Clinical and biological impact of ATP-binding cassette transporter activity in adult acute myeloid leukemia

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Received: January 31, 2022.
Accepted: July 25, 2022.
Prepublished: August 4, 2022.

https://doi.org/10.3324/haematol.2022.280676

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Abstract

Chemotherapy resistance is the main cause of treatment failure in acute myeloid leukemia (AML) and has been related to ATP-binding cassette (ABC) transporter activity. However, the links between ABC activity, immunophenotype, and molecular AML parameters have been poorly evaluated. Moreover, the prognostic value of ABC activity, when compared to new molecular markers, is unknown. Here we investigated the links between ABC activity, as evaluated by JC-1 +/- cyclosporine A assay, and immunophenotypic, cytogenetic, molecular, and targeted next-generation sequencing features in 361 AML patients. High ABC activity was found in 164 patients and was significantly associated with less proliferating disease, an immature immunophenotype (expression of CD34, HLA-DR, CD117, CD13), and gene mutations defining AML as belonging to secondary-type ontogenic groups. Low ABC activity was associated with more mature myeloid differentiation ($CD34^-$, $cyMPO^+$, $CD15^+$, $CD33^+$) or monocytic commitment ($CD64^+$, $CD4^{+weak}$, $CD14^+$), with *NPM1* mutations, *KMT2A* rearrangements, and core-binding factor gene fusions, hallmarks of the *de novo*-type AML ontogeny. ABC activity was one of the major factors we identified using a random forest model for early prediction of AML ontogeny. In the 230 patients evaluated at diagnosis and intensively treated, high ABC activity was a predictive factor for primary resistance, and in multivariate analysis including full molecular data, an independent factor for event-free survival (P=0.0370). JC-1 +/- cyclosporine A assay could be used at diagnosis to predict AML ontogeny and to complete prognosis evaluation in addition to new molecular markers.

Introduction

Acute myeloid leukemia (AML) is an aggressive malignancy that emerges from the accumulation of chromosomal and genetic events in hematopoietic stem cells (HSC) or hematopoietic stem and progenitor cells (HSPC).[1,2] Despite the recent unraveling of the mutational landscape of AML[3,4] and our better understanding of its pathophysiologic impact, AML is still associated with poor treatment efficacy and dismal prognosis.[3,4] A few genetic events like *CEBPA* bi-allelic mutations or chromosomal translocation involving the core-binding factor (CBF) are associated with good prognosis. Some other lesions like *ASXL1* or *RUNX1* mutations are associated with poor response to treatment,[5] and many events or combinations of events still have a controversial value.[4] Chemoresistance of AML cells is one of the major causes of treatment failure, but its mechanisms are not fully understood, and the precise functional links between genomic lesions and chemoresistance are not understood in most cases.

ATP-binding cassette (ABC) proteins, especially ABCB1 and ABCG2, are active transporters involved in the efflux of many xenobiotics,[6–9] including anthracyclines, from HSPC. They are highly expressed and functional in normal HSC, where they play a major role in both detoxification and protection from perturbations of the normal HSC environment.[10–12] ABC proteins can also be highly expressed and functional in AML cells, leading to direct chemoresistance.[12–14] Among ABC proteins, ABCB1 expression and

activity has a well-known prognosis value in AML. Patients with high ABCB1 activity are more likely to experience primary chemoresistance, and have lower survival probabilities.[15–19]

The prognostic value of ABC protein expression or activity has been poorly explored in comparison with recent molecular markers, and the potential links between ABC activity and other disease characteristics are not clearly described. Previous studies have shown that high ABCB1 activity is associated with a high expression of immunophenotypic markers like CD34 or CD7, and a lack of CD33 expression.[18,20,21] Specific molecular alterations have also been described in the case of high ABCB1 activity, like high *BAALC* expression, or absence of mutations in *NPM1* or *FLT3*.[18,20] This suggests that high ABCB1 activity could be a specific characteristic of AML with an early maturation blockade that is mostly associated with poor prognosis and low chemosensitivity.

The role of other ABC proteins in chemoresistance like ABCC1, ABCG1 or ABCG2 has also been described in AML,[19,22–25] and the association with further molecular characteristics has not been clearly investigated. Although it was initially developed to study ABCB1 activity, the JC-1 +/- cyclosporine A (CsA) assay also evaluates ABCG2 activity, and might evaluate the pooled activity of ABC proteins (personal data), as CsA seems to be, at least *in vitro*, a very wide-spectrum ABC inhibitor.[19]

In order to further investigate the links between ABC activity, genomic lesions, and differentiation markers, we analyzed molecular and immunophenotypic data from 361 adult AML patients and evaluated its potential prognostic value using the JC-1 assay.

