Individualized dose of anti-thymocyte globulin based on weight and pre-transplantation lymphocyte counts in pediatric patients: A single center experience

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Abstract

Anti-Thymocyte Globulin (ATG) has become standard in preventing GvHD in related and unrelated donor transplantation. There needs to be a consensus on the best administration schedule. The PARACHUTE trial reported excellent CD4 immune reconstitution (CD4 IR) using a dosing schedule based on the patient's weight and pre-conditioning absolute lymphocyte count (ALC). In 2015 we introduced the PARACHUTE dosing schedule at our center. Patients received ATG doses according to weight and ALC starting day -9. One hundred one patients were transplanted for malignant and non-malignant diseases. CD4 IR+ was seen in 81% of patients. The incidence of grade II-IV and III to IV aGvHD was 26.6% and 15.3% and 5% for cGvHD with no severe cases. We found no difference in aGvHD between donor type and stem cell sources. Five-year EFS and OS were 77.5% and 83.5%. Grade III-IV GFRS was 75.2%. CD4 IR + patients had better EFS (93.1% vs. 77.7%, p=0.04) and lower non-relapse mortality (2.7% vs. 22.2%, p=0.002). The PARACHUTE ATG dosing schedule individualized by weight and ALC results in good early immune reconstitution, low incidence of cGvHD, and favorable survival for patients with different disease groups, donor types, and stem cell sources.

Introduction

Anti-Thymocyte Globulin (ATG) is widely used during conditioning as GvHD prophylaxis in children and adults. Numerous studies have demonstrated a significant reduction in acute and chronic GvHD and improved relapse-free-GvHD-free survival (GFRS) with ATG, especially in the unrelated donor setting. (1, 2, 3). Nevertheless, there has yet to be a consensus on the best formulation, dosing, and schedule of ATG prior to transplant (4, 5, 6, 7). Pharmacokinetic studies have demonstrated wide variations among pediatric patients with slow clearance resulting in delayed immune reconstitution, increased risk of infections and transplant-related mortality, or fast clearance resulting in more GvHD (8, 9, 10). Using a validated pharmacokinetic model, Admiraal et al. concluded that a fixed 10 mg/kg dose resulted in excessive exposure for children with higher body weight and low lymphocyte count, setting the stage for individualized dosing based on these two parameters (11, 12). Children's body weight varies widely with age, and lymphocyte counts can vary from < 100/μL in patients with acute leukemia and severe combined immune deficiencies to normal levels (> 2500 /μL) in congenital anemias and inborn errors of metabolism. In the PARACHUTE phase II trial, ATG was dosed accordingly to body weight and pre-transplant absolute lymphocyte count (ALC) and administered for three or four days starting on day − 9 (13). This schedule resulted in a high rate of immune reconstitution in children, defined as a CD4 count ≥ 50/μl at day 100 post-transplant (CD4 IR), reported at 80% (95% CI 67–90) (14, 15, 16, 17). The dosing schedule proposed by the PARACHUTE trial provides a simple way to individualize ATG dosing for children in all hospital settings without the need for ATG pharmacokinetics.

The use of pre-transplant ALC to guide ATG dosing has been a topic of debate. Two recent reports found that an ALC < 500/μL conferred significantly worse survival outcomes to adults transplanted for hematologic malignancies, mainly due to an increased incidence of severe infections and non-relapse mortality (18, 19). Conversely, a study by Heelan et al. in adult patients using a fixed small dose of ATG

starting on day − 3 found no correlation between ALC measured before the first dose of ATG and outcomes (20). As previously reported, the PARACHUTE schedule may compensate for wide ALC variations, allowing patients to get a similar exposure at different weights and ALC.

Since 2015 we started the administration of individualized ATG doses according to the PARACHUTE trial in children transplanted for malignant and non-malignant diseases. The primary endpoint was to reach 80% CD4 IR+. Other endpoints of interest included a severe cGvHD incidence of < 5% and a Grade III-IV GFRS ≧ 70%. We report on outcomes, including engraftment, CD4 IR, aGvHD, cGVHD, CMV reactivation, non-relapse mortality, relapse-free GVHD-free survival (GFRS), and overall survival.

Patients and methods

We included children who received an allogeneic transplant from a matched sibling (MSD), matched unrelated donor (MUD), one antigen/allele mismatched unrelated donor (MMUD), and a single cord blood unit (CBU) from 2015 through 2021. All patients received ATG (Thymoglobulin, Sandoz) for 3 or 4 days starting on day − 9. The dose was calculated with the preconditioning weight and ALC using the PARACHUTE trial dose schedule, kindly provided by J.J. Boelens (13).

