A 5'UTR polymorphism in NT5E gene but not fludarabine systemic exposure influences HCT outcome in patients with high-risk β-thalassemia major

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Abstract

Aim Although the fludarabine (F-araA)-treosulfan based toxicity reduced conditioning regimen has improved hematopoietic cell transplantation (HCT) outcome in patients with high-risk beta-thalassemia major (TM), rejection and regimen related toxicities (RRT) are still of major concern. This study aims to assess the role of F-araA pharmacokinetics (PK) and pharmacogenetics (PG) in a uniform cohort of patients with TM. Methods All patients with TM who receiving F-araA based regimen prior to HCT between September 2010 and 2019 were enrolled in this study. F-araA plasma levels were analyzed using LC-MS/MS. Selected polymorphisms in genes encoding for the enzymes (NT5E (Ecto-5'-nucleotidase) and DCK (Deoxycytidine kinase) involved in the metabolism of F-araA were screened. The influence of F-araA PK and PG on clinical outcomes were evaluated. Results F-araA PK showed wide inter-individual variation (27 and 19 fold in F-araA AUC and CL) which was explained by a promoter polymorphism (rs2295890) in the NT5E gene. Patients carrying the NT5E promoter variant showed no graft rejection (0% vs 7.7%, p=0.07) or Sinusoidal Obstruction Syndrome (0% Vs 19%, p=0.0007) and a trend to better EFS (87.5% vs 75.7%, p=0.1). F-araA systemic exposure was not associated with HCT outcome. Conclusion Our results suggest that the NT5E promoter polymorphism could be a predictive biomarker in F-araA based HCT setting in TM, however extensive functional studies are warranted to validate the clinical utility of this finding.

A 5′UTR polymorphism in *NT5E* gene but not fludarabine systemic exposure influences HCT outcome in patients with high-risk β-thalassemia major.

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Short Title: POPPK of fludarabine in thalassemia

What is already known about this subject?

- Despite improved HCT outcome in patients with high-risk TM using F-araA based regimen, rejection and toxicities still remain a major concern.
- Limited inconclusive data on F-araA PK in non-uniform diagnoses and dose-exposure response relationship was not evaluated in patients with high-risk TM.
- Sparse data on F-araA pharmacogenomics.

What this study adds

- Dose-exposure-response relationship of F-araA was evaluated in a large uniform cohort of patients with high-risk TM undergoing HCT.
- Wide Inter-individual variation in F-araA PK, partially explained by a genetic polymorphism in *NT5E* gene.
- F-araA PK did not explain variability in HCT outcome while patients carrying *NT5E* promoter variant had improved outcome and better survival.
- Our results suggest that *NT5E* polymorphism could be a predictive biomarker in F-araA based HCT setting in TM, however extensive functional studies are warranted to validate the clinical utility of this finding.

Abstract

Aim

Although the fludarabine (F-araA)-treosulfan based toxicity reduced conditioning regimen has improved hematopoietic cell transplantation (HCT) outcome in patients with high-risk beta-thalassemia major (TM), rejection and regimen related toxicities (RRT) are still of major concern. This study aims to assess the role of F-araA pharmacokinetics (PK) and pharmacogenetics (PG) in a uniform cohort of patients with TM.

Methods

All patients with TM who receiving F-araA based regimen prior to HCT between September 2010 and 2019 were enrolled in this study. F-araA plasma levels were analyzed using LC-MS/MS. Selected polymorphisms in genes encoding for the enzymes (*NT5E* (Ecto-5'-nucleotidase) and*DCK* (Deoxycytidine kinase) involved in the metabolism of F-araA were screened. The influence of F-araA PK and PG on clinical outcomes were evaluated.

Results

F-araA PK showed wide inter-individual variation (27 and 19 fold in F-araA AUC and CL) which was explained by a promoter polymorphism (rs2295890) in the *NT5E* gene. Patients carrying the *NT5E*promoter variant showed no graft rejection (0% vs 7.7%, p=0.07) or Sinusoidal Obstruction Syndrome (0% Vs 19%, p=0.0007) and a trend to better EFS (87.5% vs 75.7%, p=0.1). F-araA systemic exposure was not associated with HCT outcome.

Conclusion

Our results suggest that the *NT5E* promoter polymorphism could be a predictive biomarker in F-araA based HCT setting in TM, however extensive functional studies are warranted to validate the clinical utility of this finding.

