CASE REPORT

Immune-mediated facial nerve paralysis in a myeloma patient post B-cell maturation antigen-targeted chimeric antigen receptor T cells

Chimeric antigen receptor (CAR) T-cell therapies targeting B-cell maturation antigen (BCMA) have changed the standard of care for multiple myeloma (MM).[1] Recently, the Food and Drug Administration approved idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel) for MM,[2,3] however, further research is needed to fully understand the long-term safety and efficacy of these treatments.

Neurotoxicity including its most common form - immune effector cell-associated neurotoxicity syndrome (ICANS) - is a potential side effect of CAR T-cell therapies. ICANS can range from confusion or headaches to seizures, coma, or death.[4] The exact cause of CAR T-mediated neurotoxicity is not fully understood, but it is thought to be related to cytokine release by CAR T in and outside of the central nervous system (CNS). In addition to ICANS, other CAR T-related neurotoxicities, such as movement disorders, cognitive impairment, and personality changes, have been described.[4] However, the pathophysiology of these is even less well understood.

Here, we describe an MM patient, without prior history of CNS involvement by her myeloma, who developed bilateral facial nerve palsy (facial diplegia) following cilta-cel. The focal neurologic deficiency correlated with a marked expansion of BCMA-targeted CAR T in the peripheral blood (PB) and recruitment of central memory-type CAR T cells into the CNS. Our study suggests mechanisms potentially resulting in CAR T CNS infiltration with neurotoxicity and ways to prevent/treat these off-tumor effects.

