Favorable pharmacokinetic and pharmacodynamic properties of gilteritinib in cerebrospinal fluid: a potential effective treatment in relapsing meningeal acute myeloid leukemia *FLT3*-ITD patients

Patients diagnosed with acute myeloid leukemia (AML) harboring internal tandem duplication (ITD) mutations in the *FLT3* gene present a higher risk of early relapses and shorter overall survival after chemotherapy.[1] Gilteritinib, a highly selective oral FLT3 inhibitor has demonstrated significant therapeutic effect in patients with relapsed or refractory *FLT3*-mutated AML with higher complete remission (CR) rates (21.1% *vs*. 10.5%) and longer median overall survival (9.3 months *vs*. 5.6 months) compared to salvage chemotherapy.[2] Gilteritinib, metabolized by CYP3A4 into inactive metabolites, has been identified *in vitro* as a P-gp substrate.[3] Gilteritinib inhibits FLT3 kinase activity and viability of cells expressing FLT3 with a half-maximal inhibitory concentration (IC_{50}) of 0.291 nM (0.16 ng/mL)[3] and 0.92-2.1 nM (0.51-1.16 ng/mL), respectively in BA/F3 cells exogenously expressing wild-type *FLT3* or *FLT3* mutants (*FLT3*-ITD, *FLT3-D835Y*, and *FLT3*-ITD-*D835Y*).[4] In a meta-analysis of 11 ECOG-ACRIN trials, central nervous system (CNS) involvement was detected in 1.1% and CNS infiltration at diagnosis was not associated with a lower rate of CR or a shorter overall survival.[5] Nevertheless, Del Principe *et al*. reported higher incidence of meningeal involvement reaching 32% at AML diagnosis, associated with a poorer outcome.[6] CNS relapses occur in 2.6-4.1% and confer a poor prognosis.[7] However, patients with CNS relapses were excluded from the gilteritinib pivotal trial.[2] Perrone *et al*. reported a relapsing medullar and meningeal AML *FLT3*-ITD patient responding to gilteritinib monotherapy,[8] however, gilteritinib CNS distribution was not assessed.

We aimed to explore the distribution and *in vitro* efficacy of gilteritinib in cerebrospinal fluid (CSF) in AML patients with CNS relapse. We report here four patients, from four French institutions treated for concomitant CNS and medullary *FLT3*-ITD AML relapse with gilteritinib and intrathecal injections of chemotherapy (IT). All patients provided informed consent to participate to this study.

Patient 1

A 63-year-old woman, was diagnosed in February 2020 with French-American-British (FAB)[9, 10] M1-AML. Cytogenetic analysis showed a normal karyotype and next-generation sequencing (NGS) analysis showed mutations of *NPM1, FLT3*-ITD, *DNMT3A, SMC3* and *KMT2D/MLL2*. CR was obtained after intensive chemotherapy (7+3 regimen) plus Midostaurin, then consolidation with intermediate dose of Cytarabine (IDAC) and Midostaurin and maintenance with Midostaurin. Fifteen months after CR, headache and radicular pain revealed a simultaneous bone marrow and CNS relapse with 2,960/mm^3 blast cells in the CSF with normal karyotype and NGS identical to February 2020 without *FLT3-TKD* mutation. Intrathecal triple therapy (ITT) (corticosteroid, Methotrexate and Cytarabine) was given with CSF blast cell clearance, followed by IDAC plus gilteritinib 120 mg once daily (QD) as consolidations in July 2021 (6 IT in all) followed by gilteritinib 120 mg QD as long-term maintenance and *in toto* encephalic irradiation. CR was obtained 4 months after CR2 and maintained at 1 year.

Patient 2

A 62-year-old woman, was diagnosed in July 2021 with an hyperleukocytic FAB M5-AML, with a normal karyotype, *NPM1* type A mutation and *FLT3*-ITD. Due to initial hyperleukocytosis, lumbar puncture (LP) with ITT was performed during induction and revealed a CNS involvement with 24 blasts/mm^3 in CSF. CR was achieved after induction chemotherapy with Daunorubicin and Cytarabin plus Midostaurin (HOVON AML 156), and two courses of consolidation with IDAC and Midostaurin. CSF examination after induction was negative. Two months after CR, she presented with delirium, psychomotor retardation and an erythematous maculopapular rash, revealing a combined dermal and CNS relapse with 5 blasts/mm^3 in CSF. IT (corticosteroid and methotrexate) injections (3 IT) and gilteritinib 120 mg QD monotherapy was started, further decreased to 80 mg QD for hepatic toxicity. In June 2022 bone marrow was positive for NPM1 and gilteritinib was increased to 120 mg twice daily (QD) as maintenance therapy with seven IT. One year after gilteritinib initiation, no blasts were detected in CSF.