Methods

Patients and samples

We retrospectively included 361 patients diagnosed with AML between 2001 and 2020 at Saint-Antoine Hospital, Paris, with available results for ABC transporter activity. Blood and bone marrow (BM) samples were collected at the time of AML diagnosis or relapse after informed consent in accordance with the Declaration of Helsinki and the local ethics committee of Saint-Antoine Hospital.

Immunophenotyping

Flow cytometric analyses were performed on erythrocyte-lysed BM or blood samples. Data were acquired on FC500 or Navios cytometers and analyzed using CXP or Kaluza softwares (Beckman Coulter, Brea, CA). Information was available in 328 of 357 cases for CD34, CD117, CD33, CD13, CD64, HLA-DR, CD36, CD14, CD4, CD19, cyMPO, CD56, CD2 and cyCD3, and in 68 of 267 cases for cyCD79a, CD7, CD3, CD15, CD11b, CD22, CD10, CD5 and CD65.

Cytogenetic and genomic analyses

Cytogenetic and fluorescent *in situ* hybridization analyses were performed as already described.[1] *FLT3*-ITD, *NPM1* mutations, *CEBPA* mutations, *CBFB-MYH11* and *RUNX1-RUNX1T1* were studied as already described.[1,18] Next-generation sequencing (NGS) analysis was performed using panels of 41 or 122 genes as already described.[1,26] For patients studied after 2019, libraries were obtained using Custom Myeloid Solution (SOPHiA GENETICS, Saint Sulpice, Switzerland) which were then sequenced using a MiSeq® sequencer (Illumina Inc.). Read alignment, variant calling, and annotation were performed using SOPHIA DDM® software 5.0.12 (SOPHiA GENETICS), with a 1% sensitivity. Variants were checked using Integrative Genomics Viewer (IGV) software v2.3. Analysis was restricted to the 41 genes shared between the panels. All variants of unknown significance with a variant allele frequency compatible with germline origin were excluded from the analyses.

ATP-binding cassette transporter functional assay

JC-1 probe assay was performed as described previously.[16,18] Briefly, cells were washed twice and resuspended in phosphate-buffered saline (PBS) containing 0.1 mM JC-1 monomer at a concentration of 500,000 cells/mL and incubated at 37°C for 15 minutes without or with a modulator (CsA, 2 mM) to assess ABC transporter activity. Cells were washed twice in cold PBS, and samples were analyzed. Cell fluorescence of the blasts was recorded on FC500 or Navios cytometers. In order to ensure that the analysis was performed on blast cells, we gated them according to CD34 and/or CD117 expression, CD45 intensity and physical characteristics (forward and side scatter channels). JC-1 uptake, reflected by the differential distribution in the presence and in the absence of CsA, was expressed as the D value ranging from 0 (no difference) to 1 (no overlap) generated by the Kolmogorov-Smirnov test. A D value ≥0.60 was considered as high ABC protein functionality (ABC^{high}).[16,18]

Statistical analyses

Statistical analyses were performed using Fisher's exact, Kruskall-Wallis, Mann-Whitney or Wilcoxon tests. Variable importance analysis was performed to rank variables by predictive power according to the Random Forest algorithm. Complete remission (CR) was defined as recovery of morphologically normal BM and normal blood count, with no evidence of extramedullary disease. Event-free survival (EFS) was measured from diagnosis to either relapse, absence of remission after one course of chemotherapy, or death with censure at time of allogeneic HSC transplantation. Overall survival (OS) was measured from the date of diagnosis to the date of death. Data was censored at last follow-up for patients still alive. Probabilities

of survival were evaluated with the Kaplan Meier method and differences between distributions were evaluated by the log-rank test. Multivariate Cox regression models were built including all variables with a *P* value <0.05 in univariate analysis, and D JC-1 value. *P* values below 0.05 were considered as statistically significant. All statistical analyses were performed using R Software, Statview V5.0 (SAS institute) and GraphPad Prism 9.

Results

Distinct acute myeloid leukemia characteristics according to ATP-binding cassette activity

Of the 361 patients in the study cohort, associations between ABC activity and other clinical or biological parameters were determined in 350 patients at the time of diagnosis, three after induction treatment failure, and eight at relapse.

Baseline patient characteristics are described in Table 1. Overall, 164 patients harbored a high ABC activity (ABC^{high}) and 197 had a low ABC activity (ABC^{low}). Median age was 63 years (range, 15-91) and was not different between ABC^{high} and ABC^{low} groups. Leukocyte count was $7.3x10^9$/L and was significantly lower in patients with ABC^{high} than in those with ABC^{low} (median 5.2 *vs.* 10.2, *P*=0.0203). BM blast count was significantly lower in ABC^{high} patients than in ABC^{low} patients (39% *vs.* 55%, *P*=0.0006). Hemoglobin, platelet, and neutrophil counts did not differ between the two groups.

