

A novel conditioning regimen of thiotepa combined with low-dose cyclophosphamide for haploidentical hematopoietic stem cell transplantation in pediatric patients with severe aplastic anemia: a prospective study from China

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Abstract

High-dose cyclophosphamide(Cy)-related cardiotoxicity(CT) during transplant conditioning in severe aplastic anemia is a life-threatening complication. Despite the adoption of low dose Cy regimens, severe CT still occurred in practice. To address it, we designed a single-arm prospective clinical trial to evaluate the efficacy and safety of a modified conditioning regimen for reducing CT in high-risk pediatric SAA patients undergoing haploidentical HSCT (haplo-HSCT). The regimen was as follows: Busulfan(Bu) 3.2mg/Kg on days -8 to -7, thiotepa(TT) 10mg/Kg on day -6, Cy80mg/Kg on days -5 to -2 and anti-thymocyte globulin(ATG) 10mg/kg on days -5 to -2. Historical high-risk SAA patients who received the traditional Bu and Cy 200mg/Kg on days -5 to -2(Bu/Cy_{high}) Regimen were enrolled as the control group. Our results demonstrated that no severe CT was observed in the novel regimen group(Bu/Cy_{low}TT). The 1-year overall Survival (OS) rate was significantly higher in BuCy_{low}TT group(100%vs.86.1%, $P=0.021$). No Graft failure (GF) or severe adverse events were observed in the Bu/Cy_{low}TT group. All patients achieved early granulocyte and platelet engraft. The incidences of grade II-IV and grade III-IV acute graft-versus-host disease (aGVHD) were comparable between the two groups (33.7% vs. 28.6%, $P=0.455$; 13.9% vs. 5.56%, $P=0.285$). Univariate analysis of 1-year OS indicated that prior ATG treatment and different conditioning regimen may affect the final outcomes. In conclusion, the BuCy_{low}TT regimen represents a promising alternative choice for pediatric SAA patients at high risk of CT undergoing haplo-HSCT.

Introduction

Severe aplastic anemia (SAA) is a life-threatening acquired bone marrow failure disorder in children. Hematopoietic stem cell transplantation(HSCT) from human leukocyte antigens(HLA) matched sibling donor(MSD) remains the first-line treatment with newly diagnosed with SAA[1]. However, MSD is unavailable for the majority of patients, and haploidentical HSCT(haplo-HSCT) have shown excellent long-term outcomes[2]. The long-term survival rate of haploidentical hematopoietic cell transplantation(haplo-HSCT) for children with AA approximately reached 90%[3]. Despite this progressions, high-dose cyclophosphamide(Cy)-induced cardiotoxicity(CT) during the conditioning phase remains a severe and life-threatening complication, which adversely impacts the final OS[3, 4].

To decrease Cy-related cardiac injury, reduced-dose Cy regimens have been developed and applied in clinical practice. However, even with a lower dose of cyclophosphamide 120mg/Kg or 100mg/Kg, severe CT and death still occur, highlighting the need for further optimization of conditioning regimens[3, 5, 6]. Thiotepa, a non-specific alkylating agent, has been confirmed to be safe and effective in the post-transplant Cy (PTCY) regimen for pediatric SAA in small-scale retrospective studies [7]. Nevertheless, it remains unknown whether thiotepa can be incorporated into the “Beijing Protocol” for haploidentical transplantation while further reducing the Cy dosage, and whether this modified regimen can effectively reduce the incidence of severe CT in high-risk patients.

To solve these questions, we conducted a prospective, single-arm clinical trial to evaluate the safety and efficacy of a thiotepa-based modified condition regimen(Bu/Cy_{low}TT) in pediatric SAA patients at high

risk of CT undergoing haplo-HSCT. We also compared the clinical outcomes with a historical control group treated with the traditional high dose Cy(BuCy_{high}) regimen, aiming to provide a novel and optimized conditioning strategy for this high-risk patient population.

METHODS

Study design and patients

This study was approved by the Ethics Committee of Peking University People's Hospital (Approval No.2024PHB399-001). All procedures adhered to the principles outlined in the Declaration of Helsinki. All recipients and guardians have signed informed consent prior to the initiation of any study-related procedures. Inclusion criteria were: 1) Diagnosis of SAA with high risk of CT; 2) age < 18 years (male or female); 3) Availability of a suitable HLA-haploidentical donor; 4) No active infection before HSCT. Exclusion criteria included: 1) Poor treatment compliance; 2) Presence of adoptive contraindications in the donor. high risk of CT before HSCT was defined as meeting two or more following criteria[4]: 1) Eastern Cooperative Oncology Group (ECOG) score (≥ 2) before transplantation; 2) Abnormal ST-T wave on 12-lead ECG; 3) hyperlipemia; 4) Calculated Cy dosage ≥ 1.8 g/m²/d. A total of 36 consecutive pediatric SAA patients were enrolled in this study, with the last follow-up date of February 1, 2026.

