blood advances

TO THE EDITOR:

Macrophage activation syndrome-like (MAS-L) manifestations following BCMA-directed CAR T cells in multiple myeloma

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The relative efficacy of autologous hematopoietic cell transplant (auto-HCT) vs chimeric antigen receptor T-cell (CAR-T) therapy in patients with diffuse large B-cell lymphoma (DLBCL) who achieve a partial remission (PR) after salvage chemotherapy is not known. Using the Center for International Blood & Marrow Transplant Research registry database, we identified adult DLBCL patients who received either an auto-HCT (2013-2019) or CAR-T treatment with axicabtagene ciloleucel (2018-2019) while in a PR by computed tomography or positron emission tomography scan. We compared the clinical outcomes between the 2 cohorts using univariable and multivariable regression models after adjustment for relevant baseline and clinical factors. In the univariable analysis, the 2-year progression-free survival (52% vs 42%; $P = .1$) and the rate of 100-day nonrelapse mortality (4% vs 2%; $P = .3$) were not different between the 2 cohorts, but consolidation with auto-HCT was associated with a lower rate of relapse/progression (40% vs 53%; $P = .05$) and a superior overall survival (OS) (69% vs 47%; $P = .004$) at 2 years. In the multivariable regression analysis, treatment with auto-HCT was associated with a significantly lower risk of relapse/progression rate (hazard ratio = 1.49; $P = .01$) and a superior OS (hazard ratio =1.63; $P = .008$). In patients with DLBCL in a PR after salvage therapy, treatment with auto-HCT was associated with a lower incidence of relapse and a superior OS compared with CAR-T. These data support the role of auto-HCT as the standard of care in transplant-eligible patients with relapsed DLBCL in PR after salvage therapy.

B-cell maturation antigen targeted chimeric antigen receptor T-cell therapy (BCMA-CAR-T) for multiple myeloma (MM) treatment is associated with immunotoxicity,[1-6] including cytokine release syndrome (CRS), neurotoxicity, and macrophage activation syndrome (MAS). MAS manifestations overlap considerably with hemophagocytic lymphohistiocytosis (HLH).[7,8] Prior studies have described individual cases of HLH/MAS in CD19-CAR-T recipients.[9,10] In a CD22-CAR-T study, HLH/MAS was reported in 32.7% of participants[11] who exhibited distinct cytokine profiles and were less responsive to tocilizumab compared with patients with CRS alone.[12] Few additional reports exist on HLH/MAS following CAR-T.

Applying traditional HLH criteria to post-CAR-T MAS is challenging because of reliance on cytopenias, which can be confounded by effects of malignancy or lymphodepletion, and fever, which can overlap with CRS.[13,14] A proposed CAR-T-related HLH/MAS definition has relied on end-organ damage,[15] which limits early detection of MAS. Therefore, we sought to develop novel criteria for MAS-like (MAS-L) disease following CAR-T.

We reviewed patients with BCMA-CAR-T MM at University of California San Francisco from 1 November 2017 through 1 May 2020 under an institutional review board-approved study. We defined MAS-L using the following criteria developed via physician consensus: (1) ferritin rise $\geq$100 μg/L/h within a 24-hour period and (2) minimum fibrinogen $<$150 mg/dL or maximum lactate dehydrogenase $>$2 times the upper limit of normal, during hospitalization (minimum 14 days) following CAR-T. We considered multiple macrophage activation markers, including triglycerides, soluble interleukin-2 receptor, and

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The full-text version of this article contains a data supplement.

