haematologica
Journal of the Ferrata Storti Foundation

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Received: June 20, 2024.
Accepted: October 9, 2024.

Citation: Florent Puisset, Emmanuel Raffoux, Lionel Ades, Tony Huynh, Raphael Itzykson, Didier Bouscary, Lorea Aguinana, Florence Rabian, Marie Sebert, Anne Vekhoff, Eolia Brissot, Mohamad Mohty, Agnes Bonnin, Alexis Genthon, Jeremie Zerbit, Lise Willems, Justine Decrocq, Adrien Contejean, Etienne Lengline, Samia Mourah, and Lauriane Goldwirt. Exposure toxicity of venetoclax in acute myeloid leukemia patients in the real-life setting: impact of high exposure on delayed neutropenia.
Haematologica. 2024 Oct 17. doi: 10.3324/haematol.2024.286062 [Epub ahead of print]

Exposure toxicity of venetoclax in acute myeloid leukemia patients in the real-life setting: impact of high exposure on delayed neutropenia

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Running head : Exposure-toxicity of venetoclax in AML patients

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Data sharing statement : Data are available upon request

Trial registration : Institutional Review Board -IRB 00006477- of HUPNVS, Paris 7 University, AP-HP, N° CER-2022-159

Ethical aspect : The study was conducted in accordance with Helsinki Declaration, and was approved by the Institutional Review Board (trial registration n° :IRB 00006477) of HUPNVS, Paris 7 University, AP-HP, N° CER-2022-159

Disclosures: No funding was received for this study. LG acts as a consultant for Abbvie. All other authors have no conflicts of interest to disclose.

Contributions: Conceptualization : LG, FP ; Data curation: FP, ER, LA, TH, RI, DB, LA, FR, MS, AV, EB, MM, AB, AG, JZ, LW, LD, AC, EL, LG ; Formal analysis, Methodology, Software, Validation, Visualization and Writing original draft : FP, ER, LA, TH, RI, DB, LA, FR, MS, AV, EB,

MM, AB, AG, JZ, LW, LD, AC, EL, LG ; Resources: FP, ER, LA, TH, RI, DB, LA, FR, MS, AV, EB, MM, AB, AG, JZ, LW, LD, AC, EL, LG; Supervision : LG; Writing review and editing: LG

Funding : no funding was received for this study

Keywords : Venetoclax, acute myeloid leukemia, exposure toxicity, pharmacokinetic, PK-PD

Venetoclax is a selective and potent B-cell lymphoma-2 (BCL-2) inhibitor that has improved clinical outcomes in combination with a hypomethylating agent (HMA) for patients with untreated acute myeloid leukemia (AML) unfit for intensive chemotherapy [1]. Venetoclax undergo hepatic metabolism involving CYP3A4 and highly sensitive to drug-drug interaction with triazoles, moderate-to-strong CYP3A4 inhibitors, which are indicated during induction phase in AML for prophylaxis and treatment of Aspergillus and Candida infections [2,3]. A 75% reduction dose of Venetoclax is therefore recommended in case of combination [4]. However, the level of dose reduction is less than the 6-fold expected reduction in venetoclax clearance, which can lead to a venetoclax overexposure despite an adapted dose. Venetoclax therapeutic drug monitoring (TDM) was therefore applied in routine practice. As the exposure-toxicity relationship remains unclear, the main objective of this study was to assess the risk of high exposure-related neutropenia beyond the first cycle of treatment in a real-life setting.

Patients followed in Hematology Department of Saint-Louis, Saint-Antoine and Cochin Hospitals of Public Assistance of Parisian Hospitals with therapeutic drug monitoring in routine care centrally performed in Saint Louis Hospital Pharmacology department between 1st January 2018 and 31st October 2021 were retrospectively selected. This retrospective study was approved by the Institutional Review Board -IRB 00006477- of HUPNVS, Paris 7 University, AP-HP institutional review board (N° CER-2022-159). Patient inclusion in the pharmacokinetic analysis and the exposure-toxicity analysis is described in Supplemental Figure 1. The exposure-response analysis was performed on the subgroup of AML patients with an evaluation of medullar blast clearance after 1 month of venetoclax (between day 20 and day 35) and with data available on toxicity during the second month of treatment.