Conditioning regimen and GVHD Prophylaxis

Briefly, the conditioning regimen was based on the classical Bu/Cy/ATG regimen, with adjustment to Cy dosage and addition of thiotepe: Busulfan (Bu) 3.2 mg/kg per day on days - 8 and - 7; thiotepe (TT) 10 mg/kg per day on day - 6; Cy 20 mg/kg per day on days - 5 to - 2 (total 80mg/Kg); rabbit anti-thymocyte globulin (rATG, Sangstat, Lyon, France, rATG) 2.5 mg/kg/day on days - 5 to - 2 (total 10mg/Kg). For the historical control group, 36 high-risk SAA patients who received the BuCy_{high} regimen at our center from January 2017 to December 2022 were enrolled, all of whom met at least two high-risk CT criteria to ensure comparability with the BuCy_{low}TT group. Bu/Cy_{high} regimen consist of Bu 0.8 mg/kg (four times) on days - 7 and - 6), Cy 50 mg/kg per day on days - 5 to - 2, and rATG 2.5 mg/kg per day on days - 5 to - 2.

All donors received granulocyte colony stimulating factor (G-CSF) on days - 3, - 2, - 1, 01 and 02, bone marrow and peripheral blood hematopoietic stem cells were harvested at day 01 and day 02, respectively. Prophylaxis for acute acute graft-versus-host disease (GVHD) included cyclosporin (CsA), mycophenolate mofetil and short-term methotrexate in both groups.

Definitions

Regimen-related CT was graded according to the Bearman criteria[8]. Severe CT was defined as Grade III or IV toxicity. Grade III defined as follows: severe ECG abnormalities with no or only partial response to

medical intervention; heart failure with no or only minor response to medical intervention; or decrease in voltage by more than 50%. Grade IV cardiotoxicity was defined as fatal toxicity.

Full donor chimerism (FDC) was defined as > 95% donor-originated cells in the recipient's bone marrow. Mixed chimerism (MC) was defined as preserving 5–95% donor-derived cells. Primary Graft Failure (GF) was defined failure to achieve the criteria of neutrophil engraftment and donor chimerism was less than 5% on day + 28. Secondary GF was defined as persistent neutropenia ($< 0.5 \times 10^9/L$) with donor chimerism $< 5\%$ after initial engraftment. Patients who developed cytopenia of two or three lineages (neutrophil count $< 0.5 \times 10^9/L$, hemoglobin < 70 g/L, and platelet count $< 20 \times 10^9/L$) for over 2 weeks with FDC were diagnosed with

poor graft function (PGF). Transplant-related Mortality (TRM) was defined as death without disease relapse. OS was defined as the time from the date of HSCT to death or the last follow up. Failure Free Survival (FFS) was defined as the time from HSCT to the time of treatment failure, including TRM, GF, and PGF.

Statistical analysis

A chi-square test and Fisher's exact test were used for categorical variables, and the Mann–Whitney U test was used to compare continuous

variables. Survival analysis was conducted by the Kaplan–Meier

method, log-rank was used for univariate analysis. The cumulative incidences aGVHD, infection and PGF were calculated using a competing risk model with death as competing event. Analyses were performed using SPSS 23.0 (SPSS, Inc., Chicago, IL, USA) and R version 3.5.2.

RESULTS

Patient Characteristics

A total of 36 patients were enrolled in the Bu/Cy_{low}TT group, and 36 historical high-risk CT patients were included in the Bu/Cy_{high} group as controls. The baseline clinical characteristics of two groups are summarized in Table 1. The age at HSCT in Bu/Cy_{low}TT group was 8 years old, which was younger than Bu/Cy_{high} group ($P = 0.031$). No significant differences were observed in other baseline variables, including the interval from diagnosis to HSCT, pre-transplant high-risk factors (dyslipidemia, prior ATG treatment, calculated Cy dosage), donor age, donor-recipient sex matching, and donor-recipient ABO matching ($P > 0.05$). The median infused mononuclear cell (MNC) dose was significantly higher in the BuCy_{low}TT group than in the control group ($12.19 \times 10^8/kg$ vs. $9.14 \times 10^8/kg$, $P < 0.001$), and the median CD34⁺ cell dose was also higher in the BuCy_{low}TT group ($4.34 \times 10^6/kg$ vs. $3.30 \times 10^6/kg$, $P = 0.01$). The median days to white blood cell (WBC) and platelet engraftment were comparable between the two

groups (WBC: 11 days vs. 12 days, $P = 0.054$; platelet: 11 days vs. 11 days, $P = 0.078$). The median follow-up time was 200 days till last follow-up time.