Conditioning regimens were based on underlying diseases. Briefly, patients with ALL received TBI + cyclophosphamide +/- thiotepa; AML, CML, and MDS patients received treosulfan, cyclophosphamide, and melphalan; SCID patients treosulfan and fludarabine; aplastic anemia patients fludarabine, cyclophosphamide +/- TBI 2 Gy and other immune deficiencies, congenital marrow failure syndromes and inborn error of metabolism received treosulfan, fludarabine, and thiotepa. GVHD prophylaxis included a calcineurin inhibitor (CNI) plus methotrexate or mycophenolate in bone marrow and peripheral blood transplant recipients and a CNI plus prednisone or mycophenolate in cord blood recipients. Patients received standard antibiotic and antifungal prophylaxis and management of febrile neutropenia. Chimerism was measured by SSO when the WBC exceeded 1000/ µl. We performed weekly qualitative testing for CMV and ADV and measured viral load in case of positive results. Patients received ganciclovir or valganciclovir according to reported guidelines (21). We measured CD4 counts on days 90, 180, 270, and 360. aGvHD and cGvHD were diagnosed and managed according to current guidelines (22). Briefly, we used steroids as the first line of therapy in aGvHD, and refractory patients received ruxolitinib or mesenchymal stem cells. cGvhD patients received sirolimus or ruxolitinib and added steroids for flare-ups. Patients with lung cGvHD or bronchiolitis obliterans received fluticasone, azithromycin, and montelukast. Extracorporeal photopheresis was not available.

The primary endpoint was immune reconstitution, defined as day 90 CD4 count > 50/ µL (CD40 IR+). Other endpoints of interest were non-relapse mortality, CMV reactivation as defined by any positive CMV test after transplantation, an incidence of II-IV and III-IV acute and chronic GvHD, relapse rate, event-free and overall survival, and GVHD free relapse-free survival (GFRS).

Statistics.

We analyzed event-free and overall survival, aGvHD and cGVHD incidence, and GFRS by Kaplan Meier. Comparisons were made with log-rank between malignant and non-malignant patients, donor types, and day 90 CD4 counts. Contingency tables were constructed for continuous variables and analyzed by the Fisher exact test. The Institutional Review Board approved the study (#220502002).

Results

Patient characteristics.

The study included 101 patients transplanted from 2015 through 2022. The median age was 6.0 years (range 0.3–17.2). Patients' diagnoses appear in Table 1. Forty-six patients had non-malignant diseases and 55 hematological malignancies (43 early and 12 advanced). Table 2 shows donor distribution, median pretransplant ALC, stem cell source, and conditioning intensity between diagnostic categories. Donor types and stem cell sources were evenly distributed among diagnostic groups. As expected, the mean ALC before conditioning was lower for malignant vs. non-malignant patients (1,395/µl vs. 2,327/µl, $p < 0.01$). The mean total dose of ATG administered was 7.4 mg/kg. Fourteen patients received < 6 mg/kg, and 24 patients > 8 mg/kg.

Immune reconstitution

Ninety-three patients were evaluated for CD4 IR (7 died before day + 90, 1 missing data). 81/93 (81%) had a day 90 CD4 count > 50/µL, reaching our primary endpoint. Table 3. shows the mean CD4 count on day 90 and the number of patients with CD4 IR + among donor groups. We found no difference in CD4 IR between donor types, conditioning intensity, or pre-transplant ALC (Fig. 1).

Non-Relapse Mortality

Eleven patients died from non-relapse causes between 21 and 219 days post-transplant. Infection was the most frequent cause (n = 4, including septicemia with multiple organ failure, Aspergillus, COVID-SARS 19), followed by aGvHD (n = 3), acute renal insufficiency (n = 2), pulmonary hemorrhage (n = 1) and transplantation associated microangiopathy (n = 1). The 24-month cumulative incidence of NRM was 11%(95% CI 1.9–29.5%). NRM was only 2.7%n in patients with CD4 IR + vs. 22.2% in those with CD4- IR. ($p = 0.002$, Fig. 2).

CMV reactivation

Twenty-seven patients (27.8%) had CMV reactivation after the transplant. The viral load range went from < 34.5 IU/µL (minimum quantifiable in our laboratory) to 15,900 IU/µl. We found no difference in CMV reactivation among donor groups (MSD 17%, MUD 30%, MMUD 33%, UCB 32%). The median day 90 CD4 in patients with CMV reactivation was 82/µl vs. 117/µl in those without CMV reactivation. ($p = 0.77$)).

