

# CD22 CAR T-cell associated hematologic toxicities, endothelial activation and relationship to neurotoxicity

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## ABSTRACT

**Background** Hematologic toxicities, including coagulopathy, endothelial activation, and cytopenias, with CD19-targeted chimeric antigen receptor (CAR) T-cell therapies correlate with cytokine release syndrome (CRS) and neurotoxicity severity, but little is known about the extended toxicity profiles of CAR T-cells targeting alternative antigens. This report characterizes hematologic toxicities seen following CD22 CAR T-cells and their relationship to CRS and neurotoxicity.

**Methods** We retrospectively characterized hematologic toxicities associated with CRS seen on a phase 1 study of anti-CD22 CAR T-cells for children and young adults with relapsed/refractory CD22+ hematologic malignancies. Additional analyses included correlation of hematologic toxicities with neurotoxicity and exploring effects of hemophagocytic lymphohistiocytosis-like toxicities (HLH) on bone marrow recovery and cytopenias. Coagulopathy was defined as evidence of bleeding or abnormal coagulation parameters. Hematologic toxicities were graded by Common Terminology Criteria for Adverse Events V.4.0.

**Results** Across 53 patients receiving CD22 CAR T-cells who experienced CRS, 43 (81.1%) patients achieved complete remission. Eighteen (34.0%) patients experienced coagulopathy, of whom 16 had clinical manifestations of mild bleeding (typically mucosal bleeding) which generally subsided following CRS resolution. Three had manifestations of thrombotic microangiopathy. Patients with coagulopathy had higher peak ferritin, D-dimer, prothrombin time, international normalized ratio (INR), lactate dehydrogenase (LDH), tissue factor, prothrombin fragment F1+2 and soluble vascular cell adhesion molecule-1 (s-VCAM-1). Despite a relatively higher incidence of HLH-like toxicities and endothelial activation, overall neurotoxicity was generally less severe than reported with CD19 CAR T-cells, prompting additional analysis to explore CD22 expression in the central nervous system (CNS). Single-cell analysis revealed that in contrast to CD19 expression, CD22 is not on oligodendrocyte precursor cells or on neurovascular cells but is seen on mature oligodendrocytes. Lastly, among those attaining

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ CD22 chimeric antigen receptor (CAR) T-cells are increasingly more used and despite an association with hemophagocytic lymphohistiocytosis (HLH)-like toxicities and coagulopathy, comprehensive study of the hematologic toxicity profile is lacking.

## WHAT THIS STUDY ADDS

⇒ Despite a relatively higher fraction of patients experiencing HLH-like toxicities and coagulopathy with CD22 CAR T-cells compared to what has been reported with CD19 CAR T-cells, clinical manifestations of bleeding were mild, self-limited and generally fully resolved with abatement of the inflammatory response without life-threatening consequences, with rare exceptions. Importantly, despite evidence for endothelial activation which has been associated with CD19 CAR T-cell associated neurotoxicity, CD22 CAR T-cell associated neurotoxicity was mild, prompting further evaluation of CD22 expression in the central nervous system (CNS).

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This manuscript provides a first analysis of CD22 expression in the CNS. The differential expression of CD22 compared with CD19 expression suggests a potential hypothesis for why neurotoxicity with CD22 CAR T-cells may be less severe despite endothelial activation.

CR, grade 3–4 neutropenia and thrombocytopenia were seen in 65% of patients at D28.

**Conclusion** With rising incidence of CD19 negative relapse, CD22 CAR T-cells are increasingly important for the treatment of B-cell malignancies. In characterizing hematologic toxicities on CD22 CAR T-cells, we demonstrate that despite endothelial activation, coagulopathy, and cytopenias, neurotoxicity was relatively mild and that CD22 and CD19 expression in the CNS differed, providing one potential hypothesis for divergent

neurotoxicity profiles. Systematic characterization of on-target off-tumor toxicities of novel CAR T-cell constructs will be vital as new antigens are targeted.

**Trial registration number** NCT02315612.

## INTRODUCTION

Hematologic toxicities, including coagulopathy, endothelial activation, and cytopenias, are a known complication of chimeric antigen receptor (CAR) T-cell therapy and can occur both in the acute and long-term setting.<sup>1-4</sup> Acute hematologic toxicities occurring within the first 28 days of treatment typically manifest in the context of cytokine release syndrome (CRS) and commonly include higher grade cytopenias and/or alterations in coagulation parameters such as prolongation of prothrombin time (PT) and activated partial thromboplastin time, elevation in D-dimer; and decrease in fibrinogen levels, often following a transient rise.<sup>2 3 5 6</sup> Coagulopathy, clinically relevant bleeding, and hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome-like toxicities following CAR T-cells have also been reported.<sup>3 6-8</sup> Furthermore, while bone marrow recovery is impacted by the effects of CAR T-cell therapy, pre-CAR T-cell bone marrow reserve and baseline inflammation may also contribute to protracted cytopenias and delayed bone marrow recovery following CAR T-cells.<sup>1 2 9</sup> Comprehensive study of such toxicities has been largely limited to CD19-targeted CAR T-cell constructs. However, as CAR T-cell targeting alternative B-cell antigens evolve, particularly as a salvage therapy, it will be important to characterize the range of toxicities associated with novel CAR T-cell constructs.

We recently reported on our experience with CD22 CAR T-cells in children and young adults with relapsed/refractory B-cell malignancies and noted that with a change in manufacturing, the incidence of HLH-like toxicities and coagulopathy increased, despite a relatively stable CRS incidence and severity, prompting dose de-escalation.<sup>10 11</sup> We also saw features of endothelial activation on this trial, which has previously been associated with CD19 CAR T-cell-related neurotoxicity.<sup>12</sup> Interestingly, however, neurotoxicity was generally both less severe and less frequently seen in our patients than what has been reported on with CD19 CAR T-cells.<sup>12-14</sup> As CD22 CAR T-cells are becoming an important treatment option, particularly for those with suboptimal response to or relapse after CD19 targeted therapies,<sup>15-17</sup> both as single agent and in combinatorial strategies, we sought to further examine the hematologic toxicity profile of CD22 CAR T-cells and associations with CRS and neurotoxicity.

## METHODS

### Patients

This phase I dose-escalation study of anti-CD22 CAR T-cells enrolled children and young adults with relapsed or refractory B-cell malignancies between the ages of 3 and 30 years. Patients who had prior CAR T-cell therapy,

immunotherapy, or hematopoietic stem cell transplant (HSCT) were eligible. Updated trial results have recently been reported.<sup>10</sup> The primary objective of this substudy was to comprehensively characterize manifestations of coagulopathy and endothelial activation alongside other hematologic toxicities observed in patients experiencing CRS and the relationship to other aspects of the toxicity profile. Patients without any CRS were excluded from this analysis to eliminate the impact of non-response/progressive disease as a confounding factor. Importantly all patients with an objective response experienced CRS. Analysis included all patients infused prior to October 20, 2019, during which period coagulopathy studies were closely monitored. All patients or guardians provided written informed consent or assent with parental permission as age appropriate (ClinicalTrials.gov).