Table 1
Clinical characteristics of patients with two regimens

Characteristics	Bu/CY _{low} TT n = 36	Bu/CY _{high} n = 36	<i>P</i>
Age(years), median(range)	8(3–16.0)	11.5(2.8-17.15)	0.031
Gender, male to female	17:19	18:18	0.814
Interval from diagnosis to HSCT(months),median(range)	20.6(0.8–119)	12.5(2-125)	0.710
Dyslipidemia, n(%)			0.059
Yes	14(37.8%)	23(62.2%)	
No	22(62.9%)	13(37.1%)	
Previous treatment,n(%)			0.233
ATG + CSA	2(5.6%)	5(13.9%)	
CSA	34(94.4%)	31(86.1%)	
Pre-calculated Cy dosage			0.114
< 1.8,n(%)	23(63.9%)	13(36.1%)	
≥ 1.8,n(%)	7(19.4%)	13(9.6%)	
Donor age(years),median(range)	36(16–50)	36(18–57)	0.846
Donor-recipient sex			0.334
match n (%)	12(33.3%)	16(44.4%)	
mismatch n (%)	24(66.7%)	20 (55.6%)	
Donor-recipient ABO match			0.845
Match n (%)	14(38.9%)	17(42%)	
Minor mismatch n (%)	10(27.8%)	10(27.8%)	
Major mismatch n (%)	10(27.8%)	7(19.4%)	
Bidirectional mismatch n (%)	2(5.6%)	2(5.6%)	
MNC(10 ⁸ /Kg)median(range)	12.19(6.69-17.0)	9.14(4.97–17.93)	0.000
CD34 + cell(106/Kg), median(range)	4.34(2.05–12.5)	3.30(0.4–15.1)	0.01
WBC engraftment day,median(range)	11(9–19)	12(9–21)	0.054
Platelet engraftment day, median(range)	11(8–25)	11(7-110)	0.078

Cardiotoxicity incidence

In BuCy_{low}TT group, no severe CT was observed within the first 28 days after HSCT. In contrast, four patients in the BuCy_{high} group developed severe CT during this period, all of whom died from cardiac complications. Among these four patients, two experienced severe CT on the day of bone marrow infusion, presenting with heart failure and large pericardial effusion; the other two experienced severe CT on the day of peripheral blood hematopoietic stem cells, with no response to glucocorticoids. Three patients died on day + 2 post HSCT, and one patient died on day + 1. The cumulative incidence of severe CT was 0% [95% CI: 0.000%] in BuCy_{low}TT group and 11.1% [95% CI: 3.45%-23.8%] in the BuCy_{high} group, with a significant difference between the two groups ($P = 0.041$, Fig. 1).

Engraftment

Except four patients who died early from severe CT in the BuCy_{high} group, all remaining patients in both groups achieved early neutrophil engraftment by day + 28 post-HSCT. The median neutrophil engraftment time was 12 days (range: 9-21 days) in BuCy_{high} group and 11 days (range: 9-19 days) in BuCy_{low}TT group ($P = 0.0054$). In BuCy_{high} group, one patient experienced delayed platelet engraftment (finally achieved on +day 110), and another patient died of thrombotic microangiopathy (TMA) with no platelet engraftment until death. No secondary GF (sGF) or PGF was observed in either group during the follow-up period.

Graft-versus-host disease

The cumulative incidences of grade II-IV aGVHD within the first 100 days post-HSCT were comparable between the BuCy_{low}TT group and the BuCy_{high} group (28.6%, 95% CI: 14.6%–44.3% vs. 33.7%, 95% CI: 18.7%–49.4%, $P = 0.455$, Fig. 2A). The cumulative incidences of grade III-IV aGVHD at 100 days were also not significantly different (5.56%, 95% CI: 0.969%–16.5% vs. 13.9%, 95% CI: 4.97%–27.3%, $P = 0.285$, Fig. 2B). No patients died of aGVHD in either group during the follow-up. Due to a median follow-up time was 206.5 days in BuCy_{low}TT group, the incidence of chronic GVHD (cGVHD) at 1 year was not calculated in this study.

Infections

No skin toxicity and mucosal injury was observed when administering Thiotepe. Since 2022, letermovir has been widely used for cytomegalovirus (CMV) for prophylaxis in our center, leading to a significant reduction in the cumulative incidence of CMV infection. The 1-year cumulative incidence of CMV infection was significantly lower in the BuCy_{low}TT group than in the BuCy_{high} group (6.25%, 95% CI: 0.362%–25.5% vs. 56.3%, 95% CI: 37.1%–71.6%, $P < 0.001$, Fig. 3A). The cumulative incidence of Epstein-Barr virus (EBV)-related post-transplant lymphoproliferative disorder (PTLD) was comparable between the two groups (10.2%, 95% CI: 1.35%–29.9% vs. 12.6%, 95% CI: 3.88%–26.7%, $P = 0.389$, Fig. 3B). No patients died of viral infections in either group during the follow-up.

