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# Impact of gene mutations on treatment response and prognosis of acute myeloid leukemia secondary to myeloproliferative neoplasms

Running title: Molecular prognosis of acute myeloid leukemia secondary to myeloproliferative neoplasms

*Venton G.<sup>1, 2\*</sup>, Courtier F.<sup>2, 3, 4\*</sup>, Charbonnier A.<sup>1</sup>, D’Incan E.<sup>1</sup>, Saillard C.<sup>1</sup>, Mohty B.<sup>1</sup>, Mozziconacci MJ.<sup>2, 3</sup>, Birnbaum D.<sup>2, 3, 4</sup>, Murati A.<sup>2, 3, 4</sup>, Vey N.<sup>1, 2, 4</sup> and Rey J.<sup>1, 2</sup>*

<sup>1</sup> *Institut Paoli-Calmettes (IPC), Département d’Hématologie*

<sup>2</sup> *Centre de Recherche en Cancérologie de Marseille, (CRCM), Inserm, U1068, CNRS UMR7258*

<sup>3</sup> *Institut Paoli-Calmettes (IPC), Département d’Oncologie Moléculaire*

<sup>4</sup> *Aix-Marseille Université, UM 105, Marseille, F-13284, France*

*\*Equal contribution*

## **Author's information:**

Geoffroy Venton, MD, PhD, Institut Paoli-Calmettes (IPC), Département d'hématologie, 232

Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail: [ventong@ipc.unicancer.fr](mailto:ventong@ipc.unicancer.fr)

Frédéric Courtier, PhD, Centre de Recherche en Cancérologie de Marseille, (CRCM), Inserm, U1068, CNRS UMR7258, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail : [frederic.courtier@inserm.fr](mailto:frederic.courtier@inserm.fr)

Aude Charbonnier, MD, PhD, Institut Paoli-Calmettes (IPC), Département d'hématologie, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.33.11.

Mail : [charbonniera@ipc.unicancer.fr](mailto:charbonniera@ipc.unicancer.fr)

Evelyne D'Incan, MD, Institut Paoli-Calmettes (IPC), Département d'hématologie, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail: [dincane@ipc.unicancer.fr](mailto:dincane@ipc.unicancer.fr)

Colombe Saillard, MD, Institut Paoli-Calmettes (IPC), Département d'hématologie, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail: [saillardc@ipc.unicancer.fr](mailto:saillardc@ipc.unicancer.fr)

Bilal Mohty, MD, Institut Paoli-Calmettes (IPC), Département d'hématologie, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail: [mohtyb@ipc.unicancer.fr](mailto:mohtyb@ipc.unicancer.fr)

Marie-Joelle Mozziconacci, MD, Institut Paoli-Calmettes (IPC), Département d'Oncologie Moléculaire, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.33.11.

Mail: [mozziconaccimj@ipc.unicancer.fr](mailto:mozziconaccimj@ipc.unicancer.fr)

Daniel Birnbaum, MD, PhD, Centre de Recherche en Cancérologie de Marseille, (CRCM), Inserm, U1068, CNRS UMR7258, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail : [daniel.birnbaum@inserm.fr](mailto:daniel.birnbaum@inserm.fr)

Anne Murati, MD, PhD, Centre de Recherche en Cancérologie de Marseille, (CRCM), Inserm, U1068, CNRS UMR7258, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail: [muratia@ipc.unicancer.fr](mailto:muratia@ipc.unicancer.fr)

Norbert Vey, MD, PhD, Institut Paoli-Calmettes (IPC), Département d'hématologie, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail: [veyn@ipc.unicancer.fr](mailto:veyn@ipc.unicancer.fr)

Jérôme Rey, MD, Institut Paoli-Calmettes (IPC), Département d'hématologie, 232 Boulevard de Sainte-marguerite, 13009 Marseille. Phone: 04.91.22.36.96.

Mail: [reyj@ipc.unicancer.fr](mailto:reyj@ipc.unicancer.fr)

Corresponding Author: Frederic Courtier, mail: [frederic.courtier@inserm.fr](mailto:frederic.courtier@inserm.fr)

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## Abstract

Acute myeloid leukemias secondary (sAML) to myeloproliferative neoplasms (MPN) have variable clinical courses and outcomes, but remain almost always fatal. Large cohorts of sAML to MPN are difficult to obtain and there is very little scientific literature or prospective trials for determining robust prognostic markers and efficient treatments.

We analyzed event-free survival (EFS) and overall survival (OS) of 73 patients with MPN who progressed to sAML, based on their epidemiological characteristics, the preexisting MPN, the different treatments received, the different prognostic groups and the responses achieved according to the ELN, and their mutational status determined by next-generation DNA sequencing (NGS). For 24 patients, we were able to do a comparative NGS analysis at both MPN and sAML phase.

After acute transformation EFS and OS were respectively of 2.9 months (range: 0-48.1) and 4.7 months (range: 0.1-58.8). No difference in EFS or OS regarding the previous MPN, the ELN2017 prognostic classification, the first-line therapy or the response was found. After univariate analysis, three genes, *TP53*, *SRSF2* and *TET2*, impacted pejoratively sAML prognosis at sAML time. In multivariate analysis, *TP53* ( $p=0.0001$ ), *TET2* ( $p=0.011$ ) and *SRSF2* ( $p=0.018$ ) remained independent prognostic factors. Time to sAML transformation was shorter in *SRSF2*-mutated patients (51.2 months, range: 14.7-98) than in *SRSF2*-unmutated patients (133.8 months, range: 12.6-411.2) ( $p<0.001$ ).

Conventional clinical factors (age, karyotype, ELN2017 prognostic classification, treatments received, treatments response, Allo-SCT...) failed to predict the patients' outcome. Only the mutational status appeared relevant to predict patients' prognosis at sAML phase.

*Key words:* Myeloproliferative Neoplasm; secondary Acute Myeloid Leukemia; Next-generation DNA sequencing; *TP53*; *TET2*; *SRSF2*

## **Background**

Leukemic transformation of BCR-ABL1-negative myeloproliferative neoplasms (MPN) occurs in 8% to 23% of primary myelofibrosis (PMF) patients in the first 10 years after diagnosis and in 4% to 8% of polycythemia vera (PV) and essential thrombocythia (ET) patients within 18 years after diagnosis and is almost always fatal <sup>1</sup>. Acute myeloid leukemias secondary (sAML) to MPN have variable clinical courses and outcomes <sup>2 3 4</sup>. Large cohorts of sAML to MPN are difficult to obtain and there is little scientific literature or prospective trials for determining the best treatment strategies.

