financial conflicts of interest to disclose. Gregory J Kato is an employee of CSL Behring. Dr. Gladwin is a co-inventor of patents and patent applications directed to the use of recombinant neuroglobin and heme-based molecules as antidotes for CO poisoning, which have been licensed by Globin Solutions, Inc. Dr. Gladwin is a shareholder, advisor, and director in Globin Solutions, Inc. Dr. Gladwin is also co-inventor on patents directed to the use of nitrite salts in cardiovascular diseases, which were previously licensed to United Therapeutics, and is now licensed to Globin Solutions and Hope Pharmaceuticals. Dr. Gladwin is a principal investigator in a research collaboration with Bayer Pharmaceuticals to evaluate riociguate as a treatment for patients with SCD.

## DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

Anusha Vittal[1], Hawwa Alao[1], Julian Hercun[1], Bashar Sharma[2], Arsalan Khan[1], Disha Sharma[1], Wilson Lee[3], Devika Kapuria[1], Matthew Hsieh[4], John Tisdale[4], Courtney Fitzhugh[4], David Kleiner[5], Elliot Levy[6], Richard Chang[6], Anna Conrey[7], Elenita Rivera[1], Amy Huang[1], Gil Ben Yakov[1], Gregory J. Kato[8], Mark T. Gladwin[9], Swee Lay Thein[7], Christopher Koh[1], Theo Heller[1]

[1]*Translational Hepatology Section, Liver Diseases Branch, NIDDK, NIH, Bethesda, Maryland, USA*
[2]*Department of Medicine, SUNY Upstate Medical University, Syracuse, New York, USA*
[3]*Department of Internal Medicine, MedStar Health Internal Medicine Program, Baltimore, Maryland, USA*
[4]*Molecular and Clinical Hematology Branch, NHLBI, NIH, Bethesda, Maryland, USA*
[5]*Laboratory of Pathology, NCI NIH, Bethesda, Maryland, USA*
[6]*Interventional Radiology, Department of Diagnostic Radiology, NIH, Bethesda, Maryland, USA*
[7]*Sickle Cell Branch, NHLBI, NIH, Bethesda, Maryland, USA*
[8]*Hematology Therapeutic area, CSL Behring, King of Prussia, Pennsylvania, USA*
[9]*Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA*

**Correspondence**
Anusha Vittal and Theo Heller, Liver Diseases Branch, NIDDK, NIH Bldg. 10 Room 5-2740, 10 Center Drive Bethesda, MD, 20892-1800, USA.
Email: anusha.vittal@nih.gov and theoh@intra.niddk.nih.gov

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## ORCID

*Anusha Vittal* https://orcid.org/0000-0002-3855-5023
*Disha Sharma* https://orcid.org/0000-0002-9352-625X
*Gregory J. Kato* https://orcid.org/0000-0003-4465-3217

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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# Blood exchange transfusion with dexamethasone and Tocilizumab for management of hospitalized patients with sickle cell disease and severe COVID-19: Preliminary evaluation of a novel algorithm

To the Editor:

Sickle cell disease (SCD) is a severe hemoglobin (Hb) disorder characterized by hemolytic anemia, recurrent painful vaso-occlusive events, and ischemia/reperfusion-driven inflammation. Acute chest syndrome (ACS) is a common and potentially life-threatening form of acute lung

injury that can be triggered by infectious conditions. Coronavirus disease 2019 (COVID-19) infection represents a significant mortality risk for SCD patients, as death or required mechanical ventilation was reported for 5.6% of patients in the largest cohort of SCD patients hospitalized for COVID-19.[1] In this cohort, ACS was described for 30% of patients.

For SCD patients hospitalized with severe COVID-19 requiring supplemental oxygen, current therapeutic strategies may include corticosteroids, the interleukin-6 (IL-6) inhibitor Tocilizumab, or anticoagulant agents. Selection of the appropriate therapies may have a critical impact on patient outcome, due to the complex relationship between the drugs' mechanism of action and SCD pathophysiology. For instance, the use of corticosteroids in SCD patients should be approached with caution, as corticosteroids are associated with an increased risk of hospitalization for vaso-occlusive crises (VOC) or ACS.[2] In contrast, Odièvre et al. reported dramatic improvement after Tocilizumab in COVID-19-related ACS in a pediatric SCD patient,[3] as was also previously described in an adult SCD patient, suggesting that IL-6 could play an essential role in ACS pathophysiology.