### CAR associated toxicities

CRS was prospectively graded using the Lee *et al* criteria,<sup>18</sup> but reconciled with the American Society of Transplantation and Cellular Therapy (ASTCT) grading, which is used in this manuscript.<sup>18</sup> Neurotoxicity symptoms were graded using Common Terminology Criteria for Adverse Events (CTCAE) V.4 guidelines as immune effector cell associated neurotoxicity syndrome (ICANS) grading was not available at study onset and cannot be retroactively applied. Specific details regarding neurotoxicity and grading have been previously reported.<sup>10</sup> CAR-associated HLH-like toxicity (carHLH) definitions used for this trial are in online supplemental table 1 and recently reported on.<sup>11</sup>

### Routine monitoring and coagulopathy definitions

Serial complete blood counts (CBCs) with differentials, coagulation studies, and acute phase reactants (eg, C-reactive protein (CRP) and ferritin) were collected in all patients. The upper limit of ferritin was 100,000 ng/mL which was used in our analysis. Additionally, after encountering bleeding manifestations in some of the first patients treated at the highest dose level, special coagulation studies were added to the study and prospectively collected. To assess for alterations in the clotting cascade, levels of protein C, S, factor VIII, antithrombin, von Willebrand factor (vWF) antigen, and vWF activity were collected in a small cohort of patients.

Coagulopathy was identified by the following parameters (adopted and revised from Toh *et al*)<sup>19</sup> across all patients with CRS: presence of bleeding (0=no, 1=yes), D-dimer (0=no increase (<0.5 µg/mL), 1=slight increase (>0.5–5 µg/mL), 2=moderate increase (>5–20 µg/mL) and 3=strong increase (>20 µg/mL)), PT (0=<3s, 1=≥3 to <6s, 2=≥6s from the upper level of normal) and fibrinogen nadir (0=≥100mg/dL, 1=<100mg/dL) (online supplemental table 2). Patients identified as coagulopathic included those with a score >5, or those with evidence of any clinically relevant bleeding (even in the absence of a score >5). Bleeding was graded by symptom and severity as per CTCAE V.4.0

### Special coagulation studies

Thromboelastography (TEG) was used in select patients to assess the contribution of coagulation factors, platelets, and fibrinogen to hemostasis by measuring clot formation and lysis in citrate anticoagulated whole blood, with or without kaolin (a contact activator of the intrinsic pathway of coagulation). To assess endothelial activation as a possible contributor to coagulopathy, we measured baseline and peak plasma thrombomodulin, tissue factor, F1+2, sE-selectin, sP-selectin, soluble intracellular adhesion molecule-1 (s-ICAM-1), and s-VCAM-1. To further determine whether endothelial activation and vascular permeability occurred in patients experiencing CRS we evaluated levels of angiopoietin-1 (Ang1), angiopoietin-2 (Ang2) and vascular endothelial growth factor (VEGF) at D7, D10, D14, and D21 after CAR T-cell infusion.<sup>20</sup>

### Correlative hematologic studies

Correlative studies evaluating markers of endothelial dysfunction were performed in select patients to assess for correlation with neurotoxicity. Plasma Ang1 and Ang2 were measured at Days 7, 10, 14 and 21 using R&D Systems ELISA Quantikine kits (Bio-Techne, Minneapolis, Minnesota, USA) according to the manufacturer's instructions (online supplemental file).

Peripheral blood smears were evaluated for platelet granularity at the following time points: pretreatment (Day -1 to -3), D0, D7, D14, D21 (online supplemental table 3).

### CD22 expression in the central nervous system

CD22 expression in the central nervous system (CNS) was performed using single-cell analysis following methods similar to those used to evaluate CD19 expression in the CNS.<sup>21</sup> Briefly, data was processed using Scanpy V.1.6 with standard workflows. Cells were filtered and counts were depth-normalized per cell to 10,000 reads, then log transformed. Data was scaled, and principle component analysis (PCA), the neighborhood graph, and uniform manifold approximation and projection (UMAP) was performed using default settings. Data was clustered using the Leiden algorithm. The indicated marker genes for each plot are shown, and the color of each cell indicates the log transformed, depth-normalized counts per cell. For expression analysis of CD22 across age, the mean expression of each postnatal time point was computed, and the Pearson correlation of mean expression and years age was calculated. Full methods can be found in the online supplemental appendix.

### Bone marrow and cytopenia assessments

Response to CAR-T cell therapy was assessed based on standard leukemia and/or lymphoma grading criteria and all patients had a bone marrow evaluation (including aspirate and biopsy) at baseline and at day +28 ( $\pm 3$  days) post-CD22 CAR-T cell infusion (online supplemental appendix). The bone marrow was also assessed for cellularity and CAR expansion. Analysis for count recovery was

restricted to those achieving complete remission (CR), all of whom experienced CRS.

Routine CBCs, including absolute neutrophil and platelet counts, were assessed in all complete responders according to CTCAE V.4.0 guidelines. Patients were defined as platelet transfusion-dependent if they received a transfusion within 7 days of their bone marrow biopsy for a platelet count  $< 25 \times 10^9/L$ , or for active bleeding. Platelet recovery was defined as a sustained platelet count  $> 50 \times 10^9/L$  for at least three consecutive days among those whose counts dropped below this threshold during the course of therapy.

### Statistical analysis

Descriptive statistics were used to summarize patient and disease characteristics. Non-parametric tests were used for all analyses. The Mann-Whitney U test was used to evaluate differences between patients stratified by presence or absence of coagulopathy. Values were captured from initiation of lymphodepletion  $\pm 2$  days to Day 28  $\pm 3$  days post CAR infusion. LDH and haptoglobin were only available from day 0 of CAR infusion  $\pm 2$  days and onward. Values that were unavailable or uninterpretable (eg, hemolysis) were not included in the analysis. Fisher's exact was used to compare categorical variables. Analysis was performed with Prism GraphPad using threshold of significance  $p < 0.05$ .