Patient 3

A 50-year-old woman, was diagnosed in March 2021 with a normal karyotype FAB M5-AML, NGS analysis of blast cells showed mutations of *NPM1* (mutant D), *FLT3*-ITD, *KRAS* and *TET2* exon 3 and 9. CR was achieved after intensive chemotherapy (7+3 regimen) plus gilteritinib (HOVON AML 156). The patient received IDAC combined

Table 1. Gilteritinib and Flt-3 ligand level in plasma and cerebrospinal fluid, protein levels in cerebrospinal fluid of patients 1 to 4.

Patient ID	Gilteritinib dose (mg, QD)	Time from gilteritinib initiation at CNS relapse (day)	Gilteritinib plasma trough concentration (ng/mL)	Gilteritinib in CSF (ng/mL)	Gilteritinib CSF to plasma ratio (%)	CSF protein level (g/L)	Flt-3L in CSF (pg/mL)	Flt-3L in plasma (pg/mL)
1	120	27	646	14.0	2.17	insufficient sampling volume	insufficient sampling volume	8,899
	120	110	763	13.3	1.74	0.64	107	6,288
2	120	7	376	10.6	2.81	0.68	<15	15
	80	40	not collected	4.9	not evaluable	1.12	<15	not collected
3	120	30	270	7.7	2.84	0.26	<15	64
	120*	60	409	6.4	1.56	0.24	<15	1,511
4	120	15	148	4.4	2.95	0.49	42	179
	120	30	227	6.6	2.89	insufficient sampling volume	insufficient sampling volume	337

*In combination with Venetoclax 400 mg once daily (QD). CSF: cerebrospinal fluid; Flt-3L: Flt-3 ligand.

with Clofarabine in consolidation. One month after CR, a neuromeningeal relapse occurred with blast cells in CSF. ITT were started (4 IT in all) and followed by gilteritinib 120 mg QD in October 2021. In November 2021, Venetoclax 400 mg QD was added.[11] Two months later, MRD on *NPM1* was undetectable with few blasts in CSF. In September 2022, a cerebral computed tomography scan showed a thalamic mass linked to the AML. The patient deceased in October 2022.

Patient 4

A 64-year-old man, was diagnosed in May 2019 with a normal karyotype FAB M5-AML, NGS analysis of blast cells displayed mutations of *NPM1* (mutant A), *DNMT3A, TET2* splice exon 5, *ASXL1* and *IDH1*. CR was achieved after induction-consolidation chemotherapy with Daunorubicin and Cytarabin followed by non-myeloablative phenoidentical bone marrow allograft. MRD *NPM1* was undetectable after 1 month. In November 2020, he presented an extramedullar and CNS relapse with *NPM1* (mutant A) and *FLT3*-ITD mutations. ITT were given with CSF blast cell clearance (4 IT). Consolidation with Azacytidine plus Venetoclax 400 mg QD, achieving incomplete cytologic response with undetectable MRD phenotype and *NMP1* mutation, was stopped after eight cycles due to hematologic toxicity. In April 2022, he was admitted for cauda equina syndrome and LP revealed a second CNS progression with 980/mm^3 blast cells in the CSF harboring *FLT3*-ITD mutation. ITT were given (6 IT) associated to gilteritinib 120 mg QD with CSF blast cell clearance. MRD *NPM1* was undetectable 1 month after gilteritinib initiation and CR maintained after 1 year.

