Safety and Efficacy of G-CSF after Allogeneic Hematopoietic Cell Transplantation Using Post-Transplant Cyclophosphamide: Clinical and In Vitro Examination of Endothelial Activation

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KEW WORDS: allo-HCT, G-CSF, endothelial activation, endothelial damage, PTCY.

ABSTRACT

Since 2021 the use of G-CSF was implemented in allo-HCT with PTCY-based prophylaxis with the aim of shortening the aplastic phase and reducing infectious complications. This study investigates the effectiveness of this change in protocol performed at our institution.

One-hundred forty-six adults undergoing allo-HCT with PTCY-based prophylaxis were included, and among them, 58 (40%) received G-CSF. The median of days to neutrophil engraftment was shorter in the G-CSF group (15 vs. 20 days, $p<0.001$). Patients receiving G-CSF had a lower incidence of day +30 bacterial bloodstream infections (BSI) than the rest (20.7% vs. 47.7%, $p<0.001$). GVHD, SOS, and TA-TMA incidences were comparable between groups, and using G-CSF did not impact on survival. Endothelial activation was investigated using EASIX and by the measurement of soluble biomarkers in cryopreserved plasma samples obtained on days 0, +7, +14 and +21 of 39 consecutive patients (10 received G-CSF) included in the study. EASIX, VWF:Ag, sVCAM-1, sTNFRI, ST2, REG3α, TM and NETs medians values were comparable in patients receiving G-CSF and those who did not.

Compared with allo-HCT performed without G-CSF, the addition of G-CSF to PTCY-based allo-HCT accelerated neutrophil engraftment contributing on decreasing BSI incidence, and without inducing additional endothelial activation.

HIGHLIGHTS

- G-CSF accelerates neutrophil stem cell engraftment contributing on reducing the incidence of bacterial bloodstream infections in patients undergoing allo-HCT with PTCY.
- The use of G-CSF in patients undergoing allo-HCT with PTCY does not increase the incidence of aGVHD, SOS or TA-TMA.
- Endothelial activation does not differ between patients undergoing allo-HCT with PTCY with or without G-CSF administration.

INTRODUCTION

The use of post-transplant cyclophosphamide (PTCY)-based prophylaxis has been progressively integrated into our program for peripheral blood (PB) allogeneic hematopoietic cell transplantation (allo-HCT) regardless of donor type (1–3). Aligned with published data, this approach has demonstrated to induce appropriate immunosuppression to allow engraftment and prevent graft-versus-host disease (GVHD) (1,3–7). However, using PTCY`s has also been linked to delayed engraftment and increased infections, mainly attributed to the negative impact of PTCY on immune reconstitution resulting in a constrained TCR repertoire, specially reported in haploidentical settings (haplo-HCT) (2,3,7–9).

In November 2021, we systematically implemented granulocyte colony-stimulating factor (G-CSF) from day +7 until neutrophil recovery to shorten the aplastic phase and reduce early infectious complications (8). G-CSF, is a key therapy in hematological settings, as blocks apoptosis, stimulates cell division, and enhances granulopoiesis, thereby reducing both the duration and severity of neutropenia to prevent infections (10–12).

Several studies point out to the crucial role of the endothelium in the initiation or the development of different early post-allo-HCT complications. During HCT, endothelial cells (EC) are activated and damaged by different factors, such as conditioning regimen, cytokines produced by the injured tissues, immunosuppressive medications, engraftment process, and allo-reactivity (10,13–17).

While the use of G-CSF post-allo-HCT has been linked to a pro-inflammatory state and the onset of vascular endothelial complications like GVHD, sinusoidal obstruction syndrome (SOS) and transplant-associated thrombotic microangiopathy (TA-TMA), other studies had yield conflicting results (13,18–24). Considering that these studies were conducted using GVHD prophylaxis protocols that did not include PTCY, we hypothesized that integrating G-CSF into PTCY-based allo-HCT protocols would be safe due to PTCY`s efficacy in mitigating GVHD. The

present study investigates the impact of implementing G-CSF in PTCY-based allo-HCT protocols, with a focus on early post-transplant endothelial activation and its clinical outcomes.

MATERIALS AND METHODS

Study Design and Patient Selection

Our retrospective analysis included 146 consecutive adults who underwent first PB allo-HCT with PTCY-based prophylaxis at Hospital Clínic Barcelona from 2020 to 2023. All consecutive patients transplanted after November 2021 received G-CSF prophylaxis. Additionally, an in vitro experimental analysis was conducted to assess prospectively the endothelial activation of cryopreserved plasma samples from 39 consecutive patients included in the entire cohort, and transplanted with (n=29) or without G-CSF (n=10) between May 2022 and July 2023.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the Hospital Clínic Barcelona (reference number: HCB/2022/0191) and conducted following the standards set forth by the Declaration of Helsinki. All patients provided informed consent for allo-HCT.

Main Allo-HCT Information and Definitions

Key information regarding procedures and definitions is summarized in **Supplementary Material (Section 1)**. Myeloablative regimens (MAC) generally combined fludarabine (40 mg/m^2/day intravenously (IV) x 4 days) with high-dose busulfan (3.2 mg/kg/day IV x 4 days), or 12 Gy of total body irradiation (TBI). Reduced intensity conditioning regimens combined standard doses of fludarabine with low-dose busulfan (3.2 mg/kg/day IV x 3 days), 8 Gy of TBI, or treosulfan (10 g/m^2 IV x 3 days). All patients undergoing haplo-HCT received 2 Gys of TBI when TBI was not included as part of the conditioning regimen.

PTCY was administered at a dose of 50 mg/kg/day on days +3 and +4 until December 2022. Since January 2023, PTCY dose was reduced to 40mg/kg daily (+3 and +4) for HLA-matched donors with the aim of further decreasing transplant toxicity. PTCY was combined with tacrolimus initiated at a dose of 0.03 mg/kg/24h IV on day +5. Mycophenolate mofetil from day +5 to day +30 was added when haplo-HCT were selected for allo-HCT. Immunosuppressant medication was maintained therapeutic until day +100 and tapered down progressively up to day +200 in the absence of GVHD.

All patients received unmanipulated T-cell replete PB stem cell (PBSC) grafts. Since November 2021, G-CSF was systematically administered at a dose of 300 µg/day from day +7 until neutrophil engraftment. The institutional antimicrobial prophylaxis and infection monitoring protocol are described in the **Supplementary Material (Section 2 and 3)**. All cytomegalovirus (CMV)-seropositive patients received letermovir (480 mg daily) until day +100 since November 2021.

Assessment Methodology of Endothelial Activation

Endothelial activation was assessed in vitro using soluble biomarkers in plasma samples from the experimental cohort and indirectly through Endothelial Activation and Stress Index (EASIX) in all study participants (14,21,24–26).

For the conduction of the experimental analysis, citrated blood samples were collected from patients on days 0, +7, +14, and +21, along with samples from 8 healthy individuals. Blood samples were centrifuged (3000g, 15min) and stored at -40ºC. The following soluble biomarkers were evaluated: von Willebrand factor antigen (VWF:Ag), soluble vascular cell adhesion molecule-1 (sVCAM-1), regenerating islet-derived 3-alpha (REG3α), soluble tumor necrosis factor receptor I (sTNFRI), soluble suppression of tumorigenicity 2 (ST2), thrombomodulin (TM), and neutrophil extracellular traps (NETs) (14,24,25,27,28). Plasma levels of circulating VWF:Ag were measured in the Atellica 360 COAG coagulometer (Siemens

149 Healthineers, Germany), by immunoturbidimetry. Plasma levels of sVCAM-1 (Sigma-Aldrich,
150 USA), REG3α (Abcam, United Kingdom), sTNFRI, ST2, and TM (R&D Systems, USA) were
151 measured by ELISA. Absorbance was read by MultiSkan Ascent (Thermo Electron, Finland).
152 NETs were determined by the quantification of circulating double-stranded DNA (dsDNA) using
153 the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Thermo Fisher, Massachusetts, USA), by
154 fluorimetry (Fluoroskan Ascent FL; Thermolab Systems, Massachusetts, USA).

