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Case Report

Defibrotide improves COVID-19-related acute respiratory distress syndrome in myeloma patients after chimeric antigen receptor T-cell treatment without compromising virus-specific and anti-myeloma T-cell responses

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DECLARATIONS

Competing Interests

P.G.R has research funding from Jazz pharmaceuticals. The remaining authors do not have any competing interests.

AUTHOR CONTRIBUTIONS

M.K. designed the study, analyzed the data, prepared figures, and wrote the manuscript. P.G.R, helped design the study, analyze data and write the manuscript. C.C.M. and A.P.R. analyzed data and helped write the manuscript. D.A. performed experiments, analyzed the data, and prepared figures.

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DATA SHARING

The data presented in this report is available upon request
The COVID-19 pandemic has caused significant morbidity and mortality, especially in patients with pre-existing comorbidities. Although the introduction of vaccines has decreased both the incidence and severity of the infection, protection is suboptimal in immunocompromised patients. We and others have shown previously that in patients with hematologic malignancies who received chimeric antigen receptor T-cell (CAR T) treatment, antibody responses to currently available vaccines are severely impaired while T-cell responses are largely maintained \(^1\text{-}^3\). Until we have broader coverage vaccines with stronger, durable immunogenicity, we will need treatments that can suppress tissue-damaging viral-induced inflammation in patients with CAR T-cell-induced immunosuppression and in particular target the endothelial pathobiology of the virus and its related endotheliitis.

Here, we describe the clinical course and correlative studies of two fully-SARS-CoV2-vaccinated patients with relapsed refractory myeloma (RRMM) who had severe COVID-19 infection shortly after their CAR-T treatments. Both remained in the ICU for prolonged periods requiring high-flow oxygen despite multipronged therapy. However, their clinical conditions improved remarkably shortly after initiation of defibrotide which was given after obtaining informed consent per institutional guidelines and which appropriately suppressed the harmful SARS-CoV-2-induced non-specific inflammatory response and related cytokine release syndrome (CRS). Importantly, our correlative studies suggested no negative impact either on the mounting of adaptive virus-specific antibody and/or T-cell responses or on the \textit{in-vivo} expansion and persistence of CAR T-cells. Both patients recovered well from their infection and their myeloma remains in deep and sustained remission. These data suggest that defibrotide has the potential to
suppress the damaging effects of the anti-COVID-19 inflammatory response while maintaining adaptive antiviral and anti-tumor immune responses.

Patient 1: A 67-year-old man with a 13-year history of IgG-lambda RRMM including upfront ASCT (Table 1) received BCMA targeting ciltacabtagene autoleucel (ciltael) CAR T for relapsed, penta-refractory disease after standard cyclophosphamide and fludarabine lymphodepleting chemotherapy in October 2022. His course was notable for grade 2 CRS which was treated with single-dose tocilizumab. After discharge, he had neutropenia that responded to G-CSF and achieved full count recovery. Approximately 1.5 months after CAR T-cell infusion, he developed fever, chills, cough, and exertional dyspnea and was readmitted with severe SARS-CoV-2 infection. He refused nirmatrelvir-ritonavir (Paxlovid), but quickly became hypoxemic, and was transferred to the ICU. CT imaging showed diffuse ground-glass consolidations and ruled out pulmonary embolism (Fig1A). For the majority of his 21-day admission, he remained symptomatic with cough and on high-flow O2 (15 days) despite a prolonged course of high-dose steroids and remdesivir but did not require intubation. After a 10-day course of i.v. steroids, he received IVIG, and one day later (on his 14th day of ICU stay), he was started on i.v. defibrotide for 7 days. Although there was some variation in FIO2 levels before the administration of defibrotide, a marked drop in FiO2, O2 flow rate, and most importantly a rapid clinical improvement only occurred after the application of the treatment (Figure 1B). After his discharge, he had bacteremias (pseudomonas and rothria) and CMV viremia which were successfully treated with appropriate antimicrobial therapy.
Immediately before his clinical recovery from COVID-19 infection, he was treated with defibrotide which is most likely what led to the improvement in clinical status. Before his CAR T treatment, he had been receiving monthly IVIG infusions, and his pre-IVIG serum IgG level was within normal limits, making this a less likely contributor. We do not use tocilizumab routinely for the treatment of COVID; however, two of the studies leading to an EUA by the FDA reported different medians of 6 and 14 days for time to significant clinical improvement. Our patient did not have a meaningful improvement and remained on high-flow oxygen during his 14-day ICU stay despite tocilizumab. Another possible explanation for his clinical improvement may be immune reconstitution. However, at 2 months, although he was not neutropenic, he still had low T-cell counts (CD4+ 138 cells/microL) and at 4 months, he only had partial immune reconstitution (CD4+ 800 cells/microL, IgA <8mg/dL). His bone marrow biopsy at 5.5 months was normocellular with trilineage hematopoiesis and no monoclonal plasma cell population was identified by morphology, immunohistochemistry or MRD (NGS). His SARS-CoV-2 PCR was positive at 6 months but subsequently cleared.

