FLT3 ligand plasma levels in acute myeloid leukemia

Pierre Peterlin, Joëlle Gaschet, Thierry Guillaume, Alice Garnier, Marion Eveillard, Amandine Le Bourgeois, Michel Chérel, Camille Debord, Yannick Le Bris, Olivier Theisen, et al.

EUROPEAN HEMATOLOGY ASSOCIATION
haematologica
Journal of the European Hematology Association

FLT3 ligand plasma levels in acute myeloid leukemia

by Pierre Peterlin, Joelle Gaschet, Thierry Guillaume, Alice Garnier, Marion Eveillard, Amandine Le Bourgeois, Michel Cherel, Camille Debord, Yannick Le Bris, Olivier Theisen, Béatrice Mahé, Viviane Dubruille, Catherine Godon, Nelly Robillard, Soraya Wuillem, Cyrille Touzeau, Thomas Gastinne, Nicolas Blin, Anne Lok, Antoine Bonnet, Steven Le Gouill, Philippe Moreau, Marie-Christine Béné, and Patrice Chevallier

Haematologica 2019 [Epub ahead of print]

Citation: Pierre Peterlin, Joelle Gaschet, Thierry Guillaume, Alice Garnier, Marion Eveillard, Amandine Le Bourgeois, Michel Cherel, Camille Debord, Yannick Le Bris, Olivier Theisen, Béatrice Mahé, Viviane Dubruille, Catherine Godon, Nelly Robillard, Soraya Wuillem, Cyrille Touzeau, Thomas Gastinne, Nicolas Blin, Anne Lok, Antoine Bonnet, Steven Le Gouill, Philippe Moreau, Marie-Christine Béné, and Patrice Chevallier. FLT3 ligand plasma levels in acute myeloid leukemia. Haematologica. 2019; 104:xxx doi:10.3324/haematol.2018.209460

FLT3 ligand plasma levels in acute myeloid leukemia

Pierre Peterlin [1,2], Joelle Gaschet [2], Thierry Guillaume [1,2], Alice Garnier [1], Marion Eveillard [2,3], Amandine Le Bourgeois [1], Michel Cherel [2,4], Camille Debord [3],Yannick Le Bris [2,3], Olivier Theisen [3], Béatrice Mahé [1], Viviane Dubruille [1], Catherine Godon [3], Nelly Robillard [3], Soraya Wuilleme [3], Cyrille Touzeau [1], Thomas Gastinne [1], Nicolas Blin [1],Anne Lok [1], Antoine Bonnet [1], Steven Le Gouill[1,2], Philippe Moreau [1,2], Marie-C Béné [2,3], Patrice Chevallier [1,2]

1-Hematology Clinic, CHU Nantes, France

2- CRCINA, INSERM, CNRS, Université d'Angers, Université de Nantes, Nantes, France.

3-Hematology Biology, CHU, Nantes, France

4-Nuclear Medicine Unit, ICO Cancer Center Gauducheau, Saint Herblain, France

Running title: sFLc kinetic profile predicts survivals in AML.

Number of Figures: 2; **Number of Tables**: 1; **Number of References**: 14

Words count: 1497

Address correspondence to:

Dr. Pierre Peterlin, MD

Service d'Hématologie Clinique

CHU Hôtel-Dieu

Place A. Ricordeau

44093 Nantes Cedex, France.

Phone: (33) 240083271; Fax: (33) 240083250; e-Mail: pierre.peterlin@chu-nantes.fr

Disclosure: All the other authors declare no potential financial conflicts.

This prospective monocentric study was designed to assess soluble Fms-like tyrosine kinase 3 ligand concentrations (sFLc) during acute myeloid leukemia (AML) treatment. Three different kinetic profiles were disclosed during induction, showing significant impact on outcomes, especially survivals.

FL is a key regulator of hematopoiesis.[1] sFLc has been shown to correlate with the extent of bone marrow aplasia after radiotherapy or chemotherapy.[2-4] Interestingly, FL is expressed by leukemic cells and might enhance proliferation through an autocrine process.[5–8] High soluble levels of FL may moreover explain resistance to FLT3 inhibitors.[9] In a previous Phase 1 study, testing a radio-immunotherapy regimen for relapsed/refractory acute lymphoblastic leukemia, we observed that only the responders displayed sustained increased sFLc.[10] Apart from this report, data regarding the prognostic impact of FL levels in leukemia are missing. We therefore designed a prospective study to investigate the potential prognostic impact of the kinetic profile of sFLc on outcome in patients with AML.

