Leukemia (2021) 35:1291–1300
https://doi.org/10.1038/s41375-020-01031-1

ARTICLE

Acute myeloid leukemia

Lomustine is beneficial to older AML with ELN2017 adverse risk profile and intermediate karyotype: a FILO study

Laetitia Largeaud[1] · Pascale Cornillet-Lefebvre[2] · Jean-François Hamel[3] · Pierre-Yves Dumas[4] · Naïs Prade[1] · Stéphanie Dufrechou[1] · Julien Plenecassagnes[5] · Isabelle Luquet[1] · Odile Blanchet[6] · Anne Banos[7] · Marie C. Béné[8] · Marc Bernard[9] · Sarah Bertoli[10] · Caroline Bonmati[11] · Luc Matthieu Fornecker[12] · Romain Guièze[13] · Lamya Haddaoui[14] · Mathilde Hunault[15] · Jean Christophe Ianotto[16] · Eric Jourdan[17] · Mario Ojeda[18] · Pierre Peterlin[19] · Norbert Vey[20] · Hacene Zerazhi[21] · Hicheri Yosr[20,22] · Ariane Mineur[23,24] · Jean-Yves Cahn[25] · Norbert Ifrah[15] · Christian Récher[10] · Arnaud Pigneux[4] · Eric Delabesse[1] · on behalf of the French Innovative Leukemia Organization (FILO)

Received: 27 April 2020 / Revised: 23 July 2020 / Accepted: 14 August 2020 / Published online: 18 September 2020

Abstract

We previously reported the benefit of lomustine addition to conventional chemotherapy in older acute myeloid leukemias with nonadverse chromosomal aberrations in the LAM-SA 2007 randomized clinical trial (NCT00590837). A molecular analysis of 52 genes performed in 330 patients included in this trial, 163 patients being treated with lomustine in combination with idarubicin and cytarabine and 167 without lomustine, identified 1088 mutations with an average of 3.3 mutations per patient. *NPM1*, *FLT3*, and *DNMT3A* were the most frequently mutated genes. A putative therapeutic target was identified in 178 patients (54%). Among five molecular classifications analyzed, the ELN2017 risk classification has the stronger association with the clinical evolution. Patients not treated with lomustine have an expected survival prognosis in agreement with this classification regarding the overall and event-free survivals. In strong contrast, lomustine erased the ELN2017 classification prognosis. The benefit of lomustine in nonadverse chromosomal aberrations was restricted to patients with *RUNX1, ASXL1*, *TP53*, and *FLT3*-ITD^{high}/*NPM1*^{WT} mutations in contrast to the intermediate and favorable ELN2017 patients. This post-hoc analysis identified a subgroup of fit elderly AML patients with intermediate cytogenetics and molecular markers who may benefit from lomustine addition to intensive chemotherapy.

Introduction

The incidence of acute myeloid leukemia (AML) increases with age, the majority of patients being diagnosed after 60 years. In these older patients, the prognosis is poor, with a median overall survival (OS) usually shorter than 1 year [1, 2]. In patients deemed fit for intensive chemotherapy, no significant improvement in outcome has been achieved except with CPX-351, a dual-drug liposomal encapsulation of cytarabine and daunorubicin recently approved for secondary AML, whereas the combination of the BCL2 inhibitor venetoclax with azacitidine improved outcome of patients unfit for chemotherapy [3]. AML have been classified initially according to cytogenetics profiles into three risk categories: favorable, intermediate, and poor [4]. Favorable cytogenetics abnormalities (core binding factor leukemias and t(15;17) translocation) have long been established as a key predictor for improved clinical outcome as opposed to patients with complex or monosomal karyotypes. The intermediate cytogenetics risk group, representing ~60% of the patients, forms a heterogeneous group [4]. Molecular analysis, especially in this latter group, is highly complementary to cytogenetics.

These authors contributed equally: Laetitia Largeaud, Pascale Cornillet-Lefebvre, Jean-François Hamel, Christian Récher, Arnaud Pigneux, Eric Delabesse

Members of the French Innovative Leukemia Organization (FILO) are listed below Acknowledgements.

Supplementary information The online version of this article (https://doi.org/10.1038/s41375-020-01031-1) contains supplementary material, which is available to authorized users.

✉ Eric Delabesse
delabesse.eric@iuct-oncopole.fr

Extended author information available on the last page of the article

Mutations in epigenetic modifiers, such as *ASXL1*, *DNMT3A*, or *TET2*, are early oncogenic events, frequently found in clonal hematopoiesis [5–7]. Moreover, *RUNX1*, *ASXL1*, and *TP53* mutations are poor prognostic markers in patients with intermediate cytogenetics risk and have been recently included in the European LeukemiaNet (ELN) 2017 prognostic classification for AML, which combines cytogenetics and molecular biology [8].

We previously reported the benefit of the addition of lomustine (also known as chlorethyl-cyclohexyl-nitrosourea) to conventional chemotherapy (idarubicin and cytarabine) for older patients with de novo AML and non-adverse cytogenetics in a randomized clinical trial that enrolled 459 patients [9]. Lomustine is an alkylating agent with a significant antileukemic activity linked to DNA damage and/or impairment of cell replication [10–12]. In the LAM-SA 2007 trial, its addition significantly improved the complete response (CR) or CR with incomplete recovery rate (84.7% vs. 74.9%, $p = 0.01$) and reduced the cumulative incidence (CI) of relapse (41.2% vs. 60.9%, $p = 0.003$) resulting in improved 2-year event-free (41% vs. 26%, $p = 0.01$) and OS (56% vs. 48%, $p = 0.02$).

Here, we report the molecular analysis of this prospective, randomized cohort of older AML patients selected for intensive chemotherapy. The specific profile of the mutations was investigated regarding their functional pathways. Finally, the impact of lomustine in this molecular landscape was investigated through five molecular classifications of AML defined mainly from younger patients [8, 13–16]. A strong benefit to the addition of lomustine was identified in a subset of patients in the ELN2017 adverse risk group [8] with intermediate cytogenetics, bearing *RUNX1, ASXL1*, *TP53*, and *FLT3*-ITD^{high}/*NPM1*^{WT} (RATFIN) mutations.

Materials and methods

Patients

The LAM-SA 2007 trial was registered at clinicaltrials.gov as NCT00590837. It involved 32 clinical centers of the French Innovative Leukemia Organization (FILO) study group that enrolled 459 patients from February 2008 to December 2011. All patients were older than 60 years and had been diagnosed with de novo AML. Patients had to be considered fit without adverse cytogenetics (defined after the analysis of 20 mitosis at least if no abnormal clone was identified) [17], promyelocytic leukemia nor isolated granulocytic sarcoma. The trial was conducted according to the Declaration of Helsinki and approved by the ethical committee of Bordeaux University Hospital and the Agence Française de Sécurité Sanitaire des Produits de Santé. All patients provided written informed consent at enrollment. Before initiation of the induction chemotherapy course, patients were registered and randomized to receive or not lomustine during induction and postinduction treatment phases [9]. The clinical analysis was performed with 424 patients as 35 were excluded, including 14 from a single center by decision of the data and safety monitoring board as a result of noncompliance with the chemotherapy regimen. Ten patients were reclassified to adverse cytogenetics, eight had myelodysplasia, two a Sorror score of 3, and one withdrew its informed consent [9]. Molecular analyses were performed centrally on samples stored at the FILOthèque, FILO tumor cell bank (DC 2009-944). DNA material was available for 330 patients (78%) with no difference of prognosis according to the DNA availability (Fig. S1).

