**International Journal of Hematology**
**Original Article**

**Title**

# Clinical features of complex karyotype in newly diagnosed acute myeloid leukemia

**Authors**

Shota Yoshida[1)], Masahiro Onozawa[1)], Naoki Miyashita[1)], Hiroyuki Kimura[1)], Shogo Takahashi[1)], Shota Yokoyama[1)], Toshihiro Matsukawa[1)], Shinsuke Hirabayashi[2)], Akio Mori[3)], Daisuke Hidaka[4)], Koichiro Minauchi[5)], Akio Shigematsu[6)], Junichi Hashiguchi[7)], Tetsuyuki Igarashi[8)], Yasutaka Kakinoki[9)], Yutaka Tsutsumi[10)], Makoto Ibata[11)], Hajime Kobayashi[12)], Yoshihito Haseyama[13)], Katsuya Fujimoto[14)], Toshimichi Ishihara[15)], Hajime Sakai[16)], Shuichi Ota[4)], Takeshi Kondo[3)], Takanori Teshima[1)]

1) Department of Hematology, Hokkaido University Faculty of Medicine, Graduate school of Medicine, Sapporo, Japan
2) Department of Pediatrics, Hokkaido University Faculty of Medicine, Graduate school of Medicine, Sapporo, Japan
3) Blood Disorders Center, Aiiku Hospital, Sapporo, Japan
4) Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan
5) Department of Hematology, Sapporo City General Hospital, Sapporo, Japan
6) Department of Hematology, Kushiro Rosai Hospital, Kushiro, Japan
7) Department of Internal Medicine/General Medicine, Kitami Red Cross Hospital, Kitami, Japan
8) Department of Hematology, Tenshi Hospital, Sapporo, Japan
9) Department of Hematology, Asahikawa City Hospital, Asahikawa, Japan
10) Department of Hematology, Hakodate Municipal Hospital, Hakodate, Japan
11) Department of Hematology, Sapporo Kosei General Hospital, Sapporo, Japan
12) Department of Hematology, Obihiro Kosei Hospital, Obihiro, Japan
13) Department of Hematology, Tonan Hospital, Sapporo, Japan
14) Department of Hematology, National Hospital Organization Hokkaido Cancer Center, Sapporo, Japan
15) Department of Hematology, Kin-ikyo Chuo Hospital, Sapporo, Japan
16) Department of Hematology, Teine Keijinkai Hospital, Sapporo, Japan

Abstract word count: 190 words
Text word count: 2704 words
The number of table: 2
The number of figures: 6
Supplemental materials: 4 tables, 2 figures

**Corresponding author**
Masahiro Onozawa, MD, PhD
Department of Hematology, Hokkaido University Faculty of Medicine, Graduate School of Medicine, Sapporo, Japan
Kita 15, Nishi 7, Kita-ku, Sapporo, JAPAN 0608638
Phone: +81-11-706-7214
Fax: +81-11-706-7823

**Abstract**

Complex karyotype acute myeloid leukemia (CK-AML) has been classified as an adverse-risk subtype. Although a few reports have further classified CK-AML as typical (including monosomy of chromosomes 5, 7 and 17 or deletion of 5q, 7q and/or 17p) or atypical, the clinical features of these subtypes in Japanese patients remain unclear. We retrospectively analyzed a total of 115 patients with CK-AML, including 77 with typical CK-AML and 38 with atypical CK-AML. Median overall survival (OS) was significantly shorter in patients with typical CK-AML than atypical CK-AML (143 days vs 369 days, P=0.009). Among patients with typical CK-AML, those with monosomy 17 or deletion of 17p had significantly shorter OS than patients without such abnormalities (105 days vs 165 days, P=0.033). *TP53* mutations were more predominant in patients with typical CK-AML than in patients with atypical CK-AML (69.7% vs 32.4%, P <0.001). Patients with typical CK-AML had a poor prognosis regardless of *TP53* mutation status. Among patients with atypical CK-AML, however, prognosis was worse for those with the *TP53* mutation than those without the mutation. In conclusion, prognosis is extremely poor for both typical CK-AML and atypical CK-AML with *TP53* mutation.

**Keywords: Complex karyotype, CK-AML, Typical CK-AML, monosomy 17, deletion of 17p, *TP53* mutation**

## Introduction

Acute myeloid leukemia (AML) is a remarkably heterogeneous disease resulting from the acquisition of chromosomal rearrangements and multiple genetic alterations [1-5]. Patients with AML who present with a complex karyotype (CK) account for 10-12% of all AML patients [6-9]. CK-AML has been consistently classified into an adverse risk group in international risk stratifications such as European LeukemiaNet (ELN) and National Comprehensive Cancer Network (NCCN) [10-14]. A few reports proposed that CK-AML can be further classified into typical CK-AML and atypical CK-AML [15-17]. The former was defined as having CK including monosomy of chromosomes 5, 7 and 17 or deletion of 5q, 7q and/or 17p, and the latter was defined as having CK without those abnormalities. Although it has been reported that patients with typical CK-AML have lower complete remission (CR) rates and shorter overall survival (OS) than those with atypical CK-AML, the clinical features of typical and atypical CK-AML remain unclear in Japanese cohort. Recently, the presence of a pathogenic *TP53* mutation (at a variant allele frequency of at least 10%) defines a new entity of AML with mutated *TP53* [10, 18]. *TP53* mutation were frequent in patients with CK-AML. Therefore, we analyzed clinical features of CK-AML and the effects of chromosomal abnormalities and genetic alterations on prognosis.

## Materials and Methods

### Patients

Hokkaido Leukemia Net (HLN) is a regional prospective cohort study registering cases of newly diagnosed AML in Hokkaido, Japan (UMIN: 000048611). We retrospectively analyzed AML cases except for acute promyelocytic leukemia registered in HLN between April 2010 and December 2021. CK-AML were further divided into typical CK-AML and atypical CK-AML. Survival curve was compared to that of patients with normal karyotype (NK) AML. This study was conducted in accordance with the Helsinki Declaration and was approved by the Hokkaido university hospital institutional review boards (#015-0344).

### Definition of complex karyotype

Definition of complex karyotype (CK) was based on the 2017 ELN guidelines. More specifically, CK was defined as 3 or more chromosomal abnormalities in the absence of the WHO-designated recurring translocations or

inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3), and AML with *BCR-ABL1* [19].

**Analysis of genetic alterations**

Bone marrow (BM) or peripheral blood (PB) samples of 113 cases with CK-AML were available for analysis of genetic alterations. We developed a compact AML panel covering mutation hot spot of 14 genes including *TP53, CEBPA, NPM1, FLT3, KIT, NRAS, KRAS, CBL, PTPN11, DNMT3A, IDH1, IDH2, RUNX1* and *ASXL1*. The sequenced region for each gene is shown in supplemental table 1 (Table S1). Multiplex polymerase chain reaction (PCR) products of genomic DNA were deep-sequenced at Research Institute for Microbial Disease, Osaka University (Osaka, Japan) using Miseq (Illumina, San Diego, CA). Only variants occurring with a variant allele frequency (VAF) of more than 10% were defined as mutations.

