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Short communication

# FLT3 ligand plasma levels have no impact on outcomes after allotransplant in acute leukemia

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ABSTRACT

*Objective:* This study was designed to assess the impact on outcomes of early soluble Fms-like tyrosine kinase 3 ligand concentrations (sFLc) in patients receiving an allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).
*Methods:* This was a prospective monocentric study including all allo-HSCT patients included in the previous FLAM/FLAL study (Peterlin et al., 2019). Blood samples collected before the start of conditioning then post-transplant were frozen, stored and tested by ELISA. The parameters considered were hematopoietic recoveries, Leukemia Free Survival and Overall Survival, acute and chronic GVHD, grade 3 or 4 acute and/or extensive chronic GVHD-free and relapse-free survival (GRFS).
*Results:* Forty-one patients were included, a total of 179 samples were assayed for sFLc. There was no impact of sFLc levels (< =median vs > median) on acute and chronic GVHD incidences, LFS, OS nor GRFS.
*Conclusion:* At variance with induction results for AML (Peterlin et al., 2019) endogenous sFLc do not appear to be a prognostic marker at the time of or after allo-HSCT. Even though the results are negatives, this is, to the best of our knowledge, the only prospective series specifically addressing the question of sFLc impact after allo-HSCT in acute leukemias.

## 1. Introduction

The cytokine Fms-like tyrosine kinase 3 ligand (FL) is a key regulator of hematopoiesis [2]. While its receptor, FLT-3, is expressed on myeloid, lymphoid and dendritic cell progenitors, FL is expressed by T cells and bone marrow (BM) stromal cells as membrane-bound FL that undergoes subsequent processing to generate sFL [3]. FL can be also expressed by tumor cells, such as leukemic cells [4]. In the recent FLAM/FLAL study, aimed to assess the impact of sFLc in ALL and AML patients, we showed that a particular sFLc kinetic profile during induction seemed to be a new powerful early prognostic parameter in AML [1]. Indeed, patients with a steady increase of sFLc during induction had significant better leukemia free (LFS) and overall (OS) survivals, suggesting that FL promotes an anti-leukemic effect. So far, the mechanisms by which sFLc kinetic profile may influence outcomes in AML remain to be elucidated. One hypothesis is that FL may be an important cytokine in the generation of effective anti-tumor immune responses by expanding or activating dendritic cells (DC) [5] or NK cells [6] in vivo. FL-generated DC or FL therapy has indeed shown already an antitumor activity in mice leukemia models [7,8].

Interestingly, FL may be also a promoter of tolerance induction prolonging the survival of transplanted solid organs in mice by expanding donor DC [9]. The same role has been suggested in other animal studies considering allo-HSCT. For example, in a dog model, FL could promote the engraftment of allo-HSCT without promoting graft-versus-host disease (GVHD) [9]. In a mouse model, Flt3 ligand therapy

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expanded host CD8alpha$^+$ DC and reduced experimental acute GVHD after transplant [10]. By contrast, another study in mice showed that FL treatment of recipients post-BM HSCT accelerated GVHD lethality [11]. As the biologic effects of FL in patients receiving allo-HSCT might differ from those observed in mice or dogs, and since no data are available so far, we were interested by studying the impact of sFLc levels or kinetics in allo-HSCT recipients.

## 2. Materials and Methods

All allo-HSCT patients included in the FLAM/FLAL study (Nantes; France, ClinicalTrials.gov NCT02693899) were considered. All patients provided informed consent. Blood samples were collected on EDTA tubes before the start of conditioning then at days 0, 30, 60 and 90/100 post-transplant frozen in 2 mL cryotubes (Dutscher ®, France) at –20 °C, stored for 23 months at most and then, tested by ELISA (DY308, R&D Systems, Minneapolis, MN). The parameters considered were hematopoietic recoveries, LFS and OS, acute and chronic GVHD, grade 3 or 4 acute and/or extensive chronic GVHD-free and relapse-free survival (GRFS). Engraftment was defined as a neutrophil recovery > 0.5 Giga/L with donor chimerism > 5%. Acute and chronic GVHD were diagnosed and graded according to standard criteria [12,13].