## RESULTS

### Patient characteristics and CAR response

Of 62 patients infused, a total of 53 patients had CRS, the latter which comprises the primary analysis cohort. Analysis plan and reasons for exclusion can be found in the online supplemental appendix. The median age of the analysis cohort was 15 years (range 4–30 years). All patients were treated for B-cell acute lymphoblastic leukemia (ALL) except one patient with diffuse large B-cell lymphoma. Thirty-six patients (68%) had undergone prior allogeneic HSCT. Thirty-three patients (62%) had received prior CD19 CAR therapy. Clinical outcomes and overall response rates for the whole cohort have been previously reported (table 1).<sup>10</sup>

### CAR associated toxicities

Among the 53 patients with CRS, 42 (79%) patients experienced grade 1–2 CRS, and 11 (21%) had grade 3–4 CRS, per ASTCT grading.<sup>18</sup> For CRS management, 6 received tocilizumab alone, 18 received tocilizumab and steroids, and 2 received only steroids. Neurotoxicity occurred in 20/53 (37.7%) patients, all of which was  $< \text{grade } 2$  with one exception (one patient with grade 4 intracranial hemorrhage (ICH) and concurrent *Bacillus cereus* sepsis) (online supplemental table 4).<sup>22</sup> Twenty-two (41.5%) patients had carHLH.

### Features of coagulopathy

#### Clinical manifestations

Among the 48 with full data for coagulopathy assessments, 18 patients comprised the coagulopathy cohort.



**Table 1** Patient characteristics

Demographics	All participants with CRS
No. of participants	53
Sex	
Male	34 (64)
Female	19 (36)
Median age, years, (range)	15 (4–30)
Diagnosis	
ALL	52 (98.1)
DLBCL	1 (1.9)
Prior HSCT	36 (68)
Prior CAR therapy	33 (62)
Evaluable for coagulopathy*	48

Data presented as No. (%) unless otherwise indicated.

\*Five patients were excluded for evaluation of coagulopathy due to lack of laboratory measurements collected in these patients for the coagulopathy parameters of fibrinogen, ferritin, D-dimer and partial thromboplastin time. These five were among the earliest to be treated in the trial and importantly had no manifestations of bleeding.

ALL, acute lymphoblastic leukemia; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; DLBCL, diffuse large B-cell lymphoma; HSCT, hematopoietic stem cell transplantation; INR, international normalized ratio; LD, lymphodepletion; LDH, lactate dehydrogenase; PCA, principle component analysis; VGEF, vascular endothelial growth factor.

This included 16 patients with bleeding manifestations, which were primarily mucosal grade 1 and 2 toxicities, and two with laboratory abnormalities without bleeding (online supplemental table 5). Thirteen patients had more than one manifestation of bleeding (figure 1A). As

previously reported,<sup>10</sup> the average time to CRS onset was day 7 (range, day 3–16 post infusion). Bleeding began a median of 6 days after onset of CRS (IQR, 3 to 7.5 days) and 1.5 days after onset of carHLH (IQR, –3 to 4.3 days) in those experiencing both bleeding and carHLH (n=14).

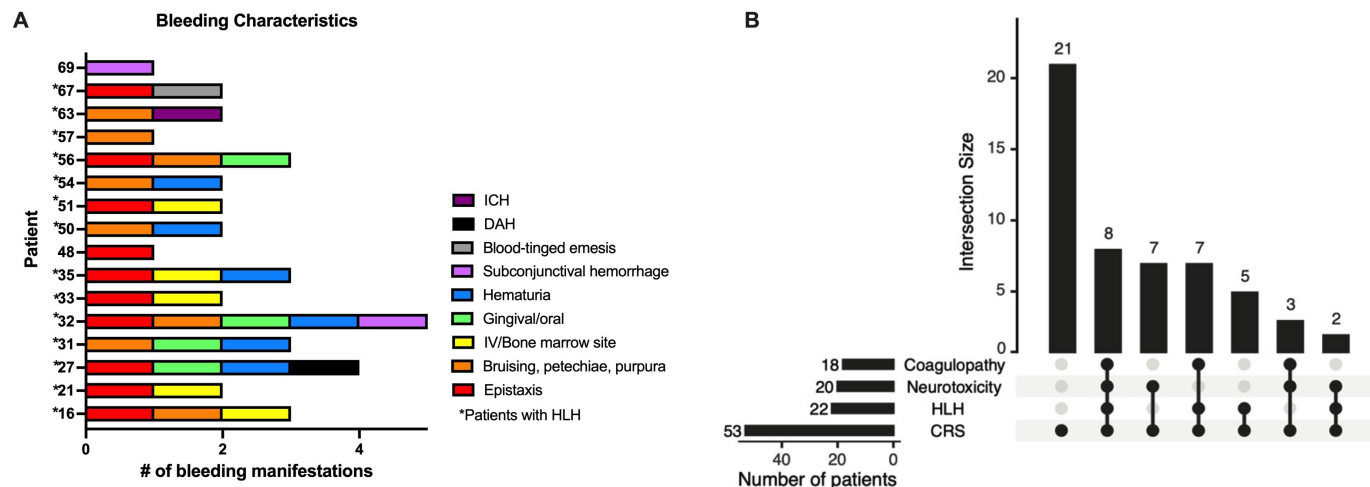
Disease severity (M1 vs >M2 marrow) prior to CAR infusion was not associated with coagulopathy (p=0.28). Grade 2 CRS or higher was associated with coagulopathy (p=0.016). Three patients developed thrombotic microangiopathy (TMA) requiring the use of eculizumab. One patient showed signs of diffuse alveolar hemorrhage (DAH), another showed signs of atypical hemolytic uremic syndrome and the third experienced ICH, though this patient was found to have *B. cereus* sepsis and has been previously described.<sup>10,23</sup> Details of their treatment course are in the online supplemental appendix.

Among the 20 patients with neurotoxicity, 11 (55%) were coagulopathic. Similarly, 15/22 (68%) patients with carHLH experienced coagulopathy (figure 1B). Bleeding manifestations were self-limited, mostly grade 1 and 2 severity, and fully resolved with abatement of the inflammatory response without life-threatening consequences, with the exception of the isolated ICH as mentioned.