Defibrotide is a multifunctional endothelial stabilizing agent that received US regulatory approval for severe hepatic sinusoidal obstruction syndrome/veno-occlusive disease (VOD/SOS) in 2016, and EMA approval in 2013. Given the high mortality rate of severe VOD/SOS, it is now broadly used in real-world practice. The postulated primary mechanism of action is the stabilization and/or reversal of endothelial cell dysfunction. Moreover, defibrotide has also been shown to abrogate the deleterious effects of CD8+ and regulatory T-cells on human microvascular endothelial cells (MVECs). These findings paved the way for the initial clinical trials in allogeneic transplant settings for
VOD/SOS. A large CIBMTR study reported a 22.1% difference in the resolution of severe VOD/SOS at day +100 \(^7\). Given its favorable effects on endothelial dysfunction \(^8\), defibrotide was also investigated in non-VOD/SOS conditions that complicate infectious and non-infectious inflammation characterized by endotheliopathy, or so-called endotheliitis \(^9,10\). In a preclinical study, defibrotide mitigated the endothelial cell injury induced by plasma from patients with COVID-19-related vasculopathies \(^11\). Specifically, MVECs were exposed to primary samples with either acute COVID-19 infection or acute TMAs in the presence and absence of defibrotide. Caspase-8 which was used as an endothelial cell activation surrogate marker was suppressed with defibrotide and signaling pathway alterations were noted \(^11\). In acute malaria, defibrotide interfered with the coagulation/inflammation cycle, inhibited TLR-mediated dendritic cell activation, and reduced interferon-gamma production \(^9\). One proposed mechanism for SARS-CoV-2-related pulmonary organ damage is the formation of neutrophil extracellular traps (NET) associated with an abundance of neutrophils in autopsy specimens from COVID-19 patients \(^12\). Interestingly, in the setting of antiphospholipid syndrome, defibrotide was demonstrated to inhibit NET formation and venous thrombosis \(^13\)-\(^15\). An open-label, single-center phase 1 study investigated the safety and efficacy of a 7-day course of defibrotide in patients with COVID-19-related acute respiratory distress syndrome (ARDS)\(^16\). Out of 12 patients treated (10 on mechanical ventilation), 9 (75%) survived all of whom had a baseline pO2/FIO2 ratio (PFR) of >125 whereas 3 patients with baseline PFR <125 died \(^16\). Although the primary mechanism of action in this setting appears to be endothelial stabilization, there is likely a multi-pronged suppression of non-specific inflammation and endotheliitis, which is suggested by the inhibition of both donor
neutrophil and T-cell trafficking resulting in reduced acute GvHD severity in a murine mismatched allogeneic transplantation model, as well as other recently derived hypotheses for modulating activated endothelium and disrupting viral infection\textsuperscript{17, 18}. Our steroid- and remdesivir-refractory patient showed rapid clinical improvement following initiation of defibrotide treatment (Figure 1B). Correlative studies demonstrated COVID-specific antibody responses which were probably enhanced by IVIG administrations (Figure 1C+D+E). Importantly, we observed robust CAR T-cell expansion favoring a central memory phenotype at month 3 (Figure 2A+B). Similarly, despite the absence of full immune reconstitution at month 2 (CD4+ <200), there were clear CD4+ and CD8+ T cell responses against the spike(S) and nucleocapsid(N) proteins at month 3 (Figure 2C+D).

**Patient 2:** A 79-year-old man with an 8-year history of IgG-kappa RRMM including upfront ASCT (Table 1) presented with cough and profound hypoxemia 45 days after ciltacel with SARS-CoV-2 infection. He was treated with Paxlovid and was refractory to two separate inpatient courses of high-dose steroids and remdesivir administered 9 days apart. During 21-day ICU course, after persistent hypoxemia requiring 7-day high-flow oxygen support, he was started on defibrotide which led to a 30% drop in FIO2 immediately after the first dose. Unlike patient 1, he never received tocilizumab and IVIG was not given until 48 hours after administration of defibrotide, a timepoint when FIO2 was already reduced by 30%. Correlative studies also confirmed robust CAR T expansion. (Fig 2E) He was discharged on 2 lpm. At 3 months, he did not require supplemental O2 and his myeloma was in remission with negative bone marrow biopsy and PET/CT imaging.
Notably, both patients shared a historical triad of ASCT, CAR T treatment and SARS-CoV-2 infection all of which are well-established risk factors for endothelial activation and subsequent vascular damage. Although there are limited data on the impact of defibrotide on immune response in acute infectious inflammatory states, our clinical observations and correlative data suggest a prompt and clinically meaningful suppression of COVID-induced inflammatory responses with well-preserved SARS-CoV-2 specific antibody and T-cell immunity as well as sustained and effective anti-myeloma CAR T-cell kinetics. Notably, no hemorrhagic events were observed in either of these patients with defibrotide treatment. Patient 1 is one year out from CAR T-cell treatment, and remains with minimal exertional symptoms, without supplemental oxygen. He is PCR-negative for SARS-CoV-2 and his myeloma is in MRD-negative (NGS) remission. Patient 2 is 5.5 months out from CAR T, his myeloma remains in remission and he does not require O2 support, as well as being PCR-negative for SARS-CoV-2. Given the complexity of CAR T-cell treatments, their unique adverse effect profiles such as CRS and potential infection profile including COVID-19, further investigation of defibrotide as a promising therapeutic option is clearly warranted.
REFERENCES


