Received: 28 July 2021 | Revised: 19 October 2021 | Accepted: 1 December 2021

DOI: 10.1002/ccr3.5209

Clinical Case Reports Open Access WILEY

CASE REPORT

Hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) following treatment with tisagenlecleucel

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Funding information
This work was partially supported by the Department of Health, Autonomous Community of Madrid grant COV20/CM, Ministry of Science and Innovation grant PID2019-107545RB-I00, Ministry of Economy and Competitiveness ISCIII-FIS grant FIS PI20/00521 co-financed by ERDF (FEDER) Funds from the European Commission, "A way of making Europe."

Abstract

Chimeric antigen receptor (CAR) T cell–related HLH/MAS is an unusual manifestation of severe cytokine release syndrome (CRS) with poor prognosis and a challenging diagnosis. The establishment of specific diagnosis criteria is essential, and the combination of several techniques for CAR T-cell follow-up, allows a more precise management of this complication.

KEYWORDS

CAR T cells, hemophagocytic lymphohistiocytosis, macrophage activation syndrome, tisagenlecleucel

Reyes Maria Martín-Rojas and Ignacio Gómez-Centurión are contributed equally to this work.

1 | INTRODUCTION

Immunotherapy with T cells genetically engineered to express CD19-specific chimeric antigen receptor (CAR) has dramatically changed the treatment of aggressive B-cell malignancies.[1,2] Two products—Tisagenlecleucel and Axicabtagene ciloleucel—have been approved by the European Medicines Agency (EMA) for the treatment of relapsed/refractory CD19+ diseases. Tisagenlecleucel (Tisa-Cel) is a CD19-targeted CAR T-cell therapy approved to treat adult patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy and children and adults up to age 25 with relapsed/refractory B-cell acute lymphoblastic leukemia (ALL).[3,4]

Despite CAR T cells can induce rapid and durable responses, this therapy is associated with specific and severe toxicities, representing an obstacle in its widespread use. Cytokine-release syndrome (CRS) is the most frequent toxicity after infusion.[5,6] This systemic inflammatory response can rarely evolve into a fulminant hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) which is associated with high mortality rates.[7,8] Diagnosis criteria for this entity have been recently proposed,[7] but discrimination between severe CRS, CAR T-related HLH/MAS, and malignancy-associated HLH/MAS can be challenging.

We describe two cases of CAR T cell–related HLH/MAS and the difficulties linked to the diagnosis and management of this unusual complication.

2 | CASES REPORT

2.1 | CASE 1

A 34-year-old male was diagnosed with a T-cell rich DLBCL with HLH/MAS at diagnosis. He received a first line of treatment with R-CHOP and high dose methotrexate as CNS prophylaxis, with end of treatment PET/CT scan showing progressive disease. He subsequently started a second-line treatment with ESHAP and autologous HSCT, but he was refractory to chemotherapy and did not proceed to HSCT. At this point, he was admitted to undergo Tisa-cel therapy in our center.

High tumor burden with progressive disease was documented after bridging therapy, with a PET/CT scan showing several hypermetabolic lymph nodes, massive hepatic, and lung infiltration with evidence of disease progression comparing these findings with previous scans. Besides, the patient presented with high LDH levels of 650 U/L.

During lymphodepletion with cyclophosphamide and fludarabine, persistent fever with neutropenia was treated with empiric broad-spectrum antimicrobials agents, without resolution. Microbiological cultures were negative and the patient did not show any infectious symptoms, attributing fever to B symptoms due to progressive disease.

On day +1, the patient continued with persistent fever and met criteria for a grade 1 CRS according to Lee et al.[5] and was therefore diagnosed as such. However, persistent fever during lymphodepletion was taken into account and the patient was initially managed as a febrile neutropenia with broad-spectrum antimicrobials, delaying CRS-targeted treatment due to clinical stability and not meeting criteria of grade 2 CRS. After ruling out infectious causes, he received tocilizumab on days +15 and +20, achieving partial response.

