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## SOME CLINICAL LESSONS FROM ONE BLOOD AND MARROW TRANSPLANTATION UNIT IN MEDELLIN, COLOMBIA

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Running title: BLOOD MARROW TRANSPLANTATION UNIT MEDELLIN

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### ABSTRACT

After more than 60 years of the first successful bone marrow transplant (BMT) by D.E. Thomas for the treatment of hematological malignant diseases and more than 46 years since the first bone marrow transplant by Alberto Restrepo-Mesa in Medellín-Colombia for the treatment of a female triplet patient with paroxysmal nocturnal hemoglobinuria and aplastic anemia, in Colombia only around 750 bone marrow transplants are performed annually. With the experience accumulated during these years by each one of us, the León XIII Clinic of the Universidad de Antioquia began the hematopoietic stem cell transplantation (HSCT) program for adults in 2014. In this review, we report some clinical lessons drawn from the different phases of the HSCT in 109 adult patients with hematological malignancies. The progression-free survival (PFS) and the five-year overall survival (OS) were for autologous stem cell transplantation (ASCT) (87% and 70%), allogeneic stem cell transplantation (Allo SCT) (50% and 40%) and haploidentical stem cell transplantation (Haplo SCT) (25% and 18%) respectively.

**Keywords:** Bone Marrow Transplantation. Hematopoietic Stem Cell Transplantation. Hematologic Neoplasms.

### INTRODUCTION

The HSCT is a treatment of some hematological malignancies with curative intention widely recognized since more of half century<sup>1,2</sup>. Medellín, with its School of Medicine Universidad de Antioquia, is a Colombian city pioneer in the solid organ transplantation and HSCT from the early 70's<sup>3</sup>.

There are available many protocols for HSCT with methodological differences that well sedimented and analyzed can permit its adaptation and implementation in areas with limited technological and economical resources.

With the accumulated experience during these years, the Clínica León XIII de la Universidad de Antioquia begun in 2014 its adult HSCT program.

In this report, we present some aspects such as the demographic of patients, CD34 mobilization and collection topics, preservation, time to engraftment, infectious, transfusional, hydroelectrolytical and immunological complications and their PFS and OS during six years of follow-up.

### PATIENTS AND METHODS

Between May 2014 and March 2020, 109 adult patients, 66 males (61%) and 43 (39%) females, median age 43 years-old (range 15 and 74 years-old), figure 1, underwent a HSCT for treating a high-risk or relapsing hematological disease at the Clínica León XIII in Medellín, Colombia. We performed 71 (65.1%) ASCT that included 43 patients with multiple myelo-

ma (60.6%), 26 patients with lymphoma (36.6%) and two patients with amyloidosis (2.8%). Twenty patients (18.4%) received an Allo SCT: 7 patients (35%) with acute myeloid leukemia (AML) and 13 patients (65%) with acute lymphoblastic leukemia (ALL). Haplo SCT were performed in 18 patients (16.5%), including 8 AML patients (44.4%) and 10 ALL patients (55.6%). Table 1.

### Conditioning regimens and prophylaxis

ASCT patients with myeloma multiple were conditioning with a single dose of melphalan (140 – 200 mg /m<sup>2</sup>). Lymphoma (HL and NHL) patients were conditioning with modified BeEAM: bendamustine 300 mg/m<sup>2</sup>, etoposide 200 mg/m<sup>2</sup>, cytarabine 200 mg/m<sup>2</sup> and melphalan 140 mg/m<sup>2</sup>. Reduced-intensity conditioning (RIC) for Allo SCT patients with ALL was fludarabine 150 m/m<sup>2</sup> and melphalan 200 mg/m<sup>2</sup>. RIC for AML patients was fludarabine 150 mg/m<sup>2</sup> and Busulfan 12.8 mg/kg iv. Haplo SCT patients received the same RIC than the Allo SCT patients, adding cyclophosphamide on day +3 and +4.

Graft vs Host Disease (GvHD) prophylaxis was cyclosporine and mycophenolate. The transplantation protocols were approved by El Comité de Trasplantes de la Clínica León XIII in 2014.