**Statistical considerations**

Fisher's exact test was used to compare categorical values and the Mann-Whitney U test was used to compare continuous values. Overall survival (OS) was measured from the date of diagnosis until the date of death from any cause using the log rank test. Statistical significance was defined as a two-tailed *P* value <0.05. The following patients were considered in univariate analysis of determinants of overall survival: patients who were 65 years or older, male gender, patients with typical CK-AML, 5,000 or more WBC count at diagnosis, intensive chemotherapy, allogeneic-hematopoietic stem cell transplantation (allo-HSCT), and *TP53* mutation. The factors associated with at least borderline significance (P<0.10) in the univariate analysis were subjected to multivariate analysis by Cox proportional hazard model. All statistical analyses were performed with EZR ver 1.52 (Jichi Medical University Saitama Medical Center), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [20].

## Results

**Patient characteristics**

CK-AML patients (N=115) accounted for 13.8% and NK-AML patients (N=345) accounted for 41.4% of the 834 non-APL AML patients. We compared the age distributions and characteristics of patients with NK-AML and those with CK-AML (Fig. S1, Table S2). In univariate analysis, patients with CK-AML were significantly

older with a lower white blood cell (WBC) count and lower percentage of BM blast cells than those with NK-AML. Percentage of AML-MRC was higher in CK-AML compared to NK-AML. Additionally, patients with CK-AML had a lower CR rate and a higher primary induction failure (PIF) rate than those with NK-AML. The 115 CK-AML cases included 77 typical CK-AML cases (67.0%) and 38 atypical CK-AML cases (33.0%). We compared the characteristics of patients with typical CK-AML and those with atypical CK-AML (Table 1). There were no significant differences between patients with typical CK-AML and those with atypical CK-AML in a median age (68 years vs 66.5 years, P=0.338), WBC count at diagnosis (3,600/μL vs 4,700/μL, P=0.217), percentage of BM blast cells at diagnosis (43.8% vs 58.4%, P=0.106), WT-1 levels in BM at diagnosis (698.2 x $10^{-4}$/K562 vs 282.5 x $10^{-4}$/K562, P=0.771), percentage of patients who received intensive chemotherapy (46.8% vs 63.2%, P=0.115), and percentage of patients who received allo-HSCT (22% vs 26.3%, P=0.813).

**Chromosomal abnormalities**

Patients with typical CK-AML had larger numbers of chromosome abnormalities (median, 9 vs 4 abnormalities; P<0.001) and larger numbers of monosomy (median, 3 vs 0 abnormalities; P<0.001) than those with atypical CK-AML. The distribution of number of gains and losses (monosomy or deletion) of each chromosomes in CK-AML is shown in Fig. 1A. Total events of monosomy or deletion of autosomal chromosomes were greater than those of chromosomal gain in CK-AML (total of 368 loss events vs 119 gain events). Patients with typical CK-AML had a larger number of monosomy, deletion and/or gain events than did patients with atypical CK-AML (median of 4 abnormalities per case vs 2 abnormalities per case, P<0.001) (Fig. 1B, C). In monosomy or deletion of chromosomes, abnormalities of chromosomes 5, 7 and 17 were the 3 most common events. In typical CK-AML patients, the numbers of patients who had monosomy or deletion of chromosomes 5, 7 and 17 were 49, 39, and 28, respectively (Fig. 2).

**Prognostic analysis by chromosomal abnormalities**

Two-year OS rates in patients with typical CK-AML, patients with atypical CK-AML and patients with NK-AML were 7.1%, 34.6% and 50.8%, respectively (Fig. 3). Median OS of patients with typical CK-AML was 143 days (95% CI: 106-183), which was significantly shorter than that of patients with atypical CK-AML (median OS of 369 days, 95% CI: 63-751, P=0.009) and that of patients with NK-AML (median OS

of 741 days, 95% CI: 565-932, P=0.005) (Fig. 3). There were no significant differences between patients with typical CK-AML and patients with atypical CK-AML in PIF (63.9% vs 50%, P=0.301), CR rate (55.6% vs 70.8%, P=0.285) and relapse rate (35% vs 47.1%, P=0.516) in patients who received intensive chemotherapy (Table 1).

Within patients with typical CK-AML, there was no significant difference between OS for patients with monosomy 5 or deletion of 5q and OS for patients without such abnormalities (148 days vs 118 days, P=0.694) (Fig. 4A). OS for patients with monosomy 7 or deletion of 7q was also similar to that for patients without such abnormalities (148 days vs 142 days, P=0.973) (Fig. 4B). However, patients with monosomy 17 or deletion of 17p had a significantly shorter OS than that for patients without such abnormalities among patients with typical CK-AML (105 days vs 165 days, P=0.033) (Fig. 4C). There were no significant differences between patients with monosomy 17 or deletion of 17p and patients without such abnormalities in PIF rate (54.5% vs 68%, P=0.475), CR rate (63.6% vs 52%, P=0.718) and relapse rate (42.9% vs 30.8%, P=0.651). However, mortality rate within 1 month of patients with monosomy 17 or deletion of 17p were significantly higher than patients without such abnormalities (20% vs 2.3%, P=0.021), excluding patients who received best supportive care. We compared patients with duplicative or triplicated chromosomal abnormalities including chromosomes 5, 7, and 17, and single chromosomal abnormalities, however there was no significant difference in OS (duplicated or triplicated deletion 148 days vs single deletion 127 days, P=0.448).

**Genetic alterations and prognostic analysis**

The most frequently mutated gene was *TP53*. *TP53* mutations were found in 65 (57.5%) of the 113 patients with samples for analysis (Fig. 5). There were 50 patients with a single *TP53* mutation and 15 patients with a double *TP53* mutation. In 50 patients with a single *TP53* mutation, 46 patients had single nucleotide substitution (45 missense mutation, 1 splice acceptor variant), and 4 patients had short indels (3 frameshift mutation, 1 in-frame mutation). Other gene mutations were less common: *IDH2* (N=5, 4.4%), *KRAS* (N=4, 3.5%), *FLT3* (N=4, 3.5%), *RUNX1* (N=4, 3.5%), *PTPN11* (N=3, 2.7%), *ASXL1* (N=3, 2.7%), *CBL* (N=2, 1.8%), *DNMT3A* (N=2, 1.8%), *KIT* (N=2, 1.8%), *NPM1* (N=2, 1.8%), *NRAS* (N=2, 1.8%), *CEBPA* (N=1, 0.9%) and *IDH1* (N=1, 0.9%) (Fig. S2). *TP53* mutations were more predominant in patients with typical CK-AML than in patients with atypical CK-AML (69.7% vs 32.4%, P <0.001 ). The frequencies of other gene mutations were not significantly different between

patients with typical CK-AML and patients with atypical CK-AML (Table S3). Fifty-two patients (80%) had *TP53* mutation as a sole mutation.

In patients with CK-AML, 2-year OS rates in patients with *TP53* mutation and patients without *TP53* mutation were 12.3% and 21.8%, respectively. Median OS of patients with *TP53* mutation was 127 days (95% CI: 67-183), which was significantly shorter than that of patients without *TP53* mutation (median OS of 302 days, 95% CI: 136-532, P=0.014) (Fig. 6A). A comparison of OS rates in patients with typical CK-AML and patients with atypical CK-AML with or without TP53 mutation is shown in Fig. 6B. Median OS periods of typical CK-AML patients with *TP53* mutation, typical CK-AML patients without *TP53* mutation, atypical CK-AML patients with *TP53* mutation, and atypical CK-AML patients without *TP53* mutation were 139 days (95% CI: 72-183), 165 days (95% CI:111-481), 77.5 days (95% CI:20-408), and 574 days (95% CI:59-1119), respectively. Median OS in atypical CK-AML patients without *TP53* mutation was significantly longer than that in patients with *TP53* mutation (P=0.017). There were no significant differences between CK-AML patients with *TP53* mutation and CK-AML patients without *TP53* mutation in PIF rate (35.5% vs 47.4%, P=0.516), CR rate (56.7% vs 67.9%, P=0.427) and relapse rate (70% vs 46.4%, P=0.109). Patient characteristics of 4 subgroup defined by CK subtype and *TP53* mutation were shown on Table S4. In univariate analysis, age of 65 years or older, male gender, typical CK-AML, WBC count of 5,000 or more at diagnosis, intensive chemotherapy, allo-HSCT, and *TP53* mutation were significant factors for OS. In multivariate analysis, typical CK-AML (HR:1.727, 95% CI:1.015-2.940, P=0.044) and having received allo-HSCT were identified as significant risk factors for poor and favorable prognosis respectively in patients with CK-AML (Table 2).