Gilteritinib trough concentration in plasma and CSF at steady state were quantified using liquid chromatography coupled to tandem mass spectrometry methods (TSQ-Altis Thermo-Fisher Scientifics, Massachusetts, USA). Protein levels in CSF were quantified by turbidimetric method (TPUC3, Cobas c703, Roche, Meylan, France). Flt-3 ligand (Flt-3L) was quantified in CSF and plasma using an enzyme-linked immunosorbant assay (Human Flt-3 Ligand Quantikine ELISA Kit, R&D Systems, Minnesota, USA). All available gilteritinib and Flt-3L levels in plasma and CSF are presented in Table 1. Plasma concentrations of the four patients were consistent with pharmacokinetics data previously described.[12] No drug-drug interaction was found here excluding patient 1 concomitantly treated with the weak CYP34A inhibitor Isavuconazole, possibly contributing to higher plasma concentrations.[12] Median CSF/plasma ratio of 2.81% was consistent in the four patients regardless of the plasma concentration and protein level in CSF, suggesting a linear correlation within the plasma concentration range observed (range, 148-763 ng/mL) and a lack of saturation phenomenon involving a weak impact of P-gp. Furthermore, in patient 3, association to Venetoclax, a known P-gp inhibitor, did not enhance gilteritinib CSF distribution.[13] This low ratio is partly explained by the low unbound fraction, in healthy subjects of 5.7%,[4] the only fraction able to cross the blood-brain barrier. Nevertheless, gilteritinib CSF concentration exceeded IC_{50} for FLT3 kinase activity in all samples.[3] Restoration of Flt-3L level in serum after starting chemotherapy has been associated with higher overall survival in AML *FLT3*-ITD patients[14] and further investigation is needed to explore Flt-3L in CSF as a prognostic biomarker. Interestingly Flt-