We propose here a treatment algorithm for SCD patients hospitalized with severe COVID-19. The aim of the algorithm is to minimize the worsening of SCD patients' clinical course related to corticosteroid therapy by proposing a prior blood exchange transfusion (BET); Tocilizumab is considered as alternative when transfusion is not recommended due to immunization against red blood cell (RBC) antigens. This algorithm was developed taking into consideration multiple aspects of SCD pathophysiology and of COVID-19 in SCD.

*Corticosteroid therapy in COVID-19 and the impact on SCD*: Multiple randomized trials indicate that systemic corticosteroid therapy reduces mortality and improves clinical outcome in patients hospitalized with COVID-19 requiring supplemental oxygen. This is probably due to the mitigation of the COVID-19-induced systemic inflammatory response that can lead to lung injury and multisystem organ dysfunction. Systemic corticosteroid therapy is notoriously associated with an increased risk of SCD exacerbation and hospitalization for VOC or ACS. The SISTER study showed that corticosteroid exposure was significantly associated with hospitalization for VOC in an SCD French cohort[2] with no COVID-19 infection. Corticosteroids have been previously shown to reduce ACS severity (especially duration of oxygen therapy and length of hospitalization) but are not considered a good treatment option, as they increase the risk of secondary VOC and early readmission.

*IL-6 inhibitors in COVID-19 and the impact on SCD*: Results of published trials provide consistent evidence that the anti-IL-6 receptor monoclonal antibody, Tocilizumab, when administered with corticosteroids, offers a modest and somehow controversial clinical benefit in certain patients with COVID-19 who are severely ill, rapidly deteriorating, with increasing oxygen needs, and who have a significant inflammatory response. Outside of the COVID-19 context, sputum IL-6 level is a well-known biomarker of respiratory inflammation in SCD patients, and has been found increased at steady state in SCD children with a reported positive correlation between sputum IL-6 level and the number of ACS episodes. Increased levels of IL-6, IL-8, CCL2, and CCL3 have also been reported in the sputum of SCD children during ACS,[4] possibly contributing to the recruitment of inflammatory cells in the lungs. Current therapeutic strategies for ACS are mainly supportive and do not target pulmonary and systemic inflammation, even though this is a major hallmark of the disease. Dramatic improvement of COVID-19-related ACS treated with Tocilizumab have been reported and appear safe and effective in SCD patients,[3] in association with the usual treatment for severe ACS. Recently, a potential benefit of Tocilizumab in SCD patients during ACS, independently of COVID-19 infection, has been published.[5] Indeed, new VOC therapeutic strategies targeting another IL (IL-1 beta) are currently being evaluated in a clinical trial, supporting the hypothesis of inflammasome activation in SCD.

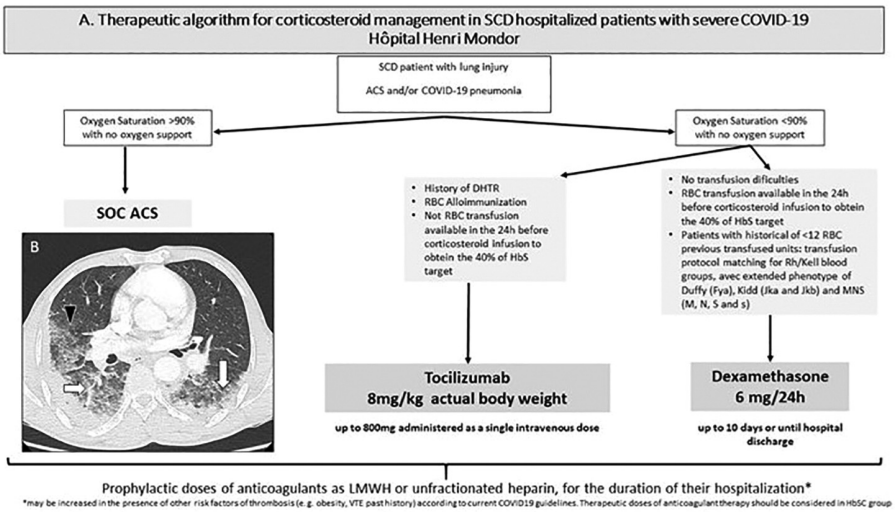
*Anticoagulant agents in SCD and COVID-19*: COVID-19 is an inflammatory condition at high risk for venous thromboembolism (VTE) as it has been associated with inflammation and a prothrombotic state accompanied by increases in fibrinogen and D-dimer. VTE, which includes pulmonary embolism (PE) and deep vein thrombosis (DVT), has been recognized as a common complication of SCD. Arlet et al. show that Sickle Hemoglobin- C Disease (SC) genotype was the only independent factor associated with a higher risk of thrombosis.[1]

