SUPPLEMENTAL TABLES

HLH-2004 Diagnostic Criteria[1]	2016 MAS Classification Criteria[5]
A. Molecular diagnosis consistent with HLH: pathologic mutations of PRF1, UNC13D, Munc18-2, Rab27a, STX11, SH2D1A, or BIRC4 OR B. Five of the 8 criteria listed below are fulfilled: 1. Fever ≥ 38.5°C 2. Splenomegaly 3. Cytopenias (affecting at least 2/3 lineages) Hgb <9 g/dL (in infants <4 weeks: Hgb <10 g/dL) PLT <100 K/mL ANC <1.0 K/mL 4. Hypertriglyceridemia (fasting, >265 mg/dL) and/or hypofibrinogenemia (<150 mg/dL) 5. Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver 6. Low or absent NK-cell activity 7. Ferritin >500 ng/mL 8. Elevated sCD25 ≥2400 U/mL	A. Fever in sJIA patient AND B. Ferritin >684 ng/mL AND C. Two of the 4 criteria listed below are fulfilled: 1. PLT ≤181 K/mL 2. AST >48 u/L 3. TG >156 mg/dL 4. Fibrinogen ≤360 mg/dL

Supplemental Table 1: Diagnostic criteria for patient inclusion. *Hgb*- hemoglobin, *PLT*- platelet count, *ANC*- absolute neutrophil count, *NK-cell*- natural killing cell, *sCD25*- soluble CD25, *sJIA*- systemic juvenile idiopathic arthritis, *AST*- aspartate aminotransferase, *TG*- triglycerides

Patient	Reintensification/Salvage Therapy	Day Started	Outcome
CCHMC 1	-Dexamethasone increased to 10 mg/m^2	84	Died post-BMT
CCHMC 4	-Dexamethasone increased to 10 mg/m^2*	35	Died post-BMT
CCHMC 9	-Emapalumab -Alemtuzumab	7 62	Survived post-BMT
CCHMC 10	-Alefacept	63	Died pre-BMT
CCHMC 13	-Anakinra -Canakinumab	52 59	Survived without BMT
CCHMC 17	-Alemtuzumab -Dexamethasone increased to 10 mg/m^2 -Tocilizumab	66 66 91	Died pre-BMT
CCHMC 18	-Dexamethasone increased to 10 mg/m^2	100	Died post-BMT
CCHMC 21	-Dexamethasone increased to 10 mg/m^2	91	Survived with BMT
CCHMC 26	-Dexamethasone increased to 10 mg/m^{2}2 -Alemtuzumab	17 17	Died post-BMT
CCHMC 27	-Dexamethasone increased to 10 mg/m^2	59	Survived with BMT
CCHMC 31	-Alemtuzumab	28	Died pre-BMT

	-Dexamethasone increased to 10 mg/m^2 -Bortezomib -Anakinra	56 59 84	
CCHMC 38	-Dexamethasone increased to 10 mg/m^2	59	Survived with BMT
CCHMC 43	-Biweekly etoposide -Dexamethasone increased to 10 mg/m^2	93 93	Survived with BMT
CCHMC 47	-Biweekly etoposide -Dexamethasone increased to 10 mg/m^2 -Alemtuzumab	28 56 56	Died pre-BMT
CCHMC 49	-Biweekly etoposide -Dexamethasone increased to 10 mg/m^2	28 28	Survived with BMT
CCHMC 50	-Dexamethasone increased to 10 mg/m^2 -Biweekly etoposide	49 52	Survived with BMT
CCHMC 55	-Dexamethasone increased to 10 mg/m^2 -Biweekly etoposide	24 38	Survived with BMT
CCHMC 64	-Canakinumab	7	Survived without BMT
CCHMC 67	-Dexamethasone increased to 10 mg/m^2	21	Survived without BMT
CCHMC 70	-Anakinra -Dexamethasone increased to 10 mg/m^2 -Ruxolitinib	19 28 31	Died pre-BMT
CCHMC 74	-Dexamethasone increased to 10 mg/m^2 -Tocilizumab -Biweekly etoposide -Emapalumab	73 78 87 100	Survived with BMT
CCHMC 75	-Biweekly etoposide -Dexamethasone increased to 10 mg/m^2 -Emapalumab	73 77 84	Survived with BMT
CCHMC 76	-Dexamethasone increased to 10 mg/m^2 -Biweekly etoposide -Emapalumab	24 25 31	Survived without BMT
CCHMC 77	-Dexamethasone increased to 10 mg/m^2 -Emapalumab	42 50	Survived with BMT
CCHMC 78	-Dexamethasone increased to 10 mg/m^2 -Emapalumab	14 80	Survived with BMT
CCHMC 80	-Dexamethasone increased to 10 mg/m^2 -Emapalumab -Biweekly etoposide	31 45 66	Survived with BMT
ACH 1	-Dexamethasone increased to 10 mg/m^2	21	Died pre-BMT

Supplemental Table 2: Patients receiving salvage therapy and associated outcomes. Salvage therapy included increasing dexamethasone to 10 mg/m^2 after 2 weeks of therapy (n=23), resuming biweekly etoposide after 2 weeks of therapy (n=9), or the use of alemtuzumab (n=6), tocilizumab (n=2), anakinra (n=3), canakinumab (n=2), emapalumab (n=7), alefacept (n=1), ruxolitinib (n=1), and bortezomib (n=1).

Patient	Age (mo's)	Underlying Trigger	Notable early organ dysfunction or TMA Features	Day 7 Unfavorable Markers/ Markers Available (%)	Cause of Death (category)	Cause of Death (specifics)	Day of Death
CCHMC 69	0	Unknown	GI bleed, liver failure, circulatory collapse	4/5 (80%)	Active HLH with MOF and infection	Serratia bacteremia with hepatic encephalopathy, and pulmonary hemorrhage	7
CCHMC 15	0	LCH	Renal failure, liver failure, circulatory collapse	4/5 (80%)	Active HLH with MOF	Hepatic, renal, and respiratory failure	9
SCMCI 13	15	EBV + MAS + genetic	None	1/5 (20%)	Active HLH with MOF and EBV	EBV viremia with severe CNS involvement	56
CCHMC 47	15	EBV	None	0/0 (0%)	Active HLH with MOF	Severe GI bleed and hypovolemic shock	61
CCHMC 10	81	Unknown	Liver failure, circulatory collapse, TMA specific features (S, H, P)	4/5 (80%)	Infection	Necrotizing fasciitis with septic shock	95
CCHMC 31	282	EBV	None	5/5 (100%)	Active HLH with MOF and EBV	Refractory HLH EBV viremia, TMA like vasculopathy with GI bleeding, renal failure, encephalopathy, and respiratory failure	95
CCHMC 32	24	Genetic + Ehrlichia chaffeenis	Circulatory collapse, TMA specific features (H)	3/5 (60%)	Active HLH with MOF and infection	Invasive CNS/systemic fungal disease	105
CCHMC 17	6	Unknown	Renal failure, circulatory collapse, TMA specific features (H)	3/3 (100%)	Active HLH with MOF and infection	Disseminated candidiasis with encephalopathy, renal failure, and severe GI bleed	117
CCHMC 70	179	EBV	GI bleed, renal failure, liver failure, TMA specific features (H, P)	2/3 (67%)	Active HLH with MOF + EBV	Chronic active EBV driven HLH & severe GI bleeding	140
ACH 5	191	MAS + RMSF	None	1/3 (33%)	Active HLH with MOF and infection	Disseminated angioinvasive aspergillosis, severe heart dysfunction & pericardial effusion	146
CCHMC 36	1	Genetic + klebsiella UTI	None	0/1 (0%)	Infection	Klebsiella pneumoniae with septic shock	149
ACH 1	11	Genetic	Renal failure, circulatory collapse, TMA specific features (S, H)	3/5 (60%)	Active HLH with MOF	Renal failure & severe heart dysfunction/cardiomegaly	375