Introduction

Hematopoietic cell transplantation (HCT) is the only proven curative modality available for patients with β-thalassemia major (TM). The ideal conditioning regimen for these patients, particularly those at high risk[1] remains to be defined. A toxicity reduced conditioning regimen containing Treosulfan (Treo), Fludarabine (F-araA) and Thiotepa has significantly improved transplant outcomes compared to the historical Busulfan/cyclophosphamide (Bu/Cy) based myeloablative regimen in patients with high-risk TM [1,2]. However, graft rejection, RRTs, and Graft Versus Host Disease (GvHD)[3,4] are still a major concern. Limited inconclusive data is available on the PK, pharmacogenetics (PG) and pharmacodynamics of F-araA [5–11] or Treo [12–19] in patients undergoing HCT with this regimen in patients with varying diagnoses. All these studies including ours have shown wide inter-individual variation in F-araA and Treo PK but none of the variables tested explained this variation. In our recent report on F-araA PK in patients with aplastic anemia/Fanconi anemia[9], a promoter polymorphism (rs2295890G>C) in the 5'ectonucleotidase (*NT5E* /CD73) gene, which is involved in the conversion of prodrug Fludarabine monophosphate to F-araA significantly explained this variation.

While the role of conditioning regimen drug exposure on HCT outcome has been extensively evaluated with respect to Bu/Cy regimen resulting in targeted dose adjustment of Bu to improve outcome[20–23], no such effort has yet been made for toxicity reduced conditioning regimen containing Treo/Flu/Thiotepa. Here we evaluated the PK and PG of F-araA and the role of these variables in influencing the inter-individual variability in PK and its influence on HCT outcome in a uniform cohort of patients with high-risk TM.

Patients and Methods

Patients:

Patients with high-risk TM receiving F-araA based conditioning regimen prior to HCT between 2010 and 2019 were recruited after obtaining written informed assent or consent from the patient/parents respectively. This study was approved by the Institutional review board. All patients were risk-stratified based on Vellore risk classification as published previously [24]. All patients received F-araA at a dose of 40mg/m^2/day x 4 days as 1hr infusion from day -5 to day -2 and Treo as 14g/m^2/day x 3 days at the rate 5g/hr from day -5 to day -3 and a single dose of Thiotepa on day -6 prior to HCT. Cyclosporine and short course methotrexate was used as GVHD prophylaxis [1].

Pharmacokinetics and pharmacogenetics of F-araA

Heparinized peripheral blood (5mL) was collected from the patients before and after the start of F-araA infusion on day -5 at specific time points (n=5). The plasma was obtained by centrifuging at 3000rpm for 5 minutes and stored at -80°C until analysis. F-araA levels in plasma samples were measured by LC-MS/MS as reported previously [9]. Similar to our previous study[9], selected polymorphisms in the *NT5E*(rs2295890) and deoxycytidine kinase, DCK (rs11544786) genes (with an allele frequency of >0.1 based on 1000 genome database or with clinical significance) encoding the rate-limiting enzymes in the F-araA metabolic pathway were screened using the pre-HCT genomic DNA by followed by Sanger sequencing.

Population pharmacokinetics (POPPK) of F-araA

Non-linear mixed effects modeling analysis was performed via Monolix (version 5.1.0) using the Stochastic Approximation Expectation-Maximization (SAEM) method. A two-compartment PK model was used to describe the data. The PK parameters estimated included clearance, CL (L/hr/m^2) and volume, V (L/m^2) along with the inter-compartmental clearance and peripheral compartment volume (Q (L/hr/m^2) and V_2 (L/m^2)). In addition, the individual post-hoc parameter values were used to estimate the area under the concentration curve (AUC). The inter-individual variability of the parameters was assumed to be log-normally

distributed. A combined additive and proportional residual error model was used with assumed normal distribution of the residuals.

The relationships between the PK parameters and covariates were described using the following model: $\vartheta = \vartheta_{Base} * \exp(\beta * \text{covariate})$. A covariate was considered significant in the Univariate analysis, if the addition of the covariate to the model reduced the objective function value (OFV) at least 3.84 units ($p < 0.05$, based on the χ^2 test for the difference in the -2 log-likelihood between two hierarchical models that differ by 1 degree of freedom).

