# Aplastic Anemia Preconditioned with Fludarabine, Cyclophosphamide, and Anti-Thymocyte Globulin

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**Background:**    Graft rejection and graft versus host disease (GvHD) have impeded the success of hematopoietic cell transplantation for severe aplastic anemia (SAA) patients. There is no sufficient data to identify the outcomes of peripheral blood stem cell transplantation (PBSCT) in SAA patients, especially for adult SAA patients. The aim of this study was to evaluate the outcomes of adult SAA patients undergoing PBSCT with the FCA regimen. The FCA regimen includes fludarabine, cyclophosphamide, and anti-thymocyte globulin (ATG).

**Material/Methods:**    We report our experience with 46 adult SAA patients who underwent PBSCT with the FCA regimen. Thirty SAA patients who received only cyclophosphamide and ATG (CA) regimen were used as controls. Complications and survival outcomes were evaluated and compared.

**Results:**    There was a significantly higher percentage of patients who achieved >95% donor chimerism by day 30 in the FCA group. The 5-year event-free survival (EFS) rate in the FCA group was higher than that in the CA group (95.4% versus 73.3%). In addition, the 5-year rejection rate (RR) in the FCA group was lower than that in the CA group (4.6% versus 23.6%). A multivariable model identified the FCA regimen as an independent factor affecting EFS and RR. However, GvHD and serious infection did not differ between the 2 groups. For patients with an unrelated donor, the FCA regimen had a higher EFS and a lower RR than the CA regimen.

**Conclusions:**    The FCA regimen for PBSCT in adult SAA patients compared favorably to the CA regimen. It can improve EFS and reduce graft rejection, especially for unrelated donor PBSCT.

**MeSH Keywords:**    **Anemia, Aplastic • Antineoplastic Combined Chemotherapy Protocols • Peripheral Blood Stem Cell Transplantation**

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## Background

Acquired severe aplastic anemia (SAA) is a life-threatening hematologic disease associated with significant morbidity and mortality. Immunosuppressive therapy and allogeneic hematopoietic cell transplantation (HCT) are effective treatments for SAA [1,2]. It was recently suggested that allogeneic transplantation using bone marrow as a stem cell source from an HLA matched-related donor should be considered first-line therapy for patients with SAA, especially for those patients under the age of 20 years, whereas immunosuppressive therapy is typically used as first-line therapy for older patients (over 40 years of age) with SAA or for younger patients who lack an HLA identical bone marrow donor [3–5]. The outcomes of HCT for treating SAA have improved dramatically over the past 2 decades due to the modification of treatment regimens, improved selection of donors by high resolution HLA matching, and better supportive care [6–9]. Recently, the number of allogeneic peripheral blood stem cell transplantations (allo-PBSCTs) has increased in the majority of centers, due to the convenient collection of grafts and faster engraftment. However, as yet, there is not sufficient data to identify the outcomes of allo-PBSCT in SAA patients, especially for adult SAA patients without matched-related donors.

The traditional preconditioning regimen for SAA patients in HCT is cyclophosphamide (Cy) in combination with anti-thymocyte globulin (ATG), which is named the CA regimen [6]. However, higher incidence of graft rejection or GvHD have been reported with CA regimens, especially among older patients and patients with an unrelated donor [6,7]. Recent data suggest that survival of SAA patients treated with HCT can be significantly improved by using a fludarabine (Flu)-based conditioning regimen [3,10–23]. However, the outcome of PBSCT with Flu-based regimens in SAA patients has not yet been defined, especially for older patients and patients with unrelated donors.

In this retrospective study, we report our experience with 46 adult SAA patients who underwent allo-PBSCT from 2010 to 2017 with Flu in combination with the CA regimen (FCA regimen), and 30 SAA patients who received only the CA regimen from 2006 to 2011 were used as historical controls. Our study demonstrated that the FCA regimen for allo-PBSCT in adult SAA patients significantly improved event-free survival (EFS) and reduced graft rejection, especially for patients with unrelated donor PBSCT.

## Material and Methods

### Patients and donor selection

This study cohort included patients with acquired SAA who underwent their first allo-PBSCT from an HLA-identical siblings or from unrelated donors at our department from January 2006 to September 2017. Eligibility criteria were 1) age older than 18 years; 2) diagnosis of acquired SAA; and 3) peripheral blood as a stem cell source. Patients who had significant functional problems in major organs, or congenital disorders were excluded, leaving 76 eligible patients in the cohort. All patients received either FCA regimen (n=46 patients) or CA regimen (n=30 patients).

Clinical information from all patients was collected. Informed consent forms were obtained from all patients before starting the conditioning therapy in accordance with the transplantation procedure. For donor selection, HLA-A, -B, -C, -DQ, and -DRB1 matching was confirmed by a high-resolution molecular method for all patients and donors. Patient eligibility criteria of the FCA group was similar to the historical control group (CA group). Patient characteristics are summarized in Table 1.

### Conditioning regimen and GvHD prophylaxis

The FCA regimen consisted of Flu (30 mg/m$^2$ on days –6 to –3), Cy (50 mg/kg/day on days –6 to –3), and ATG (Fresenius, Germany, 5 mg/kg/day on days –4 to –1). Forty-six patients (61%) received the FCA regimen, whereas the other 30 patients (39%) received a CA regimen, which included Cy (50 mg/kg/day on days –6 to –3) and ATG (Fresenius, Germany, 5 mg/kg/day on days –4 to –1).

GvHD prophylaxis consisted of cyclosporine A and methotrexate. Methotrexate was administered on day +1 after transplantation at a dose of 15 mg and on day +3 and day +6 after transplantation at a dose of 10 mg. Intravenous cyclosporine A (2–3 mg/kg daily) was administered from day –5; then switched to oral drug administration (4–5 mg/kg daily). Cyclosporine A was tapered from month +9 and discontinued by 1 year in patients with no GvHD after PBSCT.

### Stem cell collection

All donors were treated with granulocyte colony-stimulating factor (G-CSF, 10 µg/kg daily) for 4–5 days before apheresis (peripheral blood stem cells were collected). The median number of mononuclear cells infused was 7.36×10$^8$/kg (range, 3.5–13.47×10$^8$/kg) and 6.21×10$^8$/kg (range, 2.23–13.57×10$^8$/kg) in the FCA group and the CA group, respectively. The median number of CD34$^+$ cells infused was 3.12×10$^6$/kg (range, 1.789–9.96×10$^6$/kg) and 3.00×10$^6$/kg (range, 1.77–6.52×10$^6$/kg) in the FCA group and the CA group, respectively. The difference in the number of cells between the 2 groups was not statistically significant (Table 1).

