Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes

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ABSTRACT

Acute myeloid leukemias (AML) with myelodysplasia-related changes (AML-MRC) are defined by the presence of multilineage dysplasia (MLD), and/or myelodysplastic syndrome (MDS)-related cytogenetics, and/or previous MDS. The goal of this study was to identify distinct biological and prognostic subgroups based on mutations of ASXL1, RUNX1, DNMT3A, NPM1, FLT3 and TP53 in 125 AML-MRC patients according to the presence of MLD, cytogenetics and outcome. ASXL1 mutations (n=26, 21%) were associated with a higher proportion of marrow dysgranulopoiesis (mutant vs. wild-type: 75% vs. 55%, p=0.030) and were mostly found in intermediate cytogenetic AML (23/26) in which they predicted inferior 2-year overall survival (OS, mutant vs. wild-type: 14% vs. 37%, p=0.030). TP53 mutations (n=28, 22%) were mostly found in complex karyotype AML (26/28) and predicted poor outcome within unfavorable cytogenetic risk AML (mutant vs. wild-type: 9% vs. 40%, p=0.040). In multivariate analysis, the presence of either ASXL1 or TP53 mutation was the only independent factor associated with shorter OS (HR, 95%CI: 2.53, 1.40–4.60, p=0.002) while MLD, MDS-related cytogenetics and previous MDS history did not influence OS. We conclude that ASXL1 and TP53 mutations identify two molecular subgroups among AML-MRCs, with specific poor prognosis. This could be useful for future diagnostic and prognostic classifications.

INTRODUCTION

In the WHO 2008 classification, acute myeloid leukemia (AML) with myelodysplasia-related changes (AML-MRC) is defined as a distinct entity by the presence of multilineage dysplasia (MLD), and/or myelodysplastic syndrome (MDS)-related cytogenetics, and/or previously diagnosed MDS or MDS/Myeloproliferative neoplasm (MDS/MPN)[1]. The prognostic value of these three criteria is not established. The independent prognostic value of MLD is controversial and varies among different subsets of AML[2–7]. AML with MDS-related cytogenetics or previously diagnosed MDS or MDS/MPN
Table 1: Characteristics of the 125 patients with criteria for AML-MRC

|                                      | All patients (N = 125) |
|--------------------------------------|------------------------|
|                                      | N   | %      |
| Median age (years, [range])          | 71  | [18-90]|
|                                      |     |        |
| **FAB Classification**               |     |        |
| 0                                    | 6   | 5%     |
| 1                                    | 13  | 10%    |
| 2                                    | 44  | 35%    |
| 4                                    | 25  | 20%    |
| 5                                    | 9   | 7%     |
| 6                                    | 9   | 7%     |
| Unclassifiable                       | 19  | 15%    |
|                                      |     |        |
| **Previously diagnosed MDS**         | 59  | 47%    |
|                                      |     |        |
| **Multilineage dysplasia***          | 38  | 40%    |
|                                      |     |        |
| **Cytogenetics**                     |     |        |
| Normal                               | 28  | 22%    |
| Abnormal non-complex                 | 55  | 44%    |
| Complex                              | 42  | 34%    |
| Monosomal                            | 36  | 29%    |
| Non monosomal                        | 6   | 5%     |
|                                      |     |        |
| **MDS-related cytogenetics**         | 71  | 57%    |
|                                      |     |        |
| **Cytogenetic risk group**           |     |        |
| Intermediate                         | 65  | 52%    |
| Unfavorable                          | 60  | 48%    |
|                                      |     |        |
| **Treatment**                        |     |        |
| Intensive chemotherapy               | 67  | 54%    |
| Non-intensive chemotherapy           | 31  | 25%    |
| Demethylating agent                  | 19  | 15%    |
| Other                                | 12  | 10%    |
| Supportive care                      | 27  | 22%    |

* Multilineage dysplasia was evaluated on 94 patients
MDS = myelodysplastic syndrome
have often unfavorable cytogenetics, and are associated with poorer outcome than AML without criteria of AML-MRC[2,8,9]. 