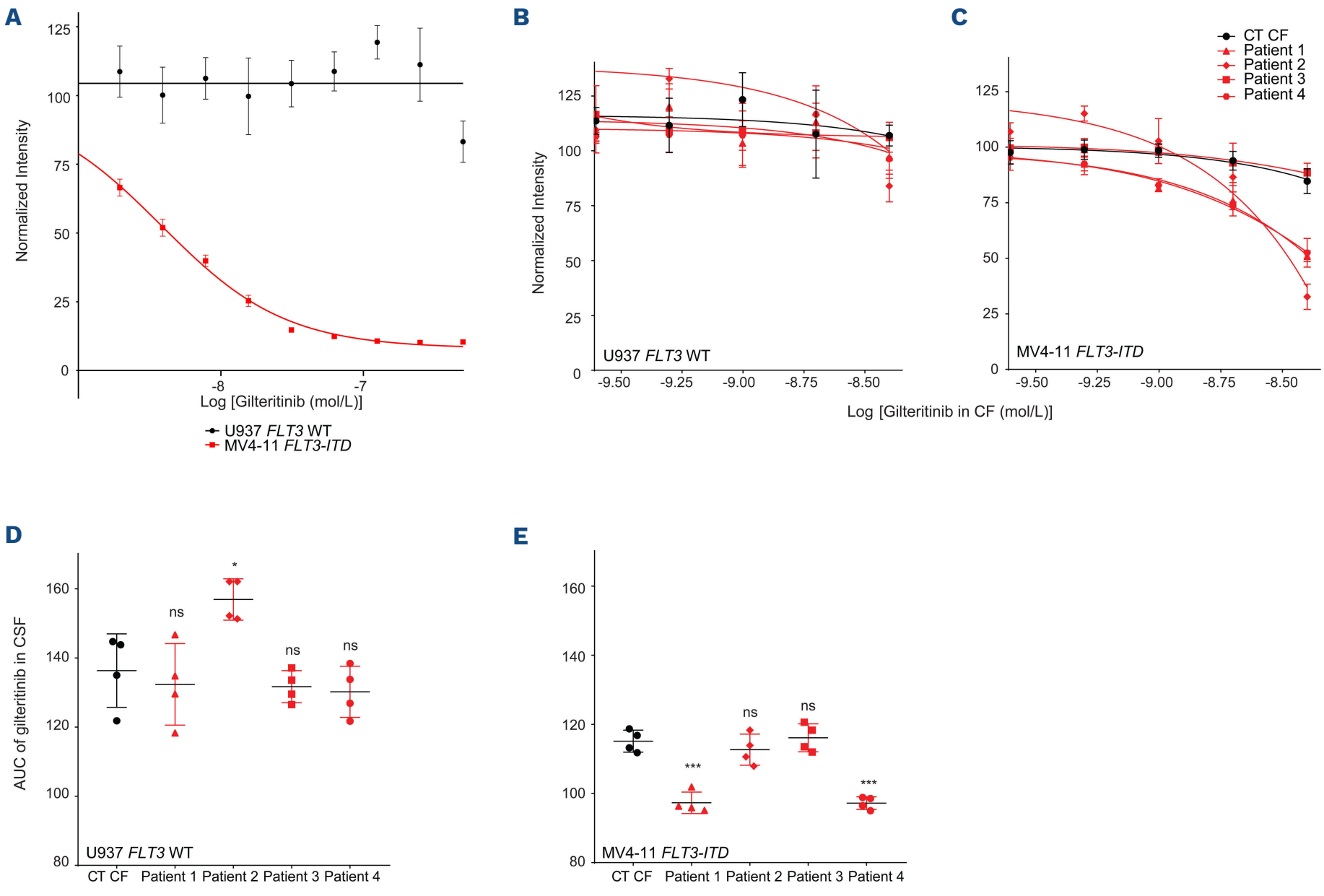

Figure 1. Response of *FLT3*-ITD-mutated (MV4-11) cells and *FLT3*-ITD wild-type cells (U937) to patient cerebrospinal fluid. (A) Response of U937 (FLT-3 negative) and MV4-11 (FLT-3 positive) cell lines treated with increasing concentrations of gilteritinib (day 3; N=7). (B) U937 cells treated *in vitro* with control cerebrospinal fluid (CSF) compared to the CSF of 4 acute myeloid leukemia (AML) patients with central nervous system (CNS) and medullary FLT3 internal tandem duplication (*FLT3*-ITD) relapse (day 5; N=4). (C) MV4-11 cells treated *in vitro* with control CSF compared to the CSF of 2 AML patients with CNS and medullary *FLT3*-ITD relapse (day 5; N=4). (D) Distribution of the area under the curve (AUC) for *FLT3* wild-type (WT) U937 cells with control CSF or CSF of 4 AML patients with CNS and medullary *FLT3*-ITD relapse for 5 days. (E) Distribution of AUC for *FLT3*-ITD MV4-11 cells with control CSF or CSF of 4 AML patients with CNS and medullary *FLT3*-ITD relapse for 5 days. Error bars represent the mean ± standard deviation of 4 technical replicates (day 5; N=4; **P*<0.05; ***P*<0.005; ****P*<0.0005).

3L level in CSF was not correlated to Flt-3L level in plasma.

MV4-11 is a cell line established from the monocytes of a 10-year-old male with AML harboring a *FLT3*-ITD mutation. U937 was obtained from a 37-year-old male with histiocytic lymphoma without a *FLT3*-ITD mutation. Both cell lines were purchased from ATCC and maintained in RPMI-1640 (Sigma-Aldrich #R8758) supplemented with 1% penicillin–streptomycin and 10% fetal bovine serum (Sigma-Aldrich) at 37°C with 5% CO_2.

In order to determine the activity of gilteritinib in the CSF of AML patients with CNS and medullary *FLT3*-ITD relapse, *FLT3*-ITD mutated (MV411) and *FLT3* wild-type (U937) AML cell lines were plated in quadruplicate and treated with increasing concentrations of patient or control CSF up to 2.2 ng/μL and normalized to MV4-11 or U937 cells with a CSF-mimicking control solution (plasma diluted 1/200th in water). Response to CSF treatment was measured using CellTiter-Glo (Promega #G7573) to determine normalized intensity of luminescence to infer cell proliferation and viability after 5 days of treatment with control CSF or gilteritinib -containing CSF samples from patients 1 to 4. The *FLT3*-ITD AML cell line, MV4-11, demonstrated a significant reduction in the area under curve (AUC) in response to increasing volume of CSF with an IC_{50} of 3.86 nM (2.13 ng/mL), in comparison with its *FLT3* WT U937 counterpart which did not show sensitivity to patient-derived CSF (Figure 1). These results confirmed that the CSF of patients 1 and 4 contains an active unbound fraction of gilteritinib which exhibited anti-leukemic properties. For patients 2 and 3, MV4-11 did not demonstrate a significant reduction in the AUC in response to an increasing volume of CSF despite the quantifiable gilteritinib concentration in the CSF.

Due to its use in combination with other therapies, gilte-

ritinib efficacy as a single agent was not assessable. Nevertheless, this report describes objective sustainable responses and pharmacodynamic proofs of gilteritinib CSF penetration in AML patients with CNS involvement. With such favorable pharmacokinetic and pharmacodynamic properties, and given the paucity of drugs active on CNS relapse of AML, our reports provide rationale for further evaluations of the use of gilteritinib in CNS involvement of AML patients.

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Contributions

LG developed the concept, supervised the research, reviewed and edited the article. NV, EL, ACH, JS, DG, TB, MK, PR, ER and LG analyzed data. NV, LK, EL, ACH, JS, DG, HS, TB, EJ-A, MK, PR, AP, ER, SM and LG performed the formal analysis, developed the methodology and software, visualized and validated data and wrote the original draft. NV, LK, EL, AC-H, JS, DG, HS, TB, EJ-A, MK, PR, AP, ER, SM and LG provided resources.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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