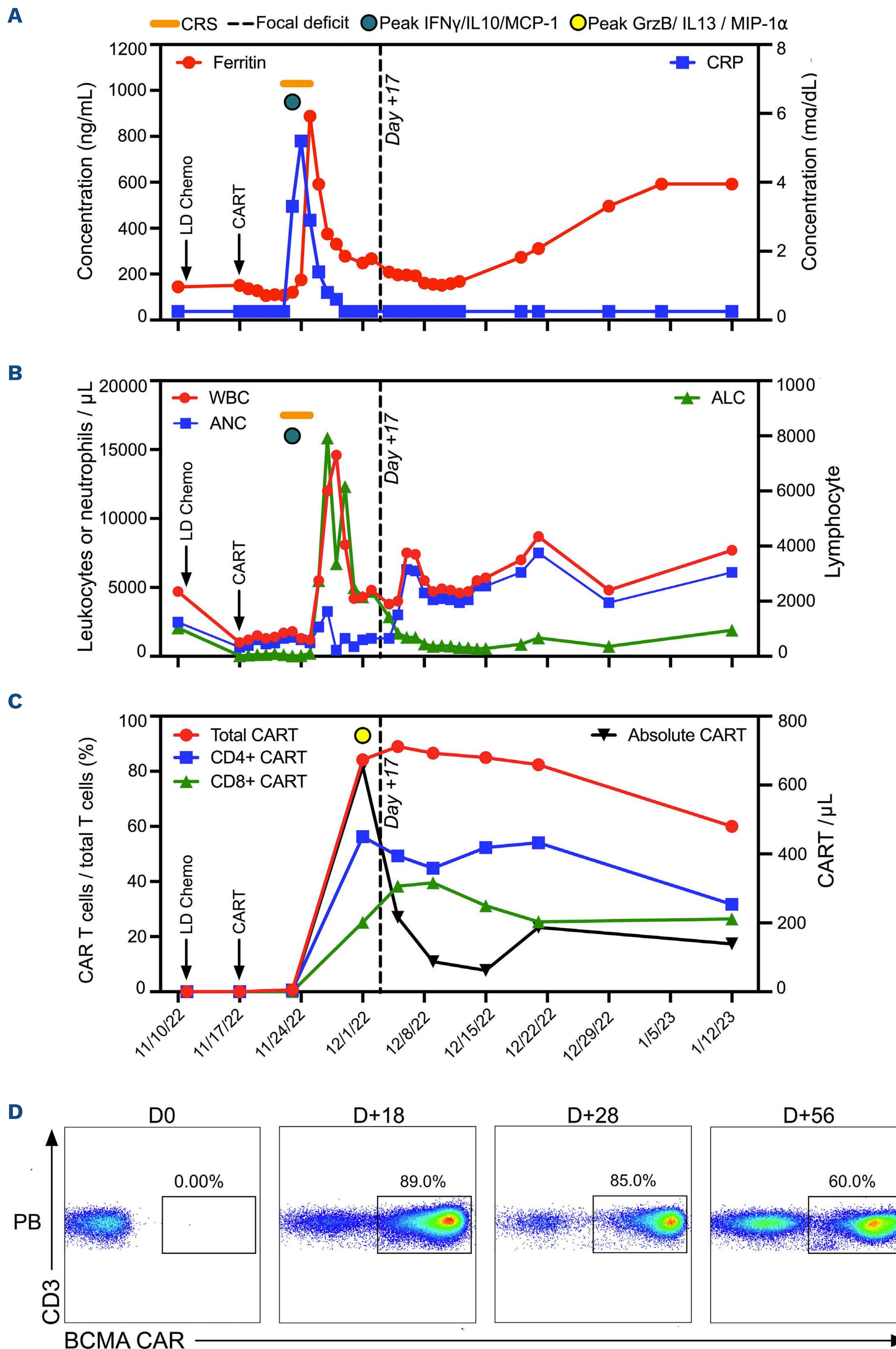
Our patient was diagnosed with immunoglobulin (Ig)G κ MM approximately 4.5 years prior to receiving CAR T. She received multiple prior treatments (induction with bortezomib/lenalidomide/dexamethasone followed by carfilzomib/lenalidomide/dexamethasone, high-dose melphalan/autologous stem cell transplant, a clinical study with an MM-dendritic cell (DC) fusion vaccine + lenalidomide as maintenance, carfilzomib/daratumumab/dexamethasone). After her most recent line of treatment, she was found to have another relapse of her myeloma with 15% plasma cells in the BM with 1q amplification and *TP53* deletion. We performed leukapheresis and decided to use daratumumab/pomalidomide/dexamethasone for post-apheresis bridging in an effort to control the myeloma during manufacturing, avoid significant myelosuppression and to allow the patient to minimize the frequency of office visits prior to admission for CAR T-cell therapy. Leukapheresis and lymphodepleting chemotherapy with cyclophosphamide/fludarabine were performed and cilta-cel CAR T were given in November of 2022 (Figure 1A-C).

Following CAR T, she developed grade 1 cytokine release syndrome (CRS) and *Enterobacter cloacae* urinary tract infection. As part of our broad CAR T-related research efforts, serum concentrations of different cytokines/chemokines were measured and we found the development of CRS to coincide with peak levels of interferon (IFN)γ, interleukin (IL)10, and monocyte chemoattractant protein-1 (MCP-1) followed by increases in C-reactive protein and ferritin (Figure 1A, E). The CRS was treated by giving one dose of tocilizumab on day +6 and two doses on day +7. The patient also received dexamethasone 10 mg twice daily on days +7 to +8 for ongoing fevers and antibiotics for her infection.

Subsequently, the patient showed recovery of her absolute lymphocyte counts (Figure 1B) paralleled by a marked expansion of BCMA-targeted $CD4^+$ and $CD8^+$ CAR T cells in her PB (Figure 1C, D). Shortly before her CAR T cells reached peak levels, the patient showed a substantial increase in granzyme B, IL13, and MIP-1α serum concentrations (Figure 1C, E). Only 2 days later (day +17), she started to complain of sudden difficulties speaking, chewing and puckering her lips.