Molecular analysis

The presence of *FLT3*-ITD was tested as described [18]. Electrophoregrams peaks were quantified using GeneMarker 2.2 (SoftGenetics, State College, PA, USA). *CEBPA* screening was performed by classical Sanger sequencing according to Pabst et al. [19]. Six recurrent and frequent mutations (*ASXL1* exon 12, *DNMT3A* exon 23, *FLT3* exon 20, *IDH1* exon 4, *IDH2* exon 4 and *NPM1* exon 12) were sequenced using next generation sequencing and a multiplex PCR amplicon based library with the following primers: qI_Halo_ASXL1_R634_F2 (CCACCACGGAGTCCTCCT), qI_Halo_ASXL1_R634_R2 (GCCTCACCACCATCACCA), qI_DNMT3A_X23_F1 (CTGGCCAGCACTCACCCT), qI_DNMT3A_X23_R1 (TGTTTAACTTTGTGTCGCTACCTCA), qI_FLT3_X20_F3 (GTTTACCATGATAACGACACAACAC), qI_FLT3_X20_R3 (GATTGCACTCCAGGATAATACACA), qI_IDH1_X4_F1 (GGCTTGTGAGTGGATGGGTAA), qI_IDH1_X4_R2 (GCATTTCTCAATTTCATACCTTGCTTA), qI_IDH2_X4_F1 (GAAAGATGGCGGCTGCAGT), qI_IDH2_X4_R2 (CACCCTGGCCTACCTGGTC), qI_NPM1_X12_F1 (GAAGTGTTGTGGTTCCTTAAC) and qI_NPM1_X12_R1 (TGGACAACACATTCTTGGCA). The library was sequenced using a MiSeq sequencer (Illumina, San Diego, CA, USA) and Miseq Reagent kit V2 (paired-end sequencing 2×150 cycles). Alignment was performed using BWA aligner and variant calling was performed using FreeBayes and Mutect2 variant callers.

An extended DNA resequencing was performed using a Illumina NextSeq500 and Haloplex HS (Agilent, Santa Clara, CA, USA) targeted on the complete coding regions of 52 genes: *ASXL1*, *ASXL2*, *ATM*, *BCOR*, *BCORL1*, *CBL*, *CCND2*, *CEBPA*, *CSF3R*, *CUX1*, *DDX41*, *DHX15*, *DNMT3A*, *EP300*, *ETV6*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *JAK2*, *KDM5A*, *KDM6A*, *KIT*, *KMT2D*, *KRAS*, *MGA*, *MPL*, *MYC*, *NF1*, *NPM1*, *NRAS*, *PHF6*, *PIGA*, *PPM1D*, *PRPF8*, *PTPN11*, *RAD21*, *RUNX1*, *SETBP1*,

SF3B1, *SMC1A*, *SMC3*, *SRSF2*, *STAG2*, *TET2*, *TP53*, *U2AF1*, *WT1*, *ZBTB7A*, and *ZRSR2*. Data were processed through two algorithms from GATK (https://software.broadinstitute.org/gatk), HaplotypeCaller (scaling accurate genetic variant discovery to tens of thousands of samples, Poplin et al. [20]) and Mutect2 [21]. The mean depth was 2,190. Identified variants were curated manually and named according to the rules of the Human Genome Variation Society (hgvs.org). Molecular data have been stored in the European Nucleotide Archive (https://www.ebi.ac.uk/ena/).

Statistics

The clinical database was frozen in June 2015 and follow-up was updated in May 2018 [9]. Statistical analyses were conducted according to intention to treat. Categorical data were presented as percentages and compared using Fisher exact tests. Continuous data were presented as mean and standard deviation and compared using Mann and Whitney tests.

The endpoints considered were OS, event-free survival (EFS), CR, and relapse. For mutation impact, OS and EFS were studied using log rank tests for equality of survivor functions and graphically represented using Kaplan–Meier curves. For model evaluation, OS and EFS were studied using Cox models, whereas CR and relapse were studied using Fine and Gray models considering death as a competing event. The prognostic value of each score was assessed through their inclusion as categorical covariates in these models.

The impact of disease severity on lomustine benefit was assessed by considering the interaction between lomustine treatment and each studied score with the allogeneic stem-cell transplantation being introduced as a time-dependent covariate. The global effects of covariates introduced in the model were assessed through likelihood ratio tests and the effects of each modality of covariates were assessed through Wald tests. The proportional hazard assumption was checked through the use of the Schoenfeld residuals analysis. The differential impact of lomustine depending on disease severity was graphically represented using Kaplan–Meier graphs.

All tests were considered as two-sided considering a type I error set to 0.05. All analyses were performed using Stata 13.1 software (StataCorp, College Station, TX, USA).

Results

Molecular landscape in older AML patients with nonadverse cytogenetics

A molecular analysis of 52 genes was performed in 330 patients included in the LAM-SA 2007 clinical trial (78% of the cohort). Cytogenetic risk was intermediate for 281 patients (203 normal and 78 other intermediate karyotypes) and favorable for 22 [15 inv(16)/t(16;16) and 7 t(8;21)] (Fig. 1). Mutations were identified in 319 patients with an average of 3.3 mutations per patient (0–12 mutations per patient; Figs. 2 and 3 and Table S1). Eleven patients have no mutation detected, including 3 with inv(16) and 1 with a *KMT2A* rearrangement.

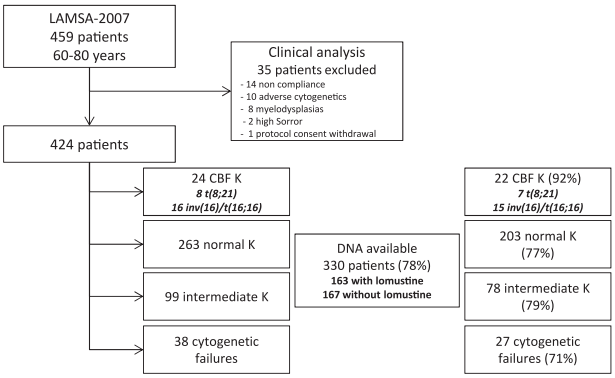

Fig. 1 LAM-SA 2007 molecular workflow. Patients included in the LAM-SA 2007 clinical trial and analyzed in the present study (K: karyotype).