Clinical Outcome :

HCT outcomes such as RRTs, engraftment, rejection, GVHD, donor-recipient chimerism status, and survival status were documented. The potential factors influencing these outcome parameters were evaluated. An absolute neutrophil count of $> 500\text{x}10^6$/L on three consecutive days was noted as neutrophil engraftment; day +28 chimerism analysis showing more than 95% of donor genetic marker patterns was considered as achieving complete chimerism (CC). Mixed Chimerism (MC) was defined as the presence of >5% residual host chimerism at any time point post HCT, whereas rejection as >90% residual host chimerism in peripheral blood as described previously [25]. The RRTs including mucositis was graded according to NCI-CTCAE V5.0 criteria [26], Hepatic Sinusoidal Obstruction Syndrome (SOS) was graded according to Baltimore criteria [27]. GVHD was graded using Glucksberg criteria [27]. Any deaths occurring within the first 100-days post HCT was regarded as Transplant Related Mortality (TRM). Early TRM (TRM D+30) and late TRM (TRM+100) are deaths occurring within 30 and 100 days post-transplantation mostly due to RRTs and infections. Event-free survival (EFS) was defined from the time of transplant to an event; an event was primary graft rejection/failure, death. Overall survival (OS) was defined as the percentage of patients who were alive at the last follow-up.

Statistical analyses:

All statistical analyses were performed by IBM SPSS statistics 21.0 (IBM Corp. Armonk, NY, USA), R Statistical software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria), and GraphPad PRISM5 software (GraphPad Software Inc, San Diego, CA, USA). Fisher's exact test and Pearson's chi-square test were used for individual parameter analysis. For testing the association between polymorphism and outcomes, we used Firth logistic regression that uses a penalized likelihood to remove much of the bias from the maximum likelihood estimates in the logistic regression model [28]. This method is best suited for the present study as the genetic variants tested are rare. Log Rank Mantel-Cox regression analysis was used for the survival analysis.

Results

Patient Demographics

Between November 2010 and 2019, 281 patients with TM underwent HCT in our centre. Of these, patients who gave consent to participate in the study, as well as those with follow-up, were enrolled for the PK study (n=169). There was no significant difference in demographics between the patients with high-risk TM enrolled in the PK study compared to the total number of patients with high-risk TM who underwent HCT during the study period. (Table-S2) Two hundred and eighty-one patients with high-risk TM underwent HCT with Thio/F-araA/Treo conditioning regimen during the study period. Their median age was 9yrs (1-25yrs). Majority of the patients belonged to class III (Class III High Risk-48%; class III Low risk-39%) and 12% of patients belonged to class II. The demographics of these patients are summarized in **Table 1.**

F-araA PopPK and PG:

F-araA PK was available for 169 patients enrolled in the study. Thirty-two (11.7%) and 19 (10.3%) patients carried variant allele for *NT5E* and *DCK* polymorphisms. The population pharmacokinetic parameters are shown in **Table 2** . The median post hoc estimated F-araA AUC and CL for the first dose was 19 (3-81) μmol*h/mL and 7 (2-38) L/h/m^2. The PopPK model estimated significant inter-individual variation (IIV) in

F-araA PK (27 and 19 fold in AUC and CL). F-araA Cl was significantly lower in patients with *NT5E*variant rs2295890 genotype (5.37 vs 7.17 L/h/m^2; p=0.001). These differences translated to significantly higher AUC in patients with variant rs2295890 genotype (26.5 vs 18.0 µM*h; p=0.01) (Figure 1). The *NT5E* variant explained 4.5% of the IIV on the clearance of F-araA. None of the other demographic/biochemical covariates including *DCK* polymorphism explained IIV in F-araA PK.

