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Consequences of hemophagocytic lymphohistiocytosis-like cytokine release syndrome toxicities and concurrent bacteremia

Katherine E. Masih[1,2], **John A. Ligon**[3], **Bonnie Yates**[3], **Haneen Shalabi**[3], **Lauren Little**[3], **Zahin Islam**[1], **Amanda K. Ombrello**[4], **Jon Inglefield**[5], **Veronique Nussenblatt**[6], **Maura Manion**[6], **Javed Khan**[1], **Nirali N. Shah**[3]

[1]Oncogenomics Section, Genetics Branch, Center for Cancer Research (CCR), National Cancer Institute (NCI), NIH, Bethesda, Maryland, USA

[2]Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK

[3]Pediatric Oncology Branch, CCR, NCI, NIH, Bethesda, Maryland, USA

[4]Inflammatory Disease Section, National Human Genome Research Institute, NIH, Bethesda, Maryland, USA

[5]Applied Developmental Research Directorate, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

[6]Infectious Disease Consult Service, National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, USA

Abstract

Serious bacterial infections (SBI) can lead to devastating complications with CD19 CAR T cells and cytokine release syndrome (CRS). Little is known about consequences of and risk factors for SBI with novel CAR T-cell constructs or with CRS complicated by HLH-like toxicities. We report on three patients with B-cell acute lymphoblastic leukemia treated with CD22 CAR T cells who developed SBI and CRS-associated HLH. Serum cytokine profiling revealed sustained

Correspondence Nirali N. Shah, Pediatric Oncology Branch, CCR, NCI, NIH, Bldg 10, Room 1W-3750, 9000 Rockville Pike MSC 1104, Bethesda, MD 20892, USA. nirali.shah@nih.gov.

Katherine E. Masih and John A. Ligon contributed equally to this work and are co-first authors.

AUTHOR CONTRIBUTIONS

Katherine E. Masih, John A. Ligon, and Nirali N. Shah conceived and designed the work; acquired, analyzed, and interpreted the data; and drafted and revised the work. Bonnie Yates, Haneen Shalabi, Lauren Little, Amanda K. Ombrello, Veronique Nussenblatt, Maura Manion, Javed Khan, and Nirali N. Shah provided patient care. All other authors (Zahin Islam, Jon Inglefield) acquired, analyzed, and/or interpreted the data, and revised the work critically for important intellectual content. All authors made substantial contributions as above and gave final approval to the version published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the ethical principles stated in the Belmont Report and the U.S. Common Rule. The clinical trial these patients were enrolled on was approved by the Central Institutional Review Board of the NCI, study number 15-C-0029. All three patients described are deceased. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

elevations well beyond CRS resolution, suggesting ongoing systemic inflammation. Heightened inflammatory states converging with SBI contribute to poor outcomes, and recognition and prevention of extended inflammation may be needed to improve outcomes.

Keywords

ALL molecular diagnosis and therapy; ALL relapse; clinical trials; cytokines; immunotherapy; infections

1 | INTRODUCTION

Chimeric antigen receptor T cells (CART) are effective for treating B-cell acute lymphoblastic leukemia (B-ALL)[1] but are associated with cytokine release syndrome (CRS) and hemophagocytic lymphohistiocytosis-like toxicities (carHLH).[1,2] CD19 CART studies have correlated CRS severity and infection,[3,4] but infectious risks of novel CART are unknown.

We report on three patients with B-ALL enrolled on the CD22 CART trial (NCT02315612) who developed serious bacterial infection (SBI) and carHLH following standard lymphodepletion (Supporting Methods) and CART infusion (Table 1, Figure 1A). In these patients, the convergence of SBI and carHLH contributed to heightened inflammatory states, predisposing patients to poor outcomes. These cases highlight the complex interplay between timing of bacteremia, CRS, immunosuppression, and the underlying inflammatory milieu in patients receiving novel CART.

2 | RESULTS

2.1 | Patient 1

A 4-year-old boy with CD19-negative relapse following tisagenlecleucel developed culture-negative febrile neutropenia (day −2 to day +1), following lymphodepletion and CD22 CART infusion. On day +7, he had fever recrudescence, coinciding with CRS onset (grade 2: fever, tachycardia, hypoxia),[5] and concurrent positive blood culture grew *Bacillus cereus*, which cleared within 24 hours on antimicrobials. He received tocilizumab (12 mg/kg, two doses) and methylprednisolone (1 mg/kg BID) for CRS, and he transferred to the intensive care unit (ICU). He defervesced and weaned off oxygen with CRS improvement, but on day +10 he developed carHLH (coagulopathy, transaminitis, hyperferritinemia) and initiated anakinra (3.3 mg/kg BID). He transferred out of the ICU on day +12 while continuing immunosuppression. He received daily cryoprecipitate for hypofibrinogenemia and had no signs of bleeding, normal PT/PTT, and platelet count remained above 50,000/μl.