Survival

A total of five patients died in the BuCy_{high} group: four died of severe early post-HSCT CT (median death time: day + 2), and one died of severe TMA combined with status epilepticus. The 1-year OS rate was 100% (95

% CI:100%–100%) in the BuCy_{low}TT group and 86.1% (95% CI:75.5%–98.2%) in the BuCy_{high} group, with a statistically significant difference ($P = 0.021$, Fig. 4A). Since no patients died of primary disease, the the FFS rate was consistent with the OS rate (Fig. 4C). The 100-day TRM rate was 0% in the BuCy_{low}TT group and 11.1% in the BuCy_{high} group, with a significant difference ($P = 0.021$, Fig. 4B).

Univariate analysis of 1-year OS (Table 2) showed that prior ATG treatment ($P = 0.017$), the BuCy_{high} regimen ($P = 0.021$), and the development of severe CT ($P < 0.001$) were significantly associated with poor survival outcomes in pediatric SAA patients. No other factors (sex, dyslipidemia, Cy dosage, donor-recipient ABO matching, MNC/CD34⁺ cell dose) were found to affect the 1-year OS (all $P > 0.05$).

Univariate analysis of 100-day TRM (Table 3) further confirmed that severe CT ($P < 0.001$), prior ATG treatment ($P = 0.017$), and the BuCy_{high} regimen ($P = 0.021$) were risk factors for early TRM. Due to the limited number of adverse events in this study, multivariate Cox regression analysis was not performed.

Table 2
Univariate analysis of 1-year OS

Factors	numbers	1-year OS	<i>P</i>
Sex			
male	35	94.3	0.593
female	37	94.6	
Previous ATG			
Yes	7	71.4	0.017
No	65	96.5	
Dyslipidemia			
Yes	28	96.4	0.913
No	39	92.3	
CTX dosage			
≥ 1.8g/m2	20	90.0	0.505
< 1.8g/m2	52	96.2	
Donor-recipient ABO match			
Match n (%)	31	93.5	0.539
Minor mismatch n (%)	20	90.0	
Major mismatch n (%)	17	100%	
Bidirectional mismatch n (%)	4	100%	
Regimen			
BU/Cy ^{high}	36	88.9	0.021
BU/Cy ^{low} TT	36	100%	
MNC			
> 12*10^8/Kg	27	96.3	0.404
< 12*10^8/Kg	54	93.3	
CD34			
> 4*10^6/Kg	32	96.9	0.251
< 4*10^6/Kg	40	90.0	

Factors	numbers	1-year OS	<i>P</i>
Sex			
Severe cardiotoxicity			
Yes	4		< 0.001
No	68		

Table 3
Univariate analysis of d100-TRM

Factors	numbers	TRM(%)	<i>P</i>
Regimen			
Bu/Cy ^{high}	36	11.1%	0.021
Bu/Cy ^{low} TT	36	0%	
Sex			
male	35	5.7%	0.593
female	37	5.4%	
Previous ATG			
Yes	7	28.6	0.017
No	65	4.5	
Dyslipidemia			
Yes	28	3.6	0.913
No	39	7.7	
CTX dosage			
≥ 1.8g/m ²	20	10.0	0.505
< 1.8g/m ²	52	3.8	
Donor-recipient ABO match			
Match n (%)	31	93.5	0.539
Minor mismatch n (%)	20	90.0	
Bidirectional mismatch n (%)	4	100%	
Regimen			
Bu/Cy _{high}	36	11.1%	0.021
Bu/Cy _{low} TT	36	0%	
MNC			
> 12*10^8/Kg	27	3.7	0.404
< 12*10^8/Kg	54	6.7	
CD34			

Factors	numbers	TRM(%)	<i>P</i>
> 4*10 ⁶ /Kg	32	3.1	0.251
< 4*10 ⁶ /Kg	40	10.0	
Severe cardiotoxicity			
Yes	4	100	< 0.001
No	68	1.5	

Discussion

In this study, we report a prospective study of a thiotepa based modified conditioning regimen(BuCy_{low}TT) with low-dose Cy for pediatric SAA patients at high risk of CT undergoing haplo-HSCT. Our results demonstrated that this novel regimen completely eliminated severe CT, significantly improved the 1-year OS rate, and maintained favorable engraftment and aGVHD profiles compared with the traditional BuCy_{high} regimen. To our knowledge, this is the first report on the incorporation of thiotepa into the G-CSF and ATG-based "Beijing Protocol" for haplo-HSCT in pediatric SAA, providing a new and safe conditioning strategy for high-risk patients.

Even though Matched sibling donor HSCT is nowadays recommended as first line therapy for SAA [1], but the unavailability of MSD for most patients limits its clinical application. Haplo-HSCT has made remarkable progress in the treatment of SAA in recent years [9], demonstrating the OS and FFS in haplo-HSCT is comparable with MSD-HSCT [2], significantly higher than those of immunosuppressive therapy [10]. In particular, G-CSF and ATG-based regimens have achieved success for haplo-HSCT in SAA with a low GF and GvHD, making haplo-HSCT great advance for pediatric SAA without MSD, especially in young patients [11].