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**Table 1.** Patient characteristics and transplantation data.

| Characteristics recipient | FCA group n=46 | | CA group n=30 | | P |
|---|---|---|---|---|---|
| Median age, yr (range) | 29 | (18–47) | 32 | (18–49) | 0.09 |
| Gender, no. (%) | | | | | 0.23 |
|    Female | 21 | (46) | 9 | (30) | |
|    Male | 25 | (54) | 21 | (70) | |
| Interval from diagnosis to HCT | | | | | 0.22 |
|    <1 yr | 27 | (59) | 22 | (73) | |
|    ≥1 yr | 19 | (41) | 8 | (27) | |
| Gender match (donor/recipient), no. (%) | | | | | 0.295 |
|    Female to Male | 10 | (22) | 9 | (30) | |
|    Others | 36 | (78) | 21 | (70) | |
| Donor type,no.(%) | | | | | 0.80 |
|    Related | 30 | (65) | 21 | (70) | |
|    Unrelated | 16 | (35) | 9 | (30) | |
| ABO blood type,no.(%) | | | | | 1.00 |
|    Matched | 23 | (50) | 14 | (47) | |
|    Unmatched | 23 | (50) | 16 | (53) | |
| HLA matching, 10/10 (HLA-A,-B,-C,-DR,-DQ), no (%) | | | | | 0.87 |
|    10/10 | 37 | (80) | 24 | (80) | |
|    9/10 | 8 | (17) | 2 | (7) | |
|    8/10 | 1 | (3) | 4 | (13) | |
| History of previous IST | | | | | 0.24 |
|    Yes | 24 | (52) | 11 | (37) | |
|    No | 22 | (48) | 19 | (63) | |
| Ferritin | | | | | 0.29 |
|    Normal | 21 | (46) | 11 | (37) | |
|    Abnormal | 25 | (54) | 19 | (63) | |
| Transfusion before HCT | | | | | |
|    Plasma-reduced blood, IU.mean (range) | 11 | (3–19) | 14 | (2–24) | 0.11 |
|    Platelet, IU.mean (range) | 8 | (1–35) | 6 | (2–15) | 0.43 |
| Median MNC, ×$10^8$/kg (range) | 7.36 | (3.5–13.47) | 6.21 | (2.23–13.57) | 0.12 |
| Median CD34$^+$, ×$10^6$/kg (range) | 3.12 | (1.789–9.96) | 3 | (1.77–6.52) | 0.36 |

MNC – indicates mononuclear cells. Values are number of cases with percents in parentheses, unless otherwise indicated.

## Study endpoints and definitions

Study endpoints included hematopoietic recovery, graft rejection, GvHD, infection, and mortality. The single nucleotide polymorphism (SNP)-polymerase chain reaction (PCR) of bone marrow aspirates at 15, 30, 60, 90, 180, and 360 days post-PBSCT was used for chimerism monitoring [24].

Primary graft rejection, which we called graft failure, was defined as no engraftment followed by severe neutropenia (absolute neutrophil count [ANC] <0.5×$10^9$/L) or the absence of donor cells in the bone marrow or peripheral blood up to day 30 after transplantation. Secondary graft rejection was defined as subsequent loss of ANC to <0.5×$10^9$/L & < 5% donor chimerism after neutrophil and/or platelet recovery. Acute GvHD (aGVHD) and chronic GvHD (cGvHD) were graded according

to consensus criteria [25,26]. Regimen-related toxicity after transplantation was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 [27,28].

Only serious infections were analyzed in this study. Serious infections were defined as infections associated with death or severe clinical compromise, and included shock or organ failure, cytomegalovirus (CMV) end-organ disease, lower respiratory tract infection with respiratory viruses, invasive molds in sinus or lung, or disseminated *Aspergillus* species infection [29].

## Statistical analysis

All data were measured from the date of PBSCT to the date of disease progression or death from any event (EFS), the date of death from any cause (overall survival, OS), the date of rejection according to the SNP-PCR of bone marrow (rejection rate [RR] including primary rejection and secondary rejection), and the date of serious infection (infection rate, [IR]). Data on patients were evaluated in May 2018. Actuarial probabilities of survival were calculated using the Kaplan-Meier method, and the log-rank test (univariate) was used to evaluate differences between groups. A Cox proportional hazard regression model was used for the multivariate analysis of prognostic factors affecting survival. SPSS 20 was used to perform the statistical analyses. All P values <0.05 indicated statistical significance. The 95% confidence interval (CI) was calculated for standard error computation.

## Results

### Patients

A total of 46 patients underwent allo-PBSCT with the FCA regimen, and 30 patients underwent allo-PBSCT with the CA regimen. The median age of patients was 29 years (range, 18–47 years) and 32 years (range 18–49 years) in the FCA group and the CA group, respectively. Fifty-one donors were a sibling of the patient, and 25 donors were unrelated to the patient. The median duration between diagnosis and PBSCT was 6.5 months (range 1–120 months). Thirty-four patients (43%) received cyclosporine A as a single immunosuppressive therapy agent before PBSCT, and only 1 patient received cyclosporine A in combination with ATG for 2 cycles. None of the patients had undergone a prior HCT. The FCA group and the CA group were comparable in terms of age, sex, time from diagnosis to HCT, donor type, ABO blood type, HLA matching, history of immunosuppressive therapy, ferritin, and transfusions before PBSCT (P>0.05, Table 1).

## Engraftment and rejection

The median number of days with an ANC ≥0.5×10$^9$/L was 14 days (range, 11–27 days) in the FCA group and 13 days (range, 8–30 days) in the CA group. Platelet recovery (≥20×10$^9$/L) without transfusion required was a median of 15 days (range, 9–31 days) in the FCA group and 15 days (range, 13–30 days) in the CA group. The difference in the engraftment time between the 2 groups was not statistically significant (P=0.57 [ANC]; P=0.56 [PLT]). Forty-one patients (41 out of 46, 89.1%) in the FCA group and 18 patients (18 out of 30 patients, 60%) in the CA group achieved >95% donor-type chimerism by day 30 post-PBSCT (P=0.004). Moreover, the donor cell percentage in bone marrow on day 30, detected by SNP-PCR, in the FCA group was higher than that of the CA group (98.8±1.1% versus 92.7±3.2%, respectively, P=0.007, 95% CI: 1.678–17.814).

