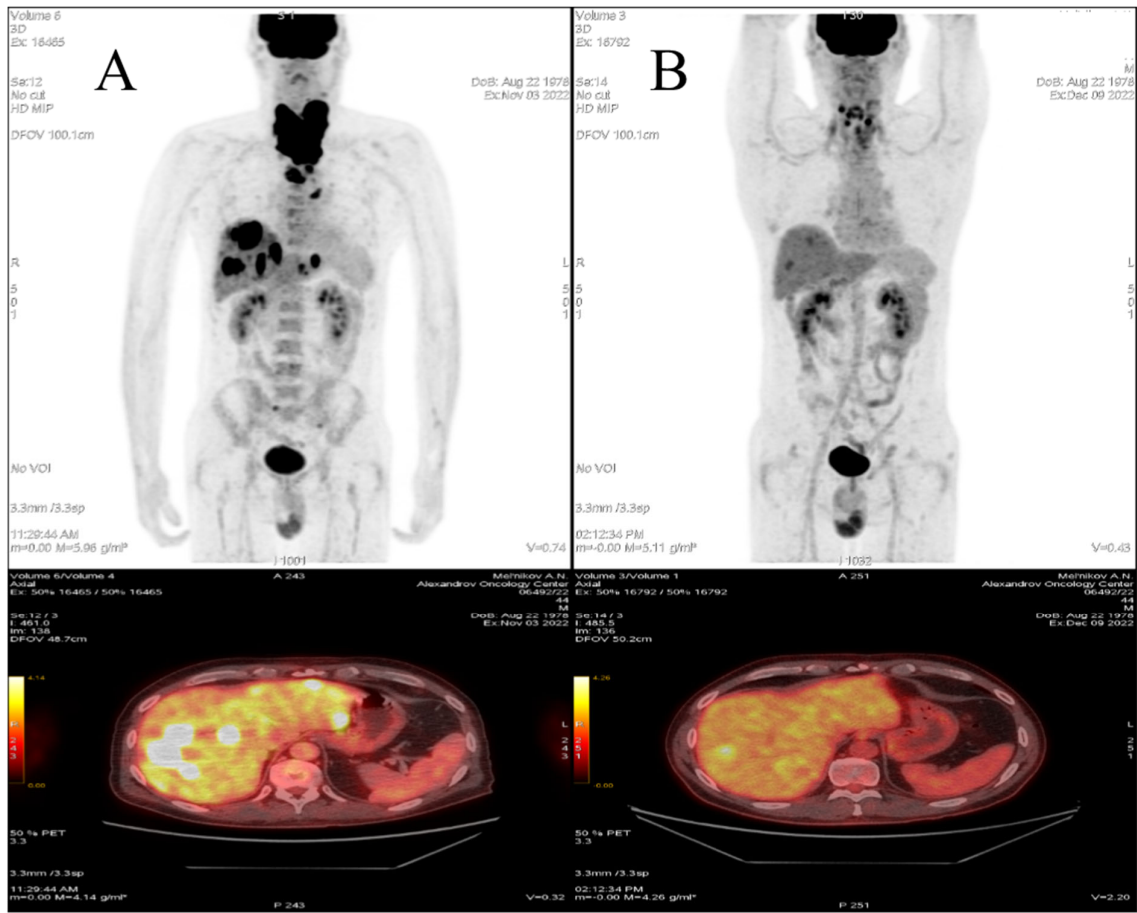


Figure 1. Patient positron emission tomography and computed tomography (PET-CT) before and after chimeric antigen receptor T cells (CAR-T) treatment. A. PET-CT before CAR-T. B. PET-CT 1 month after CAR-T.