## 3. Results

Between September 2016 and January 2018, 41 patients were allografted, including 34 AML and 7 Philadelphia positive (Ph+) ALL. Patient characteristics are shown in Table 1.

A total of 179 samples were assayed for sFLc. Pre and post-graft median sFLc levels were as follows: before conditioning (n = 39): 69 (range: 3–8821) pg/mL; day 0 (n = 39): 1599 (range: 45–7572) pg/mL; day 30 (n = 38): 74 (range: 14–3753) pg/mL; day 60 (n = 34): 101 (range: 8–2136) and day 90/100 (n = 29): 105 (range: 15–2392) pg/mL. There was no difference in terms of median sFLc concentrations between AML and ALL patients, except at day 0: 3566 (range: 1365–4550) pg/mL for the ALL group versus 1509 (range: 45–7572) pg/mL for the AML group (p = 0.002). Patients receiving a myeloablative conditioning had a higher sFLc at day 0 (median: 2563 (range:1039–4550) pg/mL vs reduced intensity conditioning: 1554 (range:45–7572) pg/mL, p = 0.03).

**Table 1**
Patient characteristics.

| | |
|---|---|
| N = 41 | Period: September 2016-May 2018<br>Median follow-up: 22.5 months (8–26) |
| Gender: male/female | 24/17 |
| Median age: years (range) | 59 (36–69) |
| *Disease* | |
| Acute myeloid leukemia (AML) | 34 |
| *ELN 2010 classification* | |
| Favorable | 3 |
| Int-1 | 13 |
| Int-2 | 8 |
| Unfavorable | 10 |
| Ph + acute lymphoblastic leukemia (ALL) | 7 |
| *Disease status* | |
| First complete remission | 37 |
| First molecular relapse | 1 |
| Active | 3 |
| *Conditioning regimen* | |
| Myeloablative | 32 |
| Reduced-intensity | 9 |
| *Donor type* | |
| Sibling | 15 |
| Matched unrelated or 9/10 mismatch | 15/2 |
| Haplo-identical | 8 |
| Cord blood | 1 |
| Relapses | 6 (all AML) |
| Deaths | 10 (all AML) |
| *Causes of death* | |
| Relapse | 5 |
| Infection | 4 |
| Other | 1 |

All patients engrafted. The median neutrophils (the first of 3 days with > 0.5 Giga/L) and platelets (the first of 3 days with platelets > 20 Giga/L without transfusion) recoveries were 16 (range: 9–23) and 10 (range: 0–45) days, respectively. No impact on hematopoietic recoveries was observed when considering sFLc (< =median vs > median) before conditioning or at day 0.

Day-100 incidences of acute grade 2 and 3–4 GVHD were 17% and 14.5%, respectively. The overall incidence of extensive chronic GVHD was 7.3%.

With a median follow-up of 22.5 months (range: 8–26) for alive patients, 2-year OS, LFS and GRFS were 75.2% (63–89), 70.8% (57–87) and 55.4% (41–73), respectively. They were 70% (56–87), 65.3% (50–84) and 45.8% (31–66), respectively for AML patients, and 100% for each outcome for ALL patients.

There was no impact of sFLc levels (< =median vs > median) on acute and chronic GVHD incidences, LFS, OS nor GRFS (Fig. 1). The same was true when considering only AML cases.

Kinetic sFLc profiles before conditioning, day 0 and day 30 could be examined for 37 patients. Most of them (n = 28, 76%) showed an increase of sFLc between conditioning and day 0, then a decrease of sFLc between day 0 and day 30. Five patients showed a constant decrease, 3 patients a stagnation of sFLc (< 100 pg/mL) and 1 patient a constant increase. There was no impact of the kinetic profile (increase/decrease vs others) on GVHD or survivals.