However, high-grade fever was associated with hyperferritinemia with ferritin levels above 10,000 ng/ml from day 0 (peak 50,000 ng/ml on day +23) together with severe cytopenias from day +10 consisting on hemoglobin levels <8 g/dl, platelets <15,000/μl and absolute neutrophil counts (ANC) of 0–100/μl, with high transfusion requirements. The patient also presented an altered liver function, with a progressive increase of ALT (from 78 U/L on day +1 up to 547 U/L on day +36) and AST levels (from 161 U/L on day +1 up to 959 U/L on day +36) as well as an increase of GGT (200 U/L on day +1, with a peak value of 576 U/L on day +20) and alkaline phosphatase levels (values around 300 U/L from day +3), and bilirubin levels increasing from day +24 (levels up to 8.2 mg/dl on day +36). Coagulopathy was also observed, including low fibrinogen levels from day +1 (113 mg/dl) with values as low as 60 mg/dl on day +18 and high D-dimer levels from 2380 ng/ml on day +1 up to 5583 ng/ml on day +36. APTT and PT also started to be increased from day +32, with an APTT of 40 s and PT of 20 s. Besides, high values of IL-15, IL-1β, GM-CSF, and IL-6 were determined and triglycerides level was 197 mg/dl. At this point, HLH/MAS was suspected and bone marrow aspiration was performed on day +22, showing hemophagocytosis and no evidence of infiltration by lymphoma.

In order to distinguish between CAR T cell–related and malignancy-associated HLH/MAS, a PET-CT was performed on day +25, showing paradoxical response. Concurrently, CAR T-cell expansion in peripheral blood (PB) was detected by flow cytometry and polymerase chain reaction (PCR). CAR T cells started to be minimally detected on day +7 (150 cells/ml), with the peak expansion observed on day +31 (2810 cells/ml). Furthermore, a lymph node biopsy was performed where CAR T cells were detected and only 4% of neoplastic lymphocytes were observed. Normal B cells were not detected. (Figure 1).

Subsequently, HLH/MAS-targeted treatment was initiated. The patient received high dose corticosteroids from day +22 onwards, with good response and fever remission

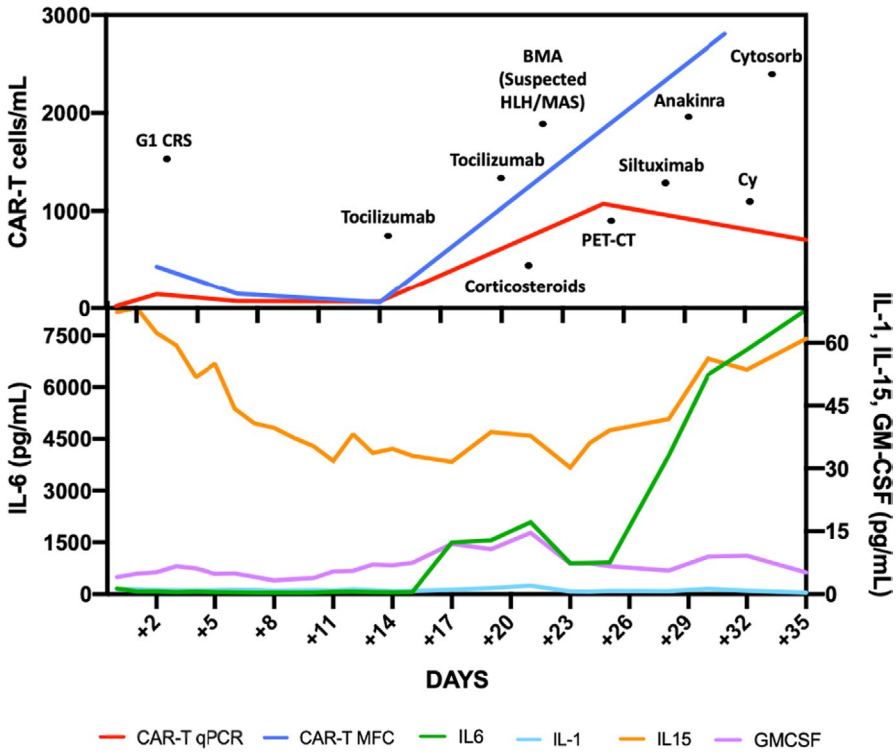

FIGURE 1 Evolution of CAR-T cells detection in peripheral blood by multiparameter flow cytometry (MFC) and quantitative PCR (qPCR), cytokines measurements and patient clinical management (Case 1). CAR-T cells detection is showed in cells/ml and cytokines in pg/ml. Normal levels: IL-6 0.16–37.7 pg/ml; IL-1 0.17–24 pg/ml; IL-15 1.25–13.1 pg/ml; GM-CSF 0.5–728.1 pg/ml. G1 CRS: Grade 1 cytokines release syndrome; BMA: Bone marrow aspirate; Cy: Cyclophosphamide

on day +24, but becoming febrile again from day +34. He was then administered Siltuximab on day +26, showing a decrease of IL-6 levels afterward. A second dose of Siltuximab was administered on day +36, showing no clinical response. Anakinra was also administered from days +29 to +32, without response. Both cyclophosphamide and hemoadsorption with extracorporeal cytokine adsorber (CytoSorb®) were initiated on day +35, with no response. Etoposide was not considered due to severe liver function impairment.