The FLAM/FLAL trial was a prospective monocentric non-interventional study conducted at Nantes University Hospital (Nantes; France). It was planned to include all consecutive adult ($\geq$18 years old) patients with AML (except pro-myelocytic AML) treated intensively in first-line therapy from May 2016 for a 18-month period. Our aim was to assess the impact of sFLc kinetic profile on outcome. Parameters considered were: refractory status after induction, relapse, progression-free (PFS) and overall (OS) survivals. AML cases were treated according to standard-of-care first-line therapy (*Supplemental file*). Younger AML patients (<60 years old) with favorable features were treated according to the LAM2006CBF trial[11] while other cases were treated within the ongoing BIG Study (www.ClinicalTrials.gov NCT02416388). All older AML patients ($\geq$60 years old) were treated according to the LAMSA2002 trial.[12] Some older AML patients with favorable features received intensive consolidation with

intermediate-dose Ara-C. Allogeneic transplantation was performed according to each protocol criteria.

sFLc (expressed in pg/mL) was assayed from thawed plasma samples by ELISA (DY308, R&D Systems, Minneapolis, MN). The latter had to be collected on days 1, 8, 15 and 22 of induction. All patients provided informed consent. The protocol had been approved by the ethical committee of Nantes University Hospital (GNEDS, Ref: RC15_0374), the French Health Ministry (Ref: 16-226) and the CNIL (Ref: 2001209v0). The trial was registered at www.ClinicalTrials.gov NCT02693899.

The prognostic value of sFLc and sFLc kinetic profile were assessed according to: i) refractory status after induction ($\geq$5% bone marrow blasts or persistent aplasia >45 days) or ii) relapse, be it morphologic ($\geq$5% bone marrow blasts after complete remission) cytogenetic (reappearance of chromosomal abnormalities), molecular (reappearance of molecular abnormalities) or immunophenotypic (reappearance of a blast population detected by flow cytometry). Parameters known to impact outcome in AML were taken into account for univariate and/or multivariate analyses: age (< vs $\geq$60 years old), blasts percentage at diagnosis (relative to median), ELN 2010 risk classification[13] and diagnosis white blood count (WBC $\leq$ or > $20x10^9$/L). Quantitative variables were described by median/range and compared by a Wilcoxon rank-sum test. Categorical variables were described as counts and percentages and compared by Wilcoxon or Fisher exact tests when appropriate. Survival probabilities are presented as percent and 95% confidence intervals (CI). For univariate analysis, PFS and OS were estimated by log rank test and Kaplan-Meier graphs. Multivariate analyses were performed using the Cox proportional-hazard model. Factors differing between the two groups in terms of distribution and factors significantly associated with outcome were included in multivariate analysis. Hazard ratios and cause-specific HRs are given with 95% confidence interval (CI). All tests were two-sided and P values <0.05 were considered

statistically significant. Statistical analyses were performed in June 2018 using the R and Medcalc (Ostend, Belguim) software packages.

Between May 2016 and January 2018, 63 AML patients were included. sFLc data were ultimately available for 62 patients. Median age was 59 years (29-71) and median follow-up for alive patients was 541 days (154-787). Patients' characteristics are shown in **Table 1**. A total of 242 samples have been analyzed during induction. Median sFLc at day 1 (n=62), day 8 (n=62), day 15 (n=59) and day 22 (n=59) were 2 (range: 0-234), 321 (range: 0-7750), 2952 (range; 0-14284) and 1390 (range: 13-16088) pg/mL, respectively. Also, results analysis disclosed three sFLc kinetic profiles: i) sustained increase from days 1 to 22 (FLI group), ii) increase from days 1 to 15, then decrease at day 22 (FLD group) and iii) stagnation of low levels all along (<1000 pg/mL from days 1 to 22, FLL group) (**Figure 1**). Twenty-six patients (42%) were classified as FLI, 22 (35%) as FLD and 14 (23%) as FLL. Three patients who achieved neutrophils recovery before day 22 with a sustained sFLc increase from days 1 to 15 were classified as FLI. Two patients who displayed sustained sFLc increase from days 1 to 15 (>1000 ng/mL), then stable sFLc were classified as FLD. Median sFLc at days 1, 8, 15 and 22 were as follows for the three groups: FLI: 2 (range: 0-234), 724 (range: 0-7750), 3673 (range: 65-14284) and 5753 (range: 1390-16088) pg/mL; FLD: 6 (range: 0-177), 1229 (range: 4-7666), 6019 (range: 1217-11640) and 684 (range: 14-9428) pg/mL; and FLL: 0 (range: 0-34), 60 (range: 0-419), 124 (range: 0-800) and 81 (range: 13-213) pg/mL. Although median sFLc were similar on day 1 (p=0,18), significant differences were observed between the three groups at days 8 (p=0,001), 15 (p<0,001) and 22 (p<0,001). Of note, median sFLc were significantly different between FLI and FLD groups only at day 22 (p<0,001).

There were no significant differences between the three groups regarding median age, ELN 2010 risk-stratification,[13] WHO classification,[14] median WBC and BM blasts percentages at

diagnosis. Also there were no differences in terms of intensive consolidation received or allograft between the three groups (**Table 1**).