### CD34+ mobilization

Autologous and allogeneic stem cells were mobilized in an outpatient basis with single dose of pegfilgrastim 6 mg SQ five days before of first of the two 13.000 ml leukapheresis scheduled. Half dose of Plerixafor, 10 mg SQ the night before to each apheresis section, was used to improve the yield of CD34+ in patients with myeloma or lymphoma at high risk of poor mobilization, especially those with history of several prior lines of therapy and low cellularity bone marrow biopsy. None ASCT patient received chemotherapy for mobilization.

CD34+ were determined in the apheresis product by flow cytometry. Autologous and allogeneic cells in bags were stored at 4°C up to six days, allowing, in the meantime, to give the conditioning regimen<sup>4</sup>. The reinfusion of the refrigerated stem cells (0 day) was carry out up to six days later and at least 24 hours after last dose of melphalan or VP16.

### Prophylaxis

After last peripheral blood stem cells harvest, patients were hyper hydrated with fluids and received diuretics to maintain a stable hydric-electrolyte bal-

ance over the administration of appropriate conditioning regimen. Patients were isolated in rooms with HEPA filters. Antivirals and antifungal therapies were administered post-SCT infusion and continued until immune recovery. Weekly monitoring CMV load and aspergillar antigenemia permitted us modify prophylaxis. No routine prophylactic antibiotics were administered. If fever developed, blood cultures were done and empiric antibiotics were started according to clinical and local microbiological guides. All patients received a single 6 mg dose pegfilgrastim on day +5. Dates of neutrophils and platelet sustained engraftment were recorded. Nutritional support was introduced when patients had grade III-IV mucositis and until its resolution. Blood products transfused were filtered and never irradiated. (GvHD) prophylaxis and treatment in allogeneic and haploidentical patients included one or several combinations of immunosupressors agents.

### Statistics

The main endpoint of the study was PFS from time of HSCT to last follow-up, relapse or death unless otherwise specified. Clinical and biological characteristics of ASCT, Allo SCT and Haplo SCT patients were compared by the Chi square test. Survival analysis was performed by the Kaplan–Meier method. The adjusted associations between baseline characteristics and treatment modality and OS was estimated by Cox regression. Statistical significance was defined as p value < 0.05. The analysis was performed with SPSS v.22.0. (SPSS lic, Chicago, IL, USA).

## RESULTS

### Stem Cell Mobilization

Autologous or allogeneic stem cell infusion will rescue patients who have received any conditioning regimen above indicated. Performing this procedure without stem cells can result in prolonged pancytopenia and the most adults would not survive to the prolonged neutropenia period. In our unit, stem cells were always extracted by two apheresis from peripheral blood mobilized with single dose pegfilgrastim in an outpatient's basis in ASCT patients or in allogeneic donors. Plerixafor, 10 mg SC before each apheresis section was given to 25 ASCT patients (35%) with myeloma or lymphoma and high risk of poor mobilization due to history of several lines of therapy and with low cellularity bone marrow biopsy<sup>5</sup>. As shown in figure 1, the allogeneic stem cell donors yielded a median of  $7.8 \times 10^6$  95% IC (7.1 – 9.6) CD34+ / kg after the scheduled leukapheresis; enough to rescue patients with allogeneic transplantation.

In autologous transplant group mobilized with pegfilgrastim only, it was yielded a median of  $4.1 \times 10^6$  95% CI (3.75 – 5.03) CD34+ / kg. In plerixafor + pegfilgrastim group it was  $5.1 \times 10^6$  95% CI (4.3 – 7.3) CD34+ / kg ( $p=0.44$ ). Figure 2.

In both transplantation settings, the CD34+ levels achieved were good enough to obtain a long-term robust engraftment.

### Engraftment

Myeloid engraftment was successful in autologous and allogeneic transplants on median day + 12.45, and day + 13.5, 95% CI (-0.37 – 3.25) ( $p=0.015$ ) respectively. Platelet transfusion independency was reached on day + 16.65 and + 21.34, 95% CI (-3.94 – 146.36) respectively ( $p=0.004$ ) being earlier in autologous transplantation as expected. No graft failure complicated this period. Table 2.

### Infections during neutropenia before engraftment

Fever was presented in about 95% of autologous patients and in almost all allogeneic patients and usually on the first week when nadir of the neutropenia reached. In addition to clinical continuous monitoring of patients, daily C-reactive protein (CRP) changes were recorded and served as an early warning of infection.