155 EASIX (creatinine (mg/dL) x LDH (U/L) / platelets ($x10^9$/L)) was retrospectively calculated in all
156 patients based on the results provided at the bloodwork collected at the pre-transplant
157 assessment (between days -30 and -7 before allo-HCT) and on days 0, +7, +14, +21, +28, +100,
158 and +180. EASIX values were transformed to base 2 logarithm to conduct the statistical
159 analysis (24,29,30).

160 **Statistical Analysis**

161 The study cohort was divided into two groups based on G-CSF administration. Descriptive
162 analysis was expressed using median ± interquartile ranges (IQR) and counts and percentages.
163 Statistical analysis was performed with parametric or non-parametric tests as needed,
164 according to Kolmogorov-Simirnov normality tests (Student`s t test / Mann-Whitney U). $\chi 2$
165 tests / Fisher`s exact tests were applied for the evaluation of frequencies among categorical
166 variables.

167 To standardize the median follow-up of consecutively included patients in the study, post-
168 transplant follow-up was censored at 1 year. Outcomes were estimated using Kaplan-Meier
169 and cumulative incidence regression analyses. Cumulative incidence analyses of infectious
170 complications accounted for death as a competing event. Cumulative incidence analysis for
171 GVHD accounted for death and relapse as competing events. Multivariate regression analysis
172 was performed including variables considered clinically relevant for the outcome investigated
173 (days to neutrophil engraftment and cumulative incidence of bloodstream infection), and using

linear and Fine-Gray multivariate regression models. All P-values were 2-sided, and $p<0.05$ indicated a statistically significant result. The statistical analysis was performed by SPSS and EZR.

RESULTS

Baseline Characteristics of the Study Cohort

Clinical information of the 146 patients included is described in **Table 1**. Overall, the median age was 53 years (range, 18-75), with 101 (69.2%) patients being males. Acute myeloid leukemia (n=47, 32.2%) the most prevalent baseline diagnosis.

The study cohort was divided into two groups according to G-CSF administration (G-CSF n=58 vs. no G-CSF n=88). Baseline characteristics were balanced between groups except for the proportion of haplo-HCT (27.7% vs. 9.1%, p=0.015), and CMV-seropositive patients who received letermovir prophylaxis (81.0% vs. 19.3%, p<0.001), that were more prevalent in patients who received G-CSF. Since patients were included consecutively, the median follow-up was shorter in the G-CSF group (7.5 vs. 22 months, p<0.001).

Engraftment Information, G-CSF Tolerance, and Early Transplant Complications

As described in **Table 2**, the median of days to neutrophil engraftment was shorter in the G-CSF group (15 vs. 20 days, p<0.001) (**Figure 1**), with no differences in the median of days to platelet engraftment (17 vs. 21 days, p=0.198). Considering these results, a complementary linear multivariate regression analysis was estimated confirming the positive association between using G-CSF and faster neutrophil engraftment (Odds ratio -2.83, p=0.013) (**Supplementary Material, Section 4**).

Three (2.05%) patients experienced primary graft failure (GF), and none of them received G-CSF (p=0.277). Secondary GF occurred in 9 (6.16%) patients with no differences according to G-

CSF administration (p=0.308). At day +60, the achievement of >95% myeloid and lymphoid donor chimerism was more frequent in the G-CSF group (granulocytes: 100% vs. 88.3%, p=0.036; lymphocytes: 80.7% vs. 55.7%, p=0.030), with no differences observed on chimerisms on days +30 and +180. Immune reconstitution was similar in both groups.

Platelet and red blood cell transfusion support was comparable between groups. The median of platelet transfusions required during the first 28 days and 100 days were 4 and 5 for patients with G-CSF, and 5 (p=0.372) and 6 (p=0.208) for those who did not. Similarly, the median of red cell transfusions required per group were 3 and 4, and 5 (p=0.176) and 7 (p=0.165), respectively.

The median duration of G-CSF treatment was 11 days (IQR: 10-13).G-CSF was well tolerated with occasional and discrete bone pain during the peri-engraftment phase. Two (3.4%) patients required G-CSF discontinuation due to the diagnosis of engraftment syndrome (ES) -1 patient- and capillary-leak syndrome (CLS) -1 case- who successfully recovered without requiring Intensive Care Unit (ICU) admission.

Grade 3-4 mucositis, and grade 3-4 neutropenic colitis were similar in both groups (31.0% vs. 20.4%, and 6.8% vs. 6.8%, respectively). Median days of transplant hospitalization (28 vs. 30, p=0.140), day +180 cumulative incidence function (CIF) of ICU admission (14.4% vs. 15.9%, p=0.790), and readmission rates (36.2% vs. 48.9%, p=0.132) were comparable between groups.

Three (2.0%) patients had SOS, and two of them received G-CSF. SOS severity of patients receiving G-CSF was mild and it successfully resolved after fluid restriction and diuretic medication. Four (2.7%) patients had TA-TMA, and three of them received G-CSF. All clinical manifestations were mild and all cases were attributed to calcineurin inhibitors toxicity.

Infectious Complications

Patients who received G-CSF had lower incidence of bacterial bloodstream infections (BSI) during the first 30 days after allo-HCT (20.7% vs. 47.7%, p<0.001). The day +30 CIF of Gram-Positive and Negative BSI were 5.2% and 13.8% in patients receiving G-CSF, and 20.5% (p=0.003) and 21.6% (p=0.193) in those who did not. As shown in **Supplementary Material (Section 5)**, among Gram-Positive and Gram-Negative BSI, *Streptococcus* (0% vs. 5.6%) and *Staphylcoccus* (8.6% vs. 11.3%), *Escherichia* (5.1% vs. 13.6%) and *Klebsiella* (1.7% vs. 3.4%) BSI were less prevalent in the G-CSF group. The median duration of targeted antibiotic treatment was shorter in patients receiving G-CSF (7 vs. 10 days, p=0.003). The impact of G-CSF implementation was additionally investigated using multivariate regression analysis confirming the positive effect of using G-CSF on the onset of this complication (HR 0.33, p<0.001) (**Table 3).**

Day +180 CIF of CMV reactivation was 10.4% in the G-CSF group and 35.2% in the no G-CSF group (p=0.002). The incidences of CMV disease, Epstein Barr virus (EBV) reactivation, Human Herpesvirus type 6 (VHH6) reactivation or disease, grade 2-4 BK-virus hemorrhagic cystitis, and respiratory viral infections were comparable between the two study groups. Lastly, a non-significant trend to higher invasive fungal infection (IFI) was observed in the G-CSF group (17.2% vs. 6.8%, p=0.075).

Graft-versus-Host Disease

As described in **Table 2** and **Figure 1**, the day +100 CIF of grades II-IV and III-IV aGVHD were 19.0% and 8.6% in patients receiving G-CSF, and 27.3% (p=0.305) and 9.1% (p=0.951) in patients not receiving it. The 1-year incidence of moderate/severe cGVHD was similar in both groups (1.9% vs. 7.7%, p=0.320). Clinical manifestations and severity of GVHD did not differ between groups. Lastly, two (1.3%) patients died secondary to steroid-refractory GVHD, one of them received G-CSF.

Main Outcomes

Post-transplant outcomes were comparable between the two groups (**Figure 2**). During the first year after allo-HCT, 15 (10.2%) patients relapsed and 13 (8.9%) died. The leading cause of death was infection in the two groups, representing the 75% and 44.4% of the primary causes of death in each group. The estimated 1-year overall survival (OS), relapse-free survival, and non-relapse mortality (NRM) were 93.1%, 82.4% and 12.5% for patients receiving G-CSF, and 89.8% (p=0.529), 79.5% (p=0.674) and 14.5% (p=0.848) for those who did not.

Dynamics of EASIX According to G-CSF

Log2-EASIX trends were examined in the 146 adults included. As illustrated in **Figure 3**, log2-EASIX increased rapidly from day 0 to day +7, peaked at day +21, and gradually declined by day +180, regardless of G-CSF use, suggesting that the onset of early endothelial activation post-allo-HCT persisted during the peri-engraftment phase. Median log2-EASIX values were comparable between groups except on day +100, where the log2-EASIX values were lower in the G-CSF group (0.70 vs. 1.45, $p<0.05$).

