FLT3 ligand kinetic profile predicts response to treatment in patients with high-risk myelodysplastic syndrome/chronic myelomonocytic leukemia receiving CPX-351: a study from the Groupe Francophone des Myélodysplasies

Here, the assessment of soluble Fms-like tyrosine kinase 3 ligand (sFL) kinetics in high-risk (HR) myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML) patients during induction therapy with CPX-351, a dual-drug liposomal encapsulation of daunorubicin and cytarabine, showed that both response and event-free survival (EFS) may be predicted by this biological marker.

Therapeutic advances in HR-MDS/CMML have so far been limited.[1,2] However, highly promising results have recently been published by our group for these patients from testing CPX-351 as first-line induction chemotherapy (GFM-CPX-MDS trial; clinicaltrials.gov 04273802), with 87% of overall response rate (ORR) and 93.5% of patients bridged to allogeneic hematopoietic stem cell transplant (allo-HSCT).[3] Here we were interested in studying the impact of kinetic profiles of two biological markers, IL-6 and sFL, as part of an ancillary analysis of this prospective study. Indeed, studies at Nantes University Hospital have shown that specific kinetic profiles of IL-6 and, mostly, sFL predict refractoriness to induction chemotherapy and, most importantly, overall survival (OS) in acute myeloid leukemia (AML).[4,5] FL is one of the key regulators of hematopoiesis.[6] Yet investigation into the role of FL in MDS or CMML has so far been poor, as only one study considered this parameter in MDS patients.[7]

Integrating the characterization of "immunome" in the stratification of the MDS/CMML setting may be also of interest to predict outcomes in both diseases as dysregulation of the immunological environment has been proven to play an important role in their pathogenesis.[8-10] For example, in MDS, higher levels of CXC10, IL-6, IL-7,[11] IL-4[12] or C-reactive protein (CRP)[13] have been reported to be associated with shorter survival. In CMML, levels of such pro-inflammatory cytokines as IL-8, TNF-α, IL-6 or IL-4 are significantly elevated, while patients with decreased levels of the immunosuppressive cytokine IL-10 present with a poorer OS.[14] Finally, high CRP values are also associated with poorer survival in CMML.[15]

This study included 31 patients (HR-MDS N=26; HR-CMLL N=5) of the GFM-CPX MDS trial. As first-line therapy, patients received intravenous CPX-351 (100 mg/m^2 cytarabine and 44 mg/m^2 daunorubicin) on days (D) 1, 3, and 5, with a second induction cycle (same daily dose on D1 and D3) if at least a partial response was not reached. Patients who responded could receive up to four monthly consolidation cycles (same daily dose on D1) or allo-HSCT. One of the secondary objectives of the trial was to evaluate the levels of sFL, IL-6 and CRP during the first cycle of CPX-351 induction chemotherapy. The trial was approved by the Comité de Protection des Personnes Sud Méditerranée ethics committee (#1994) and the French National Agency for Medicines and Health Products Safety. All patients provided informed consent for this biological sub-study. Plasma sFL concentrations (pg/mL) were evaluated using a Milliplex® cytokine assay (HCYTA-60K-01, Merck Millipore, Germany) on D1, D8, D15 and D22. Plasma IL-6 levels (pg/mL) were evaluated by ELISA (DY206, Bio-Techne, USA) on D1 and D22, as were those of CRP (mg/L, normal range 0-5; Tina-quant C-Reactive Protein IV, Cobas, Roche Diagnostics, Boulogne Billancourt, France). Both the concentrations and kinetic profile of each parameter were considered to assess their impact on outcome. Responses, including complete remission (CR), CR with incomplete hematologic recovery (CRi), morphological leukemia-free state (MLFS), relapse or stable disease (SD), as well as event-free survival (EFS) and OS were evaluated, according to European LeukemiaNet (ELN) 2017 criteria.[16] The new revised International Working Group (IWG) 2023 response criteria for HR/MDS[17] was also used. OS was defined as the time between D1 of CPX-351 administration and the date of death or last follow-up (FU). EFS was defined as the time between D1 of CPX-351 administration and the date of relapse, progressive disease from non-response after 1 or 2 cycles of CPX-351, death, or date of last FU.

