Adult patients with *de novo* acute myeloid leukemia show a functional deregulation of redox balance at diagnosis which is correlated with molecular subtypes and overall survival

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Supplementary data

Materials and methods:

We conducted a prospective cohort study of 84 adult patients at initial diagnosis of *de novo* Acute Myeloid Leukemia (AML) between June 2013 and December 2015 at Grenoble Alpes University Hospital, France. Promyelocytic AML and secondary AML were excluded. Seventy-one patients were treated according to the induction chemotherapy protocols in place at the time of sampling with anthracycline and high-dose cytarabine-based regimens. Thirteen patients received best supportive care treatment. Clinical details of the primary AML samples are provided in Supplemental Table 1. All 64 patients who participated this study gave their written informed consent. Samples for 20 patients were from Grenoble University Hospital's biobank (autorization number AC-2016-2698, subcollection 9) and therefore these patients were evaluated only for Reactive Oxygen Species (ROS). The study was approved by the ANSM (French National Agency for Medicines and Health Products Safety) and was conducted in accordance with the Declaration of Helsinki. Response to the first induction cycle was reported according to *Cheson et al.*[15]. Overall Survival was defined as the time from the date of diagnosis to the date of death due to any cause or last follow-up.

ROS production at diagnosis by hematopoietic subpopulations, by flow cytometry

ROS were measured within 2 hours of sample receipt. Cells were separated by the buffy coat method and counted. After gentle lysis of red blood cell with Versalyse, 1.5 x 10^6 cells were labelled with 10µM Dihydroethidium (DHE) (ThermoFisher Scientific) and CD45+ V450 (Becton Dickinson Biosciences). In order to construct ROS profiles for each cell population, six different ROS conditions were studied including ROS production at basal state, and after the addition of one or more ROS modulators: phorbol myristate acetate (PMA) (Sigma-Aldrich), antimycin A (AMA) (Sigma-Aldrich) / rotenone (Rot) (Sigma-Aldrich), diphenyleneiodonium (DPI) (Sigma-Aldrich) or vehicle (PBS) (ThermoFisher Scientific). Final concentrations were: PMA 80ng/mL, AMA 6µM, Rot 5µM, DPI 10µM. All reagents

were incubated 30 min with gentle mixing at 37°C, with the exception of PMA which was incubated for 15 min. The acquisitions were conducted on a FACS Canto cytometer. Tracking beads were used before each run to verify cytometer settings. Candidate populations were targeted with CD45+ antibody and Side Scatter (SSC) (supplementary Figure 1). Data analysis was performed using Becton-Dickenson FACS Diva software version 6.0. Mean fluorescence (DHE) was measured in each cell population. Measurements with at least 500 cell events per population were considered as valid. Blood cells from 65 Healthy donors (HD) were used as controls. ROS production was measured at diagnosis in bone marrow (BM) samples from 64 patients (56 receiving chemotherapy and 8 receiving palliative treatment) and in peripheral blood (PB) samples from 64 patients (53 receiving chemotherapy and 11 with palliative treatment).

Biochemical assays:

Peripheral blood was collected before any blood transfusion or the start of induction chemotherapy and transported within 2 hours on ice. Assays of glutathione, superoxide dismutase (SOD) and glutathione peroxidase (GPX) were performed spectrophotometrically. Malondialdehyde (MDA) was determined by a high-performance liquid chromatography technique. Total thiols were determined by colorimetry. Thirty blood samples from non-smoking healthy donors (HD) were used as controls. Biochemical markers were available at diagnosis for 55 patients (52 receiving chemotherapy and 3 palliative treatment).