*Transfusion in SCD*: BET in SCD is performed in an acute manner to obtain immediate benefits, such as increased oxygen-carrying capacity and improved blood flow. It can also help prevent long-term complications by replacing rigid sickled erythrocytes with normal deformable cells and by suppressing the formation of sickled erythrocytes. Stroke and ACS are examples of acute organ damage that benefit from transfusion. BET aims to reduce the percentage of sickle hemoglobin (HbS) and attain <30% of HbS. This threshold is based on expert consensus rather than findings from randomized trials, and can be achieved by repeated manual exchange transfusions (RMET) or by erythrocytapheresis, a technique that removes sickled erythrocytes but maintains baseline hematocrit. In our experience, an HbS threshold of 30%–40% may be reached after two RMET cycles (using 2 RBC transfused units in each cycle) in a homozygous SCD patient with no previous transfusion.

Delayed hemolytic transfusion reaction (DHTR) is a life-threatening complication of transfusions in SCD, which is induced by immunization against RBC antigens. Risk of DHTR can be assessed with a predictive score based on DHTR history, number of units previously transfused and immunization status before transfusion.[6]

We propose here a therapeutic algorithm (Figure 1A) for SCD patients hospitalized with severe COVID-19 treated by corticosteroid therapy, with the goal of minimizing VOC complications related to corticosteroids. The algorithm is based on DHTR risk profile and transfusion availability, and includes suggestions for VTE management.

- Before corticosteroid therapy (total daily dose dexamethasone 6 mg or equivalencies for up to 10 days or until hospital discharge) is prescribed, according to oxygen needs, a prior BET must be




**C. Sickle Cell hospitalized patients with COVID-19 treated by tocilizumab**

| SCD genotype | All patients (n = 11) | Patients Aged 16-40 years (n =5) | Patients Aged > 40 years (n = 6) |
|---|---|---|---|
| HbSS/Sβ0 | 8 (72) | 4 (80) | 4 (67) |
| HbSC | 3 (27) | 1 (20) | 2 (33) |
| Age | 40 [16,8-50] | 38,5 [16,8-37] | 45,2 [42,7-50] |
| IMC mg/kg2 | 23,9 [19,8-33,9] | 26,1 [20-33,9] | 20 [19,8-29,4] |
| Female | 6 (54) | 4 (80) | 2 (33) |
| Hydroxyurea treatment | 6 (54) | 2 (20) | 4 (67) |
| Tocilizuman infusion context | | | |
| *DHTR history* | *1 (9)* | *1 (20)* | *0* |
| *High DHTR risk* | *5 (45,5)* | *2 (40)* | *3 (50)* |
| *RBC transfusion not available* | *5 (45,5)* | *3 (60)* | *2 (33)* |
| Typical features of CT scan of COVID19 | 7 (64) | 2 (40) | 5 (83) |
| ACS | 8 (72) | 4 (80) | 4 (67) |
| Length of hospitalization stay (days) | 12,5 [6-55] | 12,5 [8-53] | 12 [6-55] |
| Intensive Care Unit admission | 8 (73) | 4 (80) | 4 (67) |
| VTE or PE | 3 (27) | 1 (20) | 2 (33) |
| Blood Transfusion | 8 (72) | 3 (60) | 5 (83) |
| Dexamethasone | 3 (27) | 2 (40) | 1 (16) |
| Mechanical Ventilation | 5 (45) | 2 (40) | 3 (50) |
| ECMO | 2 (18) | 1 (20) | 1 (16) |