## Discussion

Although the risk categorization of AML has been updated frequently, CK-AML consistently corresponds to an adverse prognosis in NCCN and 2022 ELN risk classifications [10-12]. Besides CK-AML, monosomal karyotype, monosomy 5 or deletion of 5q, monosomy 7, monosomy 17, abnormalities of 17p, mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1* or *ZESR2*, and mutated *TP53* also correspond to the adverse risk group in 2022 ELN [10].

CK-AML was classified into typical CK-AML and atypical CK-AML in a few reports [15-17]. Mrózek K, et al. reported the effects of chromosomal abnormalities and genetic alterations on prognosis in 96 patients with typical CK-AML and 40 patients with atypical CK-AML [15]. In their study, the percentage of typical CK-AML patients

with *TP53* mutation was larger than the percentage of atypical CK-AML patients with *TP53* mutation (67% vs 10%, P<0.001). Patients with typical CK-AML were older than patients with atypical CK-AML (median age of 59 years vs 53 years, P=0.007), and patients with typical CK-AML had a lower WBC count (median count of 6,000/μL vs 23,800/μL, P=0.001), lower percentage of BM blasts (46% vs 76%, P<0.001), lower CR rate (35% vs 59%, P=0.020) and lower 3-year OS rate (1% vs 23%, P<0.001) than those in patients with atypical CK-AML. Leung G, et al. also reported that the leukemia-free survival (LFS) period was significantly shorter in typical CK-AML patients with *TP53* mutation than in atypical CK-AML patients without *TP53* mutation (median LFS, 0.50 years vs 1.73 years, P =0.008) [16]. In our analysis, patients with typical CK-AML had a worse OS than that of patients with atypical CK-AML. However, there were not significant differences in age, WBC count, percentage of BM blasts at diagnosis, CR rate and PIF rate between patients with typical CK-AML and those with atypical CK-AML (Table 1). Although a panel sequence is not currently available as daily practice in Japan, typical CK-AML is defined just by chromosomal analysis and is identified as poorer prognostic group within CK-AML in our analysis.

The pathogenesis of typical CK-AML is currently unclear [21]. In our analysis of gain and monosomy or deletion of each chromosome, patients with typical CK-AML had more monosomy, deletion and/or gain abnormalities than did patients with atypical CK-AML. In monosomy or deletion of autosomal chromosomes, abnormalities of 5, 7 and 17, which belong to typical CK-AML, were the most common. Monosomy 17 or deletion of 17p, which affects the *TP53* gene locus (17p.31), results in loss of heterozygosity and a complete disruption of p53 function [17]. Monosomy 17, but not monosomy 5 and/or 7, was associated with inferior OS among patients with CK-AML [22]. In our cohort, we can also confirmed that the patients with monosomy 17 or deletion of 17p had significantly shorter OS than that of other typical CK-AML patients.

In our gene analysis, CK-AML patients with *TP53* mutation had a worse prognosis than that of patients without *TP53* mutation. However, typical CK-AML patients, especially those with monosomy 17 or deletion of 17p, had poor prognosis regardless of *TP53* mutation. Mutations of *TP53* have been shown to be associated with genomic instability, rapid gain or loss of chromosomes, and expression of various genes to maintain genomic stability, mitosis and transcriptional regulation [16]. Abnormalities of chromosome 17, where the *TP53* gene locus resides, contribute to poor prognosis, and loss of genes on 17q such as *ERBB2*, *NF1*, *RARA*, *BRCA1*, and *STAT3* might also contribute to poor prognosis [22]. These abnormalities of genes would have been

responsible for the poor prognosis of CK-AML with monosomy 17 or deletion of 17p in our cohort. *TP53* is the sole mutated gene in up to 75% of CK-AML patients with *TP53* mutation, while 25% of the patients have co-occurring mutations including *FLT3*, *NPM1*, *IDH1*, *IDH2*, *DNMT3A*, *WT1*, *RUNX1* and *RAS* mutations [23-28]. Gene analysis in our cohort showed that 52 patients (80%) had *TP53* mutation alone.

The current study has several limitations. First, this study was a study with a small number of patients and was retrospective in nature. A larger cohort is needed to evaluate the effects of chromosomal abnormalities and genetic alterations on prognosis of CK-AML. Second, we did not evaluate more patients who received VEN plus AZA in detail, although VEN in combination with conventional low-intensity drugs is an effective therapy for older AML patients [29]. VEN plus AZA may affect the prognosis of CK-AML. We should plan to accumulate more cases in the future. Recently, APR-246, which is a methylated PRIMA-1 analogue, has been reported to be one of the most promising agents for patients with *TP53* mutation. APR-246 restores wild-type p53 conformation and activity. As a result, APR-246 induces prompt apoptosis in *TP53* mutated cells. This drug in combination with traditional chemotherapeutic agents has a synergistic effect [30-32]. The prognosis of typical CK-AML and atypical CK-AML with *TP53* mutation might be improved by developing *TP53*-targeted therapy.

In conclusion, this retrospective analysis showed that the poor prognosis group of CK-AML could be further stratified into typical CK-AML and atypical CK-AML. Patients with typical CK-AML had a worse prognosis than that for patients with atypical CK-AML in a Japanese cohort. Patients with monosomy 17 or deletion of 17p had significantly shorter OS than that for other typical CK-AML patients. In gene analysis, patients with *TP53* mutation had a worse prognosis than that for patients without *TP53* mutation in CK-AML. However, typical CK-AML patients had poor prognosis regardless of *TP53* mutation. This extremely poor prognostic group of CK-AML patients with monosomy 17 or deletion of 17p can be identified before genetic testing and long survival has not been achieved by current treatments.

**Acknowledgments**

This work was partly supported by the Japan Society for the Promotion of Science (JSPS), Scientific Research (C) (20K08745; M.O.). We thank the nursing and medical staffs for their contributions to this study and their dedicated care of the patients.

**Author contributions**

S.Y. and M.O. designed the study, analyzed the data and wrote the manuscript. T. K. provided critique to the manuscript. T.T. revised and approved the manuscript. All authors contributed to the final version of the manuscript and approved it for the publication.

**Disclosure of conflict of interest**

The authors declare no competing conflict of interest.

**Figure legends**

**Fig. 1.** Distributions of number of gains and losses (monosomy or deletion) of chromosomes. A. total CK-AML, B. typical CK-AML, C. atypical CK-AML. The upper half of the axis is gain events of chromosomes (red line) and the lower half is loss events of these chromosomes (black and pink lines). The pink line shows the number of cases with -5/del(5q), -7/del(7q) and/or -17/del(17p), which is a hallmark of typical CK. Each autosomal chromosomal abnormality is shown in order of number of the chromosome from the left, and X and Y chromosomes are shown next to chromosome 22.

