

# Beneficial outcomes and epidemiologics of atypical electrophoretic profiles arising after allogeneic hematopoietic stem cell transplantation for myeloid malignancies

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# ▶ To cite this version:

Kaies Hedhli, Valentin Clichet, Amandine Charbonnier, Sandrine Castelain, Antoine Galmiche, et al.. Beneficial outcomes and epidemiologics of atypical electrophoretic profiles arising after allogeneic hematopoietic stem cell transplantation for myeloid malignancies. Current Research in Translational Medicine, 2022, 70 (1), pp.103322. 10.1016/j.retram.2021.103322 . hal-03722797

# HAL Id: hal-03722797 https://u-picardie.hal.science/hal-03722797

Submitted on 5 Jan 2024

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**BENEFICIAL OUTCOMES** AND **EPIDEMIOLOGICS** OF **ATYPICAL ELECTROPHORETIC PROFILES** ARISING AFTER **ALLOGENEIC HEMATOPOIETIC STEM** CELL TRANSPLANTATION FOR **MYELOID MALIGNANCIES** 

Running Title: Atypical electrophoresis after allogeneic transplantation

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### 2 Abstract

<u>Purpose of the study:</u> Atypical serum protein electrophoresis (SPE) profiles may arise in
patients who received allogeneic hematopoietic stem cell transplantation (allo-HSCT), but
little is known about their clinical significance. Atypical SPE combine either monoclonal and
oligoclonal components, suspected on SPE and confirmed by immunofixation. The aim of the
study is to analyse the incidence, the etiology and the clinical significance of atypical SPE
profiles in patients who received allo-HSCT.

9 Patients and methods: This retrospective study enrolled 117 patients with myeloid
10 malignancies who received an allo-HSCT between 2012 and 2018. We excluded patients with
11 lymphoid malignancies or multiple myeloma, patients presenting atypical electrophoresis
12 prior to transplantation and patients who died within 100 days post-transplant.

Results: Atypical SPE occurred in 42.7% of patients. The cumulative incidence of atypical 13 profiles was significantly higher in patients with acute Graft Versus Host Disease (GVHD, 14 p=0.019) and in patients with Cytomegalovirus (CMV) reactivation (p=0.0017). We observed 15 for the first time that atypical SPE profiles mostly occurred in patients transplanted from a 16 CMV+ donor (p=0.031). CMV reactivation preceded the occurrence of atypical SPE in the 17 majority of patients. We show that atypical SPE delay the relapse of the underlying malignant 18 disease (486 vs 189 days, p=0.006), and significantly improve overall survival (OS; 33.1 19 months vs 28.3 months, p=0.049). In both univariate and multivariate analyses, the presence 20 21 of an atypical SPE is the only factor that significantly improves OS.

<u>Conclusions:</u> The occurrence of atypical SPE profiles after allo-HSCT may reflect an adapted
 post-transplant immune response leading to favourable outcomes.

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Key words: allo-HSCT, monoclonal component, oligoclonal component, electrophoresis,
myeloid malignancies

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## 29 **1. Introduction**

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is still considered as an
 effective therapeutic option in most of the hematological malignancies. However, this
 procedure remains associated with high mortality risk, either due to direct toxicity assessed by

transplantation-related mortality (TRM) or to frequent relapses of the disease[1]. The aim of 1 2 allo-HSCT is to provide an allogeneic efficient immunotherapy to decrease the risk of relapse of the underlying disease. After hematopoietic reconstitution, biologists and clinicians often 3 4 observe monoclonal or oligoclonal components on serum protein electrophoresis (SPE) in patients who received allo-HSCT, but very little is known about their clinical relevance [2–7]. 5 These SPE profiles, considered as "atypical", are poorly described in the literature. The 6 7 incidence of these profiles is still debated, ranging from 18 to 90% [2-7]. Few studies hypothesized a relation between these profiles and reactivation of CMV and EBV viruses 8 [5,8] and/or with an aberrant immune reconstitution [2–4]. In this retrospective study, we 9 10 evaluated the incidence, the epidemiologic features and the prognostic impact of these atypical SPE profiles among 117 patients presenting myeloid malignancies who received an 11 allo-HSCT between 2012 and 2018. 12