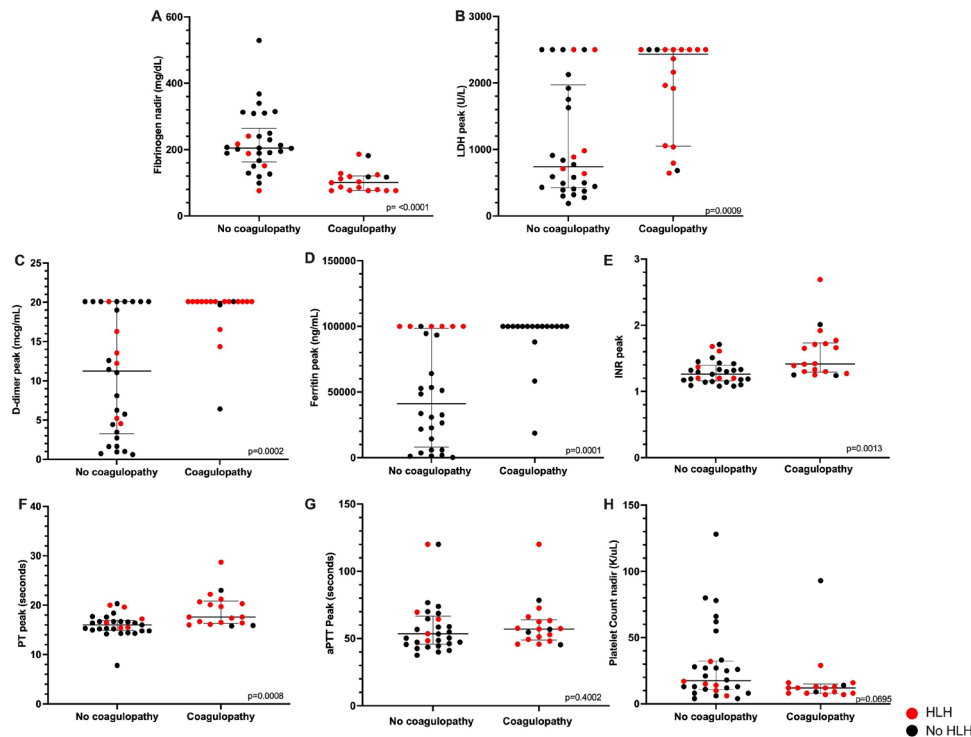
### Laboratory manifestations

Patients with coagulopathy had higher peak ferritin values (100,000 vs 41,045 ng/mL, p<0.0001) than patients who did not display any indicators of endothelial dysfunction. No differences were found between the two groups with respect to CRP or haptoglobin levels.

Patients also had lower fibrinogen nadirs (102 vs 205 mg/dL, p<0.0001), more prolonged PTs (17.60 vs 16.00 s, p=0.0008), and higher INR (1.42 vs 1.26, p=0.0013), D-dimer (20.10 vs 11.24 mcg/mL, p=0.0002), and LDH (2433 vs 741 U/L, p=0.0009) levels as compared



**Figure 1** Characteristics of patients with hematologic toxicities. (A) Bleeding manifestations of each patient who experienced clinically relevant bleeding after receiving CD22 CAR T-cell infusion. (\*) Patients with carHLH. (B) Number of patients who experienced one clinically relevant toxicity of coagulopathy, carHLH or neurotoxicity and those that had more than one manifestation. Five of the 21 patients who did not experience coagulopathy, carHLH or neurotoxicity were unevaluable for coagulopathy, but presumed to not be coagulopathic. CAR, chimeric antigen receptor; carHLH, CAR-associated HLH-like toxicity; CRS, cytokine release syndrome; DAH, diffuse alveolar hemorrhage; HLH, hemophagocytic lymphohistiocytosis; ICH, intracranial hemorrhage; IV, intravenous line site.



**Figure 2** Routine coagulation parameters comparison between those without coagulopathy and those with coagulopathy. (A) Fibrinogen nadir; (B) LDH peak; (C) D-dimer peak; (D) ferritin peak; (E) INR peak; (F) prothrombin time (PT) peak; (G) activated partial thromboplastin time (aPTT) peak; (H) platelet count nadir. HLH, hemophagocytic lymphohistiocytosis.

with those without coagulopathy (figure 2). There were no substantial differences in the observed baseline and peak levels of the anticoagulant proteins C and S, or factor VIII, antithrombin, vWF antigen, or vWF activity between the two groups.

In five patients with bleeding, TEG was performed to further characterize the nature of the coagulopathy. No patients had prolonged R times, with or without the kaolin activator. In nearly all patients, the theta angle (a measure of rate of clot formation) and maximum amplitude (MA, i.e., maximum clot firmness)—both of which depend on the contribution of fibrinogen and platelets—were abnormally low (online supplemental table 6). Despite the features of coagulopathy and bleeding in these patients, the TEG did not show evidence of hyperfibrinolysis in the EPL or LY30 parameters.

Lastly, after observance of mucosal bleeding that was seemingly out of proportion to adequate platelet counts in several patients, and gross microscopy observation of hypogranular platelets, we sought to explore platelet granularity more comprehensively to investigate the possibility of an acquired platelet dysfunction. First, we evaluated one patient's (#27) platelets by transmission electron microscopy to ascertain the status of platelet granules. This confirmed decreased dense granules of platelets (online supplemental figure 1). Subsequently, platelet granularity by standard microscopy was assessed in 25 of our patients at baseline (post-lymphodepleting (LD) chemotherapy, pre infusion), D0, 7, 14 and 21, after CD22 CAR T-cell infusion. We found the majority with CRS met criteria for hypogranularity, without a

consistent relationship to presence of coagulopathy or bleeding manifestations. Specifically, 9 of 18 (50%) were hypogranular at baseline, 15 of 21 (71.4%) on Day 0; 19 of 25 (76%) on Day 7, 19 of 25 (76%) on Day 14 and 18 of 24 (75%) on Day 21.

### Transfusion requirements

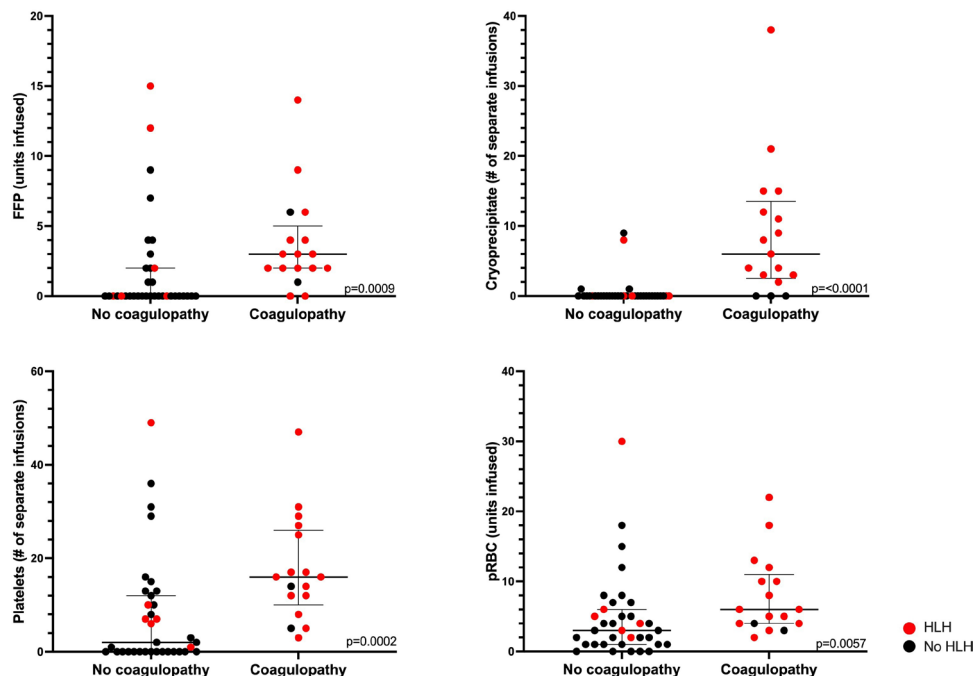
Patients with coagulopathy were also more likely to require transfusions during their treatment course. Those with coagulopathy received more packed red blood cells (6 vs 3 units infused,  $p=0.0057$ ), platelet transfusions (16 vs 2 separate transfusions,  $p=0.0002$ ), fresh frozen plasma (3 vs 0 units infused,  $p=0.0009$ ) and cryoprecipitate transfusions (6 vs 0 separate transfusions,  $p<0.0001$ ) than those without coagulopathy (figure 3).