NPM1, *FLT3*, and *DNMT3A* were the most frequently mutated genes (Figs. 2 and 3 and Table S1). *NPM1* was mutated in 113 patients (34%, 86 type A, 7 B, and 9 D), *FLT3* in 98 (30%, 76 with ITD [1–4 per patient] and 27 with other mutations including 20 of the tyrosine kinase domain), and *DNMT3A* in 88 (27%, 43 located at the Arg882 hot spot amino acid). Mutations of *IDH2* were present in 60 patients (18%, Arg140 codon in 48 patients and Arg172 codon in 12 patients) and of *IDH1* in 36 (11%). Additional therapeutic targets were found in 15 patients (*TP53* in 8, 2%; *JAK2* in 4, 1%; *KIT* in 3, 1%). Overall, a putative therapeutic target for tyrosine kinase inhibitors, IDH inhibitors or TP53 activators was identified in 178 patients (54%).

Mutation associations in older AML patients are not random

The mutations detected were analyzed using functional categories (Table S2) [16]. Mutations of DNA methylation genes were the most frequent, occurring in 178 patients (54%; Figs. 3 and 4a). *DNMT3A* mutations were strongly associated to those of *NPM1*, *IDH1*, and *IDH2* and strictly separate from *EZH2* mutations (Figs. 3 and 4a). *IDH1* and *IDH2* mutations were strictly separate from *TET2* mutations. One patient presented both *IDH1* and *IDH2* mutations (#308), the former being subclonal to the latter (variant allelic frequencies of 1% and 32%, respectively). In addition, *IDH2* and *TET2* mutations were strictly separate from *DDX41* mutations. Finally for this category, univariate analysis for patients in both study

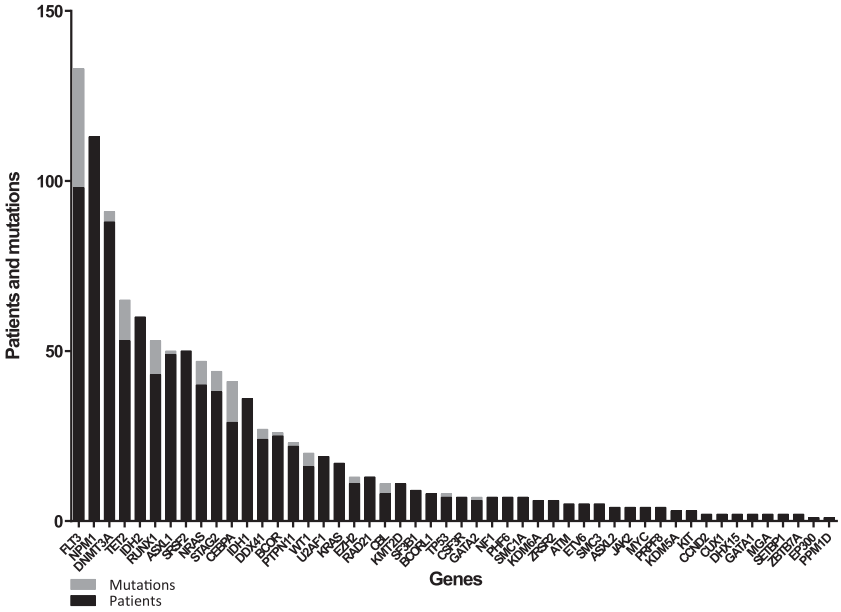

Fig. 2 LAM-SA 2007 mutation pattern. Number of mutations (gray bars) and patients with mutations (black bars) per gene.

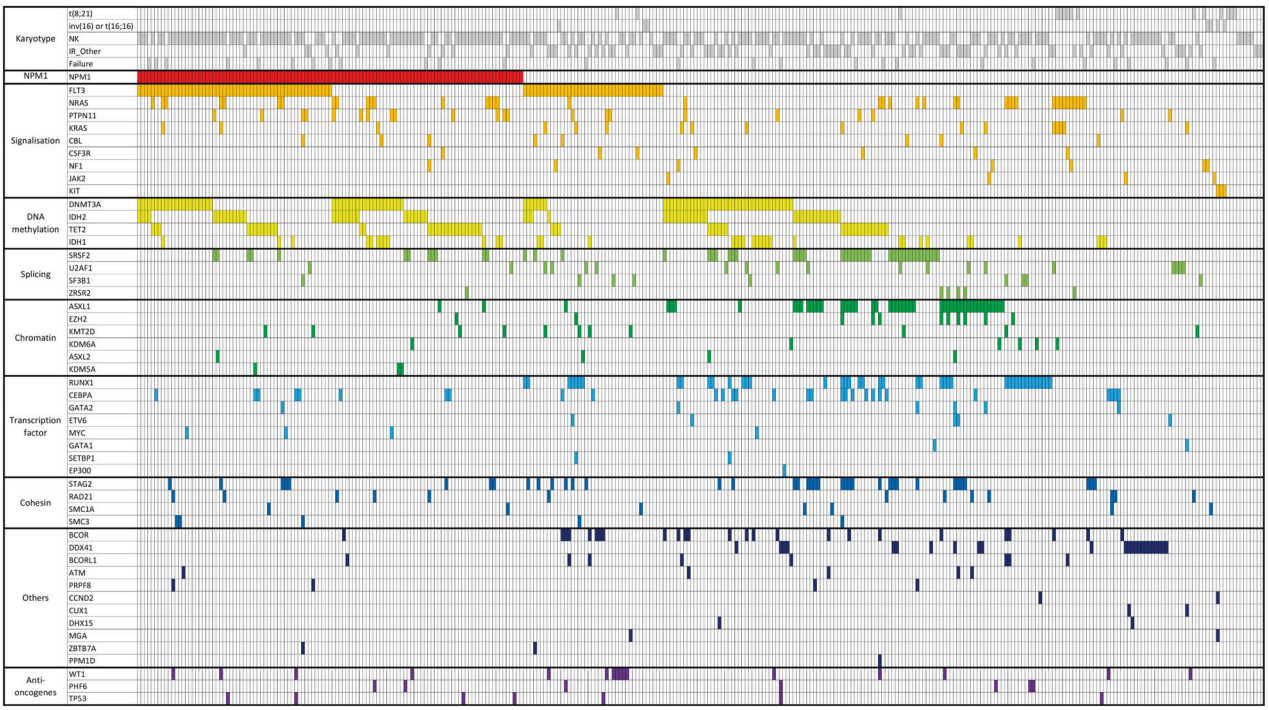

Fig. 3 Mutations associations classified as gene categories as defined by Bullinger et al. [16], *NPM1*, signalization, DNA methylation, splicing, chromatin, transcription factors, cohesin, and antioncogenes. "Other genes" regroups genes not classified in the eight previous groups (*ATM*, *BCOR*, *BCORL1*, *CCND2*, *CUX1*, *DDX41*, *DHX15*, *MGA*, *PPM1D*, *PRPF8*, *ZBTB7A*). NK normal karyotype, IR_Other intermediate karyotype other than normal.

arms showed that *IDH1* mutations were associated with a significantly worse prognosis for OS ($p = 0.021$) and EFS ($p = 0.019$; Fig. S2).