13

14 **2.** Patients and methods

# 15 Study design and clinical features

This monocentric retrospective study enrolled all adult ( $\geq 18$  years old) patients with myeloid disease who received an allo-HSCT at the Amiens-Picardie University Hospital (France) between 01/01/2012 and 31/12/2018. We excluded patients with lymphoid malignancies or multiple myeloma, due to the high frequency of monoclonal components associated with the underlying disease. Patients presenting atypical electrophoresis prior to transplantation were also excluded. We also excluded patients who died within 100 days post-transplant to avoid the TRM associated bias.

For all included patients, biological data were extracted from our laboratory management
system (DxLab Software, Medasys, le Plessis-Robinson, France). Clinical data were obtained

from the medical prescription software (DxCare Software, Medasys, le Plessis-Robinson,
 France) and from clinical observations.

3

#### 4 Serum protein electrophoresis and immunofixations

SPE was performed by capillary method at day 30, day 100, 6 months and 12 months after the 5 allo-HSCT, on the Capillarys 2 Flex piercing instrument (Sebia, Lisses, France). SPE may 6 also be performed beyond 12 months after allo-HSCT, at physician's discretion. 7 Immunofixation was systematically carried out (Hydrasys 2 system, Sebia, Lisses, France) in 8 case of any abnormality observed on electrophoresis. An atypical SPE profile was defined by 9 10 the occurrence of one or more betaglobulin or gammaglobulin peaks on the electrophoresis profile, at any time, and confirmed by the presence of monoclonal or oligoclonal bands at 11 12 immunofixation.

13

#### 14 Viral reactivation.

Patients were routinely screened for cytomegalovirus (CMV), Epstein - Barr virus (EBV) and 15 Human herpes virus6 (HHV6) reactivations by quantitative PCR in whole blood on the 16 ABBOTT m2000 chain (m2000sp + m2000rt), according to our procedures. CMV was tested 17 twice a week and EBV once a week until D100, then every 7-15 days for 1 month, then every 18 month until 6 months, then every 2 months until 1 year. HHV6 was tested once a week until 19 D100 only for haplo-identical transplants, and only in the presence of suggestive symptoms 20 for the others. We defined CMV, EBV or HHV6 reactivation as an increase of total amount of 21 22 viral DNA above the quantification threshold. In the virology laboratory of the Amiens-Picardie University Hospital, the quantification thresholds are 40 UI/mL (1.6 log) for CMV, 23 150 UI/mL (2.18 log) for EBV, 74 UI/mL (1.87 log) for HHV6A and 68 UI/mL (1.83 log) for 24 HHV6B. 25

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#### 2 Institutional authorization.

This study was approved by the institutional review board of Amiens-Picardie University
Hospital (registration number PI2020\_843\_0121.). All patients provided a written consent to
record clinical and biological data in the ProMIse databank from the European and Bone
Marrow Transplantation society.

7

### 8 Statistical analysis

9 Non-parametric Wilcoxon-Mann-Whitney test was used to compare patients with either atypical or non-atypical SPE profiles in the absence of normal distribution (verified by the 10 11 Shapiro-Wilk test), otherwise a Student T-test was performed. Qualitative variables were 12 assessed using the Chi-square test. Univariate and multivariate analyses were performed using a Cox proportional hazard regression model, and only variables that reached p<0.2 in 13 univariate analysis were considered for multivariate analysis. The onset of an atypical SPE 14 has been evaluated as a time-dependent variable. Cox model and Kaplan-Meier method was 15 16 used to estimate survival analysis. The log-rank test was used to compare the incidence and the survival curves between different groups. Time to relapse was defined as the time from 17 transplant to the date of the relapse, and Overall Survival (OS) as the time from transplant to 18 19 death or to the date of latest news. A p value <0.05 (two-tailed) was considered as statistically significant. All analyses were performed using R 4.0.2 software from R Core Team Inc 20 (1993) and XLSTAT by addinsoft version 2020.5. 21

#### 22 **3. Results**

23 Incidence of atypical SPE profiles and correlation with clinical features.

117 patients with myeloid malignancies were included in the study according to the defined
 inclusion and exclusion criteria. The clinical characteristics of the patients are summarized in
 Table I. Briefly, 76 patients were males and 41 were females (median age : 58, range : 18-73).
 All grades acute and chronic Graft-versus-Host disease (GVHD) were observed in 62 and 44
 patients respectively. 27 patients relapsed and median OS was 29.9 months.