Patients received the first cycle of CPX 351 between April 29, 2020, and February 10, 2021, and were followed until May 15, 2024. Univariate and multivariate analyses were performed in May 2024 with adjustments for potential confounding factors using the R software (version 4.4.0). All tests were two-sided. $P<0.05$ was considered statistically significant. Clinical results of the whole cohort (N=31) have already been reported.[3] None of the 31 patients had *FLT3* mutation. The characteristics of patients who could be evaluated for peripheral blood levels assays (N=28) are provided in Table 1. According to the ELN2017 criteria, the ORR (CR+CRi+MLFS) after cycle 1 regarding these 28 patients was 86% (N=24: CR+CRi N=17, MLFS N=7), while 4 patients reached SD. Three out of these 4 non-responders received a second

induction and one achieved CR after cycle 2. According to the more recent IWG 2023 criteria, the ORR after cycle 1 was slightly lower at 75% (N=21: CR N=10; + $CR_{bilineage}$ N=4; +$CR_{unilineage}$ N=1; +Crh N=4; PR N=2).

Most patients (N=26, 93%) received allo-HSCT after cycle 1 or 2. Among CR/CRi patients, 2 had relapsed and one had evolved towards MLFS status before transplant, while 2 were not transplanted because of a poor general condition. Among patients in MLFS before transplant, one had evolved towards CRi and 2 had relapsed, including one who obtained CRi after salvage regimen. Finally, among SD patients, one had obtained CR after salvage regimen before transplant. Thus, at transplant (N=26), 15 patients were in CR/CRi, 5 in MLFS, 3 in SD, and 3 with active disease. Relapse occurred in 4 patients after transplant and in one non-transplanted patient. Overall, 9 relapses were documented. Median FU, calculated using the reverse Kaplan-Meier estimator, was 40.4 months (95% Confidence Interval [CI]: 39.05-42.13). With a longer FU than in the previous report,[3] the 3-year EFS is 46% (95%CI: 31-69), and 3-year OS 68% (95%CI: 53-88) (Figure 1).

At last FU, 9 patients have died, including one non-transplanted patient who relapsed, and 8 of the 26 transplanted patients. Causes of death were: graft-*versus*-host disease (N=3), infection (N=1), relapse (N=3), multiorgan failure after graft failure (N=1). All patients experienced at least one episode of febrile neutropenia during the first induction. Achieving CR/CRi after the first induction was associated with a better 3-year EFS (59% [95%CI: 40-88] *vs*. 27% [95%CI: 10-72]; *P*=0.043), but not 3-year OS (71% [95% CI: 52-96] *vs*. 64% [95% CI: 51-100]; *P*=0.7). Patients transplanted in CR/CRi had the same 3-year OS as those transplanted in MLFS/SD/active disease (80% [95%CI: 62-100] *vs*. 55% [95%CI: 32-94]; *P*=0.13).

Median plasma sFL concentrations were 4 pg/mL (interquartile range [IQR] 3-9) at D1, 74 pg/mL (IQR 21-134) at D8, 3,267 pg/mL (IQR 199-390) at D15, and 381 pg/mL (IQR 183-460) at D22.

As in AML,[1] patients could be categorized according to three plasma sFL concentration kinetic profiles: i) sustained increase from D1 to D22 (FLI sustained increase [sFL]) group; N=17, 61%), ii) increase from D1 to D15, then decrease on D22 (FLD increase then decrease [sFL]) group; N=9, 32%), and iii) persistent Low levels (<100 pg/mL from D1 to D22, FLL persistent Low levels group [sFL]; N=2). Median plasma IL-6 concentrations were 11 pg/mL (IQR 3-292) on D1 and 180 pg/mL (IQR 21-341) on D22. An increase in IL-6 levels between D1 and D22 was observed in 19 patients (68%), a decrease in 7, while 2 patients had no IL-6 detectable at either time. Finally, median CRP concentrations were 2 mg/mL (IQR 1-4) on D1 and 40 mg/mL (IQR 18-98) on D22. An increase in CRP concentrations was observed in most patients between D1 and D22 (N=25, 96%). There was no correlation between IL-6 and CRP concentrations on D1 (*P*=0.64) or D22 (*P*=0.48). Of note, at both times, no relationship was found between IL-6 or CRP with sFL. Relapses occurred in 4, 4, and one patients in the FLI, FLD, and FLL groups, respectively.