One hundred and three patients were diagnosed with *de novo* AML (*i.e.*, no previous hematological disease and no

Table 1. Patients' characteristics.

	Total (N=361)	Low ABC activity (N=197)	High ABC activity (N=164)	*P* value
Characteristics				
Sex, Male/Female	193/169	103/94	89/75	0.7068
Median age in years (range)	63 (15-91)	62 (15-91)	64 (19-88)	0.1682
Age >60 years, N	194	101	93	0.3021
Median leukocyte, $x10^9$/L (range)	7.3 (0.5-440.3)	10.2 (0.5-344.0)	5.2 (0.5-440.3)	**0.0203**
Median hemoglobin, g/dL (range)	9.2 (3.2-14.6)	9.2 (3.2-14.6)	9.2 (4.9-14.3)	0.6121
Median platelets, $x10^9$/L (range)	58 (2-2210)	61 (2-1065)	57 (3-2210)	0.2958
Median neutrophils, $x10^9$/L (range)	0.9 (0-154,9)	1.1 (0.0-90.9)	0.8 (0.0-154.9)	0.1805
Medullary blast count, median (%)	47 (14-98)	55 (14-98)	39 (15-97)	**0.0006**
AML subtype (%)				
de novo AML, N (%)	193 (53)	119 (60)	74 (45)	**0.0005**
AML-MRC, N (%)	89 (25)	39 (20)	50 (30)	**0.0022**
AML post-MPN, N (%)	36 (10)	17 (9)	19 (12)	0.3201
Therapy-related AML, N (%)	29 (8)	16 (8)	13 (8)	0.8292
Acute biphenotypic leukemia, N (%)	3 (1)	2 (1)	1 (1)	NA
Donor Cell Leukemia, N (%)	1 (0)	0 (0)	1 (1)	NA
AML post-AA, N (%)	2 (1)	0 (0)	2 (1)	NA
AML relapse, N (%)	8 (2)	4 (2)	4 (2)	NA
Cytogenetics risk group (N=346)				
Favorable, N (%)	29 (8.5)	22 (12)	7 (5)	**0.0179**
Intermediate, N (%)	198 (57)	112 (59)	86 (55)	0.3434
Adverse, N (%)	119 (34.5)	56 (29)	63 (40)	**0.0182**

ABC: ATP-binding cassette; WBC: white blood cell; AML-MRC: acute myeloid leukemia with myelodysplasia-related changes; MPN: myeloproliferative neoplasms; AML post-AA: acute myeloid leukemia post aplastic anemia; NA: not applicable. *P* values <0.05 are in bold.

prior chemotherapy/radiotherapy). This group was associated with lower ABC activity compared to other AML subtypes (median D value 0.50 *vs*. 0.62, *P*=0.0005). AML with myelodysplasia-related changes was diagnosed in 89 (25%) patients and associated with higher ABC activity when compared to other AML subtypes (median D value 0.68 *vs*. 0.55, *P*=0.0022). One hundred and twenty-nine (36%) patients harbored a normal karyotype. Favorable cytogenetics was identified in 29 (8%) patients and associated with ABC^{low} (*P*=0.0179) whereas an adverse karyotype was identified in 119 (33%) patients and associated with ABC^{high} (*P*=0.0182).

Acute myeloid leukemia with high ATP-binding cassette activity has an immature phenotype

JC-1 assay results and blasts phenotypes were evaluated in most samples. For some of the patients, information regarding cyCD79a, CD7, CD3, CD15, CD11b, CD22, CD10, CD5 and CD65 expression in leukemic cells were missing (see details in the Methods section and *Online Supplementary Table S1*).

Expression of CD34, HLA-DR, CD117 (*P*<0.0001) and CD13 (*P*=0.0013) were more frequent in patients with ABC^{high} than in patients with ABC^{low} (Figure 1). Conversely AML associated with ABC^{low} had more frequent expression of cyMPO (*P*<0.0001), CD15 (*P*=0.0325), CD33 (*P*=0.0338), CD64 (*P*<0.0001), CD4 low intensity (*P*=0.0104), CD14 (*P*=0.0107), and CD65 (*P*=0.0122) than ABC^{low} AML. Co-expression of a lymphoid marker among CD19, CD56 and CD7 was not associated with ABC^{high} nor with ABC^{low} (*Online Supplementary Table S1*).