Atypical SPE profiles were observed in 50 out of 117 patients (42.7%), in which 18 showed oligoclonal and 32 monoclonal profiles. The median time to the onset of an atypical SPE was 127 days, and this atypical SPE persisted 430 days on average. Most monoclonal components were of IgG subtype (26/32, 81%). There was no significant difference in age, gender, type of underlying disease, use of donor lymphocyte infusion (DLI), HLA status, conditioning regimen, nor disease status at time of transplant between patients displaying either atypical or non-atypical SPE profiles.

13

#### 14 *Correlation between viral reactivations, GVHD and occurrence of atypical SPE profiles.*

15 Among the 117 patients, CMV reactivation occurred in 41 patients and EBV reactivation was observed in 74 patients, at any time. Among these patients, 8 patients showed both 16 reactivations. HHV6A/B reactivation was seen in 10 patients. We compared the frequency of 17 these viral reactivations between patients with or without atypical SPE profiles (Table II). 18 Interestingly, the incidence of CMV reactivation was significantly higher in patients with an 19 atypical SPE profile (54 vs 20.9 %, p=0.0002). No significant difference was observed for 20 EBV or HHV6 reactivations. To evaluate the correlation between CMV reactivation and the 21 22 occurrence of an atypical SPE, the time elapsed between these two events was then analyzed. The median time to CMV reactivation was 41 days. CMV reactivation preceded the onset of 23 24 the monoclonal/oligoclonal component in 24 out of 27 patients (Figure 1A). The cumulative incidence of atypical SPE profiles was significantly higher in patients displaying CMV 25

reactivation compared to the others (Figure 1B, p=0.0017). Moreover, the CMV status of both
donor and recipient prior to transplantation significantly predicts the occurrence of an atypical
SPE profile after allo-HSCT (Figure 1C, p=0.031). The cumulative incidence of atypical SPE
was significantly higher when the donor displayed a pre-transplant CMV+ status. These
results demonstrated a significant correlation between CMV reactivation and the raise of an
atypical SPE profile post allo-HSCT.

7 We then compared the incidence of acute GVHD between patients with or without atypical SPE profiles. The frequency of all grades acute GVHD was significantly higher in patients 8 with atypical SPE (Table I, 64% vs 44%, p=0.04). The median time to aGVHD onset was 40 9 10 days. Acute GVHD also preceded the onset of atypia in 29 out of 32 patients (Figure 2A). The cumulative incidence of atypical SPE profiles was significantly higher in patients with acute 11 GVHD compared to the others (Figure 2B, p=0.019). Of note, no significant difference in the 12 13 occurrence of chronic GVHD was observed between the two groups (Table I). The effect of several variables, including gender, viral reactivations, DLI use, acute and chronic GVHD, on 14 15 atypical SPE incidence was assessed by univariate and multivariate analysis (Table III). Univariate analysis indicated that CMV reactivation and acute GVHD were the only 16 parameters significantly associated with the occurrence of an atypical SPE (OR=4.44, p<0.001 17 for CMV reactivation; OR =2.33, p=0.027 for acute GVH). Further multivariate analysis 18 confirmed that CMV reactivation was the main factor significantly correlated to the 19 occurrence of an atypical SPE profile (OR=4.09, p<0.001). Considering acute GVHD, the 20 multivariate analysis was close but not significant (OR=2.07, p=0.08). Taken together, these 21 22 results demonstrated that CMV reactivation, and maybe acute GVHD, could be considered as etiological factors in the appearance of atypical SPE profiles post allo-HSCT. 23