Mutations of genes encoding tyrosine kinases and proteins of the RAS signaling pathway were identified in 51% of the patients, more frequently *FLT3* in 98 patients (30%) and *RAS* genes in 75 (23%). *RAS* mutations were secondary events as demonstrated by their very low VAF (Fig. S3). *FLT3* mutations were significantly associated with *NPM1*, *WT1*, and *SMC3* mutations (Figs. 3 and 4b) and leukocytosis ($p < 0.0001$, Fig. S4). *FLT3* mutations were strictly separate from *DDX41* mutations, *NRAS* strongly

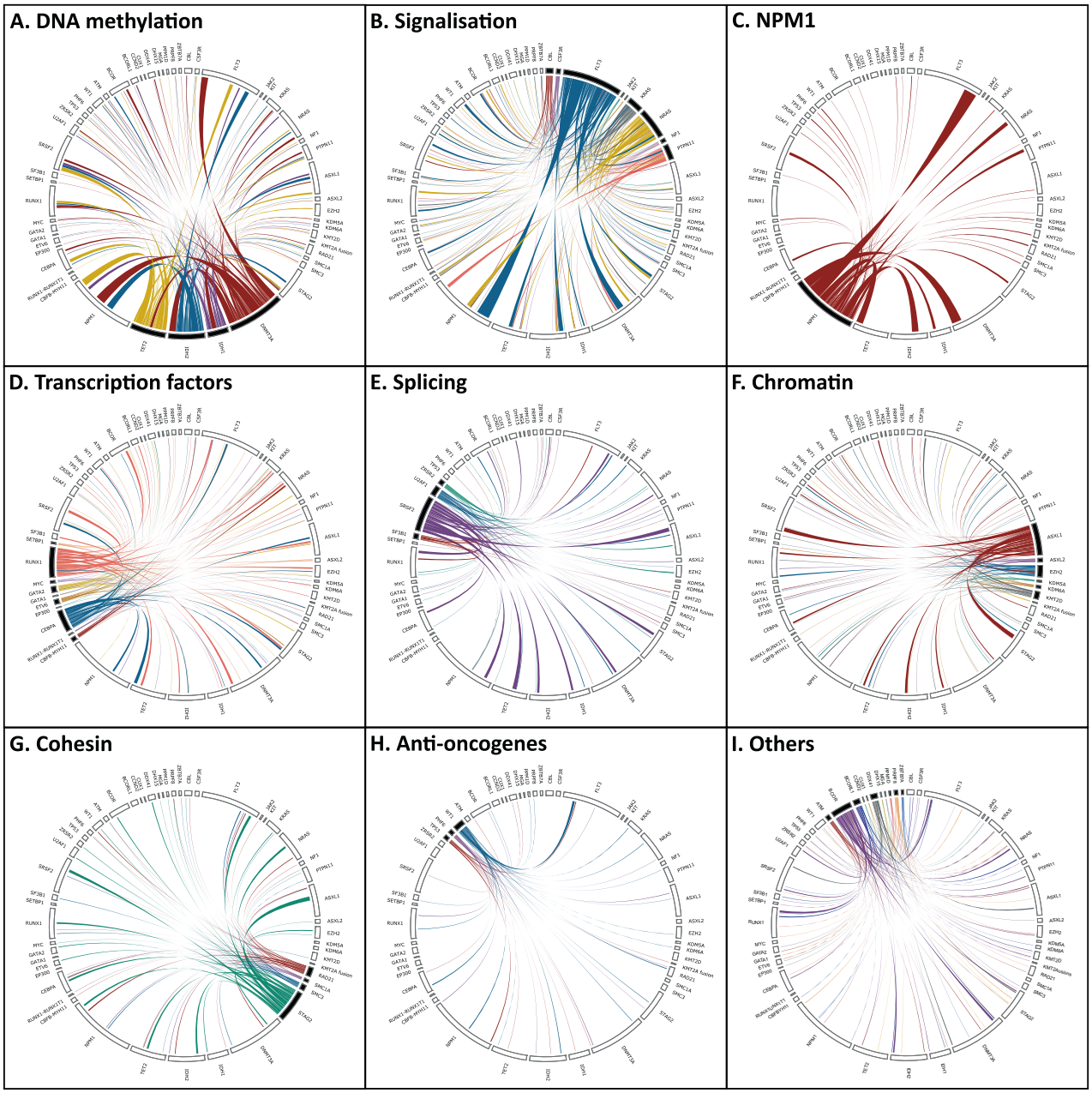

Fig. 4 Circos plots of gene categories showing the profiles of co-mutations. a DNA methylation genes (*DNMT3A*, *IDH1*, *IDH2*, and *TET2*). **b** Signaling genes (*CBL*, *CSF3R*, *FLT3*, *JAK2*, *KIT*, *KRAS*, *NRAS*, *NF1*, *PTPN11*). **c** *NPM1*. **d** Transcription factors genes (*CBFB–MYH11* and *RUNX1–RUNX1T1* fusion genes; *CEBPA*, *EP300*, *ETV6*, *GATA1*, *GATA2*, *MYC*, *RUNX1*, *SETBP1*). **e** Splicing genes (*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*). **f** Chromatin genes (*KMT2A* fusion gene; *ASXL1*, *ASXL2*, *EZH2*, *KDM5A*, *KDM6A*, *KMT2D*). **g** Cohesin genes (*RAD21*, *SMC1A*, *SMC3*, *STAG2*). **h** Antioncogenes (*PHF6*, *TP53*, *WT1*). **i** Other genes (*ATM*, *BCOR*, *BCORL1*, *CCND2*, *CUX1*, *DDX41*, *DHX15*, *MGA*, *PPM1D*, *PRPF8*, *ZBTB7A*).

separate from *STAG2* mutations and *KRAS* from *U2AF1* mutations (Figs. 3 and 4b). *FLT3*-ITD mutations were not linked to prognosis (OS, $p = 0.49$; EFS, $p = 0.82$; Fig. S5).

NPM1 mutations, present in 34% of the patients, were associated with a significantly better prognosis for OS ($p = 0.027$) and EFS ($p = 0.020$; Fig. S6). These mutations were significantly associated with *FLT3*, *DNMT3A*, *TET2*, *IDH2*, and *PTPN11* mutations (Figs. 3 and 4c) and leukocytosis ($p < 0.0001$, Fig. S4) but strictly separate from *DDX41* and *RUNX1* mutations and *CBFB–MYH11* fusion gene.

Transcription factors abnormalities were identified in 104 patients (32%), including 82 patients with intermediate cytogenetics (Figs. 3 and 4d). *CBFB–MYH11* fusions were strongly associated with *RAS* and *CSF3R* mutations. *RUNX1*

mutations were significantly associated with *EZH2*, *SF3B1*, and *BCORL1* mutations (in contrast to be significantly separate from *BCOR* mutations). *CEBPA* mutations were significantly associated with *TET2* and *STAG2* mutations and separate from *WT1* mutations, although these differences were not significant when the number of CEBPA mutations per patient (19 mono-allelic vs. 10 bi-allelic) was specifically analyzed due to a lack of power (Fig. S7).