**Fig. 2.** From top to bottom, numbers of typical CK-AML cases including -17/del(17p), -7/del(7q) and -5/del(5q) are shown. Each column represents a case showing a combination of affected chromosomes.

**Fig. 3.** Overall survival rates of patients with typical CK-AML, patients with atypical CK-AML and patients with NK-AML are shown by red, black, and green lines, respectively.

**Fig. 4.** Overall survival rates of patients with typical CK-AML stratified by type of chromosomal abnormalities are shown (A. Comparison by whether or not -5/del(5q) was included, B. Comparison by whether or not -7/del(7q) was included, C. Comparison by whether or not -17/del(17q) was included).

**Fig. 5.** Relationships between type of chromosome abnormalities and 14 gene mutations are shown. From top to bottom, 14 genes are shown in descending frequency order. Rows show cases of typical CK-AML with -17/del(17p), typical CK-AML without -17/del(17p), and atypical CK-AML.

**Fig. 6. A.** Overall survival rates of CK-AML patients with *TP53* mutation and CK-AML patients without *TP53* mutation are shown. **B.** Overall survival rates of typical CK-AML patients with *TP53* mutation, typical CK-AML patients without *TP53* mutation, atypical CK-AML patients with *TP53* mutation, and atypical CK-AML patients without *TP53* mutation are shown by black, red, green, and blue lines, respectively.

**Table 1. Comparison of the characteristics of patients with typical CK-AML and patients with atypical CK-AML**

**Table 2. Univariate and multivariate analyses of patient characteristics for overall survival**

## References

1. 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(23):2209-21. doi:10.1056/NEJMoa1516192.

2. Dohner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. N Engl J Med. 2015;373(12):1136-52. doi:10.1056/NEJMra1406184.

3. Kayser S, Levis MJ. Updates on targeted therapies for acute myeloid leukaemia. Br J Haematol. 2022;196(2):316-28. doi:10.1111/bjh.17746.

4. Hasserjian RP, Steensma DP, Graubert TA, Ebert BL. Clonal hematopoiesis and measurable residual disease assessment in acute myeloid leukemia. Blood. 2020;135(20):1729-38. doi:10.1182/blood.2019004770.

5. Liu H. Emerging agents and regimens for AML. J Hematol Oncol. 2021;14(1):49. doi:10.1186/s13045-021-01062-w.

6. Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev. 2004;18(2):115-36. doi:10.1016/S0268-960X(03)00040-7.

7. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood. 2000;96(13):4075-83.

8. Estey EH. Acute myeloid leukemia: 2019 update on risk-stratification and management. Am J Hematol. 2018;93(10):1267-91. doi:10.1002/ajh.25214.

9. Nguyen-Khac F, Bidet A, Daudignon A, Lafage-Pochitaloff M, Ameye G, Bilhou-Nabera C et al. The complex karyotype in hematological malignancies: a comprehensive overview by

the Francophone Group of Hematological Cytogenetics (GFCH). Leukemia. 2022;36(6):1451-66. doi:10.1038/s41375-022-01561-w.

10. Dohner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H et al. Diagnosis and Management of AML in Adults: 2022 ELN Recommendations from an International Expert Panel. Blood. 2022. doi:10.1182/blood.2022016867.

11. Pollyea DA, Bixby D, Perl A, Bhatt VR, Altman JK, Appelbaum FR et al. NCCN Guidelines Insights: Acute Myeloid Leukemia, Version 2.2021. J Natl Compr Canc Netw. 2021;19(1):16-27. doi:10.6004/jnccn.2021.0002.

12. Tallman MS, Wang ES, Altman JK, Appelbaum FR, Bhatt VR, Bixby D et al. Acute Myeloid Leukemia, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2019;17(6):721-49. doi:10.6004/jnccn.2019.0028.

13. O'Donnell MR, Tallman MS, Abboud CN, Altman JK, Appelbaum FR, Arber DA et al. Acute Myeloid Leukemia, Version 3.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017;15(7):926-57. doi:10.6004/jnccn.2017.0116.

14. O'Donnell MR, Abboud CN, Altman J, Appelbaum FR, Arber DA, Attar E et al. NCCN Clinical Practice Guidelines Acute myeloid leukemia. J Natl Compr Canc Netw. 2012;10(8):984-1021. doi:10.6004/jnccn.2012.0103.

15. Mrozek K, Eisfeld AK, Kohlschmidt J, Carroll AJ, Walker CJ, Nicolet D et al. Complex karyotype in de novo acute myeloid leukemia: typical and atypical subtypes differ molecularly and clinically. Leukemia. 2019;33(7):1620-34. doi:10.1038/s41375-019-0390-3.

16. Leung GMK, Zhang C, Ng NKL, Yang N, Lam SSY, Au CH et al. Distinct mutation spectrum, clinical outcome and therapeutic responses of typical complex/monosomy karyotype acute myeloid leukemia carrying TP53 mutations. Am J Hematol. 2019;94(6):650-7. doi:10.1002/ajh.25469.

17. Schoch C, Kern W, Kohlmann A, Hiddemann W, Schnittger S, Haferlach T. Acute myeloid leukemia with a complex aberrant karyotype is a distinct biological entity characterized by genomic imbalances and a specific gene expression profile. Genes Chromosomes Cancer. 2005;43(3):227-38. doi:10.1002/gcc.20193.

18. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemia: Integrating Morphological, Clinical, and Genomic Data. Blood. 2022. doi:10.1182/blood.2022015850.

19. 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(4):424-47. doi:10.1182/blood-2016-08-733196.

20. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical

444 statistics. Bone Marrow Transplant. 2013;48(3):452-8. doi:10.1038/bmt.2012.244.
445 21. Rucker FG, Dolnik A, Blatte TJ, Teleanu V, Ernst A, Thol F et al. Chromothripsis is linked
446 to TP53 alteration, cell cycle impairment, and dismal outcome in acute myeloid leukemia
447 with complex karyotype. Haematologica. 2018;103(1):e17-e20.
448 doi:10.3324/haematol.2017.180497.
449 22. Strickland SA, Sun Z, Ketterling RP, Cherry AM, Cripe LD, Dewald G et al. Independent
450 Prognostic Significance of Monosomy 17 and Impact of Karyotype Complexity in Monosomal
451 Karyotype/Complex Karyotype Acute Myeloid Leukemia: Results from Four ECOG-ACRIN
452 Prospective Therapeutic Trials. Leuk Res. 2017;59:55-64. doi:10.1016/j.leukres.2017.05.010.
453 23. Hunter AM, Sallman DA. Current status and new treatment approaches in TP53 mutated
454 AML. Best Pract Res Clin Haematol. 2019;32(2):134-44. doi:10.1016/j.beha.2019.05.004.
455 24. Kadia TM, Jain P, Ravandi F, Garcia-Manero G, Andreef M, Takahashi K et al. TP53
456 mutations in newly diagnosed acute myeloid leukemia: Clinicomolecular characteristics,
457 response to therapy, and outcomes. Cancer. 2016;122(22):3484-91. doi:10.1002/cncr.30203.
458 25. Haase D, Stevenson KE, Neuberg D, Maciejewski JP, Nazha A, Sekeres MA et al. TP53
459 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct
460 prognostic subgroups. Leukemia. 2019;33(7):1747-58. doi:10.1038/s41375-018-0351-2.
461 26. Sallman DA, Komrokji R, Vaupel C, Cluzeau T, Geyer SM, McGraw KL et al. Impact of
462 TP53 mutation variant allele frequency on phenotype and outcomes in myelodysplastic
463 syndromes. Leukemia. 2016;30(3):666-73. doi:10.1038/leu.2015.304.
464 27. Kuykendall A, Duployez N, Boissel N, Lancet JE, Welch JS. Acute Myeloid Leukemia:
465 The Good, the Bad, and the Ugly. Am Soc Clin Oncol Educ Book. 2018;38:555-73.
466 doi:10.1200/EDBK_199519.
467 28. Cancer Genome Atlas Research N, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ et
468 al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J
469 Med. 2013;368(22):2059-74. doi:10.1056/NEJMoa1301689.
470 29. DiNardo CD, Tiong IS, Quaglieri A, MacRaild S, Loghavi S, Brown FC et al. Molecular
471 patterns of response and treatment failure after frontline venetoclax combinations in older
472 patients with AML. Blood. 2020;135(11):791-803. doi:10.1182/blood.2019003988.
473 30. Perdrix A, Najem A, Saussez S, Awada A, Journe F, Ghanem G et al. PRIMA-1 and
474 PRIMA-1(Met) (APR-246): From Mutant/Wild Type p53 Reactivation to Unexpected
475 Mechanisms Underlying Their Potent Anti-Tumor Effect in Combinatorial Therapies.
476 Cancers (Basel). 2017;9(12). doi:10.3390/cancers9120172.
477 31. Ali D, Jonsson-Videsater K, Deneberg S, Bengtzen S, Nahi H, Paul C et al. APR-246
478 exhibits anti-leukemic activity and synergism with conventional chemotherapeutic drugs in
479 acute myeloid leukemia cells. Eur J Haematol. 2011;86(3):206-15. doi:10.1111/j.1600-

480 0609.2010.01557.x.
481 32. Nahi H, Merup M, Lehmann S, Bengtzen S, Mollgard L, Selivanova G et al. PRIMA-1
482 induces apoptosis in acute myeloid leukaemia cells with p53 gene deletion. Br J Haematol.
483 2006;132(2):230-6. doi:10.1111/j.1365-2141.2005.05851.x.
484

Fig. 1

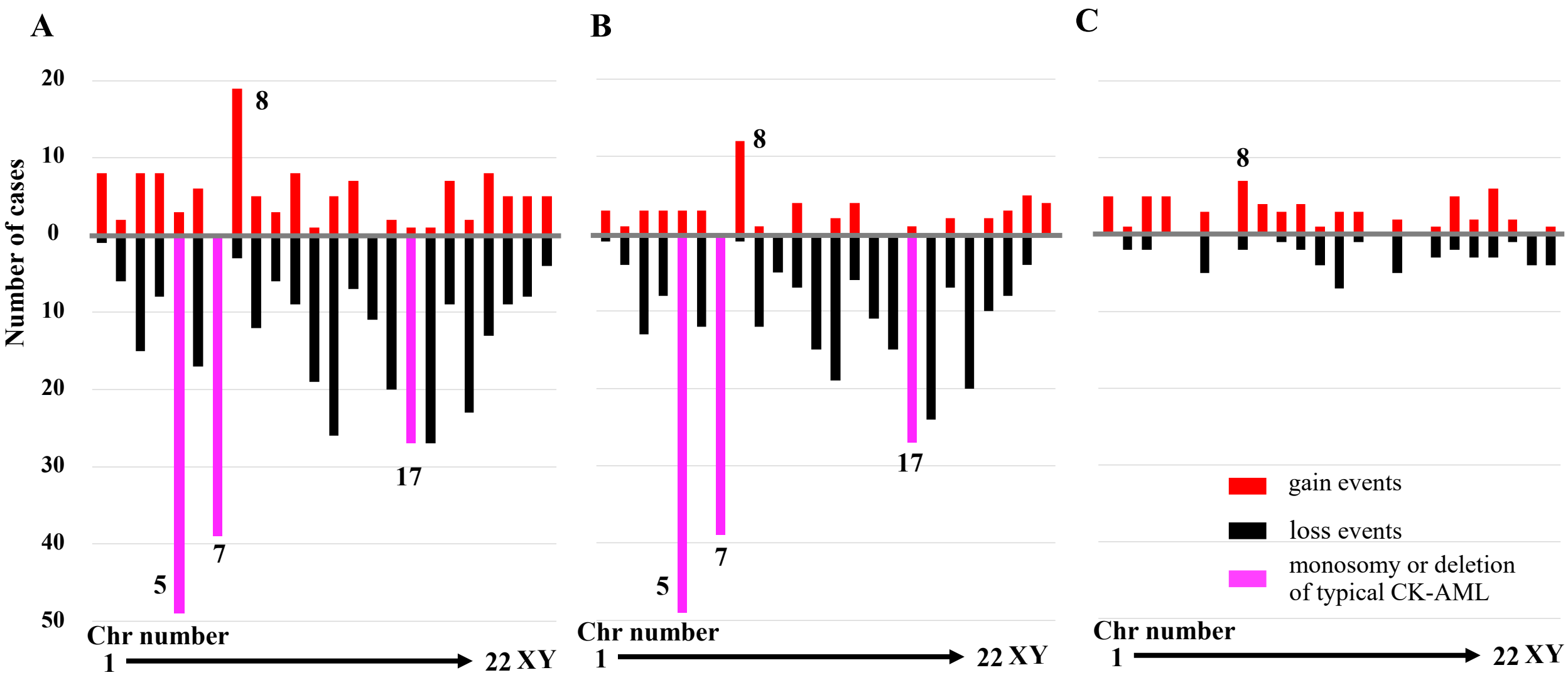

A
B
C
Number of cases
20
10
0
10
20
30
40
50
8
17
5
7
Chr number
1
22 XY
gain events
loss events
monosomy or deletion
of typical CK-AML