Relapse

The 48-month cumulative incidence of relapse was 20.6% (95% CI 4.2%-45%). Patients with early disease fared better than those with advanced (18.6% vs. 28.8%), but the difference was not significant.

Survival

With a median follow-up time of 35 months for at-risk patients (range 9–98), the 5 year cumulative EFS was 77.5% (95% CI 70%-87.8%), and OS was 83.6% (95% CI 77.2%- 89.6%). EFS for patients with malignancies was 66% (95% CI -51%-78%) and 88% (95%CI 76%- 95%) for non-malignant patients(Fig. 3a). Overall survival was significantly better for patients with CD4 IR + than those with CD IR- (93% vs. 78%, p = 0.03, Fig. 3), reflecting the higher non-relapse mortality rate.

Graft versus host disease.

The 24-month cumulative incidence of grade II-IV aGvHD for 97 evaluable patients was 26.6% (95% CI: 15.8%-42.8%) and 15.3% for grade III-IV (95% CI: 4.3% -33.7%). Figure 4 shows the incidence of grade III-IV aGVHD by donor source. As expected, MSD fared significantly better than all other donor types. We also found no difference in grade III-IV aGvHD incidence between patients who received bone marrow or PBSCs from an unrelated donor (10% and 14.6%, respectively). Five patients developed cGvHD, two mild and one moderate, within 6 to 24 months after transplant. Grades II-IV and III-IV GFRS were 64.7% (95% CI 54%-73.2%) and 75.3% (95% CI 64.9%-83.1%) respectively (Fig. 5).

Discussion

The optimal dose and ATG schedule for GvHD prophylaxis have yet to be defined. The PARACHUTE trial used individual dosing based on the patient's weight and lymphocyte count resulting in 85% CD4 IR defined by a day 100 CD4 count > 50 (13). Here we report similar results in a single-center experience. This individual ATG dose schedule is safe and results in excellent outcomes. We reached our primary endpoints: an 80% CD4 IR, < 5% severe cGvHD, and > 70% Grade III-IV GFRS. Of caveat, our study included fewer patients for subgroup analyses, and results may be impaired. Interestingly, if we had considered a fixed dose between the usually recommended 6–8 mg/kg, 14% of patients would have been overdosed, and 24% under dosed.

Our study differed from the PARACHUTE trial at the following points: patients with malignancies were 54% in our study vs. 34%, and the stem cell source was peripheral blood in 61% of the patients in our study vs. 0% in PARACHUTE. We reproduced the PARACHUTE results in the rate of CD4 IR and the correlation of delayed CD4 IR with poor outcome: those with CD4 IR+ (> 75% of the patients) had an NRM of just over 2% only. Our rate of aGvHD was higher (38% vs. 17%), with 14% of patients experiencing Grade III-IV aGvHD. Even though this could be attributed to a high rate of PBSC usage, we found no difference in incidence comparing PBSC to bone marrow recipients, contrary to previous reports. (23). CBU recipients experienced a higher aGvHD rate than reported, even though only two patients received a > 2 high-resolution mismatched CBU. Nevertheless, the incidence of cGvHD was very low, with no cases of severe disease, in line with the PARACHUTE trial. A low cGvHD incidence is particularly relevant for limited

resource settings where access to therapy, such as extracorporeal photopheresis and frontline drugs, is hampered due to unavailability and high costs. In summary, these results add to the PARACHUTE study, which only included bone marrow transplant recipients, supporting that this schedule abrogates the difference between donor types and stem cell sources.

We did not see a high incidence of relapse in malignant patients, consistent with an uncompromised graft versus leukemia effect. Even though our patient population had an extensive range of pretransplant ALC (20/µL to 12,700/µL), we found no correlation between pre-transplant ALC and immune reconstitution or survival in our patient population with a wide ALC range. This likely concerns less in vivo T cell depletion (due to lower or better predictable ATG exposure) after transplant, and the better CD4 + IR aligns with that.

Individualized, weight, and ALC-based dosing is safe in children. It provides excellent transplant outcomes, with a low rate of severe GvHD or NRM and without increasing relapse rate, applicable to all donor types and stem cell sources. It also could quickly be adopted in every hospital setting, including transplant centers in lower/middle-income countries.