When comparing the three FLs groups, nearly all refractory patients (n=6) belonged to the FLL group (FLL n=5; FLD n=1, FLI n=0, p=0.0007). Seven patients out of 14 relapsed in the FLL group (morphologic n=4, molecular n=2, immunophenotypic n=1), 7 out of 22 in the FLD group (morphologic n=4, molecular n=2, immunophenotypic n=1) and 3 out 26 (morphologic) in the FLI group. The incidence of relapse was significantly higher in the FLL group (p=0.0009).

In univariate analyses (*supplemental file*), the two-year PFS and OS were significantly better for the FLI group (79,1%±8 vs FLD 54,9%±11 vs FLS 11,4%±10, p<0001; and 80.4%±8 vs FLD 58,6%±11 vs FLL 18,6%±10, p=0.09, respectively) (**Figure 2**). There was a trend for the association of 2-year PFS (but not OS) with ELN 2010 risk stratification (favorable: 70.9%±11%, vs Int-1+Int-2: 57.1%±10% vs unfavorable 33%±13%, p=0.06). Stratification of the patients according to the median sFLc level at day +15 (2952 pg/ml) also showed significantly different 2-year PFS at 38.2%±9% for low levels vs 71.8%±8% for high levels (p=0,02). The same was true for day +22 median sFLc (1390 pg/mL) at 38.9%±9% vs 73,6%±8%, (p=0.02). Age had no impact on PFS nor OS.

In multivariate analysis (*supplemental file*) considering age, ELN stratification, day 15 and day 22 sFLc, showed that the sFLc kinetic profile remained the most powerful factor independently associated with PFS (HR: 3.62; 95%CI: 1,65-7,94, p=0,001). In our population and per our modeling, the sFLc kinetic profile was the sole factor independently associated with OS (HR: 2.60; 95%CI: 1.12-6,07, p=0.02).

Our results showed that three sFLc kinetic profiles during AML induction could be identified, which had a strong and significant impact on PFS and OS. Indeed, stable low levels of sFLc all along induction (FLL group) appear to predict not only a poor response but also a high

incidence of relapse and very poor survivals. In opposition, patients with sustained sFLc increase from days 1 to 22 (FLI group) showed better outcomes with a very low incidence of relapse. An intermediate prognosis can be deduced from a sFLc kinetic profile with unsustained increase (FLD group). This new prognostication shows better results to predict outcomes than the ELN2010 risk stratification.[13] This could be due to the fact that ELN predictors were taken into account in the management of AML patients (i.e. improving the outcome of patients with adverse criteria through adapted treatment) and thus strengthens the value of sFLc as an independent parameter.

Herein, the sFLc kinetic profiles during induction provide a strong prognostic impact. One hypothesis to explain our observation is that sustained increase of sFLc during induction reflects leukemic blasts lysis and sFL serum/plasma release. Indeed, FL is known to be expressed by leukemic cells and to stimulate the FLT3 receptor via an autocrine process that promotes leukemic cells proliferation.[5-8] This hypothesis could be strengthened by assessing soluble FLT3 receptor during blast lysis, which does not seem to have been performed so far. It may be also hypothesized that persistent leukemia suppresses the bone marrow microenvironment from producing FL and/or over regenerative cytokines.

In conclusion, the sFLc kinetic profile during induction appears to be a powerful early prognostic marker to take into account as it may help to better classify AML patients. Also, these results need to be validated in a larger cohort of AML patients.

ACKNOWLEDGEMENTS

This study was supported by a grant from the DHU Oncogreffe of Nantes.

We thank Juliette Brouazin for data management, Pauline Bargain, Lina Benaniba and Sébastien Gouard for their technical expertise with sFLc ELISA assay, and the biological resource centre for biobanking (CHU Nantes, Hôtel Dieu, Centre de ressources biologiques (CRB), Nantes, F-44093, France (BRIF : BB-0033-00040)).

AUTHORSHIP CONTRIBUTIONS

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

DISCLOSURE : All the other authors declare no potential financial conflicts.

REFERENCES

1. Tsapogas P, Mooney CJ, Brown G, Rolink A. The Cytokine Flt3-Ligand in Normal and Malignant Hematopoiesis. Int J Mol Sci. 2017;18(6).pii:E1115.

2. Bertho JM, Demarquay C, Frick J, et al. Level of Flt3-ligand in plasma: a possible new bio-indicator for radiation-induced aplasia. Int J Radiat Biol. 2001;77(6):703–712.

3. Prat M, Frick J, Laporte J-P, Thierry D, Gorin N-C, Bertho J-M. Kinetics of plasma FLT3 ligand concentration in hematopoietic stem cell transplanted patients. Leuk Lymphoma. 2006;47(1):77–80.