Although gene mutations are now a major tool for AML classification into distinct entities with specific prognosis[10–12], no molecular pattern is currently associated with AML-MRC. We hypothesized that the presence of mutations in targeted genes of interest could help identify subgroups of AML-MRC with distinct biological features and specific outcome. We had previously reported that AML-MRC have a specific mutation pattern sharing mutations found in both AML and high risk MDS and a particularly high frequency of ASXL1 mutation[13]. We report here a cohort of patients with AML-MRC for whom we analyzed the presence of mutational events according to AML-MRC criteria (MLD, cytogenetics and patient history) and identified mutation-based subgroups with specific poor outcome.

RESULTS

Patient, disease and treatment characteristics

We studied 149 patients. After morphological review, 24 patients were excluded from the main analysis because of not enough dysplasia to reach the MLD criteria. These patients were analyzed separately (Supplemental Table 1). The remaining 125 fitting the AML-MRC criteria were considered for the main analysis and their characteristics are reported in Table 1. Median age was 71 years (range: 18-90). Fifty-nine patients (47%) had a previously diagnosed MDS. Seventy-one patients (57%) had MDS-related cytogenetics, including 42 patients (34%) with complex karyotype AML (CK-AML). Ninety-four patients were evaluable for morphological dysplasia by the double centralized review. Multilineage dysplasia (MLD) was found in 38/94 patients (40%). Sixty-seven patients (54%) received intensive induction chemotherapy. Mutations were found in ASXL1 (n = 26, 21%), RUNX1 (n = 15, 12%), DNMT3A (n = 11, 9%), NPM1 (n = 4, 3%), FLT3 (n = 9, 7%) and TP53 (n = 28, 22%). No mutation was found in 47 (38%) patients (31 with non-complex karyotype NCK-AML and 16 with CK-AML).

Mutation profiles according to previous history of MDS, cytogenetics and MLD

TP53 mutations were exclusive from all other mutations and all but two (26/28, 93%) were found in CK-AML. Mutations in the other genes were almost exclusively found in NCK-AMLs (Table 2A, Figure 1). In NCK-AMLs, ASXL1 was the most frequently mutated gene (26/83, 31%). These mutations were associated with the absence of MDS-related cytogenetics (MDS-related cytogenetics: yes vs. no: 4/29, 14% vs. 22/54, 41%, p = 0.013). Other mutations were equally distributed whether MDS-related cytogenetics was present or not (Figure 1).

We analyzed the presence of mutations according to the cytogenetic risk group (Table 2B). In the 94
patients evaluable for MLD, patients with criteria for MLD had more ASXL1 mutations (MLD: 15/38, 39% vs. no MLD: 8/48, 14%, p = 0.007). We did not find any correlation between the presence of MLD and other mutations. Median percentages of bone marrow DGP, DEP and DMP in the 94 patients were 64%, 24% and 45% respectively. ASXL1 mutations were associated with higher DGP (ASXL1-mut: 75% vs. ASXL1-wt: 55%, p = 0.030) but similar DEP (ASXL1-mut: 20% vs. ASXL1-wt: 25%, p = 0.933) and DMP (ASXL1-mut: 53% vs. ASXL1-wt: 40%, p = 0.139). There was no difference in percent of morphologic dysplasia according to any other genes (Supplemental Table 2). In a linear regression analysis including the mutation status of the 6 genes, mutation of ASXL1 remained associated with higher DGP (Coefficient beta = 20, p = 0.023, Supplemental Table 3). The frequencies of these mutations were not different according to the presence or not of prior MDS or MDS/MPN.