It is appropriate to point out that CPR did not inform about a specific microbiological isolation type ( $p=ns$ ), but it was a tendency to be higher in gram-negative sepsis and frequently associated to abdominal pain (mucositis), rigors and cardiovascular instability<sup>6</sup>. Blood cultures were positive in 27% of fever episodes. Piperacillin sodium / tazobactam sodium, cefepime hydrochloride and meropenem were the antibiotics most common used at begin in accord with clinical status of each patient. If oro-esophageal mucositis appeared vancomycin was added to the regimen. De-escalation of antibiotics was done according to sensibility. Invasive fungal infections (IFI) were detected in 2 (10.5%) allogeneic and 4 (22.2%) haploidentical patients suspected with thorax CT and galactomannan and always treated with voriconazole.

### Transfusions

As we noted above, both RBC and platelet bags were not irradiated but filtered using BioR 01 Plus BS PF filter and BioP Plus BBSS PF respectively and allowing  $< 2 \times 10^5$  WBC / bag. We did not detect any case

of graft failure neither transfusional acute GvHD neither febrile reactions or bacteremia.

RBC transfusions were on average 7.7 units / patient in haploidentical transplants, 1.9 units / patient in allogeneic transplants and 1.8 units / patient in autologous transplants. In other hand, platelet by apheresis transfusional support were on average 22.1 units / patient in haploidentical patients, 12 units / patient in allogeneic transplants and 4.1 units / patient in autologous patients. So, this is confirming the grade of hematological toxicity of each one type of transplant.

### Mortality at 30 and 100 days

Mortality in the first 30 days was 5.8% and as shown in table 4, 32 (29.4%) patients died from transplanted related toxicity on the first 100 days. From these, 12 (71%) were Haplo SCT patients, 11 (61%) Allo SCT patients and 9 (13%) ASCT patients. Acute and chronic GvHD (8.1%) and relapse disease (28.1%) were the complications most frequently related with mortality. In each case there was a mixture with infectious, hemorrhagic and hydro electrolyte complications.

### Survival after relapse

For all types de HSCT, without discrimination, median PFS and OS rates were 70% and 50% at 60 months respectively. Discriminating the median PFS and OS rate at 60 months according to the type HSCT, for ASCT were 90% and 70%, for Allo SCT was 50% and 40% and Haplo SCT 25% and 18% respectively. Figures 3, 4.

### Survival after relapse

Twenty-four (22%) patients of all categories relapsed. Eighteen (75%) of them died at median of 12 months. Only six (25%) relapsed ASCT patients are alive at 6 years.

## DISCUSSION

HSCT is a treatment for some hematological malignancies with curative intention, widely recognized since more of half century<sup>1,2</sup>. Medellín, with its School of Medicine of La Universidad de Antioquia, is a Colombian city pioneer in the solid organ transplantation and HSCT from the early 70's<sup>3</sup>.

In this retrospective review we presented the clinical lessons learned from day-to-day at our Hematopoietic Transplants Unit. It shows the practical results of our protocols in order to facilitate and stimulate other colleagues working in countries with restricted health budget for developing hematopoietic transplantation units without deteriorating the quality of the hematological service provided.

The mobilization of autologous peripheral stem cells with pegfilgrastim (PEGylated GSCF) +/- plerixafor in an outpatient basis without chemotherapy was equally successful and more cost-effective for CD34 cells mobilization as it had been reported with other options widely used in this setting<sup>7,8</sup>. The collection time of apheresis began on the fifth day when the highest number of leukocytes was observed. These results confirm the importance of studying the bone marrow biopsies of some hyper treated patients before their autologous transplant in order to assess the minimal residual disease in myeloma patients and hematopoietic cellularity for planning plerixafor in low doses in advance and saving time and costs in the procedures<sup>9-12</sup>. Pegfilgrastim-induced mobilization was used too permitting the collecting of an optimal number of CD34 cells in allogeneic healthy donors<sup>10,11</sup>. There were not related complications but clinical data on HSCT will need to be studied and verified.

One or two apheresis bags harvested as described above were refrigerated at 4°C, up one week permitting conditioning regimen administration for autologous transplantation<sup>(4)</sup>. This practice is a standard in the Unit since 1992 as published. Now other HSCT Units in Latin-American report the same results. In this way, potential savings in complex facilities of cryopreservation could permit to many other Centers in the geographical area offer this treatment for benign and malignant hematological diseases<sup>12-15</sup>.