We consulted with our neurologists and on exam, she was noted to have bilateral cranial nerve VII palsy being unable to smile, puff her cheeks, frown, and form words due to facial weakness. The remainder of her neurological exam was normal; computed tomography and magnetic resonance imaging of the brain showed no pathology. Cerebrospinal fluid (CSF) collected by our neurologists on day +18 was unremarkable for infection or acute inflammation but evidenced lymphocytes consisting of large amounts of BCMA-targeted CAR T infiltrating the neuroaxis (Figure 2A). On the same day, PB levels of BCMA CAR T peaked (Figure 1C, D). Both PB (Figure 1F) and CNS (Figure 2A) CAR T consisted primarily of central memory-type T cells. PB CAR T were composed of equal proportions of $CD4^+$ and $CD8^+$ T cells (Figure 1C) while CNS-infiltrating $CD4^+$ T cells by far outnumbered $CD8^+$ T cells (Figure 2A), indicating a specific recruitment of T-helper cells. On the same day, the patient showed very high concentrations of IP-10 in the CSF (Figure 2C) which markedly exceeded IP-10 concentrations in the patient's PB (Figure 1E). Chemokine receptor CXCR3 is a ligand for IP-10 and the patient's PB CAR T showed surface expression of CXCR3 similar to their own non-CAR T (Figure 2D). However, expression levels of CCR6, which is involved in recruiting activated T cells to the brain,[5] were higher on the patient's PB CAR T cells compared to their non-CAR T (Figure 2D). Within the same

CAR T cells, expression of effector molecule granzyme B was more pronounced than within non-CAR T (Figure 2D). Finally, the patient's CAR T were uniformly positive for α4β1 integrin (Figure 2B), a receptor supporting T-cell migration across the blood-brain barrier (BBB).[5,6] As per recommendation by Neurology, the patient was



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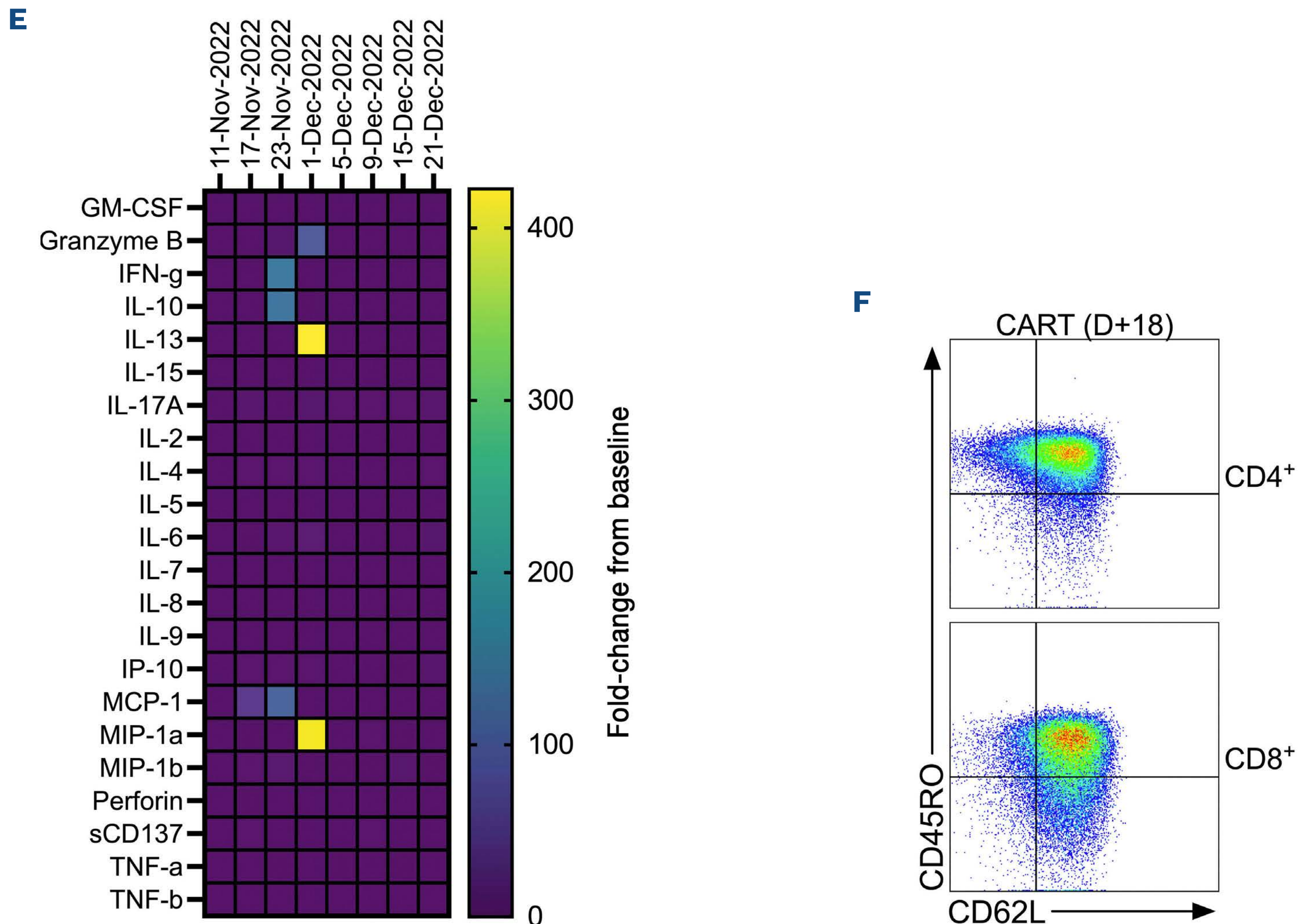