HCT outcome:

HCT outcome endpoints are listed in Table-S1. Patients were followed up for a median of 30 (0.3–108) months. Fourteen patients (5.0%) died early due to RRT and other transplantation-related complications, while 267 patients had documented engraftment (median day of engraftment was 16 days (range: 10–43 days). Post-transplant hematopoietic chimerism evaluated in all patients who were alive beyond day +28 post HCT (n=264) showed complete chimerism (CC) in 239 (90.5%), and mixed chimerism (MC) (3-97% recipient cells) in 25 (9.5%) on day +28 post HCT. Twenty-two of the 264 evaluable patients (8.3%) rejected their graft with the median time of rejection of 2 months (0.7-14.2 months). Hepatic SOS and mucositis were documented in 54 (19.2%) and 146 patients (I-2.8%, II- 25.2%, III- 22.7% IV-1.06%) respectively. Seventy-two (25.6%) patients developed acute GVHD while thirty-one (11%) had chronic GVHD. Overall, 225 (80%) patients were alive at the last follow-up and the median event-free survival (EFS) was 76.8%. Fifty-six patients died before day +100 (D+100 TRM). The major causes of death were steroid refractory GVHD (35.7%), sepsis (33.9%), and fungal infections (12.5%), SOS (10.7%), and multi-organ failure (7.1%).

Role of F-araA PK and PG on HCT outcome:

None of F-araA PK parameters was associated with OS, EFS, TRM, and RRTs in 169 patients for whom PK data was available. Interestingly, patients carrying the *NT5E* promoter variant (rs2295890) showed a trend to no rejection (0% vs 7.7%, p=0.07), better EFS (87.5% vs 75.7%, p=0.1), lower late TRM D+100 (0.3% vs 12.5%, p=0.08), better OS (89.7% vs 78%, p=0.25) and lower early TRM D+30 (0.3% vs 8.8%, p=0.2) **(Figure 2)** . Additionally, none of the patients carrying the *NT5E* promoter variant developed SOS compared to those with wild-type genotype for this variant (0% Vs 19%, p=0.0007). Logistic regression using the penalized maximum likelihood estimation method highlighted that *NT5E*promoter variant (rs2295890) has a protective effect on HCT outcome**(Table-3)** . No association was observed between DCK polymorphism and outcomes.

Discussion :

Although the toxicity-reduced conditioning regimen containing F-araA/Treo/Thiotepa has a favorable toxicity profile and has shown to improve HCT outcome in high-risk TM patients [1], graft rejection and RRTs still present a roadblock in a subset of patients[3,4]. In this first single centre study, we have evaluated the PK and PG as well as the dose-exposure-response relationship to F-araA in a large uniform cohort of patients with high-risk TM undergoing HCT.

F-araA PK has been reported previously in patients undergoing HCT for both malignant, benign conditions and in various combination[7–11,29–34]. Despite wide IIV in F-araA PK in the present study, none of the biochemical or demographic parameters explained this variability. Previous F-araA PopPK studies have identified Glomerular filtration rate (GFR) [11,34] and creatinine clearance [8,10] as significant predictors of F-araA CL. In the present study, we did not include GFR as a covariate in the PopPK model as the patients enrolled in the study had a normal renal function and all the patients received a fixed initial dose of F-araAMP. The dose of F-araA used and the PK parameters in the present study are comparable to the existing reports **(Table 4)** .

Although the dose-exposure response relationship has been explored previously for Bu [35–40], Cy [41,42], and Treo [43], no such attempt had been made for F-araA in patients with TM. Several studies have described the influence of F-araA PK in HCT outcomes, albeit majority of the studies were conducted in patients undergoing HCT for malignant conditions[6,7,10]. A recent study carried out in a mixed cohort of patients with malignant and non-malignant [34]conditions did not identify any relationship between F-araA PK and

HCT outcomes. Despite significant IIV in F-araA PK observed in the present study, none of the F-araA PK parameters was associated with HCT outcomes. This could probably be because of the decreased incidence of events such as rejection or TRM in this non-malignant condition. A recent study in F-araA PK also predicted optimal cumulative exposure of 20 mg*h/L for better EFS, lower TRM, and lower rejection[44]. However, the study cohort was heterogeneous, and the optimal exposure range was not confirmed in an independent cohort[44].

