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LETTER TO THE EDITOR

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

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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 $\geqslant 50\%$ with a myeloblast count $\geqslant 20\%$ 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 myelodysplastic syndromes (MDSs) if the percentage of myeloblasts is $\geqslant 20\%$ of non-erythroid cells but $< 20\%$ of all nucleated cells or acute myeloid leukemia (AML) if the percentage of myeloblasts is $\geqslant 20\%$ 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-AELs[3] 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.[3] 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). Statistical analyzes were done using the survival package (version 2.30) in the R software (version 2.8.0) and the correlations were calculated with the Fisher's exact test at 5% level of significance.

Median age was 61, 59 and 77 years for AMLs, AEL-MDSs and typical-MDSs, 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(5)(q15q35). 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). The main clinical and biological characteristics of the patients are presented in Supplementary Table 1.

Mutations were observed equally in AEL-MDS 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;[5–7] the high frequency in our series ($N = 10/15$) may suggest an implication in the erythroid proliferation, as recently suggested in pure erythroid leukemias.[8] 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 *CEBPA*. These results contrast with a recent report describing a high frequency of mutations in these two genes[10] 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).

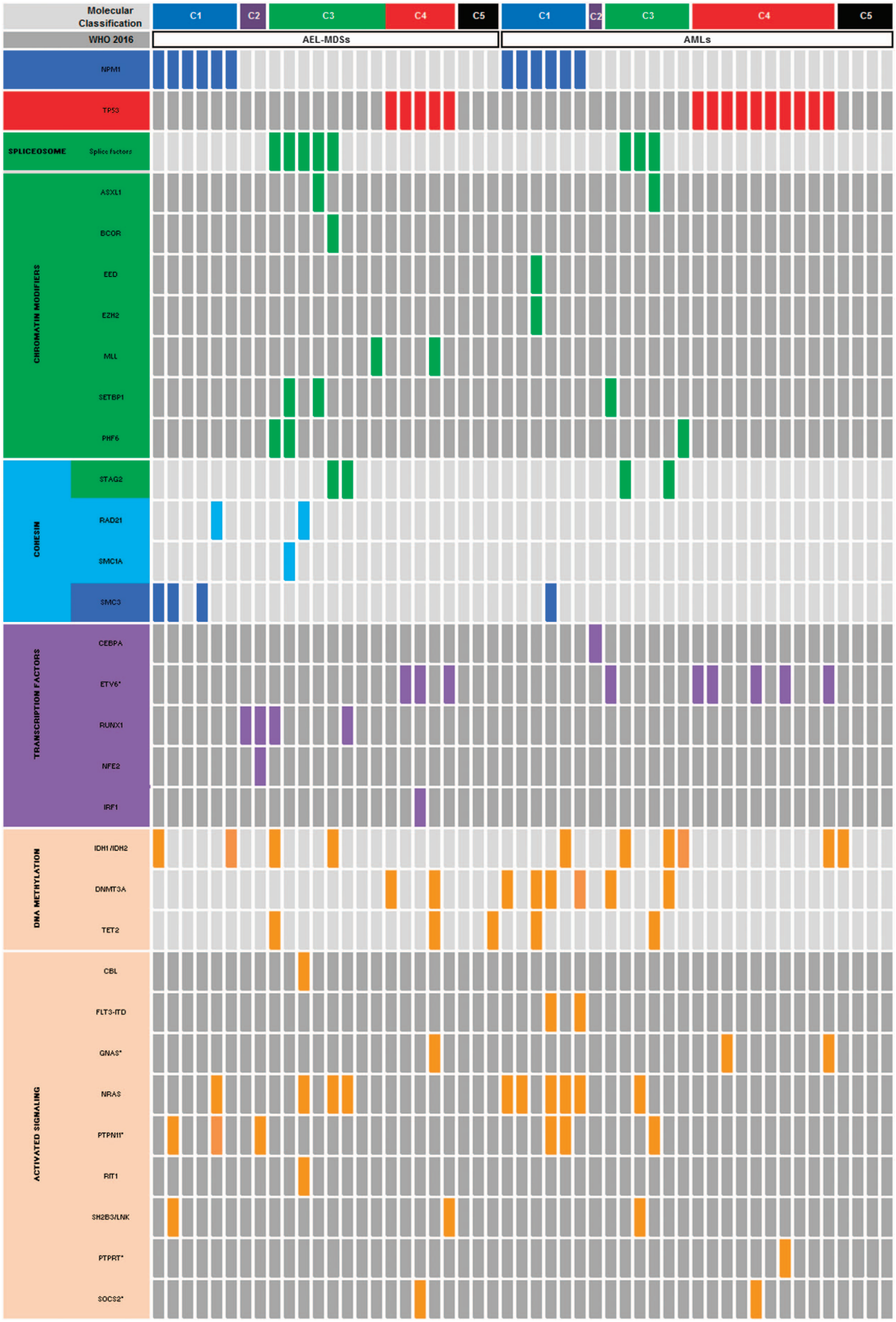

Figure 1. Mutations in a cohort of 51 ex-M6a-AML cases represented by 24 AEL-MDSs and 27 AMLs separated according to the WHO 2016: a co-mutation chart shows non-synonymous mutations in individual genes, grouped according to function, as labeled on the left and ranged according to the molecular stratification 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.