Still there are some limitations in current conditioning regimens. Even though BU/Cy regimen showed a comparable result with MSD both in adults and children[2, 9],regimen-related cardiotoxicity can not be neglected. Previous study showed that the severe cardiotoxicity is approximately 6% in both adults and children with BU/Cy regimen[4].To reduce CT, modified regimens such as Bu/Flu/Cy(Cy 100mg/Kg) based on prediction model has been developed, with significantly reduce TRM but still result in severe CT in a small subset of high-risk patients[3]. Even with a Cy dose of 100 mg/kg, severe CT and associated mortality still occur, indicating the need for further reduction of Cy dosage and optimization of conditioning regimens[3].

Cy related cardiotoxicity induces a rare lethal complication and has been proved as dose dependent. A reduce Cy dose may show a better OS, but the precise Cy dose is unclear, as Cy dose with 100mg/Kg still showed cardiotoxicity in recent study[6].In this study ,we further reduced the total Cy dose to 80mg/Kg and added thiotepa to replace Cy doses partly in CT high risk patients. Thiotepa is an alkylator agent widely used in the PTCY regimen for hematological malignancies in Europe, but its application in non-

malignant hematological diseases is limited, only a few reported in thalassemia conditioning regimens[12]. Small-scale retrospective studies have confirmed the safety and efficacy of thiotepa in the PTCY regimen for pediatric SAA, and our study further validated its value in SAA for haplo-HSCT.

The result is encouraging when compared with BU/Cy_{high} regimen. The baseline characteristics of the two groups were well matched, except for the younger median age, higher MNC, and CD34⁺ cell doses in the BuCy_{low}TT group; and these differences did not affect the final OS. The complete absence of severe CT in the BuCy_{low}TT group was the most prominent finding of this study, which was mainly attributed to the low Cy dosage (80 mg/kg). Notably, the reduction of Cy dosage and the addition of thiotepa did not compromise engraftment. All patients get full donor chimerism on day + 28, with median WBC and platelet engraftment time of 11 days, which is comparable to classic G-CSF/ATG-based regimens,[13–15], and earlier than the PT/CY regimen (median myeloid engraftment: 17–22 days)[16–18]. This favorable engraftment profile may be related to the potent immunosuppressive activity of thiotepa, which enhances donor cell engraftment without direct cytotoxicity to recipient hematopoietic stem cells. [7][19]. In contrast to previous reports of thiotepa-related extracutaneous toxicities (oral mucositis, diarrhea) in children [20], no severe extracutaneous toxicities were observed in our study, suggesting the safety of the BuCy_{low}TT regimen. As a non-malignant hematology, GF and GvHD should be widely considered[21, 22]. The incidences of grade II-IV and III-IV aGVHD were comparable between the two groups, and no GF was observed in either group, indicating that the BuCy_{low}TT regimen maintains the balance between immunosuppression and GVHD control, which is crucial for non-malignant hematological diseases.

The 1-year OS rate was 100% in Bu/Cy_{low}TT group, significantly higher than that in the Bu/Cy_{high} group, and no deaths were recorded in the Bu/Cy_{low}TT group during the follow-up. In the Bu/Cy_{high} group, 80% of mortalities were caused by severe CT, which is consistent with previous reports that severe CT is a fatal complication of HSCT with an incidence of over 10% and that mild to moderate cardiac injury occurs in more than 30% of patients[23, 24]. The significant reduction in CMV infection in the Bu/Cy_{low}TT group was mainly due to the widespread use of letermovir for CMV prophylaxis since 2022, and the incidence of EBV-PTLD was comparable between the two groups, indicating that the Bu/Cy_{low}TT regimen does not increase the risk of viral infections.

Univariate analysis identified prior ATG treatment and severe CT as independent risk factors for poor survival, which is consistent with previous clinical observations. Prior ATG treatment may induce immune tolerance and affect the engraftment and immune reconstitution of donor cells, thereby reducing survival. Severe CT is a fatal complication with a 100% mortality rate in our study, further highlighting the importance of reducing CT in high-risk patients.

This study has some limitations to date. First, it is a single-arm prospective study with a historical control group, and no concurrent randomized controlled trial was performed due to the strong preference of guardians of high-risk patients for the novel regimen. Secondly, the sample size is relatively small, and

further studies with larger sample sizes are needed to validate the results. Third, direct comparison with the Bu/Cy_{low}/Flu regimen, another modified regimen for high-risk CT patients, was not performed due to limited patient numbers, and future head-to-head studies are warranted to compare the efficacy and safety of these regimens. Finally, the median follow-up time is relatively short, and long-term follow-up is needed to evaluate the incidence of cGVHD.