Statistical analyses

Statistical analysis was performed using SPSS software version 23. Comparisons between continuous and categorical variables were made using non-parametric tests (Kruskal–Wallis or Mann–Whitney U-test). Wilcoxon test for paired samples was used for paired samples. Correlation was done using Spearman’s correlation test. Multivariate survival, relapse-free

survival and event-free survival analysis were performed on patients receiving chemotherapy using the Cox regression model. Covariates included in multivariate analyses were age, European LeukemiaNet (ELN) risk category and white blood cell count at diagnosis. To reduce the effect of extreme values, variables were log-transformed before Cox regression when their Skewness or/and Kurtosis coefficients were outside -2 and +2. Results for these models are presented as hazard ratio (HR) with 95% confidence intervals (95%CI). Kaplan Meier method was employed for OS estimates, using log-rank tests. For all the analyses, *P* values $\leqslant 0.05$ were considered statistically significant.

Supplementary data Table 1

Characteristics at diagnosis	Total patients examined N=84	Patient with induction N=71
Age at study entry - year (mean+/- sd)	60.4 +/- 16.7	56.8 +/- 15.5
White blood cells at diagnosis - per G/L (mean [min;max])	37.1 [0.5;358]	32.5 [0.5;309]
Male/ sex (n(%))	44 (52)	34 (48)
Percentage of blasts (BM) at diagnosis - (mean+/-sd)	56.4 +/- 21.8	55.2 +/- 21.7
Hemoglobin (g/L) (mean+/- sd)	94.45 +/- 20.6	93.4 +/- 19.7
ELN risk group- no (n(%))		
Favorable	29(35)	24 (34)
t(8;21) (q22;q22.1)	4(5)	4(6)
inv(16) (p13.1q22)	7(8)	5(7)
Mutated *NPM1* without *FLT3*-ITD	17(20)	14(20)
Bialleleic mutated *CEBPA*	1(1)	1(1)
Intermediate	40(48)	37(52)
Mutated *NPM1* and *FLT3*-ITD	25(30)	10(7)
Wild-type *NPM1* without *FLT3*-ITD	12(14)	24(34)
t(9;11) (p21.3;q23.3)	3(4)	3(4)
Adverse	14(17)	10(7)
Complex/monosomal karyotype	8(10)	5(7)
Wild-type *NPM1* and *FLT3*-ITD	3(4)	2(3)
Others adverse translocations (t(6;9)(p23;q34.); inv(3)(q21.3q26.2); t(v;11q23.3);t(9;22)(q34.1;q11.2))	3(4)	3(4)
Missing data	1(1)	0(0)
AML FAB subtype-no. (n(%))		
AML with minimal maturation M0	8 (10)	8 (11)
AML without maturation M1	18 (21)	15 (21)
AML with maturation M2	24 (29)	22 (31)
Acute myelomonocytic leukemia M4	20 (24)	15 (21)
Acute monoblastic/cytic leukemia M5	10 (12)	9 (13)
Acute erythroid leukemia M6	2 (2)	2 (3)
Mutation-no./total no. (n (%))		
NPM1	28(33)	24(33)
FLT3-ITD	23/81(28)	20(28)
DNMT3A	15/75 (20)	14(20)
IDH1 or IDH 2	10(12)	10(14)
Death (n(%))	-	31(44)
Complete remission (including Complete Remission with incomplete blood count recovery) (n(%))	-	53(74.6)
Follow-up period (months)	-	24.06