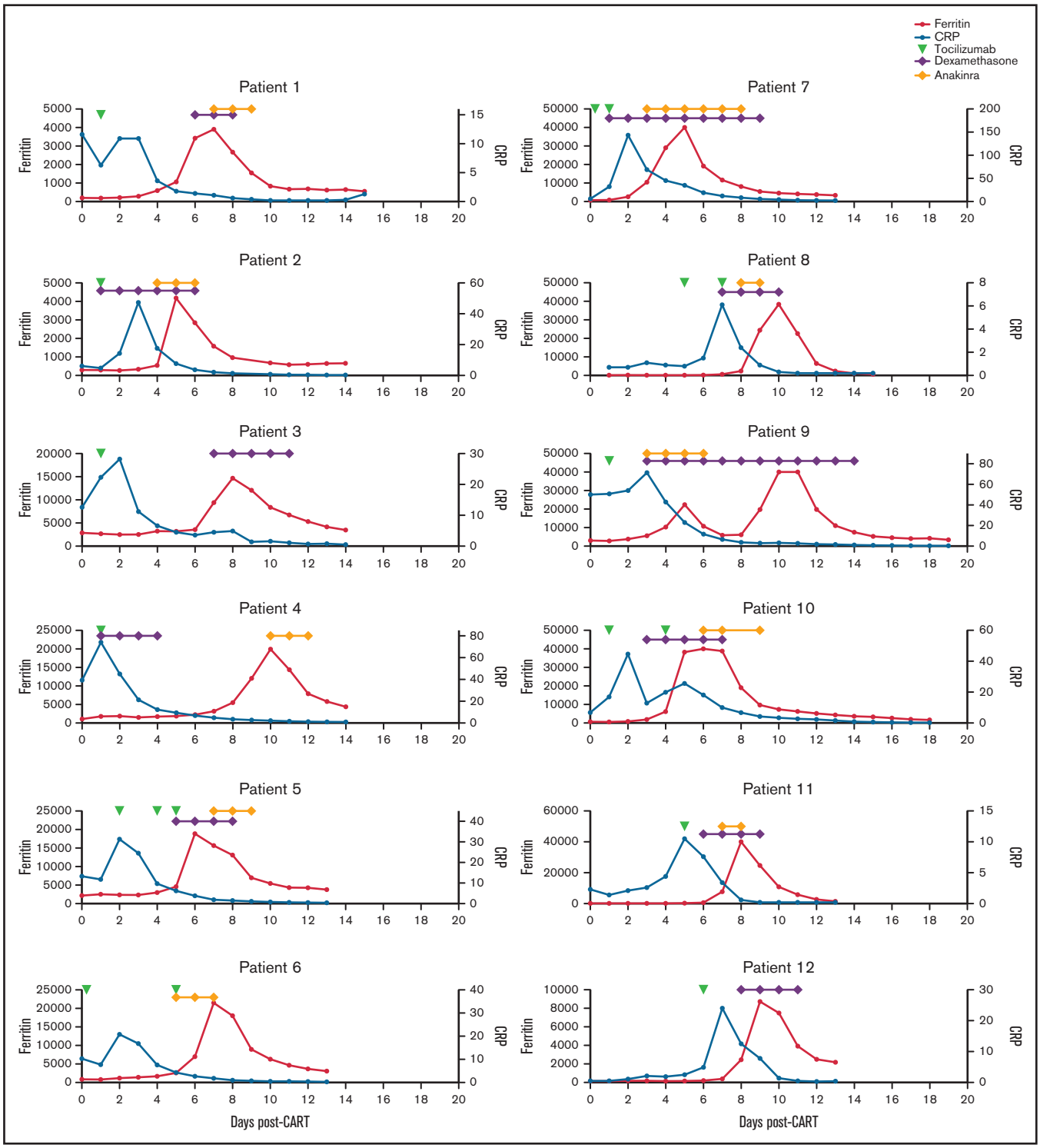

Figure 1. Ferritin, CRP, and treatment over time in patients who developed macrophage activation-like syndrome (MAS-L) following CAR T-cell therapy. Ferritin (red), C-reactive protein (CRP) (blue), and administration of tocilizumab (green triangle), systemic steroids (purple triangle), and/or anakinra (yellow) over time for the 12 patients who developed MAS-L.

natural killer cell activity, but prioritized markers with rapid turnaround time to facilitate early MAS-L identification.

Infection was defined as culture positivity, febrile neutropenia, or clinical suspicion prompting antimicrobial initiation within 30 days before CAR-T. CRS and neurotoxicity were graded per consensus criteria.[16]

Hematologic count recovery was assessed at day 30, 60, and 90 after CAR-T as previously described.[17] Disease response rate was determined per International Myeloma Working Group criteria.[18]

Continuous variables were compared using Wilcoxon rank-sum tests and binary outcomes were compared using Fisher exact tests.

Unadjusted covariate effects were evaluated using logistic regressions. Overall and relapse-free survival (OS, RFS) were estimated using Kaplan-Meier curves and compared using log-rank tests. Analysis was performed using R 3.6.3 (Vienna, Austria.)

Fifty-five patients were treated with BCMA-CAR-T for MM; 12/55 (21.8%) met MAS-L criteria. Median follow-up time was 8.8 months. Compared with historical HLH/MAS definitions, our criteria captured more patients. Using the HLH-2004[13] and CAR-T-related HLH/MAS criteria,[15] 10 (18.2%) and 1 patient (2%) met criteria, respectively. All patients who met historical criteria also met our proposed criteria.

Patients with and without MAS-L had similar disease and treatment characteristics (Table 1). A higher proportion of patients with MAS-L had an infection before CAR-T (75% vs 9.3%, $P < .001$). Median time from infection to CAR-T was 13 days (range, 8-29); there was no difference between MAS-L vs non-MAS-L patients.