Mutations of genes involved in splicing were identified in 82 patients (25%, Figs. 3 and 4e), especially *SRSF2* mutations (15%) that were significantly associated with *ASXL1*, *TET2*, *STAG2*, and *CBL* and separate from *FLT3* mutations.

Chromatin regulators were mutated in 77 patients (23%, Figs. 3 and 4f), especially *ASXL1* (15%). *ASXL1* mutations were strongly associated with *STAG2*, *SRSF2*, *EZH2*, and *ZRSR2* mutations and separate from *FLT3*, *NPM1*, and *DNMT3A* mutations. In univariate analysis, *ASXL1* mutations were associated with a very poor prognosis in our series, especially for EFS ($p = 0.0002$; OS: $p = 0.0069$; Fig. S8).

Cohesin mutations were identified in 61 patients (18%, Figs. 3 and 4g), particularly *STAG2* (12%). These mutations were significantly associated with leukopenia at diagnosis ($p < 0.0001$, Fig. S4) and mutations of *ASXL1*, *SRSF2*, *CEBPA*, *EZH2*, *ETV6*, and *GATA2* and separate from *DNMT3A* and *NRAS* mutations.

Mutations of tumor-suppressor genes were more infrequent, identified in 28 patients (8%, Figs. 3 and 4h), the most frequent being *WT1*. Its mutations were strongly associated with those of *FLT3* and separate from *RAD21* and *CEBPA* mutations. Few patients had *TP53* mutations (2%; Fig. 3), coherent with the exclusion of patient with adverse cytogenetics in this clinical trial.

The remaining genes, not classified in one of the eight categories detailed above, were found mutated in 67 patients (20%, Figs. 3 and 4i), the two most frequently mutated being *BCOR* (8%) and *DDX41* (7%). *BCOR* mutations were strongly associated to *BCORL1* mutations and separate from *NPM1*, *U2AF1*, and *RUNX1* mutations. Patients with *DDX41* mutations had a unique mutation pattern, without mutations of *FLT3*, *NPM1*, *IDH2*, nor *TET2* and were strongly associated with leukopenia at diagnosis ($p < 0.0001$, Fig. S2). They might have had a better prognosis but the small number of cases led to a lack of statistical power (Fig. S9). This group of patients deserves a specific investigation.

Prognostic significance of molecular classifications in older AML patients

Different molecular classifications for AML have been proposed, either prognostic (Patel et al. [13], Papaemmanuil et al. [15], Bullinger et al. [16], and ELN2017 [8]) or ontogenic (Lindsley et al. [14]). Their impact in the LAM-SA 2007 clinical trial was evaluated (Fig. S10). The 3-month CI of CR was 85% (95% CI: 81–89%). Papaemmanuil et al.'s and Lindsley et al.'s classifications were significantly associated with variations of CR CI (respectively, $p < 0.0001$ and $p = 0.0257$). The 3- and 5-year CI of relapse (CIR) were respectively 73% (95% CI: 68–77%) and 79% (95% CI: 74–83%). The five classifications were significantly associated with the CIR (ELN2017 [$p < 0.0001$], Bullinger et al. [$p < 0.0001$], Papaemmanuil et al. [$p < 0.0001$], Patel et al. [$p = 0.0047$], and Lindsley et al. [$p = 0.0152$]). The 3- and 5-year EFS were 23% (95% CI: 19–27%) and 17% (95% CI: 13–21%), respectively. The five classifications were also significantly associated with EFS (ELN2017 [$p = 0.0001$], Patel et al. [$p = 0.0006$], Lindsley et al. [$p = 0.0046$], Bullinger et al. [$p = 0.0096$], and Papaemmanuil et al. [$p = 0.0139$]). The 3- and 5-year OS were 39% (95% CI: 35–44%) and 24% (95% CI: 20–29%), respectively. The ELN2017 classification was strongly associated to OS ($p = 0.001$) as well as to, to a lower extent, those of Patel et al. ($p = 0.014$) and Lindsley et al. ($p = 0.050$). Overall, ELN2017 was the best molecular classification to summarize the clinical evolution of the patients included in the LAM-SA 2017 clinical trial.

Impact of lomustine in the genomic landscape of AML

We evaluated the impact of these five classifications according to the treatment, 163 patients being assigned to arm A (with lomustine) and 167 to arm B (without lomustine). Regarding CR, a significant interaction between the Papaemmanuil classification and lomustine treatment was highlighted ($p < 0.001$), lomustine being significantly associated with a lower CR rate in the CBFB–MYH11 subgroup ($p = 0.002$) and a better CR rate in the IDH2 R172K subgroup ($p < 0.001$). Regarding CIR, a significant interaction between lomustine treatment and the ELN2017 classification was also highlighted ($p = 0.027$). Lomustine is significantly associated with a lower relapse rate in the subset of the ELN2017 adverse group with a nonadverse karyotype, i.e., with RATFIN mutations ($p = 0.001$) but not in the ELN2017 favorable nor intermediate groups ($p = 0.879$ and 0.861, respectively). A significant interaction with lomustine and the risk of relapse was also highlighted using the Papaemmanuil classification ($p = 0.003$) with a lower relapse rate in the subgroup of AML with mutated chromatin and/or RNA-splicing genes ($p = 0.039$) and a higher relapse rate in patients with *RUNX1–RUNX1T1* fusion gene ($p < 0.001$). A significant interaction with lomustine was also highlighted using the Bullinger classification ($p = 0.005$) with a lower relapse rate

