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Full Length Article

Pediatric

Clinical Features, Treatment, and Outcome of Pediatric Steroid Refractory Acute Graft-Versus-Host Disease: A Multicenter Study



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Steroid-refractory acute graft-versus-host disease (SR-aGvHD) is a severe complication in pediatric allogeneic hematopoietic stem cell transplantation (HSCT). We aimed to assess clinical course and outcomes of pediatric SR-aGvHD. We performed a retrospective nationwide multicenter cohort study in the Netherlands. All patients aged 0 to 18 years who underwent transplantation between 2010 and 2020 with SR-aGvHD were included. For each patient, weekly clinical aGvHD grade and stage, immunosuppressive treatment and clinical outcomes were collected. The primary study endpoint was the clinical course of SR-aGvHD over time. As a secondary outcome, factors influencing overall survival and SR-aGvHD remission were identified using a multistate Cox model. 20% of transplanted children developed grade II-IV aGvHD, of which 51% (n = 81) was SR-aGvHD. In these patients, second-line therapy was started at a median of 8 days after initial aGvHD-diagnosis. Forty-nine percent of SR-aGvHD patients received 3 or more lines of therapy. One year after start of second-line therapy, 34 patients (42%) were alive and in remission of aGvHD, 14 patients (17%) had persistent GvHD, and 33 patients (41%) had died. SR-aGvHD remission rate was lower in cord blood graft recipients than in bone marrow (BM) or peripheral blood stem cell (PBSC) recipients (hazard ratio [HR] = 0.51, 0.27-0.94, $P = .031$). Older age was associated with higher mortality (HR = 2.62, 1.04-6.60, $P = .04$, fourth quartile [aged 13.9-17.9] versus first quartile [aged 0.175-3.01]). In BM/PBSC recipients older age was also associated with lower remission rates (HR = 0.9, 0.83-0.96, $P = .004$). Underlying diagnosis, donor matching or choice of second-line therapy were not associated with outcome. Respiratory insufficiency caused by pulmonary GvHD was a prominent cause of death (26% of deceased). Our study demonstrates that SR-aGvHD confers a high mortality risk in pediatric HSCT. Older age and use of CB grafts are associated with an unfavorable outcome. Multicenter studies investigating novel treatment strategies to prevent pediatric SR-aGvHD and inclusion of children in ongoing trials, together with timely initiation of second-line interventions are pivotal to further reduce GvHD-related mortality.

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Acute graft-versus-host disease (aGvHD) is a major complication in pediatric patients after allogeneic hematopoietic stem cell transplantation (HSCT). More than half of the patients that develop aGvHD \geq grade II do not respond to first-

line systemic corticosteroid treatment (steroid refractory [SR]) [1-3], resulting in considerable morbidity and mortality [4,5].

There is a broad choice of therapies for SR-aGvHD including mycophenolate mofetil (MMF) [6,7], TNF- α inhibitors [8-13], Janus kinase/signal transducer and activator of transcription inhibitors [14-20], $\alpha 4\beta 7$ -integrin inhibitors [21-24], T-cell inhibitors [25-27], anti-CD52 antibodies [28,29], CD25 inhibitors [30-32], IL-6 inhibitors [33-35], and mesenchymal stromal cells (MSCs) [36]. There are no prospective studies that evaluate which second-line treatment is most effective in children with aGvHD refractory to first-line high-dose corticosteroids. As a result, there is a lack of standardization in the

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management of pediatric SR-aGvHD, leading to a high variability in second-line treatment worldwide [37]. To establish more effective treatment strategies, it is important to meticulously evaluate current practices and outcomes over time.

In this retrospective multicenter cohort study, we evaluated the efficacy and safety of second-line treatment in children with grade II–IV SR-aGvHD after HSCT over the last 10 years in the Netherlands. In addition to endpoints such as aGvHD remission and survival, we report on aGvHD grade and staging in response to second-line therapy over time. This provides a detailed insight into the clinical course of SR-aGvHD in pediatric patients. Finally, we identified predictive factors for survival and SR-aGvHD remission using a multistate Cox model.

METHODS

We performed a retrospective nationwide cohort study in the 2 centers for pediatric HSCT in the Netherlands: the Willem-Alexander Children's Hospital/Leiden University Medical Center and the Princess Máxima Center for Pediatric Oncology/ Wilhelmina Children's Hospital, UMC Utrecht. All patients aged 0 to 18 years who suffered from grade II–IV SR-aGvHD between January 2010 and July 2020 were included in this study. There were no exclusion criteria.

SR disease was defined as progression of aGvHD within 3 to 5 days of first-line therapy initiation with ≥ 2 mg/kg/d of prednisone or failure to improve within 5 to 7 days after treatment initiation or incomplete response after more than 28 days of immunosuppressive treatment including steroids, according to the European Group for Blood and Marrow Transplantation–National Institutes of Health–Center for International Blood and Marrow Transplant Research (CIBMTR) Task Force position statement [38]. Data were collected by retrospective medical chart review. Onset of SR disease was determined by the treating physician's diagnosis and/or recorded disease progression of each patient, as well as medication prescription data. Substitution of the initial GvHD prophylaxis for a similar agent (cyclosporine, sirolimus, tacrolimus, MMF, or basiliximab) was not considered initiation of a new line of therapy. Only when one of these agents was added on top of already existing GvHD prophylaxis, it was considered a new line of therapy. Different therapeutic agents were categorized as combination therapy if they were started within 3 days of each other.

For each patient, weekly clinical aGvHD grade and stage were collected from the start of aGvHD until the occurrence of persistent remission, onset of chronic GVHD, or death. Both centers used the same institutional guidelines for aGvHD diagnosis and therapy. Grade and stage of aGvHD were copied from the medical chart if available, and otherwise retrospectively determined using the modified Glucksberg criteria (as used by the CIBMTR) based on percentage of affected skin reported at least weekly after physical examination by a supervising physician during grand rounds, stool volumes per m^2 body surface area recorded in daily digital nurse charts or bilirubin value available in digital laboratory records [39]. In case of missing data, the most recent known grade was imputed. Persistent remission was defined as grade 0 aGvHD (stage 0 in all organs) without relapse of aGvHD symptoms after tapering of immunosuppressive therapy [38]. Presence of chronic GVHD was determined based on the treating physician's diagnosis and medical chart review based on 2005 NIH consensus criteria [40] and categorized as either quiescent or progressive [38].