**FIGURE 1** (A) Therapeutic algorithm for corticosteroid management in SCD hospitalized patients with COVID-19 who require supplemental oxygen, depending on the severity of COVID-19 infection, ACS diagnosis, and DHTR risk. Standard of care (SOC); Low molecular weight heparin (LMWH); Sickle Cell Disease (SCD); Acute Chest Syndrome (ACS); Delayed hemolytic transfusion reaction (DHTR). (B) Computerized tomography scan of the chest of an acute chest syndrome and COVID-19-induced pneumonia. Areas of ground-glass opacities (arrows) in the lower lungs with regard to areas of consolidation, but also in the middle lobe (arrowhead). (C) Sickle cell hospitalized patients with COVID-19 treated by Tocilizumab. Data are *n* (%), or median (range). Percentages do not always equal 100% because of rounding. Around 11 patients were collected from March 13, 2020 to 17 January 2022 with a median age of 40 [17–50] years, 54% female; one pregnant woman is described. Concerning genotype HbSS was reported in 64%, HbSC in 27% and one HbS/BetaThal°; median IMC was 23.9 kg/m$^2$, Hydroxyurea (HU) treatment was reported in 62% of HbSS and HbS/BetaThal° genotype. The median length of hospitalization stay was 12.5 days [6–55], with intensive care unit admission in 73%. ACS related to COVID-19 was reported in eight patients with blood exchange transfusion (BET) performed in 75% of them and no BET in 25% due to dramatic improvement after Tocilizumab (one Jehovah's Witness patient is described). VTE or PE was reported in 27%. Tocilizumab infusion was related to history of DHTR ($n = 1$), high DHTR risk development ($n = 5$) and BET not available in the 24 h before corticosteroid infusion ($n = 5$). Corticosteroids were prescribed in 27% after BET due to infection severity despite Tocilizumab, required mechanical ventilation in 45% and extracorporeal membrane oxygenation (ECMO) in 18%. Two death were reported in a 48 years-old kidney transplant homozygous (HbSS) patient, and in a 50 years-old heterozygous (HbSC) patient with a severe PE successive to COVID-19 infection

performed using a transfusion protocol matched for Rh/Kell blood groups. An HbS target of <40% should be achieved after transfusion by RMET or erythrocytapheresis technique. We propose a transfusion protocol matched for Rh/Kell blood groups extended to Duffy (Fya), Kidd (Jka and Jkb) and MNS (M, N, S and s) phenotypes for patients with a history of <12 transfused RBC units, due to risk of DHTR development, as it was previously proposed by Narbei et al.[6]

- Tocilizumab (8 mg/kg for actual body weight up to 800 mg, administered as a single intravenous dose) is proposed as an alternative to corticosteroid therapy when transfusion is not recommended. For patients with history of transfusion difficulties (i.e., history of DHTR, anti-RBC immunization other than antibodies against Rh and Kell blood groups, in particular antibodies against FY, JK, MNS, DO), transfusion should not be performed prior to corticosteroid therapy to avoid the risk of developing DHTR. Tocilizumab was also proposed for SCD patients hospitalized for COVID-19 when corticosteroid therapy was indicated but it was not possible to perform a blood transfusion to obtain an HbS target of <40% within the first 24 h prior to corticosteroid infusion.
- Prophylactic doses of anticoagulants, such as low molecular weight heparin or unfractionated heparin, should be administrated for the duration of the hospitalization and after discharge, in the same fashion as for the non-SCD population at high risk. The standard dose of antithrombotic prophylaxis may be increased if other risk factors for thrombosis (i.e., obesity, past history of VTE) are present and according to current COVID-19 guidelines. Therapeutic doses of anticoagulant therapy should also be considered for the HbSC population.

We applied this therapeutic algorithm to our cohort of sickle cell patients hospitalized with COVID-19 and we describe here a preliminary evaluation of patients treated by Tocilizumab (Figure 1C). Around 11 patients, with a median age of 40 years [17–50], treated according to this algorithm between March 13th, 2020 and January 17th, 2022 were analyzed. Mechanical ventilation (MV) was required in 45% and extracorporeal membrane oxygenation (ECMO) in 18%. Tocilizumab infusion was related to history of DHTR ($n = 1$), high DHTR risk development ($n = 5$) and BET not available in the 24 h before corticosteroid infusion ($n = 5$). Corticosteroids were prescribed in 27% after BET due to infection severity despite Tocilizumab. Two deaths were reported: a 48 year-old kidney transplant HbSS patient with Acute Respiratory Distress Syndrome, and a 50 year-old heterozygous HbSC patient with severe PE.