Supplemental Table 3: Characteristics of patients with pre-BMT mortality. GI bleeding (within 2 weeks of treatment initiation), need for renal replacement therapy (within 1 month of treatment initiation), liver failure or hepatic encephalopathy (within 2 weeks of treatment initiation), and circulatory collapse requiring vasopressors (within 2 weeks of treatment initiation) were noted based on manual review of clinical notes. TMA features were noted if patients met ≥4 features per the

modified Jodele criteria[27] within 2 weeks of treatment initiation using HLH-2004 thresholds for anemia and thrombocytopenia. Presence of schistocytes (S), hypertension (H), and proteinuria (P) were noted as TMA specific features given overlap of anemia, thrombocytopenia, and elevated LDH with HLH. No patients had an sC5b9 level obtained during the designated timeframe. *CCHMC*- Cincinnati Children's Hospital Medical Center; *SCMCI*- Schneider Children's Medical Center of Israel; *ACH*- Arkansas Children's Hospital; *LCH*- Langerhans Cell Histiocytosis; *EBV*- Epstein bar virus; *MAS*- macrophage activation syndrome; *UTI*- urinary tract infection; *MOF*- multi-organ failure; *CNS*- central nervous system; *GI*- gastrointestinal; *S*- schistocytes present; *H*- hypertension, *P*- proteinuria.

Univariate						Multivariate	
Day	Covariate	Threshold	Percent Died	HR	p-value	HR (95% CI)	p-value
Baseline	AMC	> 0.2	0.18	1.33	0.04	ns	ns
	Bilirubin	**> 1.88**	**0.22**	**1.12**	**0.00**	**1.12 (1.04-1.2)**	**0.00**
	BUN	**> 18.94**	**0.28**	**1.05**	**0.00**	**1.05 (1.01-1.09)**	**0.01**
Day 7	Fibrinogen	> 163.97	0.17	1.01	0.03	ns	ns
	Bilirubin	> 1.4	0.21	1.14	0.00	ns	ns
	BUN	**> 20.0**	**0.30**	**1.04**	**0.00**	**1.03 (1.0-1.06)**	**0.04**
	LDH	> 533.15	0.20	1.00	0.04	ns	ns
	sCD25 Improvement	**> -48.25**	**0.22**	**1.02**	**0.00**	**1.02 (1.0-1.03)**	**0.03**
	Ferritin Improvement	> -47.34	0.16	1.00	0.01	ns	ns
Day 14	Platelet Count	< 144.25	0.17	0.99	0.04	ns	ns
	Ferritin	> 2947.69	0.16	1.00	0.03	ns	ns
	BUN	**> 20.0**	**0.26**	**1.06**	**0.02**	**1.06 (1.01-1.11)**	**0.02**
	sCD25 Improvement	> -64.41	0.18	1.00	0.05	ns	ns
Day 21	Platelet Count	< 170.0	0.18	0.99	0.04	ns	ns
	BUN	**> 17.47**	**0.21**	**1.04**	**0.02**	**1.04 (1.0-1.07)**	**0.04**
	LDH Improvement	> -67.71	0.18	1.01	0.01	ns	ns

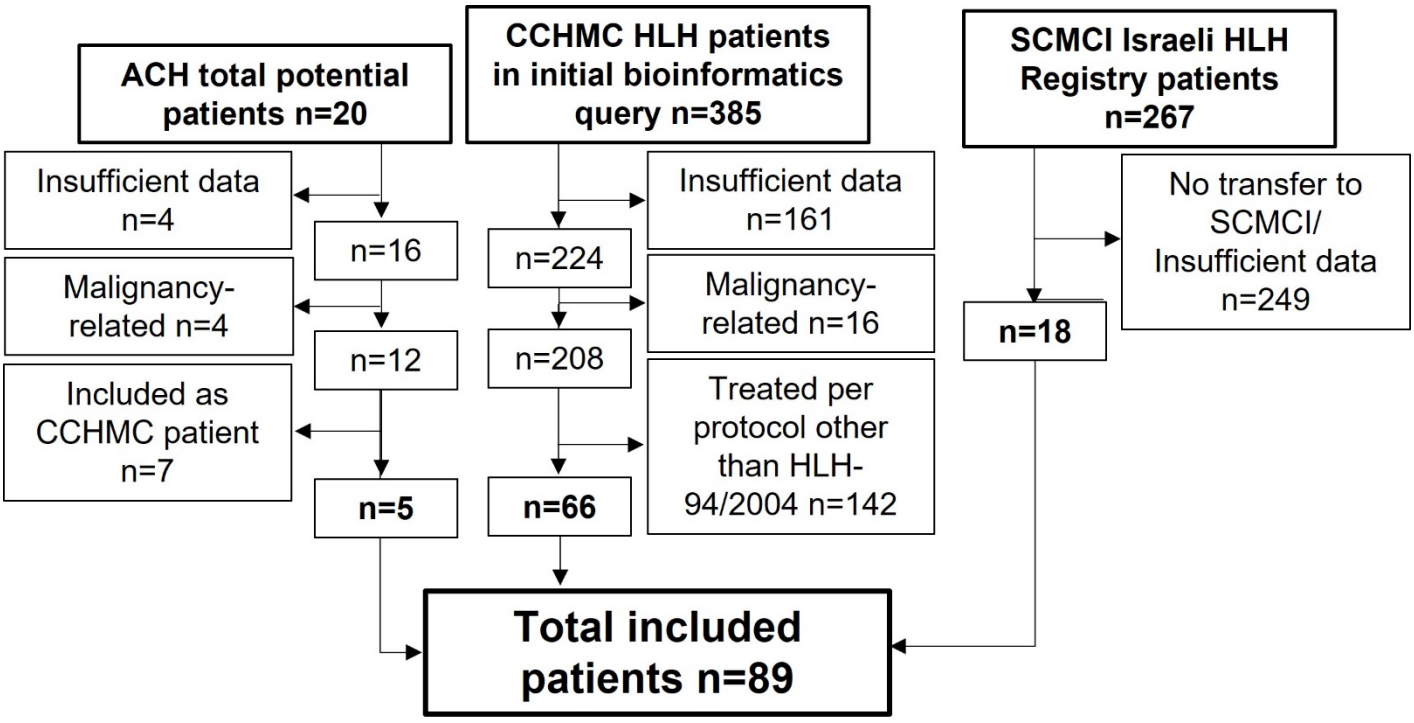
Supplemental Table 4: Cox Proportional Hazard Model assessment of prognostic markers. Missing data was imputed for each timepoint using multivariable regression. This table shows the results of running univariate (UV) and multivariate (MV) Cox model. All predictors with $p<0.05$ from UV analysis are shown. Multivariate Cox was run on the significant predictors detected from UV study. Significant MV results ($p<0.05$) are bolded, and numeric p-values are shown in the respective MV columns. *AMC* – absolute monocyte count; *BUN* – blood urea nitrogen; *LDH* – lactate dehydrogenase; *sCD25* – soluble interleukin-2 receptor; *improvement* = (difference between baseline and day X values)/baseline value; *ns* – non-significant