Despite steady improvement in clinical and laboratory parameters and without signs of neurotoxicity, on day +17, he developed suddenonset hemiparesis. Head CT revealed a large right temporofrontal hematoma and hemorrhagic foci throughout the bilateral cerebral hemispheres. He underwent emergency decompressive craniectomy and had a protracted course but remained minimally responsive. As CD22 CART are used to bridge

to hematopoietic stem cell transplant (HSCT); being unable to proceed to HSCT due to his neurologic sequelae, he passed away from CD22-negative relapse.

2.2 | Patient 2

An 18-year-old girl with post-HSCT relapse and numerous prior SBI received lymphodepletion and CART without incident. She developed grade 3 CRS (fever, tachycardia, hypotension) on day +8. She received tocilizumab (8 mg/kg) and transferred to the ICU (day +10). Following stabilization, she transferred out of the ICU (day +14), but she subsequently developed carHLH (coagulopathy, transaminitis, hyperbilirubinemia, hyperferritinemia). On day +19, she developed non-neutropenic fever that rapidly progressed to shock. Following initiation with ceftazidime, antibiotics were broadened, and she also received tocilizumab and methylprednisolone (1 mg/kg q8h). Blood cultures revealed ceftazidime-resistant *Enterobacter cloacae*. Despite initial improvement, she developed multiorgan failure and died on day +39.

2.3 | Patient 3

A 26-year-old man with relapsed B-ALL received lymphodepletion and CART without incident. On day +4, he developed grade 3 CRS (fever, hypoxia, hypotension). He received tocilizumab (8 mg/kg) and transferred to the ICU (day +5). On day +8, he developed carHLH (transaminitis, hyperferritinemia, coagulopathy, hyperbilirubinemia, hepatosplenomegaly), which was treated with a second tocilizumab dose, methylprednisolone (1 mg/kg q8h), and anakinra (1–2 mg/kg q6h). He also received broad-spectrum antibiotics for acalculous cholecystitis. He further developed atypical hemolytic uremic syndrome requiring transient renal replacement therapy (day +9) and began eculizumab (900 mg q72h) (day +16). He improved with these supportive measures and transferred out of the ICU (day +18).

On day +24 he developed febrile neutropenia, hemoptysis, and hypoxia. Chest CT identified pulmonary consolidations and bilateral ground-glass opacities. Blood cultures grew *Staphylococcus epidermidis* and *Weeksella virosa*, and bronchoscopy culture also grew *W. virosa*. Transient worsening hypoxia from infection led to increased steroid utilization. Following resolution, his restaging bone marrow demonstrated remission. He was discharged on day +46 and proceeded to allogeneic HSCT.

2.4 | Patient cytokine levels

We previously established that patients with carHLH have higher peak cytokine values compared to those without carHLH.[6] Serial evaluation of these three patients' serum cytokine levels (Figure 1 and Supplementary Figure 1) revealed that cytokines associated with CRS (IFN-γ, IL-10, IL-6, IL-8) or sepsis (IL-1β)[7] were amongst the highest values in comparison to the full CD22 CART cohort (Figure 1B). These patients had a sustained peak of several cytokines such as IL-8; or a bimodal peak of other cytokines (i.e., IL-6, IL-1β) at onset of CRS and then again with sepsis (Figure 1B).

3 | DISCUSSION

CD22 CART is an emerging treatment for relapsed/refractory B-ALL, particularly for relapse following CD19 targeting.[2,8] We previously demonstrated that carHLH was part of the CD22 CART toxicity profile,[2] but have not evaluated this toxicity in the context of concurrent bacteremia. Thus, we highlight three exceptional cases where SBI in the context of carHLH may have converged to amplify toxicity, illustrating the adverse impact of inflammation and SBI in CART recipients.

Previous series assessing infectious complications of CD19 CART concluded that infection correlates with CRS severity but not cytokine levels.[3,4] Serial cytokine profiling revealed that these three patients had a heightened and prolonged inflammatory state beyond CRS resolution, similar to what has been seen in our experience with carHLH in CD22 CART,[2,6] and their infectious outcomes suggest that this inflammatory milieu could have contributed to risk of developing SBI.

HLH-associated complications, outside of CAR T-cell therapy, have particularly poor outcomes related to heighted inflammatory toxicities on end organs, particularly with concurrent infection. These manifestations may be due to an IL-1β-driven autocrine loop.[9] Furthermore, this autocrine loop may increase IFN-γ and IL-6, which are key inflammatory regulators of CRS, and these cytokines were all elevated in our three patients.[7,9] How the dual inflammatory processes of CRS and carHLH feed into and exacerbate each other requires elucidation, but our three patients raise provocative questions regarding how SBI may further compound this storm: did SBI with CRS onset trigger carHLH (patient 1), or did carHLH predispose patients to worse outcomes with bacteremia (patients 2 and 3)?