Declarations

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Author contribution: FB and AW were responsible for the design and review of the protocol, extracting and analyzing data, and writing of the report. CSc was responsible for database management and analysis. PC, CSo, PZ, NA were responsible for gathering data and review of the manuscript. CV was responsible of reviewing the manuscript

Competing interests: The authors have no competing finantial interests in relation to the work described

Data Availability Statement: The datasets generated during/or analysed during the current study are not available due to data protection but are available from the corresponding author on reasonable request.

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Tables

Table 1. Patient´s diagnosis

Disease groups	Disease	N=101
Congenital hematologic diseases	Blackfan Diamond	5
	Agranulocytosis	2
	Chediak Higashi	1
	Familial hemophagocytic	1
Inborn errors of metabolism	X ALD	2
	Mucopolysaccharidosis type 1	1
Primary Immune deficiencies	SCID	4
	Wiskott	3
	Hyper IgM	3
	Chronic granulomatous disease	2
	APDS1	1
	GOF STAT 1	1
	Cartilage hair hypoplasia	1
	IPEX	1
	LAD	1
Hematologic acquired diseases	Severe aplastic anemia	17
ALL	High Risk CR1 or CR2	26
	CR3, Not in remission	5
AML	CR1, CR2	11
	Not in remission	1
CML	Chronic phase resistant to TKI	2
	Blast crisis in CR	1
Myelodysplasia		4
Lymphoma	Hodgkin	2
	Non-Hodgkin	2

Table 2. Patient characteristics

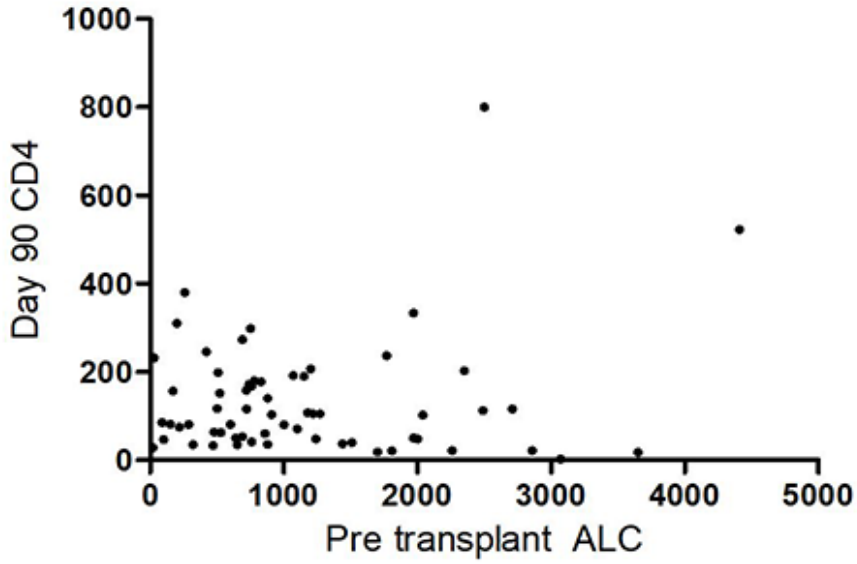
	Malignant (n=55)	Non malignant (n=46)	Total (n=101)
Age (median, range)	5(0,3-17)	5.8(0.9-17.2)	
MSD	12	11	23
MUD	16	17	33
MMUD(1 allele/Ag MM)	15	9	24
UCB (high resolution 5/8 or better)	12	9	21
Bone Marrow	10	12	22
Peripheral Blood	33	25	58
Umbilical cord blood	12	9	21
MAC	53	26	79
RIC	2	20	22
Mean pre transplant ALC	1395 (20-5950)	2327(30-12700)*	
Mean ATG dose received(mg/kg)	6.7(2-10)	1. (4.6-10)	

*(p< 000.1)

Table 3. Mean Day 90 CD4 count, CD4 IR and CMV reactivation by donor type.