4. Haidar JH, Bazarbachi A, Mahfouz R, Haidar HA, Jaafar H, Daher R. Serum Flt3 ligand variation as a predictive indicator of hematopoietic stem cell mobilization. J Hematother Stem Cell Res. 2002;11(3):533–538.

5. Meierhoff G, Dehmel U, Gruss HJ, et al. Expression of FLT3 receptor and FLT3-ligand in human leukemia-lymphoma cell lines. Leukemia. 1995;9(8):1368–1372.

6. Brasel K, Escobar S, Anderberg R, de Vries P, Gruss HJ, Lyman SD. Expression of the flt3 receptor and its ligand on hematopoietic cells. Leukemia. 1995;9(7):1212–1218.

7. Drexler HG. Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. Leukemia. 1996;10(4):588–599.

8. Zheng R, Levis M, Piloto O, et al. FLT3 ligand causes autocrine signaling in acute myeloid leukemia cells. Blood. 2004;103(1):267–274.

9. Sato T, Yang X, Knapper S, et al. FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. Blood. 2011;117(12):3286–3293.

10. Chevallier P, Eugene T, Robillard N, et al. (90)Y-labelled anti-CD22 epratuzumab tetraxetan in adults with refractory or relapsed CD22-positive B-cell acute lymphoblastic leukaemia: a phase 1 dose-escalation study. Lancet Haematol. 2015;2(3):e108-117.

11. Jourdan E, Boissel N, Chevret S, et al. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. Blood. 2013;121(12):2213–2223.

12. Pigneux A, Béné MC, Guardiola P, et al. Addition of Androgens Improves Survival in Elderly Patients With Acute Myeloid Leukemia: A GOELAMS Study. J Clin Oncol. 2017;35(4):387–393.

13. Döhner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115(3):453–474.

14. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405.

Table 1: Characteristics of Patients.

	All patients N=62	FLI group N=26	FLD group N=22	FLS group N=14	P value
Follow-up : days (range)	543 (154-787)	558 (154-787)	567 (165-757)	194 (161-488)*	
Gender : males	32 (52%)	10	10	12	0.01
Age					
Median : years (range)	59 (28-71)	62 (28-71)	58 (33-69)	57 (36-68)	0.14
<60 years n=	33 (53%)	10	14	9	0.14
ELN 2010					
Favorable n=	18 (29%)	6	9	3	0.21
Int 1+ int 2 n=	32 (52%)	16	10	6	
Adverse n=	12 (19%)	4	3	5	
WHO AML type					
NOS n=	21 (34%)	7	8	6	0.56
MDS-related n=	11 (18%)	5	2	4	
Rec cyt abn n=	25 (40%)	11	10	4	
Therapy-related n=	5 (8%)	3	2	0	
Median % of blasts at diagnosis	54 (20-94.5)	51 (20-94.5)	58 (20-94)	51 (25-68)	0.68
<median n=	32 (52%)	14	9	9	0.37
WBC at diagnosis : Median Giga/L(range)	5.8 (0.5-236)	5.8 (0.6-236)	2.3 (0.5-118)	4.9 (0.9-121)	0.69
<20 Giga/L n=	44 (71%)	18	15	11	0.77

Type of consolidation°					
Intensive n=	36 (58%)	14	15	7	0.30
Non intensive n=	18 (29%)	11	5	2	
Allograft n=	40 (64,5%)	17	13	10	0.74

Abbreviations: ELN: European leukemia net; int: intermediate; NOS: not otherwise specified; MDS: myelodysplastic syndrome; WBC: white blood count; Rec cyt abn: recurrent cytogenetic abnormality.

° n=54, excluding 6 refractory patients after induction and 2 patients did not receive consolidation (one in the FLI group and one in the FLD group.

*only three patients alive at last follow-up

FIGURE LEGENDS

Figure 1: sFLc kinetic profile of the three groups.