Patient showed no response to treatment and died from multiorgan failure on day +36. At necropsy, tumor necrosis was the predominant finding, with several lymph node conglomerates with an estimated 15% of residual tumor cells by morphology in the total cellularity of these lesions.

2.2 | CASE 2

A 22-year-old male with relapsed/refractory Philadelphia chromosome negative B-cell ALL underwent Tisa-cel therapy in 2020. The patient was diagnosed with B-ALL with a hyperploid karyotype and was initially treated with a PETHEMA *(Programa Español de Tratamiento en Hematología)* regimen, a pediatric-inspired protocol with an intensive combination of chemotherapy. However, the patient progressed within a year and was subsequently treated with blinatumomab, presenting several complications during the course of this treatment such as grade 2 CRS managed with tocilizumab and corticosteroids and infectious complications among others, which forced to discontinue this strategy. He then received a third line with inotuzumab ozogamicin but progressed during treatment. At this point, he was accepted for administration of CAR T-cell therapy.

After a bridging therapy with cyclophosphamide, vincristine, and dexamethasone, he received lymphodepletion with fludarabine and cyclophosphamide. At the time of initiation of lymphodepletion, the patient had a progressive disease with 95% of blasts in bone marrow aspirate and 70% in peripheral blood.

At the moment of Tisa-cel infusion, the patient was afebrile with a baseline ferritin of 6905 ng/ml. He developed a grade 1 CRS 6 h after infusion, progressing to grade 2 on day +2, and was treated with three doses of tocilizumab. Plasma cytokine levels showed high values of IL-15, GM-CSF, and IL-6. Both symptoms and cytokine levels rapidly improved after administration of tocilizumab.

On day +11, he presented with a new episode of fever with high flow oxygen therapy requirements. He associated impaired renal and liver function, consisting on mildly elevated AST (peak 80 U/L on day +9) and AST (peak 450 U/L on day +13), high levels of GGT (peak 720 U/L on day +17), alkaline phosphatase (peak 300 U/L on day +15), and slightly elevated bilirubin levels, up to 2.9 mg/dl on day +46. Ferritin levels were also increased, with values above 10,000 ng/ml from day +2, and maximum levels of 800,000 ng/ml on day +14. Besides, coagulopathy was observed from day +10, consisting on prolonged APTT (peak 43 s on day +13) and PT (peak 20 s

on day +13), low fibrinogen levels with a minimum of 70 mg/dl on day +13 and elevated D-dimer with a peak of 7688 ng/ml on day +18. The patient also developed pancytopenia with the lowest blood counts occurring at day +13, with hemoglobin levels of 5.7 g/dl, 7000 platelets/μl, and ANC 0/μl. CAR T-cell expansion in PB was detected (4141 CAR T/mL on day +14).

At this point, the patient met criteria of CAR T-related HLH/MAS according to Neelapu et al. as he presented with ferritin levels >10,000 ng/ml during the CRS phase of CAR T, together with grade ≥3 organ toxicities. Therefore, further studies were performed, showing hypertriglyceridemia (596 mg/dl), high levels of soluble CD25 (>10,000 pg/ml), IL-18 (>10,000 pg/ml), INF-gamma (1365 pg/ml), and TNF-alpha (241 pg/ml).

The patient was transferred to the ICU and was managed with supportive therapy, dexamethasone 20 mg/6 h from day +14, etoposide 150 mg/m^2 on days +15 and +18, and anakinra 100 mg/12 h from day +15 to day +17, with progressive improvement. Dexamethasone was slowly tapered and discontinued on day +44. Tocilizumab was not administered as, according to local protocols, we use a maximum of three doses, which the patient had already received on day +2 (Figure 2).

