

Alma Mater Studiorum Università di Bologna  
Archivio istituzionale della ricerca

CD34+ cell dose effects on clinical outcomes after T-cell replete haploidentical allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia using peripheral blood stem cells. A Study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT)

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Maffini, E., Labopin, M., Blaise, D., Ciceri, F., Gülbas, Z., Deconinck, E., et al. (2020). CD34+ cell dose effects on clinical outcomes after T-cell replete haploidentical allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia using peripheral blood stem cells. A Study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT). AMERICAN JOURNAL OF HEMATOLOGY, 95(8), 892-899 [10.1002/ajh.25826].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/917952> since: 2023-02-26

*Published:*

DOI: <http://doi.org/10.1002/ajh.25826>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Maffini E, Labopin M, Blaise D, Ciceri F, Gülbas Z, Deconinck E, Leblond V, Chevallier P, Sociè G, Araujo MC, Koc Y, Savani BN, Gorin NC, Lanza F, Nagler A, Mohty M.

*CD34+ cell dose effects on clinical outcomes after T-cell replete haploidentical allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia using peripheral blood stem cells. A study from the acute leukemia working Party of the European Society for blood and marrow transplantation (EBMT).*

Am J Hematol. 2020 Aug; 95(8): 892-899.

The final published version is available online at: [10.1002/ajh.25826](https://doi.org/10.1002/ajh.25826)

#### Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

**When citing, please refer to the published version.**

**CD34+ cell dose effects on clinical outcomes after T-cell replete haploidentical allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia using peripheral blood stem cells. A Study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT).**

Enrico Maffini,<sup>1</sup> Myriam Labopin,<sup>2-5</sup> Didier Blaise,<sup>6</sup> Fabio Ciceri,<sup>7</sup> Zafer Gülbas,<sup>8</sup> Eric Deconinck,<sup>9</sup> Veronique Leblond,<sup>10</sup> Patrick Chevallier,<sup>11</sup> Gerard Socié,<sup>12</sup> Mercedes Colorado Araujo,<sup>13</sup> Yener Koc,<sup>14</sup> Bipin N. Savani,<sup>15</sup> Norbert Claude Gorin,<sup>2,16</sup> Francesco Lanza,<sup>1</sup> Arnon Nagler<sup>2,17,18</sup> and Mohamad Mohty<sup>16,19,20</sup>

<sup>1</sup> Hematology Unit, Romagna Transplant Network, Ravenna, Italy ; <sup>2</sup> Acute Leukemia Working Party Office, Hospital Saint Antoine, Paris, France; <sup>3</sup> Assistance Publique-Hopitaux de Paris, Hospital Saint Antoine, Paris, France; <sup>4</sup> University Pierre et Marie Curie, Paris, France; <sup>5</sup> Institut National de la Santé et de la Recherche Médicale Unité Mixte de Recherche en Santé, 938, Paris, France; <sup>6</sup> Programme de Transplantation & Therapie Cellulaire, Centre de Recherche en Cancérologie de Marseille, Institut Paoli Calmettes, Marseille, France; <sup>7</sup> Ospedale San Raffaele s.r.l., Haematology and BMT, Milano, Italy; <sup>8</sup> Anadolu Medical Center Hospital, Bone Marrow Transplantation Department, Kocaeli, Turkey; <sup>9</sup> Hopital Jean Minjoz, Service d'Hématologie, Besancon, France; <sup>10</sup> Université Paris IV, Hopital la Pitié-Salpêtrière, Hematologie Clinique, Paris, France; <sup>11</sup> Centre Hospitalier Universitaire Nantes, Dept. D'Hematologie, Nantes, France; <sup>12</sup> Hopital Saint-Louis, Service d'Hematologie – BMT, Paris, France; <sup>13</sup> Hospital U. Marqués de Valdecilla, Servicio de Hematología-Hemoterapia, Santander, Spain; <sup>14</sup> Medical Park Hospitals, Stem Cell Transplant Unit, Antalya, Turkey; <sup>15</sup> Division of Hematology and Medical Oncology, Vanderbilt University Medical Center, Nashville, Tennessee, U.S., <sup>16</sup> Saint-Antoine Hospital, AP-HP, Paris, France, <sup>17</sup> Hematology and Bone Marrow Transplantation Division, Chaim Sheba Medical Center, Tel-Hashomer, Israel; <sup>18</sup> Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel, <sup>19</sup> Sorbonne University, Paris, France, <sup>20</sup> INSERM UMRs 938, Paris, France.