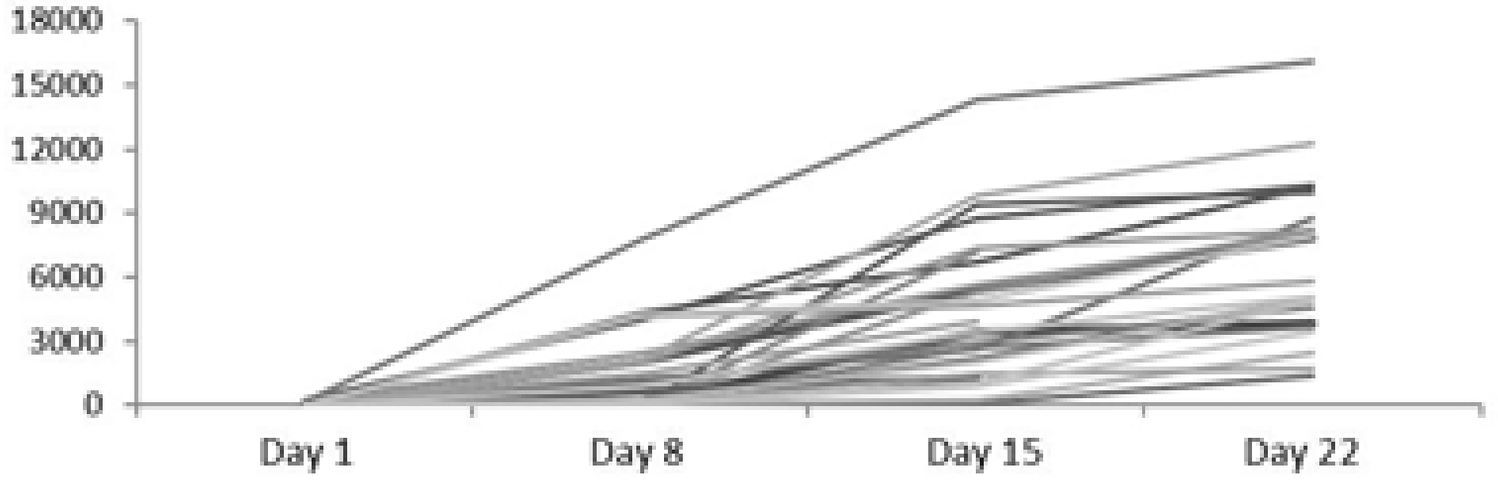
FLI: Sustained sFLc increase from day 1 to day 22 of induction (n=26).

FLD: Increase of sFLc between days 1 to 15, then decrease at day 22 of induction (n=22).

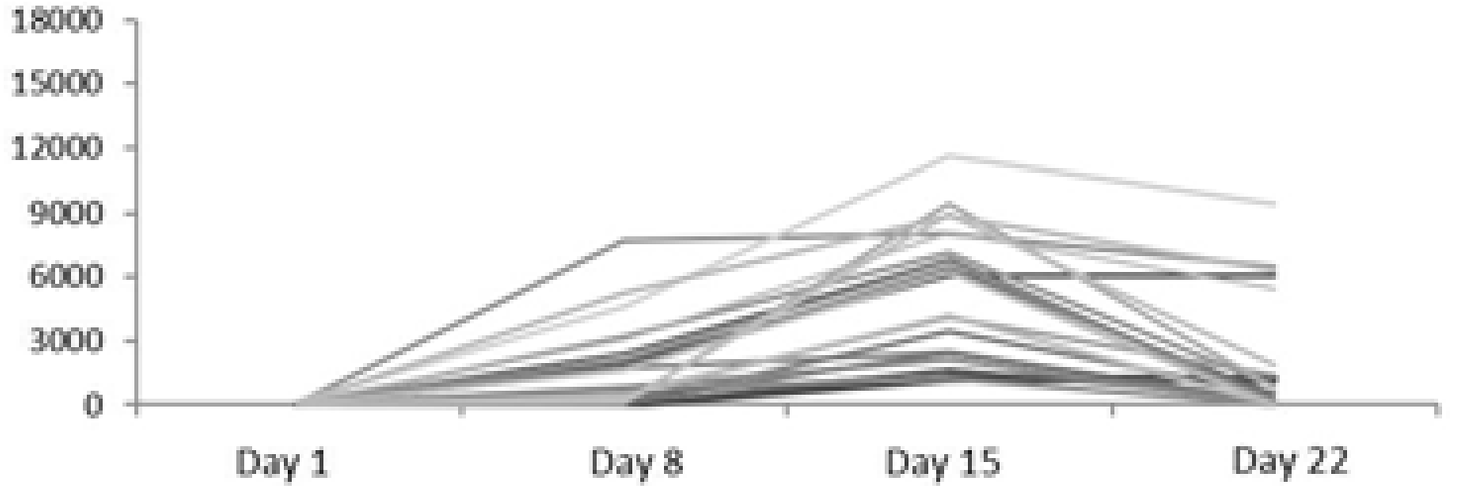
FLL: Low stable levels of sFLc (<1000 pg/mL) between day 1 and day 22 of induction (n=14).

Figure 2: PFS and OS according to three sFLc kinetic profiles.

Figure 1: FLs kinetic profile of the three groups.
FLI: Sustained FLs increase from Day 1 to Day 22 of induction (n=26).



FLD: Increase of FLs between days 1 and 15, then decrease at day 22 of induction (n=22).



FLL: Low stable levels of FLs(<1000 pg/mL) between day 1 and day 22of induction (n=14).