Conclusion

In summary, The BuCy_{low}TT conditioning regimen significantly reduced the incidence of severe CT in pediatric SAA patients at high risk of CT undergoing haplo-HSCT, while maintaining favorable engraftment, GVHD, and infection profiles. The regimen significantly improves the 1-year OS rate and eliminates early TRM related to cardiac complications. Collectively, the Bu/Cy_{low}TT regimen is a safe and effective alternative for pediatric SAA patients at high risk of CT undergoing haplo-HSCT and warrants further validation in large-scale multicenter clinical trials.

Declarations

Data availability statement: Datasets generated in this study can be obtained from the corresponding author upon a reasonable request.

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Figures

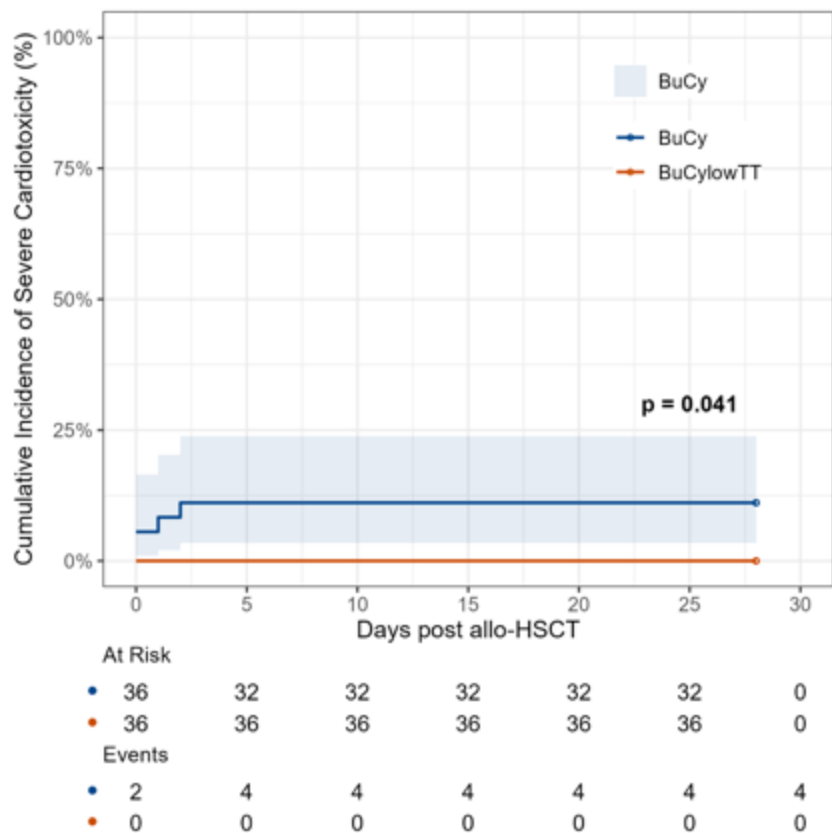


Figure 1

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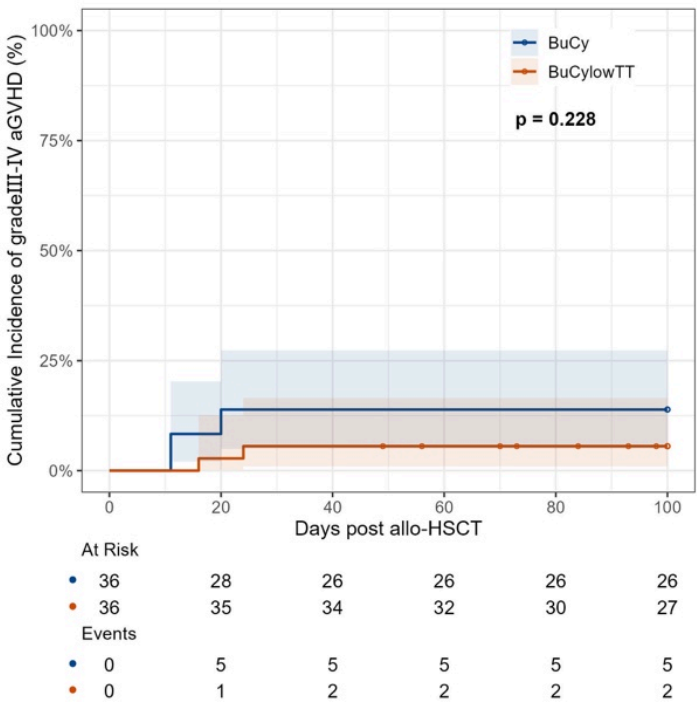
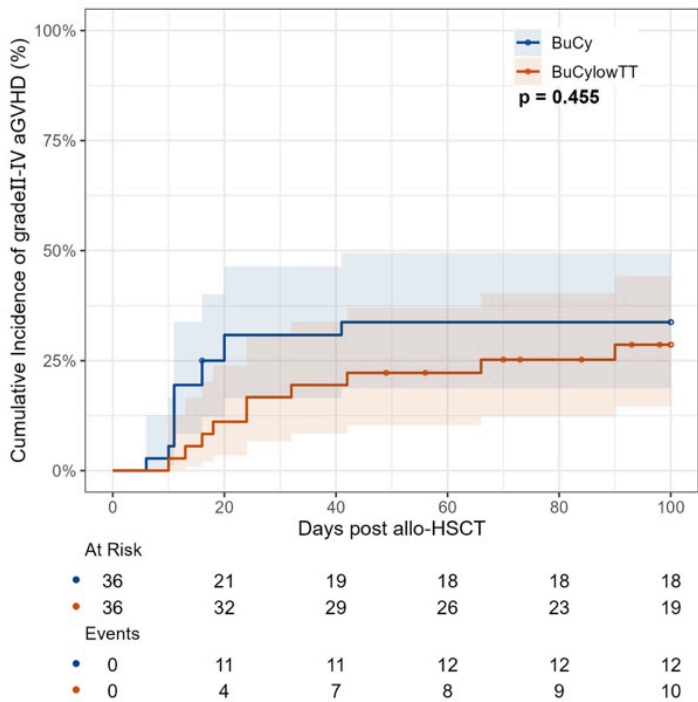