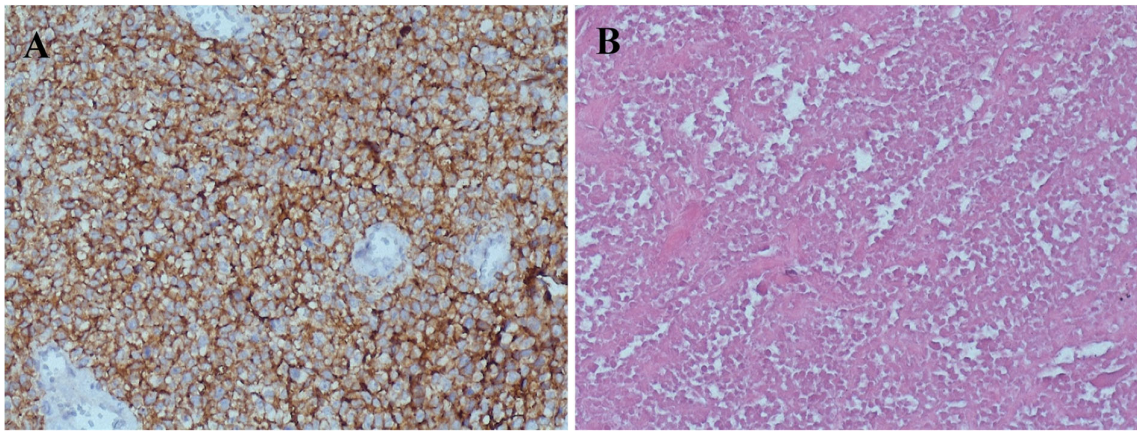


Figure 2. Pathological analysis of the patient lymph-node mass. A. Immunohistochemistry (x200) indicated strong membrane expression of CD19 on tumor cells before chimeric antigen receptor T cells (CAR-T) therapy. B. Histological examination (hematoxylin & eosin; x200) of neck tumor specimen revealed total necrosis after CAR-T therapy.

dynamics with a decrease in size and metabolic activity of all previously identified lymphoma foci. However, metabolic activity in cervical nodal conglomerates leads to an upscoring according to the Deauville scale (Deauville score 5) (**Figure 1B**).

To determine the nature of the high metabolic activity in a cervical region, an incisional biopsy was performed. Histological examination revealed total necrosis (**Figure 2B**).

The particular clinical scenario was classified as a complete remission. No further treatment was carried out.

However, PET/CT performed on day + 150 showed tumor progression due to the appearance of metabolically active mediastinal lymph nodes, pericardium. A fluorodeoxyglucose (FDG)-avid pleural effusion was observed as well (Deauville score 5) (not shown). The lymphomatous nature was confirmed by immunophenotyping of pleural fluid. Notably, malignant lymphocytes preserved CD19 expression, while the CD20 antigen escaped. Perhaps the transient response to CAR-T therapy is related to the biologically aggressive nature of Burkitt's lymphoma.

Given the patient's young age, good functional status and persistent CD19 expression on tumor cells, it was decided to perform a second infusion of anti-CD19 CAR-T cells followed by allogeneic hematopoietic stem cell transplantation (HSCT) from a matched related donor (**Figure 3**).

Despite the retreatment with tandem CAR-T infusion, the disease was rapidly progressed, and the patient eventually died on day + 7 after the second CAR-T dose in May 2023 (**Figure 3**).

Discussion

Overall, relapse/progression of Burkitt's lymphoma is uncommon, preventing meaningful analyses to develop standard therapeutic options. Different regimens were employed, including high dose methotrexate and/or cytarabine, platinum and rituximab. Most researchers recommend performing autologous/allogeneic HSCT as consolidation in patients who have achieved a second complete remission (CR2). Historical surveys from the European Society for Blood and Marrow Transplantation reported 3-year OS of 37% for adult BL patients achieving a CR2 after salvage chemotherapy and consolidated with high-dose chemotherapy (HDCT) and autologous HSCT (OS rate for patients not in complete response (CR) at the time of HDT was 7%), similarly to those undergoing allogeneic HSCT (4-year OS 37%), who experienced not insignificant transplantation-related mortality up to 30% [4]. The main problem is that patients who cannot reach CR2. One possible way to achieve remission in this group of patients may be CAR-T cell therapy.