Lab Parameter	Timepoint	Optimized Threshold	Sensitivity (%)	Specificity (%)	AUC
sCD25	Peak pre-treatment	< 3179 U/mL	36	84	0.56
	Day 7	**> 17,130 U/mL**	**50**	**96**	**0.71**
	Day 14	**> 11,588 U/mL**	**60**	**96**	**0.74**
	Day 21	> 1151 U/mL	100	41	0.67
sCD25 Improvement	**Day 7**	**<27%**	**83**	**96**	**0.92**
	Day 14	**<49%**	**80**	**91**	**0.82**
	Day 21	**<78%**	**80**	**70**	**0.71**
Ferritin	Peak pre-treatment	> 9795 ng/dL	64	57	0.51
	Day 7	> 2384 ng/dL	71	60	0.59
	Day 14	**> 2214 ng/dL**	**50**	**85**	**0.70**
	Day 21	**> 1578 ng/dL**	**88**	**74**	**0.80**
Ferritin Improvement	Day 7	<-3%	43	87	0.59
	Day 14	**<53%**	**63**	**90**	**0.79**
	Day 21	**<83%**	**100**	**58**	**0.78**
Platelet Count	Peak pre-treatment	< 35.5 K/mL	75	59	0.63
	Day 7	**< 24 K/mL**	**60**	**96**	**0.78**
	Day 14	**< 26 K/mL**	**44**	**94**	**0.70**
	Day 21	**< 45 K/mL**	**63**	**92**	**0.80**
ANC Improvement	Day 7	< 24%	88	52	0.69
	Day 14	**< -58%**	**86**	**64**	**0.72**
	Day 21	**< -32%**	**100**	**53**	**0.75**
ALC	Peak pre-treatment	> 7.132 K/mL	33	99	0.53
	Day 7	**< 0.35 K/mL**	**60**	**90**	**0.73**
	Day 14	< 1.3 K/mL	86	48	0.66
	Day 21	**< 1.78 K/mL**	**100**	**51**	**0.78**
BUN	**Peak pre-treatment**	**> 14.5 mg/dL**	**100**	**56**	**0.81**
	Day 7	**> 19.5 mg/dL**	**100**	**71**	**0.86**
	Day 14	> 19.5 mg/dL	67	74	0.69
	Day 21	**> 11.5 mg/dL**	**100**	**44**	**0.77**
LDH Improvement	Day 7	>60%	100	48	0.53
	Day 14	**>69%**	**100**	**52**	**0.70**
	Day 21	**>79%**	**100**	**65**	**0.83**

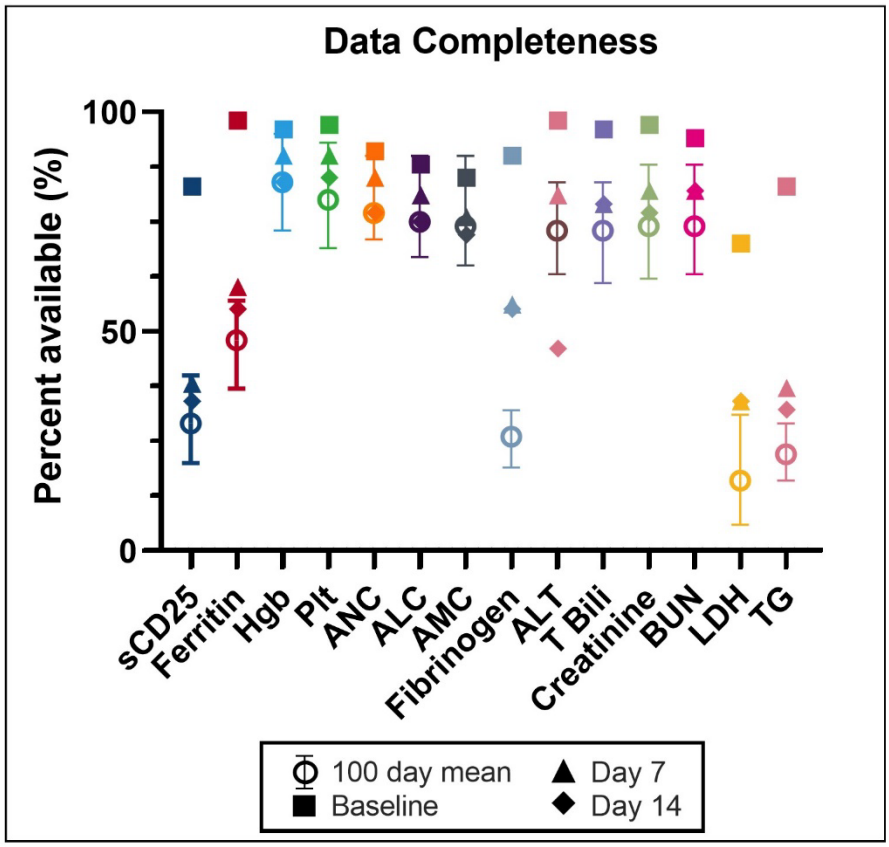
Supplemental Table 5: Significant day 7 and day 14 prognostic indicators and their ROC-derived thresholds at baseline, day 7, 14, and 21. Significance was defined by an area under the

curve (AUC) ≥0.7 of the ROC and are shown in bold. Optimized thresholds were derived from the highest Youden-index point (sensitivity + specificity-1). Non-significant markers (absolute values and their associated improvement from baseline unless listed above) included hemoglobin, absolute neutrophil count (ANC), absolute monocyte count (AMC), fibrinogen, triglycerides, total bilirubin, alanine transferase (ALT), normalized creatinine, and lactate dehydrogenase (LDH). *sCD25*- soluble CD25; *improvement* = (difference between baseline and day 7 values)/baseline value; *ANC*- absolute neutrophil count; *ALC*- absolute lymphocyte count; *BUN*- blood urea nitrogen; *LDH*- lactate dehydrogenase; *AUC*- area under the curve