Donor type (n)	Mean Day 90 CD4 (range)	CD4 IR +	CMV reactivation
MSD (21)	124 (22-249)	76%	17%
MUD (30)	184 (19-1290)	86%	30%
MMUD (24)	235(21-1985)	85%	33%
CBU (18)	183 (3-1300)	78.2%	32%
Overall	185(3-1985)	82%	28%

Figures



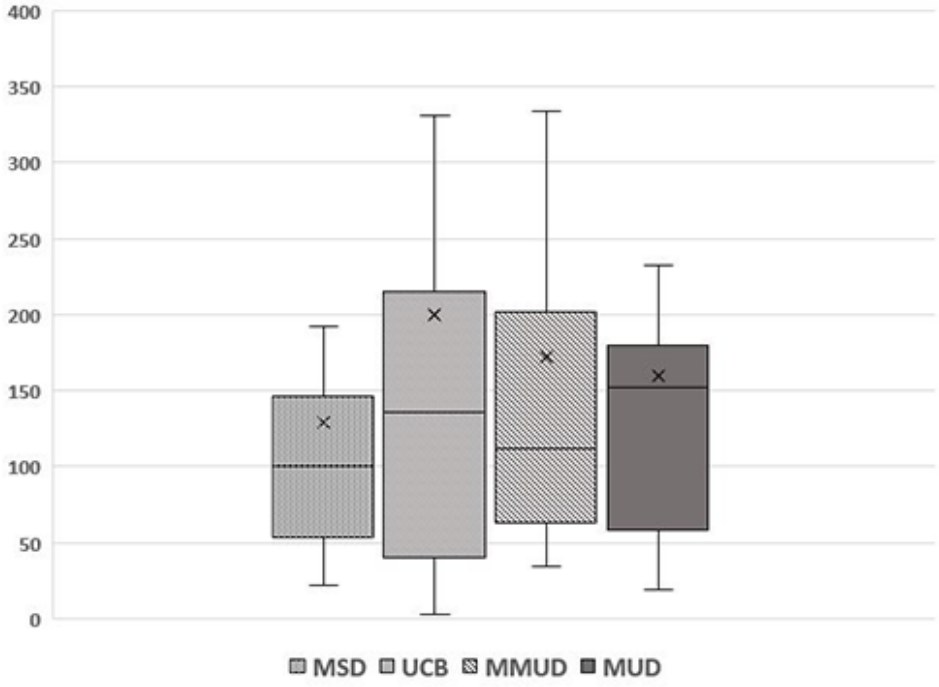


Figure 1

a) Correlation between ALC and day 90 CD4 count. b) day 90 CD4 counts by donor type.

