Population pharmacokinetics of fludarabine in patients with aplastic anemia and Fanconi anemia undergoing allogeneic hematopoietic stem cell transplantation

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is one of the curative modalities of treatment in patients with bone marrow failure conditions including aplastic and Fanconi anemia (FA). Cyclophosphamide (Cy)/anti-thymocyte globulin (ATG) is considered the standard conditioning regimen for patients with severe aplastic anemia (SAA) undergoing HSCT from a HLA-matched related/unrelated donors. Cyclophosphamide (Cy)/anti-thymocyte globulin (ATG) is considered the standard conditioning regimen for patients with severe aplastic anemia (SAA) undergoing HSCT from a HLA-matched related/unrelated donors. Although hematopoietic stem cell transplantation (HSCT) with a conditioning regimen consisting of fludarabine (F-araA) and cyclophosphamide (Cy) is associated with improved outcome in young patients with aplastic anemia (AA) and Fanconi anemia (FA), several factors limit the success of the procedure. We evaluated the population pharmacokinetics (POP PK) of F-araA and its influence on HSCT outcome in patients (n = 53) with AA and FA undergoing HSCT. Patients carrying a 5′-UTR polymorphism in NT5E gene (rs2295890 G>C) exhibited significantly lower plasma F-araA clearance compared to those with wild-type genotype (7.12 vs 5.03 L/h/m² (29%) P < 0.05). F-araA clearance was significantly higher in patients with AA compared to FA (2.46 vs 1.04 L/h/m² P < 0.0001). Of all the outcome parameters evaluated (engraftment, rejection/graft failure, GvHD, TRM, OS), high F-araA AUC (> 29.4 μg/h/L) was the only significant factor associated with the development of aGvHD by both univariate and multivariate analysis (P = 0.02). The influence of plasma F-araA levels need to be evaluated in a larger cohort of patients to propose the need for therapeutic drug monitoring.
Table 1. Patient demographics

| Characteristics (N = 53) | Median (Range) |
|-------------------------|----------------|
| Age, yrs                | 17 (3–57)      |
| Body weight, Kg         | 50 (12–89)     |
| BSA, m²                 | 1.49 (0.56–1.9) |
| Sex                     | 35 males; 18 females |

Diagnosis
- Aplastic anemia: 40
- Fanconi anemia: 13

Regimen
- F-araA/Cy: 29
- F-araA /Cy/TBI: 20
- F-araA /Cy/ATG: 4

Donor source
- Matched sibling donor: 45
- Alternate donor: 8

Stem cell source
- Bone marrow: 2
- Peripheral blood: 48

CD34 cell dose (×10⁶/kg) 9.8 (1.3–15.0)

HLA Match
- < 8: 6
- ≥ 8: 47

GvHD prophylaxis
- Cyclosporine/Methotrexate: 32
- Post T, Cy: 19
- Not Evaluable: 2

SNP frequency in F-araA metabolic pathway genes

| Gene       | SNP                  | Variant genotype | WT  |
|------------|----------------------|------------------|-----|
| NT5C2      | rs4917996A            | GT               | C   |
| CNT3       | rs7837358G            | A                | C   |
| NTSC2      | Intron rs4917996A     | G                | C   |
| hENT1      | Exon 1 rs747199G      | A                | C   |

Abbreviations: ATG = anti-thymoglobin; Cy = cyclophosphamide; F-araA = fludarabine; SNP = single-nucleotide polymorphism; Var = variant genotype; WT = wild-type genotype; yrs = years.

January 2012 and December 2014 receiving an F-araA based conditioning regimen were prospectively included in the study after obtaining written informed consent. This study was approved by the Institutional review board. All patients with AA received F-araA (30 mg/m²/day for 6 days over 1 h infusion from day −7 to −2) and cyclophosphamide (50 mg or 60 mg/kg/day for 2 days over 1-h infusion on day −3 to −2) prior to HSCT. Cyclosporine (2.5 mg/kg/dose, BD) and methotrexate as post-transplant cyclophosphamide (50 mg/kg/day for 2 days) was given as GvHD prophylaxis. Patients with FA received the same dose of F-araA, cyclosporine and methotrexate as that of AA, while the cyclophosphamide was given as 10 mg/kg/day × 2 days on day −3 to −2 and post-transplant cyclophosphamide was given at 25 mg/kg/day × 2 days as GvHD prophylaxis (Table 1).