ATP-binding cassette activity discriminates *de novo*-type acute myeloid leukemia from other ontogenic groups of acute myeloid leukemia

Concomitant ABC activity screening and cytogenetics analysis was available in 346 (96%) patients, *NPM1* status in 351, *FLT3*-ITD status in 349, *CEBPA* status in 346 and full NGS data in 291 patients. Mutational data for patients with NGS evaluation are shown in a co-mutation table available in the *Online Supplementary Figure S1*. The median number of genes mutated per patient was two (range, 0-10). The most frequently observed mutations occurred in *DNMT3A* (23%), *FLT3* (22%), *RUNX1* (20%), *TET2* (17%), *TP53* (17%), *NPM1* (17%), *NRAS* (14%), *ASXL1* (13%), *IDH2* (13%) and *IDH1* (10%).

ABC^{low} was significantly associated with the presence of *KMT2A* and CBF rearrangement, and with *NPM1* and *FLT3* (ITD or non-ITD) mutations, whereas ABC^{high} was significantly associated with mutations in *SF3B1, ASXL1, BCOR, STAG2, U2AF1, ZRSR2, RUNX1, CBL, JAK2* and *DDX41* (Figure 2A; *Online Supplementary Table S2*). Consequently, we investigated ABC activity according to AML ontogeny, as defined by Lindsley *et al.* in 2015[27]: *de novo* AML specific