There is no specific treatment for post MPN sAML. Classical AML-type induction regimen like 3+7 are used in eligible patients but the response rate is low, toxicity and mortality high with poor long term results <sup>5</sup>. Encouraging results have been obtained with hypomethylating agents but data are still limited and confirmation is warranted <sup>6</sup>. One major limit for improving treatment results is that MPN-AML are generally excluded from large multicenter AML trials and few dedicated studies have been conducted so far.

Most of BCR-ABL1-negative MPNs have recurrent somatic driver mutations in either Janus kinase 2 (*JAK2*), calreticulin (*CALR*) exon 9 or myeloproliferative leukemia virus oncogene (*MPL*). Survival and blast transformation rates differ between PV, ET, and PMF according to their specific cytogenetic abnormalities or mutational status at MPN phase <sup>1 7 8</sup>. However, little is known of the impact of mutational status at leukemic transformation time and whether it may add information to conventional prognostic stratification systems.

In a previous study, we described genetic events occurring during acute transformation of MPN<sup>9</sup>. Here, we have enriched our cohort in post MPN sAML cases and we have studied and analyzed EFS and OS of 73 patients with MPN who progressed to sAML. The objectives of this retrospective study were to compare treatment options, assess in this cohort the value of currently used response criteria, the prognostic relevance of the recently proposed ELN2017 genomic risk stratification and of gene mutations<sup>5</sup>.

## Methods

The study was approved by the institutional review board of the Paoli-Calmettes Institute (Marseille, France) and conducted in accordance with the Declaration of Helsinki. A total of 73 patients diagnosed in our institute with post-MPN sAML between January 2000 and December 2016 were analyzed.

### *Treatments received and therapeutic response*

The treatments received by the patients were classified in three groups. The first group referred to as intensive chemotherapy (IC) included the 3+7 regimen and its variants (ie daunorubicin (DNR) 90mg/m<sup>2</sup> from day 1 to day 3 associated with cytarabine (AraC) 200mg/m<sup>2</sup> from day 1 to day 7; or idarubicin (IDR) 9mg/m<sup>2</sup> from day 1 to day 4 associated with AraC 200mg/m<sup>2</sup> from day 1 to day 7 (in patients older than 65) or IDR 8 mg/m<sup>2</sup> associated with AraC 800 mg/m<sup>2</sup> from day 1 to day 5 and etoposide 150 mg/m<sup>2</sup> and from day 1 to day 3 (ICE regimen))<sup>5</sup>. The second group corresponded to azacytidine (AZA) (75mg/m<sup>2</sup>, from day 1 to day 7, every 28 days), which was given to patients non eligible for IC. The third group, the palliative care group (PC), included palliative chemotherapy such as low dose of AraC (associated or not with etoposide), 6-mercaptopurine and methotrexate, hydroxyurea or

best supportive care only. AML treatment response was evaluated according to ELN 2017 response criteria<sup>5</sup>.

### *NGS analysis*

Targeted NGS was done for 56 patients. For 24 patients NGS was done on both sAML and MPN (Supporting Information Table 1 and Supporting Information Table 2). DNAs from peripheral blood leukocytes (n=69) or bone marrow aspirates (n=11) were extracted and prepared for sequencing with Qiagen AllPrep DNA/RNA Mini Kit (Qiagen, Germany) or MACHEREY-NAGEL NucleoSpin Blood (MACHEREY-NAGEL, Germany). For each sample, a library of all coding exons and intron-exon boundaries of 79 genes, selected on the basis of their known involvement in the pathogenesis of myeloid malignancies, was constructed with HaloPlex target enrichment system (Agilent technologies, Santa Clara, USA).

Sequencing was done as previously described, on a MiSeq (Illumina) with 2x150-bp, paired-end reads according to the manufacturer's instructions. The read sequencing was aligned to human reference genome (UCSC hg19) (<http://hgdownload.cse.ucsc.edu/>) using Burrows-Wheeler Aligner.<sup>9</sup> Samples were sequenced at about 700 x coverage, which allowed the identification of missense, nonsense, splicing, frameshift and nonframeshift mutations with quantitative data on variant allele frequency (VAF). Bam files were processed according to the workflow recommended for variant analysis with GATK. Briefly, reads groups were added with PICARD tools version 1.91(1451) (<http://broadinstitute.github.io/picard/>). Local realignment and score recalibration were done using GATK version 2.5-2-gf57256b. SNVs calling was done with FreeBayes version 0.9.9 with a minimal alternate variant frequency and coverage set at 2% and 10. Indel calling was done using GATK haplotype caller version 2.5-2-gf57256b with default parameters. The variants, i.e SNVs and Indels, were annotated with



RefSeq annotation, dbsnp129, dbsnp138NonFlagged, 1000 Genome and ESP6500 population frequencies, COSMIC V68, Clinvar, and predicted effects score on the protein using the Annotate Variation Software (ANNOVAR, version 2013-11-12). Mutations predicted as "neutral" were excluded. SNVs were further filtered. Known variants found in dbsnp129 and dbsnp137 with a MAF > 1% (1000g or ESP6500) or suspected (according to data literature and VAF percentage) to be germline were removed. Finally, low frequency SNVs and indels suspected to be false positive were systematically inspected with IGV version 2.3.32.

In addition, the JAK2V617F mutation was also determined by real-time quantitative PCR; MPLW515 was detected by Sanger sequencing and CALR exon 9 mutations were determined as previously described by fragment analysis techniques followed by Sanger sequencing<sup>10</sup>.

### *Statistical Analysis*

Correlations between mutated genes and factors considered as continuous variables were calculated with the Student's t-test and Fisher's exact test for variable assessed as binary (Performed with R i386 3.3.0 software). Data sets were described with median and range as indicated. Determination of the genes of interest was done by literature selection and upon significance in univariate Cox regression analysis for survival (Wald test, Performed with R). We included in univariate analyses only genes mutated in at least 5 patients. Variables with a p-value < 0.05 in univariate analysis were tested in multivariate analysis. All statistical tests were two-sided at the 5% level of significance. Survival comparisons were done by Kaplan-Meier method and compared by log-rank (Performed with GraphPad Prism 5.01 software).