Reagents and chemicals
F-araA (Cat no: F2773) and the internal standard (IS) S-fluorocytidine (S-FC; Cat no: 543020) were purchased from Sigma-Aldrich, Bengaluru, India. The other reagents and chemicals N, N-Dimethylformamide, acetonitrile, ammonium acetate and acetic acid used were of Mass spectrometry grade from Fluka Analytical, Sigma-Aldrich Co., St Louis, MO, USA. Standards for F-araA assay were prepared in drug-free blank plasma (obtained from the Christian Medical College hospital blood bank).

Analysis of plasma F-araA using liquid chromatography-tandem mass spectrometry (LC-MS/MS)
F-araA levels in plasma samples were measured by LC-MS/MS method using a Shimadzu-Prominence UFLC consisting of binary gradient pumps (LC-20AD), autosampler (SIL-HTC), mobile phase degasser (DGU20A) and a column oven (CTO-20A) coupled with API2000 triple quadrupole mass spectrometer (ABSciex, MDS Sciex Inc., Toronto, ON, Canada). The system was managed using Analyst 1.4.2 software (ABSciex, Foster city, CA, USA). The mass spectrometry conditions were optimized with a separate external injection of 1000 ng/mL concentration of both pure F-araA and 5-FC at the rate of 10 μL/min. The parameters were adjusted to yield a maximum multiple reactions monitoring (MRM) signals (Supplementary Table S1). The Q1/Q3 for F-araA was set at 286.0/154.0 and 262.1/130.0 for internal standard, 5-FC in the positive ESI mode respectively. Chromatographic separation of the analyte was done using Syncronis C8 (2.1 × 50 mm, 5 μm, Thermo Scientific, Inc.) protected with a C8 guard column (10 × 2.1 mm, 3 μm) from the same source (Thermo Scientific, Inc., Madison, WI, USA). The LC conditions were as follows: Solvent A: 10 mL ammonium acetate (pH 5.0) and Solvent B: 100 mL acetonitrile was used as mobile phase with gradient elution of solvent B at 95% (0–0.5 min); 60% (0.5–2 min); 30% (2–4 min); 95% (4–7 min) at a flow rate of 0.25 mL/min. Retention time for F-araA was 1.26 min and the IS 1.10 min. The concentration of F-araA was expressed as ng/mL. The LLOQ was recorded to be 1 ng/mL and the method was linear for a wide concentration range from 7–700 ng/mL (1–100 μM) with mean R² = 0.99 ± 0.001 (Linearity, Accuracy and inter-day precision are as shown in Supplementary Table S2).

Sample collection and processing
Peripheral blood (5 mL) was collected in sodium heparin tubes before the start (0 h) and 1, 2, 3, 5, 7 and 24 h after the infusion of F-araA, centrifuged immediately at 3000 rpm for 5 min at 4 °C. Plasma was separated and stored at −80 °C until further analysis. The F-araA PK samples were collected on HSCT days −7, −4, −3, −2; the PK sampling began with the start of F-araA administration. Series of F-araA standards (25 μL of 1–10 μM) and F-5FC (25 μL of 250 ng/mL) were added to pre-labeled tubes containing 250 μL of drug-free blank plasma and vortexed thoroughly for 30 s. Ice cold acetonitrile was added and centrifuged at 13 000 r.p.m. for 25 min at 4 °C. The supernatants were dried under nitrogen gas at 40 °C. The residue was resuspended in 250 μL of mobile phase (10 mL Ammonium acetate pH 5.0 and 50% methanol) and the concentration was measured by liquid chromatography-mass spectrometry (LC-MS/MS) using a Shimadzu-UPLC consisting of binary gradient pumps, an autosampler (SIL-HTC), mobile phase degasser (DGU20A) and a column oven (CTO-20A) coupled with API2000 triple quadrupole mass spectrometer (ABSciex, MDS Sciex Inc., Toronto, ON, Canada). The system was managed using Analyst 1.4.2 software (ABSciex, Foster city, CA, USA). The mass spectrometry conditions were optimized with a separate external injection of 1000 ng/mL concentration of both pure F-araA and 5-FC at the rate of 10 μL/min. The parameters were adjusted to yield a maximum multiple reactions monitoring (MRM) signals (Supplementary Table S1). The Q1/Q3 for F-araA was set at 286.0/154.0 and 262.1/130.0 for internal standard, 5-FC in the positive ESI mode respectively. Chromatographic separation of the analyte was done using Syncronis C8 (2.1 × 50 mm, 5 μm, Thermo Scientific, Inc.) protected with a C8 guard column (10 × 2.1 mm, 3 μm) from the same source (Thermo Scientific, Inc., Madison, WI, USA). The LC conditions were as follows: Solvent A: 10 mL ammonium acetate (pH 5.0) and Solvent B: 100 mL acetonitrile was used as mobile phase with gradient elution of solvent B at 95% (0–0.5 min); 60% (0.5–2 min); 30% (2–4 min); 95% (4–7 min) at a flow rate of 0.25 mL/min. Retention time for F-araA was 1.26 min and the IS 1.10 min. The concentration of F-araA was expressed as ng/mL. The LLOQ was recorded to be 1 ng/mL and the method was linear for a wide concentration range from 7–700 ng/mL (1–100 μM) with mean R² = 0.99 ± 0.001 (Linearity, Accuracy and inter-day precision are as shown in Supplementary Table S2).