Table 1. Patients' characteristics and response to one cycle of CPX-351.

Characteristic	N=28
Age at diagnosis in years, median (IQR)	62.5 (58-66)
Sex	
Female/Male, N	9/19
Bone marrow blasts, %, median (IQR)	13 (11-15.25)
HR-MDS/HR-CMML, N	23/5
IPSS, N	
Intermediate 2	24
High	4
IPSS-R, N	
Intermediate	9
High	14
Very high	5
IPSS-M, N	
Low	2
Moderate low	2
Moderate high	6
High	6
Very high	12
GFM CMML model	
Low	3
Intermediate	1
High	1
CPSS-Mol, N	
Intermediate 1	1
Intermediate 2	2
High	2
Responses to cycle 1,[1] N	
CR/CRi	17
MLFS	7
SD	4
N of CPX-351 consolidation cycles, N	
1 cycle	15
2 cycles	7
3 cycles	3
4 cycles	1
allo-HSCT, N	26
Median time between cycle 1 and allo-HSCT in days (IQR)	118 (93-173)
Status at transplant, N	
CR/CRi	15
MLFS	5
SD	3
Progression	3

[1]ELN 2017 criteria. N: number; IQR: interquartile range; HR: high-risk; MDS: myelodysplastic syndrome; CMML: chronic myelomonocytic leukemia; IPSS: International Prognostic Scoring System; R: revised; M: molecular; GFM CMML model: Groupe Francophone des Myelodysplasie; CPSS-Mol: CMML-specific prognostic scoring system; Mol: molecular; CR: complete response; Cri: complete response with incomplete hematologic recovery; MLFS: morphological leukemia-free state; SD: stable disease; HSCT: hematopoietic stem cell transplantation.