Table 2: Frequencies of mutation in ASXL1, RUNX1, DNMT3A, NPM1, FLT3-ITD and TP53 in the 125 AML-MRC patients and according to the karyotypes (A) and to the cytogenetic risk groups (B)

| A       | NCK-AML (N=83) | CK-AML (N=42) | p    |
|---------|---------------|---------------|------|
|         | N  | %  | N  | %  |       |
| ASXL1   | 26 | 31%| 0  | 0% | <0.001|
| RUNX1   | 14 | 17%| 1  | 2% | 0.004 |
| DNMT3A  | 11 | 13%| 0  | 0% | <0.001|
| NPM1    | 4  | 5% | 0  | 0% | 0.243 |
| FLT3-ITD| 9  | 11%| 0  | 0% | 0.059 |
| TP53    | 2  | 2% | 26 | 62%| <0.001|

CK = complex karyotype; NCK = Non complex karyotype

| B       | Intermediate (N=65) | Unfavorable (N=60) | p    |
|---------|---------------------|--------------------|------|
|         | N  | %  | N  | %  |       |
| ASXL1   | 23 | 35%| 3  | 5% | <0.001|
| RUNX1   | 11 | 17%| 4  | 7% | 0.067 |
| DNMT3A  | 8  | 12%| 3  | 5% | 0.130 |
| NPM1    | 4  | 6% | 0  | 0% | 0.070 |
| FLT3-ITD| 8  | 12%| 1  | 2% | 0.022 |
| TP53    | 2  | 3% | 26 | 43%| <0.001|

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Outcome after intensive chemotherapy in AML-MRC patients

Among the 125 AML-MRC patients, only the 67 patients who received intensive induction chemotherapy were considered for the outcome analyses. Among them, 42 achieved CR (63%). Cytogenetic risk group, the presence of MDS-related cytogenetics, previous history of MDS or MDS/MPN, and MLD did not influence the CR rate (data not shown). The presence of an ASXL1 mutation was associated with a lower CR rate (ASXL1-mut vs. ASXL1-wt: 40% vs. 69%, p = 0.039). Other gene mutations did not influence the CR rate.

The 2-year OS was 24% in the 67 intensively treated patients. Cytogenetics did not predict outcome, with a 2-year OS of 27% and 20% in the intermediate and unfavorable groups, respectively (p=0.351, Table 3). Similarly, MDS-related cytogenetics, previous history of MDS or MDS/MPN, and MLD did not significantly influence OS (data not shown). Among the intermediate cytogenetic (IC-AML) patients, the presence of an ASXL1 mutation was associated with worse 2-year OS (14%) compared to patients without ASXL1 mutation (37%, p = 0.030, Figure 2A, Table 3).

In the unfavorable cytogenetic (UC-AML) group, TP53-mutated patients had a lower 2-year OS (9%) than TP53-wild type patients (26%, p = 0.040, Figure 2B, Table 3). In multivariate analyses adjusted for age and WBC, IC-AML with mutated ASXL1 (HR = 2.67, 95%CI = [1.15-6.24], p = 0.023) and UC-AML with mutated TP53 (HR = 5.44, 95%CI = [2.16-13.65], p < 0.001) had shorter OS than IC-AML with wild type ASXL1 (considered as reference, HR = 1). Of note, patients with UC-AML with wild type TP53 (HR = 1.14, 95%CI = [0.52-2.50], p = 0.743) had similar OS compared to those with intermediate cytogenetic and no ASXL1 mutation (considered as reference, HR = 1).

Using only the mutational status of ASXL1 and TP53 to stratify patient outcome, we found that patients who presented with either ASXL1 or TP53 mutation had worse 2-year OS (15%) than those with both ASXL1 and TP53 wild type (29%, p = 0.005, Table 3, Figure 3). In multivariate analysis including age, WBC and cytogenetics, the presence of either ASXL1 or TP53 mutation remained the only independent predictive factor associated with both lower CR rate (HR = 0.29, 95%CI = [0.09-0.89], p = 0.031) and shorter OS (HR = 2.53, 95%CI = [1.40-4.60], p = 0.002).

Patients with dysplasia but without criteria for MLD

These 24 patients were separately analyzed because they did not reach AML-MRC criteria after morphological review. Indeed, they had no previous history of MDS or MDS/MPN and no MDS-related cytogenetics. Morphological review showed dysplasia that did not reach criteria for MLD. All of them had intermediate cytogenetics and 15 (63%) had normal karyotype (Supplemental Table 1). Among these 24 patients, median percentage of DGP, DEP and DMP was 50% (range: 7-97%), 20% (range: 0-45%) et 26% (range: 0-83%), respectively. We found 4 mutations in ASXL1 (17%), 5 in RUNX1 (21%), 1 in DNMT3A (4%), 2 in NPM1 (8%),
4 in FLT3 (17%) and no TP53 mutation. ASXL1 mutated patients had a median percent of DGP of 62% (range: 35-90%) vs. 50% (range: 7-97%) in those with wild type ASXL1.