## **Results**

### ***Patient's characteristics***

Seventy three patients who developed post-MPN sAML between January 2000 and December 2016 were included and retrospectively analyzed. Twenty one patients (28.8%) had prior ET, 17 (23.3%) PV, 17 (23.3%) PMF, and 18 (24.7%) secondary myelofibrosis (sMF). Median age at AML transformation was 70 (range: 38-89). Median time to AML transformation from MPN diagnosis was 110.7 months (range: 2.6-411.2). The median number of previous treatments at MPN phase was 1 (range, 0-6). Molecular status at MPN phase was known for 51/73 patients (%). Among them, 43 (58.9%) had a *JAK2* mutation, 7 patients (9.6%) a *CALR* mutation and 1 (1.4%) an *MPL* mutation and 8 patients were triple negative (TN). At AML transformation time, *JAK2*, *CALR*, *MPN* and TN mutational status remained generally unchanged except for 7 of the 56 sequenced patients who lost their *JAK2* mutation to become TN. Patients' characteristics and mutational status are summarized in Table 1. As shown, there was no significant difference in clinical or biological characteristics according to the prior MPN type (ie ET versus PV versus MF versus sMF).

### ***Treatment and response***

As shown in Table 1, 46.6% of the patients received IC, 15.1% received AZA and 38.4% palliative treatment. EFS was respectively of 4.2 months (range: 0-48.1), 5.8 months (range: 1.9-18.9) and 1.4 months (range: 0.1-44.1) in IC, AZA and PC treatment groups. OS was respectively of 8.3 months (range: 1.3-58.8), 7.9 months (range: 2.3-24.3) and 1.8 months (range: 0.1-45.7) in IC, AZA and PC treatment groups. Patients treated by IC or AZA had a better EFS ( $p<0.0001$ ) and OS ( $p<0.0001$ ) than patients in PC, both in univariate and multivariate analysis (Supporting Information Table 3). However, as shown in Figure 1A and Supporting Information Figure S1A, no difference in EFS ( $p=0.4443$ ) or OS (0.9842) was found between IC and AZA treatment groups. IC and AZA produced similar overall response

rate (ORR) (respectively 58.8% vs. 54.6%) and day-30 mortality rate were respectively 8.8% vs.0%.

Treatment response was evaluated in the 45 patients treated actively (IC and AZA groups) according to the ELN2017 response criteria : 12 (26.7%) were in complete remission (CR), 8 (17.8%) were in complete remission with incomplete hematologic recovery (CRi), 10 (22.2%) presented a primary refractory disease (PRD), 1 patient was in partial remission (PR) (2.2%), 5 patients were in stable disease (SD) (11.1%), 3 patients (6.7 %) were in progression disease (PD), 3 deceased in aplasia (6.7%) and 3 patients were not evaluable (6.7%). OS were evaluated according to the ELN2017 response criteria. In univariate analysis, patients in CR/CRi after the first-line therapy had a better OS than patients in PRD/PR/SD/PD ( $p=0.0061$ ). However, after regression logistic, this result did not remain significant (Supporting Information Table 3). As shown in Figure 1B, after landmark analysis, OS was respectively of 9.9 months (range: 2.8-57.7) in the CR/CRi group and 5.3 months (range: 0.1-37.8) in the PRD/PR/SD/PD group ( $p=0.3622$ : NS). Among the 20 patients in CR or CRi, 9 (45%) received allogeneic stem cell transplantation (Allo-SCT) in first CR. In univariate analysis, grafted patients had a better OS (24.2 months, range: 2.9-57.7) than ungrafted patients (7.2 months, range: 2.8-25.3) ( $p=0.0036$ ). However, after regression logistic, this result did not remain significant ( $p=0.34$ : NS) (Figure 1C and Supporting Information Table 3).

### ***Prognostic factors and patients outcome***

After acute transformation EFS and OS were respectively of 2.9 months (range: 0-48.1) and 4.7 months (range: 0.1-58.8). Patients older than 65 had a lower OS (3.7 months, range: 0.2-45.7) than patients under 65 (7 months, range: 0.1-58.8) ( $p=0.0323$ ). No difference in EFS or OS regarding the prior MPN (data not shown) was found. No difference in OS was found between sAML patients with (4.9 months, range: 0.2-29) and without *JAK2* mutation (6.5

months, range: 0.1-58.8). Similarly, no difference in OS was found between sAML patients with (4.7 months, range: 1.9-45.7) and without *CALR* mutation (5.4 months, range: 0.1-58.8) (Supporting Information Figure S2).

According to ELN 2017, among the 73 sAML patients, 3 (4.1 %), 10 (13.7%) and 46 (63 %) respectively belonged to the favorable, intermediate and adverse risk category. Given the small number of patients in the favorable (Fav) (n=3) and intermediate (Int) (n=10) categories, these two groups were merged in the survival analysis. As shown in Figure 1D and Supporting Information Figure S1B, based on ELN prognostic classification, EFS was respectively of 5.3 months (range: 0-37.5) in the Fav/Int group vs. 2.9 months (range: 0.1-44.4) in the adverse group (p=0.3011). In the same way, no significant difference in OS was observed between the Fav/Int group (8.3 months, range: 0.2-39) and the adverse group (4.7 months, range: 0.2-58.8) (p=0.1846: NS). Regardless of the ELN2017 prognostic classification, no significant difference in EFS or OS was observed according to the cytogenetic status alone. OS was respectively of 4.8 months (range: 0.3-45.7) in patients with a Fav/Int karyotype vs. 6.8 months (range: 0.2-58.8) in patients with an adverse karyotype (p=0.183: NS).