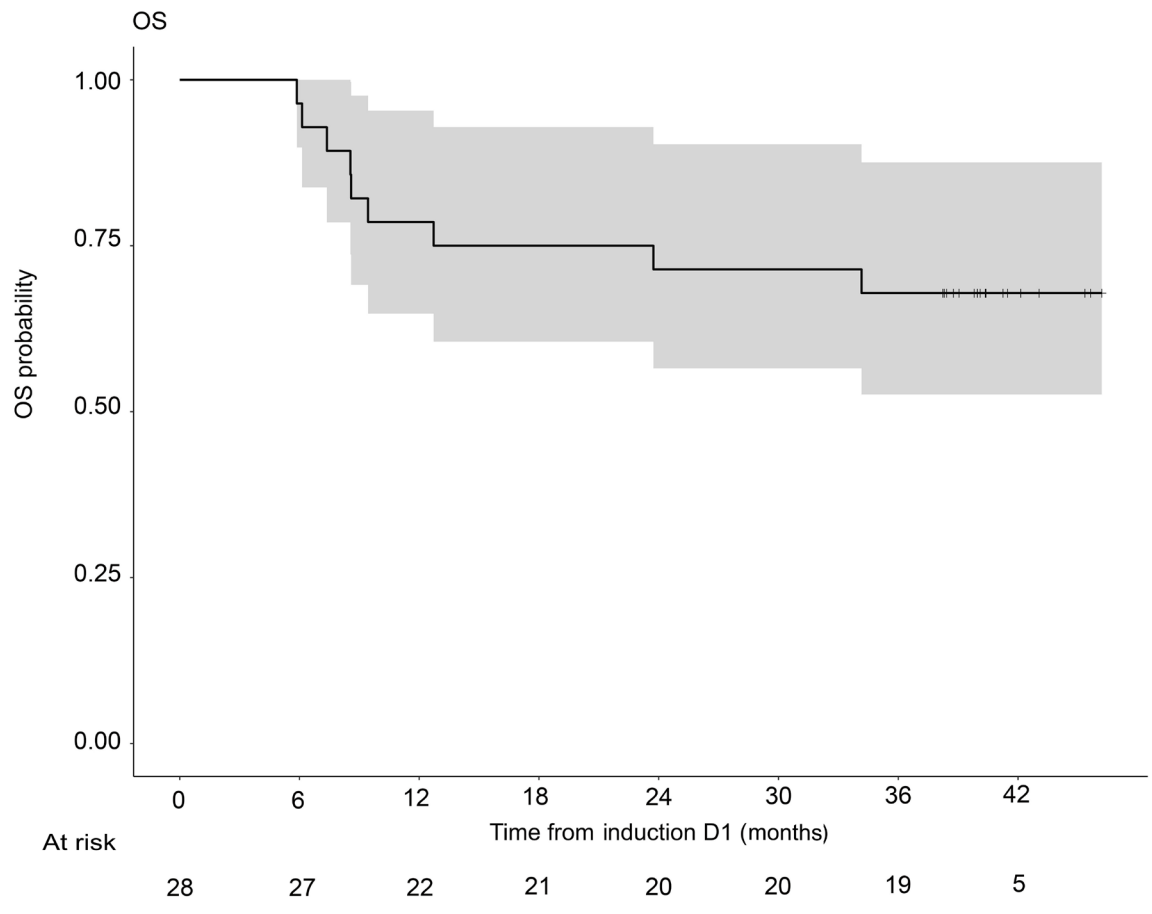

Figure 1. Overall survival of the whole cohort. OS: overall survival; D: day.