Our case report presents a patient diagnosed with refractory Burkitt lymphoma, who received multiple lines of systemic anticancer therapy and did not achieve remissions. After careful consideration, the patient received CD19 CAR-T therapy.

To the best of our knowledge, there are few reports describing the clinical efficacy of anti-CD19 CAR-T in patients with refractory BL. Thus, this article is one of the few to describe the clinical course of BL in a patient treated with anti-CD19 CAR-T in a refractory fashion.

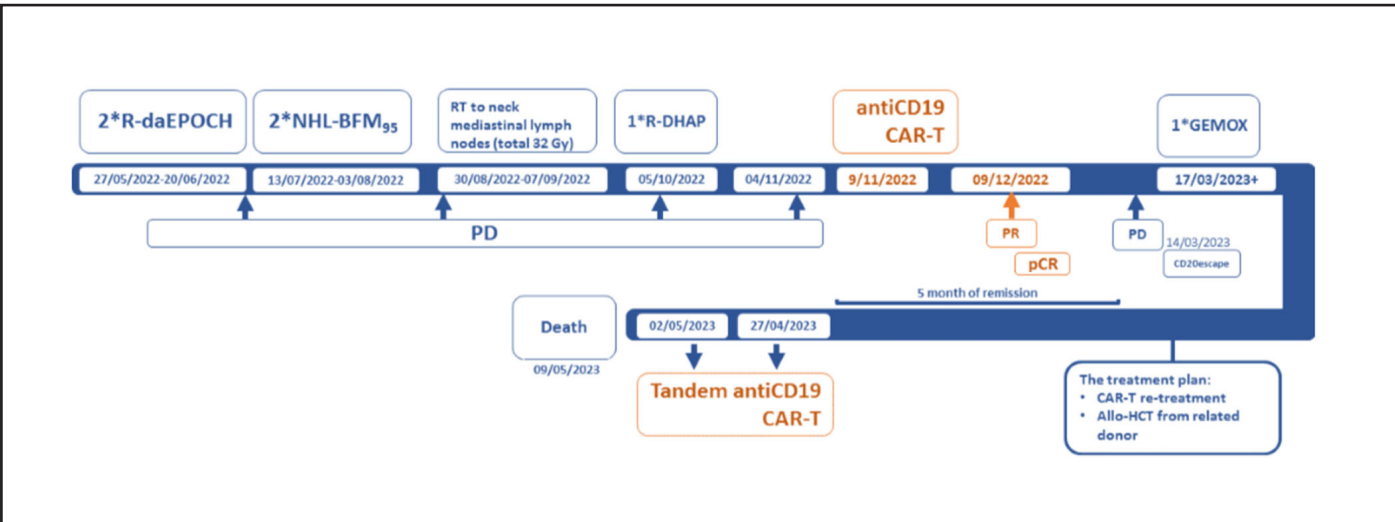


Figure 3. Patient disease and treatment course. *: Number of treatment cycles; R-daEPOCH: Dose-Adjusted Rituximab, Etoposide, Cyclophosphamide, Doxorubicin and Vincristine; NHL-BFM95: Non-Hodgkin Lymphoma Berlin–Frankfurt–Münster-95 Protocol; RT: Radio Therapy; R-DHAP: Rituximab, Cisplatin, Cytarabine and Dexamethasone; CAR-T: Chimeric Antigen Receptor T cells; PD: Progression Disease; PR: Partial Response; pCR: Pathological Complete Response; GEMOX- Rituximab, Gemcitabine, Oxaliplatin; allo-HCT: Allogeneic Hematopoietic Stem Cell Transplantation.

The literature review found several publications addressing the same issue.

Avigdor *et al.* [7] was a pioneer in the treatment of r/r BL with CAR-T. The publication described a clinical case of a 32-year-old man with disease progression after allogeneic HSCT who received anti-CD19 CAR T-cell therapy. The chimeric T-cell therapy resulted in a complete response. The patient subsequently underwent haploidentical HSCT and died due to veno-occlusive disease and sepsis on day 9 after allogeneic transplantation.