Patients who developed MAS-L followed similar disease trajectories. All 12 patients developed CRS, characterized by fever and elevated C-reactive protein (CRP), and received tocilizumab. Following tocilizumab, CRP decreased and patients developed rapid ferritin rise (Figure 1). Although many patients with MAS-L also had CRS with CRP elevation, the subsequent rapid ferritin rise was not observed (supplemental Figure 1).

Per our definition, patients with MAS-L had significantly higher maximum ferritin, higher lactate dehydrogenase, and lower fibrinogen. These patients also had significantly higher D-dimer, aspartate aminotransferase, and lower CRP (Table 1). In univariate logistic regression of patient factors and ferritin, CRP, and D-dimer before CAR T cells, only prior infection predicted MAS-L (hazard ratio, 29.2; 95% confidence interval, 5.54-154; $P < .001$) (supplemental Table 1).

During hospitalization, patients with MAS-L had lower platelet (14 vs 49 $\times$ $10^3/\mu L$, $P = .001$) and white blood cells (0.25 vs 0.50 $\times$ $10^3/\mu L$, $P = .008$) nadirs compared with patients with MAS-L; there was no difference in minimum hemoglobin or absolute neutrophil count. Similarly, at day 30 after CAR-T, fewer patients with MAS-L demonstrated hemoglobin (58.3% vs 92.1%, $P = .01$) and platelet (41.7% vs 78.9%, $P = .03$) recovery. By day 90 after CAR-T, there was no difference in count recovery for any cell line between patients who developed MAS-L vs those who did not (supplemental Figure 2).

All 12 patients with MAS-L received tocilizumab for CRS. Following tocilizumab, CRS resolved in 6 patients and persisted in 6 patients. Systemic steroids were given to treat CRS, neurotoxicity, and neurotoxicity plus MAS-L in 6, 3, and 2 patients, respectively. Of the 12 MAS-L patients, 10 received anakinra for MAS-L; following anakinra, ferritin decreased by >50% within 48 hours in 6 patients and within 72 hours in all 10 patients.

A similar proportion of patients with MAS-L vs without MAS-L developed any CRS (100% vs 84%, $P = .33$) and ≥grade 2 CRS (50% vs 50%, $P = 1$). Of the patients who developed CRS, those with MAS-L had longer CRS duration (5.4 vs 3.7 days, $P = .03$). Neurotoxicity was more common in patients with MAS-L (42% vs 14%, $P = .05$). A greater proportion of patients with MAS-L received tocilizumab (100% vs 70%, $P = .05$), steroids (92% vs 27%, $P < .001$), and anakinra (83% vs 2.3%, $P < .001$). Patients with MAS-L had longer hospitalizations (median, 21 vs 19 days; $P = .009$) and a greater proportion required intensive care unit-level care (27% vs 2%, $P = .02$).

Overall response rate was 100% vs 86% for patients with MAS-L vs those without MAS-L, respectively ($P = .05$). OS and RFS between the 2 groups were similar. One-year OS was 65.2% vs 90.6% ($P = .16$) and RFS was 35.4% vs 54.7% ($P = .37$) for patients with MAS-L vs those without MAS-L, respectively (supplemental Figure 3).

We developed simplified MAS-L criteria following BCMA-CAR-T and identified a 21.8% MAS-L incidence among 55 patients. Although patients with MAS-L required heightened monitoring, this immunotoxicity may be mitigated with steroids, anakinra, and supportive care. We also demonstrate that patients with MAS-L had similar improved disease outcomes compared with patients without MAS-L, a pattern also observed in CRS.[19,20] Our findings suggest that once initial immunotoxicity is treated, patients with MAS-L have excellent outcomes. Although ideal treatment of MAS-L remains unknown, our practice is to initiate anakinra for patients meeting MAS-L criteria.

Interestingly, we found that patients with MAS-L had significantly lower CRP compared with patients without MAS-L. This differs from CRS, where CRP correlates positively with CRS following CD19[9,21,22] and BCMA-CAR-T.[3-5] In addition, we found antecedent infections were strongly associated with MAS-L. Outside of CAR-T, infections are an established trigger for MAS/HLH[23]; the role in other post-CAR-T immunotoxicities is unknown.

The pathophysiology of MAS-L remains poorly understood. Broadly, immunotoxicity results from the inflammatory cytokine milieu produced by CAR-T and bystander macrophage activation.[24,25] It is unclear whether MAS-L exists along the continuum of CRS or represents a distinct immunologic process. Our findings, including the inverse correlation of CRP and MAS-L development, suggest the pathophysiology of MAS-L may be distinct.