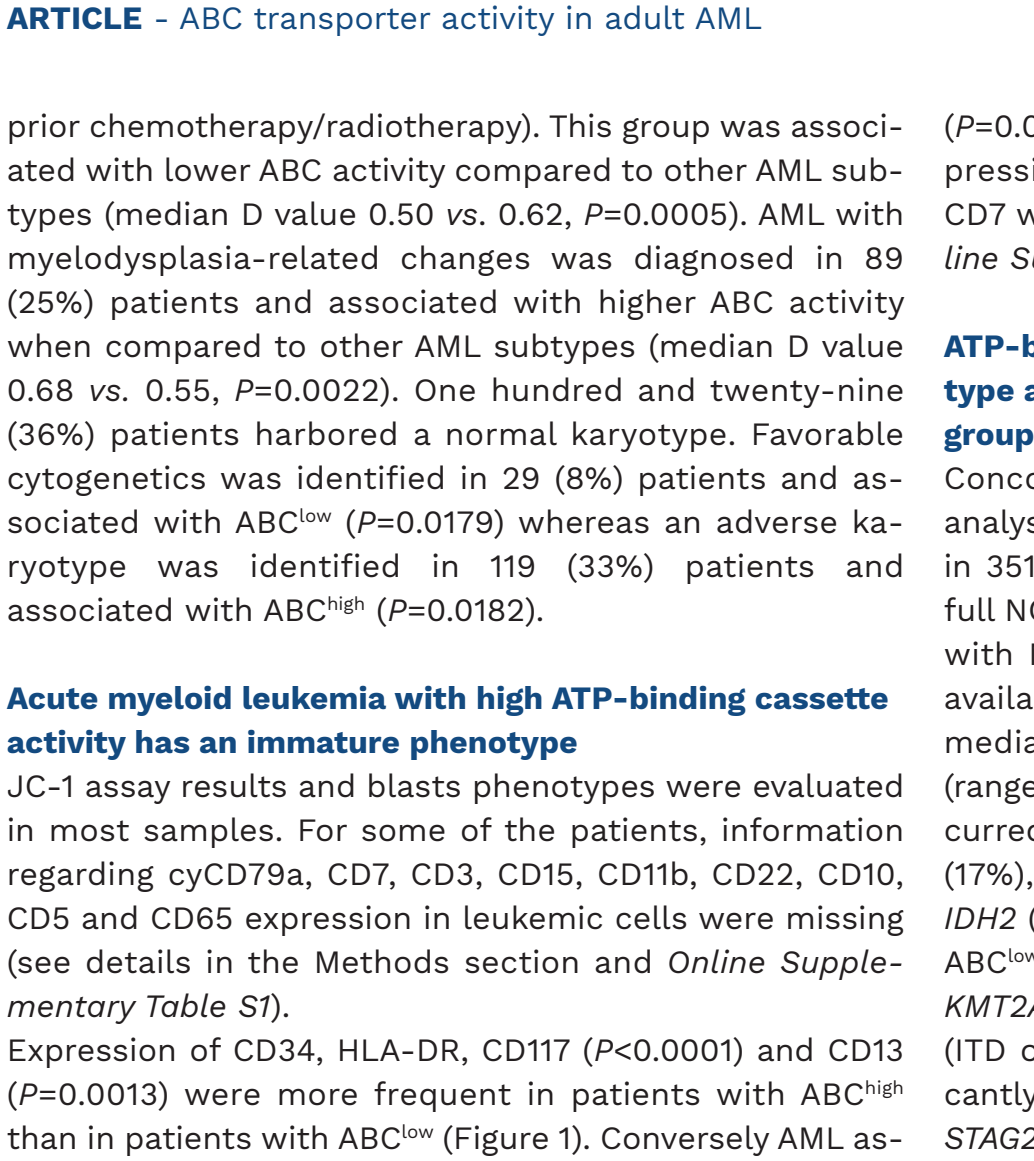


Figure 1. ATP-binding cassette activity according to the most relevant flow cytometry markers. Violin plots showing the median and the interquartile range of ATP-binding cassette (ABC) activity. For each cytometry marker patient data is represented with low ABC activity on the left and high ABC activity on the right. *P<0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001.

(with *NPM1* mutations, *KMT2A* or CBF rearrangement), *TP53*-mutated AML, secondary AML specific (with *ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1* and *ZRSR2* mutations), pan-AML (*i.e.*, other mutations).

ABC activity was lower in patients with *de novo* ontogeny (n=102) when compared to patients with *TP53*-mutated (n=47) or secondary AML specific ontogeny (n=77) (median D value +/- standard error of mean = 0.35+/-0.022, 0.75+/-0.035, 0.78+/-0.027 respectively, $P<0.0001$) (Figure 2B).

For AML diagnosis, cytology and flow cytometry results are quickly available in contrast to molecular mutations. We evaluated the importance of some data to predict de *novo* ontogeny using a random forest survival model. The top three parameters, ranked based on the mean decrease in Gini coefficient (a measure of homogeneity from 0 [homogeneous] to 1 [heterogeneous]), were percentages of cyMPO, CD34, and D JC-1 values (*Online Supplementary Figure S2*). ABC activity was one of the major factors we identified for early prediction of AML ontogeny.

In order to confirm the association between gene mutations and ABC proteins, we explored the expression levels of ABC genes known to be associated with prognosis in AML and whose functionality is putatively tested with JC-1 +/- CsA assay.[19,28–30] We explored ABC gene expression according to gene mutational status in a previously published series of 405 AML patients.[31] *ABCB1* expression was significantly lower in patients with *NPM1* or *FLT3* mutations, and significantly higher in patients with *CEBPA, TP53, GATA2, BCOR, SRSF2, JAK2* and *BCORL1* mutations. *ABCG2* expression was lower in patients with *NPM1, FLT3* or *DNMT3A* mutations and higher in patients with *TP53, PHF6, JAK2, CEBPA, GATA2, SRSF2, NF1, RUNX1* or *BCOR* mutations (*Online Supplementary Table S3*).

Prognostic value of ATP-binding cassette activity in heavily treated patients

Finally, we evaluated the prognostic value of ABC activity (considered as a continuous value) at diagnosis in 230 patients treated with intensive chemotherapy. Two hundred and sixteen patients received a 7+3 derived regimen (*i.e.*, daunorubicin or idarubicin with addition of cytarabin) and 14 received gemtuzumab ozogamicin in addition to 7+3. Allogeneic HSC transplantation in the first complete remission (CR1) or after induction failure was performed in