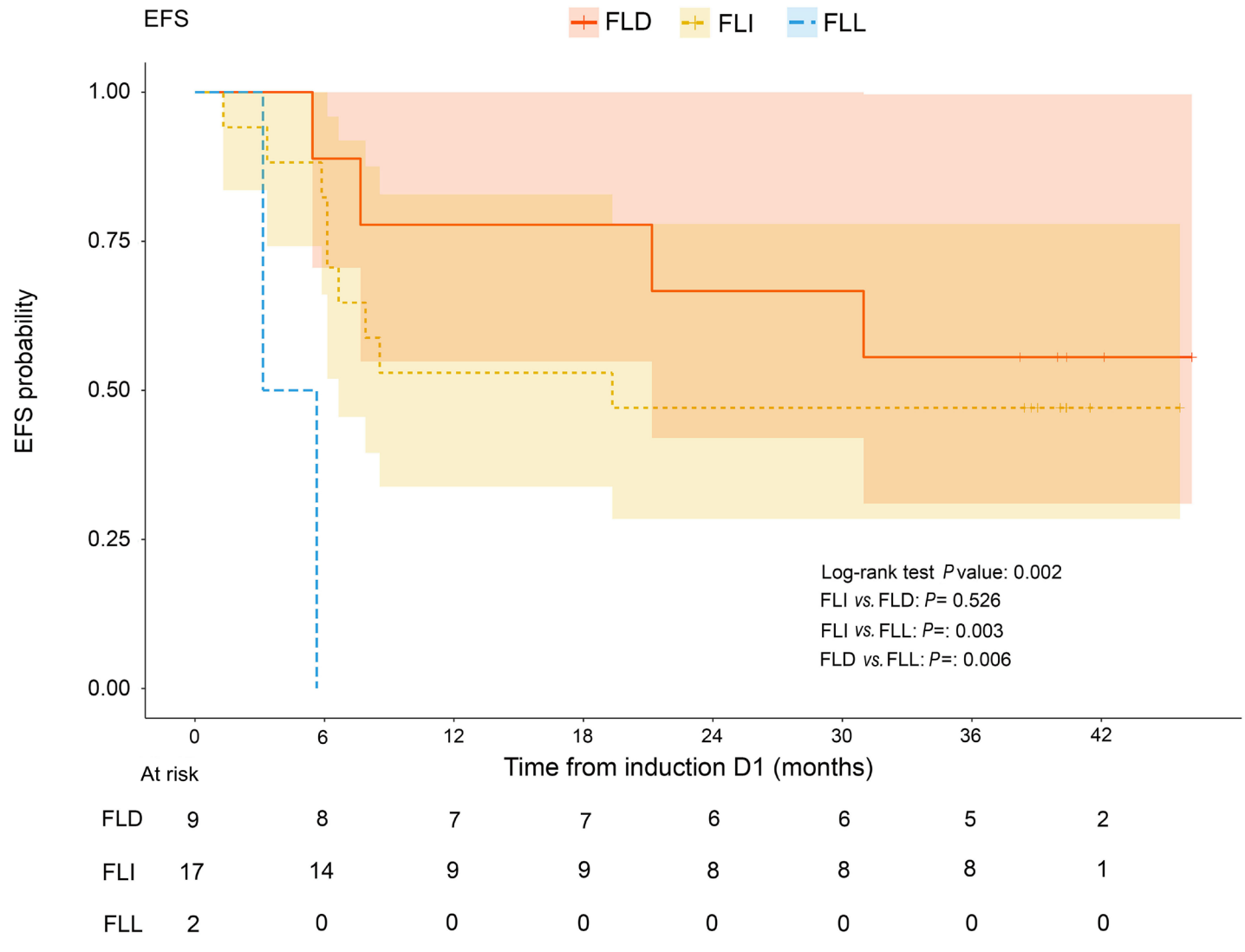

Figure 2. Event-free survival according to the three kinetic profiles of plasma soluble Flt3-ligand. EFS: event-free survival; FLD: sFL concentration increase until day (D) 15, then decrease; FLI: sFL concentration sustained increase from day 1 to day 22; FLL: sFL concentration persistent low levels.

Neither CR/CRi rates, 3-year EFS, nor OS were correlated with sFL levels (< *vs*. ≥ median) at the 4 time points considered. However, an FLD kinetic profile was significantly associated with higher CR/CRi rates (100% *vs*. 47% for FLI *vs*. 0% for FLL; *P*=0.012) and 3-year EFS (56% [95%CI: 31-100] *vs*. 47% [95%CI: 28-78] for FLI *vs*. 0% for FLL; *P*=0.002) (Figure 2).

Three-year OS was similar between the 3 sFL kinetic profile groups (FLD 67% [95%CI: 42-100] *vs*. 71% [95%CI: 52-96] for FLI *vs*. 50% [95%CI: 13-100] for FLL; *P*=0.8).

In multivariate analysis, considering age, gender, the Revised International Scoring System (R-IPSS), and sFL kinetics, an FLD profile was the only factor associated with a higher CR/CRi rate (Odds Ratio [OR]: 25.3 [95%CI: 2.02-3843]; *P*=0.004). The same analysis, using the molecular M-IPSS instead of the R-IPSS, yielded the same significant isolated value as the FLD profile (OR: 68.3 [95%CI: 3.31-13232]; *P*=0.003) (*Online Supplementary Figure S1*). The poor outcome of the 2 FLL patients seems independent of baseline characteristics as they had low and high IPSS-M risk, respectively. Plasma concentrations (< *vs*. ≥ median) and IL-6/CRP kinetic profiles had no impact on CR/CRi rates, EFS, or OS. Of note, all the 28 patients were treated with antibiotics during induction in relation with a neutropenic fever, suggesting that its influence on IL6 and CRP level disparities among patients is probably mild.

As already shown in AML,[4] the plasmatic sFL kinetic profile was found here to predict response and EFS in HR-MDS/CMML. However, OS was not impacted by this factor, essentially because allo-HSCT, that was performed in almost all patients, could clearly reverse the poor outcome linked to a HR sFL kinetic profile. In parallel, we failed to demonstrate any impact of IL6 or CRP in this series, suggesting that other markers probably contribute to the complex mechanisms of immune dysregulation and inflammation during MDS/CMML evolution.

In conclusion, our study suggests that sFL kinetics should be more widely investigated, at least in patients not eligible for allo-HSCT, as this new biomarker could help to adapt further management to the individual profile of each patient.

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Disclosures

No conflicts of interest to disclose.

Contributions

PP, JG, PF and PC are reponsible for study design. JG is reponsible for the assays. PP, PT, MPG, PYD, ST, AB, SP, MAH, TC, J-M, T-G, LD, RS, FC, LA, SD-S, MJ, PF and PC are reponsible for patient care and data collection. PP, MJ and PC are reponsible for data analyses. PP, PC and MJ prepared the manuscript. All authors have read and approved the final version of the manuscript for publication.

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Data-sharing statement

The study details are available online at clinicaltrials.gov identifier 04273802. Study data are not publicly available in order to respect participant confidentiality. Requests for sharing deidentified data should be directed to the corresponding author.

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