Matching of the stem cell donor (peripheral blood [PBSC], bone marrow [BM], or cord blood [CB]) was characterized with high-resolution HLA typing according to 10 alleles of five loci (HLA-A, B, C, DRB1, DQB1) where available. For CB transplantations without complete high-resolution-typing ($N = 5$), matching was based on serological typing for HLA-A and -B and high-resolution-typing for HLA-DRB1 (6 alleles). Donor types included matched related (10/10 HLA matching), matched unrelated (10/10 or 6/6 HLA matching) and mismatched unrelated (less than 10/10 or 6/6 HLA matching).

Furthermore, data on all lines of immunosuppressive treatment, readmissions, intensive care unit (ICU) admissions, viral reactivations, infections (excluding line associated coagulase-negative staphylococci infections), serious adverse events and complications during the first year since onset of SR disease were collected. GvHD prophylaxis regimens for BM and PBSC transplantations consisted generally of a calcineurin inhibitor (cyclosporine/tacrolimus) with or without methotrexate or MMF. In the CB transplantation setting, a combination of a calcineurin inhibitor and prednisone 1 mg/kg was used, with the addition of MMF in case of an higher anticipated aGvHD risk. Serotherapy included treatment with either anti-thymocyte globulin or alemtuzumab. Infection prophylaxes were given per protocol and included HSV prophylaxis with valacyclovir until engraftment, VZV prophylaxis until 6 to 12 months after transplantation, gut decontamination antibiotics until engraftment, and

in case of active gut aGvHD, oral yeast prophylaxis until engraftment and systemic anti-fungals for high-risk or aGvHD patients receiving more than 0.5 mg/kg steroids in combination with other lines of immune suppression. Viral reactivations were monitored by weekly viral load evaluation. Diagnosis of lower respiratory tract infections was either culture proven or presumed based on imaging. Bronchiolitis obliterans syndrome (BOS) was defined as diagnosed by typical HRCT changes, such as bronchial wall thickening, air trapping, or bronchiectasis, in the absence of signs of infection and, whenever pulmonary function testing could be done, abnormal pulmonary function test results (i.e., decrease in forced expiratory volume in 1 second $>20\%$ or in forced expiratory volume in 1 second/forced vital capacity $<70\%$) [40,41]. Medication-related complications were defined as toxicity with direct treatment consequences, either by the ceasing or switching of the medication in question or the requirement of additional therapy.

Statistical analysis was performed in R version 4.0.3 [42]. For all analyses, time was measured from onset of SR-aGvHD (i.e., start of second-line therapy). The primary study endpoint was clinical course of SR-aGvHD over time, represented by the proportions of patients with active aGvHD symptoms, patients with remission of aGvHD, patients with chronic GVHD, and deceased patients during the first year since start of second-line therapy [43].

As secondary study endpoints we aimed to investigate complication and infection rates and to identify factors influencing overall survival and SR-aGvHD remission using a multi-state Cox-regression model from the *mstate* package [44–46]. Three different states were included in this model: active GvHD, remission from aGvHD and death. Because predictive factors for death and aGvHD remission are the main interest of this study, chronic GvHD was not included as a separate state in our model. Patients who developed chronic GvHD while suffering from aGvHD remained in the "GvHD state," whereas patients who developed chronic GVHD after they had achieved aGvHD remission remained in the "remission state" for this analysis. Transition probabilities from one state to another were tested in a univariate analysis using the following covariates: age, gender, diagnosis, conditioning, stem cell source, donor type, time between aGvHD diagnosis and start of second-line therapy, type of second-line therapy, and year of transplantation, categorized as before or after January 1, 2015. Second-line therapy options were categorized as MSCs, TNF- α inhibition (infliximab or etanercept), a combination of treatment modalities ("combination therapy") or other. The statistical methodology is explained in more detail in Supplementary Material 1.

Informed consent for the use of patients' data for research purposes was collected from all included patients before HSCT. The Medical Research Ethics Committee Leiden The Hague Delft waived the need for additional specific informed consent in both centers for the analysis of the data used in the current study.

RESULTS

A total of 786 pediatric allogeneic HSCTs were performed in Leiden and Utrecht between January 1, 2010, and July 1, 2020. During this time, 158 patients (20%) suffered from grade II–IV aGvHD, which occurred after a median of 34.5 days. Of these 158 patients, 81 patients (51%) required second-line therapy because of absent or insufficient response to first-line treatment with corticosteroids. The current study focuses on these 81 SR-aGvHD patients, who all had a follow-up time until death or at least 1 year after start of second-line therapy. Patient, transplant, and aGvHD characteristics are summarized in Table 1. Initial diagnosis of aGvHD occurred for 73 SR-aGvHD patients (90%) within the first 100 days after HSCT, whereas 8 SR-aGvHD patients (10%) developed aGvHD after more than 100 days (late onset), either in the context of immunosuppression tapering ($N = 5$) or after a stem cell boost ($N = 3$). The majority of patients had grade III as their maximal aGvHD grade (56%), and the gut was the most affected organ (77% at least stage 2 gut involvement). Weekly aGvHD grade and stage were available for 1162 of 1213 (96%) evaluated weeks.

In 34 patients (42%) second-line treatment was started within 1 week after aGvHD onset, in 36 patients (44%) after 8 to 28 days and in 11 patients (14%) after more than 28 days. MSC therapy was the most frequently used second-line therapy option ($N = 38$, 47%), followed by infliximab ($N = 24$, 30%). In 12 patients (15%) second-line therapy consisted of a combination of 2 or 3 of the following agents: infliximab,