**Corresponding Author:** Maffini Enrico, MD. Viale Randi, 5, 48121, Ravenna, Italy. Phone:

00390544285621. Fax: 00390544286214. Email: [enrico.maffini@auslromagna.it](mailto:enrico.maffini@auslromagna.it)

**Abstract Word Count:** 233 **Text Word Count:** 2.678

**Tables: 3 Figures: 1**

**Running Title:** CD34+ effects on haploidentical allogeneic transplantation for acute myeloid leukemia

**Keywords:** haploidentical; allogeneic hematopoietic cell transplantation; acute myeloid leukemia; graft composition; CD34+ cells

**Abstract:**

Previous observations have reported controversial conclusions regarding cell dose and survival endpoints after allogeneic hematopoietic stem cell transplantation (HSCT). We conducted a retrospective analysis on 414 adult patients (median age 54 years, range, 18-74) with acute myeloid leukemia (AML) in first and second complete remission who received a T-cell replete allogeneic HSCT from haploidentical donors, using peripheral blood stem cells, between 2006-2018. Median number of infused CD34+ was  $6.58 \times 10^6/\text{kg}$  (range, 2.2-31.2). Graft-versus-host disease (GVHD) prophylaxis was post-transplant cyclophosphamide in 293 patients and anti-lymphocyte serum in 121 patients. Conditioning was myeloablative in 179 patients and reduced-intensity in 235 patients. After a median follow-up of 23.3 months (range, 12.1-41.8), 2-year overall survival (OS) was 64.5 % (95% CI 59.3-69.7) with leukemia-free survival (LFS) of 57.3 % (95% CI 51.8-62.7) and non-relapse mortality (NRM) of 23.3 % (95% CI 19-27.7). Grades III-IV acute GVHD day+100 incidence was 14.6 % while extensive chronic GVHD was 14.4% at 2-years. Thirteen (3.2%) patients experienced graft failure. We found the optimal CD34+/kg threshold defining high (n= 334) versus low cell dose (n= 80) at  $4.96 \times 10^6$ . Recipients of  $> 4.96 \times 10^6/\text{kg}$  CD34+ cells

experienced less NRM (Hazard ratio [HR] 0.48; 95% CI 0.30-0.76) and prolonged LFS (HR 0.63; 95% CI 0.43-0.91) and OS (HR 0.60; 95% CI 0.40-0.88) compared to those in the lower cell dose cohort. Larger cohort studies are needed to confirm these findings.

## **Introduction**

Graft cell dose may play a crucial role affecting several transplant outcomes. Historically, the absolute number of infused donor cells represented a critical step toward the achievement of a meaningful marrow engraftment after allogeneic hematopoietic stem cell transplantation (HSCT).<sup>1-5</sup> Previous studies reported conflicting conclusions regarding cell dose and survival end-points.<sup>6-11</sup> However, the vast majority of analysis conducted so far, considered myeloablative conditioning (MAC) and, particularly, reduced-intensity conditioning (RIC) allogeneic HSCT from sibling and unrelated donors, while the haploidentical setting has not been extensively studied yet. The aim of this study is to assess the impact of CD34+ cell doses in peripheral blood stem cells (PBSC) grafts on the outcome of T-cell replete haploidentical HSCT in patients with acute myeloid leukemia (AML) in complete remission (CR).

## **Methods**

### **Patients**

This is a multicenter, retrospective registry-based analysis, approved by the Acute Leukemia Working Party (AWLP) of the European Society for Blood and Marrow Transplantation (EBMT). The EBMT is a voluntary group that represents more than 600 transplant centers, mostly from European Countries. EBMT centers pay annual subscriptions to maintain the EBMT registry. Since 1990, patients have provided informed consent authorizing the use of their personal information for

research purposes. The present study analyzes the outcomes of adult patients  $\geq 18$  years, affected by AML in CR who had received a T-cell replete allogeneic HSCT from a haploidentical donor (defined as  $\geq 2$  HLA antigen mismatches), using mobilized PBSC, from 2006-2018, based on EBMT registry data. Only patients receiving a first allogeneic HSCT were included. Patients receiving an *ex vivo* T-cell depleted haploidentical HSCT were excluded from the analysis. The CD34+, CD3+ and total nucleated cells (TNC) counts were determined by the cell processing laboratories at participating transplantation centers and reported to the EBMT registry. The transplanted doses were calculated based on patients' actual body weight.

Four-hundred and fourteen adult patients (median age 54 years; range, 18-74) with AML in first (70%) and second (30%) CR were included. Seventy-three (18%) patients had secondary-AML. Eighty-seven (21%) patients had unfavorable cytogenetics, 7% had good and 59% had intermediate, while for 13% of patients, cytogenetics status was unknown. (Table S1). Median donor age was 37 years (range, 20-71). Time from hematologic disease diagnosis to HSCT was 6.3 months (range, 1.3-97.9). The Karnofsky performance status scale at the time of HSCT was  $\geq 90$  in 77% of patients. GVHD prophylaxis was post-transplant cyclophosphamide (PT-Cy)-based in 71% and anti-thymocyte globulin (ATG)-based in 29% of patients. Conditioning was MAC in 43% and RIC in 57% of patients (Table S2). Median follow-up was 23.3 months (range, 12.1-41.8).

For statistical purposes we divided patients in two cohorts based on CD34+/kg doses: the high-CD34+dose (n= 334) and the low-CD34+dose (n= 80) groups. The two cohorts differed only for donors' gender prevalence (female donors: 36.23% vs. 55% and male donors: 63.77% vs. 35%, respectively; p=0.002) and for a preponderance of female donor to male recipient combination vs.

others (20.36% vs. 32.5%, respectively;  $p=0.02$ ); all the other patients and disease characteristics were comparable in the two groups (Table 1).