Endothelial Activation and Damage and the Impact of G-CSF Administration: In Vitro Analysis

To delve into G-CSF's impact on endothelial activation, we assessed predefined biomarkers in 39 patients, 74.3% of whom received G-CSF (experimental analysis). Descriptive data of this patient subset is described in **Supplementary Material (Section 6).**

As illustrated in **Figure 3**, overall, higher endothelial activation was observed from day 0 to day +21, regardless of G-CSF administration when compared with control patients. VWF:Ag, sTNFRI, and ST2 consistently increased with no significant differences between groups. sVCAM-1 trends were superior in G-CSF group throughout all the time points but only significantly on day +21 (medians: 887.02 vs. 720.27, $p<0.05$). REG3α showed similar dynamics in both groups, with higher values than control cases only on days 0 and +7. No differences were noted in NETs values between groups or with control cases.

Plasma TM levels were lower in allo-HCT patients compared to controls, indicating endothelial injury. However, TM levels were significantly higher in the G-CSF group on days 0, +7, and +14 (medians TM on day 0: 3.51 vs. 2.47; day +7: 2.93 vs. 2.59; and day +14: 3.75 vs. 2.25, $p<0.05$), suggesting reduced endothelial injury.

EASIX analysis showed a rapid increase post-infusion, persistent elevation early post-transplant, and gradual decrease thereafter, consistent across both groups. However, differences in log2-EASIX medians between groups were noted only on day +28 (1.87 vs. 0.99, $p<0.05$).

Impact of G-CSF According to Donor Type

The impact of adding G-CSF on endothelial activation was further investigated in patients receiving grafts from HLA-matched donors and alternative donors (9/10 HLA-mismatched unrelated and haplo-HCT). Neutrophil recovery (matched: 20 vs. 15 days, $p<0.001$; alternative: 19 vs. 15 days, $p<0.001$) and BSI incidence (matched: 15.6% vs. 46.6%, $p<0.001$; alternative: 26.9% vs. 50.0%, $p=0.088$) were lower in patients who received G-CSF irrespective of donor type, **Figure 4**.

As shown in **Supplementary Material (Section 7)**, comparable medians of endothelial activation biomarkers and EASIX values were documented according to G-CSF administration in both donor groups. As observed in the entire cohort, only TM levels in HLA-matched donors showed significant differences at days 0 and +14, being higher in the G-CSF group (median TM day 0: 3.44 vs. 2.47, and median TM day +14: 3.75 vs. 1.74, $p <0.05$).

DISCUSSION

This study confirms that adding G-CSF in allo-HCT with PTCY-based prophylaxis accelerates neutrophil engraftment and reduces BSI during the peri-engraftment phase. Notably, G-CSF

did not increase endothelial activation or impact on the likelihood of post-transplant vascular endothelial complications.

G-CSF was implemented at our program in November 2021 after observing increased BSI rates during the aplastic phase with PTCY-based prophylaxis (1,4). Despite PTCY`s effectiveness in preventing GVHD, it has been associated with delayed neutrophil recovery and higher BSI incidence (43.5% compared to 28.5% with previous prophylaxis) (1,3,8,31,32). Since BSI is a potentially life-threatening complication, reducing its incidence is critical for improving transplant outcomes and decreasing medical costs (3,5,6).

Our analysis confirmed that G-CSF effectively accelerates neutrophil recovery and decreases BSI risk (3,5,6). However, G-CSF administration did not impact on immune reconstitution, transfusion support requirement, ICU admissions, OS or NRM. Interestingly, patients receiving G-CSF achieved faster day +60 chimerism suggesting that G-CSF administration might induce an additional stimulation of the stem cell graft function enhancing the achievement of a faster donor chimerism. Unexpectedly, a trend to higher incidence of IFI was observed in the G-CSF group, probably due to the higher incidence of viral respiratory infections diagnosed after November 2021 (COVID-19 pandemic).

Contrary to concerns about G-CSF-related endothelial activation, our findings indicate no significant increase in complications such as SOS, TA-TMA or acute GVHD. G-CSF has been historically associated with endothelial activation, and identified as a risk factor for the development of post-transplant vascular endothelial complications, especially GVHD (10,13,15–18,20,21,33,34). In vitro studies postulated that G-CSF exposition can induce a pro-inflammatory state followed by an activation of the JAK/STAT signaling pathway, long-lasting phosphorylation of MAPK p42/44, together with an increase in the concentration of endothelial adhesion receptors, leukocyte recruitment, and IL-6 levels (10,11,23), inducing endothelium activation and dysfunction. Since these studies were conducted in allo-HCT

317 settings without PTCY, we presuppose that PTCY`s ability to mitigate GVHD might
318 counterbalance G-CSF`s potential for endothelial damage.

319 Endothelial activation occurred similarly in all patients irrespectively of the G-CSF
320 administration. These results contrast with previous studies where consistently observed a
321 higher endothelial activation in adults receiving G-CSF, together with an increased risk for
322 endothelial vascular post-transplant complications (18–21). We hypothesize that the
323 prophylactic effect induced by PTCY-based prophylaxis on allo-reactivity during the peri-
324 engraftment phase may have mitigated the potential endothelial injury induced by G-CSF, and
325 ultimately result in comparable clinical manifestations between both groups.

326 Notably, G-CSF administration affected TM values which were lower in the G-CSF cohort than
327 in control cases and sVCAM-1 values, which were superior on day +21 in patients receiving G-
328 CSF. TM`s protective role in endothelial function makes higher TM levels beneficial (14,35,36).
329 In our cohort, the higher levels of TM in patients receiving G-CSF were particularly noted in
330 patients from HLA-matched donors. These results were potentially linked to the lower BSI,
331 CMV reactivation rates, and reduced PTCY doses (40 mg/kg/day) administered to these
332 patients after December 2022 (37). However, further confirmatory analysis would be needed.

333 On the other hand, in the HCT setting the over expression of adhesion molecules such as
334 sVCAM-1, contributes to endothelial dysfunction by inducing leukocyte recruitment and
335 transmigration through the endothelium (25). In our cohort, elevated sVCAM-1 levels on day
336 +21 in the G-CSF group suggest that G-CSF may induce subtle endothelial activation detectable
337 through this sensitive biomarker, as no clinical differences were observed at this time point.
338 Unlike previous studies, no significant differences in NETs values were found between groups,
339 likely due to the timing of measurements and the low incidence TA-TMA in our cohort (14,38).

340 Lastly, although endothelial activation during allo-HCT has been extensively investigated (21),
341 limited studies have explored how allo-HCT performed PTCY interacts with endothelium

activation and disease. Our study also provides innovative evidence on this field showing that the most remarkable increment on EC activation in PTCY-based allo-HCT patients occurred during the first 7 days after post-transplant, likely driven by stem cell allo-reactivity, PTCY administration, and tacrolimus initiation. Subsequently, this activation persisted with a slight increase around day +14 during the peri-engraftment phase.

Ultimately, endothelial activation was indirectly assessed using EASIX in both the experimental and entire cohort (26,39–41). As reported, EASIX mirrored these trends, peaking on day +21 and then declining until day +180. Notably, patients receiving G-CSF had lower EASIX values on day +100, likely due to reduced CMV reactivation rates, linked to the concurrent use of letermovir in CMV-seropositive patients.

Our study´s limitations include the cohort´s heterogeneity, including variations in PTCY doses and donor types, small sample size, and shorter follow-up for G-CSF recipients. Despite these limitations, our findings offer preliminary insights into the safety and benefits of G-CSF in PTCY-based allo-HCT. Future studies will focus on specific patient subgroups to validate these results and explore their applicability across different allo-HCT settings. Additionally, while G-CSF-related toxicities are expected to manifest early post-transplant, the actual follow-up of patients was considered adequate for presenting conclusions, although longer follow-up is essential for understanding its long-term impact. Our biomarkers analysis, using ELISA kits rather than in vitro models, also limits control over variables but provides real-world clinical insights.

In summary, our study underscores the safety and efficacy of G-CSF in patients undergoing allo-HCT with PTCY, regardless of donor type. G-CSF facilitated stem cell engraftment acceleration and reduced the incidence of BSI, without increasing endothelial activation. However, further investigations are warranted to confirm these findings and evaluate their

impact on infection-related mortality. These results are particularly relevant as PTCY becomes more widely adopted in the allo-HCT community.

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TABLE LEGENDS

Table 1. Baseline Information.

Table 2. Main Post-Transplant Information according to G-CSF.

Table 3. The impact of G-CSF implementation on BSI. Multivariate regression analysis.