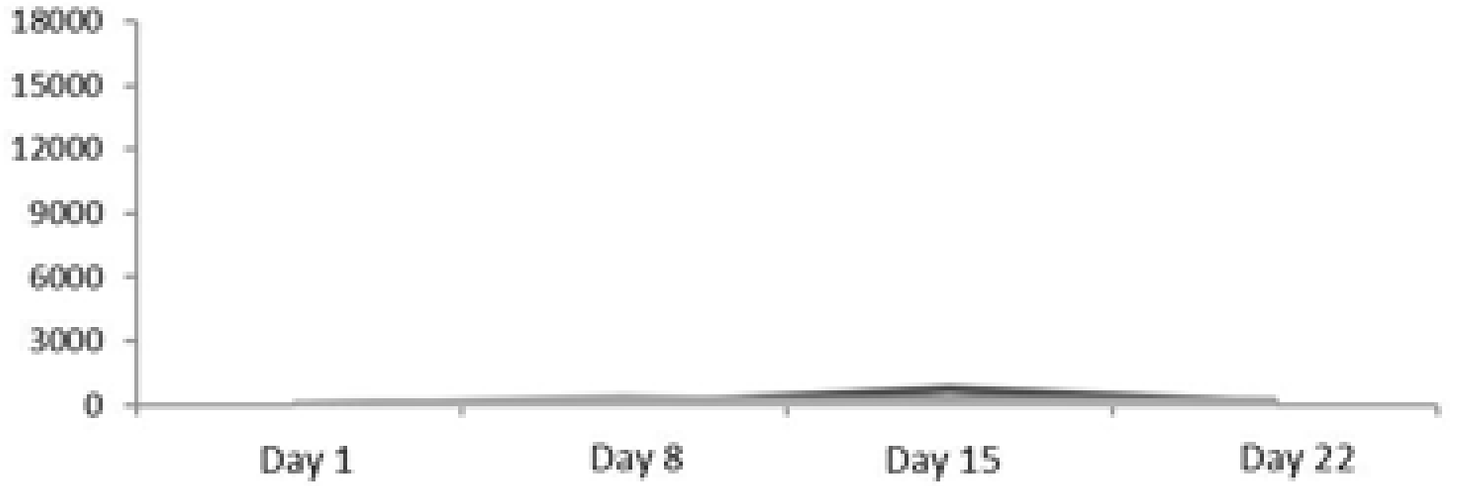
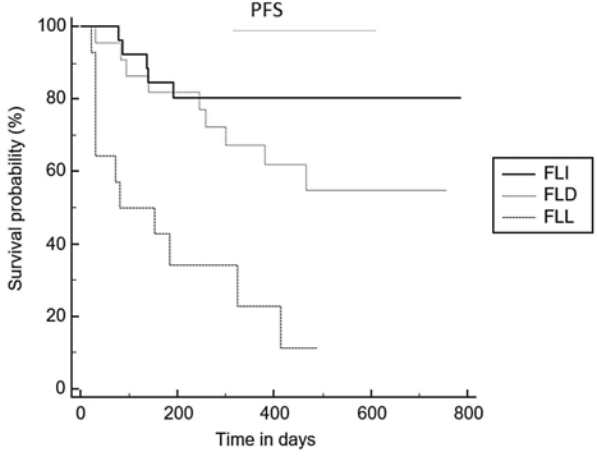
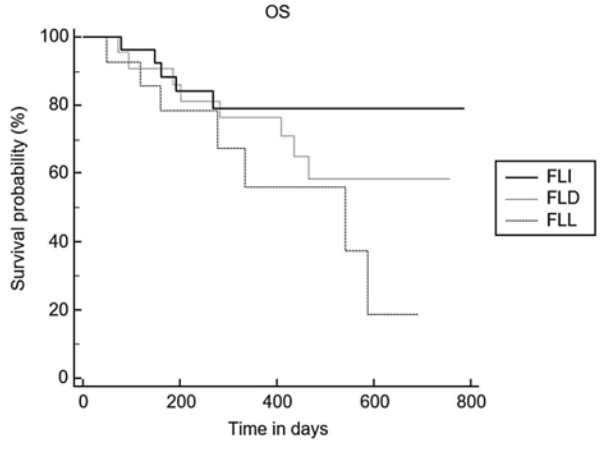


Figure 2: PFS and OS according to three sFLc kinetic profiles.



P<0.0001



P=0.09

Supplemental file

AML treatments

LAMSA2002 : Pigneux et al, 2017;35(4):387–393.

Active Comparator: B • Induction therapy Idarubicin, 8mg/m2 d1-5; Cytarabine, 100mg/m2 d1-7 and Lomustine, 200mg/m d1) If CR ou PR • maintenance therapy every 3 months = 6 courses of reinduction with : -idarubicin (8mg/m2 d1),cytarabine (100mg/m2d1-5), subcutaneously • between the courses, a continuous regimen of methotrexate and 6-mercaptopurine.	Drug: chemotherapy treatment (see arms) Induction chemotherapy + maintenance chemotherapy Other Name: Induction chemotherapy + maintenance chemotherapy
Experimental: A • Induction therapy Idarubicin, 8mg/m2 d1-5; Cytarabine, 100mg/m2 d1-7 and Lomustine, 200mg/m d1) If CR ou PR • maintenance therapy every 3 months = 6 courses of reinduction with : ○ idarubicin (8mg/m2 d1),cytarabine (100mg/m2d1-5, subcutaneously) ○ 10 to 20 mg (according to body weigh) of norethandrolone daily • between the courses, a continuous regimen of methotrexate and 6-mercaptopurine.	