### Special coagulation studies

Among 23 patients with special coagulation studies, those with coagulopathy ( $n=7$ , 30.4%) had higher peak levels of tissue factor (351 vs 213 pg/mL,  $p=0.0035$ ), prothrombin factor F1+2 (6648 vs 1247 pmol/L,  $p=0.033$ ), and s-VCAM-1 (4833 vs 2064 ng/mL,  $p=0.039$ ) (online supplemental figures 2-4; online supplemental table 7). There was no difference in peak values of thrombomodulin, 2, sE-selectin, sP-selectin at peak in those with and without coagulopathy (online supplemental table 7).

### Endothelial studies

Patients with coagulopathy had higher Ang2 levels 10 days after CAR T-cell infusion (6411 vs 3944 pg/mL,  $p=0.015$ ). There were no substantial differences in Ang1 levels



**Figure 3** Transfusions received in CD22 chimeric antigen receptor T-cell therapy comparison between those without coagulopathy and those with coagulopathy. (A) Fresh frozen plasma (FFP) (1 unit=1 FFP); (B) cryoprecipitate (# infusions, which contain varying units infused); (C) platelets (# transfusions, which could vary from 5 to 12 units of platelets/bag); (D) packed red blood cells (pRBCs) (1 unit=1 pRBC). HLH, hemophagocytic lymphohistiocytosis.

between those who had coagulopathy and those who did not (online supplemental figure 3). The Ang2:Ang1 ratio was only found to be different between the coagulopathy groups on D10 (6.25 vs 3.30,  $p=0.014$ ) but not different on any other days examined. Those with coagulopathy displayed lower levels of VEGF on D10 (10.90 vs 28.10 pg/mL,  $p=0.0019$ ) and D14 (21.60 vs 30.15 pg/mL,  $p=0.011$ ) after infusion.

#### Association of hematologic toxicities with neurotoxicity

Neurotoxicity in this cohort was relatively mild, particularly in comparison to that reported with CD19 CAR T-cells,<sup>1 12 24 25</sup> and generally manifested within 1–4 days of CRS onset. Previous data indicated that CRS severity along with hematologic toxicities and endothelial activation, particularly TMA-like manifestations associated with disruptions in the blood-brain barrier (BBB), may cause the neurotoxicity of CAR T-cells. In this regard, all three patients with TMA experienced neurotoxicity. However, despite our higher relative incidence of HLH-like toxicities and endothelial activation, our neurotoxicity profile was generally less severe.

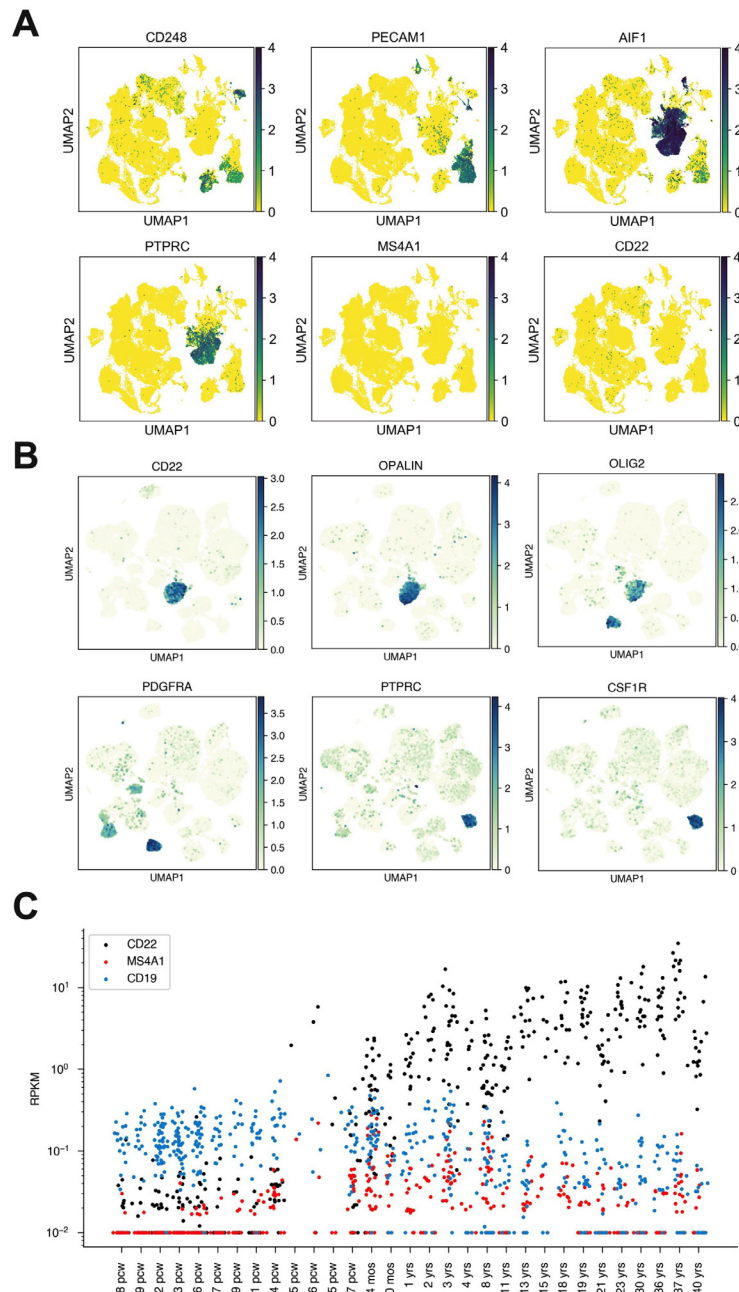
Differences in angiopoietin levels based on the presence or absence of neurotoxicity were also evaluated. Higher levels of Ang2 were seen in those with neurotoxicity at day 14 (5335 vs 3725 pg/mL,  $p=0.026$ ), but not at any other time points evaluated. Additionally, Ang2:Ang1 levels were statistically significant on D10 (6.9 vs 3.6,  $p=0.013$ ), but not D7, 14, or 21 (online supplemental figure 5).