Figure 1. CAR T-cell expansion and persistence in a myeloma patient with immune-mediated facial nerve paralysis. Time course of (A) serum ferritin and C-reactive protein (CRP) levels, (B) reconstitution of white blood cell, neutrophil, and lymphocyte counts, and (C) chimeric antigen receptor (CAR) T-cell numbers and proportions of $CD4^+$ *versus* $CD8^+$ CAR T cells after lymphodepleting chemotherapy and CAR T-cell infusion. Time points with peak serum cytokine/chemokine levels are indicated by green or yellow dots, respectively. Occurrence of facial nerve paralysis is indicated by a dotted line. (D) Dot plots showing peripheral blood CAR T cells at different time points post CAR T infusion. CAR T cells were identified by staining of the expression of the CAR on the cell surface and co-staining with anti-CD3 and other T-cell markers. (E) Serum concentrations of 22 different T-cell-related cytokines/chemokines were determined in our patient at different time points post CAR T treatment using CodePlex Secretome technology. Cytokine concentrations were quantified using the CodePlex Secretome Human Adaptive Immune Panel kit (IsoPlexis; #CODEPLEX-2L01). Analysis was performed using the Isolight instrument (Isoplexis, Branfold, CT). Automated analysis of raw data was performed using IsoSpeak software (Isoplexis, Branfold, CT). Results are shown as fold change from baseline at pre-lymphodepleting chemotherapy. (F) CAR T-cell memory subtypes were determined on day +18 post CAR T treatment by co-staining for CD45RO and CD62L. Central memory CAR T cells are shown in the right upper quadrant. Samples were collected under Institutional Review Board-approved protocol 2043GCCC (IRB HP-00091736). Plasma was generated by centrifugation at 400 G and frozen immediately at -80°C. Peripheral blood mononuclear cells were isolated using density gradient centrifugation and frozen in liquid nitrogen. Staining for flow cytometry was performed using monoclonal antibodies following manufacturer's instructions. Samples were acquired using a Miltenyi MACSQuant Analyzer 10 Flow Cytometer. Analysis of flow cytometry data was performed using FlowJo software (BD Biosciences, San Jose, CA). D: day; PB: peripheral blood.

initially started on dexamethasone 10 mg every 12 hours but was transitioned to solumedrol 1 g daily for 3 days due to worsening speech. As a result, her facial movements and speech returned to baseline. Dexamethasone was tapered slowly over the next week. By discharge, her exam had improved as compared to admission, but her symptoms had not resolved completely. Subsequently, she had a temporary re-emergence of her symptoms, requiring restarting steroids. There were no obvious correlations with serum markers for CNS damage over time, however, there seemed to be a transient post-CAR T increase in NSE and GFAP.