**Fig. 2**

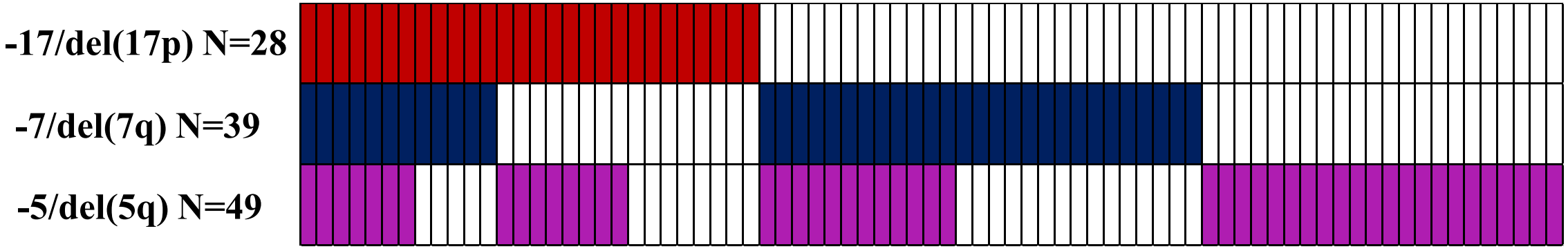
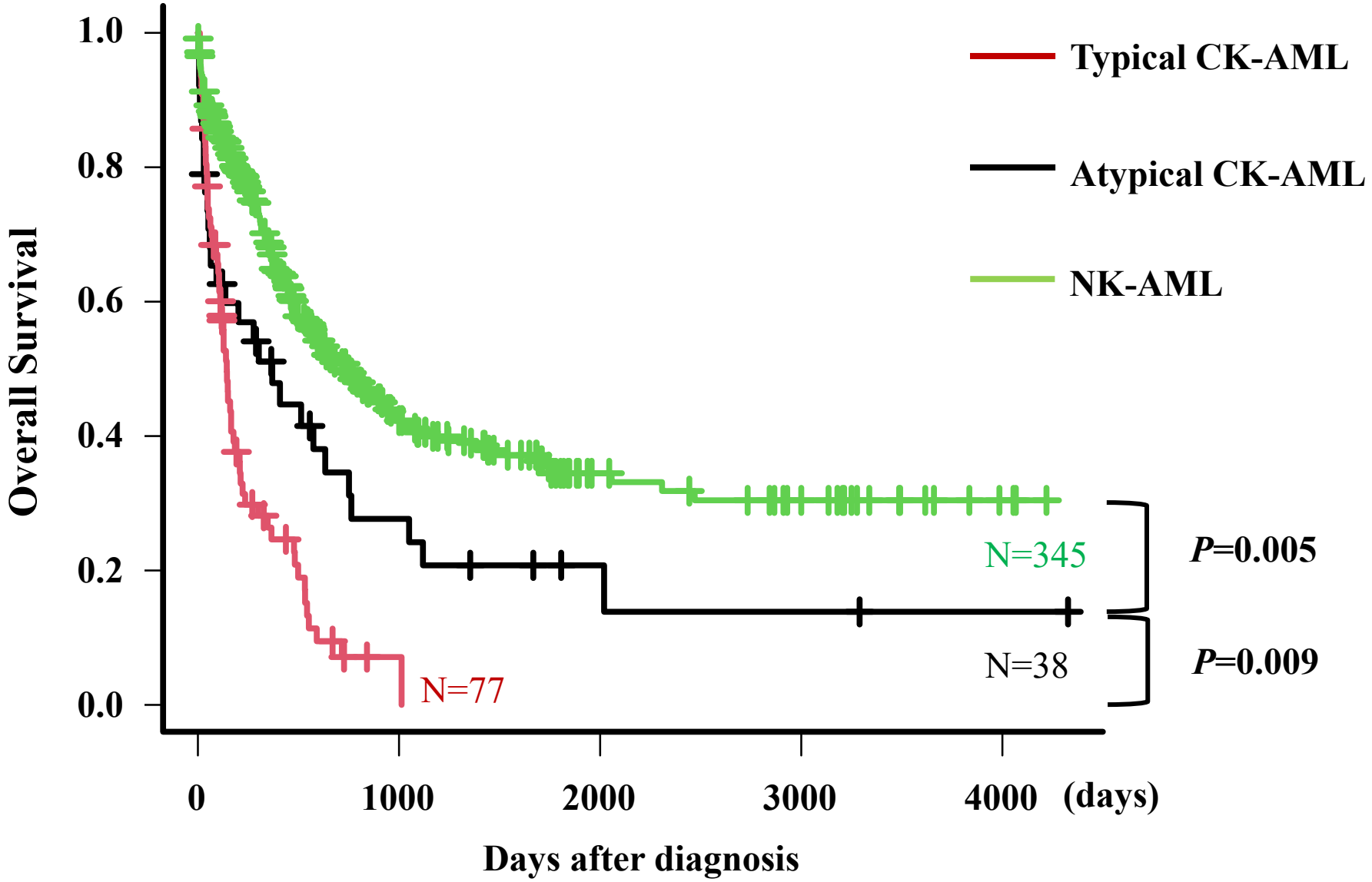


Fig. 3


1.0
0.8
0.6
0.4
0.2
0.0
Overall Survival
Typical CK-AML
Atypical CK-AML
NK-AML
N=345
N=38
N=77
P=0.005
P=0.009
0
1000
2000
3000
4000
(days)
Days after diagnosis

# Fig. 4

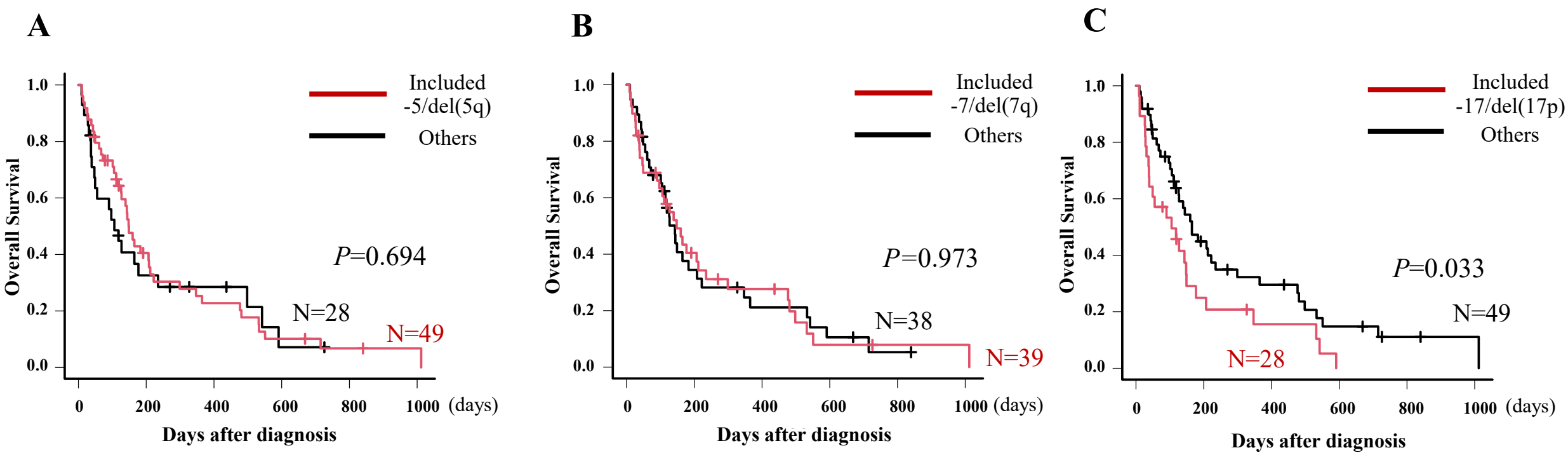

A
B
C
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
(days)
Days after diagnosis
Included
-5/del(5q)
Others
P=0.694
N=28
N=49
Included
-7/del(7q)
Others
P=0.973
N=38
N=39
Included
-17/del(17p)
Others
P=0.033
N=49
N=28