Of all 76 patients, only 1 patient in the CA group experienced primary graft rejection and that patient achieved reconstitution after immunosuppressive therapy (cyclosporine A) 3 months later. No patient in the FCA group underwent primary graft rejection. Five patients (5 out of 30 patients, 16.67%) in the CA group underwent secondary graft rejection (median 2.9 months after PBSCT, range, 1.8–4.4 months). One patient self-recovered without treatment and 1 patient received a second HCT from the same donor; both of these patients had long-term survival. The other 3 of the 5 patients were given a donor lymphocyte infusion. Of these 3 patients, 1 patient died of serious infection of the lung 2 months later and the other 2 patients survived with disease (aplastic anemia). In the FCA group, secondary graft rejection occurred in 2 patients (2 out of 46 patients, 4.35%). One of the 2 patients received a successful second HCT from a second matched-unrelated donor and ultimately achieved hematologic recovery. The other patient underwent donor lymphocyte infusion but died 1 year later because of disease that was not controlled. The 5-year RR in the FCA group was 4.6±3.1%, which was significantly lower than that in the CA group (23.6±7.8%, P=0.013; Figure 1A). Multivariate analysis revealed the FCA regimen as an independent factor affecting RR (95% CI: 0.016–0.766, P=0.026, Table 2).

## OS and EFS

The median follow-up was 54 months (range, 9–120 months). At the time of last follow-up, 45 patients (97.83%) were alive in the FCA group. The probability of 5-year OS in the FCA group was 97.4±2.5%. Twenty-seven patients (90%) in the CA group were alive, and the probability of 5-year OS was 90.0±5.5% (P=0.157; Figure 1B). In addition, the probability of 5-year EFS in the FCA group was 95.4±3.1%, which was significantly higher than that in the CA group (73.3±8.1%, P=0.006; Figure 1C). Multivariate analysis revealed the FCA regimen as
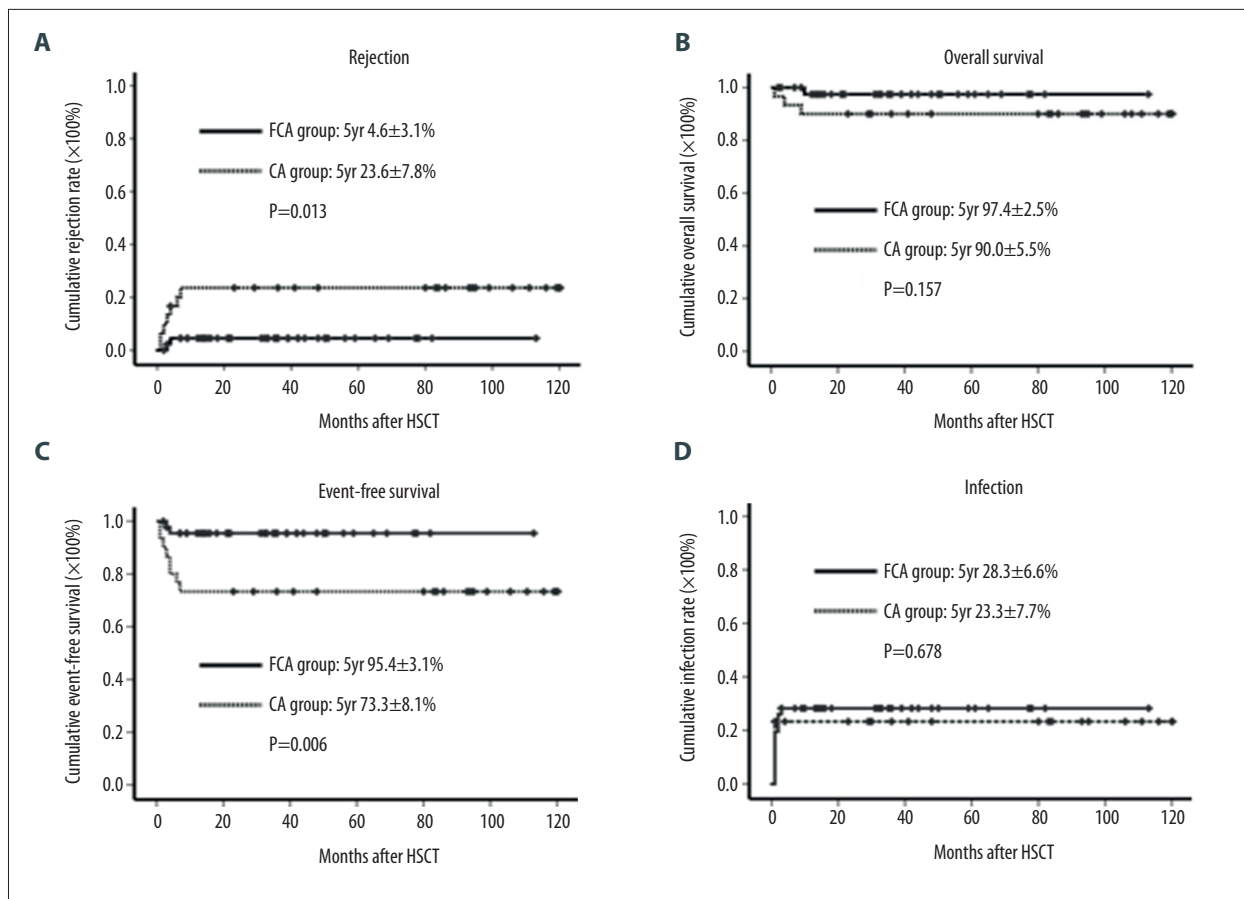
**Figure 1.** Comparison of cumulative incidence rates of (**A**) rejection rate (*P*=0.013), (**B**) overall survival (*P*=0.157), (**C**) event-free survival (*P*=0.006) and (**D**) serious infection rate (*P* = 0.678) of 46 patients in the FCA group and 30 patients in the CA group.

an independent factor affecting EFS (95% CI: 0.012–0.667, *P*=0.019, Table 2).

## Toxicity and infection

Regimen-related toxicity after PBSCT occurred in 18 patients (39%) in the FCA group and in 11 patients (37%) in the CA group. There was no significant difference between the 2 groups (*P*=0.691). The main toxicities were due to cytomegalovirus (CMV) infection (23.9% in the FCA group, 20% in the CA group), hemorrhagic cystitis (10.9% in the FCA group, 13.3% in the CA group), and pulmonary infection (10.9% in the FCA group, 6.7% in the CA group), which were successfully treated and did not cause directly mortality (Table 3).