At 1 month post CAR T, the patient's neurologic symptoms had significantly improved. Importantly, levels of PB CAR T cells had persisted over time (Figure 1C, D) and her BM evidenced substantial infiltration by $CD4^+$ and $CD8^+$ BCMA-targeted central memory CAR T (Figure 3C). Apart from some expression of TIM3, the BM-residing CAR T did not express any co-inhibitory molecules (Figure 3D), however, they were CD27-positive[7] and CD127-positive[8] (Figure 3E), indicating full functionality. Importantly, at that point in time all myeloma cells had been eradicated from the patient's BM (Figure 3A, B) and serum free light chains had normalized. At her most recent visit, approximately 6 months after CAR T, her neurologic symptoms had completely resolved without any sequalae.

Here, we describe the case of a MM patient who developed bilateral facial diplegia following cilta-cel infusion. In the

CARTITUDE-1 clinical trial using cilta-cel, neurotoxicity occurred in 21% of patients and one patient had facial nerve paralysis.[2] In the phase II KarMMa trial using idecabtagene vicleucel, neurotoxicity was reported in 18%.[3] Recently, a case of progressive movement disorder with features of parkinsonism was described after cilta-cel, associated with CAR T-cell persistence in the blood and CSF. BCMA was found to be present on neurons and astrocytes in the patient's basal ganglia, suggesting an on-target effect.[9] Our patient responded to treatment with high-dose steroids suppressing CAR T activity, however, whether the CNS toxicity was based on immediate CAR T on-target/off-tumor cytotoxicity remains to be evaluated.