Neutropenia was the criterion selected to assess the toxicity of venetoclax. Drug-related neutropenia was defined by grade ≥3 neutropenia (i.e <1000 neutrophil/mm^3 according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0) lasting at least 7 days beyond cycle 1 day 30 of venetoclax treatment, and/or with clinical impact of pursuit of treatment (dose reduction or delay in second month of treatment), and unrelated to the disease (i.e in patients with < 5% of bone marrow blasts at the first assessment performed between days 20 and 35 of venetoclax).

Blood samples were collected at any time during a dosing interval at steady state. Venetoclax quantification in plasma was performed using a validated liquid chromatography coupled to mass tandem spectrometry according to ICH M10 on bioanalytical method validation.

Venetoclax plasma concentrations were analyzed using a population pharmacokinetic approach to standardize pharmacokinetic parameter estimation method between patients with sparse data. Several structural models were tested (1 or 2 compartments) inter-individual and inter-occasion variability were assessed for all pharmacokinetics (PK) parameters. Covariates already described as influencing PK parameters were tested in our model (*i.e.* age, sex, combination with CYP3A inducer , CYP3A inhibitor, ATP Binding Cassette inhibitor or Rituximab [5]). Each covariate was tested first in univariate analysis (a decrease in objective function value (ΔOFV) of at least 3.84 chi squared test, df = 1, ($p < 0.05$) and a significant decrease in interindividual variability was used for selecting covariates). All significant covariates were added in a multivariate model, followed by backward selection. Once the final PK model validated, it was used to estimate individual PK parameters by a Bayesian approach. Then individual PK parameter were used to determine the cumulative AUC taking into account actual venetoclax daily dosing during the first 28 days. A daily average of AUC (AUCavg) was used as metric for venetoclax exposure. Model development and bayesian post-hoc estimation were performed with NONMEM version 7.4.1 (ICON Development Solutions, Ellicott City, MD), using the first-order conditional estimation with interaction estimation method [6].

Patients' clinical and biological characteristics are presented in terms of median [range] for quantitative variables and number (%) for qualitative variables. Association between drug-related neutropenia and venetoclax exposure (AUCavg) as well as with clinical characteristics at venetoclax initiation was assessed by univariate logistic regression models. Variables displaying a significant association with drug-related neutropenia in univariate analysis ($p<0.05$) were candidate for multivariate analysis and a stepwise procedure based on Akaike information criteria (AIC) minimization was conducted to compute multivariate models. Venetoclax AUCavg was considered first as a continuous variable, then an event risk threshold of AUCavg was determined by a ROC curve analysis and used as binary covariate. All analyses were carried out using R software version 4.2.4 (R Core team 2013).

A total of 123 patients (corresponding to 280 samples) were eligible for the pharmacokinetic analysis, mainly with AML (62.6%), CLL (8.9%) MM (8.1%), and MDS (8.1%) as presented in Supplemental Table 1. Among the 79 patients treated for an AML presented in table 1, there was a majority of men with high risk AML according to ELN 2017 [7]. Venetoclax was prescribed mostly as continuously daily dosing in combination with HMA, mostly concomitantly to a strong CYP3A inhibitor. The median (IQR) daily dose of venetoclax after

ramp-up reached 400 mg (200-400), whereas it was decreased to 100 mg (100-200) in case of interaction with strong CYP3A inhibitor. Among the 51 patients with available bone marrow assessment after 1 cycle, drug-related toxicity was not evaluable for 22 of them because bone marrow blasts were still ≥5% after 1 cycle. Thus, 29 patients were included in the exposure-toxicity analysis.

A one compartment with one order absorption and elimination fitted better the data than a 2-compartment model (ΔOFV = 59.1, p <0.001). Two covariates influencing apparent CL (CL/F) were kept in the model: strong CYP3A inducer (ΔOFV =20.3, p<0.001) and strong CYP3A inhibitor (ΔOFV = 124.78, p <0.001). The underlying hematological disease did not impact venetoclax pharmacokinetics (ΔOFV = 0.539, p >0.1). The combination with a strong CYP3A inhibitor led to near to five-fold decrease of CL/F, whereas a combination with a strong CYP inducer led to near to three-fold increase in CL/F. Parameters values are shown in Supplemental Table 2. The correlation between predicted and observed individual concentrations illustrates the fit of the model and therefore its ability to estimate individual pharmacokinetic parameters (Supplemental Figure 2).