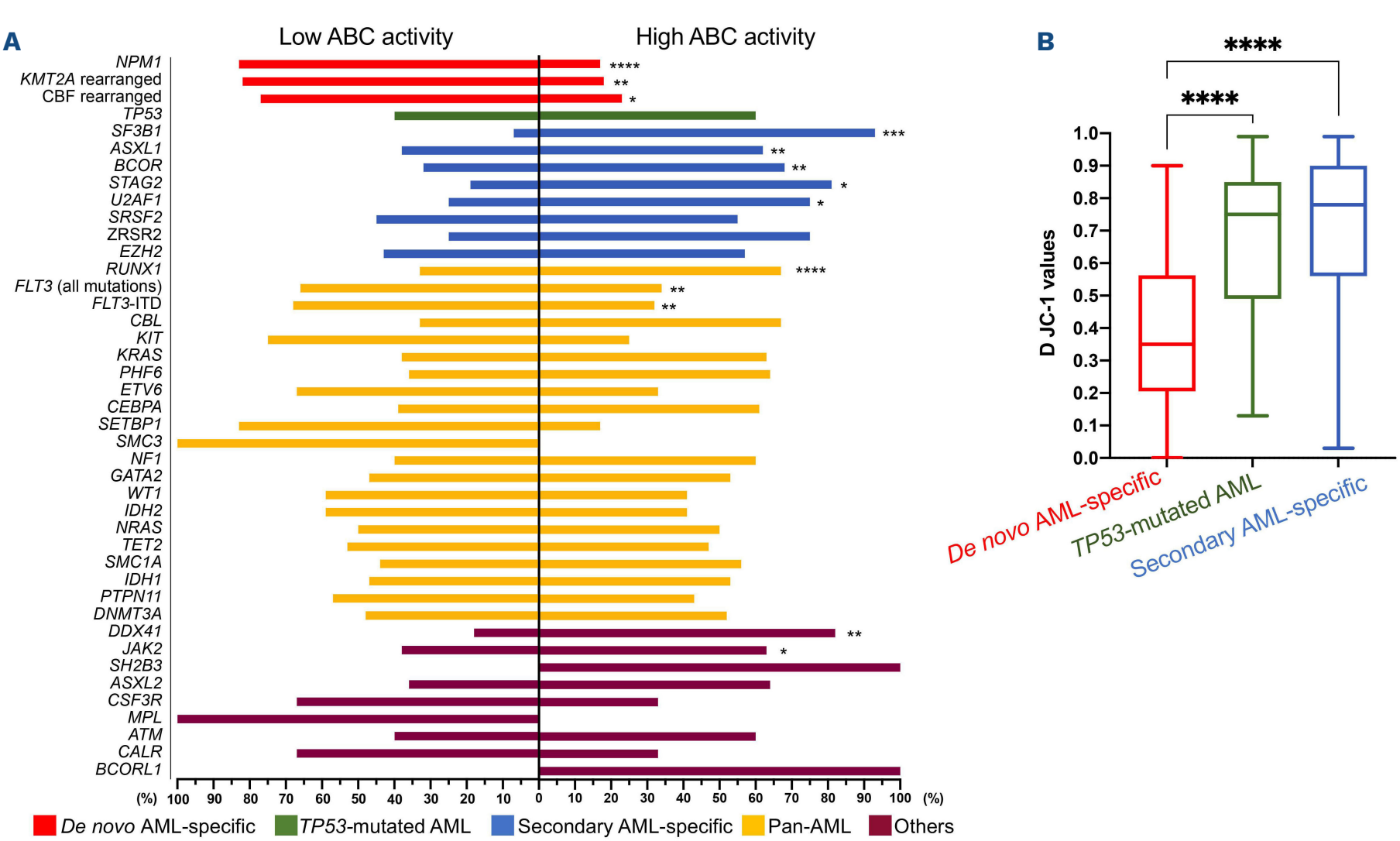


Figure 2. ATP-binding cassette activity is associated with acute myeloid leukemia ontogeny. (A) Frequency of cytogenetic or molecular lesions in the ATP-binding cassette low (ABC^{low}) group (left-hand bars) and the ABC^{high} group (right-hand bars). Mutated genes and *KMT2A* and core-binding factor (CBF) rearrangements were grouped according to the ontogenic classification from Lindsley *et al.*[27] Colors reflect ontogenic specificity of mutated genes and cytogenetic abnormalities: *de novo* acute myeloid leukemia (AML) specific (red), *TP53*-mutated AML (green), secondary AML specific (blue), pan-AML (yellow), and other genes not included in the Lindsley study, (burgundy). *$P<0.05$; **$P\leq0.01$; ***$P\leq0.001$; ****$P\leq0.0001$. (B) Box-plot of D JC-1 values distribution between de novo AML, *TP53*-mutated and secondary AML specific groups. ****$P\leq0.0001$

106 patients. Ninety patients had ABC^{high} and 140 had ABC^{low}. NGS analysis was available in 200 of 230 (87%) patients.

After one induction course, 169 of 230 patients reached CR1 and 61 of 230 (26%) experienced treatment failure. As expected, treatment failure was associated with a higher median of ABC activity (D JC-1 median value +/- standard error of mean equal to 0.57+/-0.037 in patients with treatment failure *vs.* 0.47+/-0.021, *P*=0.0224 in other patients). Other variables associated with induction failure were adverse cytogenetics (*P*<0.0001), presence of mutations in *ASXL1* (*P*=0.0002), *PHF6* (*P*=0.0003), *SETBP1* (*P*=0.0159), *IDH2* (*P*=0.02), *STAG2* (*P*=0.04) and absence of mutation in *NPM1* (*P*=0.0014).