CMV infection occurred in 11 patients in the FCA group and 5 patients in the CA group (23.9% versus 16.7%, respectively, *P*=0.199), and the median time to reactivation was 31 days after transplantation (range, 20–64 days). CMV disease occurred in only 1 patient in the CA group. More information regarding infection and toxicity rates is provided in Table 3. Two patients in the FCA group had Epstein-Barr virus (EBV)

infection; post-transplant lymphoproliferative disorder (PTLD) developed in 1 patient. The EBV infection was controlled in both patients after rituximab treatment (375 mg/m² per week). The 5-year serious infection rate after PBSCT was 28.3±6.6% and 23.3±7.7% in the FCA group and the CA group, respectively (*P*=0.157; Figure 1D).

## GvHD

In the FCA group, acute GvHD (aGvHD) was scored in 4 patients (4 out of 46 patients, 8.7%) as grade II to IV at a median time of 38 days (range, 32–49 days) after PBSCT. Compared with the FCA group, grades II to IV aGvHD appeared in 4 patients (4 out of 30 patients, 13.3%) of the CA group at a median time of 24 days (range, 19–39 days), (8.7±4.2% for the FCA group versus 13.3±6.3% for the CA group, *P*=0.51; Figure 2A). Except for 1 patient who died of transplant-related disease, 75 SAA patients survived longer than 3 months after transplantation and were eligible for evaluation for cGvHD. In the FCA group, 9 patients (19.5%) developed cGvHD at a median time of 7 months (range, 4–11 months). Compared with the FCA group, cGvHD appeared in 10 patients (34.5%) in the CA group

**Table 2.** Multivariate analyses of OS, EFS and RR among the total sample.

| Variables | OS | | EFS | | RR | |
|---|---|---|---|---|---|---|
| | Exp(B) (95% CI) | P | Exp(B) (95% CI) | P | Exp(B) (95% CI) | P |
| Age,no. | 1.363 (0.101–1.751) | 0.362 | 1.242 (0.195–7.888) | 0.819 | 1.299 (0.22–7.684) | 0.773 |
| ≤30 years | | | | | | |
| >30 years | | | | | | |
| Gender, no. | 1.526 (0.055–1.979) | 0.803 | 1.176 (0.199–6.963) | 0.858 | 1.147 (0.191–6.867) | 0.881 |
| Male | | | | | | |
| Female | | | | | | |
| Donor type, no. | 0.261 (0.013–5.161) | 0.378 | 0.169 (0.028–1.028) | 0.054 | 0.184 (0.031–1.101) | 0.064 |
| Matched related | | | | | | |
| Unrelated | | | | | | |
| ABO blood types, no. | 0.486 (0.222–1.066) | 0.316 | 0.953 (0.198–4.673) | 0.963 | 0.943 (0.199–4.757) | 0.973 |
| Matched | | | | | | |
| Unmatched | | | | | | |
| HLA disparity, 10/10, no | 0.433 (0.005–1.404) | 0.718 | 1.062 (0.171–6.604) | 0.949 | 2.157 (0.282–6.479) | 0.459 |
| 10/10 | | | | | | |
| ≤9/10 | | | | | | |
| Gender match (donor/recipient), no. | 1.775 (0.217–3.546) | 0.432 | 1.779 (0.429–7.378) | 0.428 | 1.914 (0.442–8.288) | 0.385 |
| Female to Male | | | | | | |
| Others | | | | | | |
| History of previous IST, no. | 0.166 (0.004–7.143) | 0.35 | 1.098 (0.231–5.226) | 0.907 | 0.963 (0.2–4.709) | 0.971 |
| Yes | | | | | | |
| No | | | | | | |
| Interval from diagnosis to HSCT, no. | 0.235 (0.015–3.633) | 0.3 | 1.446 (0.302–6.926) | 0.644 | 1.319 (0.387–3.9) | 0.357 |
| <1 year | | | | | | |
| ≥1 year | | | | | | |
| Conditioning regimen, no. | 0.209 (0.009–4.952) | 0.333 | 0.089 (0.012–0.667) | 0.019 | 0.112 (0.016–0.766) | 0.026 |
| FCA | | | | | | |
| CA | | | | | | |
| Acute GVHD, no. | 0.526 (0.172–5.755) | 0.343 | 0.738 (0.057–9.627) | 0.817 | 0.246 (0.197–2.141) | 0.987 |
| Yes | | | | | | |
| No | | | | | | |
| Chronic GVHD, no. | 0.376 (0.127–1.603) | 0.988 | 1.432 (0.593–3.154) | 0.149 | 1.152 (0.81–4.673) | 0.079 |
| Yes | | | | | | |
| No | | | | | | |
| Infection after HSCT, no. | 1.573 (0.062–3.999) | 0.784 | 1.337 (0.201–8.891) | 0.764 | 1.505 (0.227–9.999) | 0.672 |
| Yes | | | | | | |
| No | | | | | | |

CI – indicates confidence interval; IST – immunosuppressive therapy; HSCT – hematologic stem cell transplantation; GVHD – graft versus host disease.

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**Table 3.** Regimen-related organ toxicity rates for patients in FCA group and CA group.

| | FCA group n=46 | | CA group n=30 | | P |
|---|---|---|---|---|---|
| CMV infection | | | | | |
| Antigenemia (%) | 11 | (23.9) | 5 | (16.7) | 0.199 |
| Disease(%) | 0 | (0.0) | 1 | (3.3) | 0.395 |
| PTLD (%) | 1 | (2.2) | 0 | (0.0) | 0.605 |
| Organ toxicities, grade 3–4 | | | | | |
| Bladder (%) | 5 | (10.9) | 4 | (13.3) | 0.733 |
| Cardiac (%) | 0 | (0.0) | 1 | (3.3) | 0.395 |
| CNS (%) | 0 | (0.0) | 0 | (0.0) | |
| GI (%) | 3 | (6.5) | 1 | (3.3) | 0.294 |
| Hepatic (%) | 1 | (2.2) | 1 | (3.3) | 0.637 |
| Febrile neutropenia (%) | 4 | (8.7) | 3 | (10.0) | 0.831 |
| Pulmonary (%) | 5 | (10.9) | 2 | (6.7) | 0.697 |
| Renal (%) | 1 | (2.2) | 0 | (0.0) | 0.605 |
| Skin (%) | 1 | (2.2) | 1 | (3.3) | 0.637 |
| Veno-occlusive disease (%) | 0 | (0.0) | 1 | (3.3) | 0.395 |

