LETTER TO THE EDITOR

Revisiting gene mutations and prognosis of ex-M6a-acute erythroid leukemia with regard to the new WHO classification

Due to the lack of specific clinical and biological features, M6a-acute erythroid leukemia (M6a-AEL), defined as an erythroid/myeloid type of acute leukemia, is no longer a distinct entity in the last classification of myeloid neoplasms by the World Health Organization (WHO).1 The diagnosis of M6a-AEL was previously made if a proliferation of erythroid precursors ≥50% with a myeloblast count ≥20% when counted as a percentage of non-erythroid cells, was found in the bone marrow.2 In 2016, revision of the WHO classification, the denominator used for calculating the blasts percentage was changed from non-erythroid cells to all nucleated cells. Consequently, M6a-AELs are now either calculated the blasts percentage was changed from non-erythroid cells or acute myeloid leukemia (AML) if the percentage of myeloblasts is ≥20% of non-erythroid cells but <20% of all nucleated cells or acute myeloid leukemia (AML) if the percentage of myeloblasts is ≥20% of all nucleated cells. As for any other AMLs prior therapy, recurring WHO cytogenetic abnormalities, and criteria for AML with myelodysplasia-related changes (AML-MRC) have to be taken into consideration for classification.

By using targeted next-generation sequencing (tNGS) and array-comparative genomic hybridization (aCGH), we previously established a molecular classification of 40 M6a-AELs3 in five classes (C) based on mutations in NPM1 (C1), transcription factors (C2), splicing factors and/or chromatin modifiers (C3), TP53 (C4) or neither (C5). This classification could help in prognosis stratification. We have here re-analyzed our M6a-AEL molecular data according to 2016 WHO classification and compared them to a previously published cohort of MDS.4

After written consents obtained according to our ethical committee regulations and biobank procedures 11 new M6a-AEL patients were added to the cohort and 106 genes were sequenced by tNGS as previously described.5 The 51 ex-M6a-AELs were reclassified as either MDSs—thereafter named AEL-MDSs (N = 24)—or AMLs (N = 27). To be homogenous in terms of blasts, the comparative cohort of MDS patients—thereafter named typical-MDSs—was made of 19 MDSs with excess of blasts type 2 (MDS-EB-2), 20% when counted as a percentage of all nucleated cells or acute myeloid leukemia (AML) if the percentage of myeloblasts is ≥20% of non-erythroid cells but <20% of all nucleated cells or acute myeloid leukemia (AML) if the percentage of myeloblasts is ≥20% of all nucleated cells. As for any other AMLs prior therapy, recurring WHO cytogenetic abnormalities, and criteria for AML with myelodysplasia-related changes (AML-MRC) have to be taken into consideration for classification.

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Median age was 61, 59 and 77 years for AEL-MDSs and AMLs, respectively. AMLs comprised 9 AMLs with recurrent genetic abnormalities (AML-RGAs), 10 AMLs with myelodysplasia-related changes (AML-MRCs), 4 AML-NOS, 3 therapy-related AMLs (t-AMLs) and 1 AML unclassified due to lack of data. According to the European LeukemiaNet risk stratification by genetics (ELN 2017), the prognosis was favorable for four patients, intermediate for 10 patients and adverse for the rest of the cohort. All AEL-MDSs were MDS-EB-2, except one case with excess of blasts type 1 (MDS-EB-1). The percentage of myeloblasts was between 10 and 18 (mean = 14%) and dysplasia was observed in all of the cases. Three AEL-MDSs were therapy-related myeloid neoplasms (t-AEL-MDSs). According to the International Prognostic Scoring System Revised (IPSS-R), karyotypes were good/very good for 12 cases, intermediate for six and poor/very poor for six leading to high or very high IPSS-R risk category, except one case that fell in an intermediate category due to a del(5q)15q35. Typical-MDSs patients had between 10 and 18% myeloblasts (mean = 13%) and the erythroid component varied from 7 to 31% except for two cases (HD-0486 and HD-0982) with 45 and 40%, respectively. According to IPSS-R, karyotype was good for nine cases, intermediate for four and poor/very poor for six leading to high or very high IPSS-R risk category for most of the patients (16/19).