### ***NGS analysis and impact of the mutational status on patient's outcome***

Among the 73 sAMLs, 56 were studied by NGS. Forty three genes were mutated in the total cohort with a mean of 3.5 genes mutated per patient. The most frequently mutated genes were *JAK2* (33/56), *TP53* (20/56), *ASXL1* (14/56), *TET2* (11/56), *SRSF2* (9 /56), *DNMT3A* (8/56), *NRAS/KRAS* (8/56), *CALR* (7/56), *IDH1/2* (7/56), *EZH2* (7/56) and *RUNX1* (7/56) (Table 1 and Figure 2). The number of mutated genes per patient was not different according to the MPN type. However, at sAML phase, a different repartition of the mutations was found according to the diagnostic of MPN. Post-PMF AMLs (n=12) were more mutated in *ASXL1*

and *SRSF2* genes than post-ET/PV/sMF AMLs (n=44) (respectively 66.7% vs 13.6%,  $p<0.001$  and 50% vs 6.8%,  $p<0.01$ ). Post-ET/PV AMLs (n=31) were more mutated in *TP53* than post-PMF/sMF AMLs (n=25) (51.6% vs 16.0%,  $p<0.01$ ). No difference was found according to the MPN gene driver (*JAK2*, *CALR* or TN). Our cohort had 7 *CALR*-mutated post-MPN AMLs and 4 cases were co-mutated with *TP53*. *CALR*-mutated cases had few mutated genes (means of 2.9). They never had mutations in genes affecting signaling, and other functions such as RNA splicing or transcriptional factors were seldom altered in *CALR*-mutated post-MPN AMLs.

After univariate analysis, beside the patient's age, the front-line treatment (IC/AZA vs. PC), the treatment response (CR/CRi or not) or the performance of an Allo-SCT, three genes, *TP53*, *SRSF2* and *TET2*, impacted pejoratively sAML prognosis at acute phase. All significant factors in univariate analysis were included in the multivariate analysis. After regression logistic, *TP53* ( $p=0.001$ ), *TET2* (0.011) and *SRSF2* (0.018) mutations remained independent factors of prognosis, as well as the front-line treatment (IC/AZA vs. PC) ( $p=0.0003$ ) (Supporting Information Table 3).

As shown in Figure 3A, patients with *TP53* mutation had a shorter OS (4.4 months, range: 0.2-15.6) than patients with *TP53* wild type (WT) (6.5 months, range: 0.2-58) ( $p=0.02$ ). Concerning the impact of *TP53* mutational status in the different treatment groups, in IC treatment group, *TP53*-mutated patients had a lower OS (4.7 months, range: 1.9-14.5) than *TP53*-WT patients (11.1 months, range: 2.2-58.8) ( $p=0.0017$ ). In the AZA treatment group OS of *TP53*-mutated (8.6 months, range: 2.3-15.6) and *TP53*-WT patients (5.8 months, range: 3.3-24.3) was similar ( $p=0.451$ : NS). The OS of *TP53*-mutated patients treated with AZA was similar to that of *TP53*-WT patients treated with IC ( $p=0.25$ : NS) (Supporting Information Figure S3).

Patients with a *TET2* mutation had a shorter OS (2.3 months, range: 0.2-25.4) than patients with *TET2* WT (5.8 months, range: 0.2-58.8) ( $p=0.029$ ) (Figure 3B). In the same way, patients with *SRSF2* mutation had a shorter OS (2.7 month, range: 0.3-25.4) than patients with *SRSF2* WT (5.9 months, range: 0.2-58.8) ( $p=0.034$ ) (Figure 3C).

In addition to be an independent marker of shorter survival, *SRSF2* mutations were associated with a higher average of mutated genes per patient (4 genes mutated per *SRSF2*- mutated patient vs 2.1 in the *SRSF2* WT cohort ( $n=47$ ),  $p<0.0001$ ). In particular, *SRSF2* mutations were associated with mutations of *IDH1/2* ( $n=5/9$ ,  $p<0.0001$ ) and *ASXL1* ( $n=5/9$ ,  $p<0.05$ ).

### ***Comparative mutational analysis of MPN and sAML phases***

We had 24 pairs of MPN and matched sAML. The median time between chronic samples and acute samples was 27.8 months (range: 4.8-107.5). The molecular profiles of MPN phase samples were similar to the molecular profiles of sAML phases and we could not differentiate MPN phases to sAML phases by NGS. Overall, acute phases presented more mutations than chronic phases (4.3 mutated genes in sAML vs. 3.3 in MPN), but this was not statistically significant.

In the 24 pairs, 8 patients mutated for *SRSF2* at sAML already presented the mutation at the MPN phase. Time to sAML transformation was shorter in *SRSF2*-mutated patients (51.2 months, range: 14.7-98) than in patients with no mutation of *SRSF2* (133.8 months, range: 12.6-411.2) ( $p<0.001$ ) and accompanied by a significant mean gain of 1.8 mutated genes vs. 0.6 in *SRSF2* WT patients ( $p<0.05$ ). Two patients had acquired one mutation of *IDH1/2* and two others had acquired one mutation of *TP53* (Supporting Information Figure S4).

Eight sAML patients had a *TP53* mutation. Only 3 of these had already their mutation at the MPN phases and the variant allele frequency (VAF) was always inferior to 15%. For 2 of

these 3 patients the VAF increased to approximately 50% (the third was stable à 5% at sAML phase) and the median VAF in the 8 *TP53*-mutated patients was 34.7% (5.9-83.1). In contrast to *SRSF2*, *TP53*-mutated patients at sAML phase had a longer time to sAML transformation than *TP53* WT patients, 175 months (range: 70.1-356.7) vs. 71.1 months (range: 12.6-411.2) ( $p<0.01$ ).

Six patients had a *TET2* mutation at the sAML phase, 4 of these already presented the mutation at the MPN phase.

## Discussion

Large cohorts of post-MPN sAML are difficult to collect and few studies have been so far dedicated to the molecular evolution of MPNs<sup>8 9 11</sup>. To our knowledge, this work presents one of the three larger cohorts of the literature and the first relying on NGS analysis<sup>12 13</sup>. Leukemic transformation of MPN is classically associated with a very poor prognosis. With a median EFS of 2.9 months and a median OS of 4.7 months, our results are consistent with those previously described<sup>14</sup>.

Concerning the frontline therapy, no difference in EFS or OS was observed, between IC or AZA. In the IC treatment group the ORR was of 58.8%, consistent with those published previously<sup>13 15 16</sup>. In the same way, in the AZA treatment group the ORR was of 54.6%, consistent with or better than the results previously published<sup>6 17 18</sup>.

Patients in PC had a lower OS than those treated by IC or AZA. Obviously, in the PC group, treatment decision was frequently limited by age, performance status, and extent of competing comorbidities, which is a major bias regarding the OS. Concerning the other clinical data, no

difference in EFS and/or OS was observed according to the patient's age, the ELN 2017 prognostic classification, the karyotype, the performance or not of an Allo-SCT or the treatment response according to the ELN. In the specific framework of post-MPN sAMLs, the response achieved is better assessed by Mascarenhas' proposed criteria <sup>19</sup>. However, these responses criteria require performing regular osteomedullary biopsies to evaluate the persistence or not of residual MPN features. In our patients, no osteomedullary biopsy was performed after AML transformation and therefore none of our patients was assessable by the Mascarenhas' proposed criteria.