CMV – indicates cytomegalovirus; PTLD – post-transplant lymphoproliferative disorders; CNS – central nervous system; GI – gastrointestinal. Values are number of cases with percents in parentheses.
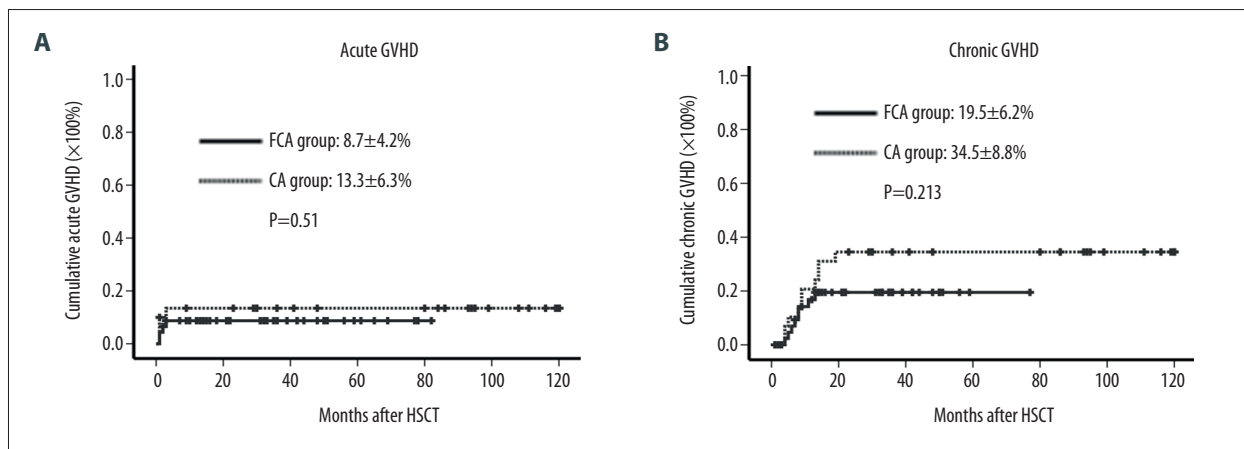


**Figure 2.** Comparison of (**A**) acute GvHD (*P*=0.51) and (**B**) chronic GvHD (*P*=0.213) of 46 patients in the FCA group and 30 patients in the CA group.

at a median time of 5 months (range, 4–9 months) (19.5±6.2% in the FCA group versus 34.5±8.8% in the CA group, *P*=0.213; Figure 2B). All cases were limited cGvHD; no extensive or severe cGvHD occurred.

## Unrelated donor PBSCT in the FCA group and CA groups

Overall, there were 25 patients out of the 76 patients included in the study who received unrelated donor PBSCT. Sixteen

patients (16 out of 46 patients, 35%) were from the FCA group and 9 patients (9 out of 30 patients, 30%) were from the unrelated donor CA subgroup. In the unrelated donor FCA subgroup, all patients (16 out of 16 patients, 100%) were alive at the last follow-up; whereas in the unrelated donor CA subgroup, 7 out of 9 patients (77.8%) were alive at the last follow-up. The probability of 5-year OS for the unrelated donor subgroups was 100% for FCA and 77.8±13.9% for CA (*P*=0.052, Figure 3A). However, the probability of 5-year EFS in the FCA
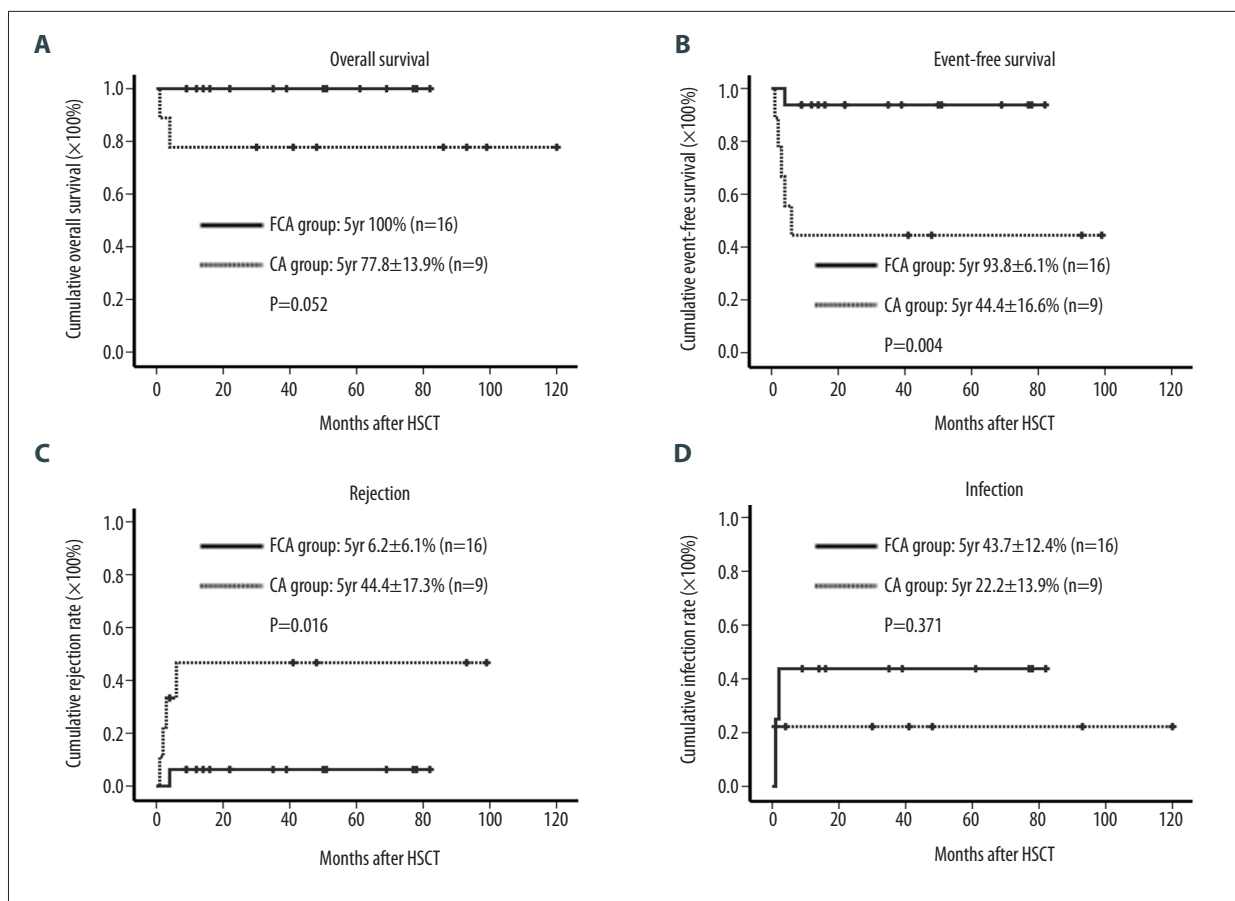
**Figure 3.** Comparison of unrelated donor PBSCT in the FCA group (n=16) and the CA group (n=9) for (**A**) overall survival (*P*=0.052), (**B**) event-free survival (*P*=0.004), (**C**) rejection rate (*P*=0.016), and (**D**) infection rate (*P*=0.371).