Screening for polymorphisms in F-AraA metabolism and transport:
Genetic variants in the NTSE gene (rs2295890) and the IS were included in the study after obtaining written informed consent. This study was approved by the Institutional review board. All patients with AA received F-araA (30 mg/m²/day for 6 days over 1 h infusion from day −7 to −2) and cyclophosphamide (50 mg or 60 mg/kg/day for 2 days over 1-h infusion on day −3 to −2) prior to HSCT. Cyclosporine (2.5 mg/kg/dose, BD) and methotrexate as post-transplant cyclophosphamide (50 mg/kg/day for 2 days) was given as GvHD prophylaxis. Patients with FA received the same dose of F-araA, cyclosporine and methotrexate as that of AA, while the cyclophosphamide was given as 10 mg/kg/day × 2 days on day −3 to −2 and post-transplant cyclophosphamide was given at 25 mg/kg/day × 2 days as GvHD prophylaxis (Table 1).

Chimerism analysis
Whole blood chimerism was evaluated by PCR amplification of the short or variable number of tandem repeats (STR/VNTR) markers followed by capillary electrophoresis (Genetic Analyzer ABI 3130) as reported previously.

F-araA PK and Population PK modeling
Non-linear mixed effects modeling analysis was performed with Monolix (version 4.3.3, LIXOFF, Batiment D, Antony, France) 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 and volume (CL (L/h/m²) and V (L/m²)) along with the inter-compartmental rate constants (k12 and k21 (1/h)). Also, the individual post hoc parameter values were used to estimate the area under the concentration curve (AUC). The inter-individual and inter-day variability of the parameters was assumed to be log-normally distributed. A 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: \( \theta = \theta_{\text{base}} \times \exp(\beta \times \text{covariate}) \). 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 (\( \Delta \text{OFV} \geq 3.84 \)) (addition of the covariate to the model reduced the objective function by 1 degree of freedom).

### Limited sampling model

A limited sampling model (LSM) for F-araA PK was developed with these data to reduce sample collection time points for future studies. Specifically, we generated data sets from our original population with subsets of 3 or 4 time points per individual chosen from the original times (1, 2, 3, 5, 7 and 24 h after the infusion). Using the maximum, a posteriori probability (MAP) estimation approach in ADAPT V10 we then estimated the individual post hoc PK for each of the 3 and 4 timepoint per individual LSMS and compared the results to the individual post hoc PK estimated using all 6 time points per individual. The LSMS were ranked by their bias and error where bias was defined as: \( \text{bias} = \sum_{i=1}^{n} \left( \frac{\theta_{\text{est}} - \theta_{\text{true}}}{\theta_{\text{true}}} \right) / n \) and error was defined as: \( \text{error} = \sum_{i=1}^{n} \left( \frac{\theta_{\text{est}} - \theta_{\text{true}}}{\theta_{\text{true}}} \right) / n \) where \( \theta_{\text{true}} \) are the individual PK parameter estimates using the 3 or 4 timepoint LSMSs.