FIGURE LEGENDS

Figure 1. Engraftment information and main early transplant complications.

Figure 2. Main post-transplant outcomes.

Figure 3. EASIX and soluble biomarkers dynamics.

Figure 4. Bacterial bloodstream infection cumulative incidence according to donor type.

Table 1. Baseline Information.

	G-CSF N=58	No G-CSF N=88	P value
Age at allo-HCT median, years (range)	54 (19-75)	53 (18-71)	0.631
Sex (%)			
Male	42 (72.4)	59 (67.0)	0.492
Baseline Diagnosis (%)			
AML	18 (31.1)	29 (33.0)	-
MDS	14 (24.1)	22 (25.0)	
MPN	3 (5.2)	4 (4.6)	
ALL	14 (24.1)	22 (25.0)	
NHL	7 (12.1)	8 (9.1)	
CML	-	1 (1.1)	
PCD	1 (1.7)	-	
Others	1 (1.7)	2 (2.3)	
Karnofsky Performance Status (%)			
<90%	14 (24.1)	22 (25.0)	0.906
HCT-CI score (%)			
≥3	8 (13.8)	23 (26.1)	0.074
Donor selection (%)			
HLA MSD	10 (17.2)	26 (29.5)	0.015
10/10 HLA MUD	22 (37.9)	32 (36.4)	
9/10 HLA MMUD	10 (17.2)	22 (25.0)	
Haploidentical	16 (27.7)	8 (9.1)	
Intensity of the conditioning regimen (%)			
Myeloablative	30 (51.7)	47 (53.4)	0.842
Reduced Intensity	28 (48.3)	41 (46.6)	
Conditioning regimen Extended (%)			
Myeloablative			
Flu-Bu (4) (+/-TBI2)	13 (22.4)	26 (29.6)	-
Flu-TBI(12Gy)	13 (22.4)	20 (22.7)	
Other	2 (3.5)	1 (1.1)	
Reduced Intensity			
Flu-Bu3 (+/-TBI2)	13 (22.4)	27 (30.7)	
Flu-TBI (8Gy)	5 (8.6)	7 (8.0)	
Flu/Treo	4 (6.9)	1 (1.1)	
Other	2 (3.5)	3 (3.4)	
Sequential RIC Allo-HCT	6 (10.3)	3 (3.4)	
GVHD Prophylaxis Extended (%)			
PTCY/TK/MMF	15 (25.9)	10 (11.4)	-
PTCY/TK	40 (68.9)	75 (85.2)	
PTCY/CyA/MMF	1 (1.7)	-	
PTCY/SIR/MMF	2 (3.5)	3 (3.4)	
CD34+ median cell dose (IQR)	6.3 (5.2-6.9)	5.8 (4.6-7.0)	0.341
Letermovir Prophylaxis (%)	47 (81.0)	17 (19.3)	<0.001
Median Follow-up			
Months (IQR)	7.5 (6.0-13.2)	22.0(9.0-31.0)	<0.001

ALL: acute lymphoblastic leukemia; allo-HCT: allogeneic hematopoietic cell transplantation; AML: acute myeloid leukemia; Bu: busulfan; Flu: fludarabine; GVHD: graft-versus-host disease; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; IQR: interquartile range; MDS: myelodysplastic syndrome; MMUD: mismatched unrelated donor; MPN: myeloproliferative neoplasm; MSD: matched sibling donor; MUD: matched unrelated donor; NHL: non-Hodgkin lymphoma; PCD: plasma cell dyscrasia; RIC: reduced intensity conditioning; TBI: total body irradiation; Treo: treosulfan.

Table 2. Main Post-Transplant Information according to G-CSF.

	G-CSF N=58	No G-CSF N=88	P value
Engraftment information			
Median days neutrophil engraftment (IQR)	15 (14-17)	20 (17-23)	<0.001
Median days platelet engraftment (IQR)	17 (13-25)	21 (14-29)	0.198
Transfusion requirements			
Median platetet transfusions (first 28 days)	4 (2-9)	5 (2-10)	0.372
Median platelet transfusions (first 100 days)	5 (2-10)	6 (2-20)	0.208
Median red blood cell transfusions (first 28 days)	3 (1-6)	5 (1-9)	0.176
Median red blood cell transfusions (first 100 days)	4 (2-10)	7 (2-15)	0.165
Graft Failure			
Primary	0	3 (3.4%)	0.277
Secondary	5 (8.6%)	4 (4.5%)	0.308
Peripheral blood chimerism			
Day +30 granulocytes			0.361
Number of determinations	29	71	
>95% donor, n (%)	29 (100%)	69 (97.1%)	
Day +30 lymphocytes			0.531
Number of determinations	11	47	
>95% donor, n (%)	7 (63.6%)	25 (53.1%)	
Day +60 granulocytes			0.036
Number of determinations	35	60	
>95% donor, n (%)	35 (100%)	53 (88.3%)	
Day +60 lymphocytes			0.030
Number of determinations	26	52	
>95% donor, n (%)	21 (80.7%)	29 (55.7%)	
Day +180 granulocytes			0.071
Number of determinations	19	19	
>95% donor, n (%)	19 (100%)	16 (84.2%)	
Day +180 lymphocytes			0.638
Number of determinations	19	18	
>95% donor, n (%)	12 (63.1%)	10 (55.5%)	
Median days of immune reconstitution (IQR)			
IgG (IgG>6.5 g/L)	154 (95-275)	171 (94-322)	0.691
CD3 (CD3>200)	185 (175-209)	186 (159-203)	0.470
CD4 (CD4>200)	202 (181-272)	223 (187-312)	0.156
CD8 (CD8>200)	197 (180-225)	191 (172-236)	0.417
Grade 3-4 mucositis, n (%)	18 (31.0)	18 (20.4)	0.729
Grade 3-4 neutropenic colitis, n (%)	4 (6.8)	6 (6.8)	0.524
TPN requirement, n (%)	13 (22.4)	13 (14.7)	0.289
Veno-Occlusive Disease, n (%)	2 (3.4)	1 (1.1)	0.335
TA-TMA, n (%)	3 (5.1)	1 (1.1)	0.144
Engraftment syndrome, n (%)	2 (3.4)	0	0.156
CLS, n (%)	1 (1.7)	0	0.397
Median duration of transplant hospitalization			
Days (IQR)	28 (22-36)	30 (26-38)	0.140
Readmission (first 180 days)			
Yes, n (%)	21 (36.2)	43 (48.9)	0.132
Days from HCT discharge to readmission (IQR)	28 (22-36)	30 (27-37)	0.141
Cumulative incidence of ICU admission [% (95% CI)]			
Day +180	14.4 (6.6-25.0)	15.9 (9.2-24.3)	0.790
Cumulative incidence infectious complications [% (95% CI)]			
Day +30 Bacterial bloodstream infection	20.7 (11.3-32.0)	47.7 (36.9-57.7)	<0.001
Day +30 Gram Positive bacterial bloodstream infection	5.2 (1.3-13.1)	20.5 (12.7-29.5)	0.003