In the context of our hematologic toxicities profiling and based on recent data using single-cell analysis, which

identified CD19 expression in human brain mural cells as a potential target for and etiology of CD19 CAR T-cell associated neurotoxicity,<sup>21</sup> we sought to explore the expression of CD22 in the CNS. We looked at expression in three public data sets: single-cell RNA expression data from human developmental brain from the Brain Initiative Cell Census Network (BICCN) consortia, single-nuclei RNA expression data from the adult brain from the Allen Brain Map, and bulk RNA sequencing data across multiple ages and brain regions from the Allen Brain-Span project. This revealed that CD22 is not expressed in neurovascular cells (mural cells, endothelial cells, or neurovascular progenitors) (figure 4A). As previously reported,<sup>26</sup> CD22 was found to be expressed in mature oligodendrocytes but not in oligodendrocyte precursor cells (OPCs), the latter of which are also perivascular<sup>27–29</sup> (figure 4B). The finding of CD22 expression in the adult brain was confirmed by the BrainSpan bulk sequencing data set (figure 4C), where we also observed a trend of increased CD22 expression across age ( $r=0.65$  across post-natal time points).

#### Bone marrow evaluation and cytopenias

Among the 62 infused patients, 43 (69.4%) achieved CR (all of whom had CRS) and were evaluated for cytopenias at D28. CRS grade did not associate with cytopenia severity. At the time of bone marrow evaluation, median absolute neutrophil count was 580 K/mcL (range, <200–2980 K/mcL). Median platelet count was 35 K/mcL (range, 12–283 K/mcL). Median bone marrow cellularity was 26.3% (range, 5–85%), with the majority being hypocellular for age. All evaluable patients had evidence

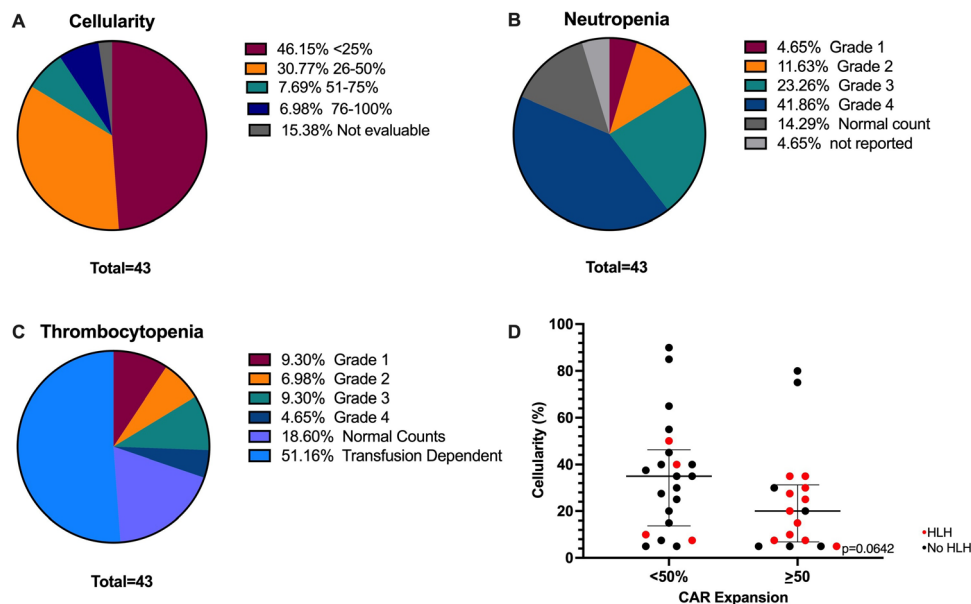


**Figure 4** Analysis of CD22 expression in human brain single-cell RNA (scRNA) sequencing data (A) UMAP plots of scRNA data from human developmental neurovascular cells showing lack of CD22 expression in neurovascular cells. Each dot is colored by the log expression level of the indicated gene. CD22 is not expressed in the CD248+ or PECAM1+ mural and endothelial clusters, nor in the AIF1+ microglia cluster. (B) UMAP plots of snRNA data from human adult brain showing strong CD22 expression in oligodendrocytes. As before, log expression of the indicated gene is shown. CD22 is highly expressed in oligodendrocytes, marked by OLIG1/2, OPALIN, PLP1, and MBP expression, but not in oligodendrocyte precursor cells, marked by OLIG1/2, CSPG4, and PDGFRA expression. Note that relatively low numbers of neurovascular (CLDN5+) cells are present. (C) CD22 expression increases with age. Bulk RNA sequencing data from different patients and brain regions are shown for each time point. Abbreviations: PDGFRA, platelet-derived growth factor receptor alpha; MBP, myelin basic protein; CSPG4, chondroitin sulfate proteoglycan 4; OPALIN, oligodendrocytic myelin paranodal and inner loop Protein; AIF1, allograft inflammatory factor 1; PTPRC4, protein tyrosine phosphatase receptor Type C; CSF1R, colony stimulating factor 1 receptor; snRNA, small nuclear RNA; OLIG1/2, oligodendrocyte transcription factor 1/2; PECAM1, platelet endothelial cell adhesion molecule 1; UMAP, uniform manifold approximation and projection

of trilineage hematopoiesis to varying degrees. Nine patients who remained transfusion-dependent at D28 were transfusion-dependent before lymphodepletion. Median CAR expansion (% of T cells that are CAR+)

was 41.5% (range, 6–84.8%). Increased CAR expansion was associated with a trend towards lower percent bone marrow cellularity ( $p=0.06$ ). Time to absolute neutrophil count (ANC) and platelet recovery could not be obtained





**Figure 5** Bone marrow recovery evaluation of bone marrow at D28 ( $\pm 3$  days) in patients who had a complete response and evaluable marrow. (A) Cellularity of the marrow (%); (B) absolute neutrophil count (C); platelet count; (D) relationship between CAR expansion and cellularity. CAR, chimeric antigen receptor; HLH, hemophagocytic lymphohistiocytosis.

for complete analysis as some patients returned to their home institutions for continuing care or proceeded to HSCT before recovery occurred (figure 5).

