EXCEPTIONAL CASE REPORT

blood advances

Fatal late-onset CAR T-cell–mediated encephalitis after axicabtagene-ciloleucel in a patient with large B-cell lymphoma

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Key Points

- Fatal neurotoxicity developed 9 months after infusion of axi-cel in a patient treated for diffuse LBCL relapse after allogeneic hematopoietic cell transplantation.
- CAR T cells found in cerebral spinal fluid in vivo and in postmortem brain tissue suggest that late neurotoxicity was directly CAR T cell mediated.

Treatment with CD19-directed chimeric antigen receptor (CAR) T cells has evolved as a standard of care for multiply relapsed or refractory large B-cell lymphoma (r/r LBCL). A common side effect of this treatment is the immune effector cell–associated neurotoxicity syndrome (ICANS). Severe ICANS can occur in up to 30% to 40% of patients treated with axicabtagene-ciloleucel (axi-cel), usually within the first 4 weeks after administration of the dose and usually responding well to steroids. We describe a case of progressive central neurotoxicity occurring 9 months after axi-cel infusion in a patient with r/r LBCL who had undergone a prior allogeneic hematopoietic cell transplant. Despite extensive systemic and intrathecal immunosuppression, neurological deterioration was inexorable and eventually fatal within 5 months. High CAR T-cell DNA copy numbers and elevated levels of interleukin-1 (IL-1) and IL-6 were found in the cerebral spinal fluid as clinical symptoms emerged, and CAR T-cell brain infiltration was observed on autopsy, suggesting that CAR T cells played a major pathogenetic role. This case of unexpected, devastating, late neurotoxicity warrants intensified investigation of neurological off-target effects of CD19-directed CAR T cells and highlights the need for continuous monitoring for late toxicities in this vulnerable patient population.

Introduction

Anti-CD19 chimeric antigen receptor (CAR) T cells, like axicabtagene-ciloleucel (axi-cel) and tisagenlecleucel, have shown great potential in treating relapsed/refractory (r/r) large B-cell lymphoma (LBCL). Among the acute toxicities of CAR T-cell treatment, the immune effector cell–associated neurotoxicity syndrome (ICANS) is of particular concern.[1-3]

ICANS, which more commonly occurs after treatment with axi-cel than with other CAR T products,[4] usually manifests as toxic encephalopathy with aphasia and confusion, sometimes including coma or seizures. The pathophysiology is not fully understood, but it has been suggested that endothelial activation by chemokines and cytokines leads to disruption of the blood-brain barrier, with subsequent CAR T-cell invasion and pericyte activation.[5,6] With appropriate supportive care and steroids, ICANS is completely reversible in most cases, although sporadic events of fatal cerebral edema have been observed.[1,5,7] Generally, ICANS occurs within the first 2 weeks after administration of axi-cel and occasionally in the third or fourth week, but only exceptionally thereafter.[3,5,6]

We describe a patient who developed fatal autoimmune encephalitis associated with CAR T-cell infiltration of the brain, with onset of symptoms 8 months after axi-cel infusion. The Diakonie Hospital Ethics Committee approved this study, which was conducted in accordance with the Declaration of Helsinki.

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Original data are available by e-mail request to the corresponding author (jung@diak-stuttgart.de).

Case presentation

A 50-year-old man was first diagnosed with diffuse large B-cell lymphoma (DLBCL) (IPI0, CNS-IPI0) in October 2013. Apart from prostate cancer (pT2c pN0 cM0, Gleason 4), which was surgically removed in October 2010 and was in complete remission, there were no preexisting diagnoses. The further course of the disease is described in supplemental Table 1. After failure of multiple lines of therapy, including allogeneic hematopoietic cell transplantation (allo-HCT), he was treated with axi-cel in August 2019 without any acute toxicity except pancytopenia.[8] Notably, he had achieved full donor chimerism at the time of leukapheresis for axi-cel production. Metabolic complete response was achieved in September and retained throughout the entire observation time.

During the first 7 months of follow-up, there were no serious complications. The patient reported having a better quality of life than in the previous 7 years. However, repeated episodes of neutropenic fever required regular injections of granulocyte colony-stimulating factor to maintain a sufficient neutrophil count.

Although our patient remained largely B-cell–depleted throughout the entire follow-up, he showed a rapid $CD4^+$ T-cell recovery in contrast to many other CD19 CAR T-cell recipients[9] (Figure 1C).