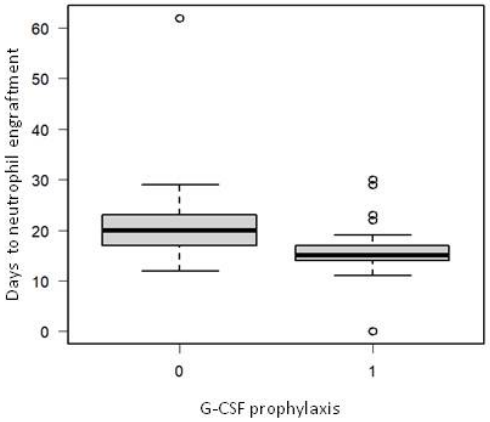
Day +30 Gram Negative bacterial bloodstream infection	13.8 (6.4-24.0)	21.6 (13.7-30.7)	0.193
Day +180 CMV reactivation	10.4 (4.2-19.9)	35.2 (25.445.2)	0.002
Day +180 CMV disease	3.4 (0.6-10.7)	8.0 (3.5-14.8)	0.277
Day +180 EBV reactivation	3.5 (0.6-10.7)	4.5 (1.5-10.4)	0.769
Day +180 VHH6 disease*	26.0 (15.4-37.8)	13.6 (7.4-21.7)	0.061
Day +180 Grade 2-4 BK-virus hemorrhagic cystitis	8.6 (3.1-17.6)	12.5 (6.6-20.4)	0.501
Day +180 Respiratory viral infection	33.3 (21.4-45.7)	26.1 (17.4-35.7)	0.481
Day +180 Fungal infection	17.2 (8.8-28.0)	6.8 (2.8-13.4)	0.075
Median duration of infections targeted antibiotic treatment			
Days (IQR)	7 (6-9)	10 (7-12)	0.003
Median days from the stem cell infusion to any grade aGVHD diagnosis			
Days (IQR)	22 (19-37)	30 (22-48)	0.087
Median days from the stem cell infusion to any grade cGVHD diagnosis			
Days (IQR)	123 (81-248)	209 (146-247)	0.139
Cumulative incidence of GVHD [% (95% CI)]			
Day +100 Grade II-IV aGVHD	19.0 (10.1-30.3)	27.3 (18.3-36.9)	0.305
Day +100 Grade III-IV aGVHD	8.6 (3.1-17.6)	9.1 (4.2-16.2)	0.951
1-Year Moderate/Severe cGVHD	1.9 (0.1-8.9)	7.7 (3.1-15.0)	0.320
Main Outcomes			
1-year Overall Survival	93.1 (82.7-97.4)	89.8 (81.3-94.5)	0.529
1-year Relapse-Free Survival	82.4 (69.8-90.1)	79.5 (69.5-86.6)	0.674
1-year Non-Relapse Mortality	12.5 (4.5-24.7)	14.5 (7.9-23.1)	0.848
1-Year Cumulative Incidence of Relapse	10.6 (3.0-23.9)	8.1 (3.5-15.0)	0.944

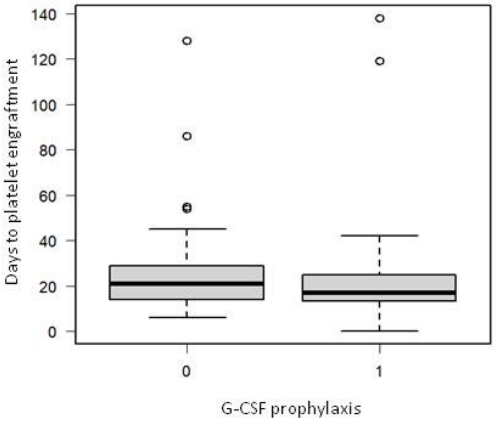
*Post-transplant follow-up has been censored at 1-year. Any event occurring after 1 year has not been accounted in the present analysis. *aGVHD: acute GVHD; cGVHD: chronic GVHD; CI: confidence interval; CLS: capillary-leak syndrome; CMV: cytomegalovirus; EBV: Epstein Barr virus; GVHD: graft-versus-host disease; HCT: hematopoietic cell transplantation; ICU: intensive care unit; IQR: interquartile range; TA-TMA: transplant-associated thrombotic microangiopathy; TPN: total parenteral nutrition; VHH6: human herpesvirus type 6.*

Table 3. The impact of G-CSF implementation on BSI. Multivariate regression analysis.

	Cumulative Incidence of BSI Hazard Ratio (95% CI)	P value
Age (continuous)	1.02 (0.99-1.04)	0.062
Mismatched donor (9/10 MMUD and haploidentical) (vs. HLA-matched)	1.33 (0.78-2.27)	0.280
HCT-CI score >3 (vs. 0-3)	0.63 (0.32-1.22)	0.180
KPS <90% (vs. 90-100%)	1.81 (1.06-3.07)	0.027
RIC (vs. MAC)	0.58 (0.30-1.13)	0.110
Grade 3-4 mucositis (vs. No)	0.91 (0.50-1.63)	0.750
G-CSF prophylaxis (vs. No)	0.33 (0.17-0.63)	<0.001

BSI: bacterial bloodstream infections; CI: confidence interval; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; KPS: Karnofsky Performance Status; MAC: myeloablative conditioning; MMUD: mismatched unrelated donor; *RIC: reduced intensity conditioning.*


Days to neutrophil engraftment
0
10
20
30
40
50
60
0
1
G-CSF prophylaxis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40
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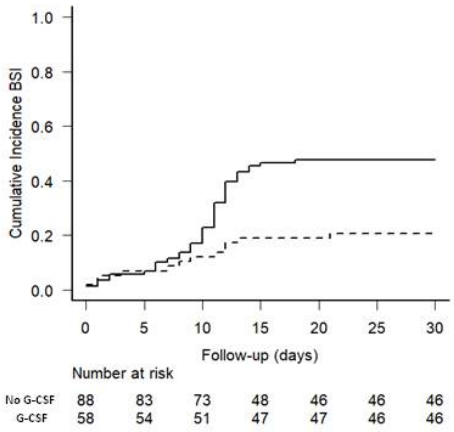
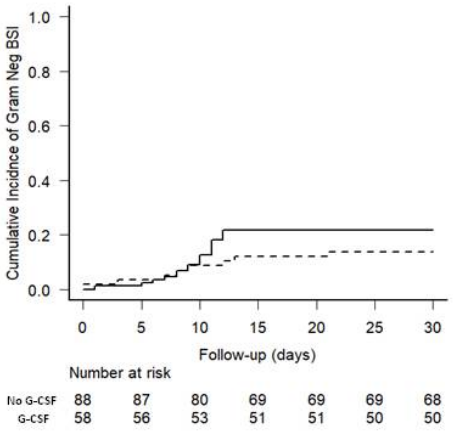
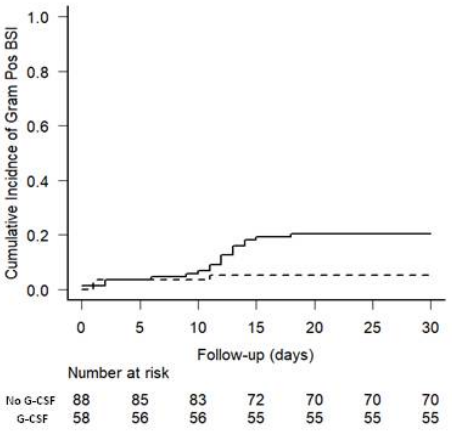
100
120
140
0
1
G-CSF prophylaxis


No G-CSF
G-CSF
Cumulative Incidence BSI
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Follow-up (days)
Number at risk
No G-CSF 88 83 73 48 46 46 46
G-CSF 58 54 51 47 47 46 46


Cumulative Incidnce of Gram Neg BSI
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Follow-up (days)
Number at risk
No G-CSF 88 87 80 69 69 69 68
G-CSF 58 56 53 51 51 50 50