Table 1
Patient, Transplant, and GvHD Characteristics

Variable	N = 81
Age at HSCT (median, IQR)	8.9 (3.0–13.9)
Sex	
Male	47 (58%)
Female	34 (42%)
Diagnosis	
Bone marrow failure	10 (12%)
Hematologic malignancy	40 (49%)
Hemoglobinopathy	4 (4.9%)
Inborn errors of immunity	16 (20%)
Inborn errors of metabolism	11 (14%)
Donor	
BM/PBSC donors	
Matched related	13 (30%)
Matched unrelated	19 (44%)
Mismatched unrelated	11 (26%)
CB donors	
Matched related	0 (0%)
Matched unrelated	2 (5.3%)
Mismatched unrelated	36 (95%)
Graft source	
Bone marrow	36 (44%)
Cord blood	36 (44%)
Cord blood + bone marrow	1 (1.2%)
Double cord blood	1 (1.2%)
Peripheral blood	7 (8.6%)
Conditioning	
Myeloablative chemotherapy	64 (79%)
Busulfan–fludarabine based	43 (53%)
Treosulfan–fludarabine based	18 (22%)
Other	3 (3.7%)
Myeloablative total body irradiation	6 (7.4%)
Reduced-intensity conditioning	11 (14%)
Busulfan–fludarabine based	4 (4.9%)
Other	7 (8.6%)
Serotherapy	
ATG (Genzyme)	56 (69%)
Alemtuzumab	6 (7.4%)
None	19 (23%)
Transplant number	
First transplant	72 (89%)
Second transplant	8 (9.9%)
Third transplant	1 (1.2%)
Stem cell boost, Yes (at d 67, d 91, and d 114 after HSCT)	3 (3.7%)
GvHD prophylaxis	
Calcineurin inhibitor (cyclosporine/tacrolimus)	7 (8.6%)
Calcineurin inhibitor + MMF/MTX	33 (41%)
Calcineurin inhibitor + prednisone	33 (41%)
Calcineurin inhibitor + prednisone + MMF	6 (7.4%)
MMF + MTX/prednisone	2 (2.5%)
Days between HSCT and aGvHD grade \geq II, median (IQR)	35 (24–55)
Days between aGvHD grade \geq II and start second-line therapy, median (IQR)	8 (5–18)
aGvHD histologically confirmed	
Yes	74 (91%)
No	7 (8.6%)
Maximum overall aGvHD grade	
II	13 (16%)
III	45 (56%)
IV	23 (28%)
Maximum skin aGvHD stage	
0–1	27 (33%)
2–4	54 (67%)
Maximum gut aGvHD stage	
0–1	19 (23%)
2–4	62 (77%)
Maximum liver aGvHD stage	
0–1	56 (69%)
2–4	25 (31%)

vedolizumab, basiliximab, MSC, etanercept, tacrolimus and/or ruxolitinib (Supplementary Table S1). 40 patients (49%) required an additional line of therapy (third or more) after second-line therapy (Supplementary Figure S1).

One year after start of second-line therapy, 34 patients (42%) were alive and in remission of SR-aGvHD and 33 patients (41%) had died. Fourteen patients (17%) were still experiencing persistent GvHD symptoms 1 year after start of second-line therapy (Table 2, Figure 1). Most patients achieved SR-aGvHD remission after more than 28 days since start of a line of therapy: 73.5% of the patients only receiving second-line therapy (25/35), 71% of the patients receiving a third-line (5/7), and all patients receiving a fourth-line or more (5/5). Respiratory insufficiency (infectious and non-infectious) and multiorgan failure from GvHD and treatment related toxicity (including sepsis) were the most frequent causes of death (35/38 total deaths) (Table 3). Noninfectious respiratory insufficiency because of BOS, idiopathic pneumonia syndrome (IPS) or suspected pulmonary GvHD contributed to 10 of 38 deaths (26%).

Using a multistate model, we performed a covariate analysis for mortality and SR-aGvHD remission rates (Figure 2, Table 4). In this covariate analysis, 2 patients who received a double CB graft and a composed graft (CB with haploidentical BM) were excluded. We found that older age was associated with higher mortality: children aged 13.9 to 17.9 (fourth quartile) had a significantly higher hazard of death compared to children aged 0.175 to 3.01 (first quartile) (hazard ratio [HR] = 2.62, 1.04 to 6.60, $P = .04$). CB graft recipients had a significantly lower chance to reach SR-aGvHD remission than BM or PBSC graft recipients (HR = 0.51, 0.27–0.94, $P = .031$) (Table 3). When modeling the interaction of graft source and age, the association between CB grafts and a lower chance of SR-aGvHD remission was even stronger (HR = 0.18, 0.06–0.51, $P = .001$). Older age was only associated with lower remission rates in children receiving BM/PBSC grafts (HR = 0.9, 0.83–0.96, $P = .004$). A graphical representation of the effects of graft source and age on clinical course is shown in Figure 3.

Over the years, preferred second-line treatment in our centers shifted from MSC monotherapy to a combination of multiple treatment modalities. There was no significant difference in outcome (survival or remission rates) between patients transplanted before or after 2015. None of the second-line treatments were significantly superior (Table 4).

Infections within the first year since start of second-line therapy were frequent, occurring in 65 of 81 patients (80%), including bacterial (54% of patients), fungal (26% of patients) and viral infections (19% of patients) and viral reactivations (52% of patients) (Supplementary Table S2). The timing of infections and viral reactivations relative to the start of second-line therapy, and the GvHD activity at that time can be found in Supplementary Table S3. BOS (14/81), thrombotic microangiopathy (TMA) (13/81), cytopenia (12/81), and renal insufficiency (10/81) were the most common noninfectious complications. In total 38 patients (47%) were admitted to the ICU at least once within the first year since start of second-line therapy. Thirty-seven patients (46%) experienced medication toxicity or an adverse drug reaction. In patients who were still alive after 1 year, the median duration of the hospital admission in which SR-aGvHD was diagnosed was 29 days. The disease burden in this population was high: of the 48 patients that were still alive 1 year after start of second-line therapy, 41 patients (85%) had experienced one or more of the following: ICU admission, readmission, chronic GvHD/BOS, relapse of underlying disease, Retransplantation, or secondary graft failure.

DISCUSSION

SR-aGvHD in pediatric HSCT patients is a severe complication with a poor prognosis. Similar to other studies [16,47,48],

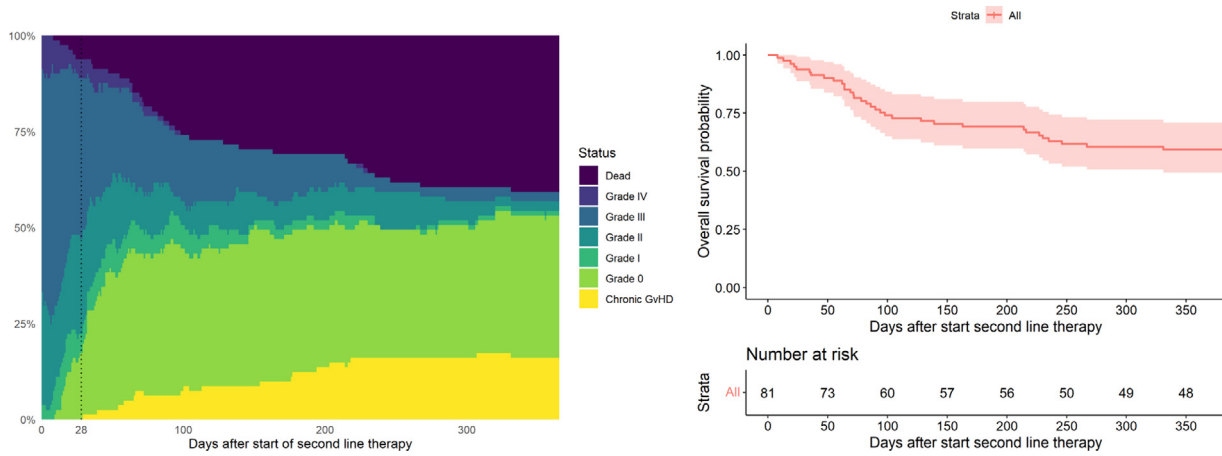


Figure 1. Clinical course since start of second-line therapy and traditional Kaplan-Meier survival plot. The proportions of patients with active GvHD symptoms, patients with remission of GvHD, patients with chronic GvHD and deceased patients during the first year since start of second-line therapy.