### End-points (and definitions)

Neutrophil engraftment was defined as the first day of an absolute neutrophil count  $>500/\mu\text{L}$  on three consecutive measurements. Platelet recovery was defined as the first day of three consecutive measurements of  $>20,000/\mu\text{L}$ , at least 7 days after the last platelet transfusion. Acute graft-vs.-host disease (GVHD) were graded according to consensus criteria.<sup>12</sup> Chronic GVHD was defined clinically by treating physicians utilizing standard criteria.<sup>13</sup> Relapse incidence (RI) was defined as disease recurrence documented by blast reappearance ( $>5\%$ ) on peripheral blood or marrow smears, or extramedullary localization by radiographic means. Non-relapse mortality (NRM) was defined as death while in continuous remission. Leukemia-free survival (LFS) was defined as the time from transplantation to relapse or death from any cause, and overall survival (OS) was defined as the time from transplantation to death from any cause.<sup>14</sup> GVHD-free/relapse-free survival (GRFS) was defined as survival free of events including grades III-IV acute GVHD, extensive chronic GVHD, relapse, or death.<sup>15</sup>

### Statistical methods

Probabilities of OS, LFS, and GRFS were calculated using the Kaplan-Meier method. Cumulative incidence was used to estimate the endpoints of NRM, RI, acute and chronic GVHD to accommodate competing risks. Relapse and death were considered as competing risks in order to assess acute and chronic GVHD incidence/rates. Univariate analyses were carried out using Gray's test for cumulative incidence functions and the log-rank test for OS, GRFS, and LFS. A Cox

proportional-hazards model was used for multivariate regression. CD34+/kg dose was first studied as a continuous variable, and then the optimal threshold defining high versus low values for CD34+/kg dose according to its impact on NRM was obtained using the Hothorn and Zeileis method.<sup>16</sup> CD3+/kg and TNC were studied only as continuous variables. All variables associated with one outcome in the univariate analysis or factors known to influence outcomes were included in the Cox model. Results were expressed as the hazard ratio (HR) with the 95% confidence interval (95% CI). All p-values were two-sided with a type 1 error rate fixed at 0.05. Statistical analyses were performed with SPSS 24.0 (SPSS Inc. Chicago, IL, USA) and R 3.4.1 [R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria] software packages.

## **Results**

### **Cell Dose and Engraftment**

Median PBSC CD34+ and CD3+ cell doses were  $6.58 \times 10^6/\text{kg}$  (range, 2.2-31.25; interquartile range [IQR]: 5.1-8.02) and  $2.60 \times 10^8/\text{kg}$  (range, 0.17-9.11; IQR: 1.84-3.63), respectively. Thirteen patients experienced graft failure while the remaining 399 engrafted uneventfully. Neutrophil engraftment at day 30 was 91.2% (95% CI, 88 - 93.6) while sustained platelets engraftment ( $>20,000/\text{uL}$  at 6-months) was observed in 83.7% (95% CI: 79.6 - 87). Median time to neutrophil and platelets engraftment was 20 (range, 10-79) and 21 days (range, 1-37), respectively. On univariate analysis, patients receiving ATG-based GVHD prophylaxis had better neutrophil (95.8% vs. 89.4%,  $p=0.02$ ) and platelet engraftment (89.9% vs. 81.3%,  $p=0.002$ ) compared with those receiving PT-Cy. Patients in the high-dose group experienced higher rates of engraftment compared to those in the low-dose group both for neutrophils (92.1% vs. 87.3%,  $p=0.005$ ) and platelets (86%



vs. 73.3%,  $p=0.001$ ) while the median time to neutrophil engraftment was 19 days (range, 10-79) in the high-dose group and 22 days (range, 14-46) in the low-dose group ( $p=0.0001$ ) (Figure S1). As for CD3 cell dose, recipients of upper quartile CD3+ cell dose had lower rates of neutrophils ( $p=0.025$ ) and platelets ( $p=0.031$ ) engraftment.

## Graft-versus-Host Disease

Incidence of grades II-IV and III-IV acute GVHD at day +100 was 32.3 % (95% CI, 27.8 - 36.9) and 14.6 % (95% CI, 11.3 - 18.2), respectively. Incidence of 2-year chronic GVHD overall and extensive was 36.3 % (95% CI, 30.9 - 41.6) and 14.4 % (95% CI, 10.7 - 18.6), respectively. The 2-year GRFS was 43.5 % (95% CI, 38 - 48.9). In the univariate analysis, patients who received ATG as GVHD prophylaxis experienced less grades II-IV acute GVHD than those in the PT-Cy cohort (HR 0.57; 95% CI, 0.37-0.88), without any differences in grade III-IV acute GVHD incidence at day +100, nor for chronic GVHD, between the two groups. Patients receiving MAC experienced more grade III/IV acute GVHD (18.9% vs. 11.9%,  $p=0.05$ ) compared with recipients of RIC. CD34+ dose did not impact the development of chronic GVHD. On multivariate analysis, female donor to male recipient combination was associated with extensive chronic GVHD incidence compared with other donor-recipient sex combinations, with CD34+ categorized according to the optimal CD34+/kg cut point of  $4.96 \times 10^6$  (HR 2.21; 95% CI, 1.23-3.97;  $p=0.008$ ). There was no statistical correlation between CD3+ or TNC and the development of acute or chronic GVHD.