Cumulative Incidnce of Gram Pos BSI
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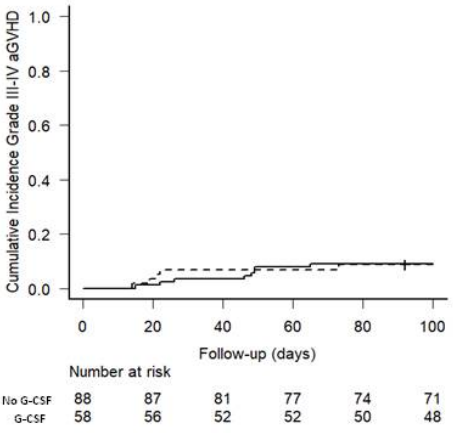
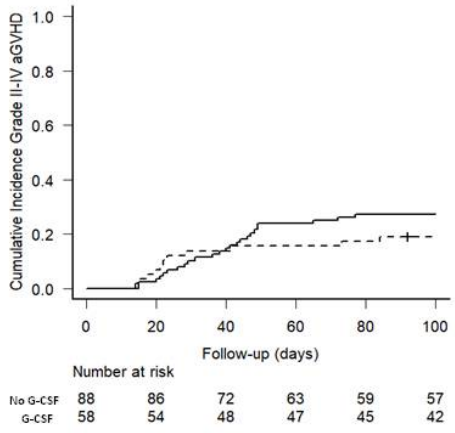
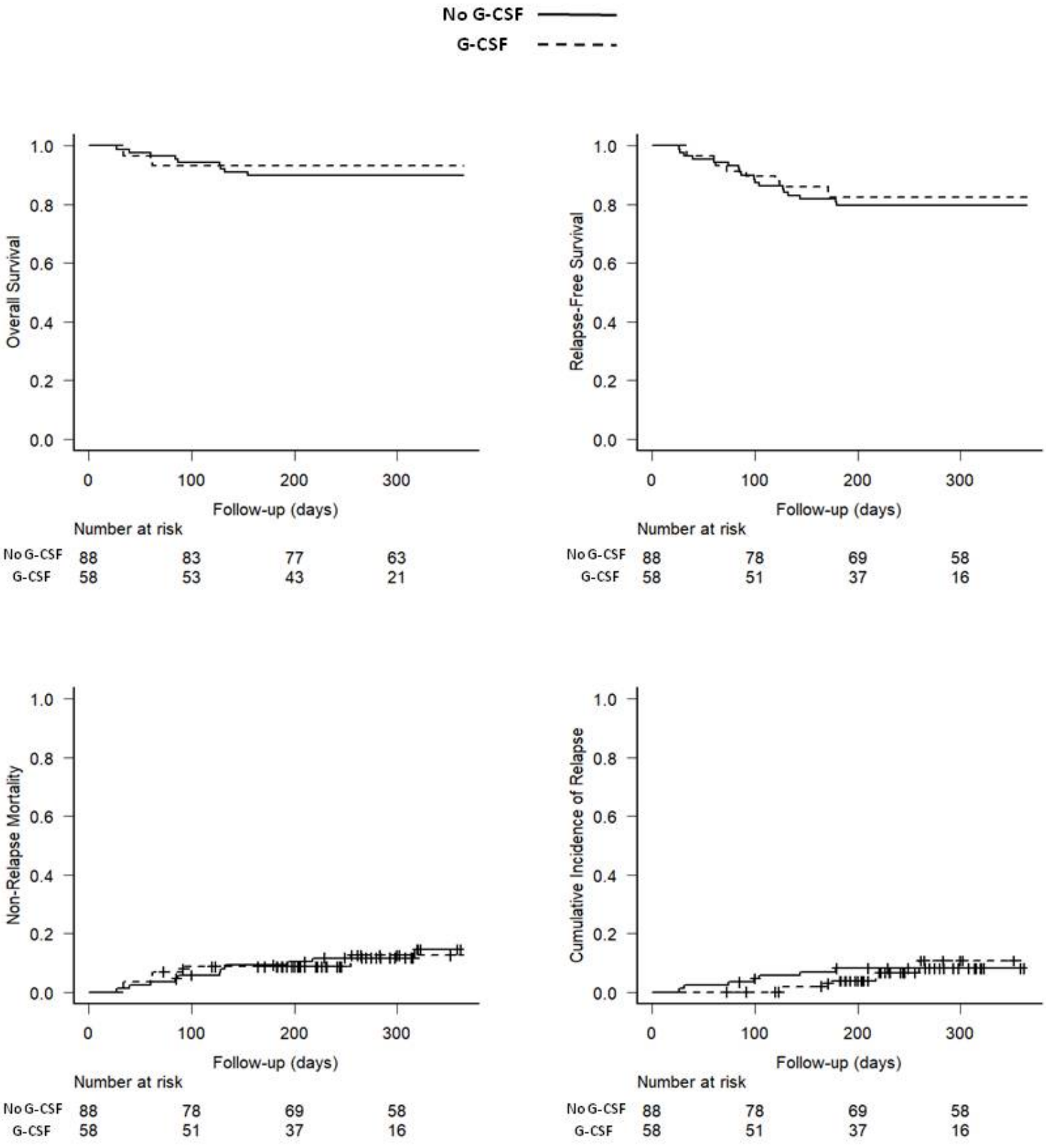
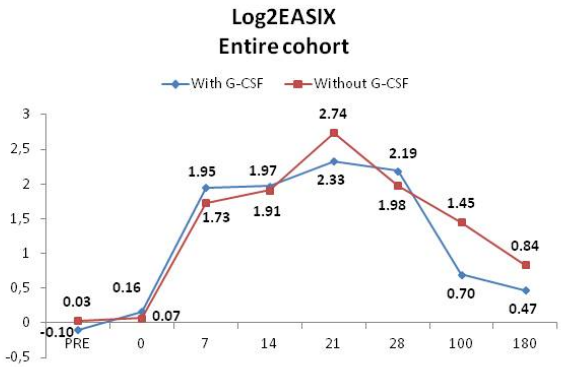
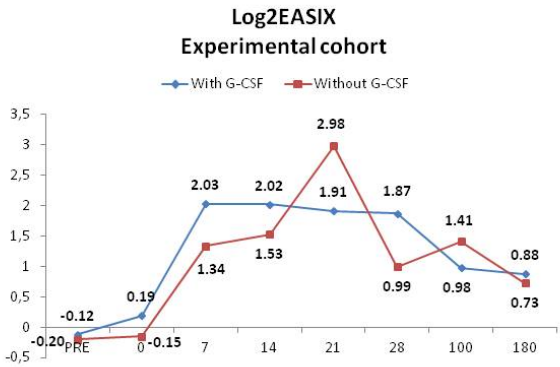
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
Follow-up (days)
Number at risk
No G-CSF 88 85 83 72 70 70 70
G-CSF 58 56 56 55 55 55 55

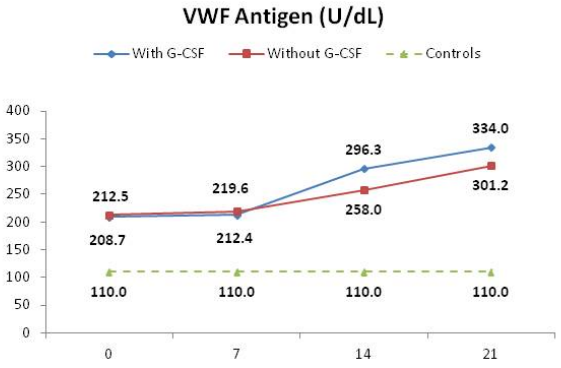

Cumulative Incidence Grade III-IV aGVHD
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Follow-up (days)
Number at risk
No G-CSF 88 87 81 77 74 71
G-CSF 58 56 52 52 50 48

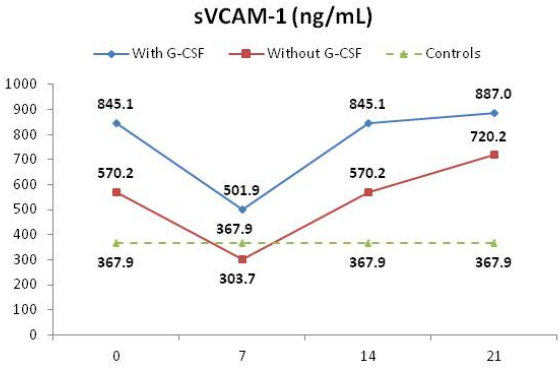

Cumulative Incidence Grade II-IV aGVHD
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Follow-up (days)
Number at risk
No G-CSF 88 86 72 63 59 57
G-CSF 58 54 48 47 45 42

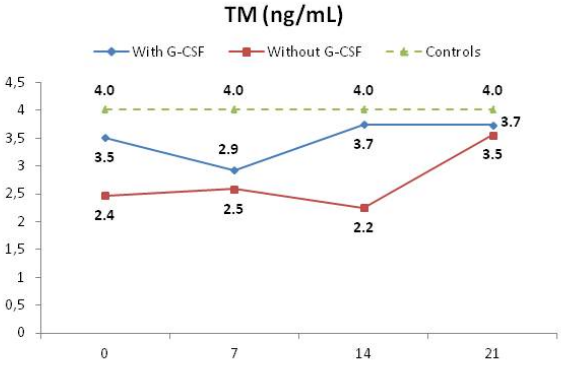

No G-CSF
G-CSF
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
Follow-up (days)
Number at risk
No G-CSF 88 83 77 63
G-CSF 58 53 43 21
Relapse-Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
Follow-up (days)
Number at risk
No G-CSF 88 78 69 58
G-CSF 58 51 37 16
Non-Relapse Mortality
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
Follow-up (days)
Number at risk
No G-CSF 88 78 69 58
G-CSF 58 51 37 16
Cumulative Incidence of Relapse
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
Follow-up (days)
Number at risk
No G-CSF 88 78 69 58
G-CSF 58 51 37 16