As proof of quality of the mobilization, harvesting and refrigeration, the CD34 counts were enough to engraftment quickly and restoring normal hematopoiesis as has been reported with others methods in the world<sup>16</sup>.

During the first month post transplantation or the neutropenic phase, the support was with antimicrobials, antifungals and antivirals as were needed and as it makes everywhere. The medullary failure post conditioning with high doses chemotherapy was supported with no irradiated blood products but both red blood and platelet but filtered using BioR 01 Plus BS PF filter and BioP Plus BBSS PF respectively and allowing  $< 2 \times 10^5$  WBC / bag. In any case we do not detected graft failure neither hyper acute GvHD neither febrile reactions or bacteremia as published by others<sup>17-19</sup>. Our experience leads us to believe that with modern leukoreduction techniques in use now, the irradiation of blood products may not be as necessary as it was once. To confirm this hypothesis, further studies are needed<sup>20,21</sup>. Mortality in this period of time was acceptably low, 5.8%.

TRM at 100 days was very similar to reported by others, 13% for ASCT, 61% in Allo SCT and 71% in Haplo SCT. Toxicity was in direct relation with HLA disparity as expected. At 5 years, OS and PFS was in autologous 87% and 70%, allogeneic 50% and 39%, haploidentical 25% and 18% respectively. Final data show acceptable numbers of PFS and OS and are very similar to reported by others anywhere and it expects for adding new era drugs as antivirals, immunosuppressors combinations and biologicals for enhancing results<sup>22-26</sup>.

In summary, this simple but systematic and comprehensive approach can permit to many hematologic services in under developed areas for offering more quantity and quality of live to patients suffering hematological malignancies.

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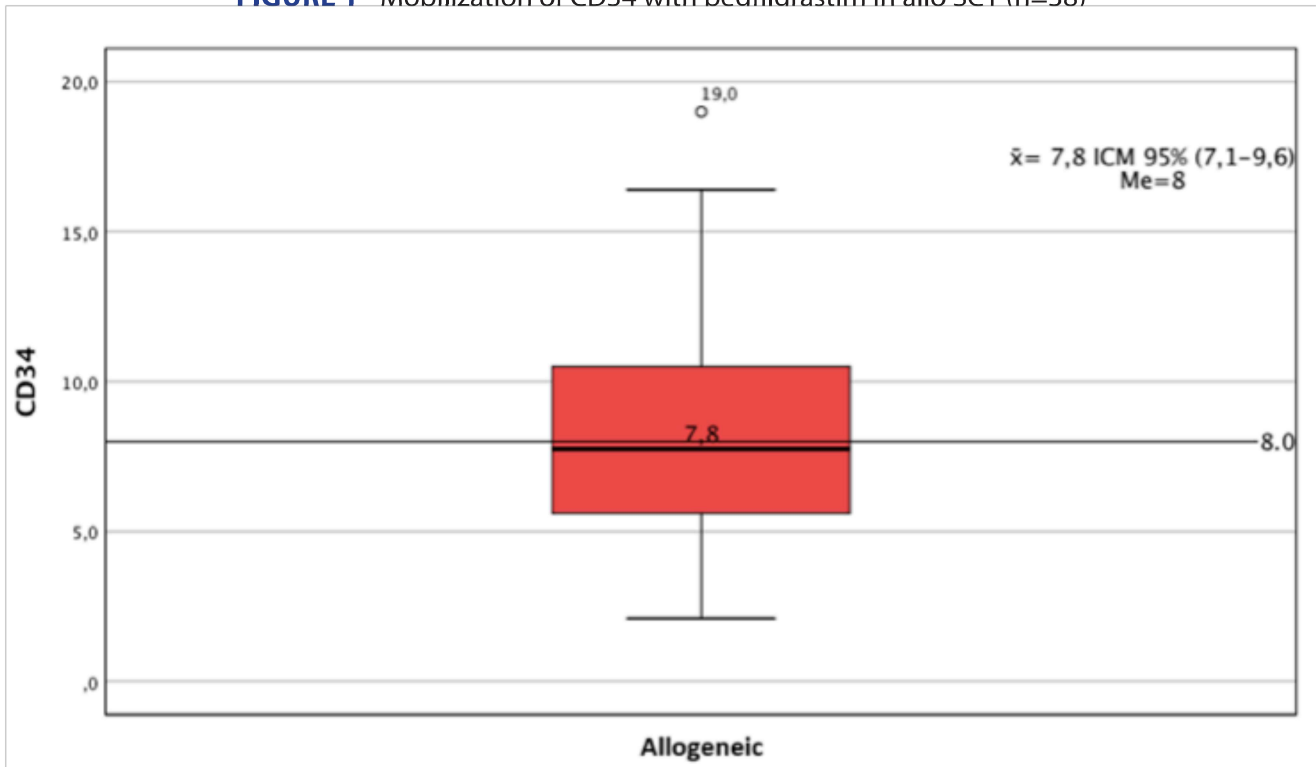
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**TABLE 1** - Demographic data of 109 patients