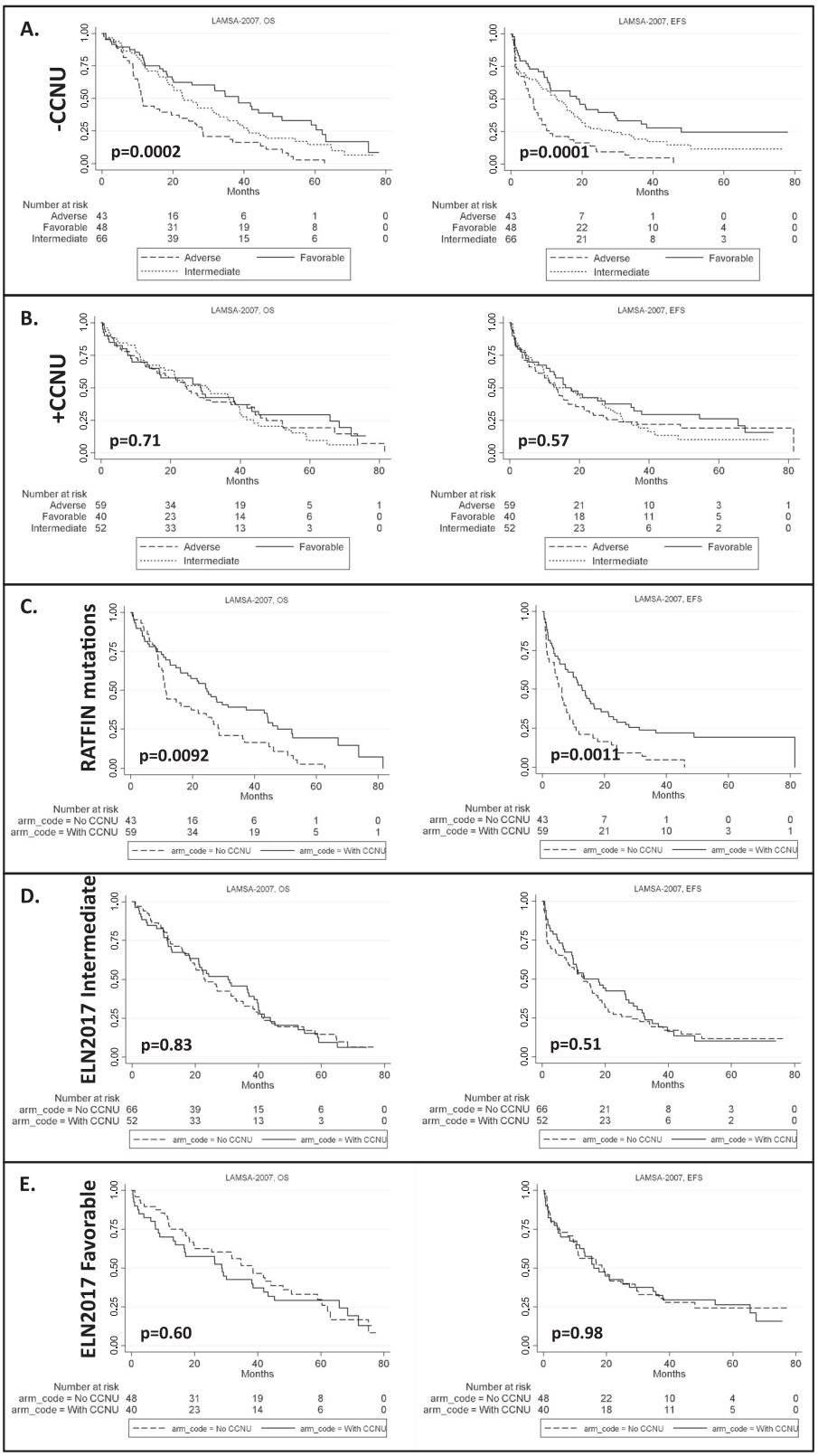

Fig. 5 Clinical responses (OS and EFS) to lomustine (lomustine) according to the ELN2017 risk classification. a ELN2017 risk classification of patients not treated with lomustine. **b** ELN2017 risk classification of patients treated with lomustine. **c** RATFIN mutated patients treated or not with lomustine. **d** ELN2017 intermediate risk classification of patients treated or not with lomustine. **e** ELN2017 favorable risk classification of patients treated or not with lomustine.

in the chromatin-spliceosome group and *TP53* mutations ($p = 0.030$ and $p < 0.001$, respectively). No interaction was observed between lomustine treatment and the classifications of Patel et al., Lindsley et al., Papaemmanuil et al., or Bullinger et al. for EFS ($p = 0.867$, 0.370, 0.232, and 0.127, respectively) nor OS ($p = 0.896$, 0.758, 0.261,

and 0.127, respectively). However, there was a significant interaction between the ELN2017 classification and lomustine ($p = 0.036$ for EFS; $p = 0.048$ for OS; Fig. 5a, b), indicating that lomustine was significantly associated with a better EFS ($p < 0.001$) and OS ($p = 0.023$) in RATFIN mutations (Fig. 5c) but not intermediate ($p = 0.162$ for EFS; $p = 0.599$ for OS; Fig. 5d) nor favorable ($p = 0.763$ for EFS; $p = 0.199$ for OS, Fig. 5e) subgroups. The advantage of the lomustine addition was stronger in patients with *TP53* and *FLT3*-ITD^{high}/*NPM1*^{WT} mutations (Fig. S11).

Discussion

This study corresponds to a post-hoc analysis of the LAM-SA 2017 phase 3 randomized trial and describes the genomic landscape of older AML patients with nonadverse cytogenetics risk selected for intensive chemotherapy. Whereas we confirmed previous studies regarding the distribution and gene-gene interactions of the most frequent mutations including *FLT3*-ITD, *NPM1*, *DNMT3A*, *RUNX1*, or *ASXL1* [22, 23], we also described a rare subgroup of patients with *DDX41* mutations, which is characterized by few co-mutations, leukopenia, and a probable better outcome in agreement with the recent publication of Sebert et al. [24].

The FILO study group has been using lomustine for decades with consistent favorable results regarding CR achievement after one course of induction and survival endpoints [9, 25]. However, adding a third cytotoxic agent to an anthracycline-cytarabine induction may increase general toxicity especially hematologic toxicity and infections. Although not significant, the early death rate was slightly increased in the lomustine arm in the LAM-SA 2017 trial [9]. Thus, defining patients who benefit most from lomustine is of considerable importance.

We assessed the prognostic impact of five recent molecular classifications [8, 13–16]. Overall, these classifications have been relatively effective in predicting EFS and risk of relapse in older AML patients although only ELN2017, Patel et al.'s, and Lindsley et al.'s classifications were associated with OS. Moreover, with regard to the main clinical endpoints, no consistent pattern of interaction between the impact of lomustine and most molecular classifications was observed except for the ELN2017 classification [8] suggesting that lomustine could benefit mostly to patients with RATFIN mutations (ELN2017 adverse risk with nonadverse risk cytogenetics) [8]. We acknowledge that this result could correspond to a type I error (i.e., a false positive result) linked to the multiplication of analyses or a true interaction between the ELN2017 score and lomustine treatment; therefore, these results should be confirmed by a prospective randomized trial in this specific subgroup of patients with RATFIN mutations.

Lomustine is an alkylating agent of the nitrosourea type that alkylates and cross-links DNA thereby inhibiting DNA and RNA synthesis [26]. DNA damage repair is mainly mediated by the O^6-methylguanine DNA methyltransferase the expression of which is very low in AML compared to other cancers [26]. Moreover, lomustine activity is cell cycle-phase nonspecific, a property not shared by anthracyclines and cytarabine, that may be important to target noncycling cells of the AML clones. Of note, lomustine is lipophilic and crosses the blood–brain barrier. The addition of lomustine may explain the absence of prognosis of *FLT3*-ITD in our series, in contrast to the well-described worse prognosis of patients with *FLT3*-ITD [27]. TP53 allows the repair of interstrand cross-links through the upregulation of the DNA repair factors XPC and DDB2 [28]. In a model of glioma, the DNA double strand breaks generated by chloroethylating nitrosourea were not repaired when *TP53* was mutated contrary to cells with normal *TP53* [28]. Nevertheless, as *RUNX1* and *ASXL1* represent the most frequent high-risk mutations in this RATFIN group, the impact of lomustine could affect these mutations through mechanisms that remain to be elucidated.