BIG STUDY (on-going study: www.ClinicalTrials.gov NCT02416388).

Experimental: R1-IDA Idarubicin	Drug: Idarubicin Induction chemotherapy : Idarubicin 9mg/m² /day, from D1 to D5 (IV, 30min) + cytarabine 200mg/m²/day from D1 to D7 (IV 24 h) Bone marrow aspirate on D15 : if medullary blasts rate < 5% → G-CSF (5 µg/kg/day) until hematopoietic recovery (PNN ≥ 1 G/L).
Active Comparator: R1-DAUNO Daunorubicin	Drug: Daunorubicin Induction chemotherapy : Daunorubicin 90mg/m²/day, from D1 to D3 (IV, 30min)

	+ cytarabine 200mg/m² /day from D1 to D7 (IV 24 h) Bone marrow aspirate on D15 : if medullary blasts rate < 5% → G-CSF (5 µg/kg/day) until hematopoietic recovery (PNN ≥ 1 G/L).
Active Comparator: R2-HDAC High dose cytarabine	Drug: HD Cytarabine Consolidation chemotherapy course (s) : -High dose cytarabine: 3g/m² /12h on D1, D3 and D5 For all patients, G-CSF (5 µg/kg/day) : SC or IV (30 min) from D8 until hematopoietic recovery (PNN ≥ 1 G/L) Up to 3 consolidation courses, depending on the patient AML risk group
Experimental: R2-IDAC Intermediate dose cytarabine	Drug: ID cytarabine Consolidation chemotherapy course (s) : -Intermediate dose cytarabine: 1.5g/m² /12h on D1, D3 and D5 For all patients, G-CSF (5 µg/kg/day) : SC or IV (30 min) from D8 until hematopoietic recovery (PNN ≥ 1 G/L) Up to 3 consolidation courses, depending on the patient AML risk group
Active Comparator: R3-MAC-MTX Methotrexate and mycophenolic acid	Drug: Methotrexate GvHD prophylaxis post allogeneic SCT : -15 mg/m² on D+1 then 10 mg/m² on D+3, D+6 and D+11 Drug: Mycophenolic acid (MPA) GvHD prophylaxis post allogeneic SCT : • 720 mg BID from D0 to D+28 for HLA-identical siblings • 720 mg BID from D0 to D+45 for 10/10 HLA allele-matched unrelated donors
Experimental: R3-MAC-MPA Cyclosporine and mycophenolic acid	Drug: Cyclosporine GvHD prophylaxis post allogeneic SCT : -Cyclosporine : 3 mg/kg /day from D-1 (IV) or 6 mg/kg/day from D-3 (PO). Not to be stopped before D100 Drug: Mycophenolic acid (MPA) GvHD prophylaxis post allogeneic SCT : • 720 mg BID from D0 to D+28 for HLA-identical siblings

	• 720 mg BID from D0 to D+45 for 10/10 HLA allele-matched unrelated donors
Active Comparator: R3-RIC-CICLO Cyclosporine	Drug: Cyclosporine GvHD prophylaxis post allogeneic SCT : -Cyclosporine : 3 mg/kg /day from D-1 (IV) or 6 mg/kg/day from D-3 (PO). Not to be stopped before D100
Experimental: R3-RIC-MPA Cyclosporine and mycophenolic acid	Drug: Cyclosporine GvHD prophylaxis post allogeneic SCT : -Cyclosporine : 3 mg/kg /day from D-1 (IV) or 6 mg/kg/day from D-3 (PO). Not to be stopped before D100 Drug: Mycophenolic acid (MPA) GvHD prophylaxis post allogeneic SCT : • 720 mg BID from D0 to D+28 for HLA-identical siblings • 720 mg BID from D0 to D+45 for 10/10 HLA allele-matched unrelated donors
Experimental: R4-VOS-IDAC Intermediate dose cytarabine and vosaroxin	Drug: vosaroxin Consolidation chemotherapy course (s) : -70 mg/m² on D1 and D4 Drug: ID cytarabine Consolidation chemotherapy course (s) : -Intermediate dose cytarabine: 1.5g/m² /12h on D1, D3 and D5 For all patients, G-CSF (5 µg/kg/day) : SC or IV (30 min) from D8 until hematopoietic recovery (PNN ≥ 1 G/L) Up to 3 consolidation courses, depending on the patient AML risk group
Active Comparator: R4-IDAC Intermediate dose cytarabine	Drug: ID cytarabine Consolidation chemotherapy course (s) : -Intermediate dose cytarabine: 1.5g/m² /12h on D1, D3 and D5 For all patients, G-CSF (5 µg/kg/day) : SC or IV (30 min) from D8 until hematopoietic recovery (PNN ≥ 1 G/L) Up to 3 consolidation courses, depending on the patient AML risk group

LAM 2006CBF: Jourdan et al, Blood 2013;121(12):2213–2223.