## DISCUSSION

Hematologic toxicities, including coagulopathy, cytopenia, bleeding, and endothelial activation, are frequently seen in the context of CRS following CAR T-cell therapy and are commonly delayed side effects of treatment.<sup>3 5 6 9</sup> The majority of the experience, however, stems from CD19 CAR T-cell constructs. With the emergence of targeting alternative antigens, the goal of this study was to characterize the hematologic toxicity profile of patients receiving CD22 CAR T-cells. This particular toxicity is of specific relevance, as concern for bleeding and coagulopathy prompted dose de-escalation on the phase I trial and there is a relatively higher incidence of carHLH with this construct.<sup>10 11</sup>

In our study 16/53 (30%) patients experienced clinically relevant bleeding, which was relatively mild (grade 1 and 2). This occurred in the setting of lower CRS severity, with only four patients having a CRS grade  $>3$ . In contrast, 137 patients from the ELIANA and ENSIGN trials reported bleeding manifestations in 26/57 (45.6%) patients with grade 3–4 CRS.<sup>4</sup> Additionally, the very low fibrinogen levels ( $<150$  mg/dL) in our patients contrasts with other studies that saw hypofibrinogenemia associated with higher CRS severity,<sup>4</sup> which may be reflective of our experience with carHLH. Indeed, hypofibrinogenemia is one of the defining criterion for primary and secondary HLH,<sup>30</sup> and coagulation abnormalities a main complication of HLH,<sup>31</sup> mechanisms which are not fully elucidated.

Prior studies of CD19-directed CAR T-cells have correlated markers of endothelial activation and vascular permeability with higher grades of CRS and neurotoxicity.<sup>12 13 32 33</sup> Based on both our experience and other CD22 CAR T-cell trials,<sup>15 16</sup> CD22 CAR T-cells appear to have lower severity CRS and generally less severe neurotoxicity despite the coagulopathy seen. We therefore evaluated various biomarkers known to be associated with endothelial cell function, activation and permeability.<sup>34</sup> While we did not see any differences in vWF or thrombomodulin, we did see higher levels of cell-surface adhesion molecule s-VCAM-1 in coagulopathic patients, indicating an increased state of endothelial activation. Although these findings suggest association of endothelial disruption with increased rates of coagulopathy and bleeding seen in our study, this did not correlate with neurotoxicity severity.

Similarly, while our results of Ang1 and Ang2 were not as strongly associated with neurotoxicity as seen with CD19 CAR T-cells,<sup>12</sup> comparisons are challenging based on differing time to onset of CRS and neurotoxicity across trials. Nonetheless in Gust *et al*,<sup>12</sup> relevant changes were seen at day 7, likely after manifestations of neurotoxicity had presented—which potentially aligns with our Day +10 and +14 post-infusion results (given the later onset of CRS). Additionally, while select serum cytokines have been associated with neurotoxicity,<sup>12</sup> our recent characterization of peak serum cytokines across CD19, CD22 and CD19/22 CAR T-cell trials revealed generally comparable elevations in cytokines with rare exception.<sup>35</sup> Exploring additional time points to align results with clinical manifestations is a next step.

Based on the single-cell analysis identification of CD19 on brain mural cells, which are known to line the BBB,



potentially implicating on-target, off-tumor toxicities from CD19 CAR T-cells,<sup>21</sup> we sought to study the expression of CD22 in the CNS. The identical analysis revealed absence of CD22 expression on brain mural cells or OPCs. Thus we hypothesize that one potential mechanism for the relatively lower incidence of severe neurotoxicity seen with CD22 CAR T-cells is that the BBB may potentially be more well-preserved following CD22 CAR T-cells than with CD19 targeting, the latter which may create a scenario more permissive of influxes of inflammatory cytokines into the CNS—despite our recent finding that CD22 CAR T-cell expansion is higher compared with either CD19 CAR T-cell-based construct.<sup>35</sup> The relevance of CD22 expression on oligodendrocytes is unknown. Provocatively challenging conventional wisdom, our experience disassociates neurotoxicity as correlating directly with endothelial activation, and rather provides data suggesting that the differential antigen expression in the CNS may provide a potential explanation for the variability in clinical manifestations, highlighting the potential value of single-cell analysis in understanding CAR T-cell toxicities with novel targets. We also show that toxicities, while frequently overlapping, may also be seen as distinct entities. Indeed, among all those with neurotoxicity, 7 of 20 (35%) had no manifestations of concurrent carHLH or coagulopathy.

Lastly, given the concern for cytopenias associated with CAR T-cells in general, we evaluated neutrophil and platelet recovery at D28. In this very heavily pretreated patient population, the majority experienced high grade cytopenias, before and after CD22 CAR T-cell infusion. The trend between higher CAR T-cell expansion and lower bone marrow cellularity may potentially be secondary to the local inflammatory response from CAR T-cell expansion, in conjunction with lower bone marrow reserve due to the impact of extensive prior therapy in this patient population. The CAR-HEMATOTOX model, which examines markers of hematopoietic reserve and baseline inflammatory markers, supports the hypothesis that patients with lower bone marrow reserves prior to therapy may have higher grade and more prolonged courses of cytopenia following CAR T-cell therapy.<sup>9</sup>

Limitations of this study include absence of laboratory data related to endothelial dysfunction and coagulation in our earliest set of patients enrolled who were treated at the lowest dose level, which did not allow for consistent collection among all participants—but who also did not experience bleeding complications. Markers of endothelial activation, such as Ang1, Ang2 and Ang2:Ang1 were generally unrevealing in our study and could not be used to validate the findings reported in other recent studies on CAR T-cell associated neurotoxicity,<sup>12</sup> possibly due to discrepant neurotoxicity findings and differences in timing of sample collection and variability in onset of clinical manifestations. Additionally, slides for evaluation of platelet granularity by standard microscopy were only available for 25 of our patients. Accurate evaluation of thrombocytopenia was hard to assess as some patients

were receiving consistent platelet transfusions to maintain a count above >30,000 K/ $\mu$ L for procedures. Lastly, comparisons of bleeding manifestations and coagulopathy to other CAR T-cell constructs is also limited based on varying grading systems for CRS and definitions for coagulopathy.

In conclusion, we comprehensively report focusing on hematologic toxicities of CD22 CAR T-cells in children and young adults with ALL and the correlation with neurotoxicity and endothelial activation—or lack thereof. As CD22 CAR T-cells are emerging as an important treatment strategy, either as single antigen or as combinatorial constructs, this report provides much needed insight into a systems-based toxicity of this construct. As HLH-like toxicities were an important confounder with coagulopathy, we anticipate that with earlier recognition and pre-emptive intervention, that both toxicities will become less challenging and more easy to manage than with these early experiences.<sup>11</sup> The differential expression of CD22 versus CD19 in the CNS is of great interest and further studies are warranted to assess the potential role of single-cell analysis in determining on-target, off-tumor toxicities.