24 Impact of atypical SPE profiles on time to relapse

To assess the clinical significance of these atypical SPE profiles, we compared the incidence of relapse in patients with and without an atypical SPE profile. We did not observe any significant difference in the cumulative incidence of relapse between the two groups (Figure 3A). Nevertheless, we can see that the incidence curve of atypical SPE was clearly shifted to the right, suggesting a longer time to relapse than in non-atypical SPE patients. We confirmed this delay in relapse showing that patients with an atypical SPE profile had a significantly longer time to relapse than patients with non-atypical profile (486 days vs 189 days, p=0.006).

8

#### 9 Impact of atypical SPE profiles on overall survival

10 A survival analysis by Cox model, considering SPE as a time-dependent variable, showed a significant difference in OS, between patients who developed an atypical SPE profile or not 11 (p=0.03). The risk of death was 2.3 times lower in patients with atypical SPE compared to 12 13 those without. Strikingly, we observed on the entire cohort that neither CMV reactivation nor acute GVHD independently impacted OS of the patients (Supplementary figure 1, p=0.721 for 14 15 CMV reactivation and p=0.966 for acute GVHD). Then, we could hypothesize that the longer OS was correlated with the presence of an atypical profile itself. Interestingly, the time to the 16 onset of atypia did not significantly impact overall survival (Supplementary figure 2). 17

Other parameters such as EBV reactivation, DLI use, and chronic GVHD did not impact OS. 18 19 In multivariate analysis, the presence of an atypical SPE remained the only factor significantly improving OS (HR=0.52, p=0.046 in univariate analysis; HR=0.53, p=0.05 in 20 multivariate analysis; Table IV). We further analysed OS between patients with and without 21 22 an atypical SPE profile, according to the HLA-matching profile between donor and recipient. We observed that patients who developed an atypical SPE profile experienced a significant 23 24 longer OS than patients with non-atypical SPE profile, except in transplantation from HLAmatched siblings (Figure 4, p=0.036). 25

1

### 2 Discussion

In this study, we show that atypical SPE profiles occurred in 42.7% of patients with myeloid 3 4 diseases who received an allo-HSCT, confirming the high incidence of such biological event after transplantation. A similar incidence has been previously described in few studies 5 6 evaluating the occurrence of SPE peaks in transplanted patients. Hammarstrom and Smith 7 described monoclonal bands in 18 out of 42 patients after bone marrow transplantation, almost exactly the same ratio as in our study (42.8%) [3]. Mitus et al. were the only ones, to 8 our knowledge, to assess the appearance of both oligoclonal and monoclonal peaks, as we did 9 10 in our study [2]. They found an incidence of 52%, which is comparable to the 42.7% observed in our cohort [2]. Other studies describe other incidence rates, in different clinical settings 11 from ours. For example, Lim et al., detected 36% of clonal gammopathies in patients 12 13 receiving a very specific conditioning [7]. Fischer et al., described an incidence of monoclonal gammopathy of 68% in a population of young children [6]. Chiusolo et al showed 14 15 abnormalities in the  $\gamma$ -region of SPE in 65.3% of patients presenting EBV reactivation after allo-HSCT [5]. 16

17 Regarding the possible etiology of such atypical SPE profiles, as in previous studies, we did
18 not find any correlation with age, gender, conditioning regimen, or type of the underlying
19 disease [2,4,8,9].

We reported here a significant correlation with CMV reactivation, which occurred in 54% of the patients developing an atypical SPE profile. These results are comparable to those of Hebart et al, who reported a 50% incidence of CMV reactivation in this context [8]. They could not establish a formal relationship between the appearance of a monoclonal peak and CMV reactivation, probably due to the low number of patients in their study (n=11)[8]. Lim et al. showed that CMV reactivation preceded the detection of an atypical SPE in most of the 1 cases[7], as we observe here. Some studies of immunocompromised patients transplanted 2 with solid organs also describe a correlation between CMV reactivation and paraprotein 3 development [10–12]. Strikingly, we demonstrate for the first time that atypical SPE profiles 4 mostly occurred in patients transplanted from a CMV+ donor. Together with the precedence 5 of CMV reactivation, these data strongly suggest that occurrence of an atypical SPE after 6 allo-HSCT highlights a clonal expansion from donor immune cells in reaction to CMV 7 reactivation.