Fig. 5

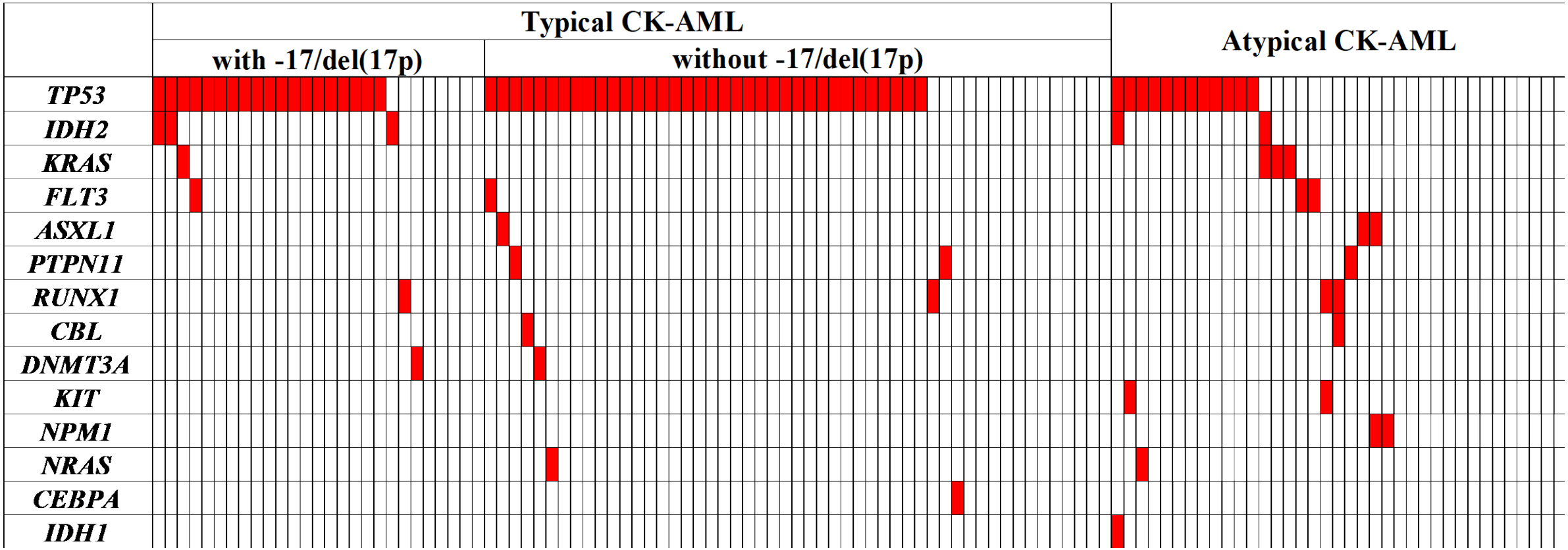
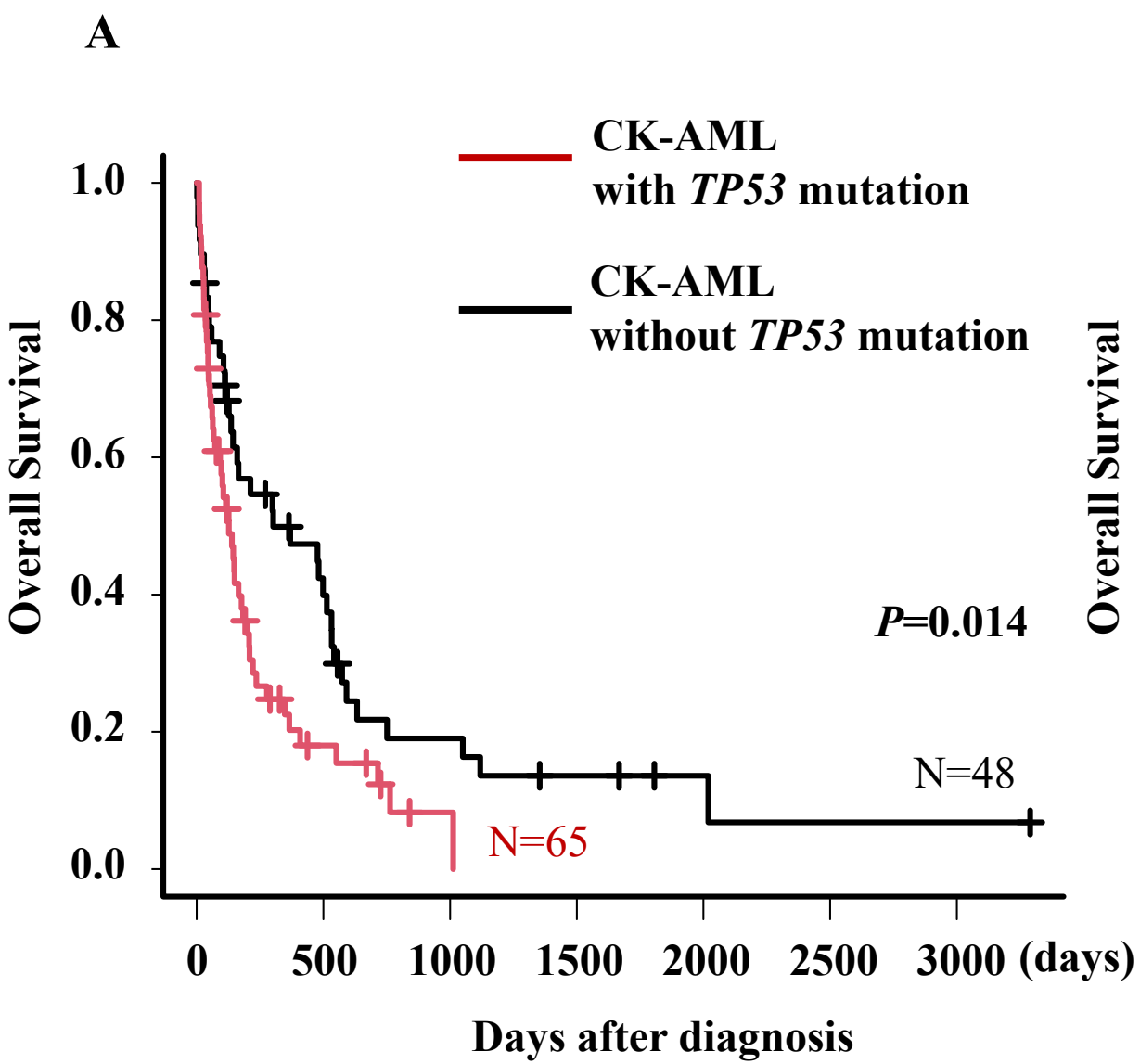

Typical CK-AML
with -17/del(17p)
without -17/del(17p)
Atypical CK-AML
TP53
IDH2
KRAS
FLT3
ASXL1
PTPN11
RUNX1
CBL
DNMT3A
KIT
NPM1
NRAS
CEBPA
IDH1

Fig. 6


A
CK-AML
with TP53 mutation
CK-AML
without TP53 mutation
P=0.014
N=65
N=48
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000 (days)
Days after diagnosis