### H SCT outcome

The influence of F-araA PK, PG and demographic factors on HSCT outcome parameters including engraftment, day 28 chimerism status, graft rejection, GvHD and overall survival, were evaluated. Neutrophil engraftment was defined as the ANC \( \geq 500 \times 10^3/\text{L} \) on three consequent lab values; Complete chimerism was defined as \( \geq 95\% \) of donor pattern in the patient’s peripheral blood and rejection was defined as more than \( 95\% \) of recipient’s pattern. GvHD was defined according to Glucksberg criteria³¹ and the transplant-related mortality (TRM) as death (excluding death from relapse) occurring within 100 days from HSCT.

### Statistical analysis

Descriptive statistics were calculated for all variables. Statistical analyses were performed by Student’s test, Mann—Whitney U-test, Kruskal—Wallis test and chi-squared analysis. Survival curves were drawn by the Kaplan—Meier method and compared by the log-rank test. The relationship of clinical features to the outcome of the procedure was analyzed by logistic regression and Cox regression analysis. The level of significance was set at 0.05 for all statistical tests. SPSS 160 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism (GraphPad software Inc., San Diego, CA, USA) were used for the analysis as appropriate. Overall the study was designed to have a power of 85% to see its effect on HSCT outcome, especially TRM and GvHD.

### RESULTS

Forty patients with AA and 13 with FA were enrolled in the study. Patients received F-araA/Cy (N = 29), F-araA/Cy/TBI (N = 20) or F-araA/Cy/ATG (N = 4) as their conditioning regimen. The patient demographics are given in Table 1.

### F-araA PK

Samples for F-araA PK analysis were collected on day \( -7, -4 \) and \( -2 \) after the start of F-araA in 7 patients. For subsequent patients, samples were collected only on day \( -7 \) and day \( -3 \) after the start of conditioning to reduce the volume of blood being drawn during conditioning. The population PK model parameters for the base model which includes BSA normalized dose are summarized in Table 2. This model is significantly better than the model with non-normalized dose (the \( -2 \text{LL} \) decreased 54.69 units; Table 3). The median post hoc estimated AUC for the first dose was \( 12.34 \mu \text{g} \cdot \text{h} \) on day \( 0 \) or 52.47 \( \mu \text{g} \cdot \text{h} \) on day \( 3 \) after the start of conditioning.

Influence of genetic variants on F-araA PK:

A single nucleotide polymorphism rs2295890 in the 5’-UTR region of the CD73/NT5E gene showed the expected/reported frequency of 0.18 in our study (Table 1). This SNP with a G>C change was found to be in complete linkage disequilibrium with four other SNPs in the same region namely, rs9450278, rs9450279, etc.

### Table 2. Population pharmacokinetics of F-araA in Aplastic anemia and Fanconi anemia

| Parameter | BSA normalized | RSE (%) | Final model | RSE (%) | P-value |
|-----------|----------------|---------|-------------|---------|---------|
| CL (L/h/m²) | 4.84 | 9.8 | — | — | — |
| WT, AA* | — | — | 7.12 | 10 | rs2295890: 3.8E – 02; Diagnosis: 2.7E-07 |
| WT, FA | — | — | 2.90 | 17 | — |
| HET/MUT, AA | — | — | 5.03 | 15 | — |
| HET/MUT, FA | — | — | 2.05 | 19 | — |
| V (L/m²) | 27.56 | 6.4 | — | — | — |
| Age on V | — | — | 21.25 \* (–0.013 \* age) | 11.8, 40.5 | 1.3E – 02 |
| k₁₂ (h⁻¹) | 0.35 | 7.3 | 0.36 | 7.6 | — |
| k₂₁ (h⁻¹) | 0.19 | 6.5 | — | — | — |
| Age on k₂₁ | — | — | 0.14 \* (0.016 \* age) | 10.1, 26.5 | 1.6E – 04 |
| p | — | — | 0.19 | 3.6 | — |
| −2 Log-likelihood | 312.34 | 2.4 | 262.51 | 2.1 | 3.9E – 08 |