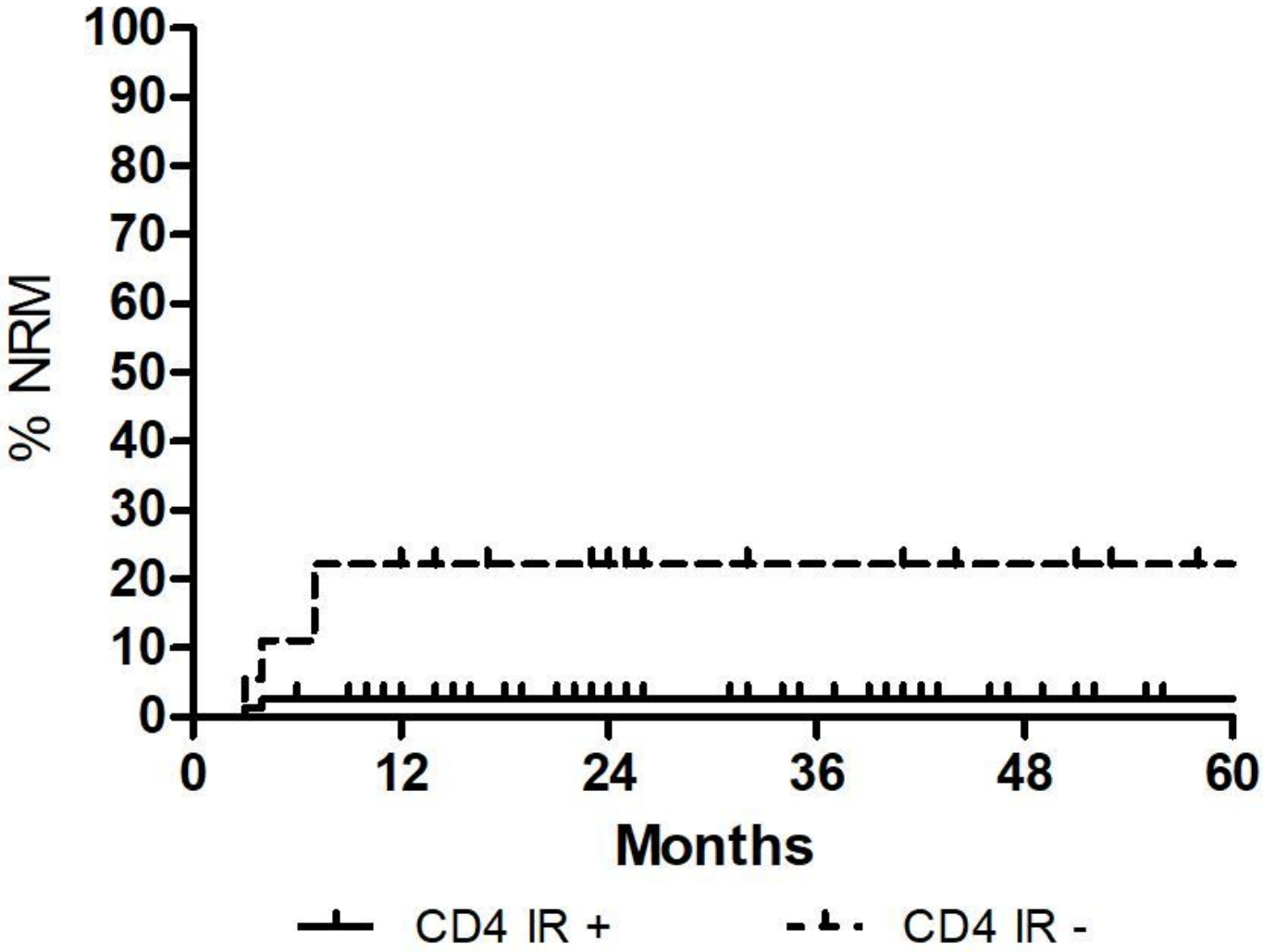


Figure 2

5-year non relapse mortality for patients with CD4 IR+ and CD4 IR-. p=0.002

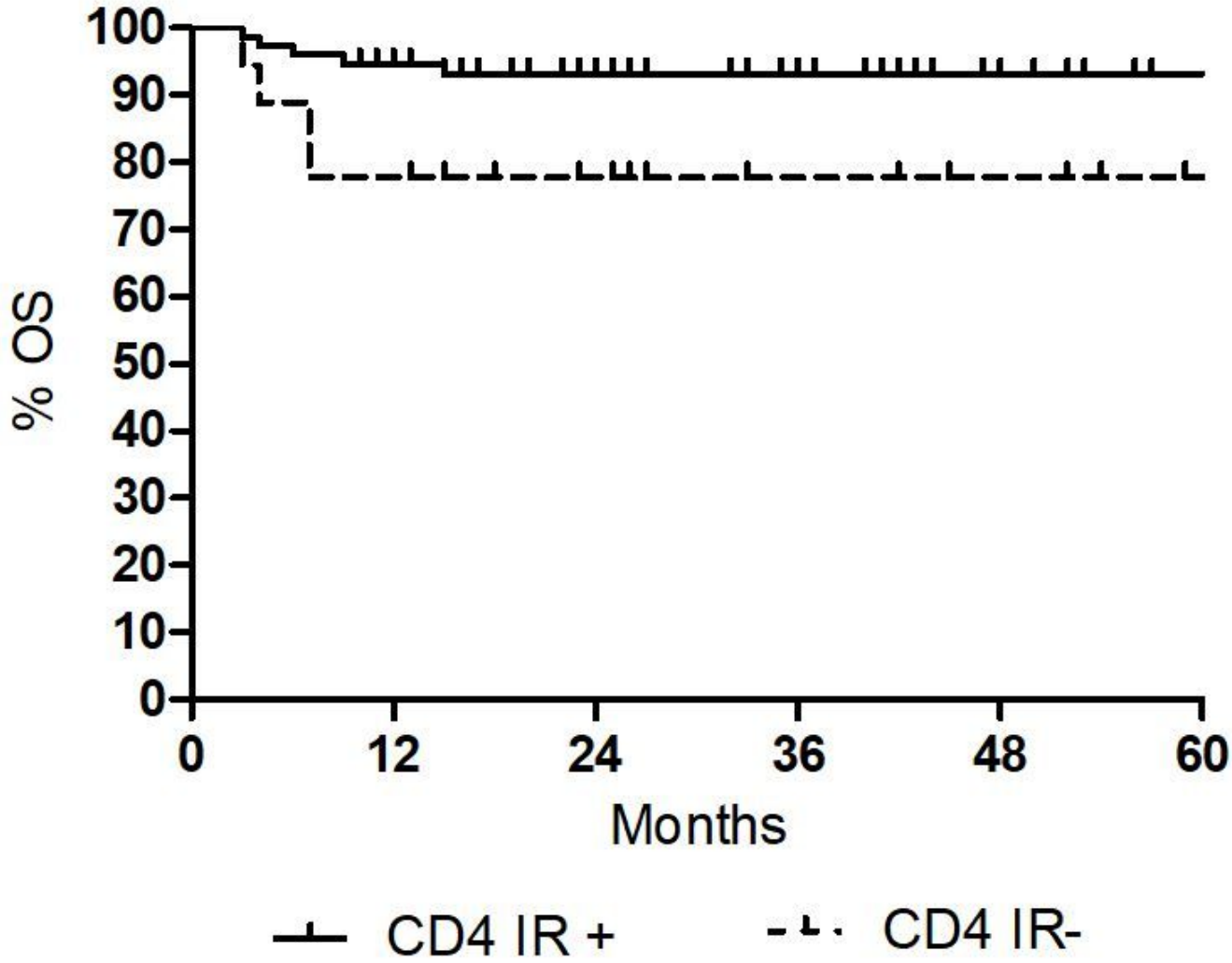


Figure 3