Figure 2

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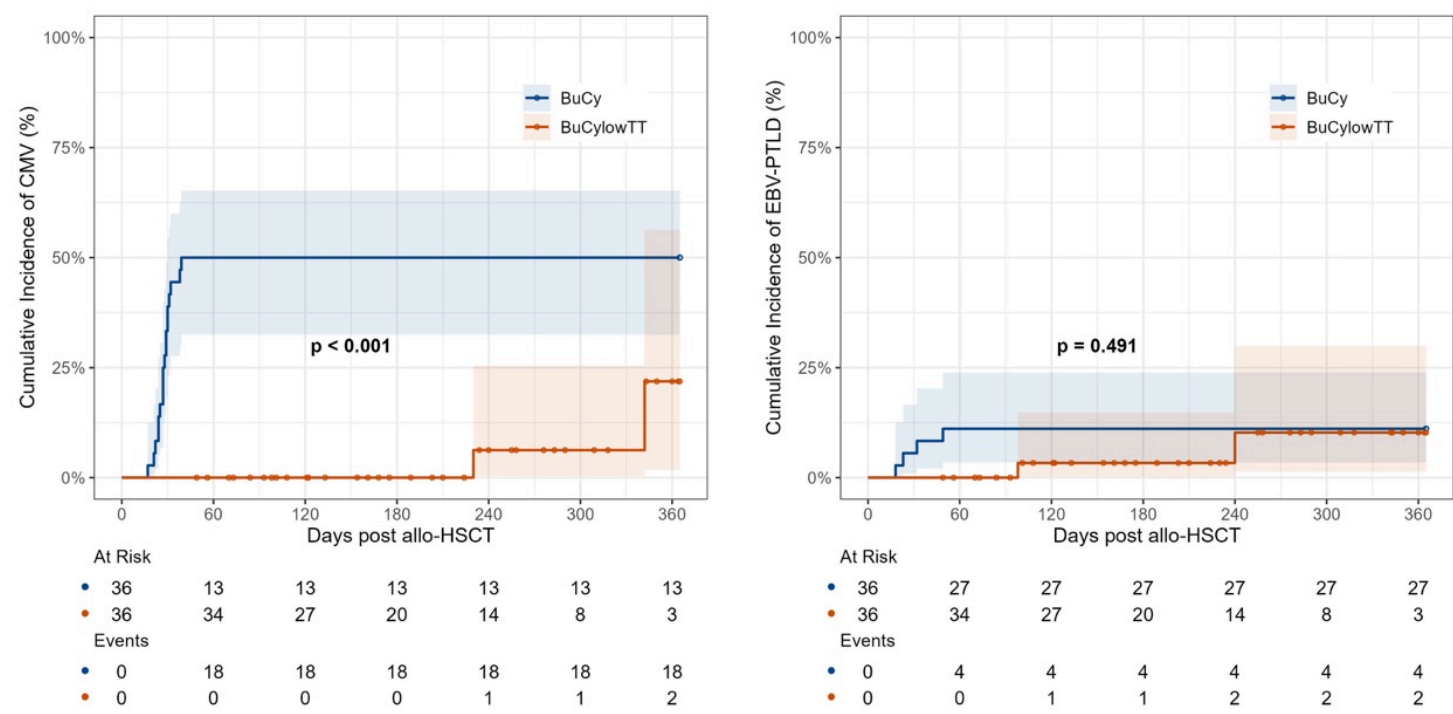