As lomustine is used during front-line treatment, the ELN2017 status must be rapidly defined for an optimal use of this drug. This may be challenging especially for *ASXL1* and *RUNX1* genes. However, we have previously shown that waiting a short period of time before induction chemotherapy is safe in AML patients and allow a molecular testing before choosing the most appropriate induction regimen [29]. A recent study has also shown an interaction between genetic profiles and gemtuzumab ozogamycin efficacy indicating that molecular stratification is useful for a rational use of targeted therapies but also of cytotoxic agents in AML patients [30].

Acknowledgements We thank physicians from participating centers as well as central and local data managers for collecting and monitoring patients' data. We thank Filothèque members for the conservation of patients samples; Catherine Lacombe, Rachid Boulghana, Laure Cabaret, Ségolène Diry, Krishshanthi Sinnadurai, and Shari Dini Mohamed.

French Innovative Leukemia Organization J.-P. Marolleau[26], A. Aleme[26], F. Orsini-Piocelle[27], N. Cadoux[27], N. Ifrah[28], M. Hunault[28], C. Marie[28], A. Al Jijakli[29], G. Lepeu[30], H. Zerazhi[30], M. Beyrne[30], A. Banos[31], S. Labarrere[31], E. Deconinck[32], M. Peria[32], A. El Yamani[33], O. Kadiri[33], B. Choufi[34], M. Brument[34], A. Pigneux[35], T. Leguay[35], P.-Y. Dumas[35], C. Berthou[36], G. Guillerm[36], G. Drugmanne[36], O. Tournilhac[37], G. Roy[37], B. Audhuy[38], S. Camara[38], D. Caillot[39], M. Grandjean[39], J.-Y. Cahn[40], C.-E. Bulabois[40], B. Fief[40], N. Vey[41], C. Ladraa[41], V. Dorvaux[42], M. Hagopian[42], N. Fegueux[43], C. Fenoll[43], V. Sabadash[43], M. Ojeda[44], C. Haby[44], F. Witz[45], C. Bonmati[45], M. Lhuire[45], J. Delaunay[46], P. Peterlin[46], L. Airiau[46], L. Mannone[47], I.

Touitou[47], E. Jourdan[48], D. Umuhire[48], M. Alexis[49], O. Michel[49], F. Dreyfus[50], D. Bouscary[50], A. Cheung[50], L. Sanhes[51], F. Touhami[51], E. Ribas[51], M. Puyade[52], M.-P. Gallego-Hernanz[52], N. Hugon[52], C. Himberlin[53], L. Maggi[53], T. Lamy[54], A. Testu[54], E. Tavernier[55], S. Marchand[55], B. Lioure[56], C. Kravanja[56], L. Benboubker[57], D. Nollet[57], M. Attal[58], C. Recher[58], A. Sarry[58], A. Lhermitte[59], G. Yrica[59], D. Schwartz[59], N. Le Montagner[59], C. Fenoll[59], V. Sabadash[59], D. Nollet[59], L. Auvray[59], R. Delepine[59], A. Fayault[59]

[26]Hematology Department, CHU Amiens, Amiens, France; [27]Hematology Department, CH Annecy-Genevois, Annecy, France; [28]Hematology Department, CHU Angers, Angers, France; [29]Hematology Department, CH Argenteuil, Argenteuil, France; [30]Hematology Department, CH Avignon, Avignon, France; [31]Hematology Department, CH Côte Basque, Bayonne, France; [32]Hematology Department, CHU Besancon, Besancon, France; [33]Hematology Department, CH Simone Veil, Blois, France; [34]Hematology Department, CH Boulogne sur Mer, Boulogne sur Mer, France; [35]Hematology Department, CHU Bordeaux, Bordeaux, France; [36]Hematology Department, CHU Brest, Brest, France; [37]Hematology Department, CHU Clermont-Ferrand, Clermont-Ferrand, France; [38]Hematology Department, Hôpitaux Civils de Colmar, Colmar, France; [39]Hematology Department, CHU Dijon, Dijon, France; [40]Hematology Department, CHU Grenoble-Alpes, Grenoble, France; [41]Hematology Department, Institut Paoli-Calmettes, Marseille, France; [42]Hematology Department, CHR Metz-Thionville, Metz, France; [43]Hematology Department, CHU Montpellier, Montpellier, France; [44]Hematology Department, CH Emile-Muller, Mulhouse, France; [45]Hematology Department, CHRU Nancy, Nancy, France; [46]Hematology Department, CHU Nantes, Nantes, France; [47]Hematology Department, CHU Nice, Nice, France; [48]Hematology Department, CHU Nimes, Nimes, France; [49]Hematology Department, CHR Orléans, Orleans, France; [50]Hematology Department, CHU Cochin, Paris, France; [51]Hematology Department, CH Perpignan, Perpignan, France; [52]Hematology Department, CHU Poitiers, Poitiers, France; [53]Hematology Department, CHU Reims, Reims, France; [54]Hematology Department, CHU Rennes, Rennes, France; [55]Hematology Department, CHU Saint-Etienne, Saint Priest En Jarez, France; [56]Hematology Department, CHU Strasbourg, Strasbourg, France; [57]Hematology Department, CHU Tours, Tours, France; [58]Hematology Department, CHU Toulouse, Toulouse, France; [59]Filo Central Office, Tours, France

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Juliusson G, Abrahamsson J, Lazarevic V, Antunovic P, Derolf A, Garelius H, et al. Prevalence and characteristics of survivors from acute myeloid leukemia in Sweden. Leukemia. 2017;31:728–31.
2. Oran B, Weisdorf DJ. Survival for older patients with acute myeloid leukemia: a population-based study. Haematologica. 2012;97:1916–24.
3. DiNardo CD, Wei AH. How I treat acute myeloid leukemia in the era of new drugs. Blood. 2020;135:85–96.
4. Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, Harrison CJ, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood. 2001;98:1312–20.
5. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20:1472–8.
6. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488–98.
7. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371:2477–87.
8. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129:424–47.
9. Pigneux A, Bene MC, Salmi LR, Dumas PY, Delaunay J, Bonmati C, et al. Improved survival by adding lomustine to conventional chemotherapy for elderly patients with aml without unfavorable cytogenetics: results of the LAM-SA 2007 FILO trial. J Clin Oncol. 2018;36:3203–10.
10. Gerson SL. Regeneration of O6-alkylguanine-DNA alkyltransferase in human lymphocytes after nitrosourea exposure. Cancer Res. 1988;48:5368–73.
11. Weiss RB, Tormey DC, Holland F, Weinberg VE, Lesnick G, Perloff M, et al. A randomized trial of postoperative five-versus three-drug chemotherapy after mastectomy: a Cancer and Leukemia Group B (CALGB) study. Recent Results Cancer Res. 1982;80:170–6.
12. Marsh JC. The effects of cancer chemotherapeutic agents on normal hematopoietic precursor cells: a review. Cancer Res. 1976;36:1853–82.
13. Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366:1079–89.
14. Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125:1367–76.
15. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374:2209–21.
16. Bullinger L, Dohner K, Dohner H. Genomics of acute myeloid leukemia diagnosis and pathways. J Clin Oncol. 2017;35:934–46.
17. Dastugue N, Payen C, Lafage-Pochitaloff M, Bernard P, Leroux D, Huguet-Rigal F, et al. Prognostic significance of karyotype in de novo adult acute myeloid leukemia. The BGMT group. Leukemia. 1995;9:1491–8.
18. LaRochelle O, Bertoli S, Vergez F, Sarry JE, Mansat-De Mas V, Dobbelstein S, et al. Do AML patients with DNMT3A exon 23 mutations benefit from idarubicin as compared to daunorubicin? A single center experience. Oncotarget. 2011;2:850–61.
19. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet. 2001;27:263–70.
20. Poplin R, Ruano-Rubio V, DePristo M A, Fennell T J, Carneiro M O, Van der Auwera G A, et al. Scaling accurate genetic variant discovery to tens of thousands of samples. bioRxiv 201178; https://doi.org/10.1101/201178.
21. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303.
22. Eisfeld AK, Kohlschmidt J, Mrozek K, Blachly JS, Walker CJ, Nicolet D, et al. Mutation patterns identify adult patients with de novo acute myeloid leukemia aged 60 years or older who respond