In April 2020, the patient reported increasing fatigue, followed by painless loss of the central visual field. Within 2 months, additional neurological symptoms appeared and rapidly progressed, whereas repeated magnetic resonance imaging (MRI) scans of the brain, analyses of the cerebrospinal fluid (CSF), and extensive neurological workup revealed no pathological findings (Table 1). Because CAR T-cell frequencies in the peripheral blood had decreased to background levels, CSF was not initially assessed for CAR T cells. Therapeutic attempts included high-dose steroids and intravenous immunoglobulins, but were largely unsuccessful.

In July 2020, the patient had completely lost vision and gradually developed dysarthria, dysphagia, ataxia, and intermittent delirium. Changes in MRI scans consistent with autoimmune encephalitis became detectable (Figure 1A) and prompted us to check the CSF for CAR T cells. A high CAR T-specific DNA copy number was found in a fresh sample (but also in a previous sample obtained shortly after the start of symptoms and analyzed post hoc),[10,11] suggesting CAR T-cell infiltration of this compartment (Figure 1C). Flow cytometry confirmed the presence of T cells in the CSF. After intrathecal administration of methotrexate, cytarabine, and dexamethasone[12] had led to only transient improvement, another MRI scan suggested posterior reversible encephalopathic syndrome (PRES), whereas the initial areas of encephalitis appeared to have improved (Figure 1B). Despite repeat intrathecal therapy, the patient's clinical status deteriorated. MRI documented resolution of PRES but showed progressive encephalitis, and CAR T-cell DNA levels in the CSF remained high.

With all other options exhausted, we attempted elimination of CSF circulating CAR T cells by intrathecal administration of alemtuzumab.[13] The symptoms markedly improved, and CSF CAR T DNA became undetectable (Figure 1C). Intrathecal alemtuzumab was stopped after 4 applications (cumulative dose, 10 mg). By that time, the patient was no longer psychotic, was able to communicate with gestures and sounds, and took part in physiotherapy and logopedic exercises. However, 2 weeks after the last dose of alemtuzumab, CAR T-cell DNA reappeared in the CSF along with rapid neurological deterioration, which was eventually fatal.

A brain autopsy showed extensive inflammation consistent with excessive immune reaction involving the brain stem, cerebellum, and limbic system. In severely affected areas, prominent gliofibrillar astrocytosis was found, associated with massive macrophage/microglia activation and diffusely dispersed infiltrates of cytotoxic T lymphocytes (Figure 1D-E). Negative SV-40 stain ruled out progressive multifocal leukoencephalopathy. DNA was extracted from the most affected areas and was found to contain CAR T-cell DNA, suggesting that the patient most likely had died of severe CAR T-cell–triggered autoimmune encephalitis.

Post hoc cytokine profiling of the CSF on day 374 revealed a cytokine pattern dominated by highly elevated levels for interleukin-1 (IL-1) and IL-6, consistent with ICANS[6] (supplemental Table 2).

Results and discussion

To our knowledge, this is the first description of severe late neurological toxicity caused by CAR T cells in the brain. One of several puzzling aspects of this case is the complete absence of neurological symptoms in the early postdose phase. For several months, the patient remained completely asymptomatic, raising the question of what could have triggered the process, seemingly out of nowhere. The patient's relatives reported that ~2 months after the CAR T-cell therapy, he underwent a significant personality change toward a much more self-assured, almost aggressively optimistic and outgoing attitude compared with a rather shy and cautious demeanor before, which in hindsight could have been an early symptom.

Two months before the onset of symptoms, the patient had received several vaccinations (diphtheria, tetanus, pertussis, polio, and *Haemophilus influenzae* type B [HiB]) which were tolerated without significant side effects. It would be difficult to determine what a vaccination can cause in a transplant recipient's immune system that has been altered by anti-CD19 CAR T-cell therapy, but a causative role of CAR T-cell–mediated immune encephalitis seems unlikely.

Another possible explanation is fludarabine toxicity. However, this side effect usually occurs within the first months after exposure and is not consistent with the imaging and histological findings in this patient.[14-16] In the context of CAR T-cell therapy, progressive multifocal leukoencephalopathy is a rare early event attributable to fludarabine.[17]

As the patient had undergone allo-HCT and complete chimerism had persisted throughout, his T lymphocytes, including the CAR T cells, were of donor origin, raising the possibility of a graft-versus-host reaction as an underlying pathologic mechanism. Although a limited number of cases of CNS GVHD are described in the literature, this concept remains controversial.[18-20] What argues against CNS GVHD in this case is the late onset, the absence of any other clinical symptoms of GVHD, and the presence of CAR T cells in CSF and brain autopsy samples.