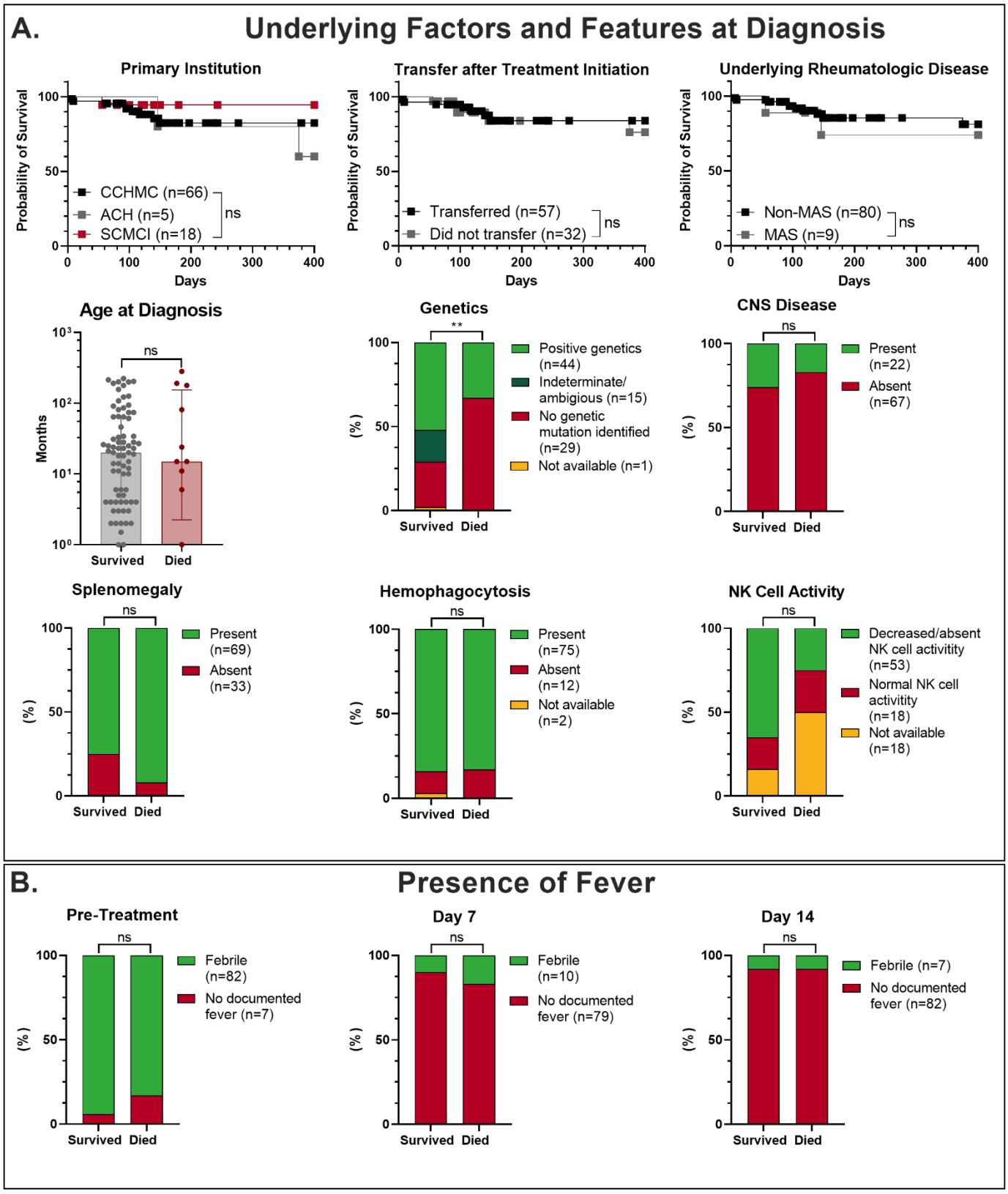
SUPPLEMENTAL FIGURES



Supplemental Figure 1: Flow-chart of patient inclusion in this study. Eighty-nine patients from three institutions met inclusion criteria. Potential patients were identified by either bioinformatic query using ICD-9 or ICD-10 diagnostic codes for HLH or MAS (288.4, D76.1) (CCHMC), Israeli HLH Registry data (SCMCI), or manual medical records search (ACH). All patients met diagnostic criteria per HLH-2004 or 2016 MAS Classification Criteria (Supplemental Table 1). Patients treated with protocols other than HLH-94 or HLH-2004, HLH secondary to malignancy, or with insufficient data were excluded. *CCHMC*- Cincinnati Children's Hospital Medical Center, *ACH*- Arkansas Children's Hospital, *SCMCI*- Schneider Children's Medical Center of Israel, *HLH*- hemophagocytic lymphohistiocytosis; *MAS*- macrophage activation syndrome