Our MAS-L criteria were based on physician consensus, a methodology with limitations as all possible laboratory values and thresholds were not analyzed given risk of overfitting in a small cohort. Our criteria require validation, which will be key in identifying additional predictors of MAS-L, including the role of CAR-T features, such as antigen binding site or costimulatory molecules. Furthermore, whether our definition applies to other CAR-T targets remains unknown. Future directions include prospective correlation with cytokines, which may identify biomarkers predictive of MAS-L development and provide insight into the underlying pathophysiology.

By defining MAS-L, future studies can better identify potential candidates for interventions. Animal models have demonstrated macrophage secretion of interleukin-1-β contributes to post-CAR-T immunotoxicity, and anakinra may mitigate this effect.[24,26] Currently, there are ongoing trials of anakinra following CAR-T (NCT04205838, NCT04148430). Stratifying outcomes by patients meeting MAS-L criteria could provide additional insight into the benefit of this and other interventions.

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Table 1. Patient, laboratory, and clinical characteristics for patients who developed MAS-L vs patients who did not

	N or median (% or range)		
	MAS-L (n = 12)	No MAS-L (n = 43)	*P*
Patient characteristics			
Median age, y	64.1 (43.3-74.2)	62.4 (33.7-77.3)	.87
Female	3 (25%)	25 (58.1%)	.06
Type of MM			
IgA-K	2 (16.7%)	8 (18.6%)	.94
IgA-L	0 (0%)	2 (4.7%)	
IgD-K	0 (0%)	1 (2.3%)	
IgG-K	6 (66.7%)	21 (48.9%)	
IgG-L	1 (8.3%)	7 (16.3%)	
LC-K	1 (8.3%)	4 (9.3%)	
High-risk cytogenetics*	8 (66.7%)	30 (69.8%)	1.0
Baseline % BM plasma cells	40 (0- 95)	25 (0-100)	.62
Baseline FLC, K/L ratio	176 (1.5-1311)	203.2 (2.2-11 054)	.62
Baseline M-protein, g/dL	2.6 (0-4.8)	1.4 (0-6.2)	.22
Median prior lines of treatment	6 (4-13)	5 (1-12)	.09
Use of bridging chemotherapy	8 (66.7%)	27 (62.8%)	1.0
Median infused CART dose ($\times 10^6$ cells)	300 (47.3-600)	300 (41.8-600)	.05
Infection before CART therapy†	9 (75%)	4 (9.3%)	<.001
Time from infection to CART, d	18 (8-29)	10.5 (9-13)	.08
Laboratory values			
Maximum ferritin, µg/L	20 707 (3903-40 000)	573 (32-40 000)	<.001
Maximum rate of ferritin rise, µg/L/h	591 (100-1279)	7.5 (0.13-1151)	<.001
Maximum triglycerides, mg/dL	475 (67-1095)	301 (124-784)	.18
Minimum fibrinogen, mg/dL	183 (91-426)	221 (117-426)	<.001
Maximum AST, U/L	153 (32-1806)	48 (18-225)	<.001
Maximum ALT, U/L	76 (29-1076)	50 (14-341)	.08
Maximum CRP, mg/L	30 (6.1-143)	60 (0.8-333)	.03
Maximum D-dimer, ng/mL	14 000 (4754-14 000)	3010 (415-14 000)	<.001
Maximum LDH, U/L	964 (260-2700)	202 (98-994)	<.001
Clinical course			
Any CRS	12 (100%)	36 (84%)	.33
Maximum CRS grade			.99
Grade 1	6 (50%)	18 (50%)	
Grade 2	6 (50%)	18 (50%)	
CRS duration, d	5 (2-9)	3 (1-9)	.03
Any neurotoxicity	5 (42%)	6 (14%)	.05
Maximum neurotoxicity grade			.74
1	2 (40%)	4 (67%)	
2	1 (20%)	1 (17%)	
≥3	2 (40%)	1 (17%)	
Received tocilizumab	12 (100%)	30 (70%)	.05
Received systemic steroids	11 (92%)	11 (26%)	<.001
Received anakinra	10 (83%)	1 (2.3%)	<.001
Hospitalized days following CAR T	20.5 (14-45)	19 (13-30)	.009
Required ICU-level care	3 (25%)	1 (2.3%)	.03

ALT, alanine transaminase; AST, aspartate aminotransferase; BM, bone marrow; FLC, free light chain; Ig, immunoglobulin; LDH, lactate dehydrogenase; WBC, white blood cell.
*High-risk cytogenetics defined as the presence of del 17p, t(4;14), or t(14,16) on pre-CAR T bone marrow biopsy.
†Infection defined as: culture positivity, febrile neutropenia, or clinical suspicion such that new antimicrobials were initiated in 30 d before receiving CAR T.

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