Table 2
Main Outcomes

Variable	N = 81
Death	
Overall	38 (47%)
28 days	5 (6.2%)
100 days	21 (26%)
1 year	33 (41%)
2 years	36 (44%)
Remission of aGvHD (alive and in remission)	
Overall (cumulative)	46 (57%)
28 days	9 (11%)
100 days	25 (31%)
1 year	34 (42%)
2 years	38 (47%)
Chronic GvHD (alive with cGvHD)	
Overall (cumulative)	22 (27%)
Progressive	10 (12%)
Quiescent	12 (15%)
28 days	0 (0%)
100 days	7 (8.6%)
1 year	13 (16%)
2 years	15 (19%)
Relapse of underlying disease	6 (7.4%)
Retransplantation	6 (7.4%)
ICU admission within first year of start second-line therapy	38 (47%)
Readmission within first year of start second-line therapy	42 (56%)

Table 3
Causes of Death

	N = 38
Multi-organ failure (GvHD and treatment related toxicity)	14
Multi-organ failure (GvHD and treatment related toxicity) and respiratory insufficiency (infectious)	1
Multi-organ failure (GvHD and treatment related toxicity) and respiratory insufficiency (suspected pulmonary GvHD)	2
Sepsis	3
Relapse of underlying disease	2
Bronchiolitis obliterans syndrome with multiple infections	3
Respiratory insufficiency (infectious)	7
Aspergillus infection	4
Other	3
Respiratory insufficiency (non-infectious)	5
Bronchiolitis obliterans syndrome	2
Idiopathic pneumonia syndrome	2
Suspected pulmonary GvHD	1
Secondary malignancy (squamous cell carcinoma)	1

47% of the 81 children with SR-aGvHD died in our study. There is a lack of evidence from prospective trials to help guide clinicians in determining which second-line treatment is most effective and safe in children with SR-aGvHD. Conducting clinical trials in this patient group is challenging. First of all, the number of patients with this condition is relatively low, hampering required statistical power to meet envisioned endpoints. Second, because of the severity of the disease and poor prognosis, multiple lines of treatment are often given concomitantly [37], possibly leading to exclusion of the trial initially enrolled in based on formulated exclusion criteria.

Because of the lack of prospective trials, it is of vital importance to carefully review current practice. Although, in general, survival rates are well reported, outcomes such as aGvHD remission are often only reported at day 28 after initiation of the investigative agent [19,49–53], which was established as the best endpoint for treatment trials [54]. In this study we provide a detailed description of the clinical course of a relatively large group of pediatric SR-aGvHD patients during the course of one year. Persistent remission occurred in only 9 patients before day 28 since start of second-line therapy in our cohort (Table 2). Many patients experienced remission of their aGvHD after day 28 since start of the most recent line of therapy, which suggests that a 28-day period is too limited for the evaluation of a therapeutic effect in SR-aGvHD.

About half of the patients in our cohort with grade II-IV acute GvHD had steroid-refractory disease, similar to earlier reports [1–3]. Most patients with steroid-refractory disease are severely affected, with 84% in our study suffering from grade III-IV GvHD. Similar to other studies [12,28,47,48,53], in our cohort patients with SR-aGvHD have a higher prevalence of liver involvement (36%) than patients with steroid-responsive aGvHD. In addition to the classical GvHD target organs being affected, many SR-aGvHD patients also suffer from other organ dysfunction, such as kidney, lung and endocrine dysfunction and cytopenia. This could either be due to these patients being generally ill, treatment toxicity, infection or direct targeting by alloreactivity. This underlines that pediatric SR-aGvHD is a multisystem disease with a high morbidity and mortality [55], associated with substantial healthcare use and costs [55,56].

In our cohort the TNF- α inhibitor infliximab and cell therapy with MSCs were most frequently prescribed, probably because of clinical studies in the 2 centers and the relatively favorable toxicity profiles [8,12,36]. The increased availability

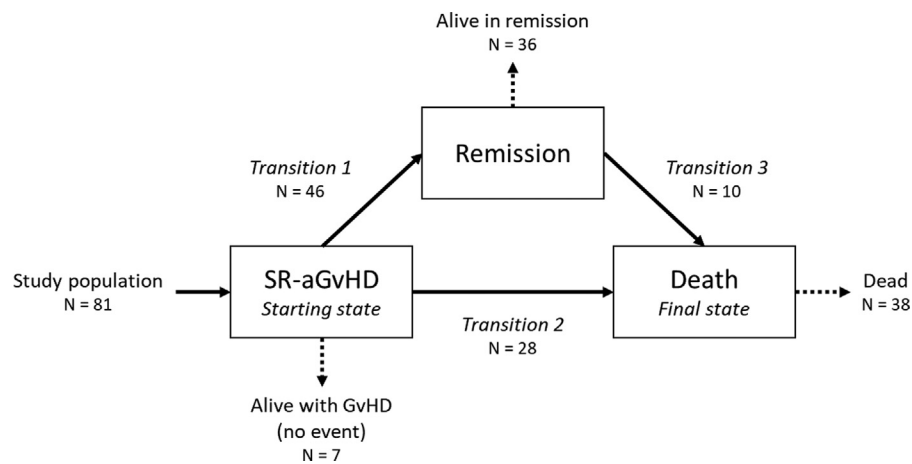


Figure 2. Multi-state survival model with transitions and transition counts. Graphical representation of the multi-state survival model used for statistical analysis of covariates. The different states are indicated by boxes. All patients start in the GvHD state and remain in this state until a new event occurs (i.e., remission of GvHD or death). The arrows indicate possible transitions to other states. Death is the absorbing or final state which means no further transitions are possible when a patient has entered this state. The number of patients entering and leaving each state are depicted at the 3 different transitions. *Dashed arrows* indicate the number of patients in that stage at the end of their follow-up.