5-year overall survival for patients with CD4 IR+ and CD4 IR-. p=0.038

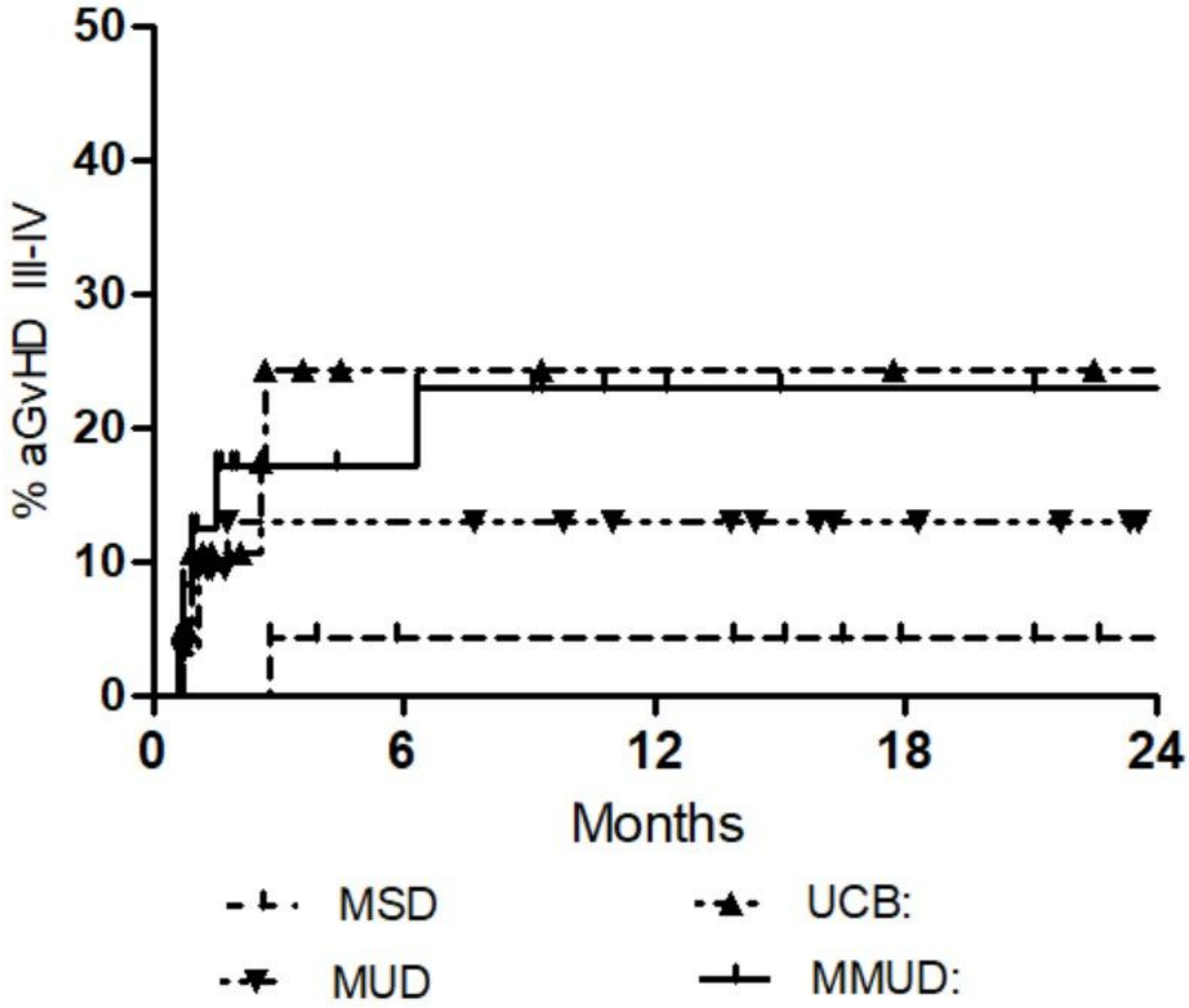


Figure 4

24-month incidence of grade III-IV aGvHD by donor type.