Genetic variants in drug-metabolizing enzymes and transporters may also contribute to PK variability, which in turn could influence HCT outcome. Similar to our previous report on F-araA PK in patients with AA/FA undergoing HCT [9], the patients carrying rs2295890 variant genotype exhibited significantly lower plasma F-araA CL compared to those with wild-type genotype in the present study **(Figure 1)** . This variant also explained 4.5% of the IIV in F-araA clearance in the POPPK model. Apart from its role in the biotransformation of F-araA[45], *NT5E/CD73* is a multifunctional ectoenzyme involved in immunosuppression [46], cancer progression[47], and tumor microenvironment[48,49]. When we compared the role of this polymorphism on HCT outcomes, we observed that patients carrying the rs2295890 variant genotype showed better OS, EFS, lower rejection and lower TRM, consistent with our previous finding in AML cohort [50]. Low *NT5E* activity has been reported to be associated with a good prognosis in many malignancies [46,51,52] probably due to the production of less adenosine that suppresses antitumor immunity and by not contributing to metastasis. The role of NT5E activity in HCT setting has not been explored except for few mice model studies, where it was suggested that low *NT5E* activity could lead to GvL/GvT (Graft Versus Leukemia/Tumor) phenomenon favoring HCT outcome, again reinstating the probable role of Adenosine 5'-triphosphate (ATP)-Adenosine axis in transplant immunology [53–56]. We could thus hypothesize that due to the reduced NT5E activity in patients carrying the variant genotype for this polymorphism, there is a lower production of adenosine and higher extracellular ATP activity, which in turn could prevent graft rejection and help in immunosuppression, eventually favoring better HCT outcome. In addition, we observed that none of the patients carrying the rs2295890 variant genotype had SOS. This could also be due to decreased *NT5E*activity in patients carrying the variant genotype for this polymorphism resulting in decreased production of adenosine, thus protecting the liver from fibrosis [57,58]. However, the exact mechanism between decreased *NT5E* activity and SOS needs to be explored further for its implication in pharmacogenetics testing as a plausible biomarker for HCT outcome.

Conclusion:

Our study demonstrates that F-araA PK does not predict HCT outcome in patients with high-risk TM. *NT5E* promoter polymorphism could be a predictive biomarker in F-araA based HCT setting in TM; however, extensive functional studies are warranted to validate the clinical utility of this finding.

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Conflict of Interest

The authors declare no conflict of interest.

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Contributors

A.A.P., E.M., P.B., J.C.P., A.S., and V.M. wrote the article; P.B. designed the research; A.A.P., E.M., P.B., J.C.P., B.B., R.S.S.I., B.R.R., E.S.E., A.K., F.N., A.A., A.V., B.G., A.S., and V.M. performed the research; A.A.P., E.M., P.B., and K.M.L. analyzed and interpreted the data. All authors contributed to the writing of the manuscript, provided critical review, and approved the final version.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1: Patient Characteristics

Parameters
Age (years)
Sex (Male/Female)
Stem Cell Source Bone marrow Peripheral Blood
HLA Match Identical Mismatch
CD34 Cell dose (x10^{6}cells/kg)
Lucarelli Classification Class I Class II Class III **Vellore Risk Classification** Class III High Risk Class III Low Risk
Donor type Matched sibling donor (MSD) Matched related donor (MRD) Matched unrelated donor (MUD)
Polymorphisms *NT5E* **(rs2295890)**[a] Homozygous reference Heterozygous variant Homozygous variant *DCK* **(rs115447**

[a] *NT5E*genotyping was performed for 271 patients

[b] *DCK* genotyping was performed for 176 patients.

NA- Not applicable

Ταβλε 2: Ποπυλατιον πηαρμαςοκινετιςς (ΠοπΠΚ) οφ Φ-αραΑ ιν β-τηαλασσεμια πατιεντς υνδεργοινγ Η̈Τ

Parameter	**Base**	**RSE (%)**	**rs2295890**	**RSE (%)**	**p-value**
CL (L/h/m^2)	6.87	4.8	7.17	5.2	
β: CL*			-0.29	43.3	0.02
V (L/m^2)	18.74	4.5	18.55	4.7	
Q (L/h/m^2)	14.21	0.2	14.46	0.2	
V_2 (L/m^2)	25.67	1.3	25.99	1.8	
σ additive (μM)	0.06	20.0	0.06	15.4	
σ prop CV%)	0.10	4.1	0.10	6.3	
-2 Log-likelihood[b]	1750.2		1739.5		0.001
IIV	**(CV%)**	**RSE (%)**	**(CV%)**	**RSE (%)**	
CL	0.57	6.6	0.56	6.8	
V	0.33	21.2	0.34	19.8	
Q	0.70	11.8	0.71	9.1	
V_2	0.40	12.8	0.44	13.5	

[a] Covariate model: ϑ*exp(β*covariate). rs2295890: 0=WT, 1=HET/MUT.