We then analyzed prognostic factors for EFS, with data censoring at the time of allogeneic HSC transplantation to focus only on chemotherapy effects. In univariate analysis, significant factors associated with shorter EFS were age (*P*=0.0059), poor cytogenetics (*P*<0.0001), and mutations in *ASXL1* (*P*=0.0025), *DNMT3A* (*P*=0.0069), *PHF6* (*P*<0.0001), *U2AF1* (*P*=0.003), *CBL* (*P*=0.0004), *SETBP1* (*P*<0.0001) and absence of *CEBPA* biallelic mutations (*P*=0.0269). There was a trend for ABC functionality to be associated with shorter EFS (*P*=0.09) (*Online Supplementary Table S4*). In multivariate analysis, parameters significantly associated with shorter EFS were adverse karyotype (*P*<0.0001), mutations in *ASXL1* (*P*=0.0359), *DNMT3A* (*P*=0.0008), *U2AF1* (*P*=0.0211), *PHF6* (*P*=0.0032), *CBL* (*P*=0.0330), *SETBP1* (*P*<0.0001) and ABC activity (*P*=0.0370) (Figure 3; *Online Supplementary Table S4*).

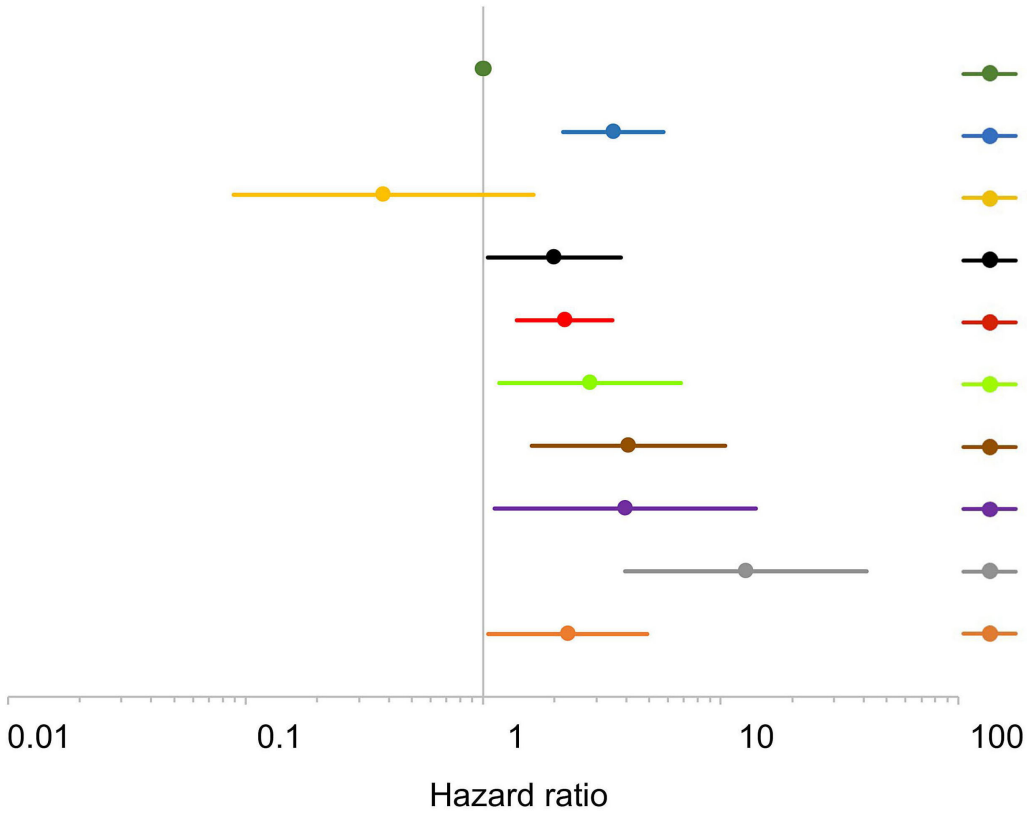
ABC activity was not predictive for OS (data not shown). When restricting survival analysis to the 125 patients with intermediate cytogenetics and full NGS data, ABC activity was neither predictive for EFS nor OS, but due to the lower number of patients and the high number of variables, the statistical power was limited.

Discussion

ABC activity is a well-known prognostic factor in AML, but most studies on its prognostic impact were performed before the NGS era.[15–19] Moreover, only a few studies comparing flow cytometry characteristics and ABC activity have been published, most of them focusing only on CD34 status.[18,20,21,24,32] Consequently, associations between ABC activity and most biologic disease parameters remain largely unknown.