Mutations were observed equally in AEL-MDSs and AML cases: 87.5% (21/24) and 85% (23/27), respectively (Fisher’s exact test P = 1, Figure 1). The number of mutations was also quite equivalent, 61 and 63 in AEL-MDSs and AMLs, respectively. The median number of mutations in AEL-MDSs and AMLs was 3 and 2, respectively. All molecular classes C1–C5 were observed in both groups and the number of patients in each class was comparable (Supplementary Figure 1a). No difference in the number of mutations by functional pathways was found between AEL-MDSs and AMLs (Fisher’s exact test, P = 0.08, Supplementary Figure 1b) in spite of a higher frequency of mutations in the cohesin complex genes in AEL-MDSs. The median variant allele frequency (VAF) in NPM1-mutated cases (C1) was similar in AEL-MDSs and AMLs (0.20 and 0.32, respectively) but the most frequent additional mutations were in the cohesin complex genes, especially in SMC3 (3/6) for AEL-MDSs, and in DNMT3A (4/6) in AMLs (Supplementary Figure 2a). Median VAF in TP53-mutated cases (C4) was 0.32 in AEL-MDSs and 0.4 in AMLs. Eighty percent of AEL-MDSs (N = 4/5) and 30% of AMLs (N = 3/10) carried two different TP53 mutations. Homozygous inactivation of TP53 was suspected in three other cases (VAF > 0.8) due to a del(17p) (N = 3) or by uniparental disomy of chromosome 17 harboring a somatic TP53 mutation (HD-2199) (Supplementary Figure 2b). In spite of a triple alteration of TP53 (double mutations and loss of heterozygosity—LOH), the VAF remained low (<0.5) for four cases suggesting either a dilution of the sample or two different clones harboring a mutation. Double TP53 mutants (sequence variant or LOH) have been described in myeloid diseases but not with the same frequency.6 The high frequency in our series (N = 10/15) may suggest an implication in the erythroid proliferation, as recently suggested in pure erythroid leukemias.7 When comparing VAFs in C3, defined as ‘secondary-type’ mutations,9 medians were not different between AEL-MDSs and AMLs, respectively 0.414 and 0.471 (Supplementary Figure 2c). Finally, we did not find any GATA2-mutations (also verified by Sanger, data not shown) in either AEL-MDSs or AMLs and only one AML patient carried a bi-allelic mutation of CEGBP4. These results contrast with a recent report describing a high frequency of mutations in these two genes10 but are in accordance with other data.9,11