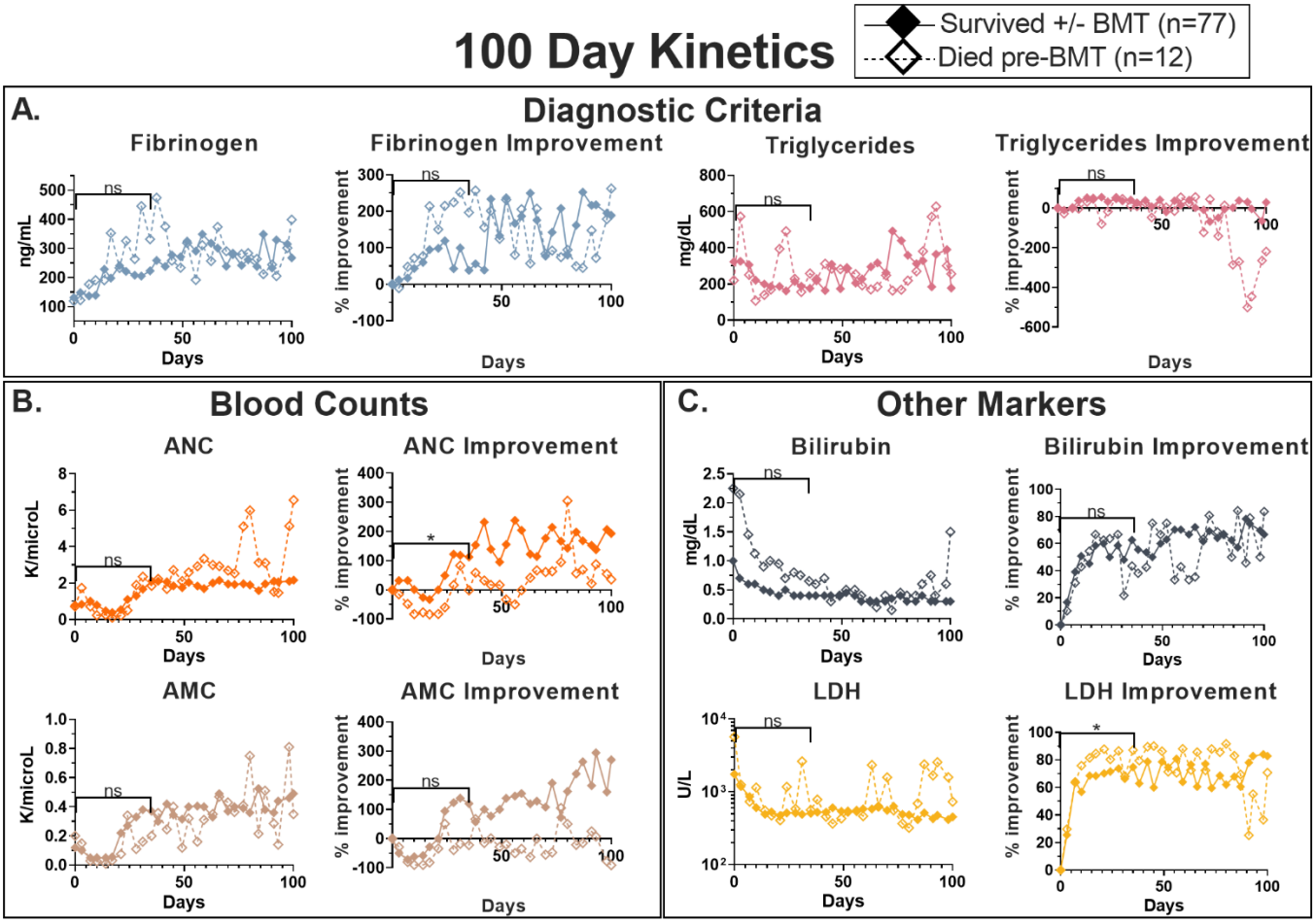


Supplemental Figure 2. Percent of labs available by individual parameters. Mean percent completeness of data over the 100-day study period plotted with 95% confidence intervals and mean percent of baseline, day 7, and day 14 data are shown. Patients were excluded at the time of death or the day BMT preparative regimen began if earlier than day 100 and percent completeness of data was calculated based on the remaining number of patients at each specified day. Baseline values were obtained up to 7 days before etoposide treatment began. Labs within 3 days of the specified timepoint were eligible for inclusion, only 1 value per patient per day was included. s*CD25*- soluble CD25; *Hgb*- hemoglobin; *PLT*- platelet count; *ANC*- absolute neutrophil count; *ALC*- absolute lymphocyte count; *AMC*- absolute monocyte count; *ALT*- alanine transaminase; *T Bili*- total bilirubin; *BUN*- blood urea nitrogen; *LDH*- lactate dehydrogenase; *TG*- triglycerides; *SD*- standard deviation.