In our cohort, no clinical data seemed able to predict sAML patients' outcome. Regarding the ELN2017 prognostic classification, not surprisingly, the majority (63%) of our sAML patients belonged to the adverse group. Interestingly, within this adverse group, two prognostic subgroups based on patients' mutational status could be clearly established. Patients with a *TP53* and/or *SRSF2* and/or *TET2* mutations had a lower OS (3.7 months, range: 0.2-25.4) than patients without any of these mutations (11 months, range: 3.4-58.8) ( $p=0.0033$ ) (Supporting Information Figure S5). Furthermore, in the favorable/intermediate group a significant difference in OS was observed between the *TET2* and / or *SRSF2*-mutated patients and patients without any of these mutations (2.2 months, range: 0.2-3.3 vs undefined median (2.2-39)) but no difference between the *TET2* and /or *SRSF2*-mutated patients of the adverse group was noted (3.7 months, range: 0.2-25.4) (data not shown).

At sAML phase, the three patients of the favorable group had mutations or cytogenetic of *de novo* AML (*NPM1* mutated, t (8; 21) and inv (16)), but only one had a *JAK2* mutation whereas all of them were *JAK2*-mutated at MPN phase. This suggests a sAML independent of the MPN disease (in particular in one of these who had antecedent non-hodgkin's lymphoma treated with alkylators). They all had a lower OS (maximum of 9 months) than



ELN would predict, perhaps because the hematopoiesis environment had been modified during the MPN phase although the patients' age (older than 70 years) may also explain these outcomes.

At MPN phase, as we had previously shown, the molecular profile of patients with MPN who evolved to sAML was different from that of patients with MPN who never transformed, and this profile was very close to the profile observed in sAML<sup>9</sup>. Gene mutations (*SRSF2*, *ASXL1*, *DNMT3A*, *IDH1/2*, *EZH2*, *TP53*...) are now known to play a role in transformation but their impact on post-MPN sAML survival has been poorly studied.

In this work, we have shown that sAML patients with mutation in *SRSF2* had a shorter OS, a shorter time to transformation to sAML and a higher gain of mutations than patients not mutated for this gene. This is coherent with our previous report of *SRSF2* as a gene that may increase the risk of transformation but may not be directly responsible for the transformation<sup>9</sup>. An *SRSF2* mutation was reported to be a negative prognostic indicator of poor survival in MF patients (at MPN phase) and to reduce leukemia-free survival<sup>20</sup>.

In addition to *SRSF2*, *TP53* and *TET2* mutations impacted pejoratively and independently sAML prognosis at acute transformation time. *TP53* and *TET2* mutations are common in post-MPN sAML and could be found at MPN phase<sup>8 21 22</sup>. *TET2* mutations are found in approximately 12% of MPN and *TP53* mutations in MPN phase correlates with age<sup>23</sup>. *TP53* mutations in MPN are often difficult to detect because of a very low VAF<sup>24</sup>. If *TP53* is well-known for its pejorative impact, *TET2* is not considered to have adverse prognostic mutations<sup>14 8</sup>. In our cohort, *TP53* mutations seemed to disadvantage patients treated with IC. In the IC group, patients with *TP53* mutation had a lower OS than patients without *TP53* mutation.

The role of *JAK2*, *MPL* and *CALR* in MPN is known but their impact in the outcome of MPN is not entirely clear, especially their role in leukemic-free survival. *CALR* was recently discovered and its prognostic impact is discussed in ET and PMF<sup>25 26 27,28</sup>. Few studies described post-MPN sAML mutated in *CALR*. In our cohort *CALR*-mutated patients represented only 12.5% of sequenced sAML. These seemed to have fewer mutations than *JAK2* or TN patients and some cellular functions were rarely affected compared to non *CALR*-mutated patients. Also, 57.1% of *CALR*-mutated patients had a *TP53* mutation, suggesting a special mechanism of transformation.

## Conclusion

In conclusion, we found that conventional clinical factors (age, karyotype, ELN2017 prognostic classification, treatments received, treatments response, Allo-SCT...) failed to predict the patients' outcome. Only the mutational status appeared relevant to predict patients' prognosis at sAML phase. Interestingly, in the patients analyzed, the mutational profile was quite stable between MPN and sAML phases. These results suggest that a more aggressive therapeutic approach could be proposed to patients with a pejorative mutational status, especially at MPN phase.

## **List of abbreviations**

sAML, secondary acute myeloid leukemia; MPN, myeloproliferative neoplasms; EFS, event-free survival; OS, overall survival; NGS, next-generation DNA sequencing; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; sMF, secondary myelofibrosis; IC, intensive chemotherapy; DNR, daunorubicin; AraC, cytarabine; IDR, idarubicin; AZA, azacitidine; PC, palliative care group; TN, triple negative; ND, not done; CR, complete response; CRi, complete remission with incomplete hematologic recovery ; PRD, primary refractory disease; SD, Stable disease; PD, progression disease; Allo-SCT, Allogeneic stem cell transplantation.

## **Declarations**

The study was approved by the institutional review board of the Paoli-Calmettes Institute (Marseille, France) and conducted in accordance with the Declaration of Helsinki.

All the authors have no conflicts of interest to declare.

All the authors have no funding to declare.

## **Contributions**

Venton G. and Courtier F. have collected the data, performed the statistical analysis and have written the paper.

Charbonnier A., D'Incan E., Saillard C. and Mohty. B have recruited the MPN-sAML patients and have corrected the paper.

Mozziconacci MJ. has performed the monitoring of JAK2, CALR, MPL and cytogenic tests in clinical routine and corrected the paper.

Murati A. and Birnbaum D. have supervised sAML NGS analysis and they have co-written the paper.

Vey N. and Rey J. have overseen the work, have recruited sAML and have co-written the paper.