Our study of 361 AML patients confirms that ABC^{high} is associated with lower blood leukocyte and BM blast counts.[20] Likewise, the most proliferative diseases have lower ABC activity than other AML, as already described.[20] These proliferative features may be related to the lower incidence of *FLT3* and *NPM1* mutations and the higher frequency of *TP53* or myelodysplastic syndrome-associated mutations in ABC^{high} AML as observed in the present work and others.[18–20,33] Moreover, we found that the other *de novo* ontogeny associated events – *i.e.*, *KMT2A*, CBF, are correlated with ABC^{low}. Interestingly these events are also frequently associated with a high initial leukocyte count.[34,35]

In this study, ABC^{low} AML had a significantly lower CD34 expression and were positive for markers of myeloid maturation such as cyMPO, CD33, CD65 and CD15 or markers of



Variable	*P* value
Age	0.1889
Cytogenetics status (poor *vs.* other)	**<0.0001**
CEBPA^{MUT} biallelic	0.1926
ASXL1^{MUT}	**0.0359**
DNMT3A^{MUT}	**0.0008**
U2AF1^{MUT}	**0.0211**
PHF6^{MUT}	**0.0032**
CBL^{MUT}	**0.0330**
SETBP1^{MUT}	**<0.0001**
ABC activity	**0.0370**

Figure 3. Forest plot of significant parameters for event-free survival in multivariate analysis. Hazard ratio with 95% confidence interval, *P* values <0.05 are in bold.

monocytic differentiation such as CD64, CD4^{+weak} and CD14. These profiles suggest a late differentiation blockade, occurring in progenitors already committed either to the granulocytic or to the monocytic lineage. Genetic events that define *de novo* ontogeny (i.e., *NPM1* mutations, *KMT2A* rearrangements, and CBF gene fusions) are known to occur in differentiated granulocytic or myelomonocytic progenitors.[34–36] Moreover, during physiological hematopoiesis, ABC proteins are mostly expressed in multipotent/immature progenitors and in HSC.[10–12] Taken together these observations suggest that *de novo* AML develops from more differentiated progenitors with lower expression of ABC proteins, and that the pathological re-acquisition of self-renewal capacities during the leukemogenesis process is not associated with the re-expression of the ABC proteins.

Conversely, ABC^{high} AML expressed mostly a CD34^{+}/CD117^{+}/HLA-DR^{+} phenotype with negative cyMPO, a combination of markers suggesting that leukemia initiating cells had been blocked very early during the myeloid differentiation. ABC^{high} activity was associated with *TP53* mutations, with events that define secondary AML ontogeny (such as mutations in *SF3B1, U2AF1* or *ASXL1*), and also with mutations in *JAK2* or *RUNX1*, all events that are known to occur in early HSPC or HSC.[37–39] This suggests that, in contrast with *de novo* AML, when leukemogenic events occur in HSC or in early progenitors, those cells could keep, among their stemness characteristics, their high ABC protein activity. Taken together, our results confirm that high ABC activity could be only a passenger characteristic reflecting a leukemogenesis process that occurred in HSC or early progenitors, as already suggested by other teams.[20]

High ABC activity is known to be associated with poor prognosis in AML, and our data confirm lower remission rates and lower EFS probabilities in multivariate models in ABC^{high} patients. This chemoresistance could be due to other disease characteristics that we found to be associated with ABC^{high} like *ASXL1* and *RUNX1*[5] mutations, or poor karyotype, rather than to a direct chemotherapy efflux mediated by ABC proteins. However, in our series, ABC activity remains an independent factor for EFS, in addition to other genetic alterations like poor cytogenetics and mutations in *DNMT3A*, *ASXL1* or others, suggesting an intrinsic role for ABC activity. Conversely, our data do not show that the poor prognosis associated with secondary-type mutations (like *SF3B1*, *U2AF1* or *ASXL1*) is only due to the higher level of ABC activity. However, our results suggest that ABC activity (as measured by JC-1 +/- CsA assay) could be a good surrogate marker for the presence of these mutations. Moreover, as this assay remains an independent prognostic factor for EFS, with a quickly available result, it could be performed systematically at diagnosis for a very fast and easy prognostic evaluation. The value of ABC activity in HSC transplantation decisions remains to be evaluated in further studies.

Conclusions

In AML, high activity of ABC proteins is associated with secondary or *TP53* molecular ontogeny, and with an early blockade of differentiation of leukemic cells. ABC activity remains an independent prognostic marker for EFS after intensive chemotherapy, using multivariate models considering full mutational data. JC-1 +/- CsA functional assay can be performed quickly in routine practice and could be used for prognostic evaluation, in addition to new molecular markers. These data should be confirmed in further studies.

Disclosures

No conflicts of interest to disclose.

Contributions

ES, LSuner, FD and PH wrote the manuscript. LSuner, LSoret and FFeger performed flow cytometry analyses. NA, CBN and HG performed cytogenetic analyses. ES, FFavale, PH and FD performed molecular biology analyses. LSuner, MC, CH, and FD performed cytological analyses. MM, AG, SL, MMohty, LH and OL provided clinical data. ES and LSuner collected all the data. ES and PH performed statistical analyses. FD and PH supervised the research. All authors contributed to manuscript revision and final approbation.

Acknowledgments

The authors would like to thank all patients who participated in this study.

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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