CAR T, especially CD4+ CAR T, have previously been shown

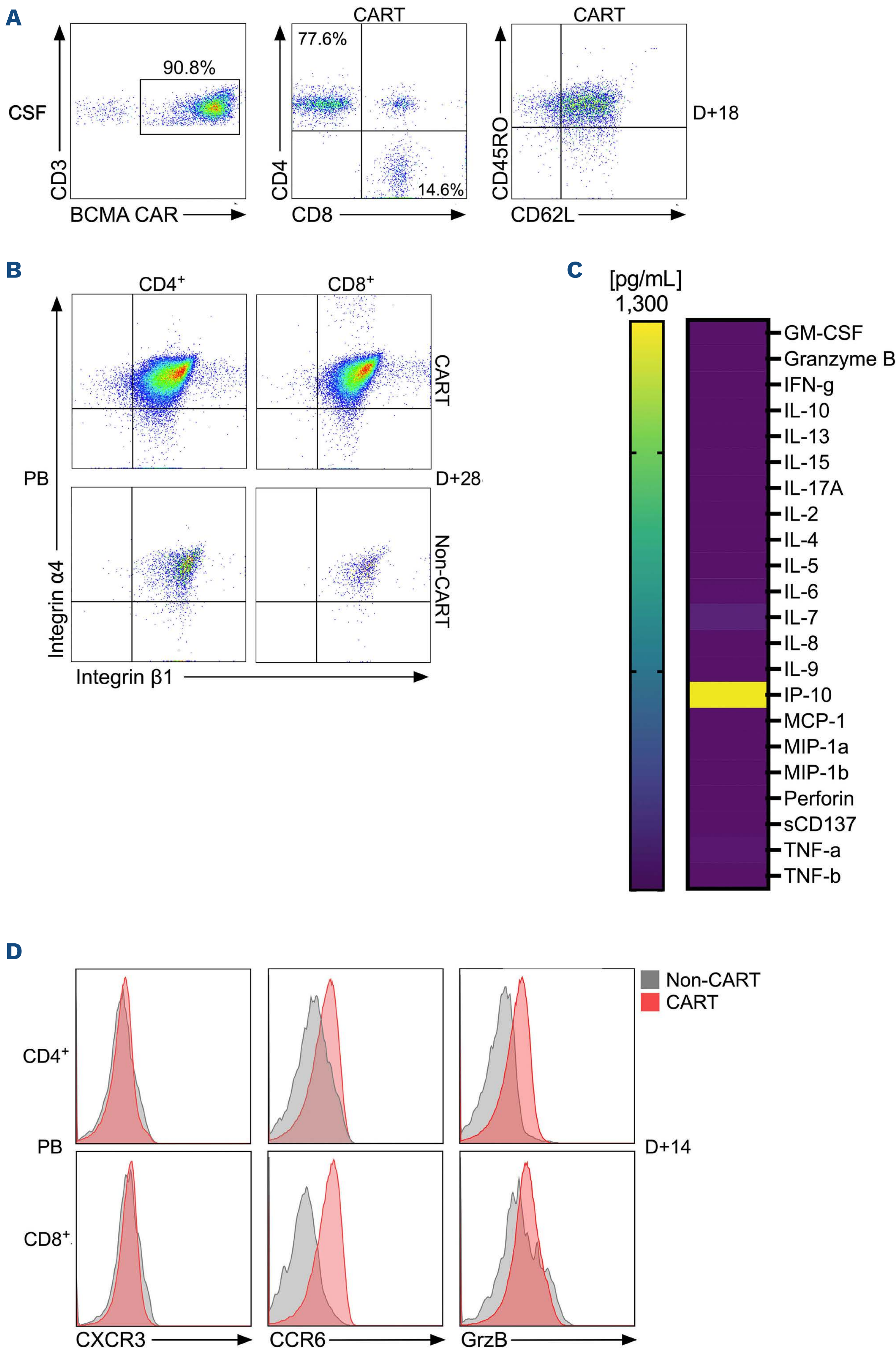

Figure 2. Central nervous system infiltration by BCMA-targeted CAR T cells in a myeloma patient with post-treatment facial nerve paralysis. (A) Proportions of CD4+ and CD8+ chimeric antigen receptor (CAR) T cells were determined in the cerebrospinal fluid (CSF) of our patient at onset of potentially immune-mediated central nervous system (CNS) toxicity using flow cytometry. Dot plots show CNS-infiltrating CAR T cells at day +18. CAR T cells were identified by staining of the expression of the CAR on the cell surface and co-staining with anti-CD3 and other T-cell markers. CAR T cell memory subtypes were determined on day +18 post CAR-T treatment by co-staining for CD45RO and CD62L. Central memory (CM) CAR T-cell are shown in the right upper quadrant. (B) Proportions of peripheral blood CAR T cells expressing $\alpha4\beta1$ integrin required for the entry of T cells into the CNS. (C) CSF concentrations of 22 different T-cell-related cytokines/chemokines were determined in our patient onset of potentially immune-mediated CNS toxicity using CodePlex Secretome technology. Results are shown as absolute concentrations in pg/mL. (D) Surface expression of receptors involved in CNS-directed homing of T cells on peripheral blood (PB) CAR T cells (red histograms) and non-CAR T (gray histograms) from our patient. In addition, cytoplasmic granzyme B was stained on day +14 post CAR T-cell treatment in both PB CAR T cells (red histogram) and non-CAR T (gray histogram) from the same patient/time point.

capable of infiltrating the CNS, e.g., in the case of CD19-targeted CAR T in patients with CNS lymphoma.[10] In this context, we consider it possible that increased numbers of CD4+ CAR T could be due to a survival advantage of these cells over CD8+ CAR T and that these cells could represented regulatory-type T cells.[11]