Despite successful management of CAR T-related HLH, he died on day +47 due to a necrotizing pancreatitis in the context of extramedular ALL progression.

3 | METHODS

Chimeric antigen receptor T-cell expansion in PB was monitored by multiparameter flow cytometry through detection of labeled CD19 in T cells (Human CD19 Protein®) and by quantitative PCR using the HIV /COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche) following manufacturer's instructions.

Tumor samples from core needle biopsies were processed using QIAamp DNA Tissue Kit (Qiagen) to detect CAR T cells.

Plasma cytokine levels (IL-6, IL-15, IL-1β, and GM-CSF) were determined with last generation ELISA multiplex (Ella®, ProteinSimple®).

Written informed consent was obtained in compliance with our institutional review board and the Declaration of Helsinki.

4 | DISCUSSION

Hemophagocytic lymphohistiocytosis/macrophage activation syndrome is an hyperinflammatory syndrome which typically consists on hyperactivation of cytotoxic T and natural killer (NK) cells and macrophages, leading to a massive cytokine production, lymphohistiocytic tissue infiltration, and immune-mediated multiorgan failure.[7,9]

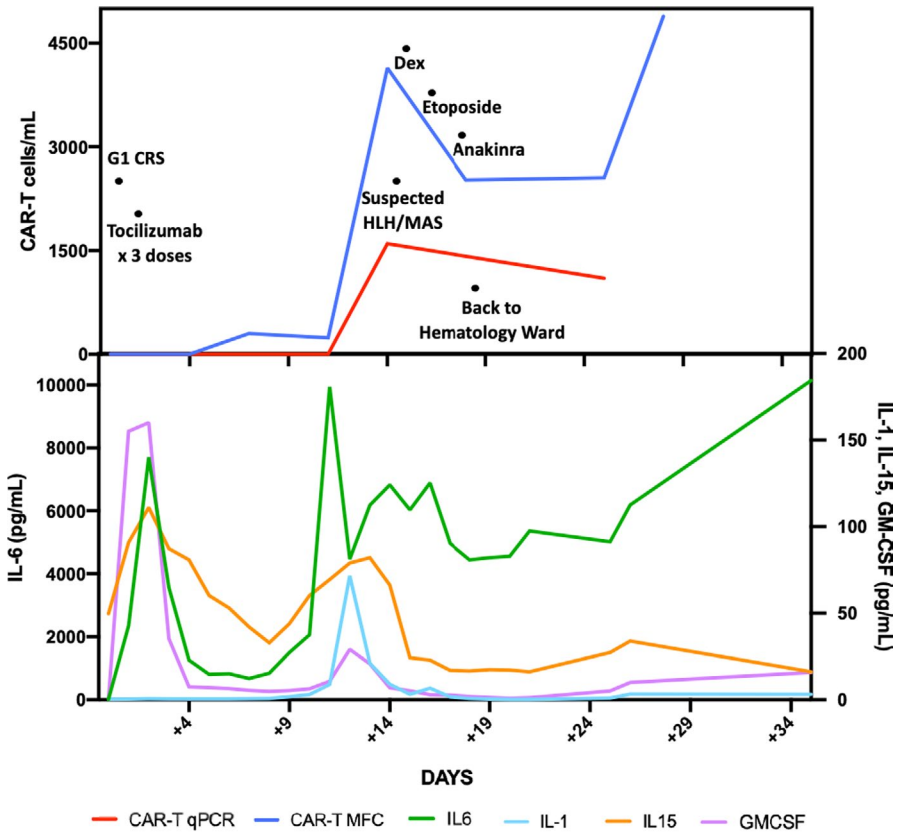

FIGURE 2 Evolution of CAR-T cells detection in peripheral blood by multiparameter flow cytometry (MFC) and quantitative PCR (qPCR), cytokines measurements and patient clinical management (Case 2). CAR-T cells detection is showed in cells/ml and cytokines in pg/ml. Normal levels: IL-6 0.16–37.7 pg/ml; IL-1 0.17–24 pg/ml; IL-15 1.25–13.1 pg/ml; GM-CSF 0.5–728.1 pg/ml.G1 CRS: Grade 1 cytokines release syndrome; Dex: Dexamethasone

CAR T-related HLH is rare, with severe and fulminant cases occurring in approximately 1% of patients receiving this treatment. However, this complication is associated with high mortality rates and a prompt diagnosis and early management is mandatory.[7]