**DISCUSSION**

We previously reported that AMLs with MRC harbor a specific mutational profile with a high proportion of ASXL1 and RUNX1 mutations and less DNMT3A, FLT3 and NPM1 mutations than AMLs without criteria of AML-MRC[13]. In this series including only patients with AML-MRC, we have evaluated the correlation between these mutations and the different criteria defining AML-MRC (previous history of MDS or MDS/MPN, MDS-related cytogenetics, and MLD). We found that none of the three MRC criteria could help identify a specific mutational profile within AML-MRC, suggesting that these criteria do not have a molecular basis, at least within the limit of the genes studied. This heterogeneity in terms of biological features as well as in prognostic significance suggests that AML-MRC is not a true distinct entity.

![Figure 2: Overall survival according to the presence ASXL1 mutation in the intermediate cytogenetic patients (A) and according to the presence of TP53 mutation in the unfavorable cytogenetic patients (B)](image)

Although they are defined as AML-MRC because of MDS-related cytogenetics, CK-AML should be considered separately because of the presence of a specific mutational profile consisting in a high frequency of TP53 mutations and the absence of other mutations. As expected, TP53 mutations predicted a worse outcome among patients with unfavorable cytogenetics, supporting that genetic stratification is useful to predict differential patient outcome although classified in the same cytogenetic risk group. This is in line with previous reports showing the close correlation between TP53 mutation, CK-AML and poor outcome[18,19]. In NCK-AML, we found that ASXL1 was the most frequently mutated gene (31%). It was the only mutation associated with the presence of MLD and a higher proportion of DGP in bone marrow. This is in agreement with previous biological reports showing the role of ASXL1 in the appearance of dysplasia and myeloid transformation[20–23]. However, the overlap between ASXL1 mutations and morphological MLD was not complete, because 39% of patients with MLD had ASXL1 mutation and 14% patients without MLD had ASXL1 mutation. Thus, there is no strong association between morphological analyses and the presence of an ASXL1 mutation. Interestingly, ASXL1 mutations were associated with the absence of MDS-related cytogenetics among the NCK-AML, as if ASXL1 mutations and karyotype with MDS-related abnormalities were mutually exclusive. Finally, there was no correlation between ASXL1 mutation and previous history of MDS or MDS/MPN.

Taken together, these results suggest that the presence of an ASXL1 mutation could be considered as an independent molecular marker of dysplasia in AML that is not redundant with the criteria defining AML-MRC. This could be helpful in the perspective of...
developing a molecular classification of AML-MRC. This statement is also supported by the presence of a specific gene expression profile associated with ASXL1-mutated AML[24]. Like TP53 stratification of outcome in UC-AML, ASXL1 mutations were associated with a shorter OS in intermediate risk patients, with a 2-year OS (14%) close to that observed in unfavorable cytogenetic patients (20%). Different studies that did not specifically focus on AML-MRC also reported this poor outcome associated to ASXL1 mutations[25,26]. Thus, stratification upon ASXL1 and TP53 mutation, which identified two distinct biological subgroups among AML-MRC, was the only significant predictor of outcome while usual cytogenetic risk classification or the different criteria defining AML-MRC (prior MDS or MDS/MPN, MDS-related cytogenetics and MLD) failed to predict patient outcome in our series. Because of the limited number of patients in some subgroups, these results need to be confirmed in larger studies.