Figure 3

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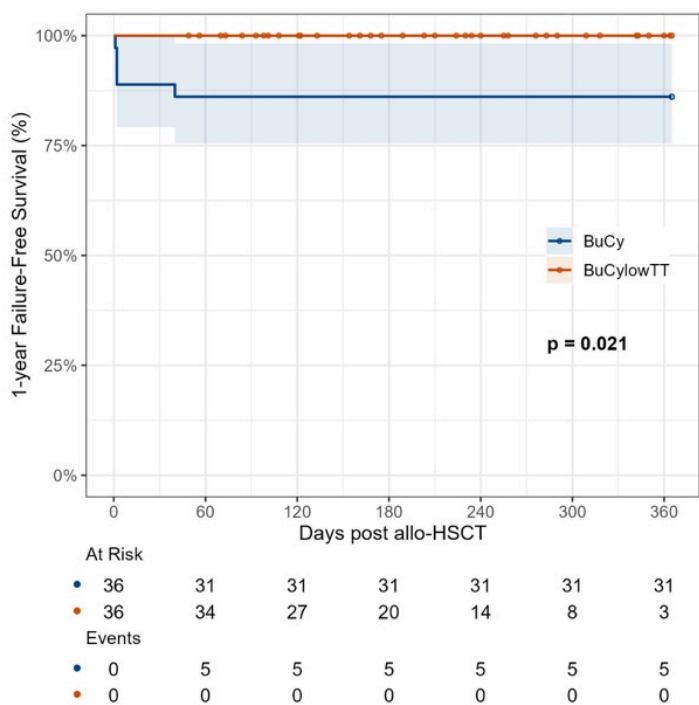
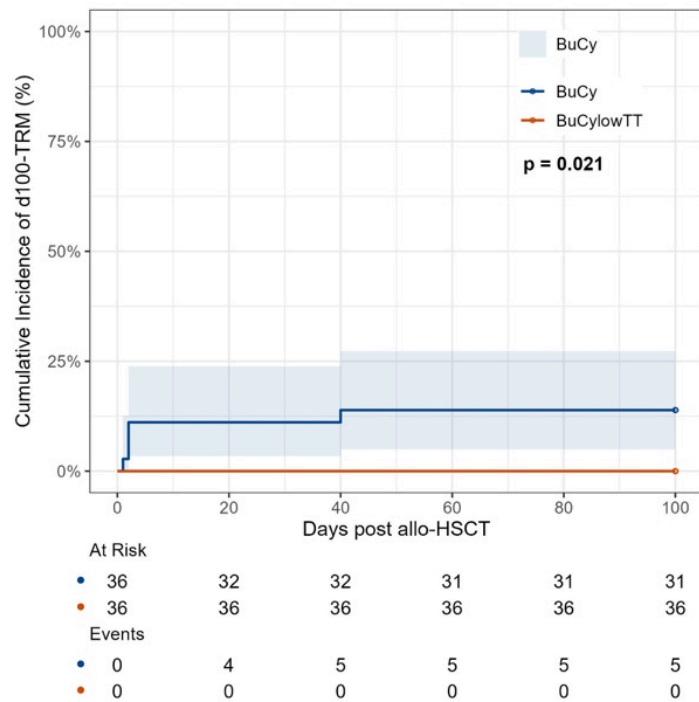
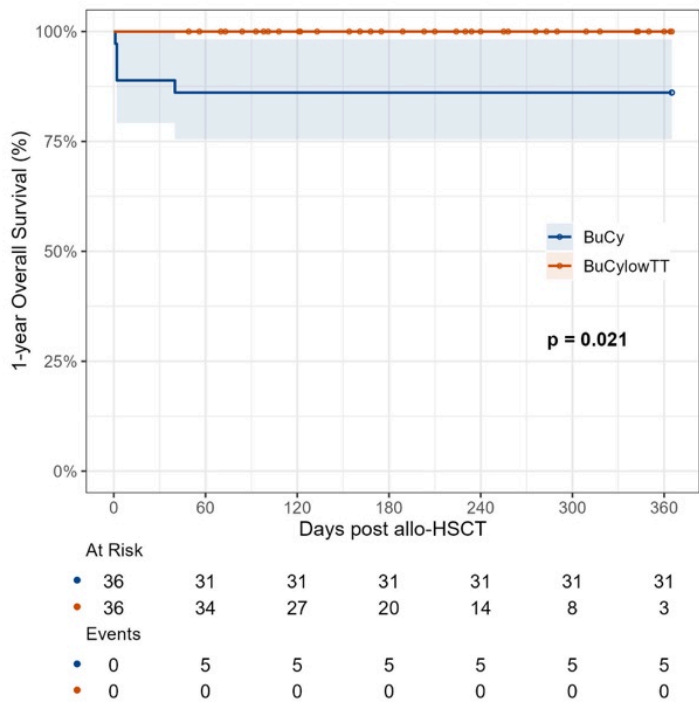


Figure 4

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