We also show that atypical SPE profiles are associated with acute GVHD. Our results confirm 8 those of two previous studies showing a correlation between the development of GVHD and 9 10 the development of a monoclonal component [2,9]. Of note, CMV reactivation and acute GVHD had no impact on OS in our study, which is unexpected. The exclusion of patients 11 who died before D100 may have erased unfavourable outcome by excluding early deaths that 12 13 are commonly observed in severe acute GVHD, or severe viral reactivation. This choice to exclude patients who died early was a necessary condition to focus on atypical SPE outcomes, 14 15 and to minimize TRM effect. As only a small number of patients had high-grade aGVHD, we analysed the effect of all-grades GVHD in this cohort, probably also explaining its non-16 adverse outcome. 17

In line with previous studies, we show that occurrence of an atypical SPE after allo-HSCT improves the OS [7,9]. This better outcome is also confirmed when classifying patients according to their HLA-matching, especially in non HLA-matched related transplantation. Whether no benefit has been shown on OS for patients transplanted from HLA-matched siblings should be weighted by their particular good prognosis, widely confirmed in the literature[13].

Finally, we also show for the first time that occurrence of an atypical SPE after allo-HSCT delays the relapse of the underlying malignant disease. First, it may explain in part the better prognosis associated with this biological event. Then, it may highlight an underlying Graft
versus Leukemia (GVL) effect which is the main purpose of allo-HSCT. This hypothesis is
strengthened by the significant correlation between atypical SPE and all grades acute GVHD
which is often thought to be the reflect of an immune response after allo-HSCT[14–16].

5 As exposure to specific post-transplant events such as CMV reactivation or acute GVHD can 6 promote immune response, we assume from our work that atypical SPE arise in reaction to an 7 immunological trigger [4,7,8,17]. CMV reactivation may lead to targeted clonal expansion of anti-CMV immune cells from previously immunised donor cells, resolving in part the higher 8 incidence of atypical SPE profiles after allo-HSCT from CMV+ donors and after CMV 9 10 reactivation. Acute GVHD may also trigger atypical SPE occurrence, possibly reflecting an 11 underlying efficient GVL effect that could explain in part the delayed time to relapse we observe here. Whatever its underlying origin, the occurrence of an atypical SPE seems to 12 improve patient outcomes. 13

14

#### 15 Conclusion

The presence of monoclonal or oligoclonal components on SPE, after allo-HSCT, may reflect an adapted post-transplant immune response, as an anti-CMV clonal expansion from preimmunized donor cells, and/or reflect both GVH and GVL activity, leading to favourable outcomes. This work provides insight to clinicians that atypical SPE profiles are common, benign and associated with an improved prognosis after allo-HSCT.

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#### 23 Acknowledgements

The authors are grateful for the financial support received from 'Ligue Contre le Cancer', and'CHU Amiens'.