For patient 1, it is plausible that the confluence of bacteremia and CRS onset lowered the threshold for carHLH development. While *B. cereus* is notorious for central nervous system manifestations,[10] delayed hemorrhage is unusual; however, the inflammatory state induced by this CART may have contributed to this atypical presentation. In contrast, for patients 2 and 3 persistent cytokine elevation preceded bacteremia, indicating that an inflammatory milieu was present despite improvement in CRS manifestations at the time of infection. This may have contributed to patient 2's rapid decompensation with bacteremia. For patient 3, treatment with eculizumab, anakinra, and steroids for carHLH may have predisposed him to this particularly rare *Weeksella* infection.[11]

Treating patients with sepsis and HLH-like features with the IL-1 receptor antagonist, anakinra, may improve outcomes.[9,12] We have increasingly used anakinra to treat this toxicity[2] and will incorporate it into pre-emptive strategies for carHLH mitigation. Akin to the positive experiences of pre-emptive tocilizumab for prevention of severe CRS,[13] we anticipate that carHLH prevention will reduce the overall inflammatory response and improve outcomes.

In conclusion, for these three patients treated with CD22 CART, SBI coinciding with CRS-associated carHLH may have resulted in poor outcomes. For patients receiving these experimental therapies, clinicians must be vigilant for evaluation of catastrophic bacteremia even after apparent CRS resolution. As novel CART emerge for patients with relapsed

disease, an extensive, consistent approach to the evaluation, treatment, and prevention of infections and mitigation of inflammatory responses will be needed to optimize outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

B-ALL	B-cell acute lymphoblastic leukemia
CAR	chimeric antigen receptor
carHLH	CAR T-cell-associated hemophagocytic lymphohistiocytosis
CRS	cytokine release syndrome
HSCT	hematopoietic stem cell transplant
ICU	intensive care unit
SBI	serious bacterial infection

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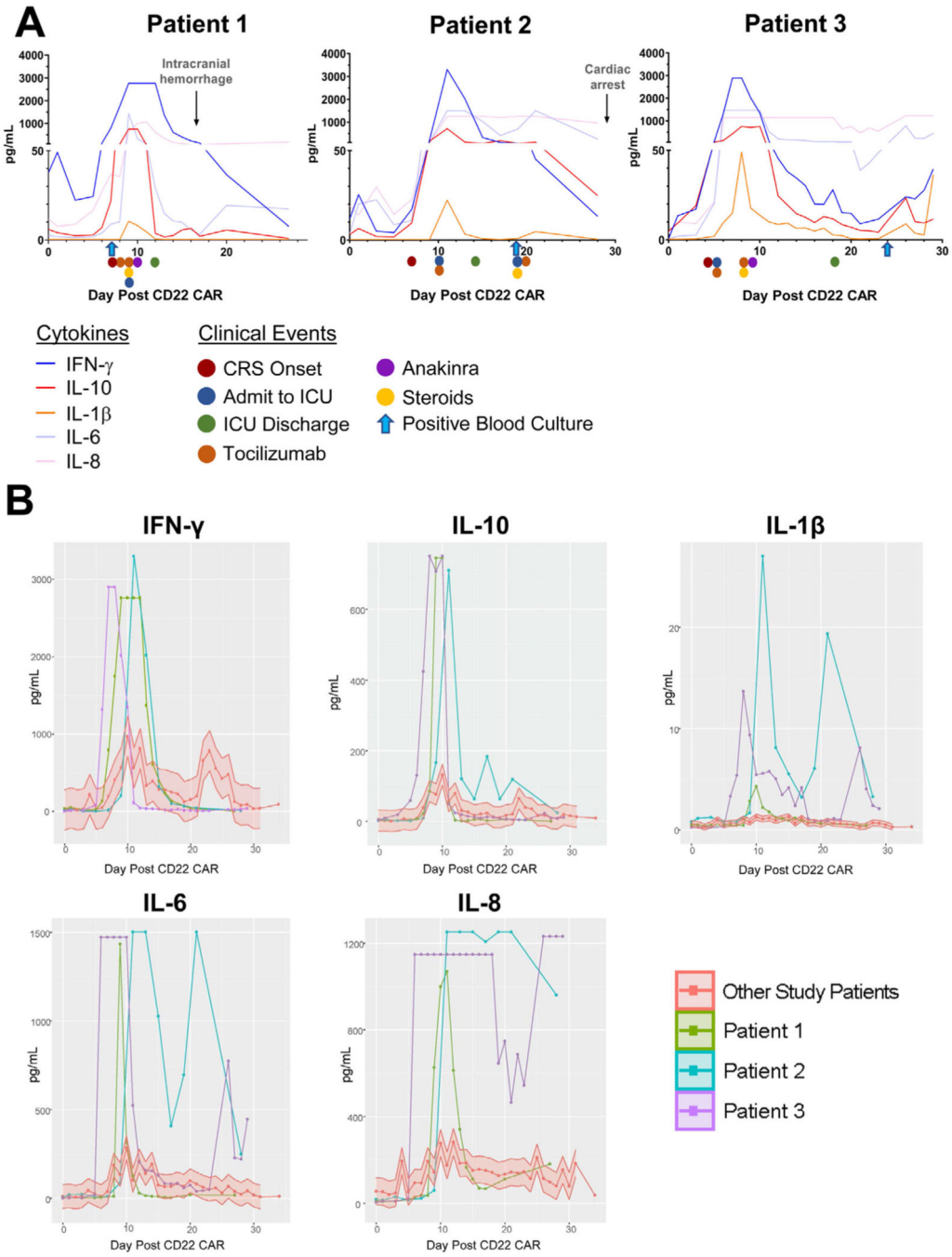