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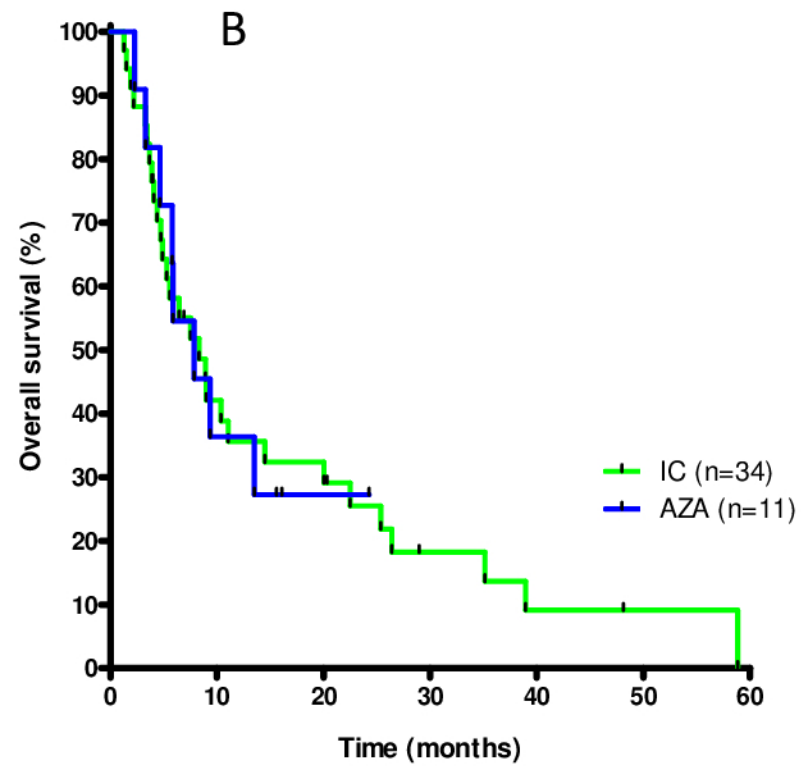
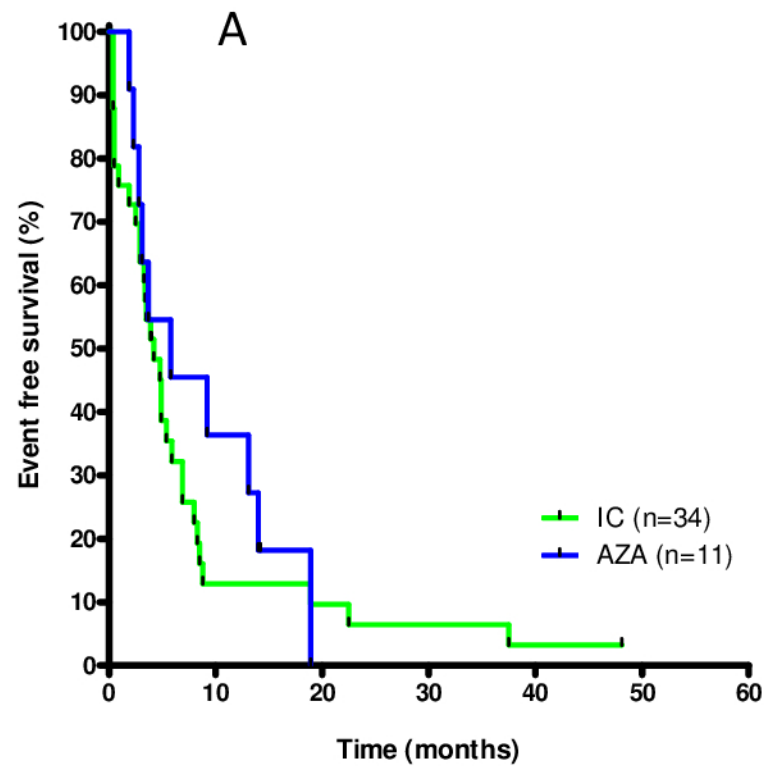
The authors would like to thank the patients whose samples were included in the study, thank the France Intergroup of Myeloproliferative neoplasms (FIM) for helpful discussions. This work was supported by Inserm, Institut Paoli-Calmettes and grants from Laurette Fugain association (2013/05).