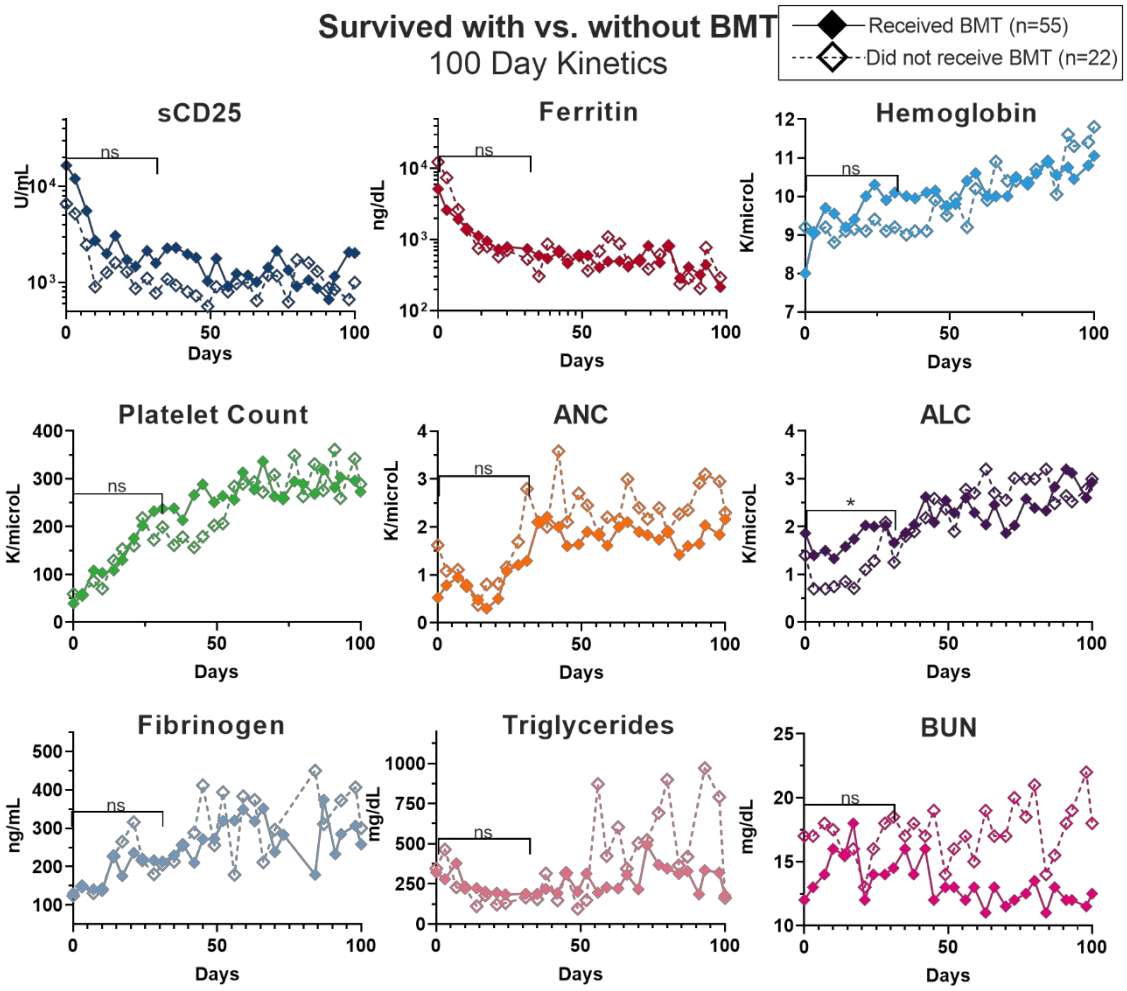


Supplemental Figure 3: Demographic, baseline, and other disease features do not correlate with pre-BMT mortality. (A) Study location, patients who transferred after starting HLH-directed therapy, and patients with an underlying rheumatologic disease or macrophage activation syndrome (MAS) displayed using Kaplan-Meier survival estimates and age at diagnosis along with other underlying factors, displayed as percentages. An underlying genetic mutation was associated with pre-BMT mortality (p-value 0.008) but all other parameters were not. (B) Fever, displayed as

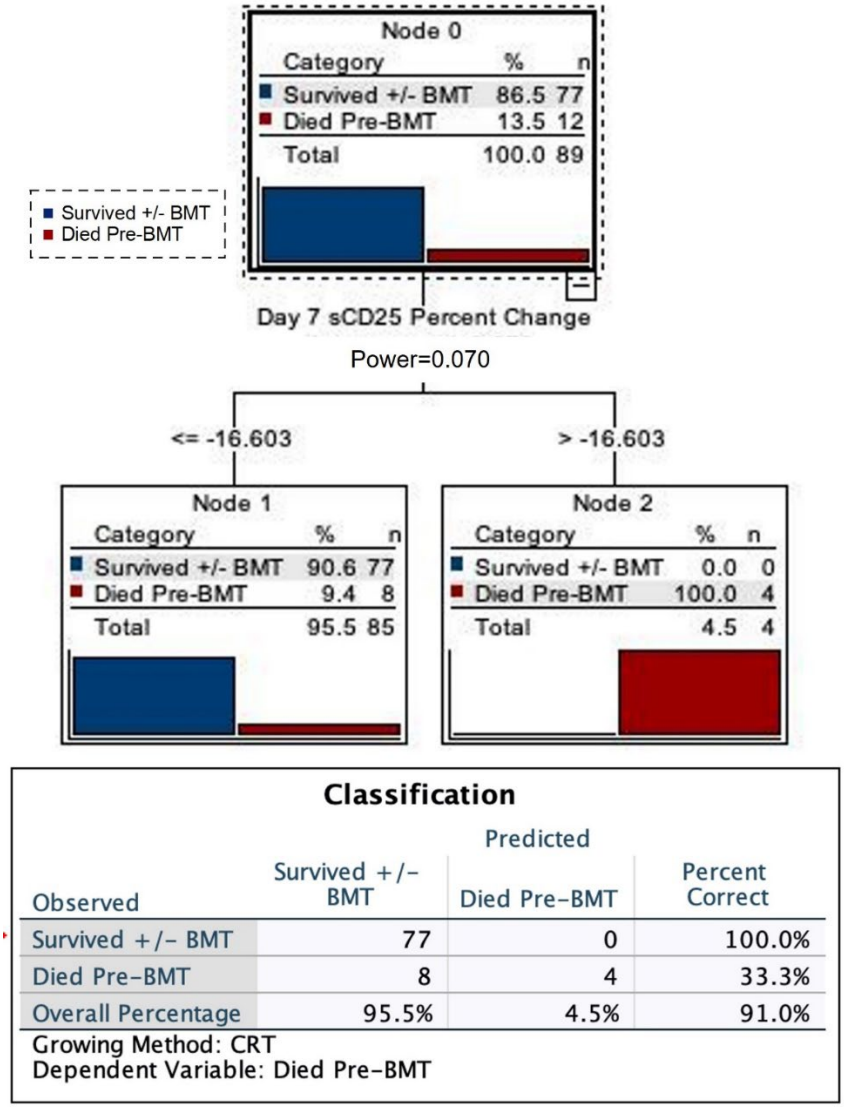
percentages, at early treatment points. The presence of fever was not significant until day 28 (p-value <0.05). Categorical variables were analyzed using Fisher's exact t-test, and continuous variables using the Mann-Whitney *U* test. Indeterminant/ambiguous genetic mutations were considered positive genetics when analyzed. ** p-value <0.01; *ns* – not significant *MAS*- macrophage activation syndrome; *CCHMC*- Cincinnati Children's Hospital Medical Center, *ACH*- Arkansas Children's Hospital, *SCMCI*- Schneider Children's Medical Center of Israel; *ns*- not significant.



Supplemental Figure 4. Response markers without significance and their associated improvement from baseline values. Non-significant **(**p-value >0.05) parameters and their improvement from baseline are plotted as median values during the first 100 days of therapy. Significance was determined using the Mann Whitney test for the first thirty-one days of treatment and data of the first 100 days of treatment are plotted. *improvement* = (difference between baseline and day X)/baseline value; *ANC*- absolute neutrophil count; *AMC* - absolute monocyte count; *LDH*- lactate dehydrogenase



Supplemental Figure 5: Survival without BMT was not correlated with response data. Kinetic data of HLH-2004 criteria and non-diagnostic unfavorable day 7 markers in patients who received BMT versus patients who survived without BMT, plotted as median values during the first 100 days of therapy. Significance was determined using the Mann Whitney test for the first thirty-one days of treatment. *sCD25*- soluble CD25; *ANC*- absolute neutrophil count; *ALC*- absolute lymphocyte count; *BUN*- blood urea nitrogen; * p-value <0.05; *ns*- not significant