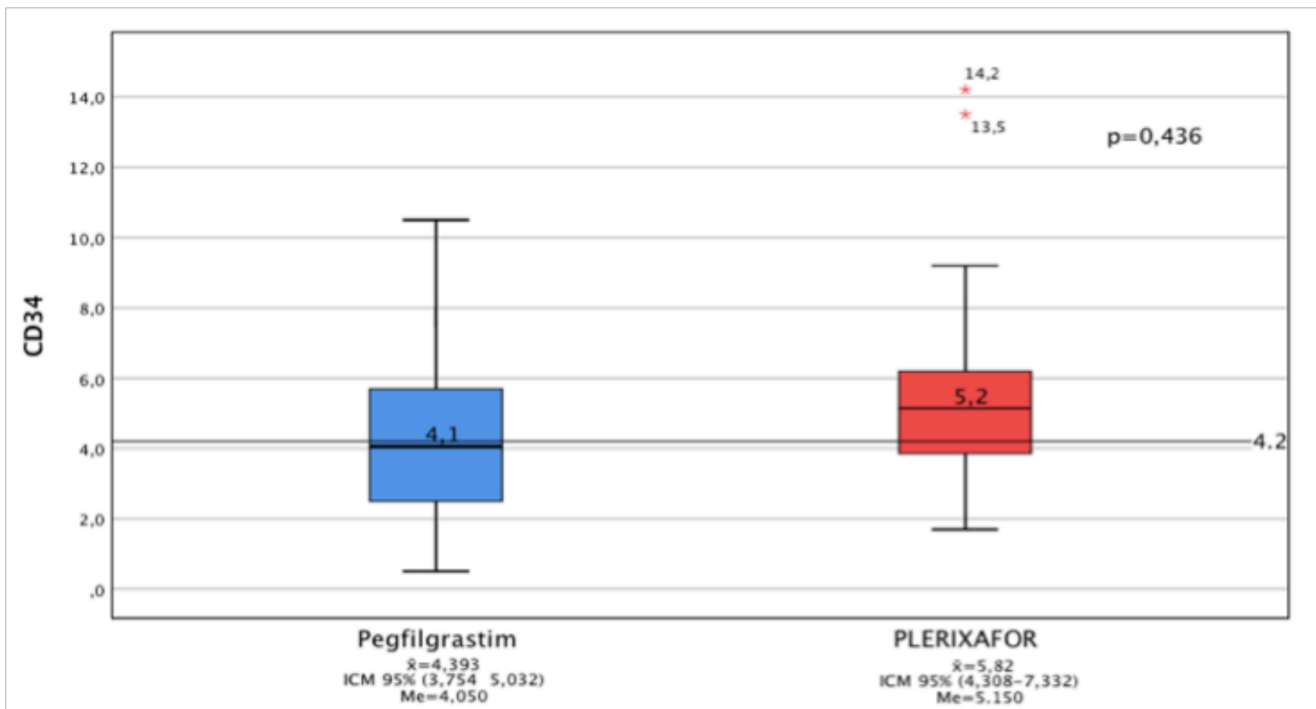
	ASCT	Allo SCT	Haplo SCT	Total
<b>GENDER</b>				
Male	42 (65%)	13 (20%)	10 (15%)	65 (100%)
Female	28 (64%)	7 (16%)	9 (20%)	44 (100%)
Total	70 (64%)	19 (18%)	19 (18%)	109 (100%)
<b>AGE (median 48.5 yo)</b>				
15-29	6 (27%)	4 (18%)	12 (55%)	22 (100%)
30-49	25 (57%)	13 (29%)	6 (14%)	44 (100%)
50-69	35 (90%)	3 (8%)	1 (2%)	39 (100%)
>70	4 (100%)	0 (0%)	0 (0%)	4 (100%)
Total	70 (64%)	20 (18%)	19 (18%)	109 (100%)
<b>DIAGNOSIS</b>				
Myeloma	43 (100%)	0 (0%)	0 (0%)	43 (100%)
Lymphoma	26 (100%)	0 (0%)	0 (0%)	26 (100%)
ALL	0 (0%)	13 (57%)	10 (43%)	23 (100%)
AML	0 (0%)	7 (47%)	8 (53%)	15 (100%)
Others	2 (100%)	0 (0%)	0 (0%)	2 (100%)
Total	71 (65%)	20 (18%)	18 (17%)	109 (100%)