Abbreviations: CL = clearance; F-araA = fludarabine; HET = heterozygous variant; IDV = inter-individual variability; IVL = inter-visit variability; MUT = mutant; RSE = residual error; WT = wild-type genotype. *Covariate effects of rs2295890 and diagnosis on clearance. The covariates are defined as follows: rs2295890 = (WT or HET/MUT); Diagnosis = AA (Aplastic Anemia or FA—Fanconi Anemia).
smallest error. 4-point sampling schedule of 1, 5, 7 and 24 h providing the bias and error in the estimates of clearance with the combinations of sampling times tested resulted in reasonable both 3- and 4-point LSMs (Supplementary Table S4). All Based on the data from this group of individuals, we evaluated Limited sampling model:

rs4599602, and rs4458647. Patients with variant genotype for rs2295890 (GC/CC) showed significantly lower plasma F-araA clearance compared to those with wild-type genotype (7.12 vs 5.03 L/h/m²; 29%; \( P = 0.0001 \)). F-araA clearance is not different between the days in AA cohort, while the F-araA clearance on first dose is significantly lower than fifth dose in FA patients \( (P < 0.0001) \); *** \( p < 0.0001 \).

Additionally, the parameters \( V \) and \( K_{21} \) increased significantly with respect to age (Table 2). The final population PK model including the covariates diagnosis (AA vs FA), rs2295890 (GG vs GC/CC), and age explained 46% of the inter-individual variability in clearance.

Limited sampling model:

Based on the data from this group of individuals, we evaluated both 3- and 4-point LSMs (Supplementary Table S4). All the combinations of sampling times tested resulted in reasonable bias and error in the estimates of clearance with the 4-point sampling schedule of 1, 5, 7 and 24 h providing the smallest error.

Table 3. –2 Log-Likelihood of each covariate model tested

| Covariates included in model | –2 Log-Likelihood |
|------------------------------|-------------------|
| Non BSA normalized base model | 376.03 |
| BSA normalized base model | 312.34 |
| rs2295890 | 301.21 |
| Age | 299.16 |
| Diagnosis | 289.87 |
| rs2295890, age | 289.35 |
| Diagnosis, age | 281.38 |
| Diagnosis, rs2295890 | 277.43 |
| Final model: diagnosis, rs2295890, age | 262.51 |

The categorical covariates are defined as follows: rs2295890 = 0—WT or 1—heterozygous variant or Mutant; Diagnosis = 0—AA/SAA/VSAA (aplastic anemia) or 1—FA (Fanconi Anemia). The covariates diagnosis and rs2295890 are included on the clearance and the covariate age is included on volume and the inter-compartmental parameter \( k_{21} \).

Figure 1. Inter-individual and inter-day variability in F-araA clearance in AA and FA patients. F-araA clearance was significantly higher in AA when compared to FA \( (P < 0.0001) \). F-araA clearance is not different between the days in AA cohort, while the F-araA clearance on first dose is significantly lower than fifth dose in FA patients \( (P < 0.0001) \); *** \( p < 0.0001 \).

Table 4. HSCT Outcome

| Outcome parameters | N | (%) |
|--------------------|---|-----|
| Engraftment | 45 | 90 |
| No | 5 | 10 |
| Not evaluable | 3 |
| Day of engraftment (days) | 15 (11–23) |
| Rejection | 4 | 8.8889 |
| No | 41 | 91.111 |
| Not evaluable | 8 |
| Chimerism | | |
| Complete | 36 | 81.818 |
| Mixed | 8 | 18.182 |
| Not evaluable | 9 |
| Mucositis | | |
| Yes | 37 | 74 |
| Grade 0–1: 17 |
| No | 13 | 26 |
| Grade 2–4: 33 |
| Not evaluable | 3 |
| aGvHD | | |
| Yes | 15 | 32.609 |
| Grade 0–1:31 |
| No | 31 | 67.391 |
| Grade 2–4:15 |
| Not evaluable | 7 |
| cGvHD | | |
| Yes | 16 | 41.026 |
| Grade 0–1:23 |
| No | 23 | 58.974 |
| Grade 2–4:16 |
| Not evaluable | 14 |
| TRM* | | |
| Yes | 16 | 30.189 |
| No | 37 | 69.811 |