Table 4
Multistate Covariates

Variable	Level	Outcome	HR	95% CI	P Value
Multistate analysis with univariate testing of covariates					
Age at transplantation		Remission	0.97	0.92-1.02	.2
		Death	1.06	1.00-1.12	.058
Age (categorized in quartiles)	0.175-3.01 years (1 st quartile)	Remission	1.0		
		Death	1.0		
	3.01-8.9 years (2 nd quartile)	Remission	1.76	0.83-3.75	.14
		Death	1.07	0.37-3.05	>.9
	8.9-13.9 years (3 rd quartile)	Remission	0.69	0.29-1.63	.4
		Death	1.46	0.54-3.97	.5
	13.9-17.9 years (4 th quartile)	Remission	0.59	0.23-1.50	.3
		Death	2.62	1.04-6.60	.04
Gender	Female	Remission	1.0		
		Death	1.0		
	Male	Remission	0.64	0.36-1.16	.14
		Death	1.09	0.56-2.14	.80
Diagnosis	Bone marrow failure	Remission	1.0		
		Death	1.0		
	Hematologic malignancy	Remission	0.77	0.31-1.91	.60
		Death	0.68	0.27-1.71	.40
	Hemoglobinopathy	Remission	1.11	0.27-4.54	.90
		Death	0	0.00-Inf	>.90
	Inborn errors of immunity	Remission	0.69	0.24-1.95	.50
		Death	0.72	0.25-2.08	.50
	Inborn errors of metabolism	Remission	0.48	0.14-1.72	.30
		Death	0.7	0.21-2.29	.60
Graft source	BM/PBSC	Remission	1.0		
		Death	1.0		
	Cord blood	Remission	0.51	0.27-0.94	.031
		Death	1.35	0.70-2.62	.40
Donor	Matched related	Remission	1.0		
		Death	1.0		
	Matched unrelated	Remission	1.05	0.44-2.53	>.90
		Death	0.51	0.18-1.42	.20
	Mismatched unrelated	Remission	0.78	0.35-1.75	.60
		Death	0.8	0.35-1.79	.60
Time until start of second line	<1 week	Remission	1.0		
		Death	1.0		
	8-28 days	Remission	1.14	0.61-2.12	.70

(continued)

Table 4 (Continued)

Variable	Level	Outcome	HR	95% CI	P Value
Second-line therapy	>28 days	Death	0.89	0.45-1.77	.70
		Remission	0.6	0.22-1.62	.30
		Death	0.61	0.20-1.81	.40
	MSC	Remission	1.0		
		Death	1.0		
	TNF-alpha inhibitor	Remission	1.35	0.69-2.64	.40
Death		0.6	0.26-1.38	.20	
Combination therapy	Remission	1.29	0.54-3.10	.60	
	Death	1.28	0.53-3.06	.60	
Other	Remission	0.81	0.24-2.77	.70	
	Death	0.92	0.27-3.14	.90	
Conditioning	MAC (chemotherapy)	Remission	1.0		
		Death	1.0		
	MAC (TBI)	Remission	1.72	0.6-4.91	.30
		Death	2.02	0.69-5.86	.20
	RIC	Remission	1.45	0.61-3.47	.40
		Death	2.06	0.89-4.78	.091
Before or after 2015	Before	Remission	1.0		
		Death	1.0		
	After	Remission	1.01	0.55-1.85	>.90
		Death	0.77	0.4-1.49	.40
Multistate analysis with interaction of age and graft source Age in cord blood grafts	Remission	0.99	0.91-1.07	0.8	
	Death	1.07	1.00-1.14	0.056	
Age in BM/PBSC grafts	Remission	0.9	0.83-0.96	0.004	
	Death	1.06	0.96-1.17	0.2	
Graft source	BM/PBSC	Remission	1.0		
		Death	1.0		
	Cord blood	Remission	0.18	0.06-0.51	0.001
		Death	1.5	0.34-6.59	0.6

of new agents is having a clear impact on treatment choices in recent years. MSCs were the only prescribed second-line therapy in the first 3 years of our cohort, whereas multiple different agents were used in the last few years. None of the specific second-line therapy options was associated with improved outcome, but this analysis is limited by the fact that therapy was highly individualized.

Complications and toxicities associated with immunosuppressive therapy in the aGvHD setting were highly prevalent in our SR-aGvHD cohort. Similar to other pediatric SR-aGvHD studies the majority of patients experienced infections or viral reactivations [19,28,48-50,52,53]. In about one third of deceased patients, infections were considered causal. TMA was a common noninfectious complication in our cohort. Because aGvHD is a risk factor for the development of TMA in both children [57,58] and adults [59], this finding was not surprising. The most frequently observed noninfectious complication in our cohort was the development of lung disease related to HSCT, such as BOS and IPS. In another pediatric SR-aGvHD cohort study BOS was also frequently observed [16], but in most studies BOS and IPS are not separately reported from general chronic GvHD. In our cohort, 26% of the patient deaths were attributed to noninfectious, HSCT-related respiratory failure. Pulmonary involvement thus represents a significant clinical challenge in pediatric SR-aGvHD patients and more research is required to understand how to manage HSCT-related lung complications to improve outcome [60].

To our knowledge only few studies identified risk factors for outcomes of SR-GvHD in children [47]. In our study, age and the use of CB grafts were associated with worse prognosis.

Older age was associated with increased mortality, and with reduced SR-aGvHD remission rates in those who received BM/PBSC grafts. In adults, older age has long been recognized as a risk factor for the development of GvHD [61], worse outcomes in HSCT overall [2,62], and higher mortality in adults with SR-aGvHD [63]. In children, the relationship between age and SR-aGvHD outcomes has not previously been reported. In addition, we found that CB grafts were associated with a lower chance of achieving remission of SR-aGvHD, irrespective of recipient age. CB grafts have generally been associated with a lower risk of GvHD in children [64], leading to the acceptance of higher levels of HLA mismatching in this setting. In most cases, aGvHD after CB transplantation develops despite GvHD prophylaxis with prednisone 1 mg/kg. As such, it may be argued that aGvHD in the CB setting is already steroid unresponsive to some extent. In adults, transplantation with a CB graft has been associated with the development of SR-GvHD [65]. However, it is still unknown why SR-aGvHD after transplantation with a CB graft is more refractory to additional immunosuppressive treatment than SR-aGvHD in a child that received a BM or PBSC graft. Because SR-aGvHD in the context of CB transplantation is associated with worse outcome, even more timely introduction of second-line treatment may be warranted in this setting. Other known predictive factors for GvHD severity, including degree of donor matching, malignancy as HSCT indication and MAC TBI conditioning [2,66-68] were not associated with worse outcomes in our SR-GvHD cohort.