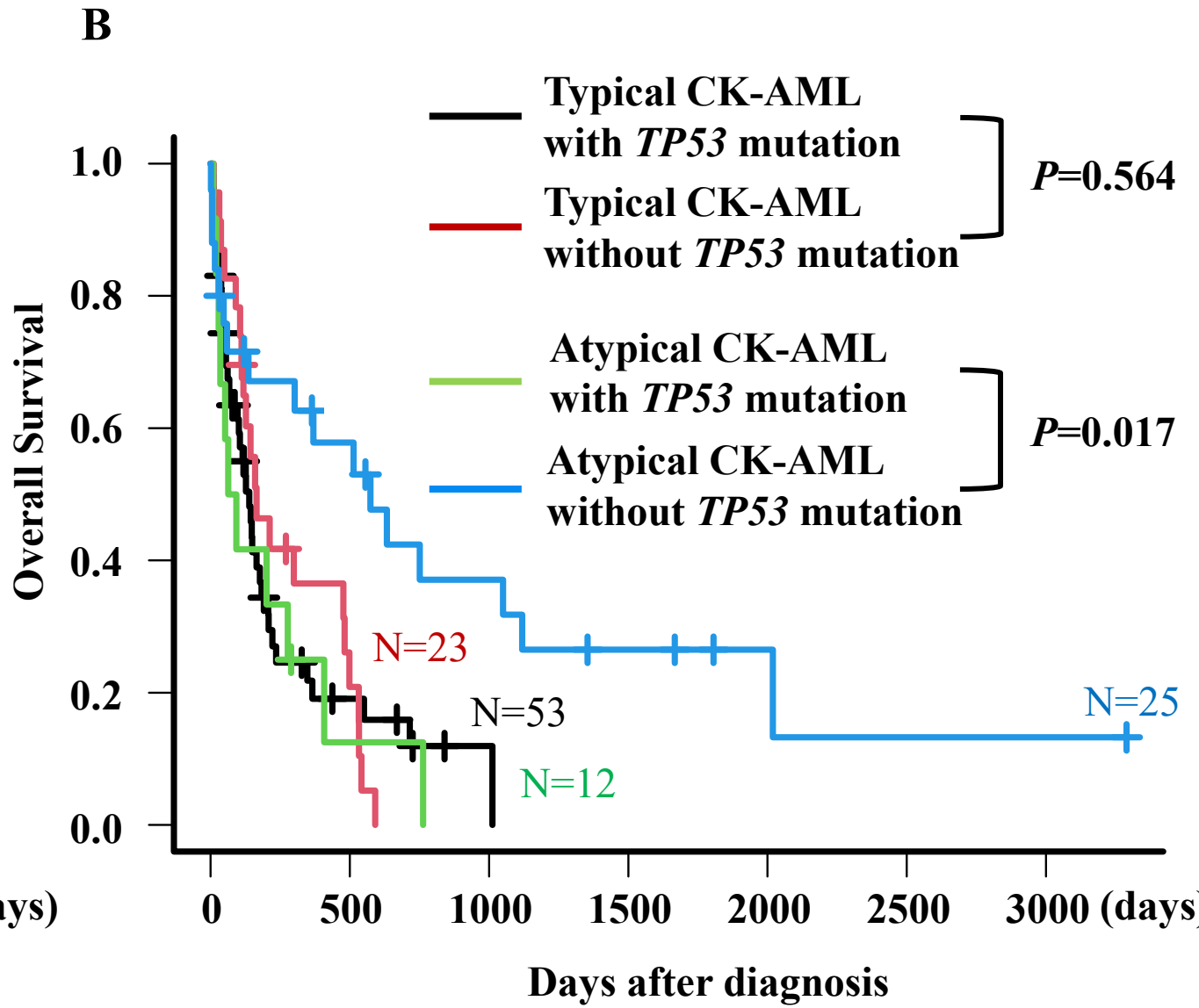

B
Typical CK-AML
with TP53 mutation
Typical CK-AML
without TP53 mutation
P=0.564
Atypical CK-AML
with TP53 mutation
Atypical CK-AML
without TP53 mutation
P=0.017
N=23
N=53
N=12
N=25
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000 (days)
Days after diagnosis

**Table 1. Comparison of the characteristics of patients with typical CK-AML and patients with atypical CK-AML**

| | Typical CK-AML (N=77) | Atypical CK-AML (N=38) | *P* value |
|---|---|---|---|
| **Median age [range]** | 68 [17-91] | 66.5 [32-89] | 0.338 |
| 65 years or older | 50 (64.9%) | 23 (60.5%) | 0.684 |
| **Gender** | | | |
| Male/Female | 46/31 | 23/15 | 1 |
| **Disease** | | | |
| AML-MRC | 72(93.5%) | 35 (92.1%) | 1 |
| t-AML | 5 (6.5%) | 2 (5.3%) | 1 |
| Myeloid sarcoma | 0 | 1 (2.6%) | 0.330 |
| **WBC (/μL) [range]** | 3,600 [700-85,800] | 4,700 [700-117,000] | 0.217 |
| **BM blast (%) [range]** | 43.8 [20-95] | 58.4 [21-98] | 0.106 |
| **BM WT-1 levels (x $10^{-4}$/K562) [range]** | 698.2 [1-6,441.7] | 282.5 [1-4,764.3] | 0.771 |
| **Number of chromosome abnormalities (/case)** | 9 [3-17] | 4 [3-20] | <0.001 |
| **Number of monosomy (/case)** | 3 [0-10] | 0 [0-6] | <0.001 |
| **Induction regimen** | | | |
| Intensive chemotherapy* | 36 (46.8%) | 24 (63.2%) | 0.115 |
| VEN+AZA | 7 (9.1%) | 1 (2.6%) | 0.268 |
| AZA | 10 (13%) | 2 (5.3%) | 0.332 |
| CAG | 14 (18.2%) | 7 (18.4%) | 1 |
| Others | 2 (2.5%) | 3 (7.9%) | 0.330 |
| BSC | 8 (10.4%) | 1 (2.6%) | 0.268 |
| **Allo-HSCT** | 17 (22%) | 10 (26.3%) | 0.813 |
| **PIF†** | 23 (63.9%) | 12 (50%) | 0.301 |
| **Achieve CR†** | 20 (55.6%) | 17 (70.8%) | 0.285 |
| **Relapse†** | 7 (35%) | 8 (47.1%) | 0.516 |
| **Cause of death** | | | |
| AML | 37 (59.7%) | 16 (59.3%) | 1 |
| Infection | 15 (24.2%) | 6 (22.2%) | 1 |
| TRM | 4 (6.4%) | 1 (3.7%) | 1 |
| Others | 6 (9.7%) | 4 (14.8%) | 1 |

Allo-HSCT: allogeneic-hematopoietic stem cell transplantation, AML: acute myeloid leukemia, AML-MRC: AML with myelodysplasia-related changes, AZA: azacytidine, BM: bone marrow, BSC: best supportive care, CAG: low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor, CK: complex karyotype, CR: complete response, PIF: primary induction failure, t-AML: therapy related AML, TRM: treatment-related mortality, VEN: venetoclax, WT-1: Wilms tumor-1 gene

*Intensive chemotherapy composed of anthracyclines and cytarabine

†PIF, Achieve CR, and Relapse were calculated for those who treated by intensive chemotherapy.

**Table 2. Univariate and multivariate analyses of patient characteristics for overall survival**

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | *P* value | Hazard ratio (95% CI) | *P* value |
| 65 years or older | 1.775 (63-165) | 0.011 | 0.720 (0.370-1.398) | 0.332 |
| Male gender | 1.657(1.078-2.547) | 0.021 | 1.470 (0.941-2.295) | 0.090 |
| Typical CK-AML | 1.895 (1.169-3.073) | 0.010 | 1.727 (1.015-2.940) | 0.044 |
| 5,000 or more WBC count at diagnosis | 1.210 (0.792-1.849) | 0.377 | – | |
| Intensive chemotherapy* | 0.564 (0.371-0.858) | 0.007 | 0.957 (0.552-1.656) | 0.876 |
| Allo-HSCT | 0.342 (0.200-0.583) | <0.001 | 0.267 (0.125-0.573) | <0.001 |
| *TP53* mutation | 1.718 (1.108-2.663) | 0.016 | 1.459 (0.908-2.345) | 0.119 |

Allo-HSCT: allogeneic-hematopoietic stem cell transplantation, AML: acute myeloid leukemia, CK: complex karyotype

*Intensive chemotherapy composed of anthracyclines and cytarabine