FIGURE 1.

Serum cytokine levels following CD22 chimeric antigen receptor (CAR) T-cell administration (day 0). (A) Clinical time course of three cases with important clinical changes indicated on appropriate days with arrows. (B) Serum cytokine levels for three patients displayed in relation to other patients with CRS in the CD22 CAR clinical trial cohort (red, shaded area representing 70% confidence interval); steroids = steroids initiated; anakinra = anakinra initiated. Serial cytokines were resulted using ELISA on 58 subjects

TABLE 1

Summary of presented case characteristics

		Case 1	Case 2	Case 3
Demographics	Infection onset in relation to CRS (day 1st positive culture)	Concurrent with CRS onset (day +7)	After CRS resolution (day +19)	After CRS resolution (day +24)
	Age/Sex	4-Year-old male	18-Year-old female	26-Year-old male
Relevant past medical history	Prior infectious disease complications	N/A	*Clostridium difficile* typhlitis bacteremia with septic shock	N/A
	Most recent line of therapy	TACL 2017–002[a] (day −44)	Vincristine (day −40)	Vincristine (day −12)
	Relapsed vs. refractory (previous lines of therapy)	Refractory (2)	Relapsed (4)	Relapsed (2)
	Prior CAR T-cell therapy	Yes (CD19)	No	No
	Prior HSCT	No	Yes (MUD)	No
	Steroids in month prior to CAR	No	Yes	Yes
	ANC at lymphodepletion start	370/μl	7420/μl	13,120/μl
	Hg at lymphodepletion start (days since pRBC transfusion)	9.4 g/dl (8)	10.7 g/dl (1)	9.9 g/dl (16)
	Plt at lymphodepletion start (days since platelet transfusion)	264,000/μl (NA)	189,000/μl (17)	228,000/μl (NA)
Infectious disease	Antibiotic at the time of infection[b]	Piperacillin/tazobactam (initiated with febrile neutropenia following CAR T-cell infusion)	Metronidazole (*C. difficile* prophylaxis)	Ciprofloxacin
	ANC at the time of infection	N/Ac	1020/μl	N/A[c]
	Hg at time of infection (days since prior pRBC transfusion)	10.4 g/dl (2)	8.4 g/dl (6)	8.6 g/dl (1)
	Plt at time of infection (days since prior platelet transfusion)	167,000/μl (NA)	53,000/μl (1)	13,000/μl (2)
	Organism(s) by blood Cx	*Bacillus cereus*	*Enterobacter clocae*	*Staphylococcus epidermidis Weeksella virosa*
	Empiric antibiotics (organism sensitive?)	Meropenem + vancomycin (Y)	Ceftazidime + amphotericin (N) → transition to meropenem within 12 hours (Y)	Meropenem + linezolid + amphotericin + posaconazole (Y)
CAR toxicities	CRS, max grade (onset)	2 (day +7)	3 (day +8)	3 (day +4)
	Peak ferritin (ng/ml) (day)	349,750 (day+11)	590,100 (day+17)	271,600 (day+10)
Clinical outcomes	Brief description	CR, but unable to proceed to HSCT; died from PD	Died from complications of sepsis	Achieved CR and proceeded to HSCT[d]

Abbreviations: ANC, absolute neutrophil count; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; Hg, hemoglobin; HSCT, hematopoietic stem cell transplant; plt, platelet count; pRBC, packed red blood cells; MUD: matched unrelated donor

[a]TACL 2017–002 (ixazomib, vincristine, doxorubicin, pegasparaginase, dexamethasone, and intrathecal methotrexate).

[b]All patients on prophylactic acyclovir, micafungin, and pentamidine.

[c]Differential not completed (total white blood cell count too low to complete differential).

[d]Patient ultimately died from complications of treatment of post-HSCT relapse).