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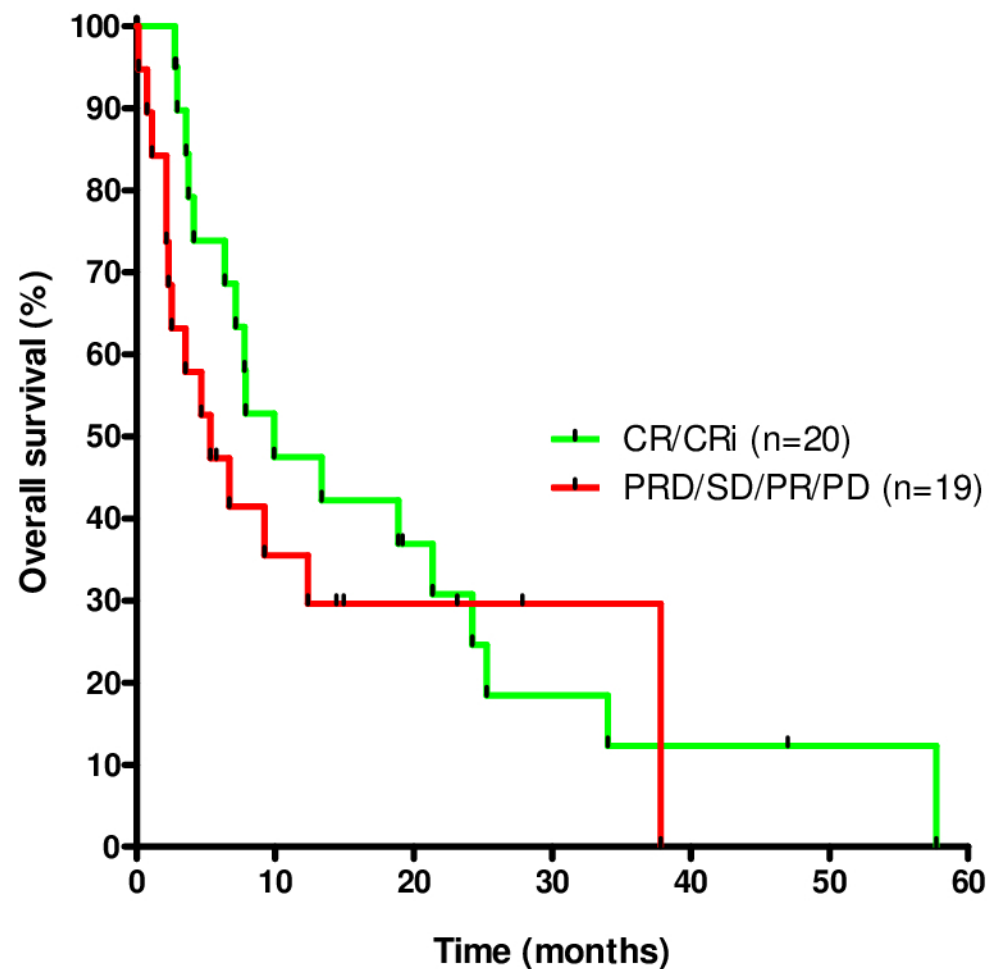
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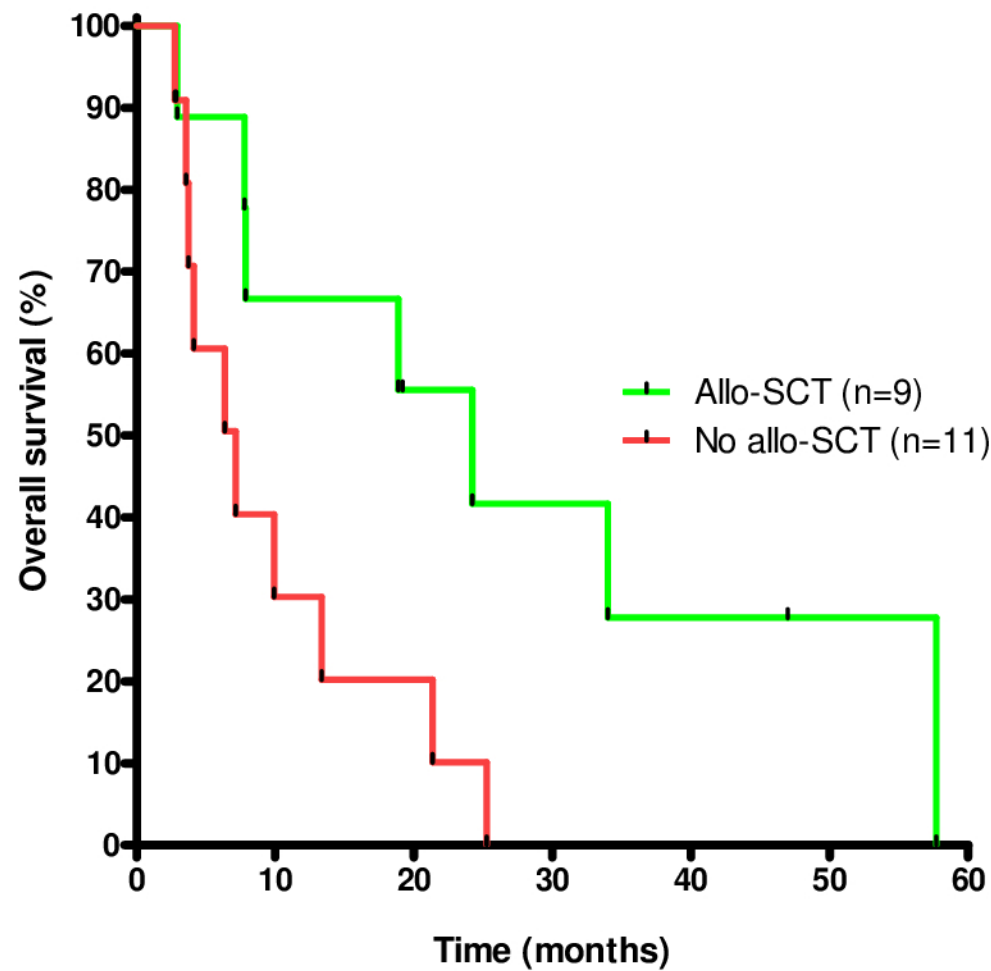


**Figure 1.** Outcome of patients according to front-line treatment: azacytidine (AZA) (n=11) or intensive chemotherapy (IC) (n= 34). **(A)** Event-free survival (0.443: NS). **(B)** Overall survival (0.9842: NS).