Tozilizumab was infused after applying the proposed algorithm in all 11 patients represented by ACS related to COVID-19 in eight (73%) of them. BET was performed in 75% of ACS, and no needed in 25% of ACS due to dramatic improvement after Tocilizumab. Two patients (18%) died, all of them aged >40 years with ACS diagnosis, HbSC genotype in one and high comorbidity (kidney transplant) in second one. In Arlet et al. work, age was identified as an independent risk factor of severity, with an 8.3-fold increase in the risk of death or intubation for patients older than 40 when compared to the 20–40 age group; and SC genotype appeared as a particularly high-risk group, with a case fatality rate of 12%.[1]

In conclusion, severity of COVID-19 infection, ACS diagnosis and history of transfusion reactions must be taken into account when considering the use of corticosteroids for the management of severe COVID-19 pneumonia in SCD patients. Blood exchange transfusion must be considered prior to dexamethasone use in order to minimize the risk of VOC complications; IL-6 inhibitors should be proposed as an alternative to dexamethasone for patients at high risk for DHTR for whom transfusion must be avoided.

## CONFLICT OF INTEREST

Pr Bartolucci discloses the following: (a) consulting agreement for F. Hoffmann-La Roche,Addmedica, Novartis, Roche, Gbt, Bluebird, Emmaus, Hemanext, Agios; (b) Lecture fees from Novartis, Addmedica, Jazzpharma; (c) Steering committee for Novartis; (d) Research support from Addmedica, Foundation Fabre, Novartis, Bluebird; (e) cofounder of Innovhem.

## DATA AVAILABILITY STATEMENT

A single investigator (GDL) collected the data from patient interviews and chart reviews between March 13th, 2020 and January 17th, 2022. Clinical data were collected using a standardized form. All consecutive adult patients with SS-homozygous and S-heterozygous (S/β, SC) SCD (age >16 years) were followed at 3 SCD referral centers in France (Sickle Cell Referral Center, Department of Internal Medicine, Henri-Mondor University Hospital- UPEC, AP-HP, Créteil; Sickle Cell Center, University of the French West Indies, CHU de Martinique; and Armand-Trousseau Hospital, Service de Pédiatrie. Center for Sickle Cell Disease, Paris, France); and 3 others Hospitals in France: Institut Universitaire du Cancer Toulouse Oncopole, Médecine Interne, Toulouse, Hôpital Saint-Vincent de Paul, Hématologie, Lille and CHU Saint-Etienne, Médecine Interne, Saint Etienne

Gonzalo De Luna[1], Anoosha Habibi[1], Marie-Hélène Odièvre[2], Henri Guillet[1], Vincent Guiraud[1], Pierre Cougoul[3], Benjamin Carpentier[4], Gylna Loko[5], Isabelle Guichard[6], Clément Ourghanlian[7], Jean Michel Pawlotsky[8], Matthieu Mahevas[9], Nicolas Limal[9], Marc Michel[9], Armand Mekontso-Dessap[10], Jean-Benoît Arlet[11], Pablo Bartolucci[1]

[1]*Sickle Cell Referral Center, Department of Internal Medicine, Henri-Mondor University Hospital- UPEC, AP-HP, Créteil, France*
[2]*Armand-Trousseau Hospital, Service de Pédiatrie, Center for Sickle Cell Disease, Paris, France*
[3]*Institut Universitaire du Cancer Toulouse Oncopole, Médecine Interne, Toulouse, France*
[4]*Department of Hematology, Hôpital Saint-Vincent de Paul, Hématologie, Lille, France*
[5]*Sickle Cell Center, University of the French West Indies, CHU de Martinique, Fort de France, France*
[6]*CHU Saint-Etienne, Médecine Interne, Saint Etienne, France*
[7]*Department of Pharmacy, CHU Henri Mondor, Pharmacie, Creteil, France*
[8]*CHU Henri Mondor, National Reference Center for Viral Hepatitis B, C and D, Department of Virology, Hôpital Henri Mondor, Université Paris-Est Creteil, Île-de-France, France*
[9]*CHU Henri Mondor, Internal Medicine, Reference center of autoimmune cytopenias, Créteil, France*
[10]*Department of Intensive Care, CHU Henri Mondor, Créteil, France*
[11]*Sickle Cell Referral Center, France, Internal Medicine Department, Georges Pompidou European Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France*