We show here that BCMA-targeted CAR T are able to cross the BBB even without CNS malignancies. Our data indicate that IP-10 produced by CNS-residing cells such as astrocytes[12] could play a role in the CNS recruitment of activated CAR T, either through CXCR3 or an alternative ligand. CXCR3 has extensively been studied with regard to T-cell recruitment during neuroinflammation. It is abundantly expressed on CNS-infiltrating T lymphocytes in multiple sclerosis patients[13] and co-ordinates migration in response to its three ligands, CXCL9/CXCL10/CXCL11.[14] Our data suggest that inhibition of IP-10 (CXCL10) could potentially represent a way to prevent CAR T neurotoxicity.

Our patient's CAR T showed high levels of CCR6 and the vasculature of the choroid plexus expresses adhesion molecules and chemokines including CCL20, the only known ligand for CCR6. CCL20/CCR6 interactions influence immune cell adhesion, rolling, and extravasation across the endothelium and pia mater. As a result, CCR6+ leukocytes

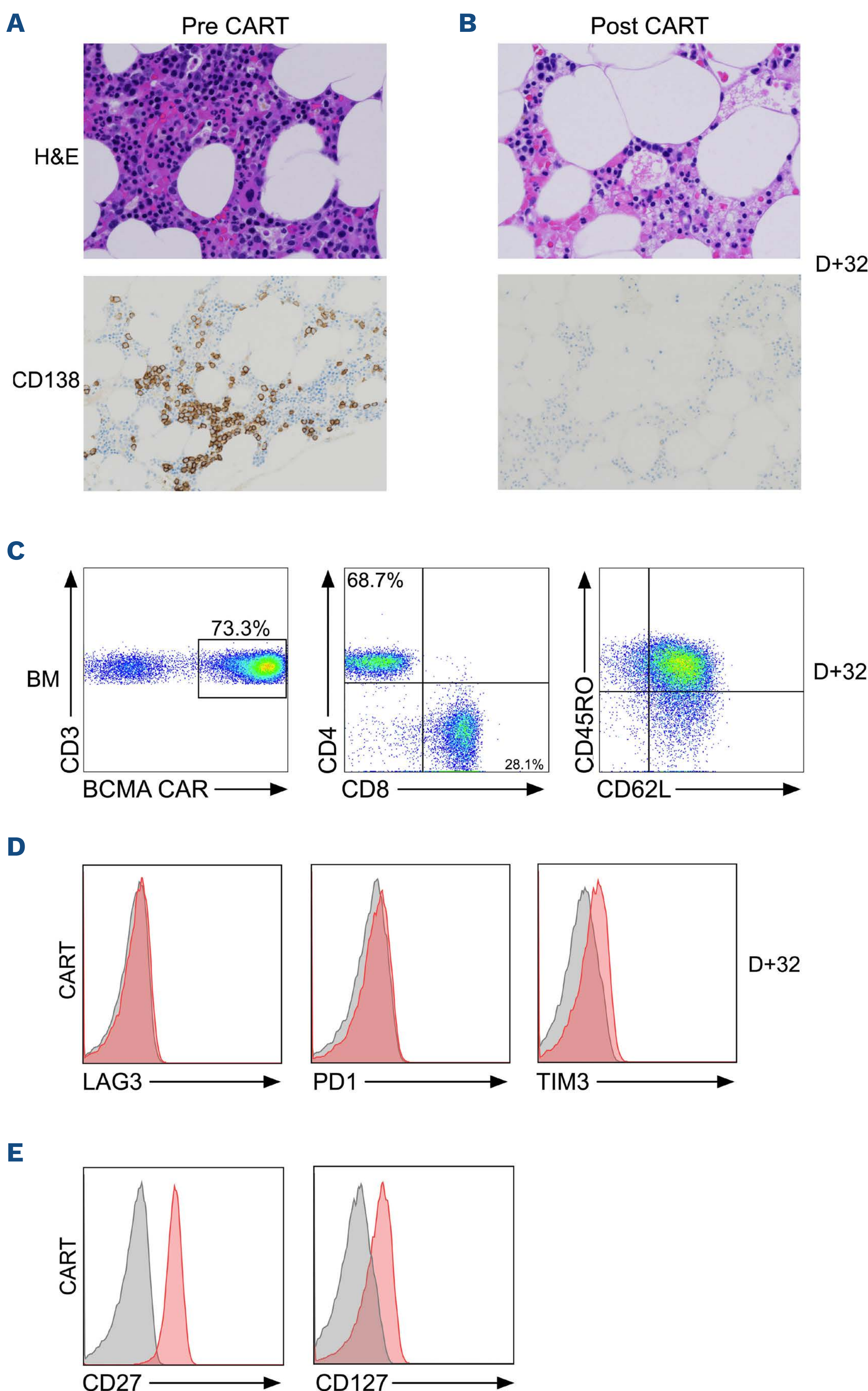

Figure 3. Infiltration by BCMA CAR T cells and eradication of myeloma cells and in the bone marrow of a patient with immune-mediated facial nerve palsy. (A) Sections from pre-chimeric antigen receptor (CAR) T-cell therapy showed a hypocellular bone marrow (BM) with trilineage hematopoiesis (hematoxilin & eosin [H&E], 400x magnification) and small clusters of plasma cells (CD138, 400x magnification) accounting for 15% of BM cellularity overall. (B) Post CAR T-cell therapy, the BM demonstrated a mild decrease in cellularity with maturing trilineage hematopoiesis (H&E, 400x magnification) and a near total absence of plasma cells (CD138, 400x magnification). (C) At the same time point, the BM showed infiltration by central memory-type CAR T cells. (D) BM-infiltrating CAR T cells did not show any significant surface expression of exhaustion markers (red histogram) by flow cytometry when compared to unstained controls (gray histogram). (E) In contrast, BM-infiltrating CAR T cells showed high levels of CD27 and CD127. Histologic sections from formalin-fixed, paraffin-embedded tissue samples underwent immunohistochemical and *in situ* hybridization staining using standard techniques.