The group was an adult population, with a ratio M/F of 1.47 and an median age 48.5 years. Multiple myeloma and lymphomas were the most common diseases in auto HSCT and Acute leukemias in allo and haplo H,

**FIGURE 1** - Mobilization of CD34 with pegfilgrastim in allo SCT (n=38)



**FIGURE 2** - Mobilization of CD34 in ASCT (n=71)



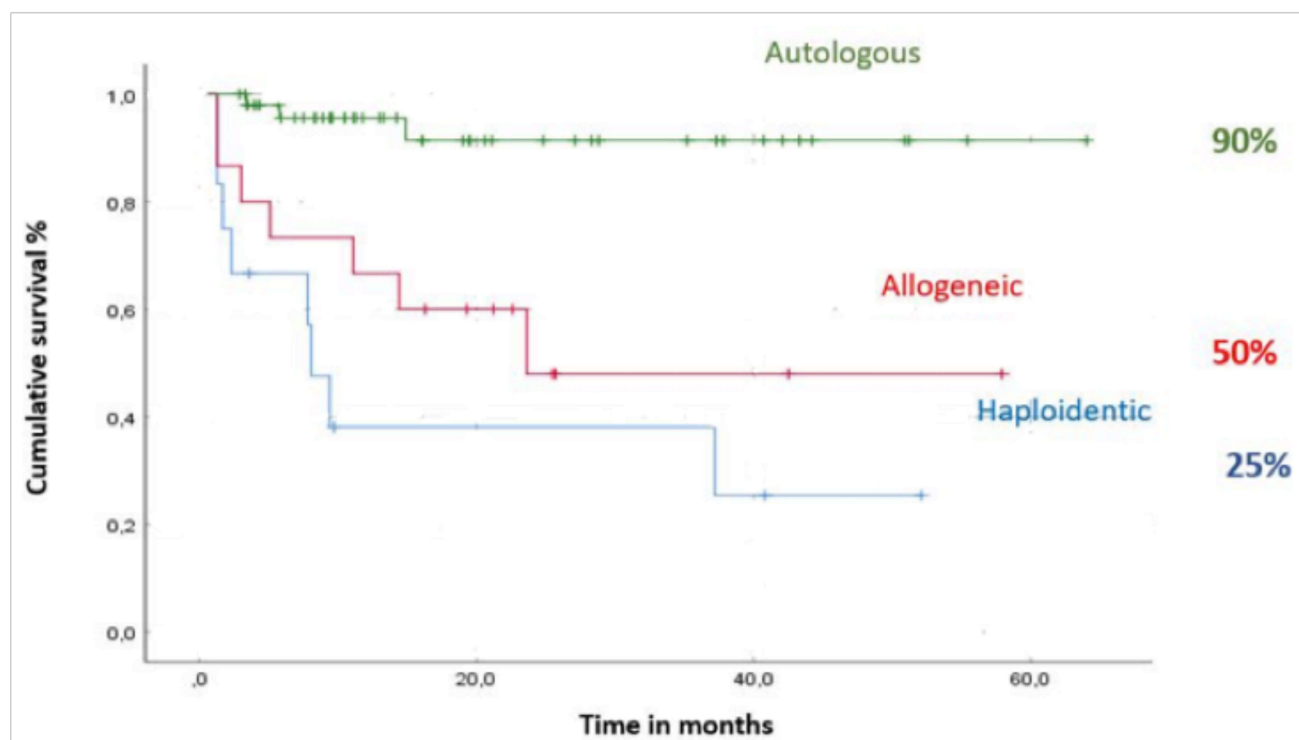
In autologous transplant group mobilized with pegfilgrastim only, it was yielded a median of  $4.1 \times 10^6$  95% CI (3.75 – 5.03) CD34+ / kg. In plerixafor + pegfilgrastim group it was  $5.1 \times 10^6$  95 IC (4.3 – 7.3) CD34+ / kg (p=0.44).