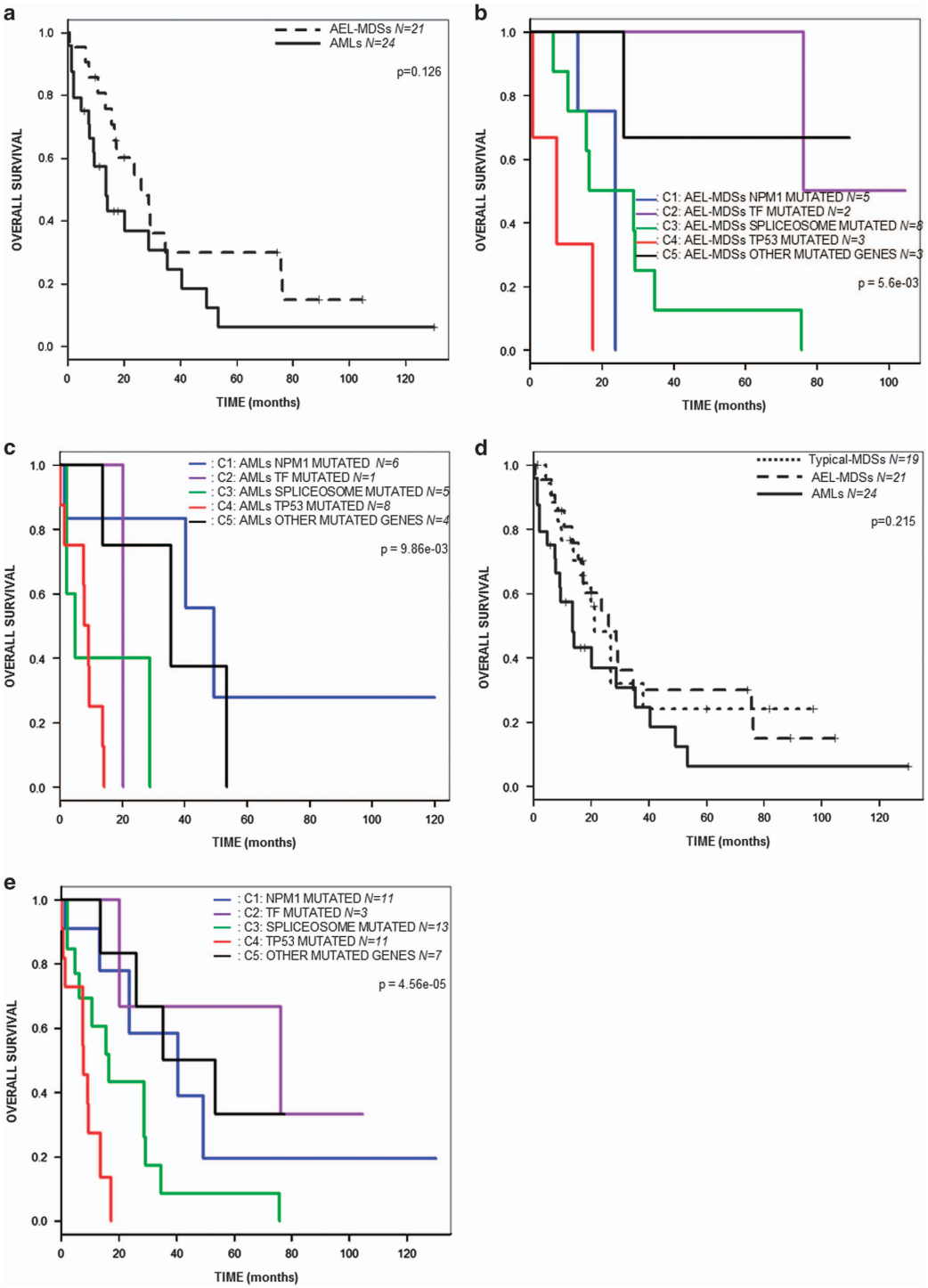

Figure 2. Kaplan–Meier estimates of overall survival (OS) in the different cohorts. For each curve the number of patients is indicated by N. (**a**) OS according to the WHO 2016 classification: AEL-MDSs (21 patients) versus AMLs (24 patients) (therapy-related AEL-MDS/AMLs were excluded). No difference 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), splicesome-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[4] (Supplementary Figure 3). Some differences were observed (Supplementary Figure 1c): (1) no *NPM1* mutations were found in the typical-MDSs (Fisher's exact test $P = 0.03$), (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.[6] A study analyzed 12 genes (*FLT3-ITD, NPM1, CEBPA, TP53, IDH1/2, DNMT3A, KRAS/NRAS, KIT* and *JAK2*) in ex-M6a-AELs, erythroid-rich MDSs (excess of blasts type 1 with $\geqslant 50\%$ bone marrow erythroid precursor) and typical-MDSs (excess of blasts type 1/2).[13] We found no difference between our AEL-MDSs and the erythroid-rich MDSs of this study (Supplementary Table 2c). When we compared our AEL-MDSs with their typical-MDS and with their ex-M6-AELs, the only difference found was with *NPM1* (Fisher's exact test, $P = 2.3e\text{-}04$ and $P = 0.01$, respectively, Supplementary Table 2c). It seems that erythroid-rich MDSs are closer to AEL-MDSs than to typical-MDSs. It is unlikely that *NPM1* mutations explain the erythroid proliferation and further studies are required.

Survival data were available for all the patients. We excluded the 6 t-AEL-MDS/AML patients from the survival analysis. The median overall survival (OS) were 13.7, 26.1 and 21.2 months for AMLs, AEL-MDSs and typical-MDSs, respectively (median follow-up was 17 months for all the patients, calculated by the reverse Kaplan–Meier method). No survival difference was found between AEL-MDSs and AMLs (Figures 2a, $P = 0.126$). Based on our molecular classification (C1–C5), no difference in OS was observed between AEL-MDSs and AMLs (Figures 2b and c). As expected, and independently of the AEL-MDS or AML distinction, *NPM1*-mutated patients had a better overall survival than *TP53*-mutated patients ($P = 0.046$ and $P = 0.009$, respectively, Figures 2b and c). Patients with isolated transcription factor mutations had a better outcome in AEL-MDSs than in AMLs ($P = 0.03$; Figure 2b) but the low number of patients does not allow a definite conclusion. When we compared the OS of AEL-MDSs, AMLs and typical-MDSs, no difference was observed ($P = 0.21$, Figure 2d) as also described in other studies.[13,14] In the typical-MDS group, OS was better 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 ($P = 4.56e\text{-}05$, Figure 2e).

In conclusion, the abandon of a specific class and the reclassification of 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,

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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N Cervera[1], N Carbuccia[1], M-J Mozziconacci[1,2], J Adélaïde[1], S Garnier[1], A Guille[1], A Murati[1,2], M Chaffanet[1], N Vey[3,4], D Birnbaum[1] and V Gelsi-Boyer[1,2]

[1]Laboratoire d'Oncologie Moléculaire, Centre de Recherche en Cancérologie de Marseille UMR1068 Inserm, Institut Paoli-Calmettes, CNRS UMR7258, Aix-Marseille Université UM105, Marseille, France;
[2]Département de BioPathologie, Institut Paoli-Calmettes, Marseille, France;
[3]Département d'Hématologie, Institut Paoli-Calmettes, Marseille, France and
[4]Aix-Marseille Université, Marseille, France
Correspondence: Dr V Gelsi-Boyer, Laboratoire d'Oncologie Moléculaire, Institut Paoli-Calmettes, 232 Bd de Sainte-Marguerite, 13009 Marseille, France
E-mail: gelsiv@ipc.unicancer.fr

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