Recently, CD19-expressing brain cells have been discussed as possible drivers of CD19 CAR T-associated neurotoxicity.[21] However, immunohistochemical analysis of the affected brain tissue did not reveal B lymphocytes or other $CD19^+$ targets.

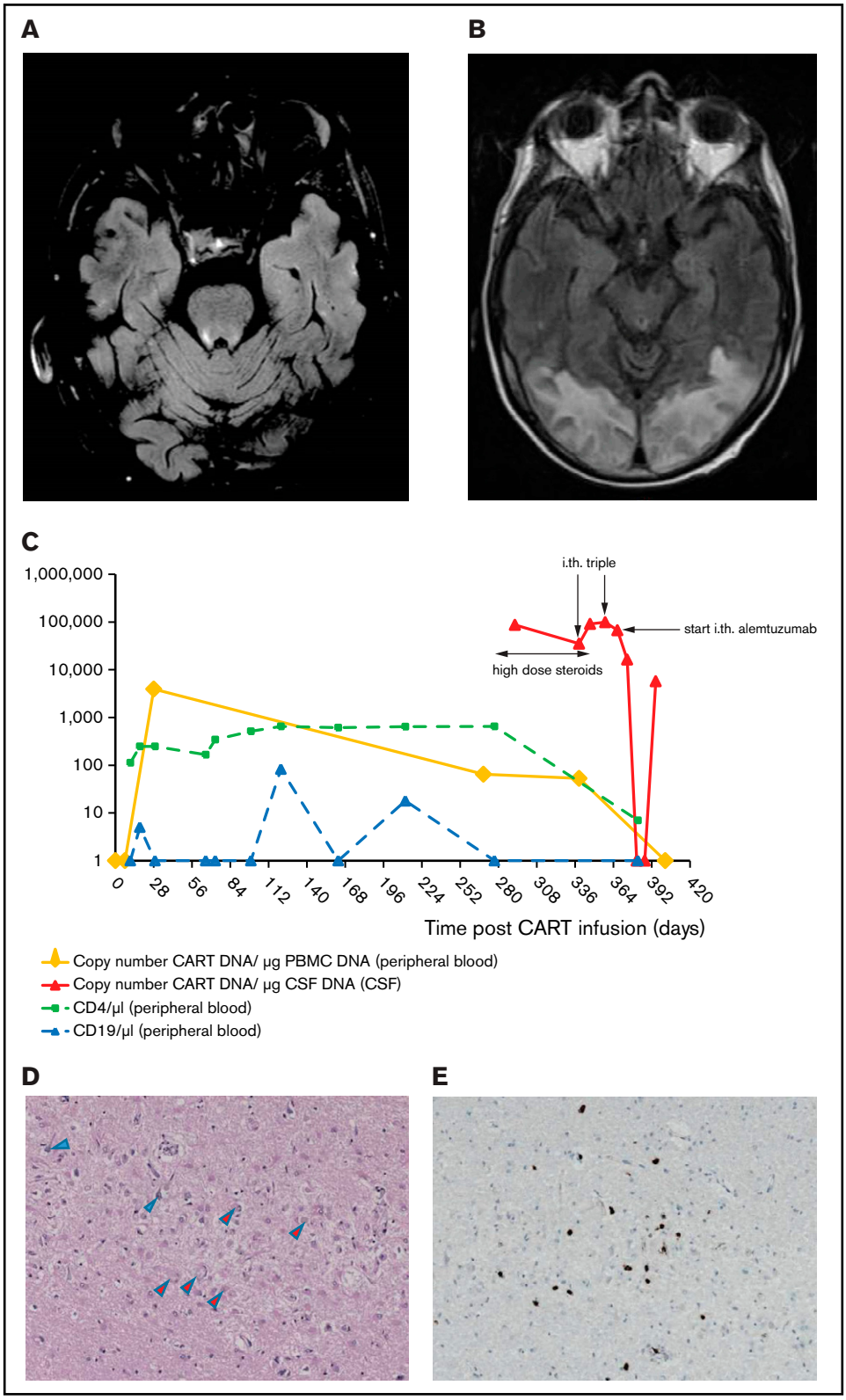

Figure 1. MRI scans, CAR T-cell kinetics in the peripheral blood, and brain histology. (A) MRI scan showing encephalitis in the brain stem and cerebellum. (B) MRI results consistent with PRES. (C) Kinetics of peripheral blood $CD4^+$ and $CD19^+$ cell counts and CAR T-cell expansion in peripheral blood and CSF. (D) Postmortem brain tissue showing severe loss of pyramidal cells in the hippocampus associated with gliofibrillar astrocytosis (blue arrowheads) and macrophage infiltration (red arrowheads). CD68 was used for staining of macrophages/microglia. (E) Postmortem brain tissue showing infiltration of the hippocampal pyramidal layer with $CD8^+$ cytotoxic T cells identified by dark brown peroxidase staining (original magnification ×100). i.th., intrathecal.