## 4. Discussion

In summary, we could monitor sFLc before conditioning and up to day 90/100 in 41 patients with allo-HSCT. As expected, median sFLc were just above to the normal level for healthy donors as reported in the literature (14 ± 39 pg/mL) [3], at a time (before conditioning and day 0, 60, and 90/100) when the patients had subnormal complete blood counts. Conversely, with the profound cytopenia that had been induced by conditioning, sFLc were significantly increased at day 0 of transplant, likely reflecting a compensatory growth factor response to stem cell deficiency [2]. Of note, the lower levels observed between day 30 and day 90/100 could be also explained by the lymphopenia observed at these times, as FL is expressed and released by T-cells [3]. Moreover, immunosuppressive drugs responsible for lymphopenia, such as cyclosporine A, has also the potential to inhibit FL release from T cells [14].

sFLc at day 0 did not influence hematopoietic recoveries, suggesting that FL administration after allo-HSCT for patients with low sFLc would not accelerate hematopoietic recovery. This has been already reported for patients receiving autologous HSCT [15]. Indeed, in this unique study, the administration of FL safely increased only the frequency and absolute counts of blood DC precursors without affecting other mature cell lineages [15]. No association was found here between sFLc and GVHD, confirming a previous report [14]. Thus, endogenous sFLc do not appear to play a role in tolerance after allo-HSCT, especially since, except at day 0, sFLc were relatively low. The beneficial effect of FL administration to increase sFLc and induce tolerance, as it is the case in some models of mice or dogs [8,9], thus still remains hypothetic in humans.

## 5. Conclusion

Finally, we investigated whether sFLc impacted relapse or survivals after allo-HSCT. No correlation was found so far, although the number

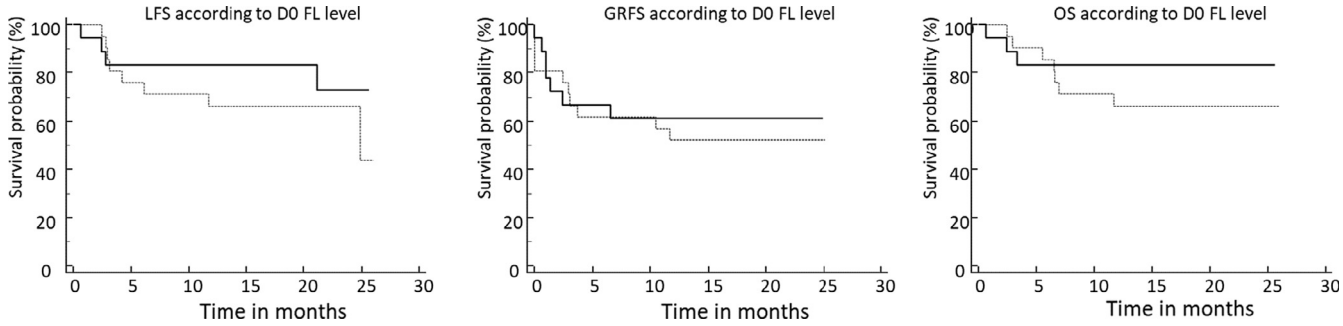


**Fig. 1.** Impact of sFLc levels at day 0 (D0) (< =median vs > median) on LFS, GRFS and OS.

of relapses is low in our study. Longer follow-up and larger cohorts are required to test this hypothesis.

In conclusion, at variance with induction results for AML [1] endogenous sFLc do not appear to be a prognostic marker at the time of or after allo-HSCT.

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## Authorship contributions

PP and PC conceived and designed the study, recruited the patients, provided clinical care, performed a bibliographic search, analyzed data, and wrote the manuscript.

JG & MC performed FLT3-L analyses and commented on the manuscript.

MCB performed statistical analyses, edited figures and helped writing the manuscript.

ME, CD, NR, YLB, OT, CG, SW performed biological analyses and commented on the manuscript.

TG, AG, ALB, BM, VD, CT, TG, NB, AL, AB, SLG, PM provided clinical care and commented on the manuscript.

## Conflict of interest

The authors declare no potential financial conflicts.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.04.015.

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