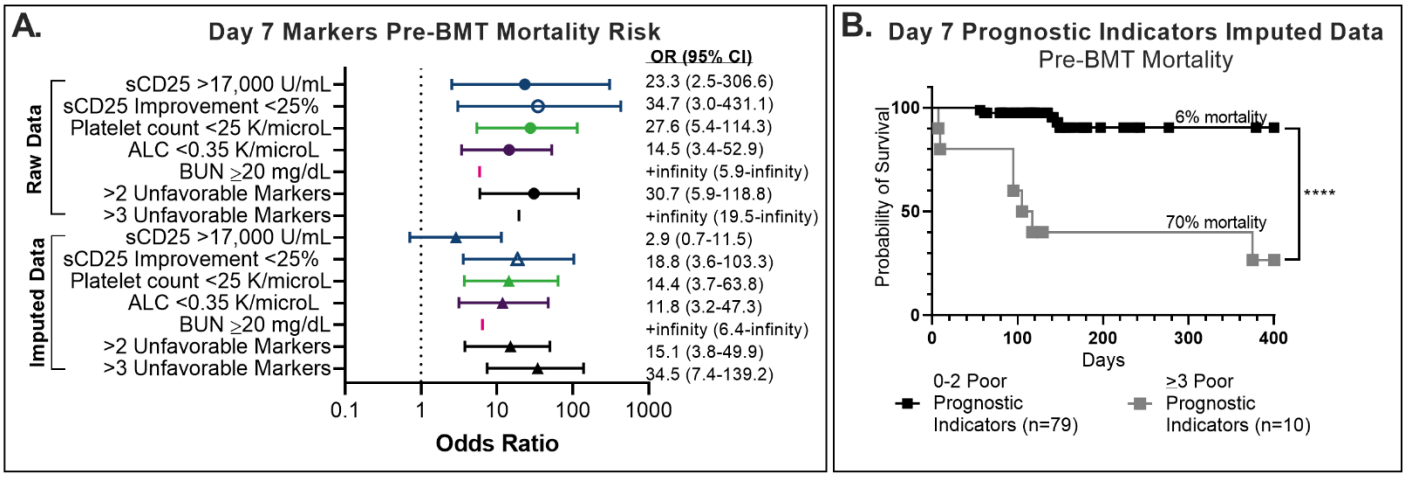


Classification

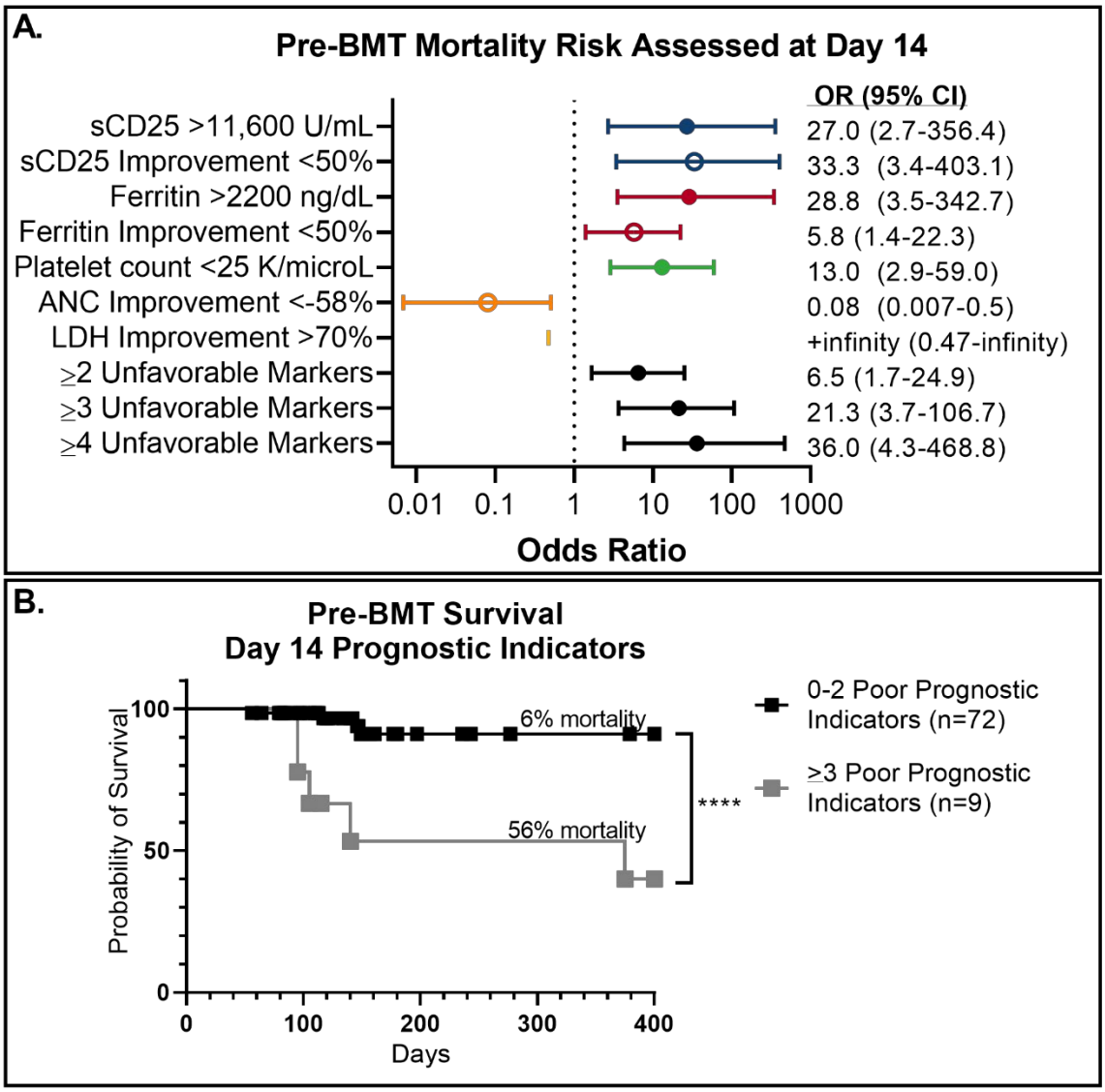
Observed	Predicted: Survived +/- BMT	Predicted: Died Pre-BMT	Percent Correct
Survived +/- BMT	77	0	100.0%
Died Pre-BMT	8	4	33.3%
Overall Percentage	95.5%	4.5%	91.0%