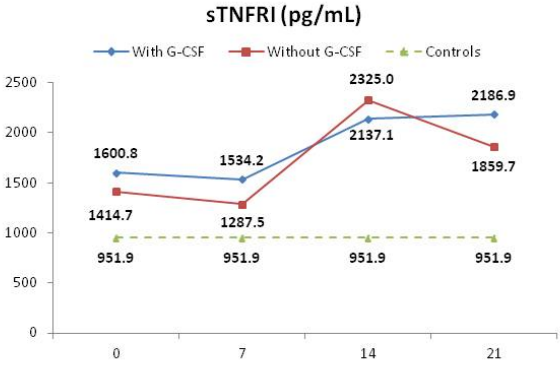

Log2EASIX
Entire cohort
With G-CSF
Without G-CSF
PRE
0
7
14
21
28
100
180
-0.10
0.03
0.16
0.07
1.95
1.73
1.97
1.91
2.74
2.33
2.19
1.98
1.45
0.70
0.84
0.47

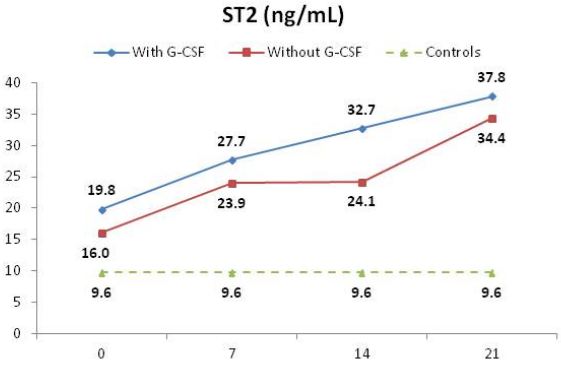

Log2EASIX
Experimental cohort
With G-CSF
Without G-CSF
PRE
0
7
14
21
28
100
180
-0.12
-0.20
0.19
-0.15
2.03
1.34
2.02
1.53
2.98
1.91
1.87
0.99
1.41
0.98
0.88
0.73

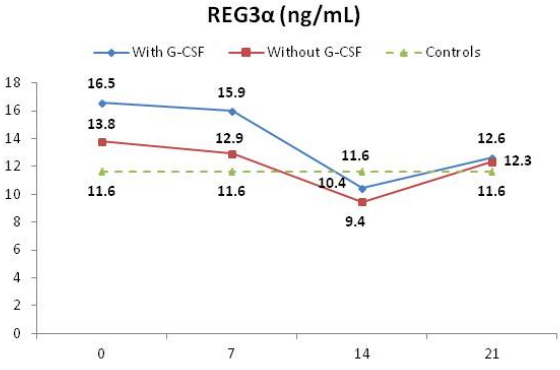

VWF Antigen (U/dL)
With G-CSF
Without G-CSF
Controls
0
7
14
21
212.5
208.7
219.6
212.4
296.3
258.0
334.0
301.2
110.0
110.0
110.0
110.0

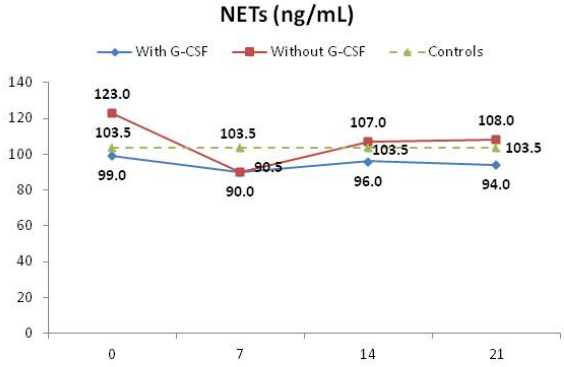

sVCAM-1 (ng/mL)
With G-CSF
Without G-CSF
Controls
0
7
14
21
845.1
570.2
501.9
367.9
845.1
570.2
887.0
720.2
367.9
303.7
367.9
367.9

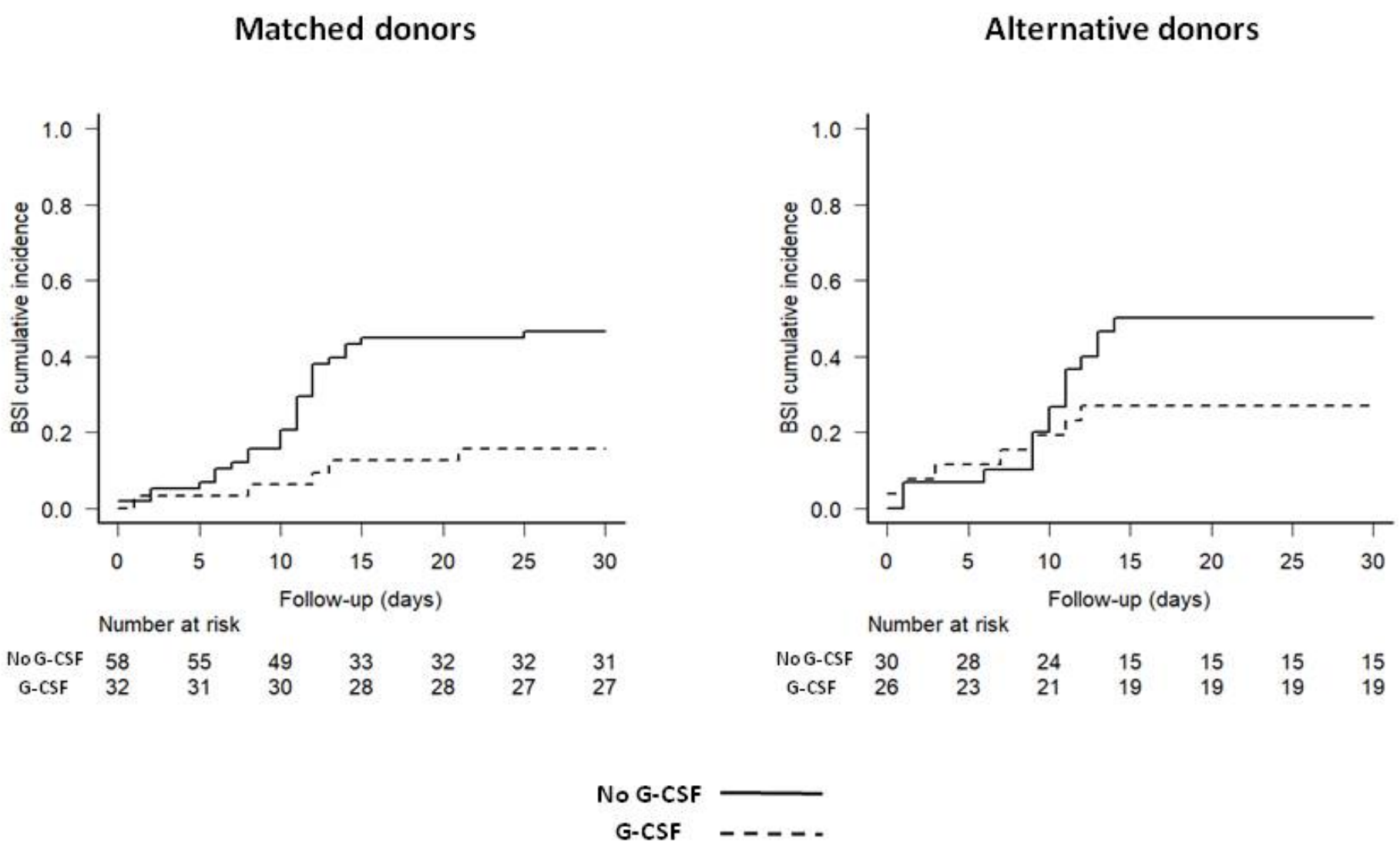

TM (ng/mL)
With G-CSF
Without G-CSF
Controls
0
7
14
21
4.0
4.0
4.0
4.0
3.5
2.4
2.9
2.5
3.7
2.2
3.7
3.5


sTNFRI (pg/mL)
With G-CSF
Without G-CSF
Controls
0
7
14
21
1600.8
1414.7
1534.2
1287.5
2325.0
2137.1
2186.9
1859.7
951.9
951.9
951.9
951.9


ST2 (ng/mL)
With G-CSF
Without G-CSF
Controls
0
7
14
21
19.8
16.0
27.7
23.9
32.7
24.1
37.8
34.4
9.6
9.6
9.6
9.6


REG3α (ng/mL)
With G-CSF
Without G-CSF
Controls
0
7
14
21
16.5
13.8
15.9
12.9
11.6
10.4
12.6
12.3
11.6
11.6
9.4
11.6


NETs (ng/mL)
With G-CSF
Without G-CSF
Controls
0
7
14
21
123.0
103.5
99.0
103.5
90.0
90.5
107.0
103.5
96.0
108.0
103.5
94.0


Matched donors
Alternative donors
BSI cumulative incidence
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
Follow-up (days)
Number at risk
No G-CSF 58 55 49 33 32 32 31
G-CSF 32 31 30 28 28 27 27
No G-CSF 30 28 24 15 15 15 15
G-CSF 26 23 21 19 19 19 19
No G-CSF
G-CSF

SUPPLEMENTARY MATERIAL:

SECTION 1. Eligibility Criteria for Allo-HCT and Donor Selection Algorithm.