enter CSF-containing ventricles and circulate through the CNS, surveying for antigen and signs of inflammation.[15] Accordingly, neutralizing CCL20 or CCR6 in mice with neuroinflammation decreased disease severity, highlighting the role of the CCL20/CCR6 axis in CNS-damaging autoimmune processes.[16]

Interactions between α4β1 integrin on effector T cells and its ligand VCAM-1 on BBB endothelial cells is a requirement for the entry of T cells into the CNS and neutralization of α4 integrin inhibits neuroinflammation and prevents T-cell recruitment into the CNS parenchyma.[17] Our findings suggest that inhibiting the function of α4β1, IP-10 and/or CCL20/CCR6 could potentially help to avoid CNS toxicity by CAR T. Future studies will further delineate the most relevant pathophysiologic mechanisms behind CAR T-related neurotoxicity and develop targeted methods to prevent and/or treat these immune-mediated side effects.

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Disclosures

SD serves on advisory boards for Bristol-Myers Squibb, Incyte, and Atara Biotherapeutics. NMH serves on advisory boards for InCyte and Kite-Gilead; and is a member of the DSMB for American Gene Technologies. The remaining authors have no conflicts of interest to disclose.

Contributions

DA designed the study, performed experiments, analyzed the data, made figures, and wrote the manuscript. PL and EP collected and processed patient samples. TI, DO, EG, and ND processed patient samples and performed experiments. YKH, HA, RK, JC, LP, JMB, KAD, KGH, AB, JAY, SD, NMH, HK, and APR analyzed data and wrote the manuscript. MEK, MK, and XF performed experiments, analyzed the data, and wrote the manuscript. TL analyzed the data, prepared figures, and wrote the manuscript.

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Data-sharing statement

Original data and protocols will be made available by the authors to other investigators upon request.

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