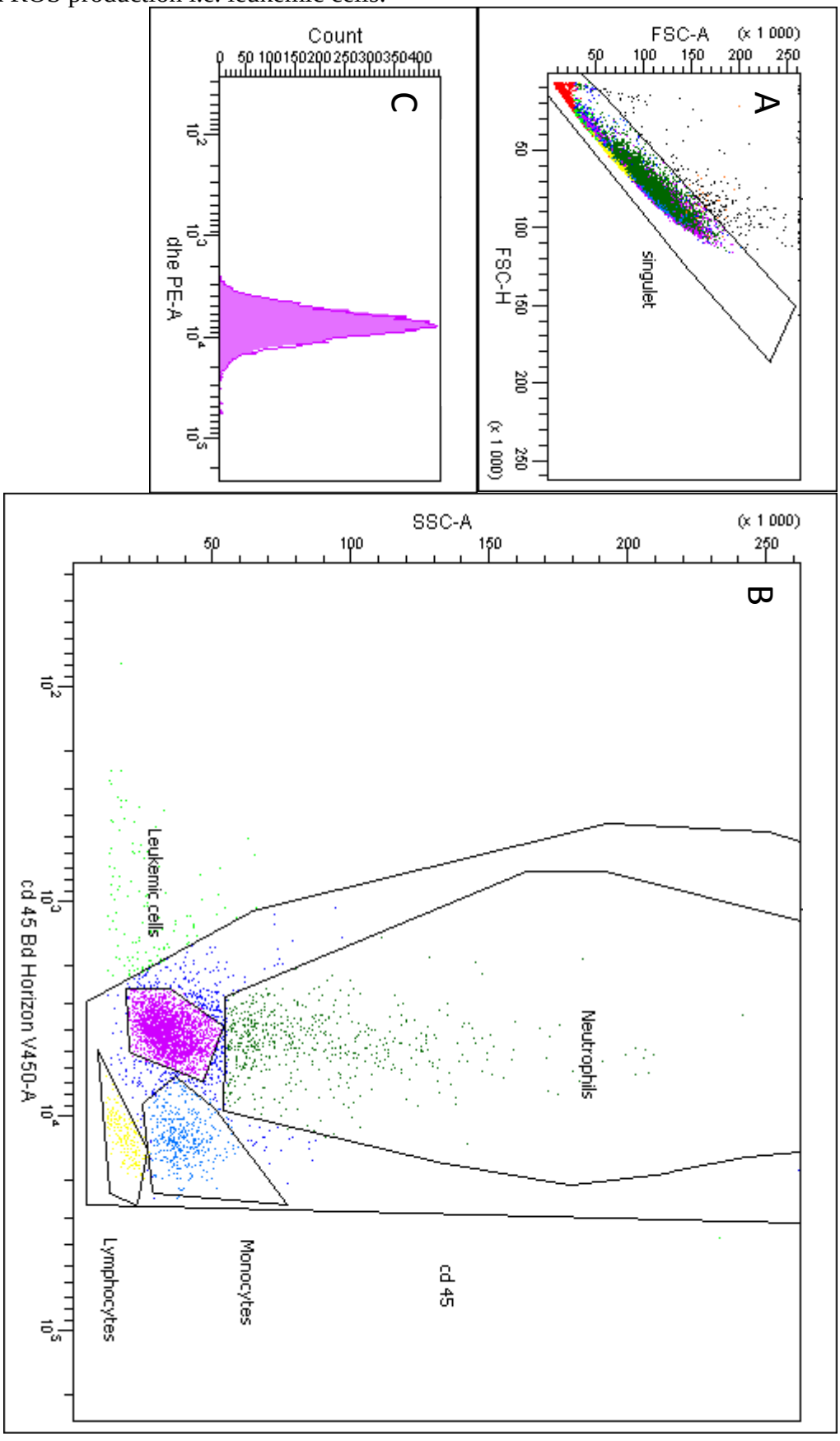
Supplementary data Table 2

Correlation between BM and PB ROS production in 54 AML patients according to ROS profile

	Spearman's rank correlation coefficient	*P* value
Basal	0.67	<0.0001
PMA+	0.71	<0.0001
DPI+	0.45	0.002
AMA/Rot+	0.64	<0.0001
DPI+ AMA/Rot+	0.75	<0.0001
AMA/Rot+PMA+	0.81	<0.0001

Supplementary data Figure 1

ROS emission among hematopoietic population : (**A**) separation between singlets and doublets; (**B**) selection of population; (**C**) Mean of fluorescence intensity after DHE staining quantified ROS production i.e. leukemic cells.



Supplementary data Figure 2

ROS production from leukemic cells according to FAB classification

ROS emission at basal state from BM **(A)** and from PB (**B**) leukemic cells according to FAB classification. ** means *P*<0.01