## Non-relapse mortality, Relapse Incidence, Survival and GRFS

After a median follow-up of 23.3 months (range, 12.1-41.8), the 2-year OS was 64.5 % (59.3-69.7) and LFS was 57.3 % (51.8-62.7). Incidence of 2-year disease RI and NRM was 19.5 % (15.2-24.2)

and 23.3 % (19.0-27.7), respectively. GRFS was 43.5 % (38.0-48.9). Since we failed to find an association between CD34+ cell dose expressed as continuous variable and major clinical outcomes, we examined it as a categorical variable, defining an optimal threshold of CD34+ cells infused, dividing patients receiving high vs. low CD34+ cells, as already mentioned above. Recipients of CD34+ dose  $> 4.96 \times 10^6/\text{kg}$  experienced lower NRM (HR 0.48; 95% CI, 0.30-0.76) and better LFS (HR 0.63; 95% CI, 0.43-0.91) and OS (HR 0.60; 95% CI, 0.40-0.88) compared with recipients of dose  $\leq 4.96 \times 10^6$  CD34+/kg, while RI did not differ between the two groups (Figure 1). Causes of NRM for both groups are reported in Table 2. There was a not significant trend towards a better GRFS for the high-dose group (HR 0.85; 95% CI, 0.60-1.18) (Figure S2). We did not observe any statistical correlation between CD3+ doses nor TNC and post-transplant clinical outcomes, categorized both as IQR and as median values.

### Other prognostic factors in Multivariate analysis

Younger patients (stratified by 10-Year Age Groups) experienced improved OS (69.9% vs. 59%,  $p=0.043$ ) due to a statistically significant trend towards lower NRM (18.9% vs. 27.7%,  $p=0.10$ ) in both univariate and multivariate analysis. Recipients of CMV-positive donors (regardless of patient CMV serostatus) presented better GRFS (HR 0.75; 95% CI, 0.55-1.01,  $p=0.058$ ) on multivariate analysis (Table 3). We observed a trend towards a worse survival (HR 1.46; 95% CI, 1.01-2.11,  $p=0.0454$ ) and LFS ((HR 1.39; 95% CI, 0.978-1.98,  $p=0.0662$ ) among patients in 2<sup>nd</sup> CR vs. those in 1<sup>st</sup> CR at the time of HSCT in multivariate analysis (data not shown).

## Discussion

The effect of CD34+ cell dose on clinical outcomes after allogeneic PBSC HSCT is still ill defined.

The majority of the studies conducted until now have been performed mostly on heterogeneous population of patients, affected by several hematologic diseases, mostly transplanted from HLA-matched sibling and unrelated donors, and recipients of both MAC and RIC.<sup>6-11</sup> As T-cell replete allogeneic HSCT performed from haploidentical donors has been in full swing in European and US Countries in the last decade, we decided to restrict our analysis to half-matched related donors.<sup>17-19</sup>

In order to limit data heterogeneity and potential analysis bias, we focused our attention only to AML patients only, considering only patients with disease in complete remission.

Our analysis showed that patients that received more than  $4.96 \times 10^6$  CD34+ cells/kg experienced prolonged survival, primarily due to a reduced NRM rate, a result that also retained statistical significance in the multivariate analysis. As we did not find any significant association between CD34+ dose as a continuous variable and post-HSCT clinical outcomes, we can conclude that CD34+ dose effect was not linear from a statistical point of view; indeed, looking at results according to percentiles, it seems that a further increase of CD34+ above  $4.96 \times 10^6$  cells/kg did not influence clinical outcomes.

Higher doses of donor CD34+ cells did not play any role in protection from disease recurrence, as we did not observe a reduction in RI rate among recipients of higher doses of CD34+ cells, probably due to the uniformity of disease remission state at the time of transplantation, conversely from previous analyses among patients with advanced disease before transplantation. The difference in disease relapse between high vs. low cell dose (36% vs. 9%,  $p=0.07$ ) recipients, among 86 patients affected by high-risk leukemia, led Perez Simon *et al.* to speculate that higher CD34+ doses could abrogate the dismal prognosis of high-risk hematologic diseases. Interestingly, recipients of