26

27 Conflict of interest : none

# 1 **References**

- [1] Tsirigotis P, Byrne M, Schmid C, Baron F, Ciceri F, Esteve J, et al. Relapse of AML
  after hematopoietic stem cell transplantation: methods of monitoring and preventive
  strategies. A review from the ALWP of the EBMT. Bone Marrow Transplant
  2016;51:1431–8. https://doi.org/10.1038/bmt.2016.167.
- [2] Mitus AJ, Stein R, Rappeport JM, Antin JH, Weinstein HJ, Alper CA, et al. Monoclonal
   and oligoclonal gammopathy after bone marrow transplantation. Blood 1989;74:2764–8.
- 8 [3] Hammarström L, Smith CI. Frequent occurrence of monoclonal gammopathies with an
  9 imbalanced light-chain ratio following bone marrow transplantation. Transplantation
  10 1987;43:447–9. https://doi.org/10.1097/00007890-198703000-00028.
- [4] Nagashima T, Muroi K, Kawano-Yamamoto C, Komatsu N, Ozawa K. Paraproteinemia
   After Hematopoietic Stem Cell Transplantation. Leuk Lymphoma 2004;45:135–7.
   https://doi.org/10.1080/1042819031000139729.
- [5] Chiusolo P, Metafuni E, Cattani P, Piccirillo N, Santangelo R, Manzara S, et al.
  Prospective Evaluation of Epstein–Barr Virus Reactivation After Stem Cell
  Transplantation: Association with Monoclonal Gammopathy. J Clin Immunol
  2010;30:894–902. https://doi.org/10.1007/s10875-010-9454-x.
- [6] Fischer AM, Simon F, Le Deist F, Blanche S, Griscelli C, Fischer A. Prospective study
  of the occurrence of monoclonal gammapathies following bone marrow transplantation
  in young children. Transplantation 1990;49:731–5. https://doi.org/10.1097/00007890199004000-00015.
- [7] Lim ZY, Ingram W, Brand R, Akthari M, Milojkovic D, Ho AYL, et al. Clonal
   gammopathies following alemtuzumab-based reduced intensity conditioning
   haematopoietic stem cell transplantation: association with chronic graft-versus-host
   disease and improved overall survival. Bone Marrow Transplant 2007;40:747–52.
   https://doi.org/10.1038/sj.bmt.1705805.
- [8] Hebart H, Einsele H, Klein R, Fischer I, Bühler S, Dietz K, et al. CMV infection after
  allogeneic bone marrow transplantations is associated with the occurence of various
  autoantibodies and monoclonal gammopathies. Br J Haematol 1996;95:138–44.
  https://doi.org/10.1046/j.1365-2141.1996.7592380.x.
- [9] Widmer CC, Balabanov S, Schanz U, Theocharides APA. Transient paraproteinemia
  after allogeneic hematopoietic stem cell transplantation is an underexplored phenomenon
  associated with graft versus host disease. Oncotarget 2017;8:106333–41.
  https://doi.org/10.18632/oncotarget.22462.
- [10] Bühler S, Laitinen K, Holthöfer H, Järvinen A, Schauman K, Hedman K. High Rate of
   Monoclonal Gammopathy among Immunocompetent Subjects with Primary
   Cytomegalovirus Infection. Clin Infect Dis 2002;35:1430–3.
- 38 https://doi.org/10.1086/344465.
- [11] Drouet E, Chapuis-Cellier C, Bosshard S, Verniol C, Niveleau A, Touraine JL, et al.
   Oligo-monoclonal immunoglobulins frequently develop during concurrent
- 41 cytomegalovirus (CMV) and Epstein±Barr virus (EBV) infections in patients after renal
   42 transplantation. Clin Exp Immunol 1999:8.
- [12] Pageaux G-P, Bonnardet A, Picot M-C, Perrigault P-F, Coste V, Navarro F, et al.
  Prevalence of monoclonal immunoglobulines after liver transplantation: Relationship
  with Posttransplant Lymphoproliferative Disorders. Transplantation 1998;65:397–400.
  https://doi.org/10.1097/00007890-199802150-00018.
- 47 [13] Shaw BE, Arguello R, Garcia-Sepulveda CA, Madrigal JA. The impact of HLA
   48 genotyping on survival following unrelated donor haematopoietic stem cell