TREATMENT DESIGN Induction course Systematic timed-sequential induction (arm A) DAUNORUBICINE (DNR): 60 mg/m2/day IV (30 min), Day 1, 2, and 3 CYTARABINE (AraC): 500 mg/m2/day Continuous infusion, Day 1 to 3 DAUNORUBICINE (DNR): 35 mg/m2/day IV (30 min), Day 8 and 9 CYTARABINE (AraC): 1 gr/m2/12 h IV (2h), Day 8, 9, and 10 Response-adapted timed-sequential induction (arm G) DAUNORUBICINE (DNR): 60 mg/m2/dayIV (30 min), Day 1, 2, and 3 CYTARABINE (AraC): 200 mg/m2/dayContinuous infusion, Day 1 to 7

Peripheral blood and bone marrow evaluation at Day 15. The following second induction course will be administered in patients with persistent marrow disease at Day 15 :

DAUNORUBICINE (DNR): 35 mg/m2/day IV (30 min), Day 16 and 17 CYTARABINE (AraC)1 gr/m2/12 h IV (2h), Day 16, 17, and 18 Persistent marrow disease at Day 15 is defined by more than 10% leukemic blasts in a non aplastic or non very hypoplastic bone marrow aspiration sample.

Salvage course In patients not reaching CR after the first induction course (either SI or TSI), a salvage course will be administered. Salvage therapy should not be initiated before Day 35 of arm A and Day 42 of arm G.

CYTARABINE (AraC) :3 gr/m2/12h IV (2h), Day 1, 3, 5, and 7 AMSACRINE : 100 mg/m2/day IV (30 min), Day 5 to 7 G-CSF lenograstim : from Day 8 until myeloid recovery (> 500 PMN/µL)

Consolidation cycles Three monthly cycles of consolidation will be administered in all patients reaching hematological CR after induction or induction + salvage.

CYTARABINE (AraC): 3 g/m2/12h IV (2h), Day 1, 3, and 5 G-CSF lenograstim : from Day 8 until myeloid recovery (> 500 PMN/µL)

Univariate analysis.

	2-year PFS	**2-year OS**
Age		
<60 years old	60% (44-80)	61.8% (43-87)
>=60 years	50·6% (34-75) p=0·36	55·5% (38-80) p=0·20
sFLc groups		
FLI	79·1% (64-87)	80·3% (66-97)
FLD	54·9% (36-82)	58·6% (39-86)
FLL	11·4% (12-66) p<0·001	18·7% (34-1) p=0·09
Median sFLc at day+15 induction		
<median(2952 pg/mL)	38·2% (23-62)	44·6% (26-76)
>=median	71·8% (56-90) p=0·02	73·3% (58-91) p=0·24
Median sFLc at day+22 induction		
<median (1390 pg/mL)	38·9% (24-63)	47·6% (29-75)
>=median	72·1% (57-90) p=0·02	73·6% (59-91) p=0·34
Higher sFLc during induction		
<median (4138 pg/mL)	40% (25-63)	48.5% (30-76)
>=median	72.9% (58-91) p=0.02	71.4% (56-90) p=0.38
ELN 2010		
Favorable	67·1% (44-1)	70·9% (52-96)
Int1 + Int2	57·1% (40-80)	61·1% (44-84)
Adverse	33% (14-74) p=0·06	42·.8% (20-88) p=0·18
% of blasts <median	50·9% (35-72)	53·2% (34-81)
>=median	60% (42-84) p=0·25	64·5% (46-88) p=0·26
WBC <20 Giga/L	53·8% (40-71)	55·7% (40-77)
>=20 Giga/L	58·3% (36-94) p=0·56	67·7% (47-97) p=0·75

Multivariate analysis

Progression-free survival	HR	95%CI	P value
sFLc groups	3·62	1·65-7·94	0·001
ELN 2010	1·74	0·98-3·10	0·05
Median sFLc at day+15	0·99	0·99-1·00	0·37
Median sFLc at day+22	1·00	0·99-1·00	0·24

Age not retained in the model.

Overall survival	HR	95%CI	P value
sFLc groups	2·0	1·12-6·07	0·02
ELN 2010	1·44	0·76-2·71	0·25
Age	1·02	0·97-1·07	0·374
Median sFLc at day+22	1·00	0·99-1·00	0·43

Median sFLc at day+15 not retained in the model.

Abbreviation: sFLc: soluble FLT3 ligand concentration.