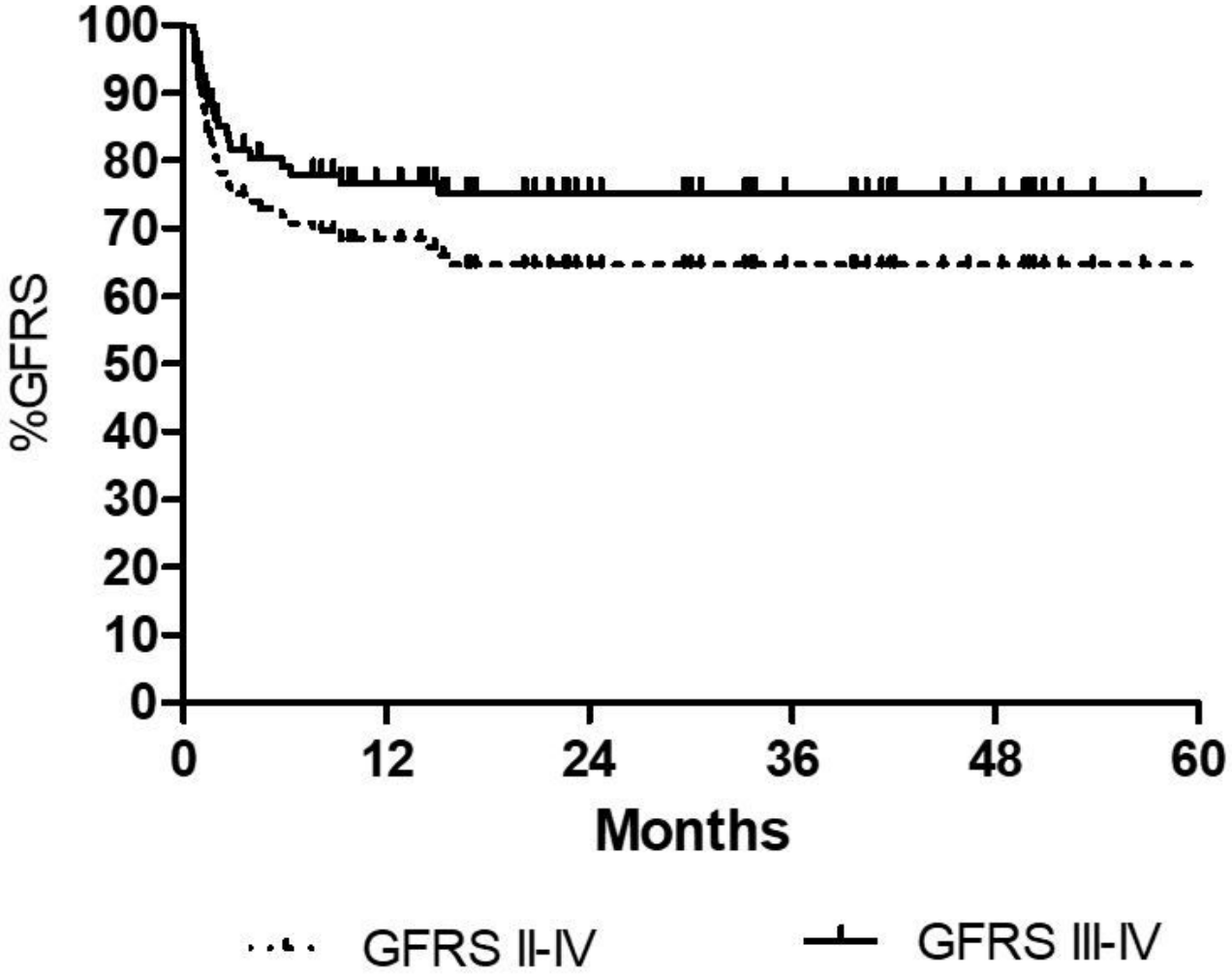


Figure 5

5-year grade II-IV and III-IV aGvHD free relapse free survival.