There are several limitations to our study. First, data were retrospectively collected, at risk of reporting bias because of missing information. Second, the studied group is

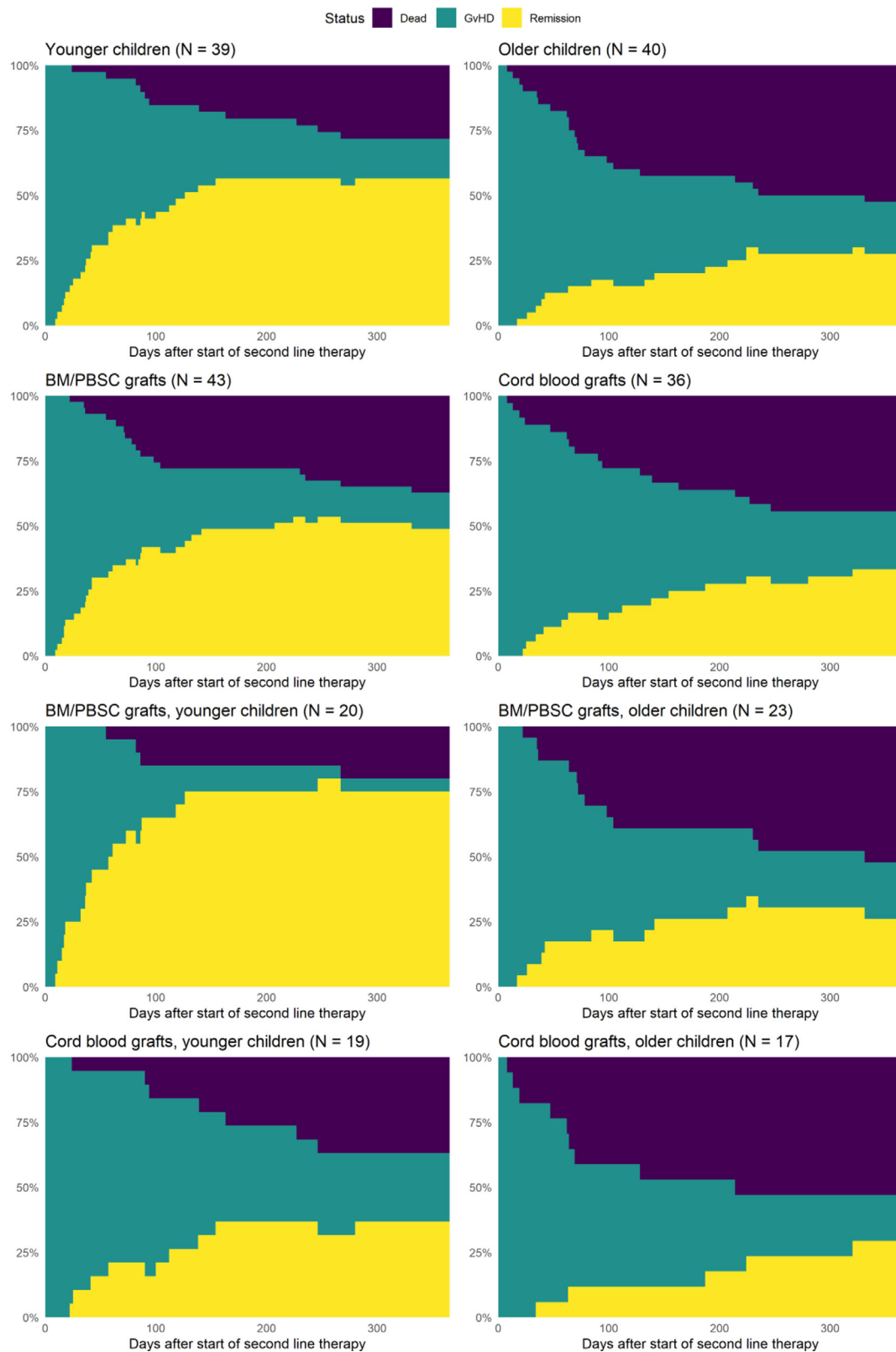


Figure 3. Clinical course since start of second-line therapy in specified subgroups. The proportions of patients with active GvHD symptoms, patients with remission of GvHD, and deceased patients during the first year since start of second-line therapy, in younger children <8.8 years versus older children \geq 8.8 years, BM/PBSC grafts and CB grafts, and combinations. Two patients who received a double CB graft and a composed graft were excluded.

heterogeneous and received multiple lines of therapy concomitantly. That, together with a relatively small study size, complicates drawing more definitive conclusions.

In conclusion, the development of SR-aGvHD in children after allogeneic HSCT is still associated with both high morbidity and mortality. Older age of the recipient at transplantation

is a risk factor for death in the whole population and in recipients of PBSC/BM grafts for lower remission rates of SR-aGVHD. In addition, we see reduced SR-aGVHD remission rates in children transplanted with a CB graft without a significant effect on survival. Choice of and time to second-line therapy were not associated with differences in outcomes. The outcomes presented in this study emphasize the unmet need for multicenter studies investigating novel therapies for pediatric patients and inclusion of pediatric cohorts on ongoing trials for SR-aGVHD. The cohort described here can serve as a reference for future studies investigating novel treatments and treatment guidelines for SR-aGVHD in pediatric patients, which it is hoped will improve the outcomes for these severely ill children.