- transplantation: Review. Br J Haematol 2010;150:251–8. https://doi.org/10.1111/j.1365 2141.2010.08224.x.
- [14] Blazar BR, Hill GR, Murphy WJ. Dissecting the biology of allogeneic HSCT to enhance
  the GvT effect whilst minimizing GvHD. Nat Rev Clin Oncol 2020;17:475–92.
  https://doi.org/10.1038/s41571-020-0356-4.
- [15] Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol 2012;12:443–58. https://doi.org/10.1038/nri3212.
- 8 [16] Markey KA, MacDonald KPA, Hill GR. The biology of graft-versus-host disease:
  9 experimental systems instructing clinical practice. Blood 2014;124:354–62.
- 10 https://doi.org/10.1182/blood-2014-02-514745.
- [17] Paloczi K. Immune reconstitution: an important component of a successful allogeneic
   transplantation. Immunol Lett 2000;74:177–81. https://doi.org/10.1016/S0165 2478(00)00270-4.
- 14

| 0  | verall Cohort (n=117)  | Grouped by SPE profiles                                     |   |   |  |
|--|--|---|---|---|--|
| Variable   |  | Atypical (n=50)   | Non atypical (n=67)   | p-value   |  |
| Sexe. Males (%)  | 76 (64.9)  | 32 (64)   | 44 (65)   | 0.9   |  |
| Age – median (range), years  | 58.16 (18-73)  | 57.1 (20.5-71.2)  | 58.3 (18-73)  | 0.94  |  |
| Pathology (%)<br>AML<br>MDS<br>PV/ET<br>Myelofibrosis<br>CML<br>PDC  | 68 (58)<br>19 (16)<br>18 (15.5)<br>6 (5.3)<br>4 (3.5)<br>2 (1.7) | 27 (54)<br>10 (20)<br>7 (14)<br>3 (6)<br>2 (4)<br>1 (2)     | 41 (61)<br>9 (13.5)<br>11 (16.5)<br>3 (4.5)<br>2 (3)<br>1 (1.5) | 0.55  |  |
| Status before transplant (%)<br>CR<br>PR<br>PD   | 53 (45)<br>16 (14)<br>25 (21)                                    | 22 (44)<br>7 (14)<br>13 (26)                                | 31 (46)<br>9 (13)<br>12 (18)                                    | 0.57  |  |
| HLA compatibility (%)<br>Matched related donor<br>Matched/Mismatched<br>unrelated donor*<br>Haploidentical donor | 38 (27)<br>62 (53)<br>17 (15)                                    | 18 (36)<br>22 (44)<br>10 (20)                               | 20 (30)<br>40 (60)<br>7 (10)                                    | 0.17  |  |
| Conditioning regimen (%)<br>MAC<br>RIC   | 21 (18)<br>96 (82)   | 10 (20)<br>40 (80)  | 11 (16)<br>56 (84)  | 0.62  |  |
| Acute GVH Disease (%)<br>Chronic GVH Disease (%)<br>DLI use (%)<br>Relapse (%)<br>OS – median (range) months     | 62 (53)<br>44 (38)<br>29 (25)<br>27 (23)<br>29.9 (3.4-85.3)      | 32 (64)<br>18 (36)<br>12 (24)<br>11 (22)<br>33.1 (5.1-85.3) | 30 (44)<br>27 (40)<br>17 (25)<br>16 (24)<br>28.33 (3.5-70.9)    | <b>0.04</b><br>0.77<br>0.9<br>0.9<br><b>0.007</b> |  |

# Table 1 : Clinical characteristics of the patients

AML : Acute Myeloid Leukemia. MDS : Myelodysplastic Syndrome. PV : Polycythemia Vera. ET : Essential Thrombocythemia. CML : Chronic Myeloid Leukemia. PDC : Plasmacytoid dendritics cells. CR : Complete remission. PD : Progressive disease. MAC : Myeloablative Conditioning RIC : Reduced Intensity Conditioning DLI : Donor-lymphocyte injection. \*including 3 HLA 9/10 mismatched transplant

# Table 2 : Viral reactivations according to the electrophoretic profile

| Parameters                | Atypical SPE (n=50) | Non atypical SPE (n=67) | p-value |
|---------------------------|---------------------|-------------------------|---------|
| CMV reactivations (%)     | 27 (54)             | 14 (20.9)               | 0.0002  |
| EBV reactivations (%)     | 35 (70)             | 39 (58.2)               | 0.25    |
| HHV6A/B reactivations (%) | 4 (8)               | 6 (8.95)                | 0.9     |

CMV : Cytomegalovirus. EBV : Epstein-Barr virus. HHV6 : Human Herpes virus 6.