favorably to standard chemotherapy: an analysis of alliance studies. Leukemia. 2018;32:1338–48.
23. Metzeler KH, Herold T, Rothenberg-Thurley M, Amler S, Sauerland MC, Gorlich D, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. Blood. 2016;128:686–98.
24. Sebert M, Passet M, Raimbault A, Rahme R, Raffoux E, Sicre de Fontbrune F, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. Blood. 2019;134:1441–4.
25. Pigneux A, Harousseau JL, Witz F, Sauvezie M, Bene MC, Luquet I, et al. Addition of lomustine to idarubicin and cytarabine improves the outcome of elderly patients with de novo acute myeloid leukemia: a report from the GOELAMS. J Clin Oncol. 2010;28:3028–34.
26. Nikolova T, Roos WP, Kramer OH, Strik HM, Kaina B. Chloroethylating nitrosoureas in cancer therapy: DNA damage, repair and cell death signaling. Biochim Biophys Acta Rev Cancer. 2017;1868:29–39.
27. Whitman SP, Maharry K, Radmacher MD, Becker H, Mrozek K, Margeson D, et al. FLT3 internal tandem duplication associates with adverse outcome and gene- and microRNA-expression signatures in patients 60 years of age or older with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Blood. 2010;116:3622–6.
28. Batista LF, Roos WP, Christmann M, Menck CF, Kaina B. Differential sensitivity of malignant glioma cells to methylating and chloroethylating anticancer drugs: p53 determines the switch by regulating xpc, ddb2, and DNA double-strand breaks. Cancer Res. 2007;67:11886–95.
29. Bertoli S, Berard E, Huguet F, Huynh A, Tavitian S, Vergez F, et al. Time from diagnosis to intensive chemotherapy initiation does not adversely impact the outcome of patients with acute myeloid leukemia. Blood. 2013;121:2618–26.
30. Fournier E, Duployez N, Ducourneau B, Raffoux E, Turlure P, Caillot D, et al. Mutational profile and benefit of gemtuzumab ozogamicin in acute myeloid leukemia. Blood. 2020;135:542–6.

Affiliations

Laetitia Largeaud[1] · Pascale Cornillet-Lefebvre[2] · Jean-François Hamel[3] · Pierre-Yves Dumas[4] · Naïs Prade[1] · Stéphanie Dufrechou[1] · Julien Plenecassagnes[5] · Isabelle Luquet[1] · Odile Blanchet[6] · Anne Banos[7] · Marie C. Béné[8] · Marc Bernard[9] · Sarah Bertoli[10] · Caroline Bonmati[11] · Luc Matthieu Fornecker[12] · Romain Guièze[13] · Lamya Haddaoui[14] · Mathilde Hunault[15] · Jean Christophe Ianotto[16] · Eric Jourdan[17] · Mario Ojeda[18] · Pierre Peterlin[19] · Norbert Vey[20] · Hacene Zerazhi[21] · Hicheri Yosr[20,22] · Ariane Mineur[23,24] · Jean-Yves Cahn[25] · Norbert Ifrah[15] · Christian Récher[10] · Arnaud Pigneux[4] · Eric Delabesse[1] · on behalf of the French Innovative Leukemia Organization (FILO)

1 Hematology Biology, Centre Hospitalier Universitaire de Toulouse, Institut Universitaire du Cancer de Toulouse Oncopôle, Université Toulouse III Paul Sabatier, Toulouse, France

2 Hematology Biology, Reims University Hospital, Reims, France

3 Department of Statistics, Angers University Hospital, Angers, France

4 Clinical Hematology, Bordeaux University Hospital, Bordeaux University, Inserm 1035, Bordeaux, France

5 Bioinformatic Department, Claudius Regaud Institute, Institut Universitaire du Cancer de Toulouse Oncopôle, Toulouse, France

6 Hematology Biology, Angers University Hospital, Angers University, Inserm, CRCINA, Angers, France

7 Clinical Hematology, Cote Basque General Hospital, Bayonne, France

8 Hematology Biology, Nantes University Hospital, Nantes, France

9 Clinical Hematology, Rennes University Hospital, Rennes, France

10 Clinical Hematology, Centre Hospitalier Universitaire de Toulouse, Institut Universitaire du Cancer de Toulouse Oncopôle, Université Toulouse III Paul Sabatier, Toulouse, France

11 Clinical Hematology, Nancy University Hospital, Nancy, France

12 Clinical Hematology, Strasbourg University Hospital, Strasbourg, France

13 Clinical Hematology, Clermont-Ferrand University Hospital, Clermont-Ferrand, France

14 FILO Tumor Bank, Pitié-Salpêtrière Hospital, Paris, France

15 Clinical Hematology, Angers University Hospital, INSERM U 892/CNRS 6299, Angers, France

16 Clinical Hematology, Institute of Oncology and Hematology, Brest University Hospital, Brest, France

17 Clinical Hematology, Nîmes University Hospital, Nîmes, France

18 Clinical Hematology, GHRMSA, Hôpital E Muller, Mulhouse, France

19 Clinical Hematology, Nantes University Hospital, Nantes, France

20 Clinical Hematology, Aix-Marseille University, Inserm, CNRS, Institut Paoli-Calmettes, CRCM, Marseille, France

21 Clinical Hematology, Avignon General Hospital, Avignon, France

22 Clinical Hematology, Montpellier University Hospital, Montpellier, France

23 Clinical Research Unit, Bordeaux University Hospital, Bordeaux, France

24 FILO, Tours University Hospital, Tours, France

25 Clinical Hematology, Grenoble University Hospital, Grenoble, France