higher doses of CD34+ cells developed more chronic GVHD (74% vs. 47%,  $p=0.02$ ) and among those, the authors observed a survival advantage in terms of both event-free survival (at a median of 43 months, 63% vs. 16%,  $p<0.0001$ ) and OS (78% vs. 28%,  $p<0.001$ ). No effect of CD3+ cells on acute or chronic GVHD was observed.<sup>20</sup> In our analysis, the severity of both grades III-IV acute and extensive chronic GVHD did not appear to be susceptible to CD34+ content within the graft, nor was the rate of RI. In a previously published analysis, Mohty *et al.* showed that, in a cohort of 100 patients transplanted from HLA-identical siblings, recipients of higher CD34+ cell doses ( $>8.3 \times 10^6$  CD34+/kg) developed more extensive chronic GVHD at 4 years (62 vs. 34% at 4-years,  $p=0.01$ ) at the cost of higher GVHD-related mortality but without any differences in relapse rates.<sup>21</sup> Gomez-Almaguer *et al.* showed that among 138 RIC HSCT recipients, those receiving more than  $5 \times 10^6$  CD34+cells/kg had prolonged 5 year OS (63.1% vs. 48.2%,  $p=0.024$ ). A trend toward prolonged OS and LFS among recipients who developed chronic GVHD, although not statistically significant, was observed.<sup>22</sup> Similarly, Sohn *et al.* showed that among 41 recipients of PBSC HCT from HLA-identical siblings, those who received  $\geq 10.5 \times 10^6$  CD34+cells/kg experienced more chronic GVHD (66.7 vs. 25.0%,  $p=0.021$ ) but less relapse rates (20.0 vs. 47.6%,  $p=0.049$ ) and superior 3 year OS (67.8 vs. 29.9%,  $p=0.043$ ).<sup>23</sup> Investigators from Fred Hutchinson demonstrated an association between CD34+ graft dose and clinically significant chronic GVHD in two different reports.<sup>24,25</sup> A recent retrospective EBMT analysis showed that the most relevant risk factors for the development of grades III-IV acute GVHD among a uniform AML patients population transplanted with PBSC using RIC were CD3+ and CD34+ (HR= 3.6, 95% CI 1.45-9.96,  $p=0.006$  and 2.65 (1.07-6.57),  $p=0.04$ , respectively).<sup>26</sup>

In 2000 Shingal *et al.* reported on the negative impact of low CD34+ cell dose on survival outcomes.<sup>27</sup> Later, in a large CIBMTR analysis of 1054 RIC recipients, Torlen and colleagues showed that recipients of low-CD34-cell dose experienced higher rates of NRM and poorer survival.<sup>28</sup> Recently, Yamamoto *et al.* demonstrated that recipients of very low CD34+ doses ( $< 1 \times 10^6$  CD34+cells/kg; n= 48) experienced inferior OS respect to those receiving low ( $1-2 \times 10^6$  CD34+; n= 377) and high ( $2-5 \times 10^6$  CD34+cells/kg; n= 2494) without significant differences in GVHD incidence, NRM and disease relapse.<sup>29</sup> If low CD34+ cell doses have been associated with inferior clinical outcomes, the effects of very high CD34+ doses have rarely been explored.<sup>30,31</sup> In a single-center study conducted on 544 patients receiving HSCT from a HLA-identical sibling (n= 227) or unrelated donor (n= 317) and conditioned with MAC (n= 292) or RIC (n= 252), Remberger *et al.* showed that very high CD34+ doses ( $> 11 \times 10^6$  CD34+/kg) were associated with lower OS (p= 0.001) due to an unexpected higher relapse incidence (p= 0.02).<sup>32</sup>

In our analysis, the combination of female donor to male recipient was associated with significant rates of extensive chronic GVHD, as reported in several previous reports<sup>33-35</sup> and further reiterated in a recent, large retrospective analysis by the CIBMTR on 11797 patients transplanted from 2008 to 2010 which found a 21% relative increase in the sub-distribution hazard of chronic GVHD (p< 0.0001) among male recipients of female donors, compared with male donors with female recipients.<sup>36</sup>

We failed to identify any significant correlation between graft-related cell products other than CD34+ and post-HSCT clinical outcomes, in contrast to previously published analyses. Martin *et al.* in a cohort of 705 patients transplanted with RIC regimens using PBSC, showed that higher TNC dose was a better predictive factor for major post-transplant outcomes in the RIC setting,

compared with higher CD34+ dose, respect to survival outcomes and GVHD incidence.<sup>37</sup> Gorin *et al.* showed similar results in their analysis conducted on 253 adult AML patients undergoing RIC regimens, with prolonged survival and higher rates of chronic GVHD among those in 2<sup>nd</sup> CR or beyond who received higher TNC within the graft. There was no correlation between CD34+ and any clinical outcomes.<sup>38</sup>

The major drawback of our study was its retrospective nature. The incorporation in the analysis of both MAC and RIC transplant platforms and different *in-vivo* T-cell depletion strategies such as ATG and PT-Cy as GVHD prophylaxis adds further difficulties to the delicate process of results interpretation. Nevertheless, we suggest that the infusion of no less than  $5 \times 10^6$  CD34+ cells/kg in T-cell replete HSCT from haploidentical donors using PBSC could be beneficial among AML patients in CR at the time of transplantation.

## **Acknowledgements:**

*Financial disclosures:* None.

*Conflicts of Interest:* There are no conflicts of interest to report.