Influence of F-araA PK on HSCT outcome:

The outcome endpoints post HSCT are detailed in Table 4. Five of the 53 patients were not evaluable for analysis of HSCT outcome due to very early death (3 patients died prior to HSCT; two patients died before engraftment could be documented). Of the 48 evaluable patients, 45 (93.75%) engrafted at a median of 15 days post HSCT (range: 11–23 days) while 3 did not engraft (6.25%). Four had secondary graft failure while 8 of the 44 (18%) evaluable patients had mixed chimerism on day 28. Grade 2–4 acute GvHD was seen in 15 of the 46 evaluable patients (32.6%) with chronic GvHD in 16 of the 39 evaluable patients (41%). Grade 2–4 mucositis was observed in 33 (66%) patients, 4 (8%) developed grade 1 mucositis and the rest 13 did not have any toxicity. The development of GvHD or mucositis was not different between AA and FA cohort. The day 100 mortality was 30%, and 32 (60%) are alive at the last follow up. None of the PK parameters or demographic variables showed any association with engraftment, mixed chimerism, rejection, overall survival or TRM. Interestingly, patients with F-araA AUC more than the 75th percentile (6/10 with F-araA AUC \( > 29.4 \mu M \times h \); vs 9/36 with F-araA AUC \( < 29.4 \mu M \times h \) had GvHD; \( P = 0.057 \)) showed trend to increased risk of developing acute GvHD. Multivariate analysis including the variables CD34+ cell dose, day of engraftment, GvHD Prophylaxis, ANC \( < 15 \) days vs \( \geq 15 \), F-araA AUC \( > 29.4 \) vs \( < 29.4 \mu M \times h \), diagnosis (FA or AA), donor source as well the polymorphisms rs2295890, rs491799 on the incidence of acute...
GvHD showed F-araA AUC > 29.4 μM*h to be significant factor (P = 0.02).

**DISCUSSION**

HSCT with F-araA based conditioning regimen has been shown to have improved the overall and event-free survival (EFS) in patients with AA/FA who are young and those who failed immunosuppressive therapy. This is the first study demonstrating the impact of a 5′ untranslated region SNP in the NT5E gene encoding ectonucleotidase enzymes on the F-araA PK variability which has never been studied before. The subjects with variant genotype are comparable to the existing reports, we observed wide inter-individual variation in F-araA PK.

Table 5. Comparison of F-araA PK with previous reports

| Sl. no | Diagnosis | N | Conditioning regimen | F-araA dose | Type of donor | AUC (μM*h) median (range) | CL (L/h/m²) median (range) | Ref |
|--------|-----------|---|----------------------|-------------|---------------|--------------------------|-----------------------------|-----|
| 1      | AML-05; CML-01; MDS-04; MF-05; CMML-01 | 16 | F-araA: days – 6 to – 2; targeted daily IV Bu days – 5 to – 2; rATG on days – 3 to – 1 | 50 mg/m²/day | MRD-11 MUD-05 | 24.8 (16.3–39.9) | — | 21 |
| 2      | ALL-06; AML-26; NHL-17; MDS-14; HL-08; CML-01; Other-15 | 87 | F-araA; Cy day – 6 | 40 mg/m²/day | MRD-22 MUD-65 | 40 mg/m²; 17.19 (7.02–40.35) | 40 mg/m²; 16.0 (6.2–36.6)L/h | 22 |
| 3      | AML-05; MM-03; MDS-03; Others-05 | 16 | F-araA: day – 6 to – 3; Bu: day – 5 to – 2 | 30 mg/m²/day | - | 21.03 (10.17–38.56) | 5.04 (2.7–10.2) | 33 |
| 4      | CML-04; MDS-38 | 42 | F-araA : 4 days; Oral Bu : 4 days | 30 mg/m²/day | MRD-16 MUD-26 | Mean: 19.1 (s.d. 7.0) | Mean: 6.3 (s.d. 2.4) | 34 |
| 5      | NHL-34; CLL-22; AML-15; MDS-10; MM-09; Others-12 | 102 | Flu: days – 4 to day – 2 to 2; 4.5 Gy TBI | 30 mg/m²/day | MRD-24 MUD-78 | 19.6 ± 4.80 (9.96–36.4) | — | 35 |
| 6      | NHL-05; HL-03; AML/MDS-03 | 11 | Flu: days – 6 to – 2; CY days – 6 and – 5; TBI day – 1 | 30 mg/m²/day | Haplo-11 | 16.4 (10.4–21.5) | — | 36 |
| 7      | MDS-18; AML-13; CML-05; CCMML-02; MF-03 | 41 | Protocol 1519 (N = 27) Flu: days – 9 to – 6; targeted oral B: day – 5 to – 2 | 30 mg/m²/day | — | — | Protocol 1519: 9.1 (8.452); Protocol 2041: 7.07 (4.40–10.76) | 37 |
| 8      | AA-40; FA-13 | 53 | Flu: day – 6 to day – 2; Cy: Day – 3 and day – 2 | 30 mg/m²/day | MSD-45 AD-08 | AA-12.34 (3.62–52.47) | AA-6.47 (1.24–22.43) | Present study |