General eligibility criteria for allo-HCT were as follows: patients older than 17 years with a Karnofsky performance score ≥60%, presenting a left ventricular ejection fraction ≥35% without significant pre-existing cardiac disease or uncontrolled arrhythmia; pulmonary function testing demonstrating a predicted diffusing capacity of carbon >40%, and liver functions tests showing total bilirubin <2.5 times normal with transaminases <3 times the upper limit of normal.

High-resolution molecular typing for HLA classes I (A, B, C) and II (DR, DQ) was performed for recipients and donors. Selecting a matched sibling donor (MSD) was always the first donor choice. In the absence of an MSD, a 10/10 HLA matched unrelated donor (MUD) followed by a 7/8 HLA mismatched unrelated donor (MMUD) were considered the second and third choice, and haploidentical donors were considered the last donor choice. Donor-specific antibodies were routinely assessed before transplant in all cases.

SECTION 2. Institutional antimicrobial prophylaxis.

Antimicrobial prophylaxis consisted of levofloxacin 500 mg daily from day +1 until neutrophil engraftment, fluconazole 400 mg daily from day +1 until day +60, acyclovir 800 mg twice daily from day +1 until 1 year after allo-HCT, either trimethoprim-sulfamethoxazole 160/ 800 mg three times per week, or inhaled pentamidine 300 mg monthly until the achievement of peripheral blood CD4+ cell count> 200 cells/mL. Since November 2021, all CMV-seropositive patients received letermovir 480 mg daily from day +7 until day +100.

SECTION 3. Infection monitoring.

Virus infection monitoring was performed in patients´ plasma samples with polymerase chain reaction. In the case of CMV a weekly or bi-weekly monitoring was performed according to the frequency of patient visits, and until withdrawal of immunosuppression. As the patients did not receive ATG, monitoring of EBV, HHV6 and BK-virus occurred according to clinical suspicion.

Fungal infection monitoring was performed in patients` plasma samples with galactomannan antigenemia weekly according to the frequency of patient visits, and until withdrawal of immunosuppression.

SECTION 4. The impact of G-CSF implementation on days from infusion to neutrophil engraftment. Multivariate regression analysis.

	Days to neutrophil engraftment Odds Ratio (95% CI)	P value
Age (continuous)	-0.05 (-0.12 - 0.02)	0.188
HCT-CI score >3 (vs. 0-3)	-0.79 (-3.03 - 1.43)	0.481
KPS <90% (vs. 90-100%)	-0.39 (-0.37 - 0.71)	0.712
Mismatched donor (9/10 MMUD and haploidentical) (vs. HLA-matched)	-0.04 (-1.87 - 1.78)	0.962
RIC (vs. MAC)	2.41 (0.07 - 4.75)	0.043
CD34+ cell dose (continuous)	-0.49 (-1.09 - 0.11)	0.111
BSI (vs. No)	2.24 (0.43 - 4.05)	0.015
Letermovir prophylaxis (vs. No)	-2.58 (-4.79 - -0.38)	0.022
G-CSF prophylaxis (vs. No)	-2.83 (-5.05 - -0.60)	0.013

BSI: bacterial bloodstream infections; CI: confidence interval; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; KPS: Karnofsky Performance Status; MAC: myeloablative conditioning; MMUD: mismatched unrelated donor; RIC: reduced intensity conditioning.

SECTION 5. Bacterial bloodstream infection data.

	G-CSF N=58	No G-CSF N=88
Day +30 Gram Positive BSI (%)	6 (10.3%)	18 (20.4%)
Microorganisms (%):		
Streptococcus	0	5 (5.6%)
Sthaphylococcus	5 (8.6%)	10 (11.3%)
Enterococcus	0	2 (2.2%)
Others	1 (1.7%)	1 (1.1%)
Day +30 Gram Negative BSI (%)	9 (15.5%)	20 (22.7%)
Microorganisms (%)		
Escherichia	3 (5.1%)	12 (13.6%)
Klebsiella	1 (1.7%)	3 (3.4%)
Pseudomonas	0	0
Others	5 (8.6%)	5 (5.6%)
Day +30 Polymicrobial BSI (%)	4 (6.8%)	4 (4.5%)

BSI: bacterial bloodstream infections.

SECTION 6. Experimental cohort descriptive information.

	G-CSF N=29	No G-CSF N=10
Age at allo-HCT median, years (range)	58 (19-75)	45 (18-71)
Sex (%)		
Male	19 (65.5)	6 (60.0)
Baseline Diagnosis (%)		
AML	9 (31.0)	3 (30.0)
MDS	7 (24.1)	1 (10.0)
MPN	1 (3.5)	-
ALL	7 (24.1)	3 (30.0)
NHL	5 (17.3)	2 (20.0)
PCD	-	-
Others	-	1 (10.0)
Karnofsky Performance Status (%)		
<90%	6 (20.7)	1 (10.0)
HCT-CI score (%)		
≥3	2 (6.9)	1 (10.0)
Donor selection (%)		
HLA MSD	6 (20.7)	4 (40.0)
10/10 HLA MUD	8 (27.6)	1 (10.0)
9/10 HLA MMUD	8 (27.6)	3 (30.0)
Haploidentical	7 (24.1)	2 (20.0)
Intensity of the conditioning regimen (%)		
Myeloablative	16 (55.2)	6 (60.0)
Reduced Intensity	13 (44.8)	4 (40.0)
CD34+ median cell dose (IQR)	6.3 (5.1-6.8)	6.7 (5.8-7.5)
Letermovir prophylaxis (%)	26 (89.7)	9 (90.0)
Median Follow-up		
Months (IQR)	7.0 (6.0-8.0)	9.5 (9.0-10.2)
Engraftment information		
Median days neutrophil engraftment (IQR)	15 (14-16)	15 (14-16)
Median days platelet engraftment (IQR)	18 (13-28)	18 (13-29)
Primary Graft Failure		
Yes	0	0
Cumulative incidence of GVHD [% (95% CI)]		
Day +100 Grade II-IV aGVHD	17.2 (6.1-33.1)	50.0 (16.3-76.8)
Day +100 Grade III-IV aGVHD	3.4 (0.2-15.2)	20.0 (2.6-49.0)
Main Outcomes		
1-year Overall Survival	83.1 (47.2-95.5)	90.0 (47.3-98.5)
1-year Relapse-Free Survival	76.3 (46.6-90.9)	90.0 (47.3-98.5)
1-year Non-Relapse Mortality	8.5 (0.4-32.9)	0
1-Year Cumulative Incidence of Relapse	15.2 (3.5-34.8)	10.0 (0.5-37.4)

*Post-transplant follow-up has been censored at 1-year. Any event occurring after 1 year has not been accounted in the present analysis. *ALL: acute lymphoblastic leukemia; allo-HCT: allogeneic hematopoietic cell transplantation; AML: acute myeloid leukemia; GVHD: graft-versus-host disease; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; IQR: interquartile range; MDS: myelodysplastic syndrome; MMUD: mismatched unrelated donor; MPN: myeloproliferative neoplasm; MSD: matched sibling donor; MUD: matched unrelated donor; NHL: non-Hodgkin lymphoma; PCD: plasma cell dyscrasia.*

SECTION 7. EASIX and some soluble biomarkers dynamics by donor type.

See the attached figure in the documents of the Supplementary Material.