Among 29 assessable patients, 20 drug-related neutropenia were observed: 18 leading to a decrease in dose intensity of venetoclax during the second cycle: as a delay of at least 7 days for venetoclax rechallenge (N=9), a decrease in number of day of venetoclax intake (N=6), a definitive venetoclax withdrawal (N=2), or a dose decrease (N=1), and 2 had a grade≥3 neutropenia lasting at least 7 days while maintaining venetoclax dose.

Higher AUCavg was significantly correlated to drug-related neutropenia considering AUCavg above the first quartile (p=0.002) or above the median value (p=0.022) (Figure 1). Based on ROC Curve analysis an AUCavg of 2.70 µg/mL.day was found to be a threshold value for higher risk of drug-related neutropenia (specificity = 88.9%, sensitivity = 75.0%). AUCavg above this threshold was significantly associated with drug-related neutropenia (OR = 24.0, p = 0.007). The secondary nature of AML was the only other covariate associated with drug-related neutropenia. The association between AUCavg above 2.70 µg/mL.day remained significant even in multivariate analysis (OR = 39.8 p=0.010) (Table 2).

In this retrospective real-life study, we observed described a statistically significant correlation between venetoclax exposure and drug-related neutropenia lasting beyond cycle 1 day 30 whereas the development studies failed to show any clear exposure-toxicity relationship. In our population pharmacokinetic model, we observed a great influence of

strong CYP3A inhibitors on CL/F by factor close to five, which was slightly lower but consistent with that of previous DDI studies [4]. The observed coefficient on CL/F was consistent with that observed in large population pharmacokinetic analysis performed in a CLL population [8]. We observed higher venetoclax AUC levels than those described by Brackmann *et al.* with the range of the first quartile of AUCavg level in our study reached the range of the third quartile Brackmann *et al.* published [9]. The venetoclax exposure we observed was also greater than those predicted by a modelling approach in case of DDI with strong CYP3A inhibitor, exceeding the maximum level of exposure assessed for safety [10]. In a post-hoc analysis using data from phase 1 to phase 3 studies, Brackmann *et al.* observed no statistically significant relationship between exposure and treatment emergent grade≥3 neutropenia but without any precision on timing of neutropenia assessment [9]. When focusing on post-remission patients included in the VIALE-A study [11], Pratz *et al.* showed a correlation between average venetoclax concentration and any grade 4 cytopenia events [12]. Despite the retrospective nature of our study, the missing data such as use of G-CSF report and the limited number of patients with complete pharmacokinetic and pharmacodynamics data, we observed a relation between high venetoclax exposure and toxicity which rise the question of a benefit of a shorter duration treatment. Response rates and overall survival obtained with 28-days regimen were comparable to those reached with 14-days regimen [13] or 7-days regimen [14]. Furthermore, Mirgh *et al.* observed a lesser hematologic toxicity without any difference in response rate with less than 21 days of venetoclax compared to 21-28 days duration [15].

Our study is to the best our knowledge the first exposure-toxicity study showing that very high level of venetoclax exposure can be reached in clinical practice leading to an increased risk of drug-related neutropenia.

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Tables

Table 1 : Patient's characteristics

Characteristic	Overall N = 51	Blasts <5%* N = 29
Age (years), median [range]	**72 [17 - 87]**	**72 [25 - 84]**
Sex		
Men, N (%)	**33 (64.7%)**	**18 (62.1%)**
Women, N (%)	**18 (35.3%)**	**11 (37.9%)**
Body weight (kg), median [range]	**69 [40 - 94]**	**67 [43 - 85]**
WHO_PS ≥2, N (%)	**14 (28.0%)**	**7 (24.1%)**
Unknown, N	**1**	**0**
Hb at baseline (g/dl), median [range]	**8.90 [6.00 - 15.00]**	**8.90 [6.90 - 13.90]**
Unknown, N	**1**	**1**
Leucocytes at baseline (10^9/L), median [range]	**3 [0 - 84]**	**2 [0 - 20]**
Unknown, N	**1**	**1**
Neutrophil count at baseline (10^9/L), median [range]	**0.70 [0.03 - 22.54]**	**0.57 [0.03 - 13.79]**
High risk AML at diagnosis (ELN 2017), N(%)	40 (78.4%)	24 (82.8%)
Refracory/relapse AML before Venetoclax, N (%)	17 (33.3%)	7 (24.1%)
Secondary AML at diagnosis, N (%)	30 (58.8%)	16 (55.2%)
Regimen, N (%)		
venetoclax-azacitidine	42 (82.4%)	24 (82.8%)
venetoclax-azacitidine-ivosidenib	1 (2.0%)	1 (3.4%)
venetoclax-cytarabine	3 (5.9%)	2 (6.9%)
venetoclax-decitabine	2 (3.9%)	1 (3.4%)
venetoclax-enasidenib	1 (2.0%)	0 (0.0%)
venetoclax-ivosidenib	1 (2.0%)	0 (0.0%)
venetoclax-midostaurine	1 (2.0%)	1 (3.4%)
N of monthly days of venetoclax, N (%)		
8	1 (2.0%)	1 (3.4%)
14	4 (7.8%)	2 (6.9%)
21	5 (9.8%)	2 (6.9%)
28	41 (80.4%)	24 (82.8%)
TDM performed during first cycle, N (%)	45 (88.2%)	27 (93.1%)
ddi with strong CYPA inhibitor (posaconazole), N (%)**	42 (82.4%)	26 (89.7%)
ddi with ABC (P-gp or BCRP) inhibitor, N (%)	44 (86.3%)	25 (86.2%)