Abbreviations: AA = Aplastic Anemia; AUC = area under the curve; FA = Fanconi Anemia; F-araA = fludarabine; PK = pharmacokinetics. Dose and the PK parameters of AA was comparable to all the studies although the underlying disease and the combination of conditioning regimen are variable among the different studies.
counterparts with wild-type genotype. NT5E, also known as CD73 is a transmembrane glycoprotein, primarily involved in the purine salvage pathway. It is shown to have a multifaceted role in the normal physiology as well in the tumor context. The gene expression of NT5E has been studied as a prognostic marker in many solid tumors and hematologic malignancies. However, its role on drug metabolism is hardly known. A report by Li et al. has shown the effect of NT5E polymorphisms and its gene expression on the cytotoxicity of thiopurine drugs. The potential functional relevance of this polymorphism is currently being evaluated in our laboratory.

A 4 timepoint LSM was developed with this data to reduce sample collection time points for future studies. Specifically, we generated data sets from our original population and validated these results to the post hoc PK estimated using all available data. The LSM time points 1.5, 7, and 24 h after infusion of F-araA were obtained with the least biased and error estimates. This model is comparable to the model developed by Salingier et al.

Although appreciable inter-individual variability is observed in this cohort, none of the PK parameters are associated with HSCT endpoints such as engraftment, graft rejection, TRM and OS except aGVHD. Long-Boyle et al. in 87 patients with varied hematologic disorders including AML, NHL or MDS showed that high F-araA exposure was associated with TRM and OS. McCune et al. in 16 patients with malignant diseases and a high dose of F-araA (50 mg/m²) also observed an association between high plasma F-araA exposure and non-relapse mortality. It is possible that, since the incidence of events such as rejection or TRM is low in our cohort, none of the PK parameters is associated with these endpoints. Entirely different from the previous study by Long-Boyle et al. we observed a higher exposure of F-araA > 29.4 μM · h to be significantly associated with the development of acute GVHD. Since the pathology of GVHD is still not exactly clear, one might think, enough ablation of the recipient lymphocytes and immunosuppression is necessary for preventing GVHD as well as rejection. Our data suggests that the F-araA PK has a therapeutic window beyond which organ damage, circumsstantial infections, release of cytokine milieu occurs eventually leading to GVHD manifestation. The observation that there is an association of F-araA exposure and GVHD development needs to be explored further since the development of GVHD in the HSCT setting is multifactorial. Measurement of both plasma F-araA and intracellular F-araATP (which is the active metabolite exhibiting the cytotoxic profile of F-araA) would be ideal to understand the efficient lymphocyte depletion and a possible mechanistic explanation for the variation observed in the patients and associations with the outcome. However with the practical difficulty in the quantification of F-araATP as studied by McCune et al., it would be interesting to study the kinetics of Cyclophosphamide (CY), which is administered along with F-araA in these patients which might bridge the gap in explaining the outcome endpoints. Further F-araA pharmacokinetics studies are warranted in a large uniform cohort of patients with various hematological disorders to arrive at the usefulness or need of therapeutic drug monitoring and personalizing the regimen.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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