**Correspondence**
Gonzalo De Luna, Sickle Cell Referral Center, Department of Internal Medicine, Henri-Mondor University Hospital- UPEC, AP-HP, Créteil, France.
Email: gonzalodeluna@gmail.com

**ORCID**
*Gonzalo De Luna* https://orcid.org/0000-0003-4138-4218
*Matthieu Mahevas* https://orcid.org/0000-0001-9182-1434
*Jean-Benoît Arlet* https://orcid.org/0000-0003-2608-302X

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# Allogeneic double-negative T cell therapy for relapsed acute myeloid leukemia patients post allogeneic hematopoietic stem cell transplantation: A first-in-human phase I study

To The Editor:

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is widely used as a potentially curative treatment option for high-risk and refractory or relapsed acute myeloid leukemia (AML) patients.[1] However, about 40% of allo-HSCT patients experiences disease relapse.[1] Traditionally, allo-HSCT patients with recurrent disease may be treated with salvage therapies such as chemotherapy, donor lymphocyte infusion (DLI), or secondary allo-HSCT.[1] However, disease relapse remains one of the main causes of death due to low response rate to the commonly used salvage therapies.[1] Hence, safe and effective treatment options are urgently needed for allo-HSCT patients.

Double-negative T cells (DNTs) are a rare subset of mature T cells that comprises 1%–5% of peripheral leukocytes and defined by the expression of CD3 but not CD4 and CD8.[2] Preclinically, ex vivo expanded donor-derived DNTs show potent activity against an array of AML targets without off-tumor toxicities toward healthy cells and tissues. Further, non-genetically modified allogeneic DNTs (allo-DNTs) fulfill the requirements of an off-the-shelf cellular immunotherapy, including not causing graft-versus-host disease (GvHD), resistance to host-versus-graft (HvG) rejection, scalability, and storability.[3] Hence, we undertook a first-in-human phase I trial (registered in the Chinese Clinical Trial Registry; ChiCTR-IPR-1900022795) to assess the feasibility, safety, and potential efficacy of healthy unrelated donor-derived allo-DNTs as a treatment for AML patients who relapsed after allo-HSCT.

In this trial, 12 patients were consecutively enrolled in accordance with the protocol (Data S1). Of these patients, one patient withdrew before the initiation of treatment owing to the absence of detectable disease, and one patient (patient #4) withdrew after the second dose of DNT infusion for personal reasons. Thus, 10 patients received three planned doses of allo-DNTs given 1 week apart (Figure 1A). The patient clinical characteristics are shown in Table S1. Among the 10 patients, 2 patients had received stem cells from human leukocyte antigen (HLA)-matched siblings, and 8 patients received umbilical cord blood transplant. The patient median age was 24.1 (range 4–44 years). The median time from allo-HSCT to relapse was 9.0 months (6.0–15.4 months), and the median time from relapse to DNT therapy was 5.1 months (1.7–11.7 months). After disease relapse, all patients discontinued immunosuppressants and received 1–5 lines of salvage therapies prior to DNT treatment as described in Table S2. Salvage therapies were stopped at least 1 week prior to preconditioning chemotherapy. DNTs were derived from third-party donors and successfully manufactured as described in Supplementary methods for all planned treatments at targeted dosage with high purity and anti-leukemic activity against AML cell lines (Figure S1). Each patient received with a total of 3 doses of third-party donor-derived DNTs at escalating doses of $5 \times 10^7$, $1 \times 10^8$ and $2–4 \times 10^8$ cells per kilogram of body weight after lymphodepleting preconditioning.

The patients were monitored for adverse events (AEs) from the time of DNT infusion until at least 30 days after the last DNT infusion or until AEs were relieved (Table S3). All DNT-treated patients showed symptoms of grade 1 or 2 cytokine release syndrome (CRS) such as fever and hypotension. The CRS symptoms were controlled within 24 h in all patients with nonsteroidal anti-inflammatory drugs such as ibuprofen to control fever. The levels of the CRS-related cytokines interleukin (IL)-6, IL-10, macrophage inflammatory protein