Growing Method: CRT
Dependent Variable: Died Pre-BMT

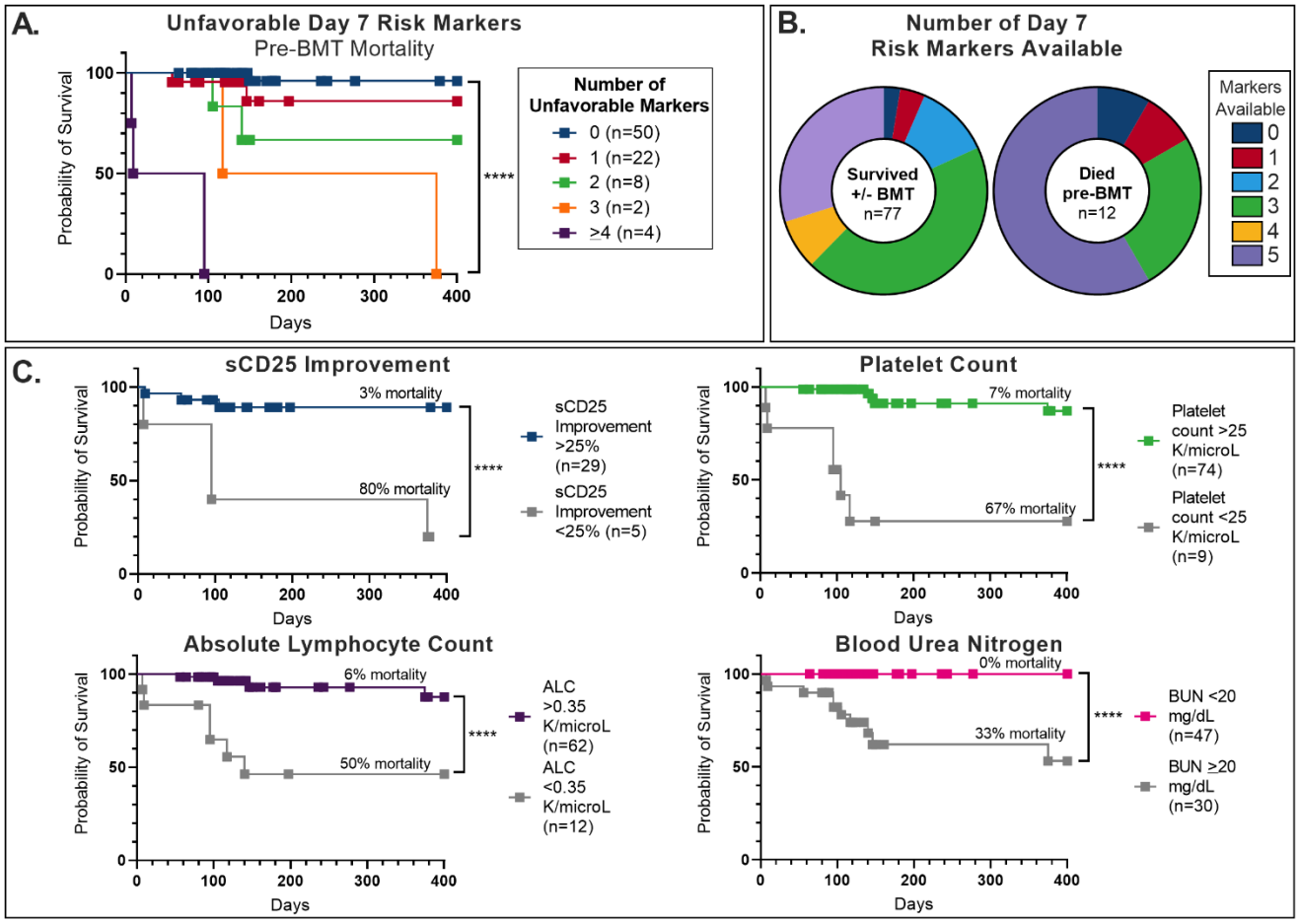
Supplemental Figure 6. Improvement in sCD25 by day 7 is the strongest single predictor of pre-BMT mortality. Classification and regression tree (CART) analysis, a predictive algorithm used in machine learning, was performed based on pre-BMT mortality as the dependent variable. Independent variables included baseline (pre-treatment), day 7, and day 14 parameters. CART growth limits were defined with the Gini method with minimum parent node cases of 3, minimum child node cases of 2, with cross-validation using 10 sample folds. All laboratory parameters (assessed at baseline and weekly thereafter) and their associated improvement from baseline values were included in this analysis. Imputed data was used to account for missing lab values. Of note, this result is identical to Figure 3, where no imputed data are used. Non-laboratory parameters included persistent fever at day 7 and 14, splenomegaly at diagnosis, presence of hemophagocytosis, and NK cell activity. CART analysis using raw data only is shown in Figure 3. *sCD25*- soluble CD25, *improvement from baseline* = (difference between baseline and day X)/baseline value; *BUN*- blood urea nitrogen



Supplemental Figure 7: Imputed data supports the predictive power of day 7 poor prognostic indicators identified in the primary analysis. (A) Forest plots of the odds ratios (OR) and 95% confidence interval (CI) of pre-BMT mortality prediction by day 7 markers using receiver operating curve (ROC) derived cutoffs (Figure 4). Use of imputed data where an observation is missing had similar pre-BMT mortality risk to raw data. An OR could not be estimated for BUN or ≥3 unfavorable markers as all patients who died prior to BMT had a BUN above the specified cutoff and no survivors had ≥3 poor prognostic markers. Imputed OR for absolute sCD25 was significant if using a threshold derived from a ROC utilizing imputed data (threshold 18,500 U/mL, OR 6.1, 95% CI 1.3-25.0), all other ROC derived thresholds were similar between raw and imputed data. (B) Kaplan-Meier pre-BMT survival estimates based on markers obtained around day 7 of therapy with imputed data showed similar outcomes to the raw data analysis (Figure 6A). *sCD25*- soluble CD25; *improvement* = (difference between baseline and day X)/baseline value; *ALC*- absolute lymphocyte count; *BUN*- blood urea nitrogen **** p-value <0.0001



Supplemental Figure 8: The presence of day 14 poor prognostic indicators identified in the primary analysis is associated with pre-BMT mortality. (A) Forest plots of the odds ratios (OR) and 95% confidence interval (CI) of pre-BMT mortality prediction by day 14 markers using receiver operating curve (ROC) derived cutoffs (Figure 4, Supplemental Table 5). Thresholds were rounded for more practical clinical use. An OR could not be estimated for LDH improvement as all patients who died prior to BMT with baseline and day 14 LDH data had an LDH improvement valuable above the specified cutoff. (B) Kaplan-Meier pre-BMT survival estimates based on the presence of 3 or more poor prognostic markers obtained around day 14 of therapy was associated with an increased risk of early mortality. Of note, 2 patients died prior to day 14 and were not included in this analysis. sCD25- soluble CD25; improvement = (difference between baseline and day X)/baseline value; ANC absolute neutrophil count; LDH- lactate dehydrogenase**** p-value <0.0001



Supplemental Figure 9: Day 7 poor prognostic indicators are predictive of pre-BMT mortality. (A) Kaplan-Meier pre-BMT survival with patients classified by the total number of day 7 unfavorable markers present. (B) Pie chart showing the frequency of available day 7 prognostic indicators between groups. Survival was not associated with the number of markers available (p-value 0.1551). (C) Pre-BMT survival estimates based on individual ROC-derived thresholds of day 7 prognostic indicators. Absolute sCD25 was predictive of pre-BMT mortality (absolute sCD25 <17,000 U/mL mortality rate 11% (n=4/35) vs. absolute sCD25 >17,000 U/mL mortality rate 75% (n=3/4), p-value 0.0017), figure not shown due to small sample size in the unfavorable group. *sCD25*- soluble CD25; *improvement* = (difference between baseline and day X)/baseline value; **** p-value <0.0001