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#### REFERENCES

- Maude SL, Laetsch TW, Buechner J, *et al.* Tisagenlecleucel in children and young adults with B-cell Lymphoblastic leukemia. *N Engl J Med* 2018;378:439–48.
- Fried S, Avigdor A, Bielora B, *et al.* Early and late hematologic toxicity following CD19 CAR-T cells. *Bone Marrow Transplant* 2019;54:1643–50.
- Wang Y, Qi K, Cheng H, *et al.* Coagulation disorders after Chimeric antigen receptor T cell therapy: analysis of 100 patients with relapsed and refractory hematologic malignancies. *Biol Blood Marrow Transplant* 2020;26:865–75.
- Buechner J, Grupp SA, Hiramatsu H, *et al.* Practical guidelines for monitoring and management of Coagulopathy following Tisagenlecleucel CAR T-cell therapy. *Blood Adv* 2021;5:593–601.
- Budno JN, Kochenderfer JN. Toxicities of Chimeric antigen receptor T cells: recognition and management. *Blood* 2016;127:3321–30.
- Ikezoe T. Advances in the diagnosis and treatment of disseminated Intravascular coagulation in haematological malignancies. *Int J Hematol* 2021;113:34–44.
- Teachey DT, Lacey SF, Shaw PA, *et al.* Identification of predictive biomarkers for cytokine release syndrome after Chimeric antigen receptor T-cell therapy for acute Lymphoblastic leukemia. *Cancer Discov* 2016;6:664–79.
- Neelapu SS, Tummala S, Kebriaei P, *et al.* Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol* 2018;15:47–62.
- Rejeski K, Perez A, Sesques P, *et al.* CAR-HEMATOTOX: a model for CAR T-cell-related hematologic toxicity in relapsed/refractory large B-cell lymphoma. *Blood* 2021;138:2499–513.
- Shah NN, Highfill SL, Shalabi H, *et al.* CD4/CD8 T-cell selection affects Chimeric antigen receptor (CAR) T-cell potency and toxicity: updated results from a phase I anti-CD22 CAR T-cell trial. *JCO* 2020;38:1938–50.
- Lichtenstein DA, Schischlik F, Shao L, *et al.* Characterization of HLH-like manifestations as a CRS variant in patients receiving CD22 CAR T cells. *Blood* 2021;138:2469–84.
- Gust J, Hay KA, Hanafi L-A, *et al.* Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive Immunotherapy with CD19 CAR-T cells. *Cancer Discov* 2017;7:1404–19.
- Mackall CL, Miklos DB. CNS endothelial cell activation emerges as a driver of CAR T cell-associated neurotoxicity. *Cancer Discov* 2017;7:1371–3.
- Santomasso BD, Park JH, Salloum D, *et al.* Clinical and biological correlates of neurotoxicity associated with CAR T-cell therapy in patients with B-cell acute Lymphoblastic leukemia. *Cancer Discov* 2018;8:958–71.
- Baird JH, Frank MJ, Craig J, *et al.* CD22-directed CAR T-cell therapy induces complete remissions in CD19-directed CAR-refractory large B-cell lymphoma. *Blood* 2021;137:2321–5.
- Pan J, Niu Q, Deng B, *et al.* CD22 CAR T-cell therapy in refractory or relapsed B acute Lymphoblastic leukemia. *Leukemia* 2019;33:2854–66.
- Pan J, Zuo S, Deng B, *et al.* Sequential CD19-22 CAR T therapy induces sustained remission in children with R/R B-ALL. *Blood* 2020;135:387–91.
- Lee DW, Santomasso BD, Locke FL, *et al.* ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant* 2019;25:625–38.
- Toh CH, Alhamdi Y, Abrams ST. Current pathological and laboratory considerations in the diagnosis of disseminated Intravascular coagulation. *Ann Lab Med* 2016;36:505–12.
- Melincovici CS, Boşca AB, Şuşman S, *et al.* Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol* 2018;59:455–67.
- Parker KR, Migliorini D, Perkey E, *et al.* Single-cell analyses identify brain mural cells expressing CD19 as potential off-tumor targets for CAR-T Immunotherapies. *Cell* 2020;183:126–42.
- Shalabi H, Wolters PL, Martin S, *et al.* Systematic evaluation of neurotoxicity in children and young adults undergoing CD22 Chimeric antigen receptor T-cell therapy. *J Immunother* 2018;41:350–8.
- Masih KE, Ligon JA, Yates B, *et al.* Consequences of hemophagocytic lymphohistiocytosis-like cytokine release syndrome toxicities and concurrent bacteremia. *Pediatr Blood Cancer* 2021;68:e29247.
- Gust J, Taraseviciute A, Turtle CJ. Neurotoxicity associated with CD19-targeted CAR-T cell therapies. *CNS Drugs* 2018;32:1091–101.
- Gardner RA, Finney O, Annesley C, *et al.* Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. *Blood* 2017;129:3322–31.
- Pluvinaige JV, Sun J, Claes C, *et al.* The CD22-IGF2R interaction is a therapeutic target for Microglial Lysosome dysfunction in Niemann-pick type C. *Sci Transl Med* 2021;13:eabg2919.
- Kirby L, Jin J, Cardona JG, *et al.* Oligodendrocyte precursor cells present antigen and are cytotoxic targets in inflammatory demyelination. *Nat Commun* 2019;10:3887.
- Niu J, Tsai H-H, Hoi KK, *et al.* Aberrant oligodendroglial-vascular interactions disrupt the blood-brain barrier, triggering CNS inflammation. *Nat Neurosci* 2019;22:709–18.
- Kishida N, Maki T, Takagi Y, *et al.* Role of perivascular oligodendrocyte precursor cells in angiogenesis after brain ischemia. *J Am Heart Assoc* 2019;8:e011824.
- Henter J-I, Horne A, Aricó M, *et al.* HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007;48:124–31.
- Valade S, Mariotte E, Azoulay E. Coagulation disorders in hemophagocytic lymphohistiocytosis/macrophage activation syndrome. *Crit Care Clin* 2020;36:415–26.
- Hay KA, Hanafi L-A, Li D, *et al.* Kinetics and biomarkers of severe cytokine release syndrome after CD19 Chimeric antigen receptor-modified T-cell therapy. *Blood* 2017;130:2295–306.
- Jiang H, Liu L, Guo T, *et al.* Improving the safety of CAR-T cell therapy by controlling CRS-related Coagulopathy. *Ann Hematol* 2019;98:1721–32.
- Page AV, Liles WC. Biomarkers of endothelial activation/dysfunction in infectious diseases. *Virulence* 2013;4:507–16.
- Shalabi H, Qin H, Su A, *et al.* CD19/22 CAR T cells in children and young adults with B-ALL: phase 1 results and development of a novel Bicistronic CAR. *Blood* 2022;140:451–63.