## **References:**

1. Storb R, Prentice RL, Thomas ED. Marrow transplantation for treatment of aplastic anemia. An analysis of factors associated with graft rejection. N Engl J Med 1977;296:61-66
2. Sierra J, Storer B, Hansen JA, Bjerke JW, Martin PJ, Petersdorf EW, Appelbaum FR, Bryant E, Chauncey TR, Sale G, Sanders JE, Storb R, Sullivan KM, Anasetti C. Transplantation of marrow cells from unrelated donors for treatment of high-risk acute leukemia: the effect of leukemic burden, donor HLA-matching and marrow cell dose. Blood 1997;89:4226-4235
3. Weaver CH, Hazelton B, Birch R, Palmer F, Allen C, Schwartzberg L, West W. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. Blood 1995;86:3961-3969

4. Dercksen MW, Rodenhuis S, Dirkson MK, Schaasberg WP, Baars JW, van der Wall E, Slaper-Cortenbach IC, Pinedo HM, Von dem Borne AE, van der Schoot CE. Subsets of CD34+ cells and rapid hematopoietic recovery after peripheral blood stem cell transplantation. *J Clin Oncol* 1995;13:1922-1932
5. Mavroudis D, Read E, Cottler-Fox M, Couriel D, Molldrem J, Carter C, Yu M, Dunbar C, Barrett J. CD34+ cell dose predicts survival, post-transplant morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies. *Blood* 1996;88:3223-3229
6. Bittencourt H, Rocha V, Chevret S, Socié G, Espérou H, Devergie A, Dal Cortivo L, Marolleau JP, Garnier F, Ribaud P, Gluckman E. Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 2002;99:2726-2733
7. Mehta J, Mehta J, Frankfurt O, Altman J, Evens A, Tallman M, Gordon L, Williams S, Winter J, Krishnamurthy J, Duffey S, Singh V, Meagher R, Grinblatt D, Kaminer L, Singhal S. Optimizing the CD34+ cell dose for reduced-intensity allogeneic hematopoietic stem cell transplantation. *Leuk Lymphoma* 2009;50:1434-1441
8. Ringden O, Barrett AJ, Zhang MJ, Loberiza FR, Bolwell BJ, Cairo MS, Gale RP, Hale GA, Litzow MR, Martino R, Russell JA, Tiberghien P, Urbano-Ispizua A, Horowitz MM. Decreased treatment failure in recipients of HLA-identical bone marrow or peripheral blood stem cell transplants with high CD34 cell dose. *Br J Haematol.* 2003;121:874-885
9. Pulsipher MA, Chitphakdithai P, Logan BR, Leitman SF, Anderlini P, Klein JP, Horowitz MM, Miller JP, King RJ, Confer DL. Donor, recipient, and transplant characteristics as risk factors after unrelated donor PBSC transplantation: beneficial effects of higher CD34+ cell dose. *Blood* 2009;114:2606-2616
10. Baron F, Maris MB, Storer BE, Sandmaier BM, Panse JP, Chauncey TR, Sorrow M, Little MT, Maloney DG, Storb R, Heimfeld S. High doses of transplanted CD34+ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft versus-host-disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. *Leukemia* 2005;19:822-828
11. Queralt Salas M, Datt Law A, Lam W, Al-Shaibani Z, Loach D, Kim D, Michelis FV, Thyagu S, Kumar R, Lipton JH, Mattsson J, Viswabandya A. Safety and Efficacy of Haploidentical Peripheral Blood Stem Cell Transplantation for Myeloid Malignancies Using Post-transplantation Cyclophosphamide and Anti-thymocyte Globulin as Graft-versus-Host Disease Prophylaxis. *Clinical Hematology International* 2019;1:105-113
12. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, Cowen EW, Dinndorf P, Farrell A, Hartzman R, Henslee-Downey J,

Jacobsohn D, McDonald G, Mittleman B, Rizzo JD, Robinson M, Schubert M, Schultz K, Shulman H, Turner M, Vogelsang G, Flowers ME. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group Report. *Biol Blood Marrow Transplant* 2005;11:945-955

13. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transpl* 1995;15:825–828

14. Kanate AS, Nagler A, Savani BN. Summary of Scientific and Statistical Methods, Study Endpoints and Definitions for Observational and Registry-Based Studies in Hematopoietic Cell Transplantation. *Clinical Hematology International*. 2019 In Press, Corrected Proof, Available Online

15. Holtan SG, DeFor TE, Lazaryan A, Bejanyan N, Arora M, Brunstein CG, Blazar BR, MacMillan ML, Weisdorf DJ. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood* 2015;125:1333-1338

16. Hothorn T, Zeileis A. Generalized maximally selected statistics. *Biometrics* 2008;64:1263-1269

17. D'Souza A, Fretham C. Current Uses and Outcomes of Hematopoietic Cell Transplantation (HCT): CIBMTR Summary Slides, 2018

18. Passweg JR, Baldomero H, Bader P, Basak GW, Bonini C, Duarte R, Dufour C, Kröger N, Kuball J, Lankester A, Montoto S, Nagler A, Snowden JA, Styczynski J, Mohty M. Is the use of unrelated donor transplantation leveling off in Europe? The 2016 European Society for Blood and Marrow Transplant activity survey report. *Bone Marrow Transplant*. 2018;53:1139-1148