**Percentage of medullar blasts after one month of venetoclax therapy.*

*** one patient received also ritonavir*

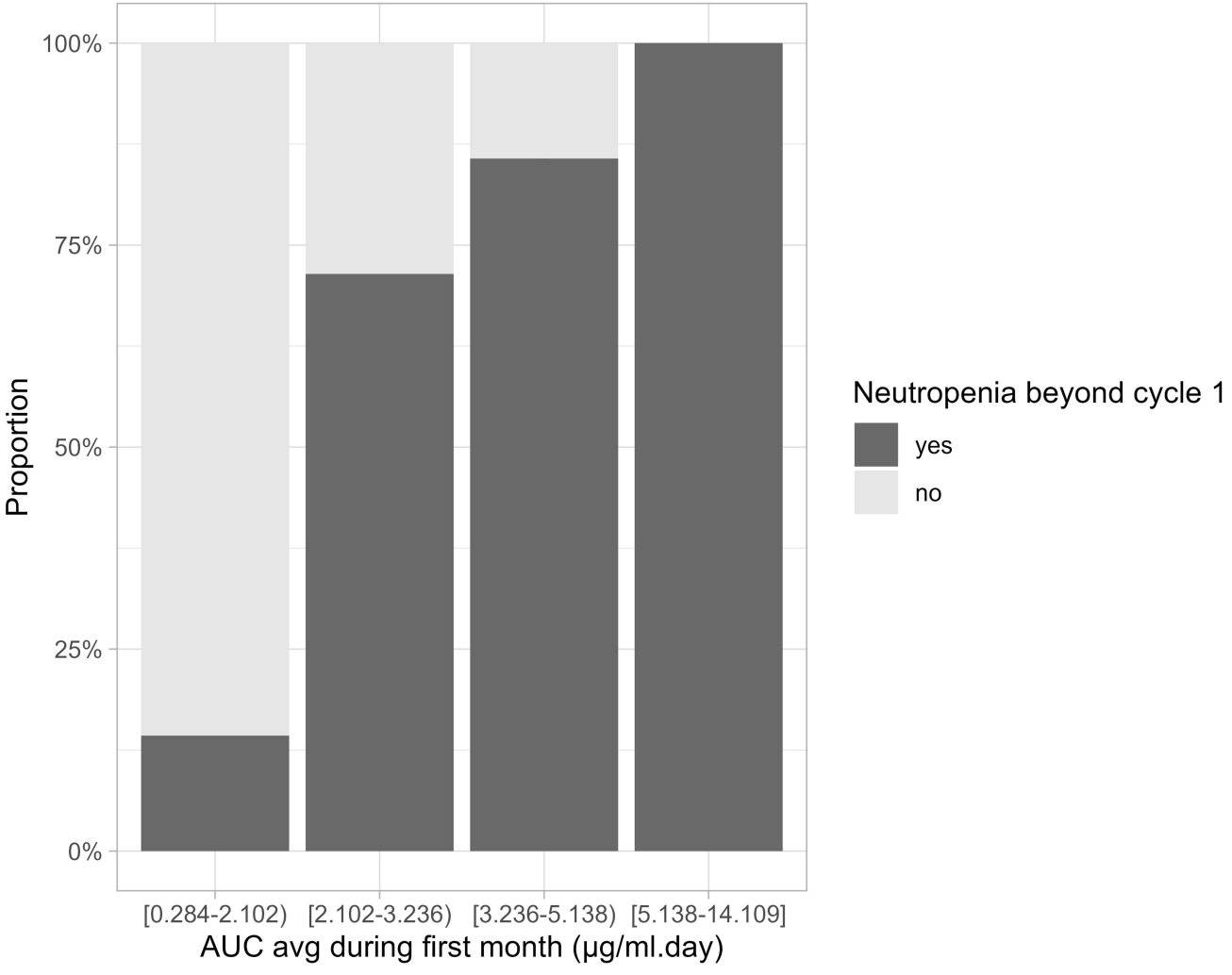
WHO_PS = World Health Organisation performance status, TDM = therapeutic Drug monitoring, ddi = drug-drug interaction ; ABC = ATP Binding Cassette