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SUPPLEMENTARY MATERIALS

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REFERENCES

- Van Lint MT, Uderzo C, Locasciulli A, et al. Early treatment of acute graft-versus-host disease with high- or low-dose 6-methylprednisolone: a multicenter randomized trial from the Italian Group for Bone Marrow Transplantation. *Blood*. 1998;92:2288–2293.
- Martin PJ, Rizzo JD, Wingard JR, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2012;18:1150–1163.
- Penack O, Marchetti M, Ruutu T, et al. Prophylaxis and management of graft versus host disease after stem-cell transplantation for hematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. *Lancet Haematol*. 2020;7(2):e157–e167.
- Ferrara JLM, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373(9674):1550–1561.
- Deeg HJ. How I treat refractory acute GVHD. *Blood*. 2007;109:4119–4126.
- Kim JG, Sohn SK, Kim DH, et al. Different efficacy of mycophenolate mofetil as salvage treatment for acute and chronic GVHD after allogeneic stem cell transplant. *Eur J Haematol*. 2004;73:56–61.
- Furlong T, Martin P, Flowers MED, et al. Therapy with mycophenolate mofetil for refractory acute and chronic GVHD. *Bone Marrow Transplant*. 2009;44:739–748.
- Sleight BS, Chan KW, Braun TM, Serrano A, Gilman AL. Infliximab for GVHD therapy in children. *Bone Marrow Transplant*. 2007;40:473–480.
- Jacobsohn DA, Hallick J, Anders V, McMillan S, Morris L, Vogelsang GB. Infliximab for steroid-refractory acute GVHD: a case series. *Am J Hematol*. 2003;74:119–124.
- Patriarca F, Sperotto A, Damiani D, et al. Infliximab treatment for steroid-refractory acute graft-versus-host disease. *Haematologica*. 2004;89:1352–1359.
- Couriel D, Saliba R, Hicks K, et al. Tumor necrosis factor- α blockade for the treatment of acute GVHD. *Blood*. 2004;104:649–654.
- Yang J, Cheuk DK, Ha SY, et al. Infliximab for steroid refractory or dependent gastrointestinal acute graft-versus-host disease in children after allogeneic hematopoietic stem cell transplantation. *Pediatr Transplant*. 2012;16:771–778.
- Rao K, Rao A, Karlsson H, Jagani M, Veys P, Amrolia PJ. Improved survival and preserved antiviral responses after combination therapy with daclizumab and infliximab in steroid-refractory graft-versus-host disease. *J Pediatr Hematol Oncol*. 2009;31:456–461.
- Spoerl S, Mathew NR, Bscheider M, et al. Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. *Blood*. 2014;123:3832–3842.
- Zeiser R, Burchert A, Lengerke C, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. *Leukemia*. 2015;29:2062–2068.
- Khandelwal P, Teusink-Cross A, Davies SM, et al. Ruxolitinib as salvage therapy in steroid-refractory acute graft-versus-host disease in pediatric hematopoietic stem cell transplant patients. *Biol Blood Marrow Transplant*. 2017;23:1122–1127.
- M González Vicent, Molina B, J González de Pablo, Castillo A, M^á Díaz. Ruxolitinib treatment for steroid refractory acute and chronic graft vs host disease in children: Clinical and immunological results. *Am J Hematol*. 2019;94:319–326.
- Abedin S, McKenna E, Chhabra S, et al. Efficacy, toxicity, and infectious complications in ruxolitinib-treated patients with corticosteroid-refractory graft-versus-host disease after hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2019;25:1689–1694.
- Laisne L, Neven B, Dalle J-H, et al. Ruxolitinib in children with steroid-refractory acute graft-versus-host disease: A retrospective multicenter study of the pediatric group of SFGM-TC. *Pediatr Blood Cancer*. 2020;67(9):e28233.
- Uygun V, Karasu G, Daloğlu H, et al. Ruxolitinib salvage therapy is effective for steroid-refractory graft-versus-host disease in children: a single-center experience. *Pediatr Blood Cancer*. 2020;67(4):e28190.
- Coltoff A, Lancman G, Kim S, Steinberg A. Vedolizumab for treatment of steroid-refractory lower gastrointestinal acute graft-versus-host disease. *Bone Marrow Transplant*. 2018;53:900–904.
- Danylesko I, Bukauskas A, Paulson M, et al. Anti- α 4 β 7 integrin monoclonal antibody (vedolizumab) for the treatment of steroid-resistant severe intestinal acute graft-versus-host disease. *Bone Marrow Transplant*. 2019;54:987–993.
- Floisand Y, Lazarevic VL, Maertens J, et al. Safety and effectiveness of vedolizumab in patients with steroid-refractory gastrointestinal acute graft-versus-host disease: a retrospective record review. *Biol Blood Marrow Transplant*. 2019;25:720–727.
- Pai V, Abu-Arja R, Auletta JJ, Rangarajan HG. Successful treatment of steroid-refractory gastrointestinal acute graft-versus-host disease with adjuvant vedolizumab therapy in a pediatric allogeneic stem cell transplant recipient. *Pediatric Blood Cancer*. 2020;67:e28298.
- MacMillan ML, Weisdorf DJ, Davies SM, et al. Early antithymocyte globulin therapy improves survival in patients with steroid-resistant acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2002;8:40–46.
- Macmillan ML, Couriel D, Weisdorf DJ, et al. A phase 2/3 multicenter randomized clinical trial of ABX-CBL versus ATG as secondary therapy for steroid-resistant acute graft-versus-host disease. *Blood*. 2007;109:2657–2662.
- Dugan MJ, DeFor TE, Steinbuch M, Filipovich AH, Weisdorf DJ. ATG plus corticosteroid therapy for acute graft-versus-host disease: predictors of response and survival. *Ann Hematol*. 1997;75(1–2):41–46.
- Khandelwal P, Emoto C, Fukuda T, et al. A prospective study of alemtuzumab as a second-line agent for steroid-refractory acute graft-versus-host disease in pediatric and young adult allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2016;22:2220–2225.
- Gomez-Almaguer D, Ruiz-Arguelles GJ, del Carmen Tarin-Arzaga L, et al. Alemtuzumab for the treatment of steroid-refractory acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2008;14:10–15.
- Funke VAM, de Medeiros CR, Setubal DC, et al. Therapy for severe refractory acute graft-versus-host disease with basiliximab, a selective interleukin-2 receptor antagonist. *Bone Marrow Transplant*. 2006;37:961–965.
- Massenkeil G, Rackwitz S, Genvresse I, Rosen O, Dörken B, Arnold R. Basiliximab is well tolerated and effective in the treatment of steroid-refractory acute graft-versus-host disease after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2002;30:899–903.
- Tan Y, Xiao H, Wu D, et al. Combining therapeutic antibodies using basiliximab and etanercept for severe steroid-refractory acute graft-versus-host disease: a multi-center prospective study. *Oncoimmunology*. 2017;6(3):e1277307.
- Drobyski WR, Pasquini M, Kovatovic K, et al. Tocilizumab for the treatment of steroid refractory graft-versus-host disease. *Biol Blood Marrow Transplant*. 2011;17:1862–1868.
- Ganetsky A, Frey NV, Hexner EO, et al. Tocilizumab for the treatment of severe steroid-refractory acute graft-versus-host disease of the lower gastrointestinal tract. *Bone Marrow Transplant*. 2019;54:212–217.
- Kolb M, Bhatia M, Madina G-G, Satwani P. Effective use of tocilizumab for the treatment of steroid-refractory gastrointestinal acute graft versus host disease in a child with very high levels of serum interleukin-6. *Pediatr Blood Cancer*. 2015;62:362–363.
- Hashmi S, Ahmed M, Murad MH, et al. Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis. *Lancet Haematol*. 2016;3(1):e45–e52.
- Lawitschka A, Lucchini G, Strahm B, et al. Pediatric acute graft-versus-host disease prophylaxis and treatment: surveyed real-life approach reveals