# Table 3: Univariate and multivariate analyses of atypical SPE incidence

|                     | Univariate analysis |            |         | Multivariate analysis |           |         |
|---------------------|---------------------|------------|---------|-----------------------|-----------|---------|
| Variable            | OR                  | 95% CI     | p-value | OR                    | 95% CI    | p-value |
| Sexe                | 0.95                | 0.44-2.06  | 0.89    |                       |           |         |
| CMV reactivation    | 4.44                | 2.01-10.23 | < 0.001 | 4.09                  | 1.81-9.58 | <0.001  |
| EBV reactivation    | 1.68                | 0.78-3.70  | 0.19    | 1.75                  | 0.76-4.14 | 0.19    |
| DLI reactivation    | 0.93                | 0.39-2.16  | 0.86    |                       |           |         |
| Acute GVH disease   | 2.33                | 1.11-5.02  | 0.027   | 2.07                  | 0.93-4.68 | 0.08    |
| Chronic GVH disease | 0.89                | 0.41-1.89  | 0.75    |                       |           |         |

OR : Odd Ratio estimated from Cox proportional regression model. CI: Confidence Interval of the estimated OR.

|                     | U    | nivariate analysis | iate analysis |      | Aultivariate analysis |         |
|---------------------|------|--------------------|---------------|------|-----------------------|---------|
| Variable            | HR   | 95% CI             | p-value       | HR   | 95% CI                | p-value |
| Atypical SPE        | 0.52 | 0.27-1.00          | 0.046         | 0.53 | 0.28-1.02             | 0.05    |
| CMV reactivation    | 1.12 | 0.47-1.69          | 0.722         |      |                       |         |
| EBV reactivation    | 0.57 | 0.31-1.05          | 0.07          | 0.65 | 0.35-1.21             | 0.18    |
| DLI use             | 0.52 | 0.23-1.16          | 0.11          | 0.52 | 0.22-1.17             | 0.11    |
| Acute GVH disease   | 0.9  | 0.5-1.6            | 0.78          |      |                       |         |
| Chronic GVH disease | 0.69 | 0.36-1.34          | 0.279         |      |                       |         |

HR : Hazard Ratio estimated from Cox proportional hazard regression model. CI: Confidence Interval of the estimated HR.

# **Figures Legends**

Figure 1 : Impact of CMV status on the development of atypical SPE. A : Time to develop an atypical SPE from CMV reactivation. Each bar represents a patient. Grey bars represent dead patients. B : Kaplan-Meier analysis of the cumulative incidence of atypical SPE in patients with or without CMV viral reactivations. C : Kaplan-Meier analysis of the cumulative incidence of atypical SPE according to CMV compatibility before transplant.

**Figure 2 : Impact of acute GVHD** on the development of atypical SPE. A : Time to develop an atypical PPE from acute GVH disease. Each bar represents a patient. Grey bars represent dead patients. B : Kaplan-Meier analysis of the cumulative incidence of atypical SPE in patients with or without acute GVH disease.

## Figure 3 : Impact of the presence of an atypical SPE on relapse.

**A** : Kaplan-Meier analysis of the cumulative incidence of relapse in patients with or without atypical SPE. **B** : Fisher test (t-test) to compare the mean time to relapse in patient with or without atypical SPE (alpha = 5%)

# Figure 4 : Impact of the presence of an atypical SPE on Overall Survival (OS).

Kaplan-Meier analysis of overall survival (OS) according to HLA compatibility between donor and recipient before the graft, in patient with or without atypical SPE.

Fig.1



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Fig.3

Α в 1400 P-value = 0.006 35% 1200 30% 1000 Time to relapse (Days) Cumulative event 25% — Atypical SPE 800 20% ---- Non atypical SPE 600 15% 400 10% P-value = 0.486 200 5% 0% 0 20 40 60 80 Non atypical SPE Atypical SPE 0 Time after stem cell transplant (months)



Time to develop an atypic SPE from acute GVHD (months)



Fig. 4