subgroup was 93.8±6.1%, which was significantly higher than that in the CA subgroup (44.4±16.6%, *P*=0.013; Figure 3B). Only 1 patient (6.2%) in the FCA subgroup experienced graft rejection after PBSCT, whereas 4 patients (44.4%) in the CA subgroup experienced graft rejection. Furthermore, the FCA subgroup had a significantly lower 5-year RR than the CA subgroup (6.2±6.1% versus 44.4±17.3%, *P*=0.016; Figure 3C).

Seven patients in the FCA subgroup and 2 patients in the CA subgroup experienced serious infection after PBSCT. The serious infection rates in the FCA subgroup and CA subgroup were 43.7±12.4% and 22.2±13.9%, respectively (*P*=0.371, Figure 3D).

## Discussion

Recently, Flu-based regimens have been introduced with promising results in SAA patients with bone marrow transplantation (BMT) [10,13]. However, the efficacy of this regimen is less well demonstrated in PBSCT, which is used more frequently in many transplantation centers, especially those patients with matched-unrelated donor in China, because the

China Marrow Donor Program only collects peripheral blood stem cells from donors [3,6,9,30–34]. We reviewed the literature and summarized reports using Flu-based regimens in both BMT and PBSCT for SAA patients published in recent years (Table 4) [18–23]. As shown in Table 4, most of these studies reported data for younger patients with BMT or PBSCT, mixed together, with 4-year or 5-year OS varying from 67.9% to 96.7%, and few studies reported the long-term outcome in large cohorts [18,19,21–23].

Since aplastic anemia is a non-malignant hematologic disease, the effect of graft versus host reaction has no benefits for patients and might lead to serious complications and even death [35]. It is well known that, compared to PBSCT, BMT results in less incidence and severity of GvHD [36,37]. As shown in Table 4, the incidences of GvHD varies greatly, ranging from 11.7% to 46.4% for aGvHD and from 16.2% to 37.9% for cGVHD. In the current study of PBSCT, however, the rates of grade II to IV aGvHD (8.7%) and cGvHD (19.5%, all limited) in the FCA group were more acceptable. This favorable result might be related to the enhancement of immunosuppression with the FCA regimen in this study.

**Table 4.** Published clinical studies of fludarabine-based regimen for stem cell transplantation in acquired severe aplastic anemia.

| Regimen | Dose | Donor | N | Stem cell source | Median engraftment, day | Survival | GVHD | Reference |
|---|---|---|---|---|---|---|---|---|
| Cy/Flu/ Thymoglobulin | Cy (50 mg/kg/d,d −9 to −6), Flu (30 mg/m$^2$/d, d −5 to −2), thymoglobulin (2.5 mg/kg/d, d −3 to −1) | Unrelated | 28 | BM (15); PB (13) | 15 | 4yr: 67.9% | aGVHD: 46.4%; cGVHD: 34.8% | 18 |
| Cy/Flu/ATG | Cy (60 mg/kg/d, d −8, −7), Flu (40 mg/m$^2$/d, d −6 to −2), ATG (2.5 mg/kg/d×3 days) | Unrelated | 29 | BM (5); PB (24) | 15 | 5yr: 96.7% | aGVHD: 41.3%; cGVHD: 37.9% | 19 |
| Cy/Flu/ Thymoglobulin | Cy (40 mg/kg/d×3 days), Flu (30 mg/m$^2$/d×5 days), thymoglobulin (2.5 mg/kg/d×3 days) | Related | 15 | BM (3); PB (11); BM+PB (1) | 13.5 | 3yr: 60% | aGVHD: 3 pts; cGVHD: 4 pts | 20 |
| Cy/Flu/ATG | Cy (50 mg/kg/d×2 days), Flu (30 mg/m$^2$/d×6 days), ATG (2.5mg/kg/d×4 days) | Related | 117 | BM (98); PB (8); BM+PB (11) | 12 | 5yr: 91.5% | aGVHD: 18.8%; cGVHD: 17.6% | 21 |
| Cy/Flu/ATG | Cy (50mg/kg/d, d −3, −2), Flu (30 mg/m$^2$/d, d −6 to −2), ATG (3 mg/kg/d, d −4 to −2) | Related (25) Unrelated (15) | 40 | BM (30); PB (10) | 15 | 4yr: 85.6% | aGVHD: 23.3%; cGVHD: 16.2% | 22 |
| Cy/Flu/ATG | Cy (50 mg/kg/d×4 days), Flu (30 mg/m$^2$/d×5 days), ATG (3 mg/kg/d×3 days) | Unrelated | 32 | BM (21); PB (11) | 18 | 5yr: 67% | aGVHD: 35.2%; cGVHD: 26.6% | 23 |
| Cy/Flu/ATG | Cy (60 mg/kg/d×2 days), Flu (30 mg/m$^2$/d×6 days), horse ATG (30–40 mg/kg/d×4 days) | Related | 5 | PB (5) | 11 | 1yr: 100% | aGVHD: 1 pts; cGVHD: 2 pts | 42 |
| Cy/Flu/ATG | Cy (60 mg/kg/d, d −7, −6), Flu (25 mg/m$^2$/d, d −5 to −1), ATG (4 mg/kg/d, d −5 to −2) | Related | 15 | CD34$^+$ selected PB (15) | 14 | 5yr: 86% | aGVHD: 13%; cGVHD: 13% | 50 |
| Cy/Flu/ATG | Cy (50 mg/kg/d, d −6 to −3), Flu (30 mg/m$^2$/d, d −6 to −3), ATG (5 mg/kg/d, d −4 to −1) | Related (30) Unrelated (16) | 46 | PB (46) | 14 | 5yr: 97.4% | aGVHD: 8.7%; cGVHD: 19.5% | Our data |

BM – bone marrow; PB – peripheral blood; Cy – cyclophosphamide; Flu – fludarabine; ATG – antithymocyte globulincytosine; GVHD – graft-versus host disease; pts – patients.