Given its hyperinflammatory nature, it is considered that CRS and HLH/MAS might belong to a similar spectrum of systemic disorders, which makes HLH/MAS diagnosis difficult, especially in the context of CRS.[7] The traditional diagnosis criteria for secondary HLH/MAS such as HLH-2004[10] and H-Score[11] are not specific, and Neelapu et al.[7] have proposed new criteria for CAR T-related HLH/MAS, considering it is crucial to promptly diagnose this complication. Both our patients met criteria of CAR T-related HLH/MAS according to Neelapu et al. Thereby, our first patient had hyperferritinemia with ferritin values >10,000 ng/ml from day 0 together with grade 3 liver toxicity from day +19 plus a histology confirmation of hemophagocytosis in the bone marrow aspirate performed on day +22. Conversely, the second patient presented with hyperferritinemia with ferritin >10,000 ng/ml from day +2 and grade 3 pulmonary affection with progressive respiratory insufficiency from day +14. The second patient showed an optimal response probably due to its early management, even if the biological differences between both cases should also be considered.

Distinction between this entity and malignancy-triggered HLH/MAS can be challenging and their management should be different. Currently, there are no generally accepted criteria for malignancy-triggered HLH/MAS.[9] In Case 1, the presence of previous HLH/MAS at lymphoma diagnosis challenged even more this differential diagnosis. However, the bone marrow aspiration performed at day +22 which showed no evidence of infiltration by lymphoma cells, together with the detection of CAR T cells in PB and a lymph node biopsy and the paradoxical response observed in the PET-CT allowed a more specific diagnosis and management in our patient. In Case 2, the fact that CAR T expansion was detected on peripheral blood was interpreted as a good response and HLH/MAS was therefore attributed to CAR T-cell toxicity rather than to malignancy.

So far, very little has been published on CAR T-associated HLH/MAS and no formal guidelines for its management exist. Most authors recommend anti-IL-6 therapy and steroids, adding etoposide if no improvement after 48h,[7,12] as etoposide selectively deletes activated T cells and suppresses inflammatory cytokine production.[9]

Anakinra, a recombinant IL-1 receptor antagonist, is an emerging treatment for CAR T-associated HLH/MAS[12] and was used in our patients. Recent reports and preclinical studies suggest that there may be a benefit in combining Anakinra with other anti-inflammatory agents.[13,14] However, due to the variability of these studies, the ideal dose schedule for this drug in HLH/MAS is still to be determined.[15]

Monoclonal antibodies may have a potential role in the management of this condition in the near future.[7] Emapalumab, an anti-IFN-gamma has been approved by the FDA for patients with refractory primary HLH/MAS and a phase two clinical trial to evaluate its efficacy in secondary HLH/MAS is undergoing, with promising interim results.[16] However, there is still no formal indication of emapalumab in secondary HLH/MAS.[17,18]

In conclusion, CAR T cell–related HLH/MAS is an unusual manifestation of severe CRS after CAR T-cell therapy, with poor prognosis, high mortality rates, and a challenging diagnosis. The establishment of specific diagnosis criteria is essential for a prompt identification of patients suffering from this complication in whom any delay in treatment can be fatal. Also, the combination of diagnosis techniques for CAR T-cell follow-up allows a more precise diagnosis and more accurate distinction between CAR T cell–related and malignancy-associated HLH/MAS, therefore granting a better-targeted treatment. However, further studies are needed to provide better preventive and treatment strategies to improve the outcome of these patients.

ACKNOWLEDGEMENTS

We would like to thank patients and their families for their collaboration toward research.

CONFLICT OF INTEREST

MK: Consultancy, Honoraria for Gilead and Novartis. RB: Speaker for Gilead (Kite). GO: Speaker for Gilead (Kite).

AUTHOR CONTRIBUTIONS

RMMR, IGC, RB, and MK Conception, design, collection and assembly of data, and manuscript writing. All authors: Provision of study materials or patients, data analysis, interpretation, and final approval of manuscript.

CONSENT

The authors confirm that informed written consent was obtained from the patient in accordance with the journal's patient consent policy.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Martín-Rojas RM, Gómez-Centurión I, Bailén R, et al. Hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) following treatment with tisagenlecleucel. *Clin Case Rep*. 2022;10:e05209. doi:10.1002/ccr3.5209