Although ASXL1 and TP53 mutations could classify distinct subgroups of AML-MRC, patients without any of these mutations still represent a heterogeneous group of AML-MRC harboring different morphological, cytogenetic and molecular features. The mutations of DNMT3A and/or NPM1, which are usually found in de novo AML and are mutually exclusive with ASXL1 and TP53 mutations, could identify patients for whom the definition of AML-MRC should be questioned. Falini et al. reported that MLD did not identify distinct biological and clinical entities among NPM1-mutated AML, with overlapping gene expression profiling and similar outcome[4]. In contrast, some patients had morphological dysplasia but not enough to reach MLD criteria, leading to their exclusion from the AML-MRC category in the absence of other criteria. Among these patients, those who presented with ASXL1 mutations might be included in the same subgroup as ASXL1-mutated AML-MRC. This supports the need of redefining AML-MRC upon molecular abnormalities.

Finally, we did not find any mutation in the 6 genes studied in 47 patient, suggesting the need to identify other molecular markers to stratify these patients. Because ASXL1 is a key regulator of the polycomb repressive complex 2 (PRC2), it is possible that AML carrying alterations of other genes involved in methylation marks via PRC2 and myeloid transformation such as EZH2[27,28], JARID2[29] or BAP1[30,31] could share characteristics of ASXL1-mutated AML. This could help identify AML-MRC through a common altered molecular pathway and could help develop future targeted treatments. An extensive mutational screening using whole genome or exome sequencing could be useful in this setting to improve the molecular characterization of these unmutated cases.

We conclude that the criteria defining AML-MRC do not identify distinct clinical and biological subgroups and do not predict outcome of patients with AML-MRC. In contrast, ASXL1 and TP53-mutated AML identify two distinct biological subgroups of AML-MRC with very poor outcome. This molecular characterization could be useful to redefine AML-MRC in a future classification aiming at merging biological characterization and specific prognostic value.

**PATIENTS AND METHODS**

**Selection criteria**

We conducted a retrospective analysis of patients from two French centers. Selection criteria for analyses were: (i) Diagnosis of AML with criteria for AML-MRC according to the WHO classification[1]: previously diagnosed MDS or MPN/MDS; and/or multilineage dysplasia (MLD); and/or MDS-related cytogenetic abnormalities (complex karyotype (CK) defined by three or more chromosomal abnormalities, -7 or del(7q); -5 or del(5q); i(17q) or t(17p); -13 or del(13q); del(12p) or t(12p); del(9q); idic(X)(q13); t(11;16) (q23;p13.3); t(3;21)(q26.2;q22.1); t(1;3)(p36.3;q21.1); t(2;11)(p21;q23); t(5;12)(q33;p12); t(5;7)(q33;q11.2); t(5;17)(q33;p13); t(5;10)(q33;q21); t(3;5)(q25;q34); (ii) Genomic DNA available for mutational analyses.

AML with inv(3)/t(3;3), t(6;9) and t(11q23) or with favorable risk according to the ELN classification (inv(16)/t(16;16), t(8;21), t(15;17), normal karyotype AML with NPM1 or CEBPA mutations) were excluded as well as therapy-related AML[14].

**Morphological analyses**

Smears of bone marrow aspirates made at diagnosis were collected and stained by May-Grünewald-Giemsa. Each smear was retrospectively analyzed by two expert cytologists in both centers. We assessed the percentage of dysplastic cells in each lineage. MLD was assessed according to the WHO classification criteria: at least 50% of dysplastic cells in at least 2 lineages. We established the percentage of dysgranulopoiesis (DGP), dyserythropoiesis (DEP) and dysmegakaryopoiesis (DMP) for each smear.

**Mutational analyses**

Direct sequencing was done using the Sanger method as previously described[15]. We searched for mutation of ASXL1 (exon 12), RUNX1 (exon 1-8), DNMT3A (exon 15-23), NPM1 (exon 12), FLT3 (internal tandem duplication ITD, exon 14-15) and TP53 (exon 4-10).
Statistical analyses

Chi-square or Fischer tests were used to compare categorical variables. We used non-parametric test (U Mann Whitney) to compare the median percentage of dysplastic cells in each lineage according to mutational status for each gene. As multivariate model, linear regression was used to find correlation between morphologic dysplasia and the presence of mutation. Surveys were calculated using the Kaplan Meier estimator [16]. Time to event started from the date of diagnosis. We compared survivals using the Log-Rank estimator [16].

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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