On the other hand, graft rejection is another key complication after allo-HCT, which happens more frequently in SAA cases, ranging from 0% to 25%, than other hematological malignancies [38–41]. Multiple transfusion is known to result in increased rates of graft rejection in SAA patients after HCT because of alloimmunization, which adversely affects survival rates [42,43]. Iron overload related to transfusion is also known to increase transplant-related complications, including graft rejection, and transplant-related mortality after HCT [44,45]. In the current study with 46 peripheral blood recipients older than 18 years of age who were preconditioned with FCA regimen, however, only 2 patients (4.35%) underwent a second graft rejection, and the estimated 5 year RR was much lower in the FCA group compared to the CA group (*P*=0.013), while the amount of transfusion and the serum level of ferritin were similar between the 2 groups. Moreover, the day 30 donor type chimerism detected by SNP-PCR was also higher in the FCA group than that in the CA group. These results indicated that the higher engraftment

property of the PB graft might play an important role in the lower RR in this cohort of adult SAA patients.

BMT with matched-unrelated donor is considered a first-line treatment approach for children with SAA who lack a matched-related donor. Lee et al. reported that matched-unrelated donor BMT conditioned with total body irradiation (TBI) and Cy regimen resulted in a 5-year OS of 88% in adult patients with SAA [46]. A recent retrospective study showed that the survival outcome of matched-unrelated BMT was not statistically inferior to that of matched-related donor BMT [47]. However, the outcome of matched-unrelated donor PBSCT for adult SAA is still unclear. Three previous studies using Flu-based regimen for matched-unrelated donor HCT (including BMT and PBSCT) with SAA patients showed that the 4-year or 5-year OS ranged from 67.9% to 96.7% (Table 4) [18,19,23]. In the current study, one-third of SAA patients (25 out of 76 patients) underwent a matched-unrelated donor PBSCT. Of these patients, the estimated 5-year OS of patients who received the FCA regimen and the CA regimen was 100% and 77.8%, respectively (*P*=0.052). The 5-year EFS for patients treated with the FCA regimen was superior to that of patients who received the CA regimen (*P*=0.013). These results can majorly be attributed to a lower RR rate in the FCA group compared to the CA group (*P*=0.016). The RR rate of the FCA group shown in this study was similar to that reported by Bacigalupo et al., with an RR of 5%

among young SAA patients who received matched-unrelated donors BMT [12]. Therefore, Flu can improve long-term survival mainly due to stronger immunosuppressive activity with lower RR rate, especially in adult patients with matched-unrelated donor PBSCT. More importantly, in our study, the stronger immunosuppressive activity of the FCA regimen was not associated with higher regimen-related toxicities or complications such as serious infections and organ toxicities (Table 3).

## Conclusions

Our findings indicated that the FCA regimen for allo-PBSCT is safe and feasible for adult patients with SAA, especially for those with a matched-unrelated donor. Compared to the CA regimen, the FCA regimen preconditioning for SAA resulted in higher rates of engraftment and EFS, fewer graft rejections, and acceptable incidence of GvHD and organ toxicity. The nature of this retrospective study which used historical controls and had a limited number of patients was a major limitation for this study. Further multicenter studies are needed to confirm this conclusion.

### Conflicts of interest

None.

## References:

1. Kojima S, Horibe K, Inaba J et al: Long-term outcome of acquired aplastic anaemia in children: Comparison between immunosuppressive therapy and bone marrow transplantation. Br J Haematol, 2000; 111: 321–28

2. Locasciulli A, Oneto R, Bacigalupo A et al: Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). Haematologica, 2007; 92: 11–18

3. Killick SB, Bown N, Cavenagh J et al: British Society for Standards in Haematology. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol, 2016; 172: 187–207

4. Bacigalupo A: How I treat acquired aplastic anemia. Blood, 2017; 129: 1428–36

5. Gupta V, Eapen M, Brazauskas R et al: Impact of age on outcomes after bone marrow transplantation for acquired aplastic anemia using HLA-matched sibling donors. Haematologica, 2010; 95: 2119–25

6. Marsh JC, Ball SE, Cavenagh J et al: Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol, 2009; 147: 43–70

7. Deeg HJ, Anasetti C, Petersdorf E et al: Cyclophosphamide plus ATG conditioning is insufficient for sustained hematopoietic reconstitution in patients with severe aplastic anemia transplanted with marrow from HLA-A, B, DRB matched unrelated donors. Blood, 1994; 83: 3417–18

8. Bacigalupo A, Socie' G, Lanino E et al: Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: A retrospective study from the EBMT-SAA working party. Haematologica, 2010; 95: 976–82

9. Champlin RE, Schmitz N, Horowitz MM et al: Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). Blood, 2000; 95: 3702–9

10. Aljurf M, Al-Zahrani H, Van Lint MT et al: Standard treatment of acquired SAA in adult patients 18–40 years old with an HLA-identical sibling donor. Bone Marrow Transplant, 2013; 48: 178–79

11. Kojima S, Matsuyama T, Kato S et al: Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: The Japan Marrow Donor Program. Blood, 2002; 100: 799–803

12. Bacigalupo A, Locatelli F, Lanino E et al: Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: A report from the EBMT-SAA Working Party. Bone Marrow Transplant, 2005; 36: 947–50

13. Maury S, Balère-Appert ML, Chir Z et al: Unrelated stem cell transplantation for severe acquired aplastic anemia: Improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica, 2007; 92: 589–96

14. Viollier R, Socié G, Tichelli A et al: Recent improvement in outcome of unrelated donor transplantation for aplastic anemia. Bone Marrow Transplant, 2008; 41: 45–50

15. Kennedy-Nasser AA, Leung KS, Mahajan A et al: Comparable outcomes of matched related and alternative donor stem cell transplantation for pediatric severe aplastic anemia. Biol Blood Marrow Transplant, 2006; 12: 1277–84

16. Tolar J, Deeg HJ, Arai S et al: Fludarabine-based conditioning for marrow transplantation from unrelated donors in severe aplastic anemia: Early results of a cyclophosphamide dose deescalation study show life-threatening adverse events at predefined cyclophosphamide dose levels. Biol Blood Marrow Transplant, 2012; 18: 1007–11

17. Maury S, Bacigalupo A, Anderlini P et al: Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: A comparison with conventional conditioning regimen. Haematologica, 2009; 94: 1312–15

18. Kang HJ, Shin HY, Park JE et al: Successful engraftment with fludarabine, cyclophosphamide, and thymoglobulin conditioning regimen in unrelated transplantation for severe aplastic anemia: A phase II prospective multi-center study. Biol Blood Marrow Transplant, 2010; 16: 1582–88