Table 2 : Logistic regression exploring the correlation between daily average of AUC (AUCavg) of venetoclax during the first month and the risk of clinically relevant toxicity at the beginning of the second month of treatment (N= 29)

Characteristic	**Univariate Analysis**			**Multivariate Analysis**		
	OR[1]	**95% CI[1]**	**p-value**	**OR[1]**	**95% CI[1]**	**p-value**
Sex (Women vs Men)	0.67	0.13, 3.49	0.6			
WHO ≥2	3.43	0.46, 70.9	0.3			
Relapsed/refactory AML	0.50	0.08, 3.16	0.4			
Secondary AML	8.17	1.48, 67.0	0.025	15.0	1.58, 385	0.039
High risk AML (ELN 2017)	0.50	0.02, 4.13	0.6			
Age > 65 years	2.40	0.45, 13.2	0.3			
AUC avg >2.70 µg/ml.day	24.0	3.29, 507	0.007	39.8	3.78, 1,472	0.010
[1] OR = Odds Ratio, CI = Confidence Interval						

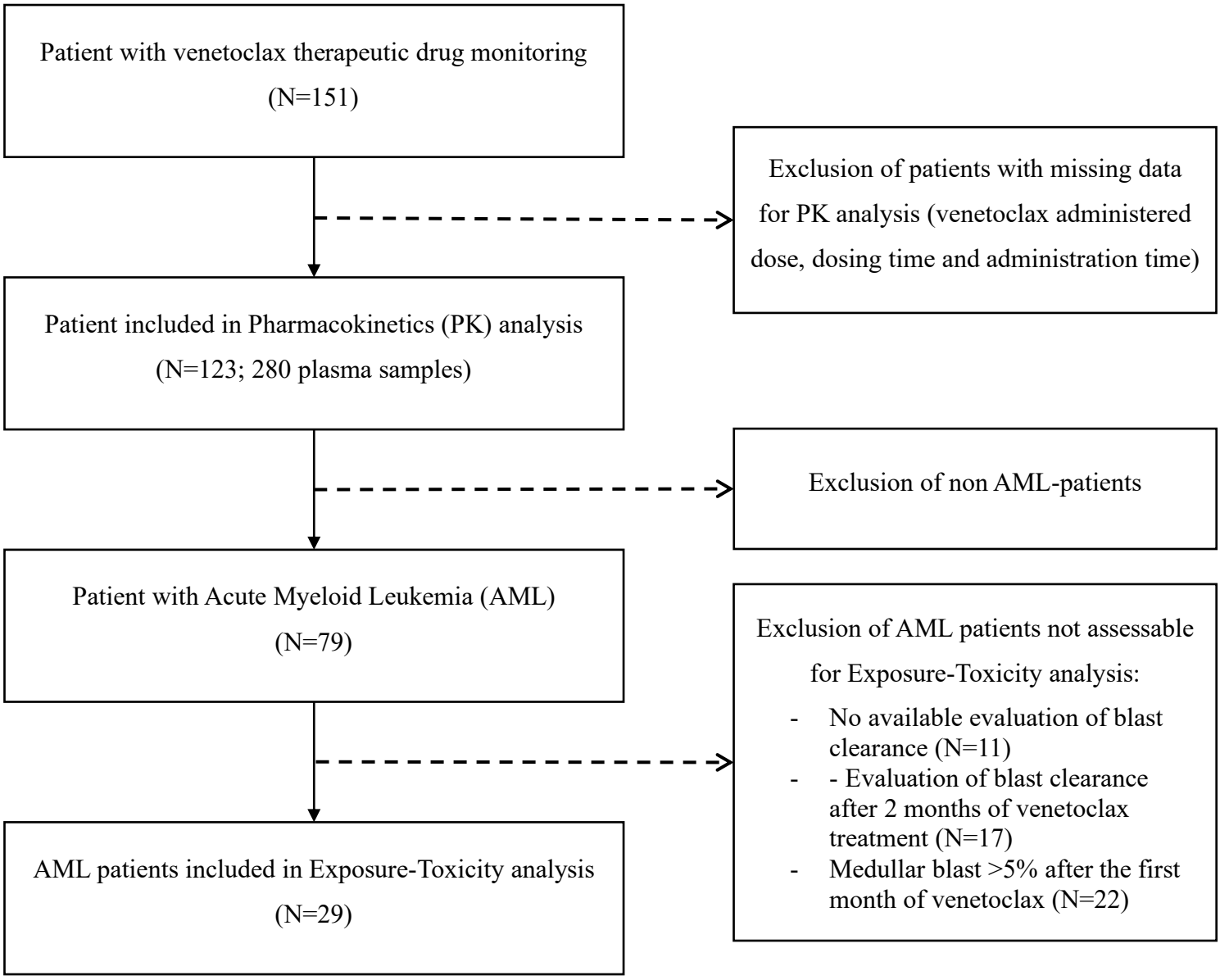
Figure legend

Figure 1: Incidence of drug related neutropenia beyond cycle 1 day 30 according to daily average of AUC (AUCavg) observed during the first month of treatment with venetoclax.


100%
75%
50%
25%
0%
Proportion
[0.284-2.102)
[2.102-3.236)
[3.236-5.138)
[5.138-14.109]
AUC avg during first month (µg/ml.day)
Neutropenia beyond cycle 1
yes
no

Supplemental data

Supplemental Figure 1. Flowchart patient inclusions in pharmacokinetic analysis and exposure-response analysis



Supplemental Table 1: Characteristics of patients with plasmatic venetoclax concentration available for the pharmacokinetic analysis.

Characteristic	N = 123
Age (years), median [IQR]	69 [59, 75]
Sex, N(%)	
Men	81 (65.9%)
Women	42 (34.1%)
Body weight (kg), median [IQR]	68 (57, 77)
Unknown	2
WHO performance status ≥2, N (%)	30 (27.0%)