Table 1: Lines of treatment prior to CAR T

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FIGURE LEGENDS

Figure 1: Clinical course and antiviral antibody titers of a myeloma patient #1 with COVID-19 related acute respiratory distress syndrome

(A) Computed tomographic (CT) images of chest for Patient 1. Axial and coronal sections display diffuse and extensive alveolar damage showing ground-glass consolidations that persist after one week of ICU treatment including high-dose steroids and remdesivir. (B) Supplemental O2 requirement of our patient while admitted to the ICU. Both FIO2 (red) and O2 flow rates (orange) are indicated. Dosing of defibrotide (blue) and IVIG (green) are indicated by vertical dashed lines. Administration of both tocilizumab (toci; black dot) and dexamethasone (dex; black line) for CRS are indicated as is administration of remdesivir (yellow line). (C) Reciprocal IgG antibody endpoint titers against SARS-CoV-2 proteins S1 (red), S2 (orange), and nucleocapsid protein (N; black) as determined by enzyme-linked immunosorbent assay (ELISA) are shown. The temporal relationship to IVIG infusion is indicated by a green dashed vertical line. (D) Quantitative immunoglobulins (IgG, IgM, IgA) over time and temporal relationship to IVIG infusion (green dashed vertical line). Absolute serum concentrations of the different immunoglobulins were measured using Human IgG, IgM, and IgA Enzyme-linked Immunosorbent Assay (ELISA) Kits (Invitrogen, Cat. No. BMS2091, BMS2098, BMS2096) as per the manufacturer’s instructions. (E) Reciprocal IgG antibody endpoint titers against microbial proteins influenza A nucleoprotein (Flu; red), tetanus toxoid (TT; orange), adenovirus type 5 hexon protein (Adv; green), varicella zoster virus envelope glycoprotein E (gE) protein (VZV; blue) and respiratory syncytial virus nucleoprotein (RSV; yellow) as determined by enzyme-linked immunosorbent assay (ELISA) are shown. The temporal relationship to IVIG infusion is indicated by a green dashed vertical line. Serum antibody responses against recombinant, full-length SARS-CoV-2 proteins or viral control proteins were determined by ELISA as previously described.
**Figure 2:** CAR T-cell expansion and anti-SARS-CoV-2 T cells in two myeloma patients with COVID-19 related acute respiratory distress syndrome. Panels A, B, C and D are the correlative studies of the first patient in the report. Panel E shows CAR T expansion characteristics of the second patient.

(A) Blood samples were collected under Institutional Review Board (IRB)-approved protocol 2043GCC (IRB HP-00091736). Peripheral blood plasma was generated by centrifugation at 400G and frozen immediately at -80°C. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation and cryopreserved in liquid nitrogen. Proportions of CD4⁺ and CD8⁺ BCMA-targeted CAR T-cells were determined in the peripheral blood of our patient at different timepoints using flow cytometry. Dot plots show percentage of CAR-expressing T cells vs. all T cells. CAR T cells were identified by staining of the expression of the CAR on the cell surface using a recombinant BCMA protein as CAR detection reagent and co-staining with anti-CD3 and other T cell markers (Supplemental Table 1). (B) CAR T-cell CD4⁺ and CD8⁺ subtypes were quantified at the different timepoints and CAR T cell memory subtypes (naïve, central memory CM, effector-memory EM, effector-type EFF) were determined by co-staining for CD45RA and CD62L. T cells specific for the S (C) and the N (D) proteins of the SARS-CoV-2 virus were identified ex vivo after short-term stimulation of total PBMC using libraries of overlapping peptides covering the complete sequence of the respective proteins. Intracellular staining of cytokines followed by flow cytometry served as a read-out assay. SARS-CoV-2-specific CD4⁺ T-cells (upper rows) were defined as TNFα/CD40L (CD154)-double positive CD3⁺CD4⁺ T-cells and SARS-CoV-2-specific CD8⁺ T-cells (lower rows) were defined as IFNγ/TNFα-double positive CD3⁺CD8⁺ T cells. (E) CAR T-cell expansion kinetics in the second patient. Dot plots show percentage of CAR-expressing T cells vs. all T cells. CAR T-cells were
identified by staining for the expression of the CAR on the cell surface using a recombinant BCMA protein as CAR detection reagent and co-staining with anti-CD3.