These results show that AEL-MDSs and AMLs are similar in terms of molecular profiles and confirm our previous observation: ex-M6a-AELs show some differences with non-erythroid-rich AMLs; they have more TP53 mutations and less DNMT3A and ASXL1 mutations (Supplementary Table 2a).
Figure 1. Mutations in a cohort of 51 ex-M6a-AML cases represented by 24 AEL-MDSs and 27 AMLs separated according to the WHO 2016: a co-mutation chart shows non-synonymous mutations in individual genes, grouped according to function, as labeled on the left and ranged according to the molecular stratification in five classes (C1–C5) indicated on the top. Mutations are depicted by colored bars, and each column represents one of the 51 sequenced cases. The colors reflect the five molecular classes.
Figure 2. Kaplan–Meier estimates of overall survival (OS) in the different cohorts. For each curve the number of patients is indicated by N. (a) OS according to the WHO 2016 classification: AEL-MDSs (21 patients) versus AMLs (24 patients) (therapy-related AEL-MDS/AMLs were excluded). No difference is seen between AEL-MDSs and AMLs. (b) OS according to our molecular stratification in AEL-MDSs: NPM1-mutated patients in blue (C1), isolated transcription factors-mutated patients in purple (C2), spliceosome-mutated patients in green (C3), TP53-mutated patients in red (C4), other mutated genes-mutated patients in dark. No significant difference in OS was observed. (c) OS according to our molecular stratification in AMLs: NPM1-mutated patients in blue (C1), isolated transcription factors-mutated patients in purple (C2), spliceosome-mutated patients in green (C3), TP53-mutated patients in red (C4), other mutated genes-mutated patients in dark. No significant difference in OS was observed. (d) OS according to the WHO 2016 classification: AEL-MDSs versus AMLs versus typical-MDS. (e) OS according to our molecular stratification in the ex-M6a-AEL patients (AEL-MDSs+AMLs): NPM1-mutated patients in blue (C1), isolated transcription factors-mutated patients in purple (C2), spliceosome-mutated patients in green (C3), TP53-mutated patients in red (C4), other mutated genes-mutated patients in dark. A significant difference in OS was observed.
The molecular profile of our AEL-MDSs and AMLs was compared to a typical-MDS cohort previously analyzed for 17 genes (Supplementary Figure 3). Some differences were observed (Supplementary Figure 1c): (1) no NPM1 mutations were found in the typical-MDSs (Fisher’s exact test $P = 0.003$), (2) mutations in spliceosome genes and ASXL1 were more frequent in typical-MDSs (Fisher’s exact test $P = 0.002$ and $P = 0.007$, respectively) (3) mutations in the CBL signaling gene was predominant in typical-MDSs (Fisher’s exact test, $P = 0.003$) whereas the number of mutated genes in signaling pathways was not different between AEL-MDSs, AMLs and typical-MDSs (Fisher’s exact test $P = 0.5$) (data not shown). These observations were confirmed in a large typical-MDS cohort$^{12}$ (Supplementary Table 2b). In the three TP53-mutated typical-MDS cases (15.8%), mutations were heterozygous, which contrasts with a recent report. A study analyzed 12 genes (FLT3-ITD, NPM1, CEBPA, TP53, IDH1/2, DNMT3A, KRAS/NRAS, their typical-MDS and with their ex-M6-AELs, the only difference between our AEL-MDSs and the erythroid-rich MDSs of this study independently of the AEL-MDS or AML distinction, NPM1 respectively, Supplementary Table 2c). It seems that erythroid-rich zygous, which contrasts with a recent report. A study analyzed 12 TP53 spliceosome genes and ASXL1 (Supplementary Figure 1c): (1) no (Fisher between AEL-MDSs, AMLs and typical-MDSs (Fisher’s exact test of mutated genes in signaling pathways was not different large typical-MDS cohort 12 (Supplementary Table 2b). In the three predicted by IPSS-R than by molecular stratification (Supplementary Figures 4a–c). Finally, and most importantly, the prognosis of ex-M6a-AELs was better predicted by the molecular classification than by the WHO 2016 classification ($P = 4.56e-05$, Figure 2e).

In conclusion, the abandonment of a specific class and the reclassification of ex-M6a-AELs in MDSs and AMLs is justified by the absence of specific features but is not supported molecularly. On the contrary, we suggest that AEL-MDSs may be considered as AMLs due to the high frequency of NPM1 mutations and TP53 double mutations. Furthermore, stratification of prognosis is better achieved by molecular than by phenotype classification. Based on the new recommendations for the diagnostic work-up of AMLs, the 2017 ELN seems more appropriate for AEL-MDSs than the IPSS-R.

**DATA AVAILABILITY**
The data sets generated during and/or analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) repository,
12 Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* 2013; **122**: 3616–3627.

13 Wang SA, Patel KP, Pozdnyakova O, Peng J, Zuo Z, Dal Cin P et al. Acute erythroid leukemia with < 20% bone marrow blasts is clinically and biologically similar to myelodysplastic syndrome with excess blasts. *Mod Pathol* 2016; **29**: 1221–1231.

14 Calvo X, Arenillas L, Luño E, Senent L, Arman M, Ramos F et al. Erythroleukemia shares biological features and outcome with myelodysplastic syndromes with excess blasts: a rationale for its inclusion into future classifications of myelodysplastic syndromes. *Mod Pathol* 2016; **29**: 1541–1551.

Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)