Table 1. Development of symptoms after axi-cel infusion, diagnostic measures, and therapeutic interventions before discovery of CAR-T-cell DNA in CSF

	Symptoms	Diagnostics	CAR T-cell DNA copy number per microgram CSF DNA	Therapeutic interventions
D+262	Loss of vision	No structural abnormalities of visual organ. Blood test results normal (including inflammatory markers [CRP, ferritin, blood sedimentation], vitamin B1/B6/B12 and folic acid, fibrinogen, triglycerides, and PCR for CMV-/ EBV-/HHV6-/ParvoB19-DNA). Brain MRI normal. CSF results normal (including analysis for HSV1/2, VZV, HHV6, borrelia, FSME, measles, *Treponema pallidum*, enterovirus, adenovirus, *Aspergillus fumigatus*, toxoplasma, mycoplasma, LCM virus and JCV). SARS-CoV-2 negative.	NA	Methylprednisolone 1 g/d for 5 d → no change
D+289	Ataxia of legs and trunk	Blood test results normal (including inflammatory markers [CRP, ferritin, blood sedimentation], fibrinogen, triglycerides, autoimmune panel). Brain MRI normal. CSF results normal (including autoimmune panel, analysis for HSV1/2, VZV, HHV6, borrelia, FSME, measles, *T pallidum*, enterovirus, adenovirus, *A. fumigatus*, toxoplasma, mycoplasma, LCM virus, and JCV). PET scan showed lymphoma in complete response, no splenomegaly. Genetic workup for LHON+ is negative.	86 783*	IV immunoglobulins ceased because of allergic reaction → no change
D+309	Wheelchair user, slight dysarthria, hearing impairment	Blood tests normal (including inflammatory markers [see D+289]). MRI of head and neck normal. CSF: 3 cells per microliter, 20% $CD3^+$; of those 31% $CD8^+$ and 68% $CD4^+$, paraneoplastic antibody panel negative. Electroneurography normal. EEG: slight unspecific general slowing. Metabolic test for porphyrias negative.	NA	Dexamethasone 40 mg/d for 4 days → no change
D+326	Dysarthria, dysphagia, myasthenia	Blood test results normal (including inflammatory markers). Test for acetyl choline receptor antibodies negative.	NA	IV immunoglobulins+100 mg prednisolone stopped because of massive pain attack → no change

CRP, C-reactive protein; D+, days after axi-cel infusion; HSV, herpes simplex virus; EBV, Epstein-Barr virus; FSME, Frühsommer (tick-borne)meningoencephalitis; HHV6, human herpesvirus 6; JCV, JC virus; LCM virus, lymphocytic choriomeningitis virus; LHON, Leber hereditary optic neuropathy; PET, positron emission tomography; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VZV, varicella-zoster virus.

*Post hoc assessment.

In summary, even though we found evidence of CAR T-cell–mediated autoimmune encephalitis, the trigger and underlying mechanisms remain unclear. Although the therapeutic effects of CAR T-cell therapies for DLBCL are promising, unexpected long-term side effects may occur and should always be considered.

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Authorship

Contribution: S.J. and P.D. collected the data and wrote the manuscript; J.G. and S.v.H. reviewed the manuscript; P.P. and R.M. supplied and analyzed the MRI slides and reviewed the manuscript; J.S. and K.K. supplied and analyzed the histological slides and contributed to the manuscript; V.D. designed, performed, and analyzed cytokine profiling; and M.S. and A.K. designed and performed the PCR assays, analyzed the data, and reviewed the manuscript.

Conflict-of-interest disclosure: P.D. has been a consultant for AbbVie, AstraZeneca, bluebird bio, Gilead, Janssen, Novartis, Riemser, and Roche and has served on the speakers' bureau for AbbVie, AstraZeneca, Gilead, Novartis, Riemser, and Roche. M.S. has received research grants from Apogenix, Hexal, and Novartis; travel grants from Hexal and Kite; and financial support for educational activities and conferences from bluebird bio, Kite, and Novartis. He has served on the advisory board member of MSD; was joint primary investigator in clinical trials for MSD, GSK, Kite, and BMS; and is cofounder of and a shareholder in TolerogenixX Ltd. The remaining authors declare no competing financial interests.

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