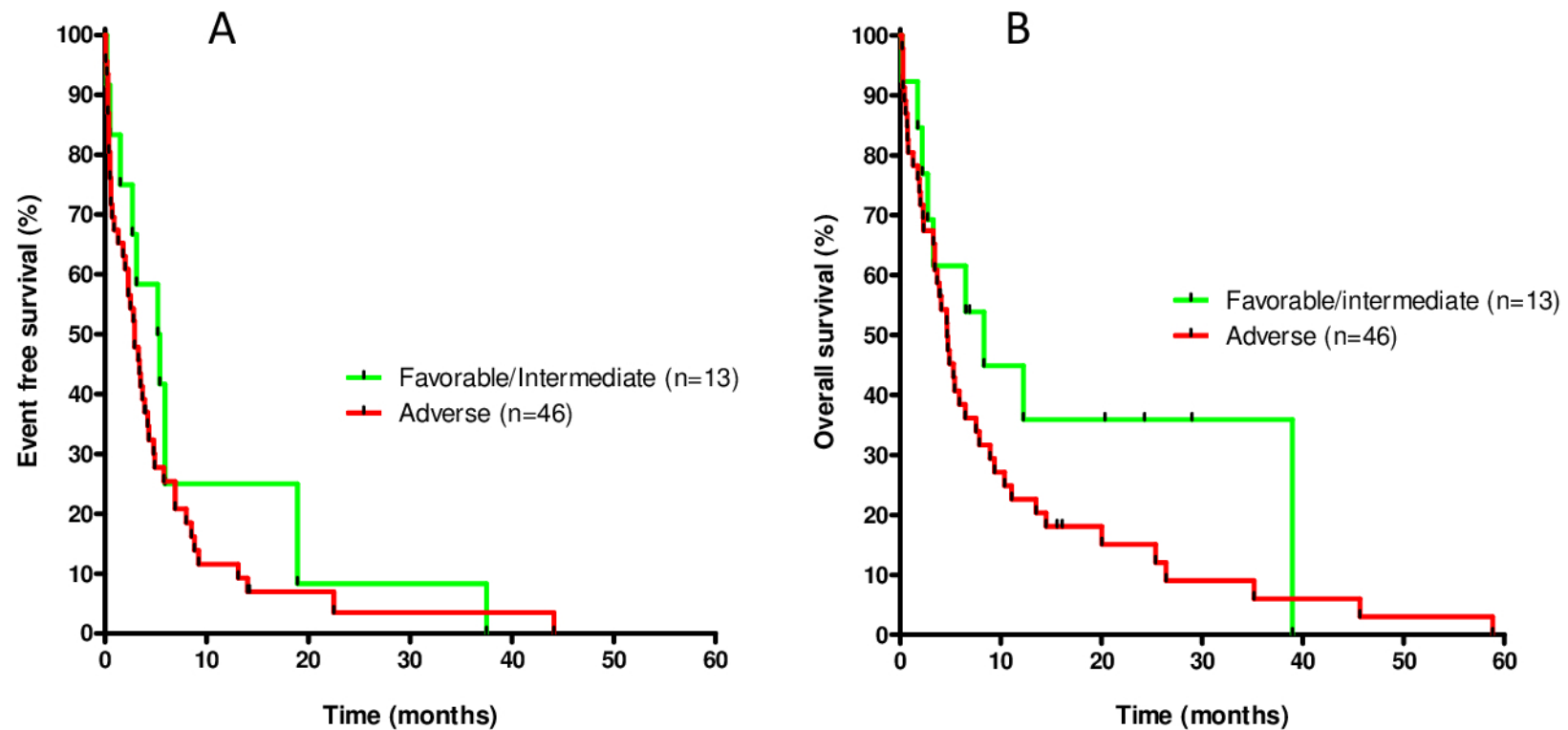




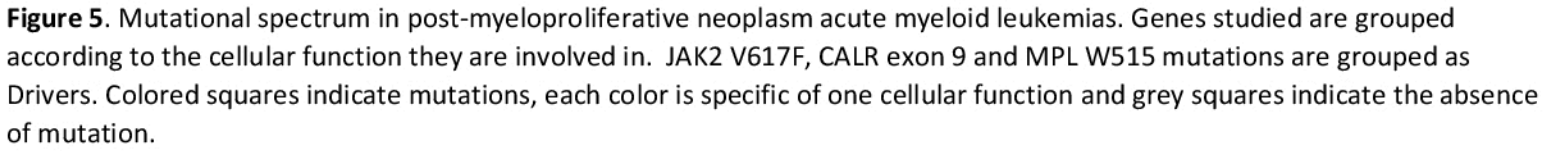
**Figure 2.** Overall survival according to ELN2017 treatment response criteria. In green, patients in complete remission (CR) or in complete remission with incomplete hematologic recovery (CRi) after the first-line treatment (n=20). In red, patients with primary refractory disease (PRD) or in partial remission (PR) or with a stable disease (SD), or in progression disease (PD) after the first-line treatment (n=19). (p=0.3622; NS)

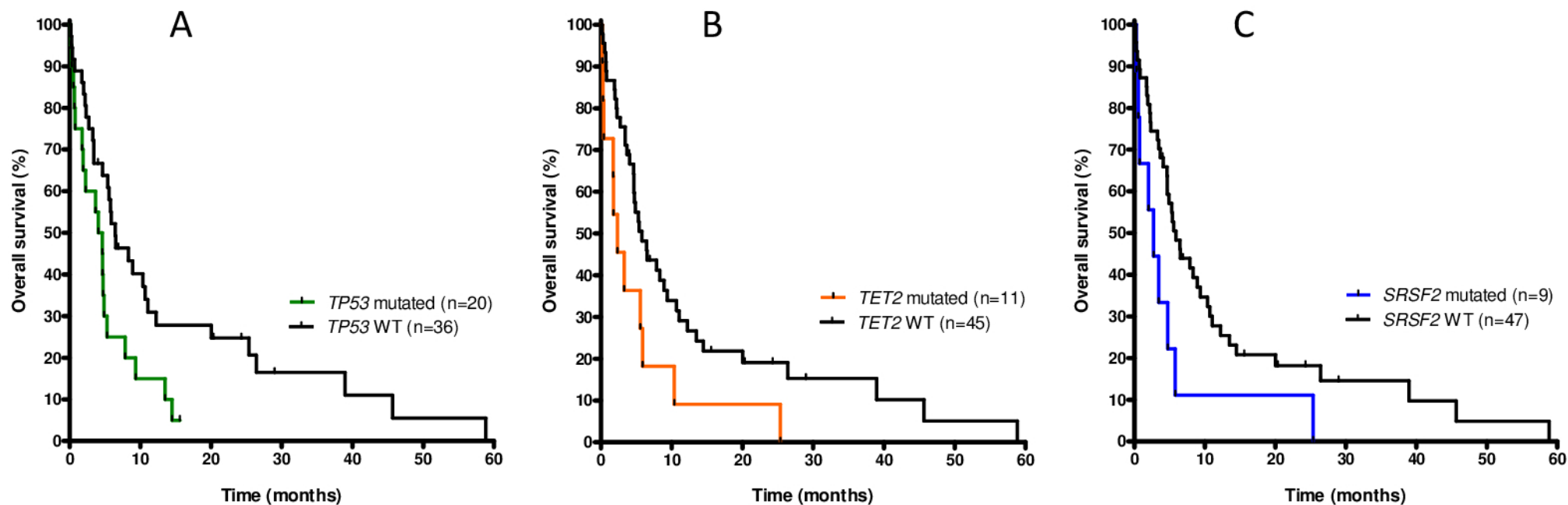


**Figure 3.** Overall survival (in months) of patients who achieved CR or CRi who received (n=9) or not (n=11) Allo-SCT (p=0.34: NS).



**Figure 4.** Outcome of patients according to ELN 2017 prognostic groups: favorable/intermediate (n=13) or adverse (n=46). **(A)** Event-free survival (p=0.3011: NS). **(B)** Overall survival (p=0.1846: NS).





**Figure 6.** Overall survival (in months) of patients according to their mutational status. **(A)** Overall survival according to *TP53* mutational status: *TP53* WT (n=36) or mutated (n=20) (p=0.001). **(B)** Overall survival according to *TET2* mutational status: *TET2* WT (n=45) or mutated (n=11) (p=0.011). **(C)** Overall survival according to *SRSF2* mutational status: *SRSF2* WT (n=45) or mutated (n=11) (p=0.018).