The results of other studies of CAR-T cell therapy in Burkitt's lymphoma are shown in **Table 1**.

Based on the published data, we believe that long-term remission is associated with HSCT manipulations ((HDCT) + autologous HSCT or allogeneic HSCT) either as consolidation or induction (**Table 1**).

The clinical dilemma regarding our patient (to treat or not to treat?) arose from the discordant results of the end-of-treatment PET/CT and the pathology report, which confirmed the necrotic nature in the area of suspected disease penetrance.

It is still unclear whether any type of consolidation is an essential or facultative part of the treatment plan. Therefore, decisions regarding treatment strategy should be based on

Table 1. Research data of BL CAR-T therapy.							
Study Name	Patient characteristics		Stem cell manipulations and timing		CAR-T product	Best post CAR-T response	OS
	Number	Age	HDCT+ auto HSCT	Allo-HSCT			
Du <i>et al.</i> 2020 [15]	5	6-10 years	NA	NA	#3-CD19, #1-CD19+CD22, #1-CD19+CD22+CD20	#5-PR	5-14 months
Zhou <i>et al.</i> 2021 [14]	6	21-34 years	NA	#1-After CAR-T	#6-CD19+CD22	#1-CR #2-PR #1-SD #2-PD	1-37 months
Wang <i>et al.</i> 2021 [8]	1	1,7 years	NA	NA	#1-CD19	#1-PR	16 months
Ye <i>et al.</i> 2022 [13]	1	61 years	Prior CAR-T	NA	#1-CD19	#1-PR	48 months
Wu <i>et al.</i> 2022 [10]	28	17-70 years	#13-After CAR-T	NA	#28-CD19+CD22	#9-PR (7 in auto HSCT subgroup) #19-SD/PD (6 in auto HSCT subgroup)	0-60 months
Cheng <i>et al.</i> 2022 [9]	1	47 years	NA	After CAR-T	#1-CD19+CD22	#1-PR	Died on +60 after CAR-T
Seitter <i>et al.</i> 2022 [12]	2	22, 45 years	NA	NA	#2-CD19	#2-CR	53 and 58 months
Zhang <i>et al.</i> 2023 [11]	1	26	NA	#1- After CAR-T	#1-CD19 +CD22	#1-CR	More than 4 years
Geerts <i>et al.</i> 2023 [16]	1	22	NA	NA	#1-CD19	#1- PD	Died on +30 after CAR-T
Footnotes: BL: Burkitt's Lymphoma; CAR-T: Chimeric Antigen Receptor T cells; OS: Overall Survival; HSCT: Hematopoietic Stem Cell Transplantation; HDCT: High-Dose Chemotherapy; NA: Not Applied; PR: Partial Response; CR: Complete Response; SD: Stable Disease; PD: Progressive Disease; #: Number of patients.							

the best available evidence and correspond to clinical data and patient preferences.

Conclusion

Our clinical case suggests that a CAR-T approach to patient with r/r BL may exist as a method of entering remission when conventional chemotherapy fails. If remission is achieved, consolidation with autologous HSCT or allogeneic HSCT is required. This result is encouraging, but further studies with more patients are definitely needed.

Conflicts of Interest

No conflicts of interest.

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Authors' Contribution Statement

DTM and SIM manufactured anti-CD19 CAR-T cell, assessed CAR-T cell persistence and expansion, and contributed to the writing of this manuscript, creating a figure and a table.

KNE assisted the patient as oncologist, contributed to the diagnosis and therapeutic recommendations, contributed to the writing of the manuscript and was the supervising.

KOA, ZKY, and AVS assisted the patient as oncologists, and contributed to the writing of the manuscript, particularly with regard to the clinical data.

DAD and ASP performed immunohistochemical studies and creating a figure.

SLP -supervision.

All authors read and approved the final version of the manuscript.

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