[b] p-value represents the significance of the change in the -2 log-likelihood (based on theχ^2 test) relative to base model

Abbreviations: RSE, relative standard error; CL, clearance; V, volume of distribution; Q, intercompartmental clearance; CV%, coefficient of variation; IIV, interindividual variation.

Table 3: Association of *NT5E* 5'UTR Polymorphism (rs2295890) and HCT outcome

Clinical Outcomes	***NT5E* 5'UTR Polymorphism (rs2295890)**	***NT5E* 5'UTR Polymorphism (rs2295890)**
	Wild type (n=239) n(%)	**Heterozygous/ Mutant type (n=32) n(%)**
Rejection	21 (7.7)	0 (0)
Incidence of SOS*	52 (19)	0 (0)
TRM D+30*	24 (8.8)	1 (0.3)
TRM D+100*	34 (12.5)	1 (0.3)
EFS	181 (75.7)	28 (87.5)
OS	190 (78)	28 (87)

Abbreviations: SOS- Sinusoidal Obstruction Syndrome, TRM- Transplant related mortality, OS-Overall Survival, EFS- Event free survival.

[a] For TRM and incidence of SOS, ORs and p-values were calculated using penalized likelihood test-Firth logistic regression method using R. Cox regression using SPSS was used in calculating ORs and p-values for EFS, OS and Rejection.

Table 4: Comparison of F-araA PK with previous reports

S.No	**Diagnosis**	**N**	**Conditioning regimen**	**Flu Dose**	**Donor type**	**AUC (uM*h) Median(range)**	**CL (L/h/m^2) Median (range)**	**Refere**
1	CML-04 & MDS-38	42	Flu : 4 days & Oral Bu : 4 days	30mg/m^2/day	MRD-16 MUD-26	Mean + SD (Range) 19.1+7 (8.0-45.2)	Mean + SD (Range) 6.3+2.4 (2.2-13.3)	[29]
2	AML-05, MF-05, CML-01 CMML- 01 & MDS- 03 AML/MDS-01	16	Flu: day -6 to -3 & Bu: day -5 to -2	30mg/m^2/day	-	21.03 (10.17-38.56)	5.04 (2.7-10.2)	[6]
3	ALL-06, AML-26, NHL-17, MDS-14, HL-08, CML-01 & Others[a]-15	87	Flu: days -6 to -2 & Cy: day-6	40mg/m^2/day (N=78) or 30-35mg/m^2/day (N=9)	MRD-22 MUD-65	40mg/m^2: 17.19 (7.02-40.35) 30-35mg/m^2 19.29 (15.1-24.56)	40mg/m^2: 16.0 (6.2–36.6)L/h 30-35mg/m^2: 11.5 (6.9–15.2) L/h	[7]

S.No	Diagnosis	N	Conditioning regimen	Flu Dose	Donor type	AUC (uM*h) Median(range)	CL (L/h/m^2) Median (range)	Refere
4	AML-05, CML-01, MDS-04, MF-05 & CMML-01	16	Flu: days -6 to -2; targeted daily IV Bu days -5 to -2 & rATG on days -3 to -1	50 mg/m^2 /day	MRD-11 MUD-05	24.8 (16.3-39.9)	-	[30]
5	-05, HL-03 & /-03	11	Flu: days -6 to -2; Cy: days -6 and -5 & TBI day -1	30mg/m^2/day	Haplo-11	16.4 (10.4 – 21.5)	-	[31]
6	MDS-18, AML-13, CML-05, CMML-02 & MF-03	41	Protocol 1519 (N=27) Flu: days -9 to -6; targeted oral Bu days -5 to -2 Protocol 2041 (N=14) Flu: days -6 to -2, targeted daily IV Bu on days -5 to -2, and rATG IV on day -3, to -1	30 mg/m^2/day 50 mg/m^2/day	-	-	Protocol 1519: 9.1 (8-45.2) Protocol 2041: 7.07 (4.40-10.76)	[32]
7	NHL-34, CLL-22, AML-15, MDS-10, MM-09, ALL- 04, MF- 03, AA- 02 HL- 02 & PNH - 01	102	Flu: days -4 to -2 & 2-4.5 Gy TBI	30 mg/m^2/day	MRD-24 MUD-78	Mean + SD (Range) 19.6±4.8 (10–36.4)	-	[33]