Unknown	12
Pathology, N(%)	
Acute Lymphoblastic Leukemia	7 (5.7%)
Acute Myeloblastic Leukemia	77 (62.6%)
Chronic Lymphocytic Leukemia	11 (8.9%)
Lymphoplasmacytic Lymphoma	1 (0.8%)
Mulitple Myeloma	10 (8.1%)
Myelodysplastic Syndrome	10 (8.1%)
Myéloïd Sarcoma	1 (0.8%)
Non Hodgkin Lymphoma	6 (4.9%)
Regimen, N (%)	
Monotherapy	4 (3.3%)
V_CD20 inhibitor	15 (12.2%)
V_cytarabine	3 (2.4%)
V_daratumumab	2 (1.6%)
V_enasidenib	1 (0.8%)
V_hypomethylating agent	83 (67.5%)
V_ibrutinib	1 (0.8%)
V_ivosidenib	1 (0.8%)
V_jak inhibitor	1 (0.8%)
V_midostaurin	1 (0.8%)
V_nelarabine	2 (1.6%)
V_proteasome inhibitor	9 (7.3%)
ddi with strong CYPA inhibitor, N (%)	75 (61.0%)
ddi with strong CYPA inducer, N (%)	11 (8.9%)
ddi with ABC (P-gp or BCRP) inhibitor, N (%)	96 (78.0%)

Supplemental Table 2. Pharmacokinetics analysis: Estimated values of pharmacokinetic parameters of the population pharmacokinetic model

Parameters	Typical Value	Relative Standard error (%)
Ka -Day^{-1}	3.73	Fixed
CL/F -L/day	204	11
Vc/F -L	132	14
Effect of strong CYP3A inhibitor on CL	0.223	11
Effect of strong CYP3A inducer on CL	2.89	19
BSV Vc/F-%	85.9	15
BSV CL/F-%	76.4	9
IOV CL/F-%	30.2	16
Proportional residual error	19.26	19

BSV : between subject variability; IOV : intra-occasion variability.

Supplemental Figure 2. Pharmacokinetics analysis: Graphical validation of population pharmacokinetic model: correlation between observed and individual predicted concentrations (left panel, R^2=0.985) ; and individual relative prediction error according to individual predicted concentrations (right panel, R^2=0.044).