19. Kang HJ, Hong KT, Lee JW et al: Improved outcome of a reduced toxicity-fludarabine, cyclophosphamide, plus antithymocyte globulin conditioning regimen for unrelated donor transplantation in severe aplastic anemia: Comparison of 2 multicenter prospective studies. Biol Blood Marrow Transplant, 2016; 22: 1455–59

20. Raut SS, Shah SA, Patel KA et al: Improving outcome of aplastic anaemia with HLA-matched sibling donor hematopoietic stem cell transplantation: An experience of Gujarat Cancer and Research Institute (GCRI). Indian J Hematol Blood Transfus, 2015; 31: 1–8

21. Shin SH, Jeon YW, Yoon JH et al: Comparable outcomes between younger (≤40 years) and older (>40 years) adult patients with severe aplastic anemia after HLA-matched sibling stem cell transplantation using fludarabine-based conditioning. Bone Marrow Transplant, 2016; 51: 1456–63

22. Kim H, Lee JH, Joo YD et al: A randomized comparison of cyclophosphamide vs. reduced dose cyclophosphamide plus fludarabine for allogeneic hematopoietic cell transplantation in patients with aplastic anemia and hypoplastic myelodysplastic syndrome. Ann Hematol, 2012; 91: 1459–69

23. Kim H, Lee KH, Kim I et al: Allogeneic hematopoietic cell transplantation without total body irradiation from unrelated donor in adult patients with idiopathic aplastic anemia: Fludarabine versus cyclophosphamide -ATG. Leuk Res, 2014; 38: 730–36

24. Shao Y, Wang JM, Gong SL et al: [A novel single nucleotide polymorphism-based method for quantitative assessment of chimerism after allogeneic stem cell transplantation.] Zhonghua Xue Ye Xue Za Zhi, 2010; 31: 92–96 [in Chinese]

25. Thomas ED, Storb R, Clift RA et al: Bone marrow transplantation (second of two parts). N Engl J Med, 1975; 292: 895–902

26. Shulman HM, Sullivan KM, Weiden PL et al: Chronic graft-versus-host syndrome in man: A long-term clinicopathologic study of 20 Seattle patients. Am J Med, 1980; 69: 204–17

27. Basch E, Reeve BB, Mitchell SA et al: Development of the National Cancer Institute's patient-reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). J Natl Cancer Inst, 2014; 106: 244

28. Hay JL, Atkinson TM, Reeve BB et al: Cognitive interviewing of the US National Cancer Institute's patient reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). Qual Life Res, 2014; 23: 257–69

29. Barker JN, Hough RE, van Burik JA et al: Serious infections after unrelated donor transplantation in 136 children: Impact of stem cell source. Biol Blood Marrow Transplant, 2005; 11: 362–70

30. Kumar R, Bonfim C, George B: Hematopoietic cell transplantation for aplastic anemia. Curr Opin Hematol, 2017; 24: 509–14

31. Ghavamzadeh A, Alimoghaddam K, Jalili M et al: Peripheral blood versus bone marrow transplant in patients with aplastic anemia, an unresolved issue. Bone Marrow Transplant, 2016; 51: 1628–30

32. Kumar R, Kimura F, Ahn KW et al: Comparing outcomes with bone marrow or peripheral blood stem cells as graft source for matched sibling transplants in severe aplastic anemia across different economic regions. Biol Blood Marrow Transplant, 2016; 22: 932–40

33. Anasetti C, Logan BR, Lee SJ et al: Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med, 2012; 367: 1487–96

34. Gómez-Almaguer D, Vela-Ojeda J, Jaime-Pérez JC et al: Allografting in patients with severe, refractory aplastic anemia using peripheral blood stem cells and a fludarabine-based conditioning regimen: The Mexican experience. Am J Hematol, 2006; 81: 157–61

35. Scheinberg P: Aplastic anemia: Therapeutic updates in immunosuppression and transplantation. Hematology Am Soc Hematol Educ Program, 2012; 2012: 292–300

36. Bacigalupo A, Socie G, Schrezenmeier H et al: Bone marrow versus peripheral blood as the stem cell source for sibling transplants in acquired aplastic anemia: Survival advantage for bone marrow in all age groups. Haematologica, 2012; 97: 1142–48

37. Schrezenmeier H, Passweg JR, Marsh JC et al: Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood, 2007; 110: 1397–400

38. Kang HJ, Shin HY, Choi HS et al: Fludarabine, cyclophosphamide plus thymoglobulin conditioning regimen for unrelated bone marrow transplantation in severe aplastic anemia. Bone Marrow Transplant, 2004; 34: 939–43

39. Chan KW, Li CK, Worth LL et al: A fludarabine-based conditioning regimen for severe aplastic anemia. Bone Marrow Transplant, 2001; 27: 125–28

40. Kumar R, Prem S, Mahapatra M et al: Fludarabine, cyclophosphamide and horse antithymocyte globulin conditioning regimen for allogeneic peripheral blood stem cell transplantation performed in non-HEPA filter rooms for multiply transfused patients with severe aplastic anemia. Bone Marrow Transplant, 2006; 37: 745–49

41. Kahl C, Leisenring W, Deeg HJ et al: Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: A long-term follow-up. Br J Haematol, 2005; 130: 747–51

42. Champlin RE, Horowitz MM, van Bekkum DW et al: Graft failure following bone marrow transplantation for severe aplastic anemia: Risk factors and treatment results. Blood, 1989; 73: 606–13

43. Gajewski JL, Johnson VV, Sandler SG et al: A review of transfusion practice before, during, and after hematopoietic progenitor cell transplantation. Blood, 2008; 112: 3036–47

44. Platzbecker U, Ehninger G, Bornhäuser M: Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem-cell transplantation. Blood, 2007; 110: 3083

45. Lee JW, Kang HJ, Kim EK et al: Effect of iron overload and iron-chelating therapy on allogeneic hematopoietic SCT in children. Bone Marrow Transplant, 2009; 44: 793–97

46. Lee JW, Cho BS, Lee SE et al: The outcome of unrelated hematopoietic stem cell transplants with total body irradiation (800 cGy) and cyclophosphamide (120 mg/kg) in adult patients with acquired severe aplastic anemia. Biol Blood Marrow Transplant, 2011; 17: 101–8

47. Bacigalupo A, Socié G, Hamladji RM et al: Current outcome of HLA identical sibling versus unrelated donor transplants in severe aplastic anemia: An EBMT analysis. Haematologica, 2015; 100: 696–702