19. McCurdy SR, Kasamon YL, Kanakry CG, Bolaños-Meade J, Tsai HL, Showel MM, Kanakry JA, Symons HJ, Gojo I, Smith BD, Bettinotti MP, Matsui WH, Dezern AE, Huff CA, Borrello I, Pratz KW, Gladstone DE, Swinnen LJ, Brodsky RA, Levis MJ, Ambinder RF, Fuchs EJ, Rosner GL, Jones RJ, Luznik L. Comparable composite endpoints after HLA-matched and HLA-haploidentical transplantation with post-transplantation cyclophosphamide. *Haematologica* 2017;102:391-400

20. Perez-Simon JA, Diez-Campelo M, Martino R, Sureda A, Caballero D, Canizo C, Brunet S, Altes A, Vazquez L, Sierra J, Miguel JF. Impact of CD34+ cell dose on the outcome of patients undergoing reduced intensity-conditioning allogeneic peripheral blood stem cell transplantation. *Blood* 2003;102:1108-1113.

21. Mohty M, Bilger K, Jourdan E, Kuentz M, Michallet M, Bourhis JH, Milpied N, Sutton L, Jouet JP, Attal M, Bordigoni P, Cahn JY, Sadoun A, Ifrah N, Guyotat D, Faucher C, Fegueux N, Reiffers J, Maraninchi D, Blaise D. Higher doses of CD34+ peripheral blood stem cells are associated with increased mortality from chronic graft-versus-host disease after allogeneic HLA-identical sibling transplantation. *Leukemia* 2003;17:869-875



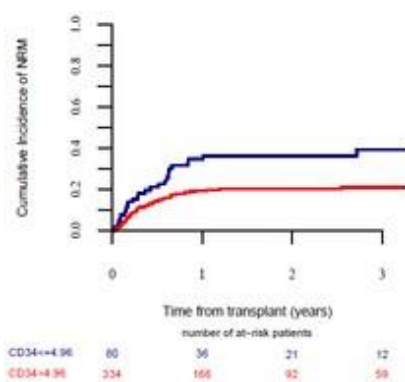
22. Gomez-Almaguer D, Gomez-Pena A, Jaime-Perez JC, Gómez-Guijosa MÁ, Cantú-Rodríguez O, Gutiérrez-Aguirre H, Martínez-Cabriaes SA, García-Rodríguez F, Olguín-Ramírez LA, Salazar-Riojas R, Méndez-Ramírez N. Higher doses of CD34+ progenitors are associated with improved overall survival without increasing GVHD in reduced-intensity conditioning allogeneic transplant recipients with clinically advanced disease. *J Clin Apher.* 2013;28:349-355
23. Sohn SK, Kim JG, Kim DH, Lee NY, Suh JS, Lee KB. Impact of transplanted CD34+ cell dose in allogeneic unmanipulated peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2003;31:967-972
24. Zaucha JM, Gooley T, Bensinger WI, Heimfeld S, Chauncey TR, Zaucha R, Martin PJ, Flowers ME, Storek J, Georges G, Storb R, Torok-Storb B. CD34 cell dose in granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell grafts affects engraftment kinetics and development of extensive chronic graft-versus-host disease after human leukocyte antigen-identical sibling transplantation. *Blood* 2001;98:3221-3227
25. Heimfeld S. Bone marrow transplantation: how important is CD34 cell dose in HLA-identical stem cell transplantation? *Leukemia* 2003;17:856-858
26. Czerw T, Labopin M, Schmid C, Cornelissen JJ, Chevallier P, Blaise D, Kuball J, Vigouroux S, Garban F, Lioure B, Fegueux N, Clement L, Sandstedt A, Maertens J, Guillermin G, Bordessoule D, Mohty M, Nagler A. High CD3+ and CD34+ peripheral blood stem cell grafts content is associated with increased risk of graft-versus-host disease without beneficial effect on disease control after reduced-intensity conditioning allogeneic transplantation from matched unrelated donors for acute myeloid leukemia - an analysis from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Oncotarget* 2016;7:27255-66
27. Singhal S, Powles R, Treleaven J, Kulkarni S, Sirohi B, Horton C, Millar B, Shepherd V, Tait D, Saso R, Rowland A, Long S, Mehta J. A low CD34+ cell dose results in higher mortality and poorer survival after blood or marrow stem cell transplantation from HLA-identical siblings: should  $2 \times 10^6$  CD34+ cells/kg be considered the minimum threshold? *Bone Marrow Transplant* 2000;26:489-496
28. Torlen J, Ringden O, Le Rademacher J, et al. Low CD34 dose is associated with poor survival after reduced-intensity conditioning allogeneic transplantation for acute myeloid leukemia and myelodysplastic syndrome. *Biol Blood Marrow Transplant* 2014;20:1418-1425
29. Yamamoto C, Ogawa H, Fukuda T, Igarashi A, Okumura H, Uchida N, Hidaka M, Nakamae H, Matsuoka KI, Eto T, Ichinohe T, Atsuta Y, Kanda Y. Impact of a Low CD34+ Cell Dose on Allogeneic Peripheral Blood Stem Cell Transplantation. *Biol Blood Marrow Transplant* 2018;24:708-716