**TABLE 2** - Engraftment day

	n	$\bar{x}$ day	p
<b>Myeloid</b>			
Allogeneic	36	13,5	0,015 ICM 95% (-3,94 a 146,36)
Autologous	69	12,45	
Total	105		
<b>Platelet</b>			
Allogeneic	34	21,34	0,004 95% (-0,37 A 3,25)
Autologous	67	16,65	
Total	101		

Myeloid and platelet engraftment was successful in autologous and allogeneic transplants. No engraftment failure was recorded.

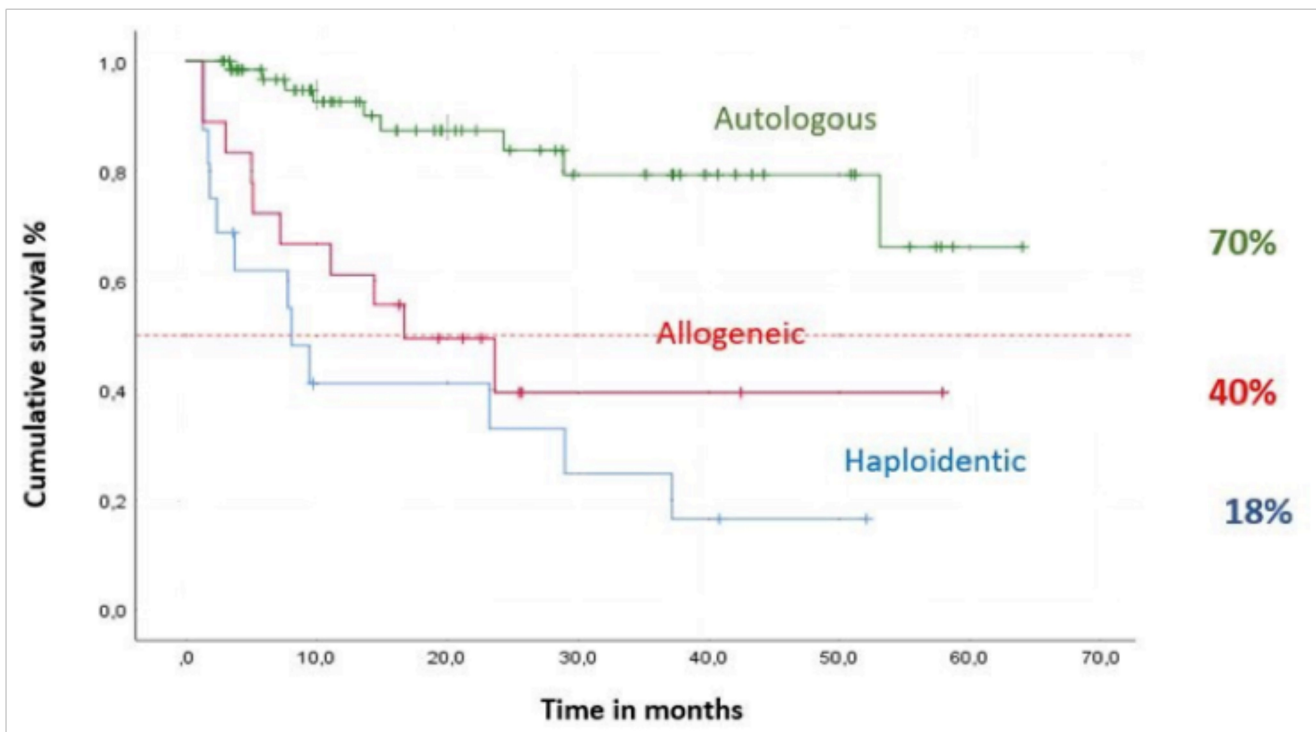
**FIGURE 3** - PFS (n=78/102)



For all types de HSCT, PFS rates was 70% for ASCT, 50% for Allo SCT and 25% for Haplo SCTat 60 months respectively.



**FIGURE 4** – OS in HSCT transplants 2014 2020



Discriminating the median OS rate at 60 months according to the type HSCT, for ASCT was 70%, for Allo SCT was 40% and for Haplo 18% respectively