S.No	Diagnosis	N	Conditioning regimen	Flu Dose	Donor type	AUC (uM*h) Median(range)	CL (L/h/m^2) Median (range)	Refere
8	AA – 40 & FA-13	53	Flu: days -6 to -2 & Cy: days -3 & -2	30mg/m^2/day	MSD-45 AD-08	20 (4-53)	4.7 (1.2-22.4)	[9]
9	HM-59, PID-18, HP-8, IMD-22, BMF-22 & EB-4	133	Bu/Flu Cy/Flu Bu/Flu/Clo Flu/ThioT/Mel Others	40 mg/m^2/day 12.5-45mg/m^2/day 0.9 - 1.33 mg/kg	MRD-38 MUD-95	13 (11.5-15.7)	3.3 (L/h/15kg)	[10]
10	AL-29, JMML/ALCL-2, SAA-3, CGD-3 OS-2 & Others[a]-4	43	Flu: day -8 to day -3 & Bu: day -8, targeted Bu from day -7 to -5	40 mg/m^2/day	MRD-6 MUD-34 Haplo- 3	15 (10.1-30.6)	6.47	[34]
11	BD-69 L-117 LY-17 MDS-32 PCD-23	258	Bu/Flu: day - 5 to day – 2 Bu/Flu/Clo in children with malignancies rATG in MUD (day - 9 for children, and-12 (for adults)	40 mg/m^2/day	-	Children 18.4 (5-36.8) Adults 22.8 (11.4-57)	3.2 (L/h/)	[11]
12	TM	169[b]	Flu: day -5 to -2 Treo: day -5 to -3 ThioT on day -6	40mg/m2/day	MSD-152 MRD- 11 MUD- 29	19 (3-81)	7 (2-38)	*Present Study*

Abbreviations: AA – Aplastic Anaemia; AL- Acute Leukemia; ALCL- Anaplastic large cell lymphoma; ALL- Acute lymphoblastic leukemia; AML -Acute Myeloid Leukemia; BD- Benign Disorder; BMF- Bone marrow failure; CGD- Chronic granulomatous disease; CLL- Chronic lymphocytic leukemia; CML- Chronic Myeloid Leukemia; CMML- Chronic myelomonocytic leukemia; EB- Epidermolysis bullosa; FA- Fanconi Anemia; JMML- Juvenile myelomonocytic leukemia; SAA- Severe Aplastic Anaemia; CGD- Chronic granulomatous disease; HM- Hematologic malignancies; HL- Hodgkin lymphoma; HP- Hemoglobinopathies; IMD- Inherited metabolic disorders; L- Leukemia; LY- Lymphoma; MDS- Myelodysplastic Syndrome; MF- Myelofibrosis; MM- Multiple Myeloma; NHL- Non-Hodgkin lymphoma; OS- Osteopetrosis; PCD-Plasma Cell Disorder; PID- Primary immune deficiencies; PNH- Paroxysmal nocturnal hemoglobinuria; TM- Thalassemia Major.

[a] Other diagnoses includes Hemophagocytic lymphohistiocytosis, Wiskott–Aldrich syndrome, Adrenoleukodystrophy and Krabbe disease.

[b] F-araA PK was evaluated only in 169 patients

Figure Legends:

Figure 1: Influence of NT5E/CD73 5'UTR polymorphism (rs2295890) in F-araA PK.

Association between rs2295890 genotype and post-hoc PK estimates- F-ara AUC (A) and Clearance (B). *Wt- Homozygous reference genotype, Het/Mut- Heterozygous and homozygous genotype, p-value was calculated by Mann Whitney U Test. Patients harboring NT5E promoter variant genotype exhibited significantly lower plasma F-araA CL and higher AUC compared to those with wild-type genotype.

Figure 2: NT5E 5'UTR variant genotype is associated with better OS, EFS, and reduced incidence of graft rejection

Kaplan-Meier survival curves showing associations between NT5E 5'UTR variant (rs2295890) genotype with Overall Survival (A), Event Free Survival (B), and graft rejection (C). Patients carrying the NT5E promoter variant genotype tended to have a better OS (p=0.25), EFS (p=0.12), and rejection-free survival (p=0.07) compared to those with wild-type genotype.