30. Singh AK, Savani BN, Albert PS, Barrett AJ. Efficacy of CD34+ stem cell dose in patients undergoing allogeneic peripheral blood stem cell transplantation after total body irradiation. *Biol Blood Marrow Transplant* 2007;13:339-344
31. Urbano-Ispizua A, Carreras E, Marin P, Rovira M, Martínez C, Fernández-Avilés F, Xicoy B, Hernández-Boluda JC, Montserrat E. Allogeneic transplantation of CD34+ selected cells from peripheral blood from human leukocyte antigen-identical siblings: detrimental effect of a higher number of donor CD34+ cells? *Blood* 2001;98:2352-2357
32. Remberger M, Torlen J, Ringden O, Engström M, Watz E, Uhlin M, Mattsson J. Effect of Total Nucleated and CD34+ Cell Dose on Outcome after Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant* 2015;21:889-893
33. Remberger M, Kumlien G, Aschan J, Barkholt L, Hentschke P, Ljungman P, Mattsson J, Svénilson J, Ringdén O. Risk factors for moderate-to-severe chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2002;8:674-682
34. Loren AW, Bunin GR, Boudreau C, Champlin RE, Cnaan A, Horowitz MM, Loberiza FR, Porter DL. Impact of donor and recipient sex and parity on outcomes of HLA-identical sibling allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006;12:758-769
35. Kumar Aj, Kim S, Hemmer MT, Arora M, Spellman SR, Pidala JA, Couriel DR, Alousi AM, Aljurf MD, Cahn JY, Cairo MS, Cutler CS, Farhan S, Gergis U, Hale GA, Hashmi SK, Inamoto Y, Kamble RT, Kharfan-Dabaja MA, MacMillan ML, Marks DI, Nakasone H, Norkin M, Qayed M, Ringden O, Schouten HC, Schultz KR, Solh MM, Teshima T, Urbano-Ispizua A, Verdonck LF, Gale RP, Hamilton BK, Majhail NS, Loren AW. Graft-versus-host disease in recipients of male unrelated donor compared with parous female sibling donor transplants. *Blood Adv* 2018;2(11):1022-1031
36. Kim HT, Zhang MJ, Woolfrey AE, St Martin A, Chen J, Saber W, Perales MA, Armand P, Eapen M. Donor and recipient sex in allogeneic stem cell transplantation: what really matters. *Haematologica* 2016;101:1260-1266
37. Martin P, Li S, Nikiforow S, Alyea EP 3rd, Antin JH, Armand P, Cutler CS, Ho VT, Kekre N, Koreth J, Luckey CJ, Ritz J, Soiffer RJ. Infused total nucleated cell dose is a better predictor of transplant outcomes than CD34+ cell number in reduced-intensity mobilized peripheral blood allogeneic hematopoietic cell transplantation. *Haematologica* 2016;101:499-505
38. Gorin NC, Labopin M, Boiron JM, Theorin N, Littlewood T, Slavin S, Greinix H, Cahn JY, Alessandrino EP, Rambaldi A, Nagler A, Polge E, Rocha V. Results of genotypical hematopoietic stem cell transplantation with reduced-intensity conditioning for acute myeloid leukemia: higher doses of stem cells infused benefit patients receiving transplants in second remission or beyond – the Acute Leukemia Working Party of the European Cooperative Group for Blood and Marrow Transplantation. *J Clin Oncol* 2006;24:3959-3966

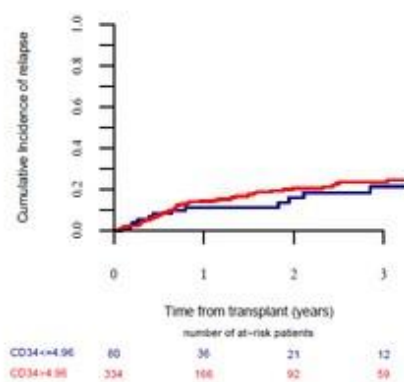
## Figure Legend

Figure 1. Non-relapse mortality (NRM), relapse incidence (RI), leukemia-free survival (LFS) and overall survival (OS) of the entire cohort of 414 AML patients receiving T-cell replete haploidentical allogeneic HSCT, stratified for high (red) *vs.* low (blue) CD34<sup>+</sup> cell dose recipients, according to the optimal threshold.

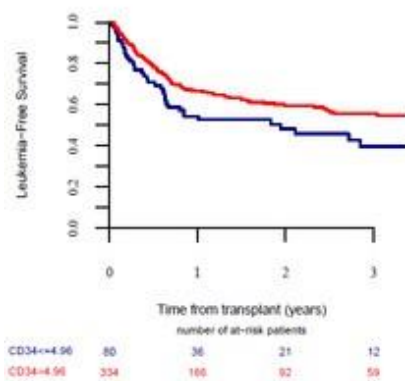
**NRM**



**RI**



**LFS**



**OS**