- dissimilarities compared to published recommendations. *Transpl Int.* 2020;33:762–772.
38. Schoemans HM, Lee SJ, Ferrara JL, et al. EBMT-NIH-CIBMTR Task Force position statement on standardized terminology & guidance for graft-versus-host disease assessment. *Bone Marrow Transplant.* 2018;53:1401–1415.
 39. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15:825–828.
 40. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant.* 2005;11:945–956.
 41. Afessa B, Peters SG. Chronic lung disease after hematopoietic stem cell transplantation. *Clin Chest Med.* 2005;26(4):571–586. vi.
 42. R Core Team. *R: A language and environment for statistical computing [Internet]*. Vienna, Austria: R Foundation for Statistical Computing; 2021.
 43. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag; 2016.
 44. de Wreede LC, Fiocco M, Putter H. The mstate package for estimation and prediction in non- and semi-parametric multi-state and competing risks models. *Comput Methods Programs Biomed.* 2010;99:261–274.
 45. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. *Stat Med.* 2007;26:2389–2430.
 46. de Wreede LC, Fiocco M, Putter H. mstate: An R package for the analysis of competing risks and multi-state models. *J Stat Softw.* 2011;38(7 SE-Articles):1–30.
 47. Berger M, Pessolano R, Carraro F, Saglio F, Vassallo E, Fagioli F. Steroid-refractory acute graft-versus-host disease graded III-IV in pediatric patients. A mono-institutional experience with a long-term follow-up. *Pediatr Transplant.* 2020;24(7):e13806.
 48. Faraci M, Calevo MG, Giardino S, et al. Etanercept as treatment of steroid-refractory acute graft-versus-host disease in pediatric patients. *Biol Blood Marrow Transplant.* 2019;25:743–748.
 49. Kurtzberg J, Abdel-Azim H, Carpenter P, et al. A phase 3, single-arm, prospective study of Remestemcel-L, ex vivo culture-expanded adult human mesenchymal stromal cells for the treatment of pediatric patients who failed to respond to steroid treatment for acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 2020;26:845–854.
 50. Tang F-F, Cheng Y-F, Xu L-P, et al. Basiliximab as treatment for steroid-refractory acute graft-versus-host disease in pediatric patients after haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2020;26:351–357.
 51. Inagaki J, Fukano R, Kodama Y, Nishimura M, Shimokawa M, Okamura J. Safety and efficacy of low-dose methotrexate for pediatric patients with steroid-refractory acute graft-versus-host disease after hematopoietic stem cell transplantation. *Ann Hematol.* 2014;93:645–651.
 52. Inagaki J, Kodama Y, Fukano R, Noguchi M, Okamura J. Mycophenolate mofetil for treatment of steroid-refractory acute graft-versus-host disease after pediatric hematopoietic stem cell transplantation. *Pediatr Transplant.* 2015;19:652–658.
 53. Kurtzberg J, Prockop S, Teira P, et al. Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for severe refractory acute graft-versus-host disease in pediatric patients. *Biol Blood Marrow Transplant.* 2014;20:229–235.
 54. MacMillan ML, DeFor TE, Weisdorf DJ. The best endpoint for acute GVHD treatment trials. *Blood.* 2010;115:5412–5417.
 55. Grabner M, Strati E, Sandman K, Forsythe A. Economic burden of acute steroid-refractory graft-versus-host disease in commercially insured pediatric patients. *J Manag Care Spec Pharm.* 2021;27:607–614.
 56. Ricci A, Jin Z, Broglie L, et al. Healthcare utilization and financial impact of acute-graft-versus host disease among children undergoing allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant.* 2020;55:384–392.
 57. Elfeky R, Lucchini G, Lum S-H, et al. New insights into risk factors for transplant-associated thrombotic microangiopathy in pediatric HSCT. *Blood Adv.* 2020;4:2418–2429.
 58. Schoettler M, Lehmann LE, Margossian S, et al. Risk factors for transplant-associated thrombotic microangiopathy and mortality in a pediatric cohort. *Blood Adv.* 2020;4:2536–2547.
 59. Vasu S, Bostic MG, Zhao Q, et al. Acute GVHD, BK hemorrhagic cystitis and age are risk factors for transplant-associated thrombotic microangiopathy in adults. *Blood Adv.* 2022;6:1342–1349.
 60. Wilhelmsson M, Vatanen A, Borgström B, et al. Adverse health events and late mortality after pediatric allogeneic hematopoietic SCT—two decades of longitudinal follow-up. *Bone Marrow Transplant.* 2015;50:850–857.
 61. Qayed M, Wang T, Hemmer MT, et al. Influence of age on acute and chronic GVHD in children undergoing HLA-identical sibling bone marrow transplantation for acute leukemia: implications for prophylaxis. *Biol Blood Marrow Transplant.* 2018;24:521–528.
 62. Friend BD, Schiller GJ. Beyond steroids: a systematic review and proposed solutions to managing acute graft-versus-host disease in adolescents and young adults. *Blood Rev.* 2022;52: 100886.
 63. Rashidi A, DeFor TE, Holtan SG, Blazar BR, Weisdorf DJ, MacMillan ML. Outcomes and predictors of response in steroid-refractory acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 2019;25:2297–2302.
 64. Rocha V, Wagner JEJ, Sobocinski KA, et al. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. *N Engl J Med.* 2000;342:1846–1854.
 65. Pagliuca S, Prata PH, Xhaard A, et al. Long-term outcomes and risk factor analysis of steroid-refractory graft versus host disease after hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2021;56:38–49.
 66. Flowers MED, Inamoto Y, Carpenter PA, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. *Blood.* 2011;117:3214–3219.
 67. Jacobsohn DA. Acute graft-versus-host disease in children. *Bone Marrow Transplant.* 2008;41:215–221.
 68. Gatza E, Reddy P, Choi SW. Prevention and treatment of acute graft-versus-host disease in children, adolescents, and young adults. *Biol Blood Marrow Transplant.* 2020;26(5):e101–e112.